© 2016. Published by The Company of Biologists Ltd | Development (2016) 143, 910-923 doi:10.1242/dev.129007

REVIEW

From single genes to entire genomes: the search for a function of nuclear organization

Ringo Pueschel[1,2,*], Francesca Coraggio[1,2,*] and Peter Meister[1,‡]

ABSTRACT

The existence of different domains within the nucleus has been clear from the time, in the late 1920s, that heterochromatin and euchromatin were discovered. The observation that heterochromatin is less transcribed than euchromatin suggested that microscopically identifiable structures might correspond to functionally different domains of the nucleus. Until 15 years ago, studies linking gene expression and subnuclear localization were limited to a few genes. As we discuss in this Review, new genome-wide techniques have now radically changed the way nuclear organization is analyzed. These have provided a much more detailed view of functional nuclear architecture, leading to the emergence of a number of new paradigms of chromatin folding and how this folding evolves during development.

KEY WORDS: Nuclear organization, Chromosome conformation capture, Epigenetics, Microscopy

Introduction

Nuclear organization, the physical structure of the genome within the nuclear space, has fascinated cellular and developmental biologists for the last 100 years. In particular, the search for a functional link between nuclear structure and function – the expression of a cell fate-specific transcriptional program – has been the focus of numerous studies. As soon as methods to stain and image chromatin had been established, early clues of such a functional organization of the nucleus were described. In the late 1920s, Emil Heitz observed two chromatin types in the nucleus of Bryophytes (mosses) (Heitz, 1928). Heterochromatin persisted following mitosis, whereas euchromatin decondensed and was no longer visible during interphase. At that time, nothing was known about the molecular nature of chromatin, although it was clear that genes were contained within it. Heitz envisioned that the different chromatin states he observed might represent functional nuclear domains, with euchromatin being 'genicly active' and heterochromatin 'genicly passive or would not contain genes' (reviewed by Zacharias, 1995). Heitz's observations were soon supported by electron microscopy (EM) studies showing the large variety of nuclear organizations in different cell types or developmental stages (Fig. 1A,Ba). Nuclear organization was, however, similar between cells of a given cell type. Underlining the correlation between cell fate and chromatin organization, the latter is nowadays one of the classical parameters used by cytologists to describe cell fate, for example upon tumor progression (Kufe et al., 2003). How changes in nuclear organization relate to transcriptional changes remains a topic of intense research.

[1]Cell Fate and Nuclear Organization, Institute of Cell Biology, University of Bern, 3012 Bern, Switzerland. [2]Graduate School for Cellular and Biomedical Sciences, University of Bern, Switzerland.
*These authors contributed equally to this work

‡Author for correspondence (peter.meister@izb.unibe.ch)

In the 1960s, microscopically described hetero- and euchromatin were biochemically purified from mammalian lymphocytes. Quantification of the transcriptional activity present in both fractions provided molecular proof of Heitz's hypothesis: although most DNA (80%) is contained in the heterochromatic fraction, most of the RNA synthesis activity is present in the remaining euchromatic 20% fraction (Frenster et al., 1963). Besides differences in transcriptional activity, hetero- and euchromatin correlate with DNA packaged by nucleosomes composed of histones carrying different modifications. Among the most common modifications, histone H3 methylated on lysine 9 and 27 correlates with heterochromatin. By contrast, transcribed chromatin is methylated on H3 lysine 4 and 36, H4 lysine 20 and 79 and acetylated on lysine 27 (Ho et al., 2014). These modifications impact on chromatin compaction as well as the interactions these histones can make with nuclear proteins, which in turn influence larger scale organizations (Zhou et al., 2011; Zhang et al., 2015).

Technological advances over the last 10 years have revolutionized the nuclear organization field. Earlier work was based on microscopic analysis of nuclear structure, by localizing genes, multi-gene complexes or entire chromosomes relative to each other or relative to nuclear landmarks. Although these techniques have defined a number of properties of nuclear structure, microscopy is limited by the number of loci that can be probed and the resolution of the imaging devices. Newly developed genomic techniques allow capturing contacts of the entire genome with nuclear landmarks as well as spatial information on how chromosomes are folded inside the nuclear space. Here, we briefly review basic organizational principles discovered using microscopy and highlight the new insights provided by genome-wide techniques as well as the functional importance of genome folds in a developmental context.

The early days: microscopy, from brightfield to fluorescence

Nuclear bodies – a wealth of structures inside the nucleus

The first revolution in the analysis of nuclear organization came with fluorescence-based microscopy (see Box 1). Starting in the late 1960s, conjugation of fluorochromes to antibodies allowed the precise localization of proteins and genes in relation to landmarks such as the nuclear periphery or the nucleolus, or particular domains stained with specific antibodies. Many large nuclear bodies, such as the nucleolus, nuclear speckles or Cajal bodies, had been observed previously using a variety of staining procedures (Cajal, 1903, 1910). Fluorescence microscopy led to the discovery of new, smaller and/or more numerous nuclear structures, such as transcription and replication 'factories', in which transcriptionally active genes or replicons cluster, respectively (Hozák et al., 1993; Jackson et al., 1993) (Fig. 1Bb). Secondly and most importantly, it allowed molecular identification of the protein and gene content of these bodies, suggesting potential functions for these sites. Many nuclear bodies have now been characterized in this way (Fig. 1Bb): speckles

Development (2016) 143, 910-923 doi:10.1242/dev.129007

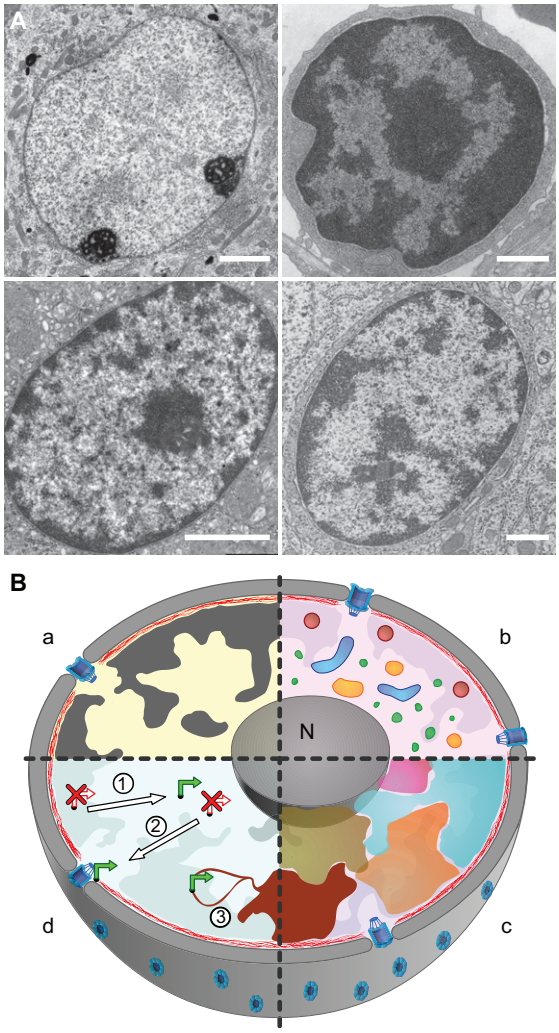

Fig. 1. Diversity and organizational principles of nuclear organization uncovered using microscopic techniques. (A) Distribution of heterochromatin and euchromatin masses shows great variability between cell types. Electron micrographs of mammalian nuclei from differentiated cells taken from various tissues. Darkly stained material is heterochromatin; lightly stained regions are euchromatin. Clockwise from upper left: a cochlear ganglion nucleus from guinea pig spirale cochleae; a rat lymphocyte nucleus; a rat parietal stomach cell nucleus; and a rat glial cell nucleus. Scale bars: 1 µm. Images taken by Dr H. Jastrow (Zentrum für Elektronenmikroskopie des Imaging Center Essen der Universität Duisburg-Essen), reproduced with permission and available online at the Electron Microscopic Atlas (http://www.uni-mainz.de/FB/Medizin/Anatomie/workshop/EM/EMAtlas.html). (B) Organization of the nucleus, as described by microscopic observations. (a) Distribution of euchromatin (light area) and heterochromatin (dark area) within the nuclear space. Heterochromatin is often clustered at the nuclear periphery or close to the nucleolus (N), with notable exceptions, such as the mammalian eye photoreceptors in which heterochromatin is centrally located. (b) A variety of microscopically identifiable domains populate the nuclear space (for space reasons, not all known domains have been represented). Red: Polycomb bodies (grouping Polycomb-repressed genes); orange: Cajal bodies (major splice sites for histone RNAs); gray: nucleolus (transcription and splicing/assembly site for the ribosomal RNAs); green: transcriptionally active genes clustered together; blue: speckles (splice assembly sites). (c) Chromosomes occupy distinct territories inside the nuclear space with little intermingling, probably owing to the polymeric properties of chromatin. (d) Observed modes of gene relocation: (1) upon developmental activation, movement from a transcriptionally silent location at the nuclear lamina (red) towards a more central area, (2) upon stress-induced activation, movement from an internal location towards the nuclear pore (blue) (3) upon developmental activation, looping out of the gene's chromosome territory to the inter-chromosomal space.

(enriched with splicing factors; Spector and Lamond, 2011), paraspeckles (organized on long non-coding RNAs; Bond and Fox, 2009), Cajal bodies (enriched for histone and small nuclear RNA genes; Morris, 2008) and PML bodies (in which diverse proteins cluster, but for which the function is still elusive; Lallemand-Breitenbach and de The, 2010). Besides these structures, many epigenetic marks form nuclear domains, characterized by clustering of similarly marked chromatin inside the nucleus, for example histone 3 lysine 27 (H3K27) methylated chromatin clusters, together with the Polycomb group proteins that deposit the mark. These so-called Polycomb bodies group Polycomb-regulated genes, presumably helping in the stable repression of these genes (Schuettengruber et al., 2007). Together, the discovery of these functionally specialized nuclear domains provided further evidence that the three-dimensional (3D) structure of the genome might indeed be involved in regulating gene expression.

General principles of nuclear organization

Besides the description of these nuclear domains, a number of general principles of nuclear organization started to emerge from microscopy studies. At the larger scale, metazoan interphase chromosomes mostly do not intermingle and instead form chromosome territories (CTs; Fig. 1Bc; reviewed by Cremer et al., 2006). Recent polymer physics modeling suggests that CTs are an intrinsic property of the DNA polymer rather than the result of a biological function (Rosa and Everaers, 2008): chromosomes are very long molecules and their complete intermingling would take more time than the lifetime of most organisms. Though the bulk of the chromosome occupies a discrete territory, the edges of CTs can intermingle, in agreement with the fact that many translocations occur between chromosomes (Branco and Pombo, 2006). In mammalian cells, CT position inside the nuclear space is correlated with gene density: gene-poor chromosomes are located closer to the nuclear periphery whereas gene-rich chromosomes are more centrally positioned (Croft et al., 1999; Bolzer et al., 2005). This chromosome-wide behavior is likely to be a consequence of the sum of individual gene-positioning effects, as differential positioning is observed within a single chromosome, with active genes located in the nuclear interior and silent ones at the nuclear rim (Kosak et al., 2007; Meister et al., 2010).

