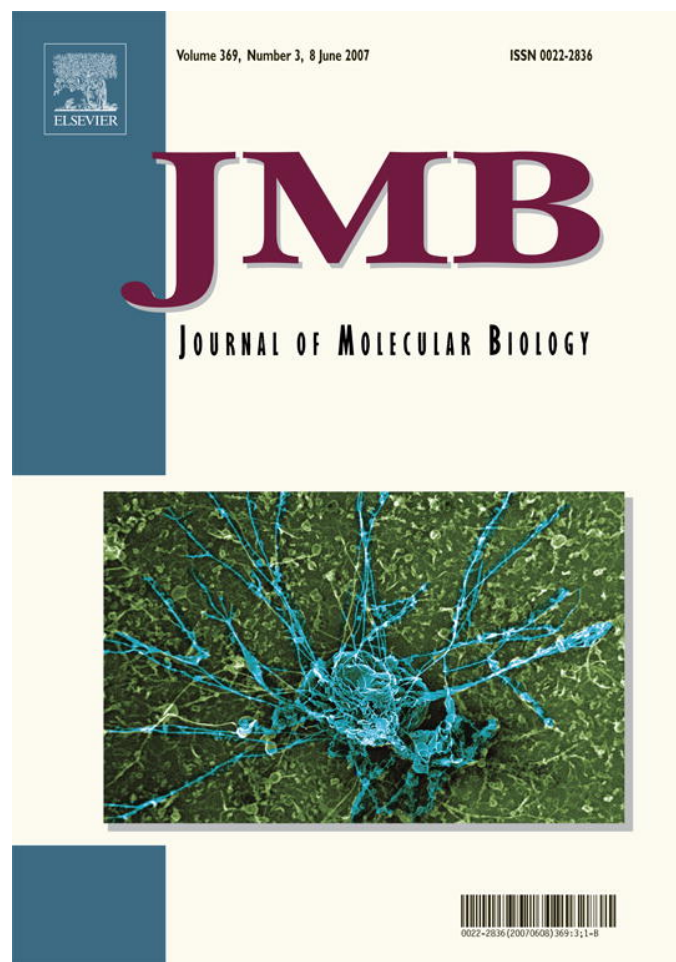


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## REVIEW

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Compartmentalization and compaction of DNA in the nucleus is the characteristic feature of eukaryotic cells. A fully extended DNA molecule has to be compacted 100,000 times to fit within the nucleus. At the same time it is critical that various DNA regions remain accessible for interaction with regulatory factors and transcription/replication factories. This puzzle is solved at the level of DNA packaging in chromatin that occurs in several steps: rolling of DNA onto nucleosomes, compaction of nucleosome fiber with formation of the so-called 30 nm fiber, and folding of the latter into the giant (50–200 kbp) loops, fixed onto the protein skeleton, the nuclear matrix. The general assumption is that DNA folding in the cell nucleus cannot be uniform. It has been known for a long time that a transcriptionally active chromatin fraction is more sensitive to nucleases; this was interpreted as evidence for the less tight compaction of this fraction. In this review we summarize the latest results on structure of transcriptionally active chromatin and the mechanisms of transcriptional regulation in the context of chromatin dynamics. In particular the significance of histone modifications and the mechanisms controlling dynamics of chromatin domains are discussed as well as the significance of spatial organization of the genome for functioning of distant regulatory elements.

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## Nucleosomes: Structural and Functional Differences

Discovery of nucleosomes in eukaryotic cells<sup>1,2</sup> formed the foundation for the modern view on chromatin. The nucleosome is formed by eight core histone molecules: a (H3)<sub>2</sub>-(H4)<sub>2</sub> tetramer and two dimers of H2A-H2B, and the 146 bp long DNA wrapped around a histone octamer. The diameter of the nucleosome disk is about 11 nm and its height is about 5.7 nm. The ordered structure of nucleosome particles has allowed their crystallization and X-ray structure analysis. The nucleosome structure is resolved with precision of 1.9 Å<sup>3</sup> and the octamer structure is resolved with even higher precision. The data of X-ray analysis clearly demonstrate that H2A-H2B dimers bind DNA on entry and exit of the nucleosomal particle, and the (H3)<sub>2</sub>-(H4)<sub>2</sub> tetramer interacts with the central part of the DNA fragment wound around the globule. Histones interact with the phosphodiester skeleton of the DNA molecule. The points of contact are spaced by 10 bp, in which the small groove of the DNA molecule is turned inside. The bases of the nucleotides are not involved in interactions with the histones; therefore the DNA interactions with nucleosome globule are not specific in relation to the DNA sequence. Still, there are DNA sequence motifs that strongly favor or disfavor nucleosome formation and positioning. This depends on rotational preferences of different dinucleotides.<sup>4,5</sup>

