tained the Xba I linker followed by sequences corresponding to the NH_2- and COOH-terminal sequences of ARF6, respectively. ARF1 cDNA was amplified with primers 5′-GCTCTAGAATGGGGAACATCTTCGCG-3′ and 5′-GCTCTAGACTACTTCTGGTTCCGGAGC-3′, which contained the Xba I linker followed by NH_2-terminal and COOH-terminal sequences of ARF1, respectively. The amplified ARF cDNAs were digested with the restriction endonuclease Xba I (Life Technologies), followed by ligation into the Xba I site of pToto10003′2J, which was previously linearized by Xba I digestion and treated with calf alkaline phosphatase. The correct sequence and orientation of the insert were confirmed by DNA sequencing. The pToto10003′2J-ARF constructs were used for recombinant virus production as described (*18*). The recombinant viruses were called SIN:ARF6 and SIN:ARF1 and were stored in aliquots at −70°C and were thawed just before use. The virus stock derived from the Sindbis vector pToto10003′2J (SIN) served as a negative control for the described experiments. The substitution mutations ARF6(T27N) and ARF6(Q67L) were created by oligonucleotide-directed mutagenesis with use of the Bio-Rad phagemid mutagenesis system that was based on Kunkel's method of mutagenesis (*19*). The cDNA sequences of ARF6 were cloned into the Xba I site of the pGC2 plasmid (*20*) as described above for pToto10003′2J. Because pGC2 contains the M13 phage replication origin, single-stranded DNA templates were produced with the use of the helper phage M13K07 and were used as templates for mutagenesis. The oligonucleotides 5′-GATCTTGTCCAGGCCGCCCACCATC-3′ and 5′-GTACAGGATTGTGTTCTTGCCGCGCG-3′ were used to generate mutations ARF6(Q67L) and ARF6(T27N), respectively. The mutations were verified by DNA sequencing. The amplified mutant cDNAs were digested with Xba I and inserted into the Sindbis vector pToto10003′2J. The plasmids were subsequently used for recombinant virus production as described above.

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22. To determine cell surface distribution of Tfn-Rs, we incubated cells with serum-free Ham's F-12 media (pH 7.4) containing 0.2% bovine serum albumin (BSA) for 1 hour to deplete cells of endogenous Tfn. Iron-loaded human Tfn was labeled with ^{125}I as described (*21*). Binding of [^{125}I]Tfn to the cell surface was conducted at 4°C for 90 min in serum-free Ham's F-12 media (pH 7.4) containing 0.2% BSA and a saturating concentration of [^{125}I]Tfn (6 μg/ml). Unbound Tfn was removed by four washes with phosphate-buffered saline (PBS) containing 0.2% BSA. Cells were scraped from the dishes and counted for radioactivity. Nonspecific binding was determined by including unlabeled Tfn (100-fold excess) and accounted for <10% of the total Tfn bound.
23. A 12–amino acid peptide, CKLTWLTSNKYS, close to the COOH-terminal end of ARF6 was coupled to rabbit serum albumin (RSA) and injected into rabbits for polyclonal antibody production (Cocalico Biologicals). Abbreviations for the amino acid residues are C, Cys; K, Lys; L, Leu; N, Asn; S, Ser; T, Thr; W, Trp; and Y, Tyr. The antiserum generated was purified by sequential chromatography on columns of activated CNBr-sepharose 4B (Pharmacia) coupled to the ARF6 peptide–RSA conjugate and protein A sepharose (Sigma).
24. Viral infection was conducted at a multiplicity of infection of 50 plaque-forming units per cell in 200 μl of PBS containing 1% fetal bovine serum (FBS). Virus adsorption was conducted at 4°C for 1 hour. The infection mixtures were replaced by 3 ml of Ham's F-12 medium (Gibco-BRL), supplemented with 5% FBS (Hyclone) and incubated at 37°C for the appropriate time periods as indicated (*18*). More than 90% of the cells were infected when the infections were done on plastic 35-*mm* tissue culture dishes. When infections were done on glass cover slips, 40 to 65% of the cell population was infected.
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26. After depletion of endogenous Tfn, binding of [^{125}I]Tfn to the cell surface was conducted as described (*22*); this was followed by four washes with PBS to remove unbound ligand. The uptake was initiated by adding prewarmed Ham's F-12 medium (pH 7.4) containing 0.2% BSA and a 100-fold excess of cold Tfn, and cells were incubated at 37°C for different times as indicated in Fig. 4. At each time point the medium was collected and the cell monolayers were washed once with PBS containing 0.2% BSA. The medium and the wash were combined and the radioactivity was determined (recycled Tfn). The concentration of cell surface–bound [^{125}I]Tfn was determined by acid stripping. Cell monolayers were washed twice with ice-cold 0.5% acetic acid and 0.5 M NaCl (pH 3.0), followed by one wash with PBS. The washes were pooled and measured for radioactivity (surface-bound Tfn). Finally, the cells were solubilized with PBS containing 1% Triton X-100 and 0.1% NaOH, and the radioactivity in the lysates was determined (intracellular Tfn). More than 90% of the radioactivity released was precipitable by trichloroacetic acid.

