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scid Cells Are Deficient in Ku and Replication Protein A Phosphorylation by the DNA-Dependent Protein Kinase

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Cell mutants of the Ku nuclear DNA-binding complex are ionizing radiation sensitive and show V(D)J recombination defects. Ku binds and activates a catalytic subunit of DNA-dependent protein kinase (DNA-PK), although the substrates for DNA-PK are unknown. We found that *scid* cell extracts were deficient in Ku phosphorylation by DNA-PK. Human chromosome 8-complemented *scid* cells, containing the human DNA-PK catalytic subunit, restored Ku phosphorylation. Likewise, radiation-induced RPA hyperphosphorylation was not completed in *scid* cells compared with control or chromosome 8-reconstituted cells. Thus, the inactivity of DNA-PK is likely responsible for the repair and recombination defects in *scid* cells.

Eukaryotic cells respond to incidents of DNA damage by combining cell cycle delays with the functioning of repair mechanisms. For damage caused by ionizing radiation (IR), double-strand break (DSB) repair is needed to overcome the major cytotoxic and mutagenic lesions formed. The interplay between DNA repair and cell cycle progression is essential for maintenance of genome stability and avoidance of cancer initiation steps, as IR damage is both mutagenic and carcinogenic (24, 43, 62).

In mammals, the DSB repair pathway bears a striking resemblance to part of the V(D)J recombination mechanism with regard to the structures of the DNA products. Likewise, mutant phenotypes attributable to specific gene lesions that affect both processes have been described. The immunodeficiency of *scid* mice is manifested by a failure to complete V(D)J recombination (55). Whereas RAG-1- and RAG-2-positive *scid* cells can relatively effectively complete recombination signal sequence joining, the mutation causes an extreme coding junction formation deficiency (26, 28, 29, 38, 42). Interestingly, *scid* cells are also sensitive to DNA damage by agents generating lethal DSBs, such as IR (3, 20, 27). In addition, three other mutational groups that were originally identified as IR^s mutants were also found to be deficient in formation of both V(D)J recombination products (35, 47, 61).

The molecular basis of DSB repair has recently been accelerated by the identification of the products of the Ku genes as important components of the mechanism. Ku is an abundant nuclear heterodimer of 70- and 86-kDa subunits that binds to DSBs in DNA, an activity that is likely relevant to DSB repair (4, 13, 18, 23, 44, 45, 67). Biochemically purified Ku tightly binds to DNA ends and alterations in a DNA sequence-independent manner. Two of the IR^s, V(D)J recombination-deficient mutational groups, *XRCC5* and *sxi-1*, are defective for Ku DNA end binding activity in in vitro assays (6, 7, 50, 51, 59). *XRCC5* group IR^s mutants are restored for IR repair and V(D)J recombination defects by introduction of the Ku large-subunit p86 gene (6, 56, 59). The *XRCC5* gene colocalizes with Ku p86 on human chromosome 2 (9, 30, 31, 59), and two *XRCC5* mutants, *sxi-2* and *sxi-3*, do not produce Ku p86 mRNA stably (6). Therefore, it is likely that Ku p86 defects are responsible for the *XRCC5* mutants.

Ku associates in cells with a large protein kinase, of at least 350 kDa (10, 36, 65), indicating a possible role for a larger protein complex in DSB repair. p350 and Ku function together as a DNA-dependent protein kinase (DNA-PK) in which p350 is the catalytic subunit (DNA-PK$_{cs}$) that is stimulated by DNA ends via Ku (16, 17, 22, 65). In fact, the p350-Ku complex association is highly stimulated by the presence of DNA ends in vitro (58). *XRCC5* mutants, deficient for Ku functions, are devoid of DNA-PK activity (19), in keeping with this pattern. Recently it was shown that *scid* group cells are defective in DNA-PK activity and complemented by a yeast artificial chromosome containing the DNA-PK$_{cs}$ gene (5, 34). DNA-PK phosphorylates numerous substrates including Ku, p53, Rb, topoisomerase II, simian virus 40 T antigen, replication protein A (RPA), and several transcription factors, in vitro (1), but the true substrates for DNA-PK have yet to be identified.

Here we show that *scid* cells are deficient in two DNA-PK activities, the ability to phosphorylate Ku in vitro and radiation-induced RPA phosphorylation in vivo. *scid* cells that were complemented by human chromosome 8 and had an introduced human DNA-PK$_{cs}$ were restored for both the Ku and RPA phosphorylation properties. The inactivity of the *scid* DNA-PK complex likely contributes to the failure in DSB repair functions, including Ku and RPA phosphorylation.

MATERIALS AND METHODS

Cell culture and radiolabeling. The cell lines ScSV3, SCH8-1, SCH8-2, NIH 3T3, and CB-1 were cultured in Dulbecco modified Eagle medium (DMEM) with 10% inactivated fetal bovine serum (FBS) containing nonessential amino acids and penicillin-streptomycin as previously described (2, 27). CB-1 fibroblasts are derived from the CB17 mouse strain, which is isogenic for *scid* (57). LAZ388, an Epstein-Barr virus-immortalized human B-cell line, was grown in RPMI plus 10% FBS. GM639 (Human Genetic Mutant Cell Repository, Camden, N.J.) was obtained from Ray Monnat. MO59J and MO59K cells were generously donated by Joan Turner (37). Ku-deficient and murine fibroblast cell lines were grown as previously described (2, 6, 7, 27).

Radiolabeling with [^{35}S]methionine was performed as follows. Exponentially growing cells (1×10^6 to 2×10^6/100-mm-diameter plate) were preincubated with 3 ml of methionine-free DMEM with 10% dialyzed FBS for 1 h and then radiolabeled with 300 μCi of [^{35}S]methionine (duPont-NEN) for 4 h. Radiolabeling with $^{32}P_i$ was conducted by washing exponentially growing cells (1×10^6 to 2×10^6/100-mm-diameter plate) in 5 ml of phosphate-free medium without serum and then replacing the medium with 3 ml of phosphate-free medium with 10% dialyzed FBS and $^{32}P_i$ (1.5 mCi; duPont-NEN) for 12 to 16 h.

Alternatively, SCH8-1 and ScSV3 cells were treated with 0 to 2,000 rad of γ

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irradiation (^{60}Co source; courtesy of Peter Mauch) prior to $^{32}P_i$ labeling as described above for 2 h.

Lysates, immunoprecipitation, and Western blotting (immunoblotting). For immunoprecipitation analysis, radiolabeled cells were washed one time in phosphate-buffered saline (PBS), trypsinized, washed in DMEM plus serum, washed in PBS in a microcentrifuge tube, and lysed by resuspension in ice-cold buffer A (50 mM Tris-Cl [pH 8.0], 0.5% Nonidet P-40, 150 mM NaCl, 10 mM EDTA, 1.33 mM phenylmethylsulfonyl fluoride, 1 μg of leupeptin per ml, 1 μg of aprotinin per ml, 1 μg of pepstatin per ml). Lysates were incubated on ice for 15 to 30 min and then spun at top speed in a 4°C microcentrifuge for 20 min. Lysates were used directly in immunoprecipitations by the addition of antibody reagents or were stored at −80°C.

