Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Cell Biology

Spandrels of the cell nucleus

Irina Solovei[1] and Leonid Mirny[2]

S.J. Gould and R. Lewontin in their famous "Spandrels paper" (1979) argued that many anatomical elements arise in evolution not due to their "current utility" but rather due to other "reasons for origin", such as other developmental processes, physical constraints and mechanical forces. Here, in the same spirit, we argue that a variety of molecular processes, physical constraints, and mechanical forces, alone or together, generate structures that are detectable in the cell nucleus, yet these structures themselves may not carry any specific function, being a mere reflection of processes that produced them.

Addresses
[1] Biocenter, Ludwig Maximilians University Munich, Planegg-Martinsried, Germany
[2] Institute for Medical Engineering and Science, Department of Physics, Massachusetts Institute of Technology, Cambridge, MA, USA

Corresponding authors: Solovei, Irina (irina.solovei@lrz.uni-muenchen.de); Mirny, Leonid (leonid@mit.edu)

Current Opinion in Cell Biology 2024, **90**:102421

This review comes from a themed issue on **Cell Nucleus (2024)**

Edited by **Evi Soutoglou** and **Noriko Saitoh**

For complete overview of the section, please refer the article collection - Cell Nucleus (2024)

Available online 23 August 2024

https://doi.org/10.1016/j.ceb.2024.102421

Introduction

In 1979, the two famous evolutionists from Harvard University, Stephen Jay Gould and Richard Lewontin, raised the issue of the correct identification of factors causing evolutionary changes, essentially supporting the Darwinian pluralistic approach [1]. The authors appealed to distinguish between reasons for adaptive feature origin and possible accompanying feature that is merely a byproduct. The authors called this phenomenon "spandrels in evolution" referring to an architectural element found in cathedrals, bridges, or arches, which simply closes space between an arch curve and the ceiling but for a long time has been considered as a specially designed architectural decor (Figure 1) [2]. Spandrels are used for ornamental effect and mosaics, yet "the architectural constraint is clearly primary", argued Gould and Lewontin. Similarly, many phenomena and structures in biology arise due to numerous constraints, historical and mechanistic, they experience (Table 1).

One of the many biological examples the authors quote is the development of male genitalia in spotted hyena females, a phenomenon attributed earlier to its signal function important for mating. Recently, however, it has been shown that the development of such penis-like structure in females is a byproduct of an increased androgen level leading to elevated female aggressiveness, which is necessary for highly competitive hyena social life [3].

Like in other fields of biology, there is a tendency to decompose nuclear organization into elements and features recognizable in the global and attribute to them specific functions. Yet, nuclear organization is driven by organizing processes — such as transcription, replication, loop extrusion, compartmentalization, and lamina anchoring — and are highly constrained, e.g. due to the polymeric nature of chromosomes, nuclear space, and shape. Here we discuss several of such very well known or recently emerged cell nuclear features, that we believe result from processes and reflect them, yet lack any functional meaning themselves and call them "spandrels of the nucleus".

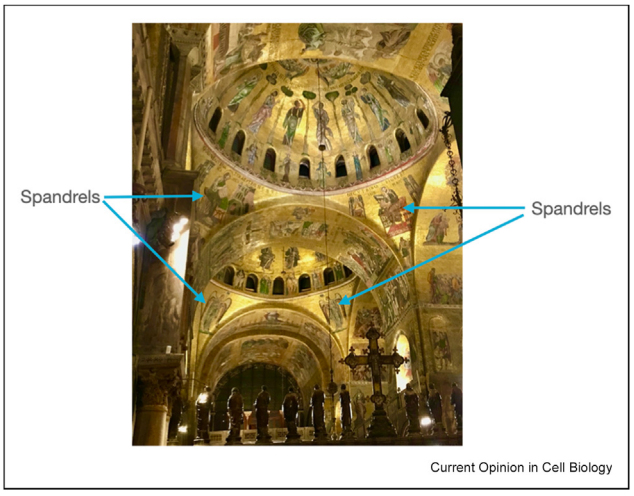
Global features of nuclear architecture

Chromosome territories

Chromosomal territoriality is a well-known paradigm in nuclear biology [4] and is listed as an important hierarchical level of genome folding in practically every review in the field, e.g. Refs. [5,6]. The paradigm in its modern shape emerged in the 1980s, contradicting the passive view from electron microscopy, which depicted chromosomes as dispersed and intermingled throughout the entire nucleus, similar to spaghetti in a bowl of broth (Figure 2a). Once observed, chromosome territoriality posed a question about its functional role.

Following mitosis, chromosomes decondense into seamless nuclear chromatin without distinguishable individual chromosomes. Only visualization of chromosomes with low-complexity paint probes reveals their positioning in the nucleus, providing a somewhat misleading territorial appearance [7]. Furthermore, in plants, yeast, and arguably in some mammalian cell types [8], chromosomes have so-called Rabl organization

Figure 1



Spandrels are triangular-shaped elements connecting the base of the dome to the support pillars (photo by Leonid Mirny).

with centromeres assembled in one nuclear location, telomeres on the opposite side, and chromosomal arms of different chromosomes stretched and spread between these sites in close contact with each other, and thus having no territorial organization [9]. Moreover, a degree of territoriality can vary significantly between cell types, most probably reflecting a duration of post-mitotic cell life and global nuclear rearrangements during cell differentiation, as has been demonstrated for mouse rod photoreceptors, olfactory and other neurons [10–12].

Another example is provided by species with Robertsonian translocations spontaneously occurring in animals with acrocentric chromosomes [13,14]. Chromosomes in these species fuse pairwise by their centromeric regions leading to the formation of new meta- or submetacentric chromosomes and new chromosome territories in interphase (Figure 2b). Although inter-chromatin interactions within new chromosomes have changed, the nuclear functions remain without any noticeable aberrations. Thus, Robertsonian mice remain viable and form huge populations [15]. Similarly, balanced translocations, not disrupting genes or regulatory elements but nonetheless changing chromosomal landscape, also do not perturb nuclear functions [16].

Although chromosomal territoriality is intuitively considered as a hindrance for translocations in somatic cells (e.g. Ref. [17]), the estimated degree of intermingling between chromosomes does not confirm this view. Thus, it was shown that gene-dense chromosomes intermingle with each other significantly stronger than gene-poor chromosomes, but they break and translocate less frequently [18].

Some close species have strongly reorganized karyotypes. For instance, the Chinese muntjac has 46 small acrocentric chromosomes, whereas the Indian muntjac – only 6 but noticeably larger chromosomes, evolved from multiple fusion events in the common muntjac-like ancestor [19]. Another striking example are gibbon species: although gibbons are close relatives of humans and great apes, their karyotypes are strongly rearranged with more than 70 translocations [20]. In both of these cases, we deal with very different chromosome territories in close species obviously not influencing their cellular or organismal physiology.

