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Spatial genomic codes

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Abstract:
The increasing variability of phenotypic traits in agricultural animal species makes it necessary to search for reliable DNA markers. Due to the poor efficiency of using clustered single-nucleotide polymorphisms (SNP) and individual genomic elements, the hierarchy of gene regulatory networks has become a relevant research area. We summarized available information on different levels of epigenetic regulation, from the linear DNA sequence and its secondary and tertiary structures to the factors outside the cell nucleus, i.e., intercellular contacts and interactions with the extracellular matrix. We also discussed the features of genomic distribution and the role of topologically associated domains (TADs), and architectural protein CTCF in chromatin loop formation. CTCF mediates protein-protein interactions and interacts with various RNA variants. It also involved in epigenetic modifications of the DNA nucleotide sequence, a target of CTCF binding. Such targeted sites are located in transposable elements (TEs). As a result of the evolutionary conservation, they are also to be found in TAD, regardless of the fact that they are delivered by species-specific TEs. CTCF and its binding sites are known to affect the structure of the mitotic spindle. They also have a certain impact on cholesterol biosynthesis, which affects the plasma membrane and cell migration. CTCF indirectly participates in the variability of intercellular contacts and interactions with the extracellular matrix. In animals, CTCF and its binding targets are involved in all levels of gene regulatory networks that maintain or change genomic expression.

Keywords: G4 quadruplexes, DNA-RNA hybrids, CTCF, chromatin loops, topologically associated domains (TAD), extranuclear factors, neoplastic transformation

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EPIGENETIC VARIATIONS AND TRANSCRIPTOME

Natural and artificial selection both target particular genomic elements for each specific biological object. Lack of information on these key genomic elements is a serious problem for molecular genetics, which prevents effective breeding of agricultural plants and animals. Genome-wide association studies (GWAS) make it possible to project phenotypic trait variability onto the genome. As they develop, they accumulate data on the correlation between the genetically complex traits and diseases in both humans and domestic animals and the genomic elements in the non-coding DNA, which control epigenetic variations in relation to coding sequences [1, 2]. Gene expression also depends on such epigenetic mechanisms as the variability of histone methylation patterns, the DNA, and splicing, as well as movements and expression of mobile genetic elements (MGE) called transposons or transposable elements (TE) [3–8].

Epigenetic modifications in eukaryotic nuclei regulate gene expression programs. Such modifications occur at different organization levels:

– methylation of the linear DNA sequence during imprinting [9];
– multiple two-way changes in chromatin packaging, e.g., formation of various chromatin loops, G4 quadruplexes,

DNA triplexes, hairpin loops, and R-loops (DNA/RNA hybrids); and

– modifications of histones, i.e., the histone code, with variability in DNA accessibility for transcription [10].

A three-way organization level includes:

– autonomous chromosome areas in the interphase nucleus;

– formation of topologically associated domains within and between chromosomes; and
– genetic relationships and location in relation to the lamina of the nuclear membrane, nucleoli, and nuclear pores.

The interphase nucleus has two compartments, A and B. Expression-inactive DNA is part of compartment B, where heterochromatin tends to segregate near the nuclear lamina under the nuclear membrane and the nucleoli. The result is specific patterns unique for each cell populations. Heterochromatization is typical of protein-interacting lamina-associated domains (LAD) enriched in long interspersed nuclear elements (LINE1) [11]. DNA between the LADs is expression-active and forms compartment A, which is believed to be enriched in retrotransposons, i.e., short interspersed nuclear elements (SINEs) [11].

The compartmentalization reflects the three-dimensional (3D) organization of the genome on a mega base scale. However, science knows about 2,000 chromatin domains that range in size from 100 kb to 1 Mb. These topologically associated domains (TADs) correspond to genomic regions with active self-interaction between different genomic elements located at different distances in the primary DNA sequence, i.e., in close physical proximity. Regulatory elements and their target genes are often located within the same TAD. Insulators protect them from interacting with genomic elements of distal TADs (Fig. 1). The regions between two TADs are enriched in insulator proteins and ubiquitously active housekeeping genes that are present in most tissues of multicellular organisms. In mammals, TAD boundaries are characterized by co-bound cohesin and architectural factor CTCF [12–14]. Accumulating evidence suggests that structural changes within TADs lead to profound variations in the expression and intergenic interactions both inside the TAD and its boundaries [15].

Multicellular organisms have a special level of genetic expression regulation, i.e., intercellular interactions represented by the architectonics of tissues and organs. Intercellular interactions affect cell differentiation, apoptosis, and ontogenesis. The 3D level of genetic organization involves many mechanisms, e.g., extrusion of chromatin loops by cohesin complexes, compartmentalization of heterochromatic domains by phase separation, direct interactions between proteins, etc. [16–17].

CHROMATIN LOOPS

The chromatin loop is an elementary unit of chromatin packaging (Fig. 2). It participates in gene expression. Disruption of chromatin loops is associated with neurological diseases [18–20].

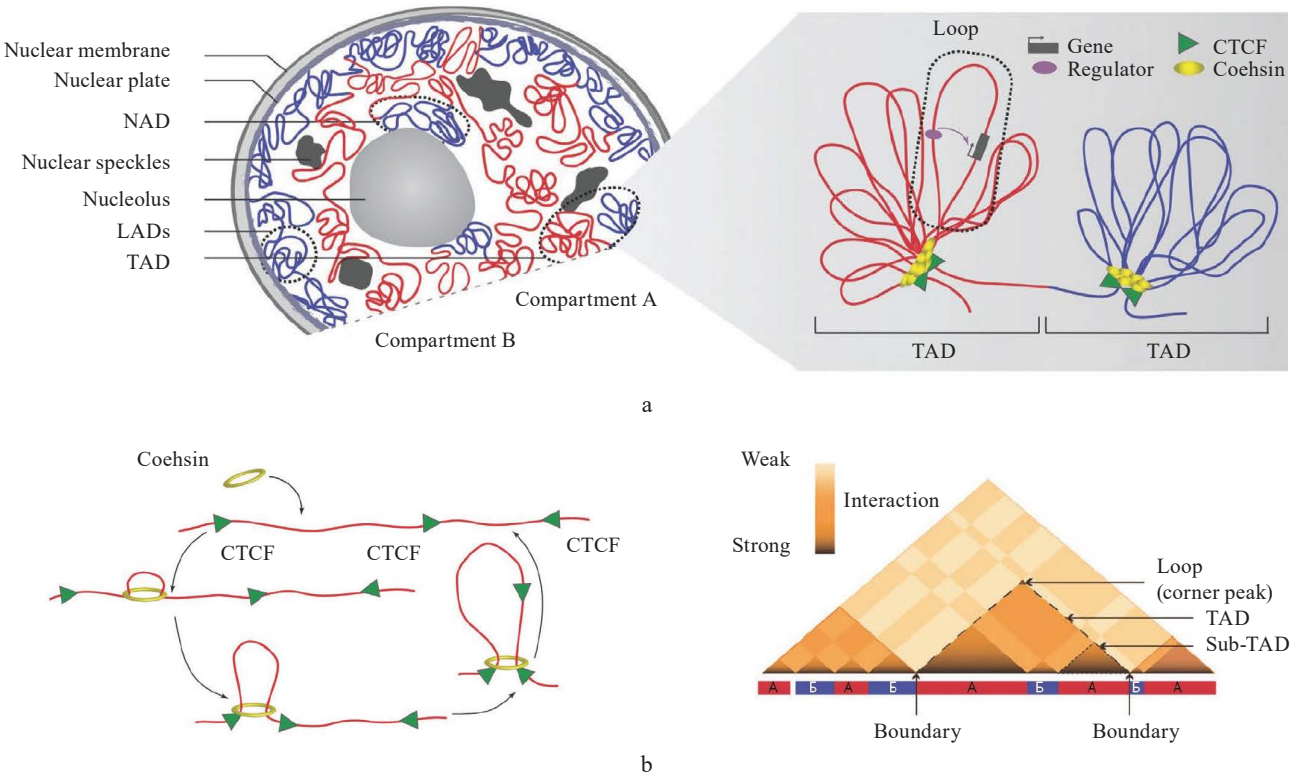

Figure 1 Three-dimensional genomic organization: (a) Compartmentalization into A (red) and B (blue) regions. DNA in compartment A often contacts with nuclear speckles while DNA in compartment B interacts with the nuclear lamina domain (LAD) and the nucleolus-associated domain (NAD). Higher resolution images show topologically associated domains (TADs). Chromatin loops within TADs are formed by cohesin and CTCF binding sites, which brings together regulatory elements (enhancers) and their targets (promoters); (b) Loop extrusion model. Cohesin extrudes chromatin through its ring-shaped structure, thus forming a loop. The loop grows until it reaches two converging CTCFs, which then block cohesin. This process is dynamic and thus can be interrupted; (c) Hi-C visualization of compartments A (red) and B (blue), loops, TADs, and sub-TADs [12].

