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A specific conformation of the territory of chromosome 17 locates *ERBB-2* sequences to a DNase-hypersensitive domain at the nuclear periphery

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Abstract. Chromatin in interphase nuclei exhibits a topology that is associated with the transcriptional state of cells. We examined the spatial, intranuclear distribution of chromosome 17 and the *ERBB-2* (*HER2/neu*) sequence thereon, relative to that of DNase-hypersensitive chromatin (DHC), in breast tumour cells exhibiting different levels of expression of *ERBB-2*. These sequences were specifically associated with the nuclear periphery, within a band of DHC. The remainder of the chromosome 17 mass showed no preferential position within the nucleus. The peripheral placement of *ERBB-2* sequences is associated with a specific conformation of chromosome 17. We propose that the conformational organization of chromosome territories might represent a fundamental control mechanism in gene expression.

Introduction

The interphase nucleus is structurally and functionally compartmentalized into regions that contain factors involved in RNA synthesis, processing and transport (Carmo-Fonseca et al. 1991; Huang and Spector 1992; Carter et al. 1993; Jiménez-Garcia and Spector 1993; Sahlas et al. 1993; Xing et al. 1993; Park and De Boni 1996). Specific chromatin domains also occupy distinct, spatial positions, the pattern of which has been associated with the transcriptional state of the cell (Borden and Manuelidis 1988; Janevski et al. 1995, 1997; Naegele et al. 1995). This led to the concept that one level of control of gene expression is exerted by spatially coupling those components that participate in transcription and RNA processing (Jackson 1991; Clemson and Lawrence 1996). It is unknown, however, whether such compartments are randomly distributed within the three-dimensional (3-D) nuclear space or are organized into a specific, non-random topology. Previous studies showed that factors involved in transcript processing, as well as spliced mRNAs, are detected in close proximity to their cognate gene (Lawrence et al. 1989; Carmo-Fonseca et al. 1991; Huang and Spector 1992; Jiménez-Garcia and Spector 1993; Xing et al. 1993, 1995; Matera et al. 1995; Huang and Spector 1996), where they form discrete "transcription factories", considered to be randomly dispersed throughout the nucleus (Fakan et al. 1976; Fakan and Puvion 1980; Zhang et al. 1994; Carmo-Fonseca et al. 1996). In contrast, other evidence and theoretical considerations suggest that there exists a non-random topology within the 3-D nuclear space, formed by a cell type-specific and cell function-specific spatial arrangement of nuclear domains (Blobel 1985; Hutchison and Weintraub 1985; de Graaf et al. 1990; Krystosek and Puck 1990; Puck and Krystosek 1992; Sahlas et al. 1993; Park and De Boni 1996).

Chromatin domains are frequently rearranged in interphase nuclei (De Boni and Mintz 1986; Park and De Boni 1991; Buchenau et al. 1997). We had postulated that this rearrangement serves to transpose transcriptionally active sequences to the nuclear periphery, in association with changes in transcriptional demands (Park and De Boni 1996). Recently, we have demonstrated that differentiation of PC12 cells is assompanied by the formation of a peripheral shell of DNase-hypersensitive chromatin (DHC) and small nuclear ribonucleoproteins apposed to the inner nuclear membrane, as the nuclear geometry changes from a prolate ellipsoid to a sphere (Sahlas et al. 1993; Park and De Boni 1996). Given that transcriptionally active sequences are sensitive to nuclease digestion, we tested the hypothesis that specific, actively transcribed sequences are positioned within this peripheral DHC shell. We examined, by dual-colour fluorescent in situ hybridization (FISH), the spatial, intranuclear distribution of *ERBB-2* (*HER2/neu*) sequences, and of chromosome 17, which carries that sequence, with respect to the peripheral DHC shell. The human breast tumour cell lines MCF-7, SK-Br-3 and ZR-75-1 were chosen as models because of the differences in copy number and in the level of expression of the *ERBB-2* sequence among these cells.

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Materials and methods

Cell culture. MCF-7, SK-Br-3 and ZR-75-1 cells obtained from ATCC (Rockville, Md.) were maintained in 25 cm^2 tissue culture flasks in their respective media (MEM, McCoy's 5a medium, and RPMI 1640 for MCF-7, SK-BR-3 and ZR-75-1, respectively) supplemented with 10% (v/v) FBS (all from GIBCO-BRL). They were subcultured weekly at 1:3 onto glass coverslips pre-treated with poly-D-lysine hydrobromide (0.1 mg/ml; ICN) and maintained for a minimum of 3 days prior to use.

Fluorescent in situ hybridization. Genomic, biotinylated probes specific to *ERBB-2* sequences were obtained by nick translation of cRCNeu1 and cRCNeu4 clones (gifts from M. Leppert, Howard Hughes Medical Institute, Salt Lake City, Utah). Labelling conditions were optimized for uniformity in probe size of 400±200 bp. To 0.5 µg of labelled probe was added 15 µl of Cy-3 conjugated chromosome 17 paint (Cambio, Cambridge, UK) and 25 µg human placental DNA. The cocktail was precipitated and redissolved in 15 µl of hybridization buffer.

Cells on coverslips were fixed for 30 min in 4% paraformaldehyde (w/v), PBS, washed three times in PBS for 5 min each washing extracted with 0.25% Triton X-100, 0.25% NP-40 in 0.2 N HCl for 30 min, then digested for 60 min with RNase A (Boehringer Mannheim; 100 µg/ml) at 37° C. This was followed by digestion with proteinase K (10 µg/ml) at 21° C for 14, 17 and 16 min, for MCF-7, SK-Br-3 and ZR-75-1 cells, respectively. These times were empirically determined based on hybridization efficiency and on preservation of morphology. To avoid distortion of morphology associated with dehydration, cells were left to equilibrate in 70% formamide, 2XSSC overnight, then denatured for 2 min at 70° C in 70% formamide, 2XSSC) and re-equilibrated in 50% formamide, 2XSSC at 0° C. (1XSSC is 0.15 M NaCl, 0.015 M sodium citrate.) 15 µl of probe cocktail was added per coverslip and cells incubated for 72 h at 37° C. After washes, probes were marked by an anti-biotin monoclonal antibody (mAb) and detected by fluorescein isothiocyante (FITC)-conjugated anti-mouse IgG (Boehringer Mannheim). Cy-3 signals (chromosome 17) and FITC signals (*ERBB-2*) were simultaneously visualized by a ZEISS LSM410 confocal microscope. The procedures employed did not result in a distortion of the nuclear morphology as determined by phase contrast microscopy (Fig. 2). In fact, the morphological preservation of each cell analysed was assured by the acquisition of corresponding phase contrast images, at the same time as the collection of fluorescent images.