During cell differentiation, many genes were found to reposition within the nucleus, and this correlated with changes in their transcriptional activity (Fig. 1Bd). A number of genes move from or to the nuclear periphery, where the nuclear lamina interacts mostly with silent genes whereas nuclear pores cluster with active chromatin (Williams et al., 2006; Kosak et al., 2007; Takizawa et al., 2008a; Meister et al., 2010). Genes were also observed to loop out of their chromosome territory upon activation (Fig. 1Bd; Mahy et al., 2002; Chambeyron and Bickmore, 2004; Chambeyron et al., 2005). Altogether, this led to a general picture, with many exceptions, of the distribution of transcriptional activity inside the nucleus: active genes are located between CTs inside the nuclear interior or in close vicinity to nuclear pores, whereas inactive genes are buried inside their CT or clustered at the nuclear periphery, in close contact with the nuclear lamina. Determinants of gene positioning remain largely unknown, although transcriptional status is clearly an important factor. However, not all promoters are able to induce relocation upon activation (Meister et al., 2010). Moreover, because modifying chromatin marks is sufficient to induce gene relocation, it appears that relocation does not depend on transcription itself, but rather on local changes in chromatin compaction induced by transcription (Tumbar et al., 1999; Tumbar

Development (2016) 143, 910-923 doi:10.1242/dev.129007

Box 1. *In vivo* gene localization techniques

Fixed cells

Fluorescence *in situ* hybridization (FISH) is the fluorescent labeling of a DNA or RNA sequence of interest, using Watson–Crick pairing of a labeled probe with its cellular homolog (Langer-Safer et al., 1982). Cells or tissues are fixed and membranes are partially solubilized to allow probe penetration into the nucleus. For DNA FISH, an additional denaturation step (heating/pH drop) is necessary to denature the double-stranded helix and allow probe hybridization. Fluorescent probes can be generated by a number of techniques, from nick translation with fluorescent nucleotides, direct crosslinking of chromophores to amino-labeled sequences or synthesis of a large number of fluorescent primers. Probe size ranges from a few hundred base pairs to entire chromosomes. A major advantage of FISH is its flexibility in terms of target sequence and fluorochrome choice (up to 23 options; Bolzer et al., 2005). However, this is an invasive approach requiring fixation and denaturation, which cannot be used to study dynamic features and is potentially prone to artifacts.

Living cells

In vivo gene-tagging techniques were designed to overcome FISH limitations. They are based on the integration of repeats of a binding site (*lacO*/*tetR*/*lexAbs*) for a bacterial transcriptional repressor close to the locus of interest and the expression of the cognate repressor (lacI/tetR/lex) fused to a fluorescent protein and targeted to the nucleus. Binding of multiple copies of the repressor to its target sites leads to the formation of a readily visible spot (Robinett et al., 1996). A similar system can be used to target genes to a given subnuclear compartment by fusing the bacterial protein with a compartment-specific protein (Andrulis et al., 1998). The recent development of 'designer' site-specific binders [TALEs (transcription activator-like effectors), zinc-finger proteins, CRISPRs (clustered regularly interspaced short palindromic repeats)] allows labeling of repetitive sequences such as microsatellites, thus overcoming the need to integrate binding sites (Miyanari et al., 2013).

and Belmont, 2001; Takizawa et al., 2008b; Therizols et al., 2014). Conversely, transcription inhibition does not seem sufficient to induce gene repositioning (Palstra et al., 2008).

Another observation suggested functional clustering of genes inside the nuclear space: transcriptionally active RNA polymerase II molecules form small, highly dynamic, dot-like structures, which group together more than one active polymerase (Ferrai et al., 2010; Cisse et al., 2013). It is still debated whether active genes cluster to create these structures, called transcriptional factories, or whether genes need to visit these physical assemblies for transcription (Cisse et al., 2013). However, a number of co-regulated genes show colocalization in a single transcription factory ('gene kissing'). 3D organization of genes inside the nuclear space might therefore impact on their transcriptional output and help regulate their expression (Kosak et al., 2007; Schoenfelder et al., 2010). These studies raise a number of questions regarding the link between the linear position of a gene along a chromosome, its location within the nucleus and its transcriptional status. For example, which nuclear position would genomic segments containing an active and a silent gene adopt (Zink et al., 2004)? What are dominant positioning effects on the chromatin polymer? What is the transcriptional effect of repositioning on nearby genes?

From snapshots to movies: dynamic features of the nuclear structures and proteins

A further refinement of fluorescence imaging techniques was the use of *in vivo* fluorescence labeling of nuclear proteins. This allowed analysis of the changes in nuclear domains over time (Misteli et al., 1997), and fluorescence bleaching techniques provided dynamic parameters such as residence time or diffusion coefficients for a number of nuclear proteins (Phair et al., 2004; Meshorer et al., 2006). This completely changed the perception of nuclear architecture: far from being composed of fixed, immobile domains, the nucleus is a highly active structure in which most chromatin components have residence times on DNA between seconds and minutes (reviewed by Misteli, 2001; Mueller et al., 2010). Direct measurement of chromatin movement itself completed the picture (Robinett et al., 1996; Heun et al., 2001; Chubb et al., 2002): not only are most chromatin-bound proteins highly dynamic, but the genome itself moves within the nuclear space, following in most cases a random walk. For example, in budding yeast a locus is able to travel across the entire nucleus (~1.5-2 µm) in less than 10 s (Heun et al., 2001). Loci in mammalian cells sample a much smaller region of the nucleus as the nuclear space is larger (Chubb et al., 2002). Such measurements, although carried out on a limited number of loci, have provided quantification of *in vivo* chromatin movement, such as compaction or displacement speed inside the nucleus (reviewed by Lanctôt et al., 2007). These parameters are essential for physical modeling of chromatin in the era of genome-wide chromatin studies. Together, microscopy studies have been instrumental in uncovering large-scale structures, understanding the functional organization of the nucleus and characterizing nuclear dynamics. Although high-throughput automated fluorescence *in situ* hybridization (FISH) and imaging techniques coupled to genome-wide RNA interference (RNAi) can uncover gene-positioning determinants (Shachar et al., 2015), imaging-based approaches reach their limits when more than a handful of loci and structures are imaged simultaneously and remain limited by the resolution of light microscopes.

Genome-wide molecular techniques for assessing nuclear organization

Overcoming these limitations, the appearance of new molecular techniques has allowed nuclear domains to be analyzed at the sequence level on a genome-wide scale. DNA adenine methylation identification (DamID; Box 2) permits molecular mapping of interactions between chromatin and any type of nuclear protein. Chromosome conformation capture techniques (C-techniques; Box 3) uncover contacts between distant genomic loci. The combination of both techniques has dramatically advanced our understanding of the structure of the genome inside the nucleus and provided new clues regarding the determinants and regulators of genome folding. In parallel, these studies have raised a number of discrepancies between microscopy and mapping data (discussed below).

DamID: how many chromatin types?

DamID was originally developed as an alternative to chromatin immunoprecipitation without the need for crosslinking and immunoprecipitation (van Steensel and Henikoff, 2000). DamID is based on the expression of trace levels of a fusion protein between a nuclear protein and the *Escherichia coli* DNA adenine methyltransferase Dam. Chromatin proximal to the fusion protein gets methylated at GATC motifs; methylated fragments can be extracted, amplified and hybridized to microarrays or sequenced. As there is no need for chromatin purification, DamID is particularly useful when the protein of interest is part of an insoluble complex, such as the nuclear lamina or nuclear pores (see below). Moreover, DamID is highly sensitive, as the procedure can be carried out with single cells and is amenable to high-throughput studies with tens of

Development (2016) 143, 910-923 doi:10.1242/dev.129007

Box 2. The DamID technique

DamID (DNA adenine methyltransferase identification) is a technique to probe the contact of nuclear proteins with DNA. It is based on the fusion of the *E. coli* adenine methyltransferase (Dam) to a protein of interest, which can be a transcription factor, a chromatin remodeler or a structural protein such as a nuclear lamin or pore subunit. Dam methylates GATCs proximal to the binding sites of the fused protein. This sequence-specific adenine modification is absent in higher eukaryotes, allowing unambiguous identification of the relevant sequences. Methylated GATCs can be identified by digesting the genome with *Dpn*I, a restriction enzyme that cleaves exclusively methylated GATC. Adapters are then ligated to the DNA fragments, before digestion of unmethylated GATCs with *Dpn*II. Fragments methylated on both ends are then amplified by PCR using a primer hybridizing to the adapter sequence. Initial experiments used dye labeling and microarray hybridization, but library sequencing is now common. As methylation by Dam depends on the accessibility of individual GATCs in a chromatinized environment, DamID is always carried out as a comparison between free Dam (fused to GFP for example) and a Dam fusion with the protein of interest. The resolution of DamID depends on the density of GATC motifs in the genome, which ranges from ∼300-1000 bp, similar to the resolution obtained with classical chromatin immunoprecipitation approaches.

fusion proteins tested in parallel (Filion et al., 2010; Kind et al., 2015).

A key achievement made using DamID has been the revision of the simple, dichotomic vision of chromatin as observed by EM. Dark-stained heterochromatin and lightly stained euchromatin greatly impacted the way chromatin types were considered until recently. Whether this crude staining could be assigned molecularly to different types of chromatin was a key question. DamID with 53 chromatin factors revealed five major types of chromatin in *Drosophila* cells (Filion et al., 2010). Three of them corresponded to heterochromatin, associated with HP1, Polycomb domains and lamina-proximal domains. Two types of euchromatin could be distinguished, grouping either housekeeping active genes or tissue-specific active genes. These results were later confirmed using chromatin immunoprecipitation with various histone marks and histone-associated proteins, depicting a similarly small number of chromatin states (Ernst et al., 2011). In both cases, the diversity of chromatin types was higher than expected by the EM-based observations, raising the question of how these molecularly characterized chromatin states correspond to the EM counterparts.

Chromosome conformation capture: contact frequencies in crosslinked chromatin

DamID allowed researchers to delineate the interaction of the genome with nuclear landmarks but is not able to capture how the linear genome folds away from these landmarks. Chromosome conformation capture techniques were specifically designed to characterize this 3D structure of the genome – the weakly characterized higher order chromatin structures (see Box 3 for the different variations of the techniques; Dekker et al., 2002; Tolhuis et al., 2002). The principle of C-techniques is to crosslink chromatin, restriction digest the cross-linked DNA before re-ligation and high-throughput sequencing. If restriction fragments distant on the linear genome get ligated together, this reflects spatial proximity of the two fragments in crosslinked chromatin. High-throughput 3C data (4C and Hi-C) do not interpret individual ligation events. These experiments rely on the statistical enrichment of contacts between restriction fragments of two genomic stretches, in particular the appearance of clusters of multiple independent ligation events between these stretches. An enrichment is then scored as a contact between genomic stretches.

Contact frequencies are often used as a proxy for the spatial juxtaposition of sequences *in vivo*. This appears to be valid in most cases in which FISH data has been used to corroborate conformation capture experiments (Simonis et al., 2006; Nora et al., 2012; Giorgetti et al., 2014; Crane et al., 2015). There is still some debate about what C-techniques are actually measuring (Gavrilov et al., 2013; reviewed by Belmont, 2014; Williamson et al., 2014) and discrepancies between laboratories might arise from the experimental system used for chromosome conformation capture

Box 3. Chromosome conformation capture-derived techniques

Chromosome conformation capture techniques (C-techniques) are based on the principle that restriction fragments can be ligated when close together (regardless of linear distance separating them). Different variations of the C-techniques exist but the initial steps are the same. Chromatin is cross-linked with formaldehyde and cut with a restriction enzyme. Fragments are ligated together, leading to ligation products between distant fragments on the linear genome.

One-to-one and one-to-many techniques

3C relies on semi-quantitative PCR with a pair of primers hybridizing near the ends of restriction fragments of interest (Dekker, 2008). When repeated for many pairs, this gives a matrix of relative ligation efficiency for all studied fragments.

The 4C methodology (circularized 3C) involves the creation of small DNA circles by another round of restriction digest and ligation (Simonis et al., 2009). These circles are amplified using inverse PCR and either hybridized to microarrays or sequenced. This approach gives a genomic view of all possible contacts between one site (often called viewpoint) and the rest of the genome at high resolution.