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DNA-Dependent Kinase (p350) as a Candidate Gene for the Murine SCID Defect

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Severe combined immunodeficient (SCID) mice are deficient in a recombination process utilized in both DNA double-strand break repair and in V(D)J recombination. The phenotype of these mice involves both cellular hypersensitivity to ionizing radiation and a lack of B and T cell immunity. The catalytic subunit of DNA-dependent protein kinase, p350, was identified as a strong candidate for the murine gene *SCID*. Both p350 and a gene complementing the SCID defect colocalize to human chromosome 8q11. Chromosomal fragments expressing p350 complement the SCID phenotype, and p350 protein levels are greatly reduced in cells derived from SCID mice compared to cells from wild-type mice.

DNA repair systems are essential in maintaining the structural integrity of genes. Unrepaired DNA damage may result in far-reaching consequences such as mutagenesis, genomic instability, tumorigenesis, and cell death. Cells have evolved distinct DNA repair pathways to cope with particular DNA lesions, and most of these pathways show conservation between lower and higher eukaryotes, such as between yeast and humans. However, DNA double-strand breaks, which confer the highest potential for genomic instability and cell death, appear to be repaired in mammalian cells by a unique pathway. The mouse mutant SCID (*1*) is the only known animal model with a deficiency in this recombination pathway. This pathway functions in V(D)J recombination, the process of assembling the immunoglobulin and T cell receptor genes from gene segments by site-specific recombination, and in DNA repair. The SCID defect in immature lymphocytes appears to be in one of the final steps of V(D)J recombination, the joining of the free DNA ends of the coding strands, whereas joining of the recombination signal sequences is relatively normal (*2*). SCID mice lack both B and T cell immunity as the result of their deficiency in V(D)J recombination, and are sensitive to ionizing radiation in all cell types, because of their deficiency in DNA double-strand break repair (*3*).

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In addition to the SCID mouse, several mutant rodent cell lines have been identified that share this dual dysfunction in DNA double-strand break repair and V(D)J recombination (*4*). Three complementation groups have been established and three human chromosomes have been identified that complement the radiosensitive phenotype. Complementation group XRCC7, represented by SCID, is complemented by human chromosome 8 (*5*, *6*); XRCC5, which includes the hamster cell lines *xrs* is complemented by human chromosome 2 (*7*); and XRCC4, which includes the hamster cell line XR-1, is complemented by human chromosome 5 (*8*).

Attempts to clone the human gene that can complement SCID by transfection of genomic fragments or complementary DNA (cDNA) libraries into mouse SCID cells have been unsuccessful. We chose the genetic approach of positional cloning, a method that is independent of the size of the gene involved. The basis of positional cloning is the analysis of the segregation through families of an inherited trait with an anonymous DNA marker. In lieu of pedigree analysis, we created "offspring" of our SCID cell line containing human chromosome 8 (SCID/hu8) by creating a panel of radiation-reduced hybrids (*9*). SCID/hu8 cells were lethally irradiated with 50 or 100 Gy of γ irradiation thereby breaking the chromosomes into many small fragments. The irradiated cells were then fused back to the radiosensitive parental SCID cell line. Hybrids were selected for the retention of the human chromosome 8 fragment containing the gene complementing the SCID defect by continuous exposure to ionizing irradiation at a low dose rate (6 cGy per hour for a total dose of 15 Gy). Recipient, radiosensitive SCID cells do not survive under these conditions (*5*). Another set of hybrids were selected for retention of a neomycin resistance gene carried on human chromosome 8. Because neomycin-resistant clones need not retain the SCID-complementing fragment of chromosome 8, the final hybrid panel contained both radiosensitive and radioresistant cell lines. After the initial round of selection, all hybrids were characterized as sensitive or resistant based on clonogenic survival after exposure to an acute dose of 8 Gy; our panel contained 24 hybrids, of which 16 were resistant and 8 were sensitive to ionizing radiation. After completion of the mapping studies all cell lines in the hybrid panel were tested again for radiation sensitivity. One cell line was identified that had reverted from a radioresistant phenotype (100D) to a radiosensitive

