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Genetics

Identification of a nonsense mutation in the carboxyl-terminal region of DNA-dependent protein kinase catalytic subunit in the *scid* mouse

[double-strand break repair/V(D)J recombination/phosphatidylinositol 3-kinase/immune development/γ-irradiation]

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ABSTRACT **DNA-dependent protein kinase (DNA-PK) consists of a heterodimeric protein (Ku) and a large catalytic subunit (DNA-PKcs). The Ku protein has double-stranded DNA end-binding activity that serves to recruit the complex to DNA ends. Despite having serine/threonine protein kinase activity, DNA-PKcs falls into the phosphatidylinositol 3-kinase superfamily. DNA-PK functions in DNA double-strand break repair and V(D)J recombination, and recent evidence has shown that mouse *scid* cells are defective in DNA-PKcs. In this study we have cloned the cDNA for the carboxyl-terminal region of DNA-PKcs in rodent cells and identifed the existence of two differently spliced products in human cells. We show that DNA-PKcs maps to the same chromosomal region as the mouse *scid* gene. *scid* cells contain approximately wild-type levels of DNA-PKcs transcripts, whereas the V-3 cell line, which is also defective in DNA-PKcs, contains very reduced transcript levels. Sequence comparison of the carboxyl-terminal region of *scid* and wild-type mouse cells enabled us to identify a nonsense mutation within a highly conserved region of the gene in mouse *scid* cells. This represents a strong candidate for the inactivating mutation in DNA-PKcs in the *scid* mouse.**

In 1983 Bosma *et al.* (1) observed a severe combined immunodeficient (*scid*) mouse in a litter of otherwise normal mice and subsequent backcrossing established the now well-known *scid* mouse. Only now are details of the molecular defect in *scid* mice beginning to emerge. *scid* mice and cell lines derived from them have an interesting and pleiotropic phenotype, with features certainly unpredicted in 1983 (2). *scid* mice fail to develop mature T and B lymphocytes due to an inability to carry out functional rearrangements of the elements encoding the immunoglobulin and T-cell receptor genes (3–6). These elements, termed the variable (V), diversity (D), and joining (J) segments, are spatially separated in germ-line cells but are rearranged into a contiguous unit during maturation of T and B cells in the process called V(D)J rearrangement (for reviews, see refs. 7–9). This process is initiated by double-strand breaks (dsbs) introduced between partially conserved recombination signal sequences (RSS) and the flanking sequence encoding the V, D, or J element (coding sequence). The rearrangement yields one junction in which the two RSS sequences are rejoined precisely and a second in which the two coding elements are rejoined. The latter junctions invariably harbor small deletions and insertions. *scid* cells manifest a SCID phenotype due to an inability to form functional coding junctions. In contrast, signal junctions are formed at near normal levels (3–6).

In lower organisms, many mutants defective in processes of genetic recombination are sensitive to ionizing radiation (10). This link prompted an examination of *scid* cell lines, which proved to be both radiosensitive and defective in their ability to rejoin DNA dsbs (11–13). A number of radiosensitive dsb-repair-defective hamster cell mutants have also been described, and analysis of these hamster mutants for their ability to carry out V(D)J recombination showed that they too harbored defects in this process (14–16). Cell fusion studies of these and *scid* cells have established the existence of three distinct complementation groups, now designated ionizing radiation groups 4, 5, and 7 (17–19). These results therefore indicate that dsb repair and V(D)J recombination utilize a common mechanism involving at least three components. *scid* cells and the hamster cell line V-3, which exhibits an identical phenotype, belong to ionizing radiation group 7 (16, 17).

Dramatic recent advances have led to the identification of the genes defective in complementation groups 5 and 7 and have established a role for DNA-dependent protein kinase (DNA-PK) in both dsb repair and V(D)J recombination (20–27). DNA-PK is a multiprotein complex that contains a DNA-end binding component, recently identified as the heterodimeric Ku protein, and a large catalytic component designated DNA-PKcs (28, 29). The larger component of Ku (Ku80) has been shown to be the product of *XRCC5*, the gene defective in ionizing radiation group 5 (20, 23), and DNA-PKcs has been identified as the product of *XRCC7*, the gene defective in group 7 mutants (24–26). DNA-PKcs is thus the product of the *SCID* gene. Mutants defective in Ku70 have not yet been described, but *XRCC6* has been designated as the gene encoding the smaller component of Ku on the assumption that such mutants will be radiosensitive (17).

