

Deep Insight Section

Spatial arrangement of the human genome and its possible functional role

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1. Introduction

In eukaryotic cell nuclei genomic DNA interacts with histones to form nucleosomes; arrays of nucleosomes are packaged into chromatin fibers that constitute chromatin in interphase cell nuclei, chromatids and chromosomes in mitotic cells. In the chromatin of the cell nucleus chromosome territories can be distinguished occupying well defined nuclear subvolumes (for review see Cremer and Cremer, 2001). Chromatin mediates gene expression in response to external or internal signals that induce complex patterns of enzyme-catalyzed chromatin modifications, such as DNA methylation by DNA methylases, histone phosphorylation by kinases, acetylation/ deacetylation of histone tails by histone- acetyltransferases and deacetylases, methylation by histone-methyltransferases, ubiquitination by Ub-ligases, etc (see e.g. Howe et al., 1999; Berger, 2001; Jenuwein and Allis, 2001). These epigenetic modifications lead to complex changes of the physico-chemical properties of chromatin, including steric effects on the chromatin structure and formation of recognition sites for other proteins. Distinct histone tail modifications can generate synergistic or antagonistic interaction affinities for chromatin-associated proteins, which in turn dictate dynamic transitions between transcriptionally active or transcriptionally silent chromatin states.

The combinatorial nature of histone amino-terminal modifications has led to the concept of a "histone code" that considerably extends the information potential of the genetic code (Strahl and Allis, 2000, Jenuwein and Allis, 2001). These results support the hypothesis that epigenetic chromatin modifications and concomitant changes of the 3D structure of chromatin from the level of chromatin loop domains via chromosomal territories (CTs) to suprachromosomal organization of the

genome are responsible for the transition of the transcriptionally silent chromatin into active chromatin states and vice versa (Cremer and Cremer, 2001).

An important role in nuclear architecture and gene silencing plays the condensed chromatin that is called heterochromatin (Heitz, 1928). Constitutive heterochromatin is virtually free of protein coding genes and mostly located in peri- and paracentromeric chromosomal subregions. Facultative heterochromatin contains silent genes. The presence of different chromocentres in cell nuclei (spatial associations of centromeric heterochromatin), "myeloid" (in monocytes and granulocytes) and "lymphoid" (in lymphocytes), was found by Alcobia et al. (2000; 2003) suggesting cell type specific and ontogenically determined organization patterns. Recruitment of genes into the close neighborhood of constitutive heterochromatin or packaging into facultative heterochromatic chromatin domains represents an important mechanism of epigenetic regulation of gene silencing (Grewal and Elgin, 2002; Francastel et al., 1999; Bartova et al., 2001). On the other hand, tissue-specific enhancers and locus control regions (LCRs) prevent active genes from being included in a region of transcriptional inactive condensed chromatin (heterochromatin) that forms during cell maturation (Bulger and Groudine, 1999; Francastel et al., 2000).

One pathway of heterochromatin formation is apparently related to histone H3 lysine (K) methyltransferases (HMTases), stably modifying histones H3 by methylating lysine at 9 position (Lachner and Jenuwein 2002). H3-K9 methylation creates a binding site for the (chromo) domain of heterochromatic HP1 proteins. These findings have suggested existence of a biochemical mechanism for induction and propagation of subdomains of facultative heterochromatin (for review see Lachner and Jenuwein 2002). Heterochromatic domains are maintained by

highly dynamic HP1 binding and, consequently, silent genes are easily accessible to individual regulatory factors (Cheutin et al., 2003; Festenstein and Aragon, 2003). The absence of HP1 in human granulocytes with highly condensed chromatin suggests another pathway of heterochromatin formation (see chapter 6.1).

Our understanding of the mechanisms of formation and the role of chromatin higher-order structures is obviously very poor to allow explanation of the large scale genome architecture and its function. Therefore, a simple description of the genome arrangement and its changes is needed in order to consider relationships and deduce mechanisms. In the subsequent chapters the following topics will be discussed :

- (i) the global structure of the human genome,
- (ii) the structure and orientation of chromosome territories in cell nuclei,
- (iii) the dynamics of the human genome arrangement,
- (iv) tethering of chromosome territories and
- (v) changes of the genome and territory structure during important cellular processes such as the cell cycle, cell differentiation, apoptosis, cell transformation etc.

2. Orderliness and Randomness in the Global Structure of the Human Genome

Visualization of CTs by fluorescence in situ hybridization (FISH) in mammalian and plant cells (Schardin et al., 1985; Pinkel et al., 1988; Cremer et al., 1988; Lichter et al., 1988) lead to intensive investigations of the structure of human genome. Studies of the arrangement of the human genome and CTs have been performed for 20 years using 2D or 3D FISH in fixed cells (for reviews see Cremer and Cremer, 2001; Parada and Misteli, 2002). Recently, experiments using incorporation of labeled precursors or GFP tagged proteins binding DNA (e.g. histone H2B-GFP) provided additional information on the structure and dynamics of the human genome (Kanda et al., 1998; Bornfleth et al., 1999; Edelman et al., 2001; Kimura and Cook, 2001; Chubb et al., 2002; Walter et al., 2003; Gerlich et al., 2003). Both 3D FISH and live cell approaches have their specific advantages and limitations and it is important to explore both approaches in parallel. For example, the 3D FISH approach is particularly suited to study the topology of a large set of active and inactive genes with respect to higher order euchromatic and heterochromatic compartments. A potential drawback of 3D FISH is represented by the fact that considerable chromatin damage produced by this procedure can be expected at the level of chromatin fibers. However, the level of preservation of the nuclear topography even after the heat denaturation step is sufficient to study the large scale chromatin topology (Solovei et al., 2002). Similar

nuclear topography has been found even after repeated FISH (Falk et al., 2002).

2.1. 3D Structure of the Human Genome in the Cell Nucleus is not Random

Investigations of the nuclear topography of centromeres started very early using indirect immunofluorescent labeling with anticentromere antibodies (Hadlaczky et al., 1986; Weimer et al., 1992) and later using confocal microscopy (e.g. Popp et al., 1990; Hulspar et al., 1994; Höfers et al. 1993; Skalnikova et al., 2000). Paracentromeric chromosome regions were found either randomly distributed in the volume of the cell nucleus or localized near the nuclear periphery. Investigations of the 3D topography of genes in cell nuclei started much later (Kozubek et al., 1997; Lukasova et al., 1997; Parreira et al., 1997). Most genes were found in the nuclear interior in striking contradiction to distributions generated from random models. In addition, tethering of genes was found and suggested as a mechanism for increased induction of chromosome aberrations in specific cases (Kozubek et al., 1997), which was confirmed in several other contributions (Kozubek et al., 1999a; Nikiforova et al., 2000; Parada et al., 2002). Systematic studies that were performed more recently involved determination of nuclear radial positions of all chromosomes (Bartova et al., 2001; Boyle et al., 2001; Boltzer et al., 2005) and also other genetic elements such as arms or other regions of chromosomes, centromeres, telomeres and some genes (Skalnikova et al., 2000; Cremer et al., 2001; 2003; Kozubek et al., 2002; Amrichová et al., 2003).

