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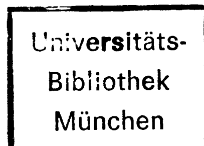
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Front Cover (Paperback): The scaffold/loop model can explain chromosome bands. This extended scaffold/loop model displays the unfolded, hypothetical chromatid fiber on the left; this fiber serves to explain the more compact metaphase chromosome on the right. (For details, see Saitoh and Laemmli, p. 757, this volume.)

Back Cover (Paperback): Electron density of the mixed sequence nucleosome core particle. (For details, see Richmond et al., p. 268, this volume.)

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Role of Chromosome Territories in the Functional Compartmentalization of the Cell Nucleus

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At the turn of this century, a few cytologists proposed the idea of a territorial organization of chromosomes in the cell nucleus of animal and plant species (Rabl 1885; Strasburger 1905; Boveri 1909) (Figs. 1A and 2A). While most of their scientific peers believed that chromosomes were temporary structures being formed de novo at the beginning of mitosis and completely dissolved in the daughter nuclei, they predicted that chromosomes would retain a structural (Rabl) and genetic (Boveri) identity throughout the cell cycle. When in 1902 Theodor Boveri and Walter S. Sutton merged cytological evidence and Mendelian genetics into the theory of chromosome heredity, Boveri's hypothesis of chromosome individuality formed the cornerstone of it (for review, see Cremer 1985). In his seminal 1909 paper, Boveri postulated that each chromosome territory would occupy a certain part of the nucleus without losing its coherence and without mixing with the other territories.

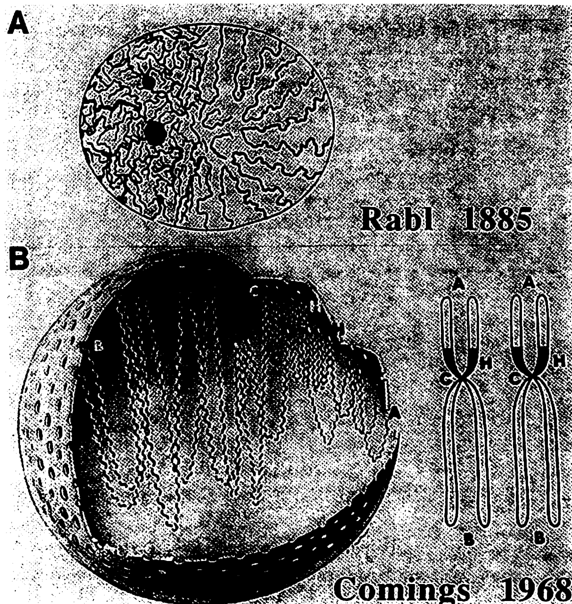

Figure 1. (*A*) Rabl's model of the interphase nucleus. "Primäre Kernfäden" (primary threads) are shown on the right side. Each thread indicates an individual chromosome territory. Numerous lateral projections originating from the primary threads are thought to form a "Kernnetz" (nuclear network) shown on the left side. (Reprinted from Rabl 1885; for further details, see Cremer et al. 1982a.) (*B*) Coming's model of the interphase nucleus (Comings 1968). Attachment sites of the chromatin fiber constituting an individual chromosome are widely separated in euchromatin but crowded together in heterochromatin (H). Consequently, the heterochromatin is a compact entity, whereas the euchromatin is strongly dispersed, resulting in a nonterritorial organization of the interphase chromosomes. Note that the attachment sites of the two homologs shown in this model nucleus are close to each other. (Reprinted, with permission, from Comings 1968.)

After a period of neglect, the concept of a territorial chromosome organization in the cell nucleus was abandoned in the 1960s and early 1970s when electron microscopic studies of cell nuclei from animal and plant species with few exceptions failed to distinguish the hypothetical chromosome territories (Wischnitzer 1973). Instead, models favoring nonterritorial chromosome arrangements became popular (Fig. 1B) (Comings 1968; Vogel and Schroeder 1974). According to the then-prevailing view, euchromatic parts of individual chromosomes in most species and cell types were strongly decondensed, in contrast to heterochromatic regions. Only a few authors still claimed "that chromosomes appear never to decondense to the point that they lose their threedimensional integrity, but remain in distinct domains throughout interphase" (Stack et al. 1977).

Two decades ago, the nucleus was still viewed by many cell biologists and biochemists as a bag with chromatin fibers from the different chromosomes intermingling in the nucleoplasm like spaghetti in a soup. Except for the nucleolus, the importance of a functional compartmentalization of the nucleus for DNA replication, gene expression, RNA processing, and transport has only emerged recently (see, e.g., Blobel 1985; Hutchison and Weintraub 1985; Gasser and Laemmli 1987; Rykowski et al. 1988; Hilliker and Appels 1989; Krystosek and Puck 1990; Manuelidis 1990; Spector 1990; Carter and Lawrence 1991; Haaf

and Schmid 1991; Laskey et al. 1991; Lamond and Carmo-Fonseca 1993; Zirbel et al. 1993). In this paper, we summarize the evidence for a territorial organization of chromosomes in the cell nucleus obtained by laser-microbeam experiments in the late 1970s and early 1980s and, more recently, by in situ hybridization experiments. Advances in three-dimensional fluorescence microscopy, in combination with the generation of DNA probes for the specific delineation of whole chromosomes, chromosome segments, and single genes by fluorescence in situ hybridization (FISH), have provided the means to study the three-dimensional organization of chromosome territories and their suprachromosomal arrangements in detail (for review, see Lichter et al. 1991). In addition, the visualization of other nuclear components, such as transcripts from individual genes and factors of the transcription and splicing machinery, has become possible at the single-cell level (see below).

We illustrate the use of these tools for studies of the volume and shape of the active and inactive X chromosome in human somatic cell nuclei, the distribution of the dystrophin gene within X-chromosome territories, the demonstration of RNA tracks, and the simultaneous visualization of chromosome territories together with components of the splicing machinery. We propose a model predicting that the surfaces of chromosome territories and a space formed between them provide a network-like three-dimensional nuclear compartment for gene expression, mRNA splicing, and transport, termed the interchromosome domain (ICD) compartment (Zirbel et al. 1993). We discuss the possibility that repulsive electric forces exerted by negative surface charges of chromosomes may provide an important mechanism for its maintenance and the confinement of factors within this space. Finally, we discuss further experimental tests of this model based on the development of multicolor FISH and further improvements of three-dimensional microscopy.

Evidence for a Territorial Interphase Chromosome Organization

To distinguish between territorial and nonterritorial models of chromosome organization in the cell nucleus, a laser UV microbeam (Cremer et al. 1974) was applied to microirradiate small subnuclear regions of living Chinese hamster cells cultured in vitro. After pulse-labeling with [^{3}H]thymidine, unscheduled DNA synthesis could be detected in the microirradiated nuclear part. When microirradiated cells were followed to the subsequent mitosis, autoradiography showed silver grains restricted to a few chromosomes (Zorn et al. 1979; Cremer et al. 1982b). The same restriction was obtained when indirect immunofluorescence with antibodies specific for UV-irradiated DNA was applied to visualize microirradiated chromatin at interphase and metaphase (Hens et al. 1983; Cremer et al. 1984). Alternatively, when a local part of the metaphase plate was microirradiated in a living cell, immunofluorescent label was observed in distinct patches in both daughter nuclei (Fig. 3) (Cremer et al. 1984). The results of these experiments clearly support a territorial organization of interphase chromosomes. Further experiments indicate a correlation between the DNA content of Chinese hamster chromosomes and the frequencies with which these chromosomes were damaged by microirradiation of a small nuclear area, a finding consistent with the idea that the relative sizes of chromosome territories would reflect their relative DNA content (Cremer et al. 1982a). Notably, homologs were rarely hit together, arguing against the idea of their close association in these somatic cell nuclei as predicted by the Comings model (Comings 1968).

