Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 4061–4065, May 1991
Cell Biology

A link between double-strand break-related repair and V(D)J recombination: The *scid* mutation

(DNA repair/x-irradiation)

ERIC A. HENDRICKSON*, XIAO-QIANG QIN*, EDWARD A. BUMP†, DAVID G. SCHATZ‡, MARJORIE OETTINGER‡, AND DAVID T. WEAVER*§

*Division of Tumor Immunology, Dana-Farber Cancer Institute, and Department of Microbiology and Molecular Genetics, Harvard Medical School, and †Joint Center for Radiation Therapy, Harvard Medical School, Boston, MA 02115; and ‡The Whitehead Institute and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139

Communicated by Arthur B. Pardee, February 14, 1991 (received for review January 10, 1991)

ABSTRACT **We show here that mammalian site-specific recombination and DNA-repair pathways share a common factor. The effects of DNA-damaging agents on cell lines derived from mice homozygous for the *scid* (severe combined immune deficiency) mutation were studied. Surprisingly, all scid cell lines exhibited a profound hypersensitivity to DNA-damaging agents that caused double-strand breaks (x-irradiation and bleomycin) but not to other chemicals that caused single-strand breaks or cross-links. Neutral filter elution assays demonstrated that the x-irradiation hypersensitivity could be correlated with a deficiency in repairing double-strand breaks. These data suggest that the *scid* gene product is involved in two pathways: DNA repair of random double-strand breaks and the site-specific and lymphoid-restricted variable-(diversity)-joining [V(D)J] DNA rearrangement process. We propose that the *scid* gene product performs a similar function in both pathways and may be a ubiquitous protein.**

Mice homozygous for the *scid* (severe combined immune deficiency) mutation lack a functional immune system but otherwise appear normal (1). The absence of B and T lymphocytes in scid mice is due to a defect in the site-specific V(D)J recombination pathway that is responsible for the somatic assembly of immunoglobulin and T-cell receptor genes. Analysis of scid variable (diversity) joining [V(D)J] recombination events has shown that large deletions, which remove all or most of the coding sequences of immunoglobulin or T-cell receptor genes, accompany the rearrangements and result in nonfunctional lymphoid cells (2–4). Furthermore, examination of model rearrangement templates (recombinant retroviruses and plasmids) introduced into scid lymphoid cells has recapitulated the aberrant deletional rearrangements (4–6). Thus, we and others have proposed that the *scid* gene product is an integral component of the V(D)J recombinase complex.

Mutations that affect site-specific and general recombination frequently also affect the pathways responsible for repairing DNA double-strand breaks (DSBs) due to chromosome damage (7). This overlap of recombination and DSB-repair pathways is presumed to result from the postulated role of double-stranded ends as structural intermediates in many types of recombination and repair (for review, see ref. 8). DSBs can also be generated by a number of DNA-damaging agents, the most common of which is ionizing radiation. X-ray-induced DSBs can stimulate chromosomal deletions and aberrant rearrangements and are lethal if not repaired (9). We show here that the similarity between recombination and DSB-repair pathways extends to mammalian cells affected by the *scid* mutation. scid cells were found to be hypersensitive specifically to agents that make DSBs. In addition, a dynamic assay for DNA repair demonstrated that the *scid* mutation severely diminished DSB repair. We propose that the *scid* gene product performs a similar function in both the V(D)J recombination and DSB-repair pathways.

MATERIALS AND METHODS

Fibroblastic Cell Lines. Fibroblast cell lines were established from scid neonates, either by spontaneous or simian virus 40-mediated immortalization. SC 3T3/W is a nonclonal population of spontaneously transformed scid fibroblasts isolated by a passage and cell density 3T3 protocol (10). SCGR-8 and SCGR-11 are clonal derivatives of SC 3T3/W cells. Alternatively, 12 clonal cell lines (SCSV-1–SCSV-12) were generated by infection of the mixed scid neonate fibroblast culture at passage 1 with wild-type simian virus 40. The doubling times and plating efficiencies of relevant scid and wild-type cell lines were as follows: SC 3T3/W, 19.5 hr and 51%; SCGR-8, 25.2 hr and 52%; SCSV-9, 27.1 hr and 36%; BALB 3T3, 31.0 hr and 64%; and NIH 3T3, 19.4 hr and 65%. There was no obvious relationship between cell-proliferation rates and radiation sensitivity.

