Nuclear architecture and the induction of chromosomal aberrations

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Abstract

Progress in fluorescence in situ hybridization, three dimensional microscopy and image analysis has provided the means to study the three-dimensional structure and distribution of chromosome territories within the cell nucleus. In this contribution, we summarize the present state of knowledge of the territorial organization of interphase chromosomes and their topological relationships with other macromolecular domains in the human cell nucleus, and present data from computer simulations of chromosome territory distributions. On this basis, we discuss models of chromosome territory and nuclear architecture and topological consequences for the formation of chromosome exchanges.

‘‘The idea of the nucleus as a bag of broken chromosome ends flapping around seeking new partners...seems no longer to be tenable from our picture of the interphase nucleus’’ (J.R.K. Savage, 1990)

1. General problem

The introduction of chromosome banding procedures in the late 1960s and of chromosome painting methods in the 1980s has made possible a wealth of studies on aberration frequencies of individual chromosomes in human lymphocytes following acute or chronic ionizing irradiation (e.g., Tanaka et al., 1983; Lloyd, 1984; Sasaki et al., 1987; Cremer et al., 1990; Popp et al., 1990a; Straume et al., 1991; Weier et al., 1991; Natarajan et al., 1991, 1992; Gray et al., 1992; Lucas et al., 1992; Popp and Cremer, 1992; Schmid et al., 1992; Lucas and Sachs, 1993; Lucas et al., 1993; Fernandez et al., 1995; Finnon et al., 1995; Tanaka et al., 1996). Various models have been proposed for the induction of chromosome aberrations which predict a linear relationship between the aberration frequency and the DNA content of the chromosomes involved (see e.g., Lucas and Sachs, 1993; Edwards et al., 1994 and references therein).
According to these models, both ends of an unrepaired double-strand break (dsb) separate and move around within the nucleus. In this way each end may independently combine with any other dsb-derived end. As a first rough approximation, this expectation has been shown to be useful (e.g., Savage and Papworth, 1982; Lucas et al., 1992). However, significant deviations have also been observed for some chromosomes. For example, the observed frequencies of chromosome interchanges which occurred spontaneously in lymphocytes of patients with Fanconi’s anemia differed from a random expectation based on chromosome DNA content (Vogel and Schroeder, 1974). Tanaka et al. (1983) reported that the number of breaks per chromosome observed in a total of 651 cells with chromosome aberrations obtained from 19 male and 20 female atomic bomb survivors was significantly less \( (p < 0.01) \) than expected from DNA-content for chromosomes 1, 2 and X, and significantly larger than expected for chromosomes 15, 18 and 22. While results obtained from the analysis of cells from persons exposed a long time ago may be influenced strongly by cellular selection, this argument appears to be less stringent in cases where first division cells were collected following irradiation. Using such conditions, a comparison of radiation-induced aberrations in human cell lines containing one to four X-chromosomes showed a strong deviation from the linear expectation (Mühlmann-Diaz and Bedford, 1994).

Deviations of observed interchanges from the linear expectation were also noted for the breakpoint distribution along a given chromosome (Sax, 1938; Caspersson et al., 1972). Significantly larger numbers of breaks than expected from a Poisson distribution were observed in a variety of autosome regions in lymphocytes from atomic bomb survivors (Tanaka et al., 1983). Chromosome damage in X-irradiated Chinese hamster primary fibroblasts was found to be more frequent in euchromatin than in heterochromatin (Slijepcevic and Natarajan, 1994a). X-ray induced aberrations preferentially mapped to G-light bands in Chinese hamster embryonic cells (Slijepcevic and Natarajan, 1994b). Using fluorescence in situ hybridization (FISH), a non-randomness of the breakpoint distribution was detected in human chromosomes 1, 2 and 4 in cells from accidentally irradiated individuals (Tucker and Senft, 1994). Recently, Folle and Obe (1995) observed a deviation from randomness in the intrachromosomal breakpoint distribution induced by restriction endonucleases. After exposure of intact Chinese hamster ovary cells to \( AluI \) and \( BamHI \), in metaphase spreads from these cells the majority of breakpoints induced by both enzymes were localized in G-light (gene rich) bands; the data indicated that nuclease sensitive sites associated with active genes were mainly responsible for the breakpoint distribution observed.

2. Experimental evidence for chromosome territories and topological implications for the formation of chromosome aberrations

The cytogenetically observed distribution of chromosome exchanges, i.e., interchanges and intra-

