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Genetics

Gene for the catalytic subunit of mouse DNA-dependent protein kinase maps to the *scid* locus

ROBERT D. MILLER*†, JOANNA HOGG*, JENNIFER H. OZAKI‡, DAVID GELL§, STEPHEN P. JACKSON§, AND ROY RIBLET‡

*Department of Biology, University of New Mexico, Albuquerque, NM 87131; ‡Medical Biology Institute, La Jolla, CA 92037; and §Wellcome/Cancer Research Campaign Institute and Department of Zoology, Cambridge University, Cambridge CB2 1QR, England

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ABSTRACT **The gene encoding the catalytic subunit of DNA-dependent protein kinase (DNA-PK$_{cs}$) has been proposed recently as a candidate gene for the mouse severe combined immune deficiency (*scid*) locus. We have used a partial cDNA clone for human DNA-PK$_{cs}$ to map the mouse homologue using a large interspecific backcross panel. We found that the mouse gene for DNA-PK$_{cs}$ does not recombine with *scid*, consistent with the hypothesis that *scid* is a mutation in the mouse gene for DNA-PK$_{cs}$.**

Developing lymphocytes undergo a process of *V(D)J* recombination in which variable (*V*), diversity (*D*), and joining (*J*) gene segments are assembled via somatic DNA rearrangement to produce complete immunoglobulin (Ig) or T-cell receptor (TCR) genes in B or T cells, respectively (reviewed in ref. 1). *V(D)J* recombination requires the cleavage of DNA at specific recombination signal sequences (RSS) that flank the *V*, *D*, and *J* coding segments and subsequent ligation of the free ends with deletion of intervening DNA. The RSS are contained on the deleted DNA segments and are ligated to form contiguous signal joints, while the *V*, *D*, and *J* segments are ligated following nucleotide excision and addition to form contiguous coding joints. One of the first indicators of the role of DNA double-strand-break repair in *V(D)J* recombination was the appearance of the mouse *scid* mutation for severe combined immunodeficiency (2). *scid* is a recessive, autosomal mutation that results in the inability to produce mature, functional lymphocytes because of failure in *V(D)J* recombination (2–4). Lymphocytes from C.B-17-*scid* mice (SCID) begin to undergo *V(D)J* recombination but fail to correctly join the coding segments, often resulting in large DNA deletions at those sites. SCID mice and SCID cells in culture have been shown to be abnormally sensitive to ionizing radiation and other agents that induce DNA breaks, suggesting that the *scid* gene product plays a more general role in DNA repair (5–7).

Further association between *V(D)J* recombination and DNA repair has been made with studies of radiosensitive Chinese hamster cell lines (8, 9). One cell line, V3, is similar to SCID cells in that it fails to correctly join the coding segments and is unable to complement the SCID phenotype when fused to SCID cells in culture, suggesting a common defect (10). Several hamster lines that fall into the same complementation group (XRCC5) are defective for *V(D)J* recombination and are deficient in an ≈80-kDa DNA end-binding protein called Ku80 (11–14). Ku80 and a 70-kDa protein (Ku70) form the Ku DNA-binding component of the DNA-dependent protein kinase (DNA-PK) complex (15–18). In addition to Ku, DNA-PK contains an ≈450-kDa catalytic subunit (DNA-PK$_{cs}$), which is a serine/threonine-protein kinase (16, 17). While the exact function of DNA-PK is not clearly understood, *in vitro* studies have shown that the complex is active only when bound to DNA and, once bound, can phosphorylate several transcription factors, suggesting a possible role in transcription regulation (19–23). Recently, the gene for DNA-PK$_{cs}$ has been proposed as a likely candidate gene for the mouse *scid* locus by several laboratories (24–26). Indeed, nuclear extracts from SCID and V3 cells lack detectable DNA-PK$_{cs}$ activity (24). Somatic cell hybrids of mouse SCID and human cells have shown that the region of human chromosome 8 containing the DNA-PK$_{cs}$ gene restores wild-type DNA repair and *V(D)J* recombination (27–30). Furthermore, the *scid*-related defects in DNA repair and *V(D)J* recombination were also complemented by transfecting V3 cells with yeast artificial chromosomes (YACs) containing the human DNA-PK$_{cs}$ gene (24).

