Chromatin compaction protects genomic DNA from radiation damage.

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Abstract
Genomic DNA is three-dimensionally organized into the nucleus, and is thought to form compact chromatin domains. Although chromatin compaction is known to be essential for mitosis, whether it confers other advantages, particularly in interphase cells, remains unknown. Here, we report that chromatin compaction protects genomic DNA from radiation damage. Using a newly developed solid-phase system, we found that the frequency of double-strand breaks (DSBs) in compact chromatin after ionizing irradiation was 5–50-fold lower than in decondensed chromatin. Since radical scavengers inhibited DSB induction in the decondensed chromatin, the condensed chromatin had a lower level of reactive radical generation after ionizing irradiation. We show that chromatin compaction also protects DNA from attack by chemical agents. Our findings suggest that genomic DNA compaction plays an important role in maintaining genomic integrity.
Introduction

Genome DNA is wrapped around histones and forms a nucleosome structure [1][2][3].

Although the higher-order chromatin structure in eukaryotic cells is not fully understood, several evidences including our recent cryo-microscopy and synchrotron X-ray scattering analyses have demonstrated that chromatin consists of irregularly folded nucleosome fibers (the 10-nm fibers) in the cells [4][5][6][7][8][9][10]. Based on these studies, we suggested that interphase chromatin forms numerous compact chromatin domains, which resemble “chromatin liquid drops”, in the interphase cells [5][9]. Notably, the chromatin liquid drops view is in line with predictions of the chromosome territory-interchromatin compartment (CT-IC) model [11][12]. In the CT-IC model, each CT is built up from a series of interconnected, megabase-sized chromatin domains, which were originally identified using a pulse labeling as the DNA replication foci [13][14][15][16] that were shown to persist stably during subsequent cell generations [17][18][19]. Recent high-throughput 3C studies such as Hi-C and 5C have also proposed the physical packaging of genome DNA which has been termed “topologically associating domains” [20], “topological domains” [21] or “physical domains” [22].

Although chromatin compaction is essential for mitosis to maintain the integrity of
genomic information, whether compact chromatin domains confer other advantages, particularly in interphase cells, has not been elucidated. In previous *in vitro* studies, DNA compaction was shown to play a key role in protection against double-strand breaks (DSBs) generated by γ-rays [23][24][25][26]. Therefore, we explored the significance of higher-order chromatin structures in the DSB generation process. Left unrepaired, DSBs caused by radiation can lead to chromosome fragmentation, chromosome loss, and the rearrangement of genetic information, events which are frequently associated with tumor formation and progression [27][28]; also see [29].

Much is already known regarding the mechanism(s) of DSB repair processes [27][28]; however, little is known regarding how the chromatin structure influences DSB induction processes, especially in the quantitative and mechanistic aspects e.g. [30][31][32], although an involvement of reactive hydroxyl radicals in the DSB induction process has been suggested [33][34][32][35][36]: Whether sensitivity to DSB induction differs for “open” chromatin configurations and inactive “condense” regions has not been resolved to date [37][38], because of the following reasons: *In vivo*, difficulty in manipulation of chromatin structure, lack of efficient damage detection system, and regional difference of DNA repair efficiency have precluded drawing a
decisive conclusion. *In vitro*, since long genomic DNA can be damaged during experimental manipulations, no efficient *in vitro* system has yet been developed for the manipulation of long chromatin and the quantitative detection of generated DSBs.

In the present study, we developed a novel system for chromatin manipulation and sensitive DNA damage detection, and succeeded in quantifying the DNA damage caused by ionizing irradiation. Importantly, the frequency of radiation-induced DSBs in the fully decondensed chromatin was 5–50-fold higher than that in the condensed chromatin, indicating the existence of a DNA damage protection mechanism that is mediated by higher-order chromatin.
Results

Development of a novel system for chromatin manipulation and DNA damage detection

