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Chromatin organization: form to function

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Recent developments in technology have made it possible to create high resolution genome-wide maps of histone marks, DNA binding proteins and physical interactions along genomic regions. Chromatin features are found together in different combinations, dividing the genome up into domains with distinct functional properties. Microscopy and chromatin conformation capture techniques have shown that the 3D structure of chromosomes is constrained by nuclear features and functional links between different parts of chromatin. These results provide insights about the 3D and domain organization of the genome and their connection to gene regulation and other nuclear functions.

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Introduction

The spatial organization and compartmentalization of the genome is likely to be of great importance for the regulation of gene expression and other nuclear functions. Recent advances in genome-wide mapping technology have allowed the inference of the 3D topology and protein packaging of the genome, giving insights into how domains and spatial organization lead to function. This review covers recent developments in this field.

Chromosome territories

Interphase chromosomes are not positioned randomly in the nucleus, but occupy spatially distinct regions, called chromosome territories (reviewed in [1]). The development of chromosome conformation capture (3C) and its derivatives allows the examination of the organization of the chromosomes and chromatin at high resolution. Briefly, the technique uses formaldehyde fixation to cross-link genomic regions that are in close proximity *in vivo*. Neighbouring DNA fragments are then allowed to form unique products via ligation [2]. With high-throughput sequencing this technique can be applied to whole genomes (Hi-C), rather than just selected loci [3]. This has enabled the creation of three dimensional models of the interactions of whole genomes (Figure 1a,b).

Data sets created by this technique confirm that there is little interplay between the chromosomes, with intra-chromosomal interactions dominating [3–5]. In *S. cerevisiae*, this bias is inversely correlated with size. Small chromosomes have relatively frequent interactions with other chromosomes, but large chromosomes mainly have self interactions [5]. A similar phenomenon is also observed in human lymphoblasts: the smaller gene-rich chromosomes, which are found in the centre of nucleus, preferentially interact. The exception is chromosome 18, which is short but gene poor, and is not enriched for inter-chromosomal interactions [3].

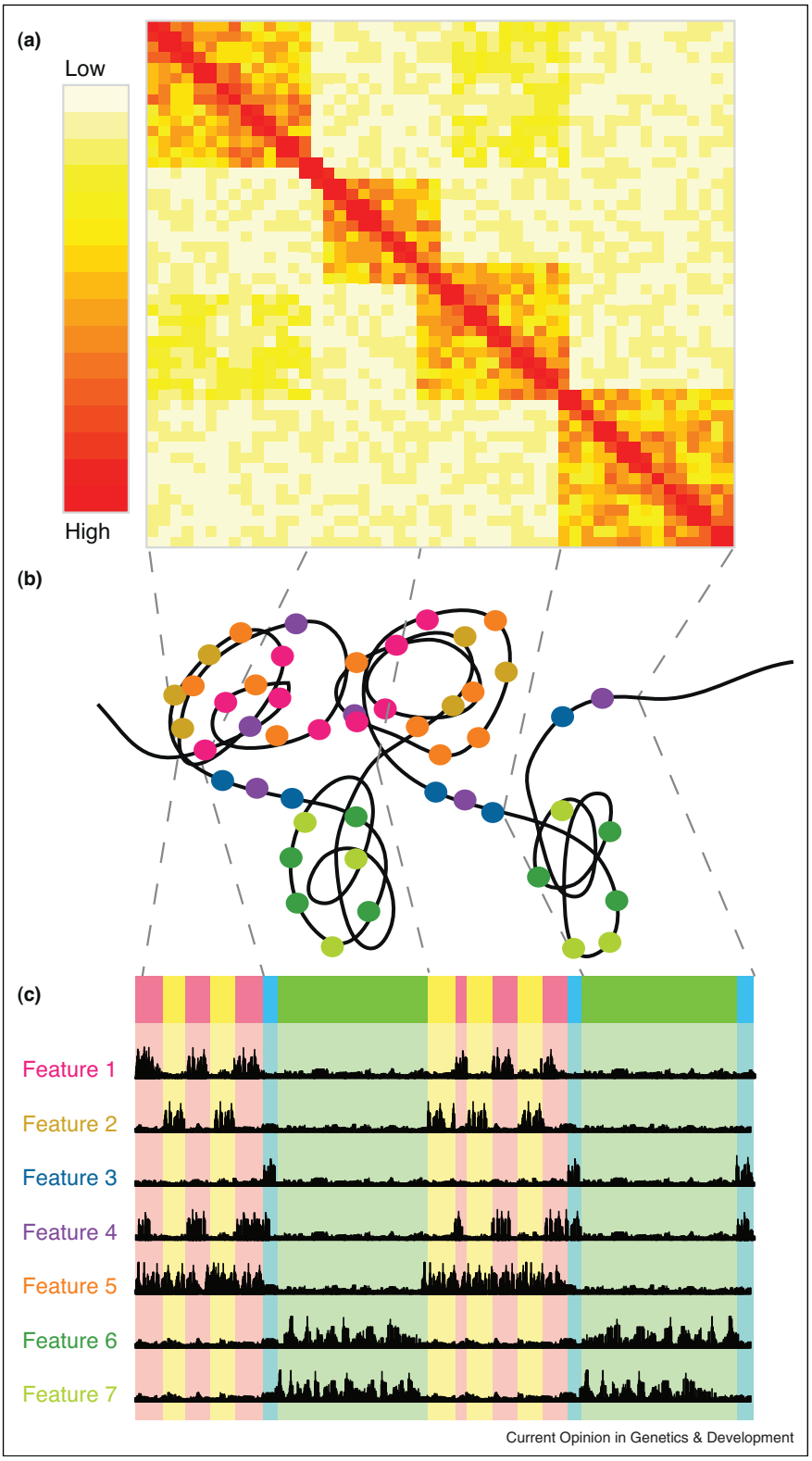
Chromosome arms also appear to have their own territories. Few interactions are observed between chromosome arms in *Drosophila* [6••,7]. A pericentric inversion that swapped two chromosomal segments to the opposite arm caused these two segments to lose contacts with their former neighboring sequences, and gain contacts with their new neighbors [6••]. This indicates that the linear structure of chromosomes dictates to a large extent which contacts are possible, and suggests that centromeres can be a barrier to interaction.