While one explanation for these findings is that the *scid* locus encodes mouse DNA-PK$_{cs}$, it is quite possible that unusual expression or overexpression of the human gene for DNA-PK$_{cs}$ in transfected mouse SCID cells in culture may overcome the *scid* defect indirectly. For example, indirect complementation has been demonstrated with other DNA repair-related defects such as ataxia-telangiectasia (A-T) in which the problem appears to involve cell cycle control rather than DNA repair directly (31). Several cDNA clones have been reported to give phenotypic complementation of A-T without being genetically linked to A-T (32). Some aspects of the recently published work associating *scid* with the gene for DNA-PK$_{cs}$ remain puzzling. The levels of DNA-PK$_{cs}$ protein in SCID cells assayed by immunoblot are significantly reduced but not completely lacking (25). Furthermore, the V3 cell YAC-transfectants containing the human DNA-PK$_{cs}$ gene express much higher levels of the human protein than is normally detectable for wild-type rodent DNA-PK$_{cs}$ (24). Thus, DNA-PK$_{cs}$ may be another component of a pathway involving the *scid* gene product or may act as a suppressor of the *scid* mutation. To address these alternative possibilities, we decided to map the mouse gene for DNA-PK$_{cs}$ and determine if it localizes to *scid*.

MATERIALS AND METHODS

Generation of the Backcross Panel. Male CAST/Ei (*Mus musculus castaneus*) mice were obtained from The Jackson Laboratory and were mated with C.B-17-*scid* mice maintained at the Medical Biology Institute. Female (C.B-17-*scid* × CAST/Ei)F_1 mice were backcrossed to male C.B-17-*scid* mice. At the time of sacrifice (3 to 4 weeks of age), each backcross mouse was typed for the SCID phenotype by examining the gross appearance of the thymus. As a result of abnormal lymphoid development, homozygous SCID mice have, at most, a tiny rudimentary thymus. Splenic histology is also abnormal

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Abbreviations: DNA-PK, DNA-dependent protein kinase; DNA-PK$_{cs}$, catalytic subunit of DNA-PK; SCID, severe combined immunodeficiency; V, variable; D, diversity; J, joining; YAC, yeast artifical chromosome.

†To whom reprint requests should be addressed.

in homozygous SCID mice because of the absence of mature lymphocytes (2). Hematoxylin/eosin-stained *scid* spleen sections appear devoid of lymphocytes and normal tissue organization, the principal cell types being fibroblasts and histiocytes. Spleen tissue from each backcross progeny was collected and preserved in 10% formalin for sectioning, staining, and examination. Spleen sections were prepared and examined to verify the SCID phenotype for all informative backcross progeny—i.e., those progeny that had a recombination between *D16Mit1* and *D16Mit33*. Genomic DNA was isolated from pooled liver and spleen tissue from each backcross progeny.

Analysis of Backcross Panel. All backcross progeny were typed for *scid* and two microsatellite DNA segments, *D16Mit1* and *D16Mit33*, which are ≈8 centimorgans (cM) apart and flank the *scid* locus. Only those progeny with a recombination between *D16Mit1*, *scid*, and *D16Mit33* were typed further for additional markers tightly linked to *scid*. No backcross progeny were found with a double recombination bracketing *scid* in the *D16Mit1*-to-*D16Mit33* interval. Genomic DNA prepared for Southern blot analysis was electrophoresed through 1% agarose gels (FMC BioProducts) after restriction enzyme digestion and was transferred to nitrocellulose filters (BA-S85, Schleicher & Schuell) for hybridization. Filters were hybridized with radiolabeled DNA probes under conditions described previously (33). All probes were plasmid inserts and were labeled by the random primer method following the manufacturer's recommendations (Stratagene). A *Taq* I restriction fragment length polymorphism for the *VpreB1* locus was typed by using the pZ121 clone (34) provided by Konrad Huppi (National Institutes of Health, Bethesda). A cDNA clone containing fragments of the human DNA-PK$_{cs}$ cDNA (clone 1 in ref. 24) was used to cross-hybridize to mouse genomic DNA under conditions similar to that referenced above except that filters were washed at low stringency with a final wash at 60°C in 0.15 M NaCl/0.015 M sodium citrate, pH 7. Identification and characterization of clones 1, 8, and 13 will be described in greater detail elsewhere (K. O. Hartley, D.G., X. Zhang, G. C. M. Smith, N. Divecha, M. A. Connelly, A. Admon, S. P. Lees-Miller, C. W. Anderson, and S.P.J., unpublished data).