Many to many

The 5C technology (carbon copy 3C) gives an overview of contacts between multiple sequences (Dostie et al., 2006). Instead of using an oligonucleotide pair, numerous oligonucleotides corresponding to the different restriction sites in the genomic region of interest are hybridized. The 5′ end of all these primers carry the same sequence as that used for PCR amplification. PCR products are either hybridized to microarrays or sequenced. The result is a matrix of contact frequencies for many sites.

All to all

For Hi-C, restriction ends are labeled using biotin-tagged nucleotides (Lieberman-Aiden et al., 2009; Rodley et al., 2009; Duan et al., 2010). After ligation, purification and shearing, ligated fragments are pulled down using biotin and sequenced. A matrix of contact frequencies between all restriction fragments in the genome can be constructed.

Importantly, one restriction fragment can only ligate once in any given haploid cell. Therefore, contact maps constructed using C-techniques are probabilistic and represent the likelihood of contact between two given fragments. Resolution of these techniques depends on the size of the restriction enzyme recognition sequence and the sequencing depth of libraries. Whereas initial studies achieved only megabase resolution, the latest study with 15 billion contact reads reaches kilobase resolution (Rao et al., 2014).

5C or Hi-C data are usually represented as color-coded log-scale contact frequency matrices, often showing only half of the symmetric matrix. Each pixel represents the one-to-one contact frequency with another region of the genome (Fig. 2B). Contact frequencies with co-linear DNA (neighboring sequences on the same chromosome) are higher than with sequences located further away (intrachromosomal contacts) or on other chromosomes (interchromosomal contacts, orders of magnitude less frequent).

Development (2016) 143, 910-923 doi:10.1242/dev.129007

and/or the biological material (Noordermeer et al., 2011; Andrey et al., 2013; Williamson et al., 2014). However, one can reasonably assume that ligation frequency observed using Hi-C and 4C approaches reflects *in vivo* contact frequency of the restriction fragments and thus their physical proximity, combined with overall chromatin compaction of the domain to which the restriction fragment belongs. In any case, functional tests, such as enhancer assays, remain the gold standard to demonstrate the reality and functional relevance of the captured contacts (Montavon et al., 2011; Andrey et al., 2013).

Among eukaryotes, Hi-C has been carried out in a number of yeast species, *Caenorhabditis elegans*, *Drosophila*, a variety of mammalian cells and *Arabidopsis*. Reassuringly, global genome organization features described using microscopy techniques were reproduced. Chromosomes are organized in territories, creating the characteristic high-contact diagonal as represented on a Hi-C map (Fig. 2B; Lieberman-Aiden et al., 2009; Sexton et al., 2012; Crane et al., 2015; Guidi et al., 2015). Centromeres and/or telomeres have a tendency to cluster for yeast, *Drosophila* and *Arabidopsis* chromosomes (the so-called Rabl configuration, named in honor of Carl Rabl who described it in 1885; Rodley et al., 2009; Duan et al., 2010; Hou et al., 2012; Sexton et al., 2012; Grand et al., 2014; Guidi et al., 2015; Mizuguchi et al., 2015). At low resolution, two major compartments are identified, a perfect reflection of Heitz's century-old microscopy observations (Fig. 2A). The first compartment comprises more open and active chromatin (compartment A, similar to euchromatin) whereas the second is more closed (or compact), harboring repressed chromatin marks (compartment B, similar to heterochromatin). These compartments cluster together inside the nucleus, with active chromatin making more interchromosomal contacts than heterochromatin (Simonis et al., 2006; Lieberman-Aiden et al., 2009; Splinter et al., 2011; Kalhor et al., 2012; Sexton et al., 2012; Nagano et al., 2013). At higher resolution, preferential clustering of chromatin marked with similar epigenetic modifications is observed, probably homologous to subnuclear domains identified using immunofluorescence (Lieberman-Aiden et al., 2009; Sexton et al., 2012). Five major types of chromatin domains could be characterized based on

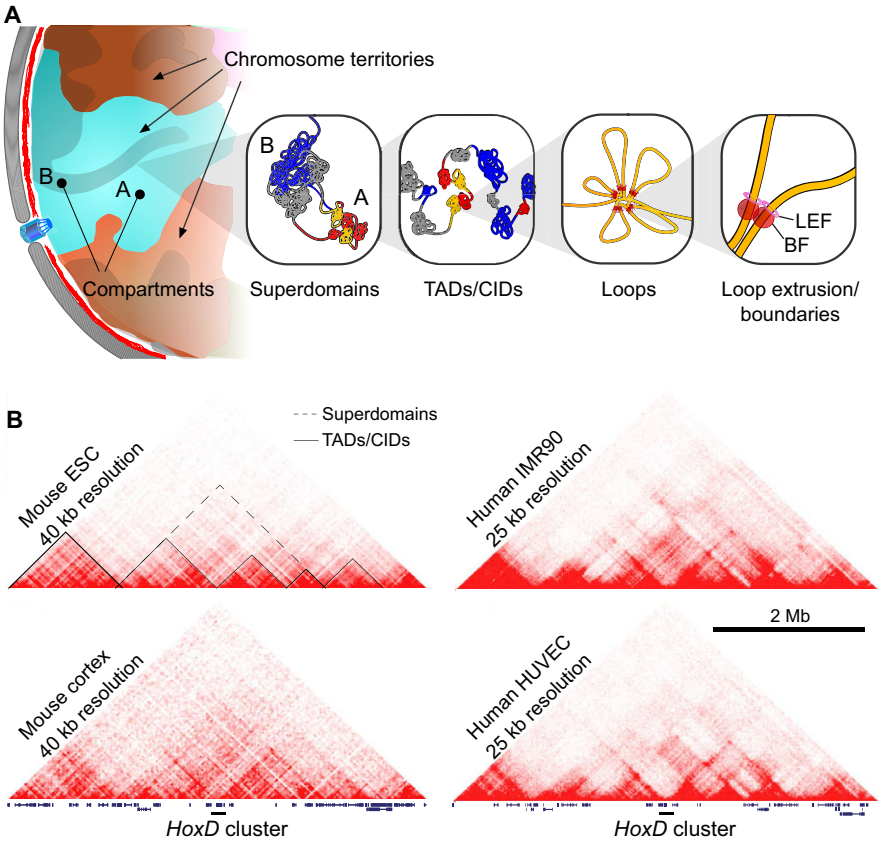

Fig. 2. Chromatin domain folding at different scales. (A) Hierarchical chromatin folding inside the nucleus, as uncovered by chromosome conformation capture. Each chromosome occupies a distinct nuclear space, termed the chromosome territory. Intrachromosomal contacts are orders of magnitude more frequently captured than interchromosomal ones. Chromosome territories can be further split into A and B compartments, transcriptionally more active or inactive, respectively. Interchromosomal contacts between domains from the same compartments (A/A, B/B) are more frequent than those between different compartments (A/B), and A compartments make more contacts than B ones. The A/B compartments are organized in superdomains, which include mostly domains belonging to the same chromatin type. Superdomains group together topologically associated domains (TADs; also known as chromosomal interaction domains or CIDS) of similar chromatin type. Inside TADs, loop formation is favored, in particular between enhancers and promoters. A number of proteins previously characterized as insulators show high enrichment between TADs and/or at the base of the loops. Loops are formed by the combined action of loop extrusion factors (LEFs, probably SMC complexes) and boundary factors (BFs, such as CTCF). (B) TADs/CIDs are conserved between cell types and paralogous regions between species. Contact matrices of a 5-Mb region centered on the *HoxD* locus in two different mouse and human cell types (ESC, embryonic stem cell; IMR90, fetal lung fibroblast; HUVEC, umbilical vascular endothelium). The TAD structure is outlined (solid lines), as well as one superdomain (dashed lines). Genes (Refseq) are shown under the contact matrices (not all transcripts shown owing to scale). Color intensity varies between experiments because the number of ligation events sequenced in the different experiments is variable. However, overall patterns of contacts are very similar between cell types of the same species as well as between species. Matrix visualization from http://promoter.bx.psu.edu/hi-c/view.php, using data from Dixon et al. (2012) and Rao et al. (2014).

Development (2016) 143, 910-923 doi:10.1242/dev.129007

clustering affinity and transcriptional activity. Three transcriptionally more 'silent' types were observed as well as two transcriptionally more 'active' types, highly reminiscent of the five chromatin types characterized using high-throughput DamID studies (Filion et al., 2010; Rao et al., 2014).

By providing a genome-wide view of nuclear organization, DamID and Hi-C have uncovered a wealth of structures that microscopy techniques were unable to characterize. The genome-wide character of these techniques gives additional statistical power to the analysis, necessary to assess the generality of these structures across cell types and species. Finally, the combination of genome-wide approaches with recent genome-editing techniques has started to demonstrate the functional importance of these recently discovered structures.

Lamina-associated domains, organization and dynamics of perinuclear heterochromatin

One of the common features of most cell types is the presence of dense heterochromatin at the nuclear periphery, contacting a dense insoluble network of intermediate filaments, the nuclear lamina (Towbin et al., 2009). Mutations in genes encoding nuclear lamins are linked to a number of human diseases called laminopathies, ranging from muscular dystrophies to lipodystrophies and accelerated aging (Gruenbaum and Foisner, 2015). These pathologies suggest that nuclear lamins and, more generally, lamina-associated heterochromatin might have a function in regulating gene expression. As discussed above, fluorescence microscopy studies demonstrated that silent genes tend to be found at the nuclear periphery. However, although the chromatin at the nuclear periphery is in general transcriptionally repressed, perinuclear localization per se is not repressive for every gene. When a genomic segment is artificially tethered to the nuclear lamina, the transcriptional modulation is gene specific, with some genes insensitive to tethering and others being repressed (Finlan et al., 2008; Kumaran and Spector, 2008; Reddy et al., 2008). The nuclear lamina is therefore thought to act as a scaffold to anchor silent chromatin at the nuclear periphery, rather than to actively repress gene transcription (Ruault et al., 2008; Towbin et al., 2009).

DamID with lamin fusions in both *Drosophila* and mammalian cells provided the first genomic view of lamina-proximal sequences (Pickersgill et al., 2006; Guelen et al., 2008). These sequences are organized in large lamin-associated domains (LADs), the sizes of which range from 0.1 to 10 megabases. In agreement with the heterochromatic aspect of lamina-proximal chromatin, LADs are mostly gene-poor, transcriptionally silent and late replicating. LAD chromatin is enriched for silent marks (H3K9 and H3K27 methylation) and deprived of active ones (Pickersgill et al., 2006; Guelen et al., 2008; Peric-Hupkes et al., 2010; Kind et al., 2013). Megabase-sized LAD sequences can autonomously direct localization to the nuclear rim when integrated in a non-LAD locus (Zullo et al., 2012; Harr et al., 2015). The mechanistic basis of LADs directing to the nuclear lamina is still debated, in particular the relative importance of specific binding motifs versus chromatin modifications. Two non-exclusive models have been proposed: a zipping structure in which LAD formation occurs from a limited number of sequences, or individual buttons/anchor points spread across the LAD. In favor of a zipping structure, individual LADs are usually very large and show long contact runs with the nuclear lamina rather than individual independent interactions sites (Kind et al., 2015). Along a single chromosome, LAD formation is coordinated even at megabase distances (Kind et al., 2015). This suggests that perinuclear attachment of one LAD or part of a LAD greatly increases the likelihood of LAD formation on other parts of the chromosome. In favor of the second model, a number of sequences in the kilobase range containing transcription repressor binding sites have been described as sufficient for perinuclear anchoring (Zullo et al., 2012; Bian et al., 2013; Harr et al., 2015). However, in all cases, targeting to the nuclear periphery depends on histone modifiers, either deacetylases or H3K9 and H3K27 methyltransferases. This suggests that the initial binding of the transcription repressor is accompanied by chromatin modifications, which in turn mediate perinuclear anchoring. This is consistent with results obtained from two genetic screens in *C. elegans*, which identified H3K9 methylation as a sufficient signal for perinuclear anchoring, and a perinuclear chromodomain protein (named CEC-4) as the methylated H3K9 anchor (Towbin et al., 2012; Gonzalez-Sandoval et al., 2015). By contrast, a number of studies have shown that histone acetylation impairs LAD formation (Pickersgill et al., 2006; Kind et al., 2013).