To examine whether the higher-order chromatin structure is directly involved in the induction of the DNA damage process, e.g., following exposure to γ-rays, we developed a novel system for chromatin manipulation and DNA damage detection (Figure 1A). To analyze DNA on the genome scale without causing physical damage, we used permeabilized nuclei that were attached to glass surfaces. Nuclei were isolated from HeLa cells and attached to poly-L-lysine-coated coverslips by gentle centrifugation (Figure 1A). Since chromatin is negatively charged, the compaction states of the nuclei and chromatin were controlled by altering the Mg$^{2+}$ concentration in the environment: In 5 mM Mg$^{2+}$, chromatin becomes highly condensed, whereas it decondenses in the absence of Mg$^{2+}$ [39][40] (Figure 1A). Based on the nuclear volume and known size of the genomic DNA, we estimated the DNA or chromatin concentration in the environment. This “solid-phase system” allowed us to perform very gentle and quantitative handling of the genome-size DNA (Figure 1A). For direct detection of DNA damage in the chromatin on the glass surface, we fluorescently labeled the DSB sites by terminal deoxynucleotidyl transferase (TdT) (TUNEL assay: [41])(Figure S1).

Thus, we detected DSBs in the chromatin without inducing additional breakages, which
distinguishes our method from the gel electrophoresis-based assay (Figure 1A).

**Chromatin compaction protects against DNA damage by γ-ray irradiation**

As shown in Figure 1B, the nuclei and associated chromatin on the glass surface were condensed in the presence of 5 mM Mg$^{2+}$ (~400 µm$^3$). In the absence of Mg$^{2+}$, the nuclei and chromatin swelled or decondensed by approximately 15-fold (~6000 µm$^3$) (Figures 1B and 1C). To check whether the decondensation process could provide DNA damage, with increasing the Mg$^{2+}$ concentration, we made the decondensed nuclei and chromatin recondensed (~450 µm$^3$) (Figure 1B) to a size comparable to that of the condensed nuclei (Figure 1C).

The three types (condensed, decondensed, recondensed) of nuclei were irradiated with various doses of $^{60}$Co γ-rays (Figure 1A). To promote the formation of a uniform compact state, which ensured the equal chromatin accessibility and equal handling between the three types, the irradiated nuclei were treated with 1 mM Mg$^{2+}$ and fixed with formaldehyde (Figure 1A). As a result, the nuclei became similar in size. DSBs in the chromatin were then detected using the TUNEL assay (Figure S1).
As shown in Figure 2A, while the DSB signal intensity in the decondensed chromatin increased in a radiation dosage-dependent manner, the condensed and recondensed chromatin showed only faint signals. The suppression effect observed for the recondensed chromatin excluded the possibility that the DNA damage was caused by the decondensation process (Figure 2A). In addition, we obtained similar results using the comet assay, which is a widely used damage detection method based on agarose gel electrophoresis [42] (Figure S2), although higher levels of DSB signals were observed even in the condensed chromatin, probably due to non-specific breakages during the manipulation steps, e.g., nuclear embedding in hot agarose. Taken together, these results indicate that the condensed chromatin is much more resistant to the γ-ray irradiation than the decondensed chromatin.

**Damage signal intensities fit well to a quadratic curve**

We next performed quantification of the DSB signals (Figure 2B). The signal intensities fitted well to a quadratic curve in this irradiation dose range: \( I = kD^2 \) (\( I \), DNA breakage; \( k \), constant; \( D \), irradiation dose). By comparing the \( k \) values, we found that the condensed chromatin had 16-fold greater damage suppression effects than decondensed chromatin (Figure 2C). The quadratic curve suggests that DSB induction is caused by
two independent single-strand DNA breaks (SSBs). Consistently, under an assumption that DSBs occurred when two random single-strand DNA breaks (SSBs) were generated within 10 bases in the double strands (Figure S3A), a simulation also showed that the number of created DSBs increased quadratically with the number of SSBs generated (Figure S3B).

**Damage protection effects in the low-dose range of irradiation**

We next focused on a lower dose range of irradiation (<100 Gy). To detect DSBs, we used an electron-multiplying charge coupled device (EM-CCD) camera that can detect single photons. Using the EM-CCD, DSB signals within the low dose range were readily detectable (Figure 3A). We found that the condensed chromatin had ~5-fold more damage suppression effects, even at 5 Gy of irradiation (Figure 3B). Moreover, in contrast with high-dose irradiation, the damage frequency increased linearly, suggesting that the DSBs were formed in a single step, which is consistent with the notion that DSBs are induced linearly with irradiation dose [29][35][37](see Discussion).

