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Defective DNA-Dependent Protein Kinase Activity Is Linked to V(D)J Recombination and DNA Repair Defects Associated with the Murine *scid* Mutation

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Summary

Murine cells homozygous for the severe combined immune deficiency mutation (*scid*) and V3 mutant hamster cells fall into the same complementation group and show similar defects in V(D)J recombination and DNA double-stranded break repair. Here we show that both cell types lack DNA-dependent protein kinase (DNA-PK) activity owing to defects in DNA-PK$_{cs}$, the catalytic subunit of this enzyme. Furthermore, we demonstrate that yeast artificial chromosomes containing the DNA-PK$_{cs}$ gene complement both the DNA repair and recombination deficiencies of V3 cells, and we conclude that DNA-PK$_{cs}$ is encoded by the *XRCC7* gene. As DNA-PK binds to DNA ends and is activated by these structures, our findings provide novel insights into V(D)J recombination and DNA repair processes.

Introduction

The only characterized example of site-specific recombination in vertebrates is the V(D)J recombination process employed by developing lymphoid cells. During V(D)J recombination, the variable regions of immunoglobulin and T cell receptor genes are created by the juxtaposition of the variable (V), diversity (D), and joining (J) segments (for reviews see Lieber, 1991; Alt et al., 1992; Gellert, 1992; Lewis, 1994). V(D)J recombination is targeted by conserved recombination signal sequences (RSSs) that flank each germline V, D, and J segment. The process is initiated by the generation of precise DNA double-stranded breaks (DSBs) between the RSS and the potential coding sequences of the segments to be joined. Subsequent processing and ligation events normally result in the joining of the two coding gene segments (coding joins) and joining of the two RSSs (RSS joins). Whereas the RSSs are generally precisely juxtaposed without loss of nucleotides, coding segment termini are usually subject to varying degrees of base deletion, addition, or both before ligation. This indicates that, although linked, the pathways generating coding and RSS joins are distinct.

V(D)J recombination employs numerous cellular components (for reviews see Lieber, 1991; Alt et al., 1992; Gellert, 1992; Lewis, 1994). Candidates for lymphocyte-specific components are the RAG1 and RAG2 proteins (Oettinger et al., 1990). Coexpression of the *RAG* genes in nonlymphoid cells confers an ability to perform V(D)J rearrangements (Oettinger et al., 1990), while ablation of either *RAG* gene in mice results in an absence of mature B and T cells due to an inability of precursor lymphocytes to initiate V(D)J recombination (Mombaerts et al., 1992; Shinkai et al., 1992).

As with site-specific recombination processes in other systems, V(D)J recombination also employs widely expressed factors, as indicated by the effects of the murine severe combined immune deficiency mutation (*scid*; for review see Bosma and Carroll, 1991). Mice homozygous for the *scid* mutation (Scid mice) are deficient in mature B and T lymphocytes owing to a V(D)J recombination defect (Schuler et al., 1986; Lieber et al., 1988; Malynn et al., 1988; Blackwell and Alt, 1989). Scid cells can initiate V(D)J rearrangement by forming precise DSBs, can join RSSs relatively normally, but are profoundly impaired in coding join formation. Furthermore, both lymphoid and nonlymphoid Scid cells are hypersensitive to killing by ionizing radiation (IR) owing to a DNA DSB repair defect (Fulop and Phillips, 1990; Biedermann et al., 1991; Hendrickson et al., 1991). Together, these observations suggest that the gene affected by the *scid* mutation is widely expressed, is a component of the cellular DNA DSB repair machinery, and is recruited to participate in DSB rejoining during V(D)J recombination.

Further evidence for a link between V(D)J recombination and DNA DSB repair came from the identification of V(D)J recombination defects in the radiosensitive Chinese hamster ovary (CHO) cell lines xrs-6, XR-1, and V3, which also manifest defects in DNA DSB rejoining (Kemp et al., 1984; Giaccia et al., 1985; Whitmore et al., 1989; Pergola et al., 1993; Taccioli et al., 1993, 1994a). These mutant cell lines define three distinct complementation groups (for review see Jeggo et al., 1991), with XR-1 and xrs-6 being assigned to groups 4 and 5, respectively. Whereas xrs-6 and XR-1 cells are drastically impaired in generating both coding

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and RSS joins, V3 cells have the Scid-like phenotype of being defective primarily in coding join formation (Pergola et al., 1993; Taccioli et al., 1994a). Consistent with this latter observation, somatic cell fusion analyses have indicated that the V3 and *scid* mutations fall into the same complementation group, which we now refer to as IR complementation group 7 (Taccioli et al., 1994a). Furthermore, human chromosome 8 has been reported to complement the DNA repair defects of both V3 and Scid cells (Itoh et al., 1993; Kirchgessner, 1993; Komatsu et al., 1993; M. Zdzienicka and P. A. J., unpublished data) and to complement the V(D)J recombination defect of murine Scid cells (Banga et al., 1994). Together, these data indicate that the murine *scid* and hamster V3 mutations reside in equivalent genetic loci.

The Ku autoimmune antigen is a nuclear DNA end-binding protein composed of two subunits of approximately 70 kDa and 80 kDa (Ku70 and Ku80, respectively), which serves as the DNA binding component of DNA-dependent protein kinase (DNA-PK). Ku functions in this regard by associating with the DNA-PK catalytic subunit (DNA-PK_{cs}), a polypeptide of approximately 450 kDa (Dvir et al., 1992; Gottlieb and Jackson, 1993; K. O. Hartley et al., unpublished data). Recently, we and others have shown that xrs-6 cells are defective in functional Ku (Getts and Stamato, 1994; Rathmell and Chu, 1994; Taccioli et al., 1994b) and lack detectable DNA-PK activity (Finnie et al., 1995). Furthermore, the characteristic radiosensitivity and V(D)J recombination defects of xrs-6 cells are complemented by expression of the *Ku80* cDNA, indicating that Ku80 is the product of the X-ray cross-complementing group 5 gene (*XRCC5*) that complements the defect in IR group 5 cells (Smider et al., 1994; Taccioli et al., 1994b).

The physiological function for DNA-PK is currently unclear, but a large body of evidence suggests that it has a role in regulating transcription. For example, in vitro studies have shown that DNA-PK can phosphorylate a variety of transcription factors (for example, Jackson et al., 1990; Anderson and Lees-Miller, 1992; Bannister et al., 1993). Furthermore, DNA-PK has been shown to repress transcription by RNA polymerase I (Kuhn et al., 1995). The recent demonstration that Ku80 is the product of the *XRCC5* gene suggests that, in addition to affecting transcription, DNA-PK_{cs} may also be intimately involved in DNA DSB repair and V(D)J recombination. In line with this suggestion, the DNA-PK_{cs} gene has been shown recently to map to the region of chromosome 8 that complements the *scid* mutation (Sipley et al., submitted). In this paper, we provide direct evidence that DNA-PK_{cs} is encoded by *XRCC7*, the gene that complements IR group 7 (V3 and Scid) mutants. These results have profound implications for our understanding of the mechanisms of DNA DSB repair and V(D)J recombination.