Investigations of the relationships between nuclear positioning of genetic elements and their other characteristics showed the correlation between gene density or activity of the chromosome region and its radial location in the cell nucleus (Croft and Brooks, 1999; Boyle et al., 2001; Cremer et al., 2001; Tanabe et al., 2002; Kozubek et al., 2002; Cremer et al., 2003). Genes of highly expressed CT regions are localized in the central parts of the cell nucleus; sequences or regions with low expression are found preferentially near the nuclear periphery. In cells with adherent growth another relationship was found - the so called size dependent positioning of chromosome territories (Sun and Yokota, 1999). This finding is currently a subject of discussions (Bolzer et al., 2005). Correlation was found between transcription activity and replication timing (Sadoni et al., 1999). It was shown that distinct higher order compartments whose DNA displays specific replication timing were stably maintained during all interphase stages. Transcriptionally competent and active chromatin was confined to a coherent compartment within the nuclear interior that comprised early replicating R-band sequences. G/C-bands were located mostly on the

nuclear periphery (including perinucleolar compartments). The average centre of the nucleus to genetic element distances were found to be element-specific, largely maintained in different cell types and even evolutionary conserved (Habermann et al., 2001; Kozubek et al., 2002; Tanabe et al., 2002; Cremer et al., 2003). It has been shown in yeast that the tethering or targeting of a silencer-flanked reporter gene to the nuclear envelope facilitates its repression (Andrulis et al., 1998). On the other hand, association with the nuclear periphery is not sufficient if the reporter construct has no silencer element (Gasser, 2001).

Radial nuclear distributions, illustrating nuclear topography of high gene density (expression) and low gene density regions of chromosomes, are shown in Fig. 1 as a superposition of a large number of measurements in central nuclear sections. The density of points represents the probability density per volume unit of genetic region occurrence at a given position. The figure demonstrates distinct radial distributions of genetic regions in cell nuclei. Highly expressed chromosome regions are found close to the centre of the cell nucleus; while regions with low expression are localized close to the nuclear membrane (Kozubek et al., 2002; Lukasova et al., 2002; Galiova et al., 2004). It is worth to note that while the density of points is

maximal in the center of the cell nucleus (Fig. 1 A), the radial distribution is near to zero in the center of the nucleus (Fig. 1 A1) owing to a very small volume of the central shell.

Thus currently available data support the view that the cell nucleus is far from being a randomly arranged bag of molecules (Croft and Brooks, 1999; Boyle et al., 2001; Cremer et al., 2001; Tanabe et al., 2002; Kozubek et al., 2002; Cremer et al., 2003). On the other hand, the high degree of variability observed among nuclei with stained genetic elements leads to the conclusion that the order in the nuclear organization is manifested rather through statistical regularities. Genetic elements are localized in concentric layers (shells) in cell nuclei which are different for various elements but similar for given element in various cell types (Kozubek et al., 2002). These layers are formed in late telophase/G1 phase and do not depend on gene expression (Ferreira et al., 1997). Decondensation of chromosomes proceeds in radial direction (Manders et al., 2003), which probably forms final structure with specific radial positions of genetic elements; this structure is maintained through the subsequent interphase (e. g. Walter et al., 2003).

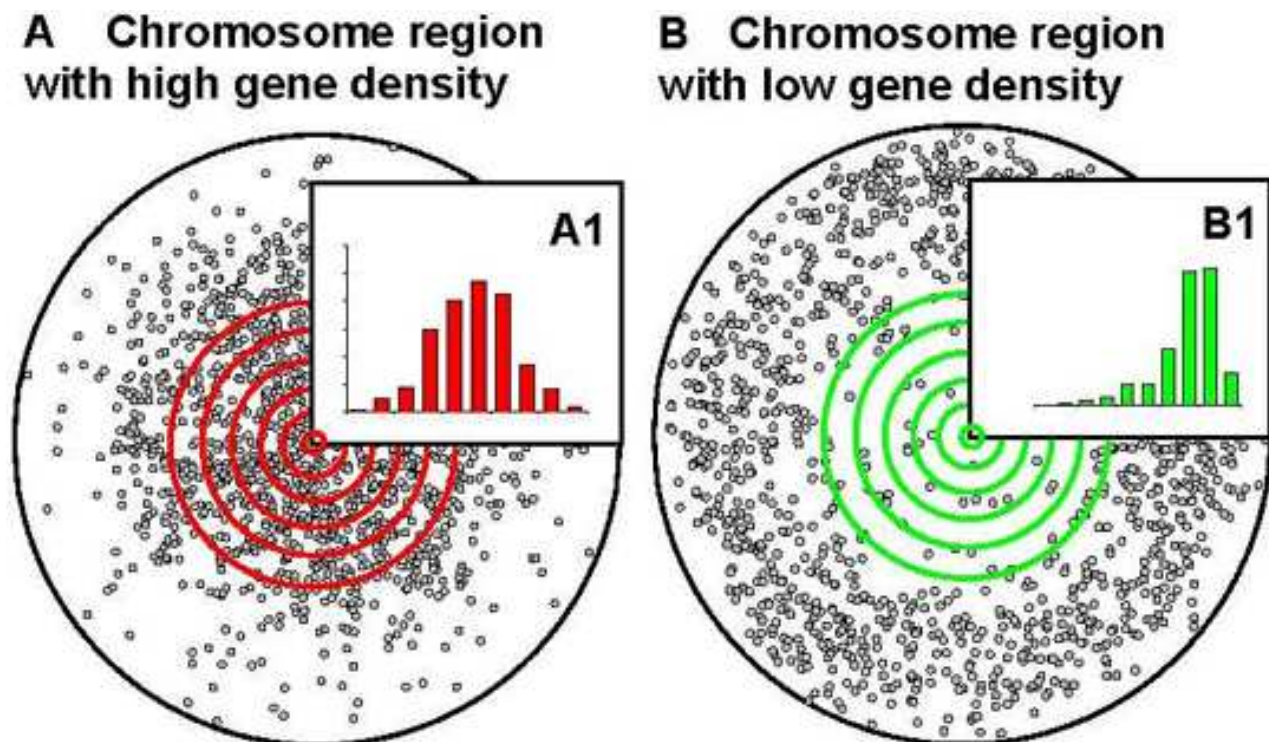


Fig. 1. Illustrative radial distributions of the positions of high gene density (A) and low gene density (B) chromosome regions in the cell nucleus. The central section of the cell nucleus is shown with x-y positions of the regions overlaid from a large number of measurements in nuclei with similar radii. The density of points represents the spatial probability density of the occurrence of the region in the particular point. The histograms in the inserts represent radial distributions, i.e. the distribution of probabilities to find the region at particular distance from the center of the nucleus. The vertical bars correspond to the number of points in the shells shown in the cell nuclei. The radial distributions are non-random and different for various genetic regions.

2.2. The Randomness in CTs Neighbourhood and Radial Symmetry of the Cell Nucleus

Mutual positioning of CTs in the cell nucleus is highly variable (Cremer and Cremer, 2001). This fact is evident for everybody from the first observation of mutual positions of two pairs of CTs (or other loci) painted by different fluorochromes. More exact investigation of this variability can be based on the determination of fluorescence weight centers of CTs in 3D space and calculation of the angle CT1-center-CT2 in the plane defined by these 3 points. Random mutual positioning of CTs is reflected in the angular distribution corresponding to the sine function (Kozubek et al., 2002). In 3D space, the most frequent angle is 90° owing to the fact that the number of possible CT positions corresponding to this angle is the largest (Fig. 2). Obviously the number of free positions for CTs will be proportional to the length of the circle perpendicular to the plane of the image corresponding to angle, that is $2 \sin(\alpha)$. Angular distributions reminding of the sine function (Fig. 2, insert) have been found for a number of different homologous and heterologous pairs of genetic elements (Kozubek et al., 2002; Amrichova et al., 2003), which suggests that in most cases CT mutual positions are random. This type of randomness in the location of CTs is largely responsible for the variability of cell nuclei.

