

Chromosome Folding: Driver or Passenger of Epigenetic State?

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Despite a growing understanding of how epigenetic marks such as histone modifications locally modify the activity of the chromatin with which they are associated, we know little about how marked regions on different parts of the genome are able to intercommunicate to effect regulation of gene expression programs. Recent advances in methods that systematically map pairwise chromatin interactions have uncovered important principles of chromosome folding, which are tightly linked to the epigenetic mark profiles and, hence, functional state of the underlying chromatin fiber.

Genes do not act independently of each other or of the genomic context in which they are present. As well as protection from the influences of surrounding repressive chromatin, many genes require control from long-range regulatory elements that may reside at megabase-scale distances in or close to unrelated genes for their appropriate expression. Furthermore, coordinately regulated genes from different chromosomes can come together at specific sites within the nucleus allowing them to share the same regulatory factors enriched at these foci. Despite our ever-growing comprehension of how epigenetic features such as histone modifications and DNA methylation determine the functional state of the chromatin they mark, such a “one-dimensional” view of the epigenome is unable to explain complex long-range control of gene expression programs. We also need to characterize the three-dimensional folding of the chromatin fiber to understand how epigenetic marks on both genes and their long-range regulatory elements intercommunicate.

Initially, chromatin interactions could only be studied in a low-throughput manner at a limited spatial resolution by light microscopy, but the field was revolutionized by the development of the 3C (chromosome conformation capture) method by Job Dekker in the Kleckner laboratory (Dekker et al. 2002). This molecular biology approach entails the fixation of chromatin in its native state, followed by restriction digestion, and religation. This generates hybrid DNA molecules from restriction fragments that may reside on different genomic locations, but are physically proximal at the time of fixation (see Fig. 5 of Dekker and Misteli 2014). 3C allows the assessment of chromatin interactions at the resolution of individual restriction fragments and has been used to show chromatin loops bringing genes and their distal regulatory elements into direct physical proximity. Importantly, these loops tend to be specific to tissues in which the distal elements exert control, suggesting that they are functional and not just a consequence of folding the genome into a small nuclear volume. However, these studies were still anecdotal and gave little information on the global interrelationship of chromosome folding and gene expression control.

The next major advance came from using high-throughput sequencing to simultaneously detect all the pairwise chromatin interactions uncovered by one 3C experiment. This “Hi-C” method was first developed in the Dekker laboratory (Lieberman-Aiden et al. 2009), but has subsequently been performed at much higher sequencing depths to derive chromatin interaction maps of *Drosophila* (Sexton et al. 2012) and mammalian (Dixon et al. 2012)

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nuclei at very high resolution. These detailed maps uncovered conserved features of chromosome folding in metazoans, which are more tightly linked to the epigenomic features of the underlying chromatin fiber than previously appreciated.

A surprising and important finding was the conserved organization of the genome into distinctly folded modules or “topological domains”; represented as squares on the interaction map (see Fig. 1), interactions between genomic regions within a domain are very strong, but sharply depleted when crossing domain borders. Strikingly, nearly all

epigenetic markers of chromatin activity (histone modifications, protein binding profiles, transcriptional output, DNase sensitivity, replication timing, etc.) correlated extremely well with topological domain organization—crossing the border from one domain to another is accompanied by a very sharp increase or decrease in the mark. In mammals, the domains are also associated with coordinately regulated genes, suggesting that the genome is functionally organized into distinct units, which are physically borne out as discretely folded chromatin modules (Fig. 1). This model implies that domains are physically segregated, but