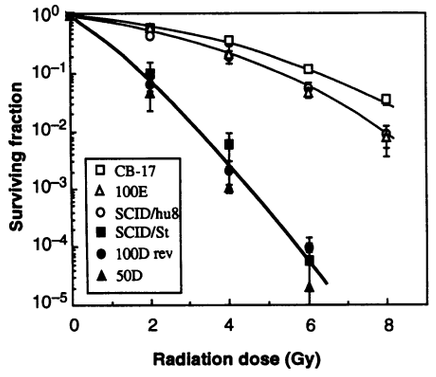

Fig. 1. Survival of SCID, SCID/hybrids and C.B-17 (the wild-type SCID-congenic parental strain) after exposure to ionizing radiation. Exponentially growing cells were irradiated with ^{137}Cs γ-rays (2.95 Gy/min). After 10 days surviving colonies were stained with crystal violet and counted. Surviving fractions were calculated from two independent experiments consisting of three plates per radiation dose. Open symbols, radioresistant lines; closed symbols, radiosensitive lines.

Table 1. Wild-type V(D)J recombination is restored in the radioresistant hybrid 100E and not in the radiosensitive 50D. The frequency of signal (pJH200) and coding (pJH290) junction formation was measured as described (*10*, *11*). Each cell line indicated was transiently transfected with expression vectors containing recombination activating genes *RAG1* and *RAG2* to induce V(D)J recombination and with one of two reporter substrates (pJH290 or pJH200). After transfection into mammalian cells, plasmids were introduced into *E. coli* and selected with ampicillin or ampicillin plus chloramphenicol. All substrates confer ampicillin resistance (Amp^R), whereas chloramphenicol resistance (Cam^R) results from V(D)J recombination of the substrate.

Cell line	Substrate	Amp^R colonies $\times 10^4$	Cam^R Amp^R colonies	Recombination frequency*
C.B-17	pJH290	110	12,000	1.0
	pJH200	37	5,900	1.4
SCID/st	pJH290	18	6	0.001
	pJH200	13	200	0.13
100E	pJH290	190	11,000	0.52
	pJH200	67	6,800	0.75
50D	pJH290	20	24	0.002
	pJH200	20	870	0.30

*Proper V(D)J joining of the doubly resistant (Cam^R Amp^R) colonies was confirmed by oligonucleotide hybridization (*11*, *12*). Recombination frequency represents the corrected number of doubly resistant colonies divided by the total number of plasmid-containing colonies (Amp^R colonies). Transfections of each reporter plasmid in the absence of RAG expression gave recombination frequencies of <0.002% in all cell lines tested. The average of duplicate transfections is presented. The results were confirmed in a second set of independent transfections.

