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INDUCTION OF ABERRATIONS IN HUMAN LYMPHOCYTES BY γ-RAYS AND FAST HEAVY IONS

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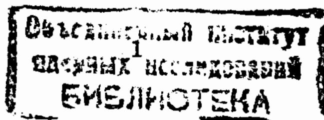
Ionizing radiation induces different lesions in the DNA molecules of mammalian cells. Among these lesions, double-strand breaks appear to be the most important ones for the induction of chromosomal aberrations (Natarajan and Obe 1978, Natarajan et al. 1986). Both the frequency and the spectrum of different aberration types depend on radiation quality and the dose of radiation. Cells with asymmetrical exchange aberrations (dicentrics, centric rings) and/or fragments are eliminated from the irradiated population during several divisions. On the contrary, cells containing so-called stable aberrations (translocations, insertions) are preserved for a long time after irradiation (Awa et al. 1978).

The frequency of dicentric chromosomes has been used for biological dosimetry owing to the possibility of their rapid scoring without banding (Lloyd et al. 1975, 1980, Evans et al. 1979). However, the use of these aberrations for biological dosimetry is complicated because the frequency of cells carrying such chromosomes decreases with time after the exposure (Awa et al. 1978, Buckton et al. 1978). Fluorescence *in situ* hybridization (FISH) with whole-chromosome painting probes allows to detect the stable chromosomal aberrations (translocations) even at low frequencies as they are usually found in exposed men. This analysis can be performed successfully at long time intervals after irradiation (Pinkel et al. 1988, Cremer et al. 1990).

There is enough evidence on the induction of translocations in lymphocytes irradiated with different doses of sparsely ionizing radiation (Cremer et al. 1990, Natarajan et al., Lucas et al. 1992, Schmid et al. 1992, Tucker et al. 1993, Nakano et al. 1993, Straume and Lucas 1993, Knehr et al. 1994, Natarajan et al. 1994, Fernandez et al. 1995). On the other hand, there are only few data (Testard et al. 1997) on these aberrations in lymphocytes irradiated by densely ionizing accelerated particles.



In this study we present a comparison of frequencies of different aberrations induced in human lymphocytes by γ-rays and accelerated nitrogen ions as detected by FISH method using whole-chromosome painting probes for the chromosomes 1 and 2 with aberrations detected in the whole genome by conventional painting technique.

Material and methods

Separation and cultivation of lymphocytes

Lymphocytes were isolated from whole heparinized blood (16–20 U/ml) of healthy donors. Plasma containing lymphocytes was obtained as supernatant after 30 min of blood sedimentation in the presence of 1% gelatine. After irradiation the cells were diluted (1:3) by RPMI-1640 medium containing 1% glutamine, 0.1 ml phytohaemagglutinin (Murex, England) per 5 ml of suspension, 1% glucose, penicillin (100 U/ml) and streptomycin (100 μg/ml). The incubation was performed at 37°C. Colchicine was added 2 h before the fixation.

Cytological preparations were made by a standard procedure, which included harvesting by centrifugation, hypotonic treatment and fixation in acetic acid-methanol (1:3). Slides for *in situ* hybridization were stored at –20°C.

Irradiation of lymphocytes with γ-rays and ^{14}N ions

Plasma containing lymphocytes was irradiated immediately after separation (G_0 phase of the cell cycle of lymphocytes) at room temperature by ^{137}Cs γ-rays (dose rate 3.6 Gy/min). The exposure to ^{14}N ions with the mean energy of 50 MeV (dose rate 6.0 Gy/min; mean value of LET was 77 keV/μm) was performed at the cyclotron U400M (Joint Institute for Nuclear Research, Dubna, Russia). Plasma containing lymphocytes was irradiated in special dishes (4 mm

depth and 12 mm in diameter) covered by polycarbonate foil (8 μm thick). The dishes containing lymphocytes were placed in a round remotely controlled container and automatically placed in the beam of ions. Several samples containing 0.4 ml of cell suspension were irradiated by the same dose of radiation to obtain 2.5 to 3 ml of cell suspension irradiated by the same dose. After irradiation, the cells were diluted with RPMI medium and incubated for 50 h (γ-irradiated samples) and 57 h (^{14}N ions irradiated samples).

FISH painting and image analysis

The *in situ* hybridization was performed using Cambio's biotinylated chromosome 1 and Oncor digoxigenated chromosome 2 painting probes to visualize both chromosomes on the same slide. The procedure was performed according to recommendations of manufactures, similarly as described by Lichter et al. (1988). The slides were viewed under a Zeiss fluorescence microscope equipped with a triple filter (AHF, Germany) specific for FITC, rhodamine and DAPI. Using a frame grabber (MuTech, Co.) images were scanned by a cooled colour videocamera of high resolution capacity (CCD camera C5310, Hamamatsu) and stored in the computer.