Antibodies used in immunoprecipitation analysis were as follows. The anti-Ku and anti-DNA-PK reagents, HT, GE2-9.5, and 111, and anti-p350 have been previously described (6, 7, 36, 51, 52, 58). HT reacts with human or rodent Ku p70 protein and coprecipitates the other Ku subunit, p86. GE2-9.5 and 111 are anti-human Ku p86 antibodies. The anti-DNA-PK_{cs} antiserum was AB145 from Carl Anderson. The anti-RPA antiserum (SSB) reacts with both human and rodent cell RPA. Following primary antibody incubation for 1 to 16 h at 4°C, antibody complexes were precipitated with 25 μl of preswollen protein A-Sepharose (Pharmacia) for 1 h. Then precipitates were washed three times in ice-cold buffer A, transferred to a new microcentrifuge tube, suspended in 25 μl of sample buffer (50 mM Tris-Cl [pH 7.0], 2% sodium dodecyl sulfate [SDS], 720 mM β-mercaptoethanol, 5 mg of bromophenol blue per ml), boiled for 5 min, and fractionated on SDS-polyacrylamide gels as specified in the text and figure legends. SDS-polyacrylamide gels were then developed by using Autofluor (National Diagnostics) as instructed by the supplier and subjected to autoradiography.

Western blots were prepared by transfer onto nitrocellulose paper (BA85; 2-μm pore size; Schleicher & Schuell) essentially as described previously (41, 49). Immunoblots were detected by using a mixture of protein A- and protein G-coupled horseradish peroxidase (Pierce) followed by a chemiluminescence system (Renaissance; Du Pont-NEN) as instructed by the suppliers.

dsDNA beads. Double-stranded DNA (dsDNA)-containing beads (dsDNA beads) were prepared as follows. Calf thymus DNA (Sigma) was sonicated to an average size of 1.5 kb. DNA coupling to cyanogen bromide-activated Sepharose (Sigma) was carried out in 0.5 M $NaPO_4$ (pH 8.4)–10 mg of sonicated DNA per ml at room temperature overnight with rocking. The excess of ligand was washed away with 0.5 M $NaPO_4$ (pH 8.4), and remaining active groups were blocked with 0.2 M glycine–0.5 M $NaPO_4$ (pH 8.4).

DNA end binding. Mobility shift gel electrophoresis was used to confirm functional Ku activity in *scid* and positive and negative control cells as described previously (6, 7). MO59J and MO59K nuclear extracts were similarly prepared. Approximately 1 ng of a 159-bp ^{32}P-labeled *Pvu*II-*Xma*I fragment of plasmid pJH290 was used for DNA end binding in the presence of a 500- to 1,000-fold excess of circular DNA. *scid* and control cell extracts for DNA end binding were prepared as described previously (21, 50), and 0.8 μg of extract was mixed with the DNA for 10 min. Gel shifts were then fractionated at room temperature as described previously (6, 7).

In vitro protein kinase assays. Cell lysates were prepared from 5×10^7 to 10×10^7 cells (10 to 20 100-mm-diameter plates) by washing cells one time in PBS, scraping each plate of cells in 1 ml of PBS, pelleting the total cell harvest, washing the cells one time in 1 ml of buffer B (10 mM Tris-Cl [pH 7.5], 25 mM KCl, 10 mM NaCl, 1.5 mM $MgCl_2$, 0.1 mM EDTA, 1.33 mM phenylmethylsulfonyl fluoride, 1 μg of leupeptin per ml, 1 μg of aprotinin per ml, 1 μg of pepstatin per ml). The total packed cell volume was then adjusted with 1 packed cell volume of buffer B and frozen on dry ice. Following thawing on ice, samples were centrifuged for 10 min at 4°C to pellet debri, and the freeze-thaw cell extract supernatant (3 to 5 μg/ml) was used immediately.

Six hundred micrograms of the freeze-thaw extract was combined with 25 μl of dsDNA beads in 200 to 400 μl of buffer B and incubated for 2 h at 4°C on a LabQuake Shaker rotator. After brief spinning to pellet dsDNA beads, the beads were washed with 200 μl of buffer B and then with 200 μl of 1× wash buffer (50 mM Tris-Cl [pH 7.5], 100 mM KCl, 10 mM $MgCl_2$). dsDNA beads were suspended in 50 μl of 1× kinase buffer, consisting of 1× wash buffer with 1.33 μM ATP and 150 μCi of [γ-^{32}P]ATP (6,000 Ci/mmol; Du Pont-NEN), and incubated with intermittent shaking at 32°C for 10 min. dsDNA beads were then washed in 200 μl of 1× wash buffer without ATP, and labeled protein was eluted from the beads in 200 μl of 1× wash buffer with 0.5 M KCl at 4°C for 1 to 2 h. Supernatants from dsDNA beads were then either directly fractionated on SDS-polyacrylamide gels or immunoprecipitated with an anti-Ku antiserum as described above.

Kinase assays from immunoprecipitates were set up as follows. LAZ388 cell lysates from buffer A were immunoprecipitated with an anti-Ku antiserum, bound to protein A-Sepharose, and washed as described above in buffer A. Protein A beads were washed with 1× wash buffer (100 μl for 25 μl of beads) and resuspended in 50 μl of 1× kinase buffer as described for dsDNA beads. Reactions were stopped by centrifugation and washing in 200 μl of 1× wash buffer without ATP, and then the mixtures were boiled in 25 μl of sample buffer in preparation for SDS-polyacrylamide gel electrophoresis (PAGE).

RESULTS

***scid* cells are deficient in Ku phosphorylation by an associated protein kinase.** DSB repair in mammalian cells is governed by the nuclear DNA end-binding DNA-PK complex. The Ku-associated DNA-PK_{cs} may control DSB repair by a requirement for its kinase activity on specific proteins also involved in the same processes. Although the relevant substrates for DNA are unknown, important substrates for DNA-PK activity may be the components of DNA-PK itself: Ku p70, Ku p86, and/or DNA-PK_{cs}. Ku is phosphorylated by biochemically purified DNA-PK (36), as are a number of additional proteins added as substrates (1). We have used the DNA binding properties of Ku to examine the activity of DNA-PK and its associated proteins by binding on dsDNA beads.