The carboxyl-terminal catalytic domain of DNA-PKcs falls into the phosphatidylinositol 3-kinase (PI 3-kinase) superfamily, so-called because of sequence similarity at the carboxyl terminus of the proteins to the 110-kDa subunit of PI 3-kinase (30). DNA-PK, however, is a serine/threonine protein kinase and does not appear to possess lipid kinase activity (30). We have isolated clones corresponding to the carboxyl-terminal region of DNA-PKcs from hamster and mouse cells and find this region is strongly conserved from human to rodents, consistent with its proposed functional significance. By analyzing the carboxyl-terminal region of DNA-PKcs from the *scid*

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Abbreviations: dsb, double-strand break; DNA-PK, DNA-dependent protein kinase; DNA-PKcs, DNA-dependent protein kinase catalytic subunit; PI 3-kinase, phosphatidylinositol 3-kinase; YAC, yeast artificial chromosome; RT–PCR, reverse transcription-coupled PCR.

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cell line, we have identified a nonsense mutation that represents a likely causative mutation in *scid* cells.

MATERIALS AND METHODS

Cell Culture. The *scid* cell lines 3T3 *scid* and SCGR11 are immortalized lines derived from neonatal *scid* mice and were obtained from J. Thacker and D. Weaver, respectively (13). The V-3 cell line was derived from AA8-4 cells and was from G. Whitmore (31). Cells were cultured in minimal essential medium (GIBCO/BRL) supplemented with nonessential amino acids and 10% fetal calf serum.

DNA and RNA Extraction. DNA from cell lines was extracted. DNA from wild-type CB-17 and CB-17 *scid* mice was extracted from spleen and skin tissue provided by G. Margison (Paterson Institute for Cancer Research, Manchester, U.K.) and A. Begg (Netherlands Cancer Institute, Amsterdam).

Poly(A)$^+$ RNA was extracted from 5×10^7 cells by using a Quickprep micro mRNA purification kit (Pharmacia).

Library Screening. The CHO cDNA library screened was derived from the Okyama–Berg library (32) and a mouse B-cell cDNA library was supplied by the Human Genome Mapping Programme. The libraries were screened by standard procedures and washed with 2× standard saline citrate (SSC)/0.1% SDS at 65°C. Low-stringency Southern hybridization to genomic DNA was carried out as described for the library screening but hybridization and washing was done at 55°C. Mouse yeast artifical chromosome (YAC) libraries were screened by PCR by L. Schalkwyk, Imperial Cancer Research Fund laboratories (London).

cDNA Synthesis and Sequencing. cDNA was synthesized from 0.5–1.0 μg of poly(A)$^+$ RNA by using oligo(dT) primers and reverse transcriptase. Reverse transcription-coupled PCR (RT–PCR) products were amplified using primers A and HCS6 (see Fig. 1), purified using a Wizard clean-up system (Promega), and directly sequenced using a Thermo Sequence Cycle sequencing kit (Amersham). The insert from library clones was cloned into pGEM-3$^+$ and -3$^-$ (Promega) and sequenced from single-stranded DNA.

PCR. PCR was carried out in 20 μl containing 2 μl of 10× PCR buffer (500 mM KCl/100 mM Tris·HCl, pH 8.3/15 mM $MgCl_2$/0.1% gelatin), 1.5 μl of all four dNTPs (each at 2.5 mM), 2–5 pmol of each oligonucleotide primer, and 0.25 unit of *Taq* polymerase (HT Biotechnology, Cambridge, U.K.). When necessary amplified products were separated on low-melting-point agarose gels, excised, cleaned using Wizard DNA clean-up system (Promega), and cloned into a T vector (33). Primers utilized are listed in Fig. 1.

Northern Blot Analysis. Poly(A)$^+$ RNA (5 μg) was size-separated by electrophoresis in 1% agarose/formaldehyde gels, transferred overnight to Hybond N (Amersham) in 20× SSC, baked at 80°C for 2 hr, and UV-cross-linked. The probe was a PCR-amplified product from the mouse cDNA library clone, pCSM1, using primers X and Y. The PCR product was gel-purified and labeled by random priming. Hybridization was carried out using standard conditions.