The formation of translocations is also driven by spatial proximity. Genome wide mapping of translocations with the Myc or IgH loci showed that when a double stranded break could not be repaired at the original site of damage, there was still a strong preference for intra-chromosomal translocations, even up to 60 MB away [8,9]. The frequency of inter chromosomal translocations was predicted by the Hi-C contact probability of the two chromosomes [10] and is also reflected in oncogenic transformations. Burkitt's lymphoma is characterized by a translocation between MYC and one of three immunoglobulin variants, all of which are on different chromosomes. MYC:IGH is the most common translocation, as IGH is spatially the closest immunoglobulin to Myc, compared to MYC:IGK which occurs less frequently and is the most distant translocation [11].

3D domain organization of chromosomes

Hi-C studies have identified general principles of chromatin organization that are conserved across species [3,5,7,12•,13]. At the coarsest level (at megabase scale), the genome is divided into two types of domains, which can be roughly characterized as transcriptionally active and inactive regions. There are minimal interactions

Figure 1



Chromatin conformation capture and chromatin feature mapping provide information about the domain and 3D structure of the genome. **(a)** Interactions between genomic regions as shown by Hi-C. Regions in close proximity are fixed and ligated together, and high throughput sequencing is used to produce a genome-wide contact matrix. The matrix shown here corresponds to cis interactions along a hypothetical genomic region, with pixel

between the two types, even when they are linearly close together on the same chromosome [3].

More recent advances in sequencing technology have allowed a higher resolution view of genome wide interactions. This showed that within the larger Mb size active and inactive domains, there are smaller nested domains. These domains contain extensive cis interactions, while contacts between such domains are much less frequent. Mice and humans share this chromatin architecture of domains and subdomains, which remain largely stable even between different cell types [12•,14••]. The boundaries between interaction domains are enriched for the insulator protein CTCF, and often mark the transition between lamina associated domains (LADs) and non-LADs in the genome, or transitions between early and late replicating regions [7,12•,14••]. Factors in the border region are partially responsible for domain separation. This was shown by the deletion of the border region between Xist-Tsix in mouse ESCs. Normally these two genes are in separate topologically associating domains (TADs), but after the border deletion, associations increase between the two domains [14••]. This shows that elements within the border region are required to block interactions. As the domains did not completely merge, factors outside the border regions are also involved in maintaining boundaries.

Chromosome organization and gene regulation

Some domains can grow or shrink as cell specific genes are activated. This is highlighted in the Hox complex, where the genes are linearly organized in order of their sequential activation. A study in mouse tissues found that when the cluster is inactive, it forms a single 3D domain separated from its flanking regions. As genes are progressively switched on it forms two domains, where the newly transcriptionally active genes transfer to the active domain [15••].