For the initial experiments, we used human LAZ388 Epstein-Barr virus-immortalized B cells, which produce functionally active Ku as evidenced by DNA end binding (6, 7). LAZ388 cells were metabolically labeled with [^{35}S]methionine, and freeze-thaw lysates were prepared as described in Materials and Methods. LAZ388 whole cell lysates were proven to contain the previously characterize 1:1 association of Ku p70 and p86 subunits by immunoprecipitation with the anti-Ku p86 monoclonal antibody (MAb) GE2-9.5 (Fig. 1A). Second, dsDNA beads were added to equal aliquots of the LAZ388 whole cell extracts in the presence of excess circular DNA or linear DNA (sonicated salmon sperm DNA) or in the absence of added DNA. Following binding and washing in low-salt buffer B, bound protein was eluted in high salt (0.5 M KCl) and immunoprecipitated with GE2-9.5. Nearly all of the Ku that could be immunoprecipitated from the whole cell lysate was also observed to bind to DNA beads in the absence of a DNA competitor (Fig. 1A). An excess of circular DNA did not appreciably interfere with Ku binding to dsDNA beads. However, the presence of excess linear DNA competed with Ku association to dsDNA beads. Thus, we infer that Ku associates with dsDNA beads via the DNA ends.

If Ku can be effectively bound to dsDNA beads, then Ku-associated proteins such as DNA-PK_{cs} may also be bound, potentially exhibiting DNA-PK activity. Protein kinase function was evaluated by in vitro phosphorylation studies in two ways. First, we prepared the dsDNA bead-bound protein fraction from LAZ388 lysates as described above. In parallel, LAZ388 lysates were immunoprecipitated with GE2-9.5 followed by protein A beads. In vitro kinase assays were run on both the dsDNA and protein A bead fractions. We found that both the p70 and p86 Ku subunits were highly phosphorylated in vitro, although p70 phosphorylation was 5- to 10-fold greater than p86 phosphorylation (Fig. 1B). The ratio of Ku p70 to p86 phosphorylation was the same in both kinase conditions. Because of the similarity in these patterns, we infer that the active kinase is associated with Ku in both contexts.

Since purification of DNA-PK ordinarily copurifies Ku, we inspected the retention of the p350 subunit of DNA-PK under the dsDNA bead assay conditions. Unlabeled GM639 cells (simian virus 40-transformed human fibroblasts) were lysed in buffer A as described in Materials and Methods. One-half of the lysed cell volume was treated by immunoprecipitation with an anti-Ku p70 antibody (HT) and then subjected to protein A-Sepharose absorption. The other half was incubated with dsDNA beads as described above. Following cofractionation of both samples on an SDS–6.5% polyacrylamide gel, protein was transferred to nitrocellulose and probed by immunoblotting with an anti-DNA-PK antiserum. We found an approximately 350-kDa protein that specifically reacted with this antiserum,

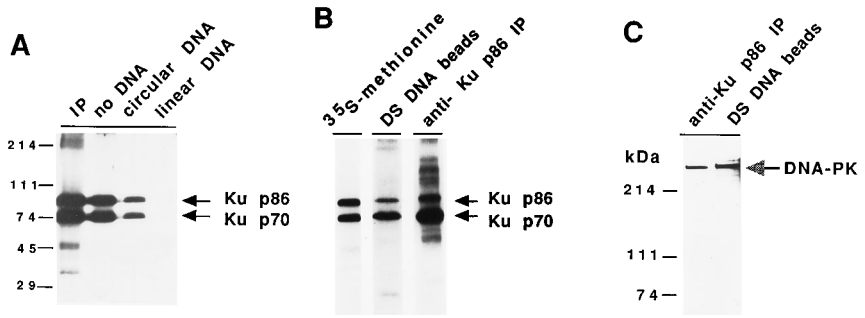

FIG. 1. Association of DNA-PK to DNA beads via the Ku-p350 interaction with DNA. (A) Following [^{35}S]methionine metabolic labeling, LAZ388 (human) B-cell lysates were bound to dsDNA beads (Materials and Methods) either in the absence of DNA or in the presence of circular DNA or linear dsDNA. The fraction of Ku bound to beads was determined for eluted proteins by immunoprecipitation with anti-Ku MAb GE2-9.5. In parallel, [^{35}S]methionine-radiolabeled LAZ388 B-cell lysates were directly immunoprecipitated with GE2-9.5 (IP). Protein was fractionated on an SDS–10% polyacrylamide gel. (B) Phosphorylation of Ku associated with dsDNA beads. Unlabeled LAZ388 cell lysates were either bound to dsDNA beads or immunoprecipitated with anti-Ku MAb 111 and then subjected to protein A-Sepharose binding. Either dsDNA beads or protein A beads were incubated with [γ-^{32}P]ATP. Following washing, Ku was eluted and immunoprecipitated with MAb GE2-9.5. Samples were fractionated on an SDS–10% polyacrylamide gel with ^{35}S-labeled and GE2-9.5-immunoprecipitated samples to display the 70- and 86-kDa Ku subunits. (C) Immunoblot of anti-p350. DNA-PK association to dsDNA beads was examined by comparing the dsDNA bead-bound fraction with Ku-associated protein in an immunoprecipitation with anti-Ku MAb GE2-9.5 and displayed on an SDS–6.5% polyacrylamide gel. Positions of molecular weight standards are indicated in kilodaltons on the left.

indicating that p350 was tightly associated with DNA beads and with Ku (Fig. 1C). The signal detected by Western blotting comigrated with p350 from [^{35}S]methionine-radiolabeled cells immunoprecipitated with the same antibody (data not shown). Importantly, the levels of p350 bound to Ku and to dsDNA beads were quantitatively similar, suggesting that all of the p350 that was bound to the dsDNA beads was likely bound via Ku. Thus, the application of dsDNA beads has great utility for examining Ku-associated DNA-PK activity.

We investigated the *scid* mutation in light of these findings because scid was a strong candidate for a Ku-associated protein, given the similarity in mutant repair and recombination phenotypes of the *scid* and Ku-deficient complementation groups (3, 6, 7, 20, 27, 56, 59). Cell lysates from *scid* and wild-type cell lines were compared for the efficiency of Ku phosphorylation in vitro. dsDNA beads were used to bind to Ku and associated proteins as described above for the human cells. We observed that dsDNA-bound protein from *scid* lysates yielded a significantly depressed level of Ku phosphorylation in vitro compared with wild-type control cells (compare results for ScSV3 and NIH 3T3 cells in Fig. 2A). Upon longer autoradiography, the *scid* samples had detectable kinase activity. We estimate an approximately 10-fold-greater level of Ku phosphorylation for wild-type cells. To confirm that *scid* cell lines had normal Ku levels, [^{35}S]methionine-radiolabeled *scid* and control extracts were immunoprecipitated with an anti-Ku antiserum (HT). ScSV3 cells yielded levels of the Ku p70-p86 complex comparable to those for mouse wild-type cell line controls (data not shown). Therefore, *scid* cells produce normal levels of Ku but are poorly able to phosphorylate Ku bound to dsDNA beads in vitro, consistent with a loss of DNA-PK activity.