RESULTS

Identification of Mouse DNA-PKcs cDNAs. cDNA clones derived from the 5′, middle, and 3′ regions of the open reading frame of the human DNA-PKcs gene were used as probes to examine mouse and hamster genomic DNA by Southern blot hybridization under low-stringency conditions (30). All probes gave weak cross-species hybridization signals, but the strongest was obtained with the 3′ probe, which included the kinase domain, indicating, as anticipated, that this is an important functional region and, therefore, highly conserved (data not shown). The most carboxyl-terminal region of the DNA-PKcs sequence [amino acid position 3921–4127 (Fig. 2)] was amplified by PCR using primers X and Y (see Fig. 1) from the 3′ clone (pCSHum1; DNA-PKcs clone 1 in refs. 30 and 24) and was found to give a strong signal when hybridized to rodent genomic DNAs. This probe was used to screen both mouse and hamster cDNA libraries, and one and four clones were detected from each library, respectively. The single mouse clone (pCSM1) and the hamster clone containing the largest insert (pCSHam5) were used as probes for Southern blot hybridization to rodent and human genomic DNAs. The intensity of the signals confirmed that the clones were of the expected rodent origin; additionally, they hybridized to the same sized bands as the human probe (data not shown). Sequence analysis of the inserts showed that their predicted products were homologues to human DNA-PKcs (Fig. 2).

PRIMER	SEQUENCE	ORIGIN	SITE
A	5′ GAC TCA AAG CCA CCT GGG AAC CTG 3′	hamster	3670
B	5′ GAG GAG CCA GTG GCT GAT GCA 3′	mouse	3918
X	5′ GA(C/T) A(G/C)I CA(C/T) C(T/T)TI AA(C/T) AA(C/T) TT(C/T) ATG 3′	degenerate	3920
Y	5′ CAT CCA IGG C(T/T)TC CCA ICC C(T/T)TC CCA 3′	degenerate	4130
C	5′ GTT AGG ATT AAT TGA GTG GCT 3′	human	3798
D	5′ TTA GCC AGT CTT TAT ATT CAC 3′	hamster	3855
E	5′ CGT TGT GCC CAT GAC CTC CAG 3′	human	3788
F	5′ CTC AGG TAA GCC GCC TTC TCC 3′	human	3299
M1	5′ GTC AGT CTC ATG TTG CCA ATG 3′	mouse	3965
M2	5′ AGT TAT AAC AGC TGG GTT GGC 3′	mouse	4059
HCS6	5′ CTG CTG TCA GTG AAG GTC TAG GAG 3′	hamster	3′UTR

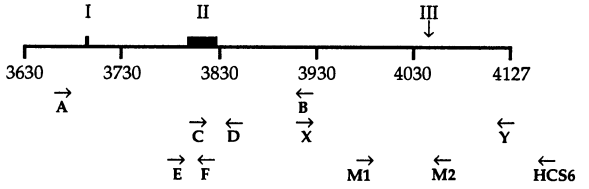

FIG. 1. Primer sequences and positions. The position of the primers corresponds to the numbering of amino acids shown in Fig. 2. I, the site of the deleted 12 bp in hamster cells (see text); II, the site of the missing exon in pCSHum1; III, the site of the mutation identified in *scid* cells.

Different Spliced Products in Human DNA-PKcs. The most notable feature of this comparison was the absence of a 31-amino acid sequence from our previously published human sequence (30). Using primers A and B (see Fig. 1), we amplified this region from rodent and human RNA by RT–PCR and from the cDNA clones derived from the three species (Fig. 3*a*). Whereas the human clone pCSHum1, which was used to derive the published human sequence, yielded a product of 645 bp (lane 4), the mouse clone pCSM1 gave a product of 738 bp (lane 5), consistent with our sequencing data. RT–PCR of human RNA produced both products (lane 1), while RNA from either rodent species yielded only the large product (lanes 2 and 3). Both products derived from human RNA were cloned and sequenced. The sequence of the smaller product was identical to our published human DNA-PKcs sequence, whereas the larger clone had a 93-bp insertion homologous to the rodent sequence (Figs. 2, boxed sequence, and 3*b* and ref. 30). Notably, the first 6 bp (GTTAGC) of the sequence missing from clone pCSHum1 closely matched the consensus sequence for a splice donor site [score of 93 by the system of Shapiro and Senapathy (34)]. In contrast, in the sequences from both rodents the first base is an adenosine instead of a canonical guanosine (ATTAGC), significantly decreasing its similarity to the donor splice site sequence. We speculated that the absence of the 31-amino acid region in a proportion of human cDNAs might result from the use of this sequence as a cryptic donor splice site (Fig. 3*b*). A prediction of this speculation is that there should be an intron at the 3′ end of the missing region. To test this, primers C and D (see Fig. 1) were used to amplify this region using genomic DNAs.

