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Forum

Higher order chromatin structures are taking shape

Chromatinstrukturen höherer Ordnung nehmen Gestalt an.

The interaction between radiation and living organisms is highly dependent on the chemical, physical and three-dimensional environment of DNA inside the cell. Each human chromosome contains a DNA thread that is highly negatively charged, very fine (2 nm diameter) and extremely long (an average chromosome is 4 cm long). It is fascinating that human cells confine 46 of these extreme molecules into the space of a micrometer-sized nucleus, and at the same time manage to establish highly regulated gene expression, DNA repair, genome duplication and rapid disentanglement and condensation for segregation during cell division. In eukaryotes this feat is made possible by the packaging of the DNA molecules into the proteinaceous matrix called chromatin.

In this Editorial I will describe our current understanding of chromatin structure and highlight its implication in the nuclear processes of gene expression, DNA repair, genome replication and segregation.

Several classes of proteins play crucial roles in chromatin formation, maintenance and function: histones, nucleosome remodelers, histone modification writers, readers and erasers, and structural maintenance of chromosome (SMC) proteins like cohesin and condensin. These activities are tightly integrated with the activities of the DNA replication, repair, transcription and segregation machineries. Besides providing for the 3D organization of DNA, chromatin is now well established as an integral part of the biochemical reactions taking place on the genome where it plays an important regulatory role and provides a signaling and information storage platform.

The basic repeating unit of chromatin is the nucleosome that organizes about 147 base pairs of DNA around a core of histone proteins [1]. The DNA is thereby wound into a coil of 1.65 left-handed superhelical turns and compacts the DNA 5-6 fold. The histone core is made up of four histone proteins that are each present in two copies. The histones H3 and H4 form two heterodimers that associate into the tetrameric core of the histone octamer, with two histone H2A/H2B heterodimers symmetrically elongating the superhelical DNA-binding path of the tetramer. The linker histone H1 binds the free linker DNA between nucleosomes, and together with the highly positively charged core histones it neutralizes about half the negatively charge of DNA while the rest of the charges of DNA is neutralized by small basic molecules as well as potassium and magnesium ions. The homeostasis of ions and histone proteins is a crucial determinant for the compaction of DNA into the cell’s nucleus.

Histones are extremely abundant and turn the linear DNA of chromosomes into a beads-on-a-string chromatin fiber. Nucleosomes are very stable structures, and DNA sequences within them are generally difficult to access by the nuclear machinery. In nucleosome-depleted regions of the genome access to the underlying DNA is facilitated by the action of nucleosome remodeling machines that space or evict nucleosomes and elicit rapid turnover of histones at active regulatory elements [2]. The generation of nucleosome-depleted regions in promoters and enhancers of genes enables the binding of transcription factors and is of paramount importance in setting the cellular gene expression program. DNaseI hypersensitivity maps and transposon-based ATAC-seq maps have revealed that nucleosome-depleted regions change in response to developmental and environmental stimuli and that they provide an important signature of the expression program and differentiation state of a specific cell [3].

Chromatin further determines the genomic landscape through posttranslational modification of histones and methylation of DNA in a CpG dinucleotide context. Specific modifications installed at specific sites in the genome mark sites of gene expression, DNA repair and initiation of replication. Histones have long N-terminal extensions that are not part of the core fold of the histone octamer. These extensions are the major substrate of a highly complex posttranslational modification system, also known as the “histone code” [4]. Acetylation, phosphorylation, methylation, ubiquitlylation and poly-ADPribosylation are just a few of a large number of modifications that serve as a means of communication between specific DNA binding factors, chromatin modifiers and the enzymatic machinery of the nucleus. In the last twenty years chromatin research has advanced from the discovery of the first histone acetyl transferase enzyme to the identification and molecular description of most players in the histone modification system, and the current focus of research is to define the role of the posttranslational modification system in physiological function and disease [5].