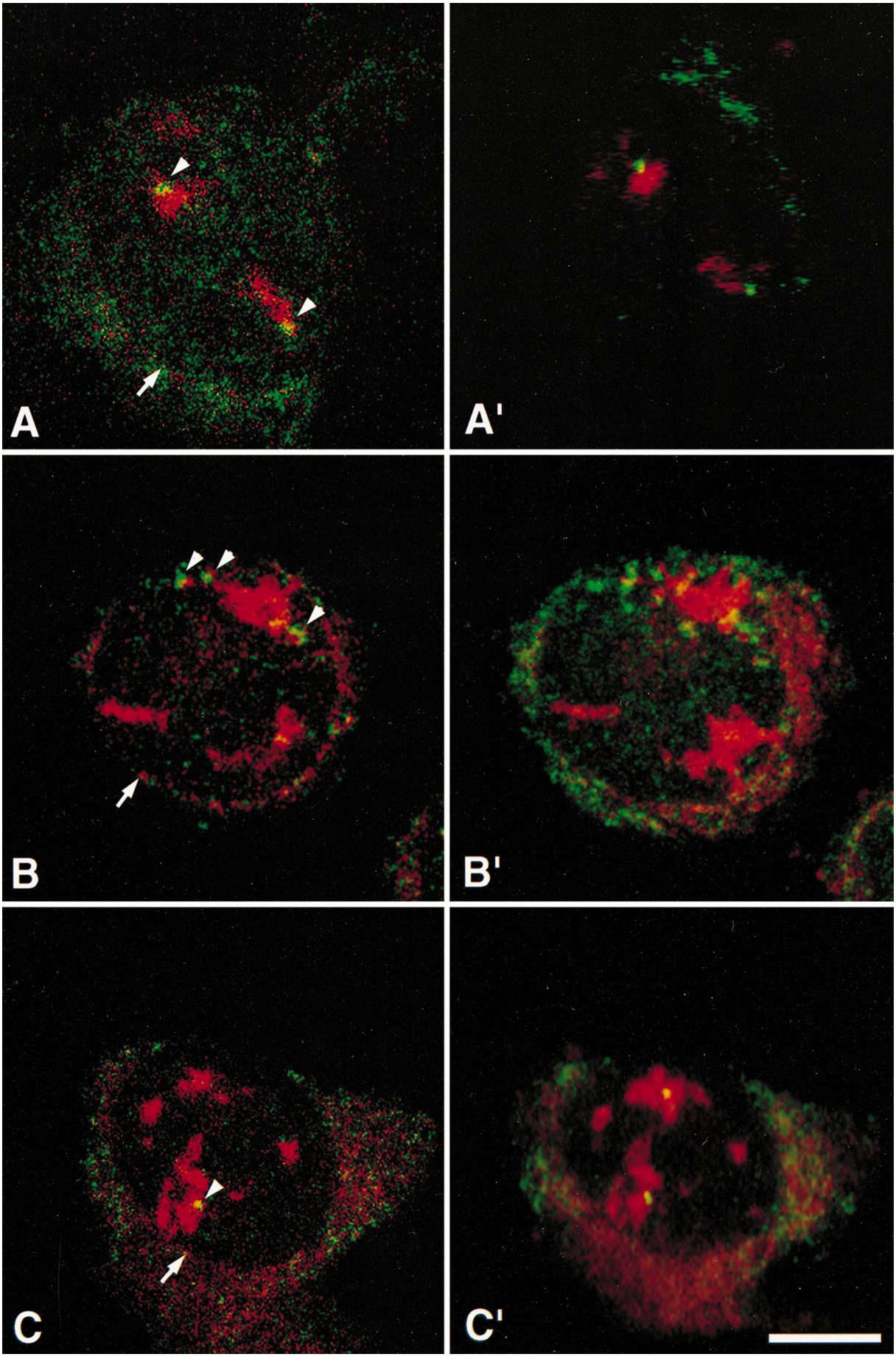
In situ nick translation. To test the hypothesis that the spatial intranuclear position of a transcribed sequence coincides with that of DHC, it would be ideal to employ dual labelling using in situ nick translation and FISH on the same cell. However, because the two techniques are incompatible, the spatial positions of *ERBB-2* sequences and DHC domains were derived for cells from separate cultures. Fixed and extracted cells (see above) were briefly rinsed in nick translation buffer (50 mM TRIS-HCl, pH 7.8, 5 mM $MgCl_2$, 10 mM β-mercaptoethanol, 10 mg/ml BSA), then incubated (1 h) in a mixture of 40 µM dNTP, 40 µM biotin-16-dUTP, 10 units/ml DNase I, 10 units/ml DNA polymerase I, in nick translation buffer. After washes in 20 mM EDTA and in PBS, cells were blocked (4% BSA in PBS), the labelled chromatin marked by in cutsation for 1 h with an anti-biotin mAb (Boehringer Mannheim; 1:100 dilution in 4% BSA) and detected by a FITC-conjugated secondary antibody.