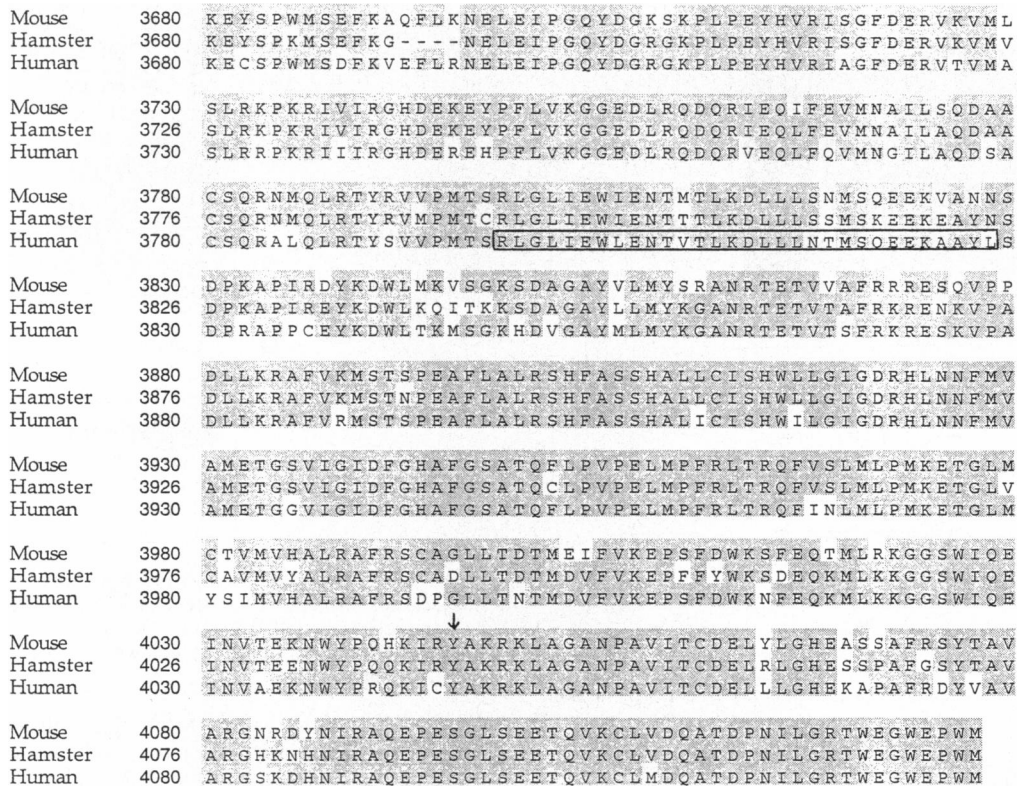

FIG. 2. Sequence comparison of the carboxyl-terminal region of mouse, hamster, and human DNA-PKcs. Shaded background indicates identical sequences. The exon missing in a fraction of human mRNAs is boxed. The arrow indicates the site of the mutation identified in *scid* cells. The numbering relates to the sequences present in this paper. This numbering is identical to that used in ref. 30 up to amino acid position 3797 and then differs due to the missing 31-amino acid region. To derive the sequence from the region of amino acids 3680–3852 from mouse (sequences present in pCSHam5 but missing in pCSM1), this region was amplified from mouse mRNA by RT–PCR using primers derived from the hamster sequence, followed by direct sequencing.

A product of approximately 1 kb was amplified from mouse genomic DNA. Cloning and partial sequencing of this product confirmed the presence of an intron at the 3′ boundary of the deleted region. A PCR product could not be obtained using primers C and D with human genomic DNA, probably explained by the presence of a very large intron at the same position. Additional PCRs using human genomic DNA also indicated the presence of an intron at the 5′ end of the deleted region. Thus primers E and F gave a product of approximately 300 bp, indicating the presence of a small intron, while primers C and F yielded the anticipated 93-bp product expected from the absence of an intron. Therefore, the missing 93-bp sequence is flanked by introns on both sides.

