Analysis of Mutations in the Human *HPRT* Gene Induced by Accelerated Heavy-Ion Irradiation

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Mutation/HPRT/Heavy-Ion Irradiation/Aberrant mRNA Splicing/High LET

Multiplex PCR analysis of HPRT($-$) mutations in human embryo (HE) cells induced by 230 keV/μm carbon-ion irradiation showed no large deletion around the exon regions of the locus gene in contrast to the irradiations at different LETs. To identify these mutations, the sequence alterations in a cDNA of *hprt* gene were determined for 18 mutant clones in this study. Missing of exon 6 was the most frequent mutational event (10 clones), and missing of both exons 6 and 8 was next most frequent event (6 clones), then base substitutions (2 clones). These characteristics were not seen in a similar analysis of spontaneous mutations, which showed base substitution (5 clones), frameshift (2 clones), missing of both exons 2 and 3 (2 clones), and a single unidentified clone. Direct sequencing and restriction enzyme digestion of the genomic DNA of the mutants which showed missing of exons 6 and 8 in the cDNA, supports the possibility that they were induced by aberrant mRNA splicing.

INTRODUCTION

Knowledge of the specificity of mutations induced by a variety of mutagens has enabled better understanding of the molecular mechanisms of mutagenesis. In the past decade, a vast amount of information has been generated concerning cell death, mutation, chromosomal aberration, etc. as affected by radiation and chemicals. Recently, mutations induced by various

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types of chemicals, UV, and conventional iozizing-radiation have been analyzed extensively at the molecular level to gain insights on the induction mechanisms; but only a few studies have been published on how the molecular structures of heavy-ion-induced mutations reflect the characteristic energy-deposition. We earlier reported several characteristics of hypoxanthine phosphoribosyltranferase (HPRT) mutations produced by carbon-ion (C-ion) irradiations of human embryo (HE) cells[1]. The initial energy of the ion was 135 MeV/n, and the linear energy transfer (LET) was adjusted to 39, 68, 124, or 230 keV/μm at the position of the cells. Deletion patterns of each exon region in the *hprt* locus was dependent on LET on analysis of the genomic DNA by multiplex PCR. Most of the mutants induced by 124 keV/μm C-ions showed deletion of almost the entire locus of the *hprt* gene, whereas none of the mutants induced by 230 keV/μm C-ions showed this deletion pattern. As pointed out, it is surprising that 230 keV/μm C-ions do not produce such large deletions, given the much greater energy deposition within their trancks.

To expand this line of investigation at molecular level, we have determined the DNA sequences of the cDNA of HPRT mutants induced by 230 keV/μm C-ions. The analyses of the genomic DNA by restriction enzyme digestion and direct sequencing also are described.

MATERIALS AND METHODS

Cells and irradiation

The establishment, culture and selection of mutants of human embryonic (HE) cells have been described elsewhere in detail[1]. In brief, human embryonic (HE) fibroblast-like cells were cultured in Eagle's minimal essential medium supplemented with 0.2 mM serine, 0.2 mM aspartate, 1 mM pyruvate and 10% fetal calf serum at 37°C in a humidified atmosphere containing 5% CO_2. C-ion irradiation was performed at different LETs after decreasing the initial energy by 135 MeV/n with the absorber. The irradiated cells were plated at a density of about 400 cells per square centimeter in medium supplemented with 40 μM 6-thioguanine to isolate mutants.

Extraction of mRNA and 1st-Strand cDNA Synthesis

Direct isolation of polyadenylated RNA and its reverse transcription to cDNA respectively were carried out with a Quick Micro mRNA Purification Kit (Pharmacia) and a First-Strand cDNA Synthesis Kit (Pharmacia). Details are given in the instruction manual for the kits. The cDNAs obtained were used in the next step, PCR amplification.

Genomic DNA Extraction

For genomic DNA extraction, 1×10^6 cells were washed twice with phosphate-buffered saline (PBS) then suspended in 100 μl of K-buffer (50 mM KCl, 10 mM Tris·HCl, 2.5 mM $MgCl_2$, pH 8.3). After adding proteinase K and Tween 20 at the repective final concentrations of 100 μg/ml and 0.5%, the suspension was heated first at 56°C for 45 min then at 95°C for 10 min. The DNA in the reaction mixture was precipitated with ethanol and stored at -80°C until use.

