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Visualize Dynamics of Chromosome Structure Formation and DNA Repair/Recombination Coupled With DNA Replication: Tight Coupled Role of DNA Replication in Chromosome Compaction and DNA Recombination

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1. Introduction

The genetic information of living organisms is carried and stored in a gene, which is a huge complex molecule involving DNA and proteins. In eukaryote cell, gene is usually higher hierarchical ordered packaged in a large complex structure called “chromosome”. The genetic information must be strictly conserved throughout life cycle, otherwise lost or false DNA information may cause malfunction of cell, cell death, impaired development or cancer prone. The genetic information must also be principally correctly transmitted to the next generations for stabilize the species. Three major cellular functions ensure maintain the complete gene information and transmit to daughter cells or next generations: (1) DNA replication; duplicates complete DNA sequence information to transmit for next cell generation, (2) DNA repair; recover structural damages of DNA and corrects false information anything else introduced in DNA molecule by intra- or extra-cellular factors and (3) Chromosome condensation; compaction of DNA molecule to architect a higher-organized packaged and compacted architecture known as “chromosomes” to assure protect and stabilize the integrity of labile DNA molecules and segregate the identical chromosome sets to daughter cells during mitosis. In addition, DNA recombination/rearrangement mechanism also plays very crucial role for various cellular function. For example, exchange genetic information between paternal and maternal homologous chromosome gene during recombination process in fertilization will promote evolution of species and prevent the gene homogeneity. Recombination of Immuno-globulin gene during lymphocyte maturation process promises the antibody diversity against vast numbers of foreign antigens. Recently accumulated evidences have strongly suggested that these mechanisms are mutually tight-coupled functions rather than they independently work. Molecular genetic studies have also provided supporting evidence for the idea that mutation in genes (HIRA/Tuple1, XCDT1, cdt1, Orc2, Orc3, Orc5, MCM2, MCM4, MCM10, RECQL4)(Maiorano et al. 2000; Nishitani et al. 2000; Pflumm and Botchan 2001; Christensen and Tye 2003; D'Antoni et al. 2004; Prasanth et al. 2004; Tatsumi et al. 2006) required for

DNA replication or DNA repair (SCC2, SMC1, SMC3, ESCO2) (Gillespie and Hirano 2004; Bekker-Jensen et al. 2006; Gandhi et al. 2006; Yoshizawa-Sugata and Masai 2009) showed abnormal phenotype in chromosome structure, inherited diseases, genomic instability or prone to cancer, or aberrant replication timing causes abnormal chromosome condensation. Cytogenetical observation of chromosome is certainly a most direct approach for elucidates how dynamics of chromosome structure formation and chromosome recombination are coupled with DNA replication. Numbers of supporting evidences have reported the relationship of DNA replication, chromosome conformation/recombination and DNA repair mechanisms. However, the results shown in these reports were mainly yielded from the observation in interphase nuclei, thus the resolution and the resulting information were still limited because chromosomes are only visible in the mitotic stage of the cell-cycle and are invisible as de-condensed in interphase. Premature chromosome condensation (PCC) technique is a unique and useful technique that allows the interphase nuclei to be visualized like as condensed form of mitotic chromosome. Conventional PCC has been carried out by cell fusion using either fusogenic viruses or polyethylene glycol (cell fusion-mediated PCC, (Johnson and Rao 1970; Pantelias and Maillie 1983). But cell fusion mediated PCC protocols are usually technically demanding and keenly depend on activity of the virus or PEG. More recently, a much easier and more rapid PCC technique using calyculin A or okadaic acid, specific inhibitors of protein phosphatases (drug-induced PCC, Gotoh et al. 1995; Gotoh and Asakawa 1996). Drug-induced PCC is now becoming much more popular and has been used in a wide range of cytogenetic studies (Durante et al. 1998; Gotoh et al. 1999; Ito et al. 2002; El Achkar et al. 2005; Gotoh and Tanno 2005; Srebniak et al. 2005; Deckbar et al. 2007; Gotoh 2007).

In this chapter, we first show the visualizing of the dynamics of chromosome structure formation and chromosome repair/recombination coupled with DNA replication in interphase nuclei, in particular by use of drug-induced PCC. We next show the spatial and temporal rearrangement of chromosome fragment after γ -ray irradiation, and this movement is tightly coupled with DNA replication. Possible hypothetical model for chromosome condensation/compaction involving the role of DNA replication and model for chromosome recombination coupled with DNA-replication will be suggested and discussed.

2. Visualizing of the dynamics of chromosome structure formation coupled with DNA replication

2.1 Background

Chromosomes condense during mitosis under a higher ordered stringent mechanism in mitotic phase, which is further divided into several sub-phases; (preprophase), prophase, prometaphase, metaphase, anaphase, telophase (these phases are called as mitosis) and followed by cytokinesis (Alberts et al. 1989). In the course of mitotic phase, number of drastic sequential transactions proceed; (1) chromatin condense to visible structure under a microscope "chromosomes" and mitotic spindle begins form (2) nuclear envelope breakdown into membrane vesicles, centriole and mitotic spindle formation followed by spindle attaches to centromere (3) kinetocore microtubules align the chromosomes at metaphase plate (4) chromosome separates and segregates to spindle poles (5) separated daughter chromatids reached at the poles, nuclear envelope re-forms (6) formation of contractile ring and cleavage furrows which constrict the cell center, cytokinesis and cell divides two daughter cells and chromosome de-condensation in divided cells and re-enter the cells in G1 phase (Alberts et al. 1989). The detailed whole mechanism is still almost

unclear, however number of molecules which involved in the mitotic events have been identified such as SMC proteins include condensin (chromosome condensation) and cohesin (chromosome cohesion of replicated chromosomes) (Swedlow and Hirano 2003), NuMA protein for spindle pole formation (Chang et al. 2009; Haren et al. 2009; Silk et al. 2009; Torres et al. 2010), nuclear lamins (Moir et al. 2000), aurora kinases in centromere function (Tanno et al. 2006; Meyer et al. 2010; Tanno et al. 2010), shugoshin and protein phosphatase 2A in chromosome cohesion (Kitajima et al. 2006; Tanno et al. 2010), cdk1 in chromosome condensation, chromosome bi-orientation (Tsukahara et al. 2010), cyclin B, cdc2, cdc25 in chromosome condensation (Masui 1974; Draetta and Beach 1988; Dunphy et al. 1988; Kumagai and Dunphy 1992), Polo and Rho in cytokinesis (Burkard et al. 2009; Wolfe et al. 2009; Li et al. 2010), and many other molecules. Dynamics in mitotic phase involves such as various elements, and numerous studies for visualize these dynamics have been reported, because these events are relatively easy observable in mitosis under microscopes. However, visualizing approaches in chromosome dynamics coupled with DNA replication is still limited, because it should be required to observe chromosomes in S-phase but chromosomes are usually invisible in S-phase as they de-condense (Gotoh et al. 1995; Gotoh and Durante 2006).

In this section, we just focus on visualize the chromosome dynamics coupled with DNA replication during S-phase progression use with drug-induced premature chromosome condensation method and we show how replicating DNA is folded to higher order chromosomes.

2.2 Materials and methods

2.2.1 Cell, chemicals and antibodies

Human normal karyotype fibroblast cell line GM05389 was from Coriell Cell Repositories (NJ, USA). Calyculin A was from Wako Chemicals (Osaka, Japan), dissolved in 100% DMSO, 100 μ M of stock solution was stored at -20°C . Cy3-dUTP was from Amersham (Uppsala, Sweden). Sizes of 425-600 μ m diameter acid-washed glass beads were from SIGMA (MO, USA).

2.2.2 Cy3-dUTP labeling by bead-loading method

Cy3-dUTP was loaded in the cells using beads loading procedure as described previously (McNeil and Warder 1987; Manders et al. 1999; Ito et al. 2002; Gotoh 2007). The procedure facilitate the Cy3-dUTP to be incorporated in the cell nucleus in very short time whereby transiently permeabilizes the cell membranes (McNeil and Warder 1987; Manders et al. 1999). GM05389 cells, seeded on a 35 mm glass-base culture dish (Iwaki, Japan) which is designed for observation under inverted microscopes, were grown exponentially and asynchronous in MEM supplemented with 15% foetal calf serum at 37°C in 5% CO_2 atmosphere with 95% humidification. For replication DNA labeling, culture medium was replaced with 37°C prewarmed 50 μ l of 10 μ M Cy3-dUTP dissolved in MEM medium, 425-600 μ m diameter glass beads were sprinkled onto the cells, tapping and rocking the dish several times, then the beads were rinsed off with PBS. It usually took a couple of minutes for complete the loading procedure. Pre-warmed (37°C) MEM was added to the culture and incubated until 10 minutes after starting of Cy3-dUTP loading. Then cells were subjected to be condensed prematurely using calyculin A, or to be immunostained.

2.2.3 Premature chromosome condensation

Immediately after Cy3-dUTP loading procedure, chromosomes were condensed prematurely using 50 nM of calyculin A. Premature chromosome condensation (PCC) using

calyculin A was done as described elsewhere (Gotoh et al. 1995; Asakawa and Gotoh 1997; Johnson et al. 1999; Ito et al. 2002; Gotoh 2007; Gotoh 2009) except that the incubation time was shortened as possible to 10 minutes to obtain a high spatial and temporal resolution, otherwise replication foci can be merged together rustling in a less spatial/temporal resolution. After incubation with calyculin A, cells were harvested, swollen in 0.075M KCl for 10 minutes at 37°C, fixed 3 times with carnoy's fixative (methanol: acetic acid = 3 vol.: 1 vol.), dropped on a glass slide and air-dried. Finally, DNA was counterstained with 200 ng/ml of DAPI, mounted with PBS and covered with a cover slip.

2.2.4 Laser scanning microscopy

Blue, green, or red confocal images were collected using a Confocal Laser Scanning microscopy system Zeiss LSM510 (Jena, Germany) equipped on an inverted epifluorescence microscope Zeiss Axiovert100M (Jena, Germany). A water immersion objective lens Zeiss C-Apochromat 63x/1.2 w corr. (Jena, Germany) was used. UV-, Ar- or He-Ne-laser was tuned at 366, 488, 568 nm to excite DAPI, FITC, Cy-3, respectively. The fluorescence signals from each fluorochrome were recorded separately in multiple scan to minimize optical cross talk. Digital images were manipulated using Zeiss LSM510 software (Jena, Germany).

