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Mutation Frequency of Plasmid DNA and *Escherichia coli* Following Long-term Space Flight on Mir

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To elucidate the biological influence of space radiation, we studied the effects of long-term space flight on mutation of the bacterial *ribosomal protein L* gene (*rpsL*). We prepared dried samples of plasmid DNA and repair-deficient and wild type cells of *Escherichia* (*E.*) *coli.* After a 40-day space flight on board the Russian space station Mir, the mutation frequencies of the *rpsL* gene were estimated by transformation of *E. coli* and by assessment of conversion of *rpsL* wild type phenotype (Sm^S) to its mutant phenotype (Sm^R). The experimental findings indicate that mutation frequencies of space samples were not significantly different from those of ground control samples in plasmid DNA and both *E. coli* strains. It may suggest that space radiation did not influence mutation frequency.

INTRODUCTION

Although the majority of space radiation consists of protons, space radiation components of high-linear energy transfer (LET) such as neutrons and heavy particles have high relative biological effectiveness[1)]. Chronic exposure at a low dose-rate is a general expectation for space. We must consider the effects of space radiation on the health of space crews in long-term flight as on International Space Station, because we have also found that pre-chronic irradiation induces a radioadaptive response in cultured human cells and mice[2)]. Although the radioadaptive phenomenon is generally noticed with low-LET radiation, there is also the latest report that the radioadaptive response can influence the outcome of neutron exposure[3)]. It still remains unclear what caused the radioadaptive response. To date, diverse biological effects of the space environment have been reported[4–12)]. All these results, however, came from short-term flights of about 2 weeks. Considering these facts, the National Space Development Agency of Japan (NASDA) used the Russian space station Mir orbiting the earth at an altitude of 400 km to study the biological effects of long-term space flight.

Here, we studied the effects of long-term space flight on the mutation of the bacterial *ribosomal protein L* gene (*rpsL*) integrated onto *E. coli* plasmid pML4 and using the wild type (KY700) and excision repair-deficient (KY706) strains of *E. coli* as a host.

MATERIALS AND METHODS

Plasmid

Plasmid DNA (pML4) were provided Dr. Yoichi Gondo (Tokai University, Japan). The pML4 contains kanamycin-resistance (Km^R) and *rpsL* genes. The *rpsL* gene encodes the S12 ribosomal protein responsible for streptomycin sensitivity (Sm^S). The pML4 plasmid is designed to carry a copy of the mutational target gene that is not expressed in the Sm^S KY700 and KY706 host strains due to the amber mutation (TAG) at the sixth codon (CAG). The plasmid is acted upon by the KY700 and KY706 strain repair systems during the non-selected growth on LB. However, it is placed back into the amber suppressor *supE44*[13)] *E. coli* HB101 strain for detection of any new mutations. Aliquots of 20 μl (total 5 μg) pML4 were placed onto each nitrocellulose sheets (12 × 12 mm, ADVANTEC, Tokyo, Japan) and vacuum-dried at 4°C overnight.

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Strains

E. coli strains of two types (KY700, Δ[*pro-lac*]*thi*, *ara*, *met*, *srlR*::*Tn10*, KY706, Δ[*pro-lac*]*thi*, *ara*, *met*, *uvrA6*, *malE*::*Tn10*) were provided Dr. Kazuo Yamamoto (Tohoku University, Japan). We employed *E. coli* strains carrying plasmid pML4. *E. coli* cells were grown in LB medium (1% Bacto tryptone, 0.5% Yeast extract, 1% NaCl, pH 7.2) at 37°C, washed and resuspended in LB medium containing 20% gelatin. Aliquots of about 200 μl (5×10^8 cells) from each E. coli culture were placed onto each of nitrocellulose sheets (12 × 12 mm, ADVANTEC, Tokyo, Japan) and vacuum-dried at 4°C overnight.

Sample preparations

The nitrocellulose sheets with adherent plasmid or *E. coli* were individually packed into polyethylene bags and stored at 4°C. Prior to the space experiment, the samples thus prepared were placed into random sections of a sample container to be loaded onto Mir Space Station. The samples were carried from Nara, Japan to Baikonur, Republic of Kazakhstan at 4°C in dark and they were loaded on Progress M-35 rocket. The space samples were carried by the Progress and kept for exposure to space radiation on the Mir Space Station for 40 days from 5 July until 14 August 1997. The temperature in the Mir Space Station was kept at 20–25°C during the flight. Control samples were prepared in the same way and kept at 22°C in a sample container on the ground.

DNA extraction

After the space flight, nitrocellulose sheets were taken at random from the sample containers that had been on Mir and from those that remained on the ground and were separately suspended in LB medium. Then a portion of the cell suspensions from each was spread and grown on an LB-plate at 37°C to count the surviving cell fraction. The remaining suspension was incubated in LB. Therefore, cell number increased to 2^{10} cells from each bacterial cell to fix induced-mutations. Plasmid DNAs were extracted from *E. coli*, then used to transform *E. coli* for analysis of the *rpsL* mutations.

