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V(D)J Recombination in Mammalian Cell Mutants Defective in DNA Double-Strand Break Repair

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V(D)J recombination has been examined in several X-ray-sensitive and double-strand break repair-deficient Chinese hamster cell mutants. Signal joint formation was affected in four mutants (*xrs 5*, XR-1, V-3, and XR-V9B cells, representing complementation groups 1 through 4, respectively) defective in DNA double-strand break rejoining. Among these four, V-3 and XR-V9B were the most severely affected. Only in V-3 was coding joint formation also affected. Ataxia telangiectasia-like hamster cell mutants (V-E5 and V-G8), which are normal for double-strand break repair but are X ray sensitive, were normal for all aspects of the V(D)J recombination reaction, indicating that X-ray sensitivity is not the common denominator but that the deficiency in double-strand break repair appears to be. The abnormality at the signal joints consisted of an elevated incidence of nucleotide loss from each of the two signal ends. Interestingly, in complementation groups 1 (*xrs 5*) and 2 (XR-1), signal joint formation was within the normal range under some transfection conditions. This suggests that the affected gene products in these two complementation groups are not catalytic components. Instead, they may be either secondary or stochiometric components involved in the later stages of both the V(D)J recombination reaction and double-strand break repair. The fact that such factors can affect the precision of the signal joint has mechanistic implications for V(D)J recombination.

The exons encoding the immunoglobulin and T-cell receptor variable-region domains are assembled during lymphocyte development by V(D)J recombination. This site-specific reaction is directed by a pair of joining signals recognized by components of the recombination activity. The consensus sequence of each signal consists of a palindromic heptamer and an AT-rich nonamer. These two elements are separated by a 12-base spacer at one signal and a 23-base spacer at the other. The recombination site for each signal is at the end of the heptamer on the side distal from the nonamer. During recombination, coding ends and signal ends are generated by recombinase-mediated cleavage at the crossover sites. The two coding ends are joined to form what is termed a coding joint, and the two signal ends are joined to form a signal joint (reviewed in reference 20). Modification can occur at the two coding ends (20, 25) in ways that are similar in some respects to modifications of chromosomal ends left after transposon excisions in eukaryotes (4, 21, 36).

Murine *scid* (3) has been shown to be defective in V(D)J recombination, defective in the repair of double-strand breaks, and sensitive to X-irradiation (1a, 2, 4a, 10, 10a, 28, 31, 32). The *scid* defect in double-strand break repair implies that the processing of coding ends in the V(D)J recombination reaction is similar to the processing of chromosomal breaks. Both processes can involve nucleotide loss, and both processes can involve nucleotide addition (22, 27).

The complexity of mechanisms involved in cellular responses to ionizing radiation is indicated by the existence of nine complementation groups identified among X-ray-sensitive rodent cell mutants (15, 37, 40). The interaction of ionizing radiation with mammalian cells produces a variety of lesions, including single-strand breaks, double-strand breaks, and base damage.

Double-strand breaks can be generated by a number of DNA-damaging agents, among which is ionizing radiation. Four complementation groups have been identified for double-strand break repair, indicating that at least four genes encode components involved in the repair of double-strand breaks (40). It is not yet established whether the murine *scid* mutant is a fifth complementation group among rodent mutants defective in double-strand break repair. Though all four double-strand break repair complementation groups show X-ray sensitivity, not all X-ray-sensitive complementation groups show a deficiency in double-strand break repair.

It has become possible to activate the V(D)J recombination reaction in nonlymphoid cells by transfecting them with the recombination-activating genes *RAG-1* and *RAG-2* (26, 30). The characteristics of the V(D)J recombination reaction in nonlymphoid cells have not previously been described in any detail. Coding-joint sequences have been described by one group (16), but the efficiency of coding joints relative to that of other V(D)J recombination products was not determined. Recently, we have characterized the V(D)J recombination reaction in detail in murine, hamster, and human fibroblasts (12) and in human lymphoid cells (6). In murine, hamster, and human fibroblasts and in human lymphoid cells, we find that coding-joint completion can be less efficient relative to signal joint completion.