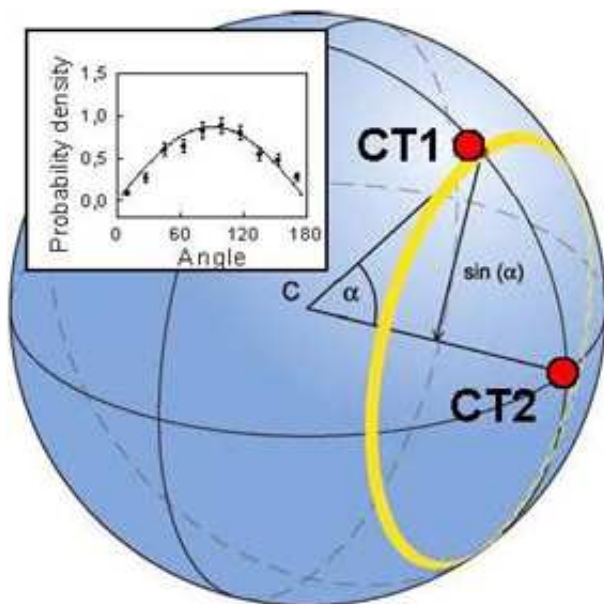


Fig. 2. Angular distributions of chromosome territories in cell nuclei. A scheme showing a model situation in which CTs (red circles) are placed on the surface of a sphere, one of them on the x-axis. The yellow sector shows part of a spherical surface corresponding to the CT1-center-CT2 angle. The area of the sector is proportional to $\sin(\alpha)$. The angular distribution in the insert shows a sine profile that corresponds to random mutual angular positioning of CTs.

It is important to note, that the existence of the radial arrangement of the cell nucleus which is mentioned in a number of articles (Tanabe et al., 2002; Cremer et al., 2003; Kreth et al., 2004) does not follow from the specificity of radial distributions (the eyes in the human face can be also characterized by some specific radial distribution but we do not say that the face has radial arrangement). In order to say that an object has radial symmetry, some periodicity in angular direction has to be observed. In the case of cell nucleus this periodicity follows from the randomness of angular distributions.

Angular randomness of chromosome positions (positions of other genetic elements) arises probably during the formation of cell nuclei in late telophase/G1 phase of the cell cycle (Walter et al., 2003). Interphase chromatin is relatively stable with restricted movements that might, however, also contribute to finally random angular chromosome positioning during several cell generations.

3. Structure and Orientation of CTs in the Cell Nucleus

The arrangement of interphase chromosomes into separate territories provides a framework for investigations of the relationship between the interphase chromosome structure and function. The basic question is whether gene expression is determined, at least in part, by the structure of the chromosome territory. The studies trying to resolve this issue are aimed at determining whether the organization of CTs is random, whether particular genomic sequences occupy special positions within chromosome territories, whether these positions differ according to the transcriptional activity of the sequences and whether genomic regions or whole individual chromosomes occupy particular compartments within the cell nucleus (Sachs et al., 1995; Belmont and Bruce, 1994; Nagle et al., 1995; Ferreira et al., 1997; Lamond and Ernschaw, 1998; Belmont et al., 1999; Cockel and Gasser, 1999; Croft and Brooks, 1999; Sadoni et al., 1999; Verschure et al., 1999; Nagle et al., 2000; Volpi et al., 2000; Chevret et al., 2000; Cremer et al., 2000; Cremer and Cremer, 2001; Sadoni et al., 2001; Tambar and Belmont 2001; Chubb et al., 2002; Walter et al., 2003; Gerlich et al., 2003).

3.1. Random-walk models

The first systematic quantitative studies of the topology of genetic elements in cell nuclei (Engh et al., 1992; Trask et al., 1993; Yokota et al., 1995; Sachs et al., 1995) lead to the conclusion that CTs could be represented by randomly walking polymers. The authors measured average spatial distances between two genetic elements (Ds) with known molecular distance (m) and showed linear dependence $Ds^2(m)$, with the simplest explanation - a random flight

polymer. In a later model of the interphase chromosome two levels of randomness were distinguished: Randomly walking loops of DNA (level 1) attached to a flexible and randomly walking backbone (level 2). This model explained behaviour of the two components of the dependence $Ds^2(m)$. Experiments were performed very carefully with high statistics using methanol 2D fixation as well as 3D paraformaldehyde fixation. In spite of the fact that the analyzed chromosome 4 and 15 territories are relatively gene poor and not highly expressed, these studies represent a basis for further thought.

3.2. CTs are Polar and Oriented in Cell Nuclei

Investigation of the higher-order compartmentalization of chromatin according to its replication timing suggested a polar orientation of early and late replicating sub-regions of chromosomes (Ferreira et al., 1997; Sadoni et al., 1999), with transcription competent and active chromatin located within the nuclear interior. Recent results have demonstrated existence of an important factor influencing nuclear location of a genetic element, which is concentration of highly expressed genes in the molecular environment of an element on the chromosome (Lukasova et al., 2002). Density of highly expressed genes in the environment can be established according to Caron et al. (2001). If a genetic element is located in a region rich in highly expressed genes, its nuclear location is close to the nuclear centre. If it is located in a region poorly populated with expressed genes, its nuclear position is more peripheral.

Regions of high expression that protrude from the more condensed parts of the chromosome located in the proximity of the nuclear membrane to the nuclear centre determine the polar character of CTs which can be directly shown by measurements of 3D positions of at least 3 genetic elements along the territory, e.g. a centromere and both telomeres. Polar nature of CTs has been directly shown for HSA 3, 8, 9, and 19 (Amrichova et al., 2003), where centromeres were localized on one side of the territory and both telomeres on the other side (Fig. 3). Chromosomes are polar independently of their positions inside cell nuclei, i.e., regardless of whether they are located near the membrane or in the centre of the cell nucleus. In addition, a majority of the polar chromosome territories are oriented in the cell nucleus with the centromere localized near the nuclear periphery and both telomeres placed in the interior of the cell nucleus. Only 5-10% of chromosome 8 and 9 territories showed the opposite orientation.

