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# mBAND analysis of chromosome aberrations in human epithelial cells induced by γ-rays and secondary neutrons of low dose rate

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ABSTRACT

Human risks from chronic exposures to both low- and high-LET radiation are of intensive research interest in recent years. In the present study, human epithelial cells were exposed *in vitro* to γ-rays at a dose rate of 17 mGy/h or secondary neutrons of 25 mGy/h. The secondary neutrons have a broad energy spectrum that simulates the Earth's atmosphere at high altitude, as well as the environment inside spacecrafts like the Russian MIR station and the International Space Station (ISS). Chromosome aberrations in the exposed cells were analyzed using the multicolor banding *in situ* hybridization (mBAND) technique with chromosome 3 painted in 23 colored bands that allows identification of both inter- and intrachromosome exchanges including inversions. Comparison of present dose responses between γ-rays and neutron irradiations for the fraction of cells with damaged chromosome 3 yielded a relative biological effectiveness (RBE) value of $26 \pm 4$ for the secondary neutrons. Our results also revealed that secondary neutrons of low dose rate induced a higher fraction of intrachromosome exchanges than γ-rays, but the fractions of inversions observed between these two radiation types were indistinguishable. Similar to the previous findings after acute radiation exposures, most of the inversions observed in the present study were accompanied by other aberrations. The fractions of complex type aberrations and of unrejoined chromosomal breakages were also found to be higher in the neutron-exposed cells than after γ-rays. We further analyzed the location of the breaks involved in chromosome aberrations along chromosome 3, and observed hot spots after γ-ray, but not neutron, exposures.

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## 1. Introduction

Biological effects from chronic exposures to either low- or high-LET radiation are of an active research interest related to human health risks [1,2]. Low-LET exposures are a concern for nuclear power plant workers, cleanup personnel in nuclear accidents or other professions involving nuclear materials. Exposures to high-LET radiation of low dose rate are also a major health concern for astronauts during space missions, as well as for miners exposed to α-particles. A particular type of high-LET radiation encountered by astronauts in space or by crew members flying airplanes at very high altitude is secondary neutrons generated due to the interaction between the primary charged particles and the atmosphere, spacecraft structure or planetary surfaces [3]. These secondary neutrons can be a significant contributor to the absorbed dose and dose equivalent received [4,5]. Inside typical spacecraft, secondary neutrons were estimated to account for as high as 30% of the dose equivalent [6].

Unlike fission neutrons that have been extensively investigated, secondary neutrons encountered in space cover a wide energy range. Comparison of the biological effectiveness of neutrons to that of low-LET radiation indicated a wide range of the relative biological effectiveness (RBE) as a function of the neutron energy. The International Commission on Radiological Protection (ICRP) has recommended the weighting factor for neutrons to be in the range of 5–20 depending upon the energy and with the peak weighting factor of 20 for energies in the range of 0.1–2 MeV [7]. The weighting factor was proposed to be a continuous function of neutron energy in a later published report by ICRP with a lower factor for thermal neutrons [8]. The RBE of neutrons also varies for different biological endpoints and different cell types. For instance, the RBE for oncogenic transformation of C3H10T1/2 cells for 70 keV fission neutrons was estimated to be 6.6 [9], while the RBE for chromosome aberrations in human lymphocytes exposed to fission neutrons of the same energy was found to be 53 [10].

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The energy spectrum of Los Alamos Neutron Science Center (LANSCE) ICE (Irradiation of Chips and Electronics) House 30L beamline is known to generate secondary neutrons with the energy spectrum similar to that measured onboard spacecrafts like the Russian MIR station and International Space Station (ISS) [6]. This spectrum is also similar to that in the atmosphere at 12,000 m altitude [11]. Previously, we reported the induction of micronuclei (MN) in human fibroblasts after exposure *in vitro* to neutrons of a dose rate of 25 mGy/h generated at the LANSCE's 30L beamline [12]. In the study, the cells were placed at the entrance or behind a 9.9 cm water column to study the effect of shielding in the protection of neutron induced damages. We found that the dose response in the MN frequency was linear for the samples with and without shielding and the slope of the MN yield behind the shielding was reduced by a factor of 3.5. Compared to the MN induction in human fibroblasts exposed to a γ source at a similar low dose rate, the RBE was found to be 16.7 and 10.0 for the neutrons without and with the 9.9 cm water shielding, respectively [12].