Within TADs, gene activation can involve the creation of loops between enhancers and promoters that are some linear distance away. In some instances, such contacting elements can be located on two different chromosomes [16]. DNaseI hypersensitive sites (DHSs) can be used to map potential regulatory sites, including promoters, enhancers and insulators. Most human promoters are linked with more than one distal DHS [16,43]. However, many occur in very few cells [3]. Therefore, bioinformatically identified loops must be verified for functional importance. For example, in erythroid cells, when β-globin is switched on, a loop between its promoter and the locus control region (LCR) is formed, involving the transcription factor GATA1 and cofactor Ldb1, which cannot directly bind DNA. Tethering Ldb1 to the β-globin promoter in GATA1 null cells, which normally cannot express β-globin, recreates the link between the promoter and the LCR, reactivating the gene [17••]. This confirms the importance of this loop for proper gene activity.

Because looping interactions are mostly stochastic, their regulatory effects may be variable from cell to cell. This was indeed observed when the human β-globin LCR was integrated into the genome of mouse cells. In liver cells this ectopic LCR was found to contact the endogenous β-globin locus located on a different chromosome with low frequency. Strikingly, in the cells where this contact occurred, high expression of one of the β-globin genes was often triggered [18•]. Although the experimental setting was somewhat artificial, these data show that contacts between regulatory elements on different chromosomes in principle can contribute to gene regulation. The stochastic nature of these contacts appears to compromise regulatory robustness.

Computational modelling of chromosome topology

Computational modelling can provide additional valuable insights into chromosome structure. A recent computer simulation study found that only a handful of basic constraints — centromeres should be attached to the spindle pole body; telomeres should be located at the nuclear periphery; and only rDNA loci can access the nucleolus — could account for much of the spatial organization of all chromosomes in S. cerevisiae, including the propensity for particular chromosomes to interact [19•]. Fascinatingly, this modelling technique was also able to predict the location of some gene territories with high correlation to fluorescence imaging derived positions. It also predicted correctly that tRNA genes and early replication sites each have significant spatial clustering [19•]. This suggests that some interactions in yeast are passively maintained by spatial constraints in the nucleus, rather than actively driven by interaction motifs.

Interaction of chromatin with nuclear landmarks

Major structural elements of the nucleus are known to interact with particular genomic regions. The nuclear lamina (NL), which lines the inner membrane of the nucleus, is thought to act as a repository for large regions of transcriptionally inactive chromatin, known as lamina

(Figure 1 legend continued) color corresponding to total number of reads. This shows that the region is divided into domains with high levels of local interactions, but also shows some interactions between domains. **(b)** A cartoon interpretation of the Hi-C data as separate clusters of DNA. **(c)** Classification of chromatin types. In this hypothetical example seven different chromatin proteins were mapped along a region of the genome. Computational algorithms can be then applied to divide the genome into different domains that have a similar composition. The proteins are shown on (b) as different colored circles, showing that the borders of different chromatin domains also often correspond with the borders of TADs.

associated domains (LADs) [20,21]. LADs are often hundreds of kilobases in length and many are evolutionarily conserved in humans and mice [22].

The vast majority of genes in LADs are repressed [20,21], and during differentiation hundreds of genes change position relative to the NL, concomitant with changes in their expression status [23]. A study in *C. elegans* showed the knockdown of their sole lamin gene shifts heterochromatin away from the nuclear periphery [24]. Similarly, a transcriptionally inactive gene cluster in *Drosophila* cells moved to the nuclear interior and became activated upon depletion of the B-type lamin [25]. In contrast, double knockout of the two B-type lamin genes in murine embryonic stem cells, which also lack Lamin A/C expression, showed that lamins are not required for self-renewal and nor did their loss affect gene expression [26]. They were however required for spindle orientation and proper organogenesis, suggesting an important role in tissue formation.

What targets LADs to the NL? H3K9 methylation has been found to have a role in the localization of heterochromatin at the nuclear periphery in *C. elegans*. The histone methyltransferase MET-2 causes monomethylation and dimethylation of H3K9, following which SET-25 deposits H3K9me3 [27•]. Without these enzymes, heterochromatic regions were released from the NL. In mice, two redundant methylases Prdm3 and Prdm16 direct H3K9 monomethylation in the cytoplasm, and in the nucleus, Suv39h enzymes cause H3K9me3 methylation [28]. Depletion of Prdm3/16 resulted in disruption of heterochromatin foci and a disorganized NL, but it is unclear whether the two phenomena are directly related.

Besides histone modifications, it is likely that certain DNA sequences also target genes to the NL, similar to the targeting of genes to nuclear pore complexes in yeast by specific DNA 'zip codes' [29]. Recently, it was suggested that GA repeats can direct certain human LADs to the NL [30], but a genome-wide study of repeats did not find GA repeats to be enriched in human LADs [20], leaving the majority of the targeting unexplained. It has also been found that DNA in LADs that are conserved between cell types is generally A/T-rich [22], but it is unknown if there is mechanism that links this sequence bias with the NL.