***scid* cells complemented with human chromosome 8 express human DNA-PK$_{cs}$.** The results presented above strongly suggested a correspondence between scid protein function and DNA-PK activity. If the *scid* gene encodes the p350 subunit of DNA-PK (DNA-PK$_{cs}$), then human chromosome 8-containing cells that complement the *scid* mutant phenotypes should also score positive for human p350 protein by [^{35}S]methionine metabolic labeling and immunoprecipitation tests. Human, but not mouse, controls were positive with the anti-p350 reagent (Fig. 3). In addition, two cell lines that were restored for *scid* DNA repair and recombination functions by addition of human chromosome 8 (SCH8-1 and SCH8-2 [2]) had abundant immunoprecipitable p350. Therefore, the human p350 subunit of DNA-PK is located on chromosome 8, where the *scid* gene had been mapped by complementation analysis. During the course of this work, others showed that the *scid* mutation closely corresponds to the absence of DNA-PK activity and that yeast artificial chromosomes containing human DNA-PK$_{cs}$ complement *scid* functions (5, 34, 48).

The chromosome 8-reconstituted *scid* cell lines were therefore appropriate for testing the reconstitution of DNA-PK activity on the Ku substrates. Extracts from the SCH8-1 human chromosome 8-complemented cell line promoted a high level of Ku phosphorylation in vitro from dsDNA beads (Fig. 2A). SCH8-1 restored both Ku p70 and p86 phosphorylation in approximately the same ratio as for wild-type control cells. In fact, the SCH8-1 phosphorylation levels consistently exceeded

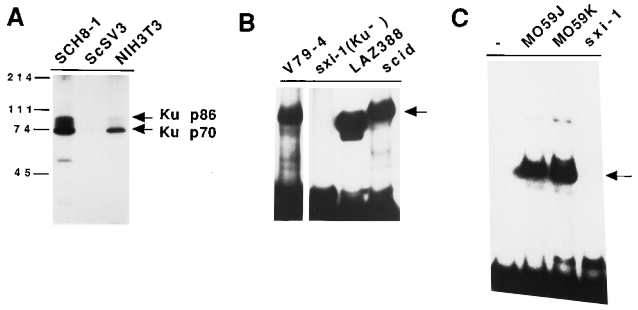

FIG. 2. Ku phosphorylation is defective in *scid* cells. (A) *scid* and control cell lysates were mixed with dsDNA beads to bind Ku and associated proteins as described in the text. Proteins bound to beads were incubated with [γ-^{32}P]ATP, eluted, and immunoprecipitated with an anti-Ku antiserum (HT), and fractionated on an SDS–10% polyacrylamide gel. Cell lines used were ScSV3 (*scid*), NIH 3T3 (wild type), and SCH8-1, a derivative of ScSV3 with an introduced chromosome 8 complementing the *scid* mutation. Sizes of markers are indicated in kilodaltons on the left. (B) Measurement of DNA end binding capacity of Ku by gel shift of *scid* and control extracts. Conditions for extract preparation, DNA labeling, and mobility gel shift were as described in Materials and Methods. All lines used were V79-4 (wild-type Chinese hamster), *xrs-1* (Ku-deficient derivative of V79-4), LAZ388 (human B cell), and 8D (*scid* mouse pre-B cell). Arrows in panels B and C denote positions of DNA end-binding complex A. (C) DNA end binding with MO59J and MO59K nuclear extracts, which are DNA-PK$_{cs}$ negative and DNA-PK$_{cs}$ positive, respectively (37).

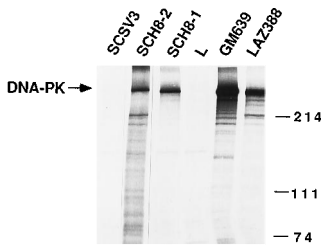

FIG. 3. The DNA-dependent protein kinase p350 gene is located on human chromosome 8. Mouse and human cell lines, including *scid* and control cells, were radiolabeled with [^{35}S]methionine as described in Materials and Methods. Cell lysates were immunoprecipitated with an anti-p350 antibody and then subjected to absorption to protein A-Sepharose, washing, boiling in buffer B, and fractionation on an SDS–6.5% polyacrylamide gel. The cell lines used were ScSV3 (*scid*), SCH8-2 and SCH8-1 (IR^r *scid*, with added human chromosome 8), L (mouse wild type), and GM639 and LAZ388 (human). Positions of protein molecular weight markers are displayed in kilodaltons on the right.

that of the wild-type mouse controls. Therefore, return of an active DNA-PK$_{cs}$ to *scid* cells by introduction of human chromosome 8 also reconstituted DNA-PK activity for the Ku substrates that are themselves involved in DSB repair.

DNA end binding does not require DNA-PK phosphorylation of Ku. DNA-PK-dependent Ku phosphorylation may be physiologically important for the biochemical properties of Ku. Specifically, Ku DNA end binding may be influenced by phosphorylation. However, we found by mobility shift gel electrophoresis that murine *scid* cells had a qualitatively normal level of the Ku DNA end-binding complex compared with V79-4 (Chinese hamster) and LAZ388 (human) cell lines (Fig. 2B). Because we were able to see some Ku phosphorylation in *scid* extracts with Ku bound to dsDNA beads (Fig. 2A), it was possible that the murine *scid* mutation was not a null mutation for the DNA-PK kinase activity. Thus, we examined another cell line that was DNA-PK deficient. The human glioblastoma cell lines MO59K and MO59J differ in that DNA-PK$_{cs}$ is expressed and not expressed, respectively (37). The absence of DNA-PK$_{cs}$ correlates with the IR sensitivity of MO59J. MO59J and MO59K had equivalent levels of DNA end binding activities in nuclear extracts (Fig. 2C). Thus, Ku DNA end binding does not require the activity of DNA-PK$_{cs}$.

Radiation-induced RPA hyperphosphorylation is defective in *scid* cells. Cells that are damaged by radiation have induced changes in the phosphorylation state of several proteins (12, 63, 64). We previously showed that the single-stranded DNA-binding protein RPA is hyperphosphorylated in a IR-inducible DNA damage response (41). The p34-kDa subunit of RPA (p34^{RPA}) was phosphorylated at multiple serine and threonine phosphorylation sites such that a mobility shift of phosphorylated products was readily detected by gel electrophoresis (41). The pattern of radiation-inducible phosphorylation was the same as that found in a normal S and G_2 phases in mammalian cells (14, 15). During the progress of our investigations, it was demonstrated that p34^{RPA} hyperphosphorylation during simian virus 40 DNA replication in vitro is controlled by DNA-PK (8). Also, DNA-PK was independently purified as an RPA kinase (46). These results motivated us to examine RPA hyperphosphorylation in *scid* and control cells to discriminate additional DNA-PK functions.

scid and mouse or human control cells were incubated overnight with $^{32}P_i$ in phosphate-free medium (Materials and Methods), a method that causes RPA hyperphosphorylation by virtue of radiation damage from ^{32}P decay. In parallel, the same cell lines were metabolically labeled with [^{35}S]methionine for 4 h. Then cell lysates were immunoprecipitated with an anti-RPA polyclonal antiserum (SSB) and fractionated on an SDS–12% polyacrylamide gel. ^{32}P-radiolabeled LAZ388 (human) cells produced an extensively hyperphosphorylated p34^{RPA} form (p36 [41]) compared with the mobility of p34^{RPA} in the absence of phosphorylation as determined by [^{35}S]methionine radiolabeling and immunoprecipitation (Fig. 4A, lanes 1 and 2). We found essentially no intermediate-mobility phosphorylated forms indicative of partial phosphorylation. *scid* and control mouse cell lines that were also labeled by [^{35}S]methionine had the expected immunoprecipitable RPA subunits of 70, 34, and 11 kDa (ScSV3 and NIH 3T3 cells; Fig. 4A, lanes 4 and 6). Examining the $^{32}P_i$-radiolabeled samples, we found that NIH 3T3 and ScSV3 cells differed significantly in the hyperphosphorylated forms of the p34 subunit that were produced (Fig. 4A). NIH 3T3 cells yielded two groups of hyperphosphorylated forms (approximately 35 and 36 kDa), in which the 36-kDa group was usually more abundant. In contrast, the *scid* cells generated only the smaller of these two forms (35 kDa) and thus had incompletely phosphorylated p34^{RPA} in the radiation-induced DNA damage response.