Results

Hamster V3 and Mouse Scid Cells Are Defective in DNA-PK Activity

Only low levels of DNA-PK activity are present in rodent cell lines compared with cell lines derived from primates (Anderson and Lees-Miller, 1992; Finnie et al., 1995). To enable an examination of DNA-PK activity in the mutant rodent cell lines, we developed a highly sensitive assay in which whole-cell extracts are microfractionated on double-stranded DNA (dsDNA) cellulose and tested for an ability to phosphorylate a consensus DNA-PK recognition motif within a peptide derived from the N-terminal transcriptional activation region of p53 (Finnie et al., 1995). A mutated version of this peptide that is no longer phosphorylated by DNA-PK was employed as a control. Consistent with previous work, we found that, whereas wild-type hamster cell lines such as AA8 contain DNA-PK activity, xrs-6 cells do not (Figure 1A). Strikingly, the V3 cell line was found to lack DNA-PK activity completely. In contrast, XR-1 cells, which are also deficient in DNA DSB repair and V(D)J recombination, contain approximately wild-type

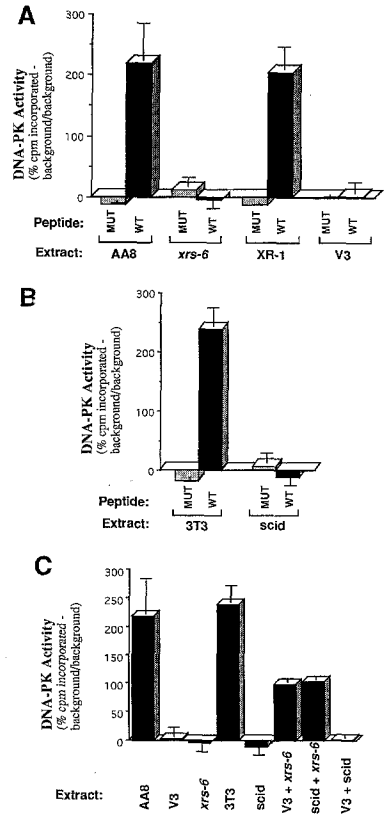

Figure 1. Cells Falling into the *scid* Complementation Group Lack DNA-PK Activity

(A) Hamster V3 and xrs-6 cells are deficient in DNA-PK. Whole-cell extracts (150 μg) derived from the hamster cell lines indicated were tested for DNA-PK activity using standard DNA-PK microfractionation/peptide assays (see Experimental Procedures). Assays were conducted in the presence of wild-type p53 peptide (WT) that is recognized effectively by DNA-PK or in the presence of a mutated peptide (MUT) that is an ineffective DNA-PK substrate.

(B) Murine Scid cells lack DNA-PK activity. Whole-cell extracts (150 μg) derived from the mouse cell lines indicated were tested for DNA-PK activity as in (A).

(C) Biochemical complementation assays. Whole-cell extracts (150 μg) or pairwise mixtures of Scid, V3, and xrs-6 extracts (150 μg plus 150 μg in each case) were tested for DNA-PK activity as in (A).

levels of DNA-PK. We also examined the mouse Scid cell line SCGRII for DNA-PK levels. Importantly, whereas control mouse 3T3 cells were found to contain DNA-PK activity, it could not be detected in extracts of Scid cells (Figure 1B). These data therefore support the results of cell fusion studies indicating that V3 and Scid cells belong to the same complementation group (IR group 7) and suggest strongly that mutations in the *scid* gene result in an absence of functional DNA-PK.

Xrs-6 Extracts Restore DNA-PK Activity to Extracts of V3 and Scid Cells

Previous studies have established that the fusion of xrs-6 cells with either V3 or Scid cells results in hybrids that are proficient in V(D)J recombination and DNA DSB repair; in contrast, V3–Scid cell hybrids are still defective in these processes (Taccioli et al., 1994a). To determine whether similar complementation behavior could be observed in vitro, we mixed pairs of extracts before performing microfractionation and DNA-PK assays. Whereas DNA-PK activity is not recovered upon mixing V3 and Scid extracts, mixing xrs-6 extracts with those of either V3 or Scid cells results in appreciable levels of DNA-PK activity (Figure 1C). This demonstrates that V3 and Scid cells are in the same biochemical complementation group, which is distinct from that of xrs-6. Since the defect in xrs-6 cells is complemented by Ku80, this implies that V3 and Scid cells are proficient in this component and suggests that they are defective in either Ku70 or DNA-PK$_{cs}$.

V3 and Scid Extracts Are Defective in Recruiting DNA-PK$_{cs}$ to DNA

Since both subunits of Ku are required for it to bind to DNA effectively and since nuclear extracts of V3 and Scid cells have been shown previously to possess Ku DNA end–binding activity (Getts and Stamato, 1994; Rathmell and Chu, 1994; N. J. F., T. M. G., and S. P. J., unpublished data), it is likely that these cells contain Ku70 that is proficient in interacting with Ku80 and mediating DNA binding. Consistent with this, V3 and Scid cells have been shown to be complemented by a gene localized on human chromosome 8 (Itoh et al., 1993; Kirchgessner, 1993; Komatsu et al., 1993; Banga et al., 1994), whereas the *Ku70* gene maps to human chromosome 22 (Cai et al., 1994). We therefore speculated that the lack of DNA-PK activity in V3 and Scid cells might stem either from deficiencies in DNA-PK$_{cs}$ or from defects in a gene that is involved in recruiting DNA-PK$_{cs}$ to DNA-bound Ku.

To investigate this possibility, we employed ultraviolet (UV) DNA–protein cross-linking, an assay that we had used previously to detect Ku and DNA-PK$_{cs}$ in crude human cell extracts (Gottlieb and Jackson, 1993). In this technique, a ^{32}P-labeled dsDNA oligonucleotide is incubated with a sample containing DNA-PK, the sample is treated with UV to cross-link DNA-bound proteins to the DNA, and then ^{32}P-tagged proteins are detected by SDS–polyacrylamide gel electrophoresis and autoradiography. Using this method with wild-type hamster AA8 and mouse 3T3 extracts, two predominant polypeptides are detected that comigrate with Ku70 and DNA-PK$_{cs}$ present in preparations of purified DNA-PK (Figure 2). That the approximately 70 kDa species is indeed Ku70 is supported by the observation that this species is diminished dramatically when extracts of xrs-6 cells are employed (T. M. G. and S. P. J., unpublished data). Significantly, although Ku70 is detected by UV protein–DNA cross-linking of V3 and Scid extracts, the approximately 450 kDa polypeptide corresponding to DNA-PK$_{cs}$ is absent in both cases. These data therefore suggest that the DNA-PK deficiencies of V3 and Scid cells result from defects in targeting DNA-PK$_{cs}$ to DNA.

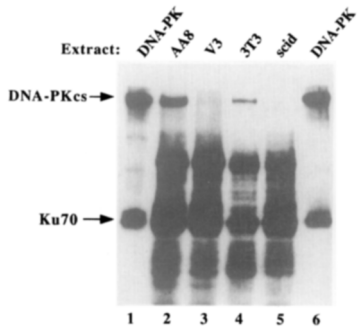

Figure 2. DNA-Bound DNA-PK$_{cs}$ Is Not Detected Using V3 or Scid Cell Extracts

Purified human DNA-PK (lanes 1 and 6) or 80 μg of whole-cell extract derived from the cell lines indicated (lanes 3–5) was incubated at 30°C for 10 min with 20 fmol of γ-^{32}P end-labeled dsDNA oligonucleotide. Reactions were then subjected to UV cross-linking and analyzed by electrophoresis on an 8% SDS–polyacrylamide gel followed by autoradiography. The positions of radiolabeled Ku70 and DNA-PK$_{cs}$ are indicated by arrows.

Purified DNA-PK$_{cs}$ Restores DNA-PK Activity to V3 and Scid Extracts

To investigate whether the inability of V3 and Scid extracts to recruit DNA-PK$_{cs}$ to DNA is due to a defect in DNA-PK$_{cs}$ itself or an inability of Ku to interact with DNA-PK$_{cs}$, we employed an essentially homogeneous preparation of human DNA-PK$_{cs}$ (Figure 3A). Consistent with previous studies (Gottlieb and Jackson, 1993), we found that, when assayed alone, purified DNA-PK$_{cs}$ is unable to be cross-linked to DNA effectively (Figure 3B). However, when purified DNA-PK$_{cs}$ is mixed with V3 or Scid extracts, it is now targeted efficiently to DNA. These data indicate that the Ku in V3 and Scid cells is capable of interacting with DNA-PK$_{cs}$ and suggest that V3 and Scid cells are defective in DNA-PK$_{cs}$ itself.