Chromosome aberrations were classified according to Savage (1975). In the case of a dicentric chromosome and a centric ring, one acentric fragment was subtracted from the number of fragments in the mitoses. The conventional analysis of chromosomal aberrations was performed in metaphase spreads prepared by air-drying method, which were stained with 3% Giemsa solution for 15 min. The relationship between the frequency of translocations detected by FISH method in chromosomes 1 and 2 and the total genomic translocation frequency, was calculated using the approach suggested by Lucas et al. (1992) assuming that there was no preference for exchange between particular pairs of

chromosomes. According to the equation derived by these authors, the frequency of translocations detected by painting F_p equals to $2.05f_p(1-f_p)F_g$ where F_g is genomic translocation frequency determined by FISH from the measured frequencies F_p using this equation, f_p is the fraction of the genome made fluorescent by FISH. f_p is equal to 0.163 for chromosomes 1 + 2 and 0.083 for chromosome 1.

Results

The frequencies of aberrations induced by γ-rays and nitrogen ions in chromosomes 1 and 2 of human lymphocytes detected by FISH and those induced by the same radiation in all genome detected by conventional staining method are shown in Table 1 and Table 2, respectively. The dose-dependences of the total number of aberrations induced by γ-rays in chromosome 1 (detected by FISH) and/or in the whole genome (detected by conventional staining) correspond to a linear-quadratic function (Fig. 1). The nitrogen ions are more efficient in the induction of aberrations. The dose-dependence curves of the number of aberrations induced by nitrogen ions in chromosomes 1, 2 and in the whole genome correspond to a linear function (Fig. 1). The results have shown that the total number of aberrations induced by ^{14}N ions in chromosomes 1 and 2 is more frequent in comparison with the induced ones in the whole genome as detected by conventional painting. The frequencies of different aberration types induced by nitrogen ions in chromosome 1 were very similar to those induced in chromosome 2 (Table 1).

The number of cells with aberrations induced in chromosome 1 by γ-rays increases with the radiation dose and reaches 74% at a dose of 7 Gy (Fig. 2a). This increase is much steeper after irradiation with nitrogen ions up to a dose of 1.5 Gy. The cells with aberrations in chromosomes 1 or 2 represent about 40%

Table 1. Aberrations in chromosomes 1 and 2 induced by different doses of γ-rays and ^{14}N ions. F – chromosome and chromatide fragments, CR – centric rings, D – dicentrics, I – insertions, T – translocations, OA – other aberrations

Radiation	Chromosome	Dose, Gy	Cells scored	Aberrant cells, %	Aberrations per 100 cells	Aberrations per 100 cells					
						F	D	CR	T	I	OA
γ-rays	1	0	150	0	0	0	0	0	0	0	0
		1	206	6,4±1,8	6,4±1,8	1,9±1,0	0,5±0,5	0	2,5±1,1	1,5±0,9	0
		3	279	19,4±2,6	19,4±2,6	4,4±1,3	2,0±0,8	0	10,5±1,9	2,5±0,9	0
		5	169	42,2±5,0	46,3±5,2	14,5±2,9	7,0±2,0	1,3±0,9	21,0±3,5	2,5±1,2	0
		7	100	74,0±8,6	83,0±9,1	27,0±5,2	10,0±3,2	2,0±1,4	40,0±6,3	4,0±2,0	0
^{14}N ions	1	0	150	0	0	0	0	0	0	0	0
		0,5	200	40,0±4,4	52,5±5,1	27,0±3,7	2,5±1,1	15,0±2,7	7,5±1,9	0,5±0,5	0
		0,75	100	35,0±5,9	43,0±6,6	11,0±3,3	4,0±2,0	11,0±3,3	13,0±3,6	4,0±2,0	0
		1,5	104	52,9±7,1	72,9±8,4	29,7±5,3	10,5±3,2	12,5+3,5	19,2±4,3	1,0±1,0	0
		2	100	53,0±7,3	90,0±9,5	36,0±6,0	9,0±3,0	22,0±4,7	22,0±4,7	1,0±1,0	0
		3	86	74,4±9,3	154,6±13,4	86,0±10,0	5,8±2,6	11,6±3,7	40,7±6,9	10,5±3,5	0
	2	0	150	0	0	0	0	0	0	0	0
		0,5	200	40,5±4,5	58,0±5,4	30,0±3,8	2,5+1,1	9,0±2,1	11,0±2,3	2,0±1,0	3,5±1,3
		0,75	100	46,0±6,8	72,0±8,5	31,0±5,6	6,0±2,4	9,0±3,0	21,0±4,6	4,0±2,0	1,0±1,0
		1,5	105	51,5±7,0	79,0±8,7	41,0±6,2	11,4±3,3	7,6±2,7	13,3±3,6	3,8±1,9	1,9±1,3
		2	104	51,9±7,0	75,0±8,5	26,0±5,0	8,7±2,9	17,3±4,1	22,0±4,6	1,0±1,0	0
		3	92	60,8±8,1	110,9±11,0	64,1±8,3	3,3±1,9	15,2±4,1	26,1±5,3	2,2±1,5	0
	1 + 2	0	150	0	0	0	0	0	0	0	0
		0,5	200	61,5±5,5	108,0±7,3	57,0±5,3	3,0±1,2	24,0±3,5	18,0±3,0	2,5±1,1	3,5±1,3
		0,75	100	61,0±7,8	106,0±10,3	42,0±6,5	10,0±3,2	20,0±4,8	28,0±5,3	5,0±2,2	1,0±1,0
		1,5	105	76,2±8,5	145,5±11,8	70,4±8,2	20,9±4,5	20,0±4,4	28,5±5,2	3,8±1,9	1,9±1,3
		2	100	77,0±8,8	162,0±12,7	61,0±7,8	16,0±4,0	38,0±6,2	45,0±6,7	2,0±1,4	0
		3	86	87,2±10,1	265,8±17,6	152,2±13,3	8,1±3,1	27,9±5,7	63,9±8,6	12,7±3,8	0