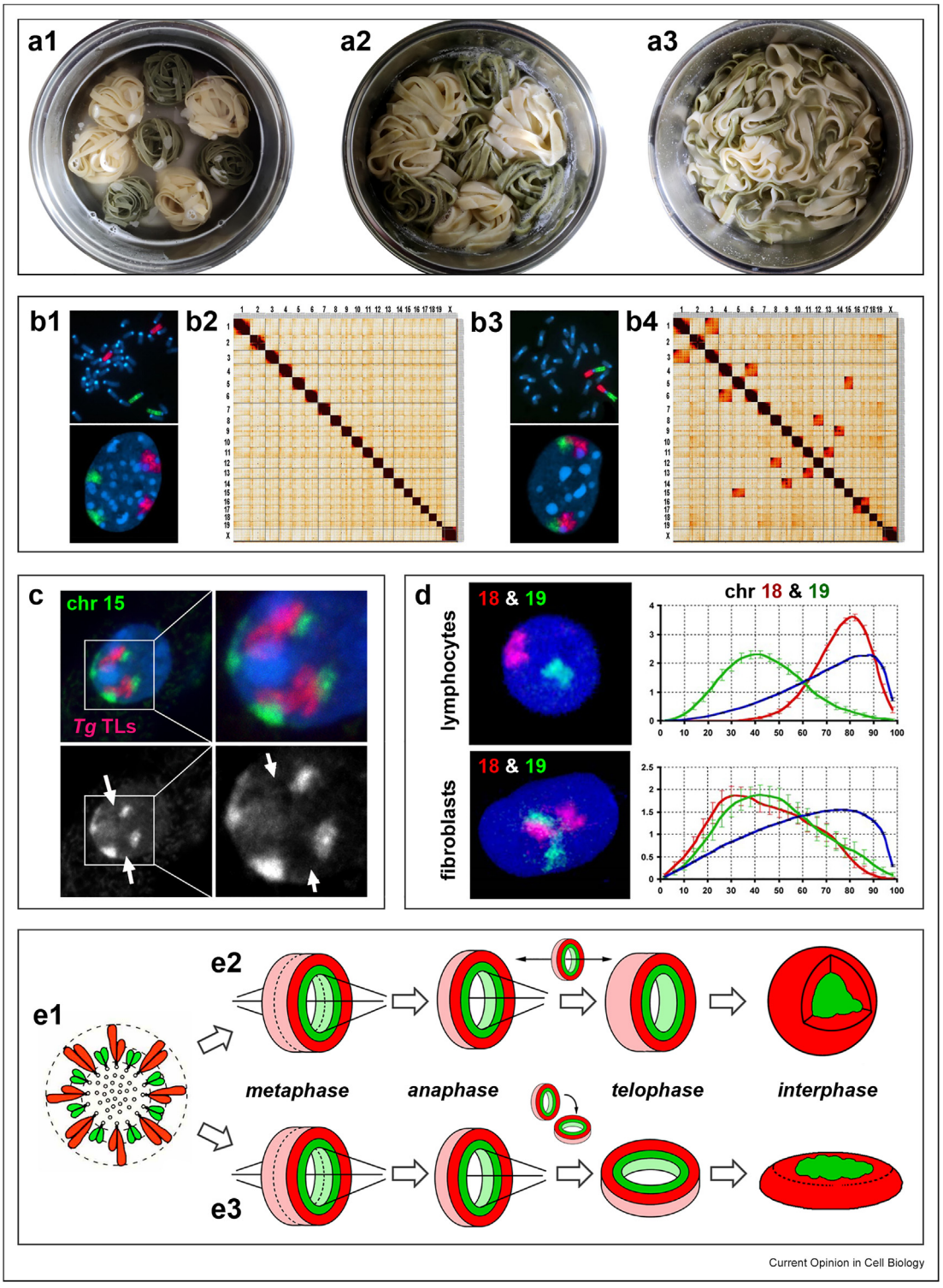
Finally, physical disruption of chromosomes without chromatin damage does not lead to any noticeable functional consequences. We have recently demonstrated that highly expressed genes form transcription loops, which in the case of long genes become stiff and

Table 1

List of discussed in the paper spandrels and their origin.

Cathedral/arches/bridges:	Byproduct of:
Spandrels as architectural element used for ornamental effect	closing the space between an arch curve and the ceiling
cell nucleus:	**byproduct/result of:**
Chromosome territories	the last mitosis, low global mobility of the interphase chromatin, and topological constraints further slowing down the mobility
Shape and positioning of interphase chromosomes	abundance and distribution of genes and LADs, geometrical constraints of a nucleus determined by a cell shape
Chromocenters	coalescence of satellite repetitive sequences during interphase
Patterns of compartmental interactions	chromatin constraints and affinities between homotypic chromatin regions
CTCF–CTCF dots	loop extrusion activity, when occluded by CTCFs
TADs	loop extrusion activity, when occluded by CTCFs
Fountains/jets	loop extrusion activity, and increased loading of extruders at specific loci
Transcription loops	intensive transcription and long transcripts
Condensates of RNAP or transcription factors	their affinity to accessible DNA, each other, and other cofactors

Figure 2



Spandrels of the nucleus on a global scale: chromosome territories.
(a), tagliatelle models of nuclear architecture in early G1 **(a1)** and in mid-interphase **(a2)**. **(a3)** depicts a contradictory to territoriality view on the chromosome arrangement in the nucleus as intermingling tagliatelle in a bowl.
(b), analysis of fibroblasts isolated from mice with normal karyotype **(b1, b2)** and with 7 Robertsonian translocations **(b3, b4)**. Chromosome paints for 4 (green) and 6 (red) chromosomes label two pairs of acrocentric chromosomes in metaphase (above) and four interphase chromosomes (below) of mouse cells with normal karyotype **(b1)**. The same probes label two arms of the derivative Robersonian chromosome 6.4 in metaphase (above) and interphase (below) cells **(b3)**. Note that the derivative 6.4 chromosome is submetacentric and consists of two arms, formed by chromosomes 6 and 4. The new

expand from harboring chromosomes. Mouse thyroglobulin gene locates on chromosome 15 and forms a huge transcription loop, which in some cells breaks the chromosome in two halves ultimately forming their own half-territories without obvious negative consequences [21] (Figure 2c).

The examples above demonstrate that the non-territorial organization of interphase chromosomes and altered territoriality due to balanced translocations, karyotype reshuffling, fusion or fission chromosomes do not alter interphase chromosome functions. Therefore, we suggest that CTs constitute a nuclear spandrel that has no specific functional role, merely representing a consequence of the last mitosis, emerging due to low mobility of the interphase chromatin.

Shape and positioning of interphase chromosomes

After the term "Chromosome Territory" was coined, much attention was paid to their shape and intranuclear positioning in various cell types and conditions. For instance, some of the mammalian chromosomes adopt a discoid flat shape, whereas others have a more spherical form [22]. Yet, an uneven distribution of both genic segments [7] and LADs (Lamina Associated Domains) [23] along chromosomes, is sufficient to explain why the positioning and shape of different chromosomes differ. Indeed, gene-poor and LAD-rich chromosomes are tethered to the nuclear envelope and thus acquire a flattened shape and remain at the nuclear periphery. Gene-rich and LAD-poor chromosomes are bound to the nuclear periphery mostly at the centromeric regions but otherwise adapt internal position simply because they lack multiple LADs [24] (Figure 2d), which is especially clearly visible in voluminous nuclei, such as neurons or stem cells [7]. Even in lobulated nuclei of human neutrophils, such radial distribution of chromosomes within single lobes is evident [25].

Many studies of chromosome distribution within an interphase nucleus were conducted on cultured cells (such as fibroblasts, epithelial cells, myoblasts, etc.) growing on a substrate and thus having a flattened shape with relatively flat nuclei (e.g. Ref. [26]). In contrast to the previous paragraph, analyses of chromosome distributions, regardless of their enrichment in genes and LADs, revealed a more central positioning of the small versus large chromosomes [27] (Figure 2d). This seemingly contradictory phenomenon, however, is caused by two unrelated factors. First, geometric centers of the large chromosomes in metaphase tend to be more peripheral than those of the smaller chromosomes [28] (Figure 2d1). This radial arrangement is inherited by anaphase and telophase cells, allowing small chromosomes to decondense closer to the 2D nuclear center and large chromosomes – closer to the 2D nuclear periphery. In addition, the large surface of flat nuclei eliminates competition of LADs for binding to the nuclear lamina [29], allowing chromosomes to decondense in places where they ended up in telophase (Figure 2d).

Therefore, the shape and nuclear positioning of interphase chromosomes are spandrels, which are determined by three pivotal functional factors/constraints: the abundance and distribution along a chromosome of (i) genes, and (ii) LADs, as well as (iii) geometrical constraints of a nucleus determined by a cell shape.

Chromocenters

Nuclei of many mammals possess so-called chromocenters (CC) formed by centromeric regions enriched in blocks of satellite DNA. In mice *Mus musculus*, such blocks consist of major satellite repeat constituting up to 10% of the entire mouse genome [30]. After mitosis, the subcentromeric heterochromatic blocks merge and form spherical CC, often inaccurately used as a proxy for heterochromatin. Number of CCs and their distribution varies between different mouse cell types or even within the same cell type – from 1 to about 40. Much of the literature is devoted to the description of quantity and positioning of CCs during differentiation and

chromosomes are also readily detectable by Hi-C – compare **(b2 and b4)** maps for all chromosomes.
(c), Tg transcription loop (red) breaks harboring chromosome 15 (green). Note that both homologous chromosomes are split in two parts with clear gap (arrows) "filled" with Tg transcription loop. For clarity, the gray scale image for chromosomes 15 is shown below the RGB image.
(d), telling example of radial distribution of human gene-rich (chr.19, green color) and gene-poor (chr. 18, red color) chromosomes in lymphocytes (upper raw) and cultured fibroblasts (low raw). Images on the left are projections through entire nuclei; graphs on the right reflect 3D relative radial distribution of the chromosome signals within a nucleus; the abscissa denotes the relative radius of 25 nuclear shells (with 0 as a nuclear center), the ordinate shows normalized relative DNA content (see Refs. [29,30] for details). In voluminous lymphocyte nuclei, gene-rich chromosome 19 sits mostly in the nuclear central area, whereas gene-poor 18 is adjacent to the periphery. In flat fibroblast nuclei, both small chromosomes 18 and 19 are central.
(e), schematics explaining seemingly contradictory chromosome distribution in spherical and flat nuclei shown in **(d)** (modified from Ref. [30]). **(e1)** depicts a polar view on metaphase chromosomes attached to a spindle; the centres of large chromosomes (red) are more peripheral than centres of small chromosomes (green). **(d2 and d3)** show inheritance of chromosome positioning from metaphase to interphase in cells with spherical **(d2)** and flat **(d3)** nuclei, respectively. When a spherical nucleus is built during late telophase and early G1, LAD-rich regions compete for the nuclear lamina and bring gene-poor/LAD-rich chromosomes (red) in the vicinity of the nuclear periphery. Gene-rich/LAD-poor chromosomes (green) are also attached to the heterochromatin rim at the nuclear or nucleolar periphery but only through several regions and thus adapt a more internal position. In flat nuclei, both gene-poor/LAD-rich and gene-rich/LAD-poor chromosomes do not compete for the lamina, which allows them to inherit their size-dependent distribution established in the metaphase. Small schematics above middle arrows symbolize transition from anaphase to telophase: in **(e2)**, arrows show bidirectional chromatin expansion of anaphase ultimately leading to a spherical nucleus; in **(e3)**, arrow shows direction of the anaphase declining to the substrate where a flattened daughter nucleus is formed.

development, in attempts to find a functional meaning of these characteristics (e.g. [31]).