Isotopic and nonisotopic in situ hybridization experiments with human genomic DNA as a probe made it possible for the first time to visualize entire human chromosome territories directly in somatic hybrid cell nuclei (Manuelidis 1985; Schardin et al. 1985; Pinkel et

Figure 2. (*A*) Strasburger's model of a tissue cell nucleus from *Galtonia candicans* (Strasburger 1905). The hypothetical territories occupied by the individual chromosomes are shown as red and blue entities. (*B*) The two X chromosomes in a metaphase spread prepared from a female human amniotic fluid cell are entirely painted. Biotinylated DNA from a library of sorted human X chromosomes was hybridized, followed by detection via FITC, and chromosomes were counterstained with propidium iodide. Adjacent interphase nuclei show two distinct and spatially separated X-chromosome territories. Note that the two territories in these hypotonically treated nuclei show a difference in condensation. (*C*) Visualization of the territories of chromosomes 2 and 8 in a human lymphocyte nucleus by two-color in situ hybridization using rhodamine and AMCA fluorochromes, respectively. Magnification, 4000 ×. The territories of chromosome 2 are clearly larger than those of chromosome 8. Note that the two adjacent territories are nonoverlapping ("border" indicated by arrows). (*D*) Schematic drawing of an optical section through a cell nucleus according to the proposed model of a functional nuclear compartmentalization (Zirbel et al. 1993). A section through a whole nucleus (*left*) and a detailed view at higher magnification (*right*) are shown. Note that the various constituents of the cell nucleus shown in this scheme are not drawn to scale. The area between the chromosome territories (*red*) constitutes the ICD compartment. Partial DNA loops extending into the ICD space are shown on the right (*red*). According to the model, active genes occur at the surface of the territories, and RNA transcripts (*blue*) are directly released into the ICD space. Transcription factors (*black*) are mostly restricted to the ICD compartment, greatly reducing the volume for searching DNA target sequences. Splicing components (*green*) are concentrated in the ICD space as well. The reticular ICD space also contains nuclear structures enriched in splicing factors like the perichromatin fibrils, interchromatin granules, and coiled bodies. Dense accumulations or binding to extended structures might yield in a "network"-like formation within the ICD space. The chromosome territories are nonoverlapping but may attach at some sites to the nuclear envelope or with each other. The ICD compartment extends between these sites of attachment and is associated with the nuclear pore complexes. Note that in an optical section, the ICD space may appear as channels. The surface of chromosome territories can be greatly increased by infoldings, as outlined within the magnified section (for details, see text).

al. 1986). In 1988, the decoration of entire human chromosomes by chromosomal in situ suppression (CISS) hybridization with chromosome-specific DNA libraries (Fig. 2B,C) confirmed the existence of chromosome territories in nuclei of both normal and tumor cells (Cremer et al. 1988; Lichter et al. 1988; Pinkel et al. 1988). Since then, a territorial organization of chromosomes has been demonstrated in a rapidly increasing number of animal and plant species (Heslop-Harrison and Bennett 1990). In addition, in situ hybridization with probes delineating chromosome segments of various sizes indicated that within the entire chromosome territory, each chromosome part forms its own distinct domain (see, e.g., Rappold et al. 1984; Lengauer et al. 1991).

In the literature, the term chromosome domain has

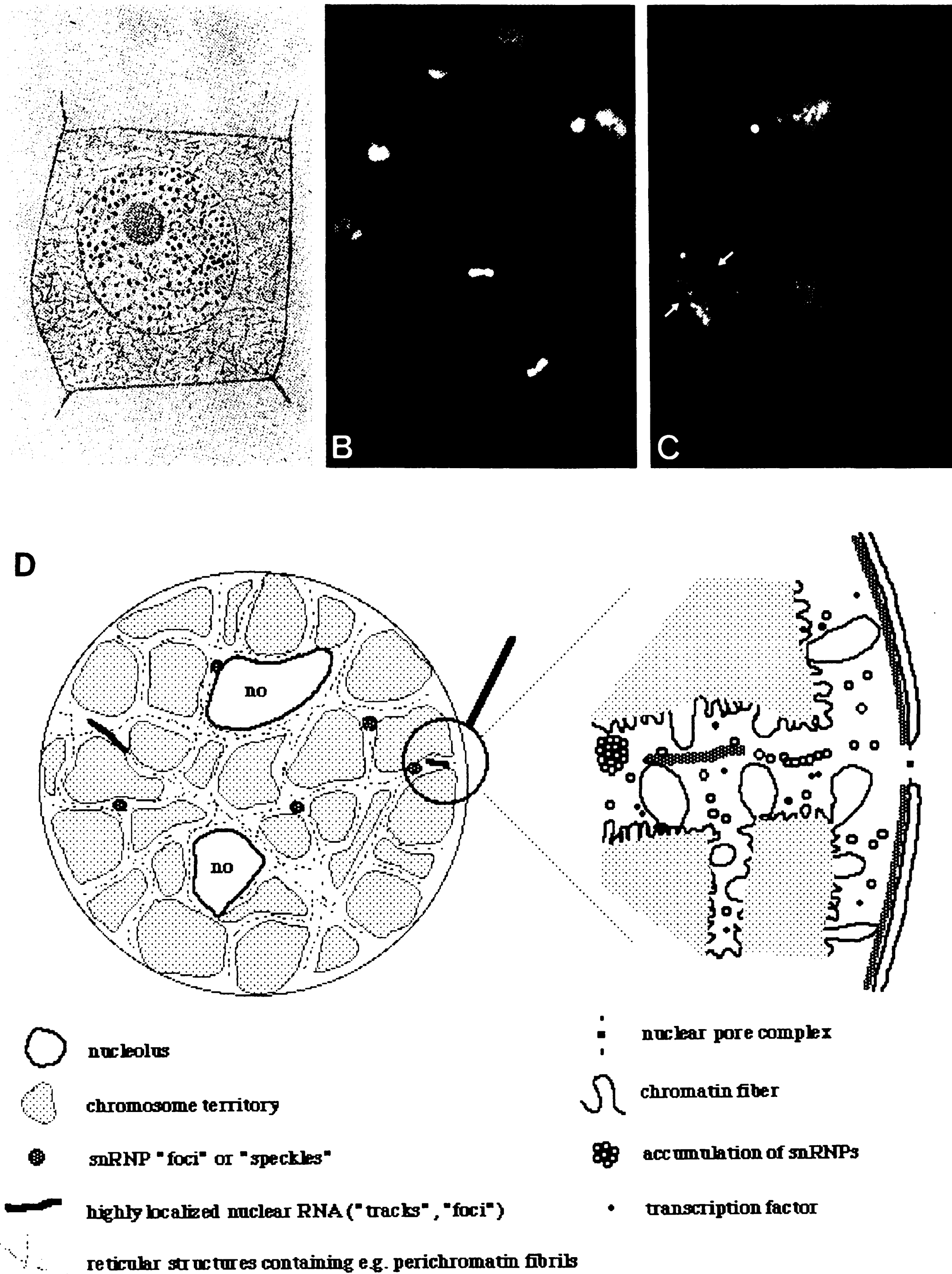

Figure 2. (*See facing page for legend.*)

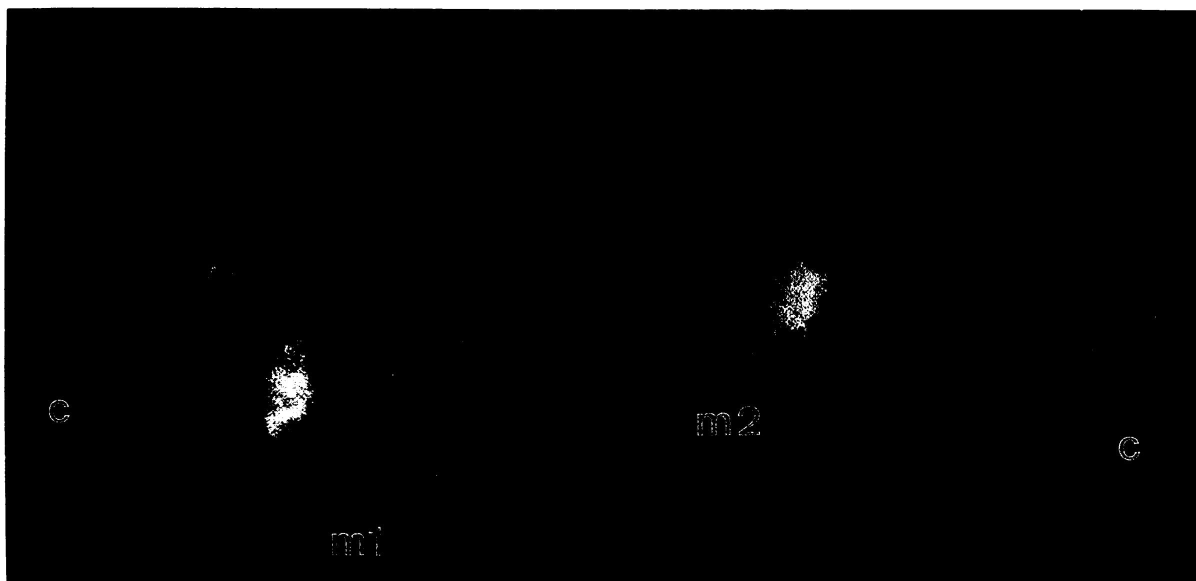

Figure 3. Two daughter cell nuclei (m1 and m2) were fixed with acetic acid/methanol, 4 hr and 25 min after laser-UV-microirradiation of a small part of the metaphase plate of a living Chinese hamster cell in a special cell culture chamber. Microirradiation did not interfere with the completion of mitosis and the formation of apparently normal daughter cell nuclei m1 and m2. Indirect immunofluorescence with antibodies against UV-irradiated DNA (for experimental details, see Cremer et al. 1984) revealed intensely labeled patches within nuclei m1 and m2. This result clearly indicates that the very few metaphase chromosomes hit by the focused UV-microbeam (focal diameter ~ 1 μm) were subsequently organized into rather compact interphase chromosome territories. The possibility remains that a few chromatin fibers would extend largely from the compact mass of such a territory into the nuclear space. Two nuclei from adjacent nonirradiated cells are indicated by c. The microphotograph was deliberately overexposed to outline the nuclear experimental and control nuclei. Magnification, 4000 ×. The help of Dr. K. Nakanishi in this experiment is gratefully acknowledged.

been used as a synonym both for chromosome territories and for chromatin domains. We use the term "chromosome territory" to indicate the domain occupied by a whole chromosome.