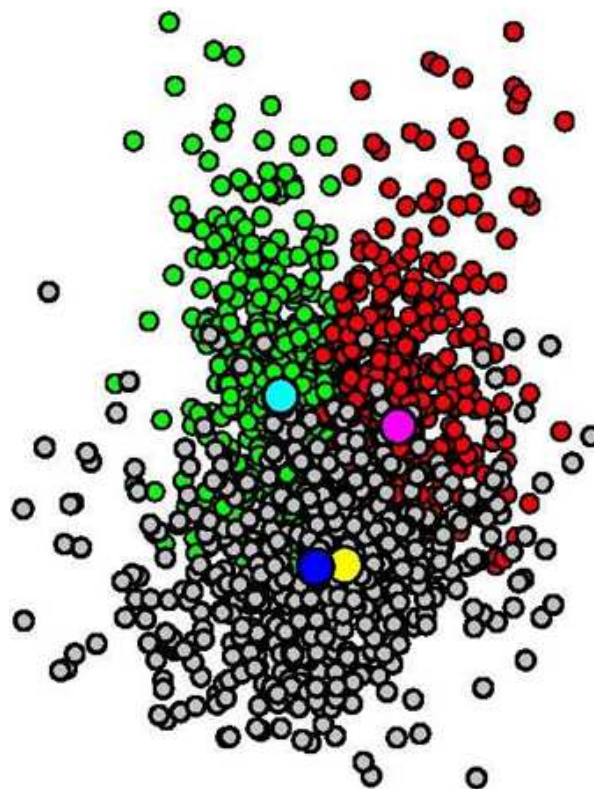


Fig. 3. 3D structure of the interphase chromosome. As a typical example, the structure of chromosome 8 in the nucleus of G0 - lymphocytes is shown. The positions of centromeres (grey circles), p-telomeres (red circles) and q-telomeres (green circles) were determined for 275 nuclei in 3D space and placed to the x-y plane in such a way that all chromosomes are superimposed to each other (with minimal deviations of the distances inside clusters of points). Pink, cyan, blue and yellow circles represent the weight centres of all measured p-telomeres, q-telomeres, centromeres and chromosome territories respectively. The figure shows directly the polarity of the chromosome territories (both telomeres are located near to each other on one pole of the chromosome territory, the centromere being on the opposite pole).

Chromosome polarity and orientation can also be deduced from experiments with induced transcription performed in fixed cells (Volpi et al., 2000) or in living cells (Tumbar and Belmont, 2001). Targeting the VP16 acidic activation domain (AAD) to an engineered chromosome site resulted in its transcriptional activation and redistribution from a predominantly peripheral to a more interior nuclear localization. Direct visualization *in vivo* revealed that the chromosome site normally moves into the nuclear interior transiently in the early G1 and again in the early S phase. In contrast, VP16 AAD targeting induced this sites permanent interior localization in the early G1. These results show that at least active CTs must be polar and oriented in cell nuclei.

4. Dynamics of the Human Genome Structure, the Cell Cycle

Stability of interphase chromatin has been suggested from early experiments already in the beginning of the 20th century (Boveri, 1909). This observation was recently confirmed by different methods in several contribution (Shelby et al., 1996; Zink and Cremer, 1998). For example, mirror images of daughter cells obtained after FISH have also lead to the conclusion that the mobility of chromatin in interphase cells is rather restricted (Kozubek et al., 1999b; Sun and Yokota, 1999). Experiments with living cells showed a high degree of stability of the interphase chromatin arrangement from G1 to G2 stages of the cell cycle and, to some extent, transmission of chromosome positions from mother to daughter cells (Gerlich et al., 2003; Walter et al., 2003). The authors admit that some intermixing that occurs within one cell division may lead to randomizing of CTs positions over several cell cycles. Constrained chromatin motion due to the likely association with nuclear compartments in human cells was shown using lacO integrant cell lines (Chubb et al., 2002). The loci at nucleoli or the nuclear periphery were significantly less mobile than other, more nucleoplasmic loci. Conserved positioning of HP1-GFP foci during interphase (Ondrej et al., submitted) is illustrated in Fig. 4; time-lapse measurements demonstrated diffusion coefficients of $1 \times 10^{-4} \text{ m}^2/\text{s}$ (similar to slowly moving loci of Chubb et al., 2002) restricted to the range of 0.1 μm ; directional movements were observed for longer time intervals.

Although CTs in the cell nucleus are relatively immobile, certain restricted movements or imprecise transitions through mitosis have been observed (Zink et al., 1998; Chubb et al., 2002; Walter et al., 2003; Gerlich et al., 2003). Movements inside CTs have also been observed in living cells (Tumbar and Belmont 2001) due to transcriptional activation. These results show that stable global interphase arrangement found by other groups (Walter et al., 2003; Gerlich et al., 2003) may be perturbed by central movement of relatively small chromosome regions. Consequently, the question arises how firmly are genetic elements attached to CTs (or the nucleus) during these movements and what is the proportion of movements of CTs as a whole and movements of genetic elements inside the territories.

Visualization of several genetic elements inside CTs has shown that their positioning can be either dependent or independent on the positioning of CTs (Amrichova et al., 2003). After rotation of the cell nucleus and transition of the weight centres of the investigated painted territories to a single point territorial distribution of genetic elements can be seen. The width of these distributions is usually narrower as compared with radial nuclear distributions. This means that the elements adhere to the territory. For example territorial distribution of q-telomeres of HSA 19 in G0-lymphocytes is narrower in comparison with nuclear distribution, which means that the q-telomere is quite firmly attached to the chromosome territory (Fig. 5).

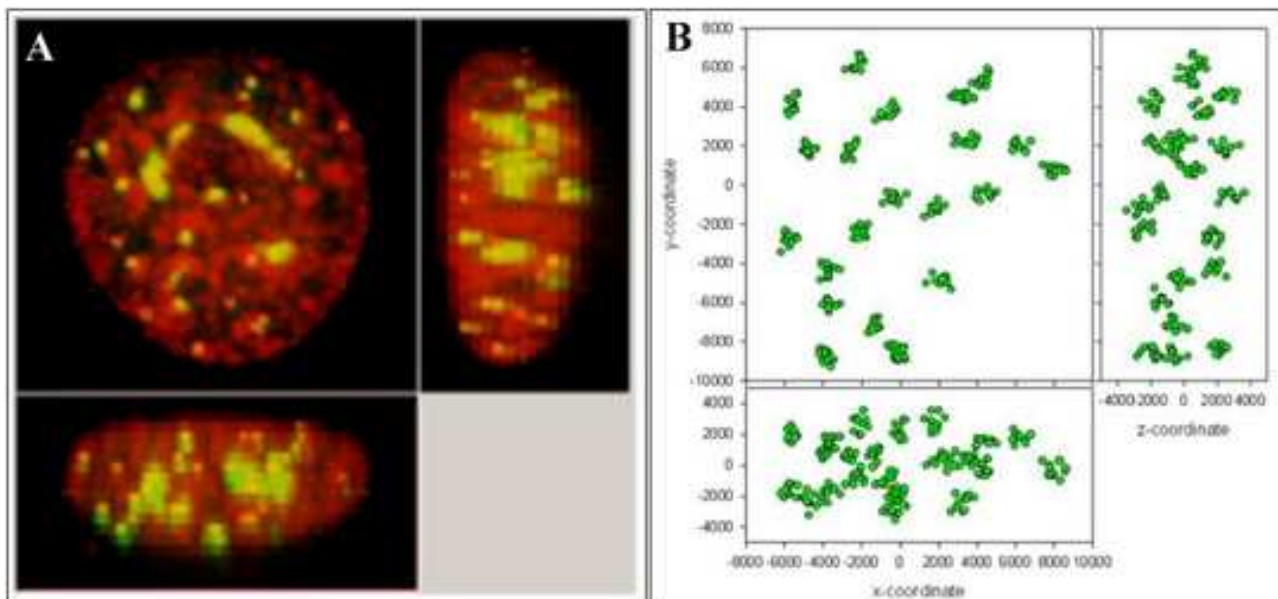


Fig. 4. The localization and movement of HP1 foci in the space of the cell nucleus. (A) XY, XZ, and YZ projections of the nucleus of MCF7 cell transfected with HP1-GFP and H2B-HcRFP is shown. (B) Positions of the HP1 foci were found by the computer at different time points (with 40 min intervals). The positions were corrected for movement and rotation of the nuclei using H2B-RFP. As a consequence of the restriction of movement of HP1 foci, the positions at different time intervals form easily distinguishable clusters.

