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Genetics

Locus specificity in the mutability of L5178Y mouse lymphoma cells: The role of multilocus lesions

(x-radiation-induced mutagenesis/x-radiation sensitivity/hypoxanthine (guanine) phosphoribosyltransferase, thymidine kinase, and Na^+,K^+-ATPase loci)

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ABSTRACT **Mouse L5178Y strain LY-S and its parental strain LY-R differ in their comparative sensitivities to the cytotoxic effects of various mutagenic agents—i.e., strain LY-S has been found to be more sensitive, less sensitive, or similarly sensitive to individual agents in comparison to strain LY-R. Nevertheless, strain LY-S has been found to be uniformly less mutable than strain LY-R at the hypoxanthine (guanine) phosphoribosyltransferase (*Hprt*) locus following treatment with x-radiation, UV radiation, or alkylating agents. In the present work we have isolated subclones of strains LY-R and LY-S that are heterozygous at the thymidine kinase (*Tk*) locus (chromosome 11). We have found that a heterozygous LY-S Tk^+/Tk^- strain shows as high or higher mutability at the *Tk* locus than do heterozygous LY-R strains following treatment with x-radiation, UV radiation, or ethyl methanesulfonate. Mutability of all heterozygous strains at the *Tk* locus is much higher than at the *Hprt* locus following treatment with these mutagenic agents, with the exception of one heterozygous LY-R strain that possesses only one chromosome 11 and that is poorly mutable at the *Tk* locus by x-radiation. On the basis of these results, we have suggested that (*i*) because of a repair deficiency, multilocus lesions are formed in the DNA of LY-S strains following treatment with radiation or alkylating agents; (*ii*) multilocus lesions lead to poor recovery of viable mutants when the target locus is closely linked to essential genes and situated on a hemizygous chromosomal region (e.g., the *Hprt* locus on the X chromosome or the *Tk* locus in strains monosomic for chromosome 11); and (*iii*) x-radiation is a relatively poor mutagen at loci situated on hemizygous chromosomal regions, in repair-efficient and repair-deficient cells, because of its tendency to form multilocus lesions.**

Mouse lymphoma strain L-5178Y-S (LY-S) was first isolated by Alexander and Mikulski (1) following a spontaneous increase in the x-radiation sensitivity of L5178Y cells growing *in vitro*. The parental strain was named L5178Y-R (LY-R) to differentiate it from the newly isolated sensitive strain. In spite of the greater sensitivity of strain LY-S to the cytotoxic effects of x-radiation and alkylating agents (2–5), strain LY-S is less mutable than strain LY-R by UV radiation and x-radiation and by alkylating agents at the hypoxanthine (guanine) phosphoribosyltransferase (*Hprt*) and Na^+,K^+-ATPase loci (4–6). In the present work we have compared the mutability of the two strains at the thymidine kinase (*Tk*) locus situated on chromosome 11 (7, 8) using heterozygous Tk^+/Tk^- strains of LY-R and LY-S. We have found the mutability of the heterozygous LY-S strain (LY-S1) to be as high or higher than that of LY-R heterozygous strains (LY-R16 and LY-R83) at the *Tk* locus following treatment with x-radiation, UV radiation, or ethyl methanesulfonate (EtMes). Mutant frequencies are much higher at the *Tk* locus than at the *Hprt* and Na^+,K^+-ATPase loci for LY-S and LY-R heterozygous strains treated with these agents, with the exception of strain LY-R83, which is poorly mutable at the *Tk* locus following treatment with x-radiation. A large proportion of the spontaneous and induced LY-S Tk^-/Tk^- mutants forms small colonies (<0.3 mm in diameter) in selective medium containing trifluorothymidine, indicating that the inactivation of the *Tk* gene in strain LY-S is often due to a lesion extending into neighboring essential genes (9–12). These results indicate that multilocus lesions are formed more often in strain LY-S than in LY-R following treatment with DNA-damaging agents. Therefore, the low recovery of *Hprt* mutants in strain LY-S may be caused by inactivation of essential genes neighboring the *Hprt* locus and present in single copies on the active X chromosome. The loss of these essential genes would render any newly induced *Hprt* mutants inviable in strain LY-S or LY-S1.