Merging of CC was first attributed to their enrichment in HP1-class proteins and affinities of chromatin with heterochromatin histone marks, but more recent studies show HP1-independent and H3K9me-independent self-association of pericentromeric regions [32–34]. The most telling example of this process is coalescence of 10–14 smaller peripheral CCs in postmitotic progenitors of rod photoreceptors into a single CC in mature rods [35]. Another example are mouse cardiomyocytes: in a proportion of their nuclei – likely due to permanent cell contractions – CCs merge in irregular spiral structure traversing the whole nucleus along its long axis (Figure 3a1).

As the case with chromosome positioning, number and distribution of CCs is geometrically constrained by nuclear shape and volume. For instance, in many flat cultured cells, such as fibroblasts or myoblasts, nuclei are also flat and possess numerous CCs (15–20) distributed almost evenly in the 2D nuclear plain. In voluminous large nuclei, such as large neurons or Sertoli cells, most of the subcentromeric regions merge in several huge CCs at the nucleolus. In strongly elongated spindle-shaped nuclei, such as in smooth muscles, CCs are distributed along the long nuclear axis (Figure 3a1).

CCs are considered as a silencing compartment, and degree of gene activity has been assessed by proximity of a gene to a CC (e.g. Refs. [36,37]). Indeed, CCs are always confined to the either nuclear, or nucleolar peripheries and thus, not surprisingly, possess a plethora of typical heterochromatic PTMs and heterochromatin-associated proteins [38]. Nonetheless, some mouse genes are preferentially silenced at the nuclear periphery, whereas others – by closeness to CCs, e.g. *Taat* and *Olfs* gene families, respectively, in olfactory sensory neurons [39]. The reason for such differentiation is not clear and tentatively can be attributed to differential folding and intranuclear positioning of harboring chromosomes.

Underlying sequence of CCs in different species, as a rule, differs, and can be either AT-, or GC-rich, depending on species-specific satellite repeats, however, in all cases CCs remain as prominent nuclear structures. Even in close species CCs can be formed by different repeats, e.g. in *M. musculus* they are built by tandems of major satellite repeat, whereas in *Mus spretus* – by tandems of minor satellite repeat [40]. Moreover, a vast number of species lack large blocks of satellite DNA in centromeric regions, e.g. human, hamster, rats, etc. (Figure 3a2). Absence of CCs, however, does not prevent positional gene silencing in these species.

What is more, ectopic manipulations of the mouse satellite repeat, leading to a reinforced binding of this sequence to the nuclear envelope (Figure 3b1), results in the destruction of spherical CCs and formation of a thin continuous layer of major satellite repeat tightly adjacent to the nuclear lamina (Figure 3b2). Nonetheless, these cells are fully viable, undergo cycling, and are capable of terminal differentiation (Figure 3b3).

Therefore, CCs as such, are the typical nuclear spandrels, a phenomenon without a specific functional meaning but being a byproduct of the coalescence of satellite repetitive sequences during building a functional nucleus.

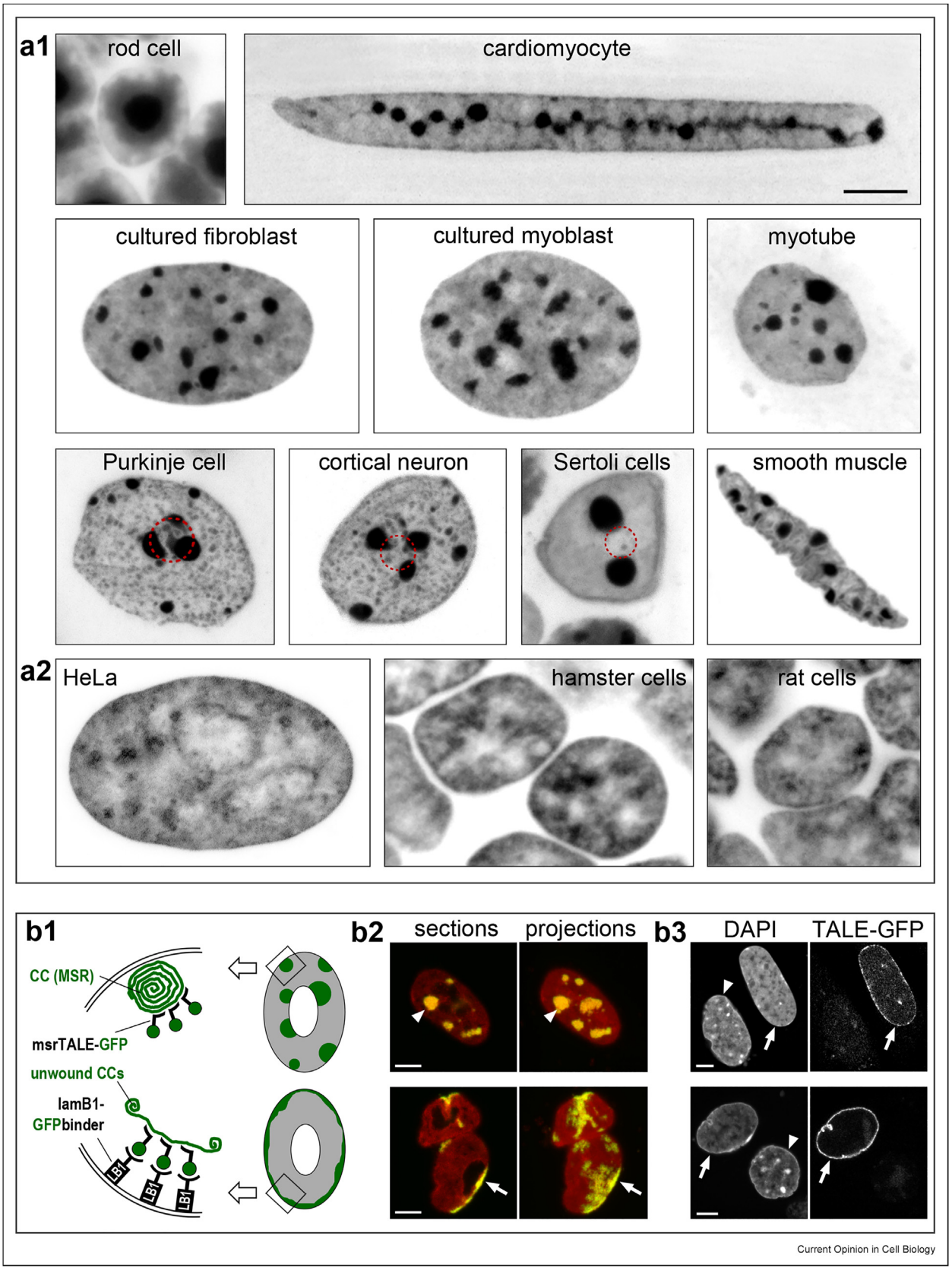
Patterns of compartmental interactions

One of the most visible patterns of interactions on Hi-C maps within and between chromosomes is the "checkerboard" that reflects chromatin compartmentalization, i.e. spatial segregation of transcriptionally active euchromatin (A) and inactive heterochromatin (B) [41]. The checkerboard pattern is the enrichment of AA and BB contacts, and the depletion of AB contacts. Recently we demonstrated that in mouse cells such spatial segregation is driven by the affinity of B regions to each other, with AA affinity being either entirely dispensable or weak [10]. Simulations with only BB affinities reproduce not only BB enrichment but also AA contact enrichments, which emerge because A regions are being displaced from parts of the nucleus occupied by B. Peripheral location of B regions is also driven by their anchoring at the nuclear lamina. These results suggest that in mouse and likely other mammalian nuclei, the pattern of interactions between large A regions is a byproduct emerging from excluded volume constraints and BB affinities. Nuclear organization in other species, however, can be driven by different mechanisms. For example, analysis of chromosome organization in the silkworm suggested that compartmentalization arises due to a complex interplay of AA affinities (stronger than BB) and non-uniform distribution of loop extruders [42].

