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Chromatin Domains and Territories: Flexibly Rigid

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ABSTRACT: The nucleus is a highly organized solid-state system, rigid and flexible at the same time, where enzymes are organized in complex processing factories. This is achieved by the organization of nuclear DNA into territories and domains, which allow compartmentalization and compaction without sacrificing accessibility. The present review discusses the implications of the organization of chromosomal domains and territories in development and carcinogenesis.

KEY WORDS: chromatin domains, chromatin territories, carcinogenesis, development

I. INTRODUCTION

In the era of molecular biology, the nucleus was subconsciously perceived by many researchers as a kind of miniature test tube where soluble enzymes and structural proteins, such as histones, would freely interact with immobile DNA. Recent studies lead us to believe that this is not the case, but rather that the nucleus is a highly organized solid-state system, rigid and flexible at the same time, where enzymes are organized in complex processing factories. Indeed, if the nuclear membrane is permeabilized and the nuclei placed in an isotonic buffer, a surprisingly small amount of nuclear proteins will diffuse into the buffer, indicating that most nuclear proteins are indeed in an insoluble state.

In the cell nucleus, DNA is organized into chromatin in a highly structured and yet flexible way: it is compacted approximately 40,000-fold and yet any part of it has to be accessible to RNA and DNA polymerases and to repair enzymes at any time in the cell cycle. This is achieved by the organization of nuclear DNA into territories and domains, which allows compartmentalization and compaction without sacrificing accessibility. Recent studies have shown that each chromosome has an allocated space within the nucleus and that specific DNA sequences are precisely positioned within the territories. Changes in the transcriptional status lead to relocalization of genes within the nucleus. Chromosomal territories are subdivided into chromatin domains, large areas that are often co-regulated (or co-repressed as is the case of heterochromatin) and contain related genes. They are separated by spacers that diminish the cross-influence of transcriptional control elements. Such spacers may contain acetylation barriers, insulators, and nuclear skeleton (matrix) anchorage sites. The domains are also flexible—they change during development, cell differentiation, and carcinogenesis, and may ensure the epigenetic regulation of gene expression.

II. CHROMOSOMAL DOMAINS AND TERRITORIES

Chromosomes were observed as distinct entities during mitosis in the early 19th century, and their disappearance during interphase remained a mystery for many years. Nonetheless, several observations were made that indicated that each chromosome occupies a distinct space in the nuclear

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volume during interphase. These "spaces" are now referred to as chromosome territories (CTs).

III. CHROMOSOMAL TERRITORIES ARISE FROM THE FUSION OF INDIVIDUAL CHROMOSOMES IN TELOPHASE

In the early development of *Xenopus laevis*, the first 12 cell divisions are extremely rapid and proceed in the absence of transcription. During this period, the cell cycle proceeds in an atypical manner. During anaphase, chromosomes became individually surrounded by a nuclear envelope forming micronuclei or karyomeres. This genomic organization permits replication to begin at early telophase before nuclear reconstruction and completion of mitosis (Lemaitre et al., 1998). During late anaphase, the karyomeres fuse to form a nucleus. Surprisingly, even after this fusion, the karyomeres keep components of their envelope, particularly the lamins (Fig. 1). This spectacular feature of *Xenopus* embryos is essentially the same in all multicellular eukaryotes; the decondensed chromosomes stay in the distinct spaces and do not intermingle within the nucleus.

IV. CHROMOSOME POSITIONING AND BORDERS OF CHROMOSOME TERRITORIES

The first hypothesis of chromosome positioning was formulated by Rabl in 1885 (Rabl, 1885). He proposed that centromere-telomere orientation of chromosomes in anaphase remains the same during interphase. Indeed, this phenomenon could be observed in *Drosophila* and plants, where centromere and telomeres of the chromosome were located on opposite sites in the nucleus (Abranches et al., 1998; Hochstrasser et al., 1986). However, such a polarized chromosome configuration is quite rare in the case of mammalian cells. Instead, a radial nonrandom distribution of mammalian chromosomes through interphase was reported recently (Cremer et al., 2001). The most gene-dense human chromosome, 19 (HSA19), appeared to be positioned in the central part of the lymphocyte nucleus, whereas HSA18, which has the lowest gene density was located at the periphery of the nucleus. There is a trend for less gene-dense chromosomes to be positioned at the nuclear periphery (Boyle et al., 2001). Moreover, within a given chromosomal territory, the gene-poor centromeric area seems to be always located at the maximal distance from the center of the nucleus (at the periphery of the nuclear layer occupied by the chromosomal territory) (Taslerova et al., 2003).