In the present study, we exposed human epithelial cells *in vitro* to γ-rays and LANSCE neutrons of low dose rate and collected the samples for chromosome analysis using the multicolor banding fluorescent *in situ* hybridization technique (mBAND). With mBAND, a series of longitudinal colored bands are generated along the axis of the chromosome [13]. In principle mBAND is capable of detecting aberrations leading to the loss or rearrangement of those colored bands and, therefore, is capable of analyzing inter- and intrachromosome aberrations simultaneously. The stable type intrachromosome aberrations that can be detected with mBAND, such as inversions, are more important than other unstable type aberrations as they are considered to be more relevant to the cancer risks.

The mBAND data are also highly useful for revealing biomarkers for the radiation quality. High-LET radiation has been predicted to induce a higher yield of intrachromosome exchanges than low-LET radiation, and a number of "fingerprints" from mBAND analysis have been proposed as a marker for the radiation quality. For instance, Hande and colleagues [14] demonstrated the potential of mBAND data as a sensitive, long-lived, quantitative and low-background biomarker for α-particle (plutonium) exposure. However, Johannes et al. [15] reported that the ratio of inter-/intrachromosome exchanges or intra-/interarm exchanges analyzed in human lymphocytes using mBAND did not represent a fingerprint of *in vitro* exposure to densely ionizing radiation. Our recent study confirmed the observation of a higher incidence of inversions in human epithelial cells after exposure to 600 MeV/u Fe ions in comparison to γ-rays [16]. However, detailed analysis of the inversion type revealed that both Fe ion and γ-ray exposures induced a low incidence of simple inversions. Half of the inversions observed in the low-LET irradiated samples were accompanied by other types of intrachromosome aberrations, and few inversions were accompanied by interchromosome aberrations. In contrast, Fe ions induced a significant fraction of inversions that involved complex rearrangements of both inter- and intrachromosome exchanges. It is apparent that further mBAND analysis is needed to fully address the issue of low- and high-LET-induced intrachromosome damages.

## 2. Materials and methods

### 2.1. Cell culture

The human mammary epithelial cell line (CH184B5F5/M10) originated from H184B5 cells, and was selected for its ability to grow in minimal essential medium supplemented with serum after irradiation with Fe ions [17]. Although this cell line has had prior radiation treatment, it has been successfully employed in several radiation assessment studies [16,18,19]. Moreover, this is one of very few cell lines that have been extensively developed over several years to successfully obtain a tissue-equivalent, three-dimensional cell model [20]. These cells are immortalized, exhibit normal G1 arrest upon attaining confluency, and exhibit both anchorage-dependent and serum-dependent growth [19]. Normal radiation-induced DNA repair-kinetics have been observed [21]. The survival curve obtained by Durante et al. [22] with X-rays for this cell line was very close to the one obtained for a different non-tumorigenic mammary epithelial cell line [23]. CH184B5F5/M10 cells contain 46 chromosomes, but translocations among several chromosomes have been identified [24]. Both copies of chromosome 3, which was chosen for the present study, were found to be normal.

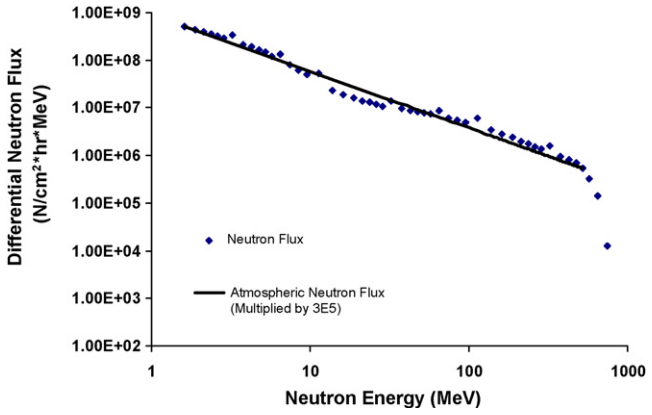


**Fig. 1.** Energy spectrum of the secondary neutrons to which the cells were exposed. The shape of the spectrum is similar to that in the upper atmosphere (multiplied by $3 \times 10^5$ as represented by the solid line) and inside the space station.

The CH184B5F5/M10 cells were grown in Dulbecco's modified Eagle medium (DMEM) (Invitrogen, Carlsbad, CA) supplemented with 10% fetal calf serum (Invitrogen) and antibiotics. They were ascertained to be free of mycoplasma by periodic testing (Bionique, Saranc Lake, NY).

### 2.2. Radiation exposure and neutron measurement

The low dose rate γ exposures were performed at NASA Johnson Space Center with a low activity Cs-137 source. The dose rate at the sample location was 17 mGy/h. T-25 flasks containing confluent cells were filled with medium, pre-warmed, and kept in a 37 °C incubator during the exposure. The exposure time was determined based on the total dose and dose rate, and lasted for several days.

