Relative locations of the centromere and imprinted SNRPN gene within chromosome 15 territories during the cell cycle in HL60 cells

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SUMMARY

Investigations of imprinted regions provide clues that increase our understanding of the regulation of gene functions at higher order chromosomal domains. Here, the relative positions of the chromosome 15 centromere and the imprinted SNRPN gene in interphase nuclei of human myeloid leukemia HL60 cells were compared, because the homologous association of this imprinted chromosomal domain was previously observed in lymphocytes and lymphoblasts. Four targets including the chromosome 15 territory, its centromere, the SNRPN gene on this chromosome, and the nucleus, were visualized simultaneously in three-dimensionally preserved nuclei using multicolor fluorescence in situ hybridization, and the spatial distributions of these probes were analyzed with a cooled CCD camera deconvolution system. We found that preferential association of SNRPN interhomologues did not occur during the cell cycle in HL60 cells, although this gene exhibited asynchronous replication and monoallelic expression in this cells. SNRPN was found to localize at the periphery of the chromosome territories, and it preferentially faced the nuclear membrane, unlike the adjacent centromeric repeat. The SNRPN gene and the centromere were located close to each other late in S phase, reflecting that these DNA segments may be compacted into the same intranuclear subcompartments with the progress of S phase and in course of preparation for the following G2 phase. Our results suggest that, although an imprinted gene has features similar to those observed with intranuclear localization of other gene coding sequences, the characteristic of mutual recognition of imprinted regions is determined by certain cellular regulation, and it is not necessary for the allele-specific features of an imprinted gene.

Key words: Multicolor FISH, Chromosome territory, SNRPN, Genomic imprinting, HL60 cell

INTRODUCTION

Genomic imprinting marks the parental origin of chromosomes and results in allele-specific changes in expression, chromatin structure, and replication (Surani, 1994; Efstratiadis, 1994; Razin and Cedar, 1994; Barlow, 1995). Many imprinted genes are clustered, and these regions are thought to be regulated by higher order chromosomal domains (Nakao and Sasaki, 1996). Therefore, investigation of the behavior of these domains provides insight into the mechanisms that regulate genomic imprinting. One approach toward such purposes is to visualize target genes and domains in the nucleus three-dimensionally and examine their nuclear dynamics under various conditions.

Recent advances in digital imaging technology, confocal microscopy, and deconvolution systems have made it possible to analyze the nuclear organization of specific genomic sequences (Agard et al., 1989; Ferguson and Ward, 1992). Information about the nuclear architecture is accumulating gradually. Chromosome painting probes can be used to visualize directly the territorial organization of each specific chromosome in interphase nuclei by fluorescence in situ hybridization (FISH; Cremer et al., 1988; Lichter et al., 1988; Pinkel et al., 1988). In mammalian cells, interphase chromosomes are generally considered to be less condensed than their mitotic counterparts, but individual chromosomes occupy discrete compartments called chromosome territories that do not overlap each other (Cremer et al., 1993; Strouboulis and Wolfe, 1996; Bridger and Bickmore, 1998). Sub-chromosomal regions, such as chromosome bands, are also compartmentalized during interphase (Visser et al., 1998; Jackson and Pombo, 1998; Zink et al., 1999), suggesting that the structures of condensed metaphase chromosomes are reflective of their organization in interphase nuclear space. Coding sequences are known to be located at the surfaces of their respective chromosome territories independent of their transcriptional status (Kurz et al., 1996). The relationships between gene activities and three-dimensional (3-D) morphologies of chromosome territories were examined with a set of active and inactive human X interphase chromosomes. Active X territories were found to have flatter shapes and larger, more irregular surfaces in comparison to the...
positions of the chromosome 15 centromere and the imprinting involves preferential association of the 15 centromere homologues. They suggested that normal segments in this domain were closer than the chromosome. From this point of view, it is implications for nuclear architecture, intranuclear structure and support the idea that transcription and genomic sequences have number of examples studied is small, these observations to be components of nucleoli. Alterations of nuclear architecture might be associated with the changes observed in tumorigenesis (Linares-Cruz et al., 1999). Although the number of examples studied is small, these observations support the idea that transcription and genomic sequences have implications for nuclear architecture, intranuclear structure and behavior of chromosomes. From this point of view, it is interesting to examine how intranuclear genome organization changes in biological phenomena, such as genomic imprinting.