Radial chromosome positioning is not observed only in human chromosomes, but it was demonstrated in the nuclei of other organisms. The gene-rich micro-chromosome territories in chicken (*Gallus domesticus*) nuclei were predominantly clustered in the nuclear interior, whereas the gene-poor macro-chromosome territories were located at the nuclear periphery (Tanabe et al., 2002). Besides, this radial arrangement of the chromosomes was precisely maintained in metaphase rosettes of embryonic fibroblasts. Such dependence of chromosome positioning on the gene density could be accounted for in terms of relative values of DNA: gene-rich chromosomes located in the nuclear interior are evidently more protected from the external influence of any toxic agents in comparison with "less precious" chromosomes with decreased gene density. In a less gene-dense chromosome any "hit" on that chromosome is less likely to hit a gene, so "important" genes may be camouflaged by junk DNA. On the other hand, positioning of chromosomes may simply reflect their average nucleotide composition. In vertebrates, most of the genes are concentrated in GC-rich isochores (Bernardi, 2000). Thus, small gene-rich chromosomes are composed for the most part of GC-rich isochores, while a significant part of gene-poor chromosomes is composed of AT-rich isochores. The supposition that GC-rich areas are targeted to the center of the nucleus and AT-rich areas to the nuclear periphery may explain the observed location of centromeres (composed of AT-rich satellite sequences) at the external part of the nuclear layer occupied by the corresponding chromosomal territory (Taslerova et al., 2003).

There is an alternative model for CT positioning referred to as relative positioning (Nagele et al., 1999; Parada and Misteli, 2002). This model suggests that chromosomes occupy "preferential" positions within the nucleus and, consequently,