In light of the above, we next tested whether addition of purified human DNA-PK$_{cs}$ could reconstitute DNA-PK activity when added to V3 and Scid extracts. As shown previously, purified DNA-PK$_{cs}$ alone has essentially no kinase activity (Figure 3C). Addition of pure DNA-PK$_{cs}$ to wild-type CHO cell extracts, however, was found to elevate kinase activity to levels much higher than obtained with wild-type rodent extracts alone (data not shown), indicating that DNA-PK$_{cs}$ but not Ku is limiting for DNA-PK activity in normal rodent extracts. Strikingly, DNA-PK activity substantially greater than that of wild-type extracts is also ob-

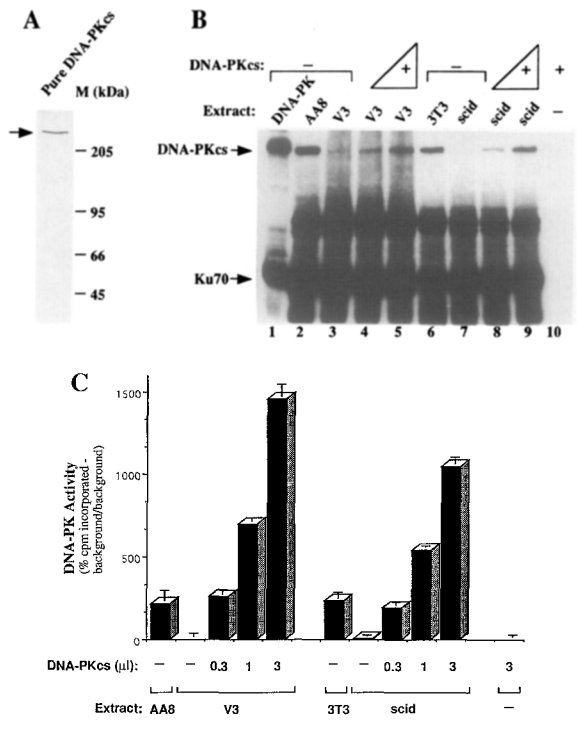

Figure 3. V3 and Scid Cells Are Defective Specifically in DNA-PK$_{cs}$

(A) Analysis of a preparation of purified human DNA-PK$_{cs}$. A sample of purified DNA-PK$_{cs}$ was electrophoresed on an 8% SDS–polyacrylamide gel and stained with silver. The location of the DNA-PK$_{cs}$ polypeptide is indicated by an arrow.

(B) Ku present in V3 and Scid cell extracts can target DNA-PK$_{cs}$ to DNA. Protein–DNA cross-linking studies were performed as in Figure 2 with 1 μl of DNA-PK (lane 1), with 3 μl of purified DNA-PK$_{cs}$ (lane 10), or with 80 μg of whole-cell extract derived from V3 or Scid cells (lanes 3–9). In addition, reactions 4 and 8 contained 1 μl of purified DNA-PK$_{cs}$, and reactions 5 and 9 contained 3 μl of purified DNA-PK$_{cs}$. The positions of the radiolabeled polypeptides corresponding to Ku70 and DNA-PK$_{cs}$ are indicated by arrows.

(C) Addition of DNA-PK$_{cs}$ to V3 or Scid cell extracts restores DNA-PK activity. DNA-PK activity assays were conducted as described in Figure 1 using whole-cell extracts (150 μg) derived from cell lines AA8, V3, 3T3, or Scid, as indicated. Where indicated, samples of purified DNA-PK$_{cs}$ were added to extracts before the microfractionation step of the procedure. The assay presented at the right-hand side of the figure was conducted with purified DNA-PK$_{cs}$ alone.

tained when DNA-PK$_{cs}$ is mixed with either V3 or Scid extracts, which by themselves do not possess kinase activity (Figure 3C). In contrast, no stimulation of kinase activity is obtained by addition of purified preparations of the Ku70–Ku80 heterodimer (data not shown), even though Ku can reconstitute DNA-PK activity when mixed with xrs-6 extracts (Finnie et al., 1995). Taken together, these results demonstrate that cells falling into IR complementation group 7 specifically lack functional DNA-PK$_{cs}$ and suggest that DNA-PK$_{cs}$ is encoded by the *XRCC7* gene.

Yeast Artificial Chromosomes Containing the DNA-PK$_{cs}$ Gene Complement the Radiosensitivity of V3 Cells

To verify that DNA-PK$_{cs}$ is the product of *XRCC7*, we wished to determine whether the defective phenotype of V3 or Scid cells could be complemented by the expression of wild-type DNA-PK$_{cs}$. Toward this end, we sought to identify yeast artificial chromosomes (YACs) containing the entire human DNA-PK$_{cs}$ gene. To do this, three YAC libraries were screened using either Southern blot hybridization or polymerase chain reaction (PCR)-based protocols. For hybridization screening, we used a mixture of three probes derived from the DNA-PK$_{cs}$ cDNA (K. O. Hartley et al., unpublished data; see Experimental Procedures); for PCR screening, primers were designed that were capable of amplifying a fragment corresponding to the 3′ untranslated region (3′UTR) of the DNA-PK$_{cs}$ cDNA. Nine YACs bearing DNA-PK$_{cs}$ sequences were thus isolated (Table 1). Two of these YACS (943-g-4 and 935-d-11), derived from the Centre d'Etudes du Polymorphisme Humain (CEPH) YAC library, contained the CEPH microsatellite markers *D8S531* and *D8S519* and were therefore identified as mapping to the region 8cen-q12. YAC 147 was also used as a probe for in situ hybridization and was found to map to the centromeric region of chromosome 8 (data not shown). This is in agreement with recent in situ hybridization studies using DNA-PK$_{cs}$ cDNA and genomic clones that map the gene to locus 8q11 (Sipley et al., submitted). Since the human gene *XRCC7*, which complements the DNA repair and V(D)J recombination defects of murine Scid cells, has also been mapped to the region 8cen-q11 (Itoh et al., 1993;

Table 1. Analysis of YACs Containing the DNA-PK$_{cs}$ Gene

YAC Name	Nomenclature in This Paper	Library Screened	Southern Blot Hybridization			Size (kb)	Location	Microsatellite Markers	γ-Ray^R/G418^R Clones
			5′ End	Middle	3′ End				
678-a-3		CEPH	Bands deleted	Intact	Intact	620			
621-f-2		CEPH	Intact	Intact	Aberrant				
943-g-4	144	CEPH	Intact	Intact	Intact	1680	8cen-q12	*D8S531, D85519*	0/4
935-d-11	145	CEPH	Intact	Intact	Intact	1270	8cen-q12	*D8S531, D85519*	0/5
18A, A2		ICI	Bands deleted	Intact	Intact				
29F, C4	147	ICI	Intact	Intact	Aberrant	300	8cen		6/6
30I, C12	148	ICI	Intact	Intact	Intact	280			2/4
35D, H8		ICI	Bands deleted	Deleted	Bands deleted				
35D, G12		ICI	Bands deleted	Deleted	Intact				

γ-Ray^R clones were those clones having a resistance to γ-rays clearly greater than the V3 parent. All γ-ray^R clones had survival levels similar to that shown by YAC 147A (Figure 4A). G418^R clones represent those clones having acquired the neomycin resistance marker from the YAC.

Kirchgessner, 1993; Komatsu et al., 1993; Banga et al., 1994), our results suggest strongly that DNA-PK$_{cs}$ is encoded by *XRCC7*.

To assess the integrity of the DNA-PK$_{cs}$ gene in the various YACs, they were analyzed by Southern blot hybridization using probes derived from the 5′, middle, and 3′ regions of the DNA-PK$_{cs}$ cDNA (Table 1). Although five YACs had obvious deletions or rearrangements in DNA-PK$_{cs}$ sequences, three YACs (144, 145, and 148) appeared, by this criterion, to contain an intact DNA-PK$_{cs}$ gene, and a fourth YAC (147) had just a single aberrant band (data not shown). A dominant selectable marker for use in mammalian cells (the bacterial aminoglycoside phosphotransferase [*agpt*] or neomycin gene) was introduced into these YACs using a retrofitting technique, and the retrofitted YACs were then transferred into V3 cells by protoplast fusion (see Experimental Procedures). Individual neomycin-resistant clones derived from these fusions were examined for radiosensitivity. As shown in Figure 4A and Table 1, correction of the radiosensitive phenotype of V3 cells is obtained for clones derived from fusions using either YAC 147 or YAC 148. Although full correction to the parental level of radioresistance is not achieved, the same magnitude of correction is observed consistently in all complementing fusion hybrids. In contrast with the results using V3 cells, when YAC 147 was introduced into xrs-6 or XR-1 mutant cells, correction of the radiosensitive phenotype was observed in neither case (data not shown). This indicates that complementation is specific for cells falling into IR group 7.