2.3 Dynamics of chromosome structure formation coupled with DNA replication

Much study has achieved to visualize the dynamics of chromosome condensation during in interphase nuclei, particularly in S-phase. However, the visualizing study on the relationship between chromosome condensation and DNA replication has still limited. Several studies tried and defined fairly well the replication foci distribution in interphase nuclei (Nakamura et al. 1986), however little is still known about how replicating DNA is folded to higher order chromosomes, because chromosomes are invisible in interphase as they de-condense.

To visualize the chromosome compaction dynamics coupled with DNA replication more precisely in S-phase nucleus, we took the advantage of the drug-induced PCC method (Gotoh et al. 1995; Asakawa and Gotoh 1997; Johnson et al. 1999; Ito et al. 2002). Individual substage of S-phase can be easily identified by typical diagnostic appearances seen in different phases of S-PCCs (Mullinger and Johnson 1983; Gollin et al. 1984; Hameister and Sperling 1984; Savage et al. 1984; Gotoh et al. 1995; Gotoh and Durante 2006). A drastic conformational change of chromosome structure formation along with the proceed of DNA replication, as shown in Figure 1 (reproduced from Chromosoma. 2007; 116(5): 453-462, Gotoh 2007), clearly revealed in PCCs following Cy3-dUTP labeling (Gotoh 2007; Gotoh 2011). Cy3-dUTP loading procedure was finished within 10 minutes followed by 10 minutes of PCC induction and fixation (for the detail of the experiment procedures, see the Materials and Methods section in Chromosoma. 2007; 116(5): 453-462, Gotoh 2007). Accordingly, only DNA replicated in this short lapse fluoresces. Thus, the observed S-PCCs in the present study reflected the replication stages at most 20 minutes (see Materials and Methods described in Gotoh 2007) before the cell fixation. (i) In early S-phase, PCCs showed a cloudy spreading mass of thin fibers like a "nebula". Numerous fine granular foci distributed homogeneously on overall the fibers (Figure 1i), which shows "beads on a string" appearance that are similar to the same named structures observed under an electron microscope (Olins and Olins 1974; Thoma et al. 1979). (ii) In middle S-phase, typical "pulverized" PCCs structure was recognized, and the size of foci increased with the number of foci decreased and distributed unevenly on chromosomes. The foci became much brighter (Figure 1j). (iii) In late S-phase, chromosomes showed mostly

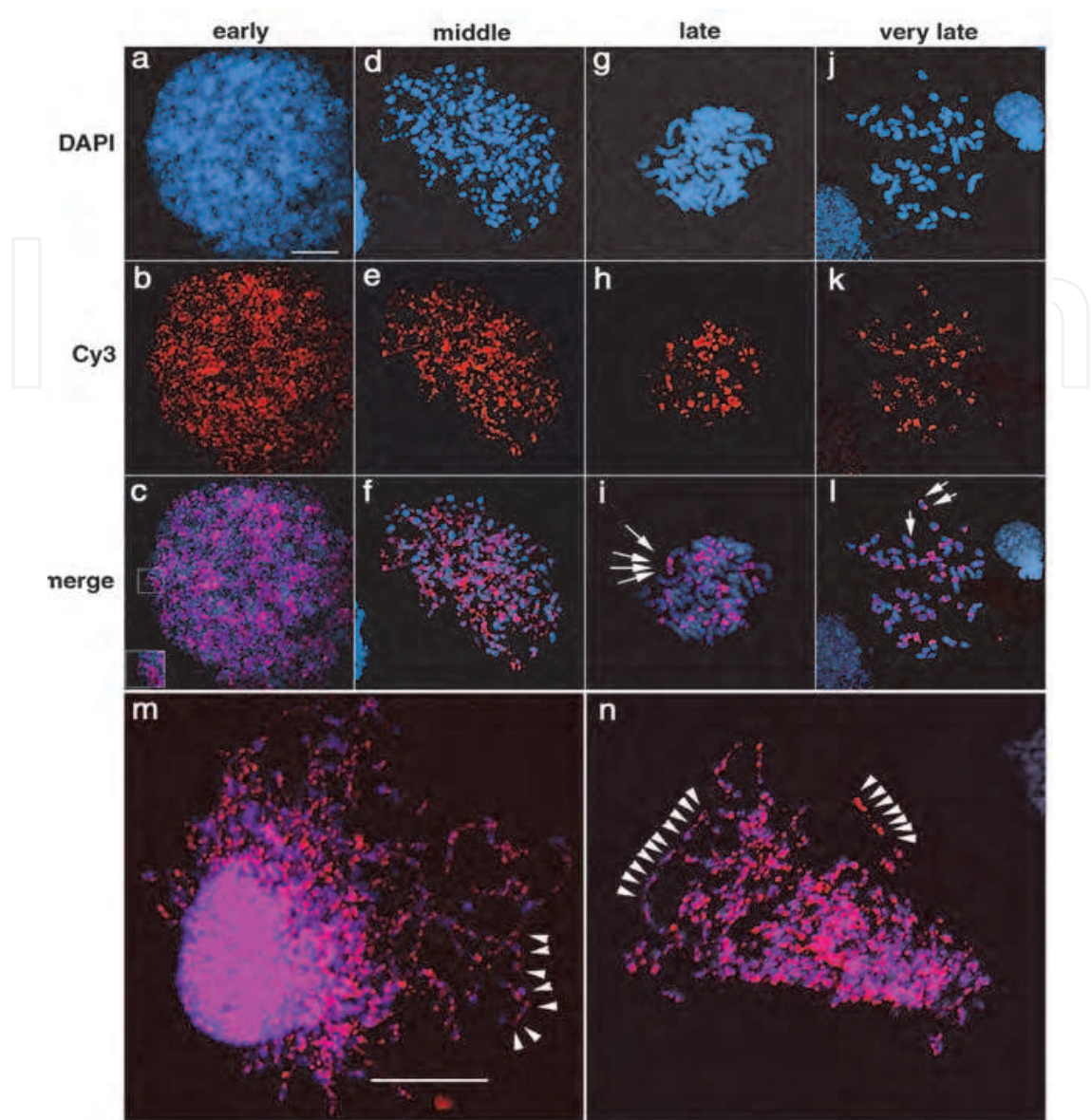


Fig. 1. (1) DNA replication regions on prematurely condensed chromosomes (PCCs) of different substages of S-phase. Ten minutes after Cy3-dUTP loading, cells were condensed prematurely using 50 nM of calyculin A (Gotoh et al. 1995). From left to right column, (a,b,c) early S-phase PCCs, (d,e,f) middle S-PCCs, (g,h,i) late S-PCCs and (j,k,l) very late S-PCCs. (a,d,g,j) DAPI counterstained DNA, (b,e,h,k) Cy3-dUTP labelled DNA replication region and (c,f,i,l) Merged image of DAPI and Cy3. Centromeric region (arrow) or telomeric region (arrowhead) replicates in very-late S-phase are indicated. (i,l) Late S- and very late S-PCCs already condensed like as mitotic chromosomes, but these PCCs were actually S-phase chromosomes because they incorporated Cy3-dUTP. G2/M chromosomes are easily distinguished from late or very late S chromosomes as G2/M chromosomes do not incorporate Cy3-dUTP (data not shown). Inset in (c) is higher magnification of the boxed portion. Scale bar, 10 μ m. (2) DNA replication regions seen on prominent fibre of PCCs. (m) early-S-phase (918 foci scored) and (n) middle S-phase (707 foci scored). Replication foci are clearly seen as 'beads on a string' structure, some of these are indicated by arrowhead. Scale bar, 10 μ m. (reproduced from Chromosoma. 2007; 116(5): 453-462, Gotoh 2007)

condensed like mitotic chromosomes. Cy3-dUTP incorporated regions were recognized as band arrays inserted in the condensed chromosome (Figure 1k, indicated by arrows). The similar appearance of replication foci aligned longitudinally on chromosomes were previously reported on metaphase of kangaroo-rat kidney PtK1 cells (Ma et al. 1998). The size of foci increased and the number decreased. (iv) In very late S-phase, the number of foci further reduced and predominantly localized at centromeric or telomeric regions (Figure 1l, indicated by arrows). These regions are actually known as satellite heterochromatic DNA regions where DNA replicates at very late S (O'Keefe et al. 1992).

2.4 The number, size and spacing of replication foci in different subphase of S

Figure 2 shows the typical “beads on a string” structure of replication foci seen in Cy3-dUTP fluoresced early S-phase PCC. The “beads on a string” structure is more evidently seen on the prominent fiber as shown in Figure 1m,n. The distance between foci was easily measured than those seen in interphase nuclei. Table 1 (reproduced from Chromosoma. 2007; 116(5): 453-462, Gotoh 2007) summarizes the number, size and the spacing of the foci of different S-phase stages. The number of foci scored maximum ~1400 in early S-phase that was much precisely than ever reported (Manders et al. 1996; Jackson and Pombo 1998; Ma et al. 1999), then decreased to ~100 in very late S-phase (Gotoh 2007). The size changing of foci during S-phase was also shown clearly (Gotoh 2007). The spacing between replication foci clusters was ranged from 85.3 kbp (0.64 μm) to 536 kbp (4.02 μm) (average, 208 kbp, 1.56 μm , Gotoh 2007) based on the assumption that 0.75 μm length of 30 nm chromatin fiber is