The secondary neutrons at LANSCE were generated by an 800 MeV pulsed proton beam that strikes a tungsten target causing spallation neutrons [25]. A sweep magnet upstream of the target area removes charged particles generated during the spallation process from the beam. The absolute neutron intensities in the energy range from 1 to 800 MeV were measured by a fission chamber and time of flight (TOF) system [26]. Fig. 1 illustrates the measured neutron spectrum presented together with the spectrum expected in the atmosphere at high altitude [11]. The dose rate of neutrons at the sample was measured to be 25 mGy/h using a Tissue Equivalent Proportional Counter (TEPC). Similar to the low dose rate γ-ray exposures, the cells in filled flasks were kept at 37 °C during the exposure period. The exposure time lasted for several hours.

### 2.3. Premature chromosome condensation (PCC)

A PCC technique was used to condense the chromosomes of cells in both G2 and metaphase, as was in the earlier reports [27,28] and in our previous study [16] in order to detect more damages, particularly for high-LET radiation exposure. Briefly, after exposure, the cells were transferred from a T-25 flask to a T-75 flask. After 24 h incubation at 37 °C, the microtubule inhibitor colcemid (Invitrogen) at a concentration of 0.03 μg/ml was added to stop the cells from progressing through the first mitosis. After further incubation at 37 °C for 8 h, the chromosomes were condensed by incubation with calyculin A (Wako Chemicals, Japan) at a concentration of 50 nM for 30 min. The cells were then incubated in 0.075 M KCl at 37 °C for 20 min and fixed in methanol/acetic acid (3:1, vol/vol) fixative solution.

### 2.4. Multicolor banding (mBAND)

Chromosomes were prepared by dropping fixed metaphase cells onto clean, wet slides which were then dried overnight. Chromosomes were hybridized *in situ* using the mBAND kit (MetaSystems, Altlussheim, Germany) for human chromosome 3 following the protocol recommended by the manufacturer. We selected chromosome 3 for analysis because it is a large chromosome with a low incidence of background damage in this cell line. Chromosome aberrations were scored using a Zeiss Axioplan2 microscope equipped with an HBO 100 mercury lamp and appropriate filter sets (Metasystems). Images were captured and processed with the Isis software (Metasystems). All types of detectable aberrations were scored.

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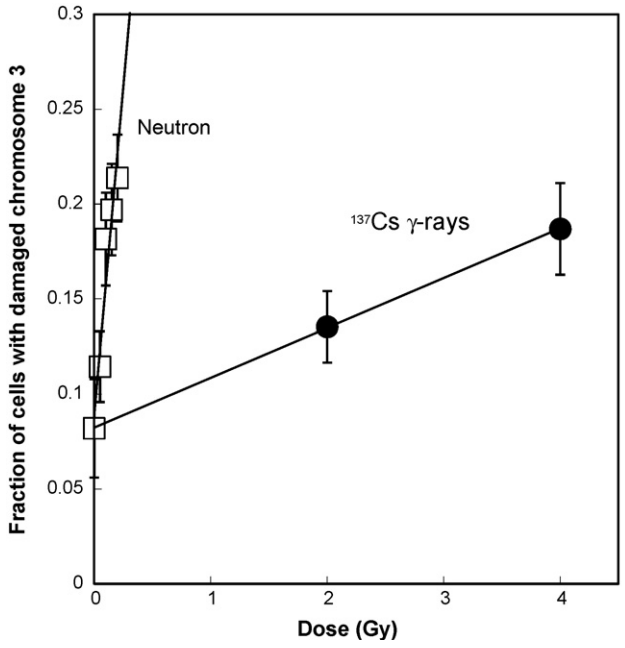


**Fig. 2.** Fractions of cells containing aberrant chromosome 3 induced by γ-rays or secondary neutrons of low dose rate. The bars represent the standard errors of the mean.

Chromosome bands in the mBAND technique are pseudocolors determined not just by the overlap of two of the five probes hybridized along the chromosome, but also by the overlaps of two probe signals of different intensities, and require careful sample preparation. For chromosome 3, a total of 23 bands can be separated with the software provided by the manufacture (Metasystems).

### 2.5. Identification of break points

When an aberration involves exchanges with the participation of chromosome 3, the location of the break, as denoted by the band number, was recorded. The relative length of each of the bands was measured in 30 chromosomes showing no aberrations. The mean value of the relative band length and the standard deviation were calculated based on the 30 measurements.

