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Complementation of the ionizing radiation sensitivity, DNA end binding, and V(D)J recombination defects of double-strand break repair mutants by the p86 Ku autoantigen

NIKOLAI V. BOUBNOV*†, KATHRYN T. HALL*†, ZACHARY WILLS*†, SANG EUN LEE‡, DONG MING HE‡, DAMIEN M. BENJAMIN‡, CHERYL R. PULASKI‡, HAMID BAND§, WESTLEY REEVES¶, ERIC A. HENDRICKSON‡, AND DAVID T. WEAVER*†‖

*Division of Tumor Immunology, Dana–Farber Cancer Institute, 44 Binney Street, Boston, MA 02115; †Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, MA 02115; §Lymphocyte Biology Section, Department of Rheumatology and Immunology, Brigham and Womens Hospital, Harvard Medical School, Boston, MA 02115; ¶Division of Rheumatology and Immunology, Thurston Arthritis Center, University of North Carolina, Chapel Hill, NC 27599; and ‡Department of Molecular Biology, Cell Biology, and Biochemistry, Division of Biology and Medicine, Brown University, Providence, RI 02912

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ABSTRACT **Two ionizing radiation-sensitive (IR^s) and DNA double-strand break (DSB) mutants, *sxi-3* and *sxi-2*, were shown to be severely deficient in a DNA end binding activity, similar to a previously described activity of the Ku autoantigen, correlating with the *xrs* (XRCC5) mutations. Cell fusions with *xrs-6*, another IR^s, DSB repair-deficient cell line, defined these *sxi* mutants in the XRCC5 group. *sxi-3* cells have low expression levels of the p86Ku mRNA. Introduction of the Ku p86 gene, but not the p70 Ku gene, complemented the IR^s, DNA end binding, and variable(diversity)joining [V(D)J] recombination signal and coding junction deficiencies of *sxi-3*. Thus, the p86 Ku gene product is essential for DSB repair and V(D)J recombination.**

DNA double-strand break (DSB) damage occurs spontaneously as errors in DNA synthesis, recombination, and mitosis or may be induced experimentally by ionizing radiation (IR) or other means. DSBs are deleterious to cells unless repaired. In mammals, DSB repair is likely to occur by a nonhomologous end joining pathway. Several mammalian mutants defective in DSB repair have been identified (reviewed in ref. 1).

Variable(diversity)joining [V(D)J] recombination of B and T cells has features of DSB repair functions. DSBs may be intermediates in V(D)J recombination (2–5). Coding junction products of V(D)J recombination are heterogeneous and can occur between coding DNA that has little or no homology (reviewed in ref. 1 and references therein). Coding junction synthesis and end joining in mammalian cells (6) may be highly similar to the repair pathway of IR-induced chromosomal DSBs.

Mutants that have combined defects in DSB repair and V(D)J recombination have been identified by different routes. *scid* (severe combined immunodeficient) mice are profoundly immunodeficient and have a defect in V(D)J recombination coding junction formation (7–10). *scid* cells are severely IR-sensitive (IR^s), specifically containing a DSB repair defect for IR damage (11–13). Similarly, the IR^s, DSB repair-deficient Chinese hamster ovary (CHO) cell mutants *XRCC5* and *XR-1* (14) were defective for recombination signal sequence (RSS) and coding joining of V(D)J recombination (15, 16). Recently we isolated additional mutants of IR-induced DSB repair in a screen for IR^s (S.E.L., D.M.H., D.M.B., C.R.P., and E.A.H., unpublished data). *xrs* cells are deficient in a DNA end binding activity (18–20). The Ku autoantigen is a heterodimer that binds to DNA ends (21). Mapping of the large subunit of Ku and xrs shows colocalization in a region on human chromosome 2 (22, 23).

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Here we show the *sxi-3* mutant of DSB repair is defective for both product formation steps of V(D)J recombination. *sxi-3* cells were found to be in the XRCC5 IR^s group and were complemented by the p86Ku gene for DNA repair and recombination functions. Thus, Ku must coordinate DSB repair and V(D)J recombination.

MATERIALS AND METHODS

Transfection and IR Cell Survival. Expression plasmids for human p70Ku and p86Ku cDNAs were constructed in SRa, a derivative of pcDL-SRa296 (24). SRa-p70Ku or SRa-p86Ku (20 μg) was transfected into *sxi-3* cells grown in 10:1 excess over pPGKhygromycin by standard calcium phosphate precipitation and hygromycin selection (Sigma, 200 μg/ml). One hundred to 200 colonies were pooled and replated for IR^s tests at 200 cells per 60-mm plate (13).

DNA End Binding. A ^{32}P-labeled *Pvu* II–*Xma* I fragment of pJH290 was prepared. Nuclear extracts, DNA end binding, and mobility shift gels were by standard methods (18, 19). Antibodies (1 μl of ascites fluid) for supershifting were directly added to the DNA end binding mixture.