Double-stranded DNA intermediates may be shared between site-directed recombination and double-strand break repair. The ability to activate the V(D)J recombination activity in nonlymphoid cells allows the analysis of the reaction in the context of X-ray-sensitive and/or double-strand break repair mutations other than murine *scid*. Recently, we have conducted such an analysis in human

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fibroblast cell lines from patients with defects in DNA metabolism, and we find that V(D)J recombination is normal (12). Alt and colleagues have described in brief (1) and in detail (35a) an analysis of three mutant lines derived from Chinese hamster cells. Some of the mutant Chinese hamster cell lines that they studied showed abnormalities in coding-joint formation. We were interested in analyzing these Chinese hamster cell mutants for inversional recombination in addition to deletional V(D)J recombination. Inversion substrates provide greater discrimination than do deletional V(D)J recombination substrates against spurious deletions created by mechanisms unrelated to V(D)J recombination. In addition, a fourth complementation group for double-strand break re pair deficiency has been described by Zdzienicka et al. (40), and we were interested in comparing the four known double-strand break repair deficiency complementation groups for V(D)J recombination with one another. Hence, using substrates that we have previously described, we have conducted a detailed analysis of six X-ray-sensitive hamster fibroblast mutant cell lines. Four of these are representatives of each of the four double-strand break repair complementation groups that have been identified in Chinese hamster cells (8, 14, 38, 40). The remaining two lines are X ray sensitive but not double-strand break repair deficient (15, 39).

MATERIALS AND METHODS

Plasmid constructions. RAG-1 and RAG-2 expression vectors were constructed by inserting RAG-1 and RAG-2 cDNA (26, 30) obtained from D. Schatz (Yale University) into the *Not*I site of a modified pCDM8 vector. The stuffer from pCDM8 was removed by *Xho*I digestion and ligation prior to cloning into the *Not*I site.

pJH200, pJH290, and pML89 have been previously described (11, 23, 33). These substrates all undergo V(D)J recombination in hamster fibroblasts. pJH200 undergoes deletion and retains a signal joint on the plasmid. pJH290 undergoes deletion and retains a coding joint. pML89 can undergo inversion and retains both coding and signal joints. pML89 can also yield the alternative product, hybrid joints (Fig. 1) (19).

Cell cultures and transfections. CHOK1 (a glycine auxotroph of CHO cells), 4364A (a proline and glycine auxotroph of CHOK1 [17]), *xrs 5* (18) and XR-1 (7, 9, 34, 35) (hamster DNA double-strand break repair-deficient mutant cell lines), and A × W-1 (a hybrid of XR-1 and a wild-type human line [8]) were obtained from A. J. Giaccia (Stanford University) and were cultured in RPMI 1640 medium. V-G8 and V-E5 (hamster ataxia telangiectasia-like mutants), the wild-type parental line V79 (39), and V79B and XR-V9B (40) were maintained in Ham's F-10 medium. V-3 cells (38), kindly supplied by A. J. Giaccia with the permission of G. F. Whitmore (Ontario Cancer Institute, Toronto, Ontario, Canada) were grown in α minimum essential medium. Media were supplemented with 10% fetal calf serum (GIBCO), penicillin (100 U/ml), and streptomycin (0.1 mg/ml). Cells were maintained at 37°C in a 5% CO_2 atmosphere, humidified to 95 to 100%. For transfection, 3×10^6 to 3.5×10^6 cells were treated with a solution of 0.5 g of trypsin per liter and 0.2 g of EDTA per liter when they reached 70% confluence, washed once with phosphate-buffered saline, resuspended in 0.5 ml of serum-free RPMI 1640 medium, and transiently transfected by electroporation with a Bio-Rad gene pulser at room temperature. Typical electroporation parameters were a voltage of 250 V, a capacitance of 960 μF, and a gap distance in the room temperature chamber of 0.4 cm. The sonicated salmon sperm carrier (250 μg, when added) and plasmid DNA (7 μg each of *RAG-1* and *RAG-2* plus 2.5 μg of substrate) were coprecipitated and always resuspended in a volume of Tris-EDTA not exceeding 20 μl. The transfections were carried out in replicate in at least two different transfection sessions. Cells were plated immediately after the transfection in complete medium. Subclones of the same cell lines behaved similarly.