Morphometric analysis. The intranuclear positions of *ERBB-2* signals and chromosome 17 signals were simultaneously recorded as serial, confocal images, at 0.5 µm increments, spanning the entire *z*-axis of the nucleus, which was usually 14 to 16 optical sections. The spatial distribution of chromosome 17 was quantified as a normalized ratio of areas of chromosome 17 per radial annulus by dividing the nuclear area at the midnuclear plane into 10 concentric annular areas at equal radial increments. The area occupied by chromosome 17 in each annulus was expressed as a fraction of the total chromosomal area in that optic section, and normalized for the area of the annulus. For quantification of the spatial, intranuclear distribution of *ERBB-2* signals, a series of projected views of the 3-D nuclear volume, rotated at 10° increments, was rendered for each nucleus from serial optical sections using ZEISS LSM software. For each signal, the view in which it was maximally displaced from the axis of rotation was considered the optimal orthogonal view of the nucleus, and was chosen for analysis. The radial vector, in this 3-D view, that connects the centroid (geometric centre) of the nucleus to the signal was measured and expressed as a fraction of that same vector extended to the nuclear envelope. The nearest distance of each signal from the nuclear envelope was similarly measured. For this, the readily discernible discontinuity in background labelling was used to define the nuclear border (arrows, Fig. 1). Comparison with the position of the nuclear envelope derived from phase contrast images of the same cells (Fig. 2), recorded at the same time, clearly shows that the error associated with this measurement is less than 0.1 µm. The position of the *ERBB-2* signal with respect to the territory of chromosome 17 was quantified by measuring the angle subtended between the two vectors that connect the centroid of the nucleus to the centroid of the chromosome and that which connects the centroid of the chromosome to the signal (Fig. 8). The spatial distribution of DHC within nuclei was recorded as serial optical confocal sections, using identical recording parameters for all cells examined. For analysis, the optical section that represented the mid-nuclear plane of each cell was converted to a binary image, at identical threshold levels, and the width of the DHC band measured along eight radial lines originating at the centroid of the nucleus, at equal angular intervals.

Results

ERBB-2 sequences were routinely detected near the nuclear envelope in all three cell types, regardless of the extent of amplification and/or overexpression (Fig. 1). Analysis of the distributions of radial distances of signals indicates that *ERBB-2* sequences are located at mean radial distances of 80%±2.0% (mean±SEM), 90%±0.6% and 79%±2.0% for MCF-7, SK-Br-3 and ZR-75-1 cells, respectively (Fig. 3). Given that the volume of a shell increases as the cube of the radius, with the outermost 20.6% of the radius representing 50% of the nuclear volume, we tested whether the observed distribution results from a random occurrence of signals within the nuclear volume or represents their actual peripheralization. The results showed, however, that the spatial distribution of the *ERBB-2* signals was not random (Fig. 4). Measurements of the positions of the *ERBB-2* signals with respect to the nuclear envelope revealed mean nearest distances of 0.91±0.10 µm (mean±SEM), 0.52±0.02 µm and 0.59±0.08 µm in MCF-7, SK-Br-3 and ZR-75-1 cells, respectively (Fig. 5).

Fig. 1A–C'. Spatial localization of *ERBB-2* sequences (*green*) and chromosome 17 (*red*) in nuclei of MCF-7 (**A, A'**), SK-Br-3 (**B, B'**) and ZR-75-1 (**C, C'**) cells. *Yellow* represents co-localization. *Arrows* point to nuclear envelope. Note co-localization of *ERBB-2* (*arrowheads*) with chromosome 17 in single confocal sections (**A, B, C**) and in stacks (**A', B', C'**). *ERBB-2* signals in stacks may appear remote from the nuclear periphery owing to the viewing angle which is different from the orthogonal view employed for quantification of distances (see Materials and methods and Fig. 5). *Bar* represents 5 µm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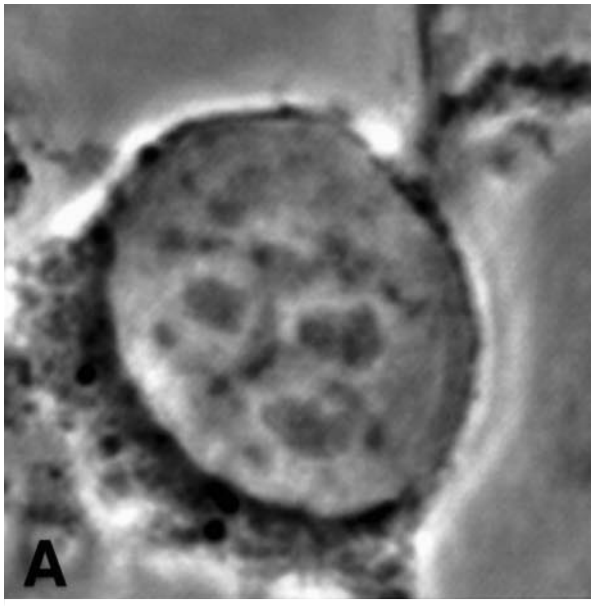