Another Hi-C pattern, referred to as micro-compartments, constitutes patches of elevated contact frequency between short (~10–50 kb) regions, becoming visible in Micro-C [43,44], deep sequenced Hi-C [45], but most pronounced in Region Capture Micro-C [73,74]. Most prominent among them are contacts between short H3K27ac stretches. This contact enrichment is also seen in average pile-ups across thousands of H3K27ac regions [46].

However, the need for averaging over thousands of regions or exceedingly deep sequencing to them, indicates that individual contacts between such regions are rare, and affinities are weak. Measurement of contact frequency for pairs of such loci using DNA FISH showed exceedingly rare, if observable at all, pairwise contacts [46]. While it is feasible that such low-affinity

Figure 3


a1
rod cell
cardiomyocyte
cultured fibroblast
cultured myoblast
myotube
Purkinje cell
cortical neuron
Sertoli cells
smooth muscle
a2
HeLa
hamster cells
rat cells
b1
CC (MSR)
msrTALE-GFP
unwound CCs
lamB1-
GFPbinder
LB1
LB1
LB1
b2
sections
projections
b3
DAPI
TALE-GFP
Current Opinion in Cell Biology

interactions can help to stabilize transient enhancer-promoter interactions separated by less than a megabase, the vast majority of patches are located at larger separation and between chromosomes. They can be too infrequent to associate with any cellular process requiring reliable outcomes.

Together these considerations suggest that while compartmentalization plays many roles, from providing optimal conditions for gene expression to supporting epigenetic memory, only part of the contact patterns that we see are causal and/or functional. Patches of AA compartmental and the vast majority of micro-compartmental interactions can arise as byproducts of constraints and affinities, and thus represent chromatin spandrels. Understanding the functional roles of compartmentalization as a phenomenon, rather than patterns it creates, is an important next goal.

Local elements of chromatin folding

Cohesin plays a central role in shaping local (<1–3 Mb) chromosome organization due to its function as a loop-extruding motor. Polymer models demonstrated that extrusion, when occluded by boundaries, can reproduce Hi-C patterns of interphase chromosomes such as TAD, CTCF–CTCF dots, and stripes [47]. Facilitated loading of loop extruders at specific sites, particularly enhancers, likely underlies the formation of a recently discovered Hi-C pattern of "jets" [48,49], or "fountains" [50,51]. Are these patterns on Hi-C maps functional or constitute loop extrusion spandrels merely reflecting the extrusion process that itself has multiple roles?

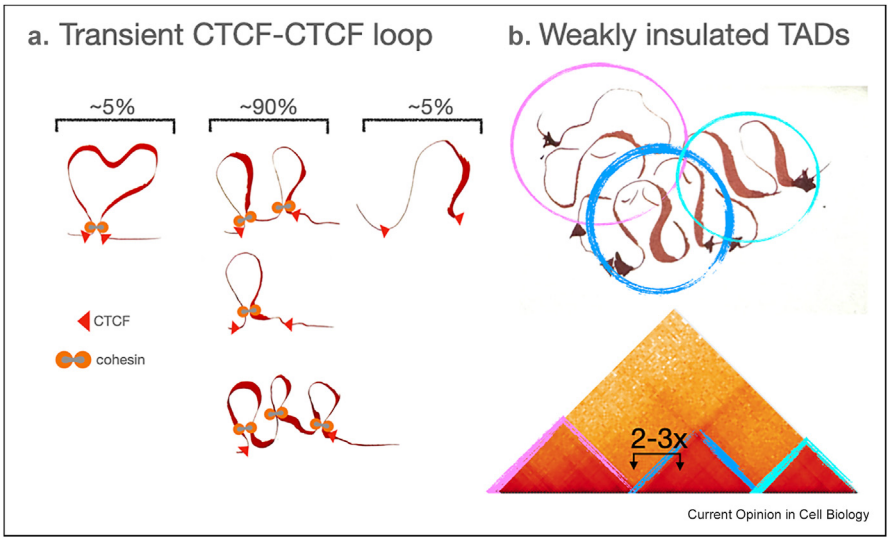
CTCF–CTCF dots are local enrichments of contact frequency on Hi-C or Micro-C maps [43,44,52,53] that represent loops formed between two CTCF sites, typically oriented towards each other [54]. Once discovered [53], they generated lots of excitement in the hope that they represent long-sought enhancer-promoter interactions. Yet, bioinformatic analysis showed a lack of enrichment for enhancers near CTCFs [53,55]. Simulations calibrated on Hi-C data predict that such CTCF–CTCF loops emerge naturally when CTCF stops cohesin yet being very transient: present for 25% and 7% of the time for a pair of CTCFs separated by 180 kb and 360 kb correspondingly [47] (Figure 4a). Recent experiments measured such CTCF–CTCF contact frequency in living cells, yielding the frequency of ~30% and 6% for CTCFs separated by 150 kb [56] and 505 kb [57] correspondingly. Live-cell imaging also measured, for the first time, the duration of such interactions yielding ~20 min, for *Fbn2* locus. The frequency and the duration together suggest that two CTCF boundaries of the *Fbn2* locus are forming a contact about once during the 24 h cell cycle. Such rare and transient CTCF–CTCF loops are unlikely to represent a functional enhancer-promoter interaction, as a typical response time of enhancer-mediated gene activation is ~10 min [58].

Together, this rareness and the lack of enrichment of enhancers at CTCFs, suggests that CTCF–CTCF dots represent structural elements emerging due to the loop extrusion activity, but have no specific function by themselves and thus represent spandrels.

TADs were first discovered as regions with increased frequency of contacts within each region, and decreased contact frequency with its neighbors [59,60]. Simulations showed that TADs emerge as a result of loop extrusion occluded by extrusion barriers such as CTCFs, as was recently observed in single-molecule experiments [61]. After functional studies demonstrated that TADs demarcate regions of enhancer activity [62,63], TADs were hailed as insulated or regulatory neighborhoods [64]. Yet available data put such a designation in question. First, Hi-C shows a rather modest (~2 fold) increase of contact frequency within TADs as compared to between TADs [65,66] (Figure 4b). Such weak insulation of contacts is unlikely to provide a reliable insulation of enhancer-promoter interactions. Second,

Spandrels of the nucleus on a global scale: chromocenters (CCs).
(a), nuclear of various mouse cell types exhibiting different arrangement of CCs after DAPI staining **(a1)**. Note that the number of CCs varies from one (rods) or two (Sertoli cells) to many in other cell types. Nuclei without CCs are exemplified by HeLa cells and neurons of two rodent species **(a2)**. Images are full or partial confocal stack projections; for clarity, DAPI images are inverted; red dash lines mark positioning of nucleoli. Scale bar, 5 μm and valid for all panels.
(b), ectopic manipulations of the mouse MSR leads to reinforced massive binding of this sequence to the nuclear envelope but does not change cell cycling or differentiation.
(b1), in cells stably expressing msrTALE conjugated with GFP, the construct targets MSR, the major component of the mouse CCs. After transient transfection of cells with chimeric protein lamina B1 conjugated with GFP-binder, spherical chromocenters lose their shape and become flattened as a result of tight tethering to the nuclear lamina.
(b2), FISH with probe for MSR in untransfected (above) and transfected (below) mESCs. Images on the left are single optical sections; images on the right are maximum intensity projections. Arrowheads point at spherical CCs; arrows – at flattened CCs. FISH signal is yellow; nuclei counterstained with DAPI (red); scale bar, 5 μm.
(b3), differentiation of transfected myoblasts into myotubes. The upper panel images show two cultured myoblasts; one exhibits a clear rim of GFP staining resulting from CC flattening after transfection (arrow); the other one has conventional CCs (arrowhead). The low panel images show nuclei of the same myotube – one with conventional CCs (arrowhead) and one transfected with flattened CCs (arrows). Images are single optical sections; scale bars, 5 μm.

