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Genetics

Gene for the catalytic subunit of the human DNA-activated protein kinase maps to the site of the *XRCC7* gene on chromosome 8

[*scid*/chromosome 8q11/double-strand break repair/variable (diversity) joining recombination]

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ABSTRACT **The DNA-activated serine/threonine protein kinase (DNA-PK) is composed of a large (≈460 kDa) catalytic polypeptide (DNA-PK$_{cs}$) and Ku, a heterodimeric DNA-binding component (p70/p80) that targets DNA-PK$_{cs}$ to DNA. A 41-kbp segment of the DNA-PK$_{cs}$ gene was isolated, and a 7902-bp segment was sequenced. The sequence contains a polymorphic *Pvu* II restriction enzyme site, and comparing the sequence with that of the cDNA revealed the positions of nine exons. The DNA-PK$_{cs}$ gene was mapped to band q11 of chromosome 8 by *in situ* hybridization. This location is coincident with that of *XRCC7*, the gene that complements the DNA double-strand break repair and V(D)J recombination defects (where V is variable, D is diversity, and J is joining) of hamster V3 and murine severe combined immunodeficient (*scid*) cells.**

DNA-activated serine/threonine protein kinase (DNA-PK) is a nuclear protein kinase that is activated *in vitro* by double-stranded DNA structures (for review, see refs. 1–4). Human DNA-PK activity copurifies with a large (>300 kDa) moderately abundant catalytic polypeptide [DNA-PK$_{cs}$ (5, 6)] but a second component, Ku, that targets DNA-PK$_{cs}$ to DNA is required for the phosphorylation of most substrates (7, 8). Ku is a heterodimeric DNA-binding protein composed of two polypeptides, one of ≈70 kDa and the other of ≈80 kDa (p70/p80); it was originally identified as a human autoantigen associated with lupus and scleroderma overlap syndromes (9). Although some sequence selectivity has been reported, most evidence indicates that Ku binds to duplex DNA ends and to DNAs with single- to double-strand transitions (10–12). DNA-PK is activated by the DNA structures that bind Ku (13) and *in vitro* phosphorylates a variety of nuclear DNA-binding regulatory proteins, including the tumor suppressor protein p53 and replication factor A (for review, see refs. 1 and 2). These findings suggest roles for DNA-PK in regulating nuclear processes and cell cycle progression in response to DNA damage or changes in DNA structure (2–4). Nevertheless, the consequences of DNA-PK-mediated phosphorylations are largely unknown.

We have undertaken cloning of the DNA-PK catalytic subunit cDNA, and these efforts revealed an ≈13.4-kb sequence containing an open reading frame of >4000 codons (K.O.H., D. Gell, H. Zhang, G. C. M. Smith, N. Divecha, M. A. Connelly, A. Admon, S. P. Lees-Miller, C.W.A., and S.P.J., unpublished data). As one approach to investigating DNA-PK function, we used fragments of the DNA-PK$_{cs}$ cDNA to isolate a segment of the DNA-PK$_{cs}$ gene‖ and to map its chromosomal location. Strikingly, we find that the DNA-PK$_{cs}$ gene maps to the site of *XRCC7* (*HYRC1*), a gene that complements ionizing radiation sensitivity, the DNA double-strand break repair defect, and V(D)J recombination deficiency (where V is variable, D is diversity, and J is joining) of murine severe combined immunodeficiency (*scid*) cell lines (14–17).