In the present study, we focused on an imprinted gene, SNRPN (small nuclear ribonucleoprotein polypeptide N), because homologous association of the different alleles of this imprinted gene domain occurs late in S phase during the cell cycle (LaSalle and Lalande, 1996). This gene is located on human chromosome 15q11-q13, and one candidate gene for the Prader-Willi syndrome/Angelman syndrome (PWS/AS; Horsthemke, 1997). LaSalle and Lalande found the allelic segments in this domain were closer than the chromosome 15 centromere homologues. They suggested that normal imprinting involves preferential association of the interhomologues of this gene region. We compared the relative positions of the chromosome 15 centromere and the SNRPN gene in interphase nuclei of human myeloid leukemia HL60 cells, because this cell line is well cultured and expected to maintain imprinting aspects of the SNRPN gene. First, we examined whether the association of homologous alleles of SNRPN was observed or not during the cell cycle in HL60 cells, after we confirmed asynchronous replication and monoallelic expression of this gene in HL60 cells. Furthermore, the relative positions of these loci during the cell cycle were analyzed with respect to the territories occupied by all of chromosome 15 to examine whether an imprinted gene has features similar to those observed with intranuclear localization of gene coding sequences. So far as we know, this is the first report that examines the relationship between an imprinted gene and a chromosome territory.

**MATERIALS AND METHODS**

**Chemicals and DNA probes**

RPNI1640, fetal bovine serum (FBS), and human Cot-1 DNA (Gibco-BRL); bromodeoxyuridine (BrDU), 4',6-diamidino-2-phenylindole (DAPI), dazacyclooctan (DABCYCO), and propidium iodide (PI; Sigma); anti-BrDU monoclonal antibody and anti-mouse antibodies conjugated with either FITC or rhodamine (MBL); and anti-biotin conjugated with Cy-3 (Jackson Lab.) were purchased from the indicated sources. Reagents used for probe labeling and signal detection for FISH were purchased from Boehringer Mannheim. Other chemicals were from Nacalai Tesque unless otherwise stated. Cosmid 93 (c93) spanning the SNRPN region and pPR541 in MHC locus were kindly provided by A. L. Beaudet (Baylor College of Medicine; Sutcliffe et al., 1994) and H. Inoko (Tokai Univ. School of Medicine), respectively. A centromere probe, pCM15, was kindly provided by A. Baldini (Baylor College of Medicine). Cy-3-conjugated chromosome 15 painting probe was purchased from Cambio (Cambridge, UK).

**Cell culture and preparation of specimens**

Human myeloid leukemia HL60 cells were cultured in RPMI1640 containing 10% FBS at 37°C in an atmosphere of 5% CO2. The HL60 cells were fractionated by centrifugal elutriation. Cultured cells (3×10⁸) were loaded onto a Beckman elutriator rotor at 2,000 rpm at 4°C with flow of 0.3% gelatin in PBS (phosphate-buffered saline). The flow rate was increased from 11 ml/minute to 26 ml/minute at every 2.5 ml/minute increment. For each flow rate, the initial 100 ml were collected, and aliquots of each fraction were analyzed by flow cytometry (FACSCalibur; Becton Dickinson). Cells were immediately fixed by the method of Kurz et al. (1996) with slight modification. Briefly, cells were suspended in PBS and fixed with 4% paraformaldehyde in PBS for 10 minutes at room temperature. After treating with 1% paraformaldehyde in 0.1 M HCl for 10 minutes, the fixed cells were permeabilized with PBS containing 0.5% Triton X-100, 0.5% saponin, and 1% paraformaldehyde for 10 minutes. Before each treatment, cells were harvested at 200 g at 4°C for 5 minutes, and the supernatant was removed. The permeabilized cells were equilibrated with 20% glycerol in PBS for 20 minutes and stored in liquid nitrogen until use.