Figure 4



Spandrels of the nucleus on a local scale: chromatin folding.
(a), An illustration of a transient CTCF–CTCF loop that is fully formed only ~5% of the time and partially extruded 90%, making CTCF–CTCF pair an unlikely candidate for reliable enhancer-promoter interactions.
(b), TADs are weakly insulated with only 2–3 fold increase of intra-TAD vs inter-TAD interactions. This makes a TAD rather unreliable for restricting enhancer-promoter interactions.

microscopy demonstrated that TADs don't correspond to "insulated" crumples of chromatin, but rather have numerous contacts with their neighbors. While median distances between loci within a TAD are smaller than those between TADs, distance distributions remain wide [67]. Finally, the notion of TADs as regulatory neighborhoods has been challenged by a recent study [68]that examined a phenomenon that they termed "border bypass", i.e. functionally important activation of a gene by an enhancer located outside of a TAD. This study demonstrated that loop extrusion can facilitate such cross-border (and cross-CTCF) interactions, yet Hi-C and microscopy consistently see insulated TADs. Given that TADs are poorly isolated regions with modest enrichment of contacts and highly variable structures, how can a TAD reliably insulate enhancer activity?

One possibility is that Hi-C and microscopy provide a rather incomplete picture due to their inability to distinguish functional interaction at the molecular scale (e.g. nucleosome-size $\sim$10 nm) from a mere proximity ($\sim$100–350 nm). Another possibility is that enhancer-promoter interactions are not so much restricted by TADs, but rather facilitated by the perpetual process of loop extrusion, with barriers targeting enhancer-promoter interactions within a TAD, whereas other chromatin contacts are not influenced. Complex extrusion-mediated communications can be potentially revealed by deeper and more focused Micro-C approaches [69,70]. Lastly, it's possible that the modest difference in contact frequency within versus between TADs are translated by transcription machinery into a much higher difference in expression. Two elegant models of multi-state promoter activation that can achieve such non-linear "reading" of contacts have been recently put forward [65,66]. Together, these arguments suggest that the way transcriptional machinery is seeing transient extrusion-mediated contacts is very different from the way we see them in Hi-C and microscopy, rendering TADs as spandrels.

Fountains/jets are recently discovered patterns on Hi-C maps, observed in mouse quiescent thymocytes [48], in early zebrafish development and mouse ESCs [50], in *C. elegans* [49,51], and in specific knock-outs [71]. Fountains are formed at active enhancers [50,51], and were proposed to represent cohesin loading sites. Yet, the functional role of such facilitated load of cohesin at enhancers remains mysterious. Importantly, observed shapes of fountains indicate two-sided extrusion by loaded cohesins, increasing contact frequency between regions flanking the loading site, but not of the enhancer where the loading initiates. One possibility is that such loading plays no particular functional role, but merely reflects higher chromatin accessibility of enhancers, hence cohesin is more likely to load there. Another possibility is that a small fraction of cohesins starts one-sided extrusion, generating contacts of the loading enhancer with nearby genes, while the vast majority of cohesins forming the fountain patterns are of no immediate functional role. Whichever scenario is more

feasible, the fountain itself is a mere reflection of the loading process, manifesting another chromatin spandrel.

TADs, CTCF–CTCF dots, and fountains can be mere reflections of the extrusion process, initiated at specific locations, and occluded by extrusion barriers. While these features may have no functional roles themselves, loop extrusion by SMCs, with their complex rule of interactions between themselves [72] and other chromatin processes [73], can play multiple functional roles in gene regulation [74,75], generation of isoform diversity [76] and somatic recombination [77].

Transcription loops

A recent microscopy study has revealed the formation of micrometer-sized loops by long and highly transcribed genes [21]. Spanning across the nuclear interior, such structures emerge as a result of the transcription, but may not have any adaptive or regulatory role. Indeed, the body of a long and highly expressed gene is covered by dense convoys of RNA polymerases, each carrying a large nascent ribonucleoprotein (RNP) particle, consisting of nascent RNA and a multitude of RNA-processing proteins. Simulations showed that the steric repulsion between such nascent RNPs can make the body of the gene incredibly stiff, similar to the phenomenon known in the polymer physics of bottlebrushes [78]. Such stiffness, in turn, forces the gene to expand from its locus and stretch across the nucleus. No adaptive argument is needed to explain the formation of such loops, because they emerge naturally due to polymer and steric phenomena, and likely constitute a transcriptional spandrel.

Condensates and other nuclear phenomena

Other nuclear phenomena can potentially be also categorized as spandrels. While nuclear condensates were hailed as the primary mechanism underlying transcription, enhancer activity, and splicing, their presence in physiological conditions and functional roles remain contested. Some may constitute functional structures, while others can be mere aggregates of inactive proteins. For example, the majority of nuclear speckles may constitute storage sites of the splicing machinery, with only a few being engaged with and formed at the sites of transcription [79]. RNAP condensates were implicated in enhancer-mediated gene activation (e.g. Ref. [80]), yet large RNAP condensates are rare (very few per cell [80]), with most RNAPs present in clusters of 1–3 according to a recent quantification [81], suggesting that very small RNAP condensates can be at the disposal for most (~5,000–10,000) transcribed genes. Similarly, condensates of transcription factors were suggested to be formed through affinities of their activation domains, implicating such condensates in transcription activation. Yet the formation of such condensates has no activating (and possibly inhibitory) effect on transcription [82]. Binding of RNAP and transcription factors to accessible DNA likely underlie the appearance of large condensates, and multivalent binding of their factors activation domains involved in recruitment of coactivators, independent of condensate formation [82,83]. Together, these evidences nominate nuclear speckles, RNAP and transcription factor condensates as spandrels of the cell nucleus.

Outlook

Examples we provide here demonstrate that many named patterns and features seen in the data can be spandrels of the cell nucleus (Table 1). They emerge as a result of specific processes or mechanisms, being as compartmentalization, loop extrusion, transcription, or their interplay, but as such may have no adaptive or regulatory function. Nonetheless, features seen in Hi-C or microscopy data are valuable in allowing to infer underlying processes, akin to the way fluctuations in the light from distant stars help detect and characterize properties and dynamics of exoplanets not visible individually.

An intriguing question is when and how a trait originating as a byproduct is harnessed by the evolution for a different function or mechanisms later on – in other words, when a spandrel is exempted from spandrels. For instance, when CCs emerged as an evident feature of the nucleus, have they later become special silencing compartments in some cell types or used to achieve desired optical properties in other cell types?

We have earlier speculated that the accumulation of repetitive sequences in mammalian genomes was driven by selective pressure to convert nuclei of nocturnal rod photoreceptors into microlenses, improving their night vision by reducing light scattering [11]. In other cell types, however, the abundant repeats in heterochromatin started to contribute to transcriptional silencing, thus converting a “spandrel” into a useful silencing compartment.

Similarly, loop extrusion, now seen across kingdoms from bacteria to multicellular eukaryotes, may have emerged as a way of resolving sister chromatids in bacteria. Later it could have gotten exapted to compact longer chromosomes, while generating spandrels of interactions between promoters, as seen in Dictyostelium, that later was harnessed to moderate promoter–enhancer interaction, VDJ recombination, DSB repair, and has potentially other functions.

Overall, patterns and features can be valuable to detect and characterize mechanisms of nuclear organization, yet naming patterns with descriptive names (e.g. domains) runs the risk of creating an illusion that

properties associated with the name (e.g. domains are isolated from each other) are associated with the object itself. Moreover many patterns seen in microscopy or Hi-C reflect mere tendencies (e.g. tendency of transcription factors to aggregate) rather than stable structures, and may or may not have any functional implications. These are some of the reasons why it is important to identify spandrels. Next time, being tempted to assign a function to a pattern, a researcher can raise her eyes and look at the ceiling of a cathedral.

Author's contribution

Conceptualization and Visualization, Conceptualization: L.M., I.S.; Writing - Original Draft: I.S., L.M.; Writing - Review & Editing: L.M., I.S.; Visualization: I.S., L.M.; Funding acquisition: I.S., L.M.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Acknowledgments

We are grateful to Silvia Garagna (Universita' degli Studi di Pavia, Pavia) for providing fibroblasts isolated from Riobertsonian mice and Job Dekker (University of Massachusetts Chan Medical School, Worcester) for Hi-C analysis of fibroblast with conventional and re-arranged karyotypes. Katharina Thanisch (Ludwig Maximilians University Munich) generated cell lines and performed experiments with ectopic manipulations in mouse ESCs and myoblasts. We are very much grateful to both reviewers who did an excellent job of careful reading our manuscript and making constructive comments and excellent suggestions, which we have appreciatively implemented. LM is supported by NSF2044895, NIH GM114190, and NIH UM1HG011536 grants; IS is supported by the Deutsche Forschungsgemeinschaft grants SPP2202/SO1054/2 (project # 422388934) and SFB106 (project # 213249687).