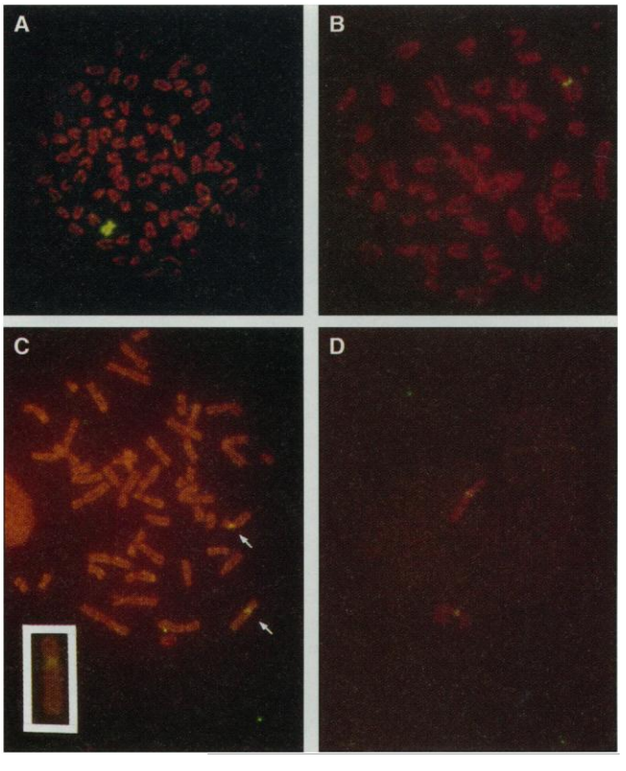

Fig. 2. FISH analysis. (**A** and **B**) SCID hybrid metaphase spreads were hybridized with biotinylated total human DNA and detected with fluorosceinated avidin. The remaining chromosomes were counterstained with propidium iodide. (**A**) SCID/hu8 hybrid, containing an intact human chromosome 8. (**B**) 100E hybrid, containing one small fragment of human chromosome 8. (**C** and **D**) Metaphase spreads from normal human diploid fibroblast cells. (**C**) Hybridization with total DNA from 100E. (**D**) Total biotinylated DNA from yeast carrying the YAC 943g4 which hybridized to 8q11.1–8q11.2. Chromosome 8 is identified by hybridization with a chromosome 8 specific probe that had been labeled with the fluorochrome Spectrum Orange (Imagenetics). Magnification was ×800 except for the inset on (C) (×1600).

phenotype (100Drev). Survival curves for representative cell lines are shown (Fig. 1).

Complementation of the SCID mutation also resulted in the restoration of wild-type levels of V(D)J recombination. Signal and coding junction formation was determined by transfection of V(D)J recombination substrates into fibroblasts from SCID, wild-type C.B-17, the radioresistant hybrid 100E, and the radiosensitive hybrid 50D (Table 1) (*10–12*). As expected, SCID fibroblasts had a vastly decreased frequency (1000-fold) of coding junction formation, measured by recombination of the pJH290 reporter plasmid. In 100E coding junctions were formed at frequencies within the range of wild-type cells (*4, 10*). The frequencies of coding junction formation in the radiosensitive hybrid 50D remained as low as in the SCID cells. There was little variation in the formation of signal junctions in the reporter plasmid pJH200. These results confirm that V(D)J recombination proficiency was restored in parallel with radiation resistance, and strongly suggest that both defects are complemented by the same gene.

Fluorescent in situ hybridization (FISH) was used to characterize the number and size of human chromosome 8 fragments present in the hybrids (*13*). The SCID/hu8 cell line (which contains a single, intact chromosome 8) and hybrid 100E (which contains a small fragment of chromosome 8) are shown (Fig. 2, A and B). To identify the region of origin of the human DNA fragments retaining *SCID*, we hybridized total biotinylated DNA from 100E, to human metaphase spreads (Fig. 2C). As the majority of 100E is murine DNA, which does not hybridize specifically, the background on this slide is increased. However, a clear signal on the pericentromeric region of chromosome 8 can be seen defining the minimal region of chromosome 8 in the 100E hybrid that contains a gene complementing the SCID defect. This localization confirms previous studies (*5, 6*). We subsequently established a more precise minimal region containing *SCID* by polymerase chain reaction (PCR) analysis for the presence or absence of anonymous markers and genes known to map to this region 8 (Table 2). The order of markers was established either by the Weissenbach map (*14*) or from the human chromosome 8 mapping consortium (*15*). Three anonymous markers (D8S531, D8S519, and D8S359) were present in all radioresistant hybrids, but in none of the sensitive hybrids. D8S531 is the marker closest to the centromere on the long arm of chromosome 8. Centromeric repeats (*16*) could be identified in all radioresistant hybrids, and in two radiosensitive hybrids (50G and 100L), thus mapping *SCID* to the long arm of chromosome 8. D8S589, the