In keeping with restoration of other *scid* mutant phenotypes by the addition of chromosome 8, we tested whether the control pattern of p34^{RPA} hyperphosphorylation may also be reconstituted. $^{32}P_i$-radiolabeled SCH8-1 cells yielded the p34^{RPA} hyperphosphorylation pattern of the wild-type mouse cell control line, CB-1, or NIH 3T3 (Fig. 4B). Thus, the RPA hyperphosphorylation property attributed to DNA-PK activity could

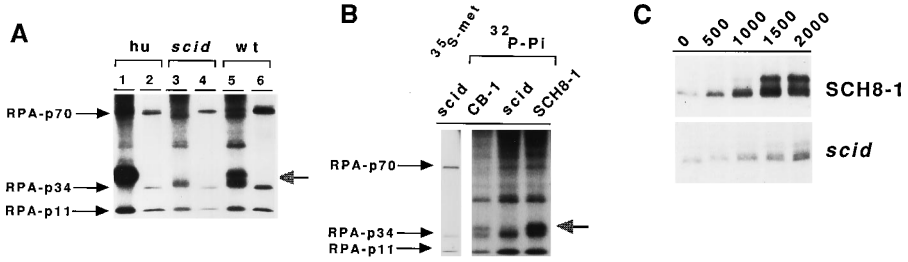

FIG. 4. RPA hyperphosphorylation is deficient in *scid* cells. *scid* and control cell lines were radiolabeled with $^{32}P_i$, which also induces radiation damage, as described in Materials and Methods. Cell lysates were immunoprecipitated with an anti-RPA antiserum (SSB). In parallel, [^{35}S]methionine-radiolabeled lysates were immunoprecipitated with SSB. (A) Samples were cofractionated on an SDS–12% polyacrylamide gel. Cell lines: human (LAZ388), lanes 1 and 2; *scid* (ScSV3), lanes 3 and 4; wild-type mouse (NIH 3T3), lanes 5 and 6. $^{32}P_i$ radiolabeling, lanes 1, 3, and 5; [^{35}S]methionine radiolabeling, lanes 2, 4, and 6. (B) $^{32}P_i$ and [^{35}S]methionine-radiolabeled samples of the indicated cells were fractionated following SSB immunoprecipitation. Shaded arrows in panels A and B denote hyperphosphorylation of p34^{RPA}. Positions of the three subunits of RPA, p70, p34, and p11, are indicated on the left. (C) SCH8-1 (IR^r) and ScSV3 (IR^s) cells were exposed to 0 to 2,000 rad of γ irradiation followed by 2 h of $^{32}P_i$ radiolabeling as described in Materials and Methods. Lysates from these samples were immunoprecipitated with an anti-RPA antiserum and fractionated as described above.

be restored in *scid* cells and correlated with the gain of an active DNA-PK kinase.

We also examined whether DNA-PK activity for RPA hyperphosphorylation could be discriminated following a single dose of γ irradiation from a ^{60}Co source (Materials and Methods). SCH8-1 and ScSV3 cells were compared by varying γ-irradiation doses between 0 and 2,000 rad followed by 2 h of $^{32}P_i$ labeling. Chromosome 8-complemented *scid* cells showed increased p34^{RPA} hyperphosphorylation under conditions in which *scid* cells were relatively unaffected (Fig. 4C). The data from both of these approaches suggest that the DNA-PK$_{cs}$ deficiency of *scid* cells impinges upon RPA hyperphosphorylation and that DNA-PK$_{cs}$ is likely one of the kinases directly phosphorylating RPA induced by irradiation. Because RPA and DNA-PK levels do not appreciably change following IR, it is likely that these effects are due to colocalization of DNA-PK and RPA on DNA.

DISCUSSION

The results presented here strongly indicate that the product of the *scid* gene is the p350 protein of DNA-PK. In conjunction with earlier work demonstrating the significance of each Ku subunit in DSB repair and recombination pathways (6, 7, 56, 59), these data point to a role for the DNA-PK$_{cs}$ subunit in the same DSB repair mechanisms as Ku. The combined molecular and biochemical data strongly argue that DNA-PK is a central multisubunit complex for DSB repair in mammals.

The *scid* mutation and DSB repair. The *scid* DSB repair and V(D)J recombination defects are complemented by yeast artificial chromosomes containing the DNA-PK$_{cs}$ gene (5). Both *scid* and *V-3* extracts are deficient for DNA-PK activity that is restored in *V-3* cells with the introduced 300-kb yeast artificial chromosome (5). Murine *scid* cells have significantly decreased DNA-PK$_{cs}$ protein levels (34, 48), but *scid* may not be a null mutation. The radiosensitive human glioblastoma cell line MO59J lacks DNA-PK$_{cs}$ mRNA and kinase activity in vitro (37). Our data are also consistent with *scid* and the p350 gene being the same gene, since we also show that they map to the same human chromosome (Fig. 3) and that two DNA-PK phosphorylation functions are complemented by the introduced human chromosome 8 (Fig. 2 and 4). The combined data make a compelling argument that the DNA-PK$_{cs}$ gene product is essential for DSB repair and V(D)J recombination functions that are deficient in the *scid* mutation.

Whereas Ku-deficient and p350-deficient mutants clearly share defects in the same pathways involving DSB repair, the differences between *scid* and Ku mutants may be instructive regarding the respective roles of DNA-PK subunits and the type of mutations analyzed thus far. The structures of certain V(D)J recombination junctions in *scid* gene rearrangements have clear differences in *scid* mutants compared with other DSB repair mutants. First, *scid* cells allow the formation of signal junction structures at nearly the wild-type efficiency and fidelity, whereas coding junction synthesis is significantly impaired (reviewed in reference 66). The Chinese hamster cell *V-3* isolate of the *scid* mutation was found to share the same bias in V(D)J recombination defects as mouse *scid* (60). Either the Ku$^-$ mutants (*XRCC5* and *sxi-1* groups) or the independent *XR-1* mutant is deficient in both product formation steps (6, 7, 47, 61). Thus, V(D)J recombination signal junction synthesis may actually have no direct requirement for DNA-PK$_{cs}$ activity. Second, the low level of pseudo-normal V(D)J rearrangements in *scid* lymphocytes frequently are composed of extended P nucleotides (33, 54). Third, in measurements of intermediates of V(D)J recombination, immature *scid* thymocytes retain hairpin coding ends rather than completed recombination products or dsDNA ends (53). This last finding led to the hypothesis that a scid function may be the resolution of hairpins as an essential step in coding end joining.