References

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest
** of outstanding interest

1. Gould SJ, Lewontin RC: **The spandrels of San Marco and the Panglossian paradigm: a critique of the adaptationist programme**. *Proc R Soc Lond B Biol Sci* 1979, **205**:581–598.

2. Gould SJ: **The exaptive excellence of spandrels as a term and prototype**. *Proc Natl Acad Sci U S A* 1997, **94**: 10750–10755.

3. Frank LG: **Evolution of genital masculinization: why do female hyaenas have such a large "penis"?** *Trends Ecol Evol* 1997, **12**:58–62.

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

5. McCord RP, Xu Y, Li H, Das P, San Martin R: **SnapShot: chromosome organization**. *Mol Cell* 2022, **82**:2350–2350.e1.

6. Perillo B, Migliaccio A, Castoria G: **Chromatin looping links gene expression to the assembly of transcription factories** (Review) *Mol Med Rep* 2024, **29**.

7. Solovei I, Thanisch K, Feodorova Y: **How to rule the nucleus: divide et impera**. *Curr Opin Cell Biol* 2016, **40**:47–59.

8. ** Hoencamp C, Dudchenko O, Elbatsh AMO, Brahmachari S, Raaijmakers JA, van Schaik T, Sedeño Cacciatore Á, Contessoto VG, van Heesbeen RGHP, van den Broek B, *et al.*: **3D genomics across the tree of life reveals condensin II as a determinant of architecture type**. *Science* 2021, **372**:984–989.
This paper presents analysis of genome folding in animal and plant species scattered through the entire eukaryotic tree of life. The authors defined the two types of 3D genome folding defined by changes in condensin II – chromosome territories and Rable-like orientation – that repeatedly appear or disappear during evolution.

9. * Tourdot E, Grob S: **Three-dimensional chromatin architecture in plants - general features and novelties**. *Eur J Cell Biol* 2023, **102**:151344.
The authors composed one of the most comprehensive reviews on 3D genome folding in plants, based on microscopy and 3C-family methods.

10. Falk M, Feodorova Y, Naumova N, Imakaev M, Lajoie BR, Leonhardt H, Joffe B, Dekker J, Fudenberg G, Solovei I, *et al.*: **Heterochromatin drives compartmentalization of inverted and conventional nuclei**. *Nature* 2019, **570**:395–399.

11. Feodorova Y, Falk M, Mirny LA, Solovei I: **Viewing nuclear architecture through the eyes of nocturnal mammals**. *Trends Cell Biol* 2020, **30**:276–289.

12. Monahan K, Horta A, Lomvardas S: **LHX2- and LDB1-mediated trans interactions regulate olfactory receptor choice**. *Nature* 2019, **565**:448–453.

13. Romanenko SA, Perelman PL, Trifonov VA, Graphodatsky AS: **Chromosomal evolution in Rodentia**. *Heredity* 2012, **108**:4–16.

14. Popescu PC: **Chromosomes of the cow and bull**. In *Advances in veterinary science and comparative medicine*. Edited by McFeely RA, Academic Press; 1990:41–71.

15. Garagna S, Page J, Fernandez-Donoso R, Zuccotti M, Searle JB: **The Robertsonian phenomenon in the house mouse: mutation, meiosis and speciation**. *Chromosoma* 2014, **123**: 529–544.

16. Warburton D: **De novo balanced chromosome rearrangements and extra marker chromosomes identified at prenatal diagnosis: clinical significance and distribution of breakpoints**. *Am J Hum Genet* 1991, **49**:995–1013.

17. Rosin LF, Crocker O, Isenhart RL, Nguyen SC, Xu Z, Joyce EF: **Chromosome territory formation attenuates the translocation potential of cells**. *Elife* 2019, **8**.

18. Bickmore WA, Teague P: **Influences of chromosome size, gene density and nuclear position on the frequency of constitutional translocations in the human population**. *Chromosome Res* 2002, **10**:707–715.

19. Mudd AB, Bredeson JV, Baum R, Hockemeyer D, Rokhsar DS: **Analysis of muntjac deer genome and chromatin architecture reveals rapid karyotype evolution**. *Commun Biol* 2020, **3**:480.

20. Müller S, Hollatz M, Wienberg J: **Chromosomal phylogeny and evolution of gibbons (Hylobatidae)**. *Hum Genet* 2003, **113**: 493–501.

21. ** Leidescher S, Ribisel J, Ullrich S, Feodorova Y, Hildebrand E, Galitsyna A, Bultmann S, Link S, Thanisch K, Mulholland C, *et al.*: **Spatial organization of transcribed eukaryotic genes**. *Nat Cell Biol* 2022, **24**:327–339.
This work shows spatial arrangement of a single gene during its transcription and is based on analysis of highly expressed and relatively long genes. Hi-C analysis, microscopy and polymer modeling demonstrated that transcribed genes form open-ended transcription loops with polymerases moving along the loops and carrying nascent RNPs. Dense decoration of transcription loops with these balky protein and RNP complexes increases gene stiffness and facilitates its extension from a harboring locus. These findings contradict the popular transcription factory model.

22. Eils R, Dietzel S, Bertin E, Schröck E, Speicher MR, Ried T, Robert-Nicoud M, Cremer C, Cremer T: **Three-dimensional reconstruction of painted human interphase chromosomes: active and inactive X chromosome territories have similar volumes but differ in shape and surface structure**. *J Cell Biol* 1996, **135**:1427–1440.

23. Guelen L, Pagie L, Brasset E, Meuleman W, Faza MB, Talhout W, Eussen BH, de Klein A, Wessels L, de Laat W, *et al.*: **Domain organization of human chromosomes revealed by mapping of nuclear lamina interactions**. *Nature* 2008, **453**:948–951.

24. Weierich C, Brero A, Stein S, von Hase J, Cremer C, Cremer T, Solovei I: **Three-dimensional arrangements of centromeres and telomeres in nuclei of human and murine lymphocytes**. *Chromosome Res* 2003, **11**:485–502.

25. Keenan CR, Mlodzianoski MJ, Coughlan HD, Bediaga NG, Naselli G, Lucas EC, Wang Q, de Graaf CA, Hilton DJ, Harrison LC, *et al.*: **Chromosomes distribute randomly to, but not within, human neutrophil nuclear lobes**. *iScience* 2021, **24**, 102161.

26. Bolzer A, Kreth G, Solovei I, Koehler D, Saracoglu K, Fauth C, Müller S, Eils R, Cremer C, Speicher MR, *et al.*: **Three-dimensional maps of all chromosomes in human male fibroblast nuclei and prometaphase rosettes**. *PLoS Biol* 2005, **3**, e157.

27. Cremer M, von Hase J, Volm T, Brero A, Kreth G, Walter J, Fischer C, Solovei I, Cremer C, Cremer T: **Non-random radial higher-order chromatin arrangements in nuclei of diploid human cells**. *Chromosome Res* 2001, **9**:541–567.

28. Habermann FA, Cremer M, Walter J, Kreth G, von Hase J, Bauer K, Wienberg J, Cremer C, Cremer T, Solovei I: **Arrangements of macro- and microchromosomes in chicken cells**. *Chromosome Res* 2001, **9**:569–584.

29. * Gholamalamdari O, van Schaik T, Wang Y, Kumar P, Zhang L, Zhang Y, Hernandez Gonzalez GA, Vouzas AE, Zhao PA, Gilbert DM, *et al.*: **Beyond A and B Compartments: how major nuclear locales define nuclear genome organization and function**. *bioRxiv* 2024, https://doi.org/10.1101/2024.04.23.590809.
The paper describes genome organization of several human cell types with respect to their shape and nuclear locales.

30. Vissel B, Choo KH: **Human alpha satellite DNA–consensus sequence and conserved regions**. *Nucleic Acids Res* 1987, **15**:6751–6752.

31. Solovei I, Grandi N, Knoth R, Volk B, Cremer T: **Positional changes of pericentromeric heterochromatin and nucleoli in postmitotic Purkinje cells during murine cerebellum development**. *Cytogenet Genome Res* 2004, **105**:302–310.

32. ** Stutzman AV, Hill CA, Armstrong RL, Gohil R, Duronio RJ, Dowen JM, McKay DJ: **Heterochromatic 3D genome organization is directed by HP1a- and H3K9-dependent and independent mechanisms**. *Mol Cell* 2024, https://doi.org/10.1016/j.molcel.2024.05.002.
Using Drosophila cells, the authors demonstrate that although both H3K9me3 and HP1alpha contribute to segregation of heterochromatin from euchromatin domains, self-association of pericentromeric heterochromatin regions occurs despite loss of H3K9-bound HP1a.