MATERIALS AND METHODS

Phage, Plasmids, and DNA Sequence Analysis. Thirteen λ phage with inserts corresponding to portions of the DNA-PK$_{cs}$ gene were identified by screening 10^6 phage from a human genomic library (Stratagene, no. 946203) with cDNA fragments corresponding to nt ≈6000 to ≈9200 of the cDNA (K.O.H., *et al.*, unpublished data). Phage representatives of five groups with different inserts are designated λgA3, λgA6, λgA10, λgA13, and λgA15 (Fig. 1); insert fragments were subcloned into pBluescript (Stratagene). Plasmid HFBCG90 [American Type Culture Collection (ATCC) no. 78049 (18)], λ Enk1 (ATCC no. 59644), a probe for the *PENK* locus (19), and λ VC28 (ATCC no. 61056), a probe for the anonymous locus *D8S41* (20), were from the ATCC. DNA sequence was obtained for both strands with the dideoxynucleotide chain-termination method by using Sequenase version 2.0 (United States Biochemical) and oligonucleotide primers. GenBank accession no. L26524 gives the sequence of the 2636-bp insert in HFBCG90; L27425 gives the sequence of a 7902-bp segment of λgA3 (Fig. 1).

Restriction Fragment Length Polymorphism Analysis. A 1361-bp segment between the last two exons in the DNA-PK$_{cs}$ genomic sequence reported here was amplified by 35 cycles of PCR with the primers 5′-TTCAGTGCCAAGAGATCTTC-CTTC-3′ (nt 6229–6252 in sequence L27425) and 5′-CCAGTG-CTTCGCGTAAGGGC-3′ (complementary to nt 7570–7589). The segment from plasmid p282 was amplified with the same leftside primer as above and 5′-GGATGTGCTGCAAGGCG-3′ from the pBluescript multiple cloning site. To control for the completeness of digestion, amplified fragment from plasmid p282 was added to a portion of the amplification products from genomic DNAs; reactions then were digested with an excess of *Pvu* II (New England Biolabs). Human tissue culture lines were as described (1), except for AG03141A and AG07066B (from the National Institute of General Medical Sciences Human Genetic Mutant Cell Repository, Camden, NJ); DNAs from human

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Abbreviations: DNA-PK, DNA-activated protein kinase; DNA-PK$_{cs}$, catalytic polypeptide of DNK-PK; V, variable; J, joining; D, diversity; CEPH, Centre d'Etude Polymorphisme Humain.

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‖The sequences reported in this paper have been deposited in the GenBank data base (accession nos. L26524 and L27425).