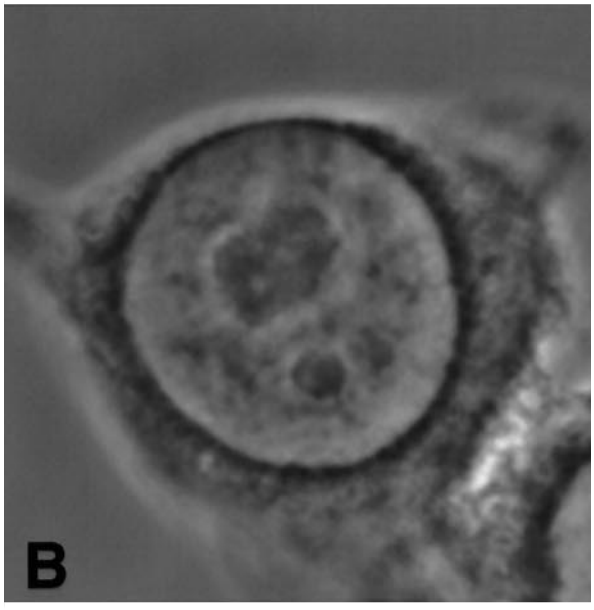

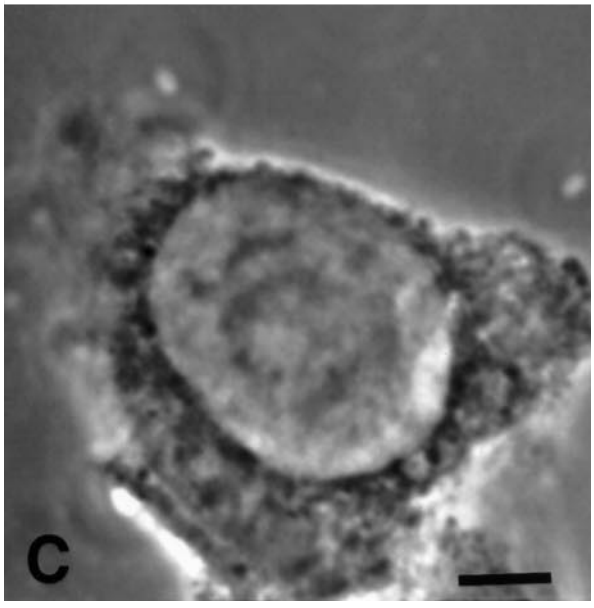

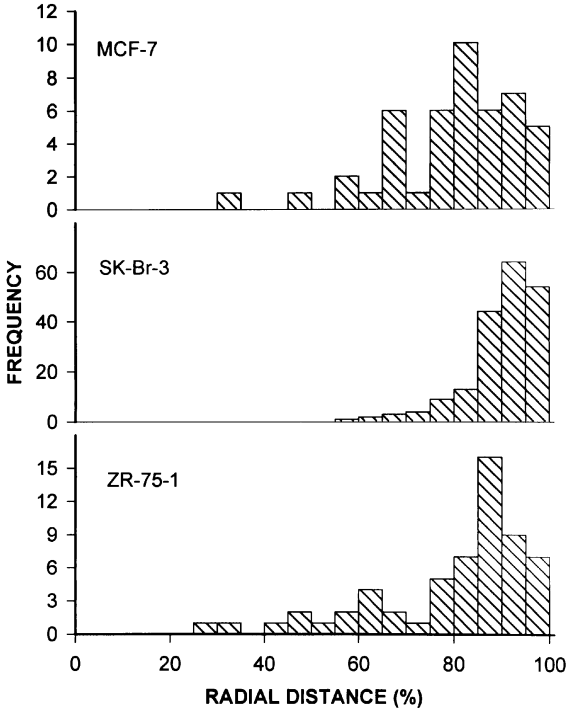

Fig. 3. Radial distributions of *ERBB-2* sequences. Note localiztion of majority of signals within peripheral 20% of the nuclear radius. MCF-7, *n*=22 nuclei, 45 signals; SK-Br-3, *n*=14 nuclei, 164 signals; ZR-75-1, *n*=27 nuclei, 55 signals

DHC, labelled by in situ nick translation, was present as a clearly defined shell, at the nuclear periphery, in all three cell types. Morphometric analysis revealed a mean width of 1.68±0.07 μm, apposed to the nuclear envelope (Figs. 5, 6), in all three cell types. While it is recognized that the extent of nucleotide incorporation during nick translation depends on labelling conditions, the width of the DHC band did not differ among the three cell types (Kruskal-Wallis, $P>0.05$) under the conditions employed here. Together with the data that show a close association of individual *ERBB-2* signals with the nuclear envelope, this indicates that 89%, 99% and 93% of the *ERBB-2* signals in MCF-7, SK-Br-3 and ZR-75-1 cells, respectively, occupy spatial positions in the nucleus that fall into this peripheral, DHC compartment (Fig. 5).

The populations of MCF-7, SK-Br-3 and ZR-75-1 cells chosen randomly for analysis displayed respective means of 2.09±0.02 (mean±SEM) (*n*=31 cells), 2.46±0.16 (*n*=18 cells) and 2.11±0.08 (*n*=30 cells) chromosome 17 signals per cell. In MCF-7 and ZR-75-1 cells, each chromosome co-labelled with one *ERBB-2* signal of reproducible size (Fig. 1A, C). In contrast, in SK-Br-3 cells, chromosome 17 territories were irregular in shape and frequently exhibited thin protrusions that extended

Fig. 2A–C. Phase contrast micrographs of the same MCF-7 (**A**), SK-Br-3 (**B**) and ZR-75-1 (**C**) cells as shown in Fig. 1. The images were acquired at the same time as the fluorescent images (Fig. 1), following all fluorescent in situ hybridization (FISH) labelling procedures, including digestion and denaturation. Note preservation of nuclear morphology. *Bar* represents 2.5 μm

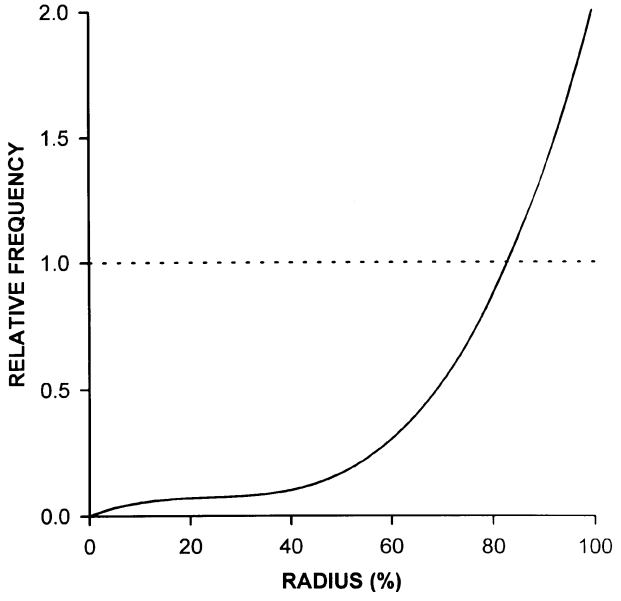

Fig. 4. Frequency of occurrence of *ERBB-2* sequences (*solid line*) normalized for increase in volume with increasing nuclear radius. Note the lower than expected frequency of occurrence of signals for a uniform distribution (*dotted line*) in the internal 50% of the nuclear volume (79.6% of radius), increasing progressively to a higher than uniform frequency in the 50% of the volume represented by the outer 20.4% of the radius. A signal density expected for a uniform distribution (*dotted line*) was derived by dividing the total number of signals, pooled from all three cell types, by the total nuclear volume. The nuclear volume was divided into 10 concentric shells at increments of 10% of the nuclear radius and the observed number of signals in each shell was expressed as a fraction of the expected number

