Constrained, Random, and Independent Motion of Texas-Red-labeled Chromatin in Living Interphase PtK2 Cells

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In this study, we observed and analyzed the sub-micron motion of interphase chromosomes in living cells, labeled with the fluorescent thymidine analogue, Texas-Red dUTP. Our approach has an advantage in that chromosomes can be analyzed with regard to the nuclear architecture. We calibrated the observed motion of fluorescence-labeled chromatin by eliminating the rotational and translational movement of living nuclei that could significantly affect chromatin motion. Mathematical analyses of chromatin motion showed that: (1) interphase chromatin in living nuclei moves randomly, and the motion is limited within a small sub-region; (2) chromatin near the nuclear envelope moves in a more limited area than does centrally located chromatin;

and (3) closely situated chromatin domains move independently of each other. Random and constrained chromatin motion in living nuclei supports the concept that interphase chromatin fibers are loose, flexible and floating in the nuclear matrix, and that chromatin anchors to the backbone of chromosomes. Moreover, that the random motions of DNA domains are independent of each other suggests that interphase chromatin arranges without structurally rigid continuity. This active motion of chromatin is consistent with dynamic biological processes, requiring chromosome motility and interactions. Additionally, the dynamic properties of interphase chromosomes may be significant in the interpretation of acquired chromosomal aberrations.

Key words: Chromatin, Interphase, Fluorescence, Living cell, Sub-micron motion

I. Introduction

Interphase chromatin is a functionally active structure associated with essential biological processes, including transcription, translocation and recombination. In contrast to the dynamic behavior of interphase chromatin, recent studies using living cells demonstrate that chromosomes are essentially immobile and remain at rest in a fixed position [2, 5, 39]. Experiments in which cell nuclei were irradiated with an ultraviolet microbeam, and the damaged nuclear part pulse-labeled with ³H-thymidine were observed subsequently, showed that interphase chromosomes were basically static and organized territorially [5]. A study using fluorescence recovery after photobleaching (FRAP) showed that interphase chromosomes are immobile over distance scales of 4 μ m [2]. Folding and extension patterns of Cy3-AP3-dUTP-labeled chro-

matin sub-domains reportedly change slowly, however they may be modified by the rotational and translational movement of the nucleus [39]. The static picture of interphase chromatin appears contrary to the more dynamic view of chromatin that might be expected from its functional roles.

Recently, fluorescence time-lapse studies of interphase living nuclei have been used to investigate sub-micron movements of interphase chromatin [18, 20, 21, 30]. Newly and nascent replicated DNA domains slowly separate from each other [20]. Some groups of fluorescence-labeled centromeres show occasional very slow movement [30]. In contrast with these studies on the slow movement of chromatin domains, Marshall *et al.* reported that chromosomes obeyed Brownian motion by time-lapse three-dimensional observation of specific chromosome regions [21].

In this study, we investigated: (1) the motion of chromatin in interphase nuclei; (2) interactions between the motions of closely situated chromosome domains; and (3) the relationship between chromatin movement and its dis-

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tribution in the nucleus. We targeted whole chromatin in interphase nuclei using a fluorescence-labeled thymidine analogue. Compared with the prior studies tracking specific regions in the nuclei [18, 21, 30], our approach has the advantage in that movement of chromatin can be analyzed with regard to nuclear architecture and topology. We introduced the fluorescent analogue of thymidine into nuclei during mid-to-late S phase. DNA domains labeled during mid-to-late S phase are regarded as representative of all chromatin in the nucleus, because both active euchromatin and inactive heterochromatin are replicated during the mid-to-late S phase [3, 13, 22–24]. To exclude cell death, we observed and analyzed only cells able to undergo mitosis. Fortunately, the fluorescence-labeled thymidine analogue, once incorporated into chromatin, was spatially very well segregated in daughter cells and also preserved during cell cycle progression [19, 39]. Moreover, we corrected the apparent motion of fluorescence-labeled chromatin by eliminating the rotational and translational movement of the living nuclei, before quantitative tracking of the movement of labeled domains, because nuclear movement significantly affects chromatin motion [39].