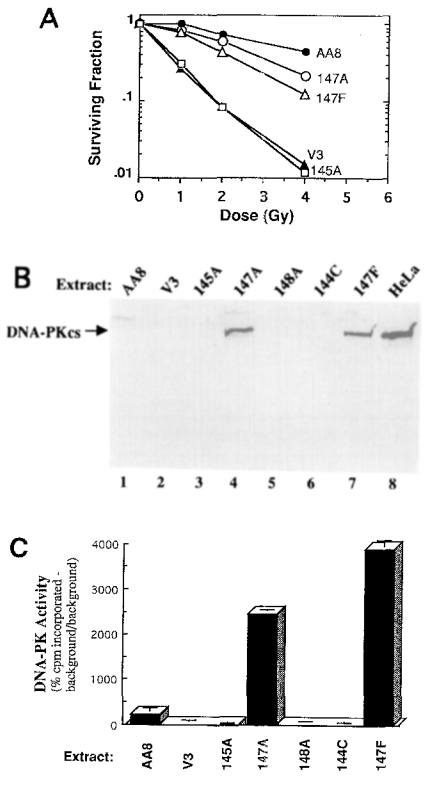

Figure 4. YACs Bearing the DNA-PK$_{cs}$ Gene Complement the Radiosensitivity of V3 Cells, Direct DNA-PK$_{cs}$ Synthesis, and Restore DNA-PK Activity to V3 Cells

(A) Survival of V3 and YAC fusion hybrids following exposure to IR. The survival data presented represent the mean of at least three experiments for each line. All complementing and noncomplementing YAC fusion hybrids gave survival levels comparable to that shown for hybrids 147A/147F and 145A, respectively.

(B) V3-derived fusion hybrids 147A and 147F that are complemented for radiosensitivity express human DNA-PK$_{cs}$. Whole-cell extracts (30 μg) derived from the indicated cell lines were subjected to Western immunoblot analysis using an antiserum raised against human DNA-PK$_{cs}$. The position of DNA-PK$_{cs}$ is indicated by an arrow.

(C) V3-derived fusion hybrids 147A and 147F contain DNA-PK activity. Whole-cell extracts derived from the cell lines indicated were analyzed for DNA-PK activity as described in Figure 1.

Next, DNA from a number of complemented and noncomplemented V3 fusion hybrids was examined for the presence and integrity of DNA-PK$_{cs}$ by PCR using the 3′UTR primers and by Southern blot hybridization using the three DNA-PK$_{cs}$ cDNA probes. By these criteria, all complementing V3 fusion hybrids appeared to carry an intact DNA-PK$_{cs}$ gene, whereas aberrations in the DNA-PK$_{cs}$ gene were evident in all noncomplementing hybrids examined (data not shown). Deletions in the DNA-PK$_{cs}$ gene were clearly evident in all the fusion hybrids derived from noncomplementing YACs 144 and 145. Perhaps reflecting the large size of these YACs (greater than 1 Mb), it appears that they are broken frequently during the protoplast fusion procedure. These results establish that correction of the radiosensitive phenotype of V3 cells can be obtained only by the introduction of YACs containing the intact DNA-PK$_{cs}$ gene and suggest strongly that DNA-PK$_{cs}$ is the product of *XRCC7*. (Though unlikely, it remains a formal possibility that *XRCC7* is distinct from but linked tightly to the DNA-PK$_{cs}$ gene.)

YACs That Direct DNA-PK$_{cs}$ Synthesis Restore DNA-PK Activity to V3 Cells

To examine whether the fusion hybrids described above express DNA-PK$_{cs}$, we analyzed extracts from these cells by Western blotting using an antiserum raised against human DNA-PK$_{cs}$ (Figure 4B). No strongly staining polypeptides were detected by this approach in extracts of either wild-type hamster AA8 cells or V3 cells. Although this could reflect lack of immunological cross-reactivity of rodent DNA-PK$_{cs}$, the absence of signal could, instead, be due to the low levels of DNA-PK in rodent as compared with human cells (Anderson and Lees-Miller, 1992; Finnie et al., 1995). When five of the V3 fusion hybrids were examined blindly, the two complementing hybrids (147A and 147F) were identified as expressing a protein that is recognized strongly by the anti-DNA-PK$_{cs}$ antiserum and that comigrates with human DNA-PK$_{cs}$ (Figure 4B). In contrast, this polypeptide is not observed in extracts derived from the noncomplementing hybrids (145A, 148A, and 144C). Thus, DNA-PK$_{cs}$ expression in V3 hybrids correlates with radiation resistance.

Next, whole-cell extracts from the same panel of V3 fusion hybrids were tested for DNA-PK activity. As with normal V3 cells, DNA-PK activity is undetectable in noncomplementing hybrids (145A, 148A, or 144C) that do not direct DNA-PK$_{cs}$ synthesis (Figure 4C). In contrast, high

DNA-PK levels are observed using extracts of the two complementing hybrids (147A and 147F) that do express DNA-PK_{cs}. Indeed, the levels of DNA-PK obtained from V3 cells containing YACs 147A or 147F are much higher than those obtained from wild-type rodent cell lines such as AA8 and are similar to DNA-PK levels derived from human cells. This is consistent with the fact that the level of DNA-PK_{cs} expression in these lines is close to that present in human cells (Figure 4B). Consonant with the observation that XR-1 cells contain active Ku, high levels of DNA-PK activity were also obtained with XR-1 hybrids containing YAC 147 (data not shown). In contrast, DNA-PK levels were undetectable in xrs-6 hybrids containing YAC 147 (data not shown), as expected from the lack of functional Ku in these cells. The fact that DNA-PK_{cs} expression and DNA-PK activity correlate with one another in the various V3 hybrids analyzed above implies that the expression of DNA-PK_{cs} alone is sufficient to restore DNA-PK activity to V3 cells. Furthermore, the correlation between restoration of DNA-PK activity and correction of the radiosensitive phenotype of V3 cells suggests that DNA-PK defects are directly responsible for the defective DNA DSB repair in this cell line.

Complementation of the Defect in V(D)J Recombination

Correction of the radiosensitive phenotype of xrs-6 cells has hitherto correlated with correction of their defect in V(D)J recombination, indicating that these two phenotypes result from a common mutation (Taccioli et al., 1993, 1994b; Smider et al., 1994; Pergola et al., 1993). We therefore assayed the five V3 hybrids described above for their ability to carry out V(D)J recombination. For this purpose, cells were transiently transfected with RAG1 and RAG2 expression constructs along with extrachromosomal V(D)J recombination substrates (Taccioli et al., 1994b). The two substrates used, pJH290 and pJH200, permit evaluation of the activity and fidelity of coding and RSS join formation when recovered from the transfected mammalian cells and analyzed in bacteria (Hesse et al., 1987).

As observed previously, we found that V3 cells, like Scid cells, are profoundly deficient (greater than 50-fold) in coding join formation, whereas they display a comparatively minor (approximately 5-fold) defect in RSS join formation (Taccioli et al., 1994a; Table 2). Analysis of the two complemented fusion hybrids (147A and 147F) revealed a recovery in the frequency of both RSS and coding join formation to at least the levels seen in wild-type cells.