Assay for V(D)J recombination with extrachromosomal substrates. The assay for V(D)J recombination with extrachromosomal substrate was performed as previously described (11). The plasmid DNA was harvested after 44 to 48 h by the rapid alkaline-sodium dodecyl sulfate lysis method and analyzed for recombinants. All plasmid substrates contain both heptamer-nonamer joining signals and the gene conferring ampicillin resistance in *Escherichia coli*. When and if the recombination occurs, the substrates undergo an excision or an inversion of a transcription terminator upstream of the *cat* gene, and this now permits transcription of the *cat* gene. The resulting product plasmid confers ampicillin and chloramphenicol resistance (designated A and C, respectively) in bacteria. The ratio of the number of double-drug-resistant colonies and the number of single-drug-resistant colonies reflects the fraction of DNA rearranged by recombination events in the fibroblasts.

Structural analysis of recombinant products. Signal joint analysis using substrate pJH200 or pML89 was conducted by digesting the plasmids with the restriction enzyme *Apa*LI and analyzing the digestion products on a 0.8% agarose gel. A precise signal joint creates a new *Apa*LI site. A subset of the imprecise joints, detected first by *Pvu*II digestion, was sequenced. The *Apa*LI digest of pML89 recombinants also permits distinction of hybrid joint events from inversion events (Fig. 2).

Coding-joint analysis using substrate pJH290 or pML89 was conducted by digesting the plasmids with the restriction enzyme *Pvu*II and analyzing the digestion products on a 5% polyacrylamide gel. The *Pvu*II fragment containing the coding joint was assessed for size relative to a coding joint of known length. All coding joints of recombinants appeared to be within 25 bp of full length. Representative coding joints were sequenced (Fig. 3).

Use of DNA replication for assessing transfection efficiency and DNA entry. Plasmids that bear a polyomavirus origin of replication will replicate in eukaryotic cells only if polyomavirus T antigen is present. In such cases, the plasmids that enter the cells efficiently replicate and V(D)J recombinants are almost exclusively detected in the replicated pool of molecules (24). Molecules that failed to enter the cells or are adherent to the outside of the cells will not replicate or undergo V(D)J recombination. Therefore, DNA replication does not significantly over- or underestimate the pool of molecules that entered the cells.

We distinguish the pool of plasmids that have replicated in the eukaryotic cells by virtue of the fact that they have lost their prokaryotic *dam* methylation at the A in GATC sites. *Dpn*I is a restriction enzyme which eliminates plasmid molecules that did not replicate and therefore retain the *dam* methylation. We designate treatment with *Dpn*I with a D. Hence, transformants arising on ampicillin plates from DNA that has been treated with *Dpn*I are designated DA. The percentage of a population of recovered plasmids that has replicated is 100 × (DA/A).