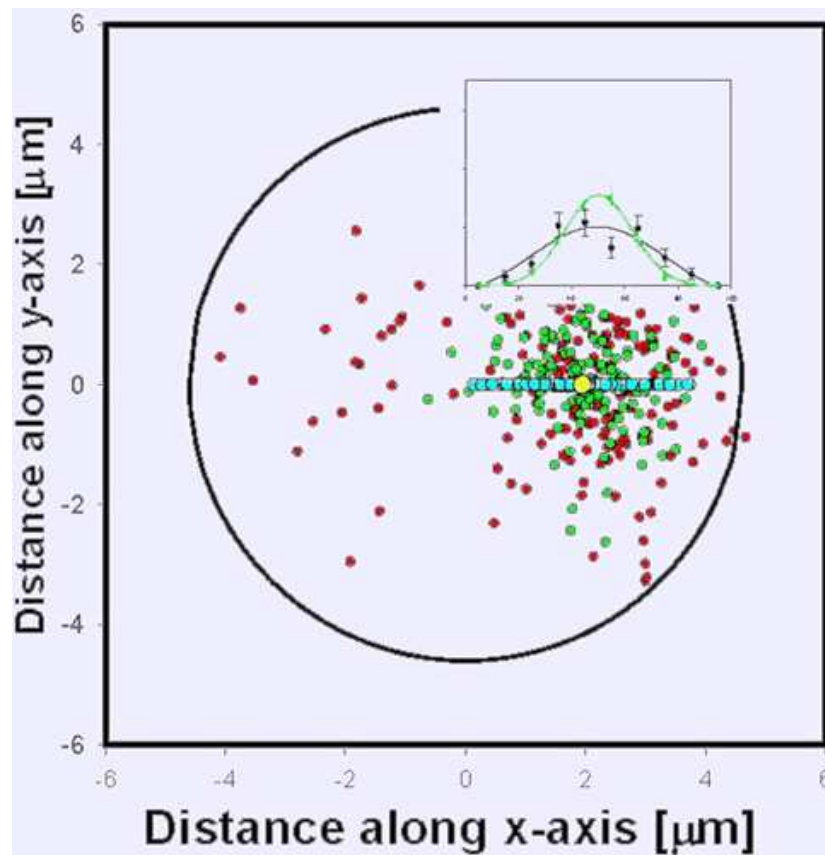


Fig. 5. The relationship between the nuclear and territorial distributions of genetic loci. After rotation of the chromosome territory (HSA 19) fluorescence weight center to x-axis, q-telomere was lowered to the plane of the image. Consequently, nuclear positioning of q telomeres (green) is shown in 2D projection. Weight centers of the territories for individual nuclei are shown as blue circles. The mean radial position of the weight centers is shown as yellow circle. The relation between nuclear and territorial distributions is shown for q-telomere in the insert. The territorial distribution is obtained for green points when the territories are shifted to the weight center. Before this operation the nuclear distribution is obtained. The territorial distribution is narrower (green line) as compared to the nuclear distribution (black line) showing a cohesion of the genetic element to the territory. The cohesion of p-telomeres to the territory is much lower (shown as red circles).

The opposite possibility is a broader territorial distribution, which means that the corresponding genetic element, not adhering to the territory; may be attached to some other nuclear structures. This extreme possibility is represented by the behaviour of the p-telomere of HSA 19 in stimulated lymphocytes which shows full independence in relation to its own chromosomal territory. These findings show that elements may be attached either to the territory or to the nucleus. This may be explained by the fact that telomeres and telomere-specific binding proteins may associate with the nuclear matrix and participate in anchoring chromosomes. In addition, the decondensation of chromatin related to high concentrations of expressed genes may cause extension of the distances between genetic elements in the cell nucleus and contribute to the relative independence of an element in relation to its territory.

In prometaphase, chromosome rosettes are formed by centromeres joining together and forming a central ring. Nagele et al. (1995) found a precise arrangement of CTs along the ring with homologous CTs being

localized on the opposite sides of the ring. These results were not confirmed by Allison and Nestor (1999) who found random positioning of CTs in metaphase rosettes. In our experiments using nocodazole to block HL-60 cells in prometaphase we were not able to reproduce the precise chromosome order described by Nagele et al., (1995), even though the positions of centromeres were not random, rather showing a trend towards a preferential order.

5. Tethering of Chromosome Territories

In some cases, non-random angular distribution of homologous and heterologous elements was found (Kozubek et al., 2002). Shorter distances than predicted by random distribution were found between BCR/BCR genes located on homologous acrocentric chromosomes or between BCR/PML belonging to heterologous acrocentric chromosomes. Acrocentric chromosomes participate in the formation of the nucleolus and this common function may influence their nuclear location and lead in some cases to mutual proximity.

Tethering of CTs is well known for centromeres that are frequently localized in chromocentres whose number per cell nucleus is in some cases substantially smaller than 46 (Alcobia et al., 2000). Centromeres are most frequently localized near the nuclear periphery or near the nucleoli. Nucleoli are thought to represent another example of inner nuclear surface where chromosomes can be attached (Sadoni et al., 1999). Association of centromeres is thought to play an important role in formation of heterochromatic foci and in gene silencing (see the section on heterochromatin).

Physiological telomere associations were found frequent in interphase nuclei of human fibroblasts (Nagele et al., 2001) and less frequently in cycling cells. This was a reason to assume that telomeric associations may be involved in the maintenance of chromosome positional stability in the interphase nucleus, especially in cells that are proliferating slowly, replicatively quiescent, or terminally differentiated. The authors thus conclude that the number of telomere associations in interphase nuclei depends on the cycling status of the cell, rather than on the individual telomeres length and telomerase activity. Using specific DNA probes, telomere association of CTs 8, 9, and 19 was investigated in Amrichova et al. (2003). No association between heterologous telomeres was found. On the other hand, homologous telomeres of CT 19 were often close to each other and signals of both telomeres (p-p or q-q) could often be identified as a single spot. This phenomenon was highly prevalent but did not depend on the stage of the cell in the cell cycle. Owing to telomere association, their central localization (Amrichova et al., 2003) and high level of gene expression in their neighborhood (Quina and Parreira, 2005), the associations of telomeres observed in the interphase nucleus might contribute, as opposed to chromocenters, for the establishment of transcription-permissive 3D nuclear compartments.

Experimental distributions of minimum distances between ABL-BCR in human lymphocytes differ from theoretical predictions (Kozubek et al., 1999a; 2002); distribution of these distances is shifted to lower values. In about 10-25% of Go-lymphocytes of 5 different healthy individuals the minimum distance between ABL and BCR genes was less than 1 μ m. No translocation between these genes was found in metaphases of stimulated lymphocytes from these individuals. The shift of distance distributions for the ABL and BCR genes was not observed for stimulated lymphocytes and HL-60 cells, even though tethering was observed for CD34+ progenitor cells. Proximity of specific chromosome regions can lead to their mutual rearrangement under some conditions, as was shown for RET/H4 (Nikiforova et al., 2000).