Another manifestation of the V3 and *scid* mutations is the presence of deletions at the normally precise RSS joins, as well as an elevation in the frequency and size of deletions at coding junctions. These phenotypes of V3 cells are also corrected in the complementing fusion clones (Figure 5; Table 2). Finally, another characteristic feature of the coding joins formed in V3 and Scid cells is the presence of abnormally large P elements (Kienker et al., 1991; Schuler et al., 1991; Taccioli et al., 1994a). This phenotype is also corrected in the complemented fusion hybrids (Figure 5), in which a maximum of two P nucleotides are found, as is observed using wild-type cells. In stark contrast with this correction, the frequency and quality of joins in the hybrids not expressing DNA-PK_{cs} retain

Table 2. Analysis of Signal and Coding Join Formation in CHO Cell Lines by Transient V(D)J Recombination Assays

		pJH200 (Signal)					pJH290 (Coding)		
Cell Line	Sensitive X-ray	DNA-PK Activity	Amp^RCam^R / Amp^R	Percent	Relative Parental Level[a]	Percent Correct Joins[b]	Amp^RCam^R / Amp^R	Percent	Relative Parental Level[a]
AA8-4(wt)	R	+	417/16,800	2.50	1	100	160/6,900	2.30	1
Mock	R	+	1/10,500	0.01	0.004	NA	0/12,450	<0.01	<0.004
V3	S	−	54/9,750	0.55	0.22	47	5/17,100	0.03	0.013
V3 147A	R	+	224/4,260	5.30	2.12	100	93/2,900	3.20	1.39
V3 147D	R	+	79/1,640	4.80	1.92	89	67/2,800	2.40	1.04
V3 147F	R	+	165/2,680	6.20	2.48	86	197/2,270	8.70	3.78
V3 145A	S	−	169/29,100	0.58	0.23	66	41/64,350	0.06	0.03
V3 144C	S	−	28/6,600	0.42	0.17	44	1/6,270	0.02	0.01
V3 144A	S	−	ND				2/9,300	0.02	0.01
V3 148A	S	−	ND				29/46,350	0.06	0.03
V3 148B	R	+	ND				169/13,800	1.22	0.52
V3 148D	R	+	ND				51/7,200	0.70	0.30
K1(wt)	R	+	147/5,550	2.60	1.04	ND	77/7,050	1.10	0.49
K1 147A	R	+	358/12,000	2.98	1.19	ND	322/13,500	2.40	1.04
xrs-6	S	−	39/90,000	0.04	0.02	ND	149/300,000	0.05	0.02
xrs-6 147δ	S	−	15/73,500	0.02	0.01	ND	21/68,700	0.03	0.01
XR-1 147T	S	+	8/50,850	0.02	0.01	ND	11/48,450	0.02	0.01

The results presented were reproduced in at least two independent transfection experiments. Abbreviations: wt, wild type; ND, not determined; S, sensitive; R, resistant.

[a] Percentage was normalized using parental cell line AA8-4.

[b] Percentage of correct RSS joins screened by digestion of the PCR products of recombination substrates pJH200 with ApaLI (see Experimental Procedures).

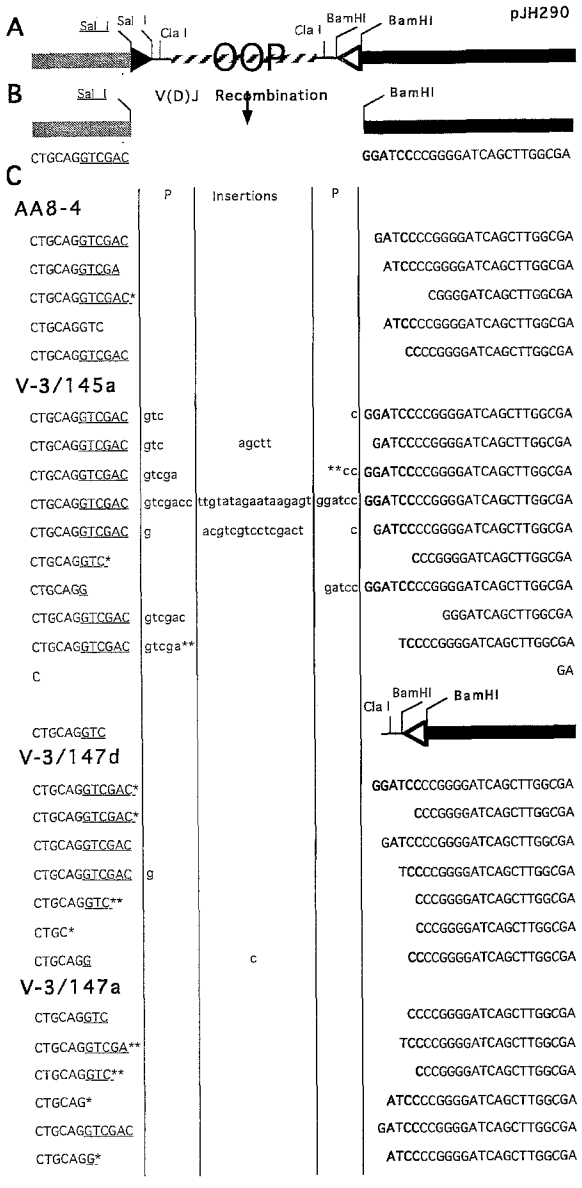

Figure 5. Nucleotide Sequence Analysis of Random Coding Joins

(A) Map of the recombinant substrate pJH290 (Hesse et al., 1987).

(B) Map and nucleotide sequence of the product of a coding join (recombined pJH290) in which no nucleotide has been excised or added.

(C) Nucleotide sequences of various recombined substrates recovered from the indicated cell lines. Asterisks represent bases that could not be assigned unequivocally. Bases inserted at the joins that are consistent with palindromic P elements (Lafaille et al., 1989) are listed under the P column depending on the end location. Extra nucleotides added at the joins are listed in the central column.

the features characteristic of V3 cells (Figure 5). As controls, we also analyzed fusion hybrids with either xrs-6 or XR-1 cells; in neither case was the V(D)J recombination defect of the mutant corrected (Table 2).

Taken together, our results show that the diverse defects identified in V3 cells can be corrected specifically by the introduction of YACs bearing the DNA-PK$_{cs}$ gene. The marked correlation between recovery of radioresistance, recovery of the defect in V(D)J recombination, the presence of DNA-PK activity, and the expression of DNA-PK$_{cs}$ in our panel of complementing and noncomplementing fusion hybrids establishes that all these end points are attributed to expression of functional DNA-PK$_{cs}$.

Discussion

V3 and Scid Cells Are Deficient in DNA-PK$_{cs}$

It has been established previously that the xrs-6 cell line, which falls into IR complementation group 5 and is defective in DNA DSB repair and V(D)J recombination, is deficient in functional Ku (Getts and Stamato, 1994; Rathmell and Chu, 1994; Smider et al., 1994; Taccioli et al., 1994b). As Ku is the DNA binding component of the DNA-PK holoenzyme (Dvir et al., 1992; Gottlieb and Jackson, 1993), xrs-6 cells consequently lack DNA-PK activity (Finnie et al., 1995). These findings suggested that DNA-PK catalytic activity may have some role in V(D)J recombination and DNA DSB repair. Here we have shown that hamster V3 and mouse Scid cells, which define IR complementation group 7, possess functional Ku but lack detectable DNA-PK activity owing to a specific absence of functional DNA-PK$_{cs}$. Furthermore, we have demonstrated that YACs containing the DNA-PK$_{cs}$ gene restore DNA-PK activity to V3 cells and complement the DNA repair and recombination defects of this cell line. Although it is not yet possible to conclude with certainty that the mutations in V3 and Scid cells actually reside in the DNA-PK$_{cs}$ gene, our data do indicate that DNA-PK$_{cs}$ plays a crucial role in V(D)J recombination and DNA DSB repair and suggest strongly that it is the product of the *XRCC7* gene. Consistent with this, we have demonstrated recently that the DNA-PK$_{cs}$ gene maps to human chromosomal locus 8q11 (Sipley et al., submitted), a chromosomal region that has been shown to complement IR group 7 cells (Itoh et al., 1993; Kirchgessner, 1993; Komatsu et al., 1993; Banga et al., 1994).

V3 hybrids containing DNA-PK$_{cs}$ YACs are complemented fully for V(D)J recombination but only partially for radiosensistivity. This contrasts with xrs-6 transfectants expressing human Ku80, which are not corrected fully for either process (Smider et al., 1994; Taccioli et al., 1994b). Since the xrs-6 transfectants that express Ku80 regain DNA-PK activity to parental levels (Finnie et al., 1995) and the complemented V3 fusion hybrids expressing human DNA-PK$_{cs}$ contain elevated DNA-PK levels in vitro, we consider it unlikely that the lack of full complementation of xrs-6 and V3 cells is due to deficiencies in kinase activity per se. Instead, these data imply that DNA-PK functions not simply as a kinase but also has other functions that are not performed effectively by the human–rodent hybrid complexes. For example, it may be that the hybrid complexes are partially deficient in mediating interactions with rodent repair components (see below).