Cell-Survival Assays with DNA-Damaging Agents. Exponentially growing scid and wild-type fibroblasts were irradiated with a 250 kilovolt peak x-ray machine at 113.6 rads/min (1 rad = 0.01 Gy) with a 0.35-mm copper filter. Cells were then subcultured in duplicate. Between 10 and 14 days after irradiation, the plates were washed with 5 ml of phosphate-buffered saline, fixed for 15 min [10% (vol/vol) HOAc/10% (vol/vol) MeOH, 3 ml] and stained for 15 min [0.4% crystal violet/20% (vol/vol) EtOH, 5 ml]. Colonies (>50 cells) were scored; colony sizes for the various cell lines were comparable. Pre-B-cell lines were irradiated in 25-cm^2 flasks and subcultured in duplicate; surviving colonies were counted 10–14 days after irradiation.

Before treatment with other DNA-damaging agents, exponentially growing fibroblasts were washed with 5 ml of serum-free medium, aspirated, and replaced with 2 ml of serum-free medium containing mutagens at various concentrations. After 1 hr at 37°C, the plates were washed in medium plus serum, subcultured, and processed as described above. For UV irradiation, plates were washed in phosphate-buffered saline, aspirated, and immediately exposed to 254 nm of UV light (0.34 $J \cdot mm^{-2} \cdot s^{-1}$) for the appropriate intervals. Medium was added back to the plates, the cells at the edge of the plate were removed with a rubber policeman, and

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: DSB, double-strand break; MMS, methyl methanesulfonate; MMC, mitomycin C; D_{37}, irradiation dose required to reduce cell survival to 37%; V(D)J, variable (diversity) joining.
§To whom reprint requests should be addressed.

the remaining cells were subcultured and processed as described above. Bleomycin (Mead/Johnson), mitomycin C (MMC, Sigma), and methyl methanesulfonate (MMS, Kodak) were prepared fresh (17).

Neutral Filter Elution. Exponentially growing fibroblasts were labeled for 44 hr with either 3 μCi of [^{3}H]thymidine (ICN, 2 Ci/mmol; 1 Ci = 37 GBq) or 1.5 μCi of [^{14}C]thymidine (Amersham, 51.4 mCi/mmol) per 100-mm plate. Four hours before the experiment, the medium was replaced with unlabeled medium. All irradiations were done on ice in the presence of 2% oxygen at a dose rate of 454.5 rads/min. Neutral filter elution was done essentially as described (11), except that the pH of the elution buffer was 9.0 instead of 9.6. After elution, the column fractions and the filters were processed for scintillation counting as described (12). Neutral filter elution data are presented as the fraction of ^{3}H-labeled DNA retained on the filter versus the fraction of ^{14}C-labeled control DNA retained on the filter. The percentage of DSBs repaired was calculated as follows: % DSB repaired = {1 − [(slope at time t − slope of unirradiated sample) ÷ (slope at 0 min of repair − slope of unirradiated sample)]} × 100 (13).

RESULTS

scid Cells Are X-Ray Sensitive. We investigated the ability of nonlymphoid scid cells to repair random DSBs in DNA generated by ionizing radiation. Fibroblast cell lines were established from scid neonates by either spontaneous or simian virus 40-mediated transformation (*Materials and Methods*). scid (SC 3T3/W, SCGR-8, and SCSV-9) and wild-type (BALB 3T3 and NIH 3T3) fibroblast cell lines were irradiated with x-rays (0–900 rads), and surviving colonies were scored. Fibroblasts from scid mice were highly x-ray sensitive relative to wild-type fibroblast controls (Fig. 1*A*). The three scid lines represented the range of x-ray sensitivities observed: the most sensitive line, SCSV-9; a typically sensitive line, SCGR-8; and the least sensitive line SC 3T3/W. Twelve other scid fibroblast cell lines had survival curves similar to SCGR-8 (data not shown). The irradiation dose required to reduce cell survival to 37% (D_{37}) is an index used to compare x-ray sensitivities. A 2-fold decrease in D_{37} values is considered significant, whereas a ≥5-fold effect represents extreme x-ray sensitivity and has only been documented for a few x-ray-sensitive cell lines (14–16). The D_{37} values of the scid lines were at least 2.5- to 7.5-fold lower than the wild-type fibroblast cell lines (Table 1). Therefore, the *scid* mutation rendered fibroblasts as sensitive to x-irradiation as any of the known x-ray-sensitive mammalian cell mutants.