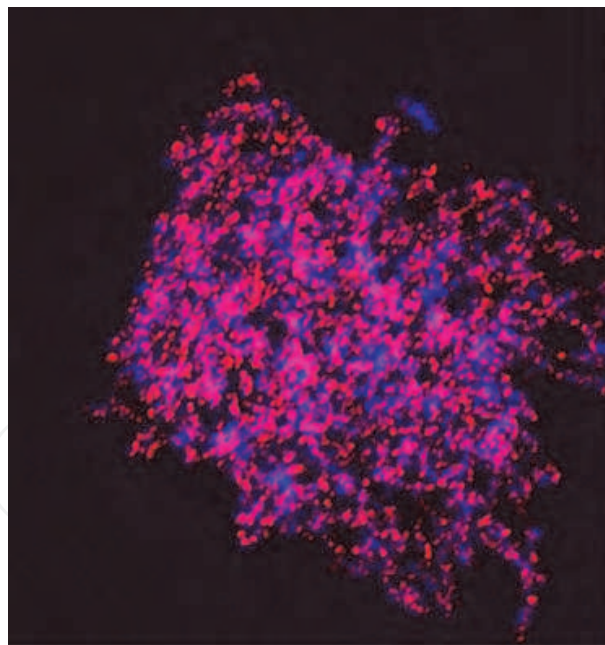


Fig. 2. Cy3 fluoresced DNA replication regions (replication foci) of early S-phase prematurely condensed chromosomes (PCCs). DNA replication regions were directly labeled with Cy3-dUTP by a bead-loading method followed by calyculin-A induced premature chromosome condensation. DNA was counterstained with DAPI. Chromosome spread shows a “cloudy nebula” appearance and, active replication regions (red) along with DNA fiber (faint blue) were seen as “beads on a string” structure. Condensed chromosome regions after finishing of DNA replication were seen as thick blue.

equivalent to 100 kbp (linear packing ratio of ~ 40:1) (Berezney et al. 2000). Fiber autoradiography of DNA labeled with [3H]thymidine revealed the synchronized synthesizing replicon of spacing distance ranging from 50-300kbp (Fakan and Hancock 1974; Edenberg and Huberman 1975; Hand 1978). Therefore the spacing of replication foci seems to vary more than previously reported values. The measure of the spacing of foci were also reported on stretched DNA fiber with detergent and calculated the average distance of foci as 144 kbp (Jackson and Pombo 1998) that is fairly good agreement with the present and the previous studies, although the intact structure of foci was no more retained in stretched fiber after lysis treatment.

Subphase of S	early	middle	late	very late
Number of foci (max~min) ^a	1046 ± 187 (1396~758)	678 ± 83 (887~601)	450 ± 132 (607~237)	153 ± 42 (245~98)
Size of foci (max~min) (µm radius) ^{b,c}	0.35 ± 0.01 (0.08 ^d ~0.62)	0.52 ± 0.06 (0.1 ^d ~0.62)	0.98 ± 0.23 (0.24~1.44)	1.1 ± 0.44 (0.32~4.1)
Spacing between foci (max~min) (µm) ^{b,c}	1.56 ± 0.68 (0.64~4.02)	1.01 ± 0.11 (0.33~1.86)	N.D. ^e	N.D. ^e

a: For each subphase, at least 12 spreads were scored., except for very late S (10 spreads to be scored were available)
b: Measured using the Zeiss LSM510 software
c: Randomly selected 20 points were measured, and the average and the error were calculated.
d: These data were beyond the resolution of optical microscope, and measured on digitized images.
e: Not done the measure because PCCs at these stages mostly condense as mitotic chromosomes thus measure the spacing seems less meaningful.

Table 1. The number, size and spacing of replication foci of different subphase of S (reproduced from Chromosoma. 2007; 116(5):453-462, Gotoh 2007)

2.5 A hypothetical chromosome compaction model coupled with DNA replication

Numbers of model for eukaryote chromosome architecture have been proposed (Marsden and Laemmli 1979; Woodcock et al. 1984; Woodcock and Dimitrov 2001; Swedlow and Hirano 2003; Kireeva et al. 2004), but they are controversial and many of things remain unclear. In addition, these models do not take account the involvement of DNA replication/transcription in chromosome packaging. DNA/RNA polymerase are known to be tightly immobilized to the replication/transcription factories (Cook 1999; Frouin et al. 2003). In the proposed model, DNA polymerase is thought to “reel in template DNA and extrude replicated DNA” (Hozak et al. 1996; Cook 1999), rather than the enzyme track along DNA template proposed in many conventional model. In Cook’s model, some kinds of mechanical tension force should be generated in the template DNA along with DNA replication goes on because the factory does not freely suspended in the nucleus but attached to nucleoskelton, consequently this force may pull and aggregate the replication foci of both side as to release the tension in DNA strand, which may resulting in formation of chromosomes as seen in mitosis. Based on the above mechanism and the observed findings obtained from chromosome structure dynamics coupled with DNA replication, Figure 3 shows a hypothetical model for the relationship of DNA replication and

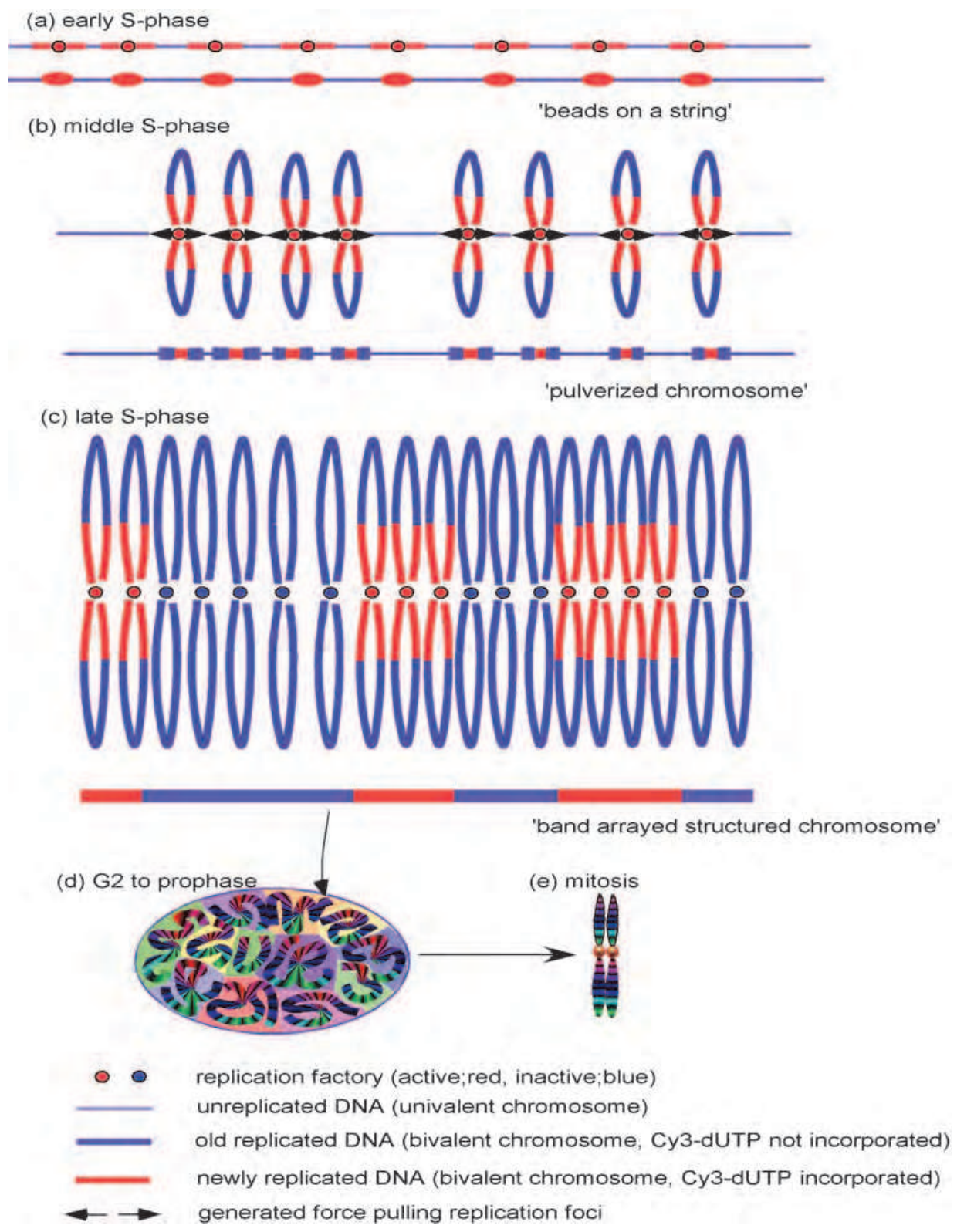


Fig. 3. A hypothetical two-dimensional model for chromosome conformational change involving DNA replication based on the models proposed by Cook (Cook 1995) or Pflumm (Pflumm 2002). (a) Early S-phase. DNA replication starts at multiple origins and proceeds bi-directionally. Early S-PCCs are seen as 'beads on a string' appearance. (b) Middle S-phase. As DNA replication proceeds, replicated DNA pass through replication factory and some tension

are generated. The generated tension may pull back the replication factories close together so as to release the tension. Replication factories may in turn fuse together and chromosomes compact. Middle S-PCCs are seen as well known 'pulverized chromosomes' appearance. (c) Late S-phase. Most of DNA finished replication and conformation was changed. Late S-PCCs are seen as 'tandem band arrayed structured chromosomes' like as mitotic chromosomes. (d) G2 to prophase. After finishing of DNA replication, chromosome conformation changed like as mitotic chromosomes, but still so elastic that packed in nucleus. Before fixation, each chromosome occupies individual chromosome territory (CT) in interphase nucleus, thus observed as compartment regions (colorized). (e) Mitosis. After prophase, chromosomes further shortening in longitudinal axis of chromosomes, consequently a straight rod shaped recognizable chromosome formed as usually seen by cytologists. For simplicity, the model is shown as two-dimensional and the scaling is arbitrary. The model intends not to depict actual events of chromosome conformation change but to help imagine how DNA replication is involved in chromosomal conformation. As the real chromosomes condense as three-dimensionally, other elements such as coiling and helical winding should be considered together to construct a stereoscopic hierarchical structure of eukaryote chromosomes (Woodcock and Dimitrov 2001; Swedlow and Hirano 2003). (reproduced from Chromosoma. 2007; 116(5): 453-462, Gotoh 2007)

chromosome conformational changes, that shows how interphase chromatin is constructed to chromosomes observed in mitosis (Figure reproduced from Chromosoma 2007; 116(5): 453-462, Gotoh 2007). During S-phase, chromosomal conformation changes as DNA replicates, and the chromosome formation would be mostly completed at the finish of DNA replication (Figure 3a,b,c). From G2 to prophase, chromosomes are still more elastic, less condensed, folded only several times and pre-aligned in interphase nuclei (Manders et al. 1999). Chromosomes, at these phases, would be observed as chromosome territories (Cremer et al. 1993; Berezney et al. 2000) (Fig. 3d). Entering in mitosis, chromosomes would condense even more as shortening the longitudinal axis to form solid and rod shape appearance of recognizable mitotic chromosomes (Manders et al. 1999) (Fig. 3e).