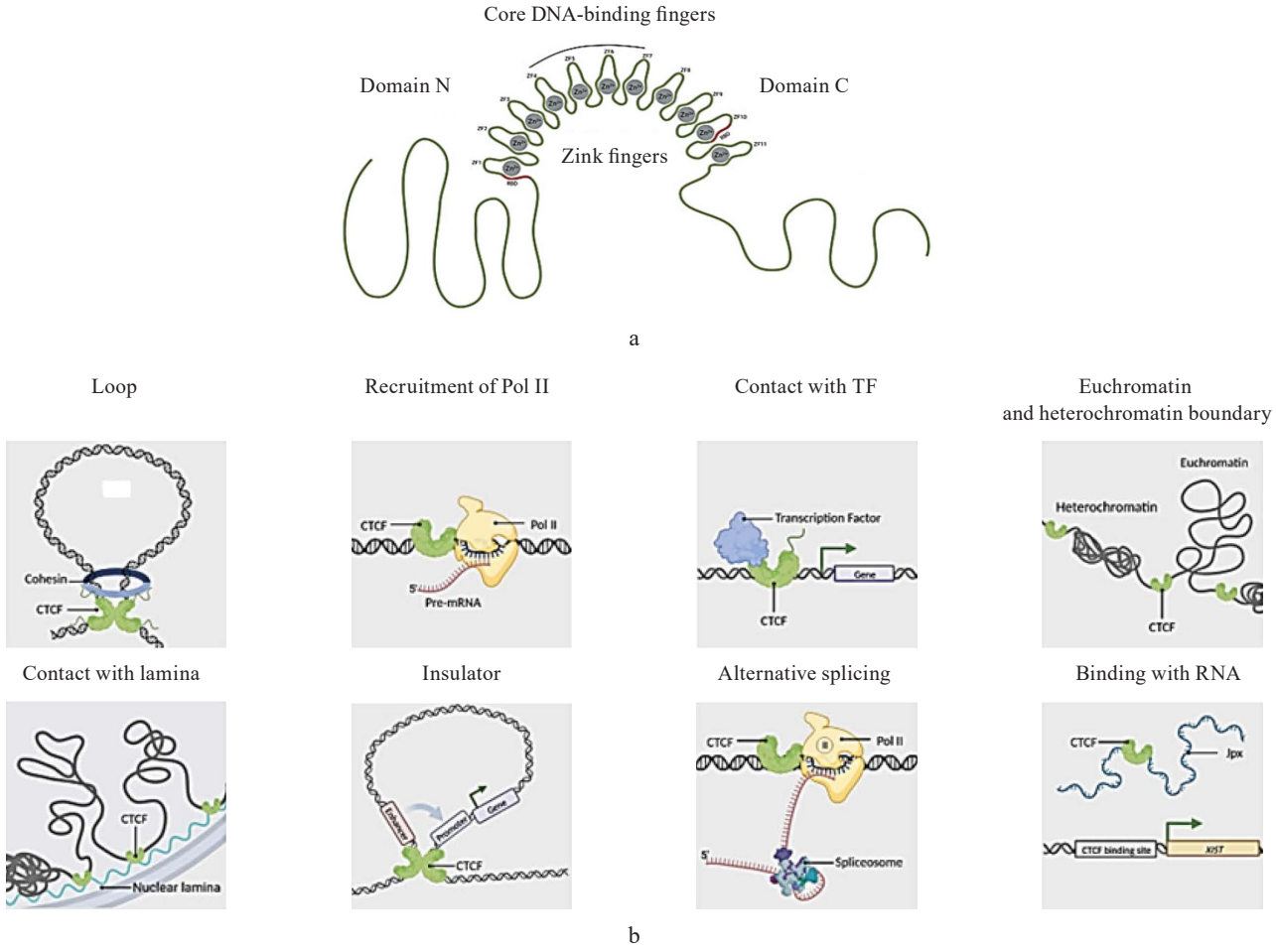

Figure 2 CTCF protein as a gene expression regulator: (a) CTCF is a protein with three domains: N-terminal domain, C-terminal domain, and a central domain with 11 zinc fingers. CTCF uses the zinc finger domain to bind to DNA. RBD – RNA-binding domain; ZF – zinc finger; (b) CTCF mechanisms: chromatin loop, recruitment of RNA polymerase II (Pol II), interaction with transcription factor (TF), defining the boundary between euchromatin and heterochromatin, anchoring the DNA to lamin, insulation, alternative splicing, and binding with RNA [23]

Loop formation usually depends on the CCCTC-binding transcription factor (CTCF), also known as architectural protein. Initially described as a negative regulator of c-Myc expression [21], it is now considered as the best studied transcription factor with C2H2-type zinc finger clusters [22].

A lot of publications report CTCF as a key protein in regulating gene expression because it can mediate between DNA and quite a number of epigenetic factors, even those with no DNA-binding domains (Fig. 3) [23]. Some post-translational modifications, e.g., glycosylation, are also known to affect the binding of CTCF protein to its DNA anchor [24].

While CTCF is a popular factor in chromatin loop formation, the process includes such independent regulatory elements as mammalian-wide interspersed repeats (MIRs) that represent an ancient family of transposable elements (TEs). They are efficient regulators and share some characteristics with tRNA-associated insulators. MIRs are enriched in genes responsible for the T-cell receptor pathway and are located at T-cell-specific boundaries between repressive and active chromatin. In this family, the anchor sequences bind to RNA Pol III and some histone modifications in a way that depends on the chromatin [25].

Tian *et al*. [26] used the method of DNA methylation dioxygenase Tet-triple knock-out (Tet-TKO) to study the effect of DNA methylation on CTCF functions. In their research, methylation differences between rich and poor domains of CpG islands (CGI) decreased, as did the CTCF binding. As a result, the TAD structure weakened and the long-range chromatin loops depleted.

Apparently, CTCF does not bind to all of its potential targets in different cell types. Not only methylation, but also interactions with various non-coding RNAs may affect this process. Many non-coding RNAs affect chromatin organization and gene expression at different levels [27]. Long non-coding RNAs (lncRNAs) participate in many cellular processes, e.g., regulate nearby and distant genes, recruit chromatin modifiers, splice, translate, etc., as well as form and regulate organelles and nuclear condensates [27–30].

Interactions between DNA and RNA yield hybrids with an R-loop or triplexes. The R-loop results in a

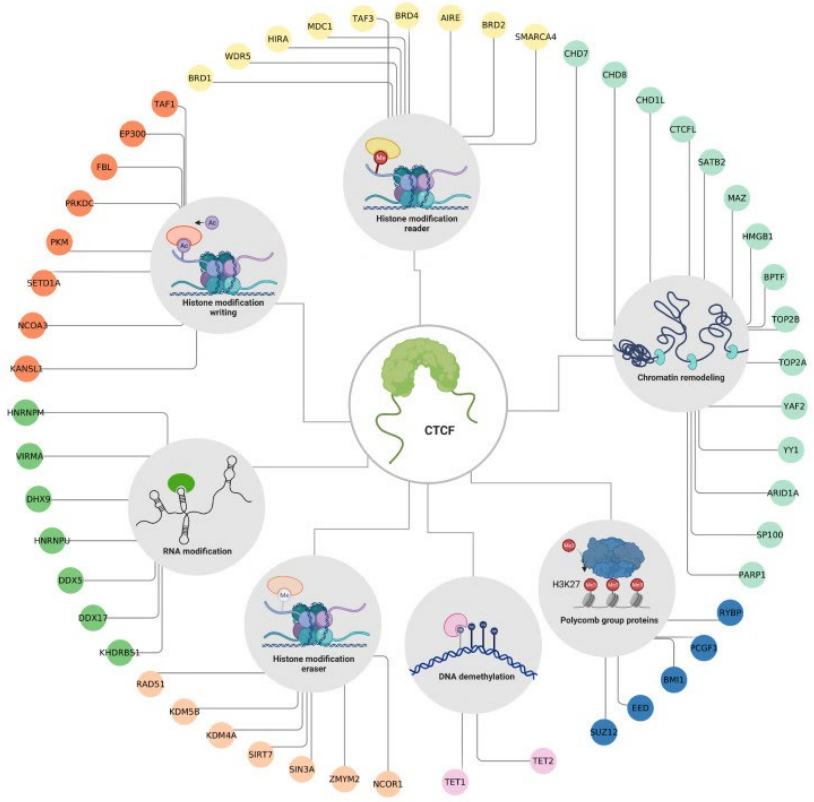

Figure 3 Interactions between CTCF and other epigenetic proteins: histone readers (yellow), chromatin remodelers (light blue), polycomb proteins (dark blue), DNA demethylation proteins (pink), protein that removes histone modifications (beige), RNA-modifying proteins (green), and histone writers (orange) [23]

three-stranded structure that consists of a DNA-RNA hybrid and a displaced DNA strand. The triplex causes a triple-helical structure within the DNA duplex major groove with a marked purine pyrimidine asymmetry across the two strands. The purine-rich DNA strand is available for Hoogsteen pairing with the third RNA strand while the pyrimidine sequence of the RNA binds to the major groove of the target DNA, parallel to the polypurine DNA strand. Should the target DNA site be located in a gene regulatory region, e.g., a promoter or an enhancer, the triplex either modulates local conformational / topological changes or recruits regulatory proteins to the portion of the lncRNA that protrudes from the triplex [31].