In Situ Hybridization and Preservation of Nuclear Topography

Little is known concerning to what extent the chromatin distribution present in nuclei in vivo can be actually deduced from fixed nuclei studied after in situ hybridization procedures (Borden and Manuelidis 1988; Scherthan and Cremer 1993). FISH often requires permeabilization steps removing nuclear proteins to facilitate probe penetration. To test the effect of such steps on the positions of the centromeres, human amniotic fluid cell nuclei were evaluated after fixation with buffered formaldehyde using kinetochore-specific antibodies. After additional permeabilization steps, including treatment with sodium iso-thiocyanate and pepsin digestion, FISH was performed with the alphoid probe p82H hybridizing to the centromeres of all human chromosomes (Mitchell et al. 1985). Avoiding any drying of the nuclei throughout the procedure, fluorescence micrographs indicated an identical distribution of the kinetochore signals before and after

Figure 4. (*A*) Paraformaldehyde-fixed amniotic fluid cell nucleus after kinetochore immunostaining with kinetochore-specific human autoantibodies detected with biotinylated anti-human IgG and avidin-FITC. (*B*) The same nucleus shows an apparently identical kinetochore immunostaining pattern as in *A* after the treatment described for *C*. (*C*) The same nucleus after NaSCN/pepsin pretreatment and FISH with α-satellite DNA probe p82H recognizing all centromeres. Note the colocalization of the hybridization signals (TRITC) of probe p82H and the kinetochore signals (shown in *B* and *C*). (*D,E*) Optical sections (xy-plane) through two HeLa cell nuclei after two-color CISS hybridization with probes delineating the dystrophin genes (*yellow*) together with their corresponding X-chromosome territories (*red*). The two dystrophin signals shown in *D*, as well as the upper signal shown in *E*, are located at the chromosome periphery (for criteria, see Fig. 8 and text), but the location of the lower dystrophin signal of *E* remains doubtful due to the strong infolding of the chromosome surface at this site. (*F–M*) Simultaneous visualization of individual chromosome territories (*red*) and Sm antigens (*green*) by in situ hybridization and indirect immunofluorescence. Digitized images of the FITC and rhodamine fluorescence were acquired separately from the same optical sections (*H*, *I* and *L*, *M*) applying confocal laser scanning microscopy. The spatial relation of chromosome territories and Sm accumulations was assessed after electronic overlay of these images (*F*, *G*, *K*). (*F*) Nucleus of a primary human foreskin fibroblast displaying the territories of chromosome 11 (*red*), which do not coincide with the speckled Sm staining pattern (*green*). Note that the upper chromosome 11 territory does not appear as a contiguous region in this section. (*G–I*) HeLa cell nucleus after visualization of the chromosomes-8 territory (*H*) and Sm antigen staining (*I*). In the overlaid image (*G*), Sm antigen foci do not occur within the chromosome territory. (*K–M*) A corresponding example showing the chromosome 7 territories (*L*) and focal Sm antigen accumulations (*M*) in a HeLa cell nucleus, as well as the overlay of both images (*K*). Bars, 5 μm.

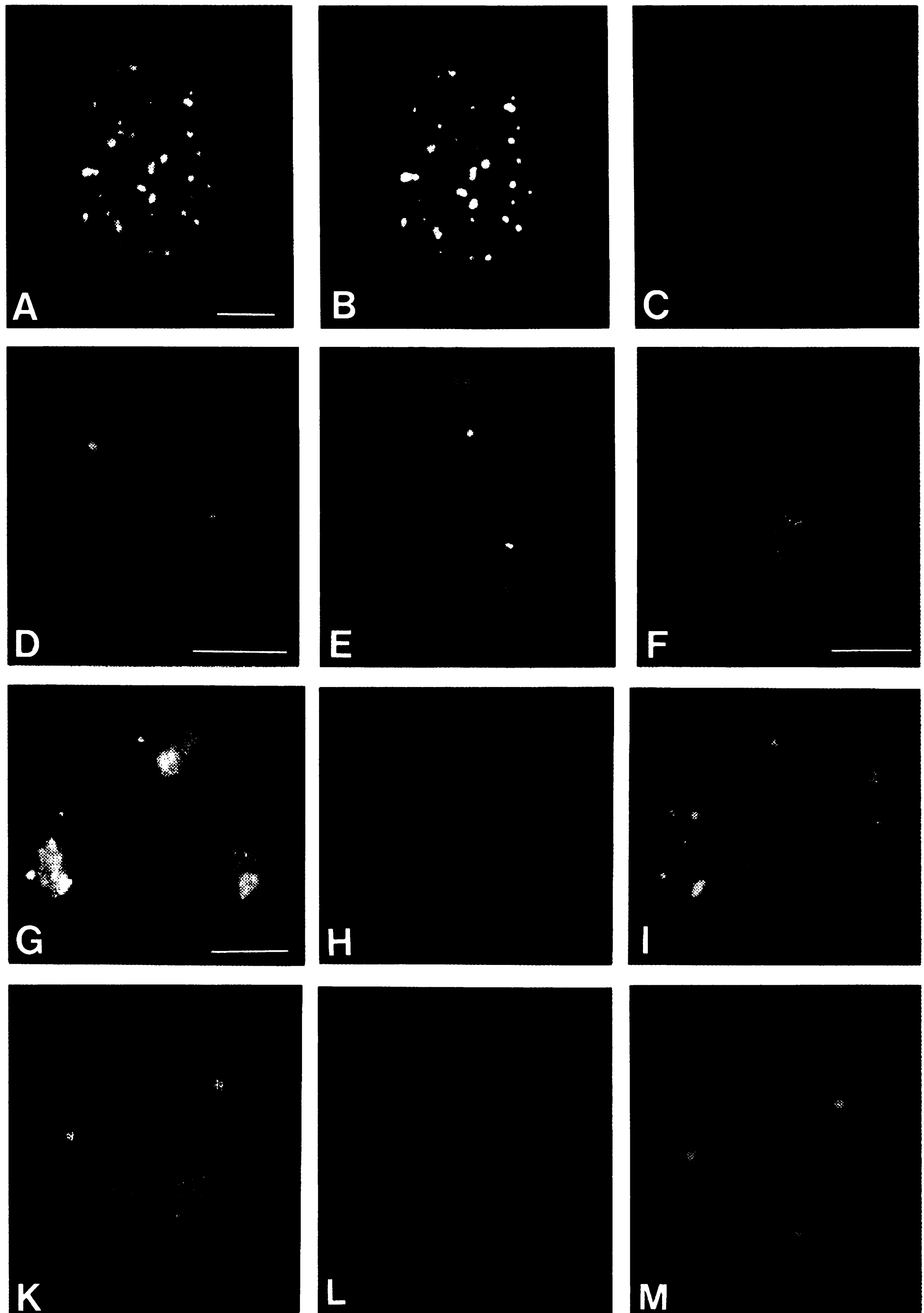

Figure 4. (*See facing page for legend.*)

FISH. Each kinetochore signal colocalized with a differently colored alphoid probe signal, indicating efficient hybridization (Fig. 4, panels A–C)(H. Scherthan and T. Cremer, unpubl.). Other experiments applying laser confocal microscopy in the Nomarski interference mode showed that parameters such as length, width, and height, as well as the position and shape of nucleoli observed in nuclei of living and fixed amniotic fluid cells, could also be maintained after pepsin digestion of formalin-fixed cells and FISH within a variation limit of a few percent (G. Paaz et al., unpubl.). These results indicate that protein/DNA cross-linking induced by formalin fixation is sufficient to maintain essential features of nuclear and chromosome topography throughout the FISH procedure, even under conditions where a large part of the nuclear proteins is removed.