## 3. Results

Table 1 lists the number of chromosome aberrations in each of the categories. Interstitial or terminal deletions contained two fragments of chromosome 3. When the number of chromosome fragments exceeded 2, the aberration was classified as a multiple deletion. Simple and complex interchromosome exchanges were identified following the traditional classification of an aberration involving three or more breaks on two or more chromosomes. A complex inversion was scored when an inversion was accompanied by other aberrations. When an intrachromosome rearrangement other than inversion, such as shift but excluding interstitial deletion, was detected, it was included in the category of "Intra-rearrangement other than inversion".

### 3.1. RBE for secondary neutrons

Since chromosome aberrations can be highly complex [29] especially for high-LET radiation, defining the frequency of aberrations in cells is not straightforward. Hence, the RBE here was evaluated only for the fraction of cells containing aberrations in chromosome 3. Fig. 2 shows the dose–response curve for the percentage of cells with aberrations involving chromosome 3, induced by γ or the secondary neutrons of low dose rate. The error bars were determined assuming Poisson's distribution of the number of aberrations. For both radiation types, the dose response appeared to be linear, as

**Table 1**
Chromosome aberration detected by mBAND analysis in chromosome 3 in human epithelial cells irradiated with low dose rate neutrons or γ-rays.

| Radiation | Dose (Gy) | Total cells scored | Cells with aberrant chromosome 3 | Aberrations | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Interstitial deletion | Terminal deletion | Multiple deletion | Inter-exchange (simple) | Inter-exchange (complex) | Simple inversion | Inversion accompanied with other aberrations | Intra-rearrangement other than inversion |
| Control | 0 | 122 | 10 | 0 | 5 | 0 | 3 | 0 | 0 | 1 | 2 |
| Neutrons | 0.05 | 332 | 38 | 0 | 18 | 5 | 9 | 4 | 1 | 0 | 3 |
| | 0.1 | 303 | 55 | 2 | 23 | 5 | 19 | 13 | 2 | 3 | 6 |
| | 0.15 | 340 | 67 | 2 | 35 | 5 | 21 | 2 | 1 | 4 | 4 |
| | 0.2 | 407 | 87 | 3 | 23 | 8 | 53 | 14 | 1 | 6 | 3 |
| γ-Rays | 2 | 384 | 52 | 0 | 10 | 3 | 42 | 2 | 1 | 0 | 1 |
| | 4 | 321 | 60 | 10 | 8 | 4 | 30 | 9 | 2 | 5 | 3 |

expected from low dose rate exposures. Comparison of the slopes of dose response yielded a RBE value of 26 ± 4. The RBE values for individual types of aberrations were not evaluated, as the linearity of the dose response data produced a weak statistical significance.

### 3.2. Distribution of break numbers in chromosome 3

The number of breaks in chromosome 3 that produced visible aberrations was analyzed. For instance, an interstitial deletion was scored as two breaks, while a terminal deletion was scored as one. Shown in Fig. 3 is the frequency distribution of breaks in the chromosome. For 2 Gy γ-rays of low dose rate, most of the damaged chromosomes resulted from misrejoining of a single break on the chromosome, and 10% of damaged cells had more than 1 break. At 4 Gy, about 40% of the damaged cells contained 2 or more breaks on chromosome 3. The break number distribution for secondary neutrons was independent of the dose (Fig. 3). The percentage of damaged cells that contained more than 1 break remained in the range of 30–40% for all neutron doses, indicating that damages from the neutron exposure were from mostly the single track effect.

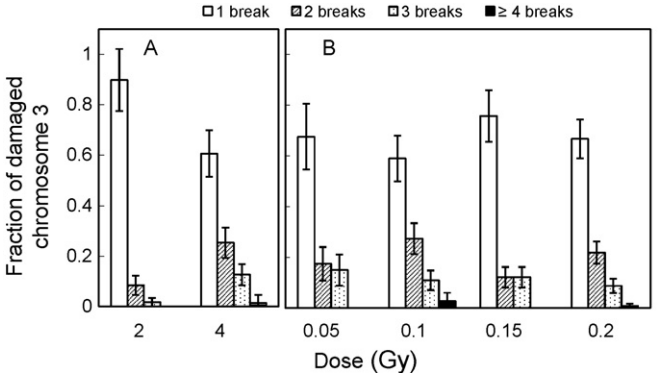


**Fig. 3.** Frequency distribution of breaks in chromosome 3 induced by γ-rays (panel A) or secondary neutrons (panel B).