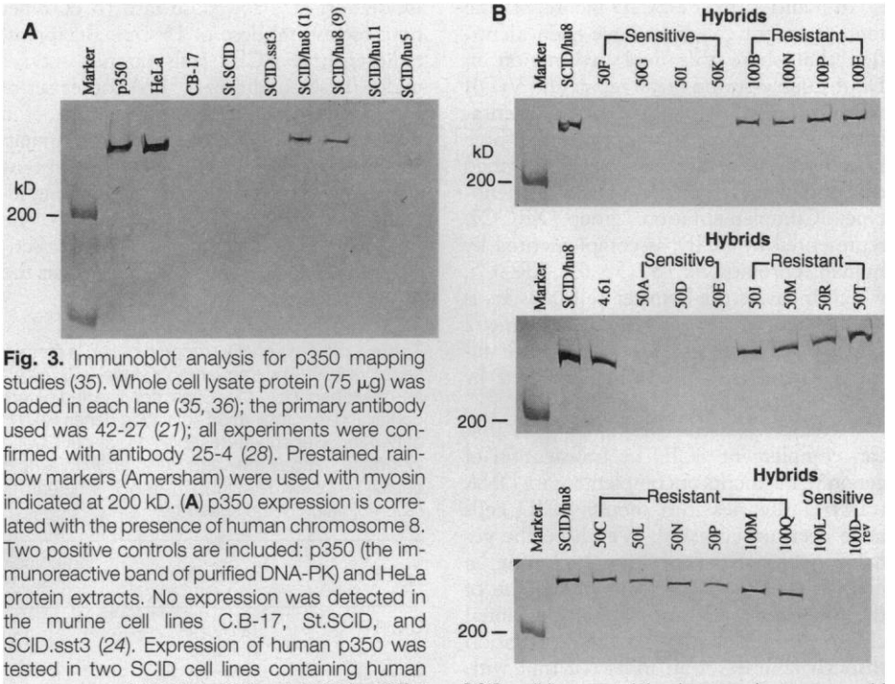

Fig. 3. Immunoblot analysis for p350 mapping studies (*35*). Whole cell lysate protein (75 μg) was loaded in each lane (*35, 36*); the primary antibody used was 42-27 (*21*); all experiments were confirmed with antibody 25-4 (*28*). Prestained rainbow markers (Amersham) were used with myosin indicated at 200 kD. (**A**) p350 expression is correlated with the presence of human chromosome 8. Two positive controls are included: p350 (the immunoreactive band of purified DNA-PK) and HeLa protein extracts. No expression was detected in the murine cell lines C.B-17, St.SCID, and SCID.sst3 (*24*). Expression of human p350 was tested in two SCID cell lines containing human chromosome 8 [SCID/hu8(1) and SCID/hu8(9)]; in a SCID cell line containing human chromosome 11 (SCID/hu11) and in a SCID cell line containing human chromosome 5 (SCID/hu5). (**B**) Mapping p350 expression through the panel of radiation hybrids. The sensitivity or resistance of all radiation hybrids to acute exposure of ionizing radiation is indicated. SCID/hu8 represents the SCID cell line containing human chromosome 8 as positive control. The radioresistant SCID/human cell hybrid 4.61, retains parts of chromosome 8 (*5*). The 100Drev represents the radiosensitive revertant of 100D.

Table 2. PCR analysis for the presence or absence of anonymous markers and genes on chromosome 8 (Chr. 8). Primers specific for chromosome 8 loci were used to verify the presence of a specific chromosome region in the radiation hybrids (*39*). The first two columns show the marker profiles for 100D reverted to the radiosensitive phenotype 100Drev. The loss of markers accompanying reversion is circled. Presence or absence of markers are represented by "+" or "−"; "nd" signifies that this marker was not determined in this cell line. Each marker was tested for non-amplification of murine SCID DNA and each experiment included SCID DNA as a negative control and SCID/hu8 DNA as a positive control. In addition, each reaction was performed at least twice for verification. The genetic distance of these loci are given according to the nomenclature of the Weissenbach map (*14*), and rely on the Weissenbach map or an integrated chromosome 8 map (*15*). Human chromosome 8 spans 159 cM and measurement is started at the tip of the short arm (*14*). Loci outside the region of interest (LPL on the distal short arm and D8S88 and D8S200 on the long arm of chromosome 8) are separated by double horizontal lines. CEN stands for centromeric repeats, and all anonymous markers are listed by their D8S numbers.