Active and Inactive X Chromosomes in Female Cell Nuclei: A Model to Study Chromatin Compaction Involved in Heterochromatinization

In 1949, Barr and Bertram discovered that a special mass of chromatin, termed the sex chromatin, could be specifically stained in female cell nuclei with cresyl violet and other dyes. The Barr body provides an easily identifiable marker for the position of the inactive X chromosome. Since then, it has been a widely held belief that the inactive X chromosome (Xi) in female cell nuclei is strongly condensed as compared to the largely decondensed active X chromosome (Xa) and, consequently, that genes contained in the heterochromatic chromatin territory may become inaccessible for factors involved in the expression of their homologs in the active chromosome. To study the problem of compaction, painted X-chromosome territories in nuclei of subconfluent human amniotic fluid cell cultures (46,XX) were investigated by light optical serial sections with a confocal fluorescence laser scanning microscope (Fig. 5). X-Chromosome territory volumes were calculated from slice volumes estimated for each light optical section (Bischoff et al. 1993). A reliable segmentation of the chromosome territory boundaries in each optical section is a prerequisite of such measurements. To eliminate the bias from subjective threshold choices, a range of equally spaced thresholds was applied to 33 sectioned nuclei. The entire threshold range was taken into account where (1) a segmentation was possible without merging of the two territories and (2) the volume of the larger X-chromosome territory was $\geq 1\%$ of the nuclear volume. As shown in Figure 6, the area of a segmented chromosome territory section strongly varies with the chosen threshold. Since the localization of the inactive X territory had not been identified in these experiments, volume ratios were determined for the larger territory divided by the smaller one. Such values should provide an upper limit for the volume ratios between the active and inactive chromosome territory. Applying nine equally spaced thresholds for the segmentation of chromosome territories in a typical amniotic fluid cell nucleus, Figure 7A shows that the volumes of the two X territories varied by a factor of about four, whereas the volume ratios varied between 1.22 and 1.35. Figure 7B provides the results of the X-territory volume ratios obtained for the 33 nuclei using 7–12 equally spaced thresholds per nucleus. Notably, for most threshold conditions (89.5%) the volume ratios were less than 2, whereas less than 3% of these ratios showed values greater than 4. Although the possible variability of Xa/Xi volume ratios at different stages of the cell cycle and in different cell types remains to be established, our data indicate that differences in the compaction of heterochromatin versus euchromatin may be more subtle than often thought.

Experiments in which the Barr body was identified prior to CISS hybridization allowed two-dimensional measurements of the maximum length (L) and the maxium width (W) of Xa and Xi. The signed rank test showed a highly significant ($p < 0.0001$) difference between the ratios L/W obtained for the two chromosomes. In agreement with other authors (Walker et al. 1991), these data support the idea of a more extended structure for Xa (Bischoff et al. 1993). A considerable increase of the surface area of Xa versus Xi could result from such a difference in chromosome territory shape. Possibly, Xa is forced into a more elongated shape as compared to Xi due to the exposure of an increased number of genes at the chromosome territory surface (see model of a functional interchromosome domain compartment described below). The size of the surface of Xa as compared to Xi may increase further, if infoldings of the chromosome territory surface occur. Such infoldings would allow an adaptation of the surface area of a given chromosome territory according to its genetic activity in a given cell type and a similar total surface area in the same cell types of different species, even when the karyotypes are largely different.

Distribution of the Dystrophin Gene in X-Chromosome Territories

Little is known about the spatial organization of coding and noncoding sequences within the chromosome territories. As an approach to study the localization of the dystrophin gene in X-chromosome territories, HeLa cells were fixed with buffered paraformaldehyde and subjected to two-color CISS-hybridization as described by Lichter and Cremer (1992) using a digoxigenin-labeled X-chromosome-specific DNA library in combination with a pool of five biotinylated genomic cosmid clones, which extend from exon 47 to exon 51 of the dystrophin gene. Volume measurements of active and inactive X-chromosomes described above were performed in hybridized nuclei subjected to pepsin digestion, but this step was avoided in the following experiments. Permeabilization was achieved with 0.5% Triton X-100 and 0.5% saponin (for further details, see Zirbel et al. 1993). Digitized optical sections of the chromosome territory (TRITC signal) and the gene (FITC signal) were obtained with a Zeiss LSM 10

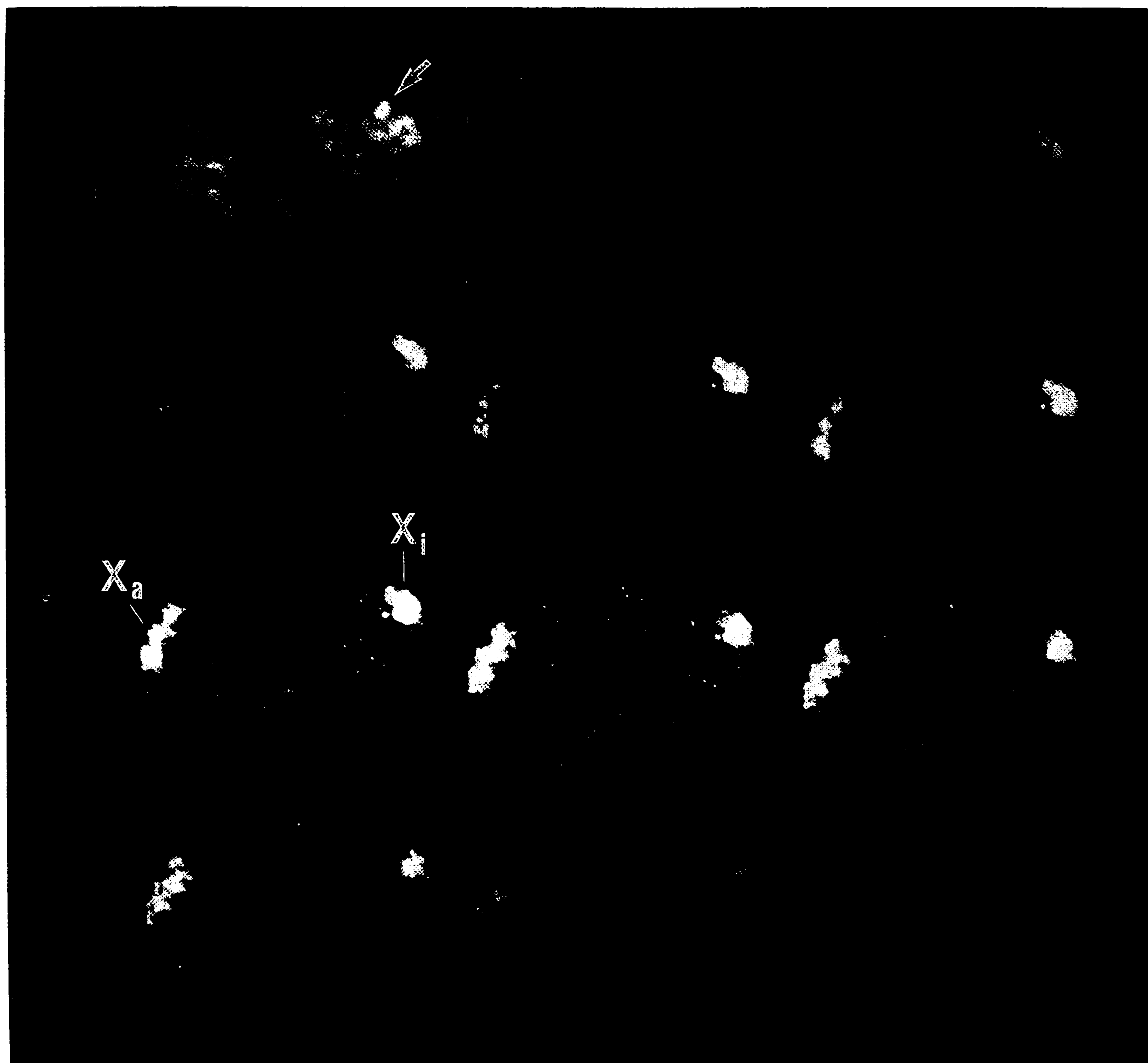

Figure 5. (*Inset*) The Barr body (indicated by arrow) in a human female amniotic fluid cell nucleus stained with DAPI was recorded with an epifluorescence microscope (Zeiss Axiophot) equipped with a Photometrics CCD camera (for details, see du Manoir et al. 1993). The same nucleus was subjected thereafter to CISS hybridization to paint the two X-chromosome territories (for details, see Bischoff et al. 1993). A panel of 11 light optical serial sections with FITC-painted territories (ordered from left to right) was recorded with a Leica confocal fluorescence laser scanning microscope. The distance between adjacent sections was approximately 300 nm. Magnification, 2000 × . One territory (X_i) shows partial colocalization with the Barr body; the other, more elongated territory reflects the active X chromosome (X_a).