The *scid* mutation promotes aberrant V(D)J rearrangement events in progenitor-stage B and T cells and in Abelson murine leukemia virus-transformed pre-B-cell lines (2, 3, 6). scid, wild-type, and scid heterozygous ($sc/+$) pre-B-cell lines were irradiated with x-rays (0–900 rads) and scored for cell survival after 2 weeks. The scid cell line 8D was clearly more radiosensitive than either $sc/+$ or wild-type control lymphoid lines at all x-ray doses tested (Fig. 1*B*). The D_{37} value for 8D

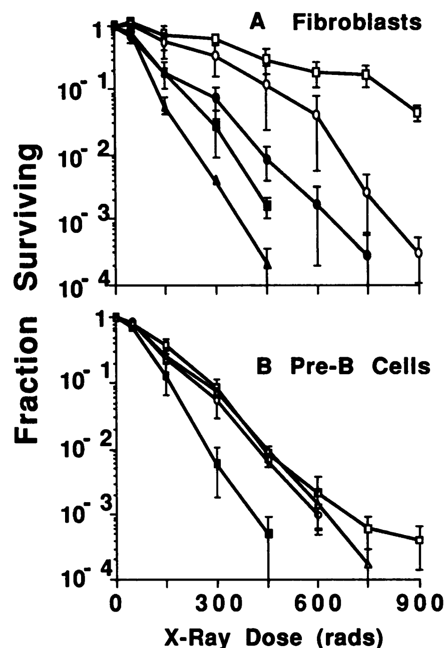

FIG. 1. scid cells are hypersensitive to x-rays. (*A*) scid and wild-type fibroblasts were exposed to the indicated doses of x-rays, and the fraction of cells surviving to form colonies was scored. □, NIH 3T3; ○, BALB 3T3; ●, SC 3T3/W; ■, SCGR-8; and ▲, SCSV-9. (*B*) scid and wild-type pre-B-cell lines were irradiated and plated at various dilutions; colonies were counted 10–14 days after irradiation. ■, Homozygous scid 8D cells; ○, homozygous wild-type 38B9; □, homozygous wild-type 300-19 cells; and △, heterozygous (*scid*/wild-type) SN-1 cells. Symbols represent the mean of two to four independent experiments, and the error bars represent the range. Profiles terminate at the dose after which survival was $<1 \times 10^{-4}$. D_{37} values in rads for the pre-B-cell lines: 8D cells, 87.5 (±17.5); SN-1 cells, 120 (±5.0); 38B9 cells, 115 (±5.0); and 300-19 cells, 147.5 (±17.5).

cells (87.5 rads) was comparable to the average level observed in scid fibroblasts (Table 1). The wild-type pre-B cells were more x-ray sensitive than wild-type fibroblasts (Fig. 1 *A* and *B*), presumably because cells of the bone-marrow compartment are generally radiosensitive. An Abelson murine leukemia virus pre-B-cell line derived from a $sc/+$ mouse (SN-1), which was phenotypically wild type for V(D)J rearrangement (6), also had a wild-type phenotype for cell viability after x-ray treatment (Fig. 1*B*). Thus, the autosomal recessive properties of the *scid* mutation were confirmed for the repair defect.

scid Cells Are Deficient in DSB Repair. Although x-rays generate several types of DNA lesions including single-stranded breaks and DSBs, the cell lethality of x-ray damage correlates specifically with the level of DSBs (9). Thus, we next examined whether the x-ray hypersensitivity of scid cells was due to a reduced ability to repair DSBs. To test this hypothesis, we used a neutral filter elution method (13), in which the extent of DNA repair in irradiated cells is measured

Table 1. Sensitivity of scid and wild-type fibroblast lines to DNA-damaging agents