Cell Fusion. DSB mutant and control cell lines were *scid* (*scSV3, V-3*) (13, 25); XRCC5 (*sxi-2, sxi-3*), *xrs-6* (26); *XR-1* (27); V79-4, and CHO. Cells were tagged with pPGKhygromycin or pPGKpuromycin by calcium phosphate transfection. Cell fusions were generated between puromycin-resistant and hygromycin-resistant cells. Cells (1×10^6) of each fusion partner were plated into a 60-mm plate for 24 hr, washed four times with serum-free F12 medium (SF12), then 0.5 ml of PEG 1500 (Boehringer Mannheim) added for 1 min, washed four times with SF12, incubated for 60–90 min at 37°C, and then incubated in F12 plus 10% fetal bovine serum (FBS), puromycin (2 μg/ml), and hygromycin (350 μg/ml). After 24 hr, fusions were replated at 10^3 cells per plate in duplicate. Twenty-four colonies of each fusion were tested for IR^r (13).

Methionine Labeling. *sxi-3, sxi-3*/p86Ku, and LAZ388 (5×10^6 cells) were preincubated with 2 ml of methionine-free medium plus 10% dialyzed FBS for 1 hr. Three hundred microcuries of [^{35}S]methionine (1 Ci = 37 GBq) was added for 5 hr. Following a phosphate-buffered saline wash, cells were lysed on ice in 200 μl of 40 mM Tris·HCl, pH 8.0/10 mM EDTA/0.5 M NaCl/0.5% Nonidet P-40 for 30 min; 100 μl of 18% PEG 8000 and 0.5 M NaCl were added, and cells were spun to remove debris. For immunoprecipitations, GE2-9.5

Abbreviations: DSB, double-strand break; IR, ionizing radiation; RSS, recombination signal sequence; V(D)J, variable(diversity)joining; scid, severe combined immunodeficient; ^{s}, sensitive (sensitivity); ^{r}, resistant (resistance); mAb, monoclonal antibody(ies); R, recombination frequency.

‖To whom reprint requests should be addressed.

(anti-human p86Ku) was added for 1 hr at 4°C; then 50 μl of protein A-Sepharose suspension was added for 1 hr. Protein A beads were then washed six times in lysis buffer, boiled in sample buffer, run on 7.5% SDS/polyacrylamide gels, developed in EN^{3}HANCE (DuPont), and exposed to x-ray film.

RNA Analysis. Chinese hamster cDNA libraries (Clontech, and generously provided by G. Gill, University of Massachusetts) were screened with human p70Ku and p86Ku probes. Lambda phage were plaque purified and cDNAs of hamster p70Ku (aa 85–555) and p86Ku (aa 39–732) were used for hybridization probes in standard Northern analysis.

V(D)J Recombination. Transfection, isolation of V(D)J recombinant products, and analysis of recombination frequencies have been described (28, 29). DNA sequencing of *sxi-3*/p86 coding junctions showed the normal extent of junctional deletion.

RESULTS

DNA End Binding Activity Is Deficient in *sxi* Mutants. The mutants *xrs-5, XR-V9B*, and *XR-V15B* (XRCC5 group) were defective for a DNA end binding activity associated with Ku (18–20). Thus, we tested *sxi-2* and *sxi-3* for DNA end binding by using mobility shift gel electrophoresis (*Materials and Methods*). *sxi-2* and *sxi-3* extracts were severely deficient in DNA end binding by this assay (Fig. 1). The parental cell line, V79-4, and a human cell line, LAZ388, produced normal levels of complex A. As a control, another DNA–protein complex (complex B) is not competitively inhibited by excess circular DNA and appears at the same level in V79-4, *sxi-2*, and *sxi-3* (data not shown; ref. 18). *xrs-6* was also deficient in DNA binding activity compared to the parental cell line, CHO (Fig. 1). Extracts from *scid* and *XR-1* cells contained the DNA end binding activity at normal levels (data not shown). Thus, *sxi-2* and *sxi-3* are missing a similar biochemical function for DNA end binding as XRCC5 group members.