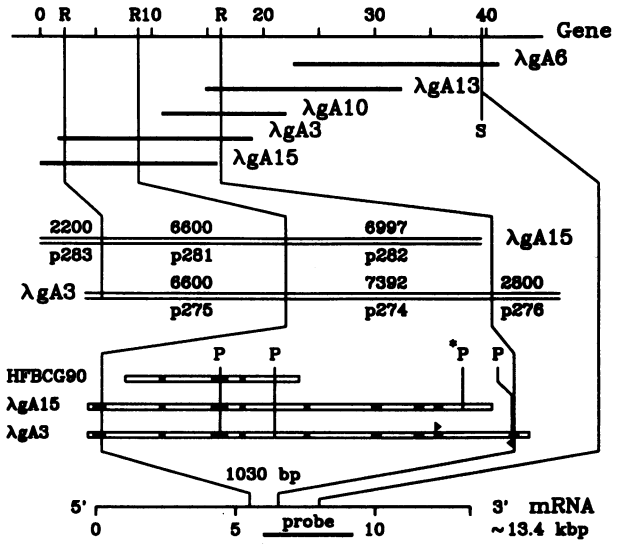

FIG. 1. Map of an ≈41-kbp segment of the DNA-PK$_{cs}$ gene. Solid lines (at the top) show the relationship and sizes of the inserts from five λ phage clones selected with probes spanning an ≈3200-bp segment of the cDNA (at the bottom). Double lines (in the center) represent the DNA-PK$_{cs}$ sequences in λ clones gA3 and gA15; the approximate sizes of *Eco*RI (R) and *Eco*RI–*Not* I restriction fragments in base pairs and the names of the corresponding subclones are indicated. Expanded representations of the sequenced 7902-bp segment (from λgA3) and corresponding segments from λgA15 and plasmid HFBCG90 are given by the lower three double lines. Exons (solid boxes), primers used for restriction fragment length polymorphism analysis (triangles), and *Pvu* II restriction sites (P) are indicated; the polymorphic *Pvu* II site (see Fig. 2) is designated *P. The 7392-bp *Eco*RI genomic fragment in subclone p274 corresponds to a 1030-bp *Eco*RI fragment in the cDNA sequence. The distance between a *Sal* I site (S) near the distal end of the genomic segment and the *Eco*RI site that is closest to the 5′ end of the gene in the sequence genomic segment is ≈30,800 bp; these sites are 2487 bp apart in the cDNA sequence.

tumor tissues were provided by M. Viola, State University of New York, Stony Brook, NY; DNAs from Centre d'Etude Polymorphisme Humain (CEPH) family founders were from O. W. McBride, National Institutes of Health, Bethesda. Mouse A9 and mouse–human hybrid cell lines containing human chromosome 7 [A9(7neo)] or 8 [A9(8neo)] (21) were from J. C. Barrett, National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Chromosomal Mapping by *in Situ* Hybridization. The position of the DNA-PK$_{cs}$ gene was determined by fluorescence *in situ* hybridization to banded metaphase chromosomes by using biotin-11-dUTP and *N*-hydroxysuccinimide- and digoxigenin-labeled probes; detection was with fluorescein isothiocyanate-conjugated avidin DCS (Vector Laboratories) and rhodamine-conjugated anti-digoxigenin antibody (22–24). Fluorescence signals were imaged separately, and gray scale images were pseudocolored and merged electronically (23).

RESULTS

Characterization of DNA-PK$_{cs}$ Genomic Clones. To begin characterizing the DNA-PK$_{cs}$ gene, we isolated genomic clones corresponding to a 3.2-kbp segment from near the center of the DNA-PK$_{cs}$ cDNA (K.O.H. *et al.*, unpublished data). Five λ phage with different inserts, based on their pattern of *Pvu* II restriction fragments, were subcloned, restriction-site-mapped, and partially sequenced. The five inserts were found to correspond to a contiguous ≈41-kbp segment of the DNA-PK$_{cs}$ gene (Fig. 1). The sequences at the ends of a 7.4-kbp *Eco*RI fragment (in plasmid p274) from phage λgA3 correspond to sequences at the ends of a 1030-bp *Eco*RI fragment from the DNA-PK$_{cs}$ cDNA. The *Eco*RI site end of the corresponding 7-kbp *Eco*RI–*Not* I fragment in plasmid p282 (from λgA15) has the same end sequence as the end of the 1030-bp cDNA fragment closest to the 5′ end of the mRNA. The sequence at the *Eco*RI site end of the 2.8-kbp *Eco*RI–*Not* I fragment in plasmid p276 is identical to sequence immediately after the distal *Eco*RI site creating the 1030-bp cDNA fragment. One end of the 6.6-kbp *Eco*RI fragment from each λ phage is identical to the cDNA sequence immediately before the proximal *Eco*RI site of the 1030-bp cDNA fragment. This information revealed the relative positions and orientations of the restriction fragments in the subclones of λgA3 and λgA15 (Fig. 1).

Next, both strands of the 7.4-kbp *Eco*RI fragment in plasmid p274 and the corresponding 7-kbp *Eco*RI–*Not* I fragment in plasmid p282 were sequenced. Over corresponding segments, both sequences are identical except for 1 nt (nt 6721 in the sequence L27425) that creates a *Pvu* II site in the λgA15 DNA that is not present in λgA3. The insert in the λgA15 DNA extends 1.6 kbp further toward the 5′ end of the DNA-PK$_{cs}$ gene than the insert in λgA3, while the insert in this phage extends 2.8 kb further into the distal part of the gene (Fig. 1).