A second notable difference between the sequences from these three species is the absence of 4 amino acids from the hamster sequence (see Fig. 2, first row). This is particularly striking since the rodents are closely related, and for other parts of the sequence they show closer sequence identity to each other (87.8% at the amino acid level) than to the human sequence (82.8%). To verify that this was not an artefact generated during construction or amplification of the cDNA library or a feature unique to cultured CHO cells, this region was amplified from a number of different mouse and hamster cell lines by RT–PCR, followed by direct sequencing. The Chinese hamster cell lines used were CHO-K1, AA8, and V-3 and the Syrian hamster kidney cell line used was BHK-1. In all cases, the sequence in this region was as shown in Fig. 2 (hamster), whereas mouse sequences from the *scid* cell line SCGR11 and 3T3 cells and the human sequence contained the extra 4 amino acids indicated.

Identification of a Mutation in *scid* Cells. The mouse *scid* cell line has been shown to have a defect in DNA-PKcs since extracts from these cells lack DNA-PK activity and material cross-reacting with anti-DNA-PKcs antibody, while possessing Ku-associated DNA-end binding activity (24–26). Additionally, V-3 cells, which belong to the same complementation group as *scid* cells, can be complemented by yeast artificial chromosomes (YACs) encoding DNA-PKcs (24). Mouse genetic studies have localized the *SCID* gene to the centromeric region of chromosome 16 (35). The DNA-PKcs gene maps to a region of human chromosome 8 that shows synteny with the centromeric region of mouse chromosome 16 (24, 25, 36). To verify the localization of the mouse DNA-PKcs, we isolated YAC clones encompassing the mouse DNA-PKcs gene by PCR screening a mouse YAC library with primers specific for the 3′ untranslated region of the mouse DNA-PKcs gene (derived from pCSM1). Verification that these YACs encode DNA-PKcs is shown by the fact that they yielded products of the expected sizes by PCR using mouse-specific DNA-PKcs primers (see, e.g., Fig. 5*b*), and one of them was able to complement V-3 cells by protoplast fusion (data not shown). Two of the YACs obtained were used as probes for *in situ* hybridization and were shown to map to the centromeric region of mouse chromosome 16 (data not shown). Our data therefore support the conclusion that the DNA-PKcs gene maps to mouse chromosome 16, which in turn supports the notion that this is the gene defective in mouse *scid* cells. These results are consistent with the work of Miller *et al.* (37) showing that the DNA-PKcs gene maps close to the *scid* locus on mouse chromosome 16 using an interspecific backcross panel of mice.

To gain further insight into the nature of the defect in *scid* and V-3 cells, we utilized pCSM1 as a probe for Northern blot hybridization to examine expression of the DNA-PKcs gene in these mutant cell lines. We found that *scid* cells contain essentially wild-type levels of DNA-PKcs mRNA while V-3 cells have very low but nevertheless detectable levels (Fig. 4).

