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Deficiency in DNA repair in mouse lymphoma strain L5178Y-S

(DNA strand break repair/filter elution/repair deficiency)

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ABSTRACT **The production and repair of radiation-induced DNA damage were measured by filter elution in strains of mouse lymphoma L5178Y cells differing in their sensitivity to ionizing radiation. The induction of radiation-induced damage, as measured by filter elution at pH 12.1, 9.6, and 7.2, was similar in the resistant strain LY-R and the sensitive strain LY-S. The repair of single-strand breaks and alkali-labile sites, as measured by filter elution at pH 12.1 at various times after irradiation, was somewhat slower in strain LY-S than in strain LY-R, although after a 20-min repair period the extent of repair was equal in the two strains. However, when filter elution was performed at either pH 9.6 or pH 7.2, the repair of x-radiation-induced damage was much less extensive in strain LY-S than in strain LY-R. We have assumed that the extent of filter elution at pH 9.6 is a measure of the occurrence of frank double-strand breaks as well as closely opposing single-strand breaks and pH 9.6-labile sites (and combinations thereof), and that the extent of elution at pH 7.2 is a measure of the occurrence of frank double-strand breaks alone. If these assumptions are correct, the results suggest that the sensitivity of strain LY-S to the cytotoxic effects of ionizing radiation is caused by a deficiency in the ability of this strain to repair frank double-strand breaks and pH 9.6-labile lesions. The repair of pH 9.6-labile lesions was temperature sensitive in strain LY-S, as previously found for cellular recovery processes in this strain. Two independent radiation-resistant variants of strain LY-S, isolated after protracted exposure of LY-S cells to low-dose-rate radiation, showed a deficiency in the repair of pH 9.6-labile lesions similar to that observed in strain LY-S. However, the repair of frank double-strand breaks was more extensive in the radiation-resistant variants than in strain LY-S and was similar in extent to that occurring in strain LY-R after a 60-min postirradiation incubation. The results suggest that there is a difference in the nature of DNA damage measured by filter elution at pH 9.6 vs. pH 7.2 and that a deficiency in the repair of pH 9.6-labile lesions does not contribute to cell lethality in the case of the radiation-resistant variants. The radiation resistance of these variants in comparison to strain LY-S may be due at least in part to recovery of the ability to rejoin frank DNA double-strand breaks.**

Mouse lymphoma strain L5178Y-S (LY-S) was isolated in 1961 by Alexander and Mikulski (1) following a spontaneous increase in sensitivity of a culture of LY5178Y cells to the cytotoxic effects of ionizing radiation. The parental culture was designated LY-R to differentiate it from the newly isolated sensitive strain. LY-S cells have been shown to be deficient in cellular recovery processes following exposure to ionizing radiation [reviewed by Beer *et al.* (2)], particularly when postirradiation incubation was carried out at 37°C (3–5). Conflicting reports have appeared concerning the difference in the ability of LY-R and LY-S cells to rejoin DNA single-strand breaks (6, 7). In the present work, we have used the technique of filter elution to measure the induction and repair of radiation-induced DNA damage in strains LY-S and LY-R. In the interpretation of the results, we have assumed that filter elution at pH 12.1 is a measure of the occurrence of single-strand breaks and alkali-labile sites, that filter elution at pH 9.6 is a measure of the occurrence of frank double-strand breaks as well as closely opposing single-strand breaks and pH 9.6-labile sites (and combinations thereof), and that filter elution at pH 7.2 is a measure of the occurrence of frank double-strand breaks alone. We found that strain LY-S is markedly deficient in the repair of frank double-strand breaks and pH 9.6-labile lesions during postirradiation incubations at 37°C. When the postirradiation incubation was carried out at 25°C, strain LY-S showed an increased ability for the repair of pH 9.6-labile lesions. Radiation-resistant variants of strain LY-S were deficient in the repair of pH 9.6-labile lesions but had recovered some ability to repair frank double-strand breaks in comparison to strain LY-S.*