PCR Amplification

PCR reaction mixture consisted of PCR buffer (50 mM KCl, 10 mM Tris·HCl pH 8.4, 1.5 mM MgCl$_2$), 1 mM of each dNTP, 2.5 units of Taq polymerase (TAKARA), and appropriate amounts of the template DNA and primers. Thirtyfive PCR cycles were performed, each of which consisted of denaturation at 94°C for 1 min, annealing at 55°C for 1.5 min, and extension at 72°C for 1.5 min. The last extension was prolonged to 8.5 min. The pairs of primers used to amplify the *hprt* cDNAs and genomic DNAs[2] are listed in Table 1.

DNA Sequencing

The sequences of the PCR amplified DNA fragments were determined with an automatic sequencer (Applied Biosystems Inc. ABI373A DNA Sequencer) using an ABI's Dye Deoxy^{TM} Cycle Sequencing Kit (401150). Sequence data were edited and compared by the use of sequence analysis software SeqEd(Applied Biosystems Inc.). Some of the primers for the DNA sequencing of the *hprt* cDNAs and genomic DNAs were the same as those used for the amplifications (Table 1).

Restriction Enzyme Digestion

PCR-amplified DNA of the intron region between exons 5 and 6 (3.51 kbp) was digested with *Eco*RI(TAKARA) or *Bam*HI (TAKARA). A 1 μg sample of genomic DNA and 12 units of enzyme were dissolved in buffer containing 50 mM Tris·HCl, 10 mM MgCl$_2$, 1 mM DTT, 100 mM NaCl then incubated at 37°C for 1 hr. The reaction products were analyzed by 1% agarose gel electrophoresis then stained with ethidium bromide.

Table 1. Primers for PCR and DNA Sequencing

a) cDNA

Purpose	Primer	Position	Sequence
PCR	HPRT01	S -58 to -42	TGCTCCGCCACCGGCTT
	HPRTC4	A 751 to 734	CTAAGCAGATGGCCACAG
PCR/	TK01	S -35 to -16	CCTGAGCAGTCAGCCCGCGC
Sequencing	TKC4	A 721 to 702	GATAATTTTACTGGCGATGT
	TK012	S -16 to 3	CGCCGGCCGGCTCCGTTATG
	TKC4'	A 701 to 682	CAATAGGACTCCAGATGTTT
Sequencing	HPRT03	S 327 to 343	GTCAACAGGGGACATAA
	HPRT04	A 318 to 302	ACAATAGCTCTTCAGTC
	HPRT05	A 453 to 433	CCTGACCAAGGAAAGCAAAGT
	HPRT06	A 176 to 159	CCTCCCATCTCCTTCATC
	HPRT07	A 118 to 102	CATGAGGAATAAACACC
	HPRT08	S 512 to 528	GTGTTGGATATAAGCCA
	HPRT09	A 558 to 542	CTTGTCTGGAATTTCAA

Numbering of positions (see ref. 16) S: Sense Strand, A: Antisense Strand

b) Genomic DNA

Purpose	Primer	Position	Sequence
PCR	ex5pF	S 31500 to 31522	AGCATCTAAAAACAAGAGTTTGG
	ex6pR	A 35096 to 35074	CACTTAATCCCCCTTCAAATGAG
	5in6pF	S 31523 to 31542	ATAATTCCTTAGGGGTTGTTA
	5in6pR	A 35028 to 35009	CATACATACCTTGCGACCTT
PCR/	ex6pF	S 34817 to 34841	GATTTTGGTGAGAATTACTGTGCTG
Sequencing	ex6pR	A 35074 to 35052	CACTTAATCCCCCTTCAAATGAG
	7in8pF	S 39805 to 39824	TAATTAACAGCTTGCTGGTG
	7in8pR	A 40084 to 40065	TAGTCAAGGGCATATCCTAC
Sequencing	5in6seq1	S 34656 to 34675	TTATAATGAAGGACAACATC
	5in6pR	A 35028 to 35009	CATACATACCTTGCGACCTT