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Fig. 1. Two color chromosome painting was performed with chromosome-specific plasmid libraries from sorted human chromosomes 7 and X (kindly provided by Dr. J. Gray, University of California, San Francisco) to formaldehyde fixed, three-dimensional intact nuclei of cultured female human amniotic fluid cells (Eils et al., 1996; for details see Lichter and Cremer, 1992). The X-specific whole chromosome composite (wcp) probe was nicktranslated with biotin-11-dUTP and detected with FITC-conjugated avidin (green fluorescence) with two rounds of signal amplification (Pinkel et al., 1986). The chromosome 7 specific wcp probe was labeled with digoxigenin and detected with a mouse anti digoxin primary antibody and a Cy3-conjugated rabbit anti mouse secondary antibody (red fluorescence). Light optical serial sections were obtained with a confocal laser scanning microscope (Leica TCS). Image analysis and 3D-reconstructions of entire chromosome territories were performed as described in detail elsewhere (Eils et al., 1995a, 1996). (a) single light optical section through a nucleus showing painted X-territory homologs (green) and chromosome 7 territory homologs (red). One chromosome X-territory is adjacent to one chromosome 7-territory. The apparent border zone is indicated by arrow. (b and c) Top (b) and bottom views (c) of 3D-reconstructions of the enveloping surfaces of the chromosome territories shown in (a) indicate that the neighboring territories occupy mutually exclusive nuclear subvolumes with some intermingling at the border zone (arrow). (d) Single light optical section through another nucleus showing the close neighborhood of a chromosome X- and a chromosome 7-territory (arrow indicates border zone). (e and f) Top (e) and bottom views (f) of 3D-reconstructions of the enveloping surfaces of the chromosome territories shown in (d) confirm that the mass of the neighboring territories is clearly separated from each other.
changes resulting from the interaction of two or
more lesions, needs to be considered in the light of
strong evidence that interphase chromosomes occupy
distinct territories (for review see Cremer et al.,
1993). In our ongoing efforts to study the three-di-
mensional organisation of chromosome territories in
cell nuclei in more detail, we have fixed and hy-
bridized human cell nuclei under conditions which
preserved their 3D-structure as much as possible.
Two color chromosome painting, laser confocal se-
rial sections and 3D-reconstructions of
neighboring/overlapping chromosome territories re-
vealed that apparent intermingling of chromatin was
limited to a border zone at the two adjacent territory
surfaces (Fig. 1; Eils et al., submitted). When genes
were visualized together with their respective chro-
mosome territory, their localization was generally
noted as a distinct signal within the territory or at its
surface, but rarely remote from it (Cremer et al.,
1993; Kurz et al., 1996, Dietzel et al., in prepara-
tion). These findings support the notion that giant chro-
matin loops which would extend from the surface of
one chromosome territory and penetrate into neigh-
boring chromosome territory are not common, at
least in the cell types and under the conditions
studied so far. In other words, present evidence
favors the view that the mass of a given chromosome
territory occupies a nuclear space mutually exclusive
from the space occupied by neighboring chromo-
some territories. We have further demonstrated that
chromosome arms (Fig. 2) and chromosome bands
(our unpublished data) form exclusive domains within
a given chromosome territory. Again laser confocal
serial sections and 3D-reconstructions have indicated
that apparent intermingling of chromatin was limited
to a border zone at the adjacent arm domain surfaces
(Dietzel et al., in preparation).

These basic findings on the organization and dis-
tribution of chromosome territories have lead to the
important consequence that only double strand breaks
dsb's) induced in a border zone at the periphery of
adjacent chromosome territories should contribute to
chromosome interchanges, while dsb's produced in
the interior of a chromosome territory (and remain-
ing inside during the time necessary for their repair)
could become involved in intrachanges. By the same
argument, intrachanges between two chromosome
arms should occur within the boundary zone of
adjacent arm domain surfaces (Savage and Papworth,
1973). In the context of this discussion the word
'interior' of a chromosome territory means chro-
matin which is not located at or near its enveloping
surface (compare Fig. 1b,c,e,f). Note that by infold-
ings, the actual surface of a chromosome can be
significantly enlarged. As a consequence, sequences
located in the chromosome 'interior' may still be
located at the surface of chromosomal subdomains
with access to such infoldings (see below, interchro-
mosomal domain (ICD) compartment model; Cremer
et al., 1995). Another basic consequence of the
existence of mutually exclusive chromosome territo-
ries is that the relative frequencies with which spe-

Fig. 2. Chromosome-arm specific microdissection probes for human chromosomes 3, 6 and 11 (Guan et al., 1996) were applied for two
color chromosome arm painting in interphase nuclei from PHA-stimulated human lymphocytes (46,XX) (for details of the chromosome
painting procedure see Lichter and Cremer, 1992). Briefly, nuclei were fixed with methanol/acetic acid (3:1, v/v) and air dried. (Note:
standard colcemid and hypotonic treatment of the lymphocyte cultures was omitted.) Probes for 3p, 6p and 11p were nicktranslated with
biotin-11-dUTP and detected with FITC-conjugated avidin (green fluorescence) with one round of signal amplification (Pinkel et al., 1986).
Probes for 3q, 6q and 11q were labeled with digoxigenin and detected with a mouse anti digoxin primary antibody and a Cy3-conjugated
rabbit anti mouse secondary antibody (red fluorescence). Nuclei were counterstained with DAPI (blue fluorescence). Microphotographs
were taken on color slide film (400 ASA Ektachrome) using a Zeiss Axioskop equipped with a Plan Neofluar 100 x /1.30 oil objective, and
a double band pass filter (AHF, Tübingen, Germany) for the simultaneous detection of FITC and Cy3 fluorescence. For better recognition
of nuclear boundaries, another brief exposure of DAPI fluorescence was performed. Nuclei exhibit two color painting of: (a) chromosome
arms 6p and 6q; (b) chromosome arms 3p and 3q; (c) chromosome arms 11p and 11q. The nuclei demonstrate variable arrangements and
shapes of chromosome territories with a striking separation of the painted chromosome arm domains in cases where both arms were in focus
and appeared side by side (several examples are denoted by arrows). Yellow color indicating potential regions of chromatin interdigitation
between adjacent arm domains is restricted to a narrow border zone in these cases. Due to the limited z-resolution of conventional
epifluorescence the possible extent of this interdigitation is difficult to judge in nuclei where the arm domains were out of focus and/or
overlapped. Laser confocal serial sections and 3D-reconstructions of chromosome arm domains providing strong evidence for the separation
of chromosome arm domains will be published elsewhere (Dietzel et al., in preparation).
specific chromosomes or chromosomal subregions are involved in interchanges (translocations) will depend on their neighborhood relationships inside the interphase nucleus: Two chromosome territories A and B which form common boundary zones of a given area more frequently with each other than with another chromosome territory C should show a correspondingly higher frequency of translocations t(A;B) than t(A;C) or t(B;C).

In the following, we shall discuss the hypothesis first proposed by Savage and Papworth (1973) that neighboring chromosome territories and their chromosome arm domains, respectively, should act on each other via their adjacent surface areas. We define 'adjacent surface areas' between chromosome territory and chromosomal domains, e.g., arm domain, band domains, operationally as those parts of the enveloping, three-dimensional surfaces of the respective structures, which are in immediate neighborhood (except for a possible interchromosomal domain space, see below). The boundary zones formed by adjacent surface areas form a specific nuclear compartment, where the illegitimate enzymatic coupling of DNA-strands required for the induction of exchange events can take place. Accordingly, an increase in the average adjacent surface area in a population of irradiated cell nuclei should increase the overall probability for translocation events. According to this view, only dsb's situated close enough to inter chromosome territory boundaries will have a chance to engage in interchange events. Consequently, most of the dsb's induced by ionizing radiation in a random manner within a chromosome territory will give rise to intrachanges, or be reconstituted in the original way. Thus, the territorial organization of chromosomes in the cell nucleus explains the low ratio of interchanges to intrachanges and reconstitutions (Edwards et al., 1994).