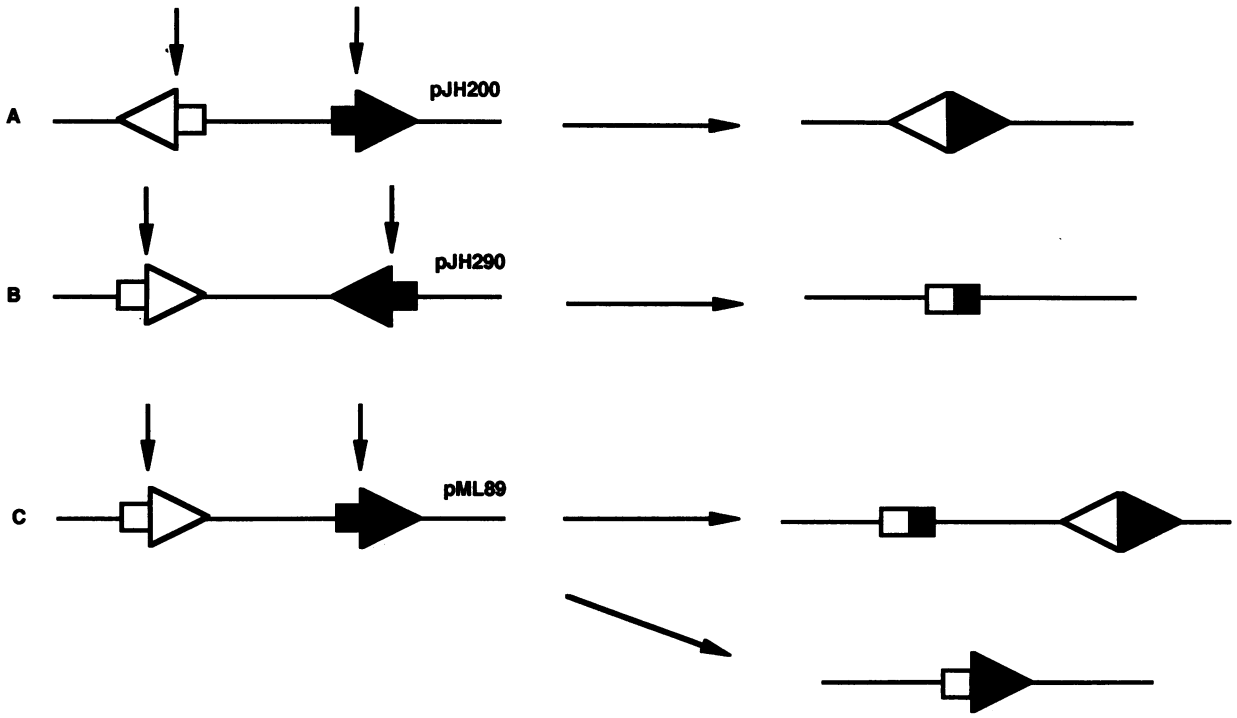

FIG. 1. Reactions tested by V(D)J recombination substrates. The recombination zone of each of three V(D)J recombination substrates is shown. The substrates contain a given recombination zone positioned within a plasmid between the *lac* promoter and the *cat* gene. The signal sequences for this site-specific reaction are depicted as triangles. A transcription terminator is positioned between the signals. The two different recombination signals are symbolized as open (12-base spacer signal) or filled (23-base spacer signal) triangles. The vertical arrows are the sites of crossover. In panel C, an inversion reaction is depicted (top line); an inversion can also yield a hybrid joint outcome (bottom line). In panels A and B, deletional reactions in which either the signal or coding joint is retained, respectively, are shown. (Reproduced from reference 10.)

RESULTS

V(D)J recombination in hamster cell mutants (XR-1 and *xrs 5*) that are DNA double-strand break repair deficient. We analyzed the mutant hamster fibroblasts XR-1 and *xrs 5* for the ability to carry out V(D)J recombination, as compared with the wild type, when activated for this reaction by transfection with *RAG-1* and *RAG-2*. The extrachromosomal substrate assay system was used because it allows determination of levels of activity and qualitative inspection of the fidelity of the signal joint and coding-joint portions of the

TABLE 1. Abnormal V(D)J recombination in double-strand break repair-defective mutants of complementation groups 1 and 2[a]

Complementation group	Cell line	Results[b] with:						
		pJH200				pJH290		
		No. of colonies		DAC/DA[c]	% Precision	No. of colonies		DAC/DA[c]
		DA	DAC[d]			DA	DAC[d]	
Wild type	4364A	22,800	66	0.3	>97	50,312	21	0.04
1	*xrs 5*	124,792	12	0.01	25	6,669	0	<0.01
2	XR-1	26,638	0	<0.004	—	1,444	0	<0.06
Complemented	A × W-1	5,206	20	0.4	>95	222,680	56	0.03

[a] Hamster wild-type and X-ray-sensitive mutant cell lines were cotransfected with RAG-1 and RAG-2 expression vectors, sonicated salmon sperm carrier DNA, and the indicated plasmid substrate. Plasmid DNA was recovered 48 h after transfection and transformed into *E. coli* to select for V(D)J recombinants and to determine the fraction of substrates recombined to product.

[b] Recombinants were checked by *Apa*LI and PvuII restriction analysis and, in selected cases, by sequencing. In pJH200 recombinants, *Apa*LI restriction demonstrates a new site at precise signal joints. In pJH290 recombinants, PvuII digestion permits assessment of deletion size in the product. The percentage of precision refers to the fraction of signal joints that showed no nucleotide loss at the signal ends. Forty, twelve, and twenty signal joints of individual recombinants from 4364A, *xrs 5*, and A × W-1, respectively, were analyzed for signal joint precision (—, not analyzed). The results are compilations from four different transfections carried out for each cell line. Ratios are weighted averages from the four transfections; the range of individual ratios was within a factor of 4 from the highest to the lowest value.

[c] Each value shown is 100 times the actual value.