MATERIALS AND METHODS

Cell Culture. LY-R and LY-S cells were originally obtained from Janusz Z. Beer (Center for Devices and Radiological Health, Federal Drug Administration, Rockville, MD). Radiation-resistant variants of strain LY-S were isolated in our laboratory following protracted irradiation of LY-S cultures at the rate of 0.02 Gy/hr (8). During the protracted exposure, the radiation resistance of some of the cultures increased, while the radiation resistance of other exposed cultures remained unchanged. Aliquots of both types of culture were plated, and single colonies were isolated and tested for their dose–response characteristics (8). The DNA repair abilities of two independent radiation-resistant clones (LY-SR and LY-S35), isolated from two separately irradiated LY-S cultures, are reported in the present work.

Cells were grown in unshaken suspension cultures at 37°C in Fisher's medium containing 0.1% Pluronic F68 (BASF Wyandotte, Parsippany, NJ), 2 mM sodium pyruvate, and 10% heat-inactivated horse serum. The cell density was maintained between 2 and 8×10^5 cells per ml. Cultures were shown to be free of mycoplasma by periodic testing according to the method described by Schneider *et al.* (9). The doubling times of the cultures were 9.8, 8.9, 9.2, and 9.1 hr for strains LY-R, LY-S, LY-SR, and LY-S35, respectively.

Cell survival, measured as colony-forming ability, was determined by plating aliquots of treated and control cultures in soft agar medium (Fischer's medium containing 0.32% Noble agar) (8). Plates were incubated for 10 days at 37°C, and visible colonies were counted by eye. Untreated cells gave plating efficiencies between 80% and 100%.

Exposure of Cells to Ionizing Radiation. In experiments in which cell survival was determined by colony formation,

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*A preliminary report of this work was given at the 34th Annual Meeting of the Radiation Research Society, April 12–17, 1986, Las Vegas, NV.

cells were suspended in growth medium in T flasks at a density of 2×10^5 cells per ml and were irradiated at room temperature at a dose rate of 0.88 Gy/min using a GE Maximar x-ray generator (250 kVp; 15 mA; half-value layer, 1.5 mm Cu). For filter elution experiments, cells were suspended in growth medium at a density of 5×10^5 cells per ml and were exposed to γ-radiation at 4°C at a dose rate of 12 Gy/min using a ^{60}Co source. Cell survival following irradiation with the ^{60}Co source under these conditions was similar to that observed following exposure of cells to equal doses of x-radiation at room temperature.

Filter Elution. Prior to each experiment, the cells were incubated with either [^{14}C]thymidine (0.02 μCi/ml; 1 Ci = 37 GBq) or [^{3}H]thymidine (0.1 μCi/ml) in the presence of 2.5 μM unlabeled thymidine for 20–24 hr at 37°C. The level of radioactive thymidine used did not affect the growth rate of the cells during the labeling period. The labeled cells were then centrifuged, washed in growth medium, and incubated for 2 hr in medium containing 10 μM unlabeled thymidine. Less than 2% of the total radioactivity of the cells and medium was present as acid-soluble material after the chase period.

Aliquots of ^{14}C-prelabeled cells were exposed to varied doses of radiation, while similar aliquots served as unirradiated controls. After the irradiation, control and irradiated cells were centrifuged and were either suspended in ice-cold medium and kept at 4°C (for 0 repair-time samples) or suspended in medium and incubated at 25°C or 37°C for various periods prior to harvest to allow repair. ^{3}H-labeled LY-R cells were exposed to a fixed dose of radiation and were used as an internal standard for each ^{14}C-labeled sample. Elution of [^{3}H]DNA did not appear to be influenced by the extent of elution of the [^{14}C]DNA. DNA single-strand breaks plus alkali-labile sites were measured by the rate of elution of DNA at pH 12.1 according to the method of Kohn *et al.* (10), as modified by Blakeley *et al.* (11). Filter elution of DNA either at pH 9.6 according to the method of Bradley and Kohn (12) as modified by Radford (13), or at pH 7.2 as described by Evans *et al.*† was also used to measure radiation-induced DNA damage.