	D_{37}				
Cell line	X-ray, rads	Bleomycin, units/ml	UV, J/mm^2	MMS, mM	MMC, μg/ml
NIH 3T3	465 (±78)	42.5 (±2.5)	4.2 (±2.4)	1.50 (±0.06)	0.75 (±0.10)
BALB 3T3	257 (±111)	69.0 (±19.0)	4.3 (±0.2)	1.17 (±0.11)	1.14 (±0.14)
SC 3T3/W	98 (±8)	21.0 (±3.0)	2.2 (±0.5)	0.91 (±0.05)	1.38 (±0.12)
SCGR-8	89 (±30)	ND	3.0 (±1.1)	ND	ND
SCSV-9	63 (±24)	18.5 (±0.5)	2.2 (±0.3)	1.06 (±0.06)	1.31 (±0.05)

All values are the means of two to four experiments (±SD). ND, not done.

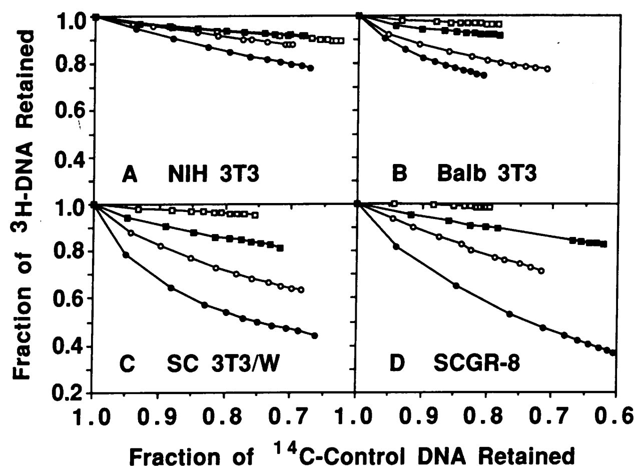

FIG. 2. Dose–response of scid and wild-type fibroblasts to x-ray-generated DSBs. Fibroblasts were labeled with [^{3}H]thymidine, irradiated with 4,000, 8,000, or 12,000 rads, incubated at 37°C for 2 hr to allow for repair of DNA damage, and then analyzed by neutral filter elution. Fragmented ^{14}C-labeled DNA was added to columns as an internal control. Neutral filter elution data are presented as fraction of ^{3}H-labeled DNA retained on filter versus fraction of ^{14}C-labeled control DNA retained on filter. X-ray dose was as follows: □, unirradiated; ■, 4,000 rads; ○, 8,000 rads; and ●, 12,000 rads.

by the relative elution of the DNA from a filter. The neutral filter elution assay has been used extensively in analysis of x-ray-sensitive mammalian cell lines (17, 18). Although the biochemical basis of neutral filter elution is still unknown and may be influenced by factors such as DNA shearing or chromatin conformation (19), the method appears to predominately measure DNA DSB lesions because the rate of elution from a filter is linearly related to the number of DSBs introduced (20). Thus, DNA that has not been irradiated or that has undergone significant repair after irradiation will elute at a much slower rate compared with irradiated and unrepaired DNA.

scid and wild-type fibroblasts were preincubated with [^{3}H]thymidine, irradiated with 4,000, 8,000, or 12,000 rads of x-rays and, after 2 hr of repair at 37°C, were then analyzed by neutral filter elution. For wild-type and scid fibroblasts, increased radiation doses resulted in a smaller percentage of the DNA being retained on the filters (Fig. 2). Significantly, however, a large fraction of the wild-type DNA was still retained on the filters, even at the highest doses, indicating appreciable DSB repair (Fig. 2 *A* and *B*). In sharp contrast, significantly less DNA from two scid fibroblast cell lines was retained on the filters at all three radiation doses (Fig. 2 *C* and *D*). After irradiation with 12,000 rads, 78.3% of NIH 3T3 and 75.0% of BALB 3T3 ^{3}H-labeled DNA was still retained on the filters, whereas only 44.2% of SC 3T3/W and 36.7% of SCGR-8 ^{3}H-labeled DNA was retained. As expected, >95% of the ^{3}H-labeled DNA from control, unirradiated samples of each line was retained on the filter, irrespective of the presence of the *scid* mutation (Fig. 2 *A–D*).