In addition to modifications of the histone tails we also find various histone isoforms that are used for specific functions. In particular for histones H3 and H2A various variants are incorporated into nucleosomes, often in non-random fashion across the genome. One of the best studied examples is the H3 isoform called cenH3 or CENP-A. This protein is incorporated specifically at centromeres, where it forms the foundation for the establishment of the kinetochore, which connects the chromosomes to the mitotic spindle during segregation of the genome. In contrast to CENP-A’s specific incorporation, the H2A variant H2AX is distributed in a random fashion across the genome and serves as the primary signaling platform to recruit and coordinate the repair machinery after damage to the DNA. It is the phosphorylated form of H2AX, γH2AX, that serves to recruit a wide range of activities including remodeling enzymes, ubiquitin ligases and Poly-ADP-polymerases (PARP) that modify local chromatin and open it up for repair. Chromatin modifications as well as their modifiers and readers have been mapped genome-wide in various organisms and cell types under a multitude of conditions using chromatin immunoprecipitation (ChIP), both in individual labs and in large scale projects like ENCODE [6]. These data provide valuable resources for further studies and are publicly available to the research community.

How are chromosomes organized at a scale beyond the nucleosome? Walther Flemming described in 1882 the organization of chromatin into metaphase chromosomes during mitosis, and in 1928 Emil Heitz observed that chromatin segregates into dense heterochromatin and weakly staining euchromatin in interphase. However, the organizational principles that span the gap between the nucleosome and these macroscopic structures have been slow to emerge, with

exciting progress made in the last decade (see cover illustration). The question of how the nucleosomes in the chromatin fiber fold has been studied extensively. In vitro, nucleosome arrays will display structural transitions from extended 10 nm fibers to tightly folded 30 nm thick fibers depending on the ionic conditions and the presence of linker histone H1 and other nucleosome binding complexes [7]. A relatively well defined structural unit is formed by four consecutive nucleosomes, and the X-ray structure of a tetranucleosome has been solved [8]. Furthermore, this structural unit has again been observed as a building block in cryo-EM structures of 12-mer and 24-mer arrays [9]. However, these in vitro structures depend on specific lengths of linker DNA and highly regular nucleosome arrays. High-resolution genome-wide nucleosome mapping studies have shown that the nucleosome positions and linker length are quite variable and thus the maintenance of pervasive uniform chromatin fiber architecture is unlikely. Furthermore, cryo-EM investigations of chromatin in the cell nucleus have failed to detect ubiquitous presence of 30-nm chromatin fibers in mammalian nuclei. These observations do not exclude that the chromatin fiber architecture varies between extended 10 nm fibers and local higher order structures such as tetranucleosomes and longer folded arrays. For example experiments using ionizing radiation and analysis of DNA fragment distribution resulting from correlated DNA breaks in live cells argue for the existence of two-start fiber folding in vivo [10]. However, the extent and functional relevance of these structures in their physiological context remains to be addressed.

How then does the chromatin fiber further fold and how does this influence the function of genomes? For mitotic chromosomes, Laemmli and Paulson have described the formation of roughly seventy kilobasepair sized loops that are organized by a scaffold [11], and this architecture is supported by recent genome-wide chromosome conformation capture data [12]. However, it has remained unclear how the chromatin fiber organizes in the interphase nucleus. Recently there has been significant progress in our understanding of chromatin higher order folding. This is in particular due to the development of chromosome conformation capture approaches (3C) and their implementation with massive parallel DNA sequencing (Hi-C) [13–15]. In combination with fluorescence in situ hybridization (FISH) imaging, Hi-C has been very powerful in describing the organization of chromatin at the kilo- to mega base pair scale. These experiments have revealed that genomes from bacteria to mammals are segregating into domains wherein segments of DNA preferentially interact with each other. In mammals a relatively specific class of these domains is called topologically associated domain (TAD) [16,17]. Interestingly, TADs are quite well defined structures and remain largely conserved during development and even between related species such as mouse and human [16]. The boundaries between domains act as insulators and correspond to insulators between enhancer elements and promoters that have been identified more than twenty years ago [18]. TAD boundaries also correspond to the limits of γH2A.X spreading upon DNA damage [19] and to replication domains [20]. These new findings suggest that chromosomal domains are functional compartments of chromosomes within which the productive contacts between genomic elements are strongly favored. A well-studied example is the action of enhancers, which seems to be limited by TAD boundaries and therefore cannot reach promoters in neighboring domains. Support for this functional role comes from very interesting studies in developing limbs [21,22]. For example, inversions or deletions affecting domain boundaries at the human WNT6/IHH/EPHA4/PAX3 locus have been shown to bring a non-developmental gene under the control of developmental regulatory elements and this miss-expression leads to malformations of digits in these individuals. Due to the advent of genome editing by CRISPR/Cas9 the function of insulator elements can now be tested rigorously. Despite their functional importance, chromosomal domains are transient