R-loops facilitate the anchoring function of CTCF during binding [32]. They form associated structures with G-quadruplexes (G4) in the CTCF binding targets. Wulfridge *et al*. [33] found R-loops and G4 together with CTCF in many regions of the murine embryonic stem cell genome. In their study, a weaker R-loop reduced CTCF binding while a deleted G4-forming motif inhibited CTCF binding and altered gene expression. Hou *et al*. [34] mentioned that sites prone to G4 formation clustered on TAD boundaries and in the transcription, factor binding sites (TFs).

lncRNA GATA6-AS1 is a triplex-forming site with purine-pyrimidine DNA tracks. Its clusters in TAD boundaries rather than in other genomic regions. Some GATA6-AS1 sites interact with CTCF and are also concentrated in CTCF-enriched genomic regions, e.g., TAD boundaries. GATA6-AS1 may be responsible for transporting CTCF protein to particular sites. Thus, some lncRNAs are able to target different genomic domains via RNA/DNA triplex formation to transport molecules, e.g., CTCF. This ability may be a universal mechanism involved in TAD formation and dynamics in 3D genomic organization [28].

CTCF participates in the sex differentiation of allelic expression during genomic imprinting in mammals. In some imprinted domains, differentially methylated sites exhibit different allelic binding to the CTCF protein with subsequent differences in cohesin retention [35].

Porcine germ cells demonstrated sex differences in the methylation of functionally different genes: 465 in males and 316 in females. The genes had sites that were homologous to retrotransposon sites. In 21%, the first intron demonstrated differentiation in methylation, which could probably affect the regulation of gene transcription [36].

The imprinting mechanism is complex and includes not only CTCF [37], but other regulators as well [37–39], e.g., interactions with long non-coding RNA (lncRNA) [40], microRNA (miRNA) clusters [41], or histone modifications [42].

Imprinting disorders in agricultural animals are known to affect the variability of some economically

valuable traits [9]. Long non-coding RNAs (lncRNAs) are part of the regulome, i.e., a set of non-coding regulatory elements of the genome that are closely associated with TEs. They regulate the dynamics of the interphase nucleus architecture. For instance, NEAT1_2 lncRNA is a granule-forming paraspeckle [29]. The non-coding RNAs, such as lncRNAs, could act as an architectural scaffold in the interphase nucleus [27].

Mammalian TEs also provide binding sites for architectural chromatin proteins, including CTCF. Choudhary *et al*. studied genomes of humans, mice, dogs, and rhesus macaques, i.e., mammalian species that diverged 30–96 million years ago. The share of TE increased from mice to primates, being slightly lower in dogs than in mice. In all four species, however, 8–37% of CTCF loop anchors and TAD boundaries came from TEs. While CTCF at TAD boundaries originated mainly from TEs, the research revealed some species-specific patterns: SINEs in mice, LTRs and DNA in humans, with dogs and rhesus macaques in between [3].

Despite species preferences, a high conservation of orthologous synteny and chromatin loop organization was observed between humans and mice [43]. Most TE-derived loop anchors in mice were generated by a few young TE subfamilies, such as B3, B2_Mm2, B3A, and B2_Mm1t. Human TEs contributed fewer orthologous loops and were distributed across more TE subfamilies than in mice. In fact, 87% of TE-derived orthologous loops in mice were discordant to human TEs and were anchored at putative ancestral CTCF binding sites. In mice, syntenic ancestral CTCF motifs were degraded or deleted, the loops being anchored at CTCF sites derived from the nearby, co-opted TEs. For example, the orthologous loop at the 5' end of Akap81 (A Kinase Anchor Protein 8-Like) is maintained in mice by a MER20 element transposed ~ 1.5 thousand pairs of nucleotides upstream of the degraded ancestral motif, which was conserved in most mammals but not rodents. When the ancestral CTCF motif derived from the 147-million-year-old MIR3 element degraded, it incapacitated the CTCF binding. The younger MER20 element, which inserted about 90 million years ago, harbored strong CTCF binding, thus providing an anchor site to maintain the conserved loop in mice. Therefore, TEs provide redundant CTCF sites and mediate them in switching their binding sites. This way, they promote conserved genome folding events in humans and mice [43].

Buckley *et al*. proposed a model where the density and distribution of genes and regulatory elements catalyzed accumulation of TEs. The conservation of synteny of genomic elements and the nuclear organization make mammalian genomes with dissimilar TEs follow similar evolutionary trajectories. [44]. However, early human embryos demonstrated significant differences in the transcription of different TEs in cell populations [45], as well as in brain cells with cell differentiations – at different stages of normal and pathological development [8].

Phenotypic traits in agricultural animal species are known to be damaged by TE insertion into the exonic, intronic, and promoter regions of various genes [46]. Cattle species demonstrated a correlation between the enrichment of CTCF binding motifs and the major quantitative trait genes (QTLs) associated with gene expression variation (eQTLs) or allele-specific expression (aseQTLs) in the transcriptomes of leukocytes and milk cells of lactating cows [47].

Assessment studies of the variability of CTCF binding motifs as a set of regulatory genomic elements can accelerate artificial selection of complex phenotypic traits [46].

The relationships between chromatin loops are complex. For example, the so-called nested loops may possess and coordinate three convergent anchor sites of the CTCF + cohesin complex in a single TAD domain [17]. Changes in AUTS2 and Calneuron 1 (Caln1) in humans provide a clear example of intra-TAD loop fusion. These two genes are separated by a distance of 1.5 Mb, but this distance changed when experimental rats and mice received cocaine. Cocaine-induced release of the Auts2-Caln1 loop increased the mRNA expression in Auts2 and Caln1 [48]. The process boosted DNA cytosine methylation, which could cause a concomitant loss of CTCF binding. The transcriptional changes could be attributed to the increase in trimethylation of the activating mark H3K4 at the Auts2 and Caln1 sites [48, 49].

Therefore, variations in primary DNA sites affect chromatin loop formation, which is closely related to the variability of gene expression.

INTER-TAD RELATIONSHIPS

The International Nucleome Consortium was initiated almost 10 years ago [50]. One of its latest meetings was held in Greece in September 2023 [51]. The Consortium strives to assess the effect of spatial and temporal genomic organization on phenotypic variability. However, its proceedings show that, to date, several important areas remain largely understudied. The list includes the patterns of TAD spatial interaction, prediction options, and the role of cytoskeletal elements, intercellular contacts, tissue architecture, etc. Apparently, TAD interactions are subject to too many regulatory factors, which may differ significantly even for two TADs and their loops, depending on the exact conditions of gene expression change.

TADs are megabase genomic domains with increased self-interaction density. Sub-TADs are smaller but more dynamic units within TADs. The initial definition of a TAD identifies its boundaries. TAD or subTAD boundaries isolate enhancers and promoters from abnormal contacts and facilitate their interactions across TADs. In addition, they locate replication origins in period S [52].

The human genome includes hundreds of thousands of CTCF binding sites (CBS). For instance, 2,898 of human HEC-1-B cells out of total 3,881 TAD boundaries contain 2–8 CBS elements. TAD boundaries are

evolutionarily conserved and associated with complex genetic traits. This feature was described by Sandoval-Velasco *et al*. [53], who studied a 52,000-year-old female woolly mammoth, the cells of which retained a certain conservation of TAD organization in Siberian permafrost.

Most interactions between enhancers and promoters occur within TADs; however, a lot of genes are controlled by distal enhancers outside of single TADs. Therefore, distal enhancers can activate target promoters across TAD boundaries. Chen *et al*. [54] reported that 21% of enhancers of key developmental genes acted across TAD boundaries, in cases of both transcription-dependent and pre-formed encounters. This finding suggests different mechanisms of enhancer-promoter interactions. Such differences were described in studies of paralogous genes [55–57]. They are part of common metabolic pathways that may have common or very similar enhancer sites and TF binding sites. Paralogous genes may participate in one and the same transcription factories, e.g., protein speckles enriched in protein-RNA clusters that facilitate the phase separation of nuclear subcompartments.

AI programs make it possible to predict changes in the 3D architecture of the interphase nucleus, e.g., formation of chromatin loops and TADs [58–60]. Keough *et al*. [60] used this method to compare human and chimpanzee genomes in enhancer sites that are active during the prenatal development of nervous system. The structural variants that were specific to humans but not to chimpanzees were associated with the changes in the 3D packaging of the genome. The process made evolutionarily conservative enhancers interact with some new domains that regulate gene expression.

Braunger *et al*. [61] detected changes in intra- and interchromosomal interactions in skin fibroblasts obtained from different age groups. They identified key transcription regulators where target genes rearrange to change their expression during aging. Correction of such changes may potentially rejuvenate cell populations.

Interchromosomal translocations during carcinogenesis receive a lot of scientific attention. They often cause intergenic fusions and chimeric proteins. Interchromosomal translocations usually involve chromosome regions that are part of common gene expression programs. Their transcription depends on the mechanism responsible for formation of transcription factories or on a set of regulatory elements [62]. By detecting such intergenic fusions at the level of nucleotide sites, medics may improve diagnostics and prognosing.

Light microscopy studies revealed that the frequency of associations between non-homologous chromosomes often coincides with typical oncomarker translocations for certain tissues. For example, interchromosomal associations between chromosomes 12 (heavy chains of immunoglobulins) and 15 (c-Myc oncogene) in bone marrow leukocytes of BALB/c mice demonstrated some translocations typical of murine plasmacytomas [63–65].