Cell Fusions and Screening of the IR^s Phenotype Places *sxi-3* and *sxi-2* in the XRCC5 Group. The above results suggested that *sxi-3* and *sxi-2* may be defective in XRCC5 gene products. We tested whether these mutants could complement other mutant cell lines (*scid, XRCC5, XR-1*) for DSB repair. *sxi-3, sxi-2*, V79-4, *scSV3, xrs-6, XR-1*, and *V-3* cells were transfected with either pPGKhygro or pPGKpuro to prepare marked cell lines that could be differentially selected (*Materials and Methods*). Cell fusions were formed between different DSB repair mutant cell lines, and following hygromycin and puromycin selection, fusion clones were evaluated for IR^s. Puromycin-resistant or hygromycin-resistant DSB repair mutant cells had the same IR^s as the untransfected mutant cell lines (data not shown). We found that fusion of *sxi-3* or *sxi-2* to V79-4 gave IR^r clones, indicating that these mutants were recessive and could be complemented for their IR^s defects (Table 1). In addition, fusion of *sxi-3* to *sxi-2* only yielded IR^s cells, suggesting that these mutants may contain defects in the same gene. IR^r clones were readily observed from each of the fusions of *sxi-3 or sxi-2* with *XR-1, scSV3*, and *V-3*. IR^r clones were examined in IR survival curves with four IR doses to confirm that these clones had been complemented for the IR^s defect of either fusion partner (data not shown). In contrast to the other complementation groups, fusions of either *sxi-2* or *sxi-3* with *xrs-6* were always IR^s (Table 1). Fusion cell lines of *sxi-3* and *xrs-6* were as defective for DNA end binding as were the parental mutants (data not shown). Therefore, *sxi-3, sxi-2*, and *xrs-6*, by definition of the lack of complementation following cell fusion, are members of the XRCC5 group.

Table 1. Complementation of IR^s of *sxi-2* and *sxi-3* by cell fusion

Cell line	V79-4	*xrs-6*	*scSV3*	*V-3*	*XR-1*	*sxi-2*	*sxi-3*
sxi-2	+	−	+	+	+	−	−
sxi-3	+	−	+	+	+	−	−

Individual fusion clones were tested for IR (450 rads; 1 rad = 0.01 Gy) cell survival. +, >20% of the cell clones expanded from the fusion were IR^r; −, 0% of the fusion cell clones were IR^r, after >20 cell fusion lines were tested.

Restoration of IR^s and DNA Binding Defects of *sxi-3* by p86Ku. We examined whether the *sxi* mutant phenotypes could be complemented by the introduction of Ku genes. *sxi-3* cells were stably transfected with the human p70Ku and p86Ku cDNAs cloned into a mammalian expression vector, SRa (*Materials and Methods*). Following selection, *sxi-3*/Ku cells were examined for IR^s in cell survival assays. We found that transfection of p86Ku restored an IR^r phenotype to *sxi-3* cells (Fig. 2). sxi-3/p86Ku cells were significantly more IR^r than *sxi-3* at every IR dose tested. *sxi-2* cells were similarly reconstituted for IR^r by p86Ku (data not shown). Transfection of pPGKhygro alone did not change the IR^s (*sxi-3H*). *sxi-3* cells were only weakly complemented by transfection of the p70Ku expression plasmid.

Increased IR^r of *sxi-3*/p86Ku cells would be expected to correlate with an increased DNA end binding capacity if a single gene were responsible for both functions. We prepared nuclear extracts from *sxi-3* cells transfected with p70Ku or p86Ku and from *sxi-3H*. With equal quantities of nuclear protein extracts examined, *sxi-3*/p86Ku cells reconstituted the level of DNA end binding observed with the V79-4 control extracts (Fig. 3*A*). *sxi-3*/p70 extracts yielded only a low level of DNA end binding activity. A mAb (111) raised against human Ku binds to an epitope of the p86 subunit (30). mAb 111 preincubated with human LAZ388 extracts caused a supershift in complex A of the DNA end binding assay (Fig. 3*B*). Complex

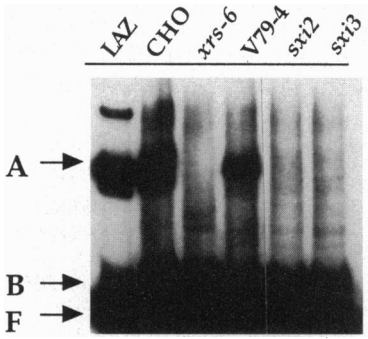

FIG. 1. DNA end binding activity is absent in *sxi-2* and *sxi-3* cells. Following incubation of nuclear extracts with [^{32}P]DNA (159 bp), samples were fractionated by mobility shift gel electrophoresis. A DNA end binding complex, A (A), is shown relative to the uncomplexed fragment (F). Complex B (B) is present in V79-4 and *sxi* mutants and is not competitively inhibited by excess linear DNA (18).

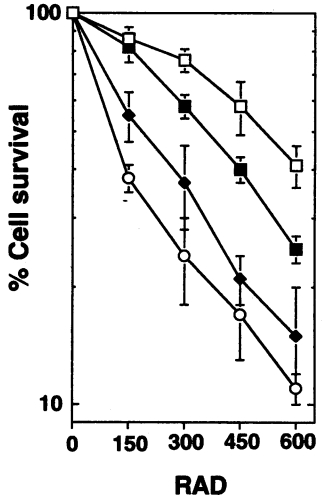

FIG. 2. Complementation of IR defect of *sxi-3* by the p86Ku gene. *sxi-3* cells were stably transfected with p86Ku, p70Ku, or pPGKhygro only. *sxi-3H* (○), sxi-3/p86Ku (■), *sxi-3*/p70Ku (◆), and V79-4 (□) cells were treated with IR (0–600 rads) and cell survival was measured.