The developmental dynamics of LADs were studied in mouse cells during the transition from undifferentiated stem cells to astrocytes. In any cell type, 1100-1400 LADs are present, with a size ranging from 40 kb to 15 Mb and covering ∼40% of the genome (Peric-Hupkes et al., 2010). When comparing different cell types, two types of LADs are detected. The vast majority of LADs are constitutive and present in all cell types, covering 33% of the genome. Constitutive LADs have a very low gene content, a high A/T element frequency and are enriched for long interspersed elements (Meuleman et al., 2013). These LADs are conserved between mouse and human, supposedly creating a fixed backbone of chromosomes at the nuclear periphery (Meuleman et al., 2013; Kind et al., 2015). The other, minority, type of LADs are facultative, and present in a cell type-specific manner (Meuleman et al., 2013). Facultative LADs contain either a single gene or multiple genes (Peric-Hupkes et al., 2010). When comparing the localization of these facultative LADs during differentiation from embryonic stem cells (ESCs) to neuronal progenitors to astrocytes, relocalization from the nuclear interior (non-LAD situation) to the nuclear periphery (LAD situation) is correlated with gene repression. Conversely, however, detachment from the nuclear lamina does not always correlate with gene activation, but in some cases appears to 'unlock' the locus or loci for transcriptional activation in a subsequent differentiation step (Peric-Hupkes et al., 2010).

DamID experiments with nuclear lamins have provided a clear framework on how the genome interacts with the nuclear periphery and started to shed light on the cell-to-cell variability of genome nuclear organization. The comparison of DamID and Hi-C data shows that the LAD boundaries are very often limits of domains defined by the latter technique (see below), suggesting a crosstalk between LADs and genome topology (Kind et al., 2015).

Multi-scale compartmentalization of chromosomes: from topologically associated domains to loops

The combination of more frequently cutting restriction enzymes and ever-deeper sequencing has allowed finer resolution of C-technique maps, revealing that chromosomes are folded into overlapping multi-scale compartments (Fig. 2A; see Table 1 for a summary of key studies). The large A and B compartments can be split into megabase-size contact domains (1-10 Mb, termed megadomains). Megadomains group together a number of smaller topologically associated domains (TADs), also called globules or chromosomal interaction domains (CIDs) (Dixon et al., 2012; Nora et al., 2012; Sexton et al., 2012; Le et al., 2013; Mizuguchi et al., 2015). The

Development (2016) 143, 910-923 doi:10.1242/dev.129007

Table 1. Chromosome conformation capture studies identifying chromatin domains folds and their boundaries

Cell type(s)	Protocol	Number of contacts sequenced ($\times 10^6$)	Resolution	Nomenclature	Number of domains identified	Size of identified domains	Domain boundary marks	References
Human lymphoblastoid (GM 6990); erythroleukemia cell line (K562)	Hi-C (*Hin*dIII, *Nco*I)	8	1 Mb	A/B compartments (chromosome territories); megadomains	ND	>200 Mb	ND	Lieberman-Aiden et al., 2009
Human GM12878 (lymphoblastoid)	Hi-C (*Hin*dIII)	22	1 Mb	A/B compartments	ND	Mean: 475 kb	Active marks: DNaseI, Pol III binding, H3K4me3, H3K9ac; silent marks: H3K27me3	Kalhor et al., 2012
Drosophila embryonic nuclei	3C-seq (*Dpn*II)	362	ND	Physical domains; A/B compartments	1169	Median: 62 kb; mean: 117 kb	CP190 chromator active marks: BEAF-32, H3K4me3; silent marks: CTCF at borders of PcG domains	Sexton et al., 2012
Mouse ESCs; neuronal progenitor cells; embryonic fibroblasts	5C (*Hin*dIII)	0.02	ND	TADs	1051	0.2-1 Mb	CTCF and cohesin	Nora et al., 2012
Mouse ESCs; human ESCs; human IMR90 fibroblasts	Hi-C (*Hin*dIII)	1700	<100 kb	Megabase-sized topological domains; LADs	2200	Median: 880 kb	15% of CTCF-binding sites; H3K9me3 (differentiated cells); TSS; housekeeping genes; tRNA genes; Alu SINE elements (humans)	Dixon et al., 2012
Drosophila Kc167 cells	Hi-C, 3C, 5C (*Hin*dIII)	373	4-20 kb	Domains	1100	Median: 61 kb; mean: 107 kb	BEAF-32, CTCF and CP190; RNAPII; transcription factors and insulator proteins	Hou et al., 2012
Mouse ESCs and ESC-derived neural precursors	5C (*Hin*dIII)	214	High	Sub-TADs	1551	Mean: 1.15 Mb	CTCF; cohesin	Phillips-Cremins et al., 2013
Male mouse splenic CD4+ Y cells	Single-cell Hi-C (*Bgl*II, *Dpn*II, *Alu*I)	190	1 Mb	*Trans*-chromosomal contacts; *cis*-contacts	1403	Mean: 1.7 Mb; median: 10.5 kb	ND	Nagano et al., 2013
Human fibroblasts (IMR90)	Hi-C (*Hin*dIII)	3400	40 kb	Promoter-enhancer contacts	11,313	100 bp-50 kb; median: 10.5 kb	ND	Jin et al., 2013
Caulobacter crescentus	Hi-C (*Bgl*II, *Nco*I)	111	ND	CIDs	23 CIDs	ND	ND	Le et al., 2013

Continued

Development (2016) 143, 910-923 doi:10.1242/dev.129007

Table 1. Continued

Cell type(s)	Protocol	Number of contacts sequenced ($\times 10^6$)	Resolution	Nomenclature	Number of domains identified	Size of identified domains	Domain boundary marks	References
Human HeLa S3 cells	5C, Hi-C (*Eco*RI, *Hin*dIII)	104	1 Mb	ND	1692	ND	ND	Naumova et al., 2013
Drosophila embryonic cells	4C-seq (*Dpn*II)	3880	ND	Promoter-enhancer contacts; TADs	1389 interactions	110 kb	Active marks: H3K27ac, H3K4me3 H3K79me3, H3K4me1 and Pol II	Ghavi-Helm et al., 2014
Arabidopsis thaliana	Hi-C (*Hin*dIII)	812	ND	A and B compartments (loose and compacted structural domains); interchromosomal clusters	10 interchromosomal clusters	ND	Active marks: H3K36me2-me3, H3K4me2-me3, H3K9ac, at LSDs; silent marks: H3K27me3 at CSDs	Grob et al., 2014
Arabidopsis thaliana	Hi-C (*Hin*dIII)	41-66	20	IHIs	10 IHIs	200-1600 kb	Silent marks: H3K9me2 and H3K27me1; negative correlation with H3K4me1/2/3	Feng et al., 2014
Human GM12878 B-lymphoblastoid cells; cell lines from human germ layers; mouse B-lymphoblasts (CH12-LX)	Hi-C (*Dpn*II, *Msp*I, *Hin*dIII, *Nco*I, *Bsp*HI)	25,000	1-5 kb	Chromatin loops	ND	Median: 185 kb	Active mark: H3K36me3	Rao et al., 2014
H1 human ESCs and four H1-derived lineages	Hi-C (*Hin*dIII)	3850	40 kb	A/B compartments; TADs	ND	ND	Active marks: H3K4me1, DHS, H3K27ac, CTCF; silent marks: H3K27me3, H3K9me3	Dixon et al., 2015
C. elegans embryonic cells	Hi-C (*Dpn*II)	824	30 kb	DCC-dependent TAD	17 TAD boundaries on X; eight are DCC dependent	1 Mb	Seven rex sites at the eight DCC-dependent TAD boundaries	Crane et al., 2015
Human cell lines	ChIA-PET	364	4 kb	CCDs	ND	ND	Cohesin, CTCF; active marks: RNAPII	Tang et al., 2015
Human ESCs	ChIA-PET	400	4 kb	CTCF-CTCF loops	ND	ND	Cohesin, CTCF	Ji et al., 2016

BEAF-32, Boundary Element Associated Factor; CCDs, CTCF-mediated chromatin contact domains; CSDs, chromosome deletions; DCC, dosage compensation complex; DHS, DNase I hypersensitive sites; IHIs, interactive heterochromatic islands; LADs, lamin-associated domains; LSDs, loose structural domains; ND, not determined; PcG, Polycomb group; Pol III, DNA polymerase III; RNAPII, RNA polymerase II; SINE, short interspersed nuclear elements; TSS (transcription start sites).

characteristic of TADs is that sequences inside TADs show higher contact frequencies between them than with sequences in neighboring TADs (this phenomenon is known as insulation). In the contact frequency matrix, TADs appear as triangles with high contact frequency values along the diagonal of the chromosome (Fig. 2B). TADs have been identified in all species examined (a number of bacterial species, many yeast species, various *Drosophila* species, *C. elegans*, mouse and human cells; Table 1 and references therein), although the domains are not as clearly defined in *Arabidopsis* (Feng et al., 2014; Grob et al., 2014). In mammals, TADs are evolutionarily conserved and present in paralogous regions of the mouse and human genome (Dixon et al., 2012; Rao et al., 2014) or in duplicated regions encompassing the *HoxA* and *HoxD* loci (Fig. 2B; Lonfat et al., 2014). The observation that the genome folds into TADs raised questions regarding the mechanisms of TAD formation and of their possible biological function.

Development (2016) 143, 910-923 doi:10.1242/dev.129007

TAD formation: internal interactions, boundaries and loop extrusion

TADs are similar to epigenetic domains defined by sets of histone modifications (Dixon et al., 2012, 2015; Nora et al., 2012; Sexton et al., 2012). However, TADs do not seem to be defined by these epigenetic marks, as mutations in key epigenetic regulators do not influence TAD structure (Nora et al., 2012; Williamson et al., 2014). The concordance between TADs and epigenetic domains might therefore be a consequence of TAD folding limiting epigenetic domains rather than the opposite (Nora et al., 2012; Williamson et al., 2014). Characterizing which sequences determine TAD formation is a challenge, considering these structures are several hundred kilobases in size. However, one can envision that TADs arise either by a set of intra-TAD interactions influencing the structure of the domain or by the creation of boundaries limiting interactions between TADs. Arguments and evidence for both hypotheses have been put forward, suggesting that a combination of both governs TAD formation.

The model of intra-TAD contacts creating the TAD structure is supported by studies of the structure of the mouse X chromosome *Tsix* TAD. Comparison of modeling and super-resolution FISH data allowed systematic interrogation of the function of each internal segment for correct TAD folding (Giorgetti et al., 2014). Two segments were found to be essential *in silico*; *in vivo*, deletion of these segments did indeed lead to TAD disruption (decrease of intra-TAD interactions) as predicted by the model. This suggests that *Xist* TAD formation is dictated by a limited number of high-interaction sites inside the TAD. Importantly, disrupting internal TAD structure leads to both TAD unfolding and higher inter-TAD contacts between the unfolded TAD and the adjacent one, suggesting the sharpness of the boundary between TADs depends on intra-TAD interactions (Giorgetti et al., 2014). Similarly, ablation of a number of factors known to create loops, such as the architectural proteins CCCTC-binding factor (CTCF) and cohesin, had a similar effect (Seitan et al., 2013; Sofueva et al., 2013; Zuin et al., 2014). The appearance of TADs by means of intra-TADs interactions is supported by theoretical studies of chromatin behavior (the strings and binders switch model; Nicodemi et al., 2008; Barbieri et al., 2012). In these models, chromatin is represented as a polymer (made of polymerized monomers), with a limited number of individual monomers with binding sites for a given factor able to bring together these specific monomers. The polymer exhibits a biphasic behavior depending on the concentration of the binding factor, with a switch-like transition between open (unfolded domain) and closed (the TAD) states.