**The protective effect in the condensed chromatin is related to the chromatin concentration, but not the level of chromatin-associated proteins**

Some previous studies have suggested that the chromatin-associated proteins
(non-histone proteins) are involved in the DNA damage induction process [30][31][32]: the more proteins carried by the chromatin, the more protective the chromatin is against ionizing irradiation. However, we found that the total levels and compositions of associated proteins were similar in the decondensed and recondensed chromatin, indicating that the protective effect in the condensed chromatin is not due to the level of associated proteins (Figure S4). Moreover, no significant irradiation-induced degradation of proteins was observed (Figure S5). These results demonstrate that chromatin compaction is important for the protection of genomic DNA from ionizing irradiation.

To confirm these observations, we performed similar experiments using isolated human mitotic chromosomes [43]. In the absence of Mg$^{2+}$, the mitotic chromosomes swelled approximately 50-fold, as compared with those in a compact state (Figures 4A and 4B). The results using chromosomes were more striking than those obtained using nuclei, in that the compact chromatin had approximately 50-fold greater resistance to γ-ray irradiation than the decondensed chromatin (Figures 4C and 4D). As the chromosomes contained fewer non-histone proteins than the nuclei (Figure S6), they were under fewer physical constraints and were more decondensed in the absence of Mg$^{2+}$. These results
provide further evidence that the protective effect is due to the chromatin concentration (volume) rather than the number of chromatin-associated proteins.

**Chromatin compaction through molecular crowding effect also has a DNA damage suppression effect**

We next used polyethylene glycol (PEG), instead of Mg$^{2+}$ ion, to induce chromatin condensation, since a high concentration of macromolecules (100–200 mg/ml) in the cells might condense chromatin though a molecular crowding effect [44][45]. The addition of 12.5% PEG increased the compaction of decondensed chromatin, with a 5-fold nuclear volume reduction (Figure S7). We found a notable inhibition effect (Figures 5A and 5B) while we considered that PEG might be contributing to the damage inhibition effect not only as a molecular crowding agent, but also as a scavenger of radicals (see below).

**Radical scavengers inhibit DNA damage caused by ionizing irradiation**

We demonstrated that the chromatin concentration plays a critical role in the suppression of DNA damage by ionizing irradiation. Since reactive radicals arising from the radiolysis of water molecules might be a major contributor to DNA damage
[33][34][32][35][36], a lower concentration of chromatin, which entails more water molecules and reactive radicals, could increase the risk of being attacked by radicals. Thus, we examined the effects of the radical scavenger dithiothreitol (DTT) on DNA damage. When the decondensed chromatin was irradiated in the presence of DTT, DNA damage was suppressed in a dose-dependent manner (Figures 5A and 5C). Since the sizes of the decondensed nuclei in the presence of 100 mM and 0 mM DTT were similar (Figure S7), the inhibitory effect observed in the presence of DTT is due to radical scavenging rather than chromatin compaction.

**Chromatin compaction protects genomic DNA from heavy ion irradiation and chemicals**

We next considered whether a protective effect of chromatin compaction was limited to ionizing radiation. To address this question, we examined the protective effect against heavy ion (carbon ion beam) irradiation [46]. We found that the compact chromatin had a 7-fold higher damage suppression effect, as compared with decondensed chromatin (Figures 5D and 5E). This demonstrates that chromatin compaction protects DNA not only from γ-rays, but also heavy ions.
We also tested the protective effect of chromatin compaction against chemical attack. Since we used ethylenediaminetetraacetic acid (EDTA) for chromatin decondensation, DNA cleavage reagents, which often contain metal ions, were not applicable to our study. Instead, we used cisplatin, which is widely used as an anti-tumor drug [47]. Cisplatin forms covalent adducts with genomic DNA, thereby interfering with DNA replication and/or transcription, and eventually leading to apoptotic cell death. We treated the compact and decondensed chromatin with cisplatin, extracted the DNA, and measured the number of cisplatin adducts using inductively coupled plasma mass spectrometry (ICP-MS) (Figure 5F). We found that DNA in the decondensed chromatin had 10-times more cisplatin adducts than the condensed chromatin (Figure 5G), suggesting that chromatin compaction protects genomic DNA from chemical-induced damage.