MATERIALS AND METHODS

Cell Culture. L5178Y cells were grown in suspension at 37°C in Fischer's medium as described (5). Tests for the presence of mycoplasma contamination were carried out every few months according to the method described by Schneider *et al.* (13).

Isolation of LY Strains Heterozygous at the *Tk* Locus. Homozygous Tk^-/Tk^- LY-R and LY-S strains (LY-TKR-5 and LY-TKS-14), induced by UV mutagenesis and isolated by selection in medium containing bromodeoxyuridine, were obtained from Janusz Z. Beer (Center for Devices and Radiological Health, Food and Drug Administration, Rockville, MD). To allow the formation of spontaneous Tk^+/Tk^- mutants, cultures of homozygous Tk^-/Tk^- cells were grown for 17 days (25–30 generations) at 37°C as described by Clive and Voytek (14). Two LY-R colonies (LY-R16 and LY-R83), isolated by virtue of their ability to grow in THMG soft agar medium [growth medium containing Noble agar (0.32%), thymidine (0.012 mM), hypoxanthine (0.04 mM), methotrexate (0.15 μg/ml), glycine (0.1 mM), penicillin (100 units/ml), and streptomycin (100 μg/ml)], showed 39–45% of the thymidine kinase activity exhibited by the homozygous LY-R Tk^+/Tk^+ strains. Since no LY-S Tk^+/Tk^- heterozygotes were isolated by this method, a culture of LY-S Tk^-/Tk^- cells was treated with 10 mM EtMes as described below. A total of 1×10^7 EtMes-treated LY-S Tk^-/Tk^- cells was plated (1×10^6 cells per dish) in the THMG soft agar medium. One LY-S colony (LY-S1) was formed and its cells exhibited 38% of the thymidine kinase activity of the homozygous Tk^+/Tk^+ LY-S strain.

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Abbreviation: EtMes, ethyl methanesulfonate.

Table 1. Thymidine kinase activity in extracts of L5178Y strains

Strain	Thymidine kinase phenotype	Thymidine kinase activity, pmol of [^{3}H]thymidine retained per μg of protein per min
LY-S1	+/−	0.088 ± 0.029
LY-R16	+/−	0.116 ± 0.018
LY-R83	+/−	0.134 ± 0.010
LY-S	+/+	0.234 ± 0.016
LY-R	+/+	0.296 ± 0.021
LY-TKS-14	−/−	0
LY-TKR-5	−/−	0

Numbers shown are the mean activities of five separately isolated clones of each strain that were cultured, harvested, and assayed for thymidine kinase activity.

Thymidine Kinase Assay. Aliquots containing 3×10^7 cells were centrifuged, resuspended in 2 ml of homogenizing medium (consisting of 0.1 M KH_2PO_4, pH 7/0.001 M EDTA/0.001 M 2-mercaptoethanol), and hand-homogenized (15 strokes) at 4°C. An aliquot was removed for the determination of protein concentration according to the method of Lowry *et al.* (15), and the remainder of the homogenate was frozen in a dry-ice bath and stored at −80°C. Samples were subsequently thawed and assayed for thymidine kinase activity by the method described by Breitman (16), in which phosphorylated thymidine is separated from the ^{3}H-labeled thymidine precursor by adsorption to DEAE filter paper disks. The measured enzyme activity increased linearly with increasing amounts of homogenate up to at least 300 μg of protein per ml. The thymidine kinase activities of the various strains are shown in Table 1.

Cleansing of Cells Prior to Experiments. Prior to experiments involving the determination of mutant frequency at the *Tk* locus, cells were incubated in THMG liquid medium for 24 hr to reduce the number of Tk^-/Tk^- cells in the starting culture, according to the method described by Clive and Spector (17).