and stochastic structures, and their mechanisms of formation and regulation are important directions of active research.

Chromosomal domains are observed to further organize and interact with each other to form a structure called compartments [14]. Domains within the same compartment share a common chromatin signature of histone modifications and bound complexes. Compartments are closely related to the classical regions of heterochromatin and euchromatin observed by electron microscopy. The wealth of knowledge on DNA-bound complexes we have these days thanks to genome-wide analysis of chromatin factors by ChIP and imaging allows us to classify chromatin more distinctly into a finer-grained spectrum of states, and depending on the approach 4-15 states are currently proposed. The most notable chromatin states are euchromatin, constitutive heterochromatin, facultative heterochromatin and null chromatin [23]. Null chromatin covers extensive regions of genomes, and is characterized by the absence of histone marks and absence of open reading frames.

SMC proteins have long been implicated in condensation and cohesion of chromosomes during mitosis [24]. However, it is becoming increasingly clear that cohesin plays an important role in transcription and in the establishment of the chromosomal domain structure [25]. Recent models proposed that cohesin is cooperating with the sequence specific DNA binding factor CTCF to extrude chromatin loops and thereby critically contributes to the formation of TADs [26]. Condensin is a key player in chromosome condensation during mitosis, and a related complex, the SMC5/6 complex is required for DNA repair and replication through difficult to replicate regions. The division of labor between the SMC complexes and their mechanism in chromatin higher order organization is a current topic of intense research efforts.

Over the last ten years the study of chromatin organization has gone genome-wide and has produced tremendous amounts of data on epigenetic marks, chromatin modifiers, the nuclear machinery and most recently higher-order genome organization. Understanding the biochemical and biophysical mechanisms behind these processes on one hand, and the functional implications of non-coding genetic and epigenetic elements on the other hand remains a major challenge in the field and promises to hold exciting new discoveries.

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References

[1] Luger K, Mader AW, Richmond RK, Sargent DF, Richmond TJ. Crystal structure of the nucleosome core particle at 2.8 Å resolution. Nature 1997;389:251–60.

[2] Becker PB, Workman JL. Nucleosome Remodeling and Epigenetics. Cold Spring Harb Perspect Biol 2013;5:a017905. doi:10.1101/cshperspect.a017905.

[3] Zentner GE, Henikoff S. High-resolution digital profiling of the epigenome. Nat Rev Genet 2014;15:814–27. doi:10.1038/nrg3798.

[4] Strahl BD, Allis CD. The language of covalent histone modifications. Nature 2000;403:41–5.

[5] Allis CD, Jenuwein T. The molecular hallmarks of epigenetic control. Nat Rev Genet 2016;advance online publication. doi:10.1038/nrg.2016.59.

