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DNA Repair Defects Associated with Chromosomal Translocation Breaksite Regions

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Using an assay that measures the removal of UV-induced pyrimidine dimers in specific DNA sequences, we have found that the *Pvt-1*, immunoglobulin H-Cα (IgH-Cα), and IgL-κ loci are poorly repaired in normal B lymphoblasts from plasmacytoma-susceptible BALB/cAnPt mice. Breaksites in these genes are associated with the chromosomal translocations that are found in >95% of BALB/cAnPt plasmacytomas. In contrast to those from BALB/cAnPt mice, B lymphoblasts from plasmacytoma-resistant DBA/2N mice rapidly repair *Pvt-1*, IgH-Cα, and IgL-κ. Further, (BALB/cAnPt × DBA/2N)F_1 hybrids, which are resistant to plasmacytoma development, carry an efficient (DBA/2N-like) repair phenotype. Analysis of allele-specific repair in the IgH-Cα locus indicates that efficient repair is controlled by dominant, *trans*-acting factors. In the F_1 heterozygotes, these factors promote efficient repair of BALB/cAnPt IgH-Cα gene sequences. The same sequences are poorly repaired in the BALB/cAnPt parental strain. Analysis of the strand specificity of repair indicates that both strand-selective and nonselective forms of repair determine repair efficiency at the gene level in nonimmortalized murine B lymphoblasts.

There is considerable evidence that chromosomal translocations play a direct role in the development of many leukemias and lymphomas. It is generally believed that these abnormal recombinations contribute to neoplastic transformation by altering the structure, function, and/or expression of proto-oncogenes or antioncogenes. Chromosomal translocations that deregulate the c-*myc* proto-oncogene are nearly universally present in two B-lymphocytic tumors: spontaneous human Burkitt's lymphoma and pristane-induced mouse plasmacytomas. In both cases, the chromosome bearing the c-*myc*/*Pvt-1* gene complex is broken and reciprocally translocated to one of the three chromosomes that carry immunoglobulin (Ig) genes.

The molecular mechanisms responsible for the formation of nonrandom chromosomal translocations are not known. Molecular analysis of the translocation breaksites in a number of mouse plasmacytomas has shown that each breaksite is unique. Thus, the mutational process is not site specific. However, the distribution of breaksites is not random. Translocation breaksites on the Ig gene-bearing chromosomes involve either heavy-chain switch sequences, most commonly the switch region of the IgH-Cα gene (Sα), or the joining region of the heavy (J_H) or kappa light chain ($J_κ$). On the c-*myc*/*Pvt-1* chromosome (chromosome 15), the breaksites in the most frequent translocations, t(12;15), occur in the nontranslated 5′ end of c-*myc* or in 5′ flanking sequences. In plasmacytomas that carry the less common t(6;15) or t(15;16) variant forms of translocation (Ig light-chain translocations), the breaksites occur in a locus far downstream (~300 kb) of c-*myc*, designated *Pvt-1* for plasmacytoma variant translocation (6, 9, 22).

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In experimental mice, plasmacytomas are selectively induced in BALB/cAnPt (B/c) mice by the intraperitoneal injection of agents such as pristane (2, 6, 10, 14-tetramethylpentadecane) or plastic shavings, which cause chronic inflammatory reactions. More than 95% of B/c plasmacytomas carry one of the aforementioned translocations. In contrast to strain B/c, most other inbred strains of mice, e.g., DBA/2N (D/2), are highly resistant to pristane-induced plasmacytomas (24, 25).

The genetic and molecular mechanisms that confer susceptibility or resistance to plasmacytoma development are not known. The inducing agent (pristane) is not believed to be genotoxic, and there are no known metabolically activated derivatives of pristane (26). However, the peritoneal granulomatous tissue induced by pristane contains numerous macrophages and other inflammatory cells. This inflammatory tissue is believed to be a source of genotoxic agents (e.g., reactive oxygen intermediates) that induce damage in resident B lymphocytes (25, 26). We have previously found that phorbol ester-activated polymorphonuclear leukocytes, isolated from pristane-treated mice, induce high levels of DNA damage in coincubated B lymphoblasts (28). Other studies (7, 11, 31, 32, 35) have shown that exposure of mammalian cells to DNA-damaging agents increases the frequency of abnormal chromosomal rearrangements (deletions, translocations or inversions, and gene amplifications). These findings suggest that DNA damage may play a role in the formation of plasmacytoma-associated chromosomal translocations.

The response of mammalian cells to DNA-damaging agents is heterogeneous. One type of variability which may be important is the rate at which DNA lesions are removed from different portions of the genome. Variation in the rate of repair between genes and even between different portions of the same gene has been found in a large number of experimental cell lines (4, 5, 13, 16, 17, 19–21). Further, the rate of repair in a given gene can differ significantly in cells

from different inbred strains of mice. For example, UV-induced DNA damage in the 5′ flank of c-*myc* is rapidly repaired in B lymphoblasts from D/2 mice, whereas this same region is poorly repaired in B cells from B/c mice (1). The poorly repaired 5′ flanking region of c-*myc* in normal B/c B lymphoblasts is a breaksite region for chromosomal translocations in some B/c plasmacytomas.

In the present study, we wished to determine whether the repair defect in B/c cells is unique to the translocation-prone region 5′ of c-*myc* or is uniformly associated with gene regions that are involved in chromosomal translocations in B/c plasmacytomas. Methodologies to analyze gene-specific repair of lesions induced by reactive oxygen intermediates have not yet been developed. However, nucleotide excision repair of cyclobutane pyrimidine dimers can be quantified with a high degree of precision at the gene level. Nucleotide excision repair is the best characterized repair pathway, and many types of DNA lesions are repaired by this mechanism. Therefore, repair of UV-induced damage was selected as a model system to study the relationships between DNA repair defects and translocation breaksite regions. Since this investigation involves the study of cellular events that may occur prior to the formation of chromosomal translocations, repair efficiency was analyzed in nonimmortalized B lymphoblasts rather than in plasmacytoma tumor cell lines.