Treatment of Cells with Radiation and Alkylating Agents. For treatment with x-radiation, aliquots of 50 ml of the cell suspensions (2×10^5 cells per ml) were irradiated in T60 flasks in growth medium at room temperature at the dose rate of 0.88 Gy/min using a GE maximar x-ray generator (250 kVp; 15 mA; a half-value layer, 1.5 mm of Cu). For treatment with UV radiation, cells were centrifuged, washed, and resuspended in phosphate-buffered saline (9 mM KCl/1.5 mM KH_2PO_4/138 mM NaCl/8 mM Na_2HPO_4, pH 7.3). Aliquots of 5 ml (containing 1–1.5 $\times 10^6$ cells per ml) were placed in 100-mm Petri dishes and exposed to 254-nm light emitted by a GE germicidal lamp (GBT5) at a fluence rate of 1.2 W/m^2, as measured with an Eppley thermopile. EtMes was dissolved in absolute ethanol immediately before use in each experiment and was added to the cells suspended in normal growth medium. The amount of ethanol in the medium of all treated and control cultures was adjusted to 1%. The cultures were incubated in the presence of EtMes for 1 hr at 37°C and then were centrifuged, washed two times, and resuspended in normal growth medium.

Assay of Cell Survival. Following treatment with radiation or alkylating agents, cell survival was measured as colony-forming ability. The cells were plated in soft agar medium (Fischer's medium containing 0.32% Noble agar), and the plates were incubated for 10 days at 37°C. Visible colonies were then counted by eye. Untreated cells gave plating efficiencies usually ranging between 80% and 100%.

Assay of Mutant Frequency. Following treatment with radiation or alkylating agents, aliquots of the treated and control cells were incubated at 37°C to allow expression of mutations. During the expression period, the total number of cells in each experimental group was kept at 4×10^6 or higher, and the cell density was maintained between 4×10^4 and 8×10^5 cells per ml to maintain exponential growth. Expression periods, found to give maximum frequencies of resistant mutants, were 2, 3, and 7 days for mutation at the *Tk*, Na^+,K^+-ATPase, and *Hprt* loci, respectively (data not shown). After the expression period, aliquots of the cells were plated for the determination of survival (as described above) and for the determination of mutant frequency in selective medium. Selective media consisted of soft agar medium containing trifluorothymidine (4 μg/ml), ouabain (0.5 mM), or thioguanine (5 μg/ml) for the assay of mutations at the *Tk*, Na^+,K^+-ATPase, and *Hprt* loci, respectively. Solutions and plates containing trifluorothymidine were kept out of direct light. The frequency of resistant mutants was not influenced by the number of cells that were plated in each selective medium (1×10^6 per plate for thioguanine and ouabain media and 3×10^5 for trifluorothymidine medium). Plates were incubated for 10–14 days at 37°C, and visible colonies were counted by eye. Mutant frequency was calculated as the number of colonies growing in the selective medium divided by the number of viable cells plated in selective medium (as determined from the plating efficiency of the cells in nonselective medium at the end of the expression period). The respective spontaneous mutation frequency was determined in each experiment using cells treated similarly to the experimental groups except for the treatment with radiation or EtMes.

Determination of Colony Size. The size of trifluorothymidine-resistant colonies was determined by using an Artek bacterial colony counter (model 870). Colonies >0.3 mm in diameter were countable by the Artek counter and were counted and subtracted from the total colony count (determined by eye) to give the number of small colonies. Manual counting of small and large colonies agreed well with the