A search of the GenBank database (release 79) revealed identity with two expressed sequence tags from clone HFBCG90, a putative cDNA clone containing a 2.6-kb insert (18). The two expressed sequence tags matched sequences 2.6 kb apart on opposite strands in our genomic sequence. That the HFBCG90 insert represents a genomic rather than a cDNA fragment was confirmed by DNA sequencing.

The sequenced DNA-PK$_{cs}$ segment is 40.6% G+C and contains 84 CpG sites. Comparison of the genomic DNA sequence with the DNA-PK$_{cs}$ cDNA sequence revealed that the 1030-bp *Eco*RI cDNA sequence is composed of nine exons (Table 1). The exons bisected by the *Eco*RI sites extend 151 bp upstream and 67 bp downstream of these sites, respectively.

A Polymorphic *Pvu* II Site Uniquely Present in λgA15. To determine the prevalence in the human population of the

Table 1. Intron–exon structure of an 8-kbp segment of the human DNA-PK$_{cs}$ gene

5′ Border			Exon size,	3′ Border			Intron size,
Intron|Exon		Exon	bp	Exon|Intron		Intron	bp
-(C/T)AG|NNN-			≈137* exon	-(A/C)AG|GT(A/G)-			1127*
Gene-cDNA†				cDNA-Gene†			
98|5464				5671|307			
-TTAĠ|ĠGGT-		E-1	208	-AAAĠ|ĠTAT-		I-1	978
1284|5672				5850|1464			
-GAAĠ|ĊTAA-		E-2	179	-TTAȦ|ĠTAA-		I-2	857
2320|5851				6019|2490			
-GCAĠ|ȦTTG-		E-3	169	-AAAĠ|ĠTAG-		I-3	254
2734|6020				6085|2801			
-ACAĠ|ȦACT-		E-4	66	-TGAĠ|ĠTGA-		I-4	1090
3890|6086				6158|3964			
-ATAĠ|ĠTTC-		E-5	73	-TCAĠ|ĠTAG-		I-5	1139
5102|6159				6307|5252			
-TCAĠ|ȦTGG-		E6	149	-ACGĠ|ĠTGA-		I-6	624
5875|6268				6445|6014			
-GCAĠ|ĠAGC-		E-7	138	-AGAĠ|ĠTGC-		I-7	213
6226|6446				6565|6347			
-TTAĠ|ĠATT-		E-8	120	-AGAĠ|ĠTAA-		I-8	1216
7562|6566				6709|7707			
-GTAĠ|ĠTCT-		E-9	144	-AACȦ|ĠTAA-		I-9	?

*Average for vertebrates (25).

†The base-pair positions at the intron–exon borders are given for the gene segment in GenBank accession no. L27425 and for the positions in the cDNA with respect to its presumptive 5′ end. Dots indicate numbered base.

nucleotide difference in the corresponding segments of plasmids p274 and p282, a 1361-bp segment of the DNA-PK$_{cs}$ gene containing this site was amplified from several tissue culture lines by PCR and analyzed for the presence of the *Pvu* II site (Fig. 2). DNAs from HeLa-S3, T98G, HUT-102, HUT-78, AG03131A, and Raji cells were completely cleaved by *Pvu* II, indicating that these cells are homozygous (+/+) for the *Pvu* II site allele, whereas half the DNA was cleaved in the segments from another six cell lines, WI-38, HPB-ALL, MOLT-3, CCRF-SB, A549, and AG07066B, indicating that these cells are heterozygous for the *Pvu* II site allele (+/−). Importantly, the 1361-bp fragment was amplified from a mouse hybrid cell line containing human chromosome 8, and this fragment was cleaved by *Pvu* II; in contrast, the parental mouse A9 cell line and another A9 hybrid line containing human chromosome 7 did not yield a comparable fragment (Fig. 2), although a slightly faster migrating pair of bands that might represent the corresponding murine sequence was amplified. These data confirm the location of the DNA-PK$_{cs}$ gene on human chromosome 8 (see below). Analysis of DNA from 12 tumors showed that 6 were homozygous for the *Pvu* II site (+/+), 4 were heterozygous (+/−), and 2 completely lacked the *Pvu* II site (−/−). Analysis of 27 CEPH family founders (Table 2) showed that 12 are homozygous for the + allele, 12 are heterozygous, and 3 lack the site. Thus, the *Pvu* II + allele is present in two-thirds (53 of the 78) of chromosome 8 samples from the 12 tumors and the 27 CEPH family founders examined.