[d] The false-positive DAC rate for substrate pJH200 in the indicated cell lines is <0.0008%. Rare DAC events that arose at a rate below 0.0008% either appeared to be unaltered substrate molecules or bore little resemblance to the input or V(D)J recombination products on the basis of restriction analysis. The false-positive DAC rate for substrate pJH290 was <0.001%. The false-positive DAC rates were determined by transfecting the substrates without *RAG-1* and *RAG-2* into the cells and analyzing them in the standard manner for recombinants.

reaction (Fig. 1). We used the deletion substrate pJH200 to inspect the signal joint portion of the reaction. In order to assess the coding-joint half of the reaction, we used the deletion substrate, pJH290, that retains the coding joint on the recombinant product plasmid.

The wild-type CHO fibroblast line 4364A was used as a representative of the wild-type phenotype. It is the parental line from which XR-1 was originally derived by mutagenesis (35). Recombination in 4364A was 0.3% for signal joint formation, as determined with substrate pJH200. Coding-joint formation in these wild-type cells was 0.04%, as determined with substrate pJH290. Hence, the efficiency of coding-joint formation is sevenfold below that of signal joint formation in the wild-type cells.

We transfected XR-1 and *xrs 5* cells and analyzed them for signal joint and coding-joint formation. We found that signal joint formation in the *xrs 5* mutant was very low, 0.01%, and coding joint formation was <0.01% (Table 1). Moreover, most (9 of 12) of the signal joints that did form showed nucleotide loss from the signal ends (Table 1 and Fig. 2A). In normal cells (e.g., 4364A), these ends usually do not lose nucleotides (Table 1). Neither signal nor coding joints were formed in XR-1 at detectable levels, even though it is clear that the DNA effectively entered the cells, because it underwent replication in the hamster cells (see Materials and Methods). The low level of recombination in the mutant lines is abnormal. However, it is the imprecision at the signal joints that is particularly noteworthy. This has previously been only seen in the murine *scid* defect (1, 23, 35a).

Does complementation of XR-1 for double-strand break repair and X-ray sensitivity also complement it for V(D)J recombination? We tested this by examining the hybrid cell line A × W-1, which is a primary cell fusion of XR-1 with the wild-type human line WI-38. This hybrid line, in which human chromosome 5 has been retained, is wild type for X-ray sensitivity and double-strand break repair (7). Examination of V(D)J recombination in this line shows that it behaves as the wild type (Table 1).

The abnormalities in V(D)J recombination in XR-1 and *xrs 5* are not apparent under all transfection conditions. When we repeated the above experiments with XR-1 and *xrs-5* but omitted the sonicated salmon sperm DNA, we found that the recombination efficiencies for signal joint formation increased, the precision at the signal joints rose to the normal range of values, and coding-joint formation rose to the levels seen in wild-type fibroblasts (Table 2). The detailed features of these experiments are as follows. For coding-joint analysis, we used the inversion substrate pML89. The advantage of using an inversion substrate is that it undergoes V(D)J recombination to yield products that bear both the signal and the coding joints (Fig. 1C). Analysis of the signal joint from an inversion documents that the rearrangement was a V(D)J recombination event. Results with substrates that undergo only deletional V(D)J recombination to retain the coding-joint half of the reaction are more difficult to interpret because coding ends normally lose 0 to 10 bp (22) and therefore the coding joints that form may have been generated by illegitimate recombination (Fig. 1B). Deletion substrates that retain the signal joint half of the V(D)J recombination reaction are readily documented as V(D)J recombination events. By comparing the ratio of the frequency of signal joint formation (using a signal joint deletion substrate) to inversional recombination (which requires both signal joint and coding-joint formation), the magnitude of the deficit in coding-joint formation can be estimated. We found that the inversion frequencies in the mutant lines were similar to the frequency in the wild-type line and that the structure of the coding joints was also normal by restriction analysis. We conclude that in the absence of large amounts of carrier DNA, XR-1 and *xrs 5* are not distinguishable from wild-type fibroblasts in conducting V(D)J recombination after transfection with *RAG-1* and *RAG-2*.