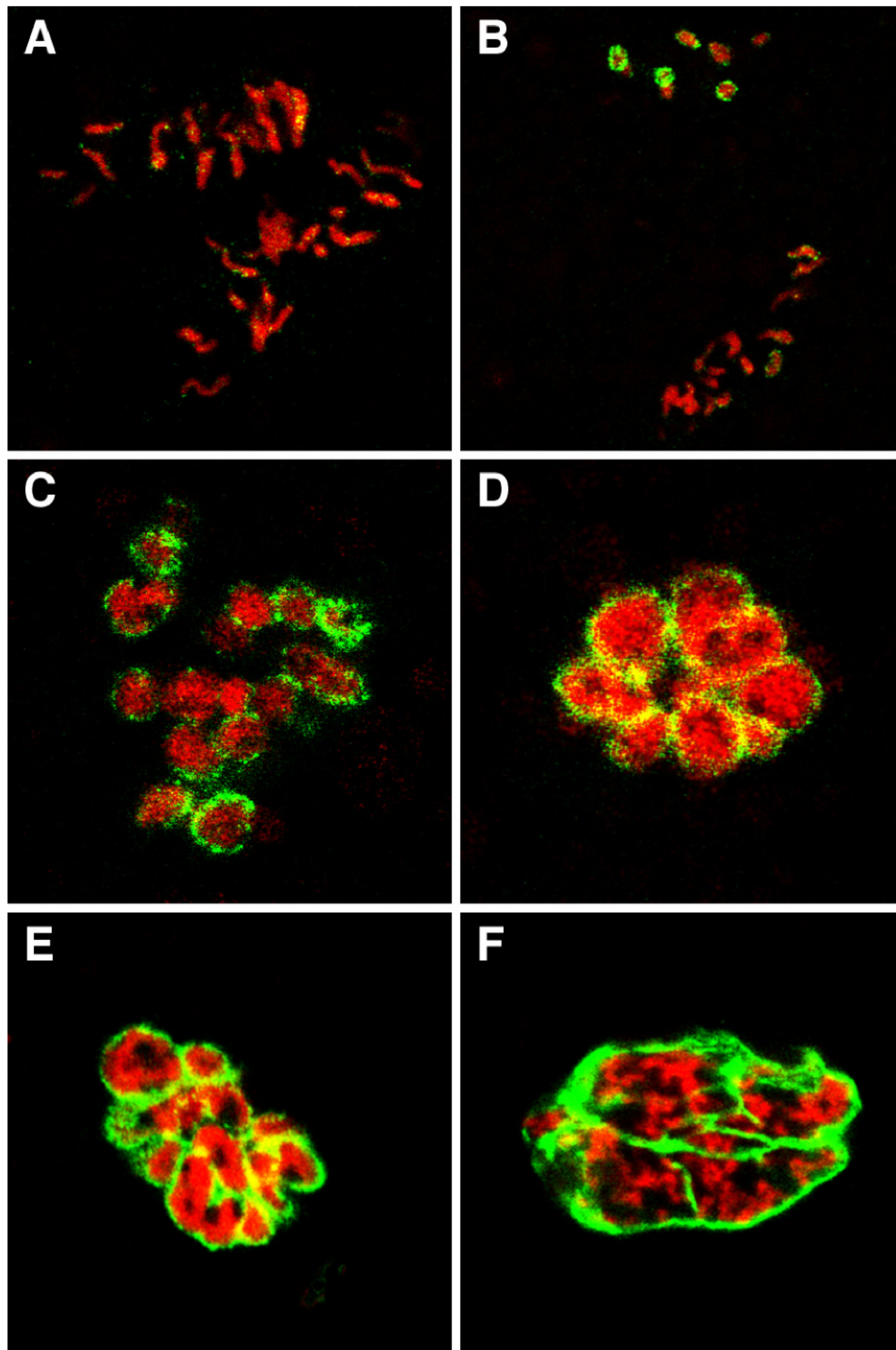

FIGURE 1. Fusion of individual karyomeres during metaphase in early development of Xenopus laevis. Note that even after fusion, individual chromosomes remain as individual entities. The karyomeres were stained with DAPI and an antilamin antibody (Lemaitre et al., 1998). Reproduced with permission from J Cell Biol 1998;142:1159–1166.

relative to each other. Only a few examples of such positioning have been demonstrated so far (Nagele et al., 1999; Parada and Misteli, 2002). In one case, human chromosomes 7, 8, and 16 were located diametrically opposite one another within the interphase nucleus of quiescent cells. This phenomenon, often referred to as mitotic preset (Parada et al., 2002), originates at metaphase, where homologues preferentially occupy opposed sites within the rosette (Nagele et al., 1999). A second example of relative chromosome positioning arose from the study of the mouse chromosomal translocations 12:14 and 14:15, which result in lymphoma. The three chromosomes participating in these translocations were preferentially positioned in close proximity to each other, and this positioning was also conserved in normal splenocytes (Parada et al., 2002).

V. CHROMOSOMAL TERRITORIES: WHAT'S INSIDE?

As yet, it is not clear what mechanisms maintain the integrity of chromosomal territories. It was originally proposed that simple electrostatic repulsion between the negatively charged chromatin areas would be sufficient to exclude the overlapping of chromosomal territories and to ensure the existence of a certain space between territories. (Zirbel et al., 1993). The compartment formed by exclusion from chromosomal territories was called the "interchromatin domain compartment [ICD]." This compartment was suggested to serve as a transport channel for the exchange of products between the chromosomal territories and the cytoplasm (via nuclear pores). Correspondingly, it was assumed that active genes were located at the surface of chromosomal territories and that the splicing machinery and the majority of pre (m) RNA is present in the ICD. Splicing speckles and mRNPs were indeed found outside chromosomal territories (for a review see Cremer et al., 1995). However, active genes are present not only at the surface but also inside chromosomal territories (see below). Furthermore, no repositioning of tissue-specific genes in connection with activation of their expression was observed (Mahy et al., 2002). The position of a given gene within a chromosomal territory (defined as a distance from the surface) seems to be constant in cells of different lineages (Mahy et al., 2002).