Numbering of positions (see ref. 16) S: Sense Strand, A: Antisense Strand

RESULTS

HPRT(−) mutant clones were isolated after C-ion irradiation at LET 230 keV/μm as well as at the different LETs used in our previous study[1]. Sequence alterations in the cDNA of 18 mutant clones at LET 230 keV/μm were determined because their mutations seemed to be unique as described in the introduction. Missing of exon 6 was the most frequent mutational event (10 clones), and missing of both exons 6 and 8 was next most frequent (6 clones) as shown in Table 2. The remaining two clones represented the base substitutions CG to TA and AT to GC at the respective positions 8 and 415. These substitutions resulted in respective amino-acid conversions from *Thr* to *Ile*, and *Thr* to *Ala*. The former conversion is located in the N-terminal region of the polypeptide backbone, which region is in the predicted substate-binding domain[3,4].

For comparison, the altered sequences of the cDNAs also were determined for 10 independent spontaneous HPRT(−) mutant clones isolated in separate experiments. Results are given in Table 3. Unlike the events for the mutations induced by 230 keV/μm C-ions, the frequent mutational events of the spontaneous mutations were base substitutions (5 clones), followed by missing of both exons, 2 and 3 (2 clones), and frameshifts (2 clones). The remaining clone remained unidentified, but it did not represent any of the missing of exons (data, not shown). The 5 base substitutions TA to GC at position 67, GC to CG at 173, GC to CG at 212, TA to CG at 548, and GC to AT at 626. Two frame-shift mutations were caused by the deletion of G at 175 or 176 and the insertion of A at a position between 490 and 491.

The incidence of cDNA mutations induced by 230 keV/μm C-ions was compared with that for spontaneous mutations (Table 4). The C-ion induced mutations consist of only two classes of mutation; base substitution and exon missing, the latter being the most frequent, accounting for

Table 2. Mutational Events Induced by 230 keV/μm C-ion

Site[a]	Incidence	Sequence context	Nucleotide Change	Mutant Clone
403–485	10	GATTGTGGAA ------------- CTTGCTGGTG exon6	−83 bp	0.2-2, 0.2-4 1–2, 1–4 1–8, 1–9 1–10, 1–13 1–17, 1–20
403–485 and 533–609	6	GATTGTGGAA ------------ CTTGCTGGTG exon6 AAGCCAGACT ------------ CATGTTTGTG exon8	−83 bp and −77 bp	1–3, 1–6 1–11, 1–15 1–18, 1–21
415	1	TGAC*ĀCTGGCA → TGACG*CTGGCA Thr Ala	AT→GC	1–1
8	1	GGCGA*CCCGCA → GGCGAT*CCGCA Thr Ile	CG→TA	1–5

[a] Mutational sites are numbered according to Jolly et al. [16], the A of the ATC codon being the first nucleotide

Table 3. Spontaneous Mutational Events

Site[a]	Incidence	Sequence context	Nucleotide change	Mutant clone
28–318	2	TGGCGTCGTC ---------------- AATGACCAGT exons 2&3	−291 bp	X0–5, CX0–4
67	1	ATT*T̄TGCATAC → ATTT*GGCATAC Cys Gly	TA→GC	CO
173	1	GATGG*GAGGCC → GATGG*CAGGCC Gly Ala	GC→CG	M–0
212	1	GGGGG*GCTATA → GGGGG*CCTATA Gly Ala	GC→CG	CO–5
548	1	TGAAA*T̄TCCGA → TGAAA*CTCCAG Ile Thr	TA→CG	Ne–20
626	1	CATTA*GTGAAA → CATTA*ATGAAA Ser Asp	GC→AT	r–10
175 or 176	1	GGGA*G*GCCATC → GGGAG*CCATG	−G	C39–3
490–491	1	CTTGCT̲GGTGA → CTTGCA*TGGTGA	+A	CX0–3
unidentified	1			Ne-1

[a] Mutational sites are numbered according to Jolly et al.[16], the A of the ATG codon being the first nucleotide

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Table 4. Comparison of Mutational Evnets

Class of mutation	Incidence	
	C-ion(230 keV/μm)	Spontaneous
Base substitution	2 (11%)	5 (50%)
Frame shift	0	2 (20%)
Missing[a]	16 (89%)	2 (20%)
exon6	10	0
exons 6 and 8	6	0
exons 2 and 3	0	2
unidentified(point mutation)	0	1 (10%)
Total	18 (100%)	10 (100%)

[a] Missing in this table refers the cDNA not the genomic DNA.

nearly 90% of the mutations. In the spontaneous mutations, in contrast, base substitution is the most frequent mutation, 50%. This indicates that the characteristics of 230 keV/μm C-ion induced mutational events differ markedly from those of spontaneous mutations.