How might these altered V(D)J recombination structures and products arise in *scid*? One model is that the inactivity of the *scid* DNA-PK$_{cs}$ disrupts downstream functions dependent on phosphorylation but does not interfere with the assembly of an inactive DSB repair complex. This could affect some product formation steps profoundly (IR DSB repair and coding junctions) and other steps weakly or not at all (signal junctions). *scid* and the DNA-PK$_{cs}$-deficient MO59J cells do have normal DNA end binding in vitro (Fig. 2). Since unrepaired chromosomes may not be able to enter DNA synthesis or mitosis, the freezing of DSB repair at such a stage may facilitate the use of secondary events to overcome the DNA-PK deficiency. For example, extended P nucleotides in *scid* would result from single-strand cleavages significantly distal to the coding ends. The detection of hairpins in *scid* lymphoid cells may have been a consequence of G_0- or G_1-arrested cells, in a manner resembling the p53-dependent DNA damage cell cycle delay (32).

DSB repair substrates for DNA-PK phosphorylation. With the association of DNA-PK activity and the *scid* mutation, a likely scenario is that protein phosphorylation modulates DSB repair and recombination pathways. This point needs to be proven by additional experimentation because none of the true substrates for DNA-PK activity are yet defined in vivo. We have shown here that a Ku-associated protein will phosphorylate both Ku subunits when associated with DNA beads (Fig. 1). Relevant to our studies, phosphorylation of both subunits of Ku has been shown in vitro with purified DNA-PK complexes (36). Also, DNA-PK$_{cs}$ is autophosphorylated and has been suggested to be inhibitory to subsequent phosphorylation (36). Phosphorylation of Ku may alter the activity of DNA-PK or influence its assembly and disassembly.

Additional factors already known to be important for IR repair and/or V(D)J recombination may also be phosphorylation substrates of DNA-PK. The other DSB repair complementation group member, XR-1, may well be associated with Ku/DNA-PK function because of the close similarity in mutant phenotypes (61). For V(D)J recombination, initiation of gene rearrangement as well as product formation steps may be influenced by protein phosphorylation by DNA-PK. The recombination-activating gene *RAG-2* is phosphorylated in vivo, and the stability of the protein is dependent on its phosphorylation status (39, 40). DNA-PK activity may regulate the transition from initiation to product formation steps of V(D)J recombination by phosphorylation of RAG proteins.

Radiation-inducible RPA hyperphosphorylation. We have shown that RPA hyperphosphorylation is disrupted by the *scid* mutation (Fig. 4), consistent with the RPA phosphorylation by DNA-PK during simian virus 40 replication in vitro (8, 46). Interestingly, p34^{RPA} hyperphosphorylation is governed by more than one protein kinase operating in a cascade (25, 41, 46). Purified cyclins/cyclin-dependent kinases (cdks) partially phosphorylate p34^{RPA} in vitro (15, 25, 41, 46), and p34^{RPA} mutations in cdk consensus sites abrogate RPA hyperphosphorylation in vitro and that induced by radiation in vivo (25, 41). These changes were discriminated by RPA mobility in SDS-PAGE, because the p34 subunit conformation is acutely affected by phosphorylation. The presence of the *scid* mutation also seems to predispose radiation-induced phosphorylation differently, as we have found that only incompletely phosphorylated products are observed in this response (Fig. 4). The *scid* p34^{RPA} products may well be phosphorylated only at the cdk

consensus sites. In the absence of DNA-PK activity, this results in only partially phosphorylated $p34^{RPA}$. Further analysis of RPA phosphopeptides will be necessary to discriminate among the phosphorylated sites. Although both DNA-PK and cyclins/cdks have now been shown to play a role in $p34^{RPA}$ hyperphosphorylation, additional kinases may also be utilized in radiation-induced or DNA replication functions of RPA.

The significance of $p34^{RPA}$ hyperphosphorylation for cell metabolism is not yet clear, even though its association with DNA-PK activity is quite strong. RPA is utilized in excision repair in vitro, possibly by its binding to sites of single-stranded DNA lesions (11). For IR repair, RPA may also be involved at repair sites containing single-stranded DNA or forming denatured DNA ends in the midst of DSB repair. DNA-PK may govern the accessibility and/or functioning of RPA complexes at these sites. Since $DNA\text{-}PK_{cs}$ and RPA interactions have been shown to be Ku independent for DNA synthesis (8), possibly Ku is not needed for DNA repair functions of RPA as well. An interesting direction for future experiments will be the role of these intriguing DNA-PK functions.