**FISH**

FISH was performed essentially as described previously (Lichter et al., 1988). Cells frozen in liquid nitrogen were washed twice with PBS. The cells were mounted onto a poly-D-lysine-coated coverslip and placed on it for 15 minutes for the fixation. Cells were equilibrated with 2× SSC for 10 minutes and then further equilibrated with denaturing solution (50% formamide in 2× SSC) for 15 minutes. Cells were then equilibrated with the hybridization solution for at least 20 minutes prior to denaturation. The nick-translated cosmid (biotinylated) and centromere (directly fluorescein labeled) probes were mixed with 3 μg of Cot-1 DNA and denatured at 80°C for 10 minutes in the hybridization solution (50% formamide (v/v), 10% dextran sulfate in 2× SSC) and preannealed at 37°C for 30 minutes. The chromosome 15 painting probe was treated at 42°C for 20 minutes and mixed with the preannealed probes just before hybridization. The cells on the coverslip were denatured for 10 minutes at 80°C, and then the above hybridization probe mixture was added. Hybridization was carried out overnight at 37°C in a moist chamber. Each coverslip was washed three times with 50% formamide in 2× SSC for 5 minutes at 42°C and then three times with 2× SSC at 60°C. The coverslips were then incubated for 30 minutes in a blocking solution (3% Block Ace (Yukijirushi), 0.1% Tween-20 in 2× SSC), and detected with Cy-3-conjugated avidin (in 1% Block Ace, 2× SSC, 0.1% Tween-20) for 30 minutes at 37°C. Then, coverslips were washed three times with 4× SSC, 0.1% Tween-20 for
FISH-based replication assay

Replication timing of cosmids clones was determined by FISH (Selig et al., 1992) with slight modification. A biotinylated probe was hybridized to methanol-acetic acid-fixed nuclei and detected with fluorescein-avidin. S phase nuclei were simultaneously visualized by incorporated BrdU following detection steps with anti-BrdU and rhodamine-conjugated antibodies. Slides were counterstained with DAPI and examined with 63× oil objective on a Zeiss Axioskop fluorescence microscope fitted with a Zeiss filter set for DAPI, FITC, and rhodamine. Signals were observed in more than 95% of interphase nuclei. Only slides with strong FISH signals and low background were scored. At least 200 S phase nuclei were scored, and their signal patterns classified as singlet-singlet (SS), singlet-doublet (SD), or doublet-doublet (DD). Only clear round signals were scored as singlets. Extremely strong, large signals, and signals with rod-like or oval-like shapes were classified as doublets. The maximal difference between the two examiners was 2.0% for SD%, and their data were averaged.

RNA-FISH

The intron probes for the RNA-FISH analysis were generated by PCR. PCR was carried out using c93 as template and the following primer sets: 5'-AGGAAAAGGAACAGGGTAAGGGAAA-3', 5'-GCAGACGCACCAGGTGACAG-3', 5'-ACGACACACAGACCCCCAG-3', 5'-GGACAGGCCTTGTTGACTGG-3') corresponding to approximately 3 kb sequences on the upstream of exon alpha of the SNRPN gene. PCR products were labeled by nick translation using digoxigenin-11-dUTP. The RNA-FISH protocol was using previously described (Dirks and Raap, 1995), with modifications. HL60 cells were fixed with 4% paraformaldehyde and 5% acetic acid for 20 minutes at room temperature. The cells were washed three times for 5 minutes with PBS and stored in 70% ethanol at −20°C. Cells were fixed onto a poly-D-lysine-coated coverslip and treated by 0.01% pepsin in 0.01% HCl for 5 minutes at 37°C, followed by a short wash in water and fixed in 1% paraformaldehyde for 5 minutes at room temperature, then washed with PBS, dehydrated in a 70, 90 and 100% ethanol series and air-dried. The hybridization mixture containing labeled intron probe and salmon sperm DNA (10 μg) in hybridization solution was mounted on the coverslip. Hybridization was carried out at 37°C overnight in a moist chamber. The coverslips were washed four times with 50% formamide in 2× SSC for 3 minutes at 37°C, and once in 2× SSC for 3 minutes at room temperature. The hybridized probe was detected with rhodamine-conjugated anti-digoxigenin Fab fragment and followed by blocking step. Finally, the coverslip was washed three times with 0.1% Tween-20 in 4× SSC for 2 minutes at 37°C, then counterstained with DAPI.