Function of DNA-PK$_{cs}$ in DNA DSB Repair and V(D)J Recombination

There are a number of ways, not necessarily mutually exclusive, that DNA-PK might function in DNA DSB repair and V(D)J recombination. One is that interactions between

the DNA-PK holoenzyme and DNA termini prevent the action of exonucleases that might otherwise lead to loss of important genetic information. Another is that the protein kinase activity of DNA-PK_{cs} is employed to regulate the activities of components of the DNA repair and recombination apparatus. Indeed, it is tempting to speculate that DNA end–restricted kinase activity provides a mechanism to ensure that the repair and recombination machineries are only activated in the vicinity of a DNA DSB. Instead of (or in addition to) working in a positive sense, phosphorylation events might also be used to inactivate components involved in early steps of repair and recombination, thereby ensuring an ordered sequence of events. In light of these suggestions, it is notable that RAG1 and RAG2 each contain consensus DNA-PK recognition motifs (Anderson and Lees-Miller, 1992; Bannister et al., 1993), suggesting that their activities might be regulated by DNA-PK-mediated phosphorylation.

Another way that DNA-PK_{cs} could promote DNA repair and recombination is by phosphorylating and inactivating transcription factors that might otherwise interfere with the assembly, functioning, or both of the DNA repair/recombination apparatus. In this regard, it may be pertinent that DNA-PK has been shown recently to repress transcription by RNA polymerase I through a mechanism that requires kinase catalytic activity (Kuhn et al., 1995). A final way in which the kinase activity of DNA-PK could be employed in promoting repair and recombination is in the activation of factors involved in triggering cellular DNA damage responses (for review see Gottlieb and Jackson, 1994). Although the available evidence indicates that Scid and xrs-6 cells do display G2 cell cycle arrest following DNA damage (Jeggo, 1985; Weibezahn et al., 1985), this does not exclude a possible role for DNA-PK in signaling the presence of a DNA DSB to the cell.

Above, we have argued that DNA-PK may play roles in DNA repair and V(D)J recombination that do not rely directly on its kinase activity. One attractive possibility is that DNA-PK could play a structural role, such as aligning DNA termini to promote their ligation. Alternatively (or additionally), DNA-PK might recruit other components of the DNA repair and recombination apparatus to the DNA. In light of its large size, it is tempting to speculate that DNA-PK_{cs} might have a role as a scaffolding protein, interacting with several other factors and holding these in appropriate orientations with respect to one another to facilitate effective DNA repair and recombination.

Implications for Differential Pathways of Coding and RSS Join Formation

The V3 and *scid* mutations severely impair coding join formation, whereas the generation of RSS joins is affected to a much lesser extent. This contrasts with xrs-6 cells lacking functional Ku, in which both RSS and coding join formations are impaired greatly. These results indicate that Ku is essential for the formation of both coding and RSS joins, whereas DNA-PK_{cs} is essential only for the former. Therefore, RSS joining can apparently occur through a DNA-PK_{cs}-independent pathway. In this case, it may be that Ku functions in DNA end protection, in end alignment, or in the recruitment of some additional factor that can substitute for DNA-PK_{cs}.

The requirement of DNA-PK_{cs} for coding but not RSS join formation is likely to relate to the known mechanistic differences between these processes; for example, the apparent requirement for further processing of coding but not RSS ends before ligation. In this regard, it is of interest to note that several lines of evidence suggest (but do not formally prove) that most coding ends pass through DNA hairpin intermediates, whereas RSS ends do not (Roth et al., 1992, 1993; Schlissel et al., 1993). Significantly, hairpin coding ends accumulate to high levels in Scid thymocytes (Roth et al., 1992), and rare coding joins from Scid cells have abnormally long P elements. Together, these findings have led to the suggestion that the *scid* mutation preferentially inhibits the processing of hairpin coding ends, with the long P elements resulting from abberent opening at sites distal to the hairpin apex.

One explanation for the differential effect of DNA-PK_{cs} on coding and RSS join formation is that DNA-PK_{cs} might be required to disassemble the recombination apparatus to make the hairpin ends accessible for further processing. Alternatively, DNA-PK_{cs} could be involved in recruiting or activating a nuclease that opens up coding end hairpin intermediates. However, a more general role in such a process is unlikely based on the finding that normal resolution takes place when hairpin substrates are transfected into Scid cells (Lewis, 1994). Finally, in addition to a potential requirement for hairpin resolution, it is also possible that DNA-PK_{cs} is required for the varying degrees of base loss, addition, or both that often occur at coding ends before they are ligated. In this regard, it is of interest to note that radiation-induced DSBs, whose repair is also affected by the V3 and *scid* mutations, frequently contain damaged bases at their ends. It may therefore be that one function for DNA-PK_{cs} in DSB repair is to mediate or control (or both) the removal of such damage to allow the ligation of broken chromosomal ends to occur effectively.

Experimental Procedures

Cell Culture and Survival Analysis

Cells and their propagation was as described previously (Jeggo et al., 1992). The V3 CHO cell line was derived from AA8-4 cells (Whitmore et al., 1989). SGRII is an immortalized fibroblast cell line derived from neonatal Scid mice (Hendrickson et al., 1991). All cells were maintained as monolayers at 37°C in a 5% CO_2 atmosphere in minimal essential medium (GIBCO) supplemented with nonessential amino acids, penicillin (100 U/ml), streptomycin (0.1 mg/ml), and 10% fetal calf serum. Survival following IR was as described previously (Jeggo et al., 1992).

Extract Preparation and Protein Purification

Whole-cell extracts were prepared as described previously (Finnie et al., 1995). In brief, pellets of approximately 1×10^7 cells were resuspended in 100 μl of extraction buffer (50 mM NaF, 20 mM HEPES [pH 7.8], 450 mM NaCl, 25% glycerol, 0.2 mM EDTA, 0.5 mM DTT, 0.5 mM PMSF, 0.5 μg/ml leupeptin, 0.5 μg/ml protease inhibitor, 1.0 μg/ml trypsin inhibitor, 0.5 μg/ml aprotinin, 40 μg/ml bestatin), then frozen on dry ice, and thawed at 30°C three times. After microcentrifugation for 7 min at 4°C, supernatants were stored at −70°C. DNA-PK_{cs} and Ku were purified from wheat germ agglutinin-depleted HeLa nuclear extracts (Jackson and Tjian, 1989) essentially as described previously (Dvir et al., 1993).

Biochemical Assays for DNA-PK and Its Components

Western blotting was performed according to the method of Jackson and Tjian (1988), and DNA-PK_{cs} was detected using the ECL system (Amersham). UV protein cross-linking and EMSAs were performed as described previously (Gottlieb and Jackson, 1993; Taccioli et al., 1994b). DNA-PK microfractionation/peptide phosphorylation assays were as described previously (Finnie et al., 1995). In brief, extract was incubated with 20 μl of preswollen dsDNA cellulose (Sigma) in a total volume of 50 μl of Z'0.05 (25 mM HEPES–KOH [pH 7.9], 50 mM KCl, 10 mM $MgCl_2$, 20% glycerol, 0.1% NP-40, 1 mM DTT). The dsDNA cellulose was then washed twice with Z'0.05 and resuspended in 50 μl of Z'0.05. Next, samples were divided into three equal aliquots, 0.5 μl of [γ-^{32}P]ATP (300 Ci/mmol) was added, and three kinase assays were conducted in parallel: one in the absence of peptide, one in the presence of 4 nmol (0.2 mM) of wild-type peptide that is a good DNA-PK substrate, and one in the presence of a mutated peptide that is an ineffective DNA-PK substrate. Reactions were then stopped and analyzed by spotting onto phosphocellulose paper, washing, and subjecting to liquid scintillation counting as described previously (Anderson and Lees-Miller, 1992). The wild-type and mutated peptides are derived from the N-terminal transcriptional activation region of murine p53 (sequences EPPL*SQE*AFADLLKK and EPPL*SEQ*AFADLLKK, respectively). All assays were performed multiple times with at least two different extract preparations, and all results presented in a particular figure were obtained in parallel. Values plotted are mean values of several experiments and were derived by subtracting the value for no peptide from the values for wild-type and mutated peptides and then dividing these two resulting figures by the no peptide value. Assays on rodent extracts typically give variability of less than 10%.