McStay [66] reported clustered gene superfamilies in different chromosomes, e.g., during nucleolus formation. Human nuclei have about 300 ribosomal genes located on five different acrocentric chromosomes. For comparison, mice have six. They have to physically converge for the preliminary assembly of ribosomes in the nucleus. Monahan *et al*. [67] located olfactory receptor genes on several different chromosomes. They combined in the same nuclear space to create an olfactosome and regulate their expression.

TADs that are located on different chromosomes can interact if assisted by housekeeping genes on TAD boundaries: as they get transcribed, they recruit ribonucleoprotein condensates. Interactions between different TADs are further facilitated by the physical forces that arise from the interaction between transcriptional condensates at these boundaries and subnuclear organelles, especially nuclear speckles [68]. Some oncological studies report a connection between pairwise aneuploidy (loss/gain) between non-homologous chromosomes. This connection emphasizes the complex hierarchy of genetic material [69].

Interchromosomal studies revealed spatial distances for intrachromosomal interactions in the range of 189 ± 95 nm; the range was greater (279 ± 163 nm) in non-homologous chromosomes [70].

GENETIC COMPOSITION OF TADS

Accumulated data on TADs, interchromosomal relationships across boundaries, and the correlations between their disturbances and various pathologies indicate multi-level hierarchical relationships between the 3D genomic organization in ontogenesis. At each level, disturbances affect the expression of many genes within and between TADs, thus leading to phenotypic consequences (Fig. 4) [71].

TAD structure is complex, dynamic, and diverse. It also depends on the functional characteristics of its genes. Abnizova *et al*. [14] studied three germ layers during gastrulation in mice. The genes with the same expression in different layers differed from those genes that varied in expression in such parameters as density and clustering. They also had a larger number of GCs in promoters and belonged to housekeeping genes. The tissue-specific genes had TADs with a relatively smaller number of genes and a reduced GC content in promoters. Their expression was predominantly regulated by distal enhancers. As for the TFs of such genes, species-specific TFs, which the authors called innovative or pioneering, were more common than in housekeeping genes. The genes transcribed in the three murine germ layers were conditionally referred to as housekeeping genes. Their TF binding sites were so close to promoters that they overlapped. The promoters had extensive GC content. The genes in TADs were so clustered that no empty space remained between them. Abnizova *et al*. [14] distinguish two groups of genes that form qualitatively different TADs: those containing developmental genes regulated by innovative TFs and cooperator genes expressed in most tissues.

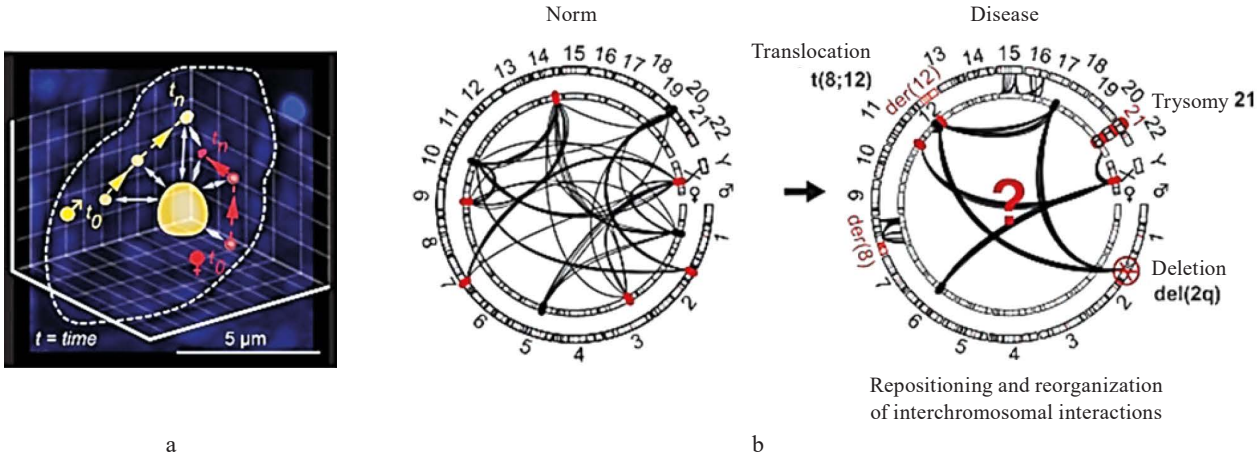

Figure 4 Interactions between non-homologous chromosomes: (a) Allele-specific loci of paternal (yellow) and maternal (red) genomes relative to the nucleolus in primordial embryonic stem cells (ESC); (b, left) Maternal and paternal alleles are in physical proximity and intertwined while they modulate tissue-specific gene regulation in the 3D nucleus space. This interaction contributes to various biological processes, DNA-RNA, proteins, biophysical properties of chromatin, 3D genomic organization, and stochastic factors; (b, right) Structural aberrations involve deletions, translocations, etc., while numerical chromosomal aberrations are represented by trisomies. Both can disrupt and reorganize the complex network of interchromosomal interactions, thus changing transcriptional programs [71].

Numerous studies report the differences in regulatory elements between the housekeeping genes and the tissue-specific genes. Roller *et al.* [72] studied four groups of mammals: primates (macaques and monkeys); rodents (mice, rats, and rabbits); pigs, horses, cats, and dogs; and marsupials (opossum). They took samples of four tissues, i.e., liver, muscles, brain, and testes, as representing three somatic tissues originating from different germ layers. The comparative analysis covered regulatory sites, i.e., promoters and enhancers, in the tissue-specific genes vs. housekeeping genes. The analysis revealed a rather high rate of evolution of tissue-specific genes, where insertions of the LINE1 retrotransposon were quite active. LINE2 as a more ancient variant occurred more often in the regulatory sites of housekeeping genes. The analysis of evolution rate in tissue-specific enhancers and promoters indicated a relatively low rate of evolution of gene expression in the brain whereas the evolution rate in testicles and liver was high. In addition, the study revealed some intraspecific conservation in the variability of regulatory systems with pronounced interspecific differences [72].

Other studies also confirmed important distinctions between TADs with genes of different functions and evolutionary origins. James *et al.* [73] reported that TAD boundaries frequently coincided with breaks in genome. Deleted boundaries depended on negative selection, suggesting that TADs may facilitate genome rearrangements and evolution. The genes co-localized within TADs in a way that depended on their evolutionary age in humans and mice. As a result, TADs were divided into two groups with different shares of older and younger genes. The division was based on whether they arose before or during vertebrate whole-genome duplications (WGDs). Evolutionarily older genes were more frequently expressed in different cell types and were more often classified as essential than younger genes. Essential genes were found responsible for growth, development, and reproduction at both the cellular and organism levels. The loss of these functions may compromise viability or adaptability. Older genes were more likely to become essential because old genes usually disappear during evolution if they are less essential. However, how some young genes become essential still remains unclear. To answer this question, James *et al.* [73] studied the TAD content ratios in old vs. young genes and essential vs. less essential young genes. In primates and rodents, recently duplicated young genes appeared to be more essential when they were located in TADs enriched in old genes and interacted with those genes that were last duplicated during WGD. Therefore, the evolutionary significance of young genes may increase if they are located in TADs with regulatory networks established by old genes [73].

Thus, the nucleus has different levels that affect the 3D organization of the genetic material, plus the fourth, ontogenetic component: modifications of the linear DNA sequence with methylation and multiple secondary structures; intra- and inter-TAD formation, dynamics, and interactions of chromatin loops; evolutionary and functional features of TAD genes. All these phenomena obviously depend on factors outside the nucleus, especially intercellular interactions.

EXTRANUCLEAR STRUCTURAL ELEMENTS AFFECTING GENE EXPRESSION

Actin is a key element of the cytoskeleton. It is one of the most conservative and widespread proteins in eukaryotic cells. Actin is present as G-actin in a monomeric globular form and as F-actin in polymeric

filamentous forms of various length. Actin is highly concentrated at the periphery of the cell, in the cytoplasm. In low concentrations, it can be found in the nucleus, where it interacts with the lamina to form a network throughout the nuclear membrane [74].

Cytoplasmic actin participates in cell motility, organelle movement, cell signaling systems, etc. The fact that the nucleus has much less actin than the cytoplasm led to discussions about its involvement in the nuclear matrix and interactions with the DNA. However, actin is now known to be part of the control of the nuclear architecture. Nuclear actin interacts with RNA polymerases I, II, and III and ribonucleoprotein transcript complexes. It prolongates transcription and transports polyribosomes. It binds to the lamins of the nuclear membrane either directly or through intermediary proteins. Nuclear actin is involved in the formation of open chromatin and TADs; it repairs DNA breaks, and controls nuclear morphology, e.g., its apical surface [74–80].

Mitotic structure also depends on the chromatin architectural protein CTCF. Targeted mutations of CTCF use clustered regularly interspaced short palindromic repeats (CRISPR) to disorganize the mitotic spindle and disrupt the anaphase chromosome segregation by forming tri- or tetrapolar spindles and chromosomes beyond the spindle pole. Therefore, CTCF is important for both correct metaphase organization and anaphase segregation [81].