3. Visualizing the DNA replication coupled spatial and temporal rearrangement of γ -ray cleaved chromosome fragment

3.1 Background

Chromosomes are easily damaged by many kinds of clastogenic agents such as ionizing irradiation (IR), ultra violet light (UV), chemicals (i.e. alkylating agents) or biological resources (i.e. some kinds of viruses) (Therman 1980). Mostly damaged chromosomes can be repaired before the cell division, some damages might be mis-repaired or remain un-repaired, resulting in chromosome aberrations (Savage 1991). These aberrations may cause cell death or cancer prone. Although the numbers of knowledge in molecular level approaches have been accumulated, little is still known about how, when and where the ends of individual chromosome fragments close and associate to form rearranged chromosomes (Savage 2000; Aten et al. 2004). It is clearly required that the cleaved fragments must contact physically to form rearranged chromosomes at some stages of the repair process (Savage 2000). Two major hypotheses are still controversial: Whether these fragments move to contact after damage (the "breakage first" hypothesis), or whether association of fragments happen only where close contact already exists at time of irradiation (the "contact first" hypothesis) (Savage 2000). As DNA is fragmented in

interphase and subsequent repair and rejoining finish until cell division, it should be required to elucidate the process occurs in interphase nuclei. Number of studies has challenged to analyze the dynamics of repair and recombination in interphase.

In this section, to elucidate how and when chromosome fragments close and association to form rearranged chromosomes in interphase, we visualized the dynamics of spatial and temporal occupation of chromosome 4 domains in γ -irradiated human peripheral blood lymphocytes in interphase nuclei and in drug-induced PCCs by means of chromosome painting method.

3.2 Materials and methods

3.2.1 Cell culture, γ -irradiation and chromosome preparation

Human peripheral blood lymphocytes (PBLs) were separated from 10 ml of whole blood from healthy donor using LymphoPrep (Becton and Dickinson, Franklin Lakes, NJ) as per supplier's protocol. PBLs were then suspended in RPMI 1640 medium (GIBCO, Grand Island, NY) supplemented with 20% foetal calf serum (Biocell Laboratory, Ramincho-Domingues, CA). To induce chromosomal breaks, aliquot of PBLs were irradiated with 10 Gy of γ -rays (^{137}Cs , 0.662 MeV, 0.9 Gy/min) using a GammaCell 40 (Atomic Energy of Canada LTD, Canada). Irradiated PBLs were then stimulated immediately with PHA-P and rIL-2 as described previously (Gotoh and Asakawa 1996), cultured at 37 °C in 5% CO_2 atmosphere with 95% humidification for different incubation time (from 24 hours to 120 hours). One hour before the each harvesting time, cellular DNA were labeled with 10 μM of BrdU (10 mM of stock solution). The same irradiation experiment of PBLs except for without PHA-P stimulation or DNA-synthesis inhibition with 2 μM aphidicholine were performed simultaneously. After harvesting, cells were swollen in 0.075M KCl hypotonic buffer and incubated for 20 minutes at 37°C. Then the swollen cells were fixed with cold Carnoy's fixative (methanol: acetic acid = 3 parts: 1part), fixed three times with the fixative and dropped on microscopic glass slides and air dried. For obtaining chromosome spreads, 50 nM calyculin A was added to the duplicated PBLs culture to induce PCC 30 minutes before harvesting the cells at each incubation time (Gotoh and Tanno 2005; Gotoh 2009). For control studies, aliquot of non-irradiated PBLs cells were harvested at either 48 hours or 72 hours after stimulation. After harvesting, cells were swollen in 0.075 M KCl for 20 minutes at 37 °C, fixed with methanol: glacial acetic acid (3: 1 vol./vol.), washed and fixed three times in the same fixative, dropped on a glass slide. Usually, the slides were stored in a desiccated box for one or two weeks for sufficient drying until chromosome painting was performed.

3.2.2 Chromosome painting

Chromosome painting was done use with human chromosome 4 painting probe (GIBCO, Grand Island, NY) per se the supplier's protocol, except for additional washing with 0.1x SSC at 60°C for 5 minutes as described previously (Gotoh and Asakawa 1996). Briefly chromosomes were denatured in 70% formamide in 2xSSC at 70°C for 3 minutes followed by dehydration in ethanol series (70, 90 and 99.5%). A 5 μl aliquot of hybridization mixture (50% formamide, 10% dextran sulphate and 100 $\mu\text{g}/\text{ml}$ carrier DNA in 2xSSC) containing probes (2 ng/ml) was denatured at 70°C for 2 minutes and loaded on a sample, covered with a cover slip (ϕ 15 mm) and sealed with a silicon rubber (Exabite normal type; GC Corp., Japan) to prevent dry up the hybridization buffer during hybridization. Hybridization was

carried out at 37°C overnight, then non-specific hybridized probes were washed out in 2xSSC at 37°C 5 minutes three times and in 0.1xSSC at 60°C 5 minutes. Cell nuclei were then counterstained with 200 ng/ml of propidium iodide (PI; Sigma, St Louis, MO) for SpectrumGreen™ labeled probe or with 200 ng/ml of diamidino-2-phenylindole (DAPI; Sigma, St Louis, MO) for SpectrumOrange™ labeled probe. The slides were mounted with 50% glycerol in PBS supplemented with 0.1% antifade (1,4-phenylene-diamine, Sigma, St Louis, MO) and observed under an epifluorescence microscope (Nikon, Japan), using B-2A or G-2 excitation-emission filter (Nikon, Japan) to visualize SpectrumGreen™ or SpectrumOrange™ fluorochrome, respectively.

3.2.3 BrdU detection for cell cycle analysis

Detection of incorporated BrdU in replicating DNA was performed as described previously (Gotoh et al. 1995; Asakawa and Gotoh 1997). After chromosome painting study, slides were immersed in 2N HCl at room temperature for 30 minutes to denature DNA and then immersed in 0.1M Na₂B₄H (pH 8.5) to neutralize the acid for a few minutes at room temperature. Slides were briefly washed twice with 0.5% Tween 20/PBS twice. Twenty µl of FITC conjugated anti-BrdU monoclonal antibody (Becton Dickinson) was diluted with 50 µl of 0.5% Tween 20/PBS and added on a glass slide, covered with a cut Parafilm sheet and incubated at room temperature for 30 minutes in a humidified chamber. Slides were washed twice with PBS and counterstained with 40 ng/ml of PI. Slides were observed under an epifluorescence microscope using a B-2A filter and images were taken using a CCD camera.

3.3 Spatial and temporal rearrangement of chromosome fragment coupled with DNA replication following γ-irradiation

3.3.1 Determination of irradiation dose for effective detection of the chromosome fragments in interphase nuclei

Human peripheral blood lymphocytes (PBLs) is an excellent system for study the irradiation induced chromosome aberrations, because *in vivo* PBLs arrest in G₀ phase (quiescent stage) and start cell cycle well synchronized until first cell division after stimulation by foreign antigens or non-specific mitogens such as LPS (lipopolysaccharides) or PHA (phytohemagglutinine). It is well known that the frequency of chromosome breakage increasing as the increase of irradiation dose (Gotoh and Asakawa 1996; Gotoh et al. 2005). Accordingly it is expected that increasing the irradiation dose will make easier to observe the fragmented chromosome domain because the number of fragmented domains will increase seen in interphase nuclei. It is, however, difficult or even impossible to obtain sufficient number of chromosomes from cells exposed high dose of ionizing irradiation, because these cells arrest in S or G₂ and do not enter in mitosis. As drug-induced PCC allows us to obtain chromosomes in cells even irradiated 40 Gy dose of γ-rays (Gotoh and Asakawa 1996; Gotoh and Tanno 2005; Gotoh et al. 2005; Wang et al. 2009; Balakrishnan et al. 2010; Lindholm et al. 2010), we used to calyculin A induced PCC method to visualize the chromosome aberration in G₂ cells after γ-irradiation, but we irradiated the cells with more milder dose of 10 Gy of γ-rays as not give severe damages to the cells. Figure 4 (reproduced from Molecular Biology International, Special Issue on “DNA in 3R: Repair, Replication, and Recombination”, Gotoh and Asakawa 2011, in press) shows the chromosome painting of human #4 chromosomes against calyculin A induced prematurely condensed chromosomes (PCCs)

of PBLs at 48 hours after 10 Gy of γ -rays irradiation. At this time point, number of mitotic chromosomes was quite very low (lower than 0.01% as shown in Table 2), which indicates that the observed chromosomes is G2-PCC of cells arrested in G2 rather than mitosis. G2-chromosomes (G2-PCC) were moderately damaged by 10 Gy of γ -irradiation, and more than 10 substantially recognizable fragments of chromosome 4 were inserted in rearranged chromosomes (indicated by arrowheads), as previously reported (Gotoh and Asakawa 1996; Gotoh and Tanno 2005; Gotoh et al. 2005). This also suggests that the cells retain repair ability of double strand breaks (DSBs) by γ -irradiation. We expected that more than 10 fragmented pieces of chromosome 4 domains can be observed in an interphase nucleus after 10 Gy of γ -rays irradiation.