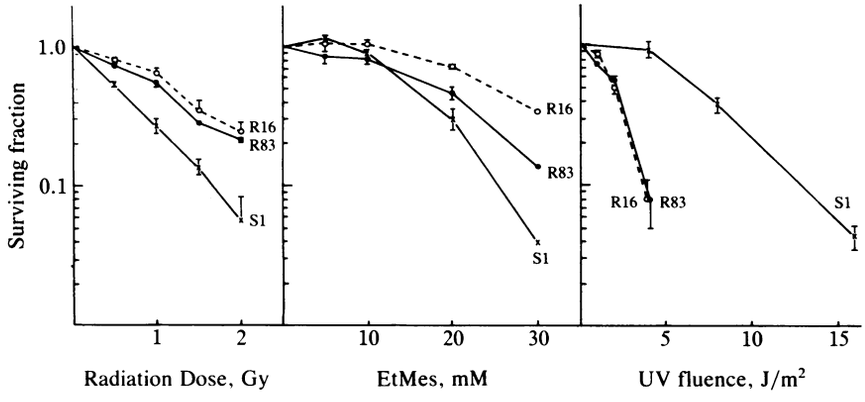

FIG. 1. Survival of heterozygous Tk^+/Tk^- strains following treatment with x-radiation (*Left*), EtMes (*Center*), or UV radiation (*Right*). Colony-forming ability was determined for cells plated immediately following treatment. Vertical bars show the standard errors of the means calculated from two to five replicate experiments. Each experiment usually involved treatment of all three strains with four different concentrations of one agent plus controls. The measurement of survival immediately following treatment thus usually entailed ≈60 plates.

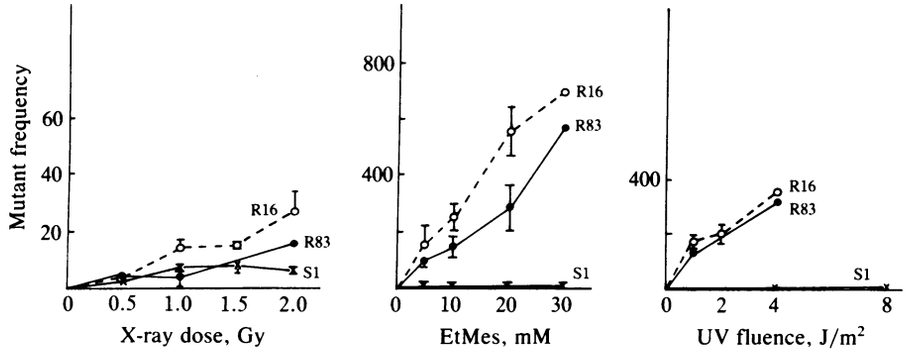

FIG. 2. Mutability of the heterozygous Tk^+/Tk^- strains at the *Hprt* locus. Mutant frequency is the number of thioguanine-resistant colonies per 10^6 viable cells. Note the difference in the scale of the ordinate in the case of treatment with x-radiation (*Left*). (*Center*) EtMes treatment. (*Right*) Treatment with UV radiation. Vertical bars show the standard errors of the means calculated from two to four replicate experiments. The spontaneous mutant frequencies have been subtracted. These frequencies per 10^6 viable cells (mean ± SEM; n = number of determinations) were as follows: LY-R83, 6.58 ± 2.03 (n = 9); LY-R16, 7.98 ± 1.21 (n = 7); LY-S1, 0.14 ± 0.13 (n = 8).

results obtained with the automatic colony counter. Small colonies are thus defined as colonies with diameters of <0.3 mm that are not counted by the Artex counter.

Banded Karyotype Analysis. Cells in the exponential phase of growth were fixed and analyzed cytogenetically as described by Hozier *et al.* (10). Two separate cultures were examined for each strain, and 15 banded metaphase spreads were examined per culture. "Blind" analyses were carried out in that the identity of each cytogenetic preparation was unknown to the cytogeneticist until the analysis was complete.

RESULTS

Sensitivity of the Heterozygous Tk^+/Tk^- Strains to the Cytotoxic Effects of X-Radiation and UV Radiation and to EtMes. As in the case of the homozygous Tk^+/Tk^+ LY-R and LY-S strains (4–6), the LY-S Tk^+/Tk^- heterozygote LY-S1 was more sensitive to the cytotoxic effects of ionizing radiation and EtMes but was more resistant to the cytotoxic effects of UV radiation than were the LY-R Tk^+/Tk^- heterozygotes LY-R16 and LY-R83 (Fig. 1).