The correlation between 3D chromatin organization and cell morphology can be ensured by the variability of the plasma membrane state. A targeted mutation of architectural proteins CTCF and CTCF pLoF was reported to affect cell migration because this mutation increased the RNA level of cholesterol biosynthesis enzymes. Migrasomes, which are extracellular vesicles on the retraction fibers of migrating cells, slowed down in their formation in CTCF pLoF cells. Hmgcs1 promoter, which encodes the cholesterol biosynthesis enzyme, did not bind with CTCF directly, However, CTCF could affect at least two key features, i.e., spatial organization and histone modification [82].

Intercellular interactions are another long-standing source of gene expression program. The epithelial-mesenchymal transition (EMT) is a good example of cell plasticity as it renders the epithelium its mesenchymal phenotypes (Fig. 5) [83].

Epithelial cells contain specialized junctional proteins, exhibit apicobasal polarity, and have limited dissociation and migration potential. In contrast, mesenchymal cells are irregular and do not form specialized adhesion complexes. Their end-to-end polarity and focal adhesions increase migratory capacity. During EMT, epithelial cells acquire mesenchymal features, which include changes in the expression of epithelial and mesenchymal markers [83].

α-Smooth muscle actin (*α*-SMA) is an actin isoform that predominates in vascular smooth muscle cells and is important for fibrogenesis. *α*-SMA expression is lower in cells during contact inhibition than in during serum starvation [84].

AIFM2 is a mitochondrion-associated apoptosis-inducing factor. It was renamed from an unidentified anti-ferroptosis gene into ferroptosis suppressor protein 1 (FSP1). It is known to protect against ferroptosis induced by deletion of glutathione peroxidase 4 (GPX4), which controls phospholipid oxidation [85].

Gene expression programs depend on the cellular environment. This fact was established on classical models

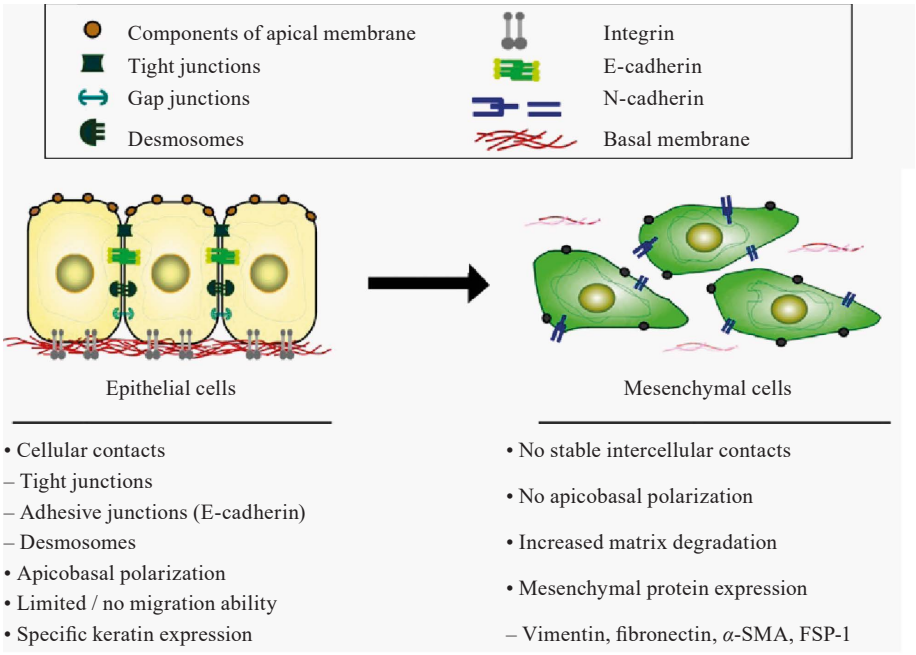

Figure 5 Key features of epithelial and mesenchymal cells

of cellular differentiation gradients, e.g., trabeculae of mammalian liver lobules. They consist of two layers of hepatocytes that polyploidize from diploids to decaploids as they move toward the central venule and change the enzymic expression. Small intestine villi are another example. They grow from a stem cell; daughter cells move to the apical apex by asymmetric differentiation, upon which they die and are exfoliated into the intestinal lumen. Based on our own unpublished research result, we know that hepatectomy of one lobule in the liver leads to polyploidization of the rest while a CH4 poisoning causes necrosis of hepatocytes around the central venule and proliferation of peripheral diploid hepatocytes. To sum it up, each cell receives differentiated signals depending on its position in the tissue architecture.

Internal and external factors that affect the asymmetry by the level of cellular differentiation of stem cell progeny in different tissues. Two daughters of one stem cell behave asymmetrically: one remains a stem cell while the other differentiates. The same happens every time they divide: one remains young while the other specializes. The process includes the state of the mitotic spindle. The transfer of old and young chromatids, histones, and cellular organelles to daughter cells is asymmetric. Thus, the gene expression of each cell depends on its position in the structure of the tissue or organ [86].

Embryonic cells can undergo neoplastic transformation in a non-embryonic environment. Tumor cells can spread out and acquire contact inhibition when placed in an embryonic environment or on a substrate that increases their adhesiveness. Sarcomas could be induced by embedding pieces of quartz glass into murine muscles. Probably, it happens because conditional morphostats stop entering cells, which prevents neoplastic transformation [87]. Inherited oncogenic traits are encoded not only by gene sequences, but also by DNA or chromosomal structures that can be changed by non-mutational mechanisms, e.g., methylation, chromatin packaging with architectural proteins and RNA, or post-translational modifications of nuclear proteins [88]. I. Berenblum, who authored the theory of two-stage carcinogenesis, reported that benign tumors develop each at its own rate under the same influences, going malignant at their own rate as well [89]. That is, each founder cell has an individual response to the same impact [90].

The individual character of the 3D cellular organization follows from the long-discussed heterogeneity of cells in the architectonics of the interphase nucleus, as well as from the potential differences in genomic organization in different sexes. Severe individual heterogeneity at the molecular level was described, in particular, in the spatial positioning of alleles of the same gene [91]. Studies in allele expression during the production of induced pluripotent stem cells revealed three groups of genes that differed in the way they coordinated their expression. They were classified as highly coordinated, semi-coordinated, or independent. Two alleles of highly coordinated genes have a similar accessibility of chromatin, enriched in such accessibility regulators as H3K4me3, H3K4me1, H3K36me3, and H3K27ac. The genes themselves have denser binding sites with enhancers, unlike the other two variants [92].

We obtained data that suggested independent segregation of haploid sets of chromosomes in mammalian somatic cells, which could change positions relative to each other in bone marrow cell populations [93]. This finding was further confirmed by data obtained after nuclear transfer of unreplicated nuclei of somatic cells at the G0/G1 stage into the metaphase cytoplasm of enucleated murine oocytes [94]. A genome sequencing analysis revealed correct segregation of homologous chromosomes into the polar body, resulting in cells with complete haploid sets of chromosomes. This, however, occurred only in the nuclei of inbred mice and never in interstrain hybrids. This finding emphasizes the importance of sequence homology between homologs. However, the same finding might indicate that haploid sets "remember" that they used to be one. The destruction of this unity in zygotes from interlinear murine crossings may reflect the organization of the cytoskeleton and the division spindle. The process is similar to chromosome ejection from the metaphase plates of one species when obtaining interspecific hybridomas.

Thus, haploid segregation is preserved in somatic cells, which indicates another level of 3D genomic organization, where haploid sets of chromosomes behave as autonomous units.

Undifferentiated stem cells are believed to be the only ones responsible for the formation of all other cells and the cause of neoplastic transformation [95]. However, tumor cells differ from the original cells by a combination of expression of both highly specialized genes and embryonic ones, e.g., in melanomas. According to C. L. Markert, neoplastic transformation is a disease of cellular differentiation, as a result of which a tumor cell differs from a normal one by an "abnormal combination of normal components" [96]. Apparently, genetic or epigenetic changes leading to neoplastic transformation must affect the cellular mechanisms involved in the organization and change of gene expression.

Some mammal species are resistant to cancer – from such large mammals as elephants and whales to such small rodents as naked mole rats, blind mole rats, and bats [97]. Naked mole rats have early two-level contact inhibition whereas blind mole rats have adapted to hypoxia and low heparanase, an endoglycosidase enzyme that breaks down heparan sulfate, which is involved in contacts between cells and their interactions with the extracellular matrix. Other cancer-resistant species differ in the activity of genes involved in other metabolic pathways but associated with the impact on intercellular contacts and relationships with the extracellular matrix.