Table 2. Aberration induced in total genome by γ–rays and nitrogen ions as detected by conventional staining analysis, CDF – chromatide fragments, CF – chromosome fragments, D – dicentrics, R – centric rings, ID – interstitial deletions, OT – other aberrations.

Radiation	Dose, Gy	Cells scored	Aberrant cells, %	Aberrations per 100 cells	Aberrations per 100 cells					
					CDF	CF	D	R	ID	OT
γ-rays	0	110	2,7±1,6	2,7±1,6	1,8±1,3	0	0,9±0,9	0	0	0
	0,5	200	12,0±2,4	13,0±2,5	1,5±0,9	2,0±1,0	5,5±1,7	1,0±0,7	3,0±1,2	0
	1	200	21,0±3,2	28,5±3,8	3,5±1,3	6,0±1,7	12,0±2,4	2,5±1,1	4,0±1,4	0,5±0,5
	2	200	47,0±4,8	76,5±6,2	4,0±1,4	13,5±2,6	31,5±4,0	7,5±1,9	19,0±3,1	1,0±0,7
	3	200	70,0±5,9	130,5±8,1	5,5±1,7	8,5±2,1	68,0±5,8	16,0±2,8	28,0±3,7	4,5±1,5
	5	200	93,0±6,8	291,0±12,1	23,0±3,4	51,5±5,1	101,0±7,1	24,0±3,5	87,5±6,6	4,0±1,4
^{14}N ions	0	200	0	0	0	0	0	0	0	0
	0,5	200	23,5±3,4	45,0±4,7	5,0±1,6	10,5±2,3	14,0±2,6	5,5±1,7	10,0±2,2	0
	0,75	100	39,0±6,2	71,0±8,4	6,0±2,4	12,0±3,5	28,0±5,3	11,0±3,3	14,0±3,7	0
	1	201	49,0±4,9	102,0±7,1	7,0±1,9	15,0±2,7	42,0±4,6	14,0±2,6	22,0±3,3	2,0±1,0
	1,5	231	59,7±5,1	131,7±7,6	8,2±1,9	20,3±3,0	56,5±4,9	18,6±2,8	26,8±3,4	1,3±0,8
	2	200	73,0±6,0	236,5±10,9	18,0±3,0	34,5±4,2	104,0±7,2	27,0±3,7	48,0±4,9	5,0±1,6
	3	200	60,0±5,5	198,5±10,0	15,0±2,7	37,5±4,3	64,5±5,7	32,0±4,0	45,0±4,7	4,5±1,5