[6] Consortium TEP. An integrated encyclopedia of DNA elements in the human genome. Nature 2012;489:57–74. doi:10.1038/nature11247.
[7] Li G, Reinberg D. Chromatin higher-order structures and gene regulation. Curr Opin Genet Dev 2011;21:175–86. doi:10.1016/j.gde.2011.01.022.
[8] Schalch T, Duda S, Sargent DF, Richmond TJ. X-ray structure of a tetranucleosome and its implications for the chromatin fibre. Nature 2005;436:138–41. doi:10.1038/nature03686.
[9] Song F, Chen P, Sun D, Wang M, Dong L, Liang D, et al. Cryo-EM Study of the Chromatin Fiber Reveals a Double Helix Twisted by Tetranucleosomal Units. Science 2014;344:376–80. doi:10.1126/science.1251413.
[10] Rydberg B, Holley WR, Mian IS, Chatterjee A. Chromatin conformation in living cells: support for a zig-zag model of the 30 nm chromatin fiber. J Mol Biol 1998;284:71–84. doi:10.1006/jmbi.1998.2150.
[11] Paulson JR, Laemmli UK. The structure of histone-depleted metaphase chromosomes. Cell 1977;12:817–28.
[12] Naumova N, Imakaev M, Fudenberg G, Zhan Y, Lajoie BR, Mirny LA, et al. Organization of the Mitotic Chromosome. Science 2013;342:948–53. doi:10.1126/science.1236083.
[13] Dekker J, Marti-Renom MA, Mirny LA. Exploring the three-dimensional organization of genomes: interpreting chromatin interaction data. Nat Rev Genet 2013;14:390–403. doi:10.1038/nrg3454.
[14] Lieberman-Aiden E, van Berkum NL, Williams L, Imakaev M, Ragoczy T, Telling A, et al. Comprehensive Mapping of Long-Range Interactions Reveals Folding Principles of the Human Genome. Science 2009;326:289–93. doi:10.1126/science.1181369.
[15] Rao SSP, Huntley MH, Durand NC, Stamenova EK, Bochkov ID, Robinson JT, et al. A 3D Map of the Human Genome at Kilobase Resolution Reveals Principles of Chromatin Looping. Cell 2014;159:1665–80. doi:10.1016/j.cell.2014.11.021.
[16] Dixon JR, Selvaraj S, Yue F, Kim A, Li Y, Shen Y, et al. Topological domains in mammalian genomes identified by analysis of chromatin interactions. Nature 2012;485:376–80. doi:10.1038/nature11082.
[17] Nora EP, Lajoie BR, Schulz EG, Giorgetti L, Okamoto I, Servant N, et al. Spatial partitioning of the regulatory landscape of the X-inactivation centre. Nature 2012;485:381–5. doi:10.1038/nature11049.
[18] West AG, Gaszner M, Felsenfeld G. Insulators: many functions, many mechanisms. Genes Dev 2002;16:271–88.
[19] Aymard F, Legube G. A TAD closer to ATM. Mol Cell Oncol 2016;3:e1134411. doi:10.1080/23723556.2015.1134411.
[20] Pope BD, Ryba T, Dileep V, Yue F, Wu W, Denas O, et al. Topologically associating domains are stable units of replication-timing regulation. Nature 2014;515:402–5. doi:10.1038/nature13986.
[21] Lonfat N, Duboule D. Structure, function and evolution of topologically associating domains (TADs) at HOX loci. FEBS Lett 2015;589:2869–76. doi:10.1016/j.febslet.2015.04.024.
[22] Lupiáñez DG, Kraft K, Heinrich V, Krawitz P, Brancati F, Klopocki E, et al. Disruptions of Topological Chromatin Domains Cause Pathogenic Rewiring of Gene-Enhancer Interactions. Cell 2015;161:1012–25. doi:10.1016/j.cell.2015.04.004.
[23] Filion GJ, van Bemmel JG, Braunschweig U, Talhout W, Kind J, Ward LD, et al. Systematic Protein Location Mapping Reveals Five Principal Chromatin Types in Drosophila Cells. Cell 2010;143:212–24. doi:10.1016/j.cell.2010.09.009.
[24] Gligoris T, Löwe J. Structural Insights into Ring Formation of Cohesin and Related Smc Complexes. Trends Cell Biol n.d. doi:10.1016/j.tcb.2016.04.002.
[25] Wendt KS, Yoshida K, Itoh T, Bando M, Koch B, Schirghuber E, et al. Cohesin mediates transcriptional insulation by CCCTC-binding factor. Nature 2008;451:796–801. doi:10.1038/nature06634.
[26] Fudenberg G, Imakaev M, Lu C, Goloborodko A, Abdennur N, Mirny LA. Formation of Chromosomal Domains by Loop Extrusion. Cell Rep 2016;15:2038–49. doi:10.1016/j.celrep.2016.04.085.