The DNA-PK$_{cs}$ Gene Maps to the Pericentric Region of Human Chromosome 8. We used two fragments corresponding to 3.2 kbp of the DNA-PK$_{cs}$ cDNA (the same fragments used to isolate the genomic clones described above) to locate the gene by fluorescence *in situ* hybridization. The fragments were labeled with biotin by nick translation and hybridized with metaphase chromosomes simultaneously with a digoxigenin-labeled probe specific for the *Alu* family of repeats (24). Analysis of 10 images localized the DNA-PK$_{cs}$ probe to the pericentric region of chromosome 8 in band q11 (Fig. 3 *A* and *B*). This position was confirmed using as probes plasmids p281 and p282, spanning ≈14 kb of the DNA-PK$_{cs}$ gene. Chromosome length measurements (23) from 25 images localized the DNA-PK$_{cs}$ gene between 32 and 38% from 8pter (the end of the short arm), which corresponds to cytogenetic region 8q11.

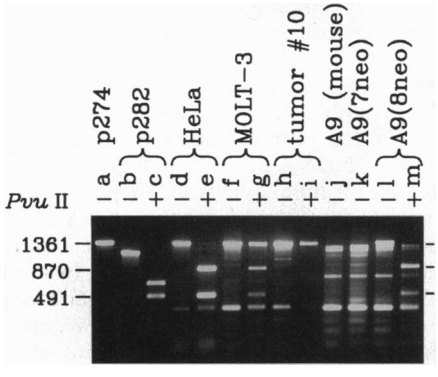

FIG. 2. Analysis of genomic DNAs for a *Pvu* II site polymorphism. The segment between two DNA-PK$_{cs}$ gene exons was amplified by PCR, and the *Pvu* II digestion products were separated by agarose (1.4%) gel electrophoresis and visualized with ethidium bromide. When the *Pvu* II site was present, digestion of the 1361-bp segment gave 870- and 491-bp fragments. Representative amplifications and digestions are as follows. Lanes: a, plasmid p274 control (no site), undigested; b and c, plasmid p282 control (*Pvu* II site present), undigested and digested, respectively; d and e, HeLa DNA undigested and digested; f and g, MOLT-3 DNA undigested and digested; h and i, DNA from a human tumor (no. 10) undigested and digested; j, mouse A9 cell DNA undigested; k, mouse hybrid A9(7neo) DNA undigested; l and m, mouse hybrid A9(8neo) DNA undigested and digested. An ≈400-bp fragment of unknown origin was amplified from both human and mouse DNAs. DNAs from human tumors and CEPH family founders were analyzed in a similar manner (see text). For simplicity, the digestion control fragment was not added to the reactions shown in this figure.

Table 2. Presence of the DNA-PK$_{cs}$ gene polymorphic *Pvu* II site in CEPH family founders

Pvu II site	CEPH family code
+/+ homozygous	133101, 133102, 134001, 134002, 134102, 134501, 134602, 136201, 136202, 137701, 137702, 140802
+/− heterozygous	133301, 133302, 133402, 134101, 134502, 134601, 134701, 134901, 135001, 135002, 140801, 141301
−/− homozygous	133202, 134702, 134902

Presence of the *Pvu* II site was determined as described in Fig. 2. The first four digits of the code are the CEPH family number; 01 as the last two digits indicates the father, and 02 is the mother.