For elution at pH 12.1, 5×10^5 ^{14}C-labeled cells and 5×10^5 ^{3}H-labeled LY-R reference cells (the latter previously exposed to 5 Gy of γ-radiation), were mixed and applied to a 2-μm-pore polycarbonate filter and lysed for 1 hr in the dark at pH 10 in the presence of 0.02 M EDTA/0.1 M glycine/2% $NaDodSO_4$/proteinase K (0.5 mg/ml). The DNA was then eluted at a flow rate of 0.04 ml/min at pH 12.1 in a buffer consisting of 0.1 M tetrapropyl ammonium hydroxide/0.02 M EDTA/0.2% $NaDodSO_4$, and samples of ≈2 ml each were collected. ^{14}C and ^{3}H were assayed by liquid scintillation counting, using Scinti Verse II (Fisher) containing 0.7% acetic acid as the scintillation fluid. The percentage of the total [^{14}C]DNA eluted in each fraction was cumulatively summed and plotted against corresponding values for the internal standard [^{3}H]DNA. Best-fit lines through the initial points were determined by regression equations, and the slopes of these lines were used as a measure of the initial elution rates. The initial elution rate has been found to depend on the single-strand length of the DNA molecules (10).

For elution at pH 9.6, aliquots of 2.5×10^5 ^{14}C-labeled cells and 2×10^5 ^{3}H-labeled LY-R reference cells (the latter previously exposed to 50 Gy of γ-radiation) were mixed and applied to a polycarbonate filter. Cells were lysed for 1 hr at room temperature at pH 9.6 in a buffer consisting of 0.05 M glycine/0.025 M EDTA/0.05 M tris(hydroxymethyl)aminomethane/2% $NaDodSO_4$/proteinase K (0.5 mg/ml). The DNA was eluted in the same buffer without proteinase K at the rate of 0.04 ml/min, and samples of 3 ml were collected for a total of 15 hr. Relative elution was calculated as the ratio of the percentage of [^{14}C]DNA eluted to the percentage of [^{3}H]DNA eluted during the 15-hr period. Radford (13) has demonstrated a linear relationship between relative elution, as defined above, and the extent of DNA double-strand breaks. We have found no marked differences using relative elution vs. initial elution rates as a measure of DNA damage (data not shown).

For elution at pH 7.2, cells were lysed for 1 hr either at pH 9.6 as described above, or at pH 7.2 in buffer consisting of 0.1 M Hepes/0.025 M EDTA/2% $NaDodSO_4$/proteinase K (0.5 mg/ml). This last solution, without proteinase K, served as the buffer for elution at pH 7.2. Other than the solutions for lysing and elution, the procedure for elution at pH 7.2 was the same as that described for elution at pH 9.6.

RESULTS

Cell Survival Following Exposure to Radiation. Survival, in terms of colony-forming ability, of strains LY-S, LY-R, and the radiation-resistant variants of strain LY-S is shown in Fig. 1. A shoulder was apparent in the dose–response curve of strain LY-R and the radiation-resistant variants of strain LY-S, but not of strain LY-S. The slope of the exponential portion of the curve was less steep for strain LY-R than for strain LY-S, while the slopes of the response curves for the radiation-resistant variants of strain LY-S were even less than that of strain LY-R. (The *Do* and *Dq* values for these strains are shown in Table 4.) The dose–response characteristics of strains LY-R and LY-S were similar to those reported earlier by us and by others (reviewed in ref. 2).