confocal laser scanning microscope. Figure 4, panels D and E, show optical sections of two HeLa nuclei with the visualization of X-chromosome territories and human dystrophin genes. To avoid any prejudice with regard to the localization of the FITC signal, a threshold yielding a reasonable segmentation of chromosome territory boundaries was first applied to the TRITC images. After electronic overlay of the TRITC and FITC images, the localization of the dystrophin gene signal within an interior or exterior part of the territory section was determined, as indicated in Figure 8. Analysis of 250 chromosome territories revealed that 208 (83%) of the gene signals were located in the exterior part, 37 (15%) of the signals were located within the interior part, and for 5 signals (2%), such a decision was not possible (see, e.g., Fig. 4, panel E). Segmentation of chromosome territories performed independently by another investigator in a subset of 30 nuclei yielded very similar results. Despite a pronounced variation in individual chromosome territory sections, the total size calculated for all exterior and interior areas of the chromosome territory sections was roughly similar (53% exterior vs. 47% interior). Accordingly, the observed distribution of the dystrophin gene signals is significantly different from the distribution expected in case of their random placement within the interior or exterior part. Notably, dystrophin signals located outside the exterior boundary of the segmented X-chromosome territories were not observed in this experiment, and only rarely (<3%) in several other experiments with different cell types including genes located on different chromosomes (A. Kurz et

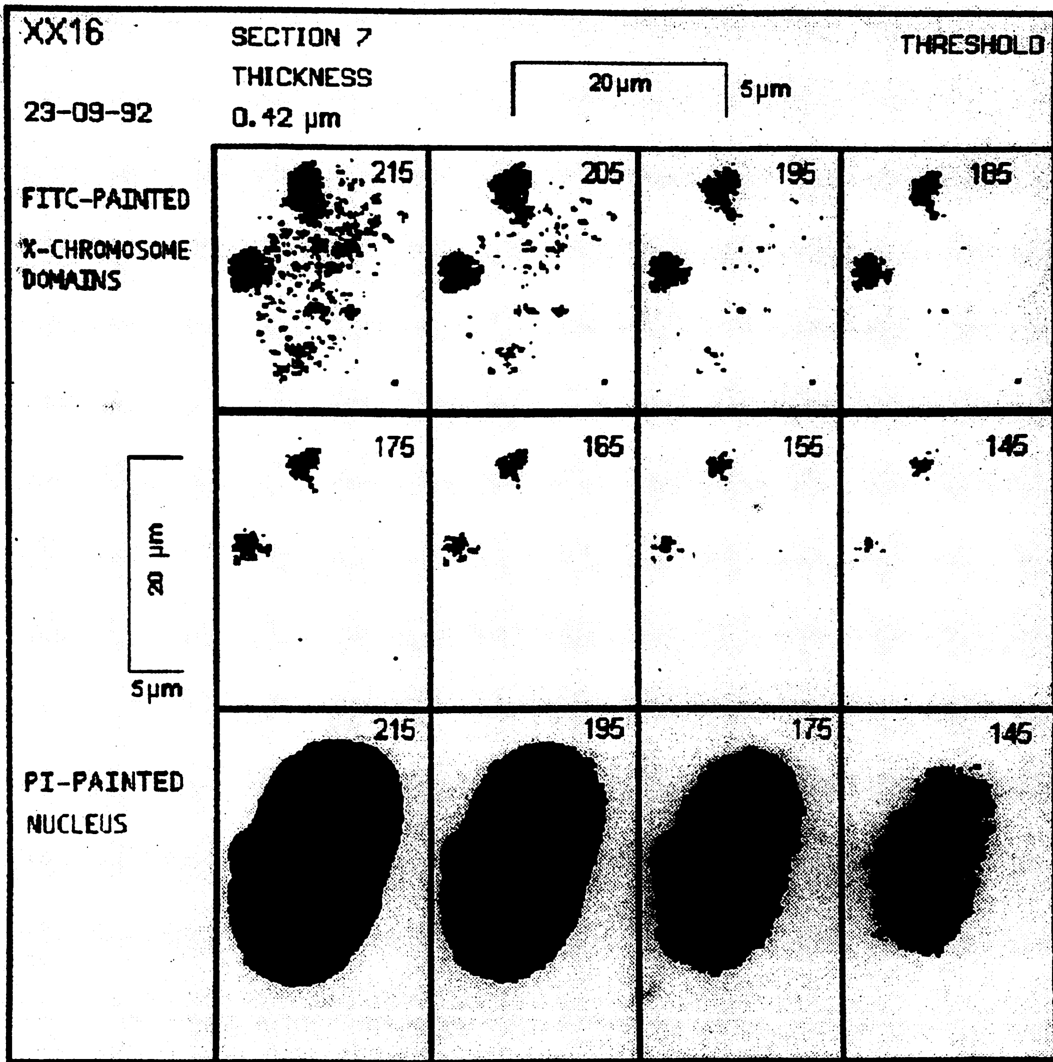

Figure 6. Digitized images from a single light optical section of a human female amniotic fluid cell nucleus (XX16, compare Fig. 7A). The X-chromosome territories are visualized by in situ hybridization via FITC fluorescence, and the nucleus is counterstained by propidium iodide (PI). The first two rows show the FITC image and the third row the PI image after application of different thresholds (215–145) chosen from a range of gray values between 0 and 256. Note that the size of the segmented territories (domains) strongly depends on the thresholding conditions (see also Fig. 7).

al., in prep.). These results indicate that individual DNA loops carrying such genes, which would extend far away from the periphery of a chromosome territory into the nuclear space, are rare events, if they occur at all in intact nuclei. The observation of such events in hypotonically treated nuclei fixed with acetic acid/ methanol may reflect artifacts. Such treatments, although useful for interphase gene mapping (for review, see Trask 1991; Lawrence et al. 1992), are less favorable for the preservation of the three-dimensional nuclear and chromosome territory structure. The dystrophin gene is known to be actively transcribed in myotubes, but it also shows low-level transcription in other cell types (Chelly et al. 1988). The level of transcription in HeLa cells is presently under investigation. A preferential distribution in the chromosome territory periphery has also been obtained for two other genes known to be active and inactive, respectively, in various cell types (A. Kurz et al., in prep.).

Visualization of RNA Tracks

Visualization of nuclear RNA by FISH has revealed that RNA transcripts occur in "track"- or "dot"-like accumulations (Lawrence et al. 1989; Berman et al. 1990; Raap et al. 1991; Huang and Spector 1992; Jiménez-García and Spector 1993). In one case, it was demonstrated that these signals were spatially associated with the corresponding gene (Xing et al. 1993). Analyses of accumulations of specific RNA transcripts using confocal laser microscopy (U. Mathieu and P. Lichter, in prep.) indicated that track-like Epstein-Barr viral transcripts observed in the Burkitt's lymphoma cell line Namalwa (Lawrence et al. 1989) were general-

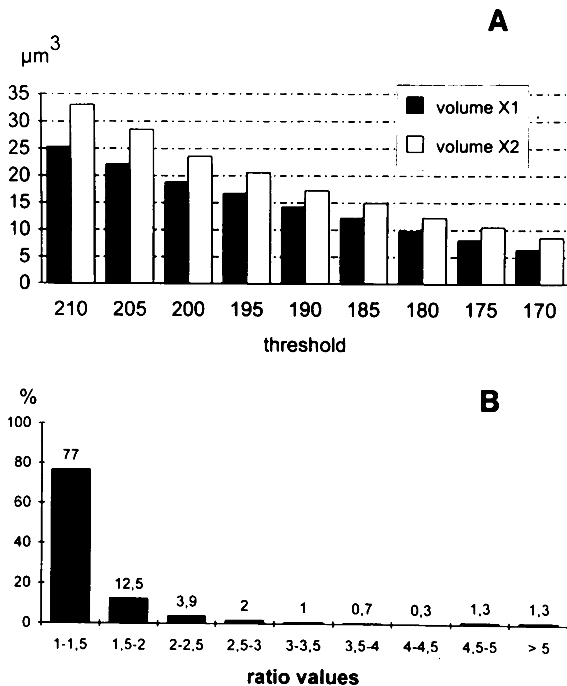

Figure 7. (*A*) A range of thresholds (cf. Fig. 6) was applied to light optical serial sections of a female human amniotic fluid cell nucleus (XX16) with painted X-chromosome territories. For each section and each threshold slice, volumes of the two territories were estimated and integrated to yield estimates of chromosome territory volumes. In these experiments the inactive X chromosome was not defined by Barr body staining prior to CISS hybridization (cf. Fig. 5). Accordingly, the volume of the larger territory (X2) was divided by the volume of the smaller territory (X1) to yield an upper limit estimate for the volume ratio X_a/X_i (for details, see Bischoff et al. 1993). (*B*) Frequencies of volume ratio values (n = 304) calculated for the larger and smaller X-chromosome territory in 33 female amniotic fluid cell nuclei using a variety of threshold conditions (cf. Figs. 6 and 7A). Note that 89.5% of the ratios in these series of nuclei were < 2.