By contrast, a number of experiments have provided data in favor of the creation of TADs by their boundaries. In flies and vertebrate cells, TAD boundaries are characterized by their enrichment for highly transcribed genes (in particular housekeeping and tRNA genes) and the associated eukaryotic chromatin marks (H3K4 and H3K36 trimethylation, Dixon et al., 2012; Sexton et al., 2012; Phillips-Cremins et al., 2013). Active chromatin was even recently suggested to be causal in the formation of TADs in the *Drosophila* genome by creating less condensed regions between TADs (Ulianov et al., 2015). In the bacteria *Caulobacter*, insertion of a highly transcribed gene inside a TAD is even able to create a new TAD boundary, splitting the original TAD in two (Le et al., 2013). Additionally, in both mammals and *Drosophila*, a number of proteins previously characterized as insulators (proteins able to separate enhancers from promoters) are enriched at the TAD boundaries (Dixon et al., 2012; Hou et al., 2012; Sexton et al., 2012). Among these, CTCF and cohesin have attracted much attention. In flies, mice, and human cells, both factors are found at TAD boundaries although not exclusively present at these (85% of CTCF-binding sites are actually inside the TADs). CTCF and cohesin ChiA-PET (chromatin immunoprecipitation paired-end sequence tag, a variation of Hi-C in which a given factor is first immunoprecipitated before the Hi-C procedure is carried out) contact maps are very similar to Hi-C maps (Ji et al., 2015; Tang et al., 2015). Furthermore, CTCF depletion decreases intra-TAD contacts and increases inter-TAD contacts, leading to a less defined yet still present boundary. Similarly, cohesin depletion weakens intra-TAD contacts, in particular long-range ones, but the overall structure and boundaries of TADs remain preserved (Seitan et al., 2013; Sofueva et al., 2013; Zuin et al., 2014). This suggests that although CTCF and cohesins are present at the TAD boundaries, these factors reinforce TAD structure by increasing intra-TAD interactions and weakening inter-TAD contacts.

High resolution Hi-C showed that CTCF-binding sites are located at the base of chromatin loops (Rao et al., 2014). These binding sites are directional and loops are observed between adjacent convergent sites, whereas they are almost absent between divergent ones (Rao et al., 2014). The colocalization of CTCF and cohesin on chromatin suggested a mechanism for loop formation. Cohesins, which are members of the structural maintenance of chromosome (SMC) complex family, create large ring-like assemblies able to accommodate chromatin inside the ring (Fig. 2A, loop extrusion factor). The ATPase activity of cohesins led to the early suggestion that these complexes can extrude chromatin to create loops (Nasmyth, 2001). Based on this original idea, a number of recent models have included the directional boundary created by CTCF-binding sites (Rao et al., 2014; Nichols and Corces, 2015; Sanborn et al., 2015). *In silico* simulations using these models were indeed able to predict loop formation *in vivo* and, conversely, targeted deletions of CTCF-binding sites led to changes in loops as predicted by the models (Sanborn et al., 2015). Whether CTCF and cohesin are loaded together and/or travel together along DNA is not known. Similarly, how CTCF creates directional boundaries remains to be determined, although DNA bending has been suggested as a possible mechanism (MacPherson and Sadowski, 2010; Alipour and Marko, 2012; Nichols and Corces, 2015). A consequence of loop formation by CTCF and cohesin is that closely located divergent CTCF-binding sites lead to looping of the two adjacent genome stretches into different TADs. Conversely, convergent CTCF motifs lead to the formation of a loop between these. Genome-wide CTCF ChIA-PET confirms that the formation of individual loops or larger TADs (composed of multiple loops) depends on the spacing and orientation of CTCF-binding sites (Tang et al., 2015). Moreover, inverting CTCF-binding site orientation at the protocadherin or β-globulin loci leads to inversion of contact domains (Guo et al., 2015). Additionally, single nucleotide polymorphism variation in the CTCF motifs leads to altered CTCF binding and, consequently, altered looping (Tang et al., 2015). Moreover, divergent CTCF sites are found at evolutionarily conserved TAD boundaries across deuterostomes (sea urchin, zebrafish, mouse and human; Gómez-Marín et al., 2015).

The formation of directional loops is an attractive model to explain TAD formation. However, CTCF is not present in all organisms in which TADs have been observed (e.g. *C. elegans*, fission yeast or *Caulobacter*). It is therefore likely that other factors are playing a similar role in those organisms, presumably together with SMC family complexes. TADs in *Schizosaccharomyces pombe* depend on cohesin, which again implies the involvement of SMC proteins and chromatin extrusion. Strikingly, in nematodes,

Development (2016) 143, 910-923 doi:10.1242/dev.129007

the formation of X chromosome-specific enhanced TADs, more clearly individualized compared with autosomal ones, follows the same logic: an SMC-like dosage compensation complex is loaded at binding sites (*rex* sites) from where it travels along the chromosome. The boundaries of the X-specific TADs are enriched for *rex* sites and deletion of a single *rex* site between two TADs leads to overlapping domains as observed by microscopy (Crane et al., 2015). How TAD reinforcement impacts on transcription remains, however, unclear.

Are TADs functional units?

As TADs are observed in almost all assayed organisms, a key question is whether these structures are functional units of the genome or a physical consequence of the polymeric nature of chromatin. In contrast to changes in the appearance of chromatin masses inside the nucleus revealed by EM, but in striking parallel to LADs, the TAD structure of chromosomes is largely invariant between different tissues (Dixon et al., 2012, 2015; Meuleman et al., 2013; Jin et al., 2013; Phillips-Cremins et al., 2013). For example, TAD boundaries are mostly the same in mouse or human embryonic stem cells (mESCs and hESCs) and differentiated cells of the same species (Fig. 2B). Similarly, TADs are almost identical in cells differentiated from hESCs (Dixon et al., 2012, 2015). The main differences between cell types are observed at the intra-TAD level, where contact frequencies decrease or increase. Decreased intra-TAD contact frequencies correlate with A-to-B compartment shifts and gene downregulation, whereas, conversely, increased intra-TAD contacts are associated with B-to-A shifts and gene upregulation (Dixon et al., 2015). Similarly, upon acute tumor necrosis factor α (TNFα) treatment, global transcriptional program changes have little effect on TAD structure (Jin et al., 2013).

Only two situations have been described in which TADs are rearranged to a greater extent, both of which involve condensin SMC complexes. The first one is the compensated X chromosome in *C. elegans* hermaphrodite animals, re-established each generation. The loading of the dosage compensation complex onto the X chromosome leads to a reinforced TAD structure with enhanced boundaries between TADs (see above). The second situation occurs during mammalian cell mitosis, during which condensin loading compacts chromosomes during prophase, leading to the mitotic chromosome structure. In metaphase, the TADs of the chromosomes completely disappear, replaced by a homogenous folding (Naumova et al., 2013). It remains unclear whether this is a consequence of the higher mitotic compaction of the chromosomes allowing more contact possibilities or whether this has a functional significance.

Even if TADs are not greatly changing between cell types and/or developmental stages, several clues point to a function of TADs in gene regulation. First, for a few tested TADs, genes inside the TAD tend to be co-regulated during development (Nora et al., 2012). Second, the majority of the long-range contacts occur within the same TAD: in fly embryos, enhancers are almost exclusively located in the same TAD as their target promoter, sometimes at very large distances, despite the small size of the genome (Ghavi-Helm et al., 2014). Similarly, in mouse, contacts between *HoxD* genes and distal enhancers occur in the same TAD (see below; Andrey et al., 2013) and, more generally, loops are restricted to within a single TAD (Jin et al., 2013; Rao et al., 2014; Ji et al. 2015; Tang et al., 2015). Early studies of the mouse Hbb genes and their respective enhancer locus control region (LCR) demonstrated that the promoter contacts the enhancer only in erythroid cells in which the globin genes are expressed (Tolhuis et al., 2002). However, this appears to be the case for a minority of genes, as only a small proportion of enhancer/promoter loops change between cell types (Ghavi-Helm et al., 2014; Rao et al., 2014). Most of these loops are invariant, as are the TADs to which they belong. Quantitatively, in human cells, from 9448 loops identified in one cell type, only 2-11% of them are different in other cell types (Rao et al., 2014). Out of these variant loops, most of them (>80%) are associated with promoters, but only 10-30% of the genes associated with these promoters show significant upregulation upon loop formation (Rao et al., 2014). Therefore, loops appear to be only loosely correlated with the transcriptional activation of the associated gene and most loops do not change upon activation or silencing (Jin et al., 2013; Ghavi-Helm et al., 2014; Rao et al., 2014). At least in *Drosophila*, promoter-enhancer loops correlate with the presence of paused polymerases (Ghavi-Helm et al., 2014). Although transcriptionally unproductive, these structures would present a dual advantage: on one hand, genes are ready for transcription triggered by the recruitment of additional transcription factors, while on the other hand these hubs sequester enhancers away from other promoters, thus impairing spurious transcriptional activation.

An additional argument in favor of a function for TADs comes from studies of human mutations leading to hand malformations (Lupiáñez et al., 2015). In individuals with such malformations, large deletions, inversions or duplications break TAD structure, in particular the boundaries between them. As a consequence, these rearrangements place genes in a different TAD, close to enhancers they should normally not interact with. Deletion of the homologous conserved genome region encompassing the TAD boundary in the mouse is sufficient to induce gene misregulation and phenocopy the human malformations. By contrast, similar-sized deletions not containing the TAD boundary are well tolerated (Lupiáñez et al., 2015). Presence of the gene in the correct TAD therefore appears to be essential for its correct expression pattern, at least in some cases.

A notable exception to the largely stable nature of enhancer/promoter loops is the *HoxD* locus. This locus, ∼70 kb large, is located at the boundary between two large TADs (>600 kb each; Fig. 2B; Dixon et al., 2012). The 13 Hox genes form a cluster that undergoes sequential expression in time and space from the 3′ to the 5′ end, thereby patterning the body along the anterior-posterior axis, as well as the proximo-distal organization of the limbs and sexual organs. During arm and forearm specification (early phase of limb development), enhancers located in the 3′ TAD drive sequential activation of the proximal *HoxD* genes. Later, during digit specification, enhancers located in the 5′ TAD drive expression of some of the same *HoxD* genes (Andrey et al., 2013). The sequential use of regulatory input from different TADs therefore allows repeated use of the same patterning genes (arm/forearm first; digits later) with a TAD-specific set of enhancers, providing an additional level of transcriptional regulation. Understanding the molecular nature of the factors necessary to switch regulatory inputs from one TAD to another would be of great interest.

In conclusion, although TAD organization appears to be largely stable during development, this stability impacts on transcription as it restricts the number of enhancers with which a specific gene promoter can engage.

Cell fate and genome organization: what is the link?

A large number of electron and light microscopy studies have documented changes in nuclear organization during development. At the scale of the entire nucleus, heterochromatin is almost absent in embryonic cells and cell fate acquisition correlates with

heterochromatin appearance (Ahmed et al., 2010; Fussner et al., 2011). At the other end of the genome size scales, differential positioning of genes in embryonic and differentiated cells has been extensively documented (Chambeyron et al., 2005; Takizawa et al., 2008a; Meister et al., 2010). In apparent contradiction with these microscopy data, the new techniques capturing genome-wide structures and localization have revealed surprisingly few changes in the 3D organization of the genome over the course of development, at both the gene scale (enhancer-promoter contacts, loops; Ghavi-Helm et al., 2014; Rao et al., 2014) and more globally (TADs; Dixon et al., 2015).