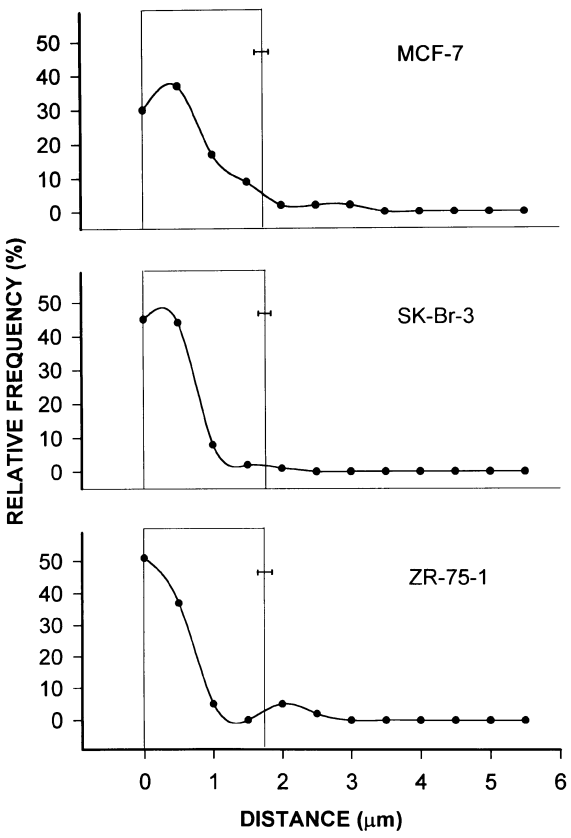

Fig. 5. Relative positions of the *ERBB-2* sequences (*curves line*) shown in Fig. 3, with respect to the nuclear envelope (0.0 μm). The majority of signals fall within the peripheral DNase-hypersensitive chromatin (DHC) domain indicated by the *width of the box*. n=10 nuclei, 78 measurements. *Error bar* represents SEM

from the main chromosomal mass (Figs. 1B, 6C). Amplification of *ERBB-2* sequences was evident in SK-Br-3 cells, with several signals routinely decorating both the main chromosomal mass as well as its protrusions (Fig. 6C, inserts). Possible clustering of sequences was also indicated by non-uniformity in signal size (Fig. 6A, C). In addition, in SK-Br-3 cells, signals were observed without apparent contact with chromosome 17 (Figs. 1B, 6A). These signals were positioned near chromosome 17. In all three cell types, *ERBB-2* signals were invariably positioned at the surface of the chromosome 17 territory.

The cells employed here showed neither an association of chromosome 17 with the nucleolus or with the nuclear periphery, nor was its spatial position, within the nuclear space, reproducible. In fact, statistical analysis of the data derived from the normalized frequency of occurrence of chromosome 17 domains within a given nuclear area failed to identify a preferential positioning of chromosome 17 within the nuclear space (Fig. 7). Given that *ERBB-2* sequences occupy positions at the nuclear periphery, while the chromosome 17 territory in toto lacks such an association, we tested the hypothesis that the *ERBB-2* sequences are placed at the nuclear periphery by a specific conformation of the chromosome 17 mass. For this we quantified the position of *ERBB-2* signals within the territory of chromosome 17. Relative angles, derived from this assay, showed that in all three cell types, *ERBB-2* signals faced the nuclear periphery, with respect to the major chromosomal mass. No differences were detectable among the three cell types (Kruskal-Wallis, $P>0.05$), with pooled data showing that 87.9% of all *ERBB-2* signals faced the nuclear periphery (Fig. 8).

Discussion

The data indicate a preferential positioning of *ERBB-2* sequences at the nuclear periphery. They also indicate that this sequence is located, in all three cell types, not only at the surface of the chromosome 17 territory, in agreement with previous reports (Cremer et al. 1993; Eils et al. 1996; Kurtz et al. 1996), but specifically at those surface of chromosome 17 that face the nuclear periphery. This is taken to indicate the presence of a conformational organization of chromosome territories.

Aneuploidy has been reported in all three cell types studied (Engel et al. 1978; Szöllösi et al. 1995; Schröck et al. 1996). The present data, however, which show a detected chromosomal complement of approximately two per nucleus in MCF-7 and ZR-75-1 cells, indicate that the results reported here are not related to alterations in the copy number of chromosome 17. Moreover, the finding of two *ERBB-2* sequences per nucleus in MCF-7 and