### 3.3. Inter- and intrachromosome exchanges

One unique advantage of the mBAND technique is the ability to analyze inter- and intrachromosome exchanges simultaneously. Since most of the aberrations are of complex type that involved both inter- and intrachromosome exchanges, we present, in Fig. 4, the fraction of cells containing interchromosomal exchanges or intrachromosomal exchanges. Interchromosome exchanges here include simple reciprocal exchanges between two chromosomes (both complete and those that appear to be incomplete) and complex type exchanges between two or more chromosomes. When a complex type aberration involves at least one exchange between chromosome 3 and an unpainted chromosome, the aberration is scored as one interchromosome exchange. Intrachromosome exchanges include any of the aberrations that involve at least

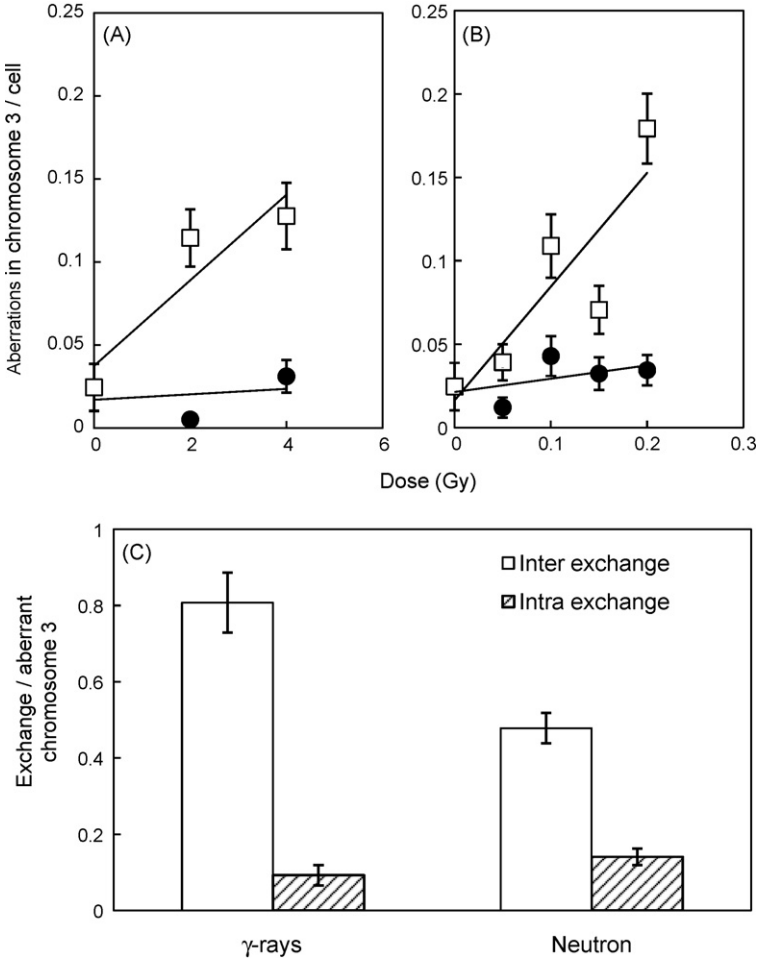


**Fig. 4.** Induction of interchromosome (open squares) and intrachromosome (filled circle) exchanges in human chromosome 3 by γ-rays (panel A) or neutrons (panel B). The pooled data from all doses are presented in panel C. Terminal deletions that were frequently found in neutron-irradiated cells were not scored as either inter- or intrachromosome exchanges, and accounted for the missing fraction in Panel C.

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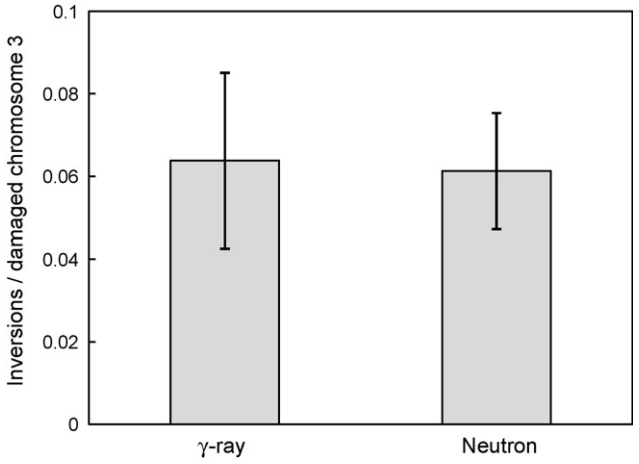


**Fig. 5.** Fraction of damaged chromosome 3 that contained inversions after γ or secondary neutron exposures. The pooled data for all doses are presented. Only a small fraction of damages were inversions even for secondary neutrons.

one exchange between two breaks on a single chromosome 3. Intrachromosome exchanges include interstitial, but not terminal deletions. A complex aberration that involves both inter- and intrachromosomal exchange types is scored as one for each type.