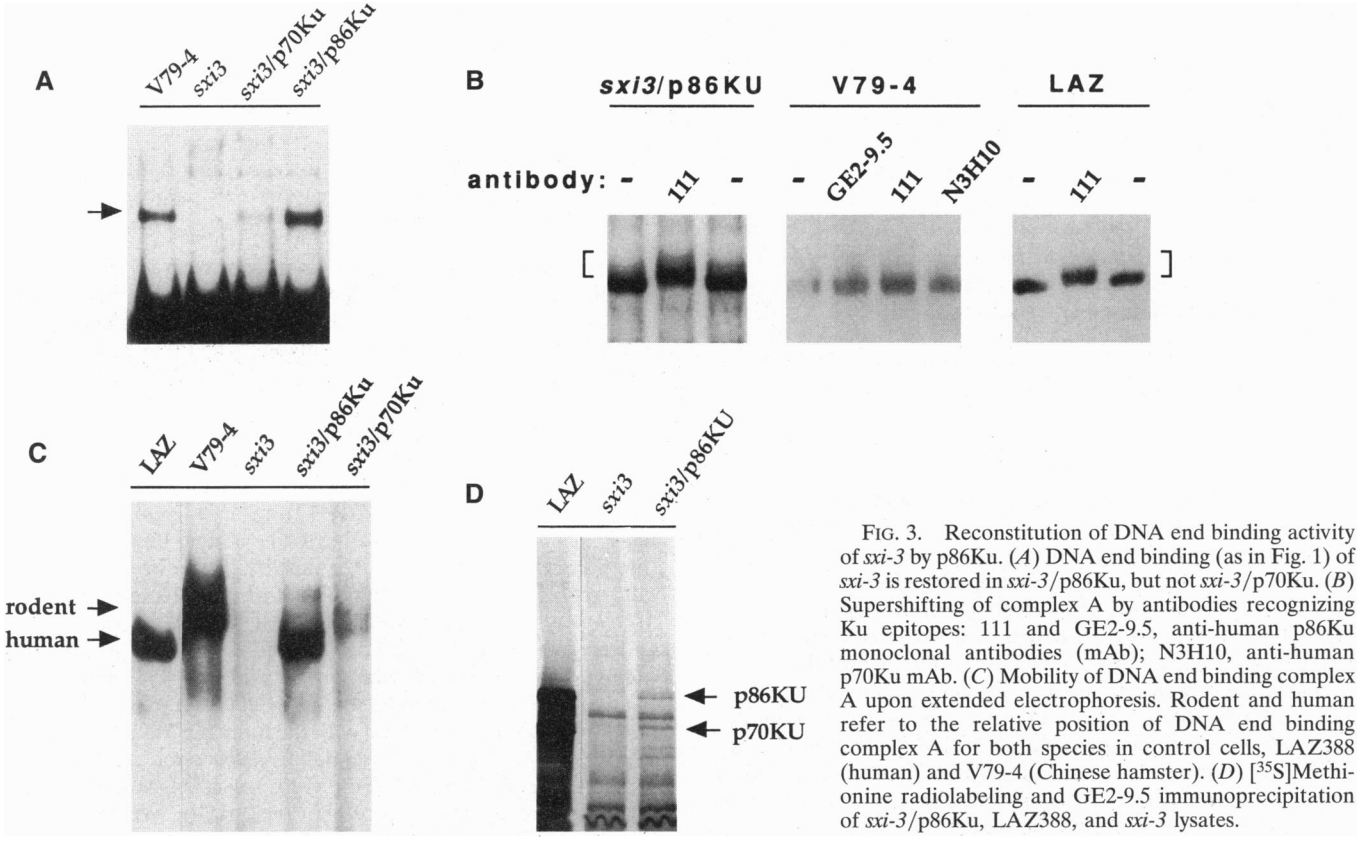

FIG. 3. Reconstitution of DNA end binding activity of *sxi-3* by p86Ku. (*A*) DNA end binding (as in Fig. 1) of *sxi-3* is restored in *sxi-3*/p86Ku, but not *sxi-3*/p70Ku. (*B*) Supershifting of complex A by antibodies recognizing Ku epitopes: 111 and GE2-9.5, anti-human p86Ku monoclonal antibodies (mAb); N3H10, anti-human p70Ku mAb. (*C*) Mobility of DNA end binding complex A upon extended electrophoresis. Rodent and human refer to the relative position of DNA end binding complex A for both species in control cells, LAZ388 (human) and V79-4 (Chinese hamster). (*D*) [^{35}S]Methionine radiolabeling and GE2-9.5 immunoprecipitation of *sxi-3*/p86Ku, LAZ388, and *sxi-3* lysates.