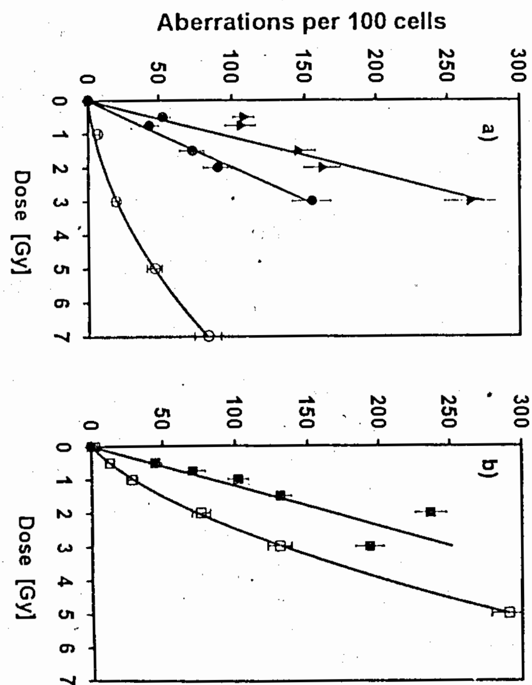


Fig. 1 Frequency of the total number of aberrations at different radiation doses detected by (a) FISH in chromosome 1 (circles), chromosomes 1+2 (triangles) and (b) by conventional staining method in the whole genome (squares). Human lymphocytes were irradiated by γ-rays (open symbols) and ^{14}N ions (filled symbols).

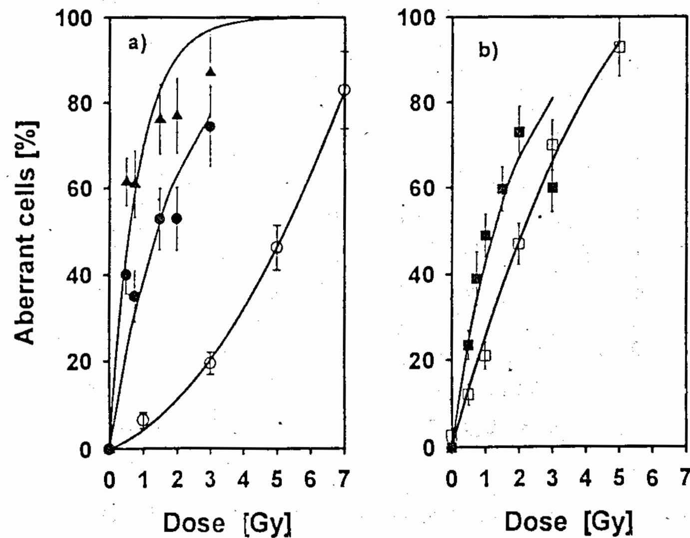


Fig. 2 Frequency of aberrant cells at different doses of γ-rays (open symbols) and ^{14}N ions (filled symbols) detected by (a) FISH in chromosome 1 (circles), chromosomes 1+2 (triangles) and by (b) conventional staining in the whole genome (squares).

of irradiated cells after the dose of 0.5 Gy and 60% or 70% at a dose of 3 Gy. At this dose the cells with aberrations in both chromosomes (1 and 2) represent about 85% of irradiated cells. The increase of the number of cells containing aberrations in the whole genome in the dependence on the dose of γ-rays and nitrogen ions detected by classical analysis is similar to that detected in chromosomes 1 and 2 by FISH (Fig. 2b).

Both types of radiation induce high frequency of translocations. This type of aberrations is the most frequent and represents about 50% of all aberrations induced by γ-rays in chromosome 1 (Table 1). The frequency of translocations induced in chromosome 1 by nitrogen ions is much higher in comparison with γ-rays. However, this type of aberrations represents only 20 to 30% of all aberrations induced in chromosomes 1 or 2 by nitrogen ions. This difference can be related to the high number of non-repaired fragments induced by nitrogen ions (Table 1). The dose-dependence of the frequency of translocations induced by nitrogen ions corresponds (as in the case of all aberrations) to a linear function while this dependence for γ-rays corresponds to a linear-quadratic one (Fig. 3).

Under the supposition that the induction of aberrations is proportional to the DNA content of individual chromosomes, the frequency of aberrations in the whole genome can be calculated from the frequencies of aberrations induced in chromosomes 1 and 2. The frequency of dicentrics calculated in this way (Fig. 4) for the whole genome is about the same as that detected by conventional staining method for different doses of ^{14}N ions. The maximum frequency corresponding to about 1 dicentric per cell is reached at a dose of 2 Gy of ^{14}N as detected by conventional staining. If the frequencies of this aberration type were calculated for the whole genome from the frequencies detected by FISH in chromosomes 1 and 2, the maximum was found at a dose of 1.5 Gy. At higher doses of ^{14}N ions, the number of dicentrics per cell decreases (Fig. 4).