CONCLUSION

The genetic code is a triplet encoding of amino acid sequences of proteins. It is the basic heredity component, but it is a very small component that occupies, for example, ≤ 2% nucleotides of the entire mammal

genome. It contributes to phenotypic variability by different levels of internal packaging, i.e., secondary DNA structures, e.g., hairpins, G4 quadruplexes, triplexes, packaging in nucleosomes, histone code, etc. The interactions between enhancers and promoters, DNA and proteins, DNA and RNA hybrids belong to genome organization level two. Level three is associated with loops, topologically associated domains (TAD), non-random integration, writing of mobile genetic elements in the genome, subdivision into hetero- and euchromatin compartments, autonomy of chromosome territories, interactions between TADs of different chromosomes, and a certain autonomy of haploid sets of chromosomes in diploid species. Level four usually implies the above-mentioned characteristics in dynamics during ontogenesis. However, it very seldom includes factors outside the nucleus, e.g., cytoskeleton, extracellular matrix, plasma membrane, intercellular contacts, etc., which affect all other levels. CTCF is an element that unifies the levels. This architectural protein possesses evolutionary conservation; its density is different on the loop boundaries and TADs of the nucleotide binding sites to this protein. CTCF is dynamic in the epigenetic variability of CTCF binding targets and post-transcriptional modifications. It is part of transcription and translation factories; it participates directly and indirectly in intercellular interactions with the extracellular matrix. All these features indicate that, apparently, its targets can create a certain framework for regulating gene expression. Studies in this sphere could contribute to a better understanding of phenotypic variability and yield approaches to manage it.

CONTRIBUTION

All the authors were equally involved in the manuscript and are equally responsible for any potential plagiarism.

CONFLICT OF INTEREST

The authors declared no conflict of interests regarding the publication of this article.

REFERENCES

1. Glazko VI, Kosovsky GYu, Glazko TT. The Sources of Genome Variability as Domestication Drivers (Review). Agricultural Biology. 2022;57(5):832–851. (In Russ.). https://doi.org/10.15389/agrobiology.2022.5.832rus
2. Glazko VI, Kosovsky GYu, Glazko TT, Fedorova LM. DNA Markers and Microsatellite Code (Review). Agricultural Biology. 2023;58(2):223–248. (In Russ.). https://doi.org/10.15389/agrobiology.2023.2.223rus
3. Choudhary MNK, Quaid K, Xing X, Schmidt H, Wang T. Widespread Contribution of Transposable Elements to The Rewiring of Mammalian 3D Genomes. Nature Communications. 2023;14:634. https://doi.org/10.1038/s41467-023-36364-9
4. Gebrie A. Transposable Elements as Essential Elements in The Control of Gene Expression. Mobile DNA. 2023;14:9. https://doi.org/10.1186/s13100-023-00297-3
5. Lawson HA, Liang Y, Wang T. Transposable Elements in Mammalian Chromatin Organization. Nature Reviews Genetics. 2023;24:712–723. https://doi.org/10.1038/S41576-023-00609-6
6. Lu JY, Chang L, Li T, Wang T, Yin Y, Zhan G, *et al.* Homotypic Clustering of L1 and B1/Alu Repeats Compartmentalizes the 3D Genome. Cell Research. 2021;31:613–630. https://doi.org/10.1038/s41422-020-00466-6
7. Glazko TT, Glazko GV, Kosovsky GYu, Zybaylov VL, Glazko VI. Interphase Nucleus and Transposable Elements (Review). Biogeosystem Technique. 2022;(9):62–76. (In Russ.). https://doi.org/10.13187/bgt.2022.2.62
8. Mustafin RN. A Hypothesis about Interrelations of Epigenetic Factors and Transposable Elements in Memory Formation. Vavilov Journal of Genetics and Breeding. 2024;28(5):476–486. (In Russ.). https://doi.org/10.18699/vjgb-24-54
9. Hubert JN, Perret M, Riquet J, Demars J. Livestock Species as Emerging Models for Genomic Imprinting. Frontiers in Cell and Developmental Biology. 2024;12:1348036. https://doi.org/10.3389/fcell.2024.1348036
10. Strahl BD, Allis CD. The Language of Covalent Histone Modifications. Nature. 2000;403:41–45. https://doi.org/10.1038/47412
11. Falk M, Feodorova Y, Naumova N, Imakaev M, Lajoie BR, Leonhardt H, *et al.* Heterochromatin Drives Compartmentalization of Inverted and Conventional Nuclei. Nature. 2018;570:395–399. https://doi.org/10.1101/244038
12. Glaser J, Mundlos S. 3D or Not 3D: Shaping the Genome during Development. Cold Spring Harbor Perspectives in Biology. 2022;14:a040188. https://doi.org/10.1101/cshperspect.a040188
13. Gamliel A, Meluzzi D, Oh S, Jiang N, Destici E, Rosenfeld MG, *et al.* Long-Distance Association of Topological Boundaries Through Nuclear Condensates. Proceedings of the National Academy of Sciences. 2022;119(32):e2206216119. https://doi.org/10.1073/pnas.2206216119
14. Abnizova I, Stapel C, Boekhorst RT, Lee JTH, Hemberg M. Integrative Analysis of Transcriptomic and Epigenomic Data Reveals Distinct Patterns for Developmental and Housekeeping Gene Regulation. BMC Biology. 2024;22:78. https://doi.org/10.1186/s12915-024-01869-2

15. Gheldof N, Witwicki RM, Migliavacca E, Leleu M, Didelot G, Harewood L, *et al.* Structural Variation-Associated Expression Changes are Paralleled by Chromatin Architecture Modifications. PLoS One. 2013;8(11):e79973. https://doi.org/10.1371/journal.pone.0079973
16. Magnitov M, de Wit E. (2024) Attraction and Disruption: How Loop Extrusion and Compartmentalisation Shape the Nuclear Genome. Current Opinion in Genetics and Development. 2024;86:102194. https://doi.org/10.1016/j.gde.2024.102194
17. Logie C. The Role of CTCF-Mediated Chromatin Looping in Enhancer-Promoter Communication. In: Witzany G, editor. Epigenetics in Biological Communication. Springer: Cham; 2024. pp. 333–355. https://doi.org/10.1007/978-3-031-59286-7_16
18. Behrends M, Engmann O. Loop Interrupted: Dysfunctional Chromatin Relations in Neurological Diseases. Frontiers in Genetics. 2021;12:732033. https://doi.org/10.3389/fgene.2021.732033
19. Osman N, Shawky AE, Brylinski M. Exploring the Effects of Genetic Variation on Gene Regulation in Cancer in The Context Of 3D Genome Structure. BMC Genomic Data. 2022;23:13. https://doi.org/10.1186/s12863-021-01021-x
20. Mitchell AC, Bharadwaj R, Whittle C, Krueger W, Mirnics K, Hurd Y, *et al.* The Genome in Three Dimensions: A New Frontier in Human Brain Research. Biological Psychiatry. 2014;75(12);961–969. https://doi.org/10.1016/j.biopsych.2013.07.015
21. Lobanenkov VV, Nicolas RH, Adler VV, Paterson H, Klenova EM, Polotskaja AV, *et al.* A Novel Sequence-Specific DNA Binding Protein Which Interacts with Three Regularly Spaced Direct Repeats of the CCCTC-Motif in the 5'-Flanking Sequence of the Chicken C-Myc Gene. Oncogene. 1990;5(12):1743–1753.
22. Maksimenko OG, Fursenko DV, Belova EV, Georgiev PG. CTCF As an Example of DNA-Binding Transcription Factors Containing Clusters of C_2H_2-Type Zinc Fingers. Acta Naturae. 2021;13(1):31–46. (In Russ.). https://doi.org/10.32607/actanaturae.11206
23. Del Moral-Morales A, Salgado-Albarrán M, Sánchez-Pérez Y, Wenke NK, Baumbach J, Soto-Reyes E. CTCF and Its Multi-Partner Network for Chromatin Regulation. Cells. 2023;12(10):1357. https://doi.org/10.3390/cells12101357
24. Tang X, Zeng P, Liu K, Qing L, Sun Y, Liu X, *et al*. The PTM Profiling of CTCF Reveals the Regulation of 3D Chromatin Structure by O-Glcnacylation. Nature Communications. 2024;15:2813. https://doi.org/10.1038/s41467-024-47048-3
25. Wang J, Vicente-García C, Seruggia D, Moltó E, Fernandez-Miñán A, Neto A, *et al.* MIR Retrotransposon Sequences Provide Insulators to the Human Genome. Proceedings of the National Academy of Sciences. 2015; 112(32):E4428–E4437. https://doi.org/10.1073/pnas.1507253112
26. Tian H, Luan P, Liu Y, Li G. Tet-mediated DNA Methylation Dynamics Affect Chromosome Organization. Nucleic Acids Research. 2024;52(4):3654–3666. https://doi.org/10.1093/nar/gkae054
27. Razin SV, Gavrilov AA. Non-coding RNAs in Chromatin Folding and Nuclear Organization. Cellular and Molecular Life Sciences. 2021;78:5489–5504. https://doi.org/10.1007/s00018-021-03876-w
28. Soibam B. Association between Triplex-Forming Sites of Cardiac Long Noncoding RNA *GATA6-AS1* and Chromatin Organization. Non-coding RNA. 2022;8(13):41. https://doi.org/10.3390/ncrna8030041
29. Zacco E, Broglia L, Kurihara M, Monti M, Gustincich S, Pastore A, Plath K, *et al.* RNA: The Unsuspected Conductor in the Orchestra of Macromolecular Crowding. Chemical Reviews. 2024;124(8):4734–4777. https://doi.org/10.1021/acs.chemrev.3c00575
30. Poller W, Sahoo S, Hajjar R, Landmesser U, Krichevsky AM. Exploration of the Noncoding Genome for Human-Specific Therapeutic Targets-Recent Insights at Molecular and Cellular Level. Cells. 2023;12(22):2660. https://doi.org/10.3390/cells12222660
31. Merici G, Amidani D, Dieci G, Rivetti C. A New Strategy to Investigate RNA: DNA Triplex Using Atomic Force Microscopy. International Journal of Molecular Sciences. 2024;25(5):3035. https://doi.org/10.3390/ijms25053035
32. Zhang H, Shi Z, Banigan EJ, Kim Y, Yu H, Bai XC, *et al.* CTCF and R-loops are Boundaries of Cohesin-Mediated DNA Looping. Molecular Cell. 2023;83(16):2856–2871. https://doi.org/10.1016/j.molcel.2023.07.006
33. Wulfridge P, Yan Q, Rell N, Doherty J, Jacobson S, Offley S, *et al.* G-quadruplexes Associated with R-loops Promote CTCF Binding. Molecular Cell. 2023;83(17):3064–3079. https://doi.org/10.1016/j.molcel.2023.07.009
34. Hou Y, Li F, Zhang R, Li S, Liu H, Qin ZS, Sun X. Integrative Characterization of G-Quadruplexes in the Three-Dimensional Chromatin Structure. Epigenetics. 2019;14(9):894–911. https://doi.org/10.1080/15592294.2019.1621140
35. Moindrot B, Imaizumi Y, Feil R. Differential 3D Genome Architecture and Imprinted Gene Expression: Cause or Consequence? Biochemical Society Transactions. 2024;52(3):973–986. https://doi.org/10.1042/BST20230143
36. Gómez-Redondo I, Planells B, Cánovas S, Ivanova E, Kelsey G, Gutiérrez-Adán A. Genome-wide DNA Methylation Dynamics During Epigenetic Reprogramming in The Porcine Germline. Clinical Epigenetics. 2021;13:27. https://doi.org/10.1186/s13148-021-01003-x