				Resistant hybrids																	Sensitive hybrids							
					100									50							50						100	
Chr. 8	Marker	Distance (cM)	D rev	D	S	B	E	M	N	P	Q	C	J	L	M	N	P	R	T	A	D	E	F	G	I	K	L
p22	LPL	~35	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-
	87	60	+	+	-	+	-	-	-	-	-	-	-	+	+	+	+	+	+	-	-	+	+	-	+	-	+
	135		+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
p12	255	64	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	+
	268/ANK	65	+	+	-	+	+	+	+	+	+	+	+	-	-	-	-	-	+	-	+	-	-	+	+	-	+
p11.2	POLB		+	+	-	+	+	+	+	+	+	+	+	-	-	-	-	-	+	-	+	-	-	+	-	-	+
p11.1	PLAT		+	+	-	+	+	+	+	+	+	+	+	-	-	-	-	-	+	-	+	-	-	+	-	-	+
q11.1	CEN		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	-	-	+
q11.2	531/519/359	66	⊖	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
	589		-	-	+	+	+	+	+	+	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-
	538		-	-	+	+	+	+	+	+	+	+	-	-	-	-	-	-	+	+	-	-	-	-	-	+	-
	524/517	67	-	-	+	+	+	+	+	+	+	+	-	-	-	-	-	-	+	+	-	-	-	-	-	+	-
	601	68	-	-	+	+	+	+	+	+	+	-	-	-	-	-	-	-	+	+	-	-	-	-	-	+	-
q12	509	70	-	-	+	-	-	+	+	+	+	+	-	-	-	-	-	-	-	+	+	-	+	-	-	+	-
	165		-	-	+	+	-	+	+	+	+	+	-	-	-	-	-	-	-	+	+	-	+	-	-	+	+
	285	72	-	-	+	+	-	+	+	+	+	+	-	-	-	-	-	-	-	+	+	-	+	-	-	+	+
	PENK		-	-	+	+	-	+	+	+	+	+	-	-	-	-	-	-	-	+	-	-	-	-	-	+	+
	260	73	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-
q22	88	~99	-	-	-	+	-	-	-	-	+	nd	-	-	-	-	-	-	-	+	+	nd	+	-	-	+	-
q23-24	200	~116	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

next most distal marker, was absent in several radioresistant hybrids, but was present in the sensitive hybrid 50K, thus clearly lying outside the region containing *SCID*. The region thus identified to contain *SCID* spans less than 2 cM, which on average would span 2 Mb in physical distance. We identified a yeast artificial chromosome (YAC), 943g4, from the library established by the Centre d'Etude du Polymorphismes Humain (*14*), which contains D8S531, D8S519, and D8S359. This YAC spans about 1700 kb and maps to 8q11 (Fig. 2D).

In addition to determining a minimal region containing *SCID*, we also investigated whether there were any known genes or proteins implicated in DNA double-strand break repair that could be candidates. Recent work in several laboratories (*17*) has identified the 86 kD subunit of the Ku protein (Ku p86) as the underlying deficiency in the radiosensitive *xrs* hamster cell lines. The Ku protein was first discovered as an autoantigen in patients with autoimmunity disorders (*18*). Ku is a heterodimer, composed of 70- and 86-kD subunits, that binds to double-stranded DNA ends, nicks, and DNA hairpins (*19*). Ku can act as a component of the DNA-dependent protein kinase, DNA-Pk, and co-purifies with the p350 catalytic subunit (*20*). DNA-Pk is a serine/threonine kinase that is absolutely dependent for activity on binding to double-stranded DNA (*21*) containing broken ends, nicks, and single-stranded gaps (*21*, *22*). Given this functional association and the fact that Ku p86 has been identified as *XRCC5*, complementing both the radiosensitive and V(D)J deficient phenotype of the *xrs* cell lines, a defect in p350 constitutes a candidate for the underlying defect in one of the other radiosensitive complementation groups.

As the cDNA sequence and map location of p350 are unknown, we used monoclonal antibodies to human p350 (*21*) to test for expression of human p350 in SCID hybrid cells containing single human chromosomes able to complement several radiosensitive phenotypes (chromosome 8 for the SCID deficiency, chromosome 5 for XR-1, and chromosome 11 for ataxia telangiectasia) (Fig. 3A). Under the reaction conditions used, no mouse p350 protein was detected, but expression of p350 protein in two independent SCID cell lines carrying human chromosome 8 was apparent. No expression was observed in cell lines carrying either human chromosome 5 or chromosome 11. Subsequently, we found 100% concordance between the radioresistance of hybrid cells and expression of p350 (Fig. 3B). One cell line, 100D, was originally categorized as radioresistant, but lacked human p350 protein. This cell line was subsequently found to have reverted to a radiosensitive phenotype. Comparing the human chromosome 8 content of 100D before and after reversion (Table 2) shows that only the contiguous markers D8S531, D8S519, and D8S359 were lost, confirming the accuracy of our mapping and the identification of these DNA sequences as markers for *SCID*.