2. Influence of the nuclear distribution of chromosome territories

The probability that dsb's formed in the same two chromosome bands (corresponding to subdomains of a few Megabase pairs) become involved in reciprocal translocations independently in two cells is about $10^{11}$ (Savage, 1990). Geometrical constraints which influence the possibility of dsb's to come together due to specific patterns of chromosome territory distributions, however, should modify such a probability. As outlined above, the formation of interchanges should occur only at sites of the enveloping surfaces of two (or more) chromosome territories which are adjacent to each other. In mitotically active human cell types, such as human amniotic fluid cells, fibroblasts and PHA stimulated lymphocytes, it has been noted that the spatial distribution of chromosome territories in the cell nucleus is variable (Emmerich et al., 1989; Popp et al., 1990b; Dietzel et al., 1995; Fig. 2), although the distribution of specific chromosome territories cannot be sufficiently explained by models of a random distribution, even taking into account geometrical constraints provided by the territory size and shape (Münk et al., 1995). This variable positioning explains the observation that a given chromosome can form interchanges with many other chromosomes.

The area of a chromosome territory surface which is attached to the nuclear envelope would not participate in translocation events. Thus, two chromosomes with the same enveloping surface area and the same number of dsb's may participate in interchanges with different frequencies, according to differences in the nuclear membrane attachment areas. If homologous chromosome territories show somatic association, the contact areas between them could also not participate in interchanges with other non-homologous chromosomes. Furthermore, chromosome territories with the same DNA-content, the same number of dsb's and the same nuclear membrane attachment areas may also provide different interchange frequencies, if their shape (and thus the size of their adjacent surface areas) is different. Furthermore, a change in the positioning of chromosome territories as a function of cell cycle and cell type (e.g., Manuelidis, 1984; Ferguson and Ward, 1992; Hulspas et al., 1994) is expected to influence the interchange frequencies between two given chromosomes.

Chromosome territory distributions appear to be influenced also by physiological variables such as the Ca$^{2+}$ level (De Boni, 1994). If so, external stimuli on the cellular membrane may trigger a chain of events (e.g., Ca waves) which eventually change the distribution of chromosome territories. As a consequence, the probability of specific interchange events would also be modified.
2.2. Models of chromosome territory organization and topological predictions for chromosome aberration formation

2.2.1. Implications of the random walk/giant loop model of chromosome territories for chromosome aberration formation

Recently, the folding of chromosome territories in nuclei of human diploid fibroblasts at G₀/G₁ was experimentally studied by measuring distances between FISH signals of DNA clones that were physically mapped along certain chromosomes (Yokota et al., 1995). The results of these measurements supported a random walk/giant loop model of chromosome territories with two organizational levels at scales > 100 kbp (Sachs et al., 1995; see also Hahnfeldt et al., 1993). The giant loops in the order of 3 Mbp were considered to be attached to a randomly folded backbone structure. This model predicts a largely random geometry of the chromosome territory as a whole. Accordingly, it has been concluded that a systematic compartmentalization of subregional chromosomal domains, for example in gene-rich and gene-poor subregions, appears unlikely and the three-dimensional localization of any given target sequence should show a large variability, i.e., the sequence may be located at the territory surface in one case and in the interior in another (Yokota et al., 1995). Furthermore, one should expect that the 3D-positioning of a given loop might change substantially in given chromosome territory simply due to thermic movements. Accordingly, almost any section of a chromosome territory would have a similar probability to be exposed at the peripheral territory surface adjacent to other chromosomes and thus from a topological point of view chromosomal interchanges should occur with similar frequencies in R- and G-bands.

Published data (Yokota et al., 1995) indicate that projected 2D interphase distances between two sites with a genomic separation > 10 Mbp vary by several micrometers. Since the typical overall diameter of a human chromosome territory is also in this range (Bischoff et al., 1993; Eils et al., 1995a, 1996), one would expect a substantial amount of intermingling between two arm domains of the same chromosome territory and by the same token between adjacent chromosome territories. These expectations seem to be in conflict with the apparently small amount of intermingling which was observed experimentally between adjacent chromosome territories, as well as chromosome arm domains (see above). Limitations of the sensitivity of present FISH protocols, however, may have led to an underestimation of intermingling giant loops.

3D-measurements of distances between chromosomal subregions of the X-chromosomes and various autosomes, including subtelomeric and centromeric regions, support a highly flexible folding (Dietzel et al., in preparation). The boundary conditions of the model of Sachs et al. (1995) fitted these 3D-data sets reasonably well for some chromosome territories and arm domains but yielded significant differences for others. In particular, we noted a strong difference in 3D-distance distributions between genes located at Xp and Xq for active and inactive X-chromosome territories (Dietzel et al., in preparation). At present the limited and – even worse – anisotropic resolution of confocal laser scanning fluorescence microscopy, which for conditions used in viewing cell specimens is in the order of some 300 and 800 nm, respectively (Rinke et al., 1996), does not allow to study chromosome territory and arm domain surfaces in sufficient detail. Advanced types of laser scanning far field fluorescence microscopes are presently being developed in several laboratories that will provide a much higher 3D-resolution (see below).