It has been considered that organization of nucleosomes and structure of histones are extremely conservative. However, this point of view appears to be oversimplified. General principle of the organization of the nucleosome particle is indeed universal, and amino acid sequence of the main histone forms of the nucleosome particle is highly conservative. At the same time, significant number of the histone variant forms encoded by separate genes have been recently described.<sup>6-8</sup> Some of these variant forms (H3.3, H2A.X, H2A.Z)<sup>9,10</sup> are not considerably different from the main forms. Two variant forms have a rather low level of homology with main forms, macro H2A (in fact represented by two forms, macro H2A1 and macro H2A2, encoded by different genes<sup>11</sup>), 64% homology with H2A in N-terminal domain;<sup>11</sup> H2A-Bbd, 42% homology with H2A;<sup>12</sup> Cid, less than 40% homology with H3.<sup>13</sup> In some cases, the presence of variant histone forms in the nucleosome particles significantly alters the architecture of the histone octamer.<sup>14-16</sup> The correlation between the structural variability of histone forms and functional characteristics of chromatin appears to be even more important. The transcriptionally active chromatin will be discussed in detail later. Here we shall mention that phosphorylated form of histone H2AX (γH2AX) is a marker for double-stranded DNA breaks.<sup>17</sup> Several variant forms of H3 are involved in formation of centromeres. Specific features of

centromeric chromatin are determined by the presence of centromere-specific H3 variant forms (CenH3s), such as CENP-A in mammals, Cid in *Drosophila*, Cse4 in budding yeast and not by satellite DNA sequences, as it was considered earlier.<sup>18,19</sup> Nucleosomes assembled with CENP-A are substantially more conformationally rigid than those assembled with histone H3 independent of DNA template. Substitution of the CENP-A centromere targeting domain comprised of loop 1 and the alpha2 helix within the histone fold is sufficient to target histone H3 to centromeres and to generate the same conformational rigidity to the initial subnucleosomal heterotetramer with histone H4 as does CENP-A.<sup>20,21</sup> Another good example of a functional significance of histone variant forms is X-chromosome inactivation in which macro H2A (a rather diverged variant of histone H2A) plays an important role. Macro H2A may replace one or both H2A within two H2A-H2B dimers participating in the formation of nucleosomal core. As demonstrated by X-ray analysis, the architecture of the histone octamer significantly changes with the replacement of one or both H2A with macro H2A. Furthermore, the whole octamer becomes more stable and NAP-1-assisted exchange of H2A-H2B dimers into intact nucleosomes becomes impossible.<sup>15</sup> According to the current model, temporal removal of H2A-H2B dimer constitutes an important step permitting RNA polymerase II to carry out transcription on chromatin templates.<sup>22,23</sup> Thus, it is easy to understand why incorporation of macro H2A into nucleosomes promotes X-chromosome inactivation.

Covalent modifications of histones substantially increase potential diversity of nucleosome particles. The main targets for modifications are localized on the N-terminal extremities of the histone amino acid chain. These sites are not included into the nucleosome globule and remain exposed on the surface of the nucleosome.<sup>3</sup> Acetylation of the lysine residues is the most well studied modification of histones. Histones H2B, H3 and H4 possess four exposed lysine residues each. Two more acetylation targets are located in the histone H2A molecule. Taking into account that all of the above histones exist in two copies in the nucleosome, and that each histone molecule can be acetylated in one or several positions, it is easy to calculate that  $6.7 \times 10^7$  versions of nucleosomes different in their histone acetylation pattern can exist in principle.<sup>24</sup> This exceeds the number of nucleosomes present in the nucleus of an average eukaryotic cell. Besides the acetylation, other covalent modifications of histones, such as methylation of lysine and arginine residues, phosphorylation of serine residues,<sup>25</sup> poly(ADP) ribosylation of glutamate residues,<sup>26</sup> ubiquitinylation and SUMOylation<sup>27</sup> are also well-characterized. Thus, potential diversity of nucleosomal particles is infinite. Nucleosomes constructed of variant and modified histone forms can differ structurally and functionally from conventional nucleosomes. The sum of signals exposed on the nucleosome surface