The DSB-repair capacity of scid cells was determined in a time-course experiment, where the rate and amount of DSB repair could be calculated. BALB 3T3, SC 3T3/W, and SCSV-9 cells were irradiated with 8000 rads and then allowed to repair for various times from 0 to 2 hr before neutral filter elution (Fig. 3*A*). With BALB 3T3 fibroblasts, as the time of repair was increased from 0 min up to 2 hr, the fraction of DNA retained on the filter steadily increased. In contrast, both scid cell lines showed a severely diminished ability to repair DSBs during the same time period (SC 3T3/W and SCSV-9, Fig. 3*A*). The 0 min time points were essentially identical for the three cell lines, suggesting that the same number of DSBs are generated in wild-type and scid cells for a given dose. The magnitude of the scid repair defect can be quantitated from the linearity of the elution profiles: the slopes are directly related to the amount of DSBs repaired (13). For wild-type BALB 3T3 fibroblasts, repair proceeded in a relatively linear fashion for ≈30 min, at which time a slower but steady increase in the percentage of DSBs repaired was seen (Fig. 3*B*). At 2 hr, >75% of the DSBs had been repaired. In contrast, SC 3T3/W and SCSV-9 showed only ≈50% as many DSBs repaired after 2 hr. A similar repair profile has been seen for other severely x-ray-sensitive mutants (11, 17, 18). These data clearly suggested that scid fibroblasts were sensitive to x-irradiation because of a reduced ability to repair DSBs.

The scid Repair Defect Is Specific for Agents That Cause DSBs. Multiple pathways are used for the repair of the diverse lesions that can occur in DNA. Although some genes are required for only one DNA-repair pathway, it is clear that others may be involved in several pathways (7). Therefore, the sensitivity of scid cell lines to other DNA-damaging

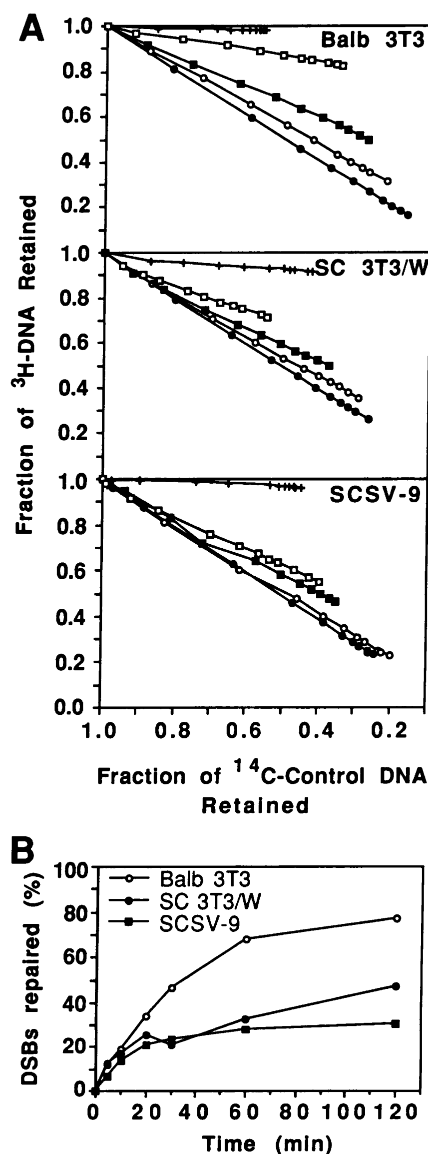

FIG. 3. DSB repair is defective in scid fibroblasts. (*A*) BALB 3T3, SC 3T3/W, and SCSV-9 fibroblasts were irradiated with 8000 rads of x-rays or left untreated. After 0–120 min of DNA repair *in vivo* at 37°C, genomic DNA was analyzed by neutral filter elution. ●, 0 min; ○, 5-min repair; ■, 20-min repair; □, 2-hr repair; and +++, unirradiated. (*B*) Percentage of DSBs repaired was calculated as described.