To demonstrate expression of the human p350 protein in our hybrid cells, we used alkaline phosphatase as the detection method. Only very brief staining of the immunoblots (1 to 2 min) was required to detect the protein in radioresistant hybrids carrying the human chromosome 8 fragment. However, when we increased the incubation time for the final stain (>15 min), we could observe faint bands in the lanes of our immunoblots containing cell extracts from the wild-type C.B-17 cell line, but not from SCID cells. It appears likely that the observed lower levels of anti-p350 signal in mouse compared to human cells represent real differences, rather than an artifact of less efficient recognition of the mouse protein by the human antibody, since DNA-PK activity is much lower in rodent cells than human cells (*23*).

To further investigate differences in p350 levels between SCID and wild-type mouse cells, we employed the more sensitive enhanced chemiluminescence (ECL) detection method. We studied the expression of mouse p350 in five SCID cell lines that were derived independently in different laboratories (*24*) and in five wild-type mouse cell lines from different strains. In all cases p350 levels in the wild-type mouse whole cell extracts were markedly lower than in the SCID/hu8 hybrid, with little variation of p350 expression among the different strains of mice (Fig. 4, top). However, expression of p350 in whole cell extracts from SCID fibroblasts was barely detectable compared to the amount seen in extracts from wild-type murine cells. To ensure that the difference observed between SCID and the wild-type cells was not an artifact of the whole cell protein preparation method used, nuclear extracts were also analyzed (Fig. 4, bottom), confirming our previous results. Although the apparent reduction in immunoreactive p350 in SCID cells could be a result of a mutation in the antigen sites of the protein, the fact that we obtained the same result with two monoclonal antibodies is consistent with a genuine deficiency in p350 protein levels. This deficiency may reflect a regulatory defect resulting in decreased steady-state levels of p350 mRNA or an increased instability of the protein.

The SCID phenotype is not absolute. Chromosomal DNA double-strand breaks in SCID cells can be repaired, albeit to a lesser extent than in wild-type cells, and DNA