To verify the map position of the DNA-PK$_{cs}$ gene, we hybridized genomic probes to metaphase chromosome spreads simultaneously with digoxigenin-labeled probes from either of two λ phage with previously mapped genomic inserts (Fig. 3 *C* and *D*). The DNA-PK$_{cs}$ gene is proximal to the proenkephalin gene *PENK* [map position 8q11–12 (26)] with respect to the centromere (Fig. 3*C*). DNA-PK$_{cs}$ and *D8S41*, an anonymous marker mapped to 8p12–q11.23, are essentially coincident (Fig. 4).

DISCUSSION

Five overlapping λ genomic clones that span a 41-kbp segment of the DNA-PK$_{cs}$ gene were isolated, and an 8-kbp portion of this segment was sequenced and found to contain nine exons and eight introns. The exon sequences match exactly the corresponding cDNA sequence, validating the accuracy of this 1248-bp cDNA segment (the 1030-bp fragment plus the ends of the first and ninth exons). The predicted intron–exon boundaries fit well with the GT–AG rule for splice site selection (25) except for the last exon–intron border, which has CA instead of AG at the exon donor site (Table 1). The average length of the nine exons is 139 bp while that of the eight introns is 795 bp; these values are close to averages for exons and introns from vertebrates, 137 bp and 1127 bp, respectively (25). An exon also was identified at a *Sal* I site near the 3′ end of the 41-kb genomic segment (Fig. 1); by using this exon as the 3′ reference point, the intron/exon length ratio is 12.4. The DNA-PK$_{cs}$ mRNA is 13.4 kb (K.O.H. *et al.*, unpublished data); thus, extrapolation suggests a gene size of 110–180 kbp and an exon content approaching 100. DNA-PK$_{cs}$ is one of the largest protein kinase catalytic polypeptides yet identified; to our knowledge, twitchin and titin, two muscle-specific polypeptides with kinase catalytic domains, are larger (27).

The DNA-PK$_{cs}$ gene was mapped to chromosome 8 band q11 by using probes derived from both a 3.2-kbp cDNA segment and genomic probes spanning a 14-kbp portion of the corresponding gene segment. The fact that hybridization was observed only with the pericentric region of chromosome 8 suggests that closely related sequences are not present elsewhere in the genome. Genes previously mapped to this region include *MOS*, the gene for a serine/threonine kinase related to the Moloney murine sarcoma viral oncogene product (28); *CEBPD*, the gene for the CCAAT/enhancer-binding transcription factor C/EBP-δ (29); and *XRCC7* (*HYRC1*), a human gene that partially complements the deficiencies of rodent cells from ionizing radiation group 7 (14).

Four of nine ionizing radiation sensitivity complementation groups of rodent cells are defective or deficient in DNA double-strand break repair (30). Three of these four—*XRCC4*