Some time ago, we proposed the "channel model of the nuclear matrix" (Razin and Gromova, 1995). According to this model, the nuclear matrix constitutes both the structural milieu for the assembly of nuclear compartments and the system of intranuclear transport pathways. These pathways were thought to span all the nuclear volume, including the space occupied by chromosomal territories. The model explained well the fact that the system of nuclear compartments remained unchanged after the solubilization of chromatin (see Berezney et al., 1995, and references therein). It also explained the preferential accessibility of the matrix-attached DNA regions to exogenous and endogenous nucleases (Gromova et al., 1995a; Lagarkova et al., 1995). Some recently published data have provided new rationales for the channel model of the nuclear matrix. First, it was demonstrated that chromosomal territories have a "sponge-like" structure, that is, they are pierced by channels of some sort, and that active genes present inside chromosomal territories are located within these channels or at their surface (Mahy et al., 2002). Second, the nature of the internal nuclear matrix became less enigmatic as the chain of lamin filaments, possibly bound to NuMA "islands," was described—both in nuclei and nuclear matrices (Barboro et al., 2002; Hozak et al., 1995; Moir et al., 2000; Neri et al., 1999; Zeng et al., 1994). It is quite possible that these filaments indicate pathways along which the transport of RNA proceeds. Indeed, these filaments appear to be so extensively covered by RNA that some of the lamin epitopes within the nuclei only become accessible after RNA digestion (Barboro et al., 2002).

VI. MAINTENANCE OF CTS THROUGH THE CELL CYCLE

It is believed that chromosome positioning is established during anaphase (Parada and Misteli, 2002) but is not conserved, with chromosomes appearing to have some translational freedom as cells enter G1 phase. A fluorescently tagged locus was shown to translocate from the nuclear periphery towards the interior, as CHO cells pro-

gressed from M-phase, before being set up near the periphery in mid-G1 (Tumbar and Belmont, 2001). A specific rearrangement of chromosomes was also demonstrated in quiescent fibroblasts upon entering G1 (Bridger et al., 2000).

However, chromosome territories exhibit very limited large-scale translational movement during late G1, S, and G2 phases (Manders et al., 1999; Zink et al., 1998). Upon entering G1, CTs seem to be quite conservative in their movement until the onset of M phase. This could readily be explained by a large excluded volume of each chromosome, relative to the limited volume of the nucleus, upon decondensation at the M/G1 interface boundary.

Two recent articles addressed the question of chromosome order conservation in mitosis. Walter et al. (2003) observed that the chromosomal territories are not maintained when the cells go through mitosis—roughly 50% of the daughter HeLa cells maintained the same chromosome order as the parent ones. In contrast, the study by Gerlich et al. (2003) suggests that the order of chromosomes is maintained throughout the cell cycle in rat kidney cells, namely, in mitosis. These apparently controversial results may be due to the cell lineages: it is known that relocalization and/or clustering of chromosomal territories may occur during differentiation (Nagele et al., 1999; Parada et al., 2002). Chromosomal territories may be highly mobile in non-differentiated cells, and maintain their relative positions in terminally differentiated cells.

VII. CTS AND TRANSCRIPTION

The size and relative position of a given chromosome territory correlates not only with DNA content but also with the overall level of transcription (Croft et al., 1999; Mahy et al., 2002). It was previously argued that chromosome territories are inaccessible for large transcription machinery (Zirbel et al., 1993). Thus transcription and RNA processing were thought to occur in the ICD compartment. In agreement with the ICD compartment model, several authors observed a correlation between an active transcriptional status of a gene and its location at the surface of the chromosomal territory. Thus, in contrast to noncoding sequences, three coding regions of the human genome were shown to have a peripheral location on corresponding CTs (Kurz et al., 1996). The correlation between transcription status and positioning was also demonstrated for a single gene within Xa and Xi territories: the ANT2 gene was actively transcribed when situated more peripherally within the Xa territory in contrast to when it was within the Xi territory in which it was silent (Dietzel et al., 1999).

However, this elegantly simple picture was complicated by several observations. First, poly(A)+ RNA, in addition to nascent RNA, has been reported to be localized deep within CTs (Abranches et al., 1998). In addition, both coding and noncoding sequences from 11p13 were shown to be located inside HSA11 (Mahy et al., 2002). These observations gave rise to the conclusion that basal transcription machinery may easily attain the interior of chromosome territories, and that large scale chromatin remodeling to position genes on the surface of a CT is not required to facilitate transcription. On the contrary, several studies indicated a role for active transcription in organizing the territories. In one case, a difference was observed in the incidence of extraction of chromatin containing the major histocompatibility complex (MHC) locus from the surface of HSA6 in the cell lines—exhibiting different expression profiles of MHC genes (Volpi et al., 2000). Inducing transcription from this region increases the incidence of chromatin looping.