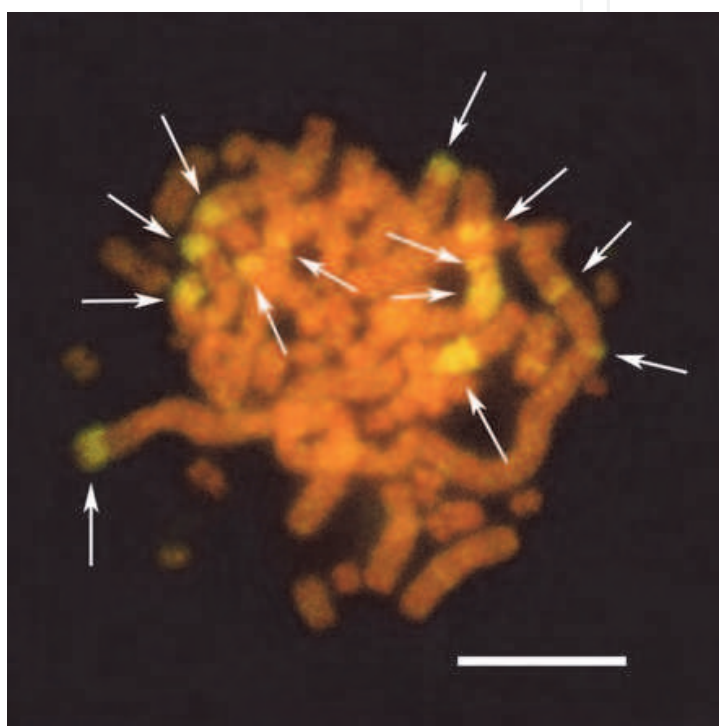


Fig. 4. Chromosome painting on a calyculin A induced PCCs at 48 hours after irradiation of 10 Gy of γ -rays. SpectrumGreen™ labeled specific probe for human chromosome 4 was used for detection of chromosomal fragments derived from chromosome 4. Chromosomes were counterstained with propidium iodide (PI). Moderate damaged chromosomes including long and short size of ones are clearly seen. Arrows indicate many fragmented pieces of chromosomes 4 which inserted in rearranged chromosomes. Scale bar : 10 μ m. (reproduced from Molecular Biology International, Special Issue on “DNA in 3R: Repair, Replication, and Recombination”, Gotoh and Asakawa 2011, in press)

3.3.2 Cell cycle analysis of control peripheral blood lymphocytes (PBLs) after mitogen stimulation

As a control study to identify the cell cycle stage of PBLs after PHA-P stimulation, we performed chromosome painting and BrdU incorporation analysis on interphase nuclei of peripheral blood lymphocytes (PBLs) without irradiation, which are shown in Figure 5 (left column, chromosome painting; right column, BrdU incorporation, reproduced from Molecular Biology International, Special Issue on “DNA in 3R: Repair, Replication, and

Sampling Time (hours)	Dose (Gy)	Treatment	S PCC (% cell)	G2/Mphase (% cell)	Interphase (% cell)
0	0 (control)	Calyculin A	0.0	0.0	100.0
		Colcemid	- **	0.0	100.0
	10	Calyculin A	0.0	0.0	100.0
		Colcemid	- **	0.0	100.0
24	0 (control)	Calyculin A	17.6	0.0	82.4
		Colcemid	- **	0.0	100.0
	10	Calyculin A	14.8	0.0	85.2
		Colcemid	- **	0.0	100.0
48	0 (control)	Calyculin A	16.3	11.5	72.2
		Colcemid	- **	1.4	98.6
	10	Calyculin A	12.1	17.4	70.5
		Colcemid	- **	0.01	99.99
72	0 (control)	Calyculin A	11.5	9.7	78.8
		Colcemid	- **	2.4	97.6
	10	Calyculin A	9.3	16.1	74.6
		Colcemid	- **	0.2	99.8

Table 2. Percentage population of PCC or metaphase obtained with calyculin A treatment (PCC) or colcemid treatment (metaphase) at different sampling time point after 10Gy of γ -irradiation. N=200 G2/M-PCC or Mitotic cells are observed in calyculin A or colemid treated cells, respectively. ** In case of colcemid treatment, S-PCC is not observable. (reproduced from Molecular Biology International, Special Issue on „DNA in 3R: Repair, Replication, and Recombination“, Gotoh and Asakawa 2011, in press)

Recombination”, Gotoh and Asakawa 2011, in press). Figure 5a and 5c show two chromosome 4 domains in interphase nuclei and Figure 5b and 5d are incorporation of BrdU in the cells of the same fields at 48 hours after PHA-P stimulation. At 48 hours after PHA-P stimulation, many PBL cells entered in S-phase, which was clearly shown by much amount of BrdU incorporation as shown by the previous study using Cy3-dUTP labeling cell (Gotoh 2007). The BrdU positive cells (proliferating cells) increased nuclear size compared with the BrdU negative cells (non-proliferating cells), which suggested the increase of DNA amount as DNA replication progressed. Two intact chromosome 4 domains were clearly recognized either in BrdU positive larger sized nuclei (enter in S-phase) or BrdU negative small nuclei (still in G0/G1-phase). At 72 hours after PHA-P stimulation, further enlarged BrdU positive nuclei were seen (Figure 5e-5h), which suggested that DNA replication progressed much more. Some nuclei enlarged much more and BrdU was less incorporated, which suggests that these cells were already in very late S phase as almost completion of DNA replication. One pair of chromosomes 4 stained with painting probe is indicated by arrows (Figure 5i). BrdU was not incorporated in the mitotic chromosomes (Figure 5j). The incorporation patterns of BrdU in replicating DNA at each cell cycle stage was the similar as described previously either using BrdU labeling (Nakamura et al. 1986; O’Keefe et al. 1992) or Cy3-dUTP labeling (Manders et al. 1999; Gotoh 2007). Two chromosomes 4 domains in a nucleus were not fully diffused over the nucleus but they occupy separated spaces each other in an interphase nucleus throughout cell cycling (Lengauer et al. 1991; Cremer et al. 1993; Savage 2000; Aten and Kanaar 2006).

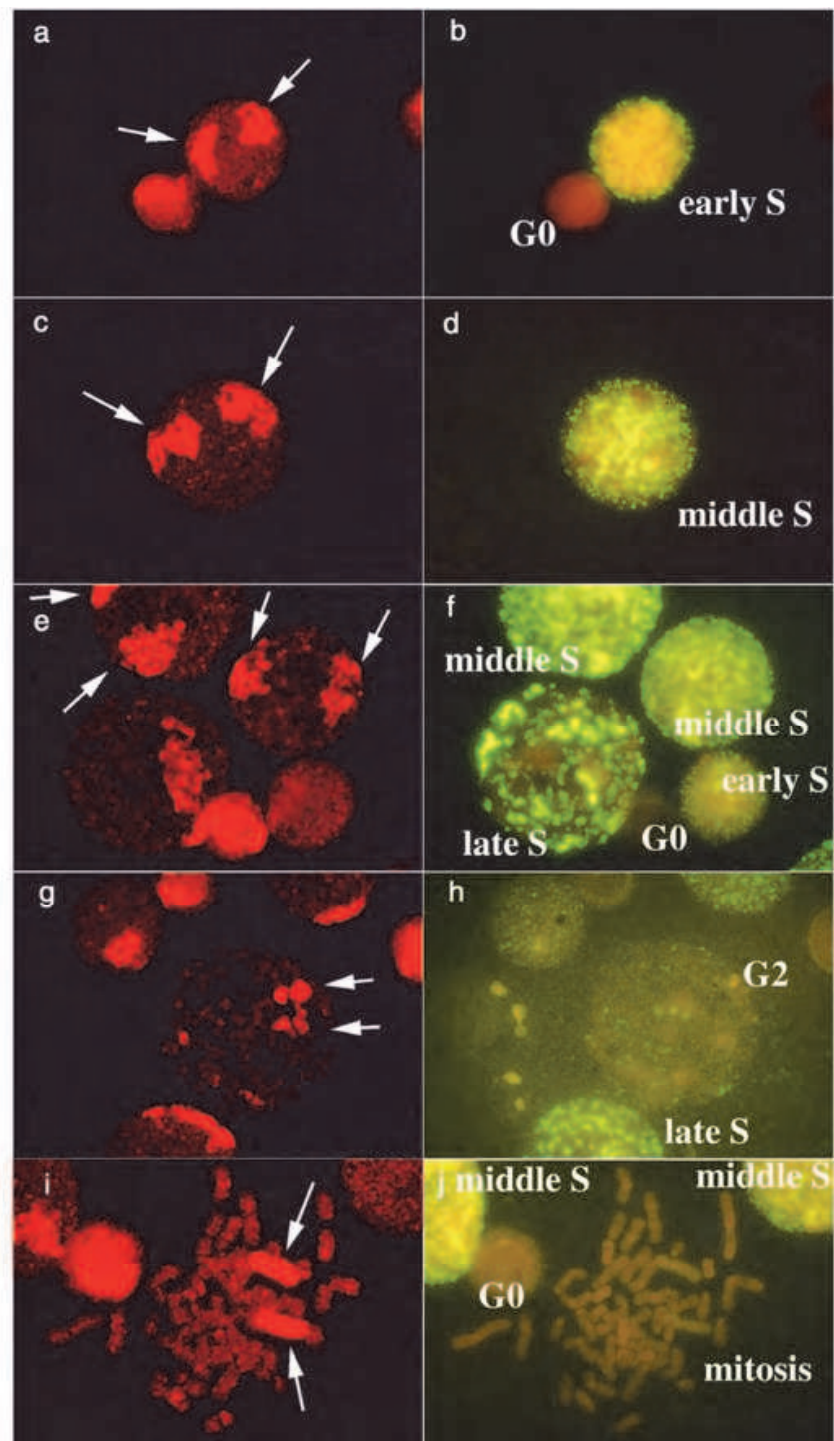


Fig. 5. Chromosome painting and incorporation of BrdU incorporation in various cell cycle stages of human peripheral blood lymphocytes stimulated with PHA-P. Left column (a, c, e, g and i): Chromosome painting using SpectrumOrange™ labeled specific probe for human chromosome 4. Arrows indicate two chromosomes 4 (i) or two chromosome 4 domains in interphase nuclei (a, c, e and g). Right column (b, d, f, h and j): Incorporation of BrdU in cells in the same field. Incorporated BrdU was detected by FITC conjugated anti-BrdU antibody. Cell cycle stage of individual cell is indicated in figures. Scale bar: 10 μm. (reproduced from Molecular Biology International, Special Issue on "DNA in 3R: Repair, Replication, and Recombination", Gotoh and Asakawa 2011, in press)