We found that (1) Texas-Red-labeled DNA domains in interphase nuclei show random motion, within a constraint area; (2) closely oriented DNA domains move independently of one another; and (3) the spatial organization and condensation of chromatin domains within nuclei affect the range of chromatin motion.

II. Materials and Methods

Cell culture and microinjection

PtK2 cells (rat kangaroo kidney epithelial cells, obtained from JCRB) were grown in 35 mm glass bottom dishes (MatTek Corporation, USA) in Ham F-12 medium supplemented with 10% fetal bovine serum (FBS; GIBCO BRL, Gaithersburg, MD, USA) at 37°C in an atmosphere of 5% CO₂. The cells were used 36–72 hr after seeding, by which time the cells were about 50–70% confluent and many mitotic cells were observed. The culture medium was replaced with HEPES (pH 7.4)-buffered Ham F-12, supplemented with 10% FBS, 1 hr before microinjection. Cell culture was continued at an air temperature of 36°C.

Texas-Red labeled-dUTP ($\lambda_{ex}/\lambda_{em}=593\text{ nm}/610\text{ nm}$, Molecular Probes Inc., Oregon, USA), diluted with an injection buffer (100 mM KCl, 10 mM NaCl, 7.8 mM K₂HPO₄, 2.2 mM KH₂PO₄, pH 7.4) [26] was microinjected into the cytoplasm of cultured PtK2 cells. About 100 living cells per dish were microinjected within about 20 min. Soon after microinjection, the culture medium was replaced with fresh HEPES-buffered medium. All these processes were performed in the darkroom where the microscope system was located. Cell culture was continued on the microscope stage in the darkroom. In this report, we used only cells which survived the microinjection, as judged by their ability for successful mitosis.

Fluorescence microscopy and image collection

Fluorescence images of labeled PtK2 cells were captured with a scientific-grade cooled CCD camera (CH250; Photometrics Ltd., AZ, USA) mounted on an inverted fluorescence microscope (Diaphoto-TMD; Nikon Inc., Tokyo, Japan) using a 100 $\times$ /NA 1.4 oil immersion lens (Nikon Inc.). The excitation shutter and CCD data collection were controlled by IPLab SPECTRUM software (Signal Analysis Corporation, Vienna, VA, USA) on a Macintosh Quadra 950 (Apple Computer Inc., USA). For fluorescence with a Texas-Red dUTP, we used a set of beam splitter and bandpass filters ($\lambda_{ex}/\lambda_{em}=590\text{ nm}/660\text{ nm}$) (Omega Optical Inc., Brattleboro, VT, USA). To minimize light toxicity against living cells and attenuation of fluorescence level, a computer controlled auto excitation shutter was used (Nikon Inc.). The whole system, except for the CCD controller and computer, was located in the darkroom at 36°C ($\pm 0.5^\circ\text{C}$).

Long term interval observation of Texas-Red dUTP-labeled nuclei

About 1 hr after microinjection, fluorescence image data were recorded. The cell culture was then continued in the darkroom for 20–30 hr. Fluorescence images of the same cells were again collected after mitosis. Separately, cells undergoing mitosis continued to grow and their images were captured at 0.5–1.0 hr intervals.

Time lapse image collection of fluorescence-labeled DNA domains in living nuclei; a short-term interval observation

Texas-Red-labeled cells passing through the mitotic phase were observed 20–30 hr after microinjection. Three different fluorescence granular patterns were observed depending on the stage of S phase at the time of microinjection [3, 13, 22–24]. We selectively acquired image data from cells showing the mid-to-late S phase pattern of fluorescence, because the spatial pattern of fluorescence domains labeled during mid-to-late S phase is thought to reflect whole chromosome structure well [3, 13, 22–24]. Thirty fluorescence image data sets per cell were collected at three seconds intervals; it took 100 msec to capture each image.