Such chromatin remodeling is believed to take place in large loci of related genes, such as the MHC locus on HSA6. It is reasonable for these families of the genes, with the same or related function, to be subjected to the same regulation (Volpi et al., 2000). Similarly the human epidermal differentiation complex (EDC) at 1q21 contains functionally related genes involved in keratinocyte differentiation. This region appears to be extended outside of the HSA1 territory in keratinocytes where the genes are actively expressed, but not in the lymphoblast where they are silent (Williams et al., 2002). Unfortunately, the authors did not study how the "extraterritorial" gene domain is located with respect to other CTs (i.e., whether it overlaps one of the CTs or is located in the ICD compartment). It is possible that looping out of the EDC domain beyond its own CT is necessary to place this gene domain in a specific nuclear compart-

ment. To this end, it may be of importance that the looped-out EDC domain was positioned close to one of the PML bodies (Williams et al., 2002). In any case, the correlation between positioning and transcriptional status described above does not seem to be a general rule. No significant alteration of the intraterritory position adopted by the WAGR locus that correlated with increased gene expression was found (Mahy et al., 2002). Genes that were inactive in lymphoblasts and fibroblasts were not relocated to the surface of the HSA11 territory when being actively transcribed, but rather remained within the CT in a similar position as neighboring ubiquitously active genes and noncoding sequences.

VIII. CHROMATIN DOMAINS AND POSITIONING OF GENES WITHIN CTS

As discussed in the previous sections, the CTs cannot be regarded as amorphous masses of decondensed chromatin. There is a clear internal organization of functional compartments within the CTs. Chromatin domains are likely to constitute the basic units for this organization. Chromatin domains were first recognized when the DNaseI sensitivity of extended genomic areas was studied (see, e.g., Groudine et al., 1983; Lawson et al., 1982). Now it becomes increasingly evident that domains can be defined by some epigenetic markers, such as by a particular type of histone modification (reviewed by Forsberg and Bresnick, 2001). The domains were traditionally regarded as relatively long (> 50 kb) pieces of DNA, characterized by a particular (and more or less uniform along the domain) mode of DNA packaging. The borders of domains were thought to be defined by special genomic elements (insulators, matrix attachment regions, etc.). Most of the conclusions relating to the domain organization of the genome were drawn based on studies of a few domains of tissue-specific genes, such as the vertebrate domains of β-globin genes. Recent studies of other genomic domains favored a conclusion that the domain organization of the eukaryotic genome is much more complex than it was traditionally thought to be. It was demonstrated that domains of tissue-specific genes may overlap with other gene domains and that house-keeping genes may be located within domains of tissue-specific genes (Chong et al., 2002). Furthermore, it was found that domains were not necessarily defined structurally (i.e., as units of DNA packaging) but may well represent integral functional units of the genome defined only by targeted interactions of regulatory elements (Dillon and Sabbattini, 2000, and references therein). For our discussion, the most important are the observations that permit a link between studies of genomic domains and studies of gene positioning within the CTs. Thus, it was demonstrated that the inactive domain of human β-globin genes (in a chromosome bearing Hispanic deletion) was located close to centromeric heterochromatin, whereas the active β-globin gene domain was located far away from the centromeres (Schubeler et al., 2000). In agreement with this finding, silencing of the λ5 promoter in mature B-cells coincided with relocation of the λ5 gene into the pericentromeric compartment (Brown et al., 1997). An active enhancer was reported to mediate relocation of a transgene away from centromeric heterochromatin (even in the absence of transcription) (Francastel et al., 1999). Finally, insulators that are thought to define boundaries of genomic domains were found to mediate a particular nuclear localization of linked genes (Gerasimova et al., 2000; Ishii et al., 2002). Thus, virtually all key regulatory elements involved in establishing genomic domains (LCRs, enhancers, insulators) also influence the positioning of genes within CTs and, speaking more generally, within nuclei. It may also be the case for the matrix attachment regions (MARs). It was shown in several studies that at least some MARs stimulate expression of transgenes, perhaps by ensuring their protection from position effects (Kalos and Fournier, 1995; Stief et al., 1989). The effect was routinely explained by creation of artificial loop-domains, that is, by spatial isolation of a transgene from the chromosomal context of the host cell (for a review, see Razin, 1996). It was shown, however, that the loop anchorage sites were preferentially accessible within CTs, possibly because of their location at the nuclear matrix channels (Gromova et al., 1995a; Razin and Gromova, 1995). It is, thus, possible that linking of a transgene to a MAR element ensures a favorable location of a transgene in respect to the intranuclear network of transport