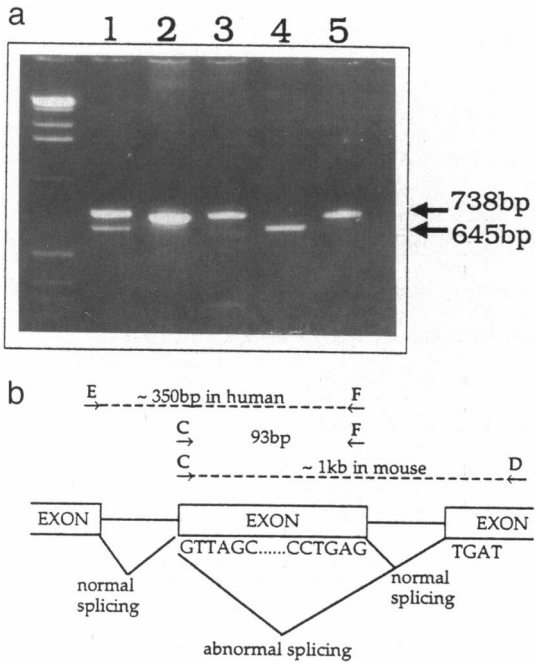

FIG. 3. Identification of a missing exon in a fraction of human cDNAs. (*a*) Amplification of the region incorporating the sequences missing in pCSHum1 from rodent and human cDNAs. cDNA derived from total human RNA (lane 1), hamster poly(A)$^+$ RNA (lane 2), mouse poly(A)$^+$ RNA (lane 3), pCSHum1 (lane 4), and pCSM1 (lane 5) was amplified using primers A and B (Fig. 1) and products were separated on 2% agarose gels. (*b*) Model of alternative splicing in human cells. The sequence GTTAGC has a score of 93 for a splice donor site (34). In hamsters or mice, this sequence is ATTAGG, which does not contain the canonical guanosine at the first position. In *b*, we have shown alternative splicing resulting from the use of the sequence GTTAGC as a splice donor site and normal splicing of the preceding intron. It is equally possible that the GTTAGC sequence might impair the use of the immediately preceding splice acceptor site and, thus, loss of the two introns and exon would occur from a single splicing event. Sizes of products amplified are as shown.

In an attempt to identify the mutation in the DNA-PKcs gene resulting in the defective phenotype of *scid* cells, we sequenced the 3′ region of *scid* DNA-PKcs mRNA by RT–PCR followed by direct sequencing. We detected 1 bp difference between *scid* and the wild-type mouse sequence, present in both alleles, at the position that corresponds to amino acid 3845 (Fig. 2) that resulted in a conservative change from Val to Met. This amino acid is different between the three species examined in this study, and we, therefore, considered it unlikely to be of any functional importance. More significantly, at a position corresponding to amino acid 4045, we identified a T → A mutation that resulted in the creation of an ochre stop codon (Figs. 2 and 5*a*). By our direct sequencing procedure, both alleles are sequenced simultaneously, and heterozygosity at a site is normally identifiable by a double sequence. We observed no evidence for the wild-type sequence at this site, suggesting that the *scid* mouse is homozygous for this mutation, a feature to be expected from the inbreeding used to establish the original mutation (1). This mutational change results in the creation of an *Alu*I restriction site, which was exploited to verify the presence of this mutation in genomic DNA from *scid* mice. Primers M1 and M2 (Figs. 1 and 5*c*) were used to amplify this region from genomic DNA extracted from a *scid* mouse, from wild-type CB-17 mice, from a putative heterozygous *scid* mouse derived from crossing a *scid* mouse with a mouse wild-type for DNA-PKcs, from the *scid* cell lines *scid* 3T3 and SCGR11, from the mouse 3T3 cell line, and from a mouse YAC encompassing DNA-PKcs. In all cases, we obtained products of 1.3 kb, indicating the presence of an intron within this region. After digestion with *Alu*I, we observed a difference in digestion products between wild-type and *scid* cells; a fragment of about 250 bp in normal cells was reduced in size in *scid* cells by about 30 bp (see Fig. 5*b*). This difference was consistent with the site of the mutation. Significantly, both bands were present in DNA from the putative *scid* heterozygote. The size of the *Alu*I fragment implied that the next *Alu*I site was in the intervening intron. The amplified product was, therefore, cloned and partially sequenced to determine the site of the intron–exon boundary and to identify the position of the first *Alu*I site within the intron sequence (from the 3′ direction). The data shown in Fig. 5*b* are entirely consistent with the results obtained, with products of sizes 241 and 211 bp detected in wild-type and *scid* cell lines, respectively. The complete absence of the wild-type fragment, therefore, verifies that *scid* cells are homozygous for this mutation.

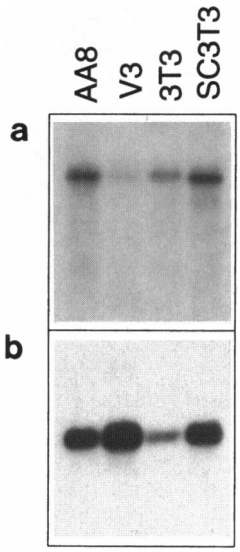

FIG. 4. Northern blot analysis of DNA-PKcs from wild-type mouse 3T3, *scid* cell line SC3T3, hamster cell line AA8, and hamster V-3 cells. In *a*, the probe was a PCR-amplified fragment from mouse DNA-PKcs clone (pCSM 1) and in *b* the probe was glyceraldehyde-3-phosphate dehydrogenase.