Oligonucleotide primers unique for the specific simple sequence-length polymorphisms (SSLP) *D16Mit1*, *D16Mit31*, *D16Mit33*, *D16Mit34*, *D16Mit56*, *D16Mit74*, *D16Mit143*, *D16Mit144*, and *D16Mit165* (refs. 35 and 36; supplemented by additional markers in the "Genetic Map of the Mouse, Database Release 9" of the Whitehead Institute/MIT Center for Genome Research) were purchased from Research Genetics (Huntsville, AL). (Note that *D16Mit31* was removed from the map and from marker lists maintained by the MIT Center for Genome Research and by Research Genetics because of mapping inconsistencies in a small cross. However, our mapping results were consistent with the original placement of *D16Mit31* on chromosome 16.) Additional DNA markers for the genomic region containing the *scid* locus were developed by subcloning fragments of YAC clones containing mouse DNA inserts. These YACs were identified by screening YAC libraries (37–40) by PCR with primers for the *D16Mit31*, *D16Mit34*, and *VpreB1* loci (unpublished results). Clones pR1, pR7.12, and pR9.10 are nonrepetitive DNA fragments isolated from gel-purified YAC DNA by PCR with 10-base oligomers. pR1 was amplified from clone yI96.G4 by using the oligonucleotide primer 5′-CGGTCACTGT-3′, and pR7.12 and pR9.10 were amplified from clone yI82.F8 by using the oligonucleotide primer 5′-GTTGGTTTAA-3′. PCR products were cloned into pCRII (Invitrogen). Clone pC117.2 is a DNA fragment generated as a subclone of the centromeric end of clone yFEZ.G5 and was isolated by digesting the YAC with the restriction enzyme *Xba* I and ligating the restriction fragments under dilute conditions. This circularized the centromeric end of the pYAC4 vector arm containing the pUC-derived *ori* and ampicillin-resistance gene and the end fragment of the mouse DNA insert.

RESULTS AND DISCUSSION

Mapping the Mouse Gene for DNA-PK$_{cs}$. Preparing genetic crosses between inbred mouse strains derived from different subspecies of *M. musculus*, such as CAST/Ei (*M. m. castaneus*), facilitates linkage analysis by increasing the probability of detecting DNA polymorphisms at desired loci. This was recently illustrated by Dietrich *et al.*, who found that the frequency of detecting differences at microsatellites increased from 50% between standard inbred strains to >90% between a standard inbred line and CAST/Ei (35). Using a large panel of interspecific backcross mice from a cross between the C.B-17-*scid* and CAST/Ei strains, we generated a high-resolution linkage map of the region of the mouse genome containing the *scid* locus. Originally *scid* was shown to map to the proximal end of chromosome 16 by linkage to the mahoganoid coat color mutation and the immunoglobulin λ light chain locus (*Igl-1*) (41). More recently we showed that *scid* is tightly linked to the B-cell surrogate light chain genes, *VpreB1* and λ5, with no recombination found in 958 backcross progeny (ref. 42 and Figs. 1 and 2). Using cDNA clones containing fragments of the human DNA-PK$_{cs}$ gene (clones 1 and 8 in ref. 24) as probes on Southern blots, we detected mouse genomic DNA restriction fragments containing the putative mouse DNA-PK$_{cs}$ gene. Clone 1 contains the region encoding the DNA-PK$_{cs}$ kinase domain and may have the highest degree of homology between the human and mouse DNA-PK$_{cs}$ genes. Clone 1 hybridizes well with a 14.1-kb *Hin*dIII fragment and weakly with a 3.9-kb *Hin*dIII fragment in the CAST/Ei genome and with 5.7- and 7.5-kb fragments in the SCID mouse genome. Of 958 backcross progeny between (C.B-17-*scid* × CAST/Ei)F$_1$ and C.B-17-*scid*, 81 were found to be recombinant between *D16Mit33* and *D16Mit1*, two microsatellite DNA segments that flank the *scid* locus. Typing all 81 of these