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REFERENCES

1. **Anderson, C. W.** 1993. DNA damage and the DNA-activated protein kinase. Trends Biochem. Sci. **18:**433–437.
2. **Banga, S., K. Hall, A. Sandhu, D. Weaver, and R. Athwal.** 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.
3. **Biedermann, K. A., J. Sun, A. J. Giaccia, L. M. Tosto, and J. M. Brown.** 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.
4. **Blier, P. R., A. J. Griffith, J. Craft, and J. A. Hardin.** 1993. Binding of Ku protein to DNA—measurement of affinity for ends and demonstration of binding to nicks. J. Biol. Chem. **268:**7594–7601.
5. **Blunt, T., N. J. Finnie, G. E. Taccioli, G. C. M. Smith, J. Demengeot, T. M. Gottlieb, R. Mizuta, A. J. Varghese, F. W. Alt, P. A. Jeggo, and S. P. Jackson.** 1995. Defective DNA-dependent protein kinase activity is linked to V(D)J recombination and DNA repair defects associated with the murine scid mutation. Cell **80:**813–823.
6. **Boubnov, N. V., K. T. Hall, Z. Wills, S. E. Lee, D. M. He, D. M. Benjamin, C. R. Pulaski, H. Band, W. Reeves, E. A. Hendrickson, and D. T. Weaver.** 1995. 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. Proc. Natl. Acad. Sci. USA **92:**890–894.
7. **Boubnov, N. V., S. E. Lee, Z. P. Wills, K. T. Hall, E. A. Hendrickson, and D. T. Weaver.** Restoration of DNA end binding, ionizing radiation resistance, and V(D)J recombination proficiency to a mammalian double-strand break repair mutant by Ku. Submitted for publication.
8. **Brush, G. S., C. W. Anderson, and T. J. Kelly.** 1994. The DNA-activated protein kinase is required for the phosphorylation of replication protein A during simian virus 40 DNA replication. Proc. Natl. Acad. Sci. USA **91:**12520–12524.
9. **Cai, Q. Q., A. Plet, J. Imbert, M. Lafagepochitaloff, C. Cerdan, and J. M. Blanchard.** 1994. Chromosomal location and expression of the genes coding for Ku p70 and p80 in human cell lines and normal tissues. Cytogenet. Cell Genet. **65:**221–227.
10. **Carter, T. H., I. S. Vancurova I., W. Lou, and S. P. DeLeon.** 1990. A DNA-activated protein kinase from HeLa cell nuclei. Mol. Cell. Biol. **10:**6460–6471.
11. **Coverley, D., M. K. Kenny, M. Munn, W. D. Rupp, D. P. Lane, and R. D. Wood.** 1991. Requirement for the replication protein SSB in human DNA excision repair. Nature (London) **349:**538–541.
12. **Devary, Y., R. A. Gottlieb, T. Smeal, and M. Karin.** 1992. The mammalian ultraviolet response is triggered by activation of Src tyrosine kinases. Cell **71:**1081–1091.
13. **deVries, E., W. van Driel, W. G. Bergsma, A. C. Arnberg, and P. C. van der Vliet.** 1989. HeLa nuclear protein recognizing DNA termini and translocating on DNA forming a regular DNA-multimeric protein complex. J. Mol. Biol. **208:**65–78.
14. **Din, S., S. J. Brill, M. Fairman, and B. Stillman.** 1990. Cell-cycle-regulated phosphorylation of DNA replication factor A from human and yeast cells. Genes Dev. **4:**968–977.
15. **Dutta, A., and B. Stillman.** 1992. *cdc2* family kinases phosphorylate a human cell DNA replication factor, RPA, and activate DNA replication. EMBO J. **11:**2189–2199.
16. **Dvir, A., S. R. Peterson, M. W. Knuth, H. Lu, and W. Dynan.** 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.
17. **Dvir, A., L. Y. Stein, B. L. Calore, and W. S. Dynan.** 1993. Purification and characterization of a template-associated protein kinase that phosphorylates RNA polymerase II. J. Biol. Chem. **268:**10440–10447.
18. **Falzon, M., J. W. Fewell, and E. L. Kuff.** 1993. EBP-80, a transcription factor closely resembling the human autoantigen Ku, recognizes single- to double-strand transitions in DNA. J. Biol. Chem. **268:**10546–10552.
19. **Finnie, N. J., T. M. Gottlieb, T. Blunt, P. A. Jeggo, and S. P. Jackson.** 1995. DNA-dependent protein kinase 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.
20. **Fulop, G. M., and R. A. Phillips.** 1990. The *scid* mutation in mice causes a general defect in DNA repair. Nature (London) **347:**479–482.
21. **Getts, R. C., and T. D. Stamato.** 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.
22. **Gottlieb, T. M., and S. P. Jackson.** 1993. The DNA-dependent protein kinase: requirement for DNA ends and association with Ku antigen. Cell **72:**131–142.
23. **Griffith, A. J., P. R. Blier, T. Mimori, and J. A. Hardin.** 1992. Ku polypeptides synthesized in vitro assemble into complexes which recognize ends of double-stranded DNA. J. Biol. Chem. **267:**331–338.
24. **Hartwell, L. H., and M. B. Kastan.** 1994. Cell cycle control and cancer. Science **266:**1821–1828.
25. **Hendricksen, L. A., and M. S. Wold.** 1994. Replication protein A mutants lacking phosphorylation sites for p34-cdc2 kinase support DNA replication. J. Biol. Chem. **269:**24203–24208.
26. **Hendrickson, E. A., V. F. Liu, and D. T. Weaver.** 1991. Strand breaks without DNA rearrangement in V(D)J recombination. Mol. Cell. Biol. **11:**3155–3162.
27. **Hendrickson, E. A., X.-Q. Qin, E. A. Bump, D. G. Schatz, M. Oettinger, and D. T. Weaver.** 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.
28. **Hendrickson, E. A., D. G. Schatz, and D. T. Weaver.** 1988. The *scid* gene encodes a *trans*-acting factor that mediates the rejoining event of Ig gene rearrangement. Genes Dev. **2:**817–829.
29. **Hendrickson, E. A., M. S. Schlissel, and D. T. Weaver.** 1990. Wild-type V(D)J recombination in *scid* pre-B cells. Mol. Cell. Biol. **10:**5397–5407.
30. **Jeggo, P., M. Hafezparast, A. F. Thompson, B. C. Broughton, G. P. Kaur, M. Z. Zdzienicka, and R. S. Athwal.** 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.
31. **Jeggo, P., M. Hafezparast, A. F. Thompson, G. P. Kaur, A. K. Sandhu, and R. S. Athwal.** 1993. A hamster-human subchromosomal hybrid cell panel for chromosome 2. Somatic Cell Mol. Genet. **19:**39–49.
32. **Kastan, M. B., Q. Zhan, W. S. El-Deiry, F. Carrier, T. Jacks, W. V. Walsh, B. S. Plunkett, B. Vogelstein, and J. A. J. Fornace.** 1992. A mammalian cell cycle checkpoint pathway utilizing p53 and *GADD45* is defective in ataxia-telangiectasia. Cell **71:**587–597.
33. **Kienker, L., W. Kuziel, and P. Tucker.** 1991. T cell receptor γ and δ gene junctional sequences in SCID mice: excessive P nucleotide insertion. J. Exp. Med. **174:**769–773.
34. **Kirchgessner, C. U., C. K. Patil, J. W. Evans, C. A. Cuomo, L. M. Fried, T. Carter, M. A. Oettinger, and J. M. Brown.** 1995. DNA-dependent kinase (p350) as a candidate gene for the murine SCID defect. Science **267:**1178–1183.
35. **Lee, S. E., C. R. Pulaski, D. M. He, D. M. Benjamin, M. J. Voss, J. Um, and E. A. Hendrickson.** 1995. Isolation of mammalian cell mutants that are X-ray sensitive, impaired in DNA double-strand break repair and defective for V(D)J recombination. Mutat. Res. **336:**279–291.
36. **Lees-Miller, S. P., Y. Chen, and C. W. Anderson.** 1990. Human cells contain a DNA-activated protein kinase that phosphorylates simian virus 40 T antigen, mouse p53, and the human Ku autoantigen. Mol. Cell. Biol. **10:**6472–6481.
37. **Lees-Miller, S. P., R. Godbout, D. W. Chan, M. Weinfeld, R. S. Day, G. M. Barron, and J. Allalunis-Turner.** 1995. Absence of p350 subunit of DNA-activated protein kinase from a radiosensitive human cell line. Science **267:**1183–1185.
38. **Lieber, M. R., J. E. Hesse, S. Lewis, G. C. Bosma, N. Rosenberg, K. Mizuuchi, M. J. Bosma, and M. Gellert.** 1988. The defect in murine severe com-