including a distinct modification pattern or presence of variant forms makes a specific epigenetic code called the histone code.<sup>28,29</sup> This code can be recognized by different proteins that regulate condensation of chromatin fibril or are involved in replication, transcription, DNA repair and other genetic functions.<sup>30</sup> The meaning of different combinations of these signals is just beginning to be decrypted. Some examples will be given in the following sections.

### Folding Nucleosome Arrays: The 30 nm Fiber

The 30 nm fiber is believed to be organized from extended nucleosomal fiber with the aid of histone H1. However the exact function of H1 in this process is not yet clear: even in the absence of linker histones there is a dynamic equilibrium between extended and compact chromatin fibers, and linker histones are necessary only to stabilize already formed compact fibers.<sup>31</sup> According to one model, the nucleosome fiber containing histone H1 is folded into a solenoid of 30 nm in diameter, which forms a superhelix with six nucleosomes per turn. This model was proposed by Finch and Klug<sup>32</sup> based on the results from electron microscopy and X-ray diffraction analysis. According to this model, the DNA surface of the nucleosomes forms the outside accessible surface of the superhelix, and the spacer DNA never passes through the central axes of the fiber.

According to the alternative "zigzag" model,<sup>33</sup> the 30 nm fiber is formed by zigzag nucleosome arrays, and the spacer DNA frequently passes through the central axes of the fiber. Recent analysis supports the zigzag model. These conclusions were based on electron microscopy analysis of rapidly frozen chromatin samples and DNA analysis in living animal cells subjected to ionizing irradiation.<sup>34-37</sup> While the thermodynamically stable solenoid structure is easily formed *in vitro*, there is no evidence for existence of this structure in living cells. Stabilization of the 30 nm fibril largely depends on the interactions between the N-terminal domains of histones in the neighboring nucleosomes.<sup>38</sup> Therefore, the structural architecture of the fiber can be changed as a result of modifications of these domains, triggering either condensation or decondensation of the 30 nm fiber.<sup>39</sup> It is especially important to underline that the 30 nm chromatin fiber can hardly be regarded as a regular structure within the framework of the zigzag model. To underline this fact it was even proposed to call it compact chromatin fiber instead of 30 nm fiber.<sup>40</sup> Model studies demonstrate that depending on ionic strength and presence of divalent cations it may become more or less compact and, consequently, the level of DNA accessibility within the 30 nm fiber can be quite different.<sup>40</sup> In agreement with this idea, recent studies demonstrated the highly dynamic nature of chromatin both at the level of individual nucleosomes<sup>41,42</sup> and at the level of higher order chromatin structures.<sup>43</sup>

### Transcriptionally Active Chromatin

Cytologists discovered the uneven distribution of DNA in the nucleus a long time ago. When the nucleus is stained by dyes that specifically bind DNA, it reveals the areas formed by densely compacted DNA and areas where DNA concentration is much lower. These areas were called "heterochromatin" and "euchromatin," respectively. It was challenging to establish association between the density of DNA compaction and its transcriptional status. It was assumed that transcriptionally active DNA should be less compacted. The first evidence to support this point of view was obtained in experiments on digestion of nuclear DNA with DNase I. It turned out that active genes were more rapidly digested compared to inactive ones.<sup>44,45</sup>