**Discussion**

In the present study, we found that condensed chromatin had 16-times (nuclei) and 50-times (chromosomes) more resistance to γ-rays than decondensed chromatin, demonstrating that damage induction depends on the chromatin concentration (or
volume) (Figures 2, 4, and 5). Importantly, this dependency suggests that the contributions of Mg\(^{2+}\) and other components in the system to the damage suppression effect are negligible. As suggested previously [48][35][26][49], the damage suppression effect of compaction is likely because the higher-concentration chromatin (compacted chromatin) has fewer water molecules per chromatin, thereby generating fewer reactive radicals. Compared with previous studies, which suggested that relaxed nuclear chromatin has 3.1–4.5-fold more DSBs than compacted chromatin e.g. [30][31][32], our results are striking and more quantitative, presumably because of the two reasons: First, the solid-phase system has the lower background (non-specific DNA breakages) signals. Secondly, in the previous systems using the agarose-embedded cells, chromatin might not decondense well. Our results allow us to evaluate the suppressive effect at the quantitative level described below.

With low-dosage \(\gamma\)-ray irradiation, the DSB frequency increased linearly (Figure 3B), in contrast to the high-dosage irradiation. This suggests that the creation of DSBs with ionizing irradiation involves a single step and two independent steps (“DSB efficiency”\(= a \times D + b \times D^2\), where \(a\) and \(b\) are constants and \(D\) is the dose). The single-step creation of DSBs (\(a \times D\)) appears to be dominant at lower radiation doses (Figure 3B),
while the two-step creation of DSBs \((b \times D^2)\) is more influential as the dose increases (Figure 2B), whereupon the single-step process becomes negligible. Our simple simulation (Figure S3C), which comprises rare single-step DSBs \((a \times D)\) and frequent two-step DSBs \((b \times D^2)\), has a profile similar to that shown in Figure 2B. In addition, the linear inhibitory effect at low irradiation doses (Figure 3B) demonstrates that the radiation dose can directly generate DSBs. This is also the case for heavy ion irradiation (Figures 5D and 5E).

It is also interesting to consider the \textit{in vivo} situation. To detect DSBs in eukaryotic cells, immunostaining to detect serine-139 phosphorylation in histone H2Ax \((\gamma H2AX)\) is commonly used \cite{27}\cite{28}, although the percentage of DSBs detected as \(\gamma H2AX\) foci and their direct relationship remain unclear \cite{50}. A number of studies demonstrated that \(\gamma H2AX\) foci occurred frequently in euchromatin regions, and less frequently in heterochromatic regions \cite{51} \cite{52} \cite{53} \cite{49} \cite{54}. Furthermore, it was demonstrated that hypotonic treatment of cells, the chromatins of which are presumably decondensed, produced more \(\gamma H2AX\) foci \cite{49}. Although these \textit{in vivo} results reflect many indirect effects, making it difficult to draw simple conclusions, they appear to be in good agreement with our \textit{in vitro} findings. For further \textit{in vivo} study, the ion microbeam \cite{55}
or UV laser microirradiation [56] approaches would be useful, both of which allow the targeted irradiation of heterochromatic and euchromatic nuclear areas and may provide possibilities for a direct comparison of the generation of DSBs in these compartments either by a TUNEL assay or by identification of γH2AX.

Since we demonstrated that more-condensed chromatins have fewer DSBs induced by γ-rays and heavy ions and less attack by chemical agents such as cisplatin, the present study indicates that chromatin compaction is advantageous, even in interphase cells (Figures 6A and 6B). This is consistent with the idea of chromatin domain models, as proposed previously [11][12][16][57][5][9] (Figure 6C). In this model, interphase chromatin forms compact chromatin domains. This compact state is a “default”. The only transcribed region, which is minimally decondensed and could be sensitive to radiation or other damages, might be looped out from the domain or at the surface of the domain (Figure 6C). This type of genome organization should be beneficial for the cells, especially in the long run.