DISCUSSION

Considerable recent evidence has indicated that mouse *scid* cells are defective in DNA-PKcs (24–26). Herein we present data that support this notion and indicate that the defect lies in the DNA-PKcs gene rather than a gene or sequences controlling its expression. (*i*) Fluorescence *in situ* hybridization analysis using a YAC encompassing the mouse DNA-PKcs gene localized the DNA-PKcs gene to the same region as the *SCID* gene, namely, mouse chromosome 16. (*ii*) We have shown that *scid* cells contain near normal levels of DNA-PKcs transcript, although another member of the same complementation group, V-3, contains decreased transcript levels. (*iii*) We have sequenced 1.35 kb of the 3′ region of DNA-PKcs cDNA from *scid* cells and have identified a mutational change that is likely to be of functional significance since it results in an ochre mutation and loss of 83 amino acids from the highly conserved carboxyl-terminal end of the protein.

DNA-PKcs is a member of the PI 3-kinase superfamily, which can be subdivided into two groups (30, 38). In the group of smaller proteins are the 110-kDa catalytic subunit of human PI 3-kinase (p110) and the yeast proteins Vps34p and Pik1p. The second group contains proteins that are much larger (greater than 200 kDa) and includes FRAP, Tor1p, Tor2p, the ATM protein, Mec1p, and the *Schizosaccharomyces pombe* Rad3 protein. These proteins have considerable sequence

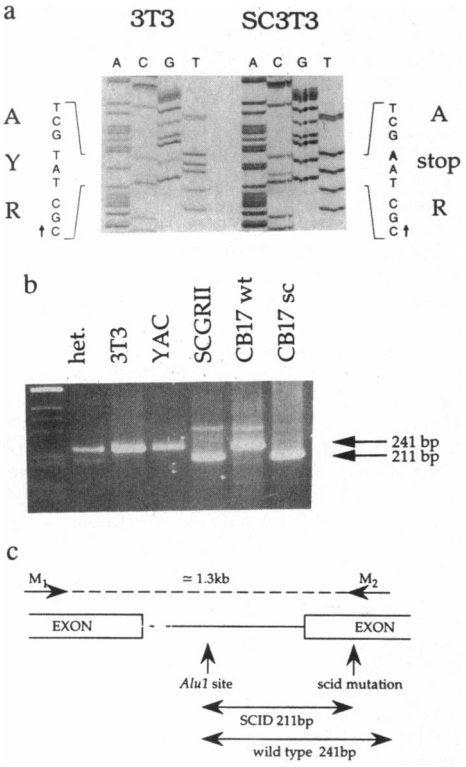

FIG. 5. Identification of a mutation in DNA-PKcs in mouse *scid* cells. (*a*) Sequence of 3T3 and *scid* SC3T3 cells obtained by RT–PCR of poly(A)$^+$ RNA and direct sequencing. (*b*) Altered restriction pattern caused by the mutation change. Genomic DNA was PCR-amplified using primers M1 and M2 and digested with *Alu*I, and the products were separated on a 3% agarose gel. In the first three lanes, but not the last three lanes, the 1.3-kb PCR product was gel-purified prior to *Alu*I digestion. The minor band present in the last three lanes, therefore, represents a spurious product amplified by the M1/M2 primers. This band was not present in other experiments carried out using the same cell lines, in which the purification step was included. (*c*) Fragments generated by *Alu*I digestion of the PCR products amplified in CB-17 and *scid* cellsusing primers M1 and M2.