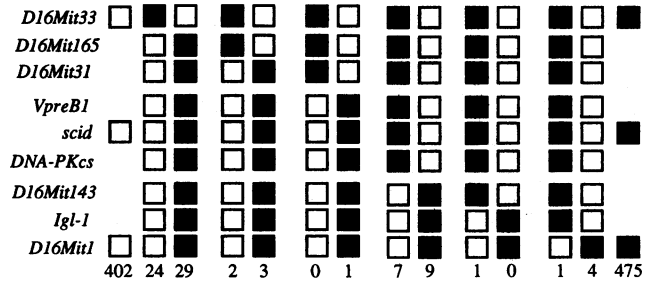

FIG. 1. Results from DNA typing the (C.B-17-*scid* × CAST/Ei)F$_1$ × C.B-17-*scid* backcross progeny in the region containing the *scid* locus. The columns represent the different chromosome genotypes inherited from the F$_1$ parent. Filled boxes indicate the presence of the CAST/Ei allele, and open boxes indicate the C.B-17-*scid* allele at the indicated loci. Values at the bottom of columns indicate the number of progeny inheriting the indicated haplotype from the F$_1$ parent.

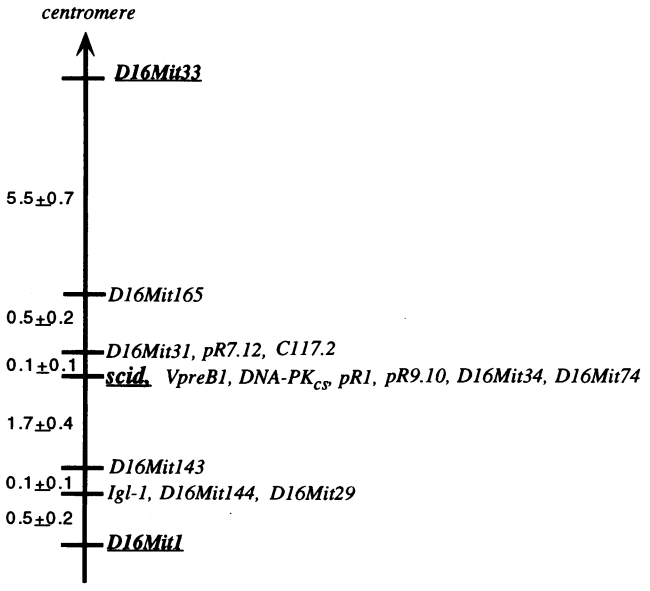

FIG. 2. Composite map of the region of mouse chromosome 16 containing the *scid* locus. Linkage distances (on the right) are expressed in centimorgans ± SE determined by using the backcross statistics function of the MAPMANAGER program version 2.5 (43). Loci on the same line are additional markers that have not been separated by recombination from those loci shown in Fig. 1. All 958 backcross progeny were typed for the loci shown in boldface letters and underlined.

recombinant backcross progeny showed that the restriction fragment-length polymorphism detected by clone 1 segregated with the SCID phenotype without exception (Fig. 1). A representative subset of the recombinant backcross progeny and parental alleles is shown in Fig. 3. The upper 95% confidence limit on the observed recombination frequency of zero is 0.3%.

In an effort to positionally clone the *scid* locus, we identified a contiguous set of YAC clones from several mouse DNA libraries (37–40) that span the region extending from *D16Mit31* to *VpreB1* (unpublished data). A single backcross progeny with a recombination between *D16Mit31* and *VpreB1* places *D16Mit31* proximal to *VpreB1* (Fig. 2) and gives orientation to the YAC contig (a contiguous sequence of DNA assembled from overlapping cloned DNA fragments). Interestingly, none of the DNA-PK_{cs} cDNA clones described (clones 1, 8, and 13 in ref. 24) hybridize to any of the YACs that make up this contig (data not shown). While there is no recombination between *VpreB1* and the gene for DNA-PK_{cs}, the latter must be distal to *VpreB1*. This places the mouse gene for DNA-PK_{cs} between *VpreB1* and *Igl-1*, both of which map to human chromosome 22. In a previous paper, we speculated that the human homologue of *scid* would likely map to chromosome 22 because of tight linkage to *VpreB1* and *λ5* (human homologue is *IGLL*) (42). However, human DNA-PK_{cs} maps to chromosome 8q11, showing a break in the conserved linkage between human chromosome 22 and mouse chromosome 16 (45). This is consistent with the report of the location of the gene for CCAAT-enhancing binding protein delta (CEBPδ) near that for DNA-PK_{cs} on human 8q11 also mapping to mouse centromeric 16 (25).