33. Larson AG, Elnatan D, Keenen MM, Trnka MJ, Johnston JB, Burlingame AL, Agard DA, Redding S, Narlikar GJ: **Liquid droplet formation by HP1α suggests a role for phase separation in heterochromatin**. *Nature* 2017, **547**:236–240.

34. Strom AR, Emelyanov AV, Mir M, Fyodorov DV, Darzacq X, Karpen GH: **Phase separation drives heterochromatin domain formation**. *Nature* 2017, **547**:241–245.

35. Solovei I, Kreysing M, Lanctôt C, Kösem S, Peichl L, Cremer T, Guck J, Joffe B: **Nuclear architecture of rod photoreceptor cells adapts to vision in mammalian evolution**. *Cell* 2009, **137**:356–368.

36. Brown KE, Baxter J, Graf D, Merkenschlager M, Fisher AG: **Dynamic repositioning of genes in the nucleus of lymphocytes preparing for cell division**. *Mol Cell* 1999, **3**:207–217.

37. Brown KE, Amoils S, Horn JM, Buckle VJ, Higgs DR, Merkenschlager M, Fisher AG: **Expression of alpha- and beta-globin genes occurs within different nuclear domains in haemopoietic cells**. *Nat Cell Biol* 2001, **3**:602–606.

38. Eberhart A, Feodorova Y, Song C, Wanner G, Kiseleva E, Furukawa T, Kimura H, Schotta G, Leonhardt H, Joffe B, *et al.*: **Epigenetics of eu- and heterochromatin in inverted and conventional nuclei from mouse retina**. *Chromosome Res* 2013, **21**:535–554.

39. Yoon K-H, Ragoczy T, Lu Z, Kondoh K, Kuang D, Groudine M, Buck LB: **Olfactory receptor genes expressed in distinct lineages are sequestered in different nuclear compartments**. *Proc Natl Acad Sci USA* 2015, **112**:E2403–E2409.

40. Wong AK, Biddle FG, Rattner JB: **The chromosomal distribution of the major and minor satellite is not conserved in the genus Mus**. *Chromosoma* 1990, **99**:190–195.

41. Mirny LA, Imakaev M, Abdennur N: **Two major mechanisms of chromosome organization**. *Curr Opin Cell Biol* 2019, **58**:142–152.

42. ** Gil J, Rosin LF, Navarrete E, Chowdhury N, Abraham S, Cornilleau G, Lei EP, Mozziconacci J, Mirny LA, Muller H, *et al.*: **Unique territorial and sub-chromosomal organization revealed in the holocentric moth Bombyx mori**. *bioRxiv* 2023, https://doi.org/10.1101/2023.09.14.557757.
3D genome architecture of the holocentric silkworm *Bombyx mori* is described and several unusual for monocentric genomes features are revealed. The study demonstrates a new type of chromatin compartmentalization caused by global affinity-mediated contacts and locally concentrated loop extrusion.

43. Krietenstein N, Abraham S, Venev SV, Abdennur N, Gibcus J, Hsieh T-HS, Parsi KM, Yang L, Maehr R, Mirny LA, *et al.*: **Ultrastructural details of mammalian chromosome architecture**. *Mol Cell* 2020, https://doi.org/10.1016/j.molcel.2020.03.003.

44. Hsieh T-HS, Cattoglio C, Slobodyanyuk E, Hansen AS, Darzacq X, Tjian R: **Enhancer-promoter interactions and transcription are largely maintained upon acute loss of CTCF, cohesin, WAPL or YY1**. *Nat Genet* 2022, **54**:1919–1932.

45. Harris HL, Gu H, Olshansky M, Wang A, Farabella I, Eliaz Y, Kalluchi A, Krishna A, Jacobs M, Cauer G, *et al.*: **Chromatin alternates between A and B compartments at kilobase scale for subgenic organization**. *Nat Commun* 2023, **14**:3303.

46. Friman ET, Flyamer IM, Marenduzzo D, Boyle S, Bickmore WA: **Ultra-long-range interactions between active regulatory elements**. *Genome Res* 2023, **33**:1269–1283.

47. Fudenberg G, Imakaev M, Lu C, Goloborodko A, Abdennur N, Mirny LA: **Formation of chromosomal domains by loop extrusion**. *Cell Rep* 2016, **15**:2038–2049.

48. * Guo Y, Al-Jibury E, Garcia-Millan R, Ntagiantas K, King JWD, Nash AJ, Galjart N, Lenhard B, Rueckert D, Fisher AG, *et al.*: **Chromatin jets define the properties of cohesin-driven in vivo loop extrusion**. *Mol Cell* 2022, **82**:3769–3780.e5.
A new Hi-C feature, "jet," has been observed in thymocytes and suggested to reflect cohesin loading these sites.

49. * Kim J, Wang H, Ercan S: **Cohesin mediated loop extrusion from active enhancers form chromatin jets in C. elegans**. *bioRxiv* 2024, https://doi.org/10.1101/2023.09.18.558239.
Observation of "jets" in Hi-C maps of *C. elegans*, demonstrating that they are formed at active enhancers.

50. * Galitsyna A, Ulianov SV, Bykov NS, Veil M, Gao M, Perevoschikova K, Gelfand M, Razin SV, Mirny L, Onichtchouk D: **Extrusion fountains are hallmarks of chromosome organization emerging upon zygotic genome activation**. *bioRxiv* 2023, https://doi.org/10.1101/2023.07.15.549120.
Chromatin "fountains" (akin to "jets") were observed at the onset of Zygotic Genome Activation in zebrafish. Fountains are the first features to emerge during development and are formed at active enhancers. Similar structures were found at mouse enhancers and were cohesin-dependent, confirmed them as cohesin-loading sites.

51. * Isiaka BN, Semple JI, Haemmerli A, Thapliyal S, Stojanovski K, Das M, Gilbert N, Glauser DA, Towbin B, Jost D, *et al.*: **Cohesin forms fountains at active enhancers in C. elegans**. *bioRxiv* 2023, https://doi.org/10.1101/2023.07.14.549011.
Fountains observed in Hi-C maps of C.elegans, demonstrated to be cohesin-dependent and controlling transcription of nearby genes.

52. Akgol Oksuz B, Yang L, Abraham S, Venev SV, Krietenstein N, Parsi KM, Ozadam H, Oomen ME, Nand A, Mao H, *et al.*: **Systematic evaluation of chromosome conformation capture assays**. *Nat Methods* 2021, **18**:1046–1055.

53. Rao SSP, Huntley MH, Durand NC, Stamenova EK, Bochkov ID, Robinson JT, Sanborn AL, Machol I, Omer AD, Lander ES, *et al.*: **A 3D map of the human genome at kilobase resolution reveals principles of chromatin looping**. *Cell* 2014, **159**: 1665–1680.

54. Vietri Rudan M, Barrington C, Henderson S, Ernst C, Odom DT, Tanay A, Hadjur S: **Comparative Hi-C reveals that CTCF underlies evolution of chromosomal domain architecture**. *Cell Rep* 2015, **10**:1297–1309.

55. Kubo N, Ishii H, Xiong X, Bianco S, Meitinger F, Hu R, Hocker JD, Conte M, Gorkin D, Yu M, *et al.*: **Promoter-proximal CTCF binding promotes distal enhancer-dependent gene activation**. *Nat Struct Mol Biol* 2021, **28**:152–161.

56. ** Mach P, Kos PI, Zhan Y, Cramard J, Gaudin S, Tünnermann J, Marchi E, Eglinger J, Zuin J, Kryzhanovska M, *et al.*: **Cohesin and CTCF control the dynamics of chromosome folding**. *Nat Genet* 2022, **54**:1907–1918.
Live-cell tracking of two CTCFs separated by 150 Kb, showing transient nature of the CTCF–CTCF loop.

57. ** Gabriele M, Brandão HB, Grosse-Holz S, Jha A, Dailey GM, Cattoglio C, Hsieh T-HS, Mirny L, Zechner C, Hansen AS: **Dynamics of CTCF- and cohesin-mediated chromatin looping revealed by live-cell imaging**. *Science* 2022.
Live-cell tracking of two CTCF loci separated by 500 Kb. Using polymer-model based inference authors detected moments when the CTCF–CTCF loop is formed. The loop was surprisingly transient (present ~5% of the time, for ~30min), and the TAD between CTCFs is highly dynamic, suggesting that CTCF–CTCF interactions are poor candidates for enhancer-promoter interactions.

58. Coulon A, Chow CC, Singer RH, Larson DR: **Eukaryotic transcriptional dynamics: from single molecules to cell populations**. *Nat Rev Genet* 2013, **14**:572–584.