Drug effects on the movement of chromatin

Following cell culture in the darkroom after microinjection, various drugs modifying cellular metabolism or architecture were added to the culture medium. We used okadaic acid (final concentrations 100 nM; Calbiochem, San Diego, CA, USA), staurosporine (20 nM; Sigma Chemical Co. St. Louis, MO, USA), sodium azide (10 mM; Nakarai Tesque, Japan), nocodazole (450 nM; Sigma Chemical Co.) and taxol (10 μM ; Sigma Chemical Co.). Thirty minutes after addition of each drug, fluorescence image data of labeled cells were acquired using the same exposure time and intervals as described above.

Sampling and processing of individual labeled domains (Fig. 1)

Time lapse data arrays were collected from 14 living cells (188 fluorescence-labeled DNA domains in total, 118 peripherally located and 70 centrally located domains) and 5 cells for each drug treatment (73 to 91 domains each). In this report, we defined "peripherally" located domains as domains located on the nuclear contour, and "centrally" located domains as the domains that do not attach to the nuclear contour (Fig. 1d). As a control, data from 45 domains from 3 fixed cells were also taken. Control cells were fixed with 4% paraformaldehyde in Buffer A (80 mM KCl, 20 mM NaCl, 15 mM HEPES, 0.2 mM Spermine, 0.5 mM Spermidine, 2 mM EDTA, 0.5 mM EGTA, pH 7.4) [34] for an hour and then rinsed with Buffer A. Four groups of closely oriented DNA domains (each group included 4–6 domains) (Fig. 1c) in living non-treated cells were selected for the analysis of correlation coefficients of chromatin motion. For motion analysis of individual labeled domains, 12 bit-digital images taken by the CCD camera were converted into 8 bit-images for further processing using Microsoft Excel (Microsoft Corporation, Redmond, WA, USA) and Mathematica (Wolfram Research Inc., USA). Macro programs for motion analysis were designed in our research group.

Fig. 1. (a) Original digital image of the nucleus labeled with Texas-Red dUTP. (b) Fluorescence-labeled DNA domains that were submitted to mathematical analysis are marked with white dots on the same image as (a). (c) Example of two groups of closely oriented domains (black and white arrows). (d) Example of peripherally (white dots) and centrally (black dots) situated domains. Bar = 10 μ m.

Fig. 2. Spatial distribution of incorporated Texas-Red-labeled dUTP. (a) Image of a labeled PtK2 cell, 1 hr after microinjection. The cell shows a mid-to-late S phase pattern of fluorescence-labeled domains. (b) Image of the daughter cells of the cell in (a). Fluorescence-labeled domains were segregated to the daughter cells and the spatial pattern of the domain is fairly well preserved. Bar = 10 μ m.

III. Results

Spatial distributions of Texas-Red dUTP-labeled DNA: a long-term interval observation

Texas-Red-labeled DNA domains were clearly observed as discrete granules in nuclei 1 hr after microinjection (Fig. 2a). Some injected cells were unable to divide (genetically dead) and their fluorescence-labeled DNA domains became motionless. To eliminate such "genetically dead" cells, we selected only cells that could pass through mitosis after the labeling process. Figure 2b shows two daughter cells that appeared to equally share fluorescence-labeled domains. Before and after mitosis the spatial distribution pattern of labeled domains did not change significantly (Fig. 2). We monitored fluorescence-labeled nuclei for up to 10 hr after cell division. The spatial distribution pattern of labeled domains was maintained during interphase (Fig. 3). We observed that a cell whose total exposure time was less than six seconds could pass through mitosis. Total exposure time for the follow-

Fig. 3. Long-term interval observation of interphase nucleus, (a) 0.5 hr, (b) 1.0 hr, (c) 1.5 hr, (d) 4.0 hr, (e) 7.0 hr, and (f) 10.0 hr after mitosis. Large-scale distribution of fluorescence-labeled domains is well maintained through the cell cycle. Bar = 10 μ m.

ing short-term interval observation was three seconds; therefore we believe that the image acquisition in the study had little influence on cell cycle progression.