A of the *sxi-3*/p86Ku extract, but not V79-4, was also supershifted by 111 (Fig. 3*B*). mAb 111 or an anti-human p70Ku mAb (N3H10) does not sufficiently cross-react with the hamster p86Ku by this assay or by immunoprecipitation (data not shown). Importantly, complex A of V79-4 cells was of reduced mobility relative to LAZ388 cells loaded in the adjacent lane (Fig. 3*C*). Complex A of *sxi-3*/p86Ku was identical in mobility to that of human extracts (Fig. 3*C*). Conversely, the low level of *sxi-3*/p70Ku complex A had the mobility of hamster extracts. Therefore, the mobility of complex A is dictated by the species of the p86Ku subunit. Human p86Ku promotes a faster mobility than hamster p86Ku. These experiments show that *sxi-3*/p86 cells contain a functionally active p86Ku subunit expressed from the transfected human gene that is a member of the DNA binding complex.

sxi-3/p86Ku cells were radiolabeled with [^{35}S]methionine to determine whether human p86Ku can complex with hamster p70Ku. We immunoprecipitated cell lysates with GE2-9.5, a mAb that specifically recognizes the human p86Ku subunit. GE2-9.5 quantitatively precipitates the p70Ku subunit by its ability to bind to p86Ku (LAZ388, Fig. 3*D*). *sxi-3*/p86Ku lysates were immunoprecipitated with GE2-9.5, and we observed a coprecipitating 70-kDa protein that is presumably the hamster p70Ku subunit (Fig. 3*D*). Equal abundances of p86 and p70 were found by this methodology. *sxi-3* cells do not have any immunoprecipitating material in the p86 and p70 regions. These data indicate that human p86Ku can stably associate with hamster p70Ku.

p86Ku mRNA Is Absent in *sxi-3*. To determine the nature of Ku defects in *sxi-3* and *sxi-2* cells, we cloned the Chinese hamster p86Ku and p70Ku genes by their ability to cross-hybridize with human p86 and p70 cDNA probes. The isolated cDNA inserts encoded highly homologous genes to the mouse and human p70Ku and p86Ku genes by DNA sequencing (data not shown). A Northern blot prepared with *sxi-3* and V79-4 poly(A)$^+$ mRNA was probed with the p86Ku hamster cDNA probe (*Materials and Methods*). V79-4 cells displayed a 2.8-kb mRNA that was not present in the *sxi-3* lane despite equal loading determined by reprobing with glyceraldehyde-3-phosphate dehydrogenase (Fig. 4). *sxi-3* and V79-4 had approximately the same levels of p70Ku mRNA. p86Ku gene expression was undetectable in *sxi-2* cell Northern blots, whereas p70Ku mRNA was at wild-type levels (data not shown).

V(D)J Recombination RSS and Coding Junction Defects of *sxi-3* Are Restored by p86Ku. We examined whether *sxi-3* and *sxi-2* affected RSS and/or coding junction formation by cotransfection of V(D)J recombination templates with RAG1 and RAG2 to stimulate recombination (28, 29). RSS junction formation, measured with pJH200, was dramatically reduced in *sxi-3* (R = 0.068% ± 0.037%) relative to V79-4 (R = 1.25% ± 0.63%) (Table 2). Only 21–44% of the RSS–RSS fusions in *sxi-3* were precise. *sxi-3* also had severely diminished coding junction formation (R = 0.016% ± 0.001%) compared to V79-4 (R = 0.73% ± 0.18%). *sxi-2* reduced RSS and coding junction formation by 27- and 17-fold, respectively (data not shown). Importantly, RSS joining was complemented by p86Ku (*sxi-3*/p86Ku, R = 1.01% ± 0.23%) relative to negative and positive controls: *sxi-3* (0.068% ± 0.037%) and V79-4 (1.25% ± 0.63%) (Table 2). *sxi-3*/p86Ku RSS joints also

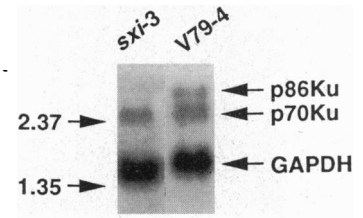

FIG. 4. Northern analysis of *sxi-3* for Ku gene expression. Poly(A)$^+$ prepared from *sxi-3* and V79-4 was evaluated for p86Ku and p70Ku expression. The Northern blot was probed sequentially with hybridization probes for p86Ku, p70Ku, and control glyceraldehyde-3-phosphate dehydrogenase (GAPDH) genes. *Escherichia coli* size markers (kilobases) are shown.