Production of Radiation-Induced DNA Damage. No marked differences were observed between the strains in the radiation-induced production of DNA damage as measured by

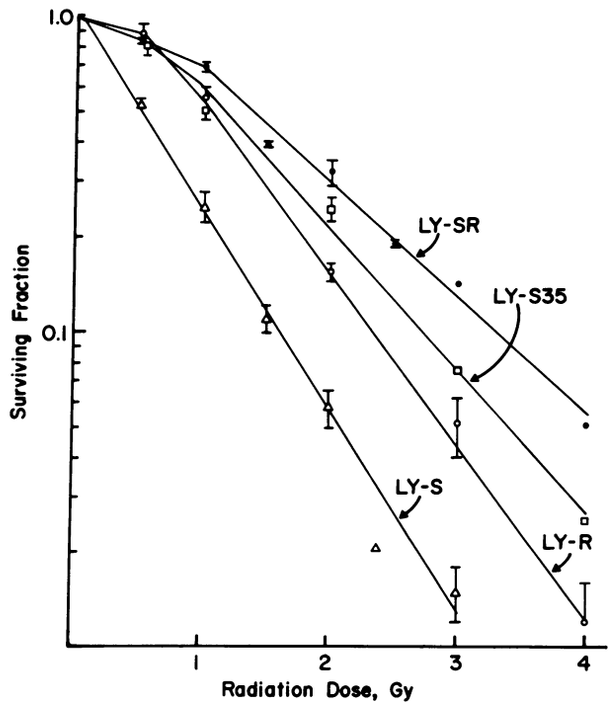

FIG. 1. Colony formation by L5178Y strains following exposure to radiation. Cell suspensions were exposed to x-radiation as described in the text and were immediately diluted and plated in soft agar medium. Colonies were counted after 10 days. Controls were treated similarly except for the radiation. Vertical bars show the SEM of replicate determinations.

†Evans, J. W., Limoli, C. L. & Ward, J. F., 34th Annual Meeting of the Radiation Research Society, April 12–17, 1986, Las Vegas, NV.

Table 1. Relationship between radiation dose and initial DNA damage as measured by filter elution

Experimental condition	Slope of dose–response curve, $\times 10^3$			
	LT-R	LY-S	LT-SR	LY-S35
1. Lyse pH 10, elute pH 12.1	173 ± 7	158 ± 9	ND	ND
2. Lyse pH 9.6, elute pH 9.6	14.4 ± 0.5	14.2 ± 0.5	12.9 ± 0.8	12.2 ± 1.5
3. Lyse pH 9.6, elute pH 7.2	12.7 ± 0.7	13.4 ± 0.8	12.4 ± 1.6	11.0 ± 1.1
4. Lyse pH 7.2, elute pH 7.2	12.4 ± 0.4	13.0 ± 1.9	13.6 ± 1.2	9.8 ± 2.3

Numbers indicate slope (y/x) of dose–response curve ± SEM of the slope ($\times 10^3$). For group 1, x = the dose in Gy and y = the initial rate of elution when the fraction of [^{14}C]DNA eluted was plotted against the fraction of standard [^{3}H]DNA eluted. For groups 2, 3, and 4, x = the dose in Gy and y = relative elution (the ratio of the fraction of [^{14}C]DNA eluted to the fraction of the standard [^{3}H]DNA eluted in a 15-hr period). The data in the table involving the elution of [^{14}C]DNA are normalized using ^{3}H-labeled LY-R cells exposed to a standard dose of radiation as an internal standard. To determine the differences in the amount of DNA eluted at the various pH values, the elution of [^{14}C]DNA was calculated without normalization. For cells receiving the highest dose of irradiation (5 Gy for group 1, and 50 Gy for groups 2–4), the fraction of [^{14}C]DNA eluted did not vary significantly between strains and averaged 98% for group 1, 51% for group 2, 34% for group 3, and 25% for group 4. The fraction of [^{14}C]DNA eluted in the case of nontreated cells did not vary between strains and averaged 23% for group 1, 15% for groups 2 and 3, and 13% for group 4. ND, not done.

filter elution at pH 12.1, pH 9.6, or pH 7.2 (Table 1). The amounts of [^{14}C]DNA eluted at pH 9.6 and at pH 7.2 are given in the footnote to Table 1. When ^{14}C-labeled cells were exposed to 50 Gy of γ-radiation and harvested immediately, the percentage of DNA eluted at pH 7.2 was 67% of that eluted at pH 9.6. Lysis of irradiated cells at pH 7.2 rather than at pH 9.6 resulted in a further 30% decrease in the percentage of DNA eluted.