59. Nora EP, Lajoie BR, Schulz EG, Giorgetti L, Okamoto I, Servant N, Piolot T, van Berkum NL, Meisig J, Sedat J, *et al.*: **Spatial partitioning of the regulatory landscape of the X-inactivation centre**. *Nature* 2012, **485**:381–385.

60. Dixon JR, Selvaraj S, Yue F, Kim A, Li Y, Shen Y, Hu M, Liu JS, Ren B: **Topological domains in mammalian genomes identified by analysis of chromatin interactions**. *Nature* 2012, **485**: 376–380.

61. * Davidson IF, Barth R, Zaczek M, van der Torre J, Tang W, Nagasaka K, Janissen R, Kerssemakers J, Wutz G, Dekker C, *et al.*: **CTCF is a DNA-tension-dependent barrier to cohesin-mediated loop extrusion**. *Nature* 2023, **616**:822–827.
In vitro single-molecule study demonstrating that CTCF is a directional barrier to extrusion. Yet it's a very weak barrier on naked DNA, becoming stronger when DNA is under tension.

62. Symmons O, Uslu VV, Tsujimura T, Ruf S, Nassari S, Schwarzer W, Ettwiller L, Spitz F: **Functional and topological characteristics of mammalian regulatory domains**. *Genome Res* 2014, **24**:390–400.

63. Lupiáñez DG, Kraft K, Heinrich V, Krawitz P, Brancati F, Klopocki E, Horn D, Kayserili H, Opitz JM, Laxova R, *et al.*: **Disruptions of topological chromatin domains cause pathogenic rewiring of gene-enhancer interactions**. *Cell* 2015, **161**: 1012–1025.

64. Hnisz D, Day DS, Young RA: **Insulated neighborhoods: structural and functional units of mammalian gene control**. *Cell* 2016, **167**:1188–1200.

65. * Zuin J, Roth G, Zhan Y, Cramard J, Redolfi J, Piskadlo E, Mach P, Kryzhanovska M, Tihanyi G, Kohler H, *et al.*: **Nonlinear control of transcription through enhancer-promoter interactions**. *Nature* 2022, **604**:571–577.
Insertion of an enhancer at different distances from a reported gene shows a distance-dependent effect of enhancer of transcription. The decay of transcription with distance does not match the decay of the contact frequency with distance. To explain this observation a model of a multi-state promoter was introduced that can explain this phenomenon by highly non-linear transcription-to-contact dependence.

66. * Xiao J, Hafner A, Boettiger AN: *How subtle changes in 3D structure can create large changes in transcription*. Cold Spring Harbor Laboratory; 2020, https://doi.org/10.1101/2020.10.22.351395.
Authors show that small changes in the enhancer-promoter contact frequency translates into large effects on transcription. A multi-state enhancer-promoter model is introduced to capture this non-linear dependence.

67. Bintu B, Mateo LJ, Su J-H, Sinnott-Armstrong NA, Parker M, Kinrot S, Yamaya K, Boettiger AN, Zhuang X: **Super-resolution chromatin tracing reveals domains and cooperative interactions in single cells**. *Science* 2018, **362**.

68. Hung T-C, Kingsley DM, Boettiger AN: **Boundary stacking interactions enable cross-TAD enhancer-promoter communication during limb development**. *Nat Genet* 2024, **56**: 306–314.

69. * Goel VY, Huseyin MK, Hansen AS: **Region Capture Micro-C reveals coalescence of enhancers and promoters into nested microcompartments**. *Nat Genet* 2023, **55**:1048–1056.
A new ultra-deep Micro-C for ~3 Mb regions reveals new patterns of interactions.

70. * Aljahani A, Hua P, Karpinska MA, Quililan K, Davies JOJ, Oudelaar AM: **Analysis of sub-kilobase chromatin topology reveals nano-scale regulatory interactions with variable dependence on cohesin and CTCF**. *Nat Commun* 2022, **13**: 2139.
Tiled Micro-C allow ultra-deep sequencing of ~1–3 Mb region revealing a variety of new patterns.

71. Liu NQ, Magnitov M, Schijns M, van Schaik T, van der Weide RH, Teunissen H, van Steensel B, de Wit E: **Rapid depletion of CTCF and cohesin proteins reveals dynamic features of chromosome architecture**. *bioRxiv* 2021, https://doi.org/10.1101/2021.08.27.457977.

72. ** Samejima K, Gibcus JH, Abraham S, Cisneros-Soberanis F, Samejima I, Beckett AJ, Pucekova N, Abad MA, Medina-Pritchard B, Paulson JR, *et al.*: **Rules of engagement for condensins and cohesins guide mitotic chromosome formation**. *bioRxiv* 2024, https://doi.org/10.1101/2024.04.18.590027.
Analysis of temporal changes in Hi-C and microscopy during chromosome compaction into mitosis allow to learn rules of engagements between four SMC complexes involved (two condensins and two cohesins) and estimations of condensin loop extrusion speeds (~1-3 kb/s) in vivo.

73. * Banigan EJ, Tang W, van den Berg AA, Stocsits RR, Wutz G, Brandão HB, Busslinger GA, Peters J-M, Mirny LA: **Transcription shapes 3D chromatin organization by interacting with loop extrusion**. *Proc Natl Acad Sci U S A* 2023, **120**, e2210480120.
Actively transcribed polymerases were demonstrated to serve as moving barrier to cohesin, suggesting that this mechanism can allow the maintenance of enhancer-polymerase contact as polymerases transcribes the gene.

74. ** Rekaik H, Lopez-Delisle L, Hintermann A, Mascrez B, Bochaton C, Mayran A, Duboule D: **Sequential and directional insulation by conserved CTCF sites underlies the Hox timer in stembryos**. *Nat Genet* 2023, **55**:1164–1175.
Authors demonstrate that sequential, directional and precisely timed activation of genes in the homeodomain locus require cohesin activity and placement of specific CTCF sites.

75. ** Kane L, Williamson I, Flyamer IM, Kumar Y, Hill RE, Lettice LA, Bickmore WA: **Cohesin is required for long-range enhancer action at the Shh locus**. *Nat Struct Mol Biol* 2022, **29**:891–897.
By recruiting activators to various enhancers of the Shh locus and monitoring Shh gene expression authors demonstrate that cohesin is required for long range enhancer-promoter interactions, while proximal enhancers (<50 Kb) do not benefit from cohesin.

76. ** Kiefer L, Chiosso A, Langen J, Buckley A, Gaudin S, Rajkumar SM, Servito GIF, Cha ES, Vijay A, Yeung A, *et al.*: **WAPL functions as a rheostat of Protocadherin isoform diversity that controls neural wiring**. *Science* 2023, **380**, eadf8440.
Authors uncover that diversity of protocadherin isoforms in individual cells rests on the loop–extrusion activity of cohesin, as regulated by Wapl.

77. Zhang Y, Zhang X, Dai H-Q, Hu H, Alt FW: **The role of chromatin loop extrusion in antibody diversification**. *Nat Rev Immunol* 2022, **22**:550–566.

78. Paturej J, Sheiko SS, Panyukov S, Rubinstein M: **Molecular structure of bottlebrush polymers in melts**. *Sci Adv* 2016, **2**, e1601478.

79. Hall LL, Smith KP, Byron M, Lawrence JB: **Molecular anatomy of a speckle**. *Anat Rec A Discov Mol Cell Evol Biol* 2006, **288**: 664–675.

80. Du M, Stitzinger SH, Spille J-H, Cho W-K, Lee C, Hijaz M, Quintana A, Cissé II: **Direct observation of a condensate effect on super-enhancer controlled gene bursting**. *Cell* 2024, **187**: 2595–2598.

81. Stein J, Ericsson M, Nofal M, Magni L, Aufmkolk S, McMillan RB, Breimann L, Herlihy CP, Dean Lee S, Willemin A, *et al.*: **Cryosectioning-enabled super-resolution microscopy for studying nuclear architecture at the single protein level**. *bioRxiv* 2024, https://doi.org/10.1101/2024.02.05.576943.

82. ** Trojanowski J, Frank L, Rademacher A, Mücke N, Grigaitis P, Rippe K: **Transcription activation is enhanced by multivalent interactions independent of phase separation**. *Mol Cell* 2022, **82**:1878–1893.e10.
Authors combine engineering of different synthetic transcription factors driving a reporter gene with real-time single-cell microscopy. They demonstrate that gene activation does not require formation of polymerase or transcription factor condensates, even for high levels of transcription activation.

83. McSwiggen DT, Hansen AS, Teves SS, Marie-Nelly H, Hao Y, Heckert AB, Umemoto KK, Dugast-Darzacq C, Tjian R, Darzacq X: **Evidence for DNA-mediated nuclear compartmentalization distinct from phase separation**. *Elife* 2019, **8**.