similarity to the PI 3-kinases at their carboxyl termini, but in fact PI 3-kinase activity has not been unequivocally demonstrated for any of them, and like DNA-PK, they probably function primarily, if not exclusively, as protein kinases (30, 39, 40). This subset of larger proteins has a highly conserved terminal 30-amino acid sequence that is absent in the smaller proteins (30, 40). Indeed, a comparison of the terminal 42-amino acid residues of the rodent and human DNA-PKcs proteins shows only a single conservative amino acid change (Fig. 2). Notably, the mutation we have identified in *scid* cells will result in loss of this extreme terminal carboxyl region but not of the PI 3-kinase domain. The function of this extreme terminal carboxyl region is not known and whether its loss would affect protein kinase activity, which is known to be ablated in *scid* cells (24, 26), is also not known. However, considering the highly conserved nature of this region, we suggest that its loss is likely to impair the function of the DNA-PKcs protein and possibly the kinase activity and, thus, represents a strong candidate for the causal mutation in *scid* cells. To verify this, it will be necessary to demonstrate that no other mutations have arisen in the DNA-PKcs cDNA of *scid* mice, which will require sequencing the entire 14-kb DNA-PKcs gene in wild-type mouse and *scid* cells. In this context it is noteworthy that the mutation is in both DNA-PKcs alleles in *scid* mice, which would not be predicted of a mutation arising subsequent to a causal mutation. Our results provide a working framework to consider the phenotype of *scid* mice and additionally provide a useful marker to identify the *scid* allele in mice. As shown in Fig. 5*b*, and in recent collaborative work (with Z. Wang) conducted blindly, we correctly identified heterozygous and homozygous *scid* mice by using genomic DNA derived from them. This result therefore provides a simple and convenient method to identify the *scid* allele in genetic crosses involving *scid* mice.

Although a prediction of our findings is that *scid* cells might possess truncated DNA-PKcs protein, we (27) and others (25, 26) have observed that *scid* cells show a large decrease in protein cross-reactive with anti-DNA-PKcs antibodies. One possibility is that loss of the carboxyl-terminal region of the protein renders it unstable. Alternatively, these antibodies, which were raised against human DNA-PKcs, may only (or predominantly) recognize epitopes in the rodent protein that are in the highly conserved carboxyl terminus. Thus loss of the carboxyl terminus may result in disappearance of most of the epitopes that are recognized by the human antibody.

scid mice show a characteristic "leaky" phenotype since approximately 15% of young adult *scid* mice and virtually all old *scid* mice develop detectable levels of serum immunoglobulin and T-cell activity (41, 42). Another observation that might be pertinent to this "leakiness" is that *scid* cells appear to retain very low but detectable levels of DNA-PKcs cross-reacting material (25, 26). If the mutation that we have identified is the causative *scid* mutation, our results provide possible explanations for these observations. One possibility is that a low level of residual DNA-PKcs protein arises in *scid* cells due to suppression of the ochre stop codon. Read through of nonsense mutations has been detected and analyzed extensively in prokaryotes and mammalian cells (43–45). Although context effects play an important role, ochre codons generally allow less read through than amber codons. Aminoglycoside antibiotics, such as G418, have been shown to enhance read through of stop codons, but we have been unable to detect any effect of G418 treatment on radiosensitivity of *scid* cells. Since only low protein levels (e.g., 1% of normal protein levels) might be expected to arise by an ochre read-through mechanism, this would be difficult to detect by current methods. Such low protein levels might, however, have an impact on V(D)J recombination during development, where only a few breaks will arise in any cell. Our results also raise possibilities to explain "leakiness" by the presence of aberrant protein in *scid* cells.

Our comparison of the carboxyl-terminal region of rodent and human DNA-PKcs has also led us to identify a pattern of splicing occurring in human cells that does not appear to occur in rodent cells. The sequence at the beginning of the exon at amino acid position 3798 (boxed in Fig. 2) is close to the consensus donor splice site sequence and consequently is occasionally recognized as a donor splice site resulting in the loss of the entire exon in a proportion of human cDNAs. Consistent with our interpretation, another sequence for the carboxyl-terminal region of DNA-PKcs has been published (GenBank accession no. U35835) that does contain the region under discussion (46). Since the region deleted includes sequences conserved between members of the PI 3-kinase superfamily, its loss is likely to result in absent or impaired kinase activity. We have observed different ratios of the two forms between different human tissues and cell lines but have not identified any pattern to these differences. Although these observations probably result from incorrect splicing, it is possible that this represents a form of alternative splicing utilized to control DNA-PKcs activity in humans. Finally, we have also observed that both Syrian and Chinese hamster cells carry a small in-frame deletion in a region of DNA-PKcs that lies outside of the conserved kinase domain. Since hamster and mouse cells have similar levels of DNA-PK activity, and similar radiosensitivities, this deletion does not appear to affect the function of the protein.

In conclusion, we have compared the carboxyl-terminal region of DNA-PKcs from human, mouse, and hamster cells and have identified a likely causative mutation in mouse *scid* cells that results in loss of the highly conserved terminal 83 amino acids from the protein.

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