For γ-rays of low dose rate, most damages in chromosome 3 involved interchromosome exchanges (Fig. 4A). The occurrence of intrachromosome exchanges was low even at the dose of 4 Gy. The fraction of cells containing intrachromosome exchanges was also low for neutron exposures in comparison to the fraction of cells containing interchromosome exchanges (Fig. 4B). Combining the data for all dose points, we found 10% of the damaged cells after γ irradiation contained intrachromosome exchanges, whereas, 25% of the damaged chromosomes after neutron irradiation contained intrachromosome exchanges (Fig. 4C).

### 3.4. Inversions

The number of aberrations containing inversions is low in the present low dose rate experiments. Of the total of 8 aberrations that contained inversions after γ irradiation, 3 aberrations were of simple type, 3 aberrations were involved with other intrachromosome exchanges, 1 was accompanied with an interchromosome exchange and 1 was accompanied with both inter- and intrachromosome exchanges. In neutron-irradiated samples, there were a total of 18 aberrations that contained inversions. Of those aberrations, 5 were simple inversions, 6 were involved with other intrachromosome exchanges, 3 were accompanied with interchromosome exchanges and 4 were accompanied with both inter- and intrachromosome exchanges.

Since the frequencies of inversions are low for both γ and neutron exposures, we present in Fig. 5 the fraction of inversions in damaged chromosome 3 that were pooled from all doses. As seen in Fig. 5, only 6 percent of the damaged chromosome 3 contained an inversion, and the percentages were statistically indifferent between γ and neutron irradiations.

### 3.5. Complex type aberrations

Aberrations involving interchromosome exchanges between chromosome 3 and other chromosomes were further divided into simple and complex types. A complex aberration was scored when it was determined to have involved exchanges of three or more breakpoints on two or more chromosomes. As shown in Fig. 6, for 2 Gy of γ-rays, most of the exchanges were of the simple type, and 4 Gy γ-rays induced more complex type aberrations. On the other hand, secondary neutrons induced a significant number of complex type aberrations even at the lowest dose of 0.05 Gy, and the fraction of complex exchanges did not appear to increase as a function of the dose. The combined fractions of simple and complex aberrations were less than 1 because a significant fraction of aberrations were terminal deletions for secondary neutrons.

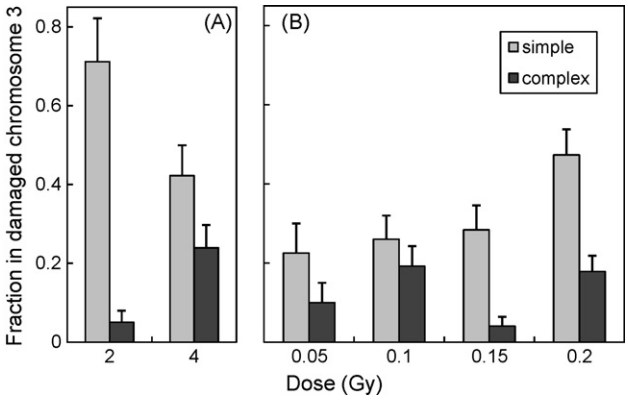


**Fig. 6.** Interchromosomal exchanges in human chromosome 3 induced by γ-rays (panel A) or neutrons (panel B) presented as simple and complex types.

### 3.6. Unrejoined breaks

Chromosome ends that did not participate apparently in either inter- or intrachromosome exchanges were scored as unrejoined breaks. A terminal deletion here was scored as two unrejoined breaks and an incomplete exchange was scored as one. In Fig. 7, we present the fraction of unrejoined breaks pooled from all doses for γ-rays or for secondary neutrons. A direct comparison showed a significantly greater fraction of unrejoined breaks after neutron exposure than γ-rays.

### 3.7. Distribution of breakpoints along chromosome 3

The position of the breaks along chromosome 3 was analyzed by identifying the band number of the double strand breaks (DSB) that is involved in chromosome aberrations. The mBAND technique used in the present study allows separation of chromosome 3 into 23 bands. The length of each of the 23 bands was measured and the expected number of breaks in these bands based on the random distribution assumption is presented in Fig. 8C. The breakpoint position distributions for both γ and neutron exposures, as shown in Fig. 8A and B, deviated from the random distribution as pre-