Our results obtained in 2D (Kozubek et al., 1999a) or 3D (Kozubek et al., 2002) show very close proximity of ABL/BCR genes (< 1 μ m) in about 15-20% of Go-

lymphocytes. Proximity of these regions might be one of the reasons for their interchanges and the formation of the Philadelphia chromosome typical of chronic myeloid leukaemia (Rabbitts, 1994).

The high frequency of interchanges induced by fast neutrons between chromosomes involved in translocations leading to most frequent haematologic malignancies also indicates the non-random arrangement of some chromosomes in cell nuclei (Lukasova et al., 1999; Cafourkova et al., 2001; Bickmore and Teague, 2002).

6. Changes of the Genome and Territory Structure During Important Cellular Processes

6.1. Cell Differentiation

Several mechanisms were considered as an explanation of structural modification of gene activity. For example Ikaros, a DNA-binding protein localized in the discrete foci of nuclei of murine B-lymphocytes, is in close association with centromeric heterochromatin. A strong correlation was found between these foci and the location of transcriptionally inactive genes (Brown et al., 1997; 1999). In addition, in the context of differentiation of human lymphocytes a discovery was made that the promoter-specific binding factor of Ikaros mediates association of cell-type-specific genes with centromeric heterochromatin. Ikaros regulates movement of the genes towards centromeric heterochromatin, whereas activated genes are released (Cockell and Gasser, 1999). Thus, gene positioning on the periphery of the chromosome territory could facilitate not only access to the transcriptional machinery (enabling gene activation), but also access to the factors inhibiting genetic expression (e.g., clusters of centromeric heterochromatin).

Spatial dynamics of selected genetic elements was studied during human blood cell differentiation with parallel monitoring of their expression dynamics (Bartova et al., 2000; Bartova et al., 2002; Galiova et al., 2004). The role of the chromatin structure in regulation of the studied gene expression was tested.

The following three hypotheses were verified:

- (i) Activated (silenced) genes change their location in the cell nucleus,
- (ii) Activity of genes correlates with their location within the corresponding chromosome territory, and
- (iii) Gene expression is regulated by the association of genes with centromeric heterochromatin.

It was found that in the process of cell differentiation genetic elements are shifted to the periphery of the cell nucleus (Bartova et al., 2000; 2002; Galiova et al., 2004). However, these changes of the nuclear organization did not correlate with alterations in genetic expression (Bártová et al., 2002).

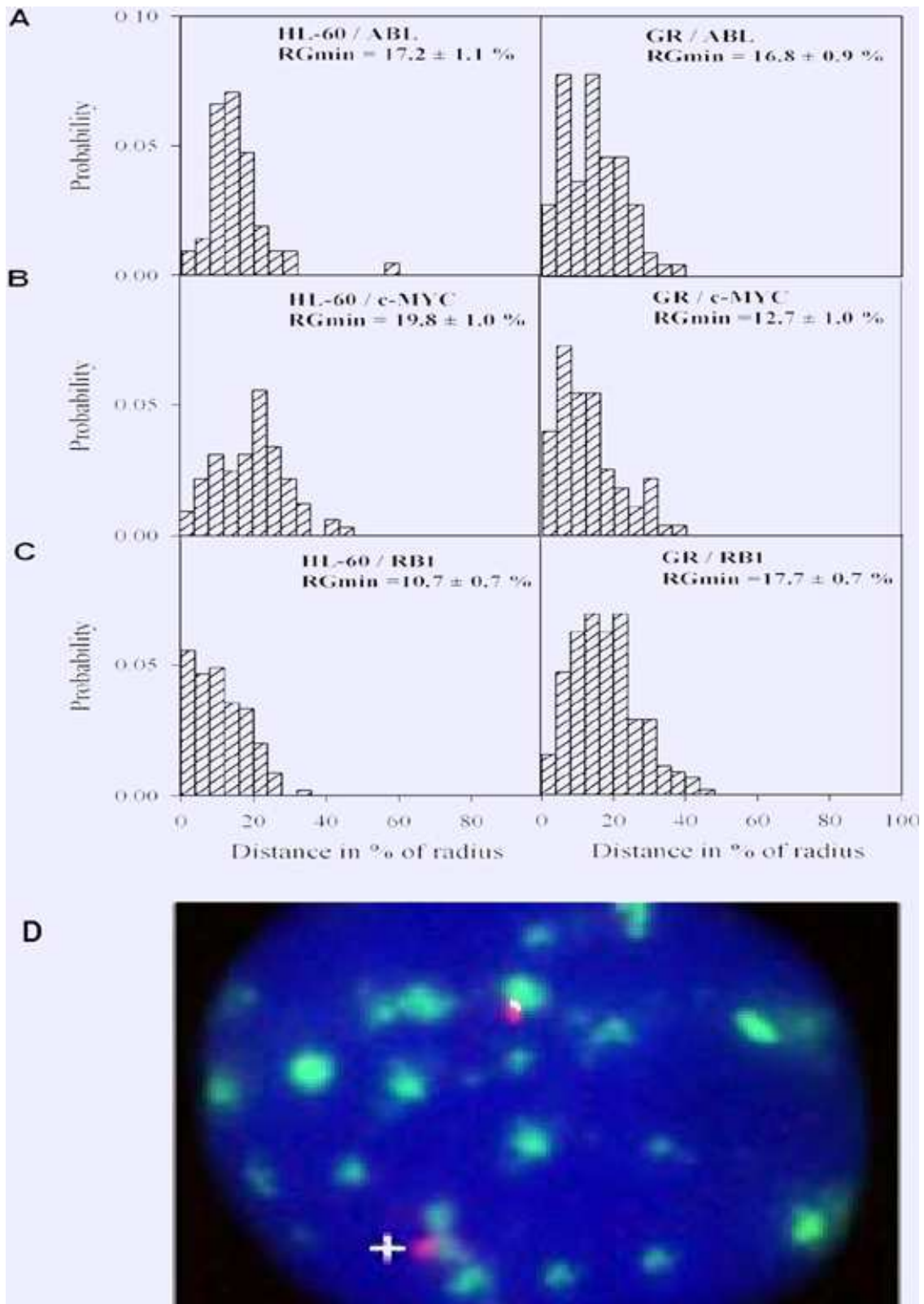


Fig. 6. Distributions of distances between genes and the nearest cluster of centromeres. Simultaneous visualisation of genes and all centromeric regions forming the chromocentres was used to determine distributions for the ABL (A) c-MYC (B) and RB1 (C) genes in undifferentiated cells (HL-60), as well as in terminally differentiated granulocytes (GR). Mean values of the distributions (RGmin) normalised to the nuclear radius and their standard errors are given in each panel. (D) An illustrative example of the nucleus of the HL-60 cell with centromeric heterochromatin (green) and the RB1 genes (red) found in close neighbourhood to one of centromeres. The RB1 gene is not expressed in HL-60 cells.

Independently of gene expression, genetic elements were located closer to the corresponding fluorescence intensity centre of chromosome territory after differentiation, which rather reflects condensation of the CTs (similar shift to the centre of CTs is also observed for centromeres). Genes were located on the periphery of CTs, unlike centromeres, found closer to the bary centre of CTs, which is in agreement with observations of Kurz et al. (1996), who found either active or inactive genes preferentially located on the periphery of CTs. The distributions of distances between the genes and the nearest centromeric heterochromatin revealed a correlation with gene activity (Fig.6). A correlation between transcriptional activities of some tissue-specific genes and their association with pericentromeric heterochromatic regions has been found in several other studies in mammalian cells (Francastel et al., 1999; Fisher and Merkschlager, 2002). Topography of different genetic loci in human peripheral blood granulocytes was investigated in Bartova et al. (2001). Nuclei of granulocytes are characterized by a segmented shape consisting of two to five lobes that are in many cases connected by a thin filament containing DNA.