Discovery of the sensitivity of the transcribed genome fraction to DNase I opened a possibility for isolation and characterization of short fragments of transcriptionally active chromatin. It was shown that an increased level of histone acetylation in nucleosome particles is a characteristic feature of active chromatin.<sup>46,47</sup> Similar observations were made when short fragments of transcriptionally active chromatin were isolated by means of affinity chromatography on columns with immobilized quicksilver.<sup>48,49</sup> It was also demonstrated *in vitro* that artificial chromatin stretches formed of pure DNA and hyperacetylated histones were also hypersensitive to DNase I, and exhibited some other features of the active chromatin isolated from nuclei.<sup>50</sup> This confirms the role of histone acetylation in organization of active chromatin. It is generally assumed that histone acetylation favors unfolding of the compact 30 nm fiber into the nucleosome string, crucial for development of increased sensitivity of active chromatin to DNase I.<sup>51</sup>

It has been considered for a long time that unfolding of the 30 nm fiber determines all specific features of the active chromatin. However, some data suggest that active chromatin may differ from inactive also at the level of individual nucleosome organization. The accumulation of nucleosomes from active chromatin fraction on a quicksilver column could possibly be due to interactions of cysteine 110 thiol groups present within histone H3 with the quicksilver ions.<sup>52</sup> To this end it is important to underline that there is no cysteinyl residue in other histones. Previously it was shown that active rDNA transcription units of *Physarum* could be selectively labeled by the sulfhydryl reagent iodoacetamidofluorescein. Thus, the SH groups of histone H3 are likely to be exposed also in non-fractionated transcriptionally active chromatin.<sup>53</sup> Later studies indicated that the histone octamer is partially unfolded to a U-like structure in nucleosomes from transcriptionally active

fraction.<sup>54,55</sup> In addition, these nucleosomes often lack one of H2A-H2B dimers.<sup>22,23</sup> It should be stressed that these changes are characteristic of nucleosomes involved in the transcription process while the overall sensitivity to DNase I and increased level of histone acetylation are characteristic of chromatin containing potentially active genes.

Recent studies have revealed a range of novel qualities of transcriptionally active chromatin. Presence of variant forms of histones and site-specific modifications of N-terminal domains of histone molecules appear to be the most prominent ones.<sup>56</sup> Nucleosomes containing the H2A.Z variant of H2A are the best studied. Taking into account a possible role of this histone in formation of the active chromatin,<sup>57,58</sup> especially in promoter regions, a nucleosome globule containing H2A.Z instead of H2A was subjected to an X-ray diffraction analysis.<sup>16</sup> Results of this analysis, performed with a resolution of 2.6 Å, indicate that the spectrum of contacts between dimer H2A.Z-H2B and tetramer (H3-H4)<sub>2</sub> differ in the nucleosome formed of histone H2A.Z and the contact spectrum inside the dimer H2A.Z-H2B itself is also altered. Moreover, the altered surface of the modified protein globule can harbor a metal ion. This appears to be very important as the majority of known chromatin remodeling complexes include a subunit containing a metal-binding domain. Besides H2A.Z, the active chromatin may contain other variant histones such as H3.3.<sup>59</sup> Replication-independent H3.3 deposition occurs on actively transcribed genes, but not on silent loci, thereby confirming its link with transcription.<sup>60</sup> Interestingly, H3.3 is distributed along the whole transcribed gene while most of modified forms of histones typical for active chromatin (see below) are concentrated close to promoter regions. This suggests that deposition of H3.3 into nucleosomes is coupled to transcription.<sup>61</sup> It is likely that H3.3 substitutes H3 in nucleosomes that are temporarily displaced during transcription and then are reassembled *de novo*.

High level of histone acetylation is one of the important features of active chromatin. Antibodies that recognize histones acetylated at different amino acid residues allowed the identification of the most distinct acetylation positions. Sites of modifications are even more important in the case of histone methylation. The level of methylation (mono di or tri) of particular lysine residues is also important. Thus, trimethylation of H3 in position K4 is characteristic of active promoter regions while dimethylation of H3 in the same position occurs elsewhere in the vicinity of active genes.<sup>62</sup> Mechanisms involved in decoding the messages written on histone tails by different modifications are far from being well understood. In case of trimethylation of H3 at position K4, it is likely that this modification attracts chromatin remodeling complexes to promoters.<sup>63</sup> Dimethylation of H3 in position K79 is also a mark of active genes<sup>64,65</sup>