In the present study, repair efficiency was analyzed in three translocation breaksite regions, *Pvt-1*, IgH-Cα, and IgL-κ, in B lymphoblasts from plasmacytoma-susceptible and -resistant strains of mice. These breaksite regions, in conjunction with c-*myc*, constitute virtually all of the sites in which chromosomal translocations are found in B/c plasmacytomas. Strain-specific differences in repair efficiency as well as differences in the DNA strand specificity of repair are discussed.

MATERIALS AND METHODS

Isotopes, enzymes, and hybridization probes. [*methyl*-^{3}H]thymidine (>80 Ci/mmol) and [^{32}P]dCTP (3,000 Ci/mmol) were purchased from Du Pont-New England Nuclear. Restriction endonuclease *Bam*HI was purchased from Bethesda Research Laboratories, Gaithersburg, Md. The bacterial enzyme T4 endonuclease V is a pyrimidine dimer-specific endonuclease with a combined glycosylase and apyrimidinic endonuclease activity. The preparation and incision activity of the T4 endonuclease V has been described elsewhere (10).

Maps of the mouse *Pvt-1*, IgH-Cα, IgL-κ, and c-*abl* genes are shown in Fig. 1. The restriction fragments analyzed for repair are indicated under the maps. The DNA probe used to detect the 18-kb *Bam*HI fragment in the *Pvt-1* locus is a 1.4-kb *Eco*RI cDNA probe, Pvt-1-1 (15). The probe used to detect the 14-kb (B/c allele) and 11-kb (D/2 allele) *Bam*HI fragments in the IgH-Cα locus is a 800-bp *Eco*RI-*Xho*I genomic fragment kindly provided by John Shaughnessy, National Cancer Institute, Bethesda, Md. The probe used to detect the 12.5-kb *Bam*HI fragment in the IgL-κ locus is a 1.8-kb *Hin*dIII-*Xba*I genomic fragment (33). The probe used to detect the 20-kb *Bam*HI fragment in the mouse c-*abl* proto-oncogene is a 1,740-bp *Hin*dIII fragment from the Abelson murine leukemia provirus, purchased from Oncor, Gaithersburg, Md. Strand-specific probes for the *Pvt-1* gene were derived from a 2-kb *Bam*HI-*Hin*dIII fragment of AJ-7, a 6.5-kb *Pvt-1* cDNA (14) cloned into M13mp18 (antisense or template strand) or M13mp19 (sense strand). Radioactive probes generated from the antisense (M13mp18) and sense

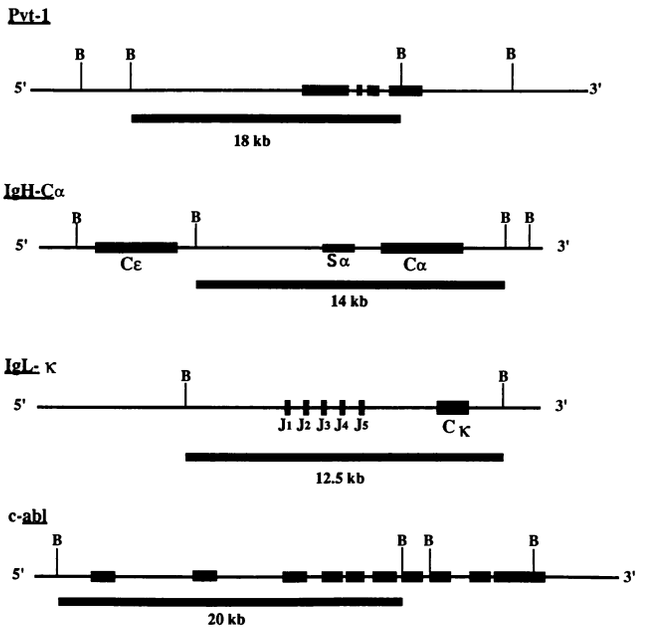

FIG. 1. Maps of the mouse *Pvt-1*, IgH-Cα, IgL-κ, and c-*abl* genes. The restriction fragments analyzed for repair are indicated by the bars under the maps. Exons are shown as solid boxes. B, *Bam*HI restriction site.

(M13mp19) strands are complementary to, and hybridize with, the transcribed and nontranscribed strands, respectively.

Cell culture. The preparation of normal mouse B lymphoblasts used in repair experiments has been previously described (1). The mice used in this study were obtained from the Laboratory of Genetics conventional mouse colony at Hazleton Laboratories, Gaithersburg, Md., under contract NOI CB-710-85. Briefly, single cell suspensions of whole spleens were cultured in RPMI 1640 (supplemented with 10% dialyzed fetal calf serum, 50 μg of lipopolysaccharide (LPS) [Difco, Detroit, Mich.] per ml, 4 mM L-glutamine, penicillin, and streptomycin) for 36 to 40 h to allow for growth and expansion of stimulated B cells. Aliquots of proliferating, nonadherent lymphoblasts were then harvested for use in repair experiments.

UV irradiation, DNA isolation, and restriction endonuclease digestion. UV irradiation of B lymphoblasts was performed as previously described (1). Cells were irradiated with UV light (predominantly 254 nm) at an incident dose rate of 1 J/m^2/s for 10 s. Cells used for determining the initial level of damage (0 h) were lysed immediately in lysis solution containing 10 mM Tris-HCl (pH 8.0)–1 mM EDTA–0.5% sodium dodecyl sulfate (SDS)–0.3 mg of proteinase K per ml. Cells used for repair analysis were resuspended in fresh, supplemented RPMI 1640 and incubated at 37°C for various periods. High-molecular-weight DNA was isolated and purified as previously described (3) and then completely digested with the *Bam*HI restriction endonuclease. DNA samples were then ethanol precipitated and resuspended in TE buffer (10 mM Tris-HCl, 1 mM EDTA). DNA concentration was determined by UV A_{260}.