agents was examined to determine the breadth of the scid DNA-repair defect. MMS (a DNA alkylating agent), MMC (a DNA cross-linking agent), and UV light (generation of pyrimidine dimers) generate single-strand DNA modifications, which are repaired by pathways different from DSB repair. scid and wild-type fibroblasts (SC 3T3/W, SCSV-9, BALB 3T3 and NIH 3T3) were tested for sensitivity to these agents by using cell-survival assays and various DNA-damaging agent concentrations. D_{37} values were calculated from the survival curves. scid fibroblasts had similar sensitivities to MMS and were slightly more resistant to MMC as compared with BALB 3T3 and NIH 3T3 fibroblasts (Table 1). On the other hand, scid fibroblasts did show a slight increased sensitivity to UV irradiation suggesting that there may be some overlap in x-ray- and UV-induced repair—perhaps in the repair of single-strand breaks. We also examined an x-ray-mimetic agent (bleomycin) that causes DSBs. As expected, the scid fibroblasts were 2- to 3.5-fold more sensitive to bleomycin than wild-type fibroblast controls (Table 1). Virtually all of the x-ray-sensitive mutant cell lines known show cross-sensitivity to bleomycin (14, 16, 18). Therefore, the *scid* gene must have a restricted role in DNA-repair pathways and appears to be primarily involved in DSB repair.

DISCUSSION

We have shown that the *scid* mutation has a general DNA-repair defect by studying the effects of several DNA-damaging agents on cell lines established from scid mice. All of these lines were acutely sensitive to x-rays. We used neutral filter elution to demonstrate that x-ray sensitivity was directly related to a deficiency in DSB repair. The defect caused by the *scid* mutation appeared specific for the DSB-repair pathway, as the scid cell lines were not hypersensitive to mutagens that introduced lesions repaired by different pathways. These experiments prompt a comparison of the DSB-repair pathway and V(D)J rearrangement.

X-Ray DSB Repair. Ionizing radiation deposits energy nonhomogeneously in a cell generating free radicals that individually stimulate base loss and strand breakage (21). Two random single-strand breakages on opposite DNA strands that are spaced nearby will produce DSBs with variable overhanging ends. The terminal 3′ nucleotides of the DSB are frequently modified and nonligatable (22). It is likely that efficient repair will require proteins that hold the ends together—specific endo- and/or exonuclease activities to trim modified residues, polymerase(s), and ligase to close the repaired strands.

The repair of DSBs in wild-type cells appeared to have a fast and a slow component: 35% of the x-ray-induced DSBs were repaired within 20 min, whereas 1.5 hr was required for the next 35% (Fig. 3*B*). This result may reflect the ease with which different types of lesions are repaired. For example, the x-ray-induced DNA breaks with modified 3′ ends may require more processing events before they can be re-ligated. scid cells appeared defective in both of these processes, although the effect was more pronounced with repair of the slower component (Fig. 3*B*).

V(D)J Rearrangement. V(D)J rearrangement requires tandem recognition of the signal sequences that flank the two coding elements involved in the recombination event. Recombination is presumed to initiate with site-specific breaks at the junction of the two coding elements and their signal sequences. The resulting four DNA ends (two coding joint and two signal joint ends) form two standard recombination products: a coding junction and a signal junction. Coding junctions usually contain deletions and/or additions of germ-line [P nucleotides (23)] and non-germ-line (N regions)-encoded residues. In striking contrast, the signal junction product generally shows little or no evidence of nucleolytic degradation or base addition in wild-type lymphoid cells (24). In scid pre-B cells, the signal junctions are formed normally on chromosomal templates (4, 5) but do show junctional deletions and additions 50% of the time for extrachromosomal templates (6). Thus, V(D)J recombination intermediates are characteristically asymmetric; coding joint ends appear to contain staggered ends and are subject to multiple processing events, whereas signal joint ends show little evidence of processing.

Common Features of DSB Repair and V(D)J Recombination. The enzymatic events (collectively termed "processing") leading to the *in vivo* rejoining of x-ray-induced DSBs and coding joint formation during V(D)J recombination are similar: exonucleolytic trimming of the DNA ends, nucleotide addition, and joining of the DNA strands. Because we have shown that the *scid* mutation affects both processing events, these similarities suggest a common function for the *scid* gene in DNA repair and V(D)J recombination. DSBs induced by x-rays are either staggered or blunt. Similarly, V(D)J recombination intermediates are hypothesized to consist of either staggered coding joint ends or blunt signal joint ends (23, 24). We postulate that the *scid* gene product is involved primarily in the resolution of those intermediates with staggered ends, consistent with the marked asymmetry of the effect of the *scid* mutation in V(D)J recombination.