bined immune deficiency: joining of signal sequences but not coding segments in V(D)J recombination. Cell **55:**7–16.
39. **Lin, W.-C., and S. Desiderio.** 1993. Regulation of V(D)J recombination activator protein RAG-2 by phosphorylation. Science **260:**953–959.
40. **Lin, W.-C., and S. Desiderio.** 1994. Cell cycle regulation of V(D)J recombination activating protein RAG-2. Proc. Natl. Acad. Sci. USA **91:**2733–2737.
41. **Liu, V., and D. Weaver.** 1993. The ionizing radiation-induced replication protein A phosphorylation response differs between ataxia telangiectasia and normal human cells. Mol. Cell. Biol. **13:**7222–7231.
42. **Malynn, B. A., T. K. Blackwell, G. M. Fulop, G. A. Rathbun, A. J. W. Furley, P. Ferrier, L. B. Heinke, R. A. Phillips, G. D. Yancopoulos, and F. W. Alt.** 1988. The *scid* defect affects the final step of the immunoglobulin VDJ recombinase mechanism. Cell **54:**453–460.
43. **Marder, B. A., and W. F. Morgan.** 1993. Delayed chromosomal instability induced by DNA damage. Mol. Cell. Biol. **13:**6667–6677.
44. **Mimori, T., and J. A. Hardin.** 1986. Mechanism of interaction between Ku protein and DNA. J. Biol. Chem. **261:**10375–10379.
45. **Paillard, S., and F. Strauss.** 1991. Analysis of the mechanism of interaction of simian Ku protein with DNA. Nucleic Acids Res. **19:**5619–5624.
46. **Pan, Z. Q., A. A. Amin, E. Gibbs, H. W. Niu, and J. Hurwitz.** 1994. Phosphorylation of the p34 subunit of human single-stranded DNA binding protein in cyclin A-activated G(1) extracts is catalyzed by CDK cyclin A complex and DNA-dependent protein kinase. Proc. Natl. Acad. Sci. USA **91:**8343–8347.
47. **Pergola, F., M. Z. Zdzienicka, and M. R. Lieber.** 1993. V(D)J recombination in mammalian cell mutants defective in DNA double-strand break repair. Mol. Cell. Biol. **13:**3464–3471.
48. **Peterson, S. R., A. Kurimasa, M. Oshimura, W. S. Dynan, E. M. Bradbury, and D. J. Chen.** 1995. Loss of the 350kD catalytic subunit of the DNA-dependent protein kinase in DNA double-strand break repair mutant mammalian cells. Proc. Natl. Acad. Sci. USA **92:**3171–3174.
49. **Petrini, J. H. J., Y. Xiao, and D. T. Weaver.** 1995. DNA ligase I mediates essential functions in mammalian cells. Mol. Cell. Biol. **15:**4303–4308.
50. **Rathmell, W. K., and G. Chu.** 1994. A DNA end-binding factor involved in double-strand break repair and V(D)J recombination. Mol. Cell. Biol. **14:**4741–4748.
51. **Rathmell, W. K., and G. Chu.** 1994. Involvement of the Ku autoantigen in the cellular response to DNA double-strand breaks. Proc. Natl. Acad. Sci. USA **91:**7623–7627.
52. **Reeves, W.** 1985. Use of monoclonal antibodies for the characterization of novel DNA-binding proteins recognized by human autoimmune sera. J. Exp. Med. **161:**18–39.
53. **Roth, D. B., J. P. Menetski, P. B. Nakajima, M. J. Bosma, and M. Gellert.** 1992. V(D)J recombination: broken DNA molecules with covalently sealed (hairpin) coding ends in scid mouse thymocytes. Cell. **70:**983–991.
54. **Schuler, W., N. R. Ruetsch, M. Amsler, and M. J. Bosma.** 1991. Coding joint formation 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.
55. **Schuler, W., I. J. Weiler, A. Schuler, R. A. Phillips, N. Rosenberg, T. W. Mak, J. F. Kearney, R. P. Perry, and M. J. Bosma.** 1986. Rearrangement of antigen receptor genes is defective in mice with severe combined immune deficiency. Cell **46:**963–972.
56. **Smider, V., W. K. Rathmell, M. R. Lieber, and G. Chu.** 1994. Restoration of X-ray resistance and V(D)J recombination in mutant cells by Ku cDNA. Science **266:**288–291.
57. **Staunton, J. E., and D. T. Weaver.** 1994. Hairpin-ended DNA integrates efficiently in *scid* fibroblasts. Mol. Cell. Biol. **14:**3876–3883.
58. **Suwa, A., M. Hirakata, Y. Takeda, S. A. Jesch, T. Mimori, and J. A. Hardin.** 1994. DNA-dependent protein kinase (Ku protein-p350 complex) assembles on double-stranded DNA. Proc. Natl. Acad. Sci. USA **91:**6904–6908.
59. **Taccioli, G., T. M. Gottlieb, T. Blunt, A. Priestly, J. Demengeot, R. Mizuta, A. R. Lehmann, F. W. Alt, S. P. Jackson, and P. A. Jeggo.** 1994. Ku80: product of the XRCC5 gene and its role in DNA repair and V(D)J recombination. Science **265:**1442–1445.
60. **Taccioli, G. E., H.-L. Cheng, A. J. Varghese, G. Whitmore, and F. W. Alt.** 1994. 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.
61. **Taccioli, G. E., G. Rathbun, E. Oltz, T. Stamato, P. A. Jeggo, and F. W. Alt.** 1993. Impairment of V(D)J recombination in double-strand break repair mutants. Science **260:**207–210.
62. **Thacker, J.** 1986. The nature of mutants induced by ionising radiation in cultured hamster cells. Mutat. Res. **160:**267–275.
63. **Uckun, F. M., G. L. Schieven, L. M. Tuel-Ahlgren, I. Dibirdik, D. E. Myers, J. A. Ledbetter, and C. W. Song.** 1993. Tyrosine phosphorylation is a mandatory proximal step in radiation-induced activation of the protein kinase C signaling pathway in human B-lymphocyte precursors. Proc. Natl. Acad. Sci. USA **90:**252–256.
64. **Uckun, F. M., L. M. Tuel-Ahlgren, C. W. Song, K. Waddick, D. E. Myers, J. Kirihara, J. A. Ledbetter, and G. L. Shieven.** 1992. Ionizing radiation stimulates unidentified tyrosine-specific protein kinases in human B-lymphocyte precursors, triggering apoptosis and clonogenic cell death. Proc. Natl. Acad. Sci. USA **89:**9005–9009.
65. **Walker, A. I., T. Hunt, R. J. Jackson, and C. W. Anderson.** 1985. Double-stranded DNA induces the phosphorylation of several proteins including the 90,000 Mr heat-shock protein in animal cell extracts. EMBO J. **4:**139–145.
66. **Weaver, D.** 1994. V(D)J recombination and double-strand break repair. Adv. Immunol. **58:**29–85.
67. **Zhang, W.-W., and M. Yaneva.** 1992. On the mechanisms of Ku protein binding to DNA. Biochem. Biophys. Res. Commun. **186:**574–579.