Separation of replicated and nonreplicated DNAs. Nonreplicated (parental) and replicated DNAs from cells labeled with 10 μM bromodeoxyuridine, 1 μM fluorodeoxyuridine, and [^{3}H]thymidine were separated as previously described

(1). Genomic DNA replication was calculated from the number of counts per minute in replicated fractions divided by the total number of counts per minute in replicated and nonreplicated fractions.

Slot blots. DNA aliquots in 200 μl of TE buffer were mixed with 20 μl of 3 N NaOH. After 30 min at room temperature, 0.2 ml of ice-cold 2 M ammonium acetate was added. DNA samples were blotted onto nitrocellulose strips, equilibrated in 2 M ammonium acetate, by vacuum filtration in a Slot blot manifold (Hoefer Scientific Instruments). The strips were then washed in 2 M ammonium acetate and baked in a vacuum oven at 80°C for 60 min. Strips were prehybridized at 45°C for 1 day in 5 ml of Hybrisol 1 (Oncor).

Unscheduled DNA synthesis. Cells suspended in phosphate-buffered saline were irradiated with 10 J of UV light per m^2 (254 nm) in six-well tissue culture plates. Immediately after irradiation, 2 ml of RPMI 1640 medium containing 10% fetal calf serum, 20 μCi of [^{3}H]thymidine, and 5 mM hydroxyurea was added to the cells. After 12 h of repair incubation at 37°C, cells were lysed overnight with 0.5 ml of lysis solution. DNA samples were then spotted on filters and acid precipitated, and the radioactivity was determined. Unscheduled DNA synthesis was measured as the difference in labeling between irradiated samples and unirradiated controls.

T4 endonuclease V treatment. Aliquots (7 μg each) of DNA from each repair time point were either treated with T4 endonuclease V, which cleaves the DNA at sites that contain pyrimidine dimers, or mock treated with buffer alone as previously described (1).

Gel electrophoresis, Southern transfer, and hybridization conditions. T4-treated and untreated DNA samples were electrophoresed in denaturing agarose gels, blotted onto nylon membranes (Oncor), and hybridized with ^{32}P-labeled DNA probes as previously described (1). After hybridization, the membranes were washed, with a final wash in 0.1× SSPE (1× SSPE is 0.18 M NaCl, 10 mM $NaPO_4$, and 1 mM EDTA [pH 7.7])–0.1% SDS at 60°C for 1 h, and exposed to Kodak XAR-5 film. The amount of hybridization to the fragment of interest was measured by scanning densitometry of the autoradiogram. Dimer frequencies and percent repair were calculated as previously described (3).

RESULTS

DNA replication after UV irradiation. The fraction of replicated DNA in samples of UV-irradiated (10 J/m^2) LPS-stimulated B lymphoblasts (Table 1) was determined as previously described (1). The total amount of replicated DNA was very small (2 to 10%) in UV-irradiated cells. More importantly, the results indicate that B/c and D/2 B lymphoblasts show no significant differences in the rate of post-UV irradiation DNA replication either in the genome overall or in two different gene regions (Table 1). Experiments comparing the rate of dimer removal in total DNA and the rate in DNA that was fractionated into replicated and nonreplicated DNAs on density gradients have shown that the small amount of replicated DNA present in DNA samples does not detectably alter measurements of repair (data not shown). Thus, the repair experiments described in this study were performed on total genomic DNA, i.e., without separation of parental DNA from replicated DNA on CsCl density gradients.

Measurements of repair kinetics in mouse B lymphoblasts. DNA repair kinetics were determined by measuring the disappearance of T4 endonuclease-sensitive sites (T4-ESS) from defined gene fragments on quantitative Southern blots rather than on a direct determination of the removal of pyrimidine dimers. The terms T4-ESS and UV dimers are used interchangeably in the text.

TABLE 1. DNA replication after UV irradiation

Strain	% Total genomic DNA replicated after[a]:				% Gene-specific DNA replicated after 12 h[b]	
	2 h	4 h	8 h	12 h	*Pvt-1*	c-*abl*
D/2	2	4	8	11	12.3	16.2
B/c	4	5	9	10	8.6	12.4

[a] Radioactively labeled genomic DNA was fractionated into replicated and nonreplicated DNAs in neutral CsCl density gradients. Percent replication was calculated from the number of counts per minute in replicated peaks divided by the total number of counts per minute in replicated peaks and nonreplicated peaks.

[b] DNA from the 12-h post-UV treatment group was separated into replicated and nonreplicated fractions in neutral CsCl density gradients, slot blotted, and probed for specific gene regions. Percent replication was calculated as follows: density of replicated band/density of nonreplicated band × percent total DNA replication.

DNA repair efficiency was analyzed in cells from three strains of mice that differ in plasmacytoma susceptibility, B/c, D/2, and (B/c × D/2)F_1 hybrids (CDF1). In each of these strains, repair efficiency was analyzed in three breaksite regions located on different chromosomes, *Pvt-1* (chromosome 15), IgH-Cα (chromosome 12), and IgL-κ (chromosome 6). The strand specificity of repair was analyzed in one of these regions (*Pvt-1*), and in another (IgH-Cα) the allele specificity of repair was analyzed. As a control, repair kinetics were also measured in the c-*abl* proto-oncogene (mouse chromosome 2) which is not involved in murine chromosomal translocations.

Repair of UV damage in the *Pvt-1* locus. The formation and removal of T4-ESS in an 18-kb *Bam*HI fragment (Fig. 1) in the *Pvt-1* locus were measured at various times after UV irradiation of cells from B/c, D/2, and CDF1 mice (Table 2). The mean dimer frequency and percent repair values shown in Table 2 are the average of repeated Southern blots from several biological experiments (see Table 2, footnote *b*). The time course of repair for each mouse strain is shown in Fig. 3A.