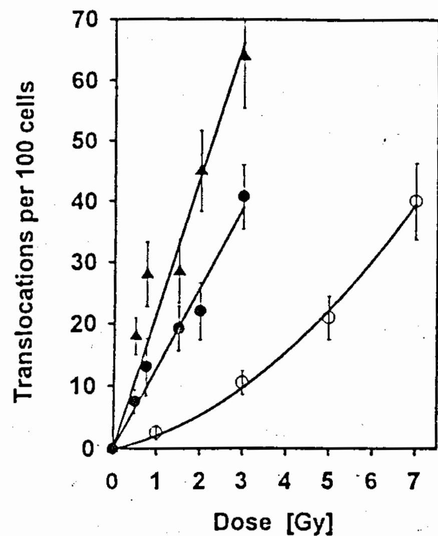


Fig. 3 Frequency of translocations at different doses of γ-rays (open symbols) and ^{14}N ions (filled symbols) detected by FISH in chromosome 1 (circles) and chromosomes 1+2 (triangles).

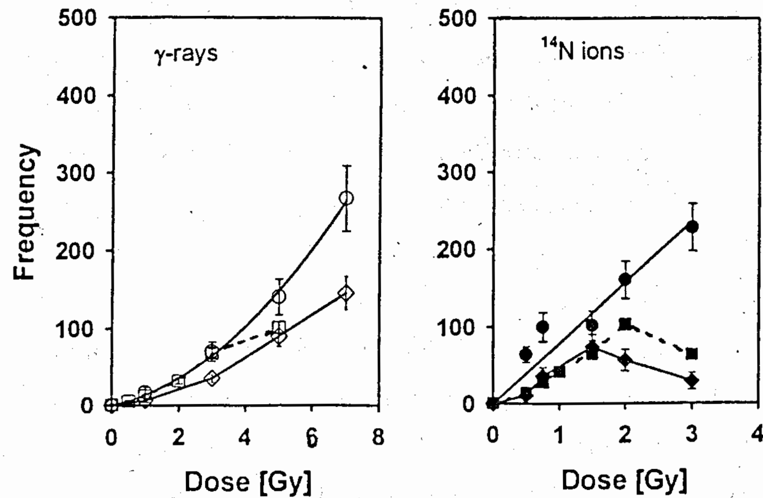


Fig. 4 Frequency of dicentrics (diamonds) and translocations (circles) at different doses of γ-rays (open symbols) and ^{14}N ions (filled symbols) detected by FISH in chromosomes 1+2 and recalculated for the whole genome according to the relation suggested by Lucas (1992). Frequencies of dicentrics detected by conventional staining (squares).

The frequencies of dicentrics for the whole genome recalculated from those induced in chromosome 1 by different doses of γ-rays are about twice lower than those detected by conventional staining at doses lower than 5 Gy (Fig. 4).

The frequencies of translocations induced in chromosomes 1 and 2 by different doses of nitrogen ions as detected by FISH are about twice higher than the frequencies of dicentrics in these chromosomes (Table 1) and this ratio remains the same if recalculated for the whole genome (Fig. 4). The translocation frequencies recalculated for the whole genome from the frequencies induced by γ-rays in chromosome 1 are about the same as the frequencies of dicentrics detected by conventional staining up to the dose of 3 Gy (Fig. 4); but they are about twice higher than the frequencies of dicentrics recalculated the same way.

A good coincidence can be seen in the frequencies of symmetrical and asymmetrical exchange aberrations (translocations — dicentrics and centric rings) at different doses of γ-rays and ^{14}N ions as detected by FISH in chromosomes 1 and 2 (Table 1). The fraction of symmetrical exchange aberrations represents 20–30% of all aberrations induced by nitrogen ions in chromosome 1 and about 17–30% in chromosome 2 in the dose range of 1–3 Gy. The asymmetrical aberrations in these chromosomes represent approximately the same fraction. However, asymmetrical aberrations represent 43–57% of the total number of aberrations detected in the whole genome by the classical method after irradiation by γ-rays and ^{14}N ions. No symmetrical exchange aberrations are included into the number of aberrations detected by conventional staining.

After irradiation with nitrogen ions we observed a big number of lymphocytes containing aberrations simultaneously in both chromosomes 1 and 2. These cells represent 32–36% of aberrant cells up to the dose of 2 Gy and 57% at the dose of 3 Gy. The cells with exchange aberrations between chromosomes 1 and 2 (translocations, dicentrics, insertions) were also observed. The number of these cells represented 3% of all cells scored. All nonreciprocal transloca-

tions and insertions were the aberrations, which had a fragment of chromosome 2 in chromosome 1.