Because all the C-ion induced mutants in this report have been certified by multiplex PCR analysis[1] as having all the exons on their genome, these deletions are considered to have occurred due to translocations of each exon region or aberrant mRNA splicing. To gain insights on the preferential mechanism of these deletions, we analyzed the intron regions upstream of exons 6 and 8 by both restriction-enzyme digestion and direct sequencing.

The intron regions for exons 5 and 6 in all the cDNA clones missing exon 6 were amplified by PCR using the primer pair ex5pF and ex6pR (Table 1) then amplified by another PCR using 5in6pF and 5in6pR (Table 1). The second amplified fragments (3.51 kbp) were digested with the restriction enzyme *Eco*RI or *Bam*HI. Figure 1 shows the results of these digestion analyses for 2 clones missing exon 6, 2 clones missing both exons 6 and 8, and a clone of wild type. All five undigested PCR-products were judged to be recovered in the expected 3.51 kbp on agarose gel electrophoresis. The scheme in the upper part of Fig. 1 represents the results of the digestions of the PCR products by *Eco*RI or *Bam*HI based on the restriction map[5,6]. Digestion by these enzymes should result in two DNA fragments; 2.78 and 0.73 kbp by *Eco*RI and 2.76 and 0.75 kbp by *Bam*HI. In fact the sizes of the digested fragments were in good agreement with the expected sizes. These results indicate that at least the entire intron-region between exons 5 and 6 has no large deletions. In other words, exon 5, the intron, and exon 6 are thought to be normally located on the *hprt* locus. We therefore speculated that the upstream region of exon 6 was not likely to be translocated.

We similarly amplified the intron region between exons 7 and 8 using PCR and a pair of primers, 7in8pF and 7in8pR (Table 1). All the DNA fragments, amplified from the genomic DNAs of the 6 mutant clones with exon 8 missing in the cDNA, were estimated to be the expected size, 280 bp, on agarose gel electrophoresis (data, not shown). This result indicates there is little possibility of the translocation of this upstream regions of exon 8.

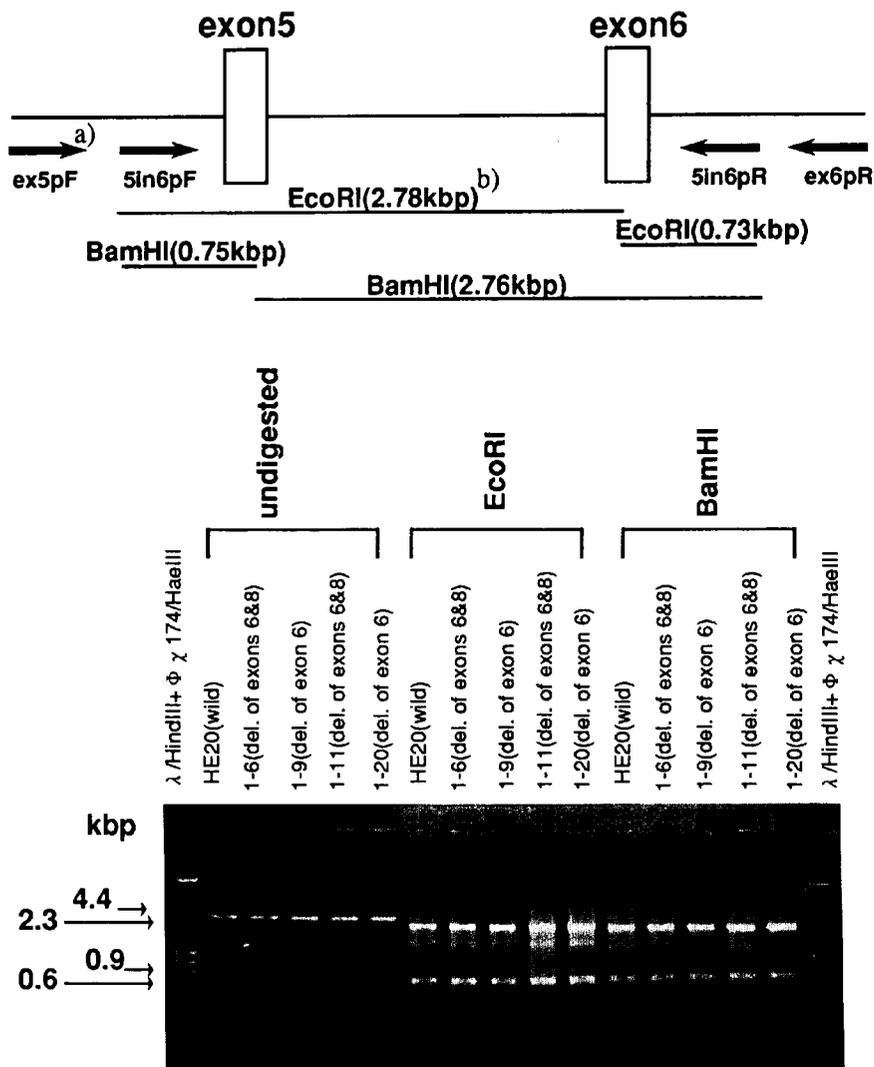Using the 3.51 kb PCR products for the upstream intron-region of exon 6 as the template