Movement of Texas-Red-labeled DNA domains: a short-term interval observation

Time-lapse movies of three-second intervals showed that fluorescence-labeled domains obviously move within a small, restricted area of living nuclei. In fixed cells, on the other hand, labeled domains were almost stable. For quantitative analysis of chromatin movement, we corrected the motion of fluorescence-labeled chromatin by eliminating the nuclear movement. The observed motion of fluorescence-labeled domains was the sum of chromosomal motion and nuclear movement [39].

Data correction of chromatin motion within nuclei

Positional measurement of an observed image in submicrometer scale are significantly affected by drifting of the specimen [10, 39] that might be caused by small movements of the specimen relative to the microscope optics. In this report we applied a well established technique known as submicrometer single-particle tracking to measure submicrometer displacements smaller than the resolution limit of the microscope system [10, 21, 26]. Primarily, we eliminate the effects of rotational and translational movements from the observed time-lapse image arrays.

Each nucleus contains fluorescence-labeled domains (Fig. 1a, b), the locations of which are denoted by $\vec{x}_i(t)$ ($t=1, 2, \dots, 30$) ($i=1, 2, \dots, N$). Here, t is the original number of an image taken every three seconds, and N is the total number of chosen domains in each nucleus. Each domain moves within the nucleus; however, the nucleus itself also moves. The motion of the nucleus from $t=1$ to $t=t$ is written as follows:

$$\vec{M}(t) = (R_{\theta(t)}, \vec{p}(t)),$$

where $R_{\theta(t)} = \begin{pmatrix} \cos \theta(t) & -\sin \theta(t) \\ \sin \theta(t) & \cos \theta(t) \end{pmatrix}$ denotes rotation and $\vec{p}(t) = (p(t), q(t))$ is the parallel movement of the nucleus. If the chromatin granule does not move within the nuclei, its expected location at time t is $\vec{a}_i(t) = R_{\theta(t)}\vec{x}_i(1) + \vec{p}(t)$. Next, we estimated the parameters θ , p , and q , that minimize E , defined by

$$E = \sum_{i=1}^N |a_i(t) - x_i(t)|^2.$$

Therefore, parameters θ , p , and q are estimated by solving the following simultaneous (nonlinear) equations

$$\partial E / \partial \theta = 0, \partial E / \partial p = 0, \partial E / \partial q = 0.$$

The movement of a granular fluorescence domain within a nucleus can be written as $\vec{u}_i(t) = (u_i, v_i)$ and is obtained by $\vec{u}_i(t) = \vec{x}_i(t) - \vec{a}_i(t)$ (Fig. 4a, b).

The following analysis of the chromosomal motion is based on $\vec{u}_i(t) = (u_i, v_i)$ calculated as above.

To evaluate the measurement precision of our experi-

ments, we also measured the motion in formaldehyde-fixed cells under identical conditions. Any motion seen in fixed cells is due to noise in the image or mechanical jitter in the microscope, and will provide an upper limit of the apparent motion attributable to measurement error. If the apparent motion was due only to measurement error, the data from living cells should closely resemble the data from fixed cells. As plotted in Fig. 5, motion in living cells (Fig. 5a) was much greater than in fixed cells (Fig. 5b), and thus cannot be simply due to measurement error. Using data from fixed cells, the standard deviation of the motion was estimated within the range of 0.19–0.27 pixel (0.017–0.024 μm), which is small relative to the displacements in living cells.