How can these two types of observations be reconciled? One clue might come from the correlation established between epigenetic domains (highlighted by combinations of histone marks) and TADs. The former are clearly changing over the course of development as a reflection of cell type-specific activation or silencing of the genes contained in these domains, but domains themselves remain largely invariant. In other words, although TADs are stable structures, their epigenetic nature changes during differentiation. These changes could impact on the type of contacts a given TAD can establish with other TADs in the genome, creating large A/B compartments, which vary greatly between cell types (Dixon et al., 2015). However, the identification of such features requires capturing a very large number of contacts as most of the identified contacts are located in *cis*. Two studies, in human and *Drosophila*, have indeed observed such preferential contacts between TADs marked with similar chromatin marks (Sexton et al., 2012; Rao et al., 2014). In favor of such a model, Hi-C with *Drosophila* salivary gland highly polyploid polytene chromosomes show a clear correspondence between the cytological banding pattern seen with both electron and light microscopy and TADs characterized by Hi-C. These very large chromosomes, which do not engage in long-range intra- or inter-chromosomal contacts, show a complete absence of inter-TAD interactions (Eagen et al., 2015).

Although the exact biological mechanism of inter-TAD clustering remains unclear, modeling with heterogeneous polymers suggests that slightly increasing self-affinity for similarly marked chromatin can indeed mimic such behavior (Jost et al., 2014). This would resolve the apparent contradiction between the highly variable eu- and heterochromatin staining observed using microscopy techniques (Fig. 1A) and the conservation of TADs across cell types as characterized using Hi-C (Fig. 2B). A clear advantage of clustering would be the stabilization of the gene expression program: silent genomic regions would be buried in other silent domains, whereas active ones would have active neighbors, resulting in the creation of a self-reinforcing feedback loop, thereby 'locking' the transcriptional program of the genome (Jencks, 1975; Meister and Taddei, 2013). Nuclear organization might therefore be an integral part of the network maintaining a stable cell fate.

Future perspectives

Almost 100 years after the initial observations of chromatin heterogeneity, the advent of genome-wide mapping techniques are now finally shedding light on the underlying sequences constituting these chromatin domains. High-throughput sequencing and ever-higher sensitivity of these techniques will allow reduction of sample size, even to the single-cell level. Once these techniques are available – some of them have already been described (Nagano et al., 2013; Kind et al., 2015) – it will be possible able to dissect cell-to-cell and developmental variability, thus untying structures resulting from stochastic assemblies governed by chromatin biophysical behavior from complexes for which formation is actively regulated. In addition, uncovering molecular determinants of nuclear organization should allow these structures to be altered in order to interrogate their transcriptional function further.

Acknowledgements

We are grateful to Dr Jastrow for the permission to use his electron microscopy images and Martin Jakob for help with the figures. We apologize to colleagues whose work could not be cited owing to space limitations.

Competing interests

The authors declare no competing or financial interests.

Funding

The Meister laboratory is funded by the Swiss National Foundation [SNF assistant professor grant PP00P3_133744/159320]; the Swiss Muscle Research Foundation (FSRMM); the Novartis Biomedical Research Foundation; and the University of Bern.

References

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

Alipour, E. and Marko, J. F. (2012). Self-organization of domain structures by DNA-loop-extruding enzymes. *Nucleic Acids Res.* **40**, 11202-11212.

Andrey, G., Montavon, T., Mascrez, B., Gonzalez, F., Noordermeer, D., Leleu, M., Trono, D., Spitz, F. and Duboule, D. (2013). A switch between topological domains underlies HoxD genes collinearity in mouse limbs. *Science* **340**, 1234167.

Andrulis, E. D., Neiman, A. M., Zappulla, D. C. and Sternglanz, R. (1998). Perinuclear localization of chromatin facilitates transcriptional silencing. *Nature* **394**, 592-595.

Barbieri, M., Chotalia, M., Fraser, J., Lavitas, L.-M., Dostie, J., Pombo, A. and Nicodemi, M. (2012). Complexity of chromatin folding is captured by the strings and binders switch model. *Proc. Natl. Acad. Sci. USA* **109**, 16173-16178.

Belmont, A. S. (2014). Large-scale chromatin organization: the good, the surprising, and the still perplexing. *Curr. Opin. Cell Biol.* **26**, 69-78.

Bian, Q., Khanna, N., Alvikas, J. and Belmont, A. S. (2013). β-Globin cis-elements determine differential nuclear targeting through epigenetic modifications. *J. Cell Biol.* **203**, 767-783.

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

Bond, C. S. and Fox, A. H. (2009). Paraspeckles: nuclear bodies built on long noncoding RNA. *J. Cell Biol.* **186**, 637-644.

Branco, M. R. and Pombo, A. (2006). Intermingling of chromosome territories in interphase suggests role in translocations and transcription-dependent associations. *PLoS Biol.* **4**, e138.

Cajal, S. R. (1903). Un sencillo método de coloración selectiva del retículo protoplásmico y sus efectos en los diversos órganos nerviosos de vertebrados e invertebrados. *Trab. Lab. Invest. Biol.* **2**, 129-221.

Cajal, S. R. (1910). El núcleo de las células piramidales del cerebro humano y de algunos mamiferos. *Trab. Lab. Invest. Biol.* **8**, 27-62.

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

Chambeyron, S., Da Silva, N. R., Lawson, K. A. and Bickmore, W. A. (2005). Nuclear re-organisation of the Hoxb complex during mouse embryonic development. *Development* **132**, 2215-2223.

Chubb, J. R., Boyle, S., Perry, P. and Bickmore, W. A. (2002). Chromatin motion is constrained by association with nuclear compartments in human cells. *Curr. Biol.* **12**, 439-445.

Cisse, I. I., Izeddin, I., Causse, S. Z., Boudarene, L., Senecal, A., Muresan, L., Dugast-Darzacq, C., Hajj, B., Dahan, M. and Darzacq, X. (2013). Real-time dynamics of RNA polymerase II clustering in live human cells. *Science* **341**, 664-667.

Crane, E., Bian, Q., McCord, R. P., Lajoie, B. R., Wheeler, B. S., Ralston, E. J., Uzawa, S., Dekker, J. and Meyer, B. J. (2015). Condensin-driven remodelling of X chromosome topology during dosage compensation. *Nature* **523**, 240-244.

Cremer, T., Cremer, M., Dietzel, S., Müller, S., Solovei, I. and Fakan, S. (2006). Chromosome territories – a functional nuclear landscape. *Curr. Opin. Cell Biol.* **18**, 307-316.

Croft, J. A., Bridger, J. M., Boyle, S., Perry, P., Teague, P. and Bickmore, W. A. (1999). Differences in the localization and morphology of chromosomes in the human nucleus. *J. Cell Biol.* **145**, 1119-1131.