2.2.2. Implications of the interchromosomal domain (ICD) compartment model for chromosome aberration formation

In contrast to the random walk/giant loop model discussed above, the ICD-compartment model (Fig. 3) predicts a highly non-random 3D-organization of chromosome territories and nuclear architecture (Cremer et al., 1993, 1995; Zirbel et al., 1993). This model proposes a hypothetical, three-dimensional network of intranuclear channels that contain the protein complexes for transcription, splicing and other essential nuclear functions, such as DNA-replication and repair. For transcription such an organization requires that genes are exposed at chromosome territory/chromosomal domain surfaces with access to the channel network. By the same argument one should expect a dynamic organization of chromosome territories and subterritory chromosomal do-
mains that allows the access of DNA-strands to the ICD-channels for replication and repair. The channels supposedly start at the nuclear pores and expand between chromosome territory surfaces, in the following termed inter chromosome territory space (Zirbel et al., 1993; for a similar view see Zachar et al., 1993). From there further branches of the channel network supposedly lead into the chromosome territory interior and further expand between chromosomal domains, such as arm domains, band domains, and individual loop domains. We have termed the entire ICD channel network together with its lining chromatin surfaces the interchromosomal domain (ICD) compartment and the interior of the entire channel network the ICD-space. The ICD compartment may also contain nuclear matrix ele-

![Inter Chromosomal Domain (ICD) Compartment Model](image)

Fig. 3. Interchromosome Domain Compartment (ICD) Model (a): Narrow ICD-channel lined by the surfaces of two genetically inactive chromosomal domains 1 and 2; c.d.s., chromosomal domain surface. In genetically inactive nuclei, channels contain few, if any proteins involved in transcription, splicing, replication or repair (symbolized by open and closed circles). The narrow ICD-channel between two genetically inactive domains are thought to be generated by electrostatic repulsive forces (Cremer et al., 1993). (b and d) Expanded ICD-channels lined by the surfaces of two transcriptionally active chromosomal domains. The interior of the channel shown in (b) contains a matrix core filament (m.c.f.). This filament provides attachment sites for a transcription complex (t.c.), a (pre)mRNA molecule, a splicing complex (s.c.) and a non-chromatin domain (n.c.d.) with unspecified function. From the surface of the left chromosomal domain a chromatin fiber loops out into the channel space. Together with the transcription complex it forms a transcriptionally active micro-domain. The channel in (d) has the same content, but lacks a matrix core filament. It ends at a nuclear pore complex (n.p.c.; ne, nuclear envelope). (c and e) The macromolecule complexes present in the ICD-channel network become highly enriched in nuclear matrix preparations due to the strong reduction of electrostatic forces to be expected under the conditions of nuclear matrix preparations (e.g., high salt conditions). Additional cross-linking may result from oxidative stress during preparation procedures and help to yield large insoluble aggregations in addition to those which are possible already present in vivo. For further details see text and Cremer et al. (1995).
ments either in a polymerized form or possibly also as individual molecules (see below; Cremer et al., 1993, 1995; Zirbel et al., 1995).

Interchromatin channels have been proposed by other authors as well (Blobel, 1985; Chai and Sandberg, 1988; Spector, 1990; Zachar et al., 1993; Kramer et al., 1994; Wansink et al., 1994, 1995; Razin and Gromova, 1995; Razin et al., 1995) although direct experimental evidence for such channels has not been presented. The ICD-compartment model provides a physicochemical explanation for the formation and topology of a channel network based on the following rationale (Cremer et al., 1993): We assume that chromosome territories and chromosomal domains are negatively charged under physiological conditions. This results in a Donnan potential between the interior and exterior of these structures and leads to repulsive electric forces between opposite surfaces of chromosome territories or subterritorial chromosomal domains. In addition to a fraction of negatively charged phosphate groups, which are not completely neutralized by histones and other positively charged molecules, negatively charged, chromatin bound non-histone proteins may lead to a further increase of the Donnan potential. We predict that a Donnan potential in the range of a few mV is sufficient to maintain ICD channels in the range of a few nm width. In inactive chromatin the channels may become empty of macromolecules and very narrow, although they should never completely collapse as long as the range of the repulsive forces between negatively charged, opposite chromosomal domains exceeds the very short range of attractive van der Waals forces (Fig. 3a).

We assume the channel width can be modulated by geometrical constraints due to the morphology of chromosome territories and chromosomal domains, by local differences in the size of repulsive forces and by Brownian movements of chromosomal domain surfaces. The assumed physical properties of the ICD-compartment allow for the enrichment and preferential transport of proteins and RNAs within the ICD-space either by channeled diffusion and/or via matrix filaments (Fig. 3b) (for a detailed discussion see Cremer et al., 1995). Consequently, channels in functionally active chromatin with ongoing transcription, DNA-replication or repair processes can strongly increase in size due to the preferential formation of the respective macromolecular domains within the channel network. Very strong enlargements of the ICD-space are compatible with the experimental observation of macromolecular domains visible as 'speckles' or 'foci' (see Spector, 1990; Zirbel et al., 1993; Cremer et al., 1993 and references therein).

2.2.3. Formation of inter- and intrachanges within the ICD-channel network

According to the ICD-compartment model, double strand breaks formed in different chromosome territories and located within (or transferred to) a common boundary zone can lead to an interchange by illegitimate repair of two ends in the interchromosome territory space. Dsb's which are illegitimately rejoined in channels located within a chromosome territory can lead to an intrachange. These considerations imply that the frequency of chromosomal exchanges (and deletions as well) should depend not only on the surface areas of adjacent territory and subchromosomal domains but also on the availability and accessibility of repair complexes at these surfaces. The model further predicts that any legitimate or illegitimate repair of dsb's induced within chromosomal domains at a given distance from ICD-channels requires the movement of these dsb's to the chromosome domain surface in order to obtain access to the DNA-repair machinery. Alternatively, one may consider the possibility that the structure of a chromosomal domain containing dsb's changes from a 'closed' to an 'open' configuration that allows access of repair factors and functional complexes to the chromosomal domain interior. Local or general changes in the structure of chromosome territories/subterritorial chromosomal domains which change the accessibility of repair complexes, e.g., by modulating the width of ICD-channels and/or by modulating the extent of intermingling of giant loops, should also change the frequency of chromosomal aberrations.