Random motion of fluorescence-labeled DNA domains is constrained

If the motion of the fluorescence-labeled domains occurs in a so-called “random walk”, then their motion can be written as follows;

$$\vec{u}(t) = \vec{u}(t-1) + \vec{\epsilon}(t),$$

where $\vec{\epsilon}(t)$ is white noise. The first difference of $\vec{u}(t)$ is

Fig. 4. Examples of the motion of fluorescence-labeled domains in an identical nucleus. (Abscissa shows time, as revealed by the sequence of acquired images; ordinate shows a distance of one axis from the original point.) (a) Original motion of domains. The three domains tended to move in the same direction together, because their motion includes nuclear movement. (b) Corrected motion of (a), eliminating the nuclear movement. Mathematical methods are described in the text. The figure shows “pure” chromosome motion within the nucleus. 1 pixel = 0.09 μm .

Fig. 5. Tracks of Texas-Red-labeled DNA domains (a) in a living cell and (b) in a fixed cell. The fluorescence-labeled DNA domain in the living cell moves in a significantly broader area than in the fixed cell. The location of the chromatin domain (u_i, v_i) is calculated based on its original data (x_i, y_i) as described in the text.

given by $\vec{u}'(t) = \vec{u}(t) - \vec{u}(t-1) = \vec{\epsilon}(t)$. $\vec{u}'(t)$ can be written as $(u'(t), v'(t))$. Here, $u'(t)$ and $v'(t)$ are independent of each other. The probability of signal conversion, defined by $u'(t)u'(t-1) < 0$ or $v'(t)v'(t-1) < 0$ is 50%. Under the condition $t=3, 4, \dots, 30$, the expected frequency of signal conversion is 14, if the motion is a true random walk. However, the observed frequency of signal conversion was 17.69 (95% confidence interval: 17.44–17.95), significantly higher than expected (Fig. 6). This means the motion of fluorescence-labeled domains is not a true random walk, but it is constrained within a limited sub-region.

We propose that the motion of DNA domains will follow

$$\begin{aligned}\vec{u}(t) &= \vec{u}(t-1) + (\vec{\epsilon}(t) - \lambda \vec{u}(t-1)) \\ &= (1-\lambda)\vec{u}(t-1) + \vec{\epsilon}(t),\end{aligned}$$

where λ is a positive constant. This formula points out that the chromatin motion at time t is influenced by the location at time $t-1$; therefore, it is constrained.

Fig. 6. The frequency distribution curves of the signal conversion. The solid line represents the observed frequency and the dotted represents line the expected frequency. The expected frequency is given by ${}_{29}C_k(1/2)^k(1/2)^{29-k}N$, where $0 \leq k \leq 29$ and sample number N is 376 in this case.

However, it also includes the uncertain component $\vec{\epsilon}(t)$, so the motion is still random in some sense. Further details about this formula and the constant λ are now under investigation.

Closely situated DNA domains move independently

We picked four groups of fluorescence-labeled domains located close to each other in the nucleus (Fig. 1c). Each group includes 4–6 domains. The average distance between 2 neighboring domains was $0.75 \mu\text{m}$ and the maximum distance was $1.2 \mu\text{m}$. Correlative coefficients about the motion of neighboring labeled domains are computed. Fourteen pairs of the closely located domains out of 17 pairs (82%) moved independently.

Range of chromatin motion depends on the position in the nucleus

We classified fluorescence-labeled domains in living cells into two categories, depending on their location in the nuclei. The motion of centrally oriented fluorescence domains was compared with that of peripherally located domains (Fig. 1d). The total moving distance of each chromatin domain, given by $\sum_{t=1}^{30} \sqrt{(u_t - u_{t-1})^2 + (v_t - v_{t-1})^2}$ was calculated. Centrally located chromatin generally tended to move in a broader region than peripherally situated domains. (Fig. 7)

Drug effect on chromatin movement

The motion of fluorescence-labeled domains in living cells without drugs was compared with that in cells to which drug was applied and that in fixed cells (Fig. 8). We calculated the total moving distance of each domain, given by $\sum_{t=1}^{30} \sqrt{(u_t - u_{t-1})^2 + (v_t - v_{t-1})^2}$. The data were examined