Granulocytes (neutrophils, basophils and eosinophils) represent an example of terminally differentiated cells with the highest possible condensation of chromatin (Alberts et al. 1994; Grigoryev and Woodcock, 1998). Different topographic types of granulocytes were distinguished on the basis of the pattern of CTs or genetic element segregation into individual lobes. Painting of the same type of chromosome in two-lobed nuclei showed a prevalence of symmetric topographic types (the homolog segregated in one lobe each). The results of the analysis of five topographic types (defined by two CTs pairs in two-lobed nuclei) have shown that symmetric topographic types for both chromosomes are significantly more frequent than predicted by simple statistics. Repeated hybridization experiments have confirmed that the occurrence of certain patterns of chromosome segregation is much higher than that predicted from the combination of probabilities. Both genes and centromeres were observed on filaments joining different lobes. The significance of individual topographic types, particularly of those observed with much higher probability than expected, is unclear.

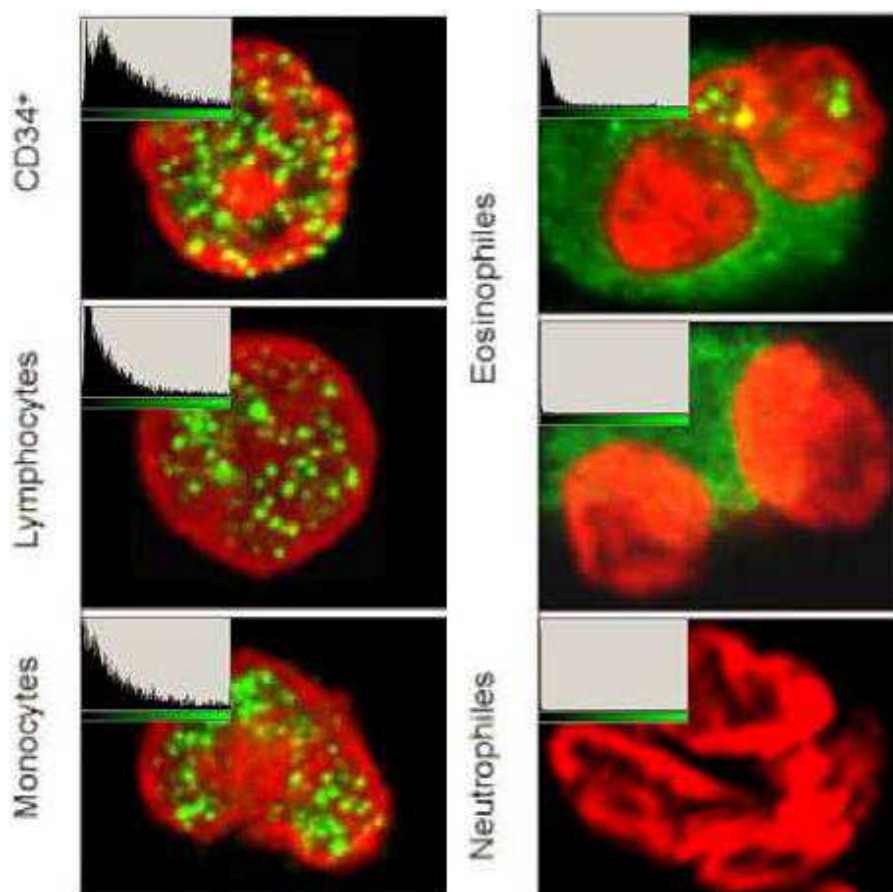


Fig. 7. Distribution of HP1 in human peripheral blood cells (lymphocytes, monocytes, eosinophiles and neutrophiles) and in human blood progenitor CD34+ cells. HP1 was found in CD34+ cells, lymphocytes and monocytes, but not in neutrophiles and some eosinophiles. The images represent the central XY cuts through the nuclei. Immunodetection was performed using FITC labeled secondary antibodies. DNA was counterstained with TOPRO-3. Inserted histograms represent the level of antibodies signals measured as the mean value of green channel intensity.

Cell differentiation represents also an interesting model for studies of alterations of the genome structure in relation to the formation of heterochromatin and histone modifications (Lukasova et al., 2005). It was shown that common heterochromatin antigenic protein markers such as HP1 and mono-, di-, and trimethylated histone H3 lysine 9 (H3K9), although present in human blood progenitor CD34+ cells, differentiated lymphocytes, and monocytes, are absent in neutrophil granulocytes and to large extent, in eosinophils (Fig. 7). Monomethylated and in particular, dimethylated H3K9 were present to variable degrees in the granulocytes of chronic myeloid leukemia (CML) patients, without being accompanied by HP1 proteins. In patients with an acute phase of CML and in acute myeloid leukemia patients, strong methylation of H3K9 and all isoforms of HP1 are detected. In chronic forms of CML, no strong correlations among the level of histone methylation, disease progression, and modality of treatment were observed. Histone methylation was found even in "cured" patients without BCR/ABL translocation, suggesting an incomplete process of developmentally regulated chromatin remodeling in the granulocytes of these patients. The absence of HP1 in human granulocytes that are characterized by highly condensed chromatin strongly suggests another mechanism of heterochromatin formation alternative to that mediated by HP1 binding to H3K9 methylated histones. Thus, at least 2 types of heterochromatin does exist, one being organized by HP1 with fast dynamics (Cheutin et al., 2003) and the other that is more compact and that is could be typical of terminally differentiated non-dividing cells.

6.2. Ionizing radiation (repair of radiation damage), apoptosis

Rearrangement of human cell homologous CTs in response to ionizing radiation was observed by Dolling et al., 1997. In this study, homologous CTs were found closer to each other after irradiation, and the authors proposed that the process of CTs pairing to facilitate recombinational repair of DNA DSBs may exist. In addition, radial movement of genetic elements was observed after irradiation of several cell lines (Jirsova et al., 2001). The spatial relationships between genetic elements returned to that of the non-irradiated controls during several hours of incubation after irradiation. The authors speculated that the changes of the large-scale chromatin structure might be related to repair processes, however, they exclude repair of DSBs by processes involving homologous recombination, because the angular distributions of homologous sequences remained random after irradiation. Radial movement was also observed by Tumbar and Belmont (2001) in live cell experiments.

Nuclear architecture of selected CTs was investigated in apoptotic nuclei of human leukaemia-affected cells

(Bartova et al., 2003). Apoptotic disorganization of chromosome territories was irregular, leading mainly to chromosomal segments of different sizes and, consequently, chromosomal disassembly was not observed at specific sites. In comparison to the control group an increased number of centromeric FISH signals were observed in prolonged confluence-treated K-562 cells induced to apoptosis. Sequential staining of the same apoptotic nuclei by the FISH and TUNEL techniques has revealed that chromosome territory segmentation precedes the formation of nuclear apoptotic bodies.