In the majority of cells irradiated with γ-rays there was only one of the both chromosomes (1 or 2) damaged. But the cells, where both homologous chromosomes 1 were damaged, represented only 2 and 3% at the doses of 5 and 7 Gy of γ-rays. The number of these cells was higher after irradiation with nitrogen ions. There were 4–7% of cells with damages in both chromosomes 1 and 7–11% of cells with damages in both chromosomes 2 after doses up to 2 Gy. The dose of 3 Gy induced damages of both chromosomes 1 or 2 in 20–30% of cells. The fraction of cells with aberrations induced in both homologous chromosomes 1 was 12–14% of all aberrant cells, the fraction induced in both chromosomes 2 was 17–22% up to the dose of 2 Gy and it increased to 30–40% at a dose of 3 Gy.

In addition, there were some cells (0.8% of all scored cells) containing dicentrics between the homologous chromosomes, especially between chromosomes 1.

Discussion

FISH method with chromosome-specific composite DNA probes has become a powerful tool for detecting structural chromosome rearrangements (Lichter et al. 1988, Pinkel et al. 1988, Cremer et al. 1990, Natarajan et al. 1991,1992, 1994, Awa et al. 1992, Gray et al. 1992, Schmidt et al. 1992, Bauchinger et al. 1993, Lucas et al. 1992, Tucker et al. 1993, 1994, 1995). This method has, however, some limitations related principally to a small number of chromosomes that can be uniquely painted at one time. For example, using the dual-colour painting, the number of these chromosomes is limited to two pairs (if all exchange aberrations between painted and non-painted chromosomes

have to be detected). Twelve separate hybridization experiments with different pairs of painting probes for specific chromosomes should be used for each radiation dose if the total spectrum of aberrations in the total human genome is to be detected.

On the other hand, chromosome aberration analysis performed with conventional cytogenetic banding technique can detect aberrations in the whole genome, but it is not able to recognize the stable exchange aberrations represented by symmetrical exchanges. From the point of view of biological dosimetry the detection of aberrations by FISH can be more sensitive for densely ionizing radiation in spite of the fact that aberrations in a restricted number of chromosomes are detected (Figs. 1, 2). Moreover, translocations detected by FISH are stable aberrations, which are potentially prelesions for cancer induction.

We tried to calculate the frequencies of translocations for the whole genome from the frequencies of these aberrations detected in chromosomes 1 and 2 by FISH method under the assumption that they are proportional to the DNA content of each chromosome. For this purpose we used the equation derived by Lucas et al. (1992) taking into account that the both chromosomes together are 16.3 % of the whole genome (Morton 1991). Our results have shown that the calculated frequencies of translocations for different doses of nitrogen ions are about twice higher than the frequencies of dicentrics detected by conventional staining (Fig. 4) as well as those calculated from FISH data for the whole genome. While the frequencies of translocations increase with the radiation dose, the frequencies of dicentrics reach the maximum (corresponding to about 1 dicentric per cell) at doses of 1.5 or 2 Gy, respectively, and then decrease. This decrease can be explained by the cell cycle prolongation of cells containing more than 1 dicentric and the later entry of these cells in the mitosis.

The number of cells with a bigger number of dicentrics increases with the dose of nitrogen ions which is manifested by a decreasing number of these

cells in the first mitosis registered at a constant time interval after irradiation. In γ-irradiated lymphocytes the frequencies of translocations calculated for the whole genome from the data obtained by FISH in chromosome 1 are very similar to the frequencies of dicentrics detected in the whole genome by conventional staining up to the dose of 3 Gy. At higher doses of radiation the frequencies of translocations increase more progressively than those of dicentrics detected by the both methods. The reason of this can be also the bigger number of dicentrics per cell what excludes these cells from detection due to their later onset of mitosis. The comparison of the frequencies of dicentrics detected in the whole genome by conventional staining with those calculated for the whole genome from the data obtained in chromosomes 1 and 2, has shown that the use of dicentrics for the molecular dosimetry does not reflect exactly the real damage of cells.