Figure. 1. Restriction enzyme digestion of the intron region between exons 5 and 6
a) PCR primers used to amplify the upstream intron-region of exon 6.
b) The expected DNA fragment sizes in *Bam*HI or *Eco*RI digestion.

DNA, a DNA sequence of about 200 base pairs, including the splice site, was determined with the primers 5in6seq1 and 5in6pR. To confirm the sequence of more restricted sites around the splice site, this region of 258 nucleotide was amplifed with primers ex6pF and ex6pR then sequenced using the same primers. The 12 samples were sequenced among the 16 mutants lacked only exon 6 or both exons 6 and 8 in thier cDNA, were analyzed. As a result, only a single mutant clone (0.2-2, see Table 2) represented the GC→TA base substitution at position 34936, the splice acceptor site. This mutation was explained by the known splicing mechanisms[7]. All the other mutant clones have maintained normal sequences (data, not shown). The entire

intron-region between exons 7 and 8 (171 bp) also was sequenced using primers, 7in8pF and 7in8pR. Four of six mutants, which lacked both exons 6 and 8 in thier cDNA, were alalyzed and were determined to have no mutations.

DISCUSSION

The analysis of genomic DNA by multiplex PCR showed that none of the *hprt*($-$) mutants induced by the C-ion of LET 230 keV/μm had a large deletion in the regions that included exons whereas most of the mutants (38/41) induced by the C-ion of 124 keV/μm had deletion of entire exons[1]. We determined the sequences in a cDNA of *hprt* gene for 18 mutations induced by the C-ion of 230 keV/μm in order to investigate this surprising fact. DNA sequences for 10 spontaneous mutations also were determined for comparison with these C-ion-induced mutations.

The C-ion-induced mutants at 230 keV/μm clearly had patterns of mutational events that differed from those of spontaneously induced mutants, despite the small size of both collections. For example, the most frequent event induced by 230 keV/μm C-ion was the exon missing in the cDNA. In contrast, only two occurrences of missing event of both exons 2 and 3 in the cDNA were found in the spontaneous collection. This kind of missing event has been frequently found in both human T-lymphocytes[8,9,10] and Chinese hamster cells[11,12]. Furthermore, only two base-substitutions, out of 18 mutations, were observed in the cDNA of the C-ion-induced mutants, in contrast to the frequent recovery (5/10) of such mutations seen in the spontaneous collection. We could identify all the *hprt*($-$) mutant clones as sequence changes in cDNA, except for one spontaneous mutant clone. This was not surprising because the mutations that affect the HPRT function are expected to be located in the coding region; whereas, mutations in the intron sequences impair enzyme activity only when they interfere with the accurate processing of transcripts[13].

