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Review article

What's in the "fold"?

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ABSTRACT

Complexity in genome architecture determines how gene expression programs are established, maintained, and modified from early developmental stages to normal adult phenotypes. Large scale and hierarchical organization of the genome impacts various aspects of cell functions, ranging from X-chromosome inactivation, stem-cell fate determination to transcription, DNA replication, and cellular repair. While chromatin loops and topologically-associated domains represent a basic structural or fundamental unit of chromatin organization, spatio-temporal organization of the genome further creates a complex network of interacting genome patterns, forming chromosomal compartments and chromosome territories. The understanding of human diseases, including cancers, auto-immune disorders, Alzheimer's, and cardiovascular diseases, relies on the associated molecular and epigenetic mechanisms. There is a growing interest in the impact of three-dimensional chromatin folding upon the genome structure and function, which gives rise to the question "***What's in the fold***?" and is the main focus of this review. Here we discuss the principles determining the spatial and regulatory relationships between gene regulation and three-dimensional chromatin landscapes, and how changes in chromatin-folding could influence the outcome of genome function in healthy and disease states.

1. Introduction

The last decade has profoundly changed our understanding of higher order chromatin architecture in the eukaryotic interphase nucleus. As a result, most current research places a strong emphasis on the folding of large chromosomes into hierarchical structures and the connection between genes and enhancers or silencers to larger chromosomal domains. The larger these chromosomal folds are in the hierarchy, the more stable they are within the cells. Chromatin organization plays an important role in gene regulation. It brought more attention to researchers when they studied that how 2 m-long eukaryotic DNA is tightly folded into a tiny 6 μm nucleus (Fig. 1). Thousands of genes and regulatory elements have been discovered through the large scale studies during the last decade, for instance; ENCODE (Encyclopedia of DNA Elements) [1], Roadmap Epigenome [2], Genome-Wide Association Studies (GWAS), International Human Epigenome Consortium, EpiGeneSys (http://www.epigenesys.eu/en/) [3], and FANTOM (Functional Annotation of the Mammalian Genome) [4]. However, there is limited information regarding how these genes and regulatory elements interact when they are structured across kilobase and megabase distances from each other. Recent advances in chromatin architectural studies shed light on the functional interplay between maintenance mechanisms of chromosome territories in the interphase nucleus and its effect on gene regulation.

4-D Nucleome was developed to conceptualize three-dimensional organization of chromatin architecture in space and time, how this organization affects gene expression and cellular function, and how changes in the organization affect normal or diseased state. [5]. Advancements in the study of genome architecture have led to the belief that genome organization in "space and time" can be a critical tool to in furthering our understanding of the complexity and intricacy of genome function. The 4D nucleome consortium aims: 1) To develop different state-of-the-art technologies to study 4D nucleome; 2) To analyze and build model databases to obtain a complete view of 4D Nucleome; and 3) To validate the functional role of various structural organizations of chromosome in the nucleus. This multidisciplinary approach should provide comprehensive and detailed views of spatially and dynamically-organized three-dimensional structures of functional genomes

Abbreviations: 3C, Chromosome Conformation Capture; Hi-C, High-throughput Chromosome Conformation Capture; TAD, Topologically Associated Domains; SE, Super Enhancers; ChIA-PET, Chromatin Interaction Analysis by Paired-End Tag Sequencing; RNA-TRAP, RNA Tagging and Recovery of Associated Proteins; GWAS, Genome Wide Association Study; SNP, Single Nucleotide Polymorphism

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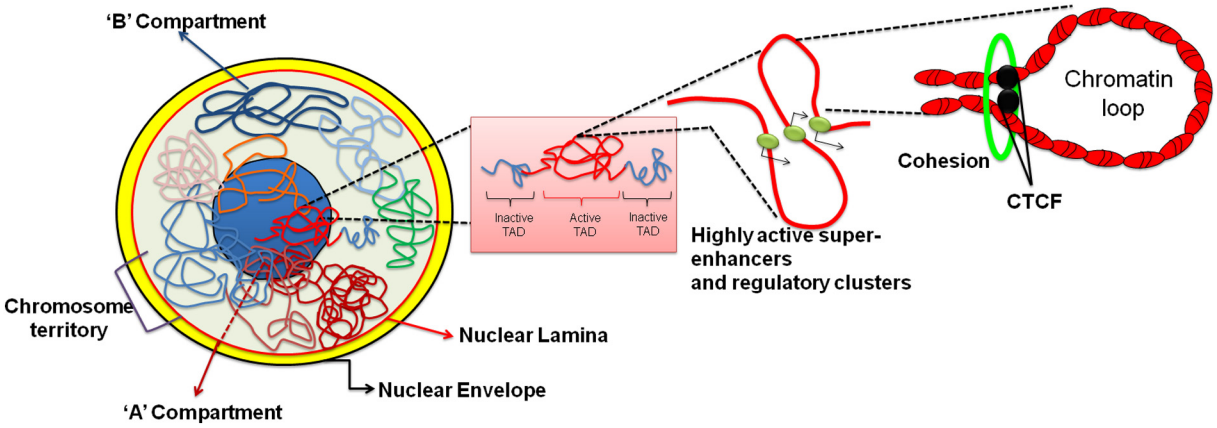

Fig. 1. Dynamic organization of higher order chromatin structure. Interphase nucleus is partitioned into chromatin loops and megabase sized topologically associated domains which further forms the basis of spatially organized chromosomal compartments, active compartment "A" and inactive compartment "B", or chromosome territories.

inside cells.