The comparison of the total number of aberrations detected by conventional staining in the whole genome with the number of aberrations detected by FISH in chromosomes 1 and 2 in lymphocytes irradiated with ^{14}N ions and in chromosome 1 of γ-irradiated ones, has shown (Fig. 1) that both types of radiation, in particular nitrogen ions, induce higher frequency of aberrations in chromosomes 1 and 2 than in the rest of genome. The both chromosomes are the largest human chromosomes and therefore they are the largest targets for ionizing radiation. However, it is possible that the detection of aberrations by two different methods in different parts of the genome can give different results. The conventional staining analysis can not detect the symmetrical exchange aberrations (translocations), what decreases the total number of aberrations detected by this method. That is why we have observed about 50–60 % of the asymmetrical exchange aberrations (dicentrics and centric rings) from the total number of aberrations detected by this method at different doses of both types of radiation. The relationship between the fractions of symmetrical and

asymmetrical exchange aberrations detected by FISH technique in chromosomes 1 and 2 of lymphocytes irradiated by γ-rays and nitrogen ions may be useful to characterize the level of symmetrical aberrations in the whole genome. Our results have shown that the fraction of symmetrical exchanges is roughly equal to the fraction of asymmetrical ones in both chromosomes of lymphocytes irradiated with ^{14}N ions. We can suppose that the same relations between these fractions will be established in the whole genome. If the amount of supposed symmetrical aberrations is added to the amount of the total aberrations detected by the conventional staining method in the whole genome, their number will increase and the size of fractions of different aberrations will decrease. Thus, the fraction of symmetrical as well as asymmetrical exchange aberrations will be only about 20–35% of all aberrations like it was found in chromosomes 1 and 2 by FISH method.

It is generally assumed that radiation-induced DNA lesions are randomly distributed among the chromosomes. However, the comparison of the total number of aberrations detected by conventional staining in the whole genome with those recalculated for the genome from the frequencies detected by FISH method in chromosomes 1 and 2, indicates that the number of aberrations is not simply proportional to the DNA content of chromosomes. It seems that there are still other factors influencing on the induction of aberrations in different chromosomes. Natarajan et al. (1994) demonstrated that condensed chromatin in Chinese hamster embryonic fibroblasts appears to be resistant to radiation-induced damage in comparison with uncondensed chromatin. This was observed earlier for human lymphocytes (Vyas et al. 1991). Differences in the kinetics of repair were also found for the chromosomes, indicating that the repair is faster in euchromatin than in heterochromatin (Slijepcevic and Natarajan 1994). Taking into account these results we can assume that the observed high

frequency of aberrations in chromosomes 1 and 2 in comparison with the rest of genome may be explained by the euchromatic structure of these chromosomes.

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References

1. Awa AA, Sofuni T, Honda T, Itoh M, Neriishi S, Otake M (1978) Relationship between the radiation dose and chromosome aberrations in atomic bomb survivors of Hiroshima and Nagasaki. J Radiat Res 19: 126–140
2. Awa AA, Nakano M, Ohtaki K, Kodama Y, Lucas J, Gray J (1992) Factors that determine the *in vivo* dose-response relationship for stable chromosome aberrations in A-bomb survivors. J Raiat Res (Tokyo) 33(suppl), 206–214
3. Bauchinger M, Schmid E, Zitzelsberger H, Braselmann H, Nahrstedt U (1993) Radiation-induced chromosome aberrations analyzed by two-colour fluorescence *in situ* hybridization with composite whole chromosome-specific DNA probes and a pancentromeric DNA probe. Int J Radiat Biol 64: 179–184
4. Buckton KE, Hamilton GE, Paton L, Langlands AG (1978) Chromosome aberrations in irradiated ankylosing spondylitis patients. In: Mutagen-Induced Chromosome Damage in Man (eds H Evans and D Lloyd) Edinburgh University Press, pp. 142–150
5. Cremer T, Popp S, Emmerich P, Lichter P, Cremer C (1990) Rapid metaphase and interphase detection of radiation-induced chromosome aberrations in human lymphocytes by chromosomal suppression *in situ* hybridization. Cytometry 11: 110–118

6. Evans HJ, Buckton KE, Hamilton GE, Carothers A (1979) Radiation-induced chromosome aberrations in nuclear-dockyard workers. Nature 277: 531–534
7. Fernandez JL, Campos A, Goyanes V, Losada C, Veiras C, Edwards AA (1995) X-ray biological dosimetry performed by selective painting of human chromosomes 1 and 2. Int J Radiat Biol 67: 295–302
8. Gray JW, Lucas JN, Pinkel D, Awa A(1992) Structural chromosome analysis by whole chromosome painting for assessment of radiation-induced genetic damage. J Rad Res (Tokyo) 33 (suppl), 80–86
9. Knehr S, Zitzelsberger H, Braselmann H, Schmid E, Bauchinger M (1994) Analysis for DNA-proportional distribution of radiation-induced chromosome aberrations in various triple combinations of human chromosomes using fluorescence *in situ* hybridization (FISH). Int J Radiat Biol 65: 683–690
10. Lichter P, Cremer T, Borden J, Manuelidis I, Ward DC (1988) Delineation of individual human chromosomes in metaphase and interphase cells by *in situ* suppression hybridization using recombinant DNS libraries. Human Genet. 80: 224–234
11. Lloyd DC, Purrott RJ, Dolphin GW, Bolton D, Edwards AA (1975) The relationship between chromosome aberrations and low LET radiation dose human lymphocytes. Int J Radiat Biol 28: 75–90
12. Lloyd DC, Purrott RJ, Reeder EJ, (1980) The incidence of unstable chromosome aberrations in peripheral blood lymphocytes from unirradiated and occupationally exposed people. Mutat Res 72: 523–532
13. Lucas JN, Awa A, Straume T, Poggensee M, Kodama Y, Nakano M, Ohtaki K., Weier HU, Pinkel D, Gray J, Littlefield G (1 992) Rapid translocation frequency analysis in humans decades after exposure to ionizing radiation. Int J Radiat Biol 62: 53–63