The most striking feature of the induced mutation was that exactly exon 6 or both exons 6 and 8 were missing from the poly A-tailed mRNA of *hprt* in a large number of mutants. Because all the C-ion-induced mutants in this report have been certified as having all the exons on their genome by multiplex PCR analysis[1], this kind of missing event considered to occur by the translocation or inversions of each exon region or by aberrant mRAN splicing. To obtain some insights into the preferential mechanism of missing event, we analyzed the upstream intron-regions of exons 6 and 8 in the *hprt* genomic DNA by restriction enzyme digestion and direct sequencing. The upstream intron-regions (3.51 kbp) of exon 6, which extends to both exons 5 and 6 themselves, of all the mutant clones that had an exon 6 missing in their cDNA was amplified by PCR. These amplified fragments then were digested with the restriction enzyme *Eco*RI or *Bam*HI. The intron region located upstream of exon 8 in the mutant clone having an event of exon 8 missing in its cDNA also was amplified. None of the sizes of the amplified DNA fragments or the restriction enzyme-digested fragments contradicted with the sizes expected from the *HPRT* restriction map, indicating there was little possibility of translocation and much aberrant mRNA splicing of exons 6 and 8.

Although much is known about the mechanism of splicing, the reasons for the selection of a particular splice site are still not well understood[10]. Alternative splicing is a mechanism widely used to vary gene expression during development and to regulate tissue specificity of gene expression. Alternative splicing involves both cis elements in the pre-mRNAs as well as transacting protein factors[7]. The mechanism of splicing involves formation of a spliceosome complex which has small ribonucleoprotein particle (U1, U2, U5 and U4/6 snRNPs) and other proteins. During splicing the splice donor site is cleaved in the consensus sequence $^C/_A$AG ↓ GU PuAGU forming a lariat structure, followed by cleavage at the 3' consensus sequence (Py)nN-PyAG ↓ G and ligation of the 5' and 3' exons. Incorrect splicings, exon skippings, or partial deletions involving 5' terminal of exons have been shown to be due mostly to mutations of various kinds at the above consensus sequences of the *hprt* locus[9,10,12,13,14,15]. Such mutations are thought to alter the normal splice sites so that splicing takes place by using the splice site of the next exon or a cryptic splice site in an exon sequence. Keeping these facts in mind, we investigated the DNA sequences of both the upstream intron-regions of exons 6 and 8 of those mutant clones which had an event of missing of exon 6 or both exons 6 and 8 in cDNA.

Only a single clone of exon 6 missing was found to represent the GC→TA transversion located in the consensus sequence of the splice acceptor site after all the upstream-regions, including the splice acceptor sites for exons 6 and 8 of the missing clones, were searched. This transversion may be the causative mutational event for missing of exon 6 from the mutant clone. Before concluding that missing of exons 6 and 8 is, at least partially, due to aberrant mRNA splicing, we must determine the origin of this aberrant splicing which may be point mutations that occur in intron regions. Several splice mutants have already been identified, in which no mutations were detected in the splice sites, and events occurring at intron regions located far from the splice sites have been suggested to be the origin of the mutations[8,9,10]. It is noteworthy that missing of both exons 6 and 8 was found in the same clone. The missing of exon 8 only is frequent in human T-lymphocytes[8,10]. Mutant clones of exon 6 missing also have been recovered in human T-lymphocytes but not as frequently as for exon 8[2,13]. To our knowledge, this is the first paper reporting the missing of two exons, the intervening exon remaining in the same *hprt* gene whereas the missing of two neighboring exons is common[2,8,10]. The possibility that missing events of both exons 6 and 8 are the results of two independent occurrences can not be ruled out as the C-ion induced the exon 6 missing and there was spontaneous missing of exon 8 in the same *hprt* locus.

In our previous report[1], the RBE values for cell death and *hprt* mutation induction relative to gamma rays showed a peak at LET 124 keV/μm. It is necessary to confirm whether the above characteristics of C-ion-induced mutations are specific to the effect of heavy-ion with a high-LET that exceeds the value that gives the RBE-peak. We also must consider the possibility that LET may not be the sole factor affecting the specificity of mutation. In other words, a quantity different from LET is required to express the status of heavy-ion that is about to lose almost all its energy and stop in the matter. Further investigations of the reasons for the very frequent missing of exons in cDNA of the above mutants also are of much interest even if they can not be proved to be related to high-LET radiation.