Fig. 7. Total distance of chromatin motion during observation is given by $\sum_{t=1}^{30} \sqrt{(u_t - u_{t-1})^2 + (v_t - v_{t-1})^2}$. Centrally located DNA domains move more extensively than peripherally located domains. * $P < 0.05$.

by t-tests. Bonferroni's correction was applied to multiple comparisons. The motion of fluorescence-labeled domains in fixed cells was significantly lower than that in living, drug-free cells. In living cells, following treatment with okadaic acid, sodium azide, or taxol, the chromosomal motion was not markedly affected. However, on staurosporine or nocodazole treatment, the chromosomes moved within a wider area than those in living, untreated cells.

IV. Discussion

In this study, we observed and analyzed minute motion of interphase chromosome in living cells labeled with a fluorescent thymidine analogue. Our approach

has the advantage in that chromosomes can be analyzed with regard to the nuclear architecture and topology. We found that: (1) interphase chromatin shows random and constrained motion; (2) the range of chromatin motion depends on the spatial organization and condensation of chromatin domains within the nucleus; and (3) closely situated chromatin domains move independently of each other.

Fluorescence-labeled chromosomes in living cells move randomly within a restricted area

Observation of time-lapse movies of labeled cells shows that fluorescence-labeled domains in nuclei move randomly. Zink *et al.* pointed out that rotational and translational movement of living nuclei affects the motion of labeled domains [39]. In our study we attempted to resolve this issue by eliminating the rotation and translation of the nuclei from the raw data before quantitative tracking of labeled domains. Our results show that chromosomal motion is constrained in the nuclear sub-region but that it is of a random nature within the sub-region.

A recent study observing the distance between two GFP spots in living yeast cells and fluorescence-labeled topoisomerase II in *Drosophila* showed that interphase chromatin undergoes constrained diffusional movement, with confinement radii of $0.3 \mu\text{m}$ in yeast and $0.9 \mu\text{m}$ in *Drosophila* [21]. Another study using FRAP showed that interphase chromatin is immobile over distance scales of $0.4 \mu\text{m}$ [2]. According to our study, the moving range of fluorescence-labeled chromatin in living cells was about $0.14 \mu\text{m}$ on average with a maximum of $0.36 \mu\text{m}$. Because chromatin movement is confined to spatial scales of less than $0.4 \mu\text{m}$, such minute movement does not contradict the immobile chromatin model expected from FRAP data. Moreover, the single chromosomal territory in an interphase nucleus is estimated at several μm in diameter, based

Fig. 8. Total distance of chromatin motion. DNA domains in fixed cells are obviously motionless, in comparison with living cells. As with living cells without drugs, in both staurosporine- and nocodazole-treated cells, fluorescence-labeled domains moved in wider areas. * $P < 0.05$, Bonferroni's correction.

on fluorescence *in situ* hybridization [7, 8, 16, 37]. The range of chromatin movement is much smaller than the reported chromosomal territory. It suggests that fluorescence-labeled DNA domains do not stray from single chromosomal territory.

We have shown that labeled chromatin motion is random, constrained, and independent of each other. These results add further information to our understanding of chromosomal architecture in interphase nuclei. The random motion of interphase chromosomes indicates that interphase chromatin is loose, flexible, and floating in the nuclear matrix [28]. Constraint of chromosomal motion also indicates that interphase chromatin is anchored to the backbone of the chromosome [12, 29]. In addition, for the first time, we have shown that closely situated domains move independently of each other. The independent motion of closely oriented DNA domains suggests that the domains have no structurally rigid continuity.