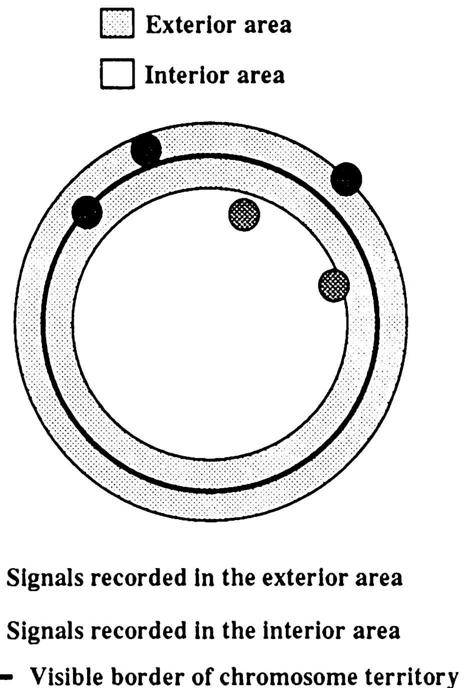

Figure 8. Schematic illustration of signal localizations in the interior and exterior areas of a chromosome territory. Signals were considered to be located in the exterior area if the center of the signal was inside or outside the segmented border of the chromosome territory by not more than the diameter of the signal. Signals located closer to the center of the territory section were considered to be located in the interior area.

ly extended from the nuclear interior to the nuclear periphery (Fig. 9), whereas dot-like accumulations of RNA transcripts observed for induced heat shock genes or human papillomaviruses in HeLa cells were preferentially located at the nuclear periphery. Simultaneous visualization of human papillomavirus (HPV 18)-RNA transcripts and the chromosome 8 territory harboring the integrated virus genome in HeLa cells indicated the preferential localization of the RNA signal at the territory surface (Zirbel et al. 1993).

Evidence for the Localization of Splicing Components at the Periphery of Chromosome Territories

Immunolabeling of major components of the splicing machinery, the splicing snRNPs containing, e.g., Sm antigen (Lerner et al. 1981) and U1, U2, U4/U6, or U5 RNA, shows—in addition to a wide distribution through the nucleus—accumulation of these snRNPs in "speckles" (Mattioli and Reichlin 1971; Northway and Tan 1972; Spector et al. 1983; Reuter et al. 1984; Spector 1984; Bachmann et al. 1986; Nyman et al. 1986; Verheijen et al. 1986) or in "foci," the latter coinciding with the coiled bodies (Carmo-Fonseca et al. 1991a,b; Raska et al. 1991; Huang and Spector 1992; Lamond and Carmo-Fonseca 1993). Human fibroblasts show Sm antigens accumulated in speckles (Fig.4, panel F), whereas HeLa cells contain between 1 and 9 foci of Sm antigen accumulations colocalizing with coiled bodies (Fig. 4, panels G–M). Speckles and foci often correspond to interchromatin granules (Monneron and Bernhard 1969; Spector et al. 1983, 1991; Fakan et al. 1984; Raska et al. 1991). On the other hand, it is known that hnRNPs as well as snRNPs coincide with perichromatin fibrils (Monneron and Bernhard 1969; Fakan et al. 1984; Raska et al. 1991). Furthermore, RNA polymerase II has been found recently to occur predominantly at the perichromatin fibrils (Raska et al. 1991; see also Spector et al., this volume). A present focus of discussion concerns the question whether the high concentrations of splicing components define the sites of active RNA processing, or sites of assembly, or storage of parts of the splicing machinery, or sites where all these functions may take place (Carmo-Fonseca et al. 1991a,b, 1992; Carter et al. 1991a, 1993; Spector et al. 1991, 1993; Huang and Spector 1992; Xing et al. 1993).

The spatial relationship of these foci to chromosome territories was analyzed in electronically overlaid optical images (see Fig. 4, panels G and K). In a series of

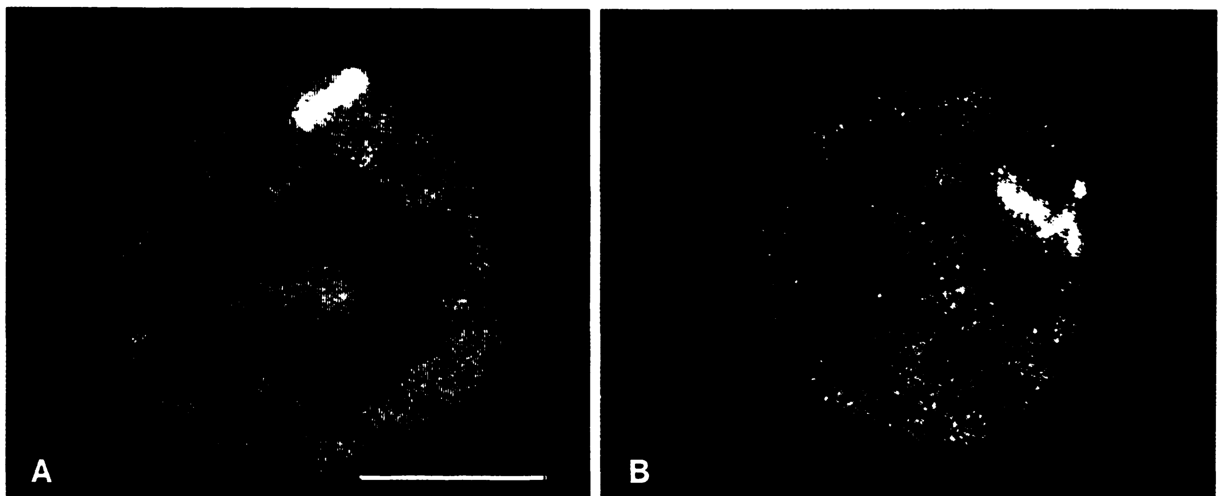

Figure 9. Confocal images of nuclear RNA tracks in two cell nuclei. The Burkitt's lymphoma cell line Namalwa contains integrated Epstein-Barr virus (EBV) DNA which is actively transcribed; the nuclear EBV RNA (visualized by FISH) often occurs in track-like accumulations (Lawrence et al. 1989). (*A*,*B*) The analysis of these EBV RNA signals in optical sections indicated the extension of these tracks from the nuclear interior to the nuclear envelope. Bar, 5 μm.

experiments, the territories of chromosomes 1, 7, 8, 16, 18, and 20 were visualized in HeLa cell nuclei together with immunostaining of the Sm antigen. For each chromosome, 45–80 nuclei were evaluated, taking into account all foci localized within the interior and exterior parts of the territory, respectively. From these foci, 93–95% were observed in the exterior part, whereas only some 50% were expected in this part in case of a random assignment (Zirbel et al. 1993). Sm accumulations present in speckles in the nuclei of human foreskin fibroblasts (Fig. 4, panel F) were analyzed in the same way. Again, more than 92% of these speckles were found at the surface of chromosome territories (Zirbel et al. 1993). The experiments described above indicate that RNA transcripts and accumulations of splicing snRNPs are basically excluded from the interior of the territories.

A Model for the Functional Compartmentalization of the Eukaryotic Cell Nucleus: The Interchromosome Domain Compartment

On the basis of the evidence described above, we propose a role for chromosome territories in the functional compartmentalization of the cell nucleus (see schematic drawing in Fig. 2). First, we postulate that the space between adjacent chromosome territories, together with the surface area of the chromosome territories, defines a three-dimensional compartment for gene expression, mRNA splicing, and transport to the nuclear pores, termed the interchromosome domain (ICD) compartment (Zirbel et al. 1993; note that chromosome domain is used as a synonym for chromosome territory). According to this model, chromatin loops with genes that are permanently or intermittently expressed in a given cell type should be located at or close to the surface area of each chromosome territory. If so, relatively small changes of the volume and shape of a given chromosome territory can have considerable effects on the number of genes that may become exposed at its surface. In this way, RNA transcripts would be directly released into the ICD compartment. Processing of RNA in a topologically highly ordered manner and transport of spliced RNA to the nuclear pores (Carter et al. 1993; Xing et al. 1993; U. Mathieu and P. Lichter, in prep.) would then occur within this compartment.