We propose that this protection by genomic DNA compaction represents a universally conserved function, from viruses to animals, to maintain genome integrity because
DNA condensation by polyamine was also observed to suppress DSB induction by γ-ray irradiation in previous in vitro studies including our own [23][24][25][26]. Maintenance of genome DNA integrity with a higher compaction would carry a selective advantage already during the evolution of prokaryotes. This selective advantage might have even been more important in early evolutionary times when the DNA repair mechanisms were preliminary. In eukaryotic organisms, the maintenance of genetic information would be especially critical in germline cells for the next generation. For example, yeast spores have ~10-fold more condensed genomic DNA than somatic cells [58]. Human primary oocytes, which take 20–40 years to complete meiosis I [59], have compact chromosomes, while the mouse and rat have highly extended (dictyate-type) chromatin in their primary oocytes [60]. Surprisingly, the primary oocytes of human are much more resistant to ionizing radiation than those of mouse and rat, which supports our hypothesis [60].

Although we emphasized the importance of the chromatin compaction in maintenance of genome DNA integrity, one might consider that chromatin compaction can interfere with DNA repair process because of less chromatin accessibility. This might be partly true [37]. However, we recently observed the local nucleosome fluctuation in living
mammalian cells and demonstrated that this nucleosome fluctuation increases chromatin accessibility, especially in compact chromatin regions [61][62]. This novel mechanism to facilitate chromatin accessibility would play an important role in the DNA repair process in the compact chromatin domains.

Finally, it is important to emphasize that our findings also provide a theoretical basis for various novel combinations of cancer therapies, e.g. [63] [64][65][66]. Since decondensed chromatin has greater susceptibility to γ-rays, heavy ions, and chemicals, histone deacetyltransferase inhibitors and other similar drugs (which decondense chromatin) would greatly enhance the cytotoxic effects of cisplatin and other DNA-damaging drugs in cancer cells, as well as of radiation therapy involving γ-rays or heavy ions.
Methods

Isolation of nuclei and chromosomes

For the isolation of nuclei, HeLa cells were maintained in Dulbecco's modified Eagle's medium (Invitrogen, Carlsbad, CA, USA) that contained 5% fetal bovine serum albumin (BSA; Nichirei Biosciences Inc., Tokyo, Japan) at 37°C in a 5% CO₂ atmosphere. Collected cells were suspended in nuclei isolation buffer (3.75 mM Tris-HCl [pH 7.5], 20 mM KCl, 0.5 mM EDTA, 0.05 mM spermine, 0.125 mM spermidine, 0.1% Trasylol, 0.1 mM phenylmethylsulphonyl fluoride (PMSF)) and centrifuged at 193 × g for 7 min at room temperature. The cell pellets were resuspended in the nuclei isolation buffer and again centrifuged at 193 × g for 7 min at room temperature. The cell pellets were then resuspended in the nuclei isolation buffer that contained 0.05% Empigen (nuclei isolation buffer+) and homogenized immediately with 10 downward strokes using a tight Dounce-pestle. The cell lysates were centrifuged at 433 × g for 5 min. The nuclei pellets were washed in the nuclei isolation buffer+ and stored at –20°C in the nuclei isolation buffer+ that contained 50% glycerol. Chromosome isolation was performed as described by Maeshima and Laemmli [43].

Solid-phase manipulation of chromatin
Isolated nuclei or chromosomes were suspended in HM buffer (10 mM HEPES-KOH [pH 7.4], 5 mM MgCl₂) and attached to poly-L-lysine-coated coverslips by centrifugation at 400 × g for 5 min. For decondensed chromatin, the nuclei or chromosomes on the coverslips were gently transferred to the buffer that contained 1 mM EDTA (pH 8.0). For recondensation, the decondensed chromatin on the coverslip was placed in HM buffer.