B cells from plasmacytoma-resistant and plasmacytoma-susceptible mice differ significantly in their abilities to repair DNA sequences in the *Pvt-1* locus. B lymphoblasts from D/2 mice rapidly remove dimers from *Pvt-1*, such that by 12 h after irradiation about 65% of the damage has been removed. In contrast, cells from B/c mice show only a limited ability to repair this gene region. Negative repair values are often detected in B/c cells and are believed to be due to the formation of abasic sites in the DNA (1). In CDF1 hybrids, DNA damage is rapidly removed from the *Pvt-1* locus (55% by 12 h after irradiation). Thus, the F_1 hybrids carry a D/2-like DNA repair phenotype.

Strand specificity of repair in the *Pvt-1* locus. To determine the strand specificity of repair in the *Pvt-1* locus, Southern blots of B/c and D/2 DNAs were hybridized sequentially with single-stranded DNA probes that were complementary to the transcribed and nontranscribed strands (Table 3). The values shown in Table 3 were averaged from repeated Southern blots as described in Table 3, footnote *b*.

In B/c cells, the removal of pyrimidine dimers in the *Pvt-1* locus is strand specific (i.e., restricted to the transcribed strand). By 12 h after irradiation, 40% of the dimers had been

TABLE 2. DNA repair in the mouse *Pvt-1* locus

Strain	Time (h) after UV irradiation	Dimer frequency[a] ($10 J/m^2$) in expt:			Mean dimer frequency[b] (SD)	% Repair[c]
		1	2	3		
B/c	0	0.70	0.67	0.89	0.75 (0.12)	0
	2	0.86	0.71	0.87	0.81 (0.09)	−8
	4	0.85	0.65	0.78	0.76 (0.10)	−1
	8	0.71	0.63	0.63	0.65 (0.05)	13
	12	0.65	0.69	0.68	0.67 (0.02)	11
D/2	0	ND	0.73	0.75	0.74 (0.01)	0
	2	ND	0.57	0.65	0.61 (0.06)	18
	4	ND	0.54	0.64	0.59 (0.07)	20
	8	ND	0.44	0.36	0.40 (0.06)	46
	12	ND	0.31	0.21	0.26 (0.07)	65
CDF1	0	ND	0.89	0.81	0.85 (0.06)	0
	2	ND	0.62	0.67	0.65 (0.04)	24
	4	ND	0.45	0.51	0.48 (0.04)	43
	8	ND	0.53	0.38	0.46 (0.11)	46
	12	ND	0.43	0.34	0.39 (0.06)	55

[a] Average number of dimers per 18-kb fragment, calculated from the percentage of fragments that had no dimers (zero class) by using the Poisson expression as previously described (1, 3). Duplicate or triplicate Southern blots from each mouse strain were analyzed for each individual experiment. ND, not determined.

[b] Average from several biological experiments. For strain B/c, the mean dimer frequency was calculated from a total of seven Southern blots from three separate biological experiments. For strains D/2 and CDF1, mean dimer frequencies were calculated from a total of six Southern blots from two separate biological experiments with each strain.

[c] Calculated by using the mean dimer frequencies as previously described (1).

removed from the transcribed strand while in the nontranscribed strand no significant removal of damage was detected. As expected, at each time point, the amount of repair for both strands (Table 2) is similar to an average value calculated from the repair of the individual strands (Table 3). Despite the ability of B/c lymphoblasts to repair the transcribed strand in *Pvt-1* (strand-specific repair), the overall level (both DNA strands) of repair in the *Pvt-1* gene unit is still very low (11% from double-stranded probing [Table 2] or 13% from the average of the strand-specific probes [Table 3]). Compared with other selectively repaired genes (21), the *Pvt-1* locus is relatively poorly repaired in B/c cells.

In contrast to B/c cells, dimers are rapidly removed from both the transcribed and the nontranscribed strands of the *Pvt-1* locus in D/2 cells (Table 3). However, there is still an element of strand specificity in D/2 cells, since repair occurs significantly faster in the transcribed strand (69%) than in the nontranscribed strand (40%). As in B/c cells, the average of the percent repair of the transcribed and nontranscribed strands in D/2 cells (Table 3) is similar to that found when repair was analyzed with a probe that detects both DNA strands (Table 2).

Repair of UV damage in the IgH-Cα locus. In B/c plasmacytomas, the reciprocal partner of translocations that occur in the 5′ end of c-*myc* is invariably one of the Ig heavy-chain loci, usually in the repetitive switch sequences (Sα) of the IgH-Cα locus. To determine whether cells from B/c mice were repair deficient in this genomic region, the rate of repair was measured in a 14-kb *Bam*HI fragment that spans the entire Cα locus (Fig. 1). Data averaged from multiple Southern blots showing the formation and removal of T4-ESS from the IgH-Cα locus in the two parental strains and the F_1 hybrids are shown in Table 4. Autoradiograms of Southern blots showing the different alleles in B/c, D/2, and the F_1 hybrid mouse cells are shown in Fig. 2. The time course of repair for each mouse strain is shown in Fig. 3B.

The initial level of damage in DNA from D/2 cells is lower than that in DNA from B/c cells because of a restriction fragment length polymorphism (Fig. 2) between these two strains which results in a 21.4%-smaller target in D/2 cells (11-kb *Bam*HI fragment) than in B/c cells (14-kb *Bam*HI fragment). B/c mouse cells are repair deficient in the IgH-Cα locus compared with cells from D/2 mice. In B/c cells, no significant removal of UV dimers from this gene region was detected. In contrast, cells from D/2 mice clearly show the ability to repair the IgH-Cα locus (Table 4).