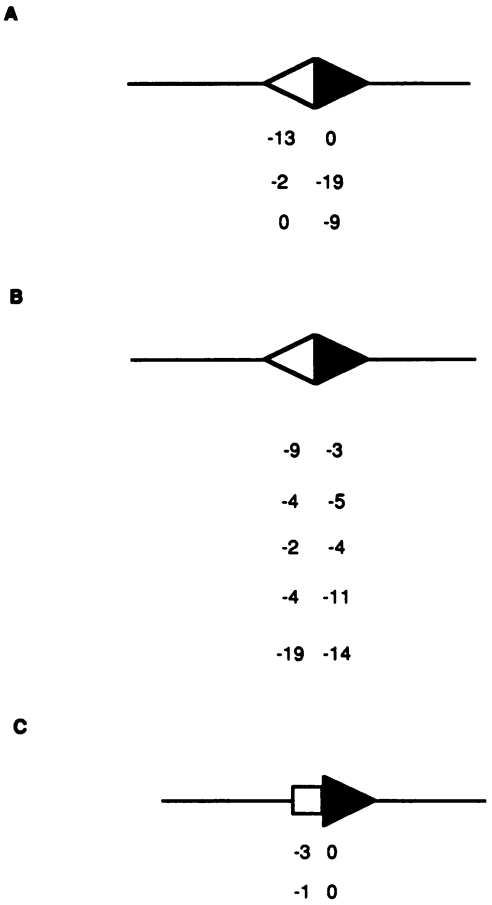

FIG. 2. Sequences of signal joints and hybrid joints from mutant cell lines. (A) The *xrs 5* mutant cell line was transfected with RAG-1 and RAG-2 expression vectors, pJH200 substrate, and salmon sperm carrier (see Materials and Methods). Recombination products were analyzed by restriction analysis and, in selected cases, by sequencing. The signal joints were abnormal (imprecise) in some cases because nucleotides were missing from one signal end or the other. The sequences of three of these imprecise signal joints are shown. The numbers indicate the bases missing at each signal end. See the legend to Fig. 1 for symbols. (B) Five imprecise signal joints from the XR-V9B mutant cell line transfected by electroporation without salmon sperm carrier. (C) Recombination of the pML89 inversion substrate in the ataxia telangiectasia-like cell lines yields both inversions and hybrid joints. Two hybrid joints are shown, the first from V-E5 and the second from V-G8. There was no nucleotide addition at any of the junctions shown in this figure.

V(D)J recombination in the hamster cell mutants V-3 and XR-V9B is abnormal. Two additional hamster fibroblast lines that are X ray sensitive and double-strand break repair deficient, V-3 (35a, 38) and XR-V9B (40), persisted in showing abnormal features in the RAG-1- and RAG-2-activated reaction, despite the more favorable transfection conditions (i.e., no carrier DNA). Both of these lines showed imprecision at the signal joint (Table 3 and Fig. 2B). The

TABLE 2. V(D)J recombination reaction when salmon sperm carrier DNA is omitted from the transfection procedure[a]

Complementation group	Cell line	Results with:						
		pJH200[b]				pML89[c]		
		No. of colonies		DAC/DA[d]	% Precision	No. of colonies		DAC/DA[d]
		DA	DAC			DA	DAC	
Wild type	4364A	12,597	513	4.1	>97	17,879	18	0.1
1	*xrs 5*	6,593	291	4.4	>98	252,517	489	0.2
2	XR-1	4,560	163	3.5	>94	319,200	389	0.1

[a] Hamster wild-type and double-strand break repair-defective mutant cell lines were cotransfected transiently with RAG-1 and RAG-2 expression vectors and the indicated substrate without salmon sperm DNA carrier. The results are compilations of four different transfections carried out for each cell line. Ratios are weighted averages from the four transfections; the range of individual ratios was within a factor of 5 from the highest to the lowest value among the different transfections of each cell line in this table.

[b] None of 40, 59, or 17 independent pJH200 recombinant products analyzed from 4364A, *xrs 5*, or XR-1, respectively, demonstrated any imprecision at the signal ends by *Apa*LI restriction analysis. The false-positive DAC rate for the deletion substrate, pJH200, in these lines (without *RAG-1* and *RAG-2* cotransfection) was <0.0008%.

[c] The inversion substrate, pML89, was used to assess inversion and hybrid formation. Junctions were analyzed by restriction analysis using *Apa*LI for signal joints and *Apa*LI and *Pvu*II for coding and hybrid joints. Eighteen pML89 recombinants of 4364A and twenty-four from *xrs 5* and XR-1 each were analyzed. Hybrids represented 20 to 40% of the pML89 recombination events. For pML89, the DAC/DA ratio indicates the inversion plus hybrid recombination frequency. The false-positive rate for pML89 was <0.005% for these lines.

[d] Each value shown is 100 times the actual value.

same results were obtained when the mutant cell line XR-V9B was transfected in the presence of salmon sperm DNA carrier; therefore, the imprecision at the signal joints did not seem to be affected by the different transfection conditions.