Optical sectioning and 3-D-image analysis

Series of light optical sections of nuclei were recorded with a cooled CCD camera (PentaMax 1317K-1, Princeton Instruments) mounted on a Zeiss Axioplan 2 MOT microscope with a Plan-Apochromat 63×/1.40 oil objective controlled by IPLab Spectrum software (Signal Analytics) on a Macintosh 9600/200MP computer. The Ludl filter wheel systems were equipped with both excitation and emission filters that were also controlled by IPLab. For sectioning, the microscope stage was lowered at 0.1 μm (input) intervals, and at each focal plane, the four fluorescent colors were imaged through respective filter combinations. The sectioned images of each color were deconvoluted separately using HazeBuster 2.0 software (VayTek) to remove haze, and the 3-D images were reconstructed using extension utilities of IPLab Spectrum and VoxBlast 2.01(VayTek). These 3-D reconstructed images were used to confirm the positioning of the probes and the territories.

RESULTS

Replication timing and expression of SNRPN in HL60 cells

Before studying the intranuclear positioning of the SNRPN gene, we examined whether this gene has general properties of genomic imprinting, i.e. asynchronous replication and monoallelic expression. First, the replication timing profile of the SNRPN gene was determined by FISH-based assay. As shown in Fig. 1A, the SNRPN gene exhibited more than 25% of asynchronous pattern (SD), although the SD% of a standard clone pPR541 was less than 10%. This confirms that the SNRPN replicates asynchronously in HL60 cells. Since the cells in S phase were selected by simultaneous detection of BrdU with different color combination with the probe, SS% and (SS + SD)% reflect the approximate time of replication of the first allele and the second allele in S phase, respectively. More than 60% for SS signal indicates that both alleles of the SNRPN gene replicate during the later half of S phase in HL60 cells.

Next, RNA-FISH using intron probes of the SNRPN gene was carried out to visualize the primary transcript in the nucleus. As shown Fig. 1B, most nuclei (79.6%) exhibited only one signal, suggesting that the imprint expression of this gene is maintained in HL60 cells, although we could not determine which parental allele of this gene was actively transcribed.

Cell fractionation and 3-D FISH imaging

To study the spatial arrangement of specific genomic sequences in the nucleus, SNRPN gene, chromosome 15 centromere, whole chromosome 15, and the nucleus were studied by multicolor FISH. A cosmids containing the SNRPN gene was labeled with biotin-dUTP and detected by anti-biotin conjugated Cy-5. The centromere probe was labeled with fluorescein-dUTP. To detect chromosome 15 in its entirety, we used a commercially-available Cy-3-conjugated painting probe. The q arm of chromosome 15 was stained.
of in S phase nuclei were classified as SS, SD, or DD. The higher SD% transcripts of the homologous alleles. (B) RNA-FISH to detect the primary examined (Fig. 2b-right). The center of the centromere probe the signal centers of each probe on the same focal plane are probes and territory is shown as an example. The locations of the rDNA genes located on the p arm of this chromosome may be associated with nucleoli. In fact, many FISH signals were observed near and around the nucleoli (data not shown).

The above three probes were hybridized to paraformaldehyde-fixed nuclei and detected by the respective color, within the nuclei which were stained with DAPI. An optical section was taken every 0.1 μm (input) for each nucleus (total 60-80 sections). Typical pseudocolored and merged images of every third focal planes with FISH signals are shown in Fig. 2a. To determine the intraterritorial locations of each fluorescence signal, only the necessary images were merged, for example, SNRPN and the territory, or the centromere and territory. In Fig. 2b-left, one focal plane merged with two probes and territory is shown as an example. The locations of the signal centers of each probe on the same focal plane are examined (Fig. 2b-right). The center of the centromere probe (marked green +) is detected within the visible border of the chromosome territories, whereas that of the SNRPN probe (marked blue +) is located at the periphery or outside of the territory. Because of the differences in sensitivity between the painting probe and the specific sequences, probes are often observed outside the territory (territory core). Therefore, the signal whose center was observed inside the territory core was considered as internal localization; the signal whose center was observed outside or boundary the territory core was considered as peripheral localization. All the sectioned images for each nucleus were surveyed to determine the classification of the FISH signals. Three-dimensional reconstruction of FISH images was performed with VoxBlast software. A representative 3-D image was shown in Fig. 2c. The 3-D images were used to confirm the positioning between probes and territories. Nucleoli are visible in the nuclei as dark regions that are not stained with DAPI. It is interesting to delineate nucleoli, because human chromosome 15 is acrocentric, and the rDNA genes located on the p arm of this chromosome may be associated with nucleoli. In fact, many FISH signals were observed near and around the nucleoli (data not shown).