ACKNOWLEDGEMENT

REFERENCES

1. Suzuki, M., Watanabe, M., Kanai, T., Kase, Y., Yatagai, F., Kato, T. and Matsubara, S. (1995) LET dependence of cell death, mutation induction and chromatin damage in human cells irradiated with accelerated carbon ions. Advances in Space Research, 18: 127–136.
2. Shimahara, H., Kato, T., Hirai, Y. and Akiyama, M. (1994) Spectrum of *in vitro hprt* mutations in T lymphocytes from atomic bomb survivors. I. Sequence alterations in cDNA. Carcinogenesis, *in press.*
3. Argos, P., Hanei, M., Wilson, J. M. and Kelley, W. N. (1983) A possible nucleotide-binding domain in the tertiary fold of phosphoribosyltrasferases. J. Biol. Chem. 258: 6540–6457.
4. Dush, M. K., Sikela, J. M., Khan, S. A., Tischfiedl, J. A. and Stambrook P. J. (1985) Nucleotide sequence and organization of the mouse adenine phosphoribosyltransferase gene: Presence of a coding region common to animal and bacterial phosphoribosyltransferases that has a variabe intron/exon arrangement. Proc. Natl. Acad. Sci. USA 82: 2731–2735.
5. Edwards, A., Voss, H., Rice, P., Civitello, A., Stegtemann, J., Schwager, C., Zimmermann, J., Erfle, H., Caskey, C. T. and Ansorge, W. (1990) Automated DNA Sequencing of the Human *HPRT* Locus. Genomics 6: 593–608.
6. Petel, P. I., Framson, P. E., Caskey, C. T. and Chinault, A. C. (1986) Fine structure of the human hypoxanthine phosphoribosyltransferase gene. Mol. Cell Biol., 6: 393–403.
7. Smith, C. W. J., Patton, J. G. and Nadal-Ginard, B. (1989) Alternative splicing in the control of gene expression. Annu. Rev. Genet., 23: 527–577.
8. Hou, S.-M., Steen, A.-M., Fält, S. and Andersson, B. (1993) Molecular spectrum of background mutation at the *hprt* locus in human T-lymphocytes. Mutagenesis 8: 43–49.
9. Rossi, A. M., Tates, A. D., van Zeeland, A. A. and Vrieling, H. (1992) Molecular analysis of mutations affecting *hprt* mRNA splicing in human T-lymphocytes in vivo. Env. Mol. Mutagenesis 19: 7–13.
10. Steingrimsdottir, H., Rowley, G., Dorado, G., Cole, J. and Lehmann, A. R. (1992) Mutations which alter splicing in the human hypoxanthine-guanine phosphoribosyltransferase gene. Nucleic Acids Res. 20: 1201–1208.
11. Zhang, L.-H., Vrieling, H., van Zeeland, A. A. and Jenssen, D. (1992) Spectrum of spontaneously occurring mutations in the *hprt* gene of V79 chinese hamster cells. J. Mol. Biol. 223: 627–635.
12. Zhang, L.-H. and Jenssen, D. (1991) Site specificity of N-methyl-N-nitrosourea-induced transition mutations in the *hprt* gene. Carcinogenesis, 12: 1903–1909.
13. Rossi, A. M., Thijssen, J. C. P., Tates, A. D., Vrieling, H., Natarajan, A. T., Lohman, P. H. M. and van Zeeland, A. A. (1990) Mutations affecting RNA splicing in man are detected more frequently in somatic than in germ cells. Mutat. Res. 244: 353–357.
14. Nelson, S. L., Giver, C. R. and Grosovsky, A. J. (1994) Spectrum of X-ray-induced mutations in the human *hprt* gene. Carcnogenesis, 15: 495–502.
15. Dorado, G., Steingrimsdottir, H., Arlett, C. F. and Lehmann, A. R. (1991) Molecular analysis of

ultraviolet-induced mutations in a *Xeroderma pigmentosum* Cell Line. J. Mol. Biol., **217**: 217–222.

16. Jolly, D. J., Okayama, H., Berg, P., Esty, A. C., Filpula, D., Bohlen, P., Johnson, G. G., Shively, J. E., Hunkapillar, T. and Friedmann, T. (1983) Isolation and characterization of a full-length expressive cDNA for human hypoxanthine phosphoribosyltransferase. Proc. Natl. Acad. Sci. USA, **80**: 477–481.