In addition to the abnormality in signal joint formation, V-3 failed to carry out coding-joint formation at a detectable level. This combination of features, imprecision at the signal joint and failure of coding-joint formation, is also seen in murine *scid* lymphocytes (1, 23, 28, 35a). Coding-joint formation by XR-V9B was normal (Table 3 and Fig. 3).

X-ray-sensitive mutants that are normal in double-strand break repair are normal in V(D)J recombination. The ataxia telangiectasia-like, X-ray-sensitive hamster fibroblast lines V-E5 and V-G8 are not deficient in double-strand break repair (40), whereas XR-1, *xrs 5*, V-3, and XR-V9B are all X ray sensitive and double-strand break repair deficient (8, 14, 38, 40). We were interested in determining whether these X-ray-sensitive cell lines demonstrated V(D)J recombination abnormalities. The results show that V-E5 and V-G8 recombine normally compared with their parental cell line, V79. None of the recombinant products analyzed by *Apa*LI digestion showed any imprecision at the signal joints. In addition, both inversions and hybrid joints were demonstrated by restriction analysis and, in selected cases, by sequence analysis (Table 4 and Fig. 2C and 3).

DISCUSSION

Our interpretation of the above data is that X-ray sensitivity itself does not correlate with abnormalities in the V(D)J recombination reaction (Table 4). However, the four double-strand break repair mutants, each from one of the four currently identified complementation groups for double-strand break repair in hamster fibroblasts, are all abnormal for V(D)J recombination under at least some conditions (Tables 1 and 3). The fact that conditions under which V(D)J recombination was normal for XR-1 and *xrs 5* could be identified indicates that the factors that correspond to these defects are not likely to be key catalytic components for the V(D)J recombination reaction (Table 2).

All four double-strand break repair-deficient lines, XR-1, *xrs 5*, V-3, and XR-V9B, showed abnormality in the precision of the signal joint at least under some conditions (Tables 1 and 3). One interpretation of these results is that signal ends may be available for end modification prior to joining and that, during this interval, these ends may suffer base

TABLE 3. Abnormal V(D)J recombination in double-strand break repair-defective complementation groups 3 and 4[a]

Complementation group	Cell line	Results with:						
		pJH200				pML89		
		No. of colonies		DAC/DA[b]	% Precision	No. of colonies		DAC/DA[b]
		DA	DAC			DA	DAC	
Wild type	AA8	10,393	149	1.4	>95	267,140	176	0.06
3	V-3	11,419	83	0.7	80	130,568	0	<0.0008
Wild type	V79B	33,098	888	2.7	>95	67,203	23	0.03
4	XR-V9B	121,486	631	0.5	27	97,166	13	0.01

[a] Cell lines were transfected as described in Materials and Methods without salmon sperm DNA carrier. The results are compilations from four different transfections carried out for each cell line. Ratios are weighted averages from the four transfections; the range of individual ratios was within a factor of 4 from the highest to the lowest value. Signal joint precision percentages are based on the restriction analysis of 20 to 26 independent pJH200 and pML89 recombinants for each of the cell lines in the table. All coding joints from V79B and XR-V9B and 20 from AA8 were analyzed by restriction analysis; representative recombinants of XR-V9B were chosen for sequence analysis (Fig. 2B and 3). Hybrids represented 20 to 40% of the pML89 recombination events. For pML89, the DAC/CA ratio indicates inversion plus hybrid recombination frequency. The false-positive DAC rate for substrate pJH200 in these lines was <0.01%, and that for pML89 was <0.0007%.

[b] Each value shown is 100 times the actual value.

	.....GGGCTGCAGGTCGA	GATCCTCTCATCGA.....
4364A	-2	-4
	-0	-2
	-2	-4
xrs 5	-4	-1
	-2	-4
	-2	-4
	-0	-2
XR-1	-0	-2
	-0	-2
	-3	-2
XR-V9B	-2	-4
	-1	-5
	-0	-2
	-0	-2

FIG. 3. Sequences of coding joints from wild-type and mutant cell lines. The coding end that was formerly attached to the 12-signal sequence in the substrate is displayed on the left, and the coding end formerly attached to the 23-signal sequence is on the right. The number of nucleotides lost from each end is shown in the columns of numbers below each end. All sequences are from independent recombinants. No nucleotide addition was seen at any of the coding joints. Many of the endpoints of nucleotide loss occur at sites of homology and therefore could be designated in multiple ways; for example, (0, −2) in line 2 could also have occurred as (−2, 0) or (−1, −1).