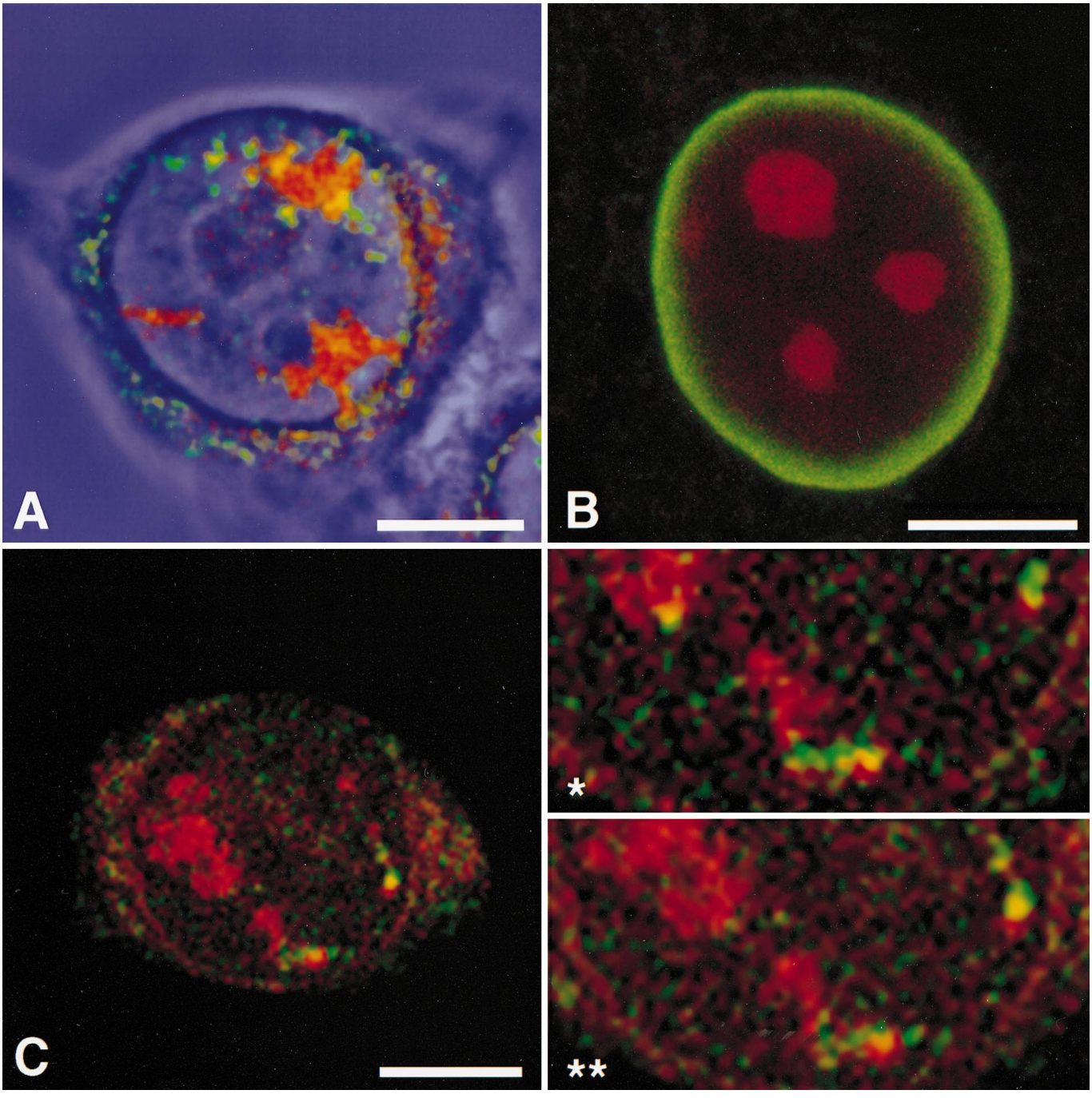

Fig. 6A–C. Confocal images showing spatial positions of *ERBB-2* sequences with respect to the chromosome 17 territory and to the nuclear DHC domain. Composite of SK-Br-3 cell (**A**) shows phase contrast image and extensions from chromosome 17 territory (*red*), decorated with *ERBB-2* sequences (*green/yellow*), the latter extending into the peripheral DHC domain, as shown in a different cell (**B**, DHC *green*; nucleoli counterstained with ethidium bromide, *red*). Some *ERBB-2* sequences appear remote from the nuclear periphery in **A** due to inclusion of sections near top and bottom of nucleus. Their peripheral position (*green/yellow*) and association with extension of the chromosome 17 territory is clearly shown in a single confocal section (**C**) and in two consecutive enlarged, confocal sections thereof (*, **). *Bar* represents ≠5 μm

ZR-75-1 cells indicates that, in agreement with other data (Kraus et al. 1988), the *ERBB-2* sequence is not amplified in these cells and supports the argument that the conformation of the territory of chromosome 17 itself might result in the placement of the sequence to the nuclear periphery.

In contrast, and in keeping with previous reports (Kraus et al. 1987, 1988), amplification of *ERBB-2* sequences was evident in SK-Br-3 cells. For this cell type, interpretation of the data is difficult owing to uncertainty of the nature of *ERBB-2* amplification. It must be considered that at least a fraction of the many sequences detect-

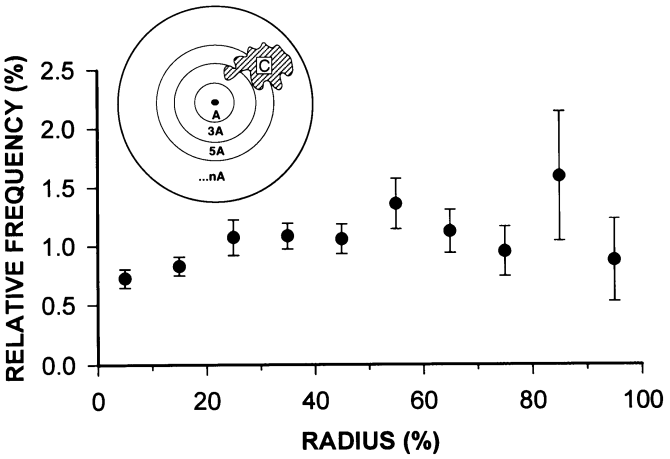

Fig. 7. Normalized ratios of chromosome 17 areas per radial annulus (mean±SEM). Ratios for each annular area, while highly variable, did not differ (ANOVA, multiple comparison test, $P<0.05$). This indicates that subdomains of the territory of chromosome 17 are present with equal probability throughout the nuclear space. For each nucleus the nuclear area at the mid-nuclear plane was divided into 10 concentric annular areas (*inset, A..nA*), at equal radial increments. For each annulus the area occupied by chromosome 17 was expressed as a fraction of the total chromosome area (*inset, C*) and normalized for the area of the annulus. n=19 nuclei (7 MCF-7, 6 SKBr-3, 6 ZR-75-1)

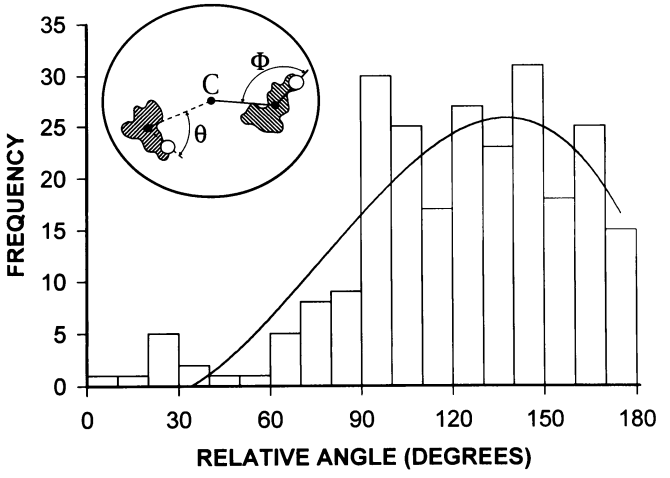

Fig. 8. *ERBB-2* sequences within the territory of chromosome 17 face the nuclear envelope. Shown are frequency histogram and third order regression (*curved line*) of the distribution of relative angles between vectors originating at the centroid (geometric centre) of the chromosome and extending to the nuclear centroid (*inset, C*) and the signal (*inset, open circle*). Angles <90° (θ) represent positions facing the nuclear centre, while angles >90° (ϕ) indicate that a signal is located on the peripheral aspect of the territory. Mean angular measurements for MCF-7, SK-Br-3 and ZR-75-1 cells were 113.1°±34.3°, 127.8±35.2° and 118.4°±31.4°, respectively. The means do not differ among the three cell types (Kruskal-Wallis, $P<0.05$). n=63 nuclei, 264 signals