Diffusible factors controlling gene expression and mRNA splicing may become concentrated in the ICD compartment due to chromatin packaging and electric surface charge phenomena at chromosome territory surfaces. This should facilitate the formation of larger complexes of factors within this compartment. At the same time, a strong decrease of the total search volume for factors involved in gene transcription (see below) should help to reduce the access times required for DNA-binding factors to reach their specific targets. Genes for which a permanent repression is required in a given cell type may be packaged into an interior part of the respective chromosome territory, thus becoming inaccessible for transcription factors available in the ICD compartment.

According to this model, we expect interchromatin granules, perichromatin fibrils, and coiled bodies to be located within the ICD compartment. It appears as an intriguing possibility that components of the splicing machinery are released from storage places to participate in the processing of RNA within the ICD compartment. The ICD compartment is assumed to be a three-dimensional space providing passages between chromosomes which are connected with each other and the nuclear pores. Dependent on their density and assembly, components located within the ICD space may or may not appear as an interconnected "network" in electron microscopic analysis. Therefore, variabilities in the density and assembly of some components, e.g.,

of the splicing snRNPs, might explain controversial findings regarding nuclear networks (Spector 1990; Carter et al. 1993).

Theoretical Considerations on Electric Forces Acting on Chromosome Territories

In this section we discuss the possibility that short-range (i.e., nm distances) and long-range (i.e., μm distances) electric forces (for textbook reviews, see Eisenberg and Crothers 1979; Hoppe et al. 1983) due to charge distribution effects of chromosome territories and other nuclear components may be involved (1) in the maintenance of the ICD compartment, (2) in the confinement of charged, diffusible factors within this space, (3) in the differential packaging of active and permanently repressed genes, (4) in the transport of electrically charged macromolecules within the ICD space, and (5) possibly even in the translational and rotational movements of whole chromosome territories.

Attracting and repulsive electric forces need to be considered, taking into account the many different charged structures and ion species contained in a nucleus and the dynamics involved in possible local and overall changes of charge distributions. Our further attempt to discuss possible electric interactions within and between chromosome territories is limited to extremely simplified model cases (C. Cremer and T. Cremer, in prep.).

Electric chromosome net charge and maintenance of the ICD space. Experiments performed with isolated metaphase chromosomes in suspension under a variety of buffer conditions have indicated an electric mobility of chromosomes on the order of $u = 1 \times 10^{-8}$ m^2/Vsec, indicating an electric net charge of one to several thousand elementary charges, depending on chromosome size (Bier et al. 1989). Chromosomal net charges can be strongly influenced by differences in pH, ionic strength, and DNA-binding proteins. For example, after binding of antibodies against z-DNA, a change in electric mobility of 30% was observed for isolated metaphase chromosomes (F. Bier and C. Cremer, unpubl.). At neutral pH, the net charge of isolated chromosomes had the same negative sign as that of purified DNA and about two thirds of its magnitude. This means that chromosomes repel each other under these conditions.

Assuming that chromosome territories have a similar electric net charge as metaphase chromosomes, the possibility is considered that repulsive Debye-Hückel forces between the surfaces of neighboring chromosome territories are sufficient to maintain an ICD space of adequate width. To be useful as a transport channel for proteins and RNA molecules, this space should have a minimum diameter on the order of 10 nm. Model calculations (C. Cremer and T. Cremer, in prep.) suggest that the formation of an ICD space with such a width due to Debye-Hückel forces is possible even in the presence of relatively high concentrations of freely movable counterions. As soon as van der Waals forces would dominate the interaction between chromatin loops exposed at the surface of neighboring chromosome territories, such loops would strongly attract each other and an ICD space would collapse at this site. On the other hand, the ICD space could be opened more widely by additional nuclear structures located between neighboring chromosome territories.

Confinement of charged macromolecules within the ICD compartment by electrical forces. Repulsive electric forces at chromosome territory surfaces could lead to a higher concentration of certain proteins or RNAs with like net charges within the ICD space as compared to the interior of the chromosome territories. A quantitative model, taking into account the net charge Q of the macromolecule and the electric potential difference U between the interior of a chromosome territory and the surrounding ICD space, predicts an exponential dependence of this relative confinement (factor concentration in the ICD space/ factor concentration in the chromosome territory interior) on $Q \times U$ at a given temperature. Thus, for macromolecules with sufficiently high negative net charge, considerable enrichments in the ICD space can be expected. According to the law of mass action, such enrichments could strongly facilitate the specific formation of multimeric protein complexes involved in gene transcription and RNA splicing within the ICD space comprising a few percent of the total nuclear space. Both the relative confinement of specific DNA-binding proteins involved in gene regulation within the ICD space and the exposure of specific DNA-binding sites toward this space would help to reduce the search volume which such factors have to explore by Brownian movements to find their specific DNA-target sites (Gasser and Laemmli 1987). At the same time, the relative exclusion of factors from the interior of chromosome territories could help to reduce nonspecific DNA-binding events and, accordingly, improve binding specificity. Repelling electric forces could also reduce the frequency of nonspecific binding events of specific DNA-binding proteins to chromatin with nonspecific DNA sequences exposed to the ICD space, while local charge distribution effects could provide a guidance to specific DNA targets (Ripoll et al. 1993). It is important to note that our model does not require a complete trapping of certain factors within the ICD compartment. A relative confinement is sufficient to bring about considerable effects in the functional compartmentalization of the cell nucleus.

Permanent repression of genes due to packaging into the interior of chromosome territories. Both the higher-order structure and the positioning of chromatin domains could possibly be affected by local changes in the charge distribution. Such effects could be provided, for example, by local changes in pH and ion concentrations, as well as the binding of specific proteins or multimeric protein complexes. It has been suggested that gene activity can be down-regulated by factors

affecting the compaction of chromatin at distinct sites. In *Drosophila*, for example, homeotic genes are permanently repressed by the action of multimeric complexes containing polycomb group proteins (Franke et al. 1992; Shaffer et al. 1993). Such higher-order chromatin structures containing repressed genes may be packaged and electrically charged in a way that contributes to the exclusion of similarly charged transcription factors. Packaging of chromatin into the interior of chromosome territories, i.e., remote from the ICD compartment, could provide a mechanism for permanently repressing the transcription of certain genes in a given cell type. Other genes which are transiently inactive or which show remaining activity at a very low level may still be located at the periphery of chromosome territories. This model predicts a cell-type-specific positioning of genes in chromosome territories and requires a mechanism to maintain the required gene positioning through the cell generations.

Considerations on physical forces affecting movements of charged molecules in the nucleus. Brownian movements of factors confined within the ICD space should be rapid enough to search this space for specific binding sites within a reasonable time. Assuming, for example, a spherical particle of 3-nm radius diffusing in a medium with 37°C and a viscosity of 0.1 kg m^{-1} sec^{-1}, corresponding to the effective viscosity of the cytoplasm in anaphase cells (Nicklas 1988), the mean displacement due to Brownian movements can be estimated to be on the order of 10 μm/minute. In addition to a random net displacement, possibilities of directional movements of charged macromolecules may also be considered. Although Debye-Hückel forces may be effective in maintaining an ICD space and in the confinement of charged macromolecules within this space, they could not explain directed movements of these factors over "long distances," e.g., movements of RNA tracks to the nuclear pores. We discuss below the question of whether "long-range" electric forces could enforce such nonrandom movements. For this purpose, one should take into consideration that chromosome territories contain a substantial amount of fixed charges, especially from the phosphate residues of the DNA, whereas in the ICD space, the amount of fixed charges would be low. This leads to a difference in the concentrations of mobile ions between interior and exterior parts of chromosome territories. As a consequence, Donnan potentials are created (estimated to be on the order of 1 mV). Depending on the concentrations of the fixed charges in different chromosome territories, of the mobile ions, and of the width of the ICD space, a potential difference may be effective not only between a territory and the surrounding ICD space, but also between two distant sites of the ICD compartment. An analogous case is provided by calculations of the equilibrium distribution of mobile ions and the resulting Donnan potential difference between the intra- and extramyofibrillar phases of rested, intact muscle fibers (Maughan and Godt 1989) Here, a fixed negative charge concentration is present in the myofibrillar phase, whereas mobile ions are present in both phases without a separating membrane. A resulting 10–20% difference in mobile ion concentrations yields a Donnan potential difference of 2.7 mV.

According to Nernst's equation, a mobile ion concentration difference of a few percent between two different sites would be sufficient to create a potential difference on the order of 0.1 mV–1 mV. Such differences may be sufficient to influence movements of charged macromolecules and possibly enforce movements of whole chromosome territories (see below). Net fluxes of ions, for example, occur when charged macromolecules together with their counterions enter or leave the nucleus via the nuclear pores. Although nuclear pores are often considered to allow a free exchange of ions between the nucleus and the cytoplasm, it is not definitively clear whether this is the case under all circumstances or whether an active transport of ions may in fact take place at the nuclear envelope. If, however, the nuclear envelope with regard to ion movements were full of holes, active ion transport at cell membranes still could generate a long-range electric field affecting movements of charged particles throughout the nucleus. Depending on the direction of local ion fluxes, a directional flux of certain species of charged molecules, such as mRNAs (Blobel 1985), could be achieved.