Mutability at the *Hprt* Locus. As with the Tk^+/Tk^+ homozygous strains (4–6), strain LY-S1 was less mutable than strains LY-R83 and LY-R16 at the *Hprt* locus following treatment with EtMes as well as with UV radiation and 2-Gy x-radiation. The difference between the strains was greater following treatment with EtMes and UV radiation than with x-radiation, reflecting the high mutability of the LY-R heterozygotes after treatment with the first two agents (Fig. 2), as shown previously with the homozygous Tk^+/Tk^+ strains (4–6).

Mutability at the Na^+,K^+-ATPase Locus. Strain LY-S1 was somewhat less mutable at the Na^+,K^+-ATPase locus than either LY-R heterozygote following treatment with EtMes, whereas mutability following treatment with UV radiation was similar in the three strains (Fig. 3). [Previous studies indicate that ouabain resistance cannot be induced by x-radiation (18–20).] The mutant frequency was much lower at the Na^+,K^+-ATPase locus than at the *Hprt* locus for the LY-R strains, and therefore, the difference in mutability between the LY-R and LY-S strains was much less at the Na^+,K^+-ATPase locus than at the *Hprt* locus, as has been observed for the homozygous Tk^+/Tk^+ LY-R and LY-S strains (5).

Mutability at the *Tk* Locus. In contrast to the low mutability of strain LY-S1 at the *Hprt* and Na^+, K^+-ATPase loci, strain LY-S1 was found to be highly mutable at the *Tk* locus following treatment with all three agents (Fig. 4). The mutability of strain LY-S1 was as high or higher than that of the two LY-R heterozygous strains (Fig. 4). Also of interest was the observation that strain LY-R83 was poorly mutable at the *Tk* locus by x-radiation, though highly mutable at this locus following treatment with EtMes and UV radiation (Fig. 4). The mutant frequency was much higher at the *Tk* locus than at the *Hprt* locus for all three strains treated with radiation or EtMes (except in the case of strain LY-R83 treated with x-radiation).

Size of Trifluorothymidine-Resistant Colonies. A greater proportion of small colonies (<0.3 mm in diameter) was formed by LY-S1 trifluorothymidine-resistant cells than by LY-R trifluorothymidine-resistant cells after treatment with all three agents (Table 2). The greater proportion of small colonies formed by trifluorothymidine-resistant mutants of strain LY-S1 in comparison to trifluorothymidine-resistant mutants of strains LY-R83 and LY-R16 apparently is not due to an inherent difference in the growth rate of the parental Tk^+/Tk^- strains, since strain LY-S1 shows a shorter doubling time in liquid medium than do the heterozygous LY-R strains (data not shown). Our designation of small colonies (<0.3 mm in diameter) is different from that used by Moore *et al.* in the mouse lymphoma mutagen assay system (12), in which small colonies are defined as colonies with diameters of $<\approx 0.6$ mm.

Banded Karyotype Analysis. As in the case of homozygous Tk^+/Tk^+ strains, each of the three heterozygotes possesses only one intact X chromosome. The banding patterns of the chromosomes 11 of the three heterozygous strains are shown in Fig. 5. Strain LY-S1 contains two chromosomes 11 that show no differences in banding in comparison to the homozygous LY-S Tk^+/Tk^+ strain. Two chromosomes 11 are present in strain LY-R16: in chromosome 11a, band B2 is larger and darker than in the homozygous LY-R Tk^+/Tk^+ strain, whereas chromosome 11b shows an enlarged band C1. Only one intact chromosome 11 is present in strain LY-R83.