Isolation and Analysis of YAC Clones

Gridded filters containing the Imperial Cancer Research Fund YAC library (supplied by Dr. H. Lehrach) were screened by a standard hybridization protocol using DNA-PK_{cs} clones 1, 8, and 13 (K. O. Hartley et al., unpublished data; these clones are available on request) as probes. The CEPH and ICI Pharmaceutical (ICI) YAC libraries (Anand et al., 1990; supplied by the Human Genome Mapping Project Resource Centre) were screened by PCR using primers 5'-CTGCAGATAGAAAGCATTACATTG-3' and 5'-TCTTGATTTAAACTCATGCTACGA-3' derived from the 3'UTR region of the DNA-PK_{cs} cDNA. Microsatellite marker information from the Genethon data base was supplied by Dr. D. Le-Paslier. YACs were modified using the vector pRAN4 as described previously (Markie et al., 1993) to facilitate their transfer to mammalian cells. Size estimation of YACs was either by the Genethon data base or by pulse field gel electrophoresis (PFGE). After retrofitting, all YACs were examined by PFGE to verify the presence of the *agpt* and DNA-PK_{cs} genes and to verify that they had not altered in size. Southern blot hybridization analysis of YACs, hybrid fusion clones, and PFGE filters was carried out by standard procedures.

Introduction of YACs into Hamster Cells by Protoplast Fusion

Yeast cells containing YACs at a density of 1×10^7 cells/ml were pelleted, washed first in ddH_2O then in 1 M sorbitol, and resuspended in 20 ml of SCE (1 M sorbitol, 0.1 M sodium citrate, 10 mM EDTA [pH 5.8]); 32 mM β-mercaptoethanol and 800 U of lyticase were added, and the cells incubated at 30°C until 75% had formed spheroplasts. The cells were gently resuspended in 1 M sorbitol, pelleted again, and resuspended in 20 ml of STC (1 M sorbitol, 10 mM $CaCl_2$, 10 mM Tris [pH 7.5]) to a concentration of 1×10^8/ml. The spheroplasts were maintained at 18°C while the mammalian cells were trypsinized, washed in serum-free (SF) medium, and resuspended at 1.5×10^6/ml. Mammalian cells were gently layered onto the spheroplasts, pelleted, and resuspended in 50 μl of SF medium; 500 μl of PEG solution (PEG 1500 [Boehringer Mannheim], 50% in 75 mM HEPES, 5 mM $CaCl_2$, 50 μM β-mercaptoethanol) was added, and after 2 min incubation, SF medium was slowly added, and the cells were left for 10 min. After gentle pelleting, cells were resuspended in growth medium and incubated at 37°C. G418 (GIBCO) (800 μg/ml) was added after 48 hr. Individual G418^R clones were subcloned after 7–10 days incubation and maintained in 600 μg/ml G418.

Transient V(D)J Recombination Assays

V(D)J recombination assays were carried out as described previously (Taccioli et al., 1993, 1994b). In brief, cells were transiently cotransfected with RAG expression vectors and V(D)J recombination substrates. Two different substrates were used; one (pJH290) allows recovery of coding joins, whereas the other (pJH200) allows recovery of signal joins (Hesse et al., 1987). Plasmid DNA was prepared after 48 hr and electroporated in Escherichia coli *MC1061*. V(D)J recombination was estimated by the relative amount of rearranged substrate (based on gain of chloramphenicol resistance [Cam^R]) compared with the total amount of recovered substrate (based on generation of ampicillin resistance [Amp^R]). Fidelity of RSS joins was assessed by digesting PCR products from recombinant substrate pJH200 with restriction enzyme ApaLI, a site that is generated only after precise fusion of heptamers (Taccioli et al., 1994b).

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References

Alt, F. W., Oltz, E. M., Young, F., Gorman, J., Taccioli, G., and Chen, J. (1992). VDJ recombination. Immunol. Today *13*, 306–314.

Anand, R., Reley, J. H., Butler, R., Smith, J. C., and Markham, A. F. (1990). A 3.5 genome equivalent multi-access YAC library: construction, characterization, screening and storage. Nucl. Acids Res. *8*, 1951–1956.

Anderson, C. W., and Lees-Miller, S. P. (1992). The nuclear serine/threonine protein kinase DNA-PK. Crit. Rev. Euk. Gene Expression *2*, 283–314.

Banga, S. S., Hall, K. T., Sandhu, A. K., Weaver, D. T., and Athwal, R. S. (1994). Complementation of V(D)J recombination defect and X-ray sensitivity of *scid* mouse cells by human chromosome 8. Mutat. Res. *315*, 239–247.

Bannister, A. J., Gottlieb, T. M., Kouzarides, T., and Jackson, S. P. (1993). c-Jun is phosphorylated by the DNA-dependent protein kinase *in vitro*: definition of the minimal kinase recognition motif. Nucl. Acids Res. *21*, 1289–1295.

Biedermann, K. A., Sun, J., Giaccia, A. J., Tosto, L. M., and Brown, J. M. (1991). *scid* mutation in mice confers hypersensitivity to ionizing radiation and a deficiency in DNA double-strand break repair. Proc. Natl. Acad. Sci. USA *88*, 1394–1397.

Blackwell, K. T., and Alt, F. W. (1989). Mechanism and developmental program of immunoglobulin gene rearrangement in mammals. Annu. Rev. Genet. *23*, 630–636.

Bosma, M. J., and Carroll, A. M. (1991). The SCID mouse mutant: definition, characterization, and potential uses. Annu. Rev. Immunol. *9*, 323–350.

Cai, Q. Q., Plet, A., Imbert, J., Lafage-Pochitaloff, M., Cerdan, C., and Blanchard, J. M. (1994). Chromosomal localization and expression of

the genes coding for Ku p70 and p80 in human cell lines and normal tissues. Cytogenet. Cell Genet. *65*, 221–227.

Dvir, A., Peterson, S. R., Knuth, M. W., Lu, H., and Dynan, W. S. (1992). Ku autoantigen is the regulatory component of a template-associated protein kinase that phosphorylates RNA polymerase II. Proc. Natl. Acad. Sci. USA *89*, 11920–11924.

Dvir, A., Stein, L. Y., Calore, B. L., and Dynan, W. S. (1993). Purification and characterization of a template-associated protein kinase that phosphorylates RNA polymerase II. J. Biol. Chem. *268*, 10440–10447.

Finnie, N. J., Gottlieb, T. M., Blunt, T., Jeggo, P., and Jackson, S. P. (1995). DNA-PK activity is absent in xrs-6 cells; implications for site-specific recombination and DNA double-strand break repair. Proc. Natl. Acad. Sci. USA *92*, 320–324.

Fulop, G. M., and Phillips, R. A. (1990). The *scid* mutation in mice causes a general defect in DNA repair. Nature *347*, 479–482.

Gellert, M. (1992). Molecular analysis of V(D)J recombination. Annu. Rev. Genet. *22*, 425–446.

Getts, R. C., and Stamato, T. D. (1994). Absence of a Ku-like DNA end-binding activity in the xrs double-strand DNA repair deficient mutant. J. Biol. Chem. *269*, 15981–15984.

Giaccia, A., Weinstein, R., Hu, K. J., and Stamato, T. D. (1985). Cell cycle–dependent repair of double-stranded DNA breaks in a gamma-ray sensitive Chinese hamster cell. Somat. Cell Mol. Genet. *11*, 485–491.

Gottlieb, T. M., and Jackson, S. P. (1993). The DNA-dependent protein kinase: requirement for DNA ends and association with Ku antigen. Cell *72*, 131–142.

Gottlieb, T. M., and Jackson, S. P. (1994). Protein kinases and DNA damage. Trends Biochem. Sci. *19*, 500–503.

Hendrickson, E. A., Qin, X.-Q., Bump, E. A., Schatz, D. G., Oettinger, M., and Weaver, D. T. (1991). A link between double-strand break-related repair and V(D)J recombination: the *scid* mutation. Proc. Natl. Acad. Sci. USA *88*, 4061–4065.

Hesse, J. E., Lieber, M. R., Gellert, M., and Mizuuchi, K. (1987). Extrachromosomal DNA substrates in pre-B cells undergo inversion or deletion at immunoglobulin V-(D)-J joining signals. Cell *49*, 775–783.