pathways. For this reason, MARs would serve as entry/nucleation sites for the transcriptional factors that might be delivered via nuclear matrix channels. To this extent, it may be of importance that many MARs did not show any insulator activity in an enhancer-blocking assay, for example, a MAR from the intergenic spacer separating two *hsp* 70 genes in *Drosophila* 87A7 locus (Kellum and Schedl, 1992). Furthermore, detailed analysis has demonstrated that MARs stimulate transgene expression but fail to make it copy number-dependent, and, thus, MARs do not insulate transgenes from signals spreading from the host cell chromatin (Poljak et al., 1994).

IX. CTS AND DEVELOPMENT

The global organization of chromatin changes during cell differentiation leading to gross changes in transcription activity of corresponding genes. For example, major changes were identified during the late stages of differentiation of pyramidal neurons from the hippocampus (Santama et al., 1996).

Tissue-specific genes can be coordinately regulated by the regulation of their spatial localization in the nucleus during development (Parreira et al., 1997). Immunoglobulin genes maintain a different but not nonrandom topography relative to each other and to the volume of the nucleus. However, this topographical distribution is independent of the transcriptional activity of the genes and is conservative in different cell types. Similar results were obtained for another type of developmentally regulated gene—proteolipid protein gene—in differentiating oligodendrocytes (Nielsen et al., 2002).

Xenopus development provides an interesting model for testing transitions in gene expression during development that could be associated with a change in the organization of specific genomic domains. Activation of zygotic transcription after the mid-blastula transition (MBT) was structurally associated to a specification of nuclear matrix attachment regions in two specific gene domains, rDNA and c-myc (Vassetzky et al., 2000). The developmental change, from apparently random (Hair et al., 1998) to specific attachments of the rDNA domain to the matrix, may be correlated to two other transitions that occur during the same development period. The first is an increase in the size of the chromatin loop (Buongiorno-Nardelli et al., 1982; Vassetzky et al., in preparation), and the second is the specification of replication origins in the same region after the mid-blastula transition (Hyrien et al., 1995). The specification of the nuclear matrix site at the onset of transcription in the embryo may help to fix the replication origin. Alternatively, the stabilization of the rDNA chromatin domain after the mid-blastula transition may permit the structural insulation of this domain for both transcription and replication. The specification of the nuclear matrix attachment regions reported in this system may be involved in the establishment of stable programs of transcription during development and may contribute to the determination of stable cell lineages in the embryo.

X. CTS AND CANCER

The nonrandom positioning of chromosomes in interphase nuclei has implications for the occurrence of chromosomal translocations in human cancers (Mitelman, 2000). The fact that there are a limited number of chromosomal translocations was the earliest indication of a nonrandom distribution of genetic material in the nucleus, because two chromosomes that undergo a reciprocal translocation should be in close contact with each other. At the moment, there are not many translocations described where the proximity of the corresponding regions are well documented. For instance, 9:22 translocation resulting in the fusion of the BCR gene on chromosome 22, with the ABL gene located on chromosome 9 and leading to chronic myeloid leukemia (Elliott and Jasin, 2002), is an example of proximal localization of translocating loci. Indeed, the analysis of the relative position of BCR and ABL genes confirmed the proximity of both loci in hematopoietic cells. In the case of the fusion of the PML and RARα resulting in promyelocytic leukemia, the proximity of the corresponding loci of chromosomes 15 and 17 was clearly shown, as well (Lukasova et al., 1997; Neves et al., 1999). Interestingly, the proximity of the corresponding regions for this translocation was not observed in all

the types of the analyzed cells (Neves et al., 1999). This observation could indicate that the pattern of chromosome localization is distinct in different tissues and perhaps makes a contribution to the frequency of the chromosome translocation (Parada and Misteli, 2002). Thus, studying the spatial organization of the interphase nucleus might provide interesting insights into chromosome rearrangements resulting in cancer.

XI. CONCLUSION

Despite the progress in genome sequencing, we still know very little about the functioning of the cell nucleus as a system. Recent studies of chromosome domains and territories revealed a new level of regulation of the genome based on the localization/translocation of chromatin within the chromosomal territories. Further studies are necessary to unveil the role of high-order chromatin organization of the nucleus in complex biological processes, such as cell differentiation or carcinogenesis.

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