Repair of DNA Single-Strand Breaks and Alkali-Labile Sites. As shown in Fig. 2, the repair of DNA single-strand breaks and alkali-labile sites was significantly slower in strain LY-S than in strain LY-R. After 20 min of postirradiation incubation, however, the extent of repair was similar in the two strains. Repair of single-strand breaks and alkali-labile sites by the two radiation-resistant variants of LY-S was similar to that observed in strain LY-S (data not shown).

DNA Repair as Measured by Relative Elution at pH 9.6. Fig. 3 shows an example of an experiment in which the relative elution at pH 9.6 was measured immediately and 60 min after exposure of cells to 50 Gy of γ-radiation. It can be seen that, in contrast to strain LY-R, little repair occurred in strain LY-S during the postirradiation incubation at 37°C. A summary of the results of experiments in which the time of the postirradiation incubation period was varied is shown in Fig.

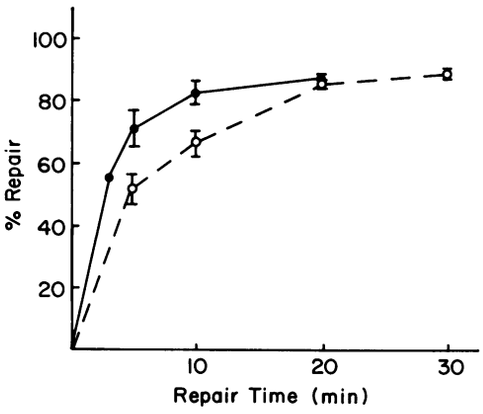

FIG. 2. Repair of DNA single-strand breaks and alkali-labile sites by LY-R (——) and LY-S (– – –) cells as a function of the postirradiation incubation time at 37°C. The percentage repair of DNA damage was calculated as $[(RO - RT)/(RO - RC)] \times 100$, where RO = the initial rate of alkaline elution of DNA from cells lysed immediately after irradiation, RT = the initial rate of alkaline elution of DNA from cells lysed after a postirradiation incubation period of T min, and RC = the initial rate of alkaline elution of DNA from control cells. The means from 1–11 experiments are plotted for each postirradiation incubation period. The vertical bars show the SEM.

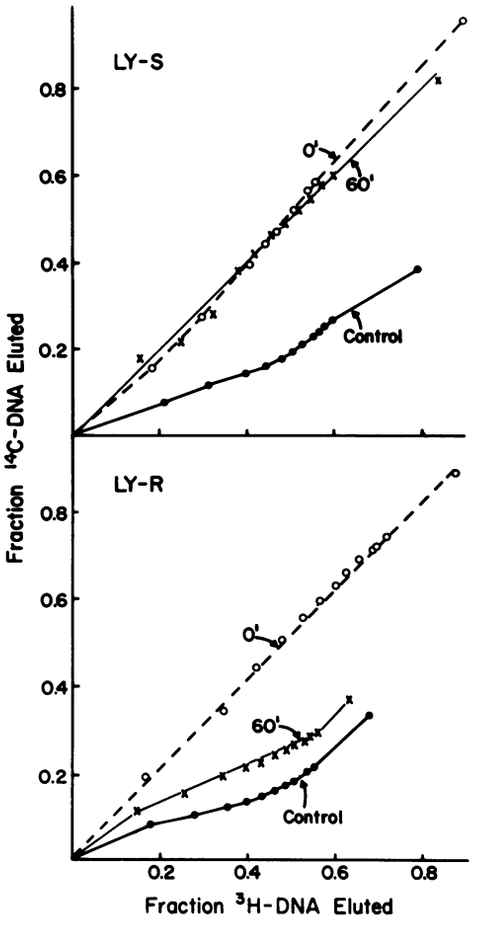

FIG. 3. Repair of DNA damage in strains LY-R and LY-S during a 60-min postirradiation incubation period at 37°C, as measured by filter elution at pH 9.6. ^{14}C-prelabeled cells were exposed to 50 Gy of γ-irradiation and were harvested, lysed, and eluted at pH 9.6 either immediately (○), or after a 60-min postirradiation incubation at 37°C (×). Controls were treated similarly except for the irradiation (●). The percentage of [^{14}C]DNA eluted is plotted against the internal standard [^{3}H]DNA from LY-R cells harvested immediately after irradiation with 50 Gy.