Itoh, M., Hamatani, K., Komatsu, K., Araki, R., Takayama, K., and Abe, M. (1993). Human chromosome 8 (p12-q22) complements radiosensitivity in severe combined immune deficiency (*scid*) mouse. Radiat. Res. *134*, 364–368.

Jackson, S. P., and Tjian, R. (1988). O-glycosylation of eukaryotic transcription factors: implications for mechanisms of transcription regulation. Cell *55*, 125–133.

Jackson, S. P., and Tjian, R. (1989). Purification and analysis of RNA polymerase II transcription factors by using wheat germ agglutinin affinity chromatography. Proc. Natl. Acad. Sci. USA *86*, 1781–1785.

Jackson, S. P., MacDonald, J. J., Lees-Miller, S., and Tjian, R. (1990). GC box binding induces phosphorylation of Sp1 by a DNA-dependent protein kinase. Cell *63*, 155–165.

Jeggo, P. A. (1985). X-ray sensitive mutants of Chinese hamster ovary cell line. Mutat. Res. *145*, 171–176.

Jeggo, P. A., Tesmer, J., and Chen, D. J. (1991). Genetic analysis of ionising radiation sensitive mutants of cultured mammalian cell lines. Mutat. Res. *254*, 125–133.

Jeggo, P. A., Hafezparast, M., Thompson, A. F., Broughton, B. C., Kaur, G. P., Zdzienicka, M. Z., and Athwal, R. S. (1992). Localization of a DNA repair gene (*XRCC5*) involved in double-strand break rejoining to human chromosome 2. Proc. Natl. Acad. Sci. USA *89*, 6423–6427.

Kemp, L. M., Sedgwick, S. G., and Jeggo, P. A. (1984). X-ray sensitive mutants of Chinese hamster ovary cells defective in double-stranded break rejoining. Mutat. Res. *132*, 189–196.

Kienker, L., Kuziel, W. A., and Tucker, P. W. (1991). T cell receptor γ and δ gene junctional sequences in *scid* mice: excessive P nucleotide insertion. J. Exp. Med. *174*, 769–773.

Kirchgessner, C. U. (1993). Complementation of the radiosensitive phenotype in severe combined immunodeficient mice by human chromosome 8. Cancer Res. *53*, 6011.

Komatsu, K., Ohta, T., Jinno, Y., Nikawa, N., and Okumura, Y. (1993). Functional complementation in mouse–human radiation hybrids assigns the putative murine *scid* gene to the pericentric region of human chromosome 8. Hum. Mol. Genet. *7*, 1031–1034.

Kuhn, A., Gottlieb, T. M., Jackson, S. P., and Grummt, I. (1995). DNA-dependent protein kinase: a potent inhibitor of transcription by RNA polymerase I. Genes Dev., *9*, 193–203.

Lafaille, J. J., DeClox, A., Bonnevil e, M., Takagaki, Y., and Tonewaga, S. (1989). Junctional sequences of T cell receptor γδ genes: implication for γδ T cell lineages and for a novel intermediate of V(D)J joining. Cell *59*, 859–870.

Lewis, S. M. (1994). The mechanisms of V(D)J rejoining: lessons from molecular, immunological, and comparative analysis. Adv. Immunol. *56*, 27–149.

Lieber, M. R. (1991). Site-specific recombination in immune system. FASEB J. *5*, 2934–2944.

Lieber, M. R., Hesse, J. E., Lewis, S., Bosma, G. C., Rosenberg, N., Mizuuchi, K., Bosma, M. J., and Gellert, M. (1988). The defect in murine severe combined immune deficiency: joining of signal sequences but not coding segments in V(D)J recombination. Cell *55*, 7–16.

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, L. B., and Alt, F. W. (1988). The *scid* defect affects the final step of the immunoglobulin VDJ recombinase mechanism. Cell *54*, 453–460.

Markie, D., Ragoussis, J., Senger, G., Rowan, A., Sansom, D., Trowsdale, J., Sheer, D., and Bodmer, W. F. (1993). New vector for transfer of yeast artificial chromosomes to mammalian cells. Somat. Cell Mol. Genet. *19*, 161–169.

Mombaerts, P., Iacomini, J., Johnson, R. S., Herrup, K., Tonewaga, S., and Papaionnou, V. E. (1992). RAG-1-deficient mice have no mature B and T lymphocytes. Cell *68*, 867–869.

Oettinger, M. A., Schatz, D. G., Gorka, C., and Baltimore, D. (1990). RAG1 and RAG2, adjacent genes that synergistically activate V(D)J recombination. Science *248*, 1517–1523.

Pergola, F., Zdzienicka, M. Z., and Lieber, M. R. (1993). V(D)J recombination in mammalian cell mutants defective in DNA double-strand break repair. Mol. Cell. Biol. *13*, 3464–3471.

Rathmell, W. K., and Chu, G. (1994). Involvement of the Ku autoantigen in the cellular response to DNA double-strand breaks. Proc. Natl. Acad. Sci. USA *91*, 7623–7627.

Roth, D. B., Menetski, J. P., Nakajima, P. B., Bosma, M. J., and Gellert, M. (1992). V(D)J recombination: broken DNA molecules with covalently sealed (hairpin) coding ends in *scid* mouse thymocytes. Cell *70*, 983–991.

Roth, D. B., Chengming, Z., and Gellert, M. (1993). Characterization of broken DNA molecules associated with V(D)J recombination. Proc. Natl. Acad. Sci. USA *90*, 10788–10792.

Schlissel, M., Constantinescu, A., Morrow, T., Baxter, M., and Peng, A. (1993). Double-strand signal sequence breaks in V(D)J recombination are blunt, 5′-phosphorylated, RAG-dependent and cell cycle regulated. Genes Dev. *7*, 2520–2532.

Schuler, W., Weiler, I. J., Schuler, A., Phillips, R. A., Rosenberg, N., Mak, T. W., Kearney, J. F., Perry, R. P., and Bosma, J. J. (1986). Rearrangement of antigen receptor genes is defective in mice with severe combined immune deficiency. Cell *46*, 963–972.

Schuler, W., Ruetsch, N. R., Amsler, M., and Bosma, M. J. (1991). Coding information of endogenous T cell receptor genes in lymphoid cells from *scid* mice: unusual P-nucleotide additions in VJ-coding joints. Eur. J. Immunol. *21*, 589–596.

Shinkai, Y., Rathbun, G., Lam, K., Oltz, E. M., Stewart, V., Mendelsohn, M., Charon, J., Datta, M., Young, F., Stall, A. M., and Alt, F. W. (1992). RAG-2-deficient mice lack mature lymphocytes due to inability to initiate VDJ rearrangement. Cell *68*, 855–867.

Smider, V., Rathmell, W. K., Lieber, M. R., and Chu, G. (1994). Restoration of X-ray resistance and V(D)J recombination in mutant cells by Ku cDNA. Science *266*, 288–291.

Taccioli, G. E., Rathbun, G., Oltz, E., Stamato, T., Jeggo, P. A., and Alt, F. W. (1993). Impairment of V(D)J recombination in double-stranded break repair mutants. Science *260*, 207–210.

Taccioli, G. E., Cheng, H.-L., Varghese, A. J., Whitmore, G., and Alt, F. W. (1994a). A DNA repair defect in Chinese hamster ovary cells affects V(D)J recombination similarly to the murine *scid* mutation. J. Biol. Chem. *269*, 7439–7442.

Taccioli, G. E., Gottlieb, T. M., Blunt, T., Priestley, A., Demengeot, J., Mizuta, R., Lehmann, A. R., Alt, F. W., Jackson, S. P., and Jeggo, P. A. (1994b). Ku80: product of the *XRCC5* gene and its role in DNA repair and V(D)J recombination. Science *265*, 1442–1445.

Weibezahn, K. F., Lohrer, H., and Herrlich, P. (1985). Double strand break repair and G2 block in Chinese hamster ovary cells and their radiosensitive mutants. Mutat. Res. *145*, 177–183.

Whitmore, G. F., Varghese, A. J., and Gulyas, S. (1989). Cell cycle responses of two X-ray sensitive mutants defective in DNA repair. Int. J. Radiat. Biol. *56*, 657–665.