37. Noordermeer D, Feil R. Differential 3D Chromatin Organization and Gene Activity in Genomic Imprinting. Current Opinion in Genetics and Development. 2020;61:17–24. https://doi.org/10.1016/j.gde.2020.03.004

38. Monk, D, Mackay DJG, Eggermann T, Maher ER, Riccio A. Genomic Imprinting Disorders: Lessons on How Genome, Epigenome and Environment Interact. Nature Reviews Genetics. 2019;20:235–248. https://doi.org/10.1038/s41576-018-0092-0

39. Hubert J-N, Demars J. Genomic Imprinting in The New Omics Era: A Model for Systems-Level Approaches. Frontiers in Genetics. 2022;13:838534. https://doi.org/10.3389/fgene.2022.838534

40. MacDonald WA, Mann MRW. Long Noncoding RNA Functionality in Imprinted Domain Regulation. PLOS Genetics. 2020;16(8):e1008930. https://doi.org/10.1371/journal.pgen.1008930

41. Malnou EC, Umlauf D, Mouysset M, Cavaillé J. Imprinted MicroRNA Gene Clusters in The Evolution, Development, and Functions of Mammalian Placenta. Frontiers in Genetics. 2019;9:706. https://doi.org/10.3389/fgene.2018.00706

42. Sanli I, Feil R. Chromatin Mechanisms in the Developmental Control of Imprinted Gene Expression. The International Journal of Biochemistry and Cell Biology. 2015;67:139–147. https://doi.org/10.1016/j.biocel.2015.04.004

43. Choudhary MN, Friedman RZ, Wang JT, Jang HS, Zhuo X, Wang T. Co-opted Transposons Help Perpetuate Conserved Higher-Order Chromosomal Structures. Genome Biology. 2020;21:16. https://doi.org/10.1186/s13059-019-1916-8

44. Buckley RM, Kortschak RD, Raison JM, Adelson DL. Similar Evolutionary Trajectories for Retrotransposon Accumulation in Mammals. Genome Biology and Evolution. 2017;9(9):2336–2353. https://doi.org/10.1093/gbe/evx179

45. DiRusso JA, Clark AT. Transposable Elements in Early Human Embryo Development and Embryo Models. Current Opinion in Genetics and Development. 2023;81:102086. https://doi.org/10.1016/j.gde.2023.102086

46. Zhao P, Peng C, Fang L, Wang Z, Liu GE. Taming Transposable Elements in Livestock and Poultry: A Review of Their Roles and Applications. Genetics Selection Evolution. 2023;55:50. https://doi.org/10.1186/s12711-023-00821-2

47. Wang M, Hancock TP, Chamberlain AJ, Vander Jagt CJ, Pryce JE, Cocks BG, *et al.* Putative bovine topological association domains and CTCF binding motifs can reduce the search space for causative regulatory variants of complex traits. *BMC Genomics*. 2018;19:395. https://doi.org/10.1101/242792

48. Engmann O, Labonté B, Mitchell A, Bashtrykov P, Calipari ES, Rosenbluh C, *et al.* Cocaine-Induced Chromatin Modifications Associate with Increased Expression and Three-Dimensional Looping of *Auts2*. Biological Psychiatry. 2017;82(11):794–805. https://doi.org/10.1016/j.biopsych.2017.04.013

49. Szabó D, Franke V, Bianco S, Batiuk MY, Paul EJ, Kukalev A, *et al.* A Single Dose of Cocaine Rewires The 3D Genome Structure of Midbrain Dopamine Neurons. 2024. https://doi.org/10.1101/2024.05.10.593308

50. Tashiro S, Lanctôt C. The International Nucleome Consortium. Nucleus. 2015;6(2):89–92. https://doi.org/10.1080/19491034.2015.1022703

51. Rey-Millet M, Bystricky K.International Nucleome Consortium. The Genome in Space and Time Comes of Age. Nucleus. 2024;15(2):2307665. https://doi.org/10.1080/19491034.2024.2307665

52. Huang H, Wu Q. Pushing the TAD Boundary: Decoding Insulator Codes of Clustered CTCF Sites in 3D Genomes. BioEssays. 2024;46(10):2400121. https://doi.org/10.1002/bies.202400121

53. Sandoval-Velasco M, Dudchenko O, Rodríguez JA, Pérez Estrada C, Dehasque M, Fontsere C, *et al.* Three-Dimensional Genome Architecture Persists in a 52,000-Year-Old Woolly Mammoth Skin Sample. Cell. 2024;187(14):3541–3562. https://doi.org/10.1016/j.cell.2024.06.002

54. Chen Z, Snetkova V, Bower G, Jacinto S, Clock B, Dizehchi A, *et al.* Increased Enhancer-Promoter Interactions During Developmental Enhancer Activation in Mammals. Nature Genetics. 2024;56:675–685. https://doi.org/10.1038/s41588-024-01681-2

55. Schoenfelder S, Sexton T, Chakalova L, Cope NF, Horton A, Andrews S, *et al*. Preferential Associations between Co-Regulated Genes Reveal a Transcriptional Interactome in Erythroid Cells. Nature Genetics. 2010;42:53–61. https://doi.org/10.1038/ng.496

56. Yan Y, Tian Y, Wu Z, Zhang K, Yang R. Interchromosomal Colocalization with Parental Genes Is Linked to the Function and Evolution of Mammalian Retrocopies. Molecular Biology and Evolution. 2023;40(12):msad265. https://doi.org/10.1093/molbev/msad265

57. Pollex T, Marco-Ferreres R, Ciglar L, Ghavi-Helm Y, Rabinowitz A, Viales RR, *et al.* Chromatin Gene-Gene Loops Support the Cross-Regulation of Genes with Related Function. Molecular Cell. 2024;84(15):822–838. https://doi.org/10.1016/j.molcel.2023.12.023

58. Fudenberg G, Kelley DR, Pollard KS. Predicting 3D Genome Folding from DNA Sequence with Akita. Nature Methods. 2020;17:1111–1117. https://doi.org/10.1038/s41592-020-0958-x

59. Liu T, Zhu H, Wang Z. Learning Micro-C from Hi-C with Diffusion Models. PLoS Computational Biology. 2024;20(5):e1012136. https://doi.org/10.1371/journal.pcbi.1012136

60. Keough KC, Whalen S, Inoue F, Przytycki PF, Fair T, Deng C, *et al.* Three-Dimensional Genome Rewiring in Loci with Human Accelerated Regions. Science. 2023;380:(6643):eabm1696. https://doi.org/10.1126/science.abm1696

61. Braunger JM, Cammarata LV, Sornapudi TR, Uhler C, Shivashankar GV. Transcriptional Changes Are Tightly Coupled to Chromatin Reorganization During Cellular Aging. Aging Cell. 2024;23(3):e14056. https://doi.org/10.1111/acel.14056

62. Schmitt AD, Sikkink K, Ahmed AA, Melnyk S, Reid D, Van Meter L, *et al.* Evaluation of Hi-C Sequencing for the Detection of Gene Fusions in Hematologic and Solid Pediatric Cancer Samples. Cancers. 2024;16(170:2936. https://doi.org/10.3390/cancers16172936

63. Glazko TT. Approaches to the Analysis of Reciprocal Chromosome Distribution in the Bone Marrow Cells of Mice. Tsitologiya. 1988;30(5):597–605. (In Russ.). https://elibrary.ru/UWLSHL