**Irradiation with γ-rays of cobalt-60 (high dose) and cesium-137 (low dose)**

For high-dose irradiation (all experiments, with the exception of that shown in Figure 3), the condensed, decondensed, and recondensed chromatins were irradiated with cobalt-60 γ-rays at 40 Gy/min at the Radiation Research Center, Osaka Prefecture University (Osaka, Japan). For low-dose irradiation (Figure 3), we irradiated the same sets of samples with cesium-137 at 1.67 Gy/min at the irradiation facility of National Institute of Genetics (NIG; Mishima, Japan). In both irradiation experiments, the coverslips with chromatin were put in a 12-well cell culture plate (Corning) with the indicated buffers and irradiated at room temperature. The applied dose was determined using a Fricke dosimeter.
TUNEL assay

After γ-ray irradiation, the condensed, decondensed, and recondensed chromatins on the coverslips were placed in HM buffer, followed by HMK buffer (10 mM HEPES-KOH [pH 7.4], 1 mM MgCl₂, 100 mM KCl), to ensure that the chromatin condensation states were uniform. The chromatins were fixed with 1% formaldehyde in HMK buffer at room temperature for 15 min. After washing with 50 mM glycine in HMK buffer and then HMK buffer, the chromatin samples were stored in HEN buffer (10 mM HEPES-KOH, [pH 7.5], 1 mM EDTA, 100 mM NaCl) at 4°C until use.

DNA damage was detected on the same assay condition using the TUNEL assay with the Click-iT TUNEL Alexa Fluor Imaging Assay (Invitrogen), according to the manufacturer’s instructions. The fluorescently labeled samples were co-stained with DAPI to visualize DNA, and mounted in PPDI (10 mM HEPES-KOH [pH 7.5], 1 mM MgCl₂, 100 mM KCl, 80% glycerol, 1 mg/ml paraphenylene diamine). For the high-dose irradiation samples (Figures. 2, 4 and 5), the microscopy images to quantify the TUNEL assay signals were acquired on the same imaging condition using an ECLIPSE E800 fluorescence microscope with a 60× objective lens (Nikon, Tokyo, Japan). For the lower dose irradiation samples (Figure. 3), we used Nikon microscope
system Ti-E with a 60× objective lens (Nikon, Tokyo, Japan) using Evolve512 EM-CCD camera (Roper). The TUNEL assay signals (Alexa488) were analyzed using the NIS-elements BR 3.10 software (Nikon) as follows: The nuclear or chromosomal regions were extracted with a threshold value of DAPI signals. Mean intensity values of Alexa488 signals in the extracted nuclear or chromosomal regions were examined. The mean intensity value in the regions at 0 Gy was used as background. After subtraction of the background signal, the obtained signal intensity values were normalized against that of the decondensed chromatin irradiated at 500 Gy. The comet assay was performed according to the manufacturer’s instructions (Trevigen, Gaithersburg, MD, USA).

**Nuclear and chromosonal volume measurements**

After fixation with 1% formaldehyde, the samples were washed with 50 mM glycine and stained with 2 nM TO-PRO-3 solution (Invitrogen) at 37°C for 30 min. After washing, Z-stack images were acquired using a LSM510 META laser scanning confocal microscope (Carl Zeiss, Wetzlar, Germany) with a 100× objective at 0.48-µm intervals. The obtained images were processed using the LSM Image Browser (Carl Zeiss) and the ImageJ software (NIH, Bethesda, MD, USA).
Protein composition and concentration analyses

The condensed, decondensed, and recondensed nuclei or chromosomes were collected by centrifugation (3000 rpm, 10 min). The pellets, which contained ~1.26 mg DNA, were completely dissolved in Laemmli sodium dodecyl sulfate (SDS) sample buffer by sonication. After boiling at 95°C for 5 min, the lysates were electrophoresed on 10–20% gradient SDS-polyacrylamide gel electrophoresis (PAGE) gels. To quantify the protein levels, BSA was used as the standard. The gel was stained with Coomassie brilliant blue R-250 (CBB) and the image was acquired by LAS-1000 (FujiFilm, Tokyo, Japan). Quantitative analysis was performed using the ImageJ software.