2.2.4. Implications of 'Nuclear matrix' models for chromosome aberration formation

We have argued that the ICD-compartment may be considered as the in vivo equivalent of biochemical nuclear matrix preparations and consequently may contain in vivo nuclear matrix constituents (see
above; Cremer et al., 1995). Accordingly, major conclusions derived from the ICD-compartment model for chromosome aberration formation should be compatible with models of aberration formation developed from nuclear matrix studies. Razin et al. (1995) have noted a close correlation between the positions of known hot spots of DNA rearrangements around the human MYC gene and the positions of matrix attachment areas. According to the ICD-model of aberration formation, one would predict a localization of the MYC gene at or near the surface of the chromosomal domain that harbors this gene and near to the in vivo nuclear matrix equivalent located in the accompanying ICD-space. Preliminary evidence obtained by FISH combined with 3D confocal microscopy and image analysis supports the view that MYC is located at the periphery of chromosome 8 territories (Eils et al., 1995b).

3. Quantitative prediction of relative translocation frequencies based on three-dimensional microscopy of chromosome territories

3.1. Rationale

The following rationale will be discussed for the formation of interchanges (translocations) between different chromosome territories. An analogous formalism may be applied also to the formation of intrachanges between parts of the same chromosome territory, as long as such a territory consists of mutually exclusive domains. In a first attempt to predict in a quantitative way relative translocation rates from territory surface ratios and nuclear distribution of territories, one may assume that the translocation rate (t-rate) of a given chromosome is proportional to the mean enveloping surface area (Aadj) which is adjacent to the surface of another chromosome territory in a given cell population:

\[ t - \text{rate} = \kappa \times \text{Aadj} \]

where t-rate is the number of interchanges involving the chromosome studied divided by the number of cells analyzed and divided by the copy number of this chromosome per cell.

Kappa is dependent on a number of biochemical, biophysical and topological factors, such as the presence and distribution of repair complexes, RNAs and DNA-sequences on the surface of a chromosome territory, the distribution of distances between two neighboring territories, diffusion coefficients, mobility of broken chromatin fibers, and dynamic changes of the topology of a chromosome territory. These variables may vary for different chromosome territories and chromosomal domains studied in different cell types and at different stages of the cell cycle.

Below, for a strongly simplified estimate we will consider only topological parameters. Let us assume as a first approximation that kappa is a constant for a given chromosome territory in a given cell type and at a distinct stage of the cell cycle. To what extent the assumption of such a simple proportionality between translocation rate and Aadj is useful has to be studied experimentally. An initial estimate of Aadj for a given chromosome territory can be obtained by measuring (and modeling, see below) the enveloping surface of that chromosome territory in a series of nuclei, neglecting its possible attachment to the nuclear membrane.

3.2. Three-dimensional measurements of the enveloping chromosome territory surface and its use in quantitative estimates

To provide experimental data for the hypothesis outlined above, we have started to measure the three-dimensional surface areas of individual, painted chromosome territories. These measurements were obtained from painted and 3D-reconstructed chromosome territories (Fig. 1; for details see Eils et al., 1995a, 1996). The surface area determined by this approach was taken as a measure for the total enveloping surface of a given chromosome territory (referred to as Aenv). Aenv does not take into account the possible extent of interdigitation of chromatin from neighboring chromosome territories and the surface area in direct contact with the nuclear membrane (and/or with a homologous chromosome territory).

As an example for the use of such 3D-measurements, an attempt is made to interpret the aberration rates observed for the X-chromosome by Tanaka et al. (1983) and Mühmann-Diaz and Bedford (1994) following the rationale of the ICD-model for aberrations...
tion formation. Surprisingly, it was found (Bischoff et al., 1993; Cremer et al., 1993; Rinke et al., 1995; Eils et al., 1995a, 1996) that in human amniotic fluid cell nuclei the active X chromosome (Xa), and the inactive X chromosome (Xi) have similar volumes. If the hypothesis is accepted that the interchange rate observed for a given chromosome territory is directly proportional to its volume, the t-rates for Xa and Xi should be very similar. This assumption, however, does not fit the experimental data described by Tanaka et al. (1983) and Mühlmann-Diaz and Bedford (1994) (see above). Most interestingly, our data demonstrate that the shape of Xi-territories was rounder and their surface areas were significantly smaller as compared to the Xa-territories. The mean enveloping surface ratio from 67 human amniotic fluid cell nuclei with identified Barr bodies between Xa and Xi was 1.40 (Eils et al., 1996).

Taking into account these differences in Xa- and Xi-territory surface areas, let us assume identical translocation rates for the X chromosome in male cells (Xm) and the active X chromosome (Xa) in female:

\[ t - \text{rate}(Xm) = t - \text{rate}(Xa) = \kappa(Xa) \times \text{Adj}(Xa). \]

To estimate the mean translocation rate for the two X chromosomes in female cells,

\[ t - \text{rate}(Xa + Xi) = [t - \text{rate}(Xa) + t - \text{rate}(Xi)] / 2, \]

it is necessary to first estimate the translocation rate of Xi alone. This may be done in the following way:

\[ t - \text{rate}(Xi) = \kappa(Xi) \times \text{Adj}(Xi) = \kappa(Xi) \times \text{Adj}(Xi) \times \text{Adj}(Xa) / \text{Adj}(Xa). \]

The factors \( \kappa(Xi) \) and \( \kappa(Xa) \) describe the probability that at a given number of dsb’s and at a given size of the mean adjacent surface area, an interchange with another chromosome territory takes place. As noted above, biochemical differences between the X-chromosomes, reflecting e.g., the degree of DNA-methylation, histone acetylation, as well as the binding of XIST RNA (Clemson et al., 1996) may also contribute to differences in the frequencies with which Xa- and Xi-territories take part in interchange events.