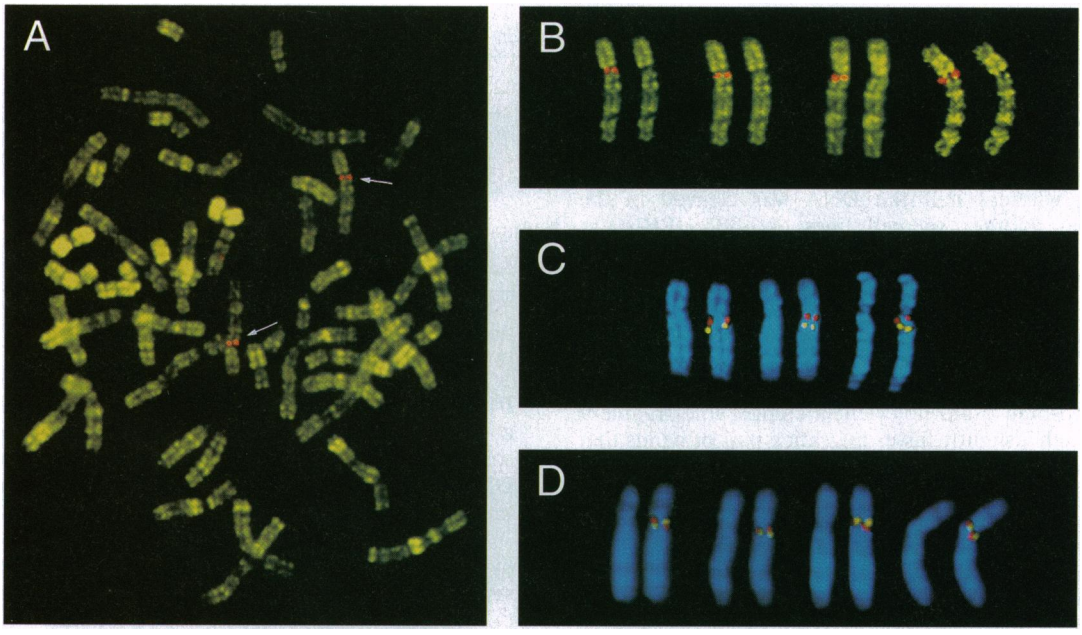

FIG. 3. Chromosomal localization of the DNA-PK$_{cs}$ gene by fluorescence *in situ* hybridization. (*A*) Metaphase spread after cohybridization with biotin-labeled plasmid cDNA clones corresponding to nt ≈6000–9200 of the DNA-PK$_{cs}$ mRNA (pseudocolored red) and a digoxigenin-labeled 48-bp oligonucleotide corresponding to the *Alu* repeat consensus sequence (pseudocolored yellow). (*B*) A montage of chromosome 8 labeled as in *A*; pairs are the same image with and without the gene-specific label. (*C*) A montage of chromosome 8 displaying digoxigenin-labeled *Enk1* (*PENK*) (pseudocolored yellow) and biotin-labeled pooled plasmids p281 and p282 (pseudocolored red). (*D*) A montage of chromosome 8 displaying digoxigenin-labeled VC28 (*D8S41*) (pseudocolored yellow) and biotin-labeled p281 and p282 (pseudocolored red).

(x-ray cross-complementing group 4), *XRCC5*, and *XRCC7* (*scid*)—also are defective in V(D)J recombination, thus linking recombination and DNA double-strand break repair in mammalian cells. The genes for the two subunits of Ku were mapped to chromosome 2 (p80) and chromosome 22 (p70) (31), and the Ku p80 polypeptide was shown to be encoded by *XRCC5*, one of the genes required for DNA double-strand break repair and V(D)J recombination (32, 33). This finding suggests that a mutation that inactivated or prevented the expression of DNA-PK$_{cs}$ might have a similar phenotype.

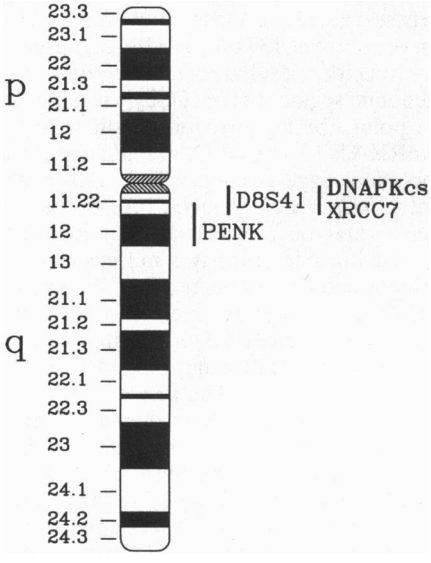

FIG. 4. Ideogram of human chromosome 8. The cytological banding pattern of human chromosome 8 and the approximate locations (vertical lines) of the DNA-PK$_{cs}$ gene, *D8S41* (as determined by this study), *PENK* (26), and *XRCC7* (*HYDRC1*) (14) are shown. The Genome Database designation for the DNA-PK$_{cs}$ gene is *PRKDC* (protein kinase, DNA-activated, catalytic subunit).