ed in this cell type might represent extrachromosomal amplifications. If those signals that, at the resolution employed, appear detached from the main chromosomal mass represent double minutes, it is noteworthy that even these sequences are spatially positioned near the nuclear periphery and, moreover, are restricted to a relatively small nuclear volume near chromosome 17 (Figs. 1B, 6A). Alternatively, it can be considered that all detected sequences are amplified within chromosome 17. The signals that appear detached might maintain connections to chromosome 17 by extensions of the latter that are nor resolvable by fluorescence microscopy. This view is consistent with the observation of the presence of elongated protrusions, some at the limit of resolution, continuous with and extending from, the main mass of chromosome 17 and extensively decorated with *ERBB-2* sequences (Fig. 6A, C).

Chromosomes have been reported to be anchored to the nucleolus and/or the peripheral nuclear lamina (Comings 1980; Hochstrasser and Sedat 1987; Glass and Gerace 1990; Sukegawa and Blobel 1993; Luderus et al. 1994; Carmo-Fonseca et al. 1996; Marshall et al. 1996). In the cells employed here, chromosome 17 territories in toto exhibited no preferential position within the nuclear space. This indicates that the observed association of *ERBB-2* sequences with the nuclear periphery does not result from an anchoring of the entire chromosome 17 territory to the nuclear periphery. Rather, the data suggest that an additional level of organization exists in the nucleus, consisting of changes in the conformation of chromosome 17 territories, and that accomodates the placing of *ERBB-2* sequences towards the nuclear periphery, into the DHC domain.

Cells with spherical nuclei display peripheral shells of DHC, proposed to include transcriptionally active sequences (Park and De Boni 1996), a finding that stands in contrast to the observation that many cell types display heterochromatin at the nuclear periphery. However, the cells employed here show no ultrastructural evidence of such peripheral heterochromatin but rather, show aggregates of heterochromatin throughout the nucleus (not shown). Moreover, given that the extent of labelling, by in situ nick translation, depends on enzyme concentrations and/or reaction duration, it might be argued that the observed labelled DNA represents remnants of incomplete digestion of the total genomic DNA content. However, when specifically assayed, the peripheral shell of DHC was routinely the first site to be labelled with increasing concentrations of DNase I, indicating that these sites do indeed represent the most sensitive domain. Moreover, the data are consistent with the report that the promoter associated with the *ERBB-2* sequence in MCF-7 and ZR-75-1 cells contains DNase-sensitive sites (Scott et al. 1994). While an association of actively transcribed sequences with this peripheral shell of DHC would ideally be testable by dual labelling employing in situ nick translation and FISH, the two techniques are incompatible. Nevertheless, the observation that the majority of *ERBB-2* sequences are positioned within this DHC domain at the nuclear periphery strengthens the view that this DHC shell represents a transcriptionally competent compartment.

While the data reported here were derived from three cell lines, the peripheral positioning of active sequences as well as the conformational organization of chromo-

some territories might well apply generally to transcriptionally active sequences and thus might represent a fundamental control mechanism in eukaryotic gene expression.

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References

Blobel G (1985) Gene gating: a hypothesis. Proc Natl Acad Sci USA 82:8527–8529

Borden J, Manuelidis L (1988) Movement of the X chromosome in epilepsy. Science 242:1687–1691

Buchenau P, Saunweber H, Arndt-Jovin DJ (1997) The dynamic nuclear redistribution of an hnRNP K-homologous protein during *Drosophila* embryo development and heat shock. Flexibility of transcription sites in vivo. J Cell Biol 137:291–303

Carmo-Fonseca M, Tollervey D, Pepperkok R, Barabino SML, Merdes A, Brunner C, Zamore PD, Green MR, Hurt E, Lamond AI (1991) Mammalian nuclei contain foci which are highly enriched in components of the pre-mRNA splicing machinery. EMBO J 10:195–206

Carmo-Fonseca M, Cunha C, Custodio N, Carvalho C, Jordan P, Ferreira L (1996) The topography of chromosomes and genes in the nucleus. Exp Cell Res 229:247–252

Carter KC, Bowman D, Carrington W, Fogarty K, McNeil JA, Fay FS, Lawrence JB (1993) A three-dimension view of precursor messenger RNA metabolism within the mammalian nucleus. Science 259:1330–1335

Clemson CM, Lawrence JB (1996) Multifunctional compartments in the nucleus: insights from DNA and RNA localization. J Cell Biochem 62:181–190

Comings DE (1980) Arrangements of chromatin in the nucleus. Hum Genet 53:131–143

Cremer T, Kurz A, Zirbel R, Dietzel S, Rinke B, Schröck E, Speicher MR, Machieu U, Jauch A, Emmerich P, Scherthan H, Reid T, Cremer C, Lichter P (1993) Role of chromosome territories in functional compartmentalization of the cell nucleus. Cold Spring Harbor Symp Quant Biol LVIII:777–792

De Boni U, Mintz A (1986) Curvilinear, three-dimensional motion of chromatin domains and nucleoli in neuronal interphase nuclei. Science 234:863–866

de Graaf A, Van Hemert F, Linnemans WAM, Brakenhoff J, De-Jong L, Van Renswoude J, Van Driel R (1990) Three-dimensional distribution of Dnase I-sensitive regions in interphase nuclei of embryonal carcinoma cells. Eur J Cell Biol 52:135–141

Eils R, Dietzel S, Bertin E, Shröck E, Speicher MR, Reid T, Robert-Nicoud M, Cremer C, Cremer T (1996) Three-dimensional reconstruction of painted human interphase chromosomes: active and inactive X chromosome territories have similar volumes but differ in shape and surface structure. J Cell Biol 135:1427–1440

Engel LW, Young NA, Tralka TS, Lippman NE, O'Brien SJ, Joyce MJ (1978) Establishment and characterization of three new contiguous cell lines derived from human breast carcinomas. Cancer Res 38:3352–2264