Table 2. V(D)J recombination deficiencies of *sxi-3* mutant and complementation by p86Ku

Cell line	Exp.	Amp^{r}*	Amp^{r} + Cam^{r}†	R, %‡	Average§	ApaL1^{s}¶
			RSS joining (pJH200)			
V79-4	1	334,000	3200	0.96	1.25 ± 0.63	19/21
	2	294,000	2400	0.82		
	3	204,000	4040	1.98		
sxi-3	1	9,000	5	0.056	0.068 ± 0.037	8/18
	2	23,000	25	0.109		
	3	5,100	2	0.039		
sxi-3/p86Ku	1	17,000	128	0.75	1.01 ± 0.23	21/21
	2	48,100	545	1.13		
	3	25,800	300	1.16		
sxi-3/p70Ku	1	12,800	20	0.16	0.15 ± 0.065	4/ 8
	2	13,100	28	0.21		
	3	6,200	5	0.081		
			Coding joining (pJH290)			
V79-4	1	129,000	1200	0.93	0.73 ± 0.18	
	2	143,000	800	0.56		
	3	67,000	410	0.61		
	4	26,200	215	0.82		
sxi-3	1	109,000	18	0.017	0.016 ± 0.001	
	2	68,000	10	0.015		
sxi-3/p86Ku	1	319,000	1240	0.39	0.39 ± 0.01	
	2	203,000	765	0.38		
sxi-3/p70Ku	1	77,000	20	0.026	0.040 ± 0.019	
	2	38,000	20	0.053		

*Ampicillin (100 μg/ml)-resistant bacterial colonies.
†Cam^{r} + Amp^{r}, chloramphenicol (20 μg/ml)- and ampicillin (100 μg/ml)-resistant colonies.
‡[(Cam^{r} + Amp^{r})/Amp^{r}] × 100.
§Average ± SD.
¶% correct joins determined from ApaL1 restriction digests.

formed with an increased precision, as assessed by ApaL1^{s}. In contrast, *sxi-3*/p70Ku showed only marginal complementation of RSS junction frequency and fidelity. The R values of coding junctions were also complemented in *sxi-3*/p86Ku cells (R = 0.39% ± 0.01%) compared to *sxi-3* (R = 0.016% ± 0.001%). In this case, V79-4 controls yielded higher values for pJH290 (0.73% ± 0.18%). *sxi-2*/p86Ku cells that were IR^{r} and DNA end binding-positive (data not shown) also restored RSS and coding joining proficiency (R = 0.80% ± 0.49% and 0.86% ± 0.18%) relative to *sxi-2* (R = 0.045% ± 0.014% and 0.053% ± 0.035%). Thus, p86Ku complements the V(D)J recombination deficiency like the IR^{s} and DNA end binding properties of *sxi-3* and *sxi-2*.

DISCUSSION

This study defines *sxi-3* and *sxi-2* as members of the XRCC5 group, deficient in a DNA end binding activity and defective in RSS and coding junction product synthesis of V(D)J recombination. Introduction of the p86Ku gene complemented all of the mutant phenotypes, including IR, DNA end binding, and RSS and coding junction formation of V(D)J recombination (Figs. 2 and 3, Table 2, and data not shown). These data identify p86Ku as an essential factor of V(D)J recombination and DSB repair.

Several experiments strongly suggest that the 86-kDa Ku gene product is directly responsible for the DSB repair, V(D)J recombination, and DNA end binding defects of these *sxi* mutants. We have demonstrated a very strong correlation between the repair and recombination characteristics of *sxi-3*/p86Ku (Fig. 2 and Table 2) and the biochemical properties of DNA end binding complexes that were shown to be absent in *sxi-3* (Fig. 3). Our observations are in concert with previous data indicating that the DNA end binding complex is Ku in mammalian cells. Purified Ku, consisting of only the 86- and 70-kDa subunits, binds to DNA ends *in vitro* and has similar properties to the DNA end binding complex that we have described here (32). Supershifting with an anti-human p86Ku antibody and the mobility of the reconstituted DNA binding complex indicated that human p86Ku is a constituent of the complex found in *sxi-3*/p86Ku cells (Fig. 3 *B* and *C*). Independent of the biochemical data, p86Ku mRNA was not detectably produced in *sxi-3* and *sxi-2* and these cell lines do not complement one another in cell fusions (Table 1). Importantly, human p86Ku colocalizes with *xrs*, another XRCC5 mutant (23). *xrs* cells are restored for IR^{s} and V(D)J recombination defects by microcell-mediated transfer of human chromosome 2 (15, 33). During the publication of this work, the repair and recombination defects of *xrs-6* cells were shown to be complemented by p86Ku (34). Our work with *sxi* mutants corroborates these findings. We also found that *xrs-6* could be reconstituted for V(D)J recombination RSS and coding junction formation by p86Ku, but not p70Ku, further supporting the correlation between XRCC5 mutants and p86Ku.