while H3 methylation at position K27 is a mark of inactive chromatin.<sup>66,67</sup> The significance of different modifications of H3 at position K9 is less clear. Acetylation at this position is typical for active chromatin<sup>25,68</sup> and deacetylation constitutes an important step of heterochromatin formation. Methylation of histone H3 at K9 was considered for quite a long time as a modification necessary for heterochromatin formation.<sup>69-71</sup> However, more recently this modification was also detected in active genes.<sup>72,73</sup> Thus, interpretation of histone code is a difficult task. It is likely that there is no single decisive modification and combinations of different modifications define the chromatin state. In agreement with this idea, genome-wide analysis of histone modification profiles demonstrated that modifications typical of active and inactive chromatin were present in the same genomic areas, although there was a higher probability of clustering of modifications typical for either active or inactive chromatin.<sup>74</sup>

Importantly, variant histones can also be post-transcriptionally modified. Some of these modifications are directly related to the potentiating of chromatin for transcription. Thus, acetylation of H2A.Z is typical for active promoters while non-acetylated H2A.Z can be found in promoters of both active and inactive genes.<sup>75,76</sup>

## Chromatin Domains and Regulation of Transcription

Regulation of transcription occurs at several levels in eukaryotic cells. Our goal here is to analyze the regulatory systems acting at the chromatin level. It should be mentioned that there are at least two steps of gene activation: unfolding of chromatin and activation of promoters. The "chromatin domain" can be defined as a rather large genome area inside which changes of the chromatin fiber folding occur independently of the flanking regions (see Razin *et al.*,<sup>77</sup> for a detailed discussion). Chromatin domain can include one or several genes; activation of the domain converts all the constituting genes to be potentially active. In many cases, chromatin activation correlates with transition from a DNase-resistant to DNase-sensitive configuration of chromatin. The crucial event of this process is the domain-wide acetylation of histones.<sup>78-81</sup> Most of the histone acetyltransferases (histone acetylases) contain a so-called bromodomain that recognizes acetylated lysine residues.<sup>82</sup> This enables the ability for progressive acetylation. Histone acetylases would be linked to already acetylated nucleosomes and would produce acetylation of the neighboring non-acetylated nucleosomes. Theoretically the process can go on without interruption before progressive acetylation reaches a barrier. This can be an insulator or a heterochromatic region.<sup>77,83</sup> Some specific event triggers the process of progressive acetylation. Local acetylation of histones inside the regulatory sequences can serve as such a

stimulus.<sup>79,84–89</sup> It should be mentioned that many transcription factors can attract histone acetylases and factors of chromatin remodelling.<sup>90</sup> More detailed information on the matter can be found in reviews.<sup>91,92</sup> Resuming the discussion about formation of transcriptionally active chromatin domains we should mention the Travers hypothesis.<sup>93</sup> According to this hypothesis, the elongating RNA polymerase II complex is a vehicle for transportation of histone acetylases, chromatin remodeling factors and other proteins necessary for formation of active domains along the chromatin domain. Thus a low-level transcription of chromatin domains might be necessary for their activation. Recent experimental evidence strongly supports this model. Long intergenic or full domain transcripts were found in many genomic areas.<sup>88,94–97</sup> It was demonstrated that in  $\beta$  globin gene domains of different vertebrates, RNA polymerase II was recruited to locus control regions and actually started transcription of the whole downstream domain.<sup>98,99</sup> Progressive modification of histones linked to polymerase II transcription was demonstrated in several studies.<sup>100–103</sup> Finally, it was demonstrated that domain-wide transcription is absolutely necessary for activation of chromatin domains in several genomic regions.<sup>96,99,104–106</sup>

Activation of a chromatin domain correlates with its transition from a DNase-resistant to a DNase-sensitive form. However some vast genome regions always keep an open DNase-sensitive configuration. These regions harbor tissue-specific genes and gene clusters along with the house-keeping genes. For a long time it was unclear whether tissue-specific genes in the open areas possess some specific chromatin structure. This question was answered by comparing histone acetylation pattern in the vertebrate alpha-globin gene domain and the overlapping housekeeping gene. In erythroid cells, alpha-globin domain is characterized by increased level of the acetylated lysine residue K5 in histone H4.<sup>107</sup> This modification does not influence the DNase-sensitivity; it should perform some other function.