6.3. Haemoblastoses and Cancer

Radial and angular distributions have been measured in both normal and tumour cell lines with similar results. Distributions of ABL and BCR are very similar in bone marrow cells, in Go and stimulated lymphocytes, HL-60 cells, HT-29 colon cancer cells and also in nuclei of colon tissue and CML patients (Koutna et al., 2000). Radial distributions of EWSR1 and FLI1 genes are similar for Go and stimulated lymphocytes as well as for Ewing sarcoma cells (Taslerova et al., 2003). In mouse lymphoma cells two translocated CTs were preferentially positioned in close proximity to each other (Parada et al., 2002). The relative positions of the chromosomes involved in these translocations are close even in normal splenocytes. These observations demonstrate the fact that relative arrangement of CTs in the interphase nucleus can be conserved between normal and cancer cells (see also Cremer et al., 2003). Specific translocations that are (casually) related to some types of leukaemia provide chimeric chromosomes and their nuclear location can be investigated. For example, in Ewing sarcoma cells, radial positions were measured for EWSR1, FLI1 and fusion genes (Taslerova et al., 2003). The radial positions of both fusion genes are shifted compared with the radial positions of non-aberrant EWSR1 and FLI1 genes. While HSA 11 fusion gene is shifted more centrally, HSA 22 fusion gene lies towards the periphery. Thus, both fusion genes are located approximately midway between EWSR1 and FLI1 genes in Ewing sarcoma cells (Fig. 8). The different location of the fusion genes might be explained by the substitution of a small part of HSA 11 for a larger part of HSA 22 and vice versa. The central nuclear location of HSA 22 correlates with its high gene density. Thus, the transfer of a part of HSA 22 with high gene density to HSA 11 causes relocation in the central direction of the translocation neighborhood of chimeric HSA 11. On the other hand, the translocation neighborhood of chimeric HSA 22 is shifted towards the nuclear periphery. In CML the position of the chimeric chromosome is similar to that of both ABL and BCR loci (Fig. 8).

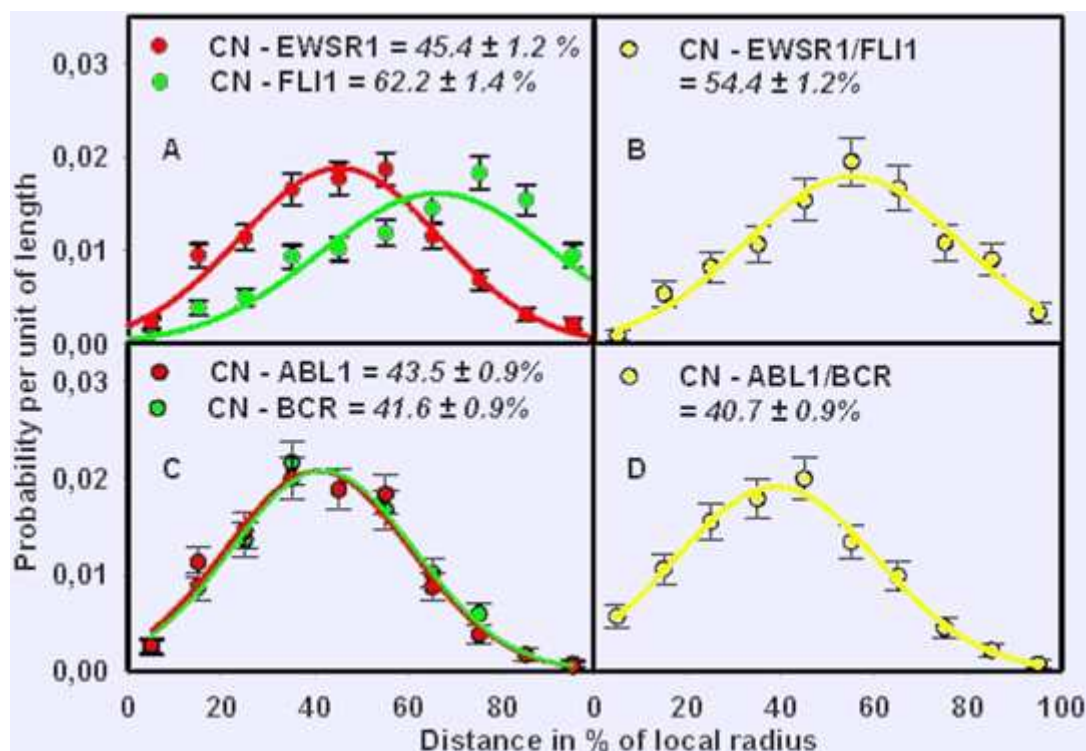


Fig. 8. Comparison of radial distributions of (A) the EWSR1, FLI1 genes and (B) the EWSR1/FLI1 fusion gene in Ewing sarcoma cell nuclei with radial distributions of (C) the ABL1, BCR genes and (D) the ABL1/BCR fusion gene in bone marrow cell nuclei of patients suffering from CML. The distributions of the fusion genes represent an average of both original gene distributions. CN - center of nucleus.

For the purpose of finding the influence of increased gene expression and amplification in colorectal carcinoma on the chromatin structure nuclear distances between two BAC clones with short genomic separation (1-2 Mb) were measured (using the method called spectral microscopy) and compared between tumor and parallel epithelial cells of 6 patients (Lukášová et al., 2004). Larger nuclear distances were found for tumor as compared with epithelial cells for the same genomic separation. The ratio of the mean nuclear distance between the loci in tumor and epithelium decreased with the mean degree of amplification of genetic loci. Similarly, distances between two exons of dystrophin gene were substantially longer for active X-chromosome as compared to inactive one (Falk et al., 2002). Substantial changes in distances between neighboring loci after locus activation were observed for Hoxb complex (Chambeyron and Bickmore, 2003; Chambeyron et al., 2004).

7. Conclusions

There are several principles of the genome organization in interphase nuclei of human cells that involve a combination of random and nonrandom processes: Genetic elements are localized in concentric layers (shells) in cell nuclei which are different for various elements but similar for given element in various cell types. This arrangement arises during chromosome

decondensation in late telophase/G1 phase and forms a general framework for gene expression with more decondensed and consequently centrally located regions with high gene expression.

The angular positioning of chromosomes (and consequently of all other genetic elements) is random and consequently the neighborhood of territories may consist of any combination of other territories. This is the reason for radial arrangement of the human genome. In relatively rare cases, chromosome territories can be tethered (e.g. acrocentric chromosomes in nucleoli), which may contribute to higher incidence of specific chromosome exchanges.

Chromosome territories consist of chromatin that is relatively stable during interphase with restricted diffusive movement of individual genetic elements. The most stable structure represents heterochromatin that provides silencing of genes. Binding of HP1 protein to methylated H3 histone contributes to the formation of heterochromatin (Cheutin et al., 2003). The HP1 protein is absent in human granulocytes (Lukasova et al., 2005), which strongly suggests that some other mechanism (protein) participates in heterochromatin formation and gene silencing.

Randomness in the arrangement of the subregions of a chromosome territory corresponds to the random-walk polymer, however, on the global scale the arrangement of the chromosome territories is nonrandom. Chromosome territories are polar and oriented in the

cell nuclei with more condensed and mostly silent subdomain on the periphery and less condensed and active subdomain in the central parts of the cell nucleus.

The arrangement of the human genome undergoes changes during the cell cycle, cell differentiation, apoptosis, cell transformation and other cellular processes. Condensation of chromatin in mitosis obviously leads to less random but in the same time less functional structure. Cell differentiation and transformation are accompanied by relatively subtle changes in the large-scale genome structure with conserved the main features described above. These changes are, however, of a great importance for gene expression and cell function.

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