Our data also strongly suggest that the 70-kDa Ku gene product is not mutated or altered in the XRCC5 group, even though the DNA end binding complex that includes the p70Ku gene product is absent (20). Introduction of the p70 Ku subunit was unable to significantly increase the level of complementation of IR^{s}, V(D)J recombination product formation, or DNA end binding (Figs. 2 and 3 and Table 2). Our preliminary data suggest that *sxi-3, sxi-2*, and *xrs* normally produce the p70Ku subunit because p70Ku mRNA is expressed as in control cells. At least for *sxi-3* cells, a p70Ku protein is produced and only appears in the DNA end binding complex when a functional p86Ku is also present (Fig. 3).

V(D)J Recombination Complementation Groups. The V(D)J recombination pathway requires gene products that are lymphoid cell-restricted as well as several that are expressed in other cell types and may be ubiquitous (1). To date, three complementation groups (scid, XR-1, and XRCC5) required in the combined DNA repair and V(D)J recombination func-

tions have been reported. Several criteria, including those in this study, support the conclusion that three separate genes are represented in these groups. *sxi-3* and *sxi-2* are distinct from *scid* and *XR-1* based on the IR complementation tests from the cell fusions (Table 1). Likewise, the determination of V(D)J recombination properties of *sxi-3* and *sxi-2* differentiate these mutants from *scid*. We observed that coding and RSS junction formation were defective for both mutants (Table 2). This same recombination feature of both junctions being affected had previously been reported for *xrs-6* and *xrs-5* (15, 16). For *scid* and *V-3*, there is a selective deficiency in coding junction formation (9, 25, 35). Because there are now established to be similar repair and recombination properties of four independent mutants in XRCC5 group compared to the two mutants of the scid group, Ku and scid gene products are very likely not to be allelic variations of the same gene. Furthermore, *XR-1* and *scid* cells have normal levels of the DNA end binding activity (refs. 18–20 and this study), and p86Ku and p70Ku genes map to different chromosomes from *scid* or *XR-1* (1).

Because Ku exists as a heterodimer in cells, p70Ku mutations may be defective in DSB repair and V(D)J recombination. The 70-kDa subunit binds to DNA, even in the absence of p86Ku (refs. 21 and 36 and references therein). Therefore, p70Ku may provide the DNA binding activity critical to these functions. Ku is associated with a protein kinase (DNA-PK) in mammalian cells (37, 38). The kinase is activated by DNA ends, presumably via the associated Ku heterodimers. Tethering of an activatible protein kinase is very likely to have important ramifications for DSB repair and V(D)J recombination. DNA-PK may phosphorylate repair and recombination proteins to modulate their activity.

Ku Function in DSB Repair and V(D)J Recombination. Ku could be used at initiation or at product formation steps in V(D)J recombination. Defects at both stages of the reaction may be expected to decrease the R value for detecting either of the recombination products. Our data and previous reports are strongly supportive of a role of Ku in V(D)J recombination product formation instead of the initiation of V(D)J recombination. Ku associates with ends of DNA with high affinity (39). Although the actual end structures for V(D)J recombination and DSB repair are not documented fully, in both cases these ends are likely to have significant variability. Importantly, Ku is able to recognize many variations in DNA structure by binding to the transition of single-stranded and double-stranded DNA or hairpins (17, 31, 40, 41). Thus, Ku is more likely to play a role at intermediate stages of V(D)J recombination, where DSBs are formed and need to be repaired. For DSB repair, the appearance of damaged DNA ends may signal the recognition by Ku.

Ku may associate with the chromosomal regions of V(D)J recombinase activity by passive copositioning on the DNA or by association with other recombination components. Ku can translocate along DNA to reach a DSB (17, 40, 41). Second, due to its strong affinity for DNA ends, Ku complexes could displace other proteins from DNA ends in the reaction. The substitution of Ku complexes for preexisting structures may serve to prepare the DNA ends for processing or joining. Because coding ends and RSS ends are metabolized differently, additional factors such as scid and XR-1 must contribute. Identification of one Ku subunit as an important player in these processes will accelerate the understanding of repair and recombination pathways overall.