Spatial organization of genome and its dynamic nature can be deciphered by the use of various imaging techniques, Chromosome Conformation Capture technique, 3C, and its derivatives (4C, 5C and Hi-C), ChIA-PET and RNA TRAP. With the advent of high-resolution 3C techniques such as Hi-C, it is possible to read complex and dynamic chromatin structures down to 5 kb resolution. Furthermore, computational analysis and new simulation methods have proven helpful in understanding how the three-dimensional structure of chromatin changes in response to a given stimuli [6]. These technical advancements have allowed us to better understand the "dynamic" nature of chromatin within a cell nucleus and have led to the identification of different entities of chromatin architecture, including Super Enhancers, TADs (Topologically Associated Domains), nuclear subdomains such as LADs (Lamin Associated Domains) and NADs (Nucleolar Associated Domains). Studies involving genome-editing techniques such as CRISPR-Cas9 and mutational studies further help probe the functional aspect of altered looped chromatin in disease phenotypes. In this review we summarize our current knowledge of three-dimensional chromatin organization, the architectural components involved in maintaining the epigenome in active or repressive state, and the functional impact it has on gene regulation and complex phenotypes. In addition, we provide the latest updates in the field of higher order chromatin architecture in "health" and "disease", and propose prospective lines of inquiry which may further our understanding of the unique principals of gene regulation.

2. Higher order chromatin structure-function relationship under physiological conditions

Cell nucleus is a highly compartmentalized domain in which higher order chromatin architecture can be described by the hierarchy of chromatin fibers built from the nucleosomes and further extended into the form of chromosome territories, chromatin domains and finally into chromosomes [7]. Chromosome topology may also be described as open or closed, based on its spatial organization. Using novel Chromosome Conformation Capture technique, Hi-C, local packaging of human chromatin (up to 10 Mb) was found to be consistent with "fractal" molecule which can be described as topologically maintained, unentangled, dynamic chromatin loop [8]. The defined topology of nuclear structure at a given space and time plays an important role in functional aspect of the genome in terms of transcription, splicing, replication and DNA repair. Fractal properties of chromatin have profound effect on its spatial arrangement, which explains the direct correlation of accessible surface area for transcription factors to its local chromatin density [9]. Previous findings have revealed that changes in physical structure of chromatin (local density and folding) even at nanoscale range can influence chromatin accessibility and thus gene expression [10,11]. A multitude of chromatin modifiers and genome organizers are involved in maintaining nuclear architecture, determining the structural and functional aspect of genome integrity and stability. Defects in global chromatin organization are highly relevant determining the pathological aspect of the cell function.

2.1. CTCF and cohesion are the master regulators of higher order chromatin architecture

CTCF (CCCTC binding factor), ubiquitously expressed and highly conserved DNA binding zinc finger protein, serves as a transcriptional regulator and epigenetic modifier [12]. Depending on the functional aspect of the gene, CTCF can act as a transcriptional repressor or activator [13] or an insulator [14]. Given its multifunctional role in gene regulation and chromatin organization, CTCF is considered to be a master regulator in genome organization. CTCF has a very well established role in chromatin folding at β-globin locus, H19-IGF2 locus and in antigen receptor loci. Hi-C studies have shown enriched CTCF sites at borders between the Topologically Associated Domains (TADs), as well as between the LADs (Lamina Associated Domains), suggesting it plays a role in organizing chromatin structure at 3D level [15].

CTCF maintains chromosomal topology by binding to various sites in intergenic regions, intragenic regions or near gene promoters [16]. CTCF, along with Cohesin, spatially organizes mammalian genome into chromatin loops [17]. Cohesin is an essential component for the maintenance of chromosome topology through the cell cycle, but its higher expression in post-mitotic cells suggests its emerging role in other cellular functions. Cohesin is a ring shaped complex made of three subunits (SMC1, SMC2 and Rad21) which entrap DNA into its lumen [18]. Genome-wide studies have suggested that Cohesin is highly enriched at CTCF binding sites and involved in the formation and/or maintenance of chromatin loops [19], and can create boundaries between Topologically Associated Domains (TADs) at megabase levels (12) which are considered to be autonomous transcriptional units [12] and have higher interaction frequencies within the clusters. Conditional deletion of Cohesin complex does not affect the enhancer compartmentalization, but it can affect interaction frequencies between enhancers. Taken together, CTCF and Cohesin provide spatial and regulatory compartmentalization of mammalian genome.

Chromatin Conformation Capture studies and 3D-FISH has shown that two layers of spatial conformation occurs at Immunoglobulin locus during V(D)J recombination and class switch recombination in pro-B cells which require coordination between multiple factors. While YY1 directly regulates Class Switch Recombination by binding to intronic