Repair efficiency in the IgH-Cα locus was also analyzed in B lymphoblasts from CDF1 mice. Since the probe used for the IgH-Cα locus detects both the 14-kb B/c allele and the 11-kb D/2 allele (Fig. 2), it is possible to analyze repair in both the B/c and the D/2 alleles separately within the same CDF1 cells. The initial level of damage in each of the CDF1 IgH-Cα alleles is similar to those in the corresponding alleles in the two parental strains. However, in the F_1 hybrids both the B/c and the D/2 alleles are repaired at rates that are similar to the rate of repair in the D/2 parental strain.

Repair of UV damage in the IgL-κ locus. The reciprocal partner of translocations that involve breaksites in the *Pvt-1* locus is invariably the region containing the joining gene segments of one of the Ig light-chain loci, usually J_κ. To determine whether cells from B/c mice were repair deficient in this genomic region, the rate of repair was measured in a 12.5-kb *Bam*HI fragment in the IgL-κ locus (Fig. 1). Data averaged from multiple Southern blots showing the formation and removal of T4-ESS in the two parental strains and the F_1 hybrids are shown in Table 5. The time course of repair for each mouse strain is shown in Fig. 3C.

As in the two breaksite regions described above, B/c lymphoblasts are repair deficient in comparison to D/2 lymphoblasts. In B/c cells UV dimers are not repaired in IgL-κ, while in D/2 cells 40% of the dimers are removed by 12 h after UV irradiation. In the F_1 hybrids, the IgL-κ gene is also rapidly repaired (42% by 12 h after irradiation). As with the other gene regions analyzed, CDF1 mice carry a D/2-like repair phenotype.

DNA repair kinetics in total genomic DNA in mouse B lymphoblasts. It is well established that rodent cells have little or no overall repair of cyclobutane pyrimidine dimers in total genomic DNA. As expected, measurements of unscheduled DNA synthesis indicated that LPS-stimulated lymphoblasts show little or no ability to repair bulk DNA. No significant differences between the abilities of UV-irradiated B/c and D/2 B lymphoblasts to repair total genomic DNA were detected ($[0.0 \pm 0.97] \times 10^3$ and $[0.7 \pm 0.64] \times 10^3$ cpm/10^6 cells, respectively; cells were irradiated at 10 J/m^2 and allowed to repair for 12 h). In contrast to B/c and D/2 cells, the repair-proficient human cell line GM38A, which was used as a positive control, showed a high level of unscheduled DNA synthesis (15.3×10^3 cpm/10^6 cells).

Repair of UV damage in the c-*abl* proto-oncogene. As an additional control, the rate of repair was measured in a 20-kb *Bam*HI fragment in the mouse c-*abl* gene, which is not involved in murine chromosomal translocations (Fig. 1). Data from multiple Southern blots showing the formation and removal of T4-ESS from the c-*abl* gene in the two parental strains and the F_1 hybrids are shown in Table 6. The time course of repair for each mouse strain is shown in Fig. 3D.

TABLE 3. Strand specificity of repair in the *Pvt-1* locus[a]

Strain	Strand probed	Time (h) after UV irradiation	Dimer frequency (10 J/m^2) in expt: 1	Dimer frequency (10 J/m^2) in expt: 2	Mean dimer frequency[b] (SD)	% Repair
B/c	Template	0	0.78	0.72	0.75 (0.04)	0
		2	0.79	0.61	0.70 (0.13)	7
		4	0.76	0.54	0.65 (0.16)	13
		8	0.58	0.52	0.55 (0.04)	27
		12	0.45	0.44	0.45 (0.01)	40
	Nontemplate	0	0.83	0.75	0.79 (0.06)	0
		2	0.84	0.73	0.79 (0.08)	0
		4	0.84	0.76	0.80 (0.06)	−1
		8	0.88	0.78	0.83 (0.07)	−5
		12	0.87	0.88	0.88 (0.01)	−11
	Average for the 2 strands	0			0.77	0
		2			0.75	3
		4			0.73	5
		8			0.69	10
		12			0.67	13
D/2	Template	0	0.70	0.69	0.70 (0.01)	0
		2	0.67	0.45	0.56 (0.16)	20
		4	0.53	0.52	0.53 (0.01)	24
		8	0.33	0.26	0.30 (0.05)	57
		12	0.22	0.21	0.22 (0.01)	69
	Nontemplate	0	0.84	0.78	0.81 (0.04)	0
		2	0.81	0.52	0.67 (0.20)	17
		4	0.62	0.67	0.65 (0.04)	20
		8	0.58	0.30	0.44 (0.20)	46
		12	0.46	0.51	0.49 (0.04)	40
	Average for the 2 strands	0			0.76	0
		2			0.62	18
		4			0.59	22
		8			0.37	51
		12			0.36	53

[a] Dimer frequency (per 18-kb fragment), mean dimer frequency, and percent repair were calculated as for Table 2.
[b] Average of four repeated Southern blots from two separate biological experiments with each strain.

In contrast to the *Pvt-1*, IgH-Cα, and IgL-κ loci, B cells from both B/c and D/2 mice efficiently repair the c-*abl* gene. The rate of repair is slightly, but significantly, faster in D/2 cells than in B/c cells, indicating that these two strains do show some difference in their abilities to repair c-*abl*. However, in contrast to the gene regions that are associated with translocation breaksites, B/c cells are able to repair a significant amount of the damage in the c-*abl* gene (38% by 12 h). Thus, B/c cells do not show the same marked deficiency in their ability to repair the c-*abl* gene as they do in their ability to repair the other gene regions analyzed. In the F_1 hybrids, the c-*abl* gene is also rapidly repaired (57% by 12 h after irradiation).

DISCUSSION

Strain-specific differences in repair efficiency in translocation breaksite regions. Using an assay that measures the removal of UV-induced T4-ESS from specific gene regions, we have shown that the *Pvt-1*, IgH-Cα, and IgL-κ loci are repaired differently in normal, nonimmortalized B lymphoblasts from B/c and D/2 mice. In cells from D/2 mice, 65, 40, and 40% of the dimers are removed from *Pvt-1*, IgH-Cα, and IgL-κ, respectively, by 12 h after irradiation. In B cells from B/c mice, little or no removal of dimers was detected in these gene regions.