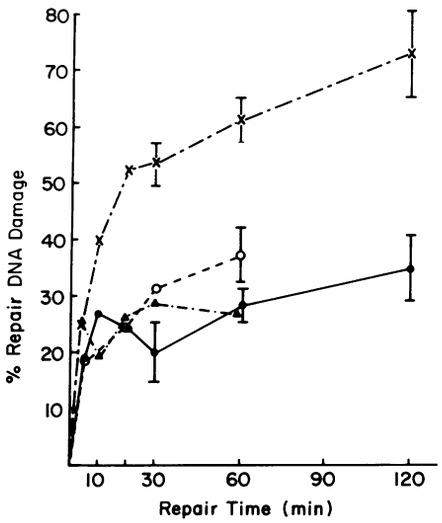

FIG. 4. Repair of DNA damage as measured by filter elution at pH 9.6 at various times during postirradiation incubation at 37°C in strain LY-R (×), strain LY-S (●), strain LY-SR (○), and strain LY-S35 (▲). The percentage repair of DNA damage was calculated as described in Fig. 2, substituting relative elution at pH 9.6 for the initial rate of alkaline elution. The means from 1–20 experiments and the SEM are plotted as in Fig. 2.

4. The extent of DNA repair during the first 5 min of postirradiation incubation at 37°C was similar in all of the strains. However, after postirradiation incubation periods of 10 min or longer, DNA repair was much more extensive in strain LY-R than in strain LY-S or the radiation-resistant variants. Extension of the postirradiation incubation period for up to 6 hr did not eliminate the difference in DNA repair between strain LY-R and strain LY-S or the radiation-resistant variants (data not shown). To determine whether repair was inhibited in strain LY-S by the procedures used for processing the cells for filter elution, repair in strain LY-S was measured in cultures in which all centrifugation and cooling steps between the chase period and the end of the postirradiation incubation were eliminated. No increase in repair was observed under these conditions (data not shown).

When postirradiation incubation was carried out at 25°C, DNA repair in LY-S cells, as measured by elution at pH 9.6, was more extensive than at 37°C (Table 2). The results in Table 2 also show that following postirradiation incubation at 25°C, DNA repair was similar in strains LY-R and LY-S, since at 25°C repair decreased for strain LY-R and increased in strain LY-S in comparison to incubation at 37°C. This temperature sensitivity of DNA repair was not observed in the case of the radiation-resistant variants of strain LY-S, or in the repair of single-strand breaks and alkali-labile sites by strain LY-S (Table 2).

DNA Repair as Measured by the Relative Elution at pH 7.2. A deficiency in DNA repair during postirradiation incubation at 37°C in the case of strain LY-S was also apparent when the cells were lysed at pH 9.6 and filter elution was carried out at pH 7.2 (Table 3). In contrast to the results obtained with strain LY-S when filter elution was carried out at pH 9.6, no temperature sensitivity was observed in the repair of damage measured by filter elution at pH 7.2, in that the extent of repair at 25°C was equal to or lower than the extent of repair at 37°C for all strains (data not shown). When damage was measured by elution at pH 7.2, the radiation-resistant variant LY-SR exhibited DNA repair similar to that of strain LY-R during a 60-min postirradiation incubation period at 37°C (Table 3). When both lysis and filter elution were carried out at pH 7.2, DNA repair was slower in the radiation-resistant variants than in strain LY-R, but after 60 min of postirradiation incubation the extent of repair was similar to that occurring in strain LY-R (Table 3).