To investigate cell-cycle dependent localizations of genomic sequences in the nucleus, cultured HL60 cells were fractionated by centrifugal elutriation. This method was used instead of cell synchronization to avoid the effects of drugs necessary to control the cell cycle. Four fractions were pooled, and aliquots from each fraction were analyzed for the cell cycle profile by flow cytometry. As shown in Fig. 3, fractions A, B, C, and D were rich in G1, S, late S, and G2/M phase cells, respectively. Because M phase cells in the G2/M rich fraction were excluded from these analyses, the fractions were designated G1, S, late S, and G2. After the elutriation, cells were fixed immediately in paraformaldehyde as described previously (Kurz et al., 1996). We used a protocol that did not include alcoholic and protease treatments to better preserve the intranuclear organization of the genomic sequences.

**Preferential association of SNRPN homologues does not occur in HL60 cells**

LaSalle and Lalande (1996) reported homologous association of the oppositely imprinted human chromosome 15q11-q13 region in late S phase. They observed a marked increase in the number of nuclei with closely-associated 15q11-q13 region (within 2 μm) in late S phase, even compared with the chromosome 15 centromere. They also showed that the imprinted status is important for this association. Since SNRPN in this locus has been confirmed to show monoallelic expression and asynchronous replication in HL60 cells (Fig. 1A and B), we expected that association of SNRPN homologues would be observed during late S phase in most nuclei. Therefore, we compared the interhomologue distance of SNRPN with that of the centromere in HL60 cells for the SNRPN and centromeric probes, we scored the number of nuclei in which the distance between the one pair of the homologues was closer than the distance between the other pair in the same nucleus (Fig. 4). The specific association of SNRPN homologues in late S phase was not significant statistically (P>0.3). The results showed that there were no significant differences in relative distances during the cell cycle for either probe. Although HL60 cells maintain imprinting of SNRPN, we could not find any specific association of this
Imprinted SNRPN within chromosome territories

locus. In contrast, interhomologues of both SNRPN and the centromere were observed in the nuclear peripheries and apart from each other in most cases (data not shown).

Distribution of chromosome 15 centromeres or SNRPN genes within the chromosome territories

Although the number of examples are not many, gene-coding sequences were shown to be located at the surface of the chromosome territories independent of their transcriptional activities, and non-coding sequences were found randomly distributed or localized in the interior of the chromosome territories (Kurz et al., 1996). Here, the distributions of the chromosome 15 centromere and SNRPN gene within the chromosome territories were examined for each fraction of the cell cycle, to know whether these DNA segments have such features or not. It is also interesting to compare the intranuclear positioning of physically adjacent DNA segments with different properties, because the human SNRPN gene is located on chromosome 15q11-q13 close to the centromere, and both of these target sequences are spaced closely as detected by FISH. The images in Fig. 5 are examples of deconvoluted, merged images on the same focal plane. Only patterns for the SNRPN signal is demonstrated for easier view. The relative position of SNRPN or the centromere within the chromosome territory was examined on the sectioned images, and the number of territories with the patterns in the upper three images in the figure (a-c) were scored. The image (a) shows the FISH signal of SNRPN (or the centromere) probe located on the periphery of the chromosome territory and facing the nuclear membrane. In the lower part of this figure, the frequency of this pattern to the total number of cells scored is calculated and shown as a red bar for each cell fraction. The image (b) shows a FISH signal located on the periphery of the territory and facing the interior of the nucleus. The percentage

Fig. 2. Three-dimensional analysis of the centromere and SNRPN within chromosome 15 territories. (a) Examples of optical section images by a deconvolution system. Sixty to eighty sections obtained every 0.1 μm (input) were collected for each nucleus. The four fluorescent signals were imaged in each focal plane using a filter wheel system. Each set of collected images was deconvoluted, and the deconvoluted images on the same focal plane were merged and pseudocolored. Chromosome 15 painting probe (Cy-3; red), c93 SNRPN probe (Cy-5; blue), pCM15 centromere probe (fluorescein; yellow/green), and DAPI-stained DNA (gray). Fifteen images with 0.3 μm (input) intervals of the representative nucleus are shown in the figure. (b-left) Example of a chromosome territory (red) after merging of the centromere (yellow/green) and SNRPN (blue) images acquired from the same focal plane. (b-right) Same image as in b-left is represented in gray scale for the territory. Centers of the centromere and SNRPN signals are marked as green and blue (+), respectively, to determine their intraterritorial localizations. In this case, the centromere and SNRPN was considered as interior and periphery of the territory, respectively, because the center of the centromere is inside the territory, but that of SNRPN is at the boundary or exterior the territory. (c) Representative 3-D image generated by a deconvolution system. The same pseudocolors as the above are used. The left image was rotated counter-clockwise from the z-axis (c-right).
of cells with this pattern is indicated as an orange bar in the figure. The image (c) is a pattern that the FISH signal is detected in the territorial volume. This percentage is shown as a blue bar in the figure. As clearly shown, the centromere probe preferentially localizes in the territorial volume during the cell cycle (blue, average, 79%). The SNRPN gene localized in the more peripheral part of the chromosome territory than the centromere independent of the cell cycle stage ($P<0.0001$). The average was 56% for SNRPN but 21% for the centromere.