The q11 region of human chromosome 8 has not been associated with a human disease, nor does the region contain a known locus for radiation sensitivity; however, chromosome 8 partially corrects the ionizing radiation sensitivity and V(D)J recombination deficiency of cells from the *scid* mouse (15, 16), and the responsible human gene was localized to band q11 (14). The *scid* mutation prevents normal B- and T-cell development, presumably by inactivating or preventing expression of a component(s) required to resolve DNA structures produced during V(D)J recombination (34, 35), and this component also is required for repairing DNA double-strand breaks (36–39). DNA-PK is activated by DNA ends and structures with single- to double-strand transitions (13), precisely the structures generated during recombination and by ionizing radiation. Although DNA-PK is 50–100 times less abundant in rodent cells than in human cells (1), a peptide-based "pull-down" assay was used to show that DNA-PK activity is deficient in extracts of hamster xrs-6 (*XRCC5* defective) and V3 cells, and in murine *scid* cells (17, 40). In the latter two cases, the absence of activity is due to a specific deficiency in DNA-PK$_{cs}$ (17). Furthermore, activity was restored by yeast artificial chromosomes containing the entire human DNA-PK$_{cs}$ gene. Although the *scid* mutation has not been identified, our estimate for the size of the DNA-PK$_{cs}$ gene suggests that not many other genes can be present on the complementing yeast artificial chromosomes. Thus, these findings imply that DNA-PK$_{cs}$ is the product of *XRCC7* and suggest that the murine *scid* and hamster V3 mutations lie within the DNA-PK$_{cs}$ gene.

The inability to develop functional B and T cells is a severe affliction that may explain why defects in DNA-PK$_{cs}$ have not been linked with a human disease or the q11 region of chromosome 8. We do not yet know what role DNA-PK plays in DNA double-strand break repair and V(D)J recombination, but at least three can be imagined. (*i*) DNA-PK could be directly involved in regulating the activities required to rejoin separated DNA strands, and a need to interact with an extended complex containing both DNA ends might account for the large size of the DNA-PK$_{cs}$ polypeptide. (*ii*) DNA-PK

may inhibit nuclear activities that might interfere with DNA double-strand break repair or the resolution of recombination structures. Consistent with the latter idea, DNA-PK recently was found to inhibit RNA polymerase I transcription from linear templates (41). DNA-PK also is capable of phosphorylating the C-terminal domain of the large subunit of RNA polymerase II *in vitro* (42) and might similarly act to block RNA polymerase II-mediated transcription. Furthermore, DNA-PK hyperphosphorylates replication protein A (43), and a similar hyperphosphorylation is observed after treating cells with DNA damage-inducing agents (44, 45). (*iii*) DNA double-strand breaks induce the accumulation of p53, which leads to an arrest of cell cycle progression in late G_1 phase (46). The upstream factors that detect DNA breaks and signal the accumulation of p53 have not been identified, but DNA-PK could be involved. Consistent with this possibility, DNA-PK phosphorylates Ser-15, a site conserved among mammalian p53 proteins (47), and changing this site to alanine increases the half-life of the mutant p53 (48). It is not known, however, whether phosphorylation at this site contributes to the transient stabilization of p53 that occurs after DNA damage. Furthermore, there may be insufficient DNA-PK in rodent cells to rapidly produce a checkpoint signal in response to DNA strand breaks. A need to detect DNA breaks rapidly may have provided the selective force that led to the significantly higher levels of DNA-PK expression seen in somatic cells of longer lived primates.

Note. Kirchgessner *et al.* (49) and Peterson *et al.* (50) also recently reported that DNA-PK_{cs} expression is reduced in murine *scid* cells and that expression is restored by DNA from the centrimeric region of human chromosome 8.

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