DISCUSSION

The experiments described above in which the results of filter elution at pH 9.6 and pH 7.2 were compared were suggested by Evans *et al.*,† who reported results indicating that elution at pH 7.2 measures frank double-strand breaks, while elution at pH 9.6 measures both frank double-strand breaks and lesions converted to double-strand breaks at pH 9.6. The conclusion was based on their finding that DNA digested with the restriction enzyme *Pvu* II eluted at the same rate at pH 7.2 and pH 9.6, whereas the DNA from irradiated cells eluted 1.6 times faster at pH 9.6 than at pH 7.2. Assuming that these conclusions are correct, we suggest that the results of the present paper indicate that (*i*) strain LY-S is deficient in the repair of both frank double-strand breaks and pH 9.6-labile lesions as compared to strain LY-R when postirradiation incubation is carried out at 37°C, thus explaining the greater radiation sensitivity of strain LY-S relative to strain LY-R; (*ii*) the repair of pH 9.6-labile lesions in strain LY-S is temperature sensitive—i.e., more repair occurs at 25°C than at 37°C, corresponding to the temperature sensitivity of cellular recovery processes occurring in this strain; and (*iii*) variants of strain LY-S, which are more resistant to the cytotoxic effects of irradiation than either strain LY-S or strain LY-R are deficient in the repair of pH 9.6-labile lesions but appear to have recovered some ability to repair frank double-strand breaks. A summary of the comparative characteristics of the various LY strains is shown in Table 4.

Table 2. Effect of temperature on DNA repair

Experimental condition	Strain	% repair 25°C	% repair 37°C
1. Repair of DNA damage after exposure to 5 Gy and a 10-min postirradiation incubation. Lysis at pH 10, elution at pH 12.1.	LY-R	61	83 ± 3
	LY-S	32	66 ± 3
2. Repair of DNA damage after exposure to 50 Gy and a 60-min postirradiation incubation period. Lysis and elution at pH 9.6.	LY-R	50 ± 6	61 ± 4
	LY-S	44 ± 6	28 ± 3
	LY-SR	29	37 ± 5
	LY-S35	18	26 ± 3

The % repair was calculated as described in the legend to Figs. 2 and 4. Results of replicate experiments were averaged, and the means ± SEM are shown.

Table 3. Repair of DNA damage at 37°C as indicated by filter elution at pH 7.2

Experimental condition	Strain	% repair 10 min	% repair 60 min	% repair 240 min
1. Lyse pH 9.6, elute pH 7.2	LY-R	48 ± 8	50 ± 6	83 ± 14
	LY-S	18 ± 6	24 ± 3	41 ± 8
	LY-SR	20 ± 6	44 ± 8	70 ± 5
	LY-S35	25 ± 3	28 ± 5	51 ± 11
2. Lyse pH 7.2, elute pH 7.2	LY-R	25 ± 2	25 ± 5	
	LY-S	12 ± 3	10 ± 2	
	LY-SR	17 ± 0	25 ± 8	
	LY-S35	14 ± 4	25 ± 3	

The % repair of DNA damage after exposure of the cells to 50 Gy of γ-radiation was calculated as indicated in the legend to Fig. 4. Results of replicate experiments were averaged, and the means ± SEM are indicated.

Table 4. Summary of the comparative characteristics of LY strains

Strain	*Do*, Gy	*Dq*, Gy	Ability to repair DNA damage: SSB and alkali-labile sites	pH 9.6-labile lesions 25°C	pH 9.6-labile lesions 37°C	Frank DSB
LY-R	0.80	0.48	+++	++	+++	+++
LY-S	0.66	0.05	++	++	+	+
LY-SR	1.19	0.55	++	+	+	++
LY-S35	0.98	0.48	++	+	+	++

The nature of the lesion is assumed from the elution characteristics, which were (*i*) for SSB (single-strand breaks) and alkali-labile sites: lysis at pH 10 and elution at pH 12.1; (*ii*) for pH 9.6 labile lesions: lysis and elution at pH 9.6; and (*iii*) for frank DSB (double-strand breaks): lysis and elution at pH 7.2. The repair capabilities of strains LY-S, LY-SR, and LY-S35 are shown in comparison to strain LY-R. *Do* is the dose that reduces survival by $1/e$ and is equal to the reciprocal of the slope of the exponential portion of the survival curve. *Dq* is the intercept of the back-extrapolated exponential portion of the survival curve with the abscissa at a surviving fraction of 1.0.