Considerations on physical forces affecting movements of chromatin within the nucleus. Cell-type-specific chromosome arrangements have been demonstrated in various species (for review, see Hilliker and Appels 1989; Manuelidis 1990; Lichter et al. 1991). Chromosomes and chromosomal subregions, respectively, can move and possibly even rotate in cell nuclei during interphase and in postmitotic cells (Manuelidis 1984; Ferguson and Ward 1992; Hiraoka et al. 1993). In nuclei of cultured rat spermatocytes, time-lapse cinemicrographs have indicated that the chromosomes seem to move around several axes in numerous different planes (Parvinen and Söderström 1976).

Complex interactions between chromosomes and their neighborhood during mitosis and interphase could result in random positional changes. For example, in human amniotic fluid cell colonies of a few hundred cells, the variability of the positioning of chromosome-specific heterochromatin sites was apparently the same as the variability observed in whole cultures, although in two-dimensional nuclear projections, smaller chromosomes were located more centrally than larger ones (T. Volm et al., in prep.). The rapid loss of the initial chromosome arrangement during clonal growth of a given cell indicates that cell-type-specific chromosome arrangements have to be established and maintained by specific forces. Although a number of proteins required for proper chromosome distribution in mitosis and meiosis have been identified, including microtubule motor proteins (for review, see Endow 1993), the mechanisms by which specific chromatin movements take place in the cell nucleus are still unknown. In principle, such movements might be enacted by micro-

tubules or microfilaments with anchoring points at chromosomes, a non-chromatin matrix, the nuclear envelope, and possibly even structures located outside the nucleus. Evidence for such mechanisms, however, is lacking so far. Therefore it seems appropriate to consider the possibility that long-range electric forces as discussed above for the movement of charged macromolecules might be strong enough to move even whole chromatin structures.

A simple model (C. Cremer and T. Cremer, in prep.) suggests that an energy-dependent mechanism maintaining long-range differences of intranuclear charge concentrations on the order of a few percent would be sufficient to produce a nuclear electric field capable of moving chromosome territories over micrometer distances within a few hours. In this model, it is assumed that the nuclear matrix is a dynamic structure which should not inhibit chromosome territory movements per se. In analogy with viscosity, the overall resistance to such movements could be described summarily by an "effective nuclear resistance factor," η_{nucl}, which should be understood as a purely descriptive term. It would result from frictional forces between the surfaces of chromosome territories and other nuclear structures, their plasticity and position (e.g., within the nuclear interior or attached to the nuclear envelope). An estimate for η_{nucl} can be derived from experiments with "optical tweezers" used to move nucleoli and other intranuclear particles within the nuclei of living algae cells (Ashkin and Dziedzic 1989). Estimates derived from such experiments indicate that η_{nucl} could be on the order of 50 kg m^{-1} sec^{-1}. This value is about 70,000 times larger than the viscosity of water at 37°C and about 500 times larger than the viscosity of cytoplasm during anaphase (Nicklas 1988). Taking into account such a range of η_{nucl}, a long-range electric gradient due to an effective potential difference of 1 mV, for example, between two opposite poles of a nucleus could move a mobile chromosome territory from one side of the nucleus to the other, whereas other chromosome territories being fixed within the nucleus would remain behind. Such a specific movement of a single chromosome takes place, for example, in spermatids of *Urodela* (Schmid and Krone 1976). Appropriate differences in the concentrations of fixed charges at different sites of chromosome territories could also result in rotational movements under the influence of an external electric field on the order of 10 $\mu V/\mu m$. Whether the predicted effects of long-range electric fields on chromosome movements can be demonstrated remains to be seen. However, the predictions of the model are, in principle, testable by electrophysiological approaches.

Multicolor FISH and Improvements of Three-dimensional Digital Fluorescence Microscopy: New Tools to Study the Chromosomal and Suprachromosomal Organization of the Cell Nucleus

Multicolor FISH can now be used to visualize several chromosomes and chromosomal subregions simultaneously (Nederlof et al. 1990; Ried et al. 1992; Lengauer et al. 1993), providing ample opportunities to test aspects of the chromosomal and suprachromosomal nuclear topography. For example, the multicolor delineation of a chromosome territory with several coding and noncoding DNA segments should allow for the testing of predicted nonrandom and possibly cell-type-specific organization of chromosome territories. A major concern at present relates to the limited spatial resolution of light microscopy. Confocal laser scanning fluorescence microscopy (Cremer and Cremer 1978; Brakenhoff et al. 1985; Wijnaendts van Resandt et al. 1985; for review, see Shotton 1989) has improved the resolution along the optical axis as compared to conventional epifluorescence microscopy. Still, the z-resolution is reduced by a factor of 2–3 as compared to the lateral resolution. It has been speculated that the microscopic resolution could be increased beyond the limits of Abbe-type arrangements by combining confocal fluorescence detection and 4π illumination with coherent light (Cremer and Cremer 1978). Recently, a prototype of a 4π-confocal microscope with two opposing objective lenses of high numerical aperture has been developed (Hell and Stelzer 1992a). It is expected that such a microscope will allow an axial resolution at 700 nm excitation wavelength on the order of 100 nm (Hell and Stelzer 1992b). Since absorption is almost confined to the observed volume in the case of two-photon excitation, photodamage outside this volume can be minimized, especially if pulsed infrared laser light is used (Denk et al. 1990).

Improved distance measurements in the three-dimensional nuclear space can be obtained even with a conventional epifluorescence microscope (Bradl et al. 1992). For this purpose, nuclei hybridized with chromosome-specific DNA probes in suspension can be sucked into a quartz capillary. A mechanical device allows the capillary to turn around under an epifluorescence microscope. The refraction indices of the fluids inside and outside the capillary are carefully adjusted to the refractory index of the capillary to minimize optical aberrations. Images of nuclei at different angles can be obtained using high magnification/high aperture objectives and a cooled black and white CCD camera. For measurement of the distance between two targets, the nucleus is turned around until both targets are in focus, and their distances are maximized on the monitor (Fig. 10). In this way, any intranuclear distance can be measured in the focal plane providing the highest possible optical resolution. In the future it may be possible to combine this approach with confocal scanning microscopy and to reconstruct three-dimensional nuclear images from a series of optical sections taken at certain rotation angles (light microscopic axial tomography).

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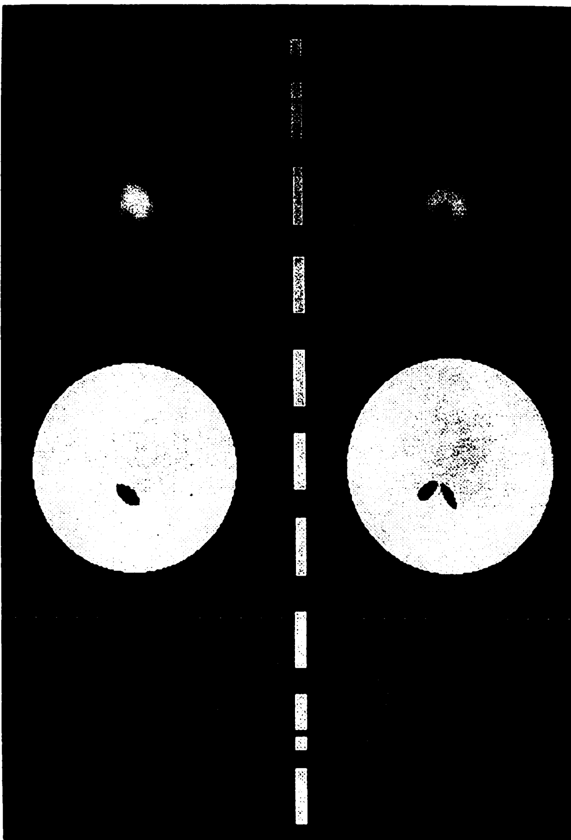

Figure 10. Nucleus of a PHA-stimulated human lymphocyte hybridized in suspension with a biotinylated chromosome-15-specific repetitive DNA probe (D15Z1) detected with FITC-conjugated avidin. Digitized images of the nucleus in a quartz capillary were recorded (see text). In the recording shown on the left, only one signal is apparent, whereas after a turn of the capillary by 60°, two signals can clearly be resolved. This approach can help to perform distance measurements between two signals with the same accuracy in all three dimensions of the nuclear space.

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