In conclusion, we have demonstrated that the mouse homologue of the human gene for DNA-PK_{cs} is tightly linked to the *scid* locus in the mouse genome. These results give strong support to the hypothesis drawn from recent complementation studies that the SCID phenotype is due to a defect in DNA-PK_{cs} function. Final proof will require cloning of the mouse DNA-PK_{cs} gene and identification of the alterations in the mutant *scid* allele. To date, however, the combination of lower expression of DNA-PK_{cs} in rodent cells compared with human cells (26) and the weak hybridization of the human DNA-PK_{cs} cDNA to rodent DNA has made identification of the mouse or hamster homologue difficult. Screening of cDNA libraries has been unproductive, and chromosome walking has not yet reached the *scid* locus.

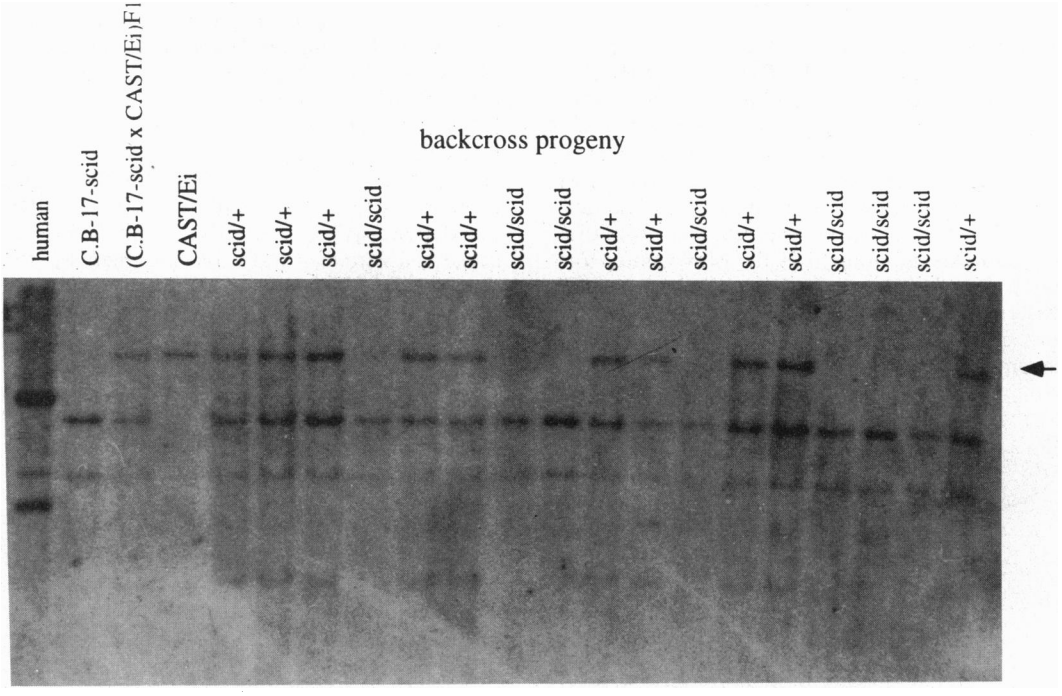

FIG. 3. The CAST/Ei restriction fragments segregate with the wild-type phenotype. Shown is an autoradiograph of a Southern blot containing DNA from human, parental mouse strains, and a representative panel of backcross progeny hybridized with clone 1. The *scid* genotype of the backcross progeny is shown above the lane. Arrow indicates the segregating 14.1-kb DNA fragment. The weakly hybridizing 3.9-kb CAST/Ei restriction fragment was not always visible and is not visible in the CAST/Ei parental lane shown in this autoradiograph.

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