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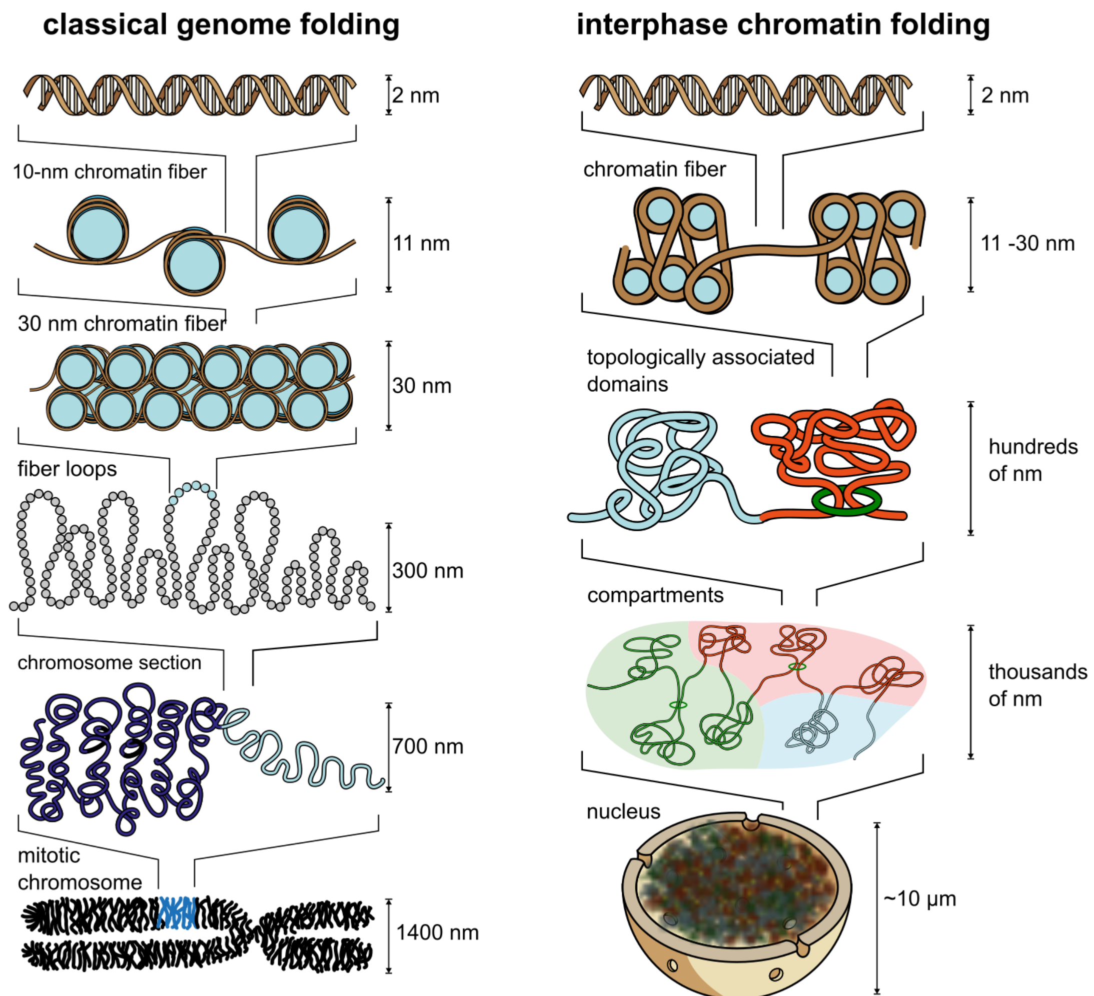

Cover illustration:

Comparison of the classical chromosome fiber folding model and the emerging chromatin folding model for the interphase nucleus. Particularly interesting is the concept of domains as organizational units of chromosomes.

Vergleich des klassischen Models der Chromosomenfaltung mit dem neuesten Stand des Models für die Faltung des Chromatins in der Interphase. Besonders hervorzuheben ist das Konzept von chromosomalen Domänen als Organisationseinheiten.