Activation of chromatin domain ensures only the potential ability for transcription of the genes inside. Further events happen at the level of individual promoters. Recent data suggest that RNA polymerase II pre-initiation complex is formed on a nucleosome-free promoter.<sup>108,109</sup> Several mechanisms perform this transition. In some cases, binding of chromatin remodeling factors to the promoter region is sufficient to discard nucleosomes from the promoter. Tissue-specific transcription factors are involved in the process.<sup>90,110</sup> The local histone acetylation including the histone H2A.Z acetylation in promoter regions is also important. H2A.Z is present both in active and inactive promoters.<sup>58,75,76,84</sup> It was reported that promoter flanked by two nucleosomes that contain H2A.Z maintained the nucleosome-free status.<sup>58</sup> It should be mentioned that the H2A.Z histone is included into nucleosomes by a replication-independent pathway.<sup>58</sup>

This enables the possibility for selective activation of promoters *via* site-specific inclusion of this histone in the nucleosomes in the course of cell differentiation, or *via* some exogenous signals.

### Gene Regulation by Three-Dimensional (3-D) Chromatin Conformation

Experimental evidence in eukaryotic cells suggests that bending and looping of chromatin facilitates specific genomic interactions over distance.<sup>111,112</sup> These interactions may occur between transcription activators bound to enhancers and transcription machinery at the promoter. Development of chromosome conformation capture (3C) methodology gave a new dimension to the studies of gene regulation.<sup>113</sup> It allowed the study of spatial organization of gene domains by measuring long-range interactions between different chromatin segments within the domain. 3C analysis of the *Ifng* gene domain in CD4+ T helper (Th) cell subsets showed that the domain adopts a specific looped conformation in immune T cells undergoing activation and differentiation.

Differentiation of naïve CD4+ T cells into functionally specialized T-helper 1 (Th1) and T helper 2 (Th2) cell lineages represents a simple and efficient model to study regulation of gene expression in the context of spatial chromatin organization. The Th cell subsets originate from a common naïve precursor. When cultured under specific conditions that favor either Th1 or Th2 differentiation pathway, the cells mature into committed effector Th1 and Th2 subsets that result in mutually exclusive cytokine profiles.<sup>114</sup> Th1 cells transcribe the signature *Ifng* gene, but Th2 cells silence the *Ifng* gene, and coordinately transcribe the *IL4*, *IL13*, and *IL5* genes. Activation of naïve T cells through their T cell receptor (TCR) induces rapid expression of the *Ifng* gene. Transcription of the *Ifng* gene in these cells corresponds to a spatial conformation that brings distal regions of the domain into close proximity to the promoter/first intron. As cells differentiate into Th1 and Th2 effector cell subsets, the conformation in the *Ifng* gene begins to change and display common features and specific characteristics that distinguish each cell subset.<sup>115</sup> Th1 cells, that produce high levels of IFN- $\gamma$ , display the most open conformation, but Th2 cells that do not express *Ifng* display a tightly closed conformation. Analysis of the gene conformation in undifferentiated bone marrow precursor cells and not yet mature unactivated T cells, in which transcription of the *Ifng* gene is repressed, showed the presence of a linear-like spatial organization. The switch from a linear to a loop conformation in the *Ifng* gene takes place after Th cells activation.<sup>116</sup>

Thus, the formation of the loop structure in the *Ifng* gene around the promoter/first intron allows the gene, transcription machinery, and distal

enhancers and silencers to achieve close spatial proximity for interaction. Interestingly, the RNA Pol II remains recruited to the *Ifng* gene promoter in all three functionally different Th cell subsets: IFN- $\gamma$  producing Th1, IFN- $\gamma$  silent Th2 and activated, but not in fully differentiated neutral Th cells that produce negligible amount of IFN- $\gamma$  cytokine (E.R.E. *et al.*, unpublished results) thus making them ready to initiate transcription. The studies of *IL4* cytokine gene locus and  $\beta$ -globin genes showed that a poised loop conformation serves for rapid transcription in response to specific stimuli.<sup>117–119</sup>

The sites of interaction along the gene that come into physical contact in the looped conformation are mapped to the areas of high human/mouse sequence conservation, DNase I hypersensitive sites HS<sup>114</sup> or, in some cases, to conserved non-coding sequences (CNS) described as long-range regulatory regions.<sup>120</sup> A more opened chromatin conformation in the gene in Th1 cells had fewer sites of interaction than more closed, tighter conformation in Th2 cells. This supports the notion that activation or silencing of genes can be achieved *via* rearrangement of the sites of interaction within a gene domain, by “delivering” regulatory elements to promoters over distance.