In case that \( \kappa(Xi) = \kappa(Xa) = \kappa \), it follows from \( \kappa \times \text{Adj}(Xa) = t - \text{rate}(Xa) \) that

\[ t - \text{rate}(Xi) = (t - \text{rate}(Xa)) \times (\text{Adj}(Xi) / \text{Adj}(Xa)). \]

The mean surface areas \( \text{Adj}(Xi) \) and \( \text{Adj}(Xa) \) adjacent to non-homologous chromosome territories can be obtained from the measured enveloping surface areas \( \text{Aenv}(Xi) \) and \( \text{Aenv}(Xa) \), if the attachment areas of Xi and Xa to the nuclear membrane (and/or to each other) are known (factors \( q(Xi) \) and \( q(Xa) \)):

\[ \text{Adj}(Xi) = q(Xi) \times \text{Aenv}(Xi); \]
\[ \text{Adj}(Xa) = q(Xa) \times \text{Aenv}(Xa). \]

To account for the strong association of the Barr body with the nuclear membrane, one may (as a first approximation) assume that the surface area fraction of Xi participating in translocations is considerably smaller than the surface area fraction of Xa, e.g., \( q(Xi)/q(Xa) = 0.5 \). Under these assumptions, \( t - \text{rate}(Xi) = (t - \text{rate}(Xa)) \times 0.5 / 1.4 \)

follows for the translocation rate of the inactive X-chromosome. Alternatively, if we assume that \( q(Xi) = q(Xa) \), we obtain

\[ t - \text{rate}(Xi) = t - \text{rate}(Xa) / 1.4. \]

A re-examination of the data given by Tanaka et al. (1983) using the rationale outlined above suggests that the significantly lower breakpoint numbers observed for X-chromosomes can be explained assuming (i) that \( \kappa(Xi) = \kappa(Xa) \), \( q(Xi)/q(Xa) = 0.5 \), and (ii) that the measured three-dimensional surface ratio \( \text{Aenv}(Xa)/\text{Aenv}(Xi) = 1.40 \).

As a second example, we have reanalyzed the data on X-aberrations (exchanges, deletions) per cell reported by Mühlmann-Diaz and Bedford (1994) assuming that the number of deletions can be neglected. Using the same reasoning as outlined above, the number of X-aberrations per cell (T) may be estimated as

\[ T(46,XY) = T(Xa); \]
\[ T(46,XX) = T(Xa) + T(Xi); \]
\[ T(47,XXX) = T(Xa) + 2T(Xi); \]
\[ T(49,XXXXY) = T(Xa) + 3T(Xi). \]

Assuming again a value of 1.4 for the ratio between the enveloping mean surfaces of Xa and Xi (see above), q values between 1 and 0.5, and \( \kappa(Xi) \) equal to or smaller than \( \kappa(Xa) \) by a factor 2 at most (in case of the cell line with 4X), the experimental data obtained by Mühlmann-Diaz and Bedford were found to be compatible with the model outlined.
above, without further assumptions, e.g., concerning compaction of the heterochromatic X, cellular selection etc.

3.3. Perspectives of 3D-measurements of chromosome territory organization and nuclear architecture for the study of aberration formation

The above cases should only serve as examples, how chromosomal aberration rates from experimental data can be compared with data deduced from model assumptions based on our present and still crude knowledge of chromosome territory organization. For the X-chromosome, the translocation rates following irradiation should be measured separately for Xa and Xi. Furthermore, experimental data are essential that quantitate the extent of the DNA-strand coupling efficiency (included in the term \( K \)) with regard to the biochemical status of a X-chromosome territory (e.g., methylation, XIST-RNA binding).

More detailed comparisons with measured chromosome translocation rates in general are presently not possible due to the lack of models based on an extensive knowledge of the dynamics of chromosome territory morphology and distribution for a given cell type. For example, to develop such models and test their validity for the formation of interchanges between specific sequences (e.g., abl and bcr; Tkachuk et al., 1990) in more detail, it is necessary to measure the frequency distribution of 3D-distances for such sequences. To test the validity of the these models for the intrachromosomal breakpoint distribution, it is necessary to measure the relative 3D-positions of the respective sequences (e.g., R-bands, G-bands; specific active and inactive genes etc.) within the chromosome territory with appropriate precision. The desired precision is presently impaired by the limited ‘practical’ 3D-resolution of present confocal laser scanning fluorescence microscopes (CLSM) (see above). A human chromosome territory typically has a diameter of a few micrometers, while a CLSM – due to its impaired axial resolution (see above) – provides a 3D-resolution of about 0.8 micrometers only (Rinke et al., 1996). While a somewhat more precise localization of 3D-gene positions can be achieved to a certain degree by taking into the point spread function of the microscope and statistical analysis, the development of far field light microscopical procedures with improved 3D-resolution is an urgent need for future studies. Such procedures have been conceived (Cremer and Cremer, 1978; Hell and Kroug, 1995) and in part already realized (Bradl et al., 1992, 1994; Hell and Wichmann, 1994; Hell et al., 1994; Hänninen et al., 1995). It is expected that future far field light microscopy will provide an effective 3D-distance resolution color < 100 nm between fluorescence labeled targets of the same. The precision of the relative localization of a given target as compared to the localization of reference targets may become even better, i.e., down to a few nucleosome diameters only.

4. Quantitative estimates based on model calculations of nuclear structure

4.1. Simple geometrical models

The general dependence of the interchange frequency per chromosome \((t\)-rate) on the surface area of autosomes can easily be estimated for a few highly simplified alternatives: Chromosome territories are assumed to have simple geometrical forms and each territory is assumed to be attached to the nuclear envelope and to homologous territories with a constant fraction of its surface.

(a) If autosomes are represented by rod like structures, i.e., by cylinders of equal diameter with length proportional to DNA content (J. Aten, personal communication), the chromosome territory surface area increases about linearly with its DNA content.

(b) The same relationship as in (a) is obtained, if chromosome territories are represented by volumes with equal, constant cross sections along length (e.g., hexagons, Savage and Papworth, 1973) and with lengths proportional to DNA content.

In cases (a) and (b) the \( t\)-rate can also be approximated by a linear function of the DNA content as in models which were proposed for the induction of chromosome translocations without taking into account the existence of chromosome territories.