These results are consistent with the findings of Kurz et al. (1996). Allelic differences in the positions within the territories were not evident (data not shown). Furthermore, the SNRPN gene preferentially faces the nuclear membrane particularly in the late S and G2 phases. For SNRPN, 58% and 71% of the FISH signals located on the periphery of the chromosome territories face the nuclear membrane in the late S and G2 phases, respectively, while these values are 32% and 37% for the centromere ($P>0.6$ for G1 and S; $P=0.01$ for late S; $P=0.001$ for G2). Relative positions of the SNRPN and centromere FISH signals in the nucleus were not significant during the cell cycle (data not shown).

**Cell cycle-dependent association of SNRPN and chromosome 15 centromere in HL60 cells**

The distance between the SNRPN and centromere signals was compared across the cell cycle. As shown in Fig. 6, four FISH signal patterns for SNRPN and the centromere were observed: (a) both probes colocalized, (b) both probes overlapped, (c) both probes were separated but were within one signal distance, or (d) both probes were separated by more than one signal distance. Both the FISH signals were close to each other during late S and G2 phases, as compared with G1 and S phases ($P<0.001$, Fig. 5; purple and orange bars). This result suggests that these sequences are associated in late S phase. This may be more understandable if it is taken into account that...
consideration that the late S fraction was not well-fractionated and that the G2 fraction contains many cells in late S phase (see Fig. 3). Because the map positions of SNRPN and the centromere are close on chromosome 15, this genomic region may be more extended in early S and more condensed in late S phase. It is possible that these DNA segments are compacted into the same intranuclear subdomains in the course of preparation for the normal G2 phase.

DISCUSSION

We analyzed the cell-cycle dependent spatial arrangement of the imprinted SNRPN gene on human chromosome 15 in human myeloid leukemia HL60 cells, because this gene is on a well-characterized chromosomal domain, the PWS/AS critical region, and a relationship between the genomic function and the intranuclear behavior has been suggested (LaSalle and Lalande, 1996). We asked, first of all, whether the imprinted status of SNRPN is necessary and sufficient for the homologous association of this gene locus. As shown in Fig. 1, HL60 cells were confirmed to maintain its monoallelic expression and asynchronous replication, which are general properties of imprinted genes. We expected that this gene pairs between homologues in a specific stage of the cell cycle as previously observed. However, in the present study, the association of homologous alleles of SNRPN was not observed during the cell cycle in HL60 cells (Fig. 4). The results of this study contradict a report of homologous association of this imprinted chromosomal domain in lymphocytes and lymphoblasts (LaSalle and Lalande, 1996). Our results suggest that the characteristic mutual recognition of imprinted regions is determined by specific cellular regulation, and is not necessary for the allele-specific features of an imprinted gene. Another group also found preferential pairing of the imprinted region on distal mouse chromosome 7 in fibroblast cells (Riesselmann and Haaf, 1999), although their observation was not so significant. Long-time cultured cells like HL60 might lose this sort of specific features of imprinted domains.