64. Righolt CH, Wiener F, Taylor-Kashton C, Harizanova J, Vermolen BJ, Garini Y, *et al.* Translocation Frequencies and Chromosomal Proximities for Selected Mouse Chromosomes in Primary B Lymphocytes. Cytometry. 2011;79A(4):276–283. https://doi.org/10.1002/cyto.a.21038

65. Kovalchuk AL, Kim JS, Janz S. *Eμ*/*Sμ* Transposition into *Myc* is Sometimes a Precursor for T(12;15) Translocation in Mouse B Cells. Oncogene. 2003;22:2842–2850. https://doi.org/10.1038/sj.onc.1206345

66. McStay B. Nucleolar Organizer Regions: Genomic 'Dark Matter' Requiring Illumination. Genes Development. 2016;30:1598–1610. https://doi.org/10.1101/gad.283838.116

67. Monahan K, Schieren I, Cheung J, Mumbey-Wafula A, Monuki ES, Lomvardas S. Cooperative Interactions Enable Singular Olfactory Receptor Expression in Mouse Olfactory Neurons. Elife. 2017;6:e28620. https://doi.org/10.7554/eLife.28620

68. Gamliel A, Meluzzi D, Oh S, Jiang N, Destici E, Rosenfeld MG, *et al.* Long-distance association of Topological Boundaries Through Nuclear Condensates. Proceedings of the National Academy of Sciences. 2022;1199320;e2206216119. https://doi.org/10.1073/pnas.2206216119

69. Ozery-Flato M, Linhart C, Trakhtenbrot L, Izraeli S, Shamir R. Large-Scale Analysis of Chromosomal Aberrations in Cancer Karyotypes Reveals Two Distinct Paths to Aneuploidy. Genome Biology. 2011;12:R61. https://doi.org/10.1186/gb-2011-12-6-r61

70. Maass PG, Barutcu AR, Weiner CL, Rinn JL. Inter-chromosomal Contact Properties in Live-Cell Imaging and in Hi-C. Molecular Cell. 2018;69(15):1039–1045. https://doi.org/10.1016/j.molcel.2018.02.007

71. Maass PG, Barutcu AR, Rinn JL. Interchromosomal Interactions: A Genomic Love Story of Kissing Chromosomes. Journal of Cell Biology. 2019;218(1):27–38. https://doi.org/10.1083/jcb.201806052

72. Roller M, Stamper E, Villar D, Izuogu O, Martin F, Redmond AM, *et al.* LINE Retrotransposons Characterize Mammalian Tissue-Specific and Evolutionarily Dynamic Regulatory Regions. Genome Biology. 2021;22:62. https://doi.org/10.1186/s13059-021-02260-y

73. James C, Trevisan-Herraz M, Juan D, Rico D. Evolutionary Analysis of Gene Ages Across Tads Associates Chromatin Topology with Whole-Genome Duplications. Cell Reports. 2024;43(4):113895. https://doi.org/10.1016/j.celrep.2024.113895

74. Fernandez MK, Sinha M, Zidan M, Renz M. Nuclear Actin Filaments – a Historical Perspective. Nucleus. 2024;15(1):2320656. https://doi.org/10.1080/19491034.2024.2320656

75. Venit T, Xie X, Percipalle P. Actin in the Cell Nucleus. In: Victor JM, editor. Nuclear Architecture and Dynamics. Boston: Academic Press; 2018. p 345–367.

76. Mahmood SR, Xie X, Hosny El Said N, Venit T, Gunsalus KC, Percipalle P. β-actin Dependent Chromatin Remodeling Mediates Compartment Level Changes in 3D Genome Architecture. Nature Communications. 2021;12:5240. https://doi.org/10.1038/s41467-021-25596-2

77. Wollscheid HP, Ulrich HD. Chromatin Meets the Cytoskeleton: The Importance of Nuclear Actin Dynamics and Associated Motors for Genome Stability. DNA Repair. 2023;131:103571. https://doi.org/10.1016/j.dnarep.2023.103571

78. Sen B, Xie Z, Thomas MD, Pattenden SG, Howard S, McGrath C, *et al.* Nuclear Actin Structure Regulates Chromatin Accessibility. Nature Communications. 2024;15:4095. https://doi.org/10.1038/s41467-024-48580-y

79. Zhuo L, Stöckl JB, Fröhlich T, Moser S, Vollmar AM, Zahler S. A Novel Interaction of Slug (SNAI2) and Nuclear Actin. Cells. 2024;13(8):696. https://doi.org/10.3390/cells13080696

80. Marshall-Burghardt S, Migueles-Ramírez RA, Lin Q, El Baba N, Saada R, Umar M, Mavalwala K, *et al*. Excitable Rho Dynamics Control Cell Shape and Motility by Sequentially Activating ERM Proteins and Actomyosin Contractility. Science Advances. 2024;10(36):eadn6858. https://doi.org/10.1126/sciadv.adn6858

81. Chiu K, Berrada Y, Eskndir N, Song D, Fong C, Naughton S, *et al.* CTCF is Essential for Proper Mitotic Spindle Structure and Anaphase Segregation. Chromosoma. 2024;133:183–194. https://doi.org/10.1007/s00412-023-00810-w
82. Kaczmarczyk LS, Levi N, Salmon-Divon M, Gerlitz G. The CTCF-H3K27me3 Axis Supports Melanoma Cell Migration by Repressing Cholesterol Biosynthesis. 2023. https://doi.org/10.1101/2023.02.23.529650
83. Lee K, Nelson CM. New Insights into The Regulation of Epithelial-Mesenchymal Transition and Tissue Fibrosis. International Review of Cell and Molecular Biology. 2012;294:171–221. https://doi.org/10.1016/B978-0-12-394305-7.00004-5
84. Andrianto A, Sudiana IK, Suprabawati DGA. α-Smooth Muscle Actin as Predictors of Early Recurrence in Early-Stage Ductal Type Breast Cancer After Mastectomy and Chemotherapy. Iranian Journal Pathology. 2024;19(1):67–74. https://doi.org/10.30699/ijp.2023.2004468.3126
85. Doll S, Freitas FP, Shah R, Aldrovandi M, da Silva MC, Ingold I, *et al.* FSP1 is a glutathione-independent ferroptosis suppressor. Nature. 2019;575:693–698. https://doi.org/10.1038/s41586-019-1707-0
86. Chao S, Yan H, Bu P. Asymmetric Division of Stem Cells and its Cancer Relevance. Cell Regeneration. 2024;13:5. https://doi.org/10.1186/s13619-024-00188-9
87. Baker SG, Soto AM, Sonnenschein C, Cappuccio A, Potter JD, Kramer BS. Plausibility of Stromal Initiation of Epithelial Cancers Without a Mutation in The Epithelium: A Computer Simulation of Morphostats. BMC Cancer. 2009;9:89. https://doi.org/10.1186/1471-2407-9-89
88. Zhu S, Wang J, Zellmer L, Xu N, Liu M, Hu Y, *et al.* Mutation or Not, What Directly Establishes a Neoplastic State, Namely Cellular Immortality and Autonomy, Still Remains Unknown and Should Be Prioritized in Our Research. Journal of Cancer. 2022;13(9):2810–2843. https://doi.org/10.7150/jca.72628
89. Berenblum I, Shubik P. An Experimental Study of The Initiating State of Carcinogenesis, and a Re-Examination of The Somatic Cell Mutation Theory of Cancer. British Journal of Cancer. 1949;3(1):109–118. https://doi.org/10.1038/bjc.1949.13
90. Foulds L. Multiple etiologic factors in neoplastic development. Cancer Research.1965;25(8):1339–1347.
91. Finn EH, Pegoraro G, Brandão HB, Valton AL, Oomen ME, Dekker J, *et al.* Extensive Heterogeneity and Intrinsic Variation in Spatial Genome Organization. Cell. 2019;176(6):1502–1515. https://doi.org/10.1016/j.cell.2019.01.020
92. Ayyamperumal P, Naik HC, Naskar AJ, Bammidi LS, Gayen S. Epigenomic States Contribute to Coordinated Allelic Transcriptional Bursting in iPSC reprogramming. Life Science Alliance. 2024;7(4):e202302337. https://doi.org/10.26508/lsa.202302337
93. Glazko TT, Glazko VI. Segregation Of Haploid Sets of Chromosomes in Diploid Cells of a Number of Mammalian Species. Cifra. Biology. 2024;2:2. (In Russ.). https://doi.org/10.60797/BIO.2024.2.1
94. Mikhalchenko A, Gutierrez NM, Frana D, Safaei Z, Van Dyken C, Li Y, *et al.* Induction of Somatic Cell Haploidy by Premature Cell Division. Science Advances. 2024;10(10):eadk9001. https://doi.org/10.1126/sciadv.adk9001
95. Cho CJ, Brown JW, Mills JC. Origins of Cancer: Ain't It Just Mature Cells Misbehaving? EMBO Journal. 2024; 43(13):2530–2551.
96. Markert CL. Neoplasia: a disease of cell differentiation. Cancer Research. 1968;28(9):1908–1914.
97. Trivedi DD, Dalai SK, Bakshi SR. The Mystery of Cancer Resistance: A Revelation Within Nature. Journal of Molecular Evolution. 2023;91:133–155. https://doi.org/10.1007/s00239-023-10092-6

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