Determination of rpsL mutations

E. coli HB101 (Km^S and Sm^R) cells were transformed by electroporation using a Gene-Pulser II (Bio-Rad Lab., CA, USA) with plasmid DNA and extracted plasmid DNA from *E. coli* cells. Transformed *E. coli* cells were spread both on Km-containing plates and on Km plus Sm-containing plates, and grown at 37°C for 20 h. The forward mutation frequency was estimated from the ratio of the numbers of Km^R and Sm^R colonies to those of total Km^R transformant colonies. The background mutation frequency was estimated with *E. coli* carrying intact pML4.

Statistical analysis

Levels of significance were calculated using Student's *t*-test. $P<0.05$ was considered significant.

RESULTS AND DISCUSSION

The space experiment was carried out with plasmid DNA. We also used two kinds of *uvrA*⁻ strain of *E. coli* as compared with the wild type strain, because the *uvrA*⁻ strain yields a higher frequency for radiation-induced mutation. The biological response might be induced directly, therefore, not but indirect action for survival and mutation frequencies. After the 40-day Mir mission, 6–10 samples on nitrocellulose sheets were selected at random and examined for survival and *rpsL* mutation.

The mutation frequencies of plasmid DNA exposed to space radiation for 40 days are shown in Table 1. There was no significant difference observed between the mutation frequencies of the space samples and ground control samples.

Table 1. Mutation frequencies of plasmid DNA exposed to space radiation for 40 days

	plasmid DNA ($\times 10^{-5}$)	
on earth	0.75 ± 0.03	ns
in space	0.75 ± 0.02	

Values represent means ± SD. n=6–12.
ns; not significant ($p>0.05$ by student's *t* test).

The survival rate of space flight and ground control *E. coli* cells were almost the same and 3–4 % compared to the pre-flight sample (Table 2). There was no significant difference between wild type (KY700) and excision repair-deficient (KY706) strains of *E. coli* (Table 2). From these results, it was thought that the preservation period had a greater influence on the survival rate than space radiation. We could analyze the *rpsL* mutation frequencies because more than 1×10^6 cells survived in all samples (Table 2), which was sufficient to estimate the mutation frequencies.

The mutation frequencies of extracted plasmid DNA from *E. coli* cells exposed to space radiation for 40 days are shown in Table 3. There were no significant differences observed between the mutation frequencies of space samples and ground control samples regardless of repair activ-

Table 2. Survival number in *E. coli* cells exposed to space radiation for 40 days

	KY700 (wild)				KY706 (*uvr*⁻)			
	n	cells ($\times 10^7$/sample)	survival		n	cells ($\times 10^7$/sample)	survival	
before flight	4	9.18 ± 0.11	1.00		4	8.86 ± 0.11	1.00	
on earth	16	0.35 ± 0.11	3.79×10^{-2}	ns	28	0.35 ± 0.09	4.00×10^{-2}	ns
in space	15	0.36 ± 0.15	3.92×10^{-2}		15	0.34 ± 0.17	3.86×10^{-2}	

Values represent means ± SD. n = 6–12.
ns; not significant (p>0.05)

Table 3. Mutation frequencies of extracted plasmid DNA from *E. coli* cells exposed to space radiation for 40 days

	plasmid DNA($\times 10^{-5}$)			
	KY700 (wild)		KY706 (*uvr*⁻)	
on earth	1.07 ± 0.21	ns	1.03 ± 0.17	ns
in space	0.99 ± 0.08		1.09 ± 0.23	

Values represent means ± SD. n=6–12.
ns; not significant (p>0.05 by student's t test).

ity. There was almost no effect of space radiation on survival and mutation frequency during space flight for 40 days; it is suggested that this level of space radiation had not influenced them.

In related experiments, it has been reported that the space station Mir was exposed to many kinds of space radiation containing high-LET of 10–21 mGy during the 40-day space flight (0.25–0.51 mGy/day) as measured using physical dosimeters[14]. About 50% of the mutations induced by heavy particle irradiation in the Chinese hamster *hprt* locus resulted from total gene deletions[15,16]. High-LET radiation is known to induce large interstitial deletions. However, the number of mutants with gross structural changes might have been greatly underestimated in the present study, because the pML4 plasmid was not designed to screen large deletion mutations[17]. Although the averaged mutation frequency did not demonstrate any significant difference between space samples and ground control samples, we found that certain space samples showed higher mutation frequencies than ground samples of *B. subtilis*[18] and *S. cerevisiae*[19]. The mutant samples showed a large deletion[18,19], suggesting that space radiation containing high-LET might have caused deletion-type mutations. Further analysis is necessary to measure the exact dosimetry for each sample using CR39, which records the tracks of space radiation three-dimensionally.

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