Simulation

The simulation was conducted as follows using Microsoft Visual C++. Two random numbers (SSBs) from 1 to 100,000 (100 kb) were generated independently for strand a and strand b (Figure S3A). If the difference between the two numbers on the two strands was within 10, we counted the pair as a DSB (Figure S3A). We repeated this process at the indicated times and plotted the number of DSBs against the number of SSBs (Figure S3B). The number of created DSBs was quadratically increased with the number of generated SSBs. For Figure S3C, two random numbers (SSBs) from 1 to
100,000 (100 kb) were again generated independently for strand a and strand b. Every 50 SSBs, a DSB was also created at random.

Carbon ion beam irradiation

Heavy ion treatment was performed using the Heavy Ion Medical Accelerator in Chiba (HIMAC) at the National Institute of Radiological Sciences (NIRS). The accelerated ions used in this study were carbon ions (290 MeV/n). Details concerning the beam characteristics of the carbon ion beams, biological irradiation procedures, and dosimetry have been provided previously [67][68].

Cisplatin treatment of condensed and decondensed chromatin

Isolated nuclei (~1 × 10⁷) were suspended in HM buffer or 1 mM EDTA containing buffer and treated with cisplatin at 2 mM overnight at room temperature. After five washes with HMK buffer, half of the samples were subjected to inductively coupled plasma mass spectrometry (ICP-MS) (chromatin fractions). DNA was extracted from the remaining samples and subjected to ICP-MS (DNA fractions). We performed ICP-MS analyses using ELAN DRC II (PerkinElmer, Waltham, MA, USA).

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Figure Legends

Figure 1

Schematic representation of a “solid-phase” system for chromatin manipulation and DNA damage detection. (A) The nuclei and their chromatins on the glass surface become condensed in the presence of 5 mM Mg$^{2+}$ (left, see also Panel B) and become decondensed in the absence of Mg$^{2+}$ (center, see also Panel B). With increasing Mg$^{2+}$ concentration, the decondensed nuclei and chromatins become recondensed (right, see also Panel B). Since chromatin compaction by cations is a type of phase-transition process [39], the two conditions (with 5 mM Mg$^{2+}$ and without Mg$^{2+}$) are sufficient to reconstruct the chromatin condensation and decondensation states. The three types of nuclei were irradiated with $^{60}$Co $\gamma$-rays at varying dosages of irradiation. The irradiated nuclei were treated with a buffer that contained 1 mM Mg$^{2+}$, and the DSBs in the chromatin were detected using the TUNEL assay [41] (bottom). (B) Microscopy images of the condensed, decondensed, and recondensed nuclei with DNA staining (DAPI). Bar, 10 $\mu$m. (C) Volumes of the condensed (417.7 ± 91.0, N = 50), decondensed (6196.6 ± 1310.3, N = 50), and recondensed nuclei (Recondensed; 478.6 ± 98.7, N = 50) (see Methods). The standard deviation is shown as an error bar.
Chromatin compaction suppresses DNA damage by γ-ray irradiation. (A) DSB signal detection based on the TUNEL assay. For each radiation dose, DNA staining (left) and DSB signals (right) are shown. In the decondensed nuclei (2nd row), a prominent increase in DSB signal is observed in a dose-dependent manner. However, the condensed (1st row) and recondensed (3rd row) nuclei show only faint signals. Note that their sizes become similar because the three types of irradiated nuclei were treated with a buffer that contained 1 mM Mg²⁺, so as to drive them into a uniform compact state and to ensure the equal chromatin accessibility and equal handling between the three types. Bar, 10 μm. (B) Quantification of the detected DSB signals. The plotted normalized signal intensities and irradiation doses fit well to the quadratic curve: $I = kD^2$, where $I$, $D$, and $k$ are DNA breakage by irradiation, irradiation dose, and the constant, respectively. The formula for each condition is shown in the graph. For each point, $N > 150$. (C) The relative DNA damage frequency upon exposure to 500 Gy of irradiation for each condition is shown as a bar graph (Condensed, 0.06 ± 0.03, $N > 100$; Decondensed, 1.0 ± 0.19, $N > 100$; Recondensed, 0.06 × 0.03, $N > 100$). Error bar shows standard deviation.
Figure 3