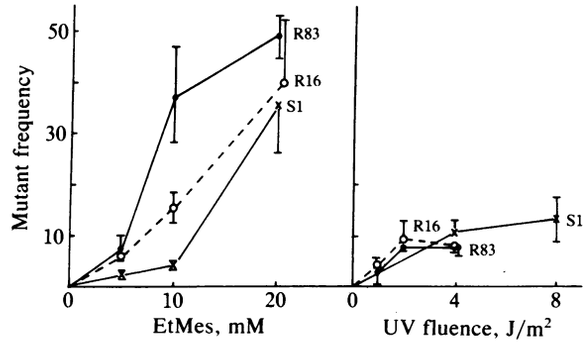

FIG. 3. Mutability of the heterozygous Tk^+/Tk^- strains at the Na^+,K^+-ATPase locus. Mutant frequency is the number of ouabain-resistant colonies per 10^6 viable cells. (*Left*) EtMes treatment. (*Right*) Treatment with UV radiation. Vertical bars show the standard errors of the means calculated from two to five replicate experiments. Spontaneous mutant frequencies have been subtracted. These frequencies per 10^6 viable cells (mean ± SEM; n = number of determinations) were as follows: LY-R83, 1.02 ± 0.39 (n = 5); LY-R16, 0.15 ± 0.17 (n = 4); LY-S1, 0.30 ± 0.18 (n = 5).

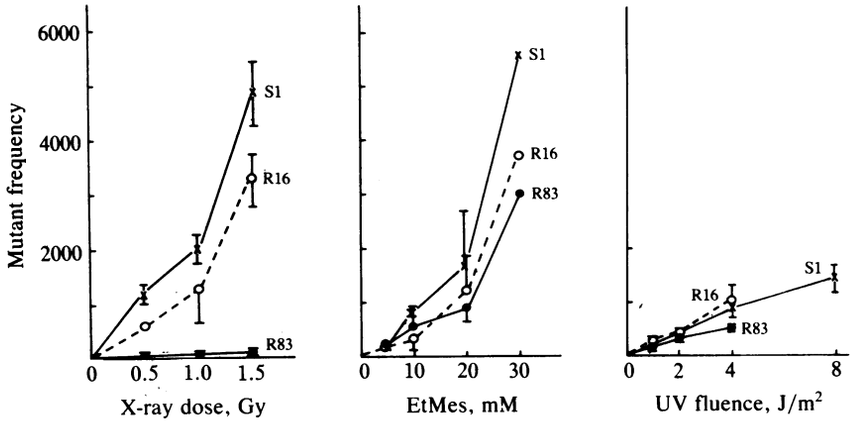

FIG. 4. Mutability of the heterozygous Tk^+/Tk^- strains at the *Tk* locus. Mutant frequency is the number of trifluorothymidine-resistant colonies per 10^6 viable cells. (*Left*) Treatment with x-radiation. (*Center*) EtMes treatment. (*Right*) Treatment with UV radiation. Vertical bars show the standard errors of the means calculated from two to five replicate experiments. Spontaneous mutant frequencies have been subtracted. These frequencies per 10^6 viable cells (mean ± SEM; n = number of determinations) were as follows: LY-R83, 17.0 ± 5.6 (n = 7); LY-R16, 106 ± 20 (n = 8); LY-S1, 143 ± 24 (n = 12).

This chromosome has a banding pattern similar to strain LY-R16 chromosome 11a with regard to the enlarged B2 band. Preliminary results indicate that the *Tk* gene is located between bands B2 and D, corresponding approximately to bands B5 and D of chromosome 11 in the L5178Y Tk^+/Tk^- 3.7.2C cell line (22).

DISCUSSION

The results presented above show that the Tk^+/Tk^- heterozygotes behave similarly to the Tk^+/Tk^+ homozygous strains with regard to (*i*) their sensitivity to the cytotoxic effects of x-radiation and UV radiation and EtMes and (*ii*) the low mutability at the *Hprt* locus of strain LY-S1 in comparison to the two LY-R heterozygotes. In contrast, strain LY-S1 is highly mutable at the *Tk* locus, showing a mutation frequency following treatment with all three agents equal to or greater than the mutability of the two LY-R heterozygotes. Thus, the mutability of strain LY-S1, as well as the difference in mutability between strains LY-R and LY-S, is locus specific.