3.3.3 Visualizing spatial and temporal dynamics of γ -ray cleaved chromosome fragments in interphase nuclei

We next studied the spatial and temporal distribution of chromosomes 4 domains after γ -rays irradiation. Immediately after 10 Gy of γ -irradiation, PBLs were stimulated with T-cell specific mitogen PHA-P and cells were harvested at different culture time as control study (every 24 hour up to 72 hours; usually 48 hours incubation time is enough to obtain sufficient number of G2-PCCs even in cells γ -irradiated of 10 Gy). Table 2 (reproduced from Molecular Biology International, Special Issue on “DNA in 3R: Repair, Replication, and Recombination”, Gotoh and Asakawa 2011, in press) shows the percentage population of PCC or mitotic cell observed in calyculin A or colcemid treated cell samples. At 48 or 72 hours after γ -ray irradiation, more than 10 % of G2/M PCCs were obtained and chromosomes were highly damaged as shown in Figure 4, but number of mitotic cells was very low. As shown in Table 2, control un-irradiated human PBLs started cell cycle from G0 and at 48 hours after stimulation, 1.4% of cells reached in mitosis but others still in G2 or earlier stages even the case of un-irradiated cells. Therefore, the observed chromosome aberration seen in G2-PCCs is before first mitosis after irradiation. Figure 6 (reproduced from Molecular Biology International, Special Issue on “DNA in 3R: Repair, Replication, and Recombination”, Gotoh and Asakawa 2011, in press) shows the dynamics of chromosomal domain transition through the cell cycling. At 24 hours after irradiation, mostly cells did not enlarge their size and seemed to be still in G1 stage (population of stimulated or un-stimulated cell is shown in Table 2). At this stage, the cells showed two intact (un-fragmented) chromosome domains (Table 3, Figure 5a), that is quite the same as observed in G0/G1 nuclei of control un-irradiated PBLs cells. At 48 hours after irradiation, some cells slight enlarged their size, which suggests that these cells entered in S-phase. As cell enlarged, two chromosomal domains began slightly enlarged (Figure 5b), but still kept in intact form as a control S-phase PBLs nuclei as shown in Figure 4. However, in a small population (6.3%) of cells, chromosomal domains seemed somehow coarse appearance or rather seemed fragmented (Figure 5c), which suggested that chromosome domains fragments started separation and dispersion. Such phenomenon was not totally observed in control PBLs. At 72 hours after γ -irradiation (Figure 6d-6f), the number of enlarged cell became much more as 60.3%. The degree of separation and dispersion of chromosomal fragments was much more clearly in 58.5% of cells as the increase of the cell nuclear size.

Sampling Time(hours)	Non-ploliferating cells (small sized cell)		ploliferating (blastcytes) cells (enlarged sized cell)	
	fragmentation (-)	fragmentation (+)	fragmentation (-)	fragmentation (+)
0	100	0	0	0
24	95.4	0	4.6	0
48	42.6	0	51.1	6.3
72	39.7	0	1.8	58.5

Table 3. Population of non-proliferating or proliferating (blastcytes) pheripheral blood lymphocyte stimulated by PHA-P, with or without fragmentation of chromosome domain at different sampling time after irradiation of 10 Gy of γ -rays were scored. N=200. (reproduced from Molecular Biology International, Special Issue on “DNA in 3R: Repair, Replication, and Recombination” , Gotoh and Asakawa 2011, in press)

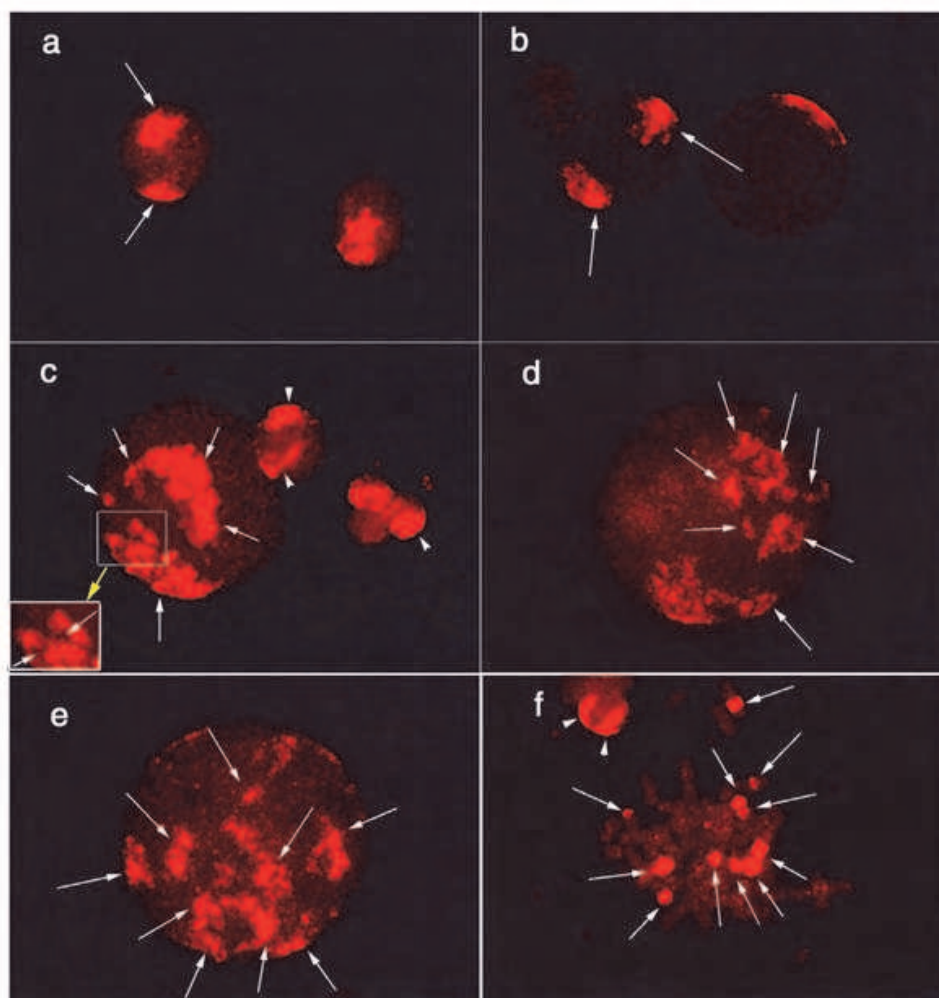


Fig. 6. Chromosome 4 painting on nuclei of peripheral blood lymphocytes irradiated with 10 Gy of γ -rays. Chromosome painting with SpectrumOrange™ labeled probe for chromosome 4. (a and b) Arrows indicate chromosome 4 domains. (c) Arrows indicate some of the fragments of chromosome 4. Note that the cells not enlarge their size (i.e. G0 arrested cells) have two un-fragmented chromosome domains (indicated by arrowheads). (Inset in c) Magnifying the white line boxed region, arrows indicate gaps between fragmented domains. (d-f) Arrowheads indicate some of the chromosome 4 fragments. Arrowheads in Figure 3f indicate two un-fragmented chromosome domains in the cells which not enlarge their size (i.e. G0 arrested cells). Scale bar: 10 μ m. (reproduced from Molecular Biology International, Special Issue on “DNA in 3R: Repair, Replication, and Recombination”, Gotoh and Asakawa 2011, in press)

According to the nuclear size, the degree of condensation of chromosome domain (also described by Mukherjee et al. 1992) and the degree of dispersion of the fragments, we could order the temporal dynamic series of chromosome domain diffusion as from Figure 6d to 6f. Figure 6d shows the further progress moving, dispersion and separation of the fragmented chromosome. Furthermore, as shown in Figure 6e, individual small fragmented chromosome domain completely separated over the nucleus. At 48 hours after irradiation, some cells already reached in G2 stage (Gotoh and Tanno 2005). Figure 6f shows an example of calyculin induced G2-PCCs, which clearly shows severely damaged chromosomes. Many

pieces of fragmented chromosome 4 were inserted (shown by arrowheads) in highly aberrant chromosomes.

3.3.4 Spatial and temporal transition of chromosome fragment is coupled with DNA replication

We noticed that about 40% of cells did not enlarge their size, which suggested that these cells were not proliferated and arrested in G₀-stage. These cells might be B-cells, monocytes or natural killer cells, which are consist of mononuclear cells in peripheral blood. Because we stimulated the MNCs with T-cell specific mitogen PHA-P, the cells except for T-cells were not stimulated and thought to arrest in G₀ stage. Stimulation failed T-cells also involved in this small sized cell population. Surprisingly and unexpectedly, two chromosome domains were kept as two un-fragmented (see like as two intact domains) in these non-proliferated cells and did not diffuse over the nucleus (indicated by arrowheads in Figure 6c and 6f), although these cells must be received the same 10 Gy dose of γ -rays as proliferating cells. We did not see any un-stimulated cells having fragmented chromosome domain. We therefore hypothesized that spatial and temporal redistribution of γ -irradiated chromosome fragments might be coupled with cell proliferation.

To confirm this hypothesis, we γ -irradiated PBLs as same as the above experiment (i.e. 10 Gy of irradiation dose) but stimulation with PHA-P was omitted. At 48 or 72 hours after irradiation, more than half of cells were lost presumably because of interphase death caused by lack of mitogen stimulation, however some cells were still alive. Figure 7a (reproduced from Molecular Biology International, Special Issue on "DNA in 3R: Repair, Replication, and Recombination", Gotoh and Asakawa 2011, in press) shows chromosome painting against these un-stimulated cells. The size of cell and nucleus did not increase and the chromosome 4 domains retained as intact. This finding also seems to support that spatial redistribution of fragmented chromosomes in interphase nuclei is tightly coupled with cell cycle.

We next questioned whether the spatial redistribution of fragmented chromosomes in S-phase is coupled with DNA replication. We irradiated PBLs with 10 Gy of γ -rays, stimulated with PHA, but cellular DNA replication was inhibited by 2 μ M of aphidicholin. As the same as observed in the irradiation experiment of PBLs without PHA-P stimulation, chromosomal domains were kept as un-fragmented, in spite of received 10 Gy dose of γ -ray exposure as PHA stimulated cells (Figure 7b). According to these results, we concluded that nuclear movement that causes spatial redistribution of the fragments was tightly coupled with cell cycling and in particular with DNA replication.