14. Morton NE (1991) Parameters of the human genome. Proc Natl Acad Sci USA 88: 7474–7476

15. Nakano M, Nakashima E, Pawel DJ, Kodama Y, Awa A (1993) Frequency of reciprocal translocations and dicentrics induced in human blood lymphocytes by X-irradiation as determined by fluorescence *in situ* hybridization. Int J Radiat Biol 64: 565–569

16. Natarajan AT, Obe G (1978) Molecular mechanisms involved in the production of chromosomal aberrations. Utilization of Neurospora endonuclease for the study of aberration production in G_2 stage of the cell cycle. Mutat Res 52: 137–149

17. Natarajan AT, Darroudi F, Mullenders LHF. Meijers M (1986) The nature and repair of DNA lesions that lead to chromosome aberrations induced by ionizing radiation. Mutat Res 160: 231–236

18. Natarajan AT, Vyas RC, Wiegant J, Curado MP (1991) A cytogenetic follow-up study the victims of a radiation accident in Goiania (Brazil). Mutat Res 247: 103–111

19. Natarajan AT, Vyas RC, Darroudi F, Vermeulen A (1 992) Frequencies of X-ray induced chromosome translocations in human peripheral lymphocytes as detected by *in situ* hybridization using chromosome specific DNA libraries. Int J Radiat Biol 61: 53–63

20. Natarajan AT, Ballajee AS, Boei JJWA, Chatterjee S, Darroudi F, Grigorova M, Noditi M, Oh HJ, Sliepcevic P, Vermeulen S (1994) Recent developments in the assessment of chromosomal damage. Int J Radiat Biol 66: 615-623

21. Pinkel D, Landegent J, Collins C, Fuscoe J, Segraves R, Lucas J, Gray J (1988) Fluorescence *in situ* hybridization with human chromosome-specific libraries: Detection of trisomy 21 and translocations of chromosome 4. Proc Natl Acad Sci USA 85: 9138–9142

22. Savage JRK (1975) Classification and relationships of induced chromosomal structural changes. J Med Genet 12: 103–122

23. Schmid E, Zitzelsberger H, Braselmann H, Gray JW, Bauchinger M (1992) Radiation-induced chromosome aberrations analyzed by fluorescence *in situ* hybridization with a triple combination of composite whole chromosome-specific DNA probes. Int J Radiat Biol 62: 673–678

24. Sliepovic P, Natarajan AT (1994) Distribution of X-rays induced G_2 chromatid damage among Chinese hamster chromosomes: Influence of chromatin configuration. Mutat Res 323: 113–119

25. Straume T, Lucas JN (1993) A comparison of the yields of translocations and dicentrics measured using fluorescence in situ hybridization. Int J Radiat Biol 64: 185–187

26. Testard I, Dutrillaux B, Sabatier L (1997) Chromosomal aberrations induced in human lymphocytes by high-LET irradiation. Int J Radiat Biol 72: 423–433

27. Tucker JD, Ramsey MJ, Lee DA, Minkler JL (1993) Validation of chromosome painting as a biodosimeter in human peripheral lymphocytes following acute exposure to ionizing radiation *in vitro*. Int J Radiat Biol 64: 27–37

28. Tucker JD, Senft JP, (1994) Analysis of naturally occurring and radiation-induced breakpoint location in human chromosomes 1, 2 and 4. Radiat Res 140: 31–36

29. Tucker JD, Lee DA, Moore DH (1 995) Validation of chromosome painting. II. A detailed analysis of aberrations following high doses of ionizing radiation *in vitro*. Int J Radiat Biol 67: 19–28

30. Vyas RX, Darroudi F, Natarajan AT (1991) Radiation induced chromosomal breakage and rejoining in interphase-metaphase chromosomes of human lymphocytes. Mutat Res 249: 29–35

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