Dekker, J. (2008). Mapping in vivo chromatin interactions in yeast suggests an extended chromatin fiber with regional variation in compaction. *J. Biol. Chem.* **283**, 34532-34540.
Dekker, J., Rippe, K., Dekker, M. and Kleckner, N. (2002). Capturing chromosome conformation. *Science* **295**, 1306-1311.
Dixon, J. R., Selvaraj, S., Yue, F., Kim, A., Li, Y., Shen, Y., Hu, M., Liu, J. S. and Ren, B. (2012). Topological domains in mammalian genomes identified by analysis of chromatin interactions. *Nature* **485**, 376-380.
Dixon, J. R., Jung, I., Selvaraj, S., Shen, Y., Antosiewicz-Bourget, J. E., Lee, A. Y., Ye, Z., Kim, A., Rajagopal, N., Xie, W. et al. (2015). Chromatin architecture reorganization during stem cell differentiation. *Nature* **518**, 331-336.
Dostie, J., Richmond, T. A., Arnaout, R. A., Selzer, R. R., Lee, W. L., Honan, T. A., Rubio, E. D., Krumm, A., Lamb, J., Nusbaum, C. et al. (2006). Chromosome Conformation Capture Carbon Copy (5C): a massively parallel solution for mapping interactions between genomic elements. *Genome Res.* **16**, 1299-1309.
Duan, Z., Andronescu, M., Schutz, K., McIlwain, S., Kim, Y. J., Lee, C., Shendure, J., Fields, S., Blau, C. A. and Noble, W. S. (2010). A three-dimensional model of the yeast genome. *Nature* **465**, 363-367.
Eagen, K. P., Hartl, T. A. and Kornberg, R. D. (2015). Stable chromosome condensation revealed by chromosome conformation capture. *Cell* **163**, 934-946.
Ernst, J., Kheradpour, P., Mikkelsen, T. S., Shoresh, N., Ward, L. D., Epstein, C. B., Zhang, X., Wang, L., Issner, R., Coyne, M. et al. (2011). Mapping and analysis of chromatin state dynamics in nine human cell types. *Nature* **473**, 43-49.
Feng, S., Cokus, S. J., Schubert, V., Zhai, J., Pellegrini, M. and Jacobsen, S. E. (2014). Genome-wide Hi-C analyses in wild-type and mutants reveal high-resolution chromatin interactions in Arabidopsis. *Mol. Cell* **55**, 694-707.
Ferrai, C., de Castro, I. J., Lavitas, L., Chotalia, M. and Pombo, A. (2010). Gene positioning. *Cold Spring Harb. Perspect. Biol.* **2**, a000588.
Filion, G. J., van Bemmel, J. G., Braunschweig, U., Talhout, W., Kind, J., Ward, L. D., Brugman, W., de Castro, I. J., Kerkhoven, R. M., Bussemaker, H. J. et al. (2010). Systematic protein location mapping reveals five principal chromatin types in Drosophila cells. *Cell* **143**, 212-224.
Finlan, L. E., Sproul, D., Thomson, I., Boyle, S., Kerr, E., Perry, P., Ylstra, B., Chubb, J. R. and Bickmore, W. A. (2008). Recruitment to the nuclear periphery can alter expression of genes in human cells. *PLoS Genet* **4**, e1000039.
Frenster, J. H., Allfrey, V. G. and Mirsky, A. E. (1963). Repressed and active chromatin isolated from interphase lymphocytes. *Proc. Natl. Acad. Sci. USA* **50**, 1026-1032.
Fussner, E., Djuric, U., Strauss, M., Hotta, A., Perez-Iratxeta, C., Lanner, F., Dilworth, F. J., Ellis, J. and Bazett-Jones, D. P. (2011). Constitutive heterochromatin reorganization during somatic cell reprogramming. *EMBO J.* **30**, 1778-1789.
Gavrilov, A. A., Gushchanskaya, E. S., Strelkova, O., Zhironkina, O., Kireev, I.I., Iarovaia, O. V. and Razin, S. V. (2013). Disclosure of a structural milieu for the proximity ligation reveals the elusive nature of an active chromatin hub. *Nucleic Acids Res.* **41**, 3563-3575.
Ghavi-Helm, Y., Klein, F. A., Pakozdi, T., Ciglar, L., Noordermeer, D., Huber, W. and Furlong, E. E. (2014). Enhancer loops appear stable during development and are associated with paused polymerase. *Nature* **512**, 96-100.
Giorgetti, L., Galupa, R., Nora, E. P., Piolot, T., Lam, F., Dekker, J., Tiana, G. and Heard, E. (2014). Predictive polymer modeling reveals coupled fluctuations in chromosome conformation and transcription. *Cell* **157**, 950-963.
Gómez-Marín, C., Tena, J. J., Acemel, R. D., López-Mayorga, M., Naranjo, S., de la Calle-Mustienes, E., Maeso, I., Beccari, L., Aneas, I., Vielmas, E. et al. (2015). Evolutionary comparison reveals that diverging CTCF sites are signatures of ancestral topological associating domains borders. *Proc. Natl. Acad. Sci. USA* **112**, 7542-7547.
Gonzalez-Sandoval, A., Towbin, B. D., Kalck, V., Cabianca, D., Gaidatzis, D., Hauer, M. H., Geng, L., Wang, L., Yang, T., Wang, X. et al. (2015). Perinuclear anchoring of H3K9-methylated chromatin stabilizes induced cell fate in C. elegans embryos. *Cell* **163**, 1333-1347.
Grand, R. S., Pichugina, T., Gehlen, L. R., Jones, M. B., Tsai, P., Allison, J. R., Martienssen, R. and O'Sullivan, J. M. (2014). Chromosome conformation maps in fission yeast reveal cell cycle dependent sub nuclear structure. *Nucleic Acids Res.* **42**, 12585-12599.
Grob, S., Schmid, M. W. and Grossniklaus, U. (2014). Hi-C analysis in Arabidopsis identifies the KNOT, a structure with similarities to the flamenco locus of Drosophila. *Mol. Cell* **55**, 678-693.
Gruenbaum, Y. and Foisner, R. (2015). Lamins: nuclear intermediate filament proteins with fundamental functions in nuclear mechanics and genome regulation. *Annu. Rev. Biochem.* **84**, 131-164.
Guelen, L., Pagie, L., Brasset, E., Meuleman, W., Faza, M. B., Talhout, W., Eussen, B. H., de Klein, A., Wessels, L., de Laat, W. et al. (2008). Domain organization of human chromosomes revealed by mapping of nuclear lamina interactions. *Nature* **453**, 948-951.
Guidi, M., Ruault, M., Marbouty, M., Loïodice, I., Cournac, A., Billaudeau, C., Hocher, A., Mozziconacci, J., Koszul, R. and Taddei, A. (2015). Spatial reorganization of telomeres in long-lived quiescent cells. *Genome Biol.* **16**, 206.
Guo, Y., Xu, Q., Canzio, D., Shou, J., Li, J., Gorkin, D. U., Jung, I., Wu, H., Zhai, Y., Tang, Y. et al. (2015). CRISPR inversion of CTCF sites alters genome topology and enhancer/promoter function. *Cell* **162**, 900-910.
Harr, J. C., Luperchio, T. R., Wong, X., Cohen, E., Wheelan, S. J. and Reddy, K. L. (2015). Directed targeting of chromatin to the nuclear lamina is mediated by chromatin state and A-type lamins. *J. Cell Biol.* **208**, 33-52.
Heitz, E. (1928). Das heterochromatin der moose. *Jahrb. Wiss. Botanik* **69**, 762-818.
Heun, P., Laroche, T., Raghuraman, M. K. and Gasser, S. M. (2001). The positioning and dynamics of origins of replication in the budding yeast nucleus. *J. Cell Biol.* **152**, 385-400.
Ho, J. W. K., Jung, Y. L., Liu, T., Alver, B. H., Lee, S., Ikegami, K., Sohn, K.-A., Minoda, A., Tolstorukov, M. Y., Appert, A. et al. (2014). Comparative analysis of metazoan chromatin organization. *Nature* **512**, 449-452.
Hou, C., Li, L., Qin, Z. S. and Corces, V. G. (2012). Gene density, transcription, and insulators contribute to the partition of the Drosophila genome into physical domains. *Mol. Cell* **48**, 471-484.
Hozák, P., Hassan, A. B., Jackson, D. A. and Cook, P. R. (1993). Visualization of replication factories attached to a nucleoskeleton. *Cell* **73**, 361-373.
Jackson, D. A., Hassan, A. B., Errington, R. J. and Cook, P. R. (1993). Visualization of focal sites of transcription within human nuclei. *EMBO J.* **12**, 1059-1065.
Jencks, W. P. (1975). Binding energy, specificity, and enzymic catalysis: the circe effect. *Adv. Enzymol. Relat. Areas Mol. Biol.* **43**, 219-410.
Ji, X., Dadon, D. B., Powell, B. E., Fan, Z. P., Borges-Rivera, D., Shachar, S., Weintraub, A. S., Hnisz, D., Pegoraro, G., Lee, T. I. et al. (2015). 3D chromosome regulatory landscape of human pluripotent cells. *Cell Stem Cell*.
Jin, F., Li, Y., Dixon, J. R., Selvaraj, S., Ye, Z., Lee, A. Y., Yen, C.-A., Schmitt, A. D., Espinoza, C. A. and Ren, B. (2013). A high-resolution map of the three-dimensional chromatin interactome in human cells. *Nature* **503**, 290-294.
Jost, D., Carrivain, P., Cavalli, G. and Vaillant, C. (2014). Modeling epigenome folding: formation and dynamics of topologically associated chromatin domains. *Nucleic Acids Res.* **42**, 9553-9561.
Kalhor, R., Tjong, H., Jayathilaka, N., Alber, F. and Chen, L. (2012). Genome architectures revealed by tethered chromosome conformation capture and population-based modeling. *Nat. Biotechnol.* **30**, 90-98.
Kind, J., Pagie, L., Ortabozkoyun, H., Boyle, S., de Vries, S. S., Janssen, H., Amendola, M., Nolen, L. D., Bickmore, W. A. and van Steensel, B. (2013). Single-cell dynamics of genome-nuclear lamina interactions. *Cell* **153**, 178-192.
Kind, J., Pagie, L., de Vries, S. S., Nahidiazar, L., Dey, S. S., Bienko, M., Zhan, Y., Lajoie, B., de Graaf, C. A., Amendola, M. et al. (2015). Genome-wide maps of nuclear lamina interactions in single human cells. *Cell* **163**, 134-147.
Kosak, S. T., Scalzo, D., Alworth, S. V., Li, F., Palmer, S., Enver, T., Lee, J. S. J. and Groudine, M. (2007). Coordinate gene regulation during hematopoiesis is related to genomic organization. *PLoS Biol.* **5**, e309.
Kufe, D. W., Pollock, R. E., Weichselbaum, R. R., Bast, R. J., Gansler, T. S., Holland, J. F. and Frei, E. (2003). *Holland-Frei Cancer Medicine*, 6th edn. Hamilton, ON: BC Decker.
Kumaran, R. I. and Spector, D. L. (2008). A genetic locus targeted to the nuclear periphery in living cells maintains its transcriptional competence. *J. Cell Biol.* **180**, 51-65.
Lallemand-Breitenbach, V. and de The, H. (2010). PML nuclear bodies. *Cold Spring Harb. Perspect. Biol.* **2**, a000661.
Lanctôt, C., Cheutin, T., Cremer, M., Cavalli, G. and Cremer, T. (2007). Dynamic genome architecture in the nuclear space: regulation of gene expression in three dimensions. *Nat. Rev. Genet.* **8**, 104-115.
Langer-Safer, P. R., Levine, M. and Ward, D. C. (1982). Immunological method for mapping genes on Drosophila polytene chromosomes. *Proc. Natl. Acad. Sci. USA* **79**, 4381-4385.
Le, T. B. K., Imakaev, M. V., Mirny, L. A. and Laub, M. T. (2013). High-resolution mapping of the spatial organization of a bacterial chromosome. *Science* **342**, 731-734.
Lieberman-Aiden, E., van Berkum, N. L., Williams, L., Imakaev, M., Ragoczy, T., Telling, A., Amit, I., Lajoie, B. R., Sabo, P. J., Dorschner, M. O. et al. (2009). Comprehensive mapping of long-range interactions reveals folding principles of the human genome. *Science* **326**, 289-293.
Lonfat, N., Montavon, T., Darbellay, F., Gitto, S. and Duboule, D. (2014). Convergent evolution of complex regulatory landscapes and pleiotropy at Hox loci. *Science* **346**, 1004-1006.
Lupiáñez, D. G., Kraft, K., Heinrich, V., Krawitz, P., Brancati, F., Klopocki, E., Horn, D., Kayserili, H., Opitz, J. M., Laxova, R. et al. (2015). Disruptions of topological chromatin domains cause pathogenic rewiring of gene-enhancer interactions. *Cell* **161**, 1012-1025.
MacPherson, M. J. and Sadowski, P. D. (2010). The CTCF insulator protein forms an unusual DNA structure. *BMC Mol. Biol.* **11**, 101.
Mahy, N. L., Perry, P. E. and Bickmore, W. A. (2002). Gene density and transcription influence the localization of chromatin outside of chromosome territories detectable by FISH. *J. Cell Biol.* **159**, 753-763.
Meister, P. and Taddei, A. (2013). Building silent compartments at the nuclear periphery: a recurrent theme. *Curr. Opin. Genet. Dev.* **23**, 96-103.