The surface of the nucleolus is also a repository for heterochromatin. Genome-wide surveys have identified nucleolus associated domains (NADs) on most human chromosomes [31,32]. Interestingly, there is considerable overlap between NADs and LADs. It is not known if the same factors are involved in sequestering heterochromatin at the nucleolus and at the NL, but the overlap suggests that shared mechanisms may be at work.

Defining chromatin domains by protein composition

Principles of chromosome organization have also been revealed by the systematic mapping of genome-wide occupancy patterns of histone modifications and chromatin proteins. Recently several groups have used collections of these maps, across several species and many cell types, to segment the genome into different chromatin domains (Table 1) (Figure 1b,c). Analysis of binding maps of 53 chromatin proteins in *Drosophila* Kc167 cells [33] indicated that the genome is covered by five principal chromatin types, including a new repressive chromatin type, BLACK, that covers around half the genome and lacks classical heterochromatin markers. These chromatin types also correspond reasonably well with TADs, with the borders between chromatin domains roughly matching the borders between TADs [7].

Table 1

Genome wide surveys of chromatin domain types

Species	Cell type	# of factors/marks	# of types	Features described	Reference
D. melanogaster	Kc	53 proteins	5	Repressed, tissue specific active, broadly expressed active, polycomb	[33]
D. melanogaster	S2, BG3	18 histone marks	9	Active, transcriptional elongation, intronic, X chr, heterochromatin, polycomb, repressed	[36]
H. sapiens	CD4 T cells	38 histone marks + H2AZ, PolII, CTCF	51	Promoter, transcribed, active intergenic, repressed, repetitive	[37]
H. sapiens	9 cell lines	9 histone marks + CTCF	15	Promoter, enhancer, insulator, transcription, polycomb, repressed, repetitive	[38]
H. sapiens	6 cell lines	8 histone marks, PolII, CTCF, DNase hypersensitive sites	7	CTCF, enhancer, transcribed, repressed, promoter	[39]
H. sapiens	5 cell lines	117 TFs	6	Binding active or inactive; high or low cobinding; regulator regions proximal or distal to genes.	[35]
H. sapiens	ES, K562	29 chromatin features	4	Initiation, transcript, polycomb, heterochromatin	[40]
A. thaliana	Seedlings	12 histone marks	8	Active, repressive, repetitive, intergenic	[41]
C. elegans	Embryos, L3 larvae	21 chromatin features	3	Active, repressive, dosage compensation	[42]
C. elegans	Embryos, L3 larvae	28 chromatin features	5	Active, repressive, X chromosome	[34]

While there is some overlap, each protein mapping survey has identified a different set of chromatin domains. The domains range in resolution from chromosome scale repressive versus active domains [34], to features mapping to TADs and LADs [33], and to promoter, exon and intron specific states [35]. The selection of factors analysed is linked with the types of domains identified. For example, H3K9ac and H3K4me2/me3 are localized to transcription start sites and inclusion of these marks can help resolve these features [36]. However, the clustering of particular combinations of smaller domains along the genome has been shown to create larger domain types [36]. This shows that new information can be found when looking at the genome at different scales.

Conclusion and future perspectives

The recent production of many genome-wide datasets has resulted in a better understanding of the organization of interphase chromosomes, both in terms of three-dimensional structure and in terms of domains of similar chromatin features. The next challenge will be to unravel the cause-consequence relationships: to what extent do spatial folding and domain organization matter for gene regulation? New high throughput methods to query the effects of different genomic contexts on transcription will be an important tool for addressing such questions.

The large variation in the frequency of detection of different chromatin conformations suggests that not all interactions are equally important. Furthermore, the stochastic nature of these conformations and states poses the question of how robust gene regulation is achieved. Development of new single cell techniques to study these phenomena — ideally genome-wide — will be necessary to tackle these issues.

Many of the chromatin domains that have been identified have sharp boundaries, and while recent work on insulators has made some progress in understanding what maintains these boundaries, we cannot explain them all. Techniques which can manipulate these domains, including systematic disruptions of putative boundary elements by genetic manipulation, will help to explore the functional importance of different types of elements in different contexts.

With the rapidly expanding pool of publicly available data mapping the state of chromatin, the future looks bright for the study of chromatin organization. This will aid in understanding the molecular mechanisms controlling chromatin organization, and the role of genome organization in regulating gene regulation and other nuclear functions.

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