Several models have been proposed for the functional architecture of interphase chromosomes. In an electron-microscopic study, Horowitz *et al.* showed that three-dimensional asymmetric zig-zag of nucleosomes and linker DNA represent a basic chromatin folding [14]. The channel model of nuclear matrix structure [28] and the inter-chromosomal domain compartment model [4] have been introduced as a hypotheses for a functional nuclear architecture. Sachs *et al.* proposed the random walk/giant loop model, which predicts giant loops of several mega base pairs of DNA attached to a highly flexible backbone [29, 35, 38]. Giant loops are flexible loops of DNA whose connecting points lie on a random walk. The random walk/giant loop model is consistent with our data, because under this model, chromatin domains can move randomly within the limitation of the backbone, to which flexible loops attach and also because fluorescence-labeled granules belonging to different but closely oriented loops would be expected to move independently.

Relationship between chromosomal motion and its location in the nuclei

Labeled domains located centrally in the nucleus move within meaningfully larger areas than peripherally situated domains. This could be the result of differences in chromatin condensation, because inactive heterochromatin tends to locate around the nuclear membrane and active euchromatin is primarily located in the central part of the nucleus [3, 5, 12, 23, 24]. On the other hand, near the nuclear periphery, chromatin has binding sites interacting with lamin that forms a filamentous meshwork at the inner surface of the nuclear envelope [31]. The connection between chromatin and the nuclear lamina could modify the chromatin movement and may cause peripherally located DNA domains to be less dynamic.

Effect of drugs modifying chromatin condensation

When staurosporine was applied to PtK2 cells, fluorescence-labeled domains moved more widely.

Staurosporine, a microbial inhibitor of protein kinases, causes chromatin decondensation [1, 25, 32]. Although we could not observe decondensation of the chromatin under phase-contrast microscopy, staurosporine may loosen the chromosome leading to extensive movement of chromatin. This result is consistent with the consequences previously described. We expected that okadaic acid, an inhibitor of protein phosphatase [11, 15, 33], would make DNA domains less mobile, however, it did not significantly affect the chromatin motion.

Is chromosome motion affected by microtubules or ATP?

We found that nocodazole, an inhibitor of microtubule polymerization [6], caused fluorescence-labeled DNA domains to move in wider areas. Microtubules are one of the main components of the cellular architecture. Another investigator observed that DNA domains in nocodazole-treated yeast moved widely [21]. Nocodazole influences chromatin motion, possibly through nuclear structure, but the details remain unclear. We had expected that taxol, an inhibitor of microtubule depolymerization [6], would have the opposite effect to nocodazole on the range of chromosomal motions. However, no effect of taxol on chromatin motion was clearly observed. Sodium azide, which decreases cytoplasmic ATP levels [36], also had no clear effect on chromatin motion. These results are consistent with the motion of fluorescence-labeled domains in nuclei being energy-independent as previously reported [21].

Dynamic biological processes in interphase nuclei such as transcription, DNA replication, and translocation suggest an active model of interphase chromosomes, although the concept of immobile, static interphase chromatin had been widely accepted [2, 5, 39]. This study also revealed that the spatial distribution of fluorescence-labeled DNA domains does not change significantly and interphase chromatin is essentially static. From the point of view of gene conservation and preservation of nuclear structure, the stability of interphase chromatin is reasonable. Short-term interval observations, however, showed minute motion of DNA domains in interphase chromatin. Such minute motion of interphase chromosome is consistent with the functionally active nature of interphase chromatin, where processes such as DNA replication and translocation occur.

Even in interphase, positioning of specific chromosomal domains change, depending on the cell cycle stage [9, 17]. It has been debated whether there is a relationship between chromosomal activity and location within chromatin territory [4, 16, 37]. Further investigation is needed regarding any relationship between the motion of DNA domains and cell cycle stage in interphase as well as chromatin territory and nuclear architecture. The dynamic properties of DNA domains should be examined in the interpretation of acquired chromosomal abnormalities, such as chromosomal dislocations, rearrangements and carcinogenesis.

V. References

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