3.4 Hypothetical model of chromosome repair/recombination coupled with DNA replication

Two possible models have been proposed for explain how chromosome fragments close and associate to form radiation-induced rearranged chromosomes. Obviously, radiation-induced chromosome fragments must "touch" at some stage of the repair process (Savage 2000). One model is that fragmented chromosomes move to contact with another apart from fragment after breakage by irradiation ("breakage first" hypothesis or post-dispersal rejoining, Figure 8 (1) a and Figure 8 (2) a, figures reproduced from Molecular Biology International, Special Issue on "DNA in 3R: Repair, Replication, and Recombination", Gotoh and Asakawa 2011, in press). Another model is that fragment association occurs only where close contact already exists. Fragments adjacent to another fragment have rejoined each other and moved away ("contact first" hypothesis or pre-dispersal rejoining, Figure 8 (1) b and Figure 8 (2) b,

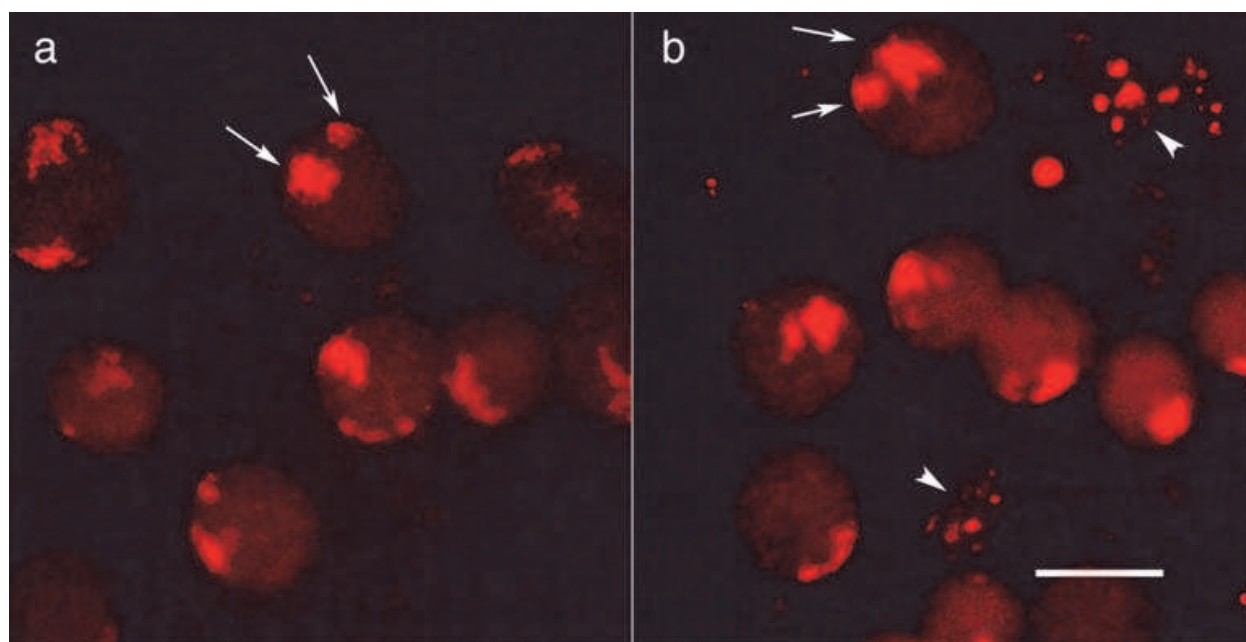


Fig. 7. (a) Chromosome painting against 10 Gy dose of γ -irradiated nuclei without stimulation by PHA-P, therefore cells yet arrested in G0 at 48 hours after irradiation. Two un-fragmented chromosome 4 domains are clearly visible (indicated by arrows). (b) Chromosome painting against 10 Gy dose of γ -irradiated cell nuclei, but DNA synthesis was inhibited by 2 μ M of aphidicholin. Arrows indicate one pair of un-fragmented chromosome 4 domain. Arrowheads indicate apoptotic fragmented nuclei, which is possible caused by DNA synthesis inhibition by aphidicholin. Scale bar: 10 μ m. (reproduced from Molecular Biology International, Special Issue on "DNA in 3R: Repair, Replication, and Recombination", Gotoh and Asakawa 2011, in press)

figures reproduced from Molecular Biology International, Special Issue on "DNA in 3R: Repair, Replication, and Recombination", Gotoh and Asakawa 2011, in press). In the case of the latter model, rejoining might occur limited to among those fragments adjacent to each other at G0/G1 phase. Recently accumulated evidences indicate that the radiation-induced exchanges cannot be possible between very far apart DNA strand breaks in chromosomes (Savage 2000). In the present report, quiescent G0 lymphocytes were γ -irradiated with 10 Gy of γ -rays, and chromosome type aberrations (i.e. acentric fragments, di-centric chromosome or translocations) are exclusively observed in G2-PCC at either 48 hours or 72 hours after irradiation. The reason for formation of chromosome type aberrations in irradiated quiescent lymphocytes is that damages were introduced in G0/G1 cell, repair or mis-rejoining of univalent chromosomes finishes before duplication, then DNA duplicates and result in bivalent chromosome type aberrations (Savage 1975). In this context, contact and association of cleaved chromosome ends should be finished before the cells enter in S-phase for the case of chromosome type aberrations formation. As the chromosome domain occupies the fixed space apart away in nucleus until S-phase starts as shown in Figure 5 and Figure 6, "breakage-first" hypothesis seems less likely to responsible in chromosome type aberration formation. Because chromosome fragment must move before begin of S-phase, but chromosome fragment move actually after S-phase starts. Thus the results reported here seems strongly support the "contact first" hypothesis in chromosome type aberration

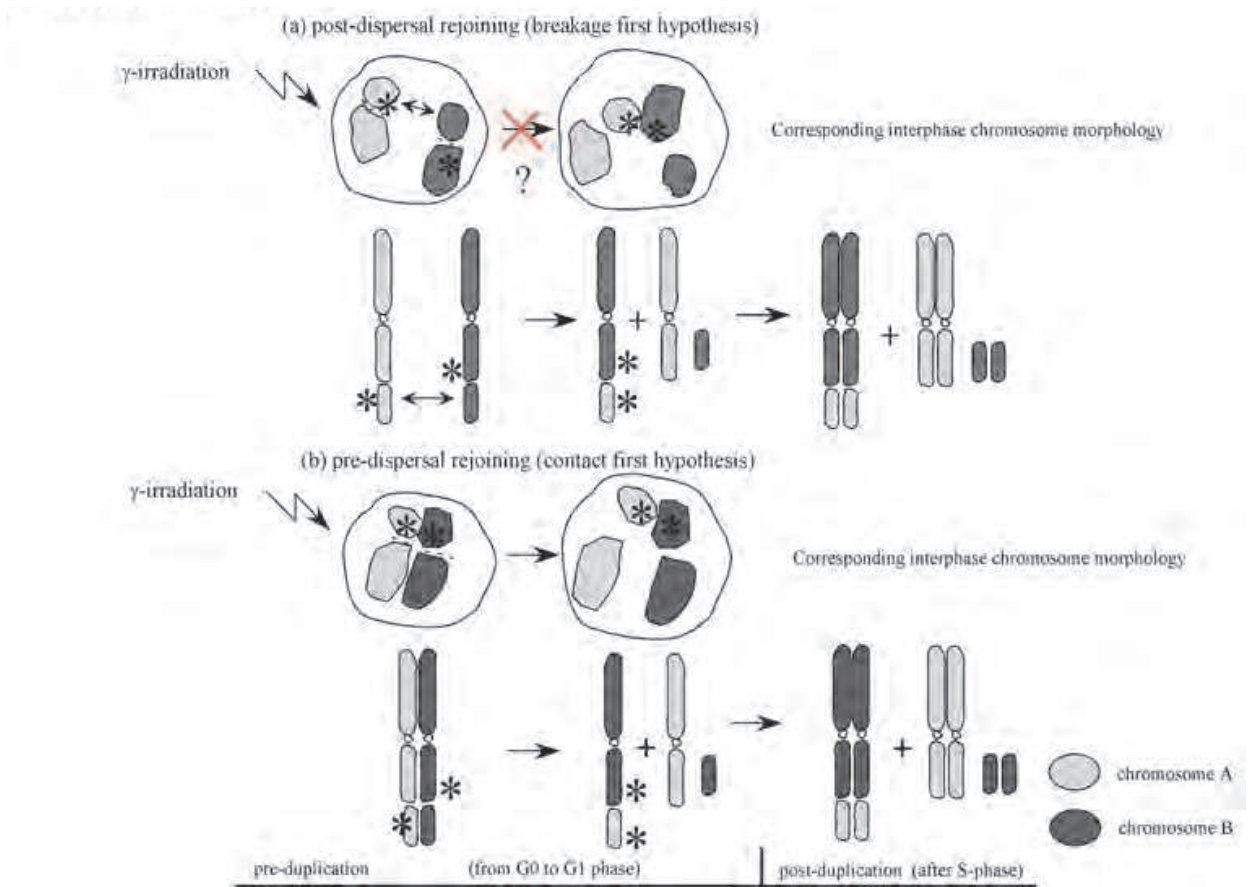


Fig. 8. Two models for chromosome cleavage and re-union involving nuclear movement progress in interphase nuclei

(1) Chromosome type aberration. Chromosome type aberration is introduced by DSBs in un-replicated univalent chromatid (Savage 1975). Therefore, chromosome domain is fragmented by DSBs. (a) Chromosome-end rejoining occurs after dispersion of chromosome fragments (post-dispersal rejoining or dynamic “breakage-first” model). In this case, chromosomes were cleaved by γ -irradiation and the fragments moved away. Then the fragments moved to touch and rejoin with fragmented chromosomes. However, breakage-first model does not seem likely to happen, because association should be finished before chromosome duplication. However, chromosome fragments may not move before DNA replication starts. (b) Rejoining occurs before chromosomal fragments dispersed (pre-dispersal rejoining or static “contact-first” model). In this case, chromosome rejoining occurs among the fragments that contact physically at the irradiated time (i.e. G0/G1 phase). Then recombined chromosomes moved away.