DSB damage protection effects in low-dose range. (A) For the sensitive detection of DSB signals, an EM-CCD camera was used. For each radiation dose, DNA staining (left) and DSB signals (right) are shown. Note that the DSB signals in the low-dose range are readily detectable in the decondensed chromatin (2nd row). Bar, 10 µm. (B) Quantification of the detected DSB signals in the low-dose range. Normalized signal intensities and irradiation doses are plotted. Error bar shows the standard deviation. Consistent with the high-dose irradiation results, the condensed chromatin has considerable damage suppression effects. Note that DSB signals seem to increase linearly at low dose irradiation.

Figure 4

Chromatin compaction in mitotic chromosomes suppresses the DNA damage caused by γ-ray irradiation. (A) Microscopy images of the condensed (left), decondensed (center), and recondensed (right) mitotic chromosomes with DNA staining (DAPI). Bar, 10 µm. (B) Volumes of the condensed, decondensed, and recondensed chromosomes (see Methods). The standard deviation is shown as an error bar. (C) DNA damage detection using the TUNEL assay. For 0 Gy of irradiation (left two columns) and 500 Gy of...
irradiation (right two columns), DNA staining (left image) and DSB signals (right image) are shown. Bar, 10 µm. In the decondensed chromosomes (2nd row), a strong increase in DSB signal intensity is observed. However, the condensed (1st row) and recondensed (3rd row) chromatins show only faint. (D) Quantification of the detected DSB signals. DNA damage frequency for each condition is shown as a bar graph. The error bar represents the standard deviation.

Figure 5

Effects of molecular crowding and radical scavengers on damage suppression (A-C) and protection from attack by heavy ions or chemicals (D-G). (A) DSB signals in the chromatins under various conditions. For each condition, DNA staining (1st row) and DSB signals (2nd row) are shown. Addition of PEG or DTT to the decondensed nuclei has strong DNA damage suppression effects. Bar, 10 µm. (B) (C) Quantification of the DSB signals after the addition of PEG or DTT. The error bar represents the standard deviation. For each point, N = ~300. (D) DSB damage protection effect against heavy ion irradiation. For each radiation dose, DNA staining (left) and DSB signals (right) are shown. (E) Quantification of the detected DSB signals. Normalized signal intensities and irradiation doses are plotted. The error bar shows the standard deviation. Note that
DSB signals increase linearly under this condition. Bar, 10 μm. (F) Treatment of the condensed and decondensed chromatin with cisplatin. Schematic representation of the experiment. (G) The amount of cisplatin bound to chromatin (nuclei) and extracted DNA were quantitatively measured by ICP-MS. Note that the DNA in the decondensed chromatin has 10-fold more cisplatin adducts than the condensed chromatin.

Figure 6
Damage suppression and the compact chromatin domain model. (A) Reactive radicals arising from radiolysis of water molecules by irradiation are major contributors to the damaging of decondensed chromatin. In addition, decondensed chromatin is more accessible to chemicals (marked with “Pt”). (B) A higher density of chromatin with fewer water molecules means that there is less risk of being attacked by hydroxyl radicals. The situation is also effective to protect DNA from the binding of cisplatin. (C) The proposed compact chromatin domain model. We assume that the condensed state is a “default” and only the transcribed region, which is being decondensed and is sensitive to the radiation damage, might be looped out from the domain or at the surface of the domain, so as to minimize the risk of radiation or chemical damage.
References


Figure 1

Human Nuclei

Attach on coverslips

5 mM Mg²⁺

- Mg²⁺

5 mM Mg²⁺

Condensed

Decondensed

Recondensed

DNA damage detection by TUNEL assay

B

C

Nuclear Volumes (μm²)

Condensed

Decondensed

Recondensed
Figure 2
Figure 3
Figure 4
Figure 5
Figure 6