Meister, P., Towbin, B. D., Pike, B. L., Ponti, A. and Gasser, S. M. (2010). The spatial dynamics of tissue-specific promoters during C. elegans development. *Genes Dev.* **24**, 766-782.
Meshorer, E., Yellajoshula, D., George, E., Scambler, P. J., Brown, D. T. and Misteli, T. (2006). Hyperdynamic plasticity of chromatin proteins in pluripotent embryonic stem cells. *Dev. Cell* **10**, 105-116.
Meuleman, W., Peric-Hupkes, D., Kind, J., Beaudry, J.-B., Pagie, L., Kellis, M., Reinders, M., Wessels, L. and van Steensel, B. (2013). Constitutive nuclear lamina-genome interactions are highly conserved and associated with A/T-rich sequence. *Genome Res.* **23**, 270-280.
Misteli, T. (2001). Protein dynamics: implications for nuclear architecture and gene expression. *Science* **291**, 843-847.
Misteli, T., Cáceres, J. F. and Spector, D. L. (1997). The dynamics of a pre-mRNA splicing factor in living cells. *Nature* **387**, 523-527.
Miyanari, Y., Ziegler-Birling, C. and Torres-Padilla, M.-E. (2013). Live visualization of chromatin dynamics with fluorescent TALEs. *Nat. Struct. Mol. Biol.* **20**, 1321-1324.
Mizuguchi, T., Barrowman, J. and Grewal, S. I. S. (2015). Chromosome domain architecture and dynamic organization of the fission yeast genome. *FEBS Lett.* **589**, 2975-2986.
Montavon, T., Soshnikova, N., Mascrez, B., Joye, E., Thevenet, L., Splinter, E., de Laat, W., Spitz, F. and Duboule, D. (2011). A regulatory archipelago controls Hox genes transcription in digits. *Cell* **147**, 1132-1145.
Morris, G. E. (2008). The Cajal body. *Biochim. Biophys. Acta* **1783**, 2108-2115.
Mueller, F., Mazza, D., Stasevich, T. J. and McNally, J. G. (2010). FRAP and kinetic modeling in the analysis of nuclear protein dynamics: what do we really know?. *Curr. Opin. Cell Biol.* **22**, 403-411.
Nagano, T., Lubling, Y., Stevens, T. J., Schoenfelder, S., Yaffe, E., Dean, W., Laue, E. D., Tanay, A. and Fraser, P. (2013). Single-cell Hi-C reveals cell-to-cell variability in chromosome structure. *Nature* **502**, 59-64.
Nasmyth, K. (2001). Disseminating the genome: joining, resolving, and separating sister chromatids during mitosis and meiosis. *Annu. Rev. Genet.* **35**, 673-745.
Naumova, N., Imakaev, M., Fudenberg, G., Zhan, Y., Lajoie, B. R., Mirny, L. A. and Dekker, J. (2013). Organization of the mitotic chromosome. *Science* **342**, 948-953.
Nichols, M. H. and Corces, V. G. (2015). A CTCF code for 3D genome architecture. *Cell* **162**, 703-705.
Nicodemi, M., Panning, B. and Prisco, A. (2008). A thermodynamic switch for chromosome colocalization. *Genetics* **179**, 717-721.
Noordermeer, D., Leleu, M., Splinter, E., Rougemont, J., De Laat, W. and Duboule, D. (2011). The dynamic architecture of Hox gene clusters. *Science* **334**, 222-225.
Nora, E. P., Lajoie, B. R., Schulz, E. G., Giorgetti, L., Okamoto, I., Servant, N., Piolot, T., van Berkum, N. L., Meisig, J., Sedat, J. et al. (2012). Spatial partitioning of the regulatory landscape of the X-inactivation centre. *Nature* **485**, 381-385.
Palstra, R.-J., Simonis, M., Klous, P., Brasset, E., Eijkelkamp, B. and de Laat, W. (2008). Maintenance of long-range DNA interactions after inhibition of ongoing RNA polymerase II transcription. *PLoS ONE* **3**, e1661.
Peric-Hupkes, D., Meuleman, W., Pagie, L., Bruggeman, S. W., Solovei, I., Brugman, W., Gräf, S., Flicek, P., Kerkhoven, R. M., van Lohuizen, M. et al. (2010). Molecular maps of the reorganization of genome-nuclear lamina interactions during differentiation. *Mol. Cell* **38**, 603-613.
Phair, R. D., Gorski, S. A. and Misteli, T. (2004). Measurement of dynamic protein binding to chromatin in vivo, using photobleaching microscopy. *Methods Enzymol.* **375**, 393-414.
Phillips-Cremins, J. E., Sauria, M. E. G., Sanyal, A., Gerasimova, T. I., Lajoie, B. R., Bell, J. S. K., Ong, C.-T., Hookway, T. A., Guo, C., Sun, Y. et al. (2013). Architectural protein subclasses shape 3D organization of genomes during lineage commitment. *Cell* **153**, 1281-1295.
Pickersgill, H., Kalverda, B., de Wit, E., Talhout, W., Fornerod, M. and van Steensel, B. (2006). Characterization of the Drosophila melanogaster genome at the nuclear lamina. *Nat. Genet.* **38**, 1005-1014.
Rao, S. S. P, Huntley, M. H., Durand, N. C., Stamenova, E. K., Bochkov, I. D., Robinson, J. T., Sanborn, A. L., Machol, I., Omer, A. D., Lander, E. S. et al. (2014). A 3D map of the human genome at kilobase resolution reveals principles of chromatin looping. *Cell* **159**, 1665-1680.
Reddy, K. L., Zullo, J. M., Bertolino, E. and Singh, H. (2008). Transcriptional repression mediated by repositioning of genes to the nuclear lamina. *Nature* **452**, 243-247.
Robinett, C. C., Straight, A., Li, G., Willhelm, C., Sudlow, G., Murray, A. and Belmont, A. S. (1996). In vivo localization of DNA sequences and visualization of large-scale chromatin organization using lac operator/repressor recognition. *J. Cell Biol.* **135**, 1685-1700.
Rodley, C. D. M., Bertels, F., Jones, B. and O'Sullivan, J. M. (2009). Global identification of yeast chromosome interactions using Genome conformation capture. *Fungal Genet. Biol.* **46**, 879-886.
Rosa, A. and Everaers, R. (2008). Structure and dynamics of interphase chromosomes. *PLoS Comput. Biol.* **4**, e1000153.
Ruault, M., Dubarry, M. and Taddei, A. (2008). Re-positioning genes to the nuclear envelope in mammalian cells: impact on transcription. *Trends Genet.* **24**, 574-581.
Sanborn, A. L., Rao, S. S. P., Huang, S.-C., Durand, N. C., Huntley, M. H., Jewett, A. I., Bochkov, I. D., Chinnappan, D., Cutkosky, A., Li, J. et al. (2015). Chromatin extrusion explains key features of loop and domain formation in wild-type and engineered genomes. *Proc. Natl. Acad. Sci. USA* **112**, E6456-E6465.
Schoenfelder, S., Sexton, T., Chakalova, L., Cope, N. F., Horton, A., Andrews, S., Kurukuti, S., Mitchell, J. A., Umlauf, D., Dimitrova, D. S. et al. (2010). Preferential associations between co-regulated genes reveal a transcriptional interactome in erythroid cells. *Nat. Genet.* **42**, 53-61.
Schuettengruber, B., Chourrout, D., Vervoort, M., Leblanc, B. and Cavalli, G. (2007). Genome regulation by polycomb and trithorax proteins. *Cell* **128**, 735-745.
Seitan, V. C., Faure, A. J., Zhan, Y., McCord, R. P., Lajoie, B. R., Ing-Simmons, E., Lenhard, B., Giorgetti, L., Heard, E., Fisher, A. G. et al. (2013). Cohesin-based chromatin interactions enable regulated gene expression within preexisting architectural compartments. *Genome Res.* **23**, 2066-2077.
Sexton, T., Yaffe, E., Kenigsberg, E., Bantignies, F., Leblanc, B., Hoichman, M., Parrinello, H., Tanay, A. and Cavalli, G. (2012). Three-dimensional folding and functional organization principles of the Drosophila genome. *Cell* **148**, 458-472.
Shachar, S., Voss, T. C., Pegoraro, G., Sciascia, N. and Misteli, T. (2015). Identification of gene positioning factors using high-throughput imaging mapping. *Cell* **162**, 911-923.
Simonis, M., Klous, P., Splinter, E., Moshkin, Y., Willemsen, R., de Wit, E., van Steensel, B. and de Laat, W. (2006). Nuclear organization of active and inactive chromatin domains uncovered by chromosome conformation capture–on-chip (4C). *Nat. Genet.* **38**, 1348-1354.
Simonis, M., Klous, P., Homminga, I., Galjaard, R.-J., Rijkers, E.-J., Grosveld, F., Meijerink, J. P. P. and de Laat, W. (2009). High-resolution identification of balanced and complex chromosomal rearrangements by 4C technology. *Nat. Methods* **6**, 837-842.
Sofueva, S., Yaffe, E., Chan, W.-C., Georgopoulou, D., Vietri Rudan, M., Mira-Bontenbal, H., Pollard, S. M., Schroth, G. P., Tanay, A. and Hadjur, S. (2013). Cohesin-mediated interactions organize chromosomal domain architecture. *EMBO J.* **32**, 3119-3129.
Spector, D. L. and Lamond, A. I. (2011). Nuclear speckles. *Cold Spring Harb. Perspect. Biol.* **3**, a000646 .
Splinter, E., de Wit, E., Nora, E. P., Klous, P., van de Werken, H. J. G., Zhu, Y., Kaaij, L. J., van IJcken, W., Gribnau, J., Heard, E. et al. (2011). The inactive X chromosome adopts a unique three-dimensional conformation that is dependent on Xist RNA. *Genes Dev.* **25**, 1371-1383.
Takizawa, T., Gudla, P. R., Guo, L., Lockett, S. and Misteli, T. (2008a). Allele-specific nuclear positioning of the monoallelically expressed astrocyte marker GFAP. *Genes Dev.* **22**, 489-498.
Takizawa, T., Meaburn, K. J. and Misteli, T. (2008b). The meaning of gene positioning. *Cell* **135**, 9-13.
Tang, Z., Luo, O. J., Li, X., Zheng, M., Zhu, J. J., Szalaj, P., Trzaskoma, P., Magalska, A., Wlodarczyk, J., Ruszczycki, B. et al. (2015). CTCF-mediated human 3D genome architecture reveals chromatin topology for transcription. *Cell* **163**, 1611-1627.
Therizols, P., Illingworth, R. S., Courilleau, C., Boyle, S., Wood, A. J. and Bickmore, W. A. (2014). Chromatin decondensation is sufficient to alter nuclear organization in embryonic stem cells. *Science* **346**, 1238-1242.
Tolhuis, B., Palstra, R.-J., Splinter, E., Grosveld, F. and de Laat, W. (2002). Looping and interaction between hypersensitive sites in the active beta-globin locus. *Mol. Cell* **10**, 1453-1465.
Towbin, B. D., Meister, P. and Gasser, S. M. (2009). The nuclear envelope – a scaffold for silencing?. *Curr. Opin. Genet. Dev.* **19**, 180-186.
Towbin, B. D., González-Aguilera, C., Sack, R., Gaidatzis, D., Kalck, V., Meister, P., Askjaer, P. and Gasser, S. M. (2012). Step-wise methylation of histone H3K9 positions heterochromatin at the nuclear periphery. *Cell* **150**, 934-947.
Tumbar, T. and Belmont, A. S. (2001). Interphase movements of a DNA chromosome region modulated by VP16 transcriptional activator. *Nat. Cell Biol.* **3**, 134-139.
Tumbar, T., Sudlow, G. and Belmont, A. S. (1999). Large-scale chromatin unfolding and remodeling induced by VP16 acidic activation domain. *J. Cell Biol.* **145**, 1341-1354.
Ulianov, S. V., Khrameeva, E. E., Gavrilov, A. A., Flyamer, I. M., Kos, P., Mikhaleva, E. A., Penin, A. A., Logacheva, M. D., Imakaev, M. V., Chertovich, A. et al. (2015). Active chromatin and transcription play a key role in chromosome partitioning into topologically associating domains. *Genome Res.* **26**, 70-84.
van Steensel, B. and Henikoff, S. (2000). Identification of in vivo DNA targets of chromatin proteins using tethered dam methyltransferase. *Nat. Biotechnol.* **18**, 424-428.
Williams, R. R. E., Azuara, V., Perry, P., Sauer, S., Dvorkina, M., Jørgensen, H., Roix, J., McQueen, P., Misteli, T., Merkenschlager, M. et al. (2006). Neural

induction promotes large-scale chromatin reorganisation of the Mash1 locus. *J. Cell Sci.* **119**, 132-140.

Williamson, I., Berlivet, S., Eskeland, R., Boyle, S., Illingworth, R. S., Paquette, D., Dostie, J. and Bickmore, W. A. (2014). Spatial genome organization: contrasting views from chromosome conformation capture and fluorescence in situ hybridization. *Genes Dev.* **28**, 2778-2791.

Zacharias, H. (1995). Emil Heitz (1892–1965): chloroplasts, heterochromatin, and polytene chromosomes. *Genetics* **141**, 7-14.

Zhang, T., Cooper, S. and Brockdorff, N. (2015). The interplay of histone modifications - writers that read. *EMBO Rep.* **16**, 1467-1481.

Zhou, V. W., Goren, A. and Bernstein, B. E. (2011). Charting histone modifications and the functional organization of mammalian genomes. *Nat. Rev. Genet.* **12**, 7-18.

Zink, D., Amaral, M. D., Englmann, A., Lang, S., Clarke, L. A., Rudolph, C., Alt, F., Luther, K., Braz, C., Sadoni, N. et al. (2004). Transcription-dependent spatial arrangements of CFTR and adjacent genes in human cell nuclei. *J. Cell Biol.* **166**, 815-825.

Zuin, J., Dixon, J. R., van der Reijden, M. I. J. A., Ye, Z., Kolovos, P., Brouwer, R. W., van de Corput, M. P. C., van de Werken, H. J. G., Knoch, T. A., van IJcken, W. F. J. et al. (2014). Cohesin and CTCF differentially affect chromatin architecture and gene expression in human cells. *Proc. Natl. Acad. Sci. USA* **111**, 996-1001.

Zullo, J. M., Demarco, I. A., Piqué-Regi, R., Gaffney, D. J., Epstein, C. B., Spooner, C. J., Luperchio, T. R., Bernstein, B. E., Pritchard, J. K., Reddy, K. L. et al. (2012). DNA sequence-dependent compartmentalization and silencing of chromatin at the nuclear lamina. *Cell* **149**, 1474-1487.