Fakan S, Puvion E, Spohr G (1976) Localization and characterization of newly synthesized nuclear RNA in isolated rat hepatocytes. Exp Cell Res 99:155–164

Fakan S, Puvion E (1980) The ultrastructural visualization of nuclear and extranucleolar RNA synthesis and distribution. Int Rev Cytol 65:255–299

Glass JR, Gerace L (1990) Lamins A and C bind and assemble at the surface of mitotic chromosomes. J Cell Biol 111:1047–1057

Hochstrasser M, Sedat JW (1987) Three-dimensional organization of *Drosophila melanogaster* interphase nuclei. II Chromosome spatial organization and gene regulation. J Cell Biol 104: 1471–1483

Huang S, Spector DL (1992) U1 and U2 small nuclear RNAs are present in nuclear speckles. Proc Natl Acad Sci USA 89:305–308

Huang S, Spector DL (1996) Intron-dependent recruitment of pre-mRNA splicing factors to sites of transcription. J Cell Biol 133:719–732

Hutchinson N, Weintraub H (1985) Localization of DNase I-sensitive sequences to specific regions of interphase nuclei. Cell 43:471–482

Jackson DA (1991) Structure-function relationships in eukaryotic nuclei. Bio Essays 13:1–10

Janevski J, Park PC, De Boni U (1995) Organization of centromeric domains in hepatocytes nuclei: rearrangement associated with de novo activation of the vitellogenin gene family in *Xenopus laevis.* Exp Cell Res 217:227–239

Janevski J, Park PC, De Boni U (1997) Changes in morphology and spatial position of coiled bodies during NGF-induced neuronal differentiation of PC12 cells. J Histochem Cytochem 45: 1523–1531

Jiménez-Garcia LF, Spector DL (1993) In vivo evidence that transcription and splicing are coordinated by recruiting mechanism. Cell 73:47–59

Kraus MH, Popescu NC, Armsbaugh SC, King CR (1987) Overexpression of the EGF receptor-related proto-oncogene erbB-2 in human mammary tumor cell lines by different molecular mechanisms. EMBO J 6:605–610

Kraus MH, Di Fiore PP, Pierce JH, Aaronson SA (1988) Different mechanisms are responsible for oncogene activation in human mammary neoplasia. In: Lippman ME, Dickson RB (eds) Breast cancer: cellular and molecular biology. Kluwer, Boston, pp 49–66

Krystosek A, Puck TT (1990) The spatial distribution of exposed nuclear DNA in normal, cancer, and reverse-transformed cells. Proc Natl Acad Sci USA 87:6560–6564

Kurtz A, Lampel S, Nickolenko JE, Bradl J, Benner A, Zirbel RM, Cremer T, Lichter P (1996) Active and inactive genes localize preferentially in the periphery of chromosome territories. J Cell Biol 135:1195–1205

Lawrence JB, Singer RH, Marselle LM (1989) Highly localized distribution of specific transcripts within interphase nuclei visualized by in situ hybridization. Cell 57:493–502

Ludérus ME, den Blaauwen JL, de Smit OJ, Compton DA, van Driel R (1994) Binding of matrix attachment regions to lamin polymers involves single stranded regions and the minor groove. Mol Cell Biol 14:6297–6305

Marshall WF, Dernburg AF, Harmon B, Agard DA, Sedat JW (1996) Specific interactions of chromatin with the nuclear envelope: positional determination within the nucleus in *Drosophila melanogaster*. Mol Biol Cell 7:825–842

Matera AG, Frey MR, Margelot K, Wolin SL (1995) A perinucleolar compartment contains several RNA polymerase III transcripts as well as the polypyrimidine tract-binding protein, hnRNP I. J Cell Biol 129:1181–1193

Naegele R, Freeman T, McMorrow L, Lee H (1995) Precise spatial positioning of chromosomes during prometaphase: evidence for chromosomal order. Science 270:1831–1835

Park PC, De Boni U (1991) Dynamics of nucleolar fusion in neuronal interphase nuclei in vitro: association with nuclear rotation. Exp Cell Res 197:213–221

Park PC, De Boni U (1996) Transposition of DNase hypersensitive chromatin to the nuclear periphery coincides temporally with nerve growth factor-induced up-regulated of gene expression in PC12 cells. Proc Natl Acad Sci USA 93:11646–11651

Puck TT, Krystosek A (1992) Role of cytoskeleton in genome regulation and cancer. Int Rev Cytol 132:75–108

Sahlas DJ, Milankov K, Park PC, De Boni U (1993) Distribution of snRNPs splicing factor SC-35 and actin in interphase nuclei: immunocytochemical evidence for differential distribution during changes in functional states. J Cell Sci 105:347–357

Schröck E, du Manoir S, Veldman T, Schoell B, Weinberg J, Ferguson-Smith MA, Ning Y, Ledbedder DH, Bar-Am I, Soenksen D, Garini Y, Reid T (1996) Multicolor spectral karyotyping of human chromosomes. Science 273:494–497

Scott GK, Daniel JC, Xiong X, Maki RA, Kabat D, Benz C (1994) Binding of an ETS-related protein within the DNase I hypersensitive site of the HER2/neu promoter in human breast cancer cells. J Biol Chem 269:19848–19858

Sukegawa I, Blobel G (1993) A nuclear pore complex protein that contain zinc finger motifs, binds DNA and faces the nucleoplasm. Cell 72:29–38

Szöllösi J, Balazs M, Feuerstein BG, Benz CC, Waldman FM (1995) ERBB-2 (HER2/neu) gene copy number, $p185^{HER-2}$ overexpression, and intratumor heterogeneity in human breast cancer. Cancer Res 55:5400–5407

Xing Y, Johnson CV, Dobner P, Lawrence JB (1993) Higher level organization of individual gene transcription and RNA splicing: integration of nuclear structure and function. Science 259:1326–1330

Xing Y, Johnson CV, Moen Jr PT, McNeil JA, Lawrence JB (1995) Non-random gene organization: structural arrangements of specific pre-mRNA transcription and splicing with SC-35 domains. J Cell Biol 131:1635–1647

Zhang G, Taneja KL, Singer RH, Green MR (1994) Localization of pre-mRNA splicing in mammalian nuclei. Nature 372:809–812