The presence of more than one active *Hprt* gene in strain LY-S1 (and strain LY-S) could explain the low mutability of this strain at the *Hprt* locus. Although these strains exhibit only one recognizable X chromosome, the possibility of the presence of a second active *Hprt* gene on a marker chromosome has not been eliminated. However, the comparative enzyme activity of LY-R and LY-S cells (6) as well as the less-marked difference in mutability between the strains following treatment with x-radiation (4, 5, 23) indicate that other explanations are necessary to account for the low mutability of strain LY-S (and strain LY-S1) at the *Hprt* locus. We therefore suggest that a repair deficiency is responsible for the poor mutability of strain LY-S at the *Hprt* locus, as discussed below.

Strain LY-S (and its heterozygous Tk^+/Tk^- subclone LY-S1) is more sensitive to the cytotoxic effects of x-radiation and EtMes than are the LY-R strains. Further, in contrast to strain LY-R, strain LY-S has been found to lack split-dose recovery when exposed to x-radiation (3, 24), and its survival is less dose-rate dependent than is the case for strain LY-R (23). In addition, strain LY-S shows a higher incidence of chromosome aberrations following treatment with x-radiation than does strain LY-R (25). These characteristics of strain LY-S are consistent with the suggestion that LY-S cells are deficient in repair. This repair deficiency could result in the occurrence of multilocus lesions following treatment of LY-S cells with mutagens that ordinarily are not clastogens in repair-efficient cells and that do not form multilocus lesions in strain LY-R. The preponderance of small colonies formed by LY-S1 Tk^-/Tk^- mutants induced by a variety of mutagens is in agreement with this hypothesis, as is the previous finding of de Serres *et al.* (26) that two excision-repair deficient mutants of *Neurospora crassa* show a decrease in missense mutations and an increase in noncomplementing mutations (involving multilocus lesions) following treatment with UV radiation, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, or 4-nitroquinoline-1-oxide.

Although strain LY-S is more resistant to the cytotoxic effects of UV radiation than strain LY-R, UV-induced trifluorothymidine-resistant cells form a larger proportion of small colonies in trifluorothymidine medium in the case of strain LY-S than strain LY-R (Table 2). Therefore, it is possible that some type of UV-induced mutagenic lesion is not repaired normally in strain LY-S and that multilocus lesions are induced in this strain by UV radiation as well as by EtMes and x-radiation. Because these cells contain only one active X chromosome, lesions affecting essential genes neighboring the *Hprt* locus would result in a low recovery of viable thioguanine-resistant mutants.

With regard to the Na^+,K^+-ATPase locus, base-change mutations are necessary to inactivate the ouabain-binding site without inactivating ATPase activity. As expected, the recovery of viable mutations at this locus is lower in both strains than at the *Hprt* locus (where inactivation of the gene

Table 2. Colony size of trifluorothymidine-resistant mutants

Agent and treatment	LY-S1		LY-R16		LY-R83	
	Total colonies counted	% small colonies	Total colonies counted	% small colonies	Total colonies counted	% small colonies
Control (spontaneous)	5100	48	800	12	119	8
X-radiation, 0.5 Gy, 0.9 Gy/min	7600	61	900	18	188	17
UV radiation, 4 J/m², 1.2 W/m²	1000	39	350	15	101	15
EtMes, 10 mM, 1 hr	2250	49	750	25	1100	26

Colonies larger than 0.3 mm were counted with an Artek colony counter, and total colonies were counted by eye. The number of colonies smaller than 0.3 mm was divided by the total number of colonies. The designation of small colonies as those with diameters of <0.3 mm differs from the designation used in the mouse lymphoma mutagen assay system, in which small colonies are defined as those with diameters of <≈0.6 mm (12).'