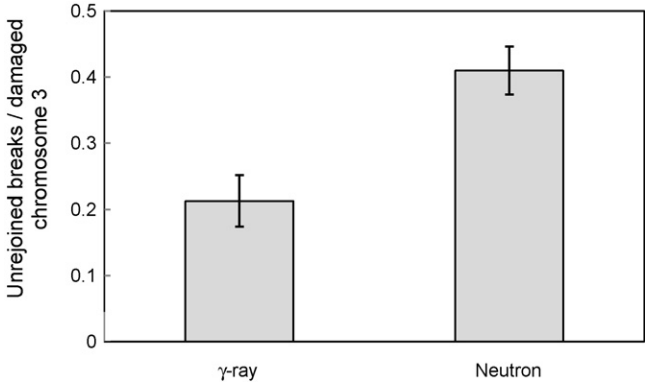


**Fig. 7.** Fraction of damaged chromosome 3 in γ- or neutron-irradiated cells that contained only breaks that did not participate apparently in either inter- or intrachromosome exchanges. The pooled data for all doses are presented.

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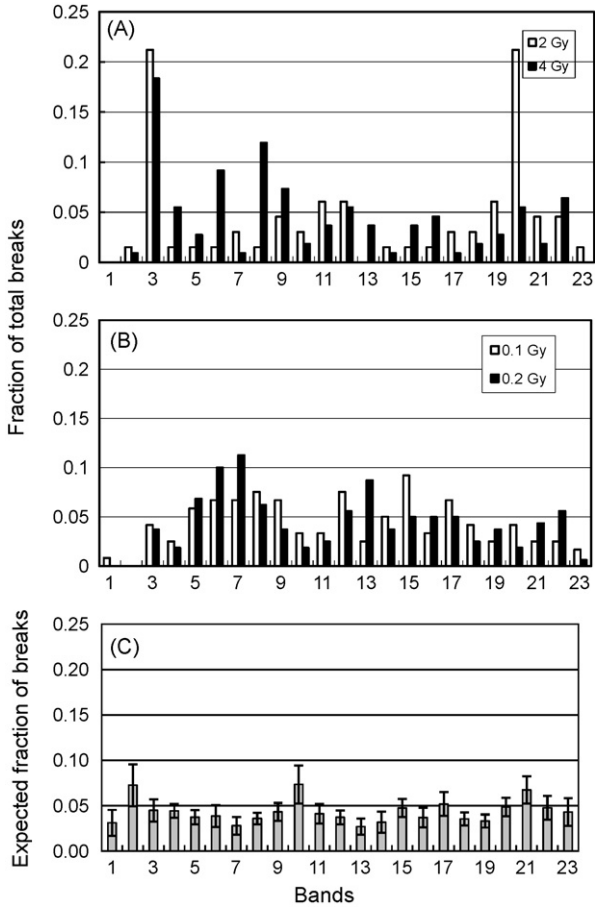


**Fig. 8.** Observed break points in the banded chromosome 3 following irradiation with γ-rays (panel A) or neutrons (panel B). Expected distribution from bands length is shown in panel C.

dicted from the length of the band (Fig. 8C). The difference of the breakpoint distributions between these two radiation types was also significant. For γ-rays, hot spots were found close to the end of the chromosome, whereas the variation of the number of breaks among the bands was relatively smaller, in comparison to γ-rays.

## 4. Discussion

Understanding the health consequences from chronic exposures to low doses of both low and high-LET radiation is a major research focus in recent years. In the present study, we investigated damages in chromosome 3 of human epithelial cells after *in vitro* low dose rate exposures to γ-rays or secondary neutrons of a broad spectrum using the mBAND technique. Our analysis of inversions using mBAND for low dose rate γ irradiation was one of the few studies addressing this stable type of chromosome aberrations from chronic radiation exposures *in vitro*, whereas, our neutron experiments appeared to be one of the very few investigations of the direct biological effects of secondary neutrons encountered inside the International Space Station as well as in the upper atmosphere.

### 4.1. Low dose rate γ exposure

Low-LET radiation has been known to induce relatively low levels of intrachromosome exchanges in lymphocytes that were collected from exposed individuals [14] or in lymphocytes exposed *in vitro* [14,15,30,31]. In comparison to our previous high dose rate γ-ray data [16], 4 Gy γ-rays of low dose rate in the present study induced about the same total damages as 2 Gy γ-rays of high dose rate. Our earlier results demonstrated that most of the intrachromosome exchanges including inversions occurred only after 4 Gy acute γ exposures [16]. Our present results further demonstrated that even at a dose as high as 4 Gy, low dose rate γ-rays are ineffective in inducing inversions.