The temperature sensitivity of the repair of pH 9.6-labile lesions in strain LY-S at 37°C vs. 25°C corresponds to the temperature sensitivity of cellular recovery occurring during postirradiation holding periods (3, 5) and between fractionated doses of irradiation (4, 5, 14) in strain LY-S. A deficiency in the repair of pH 9.6-labile lesions and/or frank double-strand breaks at 37°C may also explain the limited dose-rate dependence of the survival of strain LY-S (15) and the increased induction of chromosome aberrations in strain LY-S following irradiation in comparison to strain LY-R (16).

An association between the lack of repair of DNA double-strand breaks (as measured by sedimentation rate in sucrose gradients following lysis at pH 8), the lack of the recovery of colony-forming ability between split radiation doses, and the absence of dose-rate dependence, has been demonstrated in a radiation-sensitive strain of *Saccharomyces cerevisiae, rad52* (17–19). Similarly, radiation-sensitive Chinese hamster ovary strains isolated by Jeggo and Kemp (20) have been shown to be deficient in the repair of DNA double-strand breaks (as measured by filter elution at pH 9.6) (21) and to lack dose-rate dependence (22). Another radiation-sensitive Chinese hamster ovary strain, isolated by Stamato *et al.*, showed a marked cell-cycle variation in radiation sensitivity, which was correlated with variation in the repair of DNA double-strand breaks (as measured by filter elution at pH 9.6) at different times in the cell cycle (23, 24). A deficiency in the repair of pH 8- or pH 9.6-labile lesions and/or of frank double-strand breaks thus appears to be related to a deficiency in cellular repair processes in these radiation-sensitive cells.

The radiation-resistant variants of strain LY-S appear to have regained the ability to rejoin frank double-strand breaks, although the rate of repair was less than that occurring in strain LY-R. Strain LY-SR appears to be more proficient than strain LY-S35 in the repair of damage measured in cells lysed at pH 9.6 and eluted at pH 7.2. Possibly strain LY-SR has regained the ability to repair a fraction of pH 9.6-labile lesions. Since the variants exhibit more resistance to the cytotoxic effects of radiation than does strain LY-R (Fig. 1), pH 9.6-labile lesions do not appear to contribute to radiation-induced lethality in these variant strains. This discrepancy could be due to the high doses required to demonstrate pH 9.6-labile lesions vs. the low doses required to demonstrate changes in survival. However, in spite of the difference in dose levels, the correlation of temperature sensitivities of the molecular and cellular repair processes suggests that the pH 9.6-labile lesions contribute to lethality in strain LY-S. Possibly pH 9.6-labile lesions are converted to frank double-strand breaks *in vivo* and contribute markedly to lethality only in strains that are deficient in double-strand break repair.

The deficiency in repair exhibited by strain LY-S could be caused either by an alteration in a repair enzyme or by the occurrence of DNA degradation, which obscures simultaneous DNA repair processes, as has been reported in the related strain LY5178Y-S/S 10 hr or more after irradiation (25–27). For instance, it is possible that in strain LY-S at 37°C (but not at 25°C), pH 9.6-labile lesions are enzymatically converted to nonrepairable lesions; that these lesions are repairable in strain LY-R; and that in the radiation-resistant variants the enzymatic conversion does not occur at either 25°C or 37°C.

Note. Wlodek and Hittelman (28) have also reported that strain LY-S exhibits a deficiency in the repair of DNA double-strand breaks, as measured by filter elution at pH 9.6.

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