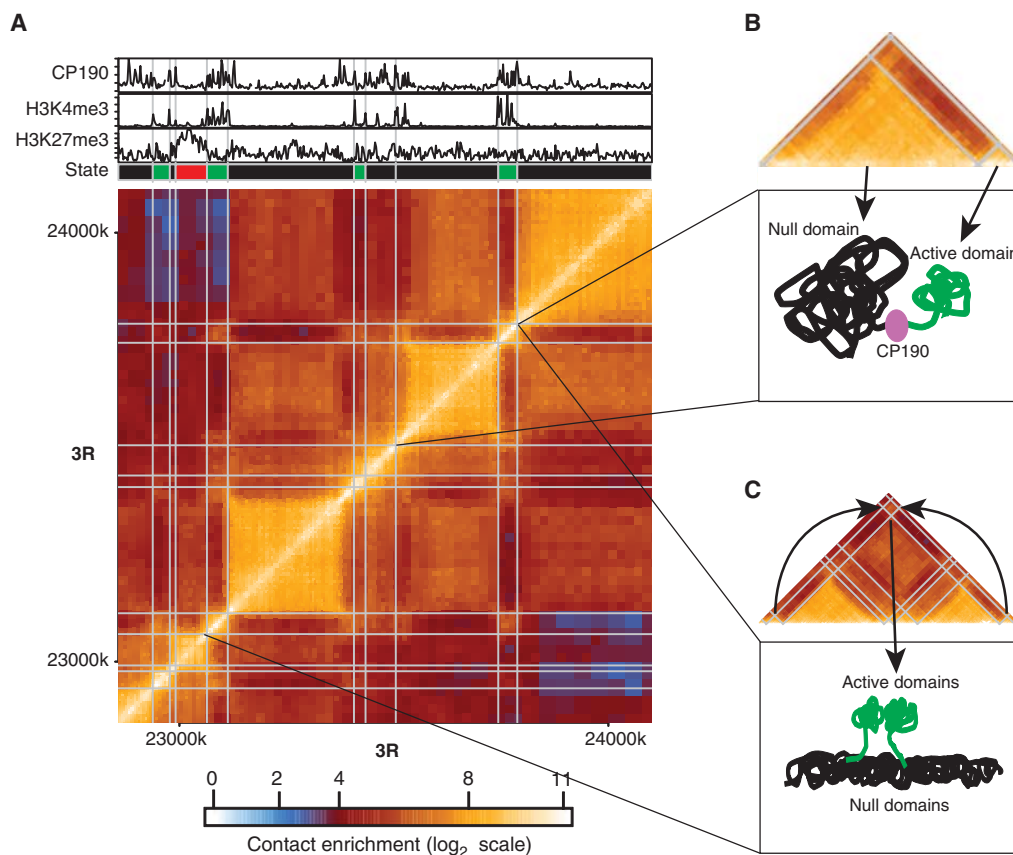


Figure 1. Spatial organization of the epigenome. (A) Chromatin interaction map for an ~ 1 Mb region of the genome in *Drosophila* embryos (data from the laboratories of Giacomo Cavalli and Amos Tanay). The interaction strength between two specific genomic regions is given by the heat map corresponding to their coordinates on the x - and y -axes. The presence of distinct topological domains is indicated by the patterns of squares on the heat map diagonal, with steep decreases in interaction strength when passing beyond the domain. The topological domain borders are denoted by gray lines. The linear profiles for binding of the insulator protein, CP190, and the histone modifications, H3K4me3 and H3K27me3, are shown above the map, along with a color-coding for the epigenetic “state” of each topological domain (“null” domains, devoid of known epigenetic marks are indicated in black; “active” domains, marked by H3K4me3, are indicated in green; domains repressed by the mark H3K27me3 are indicated in red). Key functional organization principles of the genome are shown in the insets. (B) A subset of the interaction map, rotated by 45° , is shown to highlight the topological domain organization of the genome. This inset shows a large null domain, physically segregated from a small active domain, with the border between them containing a binding site for the insulator protein CP190 (shown schematically below). (C) A specific interdomain interaction between two small active domains, kept separate from the surrounding repressive chromatin, is highlighted in a subset of the interaction map and schematically shown below the map.

also that this segregation prevents functional communication across domain borders. In support of this, the well-characterized regulatory elements acting over megabase distances have all been found to be present in the same topological domains as their target genes. Moreover, domain borders are enriched in the binding of insulator proteins (such as CTCF [CCCTC-binding factor] in mammals, or CP190 in *Drosophila*), which are factors that have been genetically characterized to shield genes from the influence of surrounding chromatin. It should be noted, however, that many insulator binding sites do not define topological domain borders; the genetic and epigenetic features distinguishing these sites from true domain borders will be an interesting topic of research in the near future.

In addition to a tight link between local folding of chromatin and its underlying epigenetic state, there is also a striking interplay between long-range, intertopological domain interactions and chromatin activity. The first Hi-C study in human cells identified two fundamental types of chromatin based on their interaction patterns: Type A, characterized by hallmarks of transcriptionally active chromatin, preferentially formed interactions with other type-A domains; type-B chromatin, which was transcriptionally repressed, formed strongest interactions with other type-B domains, and there were greatly reduced interactions between type-A and type-B chromatin. Such a model is consistent with observed clustering of coexpressed genes in shared transcriptional foci within the nucleus. The higher-resolution Hi-C maps are also consistent with the two-state chromatin model, but they extend it to show that topological domains form a basic unit of higher-order chromatin folding, with interdomain contacts predominantly occurring between active domains or between inactive domains, with little intermixing. Thus, although local epigenetic marks, long considered a proxy of gene activity, reflect the local topology of the chromatin fiber, they may also play a substantial role in global chromosome folding.

Thanks to these initial maps of genome configurations, the intimate link between genome structure, epigenetic state, and functional output is now clear, although the question of whether structure defines function or vice versa remains unresolved. Recent elegant experiments have provided evidence for chromatin loops as a cause of transcriptional activation rather than a consequence (Deng et al.

2012). Similar experiments may also be expected to assess the causal role of topological domains in regulating genome function. With ever-decreasing sequencing costs, it is reasonable to predict that more chromatin contact maps will be produced at increasing resolution and in a larger repertoire of cell types and experimental conditions. As a result, epigenomic profiling could become three-dimensional. Regions of histone modifications may no longer be viewed as tracks on conventional genome browsers, but could instead be viewed in the more physiological context of their nuclear neighbors. We anticipate that this will allow a much greater understanding of how entire gene expression programs are regulated—for example, allowing better in silico predictions of how mutations (e.g., previously uncharacterized disease-linked single-nucleotide polymorphisms) or transgenic insertions (e.g., during gene therapy) affect transcription of seemingly unrelated genes.

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