Next, we collected general observations on the relative positions of gene-coding and repetitive sequences within the chromosome territories or the nuclei using the chromosome 15 centromere and SNRPN as examples. We delineated four targets stained with different colors simultaneously with a deconvolution system. In contrast with the centromere, we found that the SNRPN gene is localized in the periphery of the chromosome territories and preferentially faces the nuclear membrane in late S and G2 phases (Fig. 5). The peripheral localization of SNRPN in the chromosome territories was consistent with the findings of Kurz et al. (1996) that genes are located at the surface of their respective chromosome territories independent of their transcriptional status. Although this gene shows imprinted expression, we could not find allelic differences in position within the territories in individual nuclei (data not shown). Localization of a gene and its primary transcript near the nuclear membrane may be responsible for prompt transport of the processed RNA through the nuclear pores. DNA replication may be another explanation for the peripheral localization because both alleles of SNRPN replicate asynchronously but late in S phase (Gunaratne et al., 1995, Fig. 1A), and DNA replication usually occurs at the periphery of the nucleus in the later half of S phase.

The boundaries of territories and nuclei are an important point. Kurz et al. (1996) characterized active and inactive genes by considering a visible border of the territory exterior region. Eils et al. (1996) analyzed the shape and surface structures of the active and inactive X chromosome territories using 3-D Voronoi tessellation procedure. In HL60 cells, the shape of the nucleus varies. Therefore, to examine the homologous association, it was considered more accurate to compare the relative positions of two signals than to determine the absolute distance of FISH signals from the center or the edge of the nucleus. The shapes of territories are also complex and variable, and the surfaces of territories are not smooth. Even if a gene was observed within a territory, some factors could be accessible to it through a hollow on the territories (Clemson et al., 1996). The difference in sensitivities between the cosmid and the painting probes should also be considered. In this point of view, it may be reasonable to regard the painted regions as territorial cores, because FISH signals of SNRPN are often observed outside the painted territories. In fact, we determined intraterritorial localization of the probes based on this territory core (Fig. 2b).

We observed that SNRPN and the centromere are located close to each other particularly late in S phase (Fig. 6). Since SNRPN maps to chromosome 15q11-q13, it is close to the centromere. Therefore, it is not surprising that their FISH signals are adjacent to each other in interphase nuclei; in fact, approximately 90% of the signals were detected within one signal distance (see the green bars in Fig. 6). However, as shown in Fig. 6, 49% and 44% of both probes were associated with or overlapped each other during late S and G2 phases, respectively. This is very high because late S fraction contains many cells in G2 stage and vice versa (see Fig. 3), indicating that this genomic region is more extended in early S phase and more condensed in late S phase. Although it has been suggested that the organization of chromatin segments within a chromosome territory is random (van den Engh et al., 1992; Yokota et al., 1995), these two loci may be compacted into the same intranuclear sub-compartments with the progress of S phase. The assembly of these loci into a compartment after DNA replication may be in preparation for the following normal G2 and division stage. In recent model, DNA polymerases are immobilized by attachment to larger structures, and concentrated in discrete ‘replication factories’, where they reel in their templates and extrude newly made nucleic acids (Cook, 1999). Generally, a large number of small foci are observed early in S phase, while a relatively small number of assembled foci are observed in the middle and late S phases (Nakayasu and Berezney, 1989; Fox et al., 1991; O’Keefe et al., 1992). Since both alleles of SNRPN and the centromere replicate in the later half of S phase, it might be possible that both regions assemble into the same replication domains before or after DNA replication. In late S phase, replicating regions and flanking-replicated regions might be collected into larger replication factories. To clarify the meaning of this compartmentalization, it is necessary to examine the positioning of these probes in relations to various intranuclear structures.

As demonstrated in Fig. 1, SNRPN imprinted expression and asynchronous replication are maintained in HL60 cells.
Although SNRPN and the centromere tend to colocalize or overlap in late S phase, a number of cells showed differences of FISH signal patterns in the homologues during the cell cycle. In 30 to 40% of the nuclei, SNRPN and the centromere on one chromosome were colocalized, while they were separated on the other chromosome (data not shown). Unfortunately, polymorphic markers to identify the alleles did not work in this cells (Kitsberg et al., 1993; Ritchie et al., 1998). Although the parental origins of the alleles were not determined in this study, these results may reflect allelic differences in the chromatin structure of this imprinted region. As mentioned before, allelic differences in intraterritorial locations of SNRPN was not found. So, this allelic difference may be correlated with DNA replication timing.

Imprinted regions are thought to be regulated by higher-order chromosomal domains (Nakao and Sasaki, 1996). Therefore, it is interesting how intranuclear genome organization is related to this biological phenomenon. The present study is a first step to clarifying this important biological problem as well as to understanding that the intranuclear behaviors of the genomic sequences have biological significance.

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