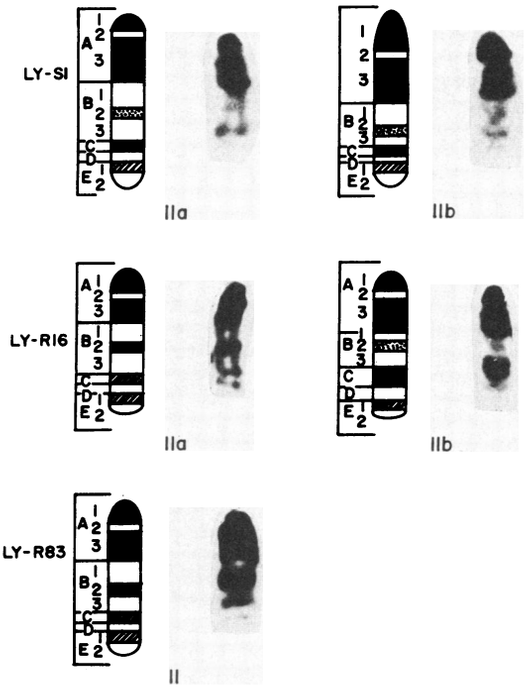

FIG. 5. Banding patterns of chromosome 11 in the three heterozygous Tk^+/Tk^- strains. Photographs of five individual chromosomes 11 from each strain were cut out, mounted, rephotographed, and enlarged six times for measurements. The total length of each chromosome and the width of each distinguishable band were measured and named in accordance with guidelines set by the Committee on Standardized Genetic Nomenclature for Mice (21). Schematic representations were constructed by using the LisaDraw graphics program and an Apple Lisa computer.

by any type of single-locus lesion should not affect viability in normal medium).

With regard to the *Tk* locus, we suggest that cells bearing multilocus lesions involving *Tk* and its neighboring genes on chromosome 11 are subject to a much higher recovery than in the case of multilocus mutations in the vicinity of the *Hprt* locus, because of the presence of the homologous chromosome 11 (theoretically normal except for its inactive *Tk* gene). A higher mutability at the *Tk* locus than at the *Hprt* locus has also been observed by Clive *et al.* (9) and Moore and Clive (27) in L5178Y cells (strain 3.7.2C). The small colonies formed by cells harboring multilocus lesions in one chromosome 11 reflect slow growth of these cells (12, 28, 29), possibly due to dosage effects in essential genes linked to the *Tk* locus. Clive *et al.* have suggested that the absence of small colony-forming $Hprt^-$ mutants in L5178Y strain 3.7.2C treated with agents causing multilocus lesions is due to the nonviability of these mutants (28). Analogous to cells containing multilocus lesions on the X chromosome, strain LY-R83, which possesses only one chromosome 11, is poorly mutable at the *Tk* locus by x-radiation, an agent known to produce multilocus lesions (30–38). It appears that the poor recovery is caused by multilocus lesions involving a target locus on a chromosome present as a single homolog. Similar results were obtained previously by Webber and de Serres (39), who reported that multilocus lesions are lethal in haploid strains of *N. crassa*.

In conclusion, we suggest that because of a repair deficiency, strain LY-S is subject to the formation of multilocus lesions following treatment with radiation or alkylating agents. These lesions lead to poor recovery of viable mutants when the target locus is closely linked to essential genes and situated on a hemizygous chromosomal region (e.g., the *Hprt* locus on the X chromosome). In contrast, a high recovery of multilocus LY-S mutants is obtained at the *Tk* locus because of the presence of active copies of the linked essential genes on the homologous chromosome 11. We further suggest that x-radiation is a relatively poor mutagen at loci linked to essential genes and situated on a hemizygous chromosomal region in repair-proficient and repair-deficient cells because of its tendency to form multilocus lesions.

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