The *scid* gene product may regulate a trimming activity or stabilize broken ends of chromosomes involved in DNA repair or V(D)J recombination. Single-stranded DNA ends generated during V(D)J recombination may be obligatory for the exonucleolytic trimming seen at coding junctions, perhaps by providing a binding site for an exonuclease (23). These coding ends are presumably the targets for the *scid* mutation because extensive deletions are observed preferentially in coding strands (4–6, 25). It is interesting to note that the exonuclease activity associated with the repair of x-ray damage in wild-type cells probably removes only three to four nucleotides per x-ray lesion, as calculated from the incorporation of nucleotides during repair (26). Similarly, the coding joint ends of wild-type V(D)J recombination intermediates are not deleted >one to five nucleotides, on average, indicating that the extent of nuclease action is controlled or that joining is efficient enough to limit extent of digestion. An exonuclease potentially used in V(D)J recombination would require a 3′-to-5′ specificity to degrade P (germ-line) nucleotides. Because most 3′ ends of radiation-damaged DNA are modified such that they are no longer substrates for DNA ligase (22), the DSB-repair-associated exonuclease must also have a 3′-to-5′ activity.

Although coding junctions are characteristically heterogeneous, signal junctions generally form by the exact fusion of the two heptamer–nonamer signal sequences. Because these signal ends show no evidence of nuclease digestion, P nucleotide addition or N base addition, the strands may be protected in a protein complex, such as the V(D)J recombinase. This complex presumably is also what makes these ends refractory to the *scid* mutation and may be responsible for the ligation of these strands. Thus, signal joint formation may represent a special case of DSB repair.

DNA-Repair Defects and Immunodeficiencies. Except for the pronounced immune deficiency, scid mice are phenotypically normal (1). Given the severity of the DSB defect documented above for scid cell lines, it is surprising that more effects of the mutation have not been observed in scid animals. However, the *scid* mutation is "leaky" in its effect on V(D)J recombination (4, 27). From the neutral elution profiles (Figs. 2 and 3) scid cells clearly have a limited ability to repair DSBs. This level of repair may be sufficient for scid animals in a pathogen-free environment. Bone-marrow stem cells from scid mice have recently been shown to be hypersensitive to x-rays, and primary kidney fibroblasts showed

2-fold higher levels of micronuclei after x-ray irradiation (28). We have similarly observed increased levels of micronuclei after x-ray irradiation (X.-Q.Q., E.A.H., and D.W., unpublished observations). These combined data indicate that the DSB-repair role that we have described here for the *scid* gene may be important in several tissues for the survival of a wild-type mouse.

Several other mammalian DNA-repair-defective mutants have been characterized that also have associated immunodeficiencies. In humans, ataxia telangiectasia (29) and Bloom syndrome (30) are associated with immunodeficiencies and greatly increased incidence of cancer and have suspected defects in DNA repair. Ataxia telangiectasia lymphoid cells have elevated frequencies of chromosomal translocations, coincident with sites of immunoglobulin and T-cell receptor V(D)J rearrangement (29). Thus, other gene products may effect both DNA repair and V(D)J recombination. Likewise, other DNA recombination or modification events that have been previously considered to be lymphoid specific (class switching and somatic mutation) may also incorporate the use of DNA-repair proteins.

We gratefully thank Drs. David Norris, Terry Orr-Weaver, John Petrini, and Michel Streuli for their critical reading of the manuscript and their helpful suggestions. We thank Dr. Mike McCune for scid mice and Dr. Melvin Bosma for SN-1 cells. We thank Dr. Hatsumi Nagasawa for her generous guidance during the UV irradiation experiments. E.A.H. was supported by a U.S. Public Health Service Postdoctoral Fellowship (5F32HD07034) from the National Institutes of Health. D.T.W. was supported, in part, by Public Health Service Grant GM39312 from the National Institutes of Health and a American Cancer Society Junior Faculty Award.