loss. The double-strand break repair mutants may involve decreases in factors that protect DNA ends from exonucleolytic events or an increase in factors that conduct exonucleolytic reactions. A variety of lines of investigation are consistent with the possibility that signal ends are available for end modification prior to signal joint completion. First, free signal ends have been found in the fetal thymus during T-cell receptor gene rearrangement (29). Second, nucleotide addition at signal ends, probably due to terminal transferase, is common (22). Third, the efficiency of signal joint completion appears to be less than 100% (5, 6). And fourth, coding joints appear to form under conditions where signal joints are unlikely to form efficiently (23, 33). All of these features are consistent with the possibility that there is not an efficient and obligate mechanistic resolution of signal joints. This would then leave them available for end modification by enzymatic activities that are not intrinsic in any way to the V(D)J recombination reaction. Such extrinsic factors could function in any of a wide array of other DNA metabolic activities.

There is some feature of the sonicated salmon sperm DNA that may affect V(D)J recombination or the transfection procedure (Tables 1 and 2). At this point, it is difficult to know how the carrier DNA is adversely affecting precision at the signal joint. Among the many possibilities, the free ends of the carrier DNA might be titrating out a DNA end-binding protein, for example. However, what may be more important is that the reaction in XR-1 and *xrs 5* appears to be the same as that in the wild type in the absence of carrier, and this weighs against a key catalytic role in V(D)J

TABLE 4. V(D)J recombination in X-ray-sensitive mutants which are not double-strand break repair defective[a]

Cell line	Results with:						
	pJH200				pML89		
	No. of colonies		DAC/DA[b]	% Precision	No. of colonies		DAC/DA[b]
	DA	DAC			DA	DAC	
V79	414,302	13,224	3.2	>95	1,016,880	397	0.4
V-E5	20,368	1,688	8.2	>95	186,656	795	0.4
V-G8	81,548	5,650	6.9	>95	228,000	1,492	0.6

[a] Ataxia telangiectasia-like mutants V-G8 and V-E5 and the wild-type parental cell line V79 were transfected as described in Materials and Methods without salmon sperm DNA carrier. The results are compilations from four different transfections carried out for each cell line. The results were essentially the same when carrier was used (data not shown). Twenty recombinants were analyzed for signal joint precision and for coding-joint deletion size for each of the cell lines. For pML89, the DAC/DA ratio indicates inversion plus hybrid recombination frequency. The false-positive DAC rate for substrate pJH200 in these lines was <0.01%, and that for pML89 was <0.001%. Ratios are weighted averages from the four transfections; the range of individual ratios was within a factor of 3 from the highest to the lowest value.

[b] Each value shown is 100 times the actual value.

recombination for the products of the affected genes in these mutant cells.

In addition to the abnormalities described above for signal joint formation, in one of the four double-strand break repair mutants, V-3, coding-joint formation is also abnormal, as previously reported (35a). The abnormality is observed as a larger deficit in inversions (relative to signal joints) than is found in wild-type cells. In the wild-type hamster line AA8, inversions are 23-fold less frequent than signal joint deletion. In V-3, the deficit is at least 875-fold. Signal joint imprecision and a relative deficit of coding joints is also seen in *scid* lymphocytes (1, 23, 35a). It is possible that *scid* and V-3 mutants are affected in the same DNA activity, or they could be affected along the same DNA end-processing pathway (1, 35a). Just as the studies here suggest that multiple extrinsic factors are affecting the signal end resolution process, one cannot rule out the possibility that multiple extrinsic factors also affect the coding-end resolution process.

These observations will be useful as the proteins involved in V(D)J recombination and double-strand break repair are isolated and characterized. Interpretation of the physiologic fidelity of their cell-free activities will rely on descriptions of the features of the reaction within the cell.

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ADDENDUM IN PROOF

While this article was in press, F. W. Alt's laboratory published an article, in addition to references 1 and 35a, about V(D)J recombination in the first two complementation groups of Chinese hamster fibroblasts (G. E. Taccioli, G. Rathbun, E. Oltz, T. Stamato, P. Jeggo, and F. W. Alt, Science **260**:207–210, 1993).

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