(c) In the hypothetical case of spherical chromosome territories with a volume proportional to DNA content, the surface area (and thus the \( t\)-rate) would be proportional to \((DNA\ content)^{2/3}\).
(d) More elaborate geometrical models take into account that the distribution of chromosome territories in the nucleus has to be compatible with the principle of mutual exclusiveness and that the distribution of point like targets belonging to a given territory is restricted by geometrical constraints resulting from the chromosome territory extension. Monte Carlo simulations performed on this basis assuming spherical chromosome territories with a volume proportional to their DNA content resulted in strong deviations of point like target distributions from models simply assuming a random distribution of points (Miinkel et al., 1995). Such a model implies that translocation rates do not depend solely on the DNA content of chromosome territories but also on the territory distribution enforced by geometrical constraints.

4.2. Computer simulations of nuclear architecture for estimates of relative translocation rates: a first approach

The progress in computer sciences has made possible to estimate relative translocation rates using more sophisticated models for chromosome territories.

4.2.1. Implications of the spherical subdomain model of nuclear architecture

Relative chromosome territory surface size (Aenv) estimates were obtained by computer simulations of

![Computer simulation of nuclear architecture using the 'spherical subdomain' model](image)

Fig. 4. Computer simulation of nuclear architecture using the 'spherical subdomain' model (a) 3D-computer representation of a simulated human cell nucleus. The 46 simulated model chromosome territories (represented by a total of 6032 spherical elements) are shown individually in false colors. A spherical envelope (not drawn) provides a geometrical constraint that keeps the chromosome territories together within the boundaries of the spherical model nucleus also resulting in certain deformations of the territory shapes. (b) Median section through the model nucleus shown in (a): Grey color indicates the overlapping zones between different model chromosome territories. These overlapping zones are the consequence of the non-rigid, repulsive interaction potentials used (Miinkel et al., manuscript in preparation). Intranuclear 'gaps' free of model territory elements are indicated by black areas. (c) Section through nucleus at 1/3 of height and (d) at 2/3 of height (compare arrows in (a)).
models of entire human cell nuclei using the following approach (Münkel et al., in preparation): In the starting configuration, the 46 territories of a male human nucleus were simulated as compact, globular structures composed of small spherical elements held together by weak attractive forces. The number of elements of each model chromosome ‘territory’ was chosen to be proportional to its DNA content. The largest ‘territory’ (No. 1) was composed of up to 250 spherical elements. Thus, in this ‘spherical subdomain model’, one element (‘subdomain’) corresponds to a size of about one Megabasepair (compare Morton, 1991). All these (so far globular) model chromosome territories were then placed in a random position into a spherical volume representing the volume of the cell nucleus. To get a reasonable acceptance rate for this procedure, all model chromosome territories were diminished by a common factor so that they became small enough not to overlap with each other or with the border of the ‘nuclear volume’. Then the elements were enlarged step by step until the model chromosome territories regained their original volumes. All elements were allowed to move according to the actual forces derived from polymer modeling. As a consequence, the final position, form and surface size of these model chromosome territories deviated from the initial globular configuration; furthermore, the position of a model chromosome territory (as defined by its barycenter) also changed. The complete simulation of one model nucleus, using a total of 6032 elements (250 for one chromosome 1 model territory), took about several hours on a modern workstation, all 384 nuclei about 2 days on a parallel computer (Fig. 4a). The simulated non-rigid, repulsive interaction potentials between elements of different territories resulted in relative small overlapping border zones between the model chromosome territories (Fig. 4b–d). Note that constraints in the simulated chromosome territory architecture led to the formation of chromatin free gaps of variable size between the model chromosome territories (see black areas in Fig. 4b–d). In the context of the ICD-compartment model such gaps may be considered as extended sections of the ICD-channel network. These gaps could provide space for the formation of foci (Zirbel et al., 1993) or speckled domains, e.g., SC35-domains (Xing et al., 1995). Genes exposed at chromosome territory surfaces (see above) could be located at the border of these speckled domains (Xing et al., 1995). So far, a total of 384 nuclear configurations was obtained in this way. From these configurations, for each model chromosome territory estimates (in arbitrary units) were obtained for means of the surface area, the roundness, and the distance of the barycenter from the nuclear center. Furthermore, the average contact surface of a given model chromosome territory with any other model chromosome territory was calculated (Fig. 5).

An increase of the mean enveloping surface areas of the model chromosome territories with the exponent 2/3 was found as in the simple geometrical model C; the average contact surface of a model chromosome territory A with a model chromosome territory B increased with the number of elements (DNA content) of both A and B. Differences were obtained also for the mean distances of the model chromosome territories from the border of the spherical nuclear ‘volume’, and for the mean roundness of the territories, as a function of their element number (proportional to DNA content).

Finally, the probability was determined that two territories hit by a randomly placed straight line were adjacent to each other. From this, a ‘relative mean

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**Fig. 5.** Quantitative estimates of relative contact surface areas, roundness, and nuclear positions of model chromosome territories according to the ‘spherical subdomain’ model (see text). (a) Average relative contact surface areas (arbitrary units) between individual model chromosome territories. On the two abscissas, the chromosome numbers (autosomes 1–22; 23 (X); 24 (Y)) are given. On the ordinate, for each homolog-homolog combination, the average contact surface area obtained from 384 spherical model nuclei with the same radius is presented. (b) The mean roundness (± standard deviation) obtained for the individual model chromosome territories in (a) is given as a function of the relative chromosomal DNA-content (Morton, 1991; No. 1 = 1.0) proportional to the number of elements used in the simulation of each model chromosome territory. For each simulated model chromosome territory, the roundness R (ordinate) was determined from the three moments of inertia \( \lambda_1 \geq \lambda_2 \geq \lambda_3 \), with \( R = (\lambda_2 \times \lambda_3)^{1/3} / \lambda_1^{1/3} \). The greater the deviation from a spherical form, the smaller is the roundness R. (c) The average mean distance (± standard deviation) from the barycenters of the model chromosome territories to the nuclear center of the 384 simulated model nuclei is given (in units of nuclear radius). Abscissa: Relative DNA-content (see b).
Fig. 6. Abcissa: Relative DNA-content of chromosomes (see Fig. 5) Ordinate: Computed translocation rates obtained from the 'spherical subdomain' model (compare Fig. 4Fig. 5 and text). For comparison, translocation rates are also shown for simple geometrical models (see text) yielding a linear relationship (solid line) or a relationship where translocation rates increase with the exponent 2/3 of the chromosome territory DNA content (dashed line). Note that the expected differences for the three models are small assuming random distributions of chromosome territories (i.e., distributions solely affected by geometrical constraints; see also Münkell et al., 1995).