Despite the clear differences in these cells in their abilities to repair *Pvt-1*, IgH-Cα, IgL-κ, and as shown previously (1), the 5′ flank of c-*myc*, B/c lymphoblasts do not appear to carry a general defect in their ability to excise pyrimidine dimers. Unlike the aforementioned genes, B/c cells are able to repair a significant amount of the damage induced in the c-*abl* proto-oncogene, albeit at a rate slightly slower than that in D/2 cells. We have previously reported that the rates of repair in the dihydrofolate reductase housekeeping gene and the 3′ portion of c-*myc* are similar in cells from the two strains of mice (1). Thus, the repair deficiency in B/c B cells appears to be confined to specific gene regions.

***trans*-acting factors control repair efficiency in translocation breaksite regions.** The rates of repair in the *Pvt-1*, IgH-Cα, and IgL-κ loci were also analyzed in CDF1 hybrids. CDF1 hybrids carry a plasmacytoma-resistant phenotype like that of the D/2 parental strain. In the F_1 hybrids, all three of these genes were rapidly repaired, in agreement with the results obtained with cells from the D/2 parental strain. This demonstrates that an efficient repair phenotype is the dominant trait, as is plasmacytoma resistance.

TABLE 4. DNA repair in the mouse IgH-Cα locus[a]

Strain (allele size [kb])	Time (h) after UV irradiation	Dimer frequency (10 J/m²) in expt: 1	2	3	Mean dimer frequency[b] (SD)	% Repair
B/c (14)	0	0.54	0.60	0.63	0.59 (0.05)	0
	2	0.48	0.61	0.62	0.57 (0.08)	3
	4	0.47	0.66	0.61	0.58 (0.10)	2
	8	0.54	0.56	0.62	0.57 (0.04)	3
	12	0.51	0.54	0.68	0.57 (0.09)	3
D/2 (11)	0	ND	0.49	0.41	0.45 (0.06)	0
	2	ND	0.39	0.36	0.38 (0.02)	16
	4	ND	0.33	0.32	0.33 (0.01)	27
	8	ND	0.32	0.33	0.33 (0.01)	27
	12	ND	0.29	0.24	0.27 (0.04)	40
CDF1 × BALB/c (14)	0	ND	0.62	0.58	0.60 (0.03)	0
	2	ND	0.56	0.61	0.59 (0.04)	2
	4	ND	0.49	0.40	0.45 (0.06)	25
	8	ND	0.47	0.34	0.41 (0.09)	32
	12	ND	0.35	0.38	0.37 (0.02)	38
CDF1 × D/2 (11)	0	ND	0.49	0.46	0.48 (0.02)	0
	2	ND	0.38	0.43	0.41 (0.04)	15
	4	ND	0.33	0.28	0.31 (0.04)	35
	8	ND	0.41	0.25	0.33 (0.11)	31
	12	ND	0.27	0.15	0.21 (0.08)	56

[a] Dimer frequencies in the 14-kb B/c allele and the 11-kb D/2 allele, mean dimer frequency, and percent repair were calculated as for Table 2. ND, not determined.

[b] For strain B/c, the mean dimer frequency was calculated from a total of six Southern blots from three separate biological experiments. For strains D/2 and CDF1, mean dimer frequencies were calculated from four repeated Southern blots from two separate biological experiments with each strain.

Analysis of repair in the F_1 hybrids also demonstrates that strain-specific repair differences are a function of the cellular milieu rather than a function of the origin or primary sequence of the DNA. B/c and D/2 mice are polymorphic at the IgH-Cα locus. In the F_1 hybrids, both the B/c and the D/2 alleles are detected and can be separately analyzed for repair efficiency. In the B/c parental strain IgH-Cα gene sequences are not repaired, while in the F_1 hybrids the same B/c sequences are efficiently repaired. This demonstrates that *trans*-acting factors, expressed by dominant D/2 genes, can overcome the repair block in B/c IgH-Cα sequences.

Molecular mechanisms involved in controlling strain-specific differences in the fine structure of repair. Several studies have shown that pyrimidine dimers are repaired more rapidly in actively transcribed genes than in inactive genes (16, 17, 23). The association between transcription and repair at the gene level is further supported by the findings that repair enzymes are selectively directed to the template strand of the DNA in organisms as diverse as yeasts, bacteria, and mammals (20, 21, 29) and by the isolation of a transcription-repair coupling factor from *Escherichia coli* cells (27). However, transcription-coupled repair mechanisms do not appear to play a central role in determining strain-specific repair differences in B lymphoblasts. B/c and D/2 B lymphoblasts do not differ in their steady-state levels of RNA transcripts in the *Pvt-1* gene (data not shown). Extensive analysis of run-on transcripts in both the c-*myc* and the *Pvt-1* genes has shown no strain-specific differences in transcriptional activity (unpublished data). Further, analysis of repair in the template and nontemplate strands of the *Pvt-1* gene suggests that there are two types of repair of UV dimers in mouse B lymphoblasts, template strand specific and strand independent (non-template specific).

In B/c cells, repair enzymes are directed only to the template strand of the *Pvt-1* gene. This indicates that B/c cells utilize only template strand-specific repair mechanisms to remove damage from the *Pvt-1* gene. It is reasonable to assume that this form of repair may be coupled to RNA transcription. In D/2 cells, dimers are rapidly removed from both the template and the nontemplate strands of the *Pvt-1* gene. This is clearly different from the pattern of repair activity in B/c cells and results in a more complete removal of dimers from the *Pvt-1* gene unit in D/2 cells. However, D/2 cells also show a strand bias of 29% for the template strand (40% in the nontemplate strand versus 69% in the template strand). In terms of strand specificity, D/2 cells cannot be described as being either wholly strand specific or non-strand specific. Rather, repair appears to be a combination of both strand-selective and nonselective processes.