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1. Weaver, D. (1994) *Adv. Immunol.* **58,** 29-85.
2. Roth, D. B., Nakajima, P. B., Menetski, J. P., Bosma, M. J. & Gellert, M. (1992) *Cell* **69,** 41–53.
3. Roth, D. B., Menetski, J. P., Nakajima, P. B., Bosma, M. J. & Gellert, M. (1992) *Cell* **70,** 983–991.
4. Schlissel, M., Constantinescu, A., Morrow, T., Baxter, M. & Peng, A. (1993) *Genes Dev.* **7,** 2520–2532.
5. Roth, D. B., Zhu, C. & Gellert, M. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 10788–10792.
6. Roth, D. B., Chang, X.-B. & Wilson, J. H. (1989) *Mol. Cell. Biol.* **9,** 3049–3057.
7. 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.
8. Hendrickson, E. A., Schatz, D. G. & Weaver, D. T. (1988) *Genes Dev.* **2,** 817–829.
9. 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.
10. Malynn, B. A., Blackwell, T. K., Fulop, G. M., Rathbun, G. A., Furley, A. J. W., Ferrier, P., Heinke, L. B., Phillips, R. A., Yancopoulos, G. D. & Alt, F. W. (1988) *Cell* **54,** 453–460.
11. Fulop, G. M. & Phillips, R. A. (1990) *Nature (London)* **347,** 479–482.
12. Biedermann, K. A., Sun, J., Giaccia, A. J., Tosto, L. M. & Brown, J. M. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 1394–1397.
13. Hendrickson, E. A., Qin, X.-Q., Bump, E. A., Schatz, D. G., Oettinger, M. & Weaver, D. T. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 4061–4065.
14. Jeggo, P. (1990) *Mutat. Res.* **239,** 1–16.
15. Taccioli, G. E., Rathbun, G., Oltz, E., Stamato, T., Jeggo, P. A. & Alt, F. W. (1993) *Science* **260,** 207–210.
16. Pergola, F., Zdzienicka, M. Z. & Lieber, M. R. (1993) *Mol. Cell. Biol.* **13,** 3464–3471.
17. Zhang, W.-W. & Yaneva, M. (1992) *Biochem. Biophys. Res. Commun.* **186,** 574–579.
18. Rathmell, W. K. & Chu, G. (1994) *Mol. Cell. Biol.* **14,** 4741–4748.
19. Getts, R. C. & Stamato, T. D. (1994) *J. Biol. Chem.* **269,** 15981–15984.
20. Rathmell, W. K. & Chu, G. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 7623–7627.
21. Mimori, T. & Hardin, J. A. (1986) *J. Biol. Chem.* **261,** 10375–10379.
22. Hafezparast, M., Kaur, G. P., Zdzienicka, M., Athwal, R. S., Lehmann, A. R. & Jeggo, P. A. (1993) *Somat. Cell Genet.* **19,** 413–421.
23. Cai, Q. Q., Plet, A., Imbert, J., Lafagepochitaloff, M., Cerdan, C. & Blanchard, J. M. (1994) *Cytogenet. Cell Genet.* **65,** 221–227.
24. Takebe, Y., Seiki, M., Fujisawa, J., Hoy, P., Yokota, K., Arai, K., Yoshida, M. & Arai, N. (1988) *Mol. Cell. Biol.* **8,** 466–472.
25. Taccioli, G. E., Cheng, H.-L., Varghese, A. J., Whitmore, G. & Alt, F. W. (1994) *J. Biol. Chem.* **269,** 7439–7442.
26. Jeggo, P. A. & Kemp, L. M. (1983) *Mutat. Res.* **112,** 313–327.
27. Stamato, T. D., Weinstein, R., Giaccia, A. & Mackenzie, L. (1983) *Somat. Cell Genet.* **9,** 165–173.
28. Boubnov, N. V., Wills, Z. P. & Weaver, D. T. (1993) *Mol. Cell. Biol.* **13,** 6957-6968.
29. Oettinger, M. A., Schatz, D. G., Gorka, C. & Baltimore, D. (1990) *Science* **248,** 1517–1523.
30. Reeves, W. (1985) *J. Exp. Med.* **161,** 18–39.
31. Falzon, M. & Kuff, E. (1992) *Nucleic Acids Res.* **20,** 3784.
32. Griffith, A. J., Blier, P. R., Mimori, T. & Hardin, J. A. (1992) *J. Biol. Chem.* **267,** 331–338.
33. Jeggo, P., Hafezparast, M., Thompson, A. F., Broughton, B. C., Kaur, G. P., Zdzienicka, M. Z. & Athwal, R. S. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 6423–6427.
34. Taccioli, G., Gottlieb, T. M., Blunt, T., Priestly, A., Demengeot, J., Mizuta, R., Lehmann, A. R., Alt, F. W., Jackson, S. P. & Jeggo, P. A. (1994) *Science* **265,** 1442–1445.
35. Hendrickson, E. A., Schlissel, M. S. & Weaver, D. T. (1990) *Mol. Cell. Biol.* **10,** 5397–5407.
36. Chou, C., Wang, J., Knoth, M. & Reeves, W. (1992) *J. Exp. Med.* **175,** 1677–1684.
37. Gottlieb, T. M. & Jackson, S. P. (1993) *Cell* **72,** 131–142.
38. Dvir, A. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 11920–11924.
39. Blier, P. R., Griffith, A. J., Craft, J. & Hardin, J. A. (1993) *J. Biol. Chem.* **268,** 7594–7601.
40. Paillard, S. & Strauss, F. (1991) *Nucleic Acids Res.* **19,** 5619–5624.
41. deVries, E., van Driel, W., Bergsma, W. G., Arnberg, A. C. & van der Vliet, P. C. (1989) *J. Mol. Biol.* **208,** 65–78.