It has been reported that gene families, comprised of several coordinately regulated genes and a locus control region (LCR), can adopt a looped 3-D spatial conformation forming a so-called chromatin hub.<sup>112</sup> The best examples are coordinately expressed  $\beta$ -globin genes, *IL4* cytokine genes and TCR beta gene loci that are stretched along the thousands of DNA base-pairs. In such conformations, transcriptionally active genes in the loci interact with LCR, whereas transcriptionally silent genes are looped out of the chromatin hub. Moreover, direct inter-chromosomal interactions were also demonstrated experimentally for the cytokine genes, positioned on different chromosomes and expressed within a common cell lineage.<sup>121,122</sup> The inter-chromosomally interacting loci were found in transcriptionally active environment in the nucleus.

These observations definitely change the concept of gene regulation from one-dimensional into three-dimensional.<sup>115,120</sup> The above inter- and intra-chromosomal interactions are likely to be a general phenomenon occurring at multiple genetic loci. The implication of such interactions is not only in positive or negative regulation of gene expression in the same locus, but also in a locus on another chromosome. Interchromosomal associations could be particularly important for coordinate or alternative gene regulation occurring at multiple genetic loci, and possibly cause chromosomal translocations in disease and malignancy.<sup>123,124</sup> Spatial colocalization of interacting loci of coordinately expressed genes, positioned on different chromosomes affects their nuclear localization and association with euchromatin within an active chromatin domain.<sup>118,121,125</sup> However, the extent and exact role of spatial gene organization in overall efficiency

of gene expression in relation to other factors involved remains to be determined.

## Spatial Organization of the Genome in Eukaryotic Cells Might be Supported by DNA Interactions with the Nuclear Matrix

It has long been known that the eukaryotic genome is organized into loops fixed at the nuclear matrix. The functional significance of this organization (beyond the purpose of DNA compaction) still constitutes a subject of discussions (for a review see Razin & Gromova<sup>126</sup>). This was to a large extent due to the absence of a reliable method for mapping DNA loops.<sup>127</sup> Some time ago we have suggested to use topoisomerase II-mediated DNA loop excision for construction of long-range maps of genome organization into loops attached to the nuclear matrix.<sup>128–130</sup> An individual loop mapped by topoisomerase II-mediated DNA loop excision could be directly visualized upon hybridization of the corresponding BAC clone with histone-depleted nuclei (nuclear halos). Thus it was demonstrated that DNA domain mapped as a loop using the above-mentioned biochemical approach did correspond to the loop visible on cytological preparations.<sup>131</sup> Further studies allowed the demonstration that while organization of DNA into loops changes during development,<sup>132</sup> it is relatively static in somatic cells. Inspection of a number of histone-depleted nuclei permitted the conclusion that partitioning of DNA into loops and matrix attachment regions (that could be fairly long) is identical in the majority of somatic cells.<sup>131,133,134</sup> Obviously, the existence of relatively stable three-dimensional organization of genomic DNA that is not directly related to ongoing functional processes might have important impact on regulation of gene expression. Indeed, the sites of DNA attachment to the nuclear matrix may be located far from each other on the DNA chain and at the same time be in direct proximity in the nuclear space. This may greatly facilitate the probability of formation of complexes between distant regulatory elements. Indeed, such interaction has recently been demonstrated for the murine gamma-interferon gene locus delimited by two nuclear matrix attachment sites (MARs).<sup>116</sup> To this end it is important to mention that matrix attachment regions frequently colocalize with replication origins,<sup>135,136</sup> enhancers,<sup>137–139</sup> insulators<sup>140,141</sup> and other regulatory elements. Thus, a new as yet uncharacterized epigenetic mechanism might operate at the level of genome organization into DNA loops.

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