One possible explanation for this combined type of repair is that both template-specific and strand-independent (non-template-specific) repair mechanisms are utilized in D/2 cells. Template-specific repair mechanisms are implicated because of the strand bias in D/2 cells. Template-specific repair suggests that a portion of the repair in *Pvt-1* may be due to transcription-coupled repair mechanisms. Strand-independent mechanisms are also implicated because we have previously found a gene region in D/2 cells where dimers are repaired by a strand-independent mechanism. In the 5′ flank of c-*myc*, both DNA strands are rapidly repaired. However, in contrast to *Pvt-1*, there is no strand bias in the 5′ flank of c-*myc* (1); rather, both the template and the nontemplate strands are repaired at the same rate. This rate is the same as that in the nontemplate strand in the *Pvt-1* locus. Thus, the pattern of repair in the *Pvt-1* locus suggests that there is an accelerated repair of the template strand which is superimposed on an already efficient strand-independent repair process. This combined type of repair may be

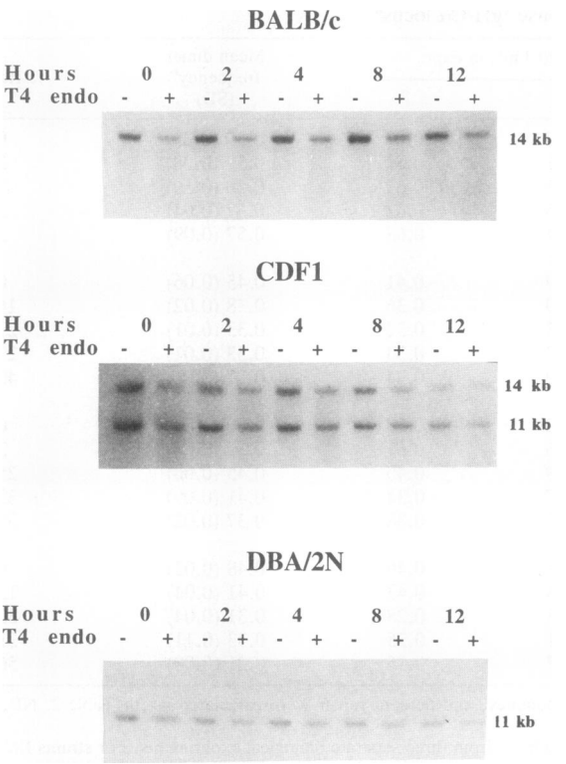

FIG. 2. Autoradiograms of Southern blots probed for the IgH-Cα gene. DNAs isolated from UV-irradiated B lymphoblasts from D/2, CDF1, and B/c mice were digested with *Bam*HI, separated on denaturing agarose gels, and transferred to support membranes as described in Materials and Methods. The locations of the B/c (14-kb) and D/2 (11-kb) alleles are indicated on the right. DNA from cells that were allowed to repair DNA damage for the indicated times was either treated with T4 endonuclease (endo) V (+) or mock treated with buffer alone (−).

the murine equivalent of the different types of repair that can be superimposed in the adenosine deaminase gene in human severe combined immunodeficiency and wild-type cells (34).

It is thought that the biological function of template strand-specific repair is to promote cell survival by facilitating the rapid recovery of RNA synthesis after DNA damage. There is reason to believe that strand-independent repair mechanisms may also serve a biological function—to maintain genetic stability rather than to promote cellular survival. Recent studies in rodent cells have shown that selectively repaired genes (template strand restricted) can be readily mutagenized by an accumulation of premutagenic lesions in the poorly repaired, nontemplate strand (8, 18, 36). This indicates that selective repair mechanisms do not provide cells with an adequate means of maintaining genetic stability. The directed repair of certain nontemplate sequences, as in D/2 cells, is one mechanism that murine cells may employ to counteract the mutagenic effects of template strand-restricted repair.

Correlations between DNA repair processes and abnormal Ig gene rearrangements. *Pvt-1*, IgH-Cα, IgL-κ, and the 5′ flank of c-*myc* are all hotspots for translocation breaksites in B/c plasmacytomas. The formation of chromosomal translocations that activate c-*myc* is believed to be a critical event in the development of plasmacytomas. The finding that all four of these gene regions are poorly repaired in B/c lym-