formation in γ -irradiated lymphocytes. Nikiforova et al. reported the inversion aberration of radiation-induced thyroid cancer (Nikiforova et al. 2000). They concluded that radiation-induced interchange between RET and H4 gene is presumably due to positional dependent and thus supports “contact first” hypothesis. Recently, Durante et al. showed that the exchange process was highly dependent on chromosome localization in nucleus or chromosome aberration was highly dependent on cell position and irradiation geometry (Durante et al. 2010). Therefore, their result also seems to support the “contact first” hypothesis. Contrary, clustering of fragmented domains after irradiation exposure was visualized using γ H2-AX, which suggests the “breakage-first” hypothesis is dominant in

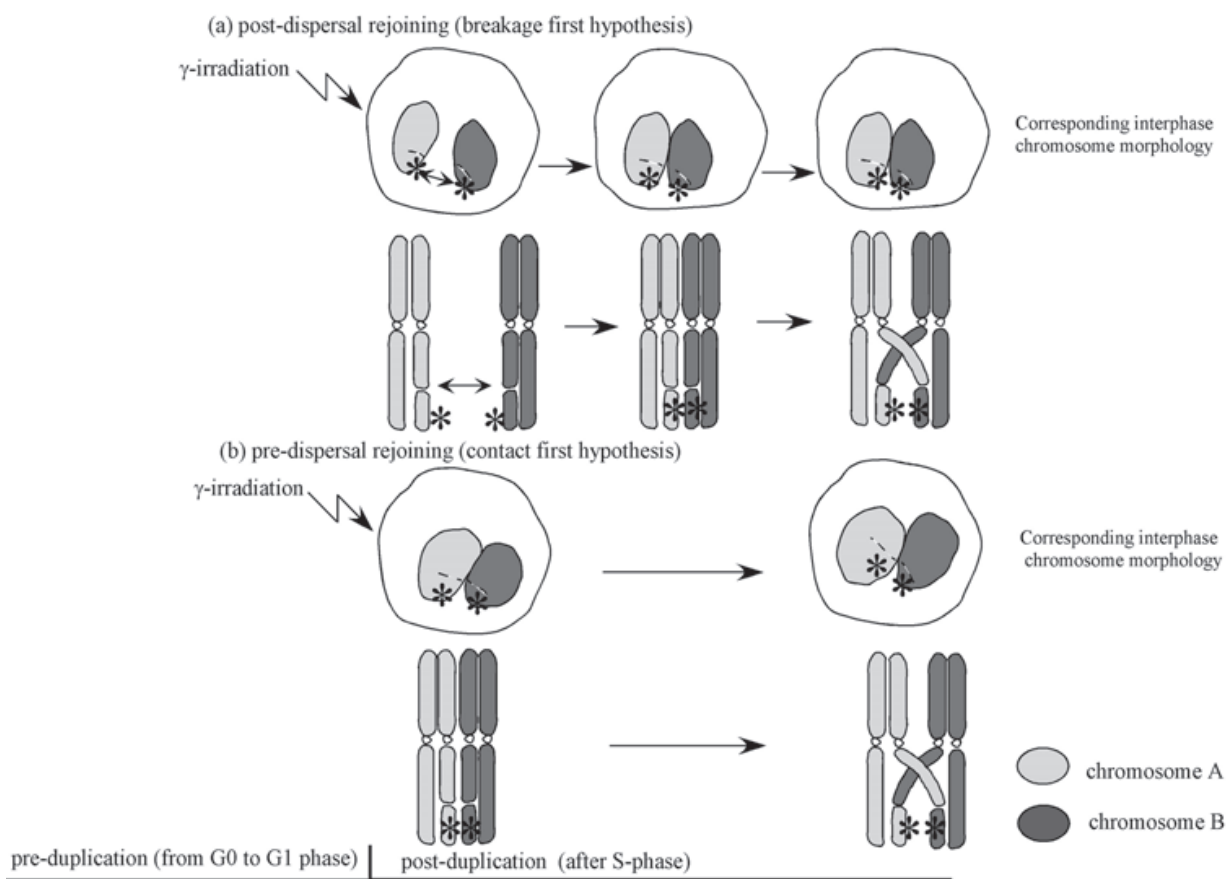


Fig. 8. Two models for chromosome cleavage and re-union involving nuclear movement progress in interphase nuclei

(2) Chromatid type aberration. Chromatid type aberration is introduced in the cells after S-phase by the DSBs only one of the sister chromatid of bivalent chromosome (Savage 1975). Chromatid does not apart away, because the cleaved chromatid coheres with opposite sister chromatid. "Breakage-first" model (a) and "contact-first" (b) model may both responsible in chromatid type aberration formation, because the nuclear movement already exists in the cells after DNA replication starts. Asterisks (*) indicate the chromosome ends, which will later contact and rejoin. Following points are for the simplicity: only one DSBs per chromosome are illustrated and indicated by dotted line. Many types of chromosome or chromatid type aberrations are known to be produced after ionizing irradiation exposure, but only typical type of aberrations are illustrated. Illustration is shown as a 2-D image for simplification, however dispersal and rejoining of chromosome proceed in 3-D, of course. (reproduced from Molecular Biology International, Special Issue on "DNA in 3R: Repair, Replication, and Recombination", Gotoh and Asakawa 2011, in press)

exchange process (Aten et al. 2004). Which mechanism works principle in chromosome ends association may be depend on the existence of DNA replication coupled nuclear movement. If replication coupled nuclear movement exists (i.e. S-phase or later), both "breakage first" and "contact first" hypothesis may responsible. Aten et al.'s report seems to support this idea because they observed exchange formation in α -particle irradiated cycling HeLa cells (Aten et al. 2004). On contrast, "contact first" hypothesis only may contribute for chromosome ends joining if not nuclear movement occurred (i.e. before S-phase). It is, however still controversy, which mechanism is dominantly work or both are simultaneously occur, and should be elucidated in the future.

The finding also implies other aspects as follows: 1) Generally it is believed that cycling cells are more sensitive to ionizing radiation or other agents that causes DNA strand break. Existence of S-phase coupled kinetics that drives fragmented chromosomes diffuse over nuclei may be one explanation for such phenomenon. After receiving damages, cell must repair the damages. If nuclear movement begin before the damage repaired completely, unrepaired fragments may be separated away from each other, which gives the cells irreversible chromosome aberrations which in turn resulting in cell death or prone cancer. 2) By analysis of unstable type chromosomal aberration (such as di-centric chromosome or acentric fragment) induced by irradiation, it is known that quiescent PBLs survive more than 10 years in vivo (Buckton et al. 1967). PBLs that are not stimulated by foreign antigens are thought to be in resting state in vivo for long time. Cleaved DNA fragments are not separated and dispersion did not occur until cells enter in S-phase. So the PBL cells at resting state are stable and may survive for a long period in vivo.

4. Summary and conclusion

To visualize the dynamics of chromosome structure formation coupled with DNA replication, Cy3-dUTP direct-labeled active replicating DNA was observed in prematurely condensed chromosomes (PCCs) utilized with drug-induced premature chromosome condensation (PCC) technique that facilitates the visualization of interphase chromatin as well as condensed chromosome form. S-phase PCCs revealed clearly the drastic dynamic transition of chromosome formation during S-phase along with the progress of DNA replication; from a “cloudy nebula” structure in early S-phase to numerous number of “beads on a string” in middle S-phase and finally to “striped arrays of banding structured chromosome” in late S-phase like as usual observed mitotic chromosomes. Drug-induced PCC clearly provided the new insight that eukaryote DNA replication tightly coupled with the dynamics of chromosome condensation/compaction for construction of eukaryote higher ordered chromosome structure.

To elucidate how and when individual chromosome fragments close and association to form rearranged chromosomes in interphase, we next visualized the dynamics of spatial and temporal occupation of chromosome 4 domains in γ -irradiated human peripheral blood lymphocytes in interphase nuclei and in drug-induced PCCs by means of chromosome painting method. After γ -irradiation exposure, breakage and dispersion of chromosome 4 domains starts when the cells entered in S-phase. This S-phase dependent dynamics was however not seen in un-stimulated cells or in the cells of which DNA replication was inhibited by aphidicholin. Thus the results seem to support “contact first” hypothesis for association of chromosome ends in chromosome type aberration formation. Spatial and temporal dynamics of chromosome fragments may tightly coupled with DNA replication, and this dynamic might be drive chromosome rearrangement.

Therefore, these findings presented in this chapter strongly suggested that DNA replication, rejoining/recombination and chromosome structure formation are tightly coupled mechanism in eukaryote cells.

5. Condolences

During preparation of this chapter, an enormously big Earthquake and Tsunami disaster attacked Japan 11th March 2011, by which a lot of people have been killed or lost. I lamentably dedicate this article to all of the victims.

6. Acknowledgement

I wish to express my thank to Prof. Aleksandar Lazinica and Prof. Viktorija Zgela for giving me an opportunity to write this manuscript.

7. References

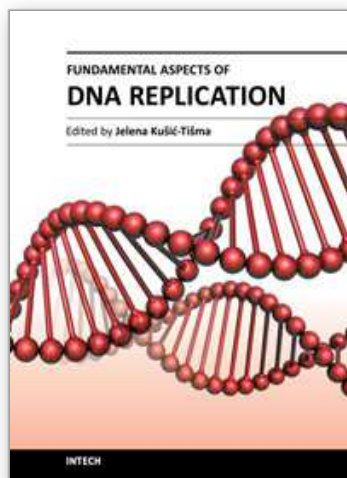
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DNA replication, the process of copying one double stranded DNA molecule to produce two identical copies, is at the heart of cell proliferation. This book highlights new insights into the replication process in eukaryotes, from the assembly of pre-replication complex and features of DNA replication origins, through polymerization mechanisms, to propagation of epigenetic states. It also covers cell cycle control of replication initiation and includes the latest on mechanisms of replication in prokaryotes. The association between genome replication and transcription is also addressed. We hope that readers will find this book interesting, helpful and inspiring.

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