translocation rate' (plus/minus standard deviations) was calculated for each territory (Fig. 6). Again, the deviations from the predictions of the purely geometrical models (a–c) were relatively small. Nonetheless, the ‘spherical subdomain model’ appears to be preferable, since it takes into account mutual exclusiveness of territories as well as variability of shape and nuclear distribution.

4.2.2. Computer simulation of chromosome territories according to the random walk/giant loop model

Computer simulations of entire chromosome territories (Ch. Münkell and J. Langowski, unpublished results) using the principles of the random walk/giant loop model (Sachs et al., 1995; Yokota et al., 1995) confirmed the qualitative expectations drawn above concerning a high degree of intermingling between chromosome arm territories, as well as a random positioning of individual sections on the enveloping surface of a territory. To reconcile the general features of the random walk/giant loop model with the observed low degree of chromosome arm intermingling and with the observed non-randomness of intrachromosomal breakpoint distribution, the model was modified substantially (Ch. Münkell and J. Langowski, unpublished results), using the principle of mutual exclusion of chromatin fibers. Instead of a structurally continuous backbone as suggested by Sachs et al. (1995), it was sufficient for the modeling of chromosome territories to assume (i) that giant loops are held together at their basis by linker proteins, and (ii) that individual loops are connected by linker DNAs of appropriate length. With this modified model, it was possible to estimate the enveloping volumes and surface areas of a ‘territory’ and of its ‘arms’ and predict a low degree of intermingling between the chromosome arm territories. Furthermore, it became possible to reconcile the findings of a more peripheral positioning of genes within a chromosome territory (see above) by assuming that the gene poor G-band domains are formed by smaller and more frequent giant loops than the gene rich R-band domains.

Eventually, advanced computer simulations of chromosome territory and nuclear architecture will provide quantitative topological predictions for the distributions of breakpoints involved in chromosome exchanges. Such improved models need also to take into account the possibility that chromosome territory and nuclear architecture may be influenced by the formation of double strand breaks and resulting repair processes.

5. Conclusions and suggested experiments

Half a century ago, H.J. Muller (1941) formulated an important principle concerning the relationship between nuclear structure and chromosomal aberrations, stating that the distance between broken chromosome ends will be of great importance. A large part of following models for aberration induction was based explicitly or implicitly on a model of the mammalian cell nucleus summarized by Comings (1968) where the euchromatin-fibers constituting each interphase chromosome are distributed over a major part of the nuclear volume and thus intermingle to a very large extent. More recently, it was demonstrated that each chromosome in a mammalian cell nucleus, as well as in nuclei of other eukaryotes,
occupies a limited part of the nuclear volume, forming a chromosome territory. Thus, the old idea of the cell nucleus as an architecture consisting of mutually exclusive parts was validated. Accordingly, the idea of the irradiated nucleus as a bag of broken chromosome ends moving around in a statistical way seeking new partners, or being fixed by protein constraints but otherwise interacting in a purely random way, had to be revised.

In the above contribution the present experimental basis for a territorial chromosome architecture in the cell nucleus was described. Several examples were presented, how such data could be used to estimate relative aberration rates for specific chromosomes. Furthermore, computer simulated models of chromosome territory and nuclear architecture were outlined with the potential to offer an advanced level of precision in the prediction of relative aberration rates. The rapid progress in parallel computing will make it eventually possible to simulate the nuclear distribution of specific chromosomal territories, arm domains and giant loop domains taking into account dynamic structural changes and movements of these structures.

For further tests of the implications of chromosome territories for the induction of chromosome exchanges, it is essential to measure surface areas of chromosome territories, of arm domains, and of still smaller domains, such as band domains or even individual loop domains, and to study the possible expansion of an enveloping surface by chromatin interdigitation and intermingling, as well as the fraction of the chromosome territory surface of a given chromosome territory associated with the nuclear envelope and with the surfaces of other homologous and non-homologous chromosome territories. It is also necessary to measure the target distribution (e.g., of active and inactive genes) within the chromosome territories themselves. Since chromosome territory organization likely depends on cell type, stage of the cell cycle, repair activities etc., such investigations need to be performed with explicit reference to these parameters. At the same time, a much larger body of experimental data for irradiation induced exchange rates can be expected from the combination of FISH, especially combinatorial multi-fluor FISH (Speicher et al., 1996) and automated evaluation procedures (Hausmann et al., 1991; Cremer et al., 1992; Fantes et al., 1995). Eventually, the experimental and theoretical elucidation of the three-dimensional and (considering the time scale as well) four-dimensional chromosome territory and nuclear architecture combined with the high precision monitoring of specific metaphase aberration frequencies should lead to an improved understanding of the role of the functional nuclear architecture not only in biological dosimetry but also in the induction of cancer related chromosome aberrations.

Acknowledgements

This work was supported by grants from the Deutsche Forschungsgemeinschaft and the European Community (Biomed 2) to C.C. and T.C. We are particularly grateful to Drs. J.A. Aten (Amsterdam), U. Hagen (Munich), A.T. Natarajan (Leiden), G. Obe (Essen), R. Sachs (Berkley), J.R.K. Savage (Didcot), and K. Tanaka (Hiroshima) for stimulating discussions. We also gratefully acknowledge the help of J. Rauch in the editing of the manuscript.

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