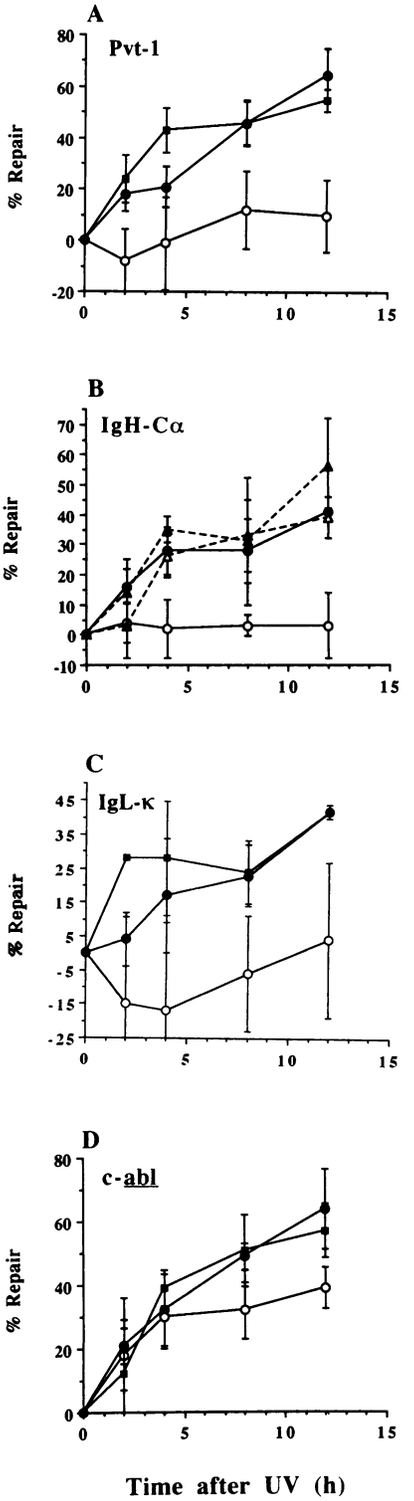

FIG. 3. Removal of dimers as a function of time in B lymphoblasts from D/2, B/c, and CDF1 (F_1) mice. Percent repair was calculated from the mean dimer frequencies shown in Tables 2 (*Pvt-1*), 4 (IgH-Cα), 5 (IgL-κ), and 6 (c-*abl*) as previously described (1). The data are averages (with standard deviations [bars]) of multiple Southern blots. ●, D/2; ○, B/c; ■, CDF1; △, CDF1, B/c allele; ▲, CDF1, D/2 allele.

TABLE 5. DNA repair in the mouse IgL-κ locus[a]

Strain	Time (h) after UV irradiation	Dimer frequency (10 J/m²) in expt: 1	2	3	Mean dimer frequency[b] (SD)	% Repair
B/c	0	0.49	0.48	0.50	0.49 (0.01)	0
	2	0.69	0.43	0.57	0.56 (0.13)	−14
	4	0.72	0.50	0.50	0.57 (0.12)	−16
	8	0.60	0.52	0.44	0.52 (0.08)	−6
	12	0.50	0.34	0.58	0.47 (0.12)	4
D/2	0	ND	0.45	0.49	0.47 (0.03)	0
	2	ND	0.40	0.49	0.45 (0.06)	4
	4	ND	0.43	0.35	0.39 (0.06)	17
	8	ND	0.38	0.34	0.36 (0.03)	23
	12	ND	0.27	0.28	0.28 (0.01)	40
CDF1	0	ND	0.64	0.70	0.67 (0.04)	0
	2	ND	0.48	0.50	0.48 (0.03)	28
	4	ND	0.47	0.49	0.48 (0.01)	28
	8	ND	0.54	0.49	0.51 (0.04)	24
	12	ND	0.40	0.38	0.39 (0.01)	42

[a] Dimer frequency per 12.5-kb fragment, mean dimer frequency, and percent repair were calculated as for Table 2. ND, not determined.
[b] For strain B/c, the mean dimer frequency was calculated from a total of six Southern blots from three separate biological experiments. For strains D/2 and CDF1, mean dimer frequencies were calculated from four repeated Southern blots from two separate biological experiments with each strain.

phoblasts demonstrates a consistent correlation between repair deficiency and translocation breaksite regions.

Associations between DNA repair defects and error-prone Ig gene recombination have been described for other experimental systems. Several studies have shown that mutations that confer DNA double-strand break repair defects in CHO cells (30) and in *scid* mice (2, 12) also impair Ig gene recombination. These double-strand break repair defects prevent the accurate rejoining of both coding and signal junctions and lead to immunodeficiency in *scid* mice.

In contrast to *scid* mice, B/c mice are not immunodeficient, and errors in Ig gene recombination are detected only in mice given plasmacytoma-inducing agents. Further, B/c mice do not carry an overall defect in excision repair (1; also this study) or in single-strand break repair (27a) and show only a slightly slower rate of double-strand break repair than that in D/2 mice (27a). Rather, the repair defect in B/c cells appears to involve the inability of repair enzymes to gain access to several gene regions that are involved in chromosomal translocations. This suggests that, in B/c cells, the relationship between DNA repair mechanisms and chromosomal translocations is indirect. One possibility is that defective repair mechanisms cause an accumulation of lesions in specific regions of the genome. This accumulation of damage may secondarily predispose such regions to abnormal recombinations by altering the activity or binding affinity of repair or recombination complexes or by creating nicks or breaks in the DNA. Alternatively, it is possible that the repair deficiency in B/c cells is simply a marker of other differences in chromatin structure which are associated, strain specifically, with translocations. These differences in chromatin structure could have secondary effects on the accessibility of these regions to repair enzymes. Nonetheless, the finding that DNA repair efficiency is closely correlated with translocation breaksites indicates that the accessibility of specific gene regions to DNA repair enzymes may be an important biomarker of genomic instability.

TABLE 6. DNA repair in the mouse c-*abl* locus[a]

Strain	Time (h) after UV irradiation	Dimer frequency (10 J/m²) in expt: 1	2	3	Mean dimer frequency[b] (SD)	% Repair
B/c	0	0.81	0.84	0.82	0.82 (0.02)	0
	2	0.74	0.83	0.57	0.71 (0.13)	18
	4	0.63	0.75	0.48	0.62 (0.14)	30
	8	0.63	0.65	0.48	0.58 (0.09)	32
	12	0.55	0.54	0.45	0.51 (0.06)	39
D/2	0	ND	0.71	0.72	0.72 (0.01)	0
	2	ND	0.53	0.60	0.57 (0.05)	21
	4	ND	0.42	0.55	0.49 (0.09)	32
	8	ND	0.34	0.39	0.37 (0.04)	49
	12	ND	0.32	0.19	0.26 (0.09)	64
CDF1	0	ND	0.84	0.79	0.82 (0.03)	0
	2	ND	0.60	0.59	0.60 (0.01)	27
	4	ND	0.56	0.47	0.52 (0.06)	37
	8	ND	0.47	0.32	0.40 (0.11)	51
	12	ND	0.31	0.27	0.35 (0.06)	57

[a] Dimer frequency per 20-kb fragment, mean dimer frequency, and percent repair were calculated as for Table 2. ND, not determined.
[b] For strain B/c, the mean dimer frequency was calculated from a total of six Southern blots from three separate biological experiments. For strains D/2 and CDF1, mean dimer frequencies were calculated from four repeated Southern blots from two separate biological experiments with each strain.

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