1. Bosma, G. C., Custer, R. P. & Bosma, M. J. (1983) *Nature (London)* **301**, 527–530.
2. Schuler, W., Weiler, I. J., Schuler, A., Phillips, R. A., Rosenberg, N., Mak, T. W., Kearney, J. F., Perry, R. P. & Bosma, M. J. (1986) *Cell* **46**, 963–972.
3. Hendrickson, E. A., Schatz, D. G. & Weaver, D. T. (1988) *Genes Dev.* **2**, 817–829.
4. Hendrickson, E. A., Schlissel, M. S. & Weaver, D. T. (1990) *Mol. Cell. Biol.* **10**, 5397–5407.
5. Ferrier, P., Covey, L. R., Li, S. C., Suh, H., Malynn, B. A., Blackwell, T. K., Morrow, M. A. & Alt, F. W. (1990) *J. Exp. Med.* **171**, 1909–1918.
6. Lieber, M. R., Hesse, J. E., Lewis, S., Bosma, G. C., Rosenberg, N., Mizuuchi, K., Bosma, M. J. & Gellert, M. (1988) *Cell* **55**, 7–16.
7. Friedberg, E. C. (1988) *Microbiol. Rev.* **52**, 70–102.
8. Orr-Weaver, T. L. & Szostak, J. W. (1985) *Microbiol. Rev.* **49**, 33–58.
9. Radford, I. R. (1986) *Int. J. Radiat. Biol.* **49**, 611–620.
10. Todaro, G. J. & Green, H. (1963) *Annu. Rev. Genet.* **17**, 299–313.
11. Kemp, L. M., Sedgwick, S. G. & Jeggo, P. A. (1984) *Mutat. Res.* **132**, 189–196.
12. Bradley, M. O. & Kohn, K. W. (1979) *Nucleic Acids Res.* **7**, 793–804.
13. Blakely, W. F., Ward, J. F. & Joner, E. I. (1982) *Anal. Biochem.* **124**, 125–133.
14. Jeggo, P. A. & Kemp, L. M. (1983) *Mutat. Res.* **112**, 313–327.
15. Jones, N. J., Cox, R. & Thacker, J. (1987) *Mutat. Res.* **183**, 279–286.
16. Stamato, T. D., Weinstein, R., Giaccia, A. & Mackenzie, L. (1983) *Somatic Cell Genet.* **9**, 165–173.
17. Giaccia, A., Weinstein, R., Hu, J. & Stamato, T. D. (1985) *Somatic Cell Mol. Genet.* **11**, 485–491.
18. Zdzienicka, M. Z., Tran, Q., van der Schans, G. P. & Simons, J. (1988) *Mutat. Res.* **194**, 239–249.
19. Wlodek, D. & Olive, P. L. (1990) *Radiat. Res.* **124**, 326–333.
20. Radford, I. R. & Hodgson, G. S. (1985) *Int. J. Radiat. Biol.* **48**, 555–566.
21. Mozumder, A. & Magee, J. L. (1966) *Radiat. Res.* **28**, 203–214.
22. Henner, W. D., Grunberg, S. M. & Haseltine, W. A. (1983) *J. Biol. Chem.* **258**, 15198–15205.
23. Lafaille, J. J., DeCloux, A., Bonneville, M., Takagaki, Y. & Tonegawa, S. (1989) *Cell* **59**, 859–870.
24. Lewis, S. & Gellert, M. (1989) *Cell* **59**, 585–588.
25. Blackwell, T. K., Malynn, B. A., Pollock, R. R., Ferrier, P., Covey, L. R., Fulop, G. M., Phillips, R. A., Yancopoulos, G. D. & Alt, F. W. (1989) *EMBO J.* **8**, 735–742.
26. Frances, A. A., Snyder, R. D., Dunn, W. C. & Regan, J. D. (1981) *Mutat. Res.* **83**, 159–169.
27. Bosma, G. C., Fried, M., Custer, R. P., Carroll, A., Gibson, D. M. & Bosma, M. J. (1988) *J. Exp. Med.* **167**, 1016–1033.
28. Fulop, G. M. & Phillips, R. A. (1990) *Nature (London)* **347**, 479–482.
29. McKinnon, P. J. (1987) *Hum. Genet.* **75**, 197–208.
30. German, J. (1983) in *Chromosome Mutation and Neoplasia*, ed. German, J. (Liss, New York), pp. 347–357.