Among the few reported studies that focused on the effects of dose rate on intrachromosome exchanges, Karthikeya-Prabhu et al. compared inter-arm and intra-arm chromosome aberration frequencies as measured by the occurrence of dicentrics and double minutes in human lymphocytes after *in vitro* exposure to γ-rays of 1.0, 0.1 and 0.0014 Gy/min, and reported that the ratios of inter- to intra-arm exchanges were significantly different between the lowest dose rate and other two higher dose rates [32]. Further analysis of the present data will be made to compare the dose rate effect in the induction of inter- or intra-arm exchanges.

### 4.2. Neutron RBE

Studies of the biological effects from exposures to secondary neutrons encountered in space have been rare [33]. The neutrons of a broad spectrum in the present study presented a RBE for total chromosome exchanges of $26 \pm 4$ in comparison to the present low dose rate γ data. RBE values for neutrons vary in a wide range depending upon the neutron energy and the biological endpoint [10]. In a previous study of micronuclei induction in human fibroblasts exposed to the same sources and γ-rays and neutrons of low dose rate, we reported a RBE value of 16.7 [12]. Our current RBE value is higher than those for chromosome aberrations induced from high-LET charged particle exposures [34]. The current RBE value is also higher than the average quality factor of 17.4 that was calculated from the neutron spectrum using the ICRP-60 quality factor [6]. Exposing human lymphocytes to 2 Gy neutrons of a mean energy of 5.7 MeV, Johannes et al. reported a similar percentage of cells with aberrant chromosome 5 as in the cells exposed to 4 Gy X-rays [15], which should have also resulted in a lower RBE value. Comparing to the studies with fission neutrons, the secondary neutrons in the present study contained more particles at lower energy, which may have contributed in part to the higher RBE value than those reported in other neutron studies.

### 4.3. Chromosome signature of radiation quality

Previously, Brenner and Sachs proposed that the ratio of inter- to intrachromosome exchanges, or the *F* ratio as measured by the frequency of dicentrics and centric rings, can be used as a biosignature of radiation quality [35]. Subsequently, other yield ratios have also been suggested [36]. While experimental verification of the F ratio has been inconclusive and appeared to depend on the method by which the chromosomes are collected [37,38], Hande et al. demonstrated a significantly higher yield of inversions detected in the lymphocytes of individuals exposed to high-LET α-particles in comparison to victims exposed to γ-rays using mBAND [15]. This *in vivo* observation was supported by Janet et al. [31] in an *in vitro* α-particle study. However, using the mBAND technique, Johannes et al. reported no such distinction of the ratio of inter- to intrachromosome exchanges between low and high-LET radiation in human lymphocytes exposed *in vitro* [15]. In the present study, we found that, for human epithelial cells, secondary neutrons of low dose rate induced a higher fraction of intrachromosome exchanges than γ-rays, but the fractions of inversions observed between these two radiation types were indistinguishable. Johannes et al. also reported a lack of distinction of the ratio of intra- to interarm exchanges between low and high-LET [15], but our present data were insufficient to support the observation.

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In addition, our present data support the use of the ratio of complex to simple exchanges as a signature of radiation quality as has been previously demonstrated [15,39,40]. The fraction of unrejoined chromosomal breaks after neutron exposures was also higher than that for γ-rays. This unrejoined breakage result was different from that reported by Formina et al. for high energy neutrons analyzed using telomere probes [41], but similar to the findings for other high-LET charged particles [39]. It should be noted that the mBAND technique presents several limitations. The average length of each band is about 10 Mbp and so the technique is unable to detect intrachromosome exchanges between two breaks separated by a genomic distance of less than that size. Hence, the frequency of interstitial deletions, or double minutes, as detected by mBAND may differ from those reported using the traditional Giemsa staining.

### 4.4. Breakpoint distribution

The technique of mBAND has the unique advantage to measure the breakpoint location along the chromosome in a precision that is unmatched with other traditional banding techniques [13]. In the present study, we measured the breakpoint locations in each of the 23 bands. As seen in Fig. 8, both γ-rays and secondary neutrons of low dose rate appeared to produce non-random breakpoint distribution which is consistent with the finding reported in the literature using mBAND or other banding techniques [13,42–44]. Due to the low numbers of break points in each of the bands, the variation between different doses of the same radiation type was large. Nevertheless, two bands (the 3rd and the 20th bands) that are close to either end of the chromosome appeared to be hot spots for γ-rays, but not for secondary neutrons. On the other hand, the breakpoint distribution for neutrons appeared to be clustered in the regions centered at Band 7. It should be noted that the number of total breakpoints in the data presented in Fig. 8 were similar between γ and neutrons. The detailed distinctions of the breakpoint distribution between low and high-LET radiation will be presented elsewhere.

## Conflict of interest statement

None declared.

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