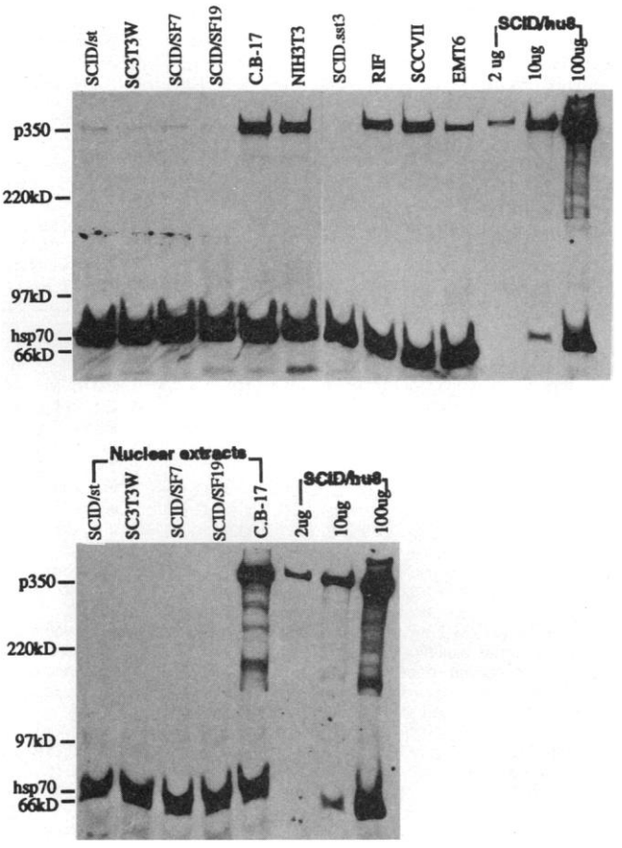

Fig. 4. Immunoblot analysis of p350 expression in SCID and wild-type mouse fibroblast and tumor cell lines (*21*). (**Top**) Total cell extracts (100 μg) or (**bottom**) nuclear extracts (50 μg) (*37*) were loaded in each lane; after gel electrophoresis and blotting the membrane was cut in half and incubated with antibody 42-47 (upper half of each blot) (*21*) for p350 expression or with monoclonal antibody N6 hsp70 (*36*) (lower halves) as a control for equal loading. N6 was a gift from W. Welch. Gels were also stained after the transfer with Coomassie blue as a further control for equal loading. Band detection was by ECL (Amersham), with anti-mouse immunoglobulin G conjugated to horseradish peroxidase. Experiments were repeated with the antibody 18-2 (*21*) against p350 (*28*) to confirm the results. Different amounts of protein isolated from whole cell extracts of SCID/hu8, the SCID cell line containing chromosome 8 were loaded as a positive control and a semiquantitative indicator.

double-strand breaks in extra-chromosomal substrates are repaired as efficiently as in wild-type cells (*25*). The immune-deficient phenotype of SCID has also been described as "leaky," since on rare occasions coding junctions are formed during the rearrangement of the immunoglobulin genes (*26*). In addition, recent experiments have shown the restoration of V(D)J recombination in the T cell receptor β-chain locus (TCRβ) after a single treatment of newborn SCID mice with 1 Gy of ionizing radiation (*27*). These observations fit a model in which the SCID phenotype results from a defect in regulation of p350 expression, rather than from a mutation in the coding region that interferes with the enzyme activity. However, we have not been able to observe the induction of p350 after irradiation in either SCID or wild-type mouse fibroblasts (*28*). Identification of the mutation underlying the reduced levels of p350 must await cloning and sequence analysis of the murine p350 gene.

DNA-PK phosphorylates many substrates in vitro including transcription factors (Sp1, Oct 1, Oct 2, SRF, Fos, and Jun) and proteins implicated in the response of cells to DNA damage (p53, Ku, and replication protein A) (*23*). A deficiency in DNA-PK activity in SCID cells could therefore result in a failure to elicit the appropriate cellular response to DNA damage. It is also possible that the p350 subunit could associate with enzymes that repair DNA damage, such as polymerase and ligase. Alternatively p350 may contain a structural domain, that changes the chromatin formation around a DNA lesion to enable the repair complex to join the DNA ends. This scenario could explain why SCID cells efficiently repair DNA double-strand breaks on an extra-chromosomal substrate, but not in the chromosomal context.

The SCID defect has been mapped by genetic linkage analysis to a region on mouse chromosome 16 lying close to the centromere flanked by the loci for *Igl* (Immunoglobulin light chain), and *Prm* (protamine) (*29*). As no synteny between human chromosome 8 and mouse chromosome 16 has previously been reported, it was not inherently obvious that a gene on human chromosome 8q11 would be the homolog of the murine *SCID*. The genes encoding plasminogen activator (*PLAT*) and polymerase β (*POLB*), which lie on the short arm of human chromosome 8 close to the centromere have been shown to map to mouse chromosome 8 (*30*). The *mos* oncogene, known to be close to the centromere on the long arm of human chromosome 8, maps to mouse chromosome 4 (*31*). However, we were able to fine-map the gene for *CEBPδ* (CCAAT-enhancing binding protein δ), which has been cloned and mapped to the pericentromeric region of human chromosome 8 (8p11-q11, between *PLAT* and *MOS*) (*32*). Our analysis indicates that *CEBPδ* maps to 8q11, approximately 4 cM proximal to MOS. The CEBPδ gene is localized on the YAC943g4, which, as previously stated, also contains the three anonymous DNA markers closest to *SCID* (*33*). The murine CEBPδ gene has been mapped to mouse chromosome 16 in close linkage to the *Igl* locus (*34*). Thus, we have established a new synteny group between human chromosome 8q11, containing the p350 and CEBPδ genes, and the centromeric region of mouse chromosome 16 at the position of the *SCID* locus. This provides further evidence for our conclusion that a gene on chromosome 8q11 is the human homolog of the murine *SCID* and supports the proposal that p350 is a candidate for *SCID*.

Note added in proof: Since completion of this work we learned of similar findings to be published by the groups of P. Jeggo, S. Jackson, and F. Alt; and by the group of D. Chen.

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physically mapped on chromosome 8. We tested this novel marker through our radiation hybrid panel. The location of D8S359 is undistinguishable in our panel from D8S531 and D8S519. All three markers are contained on YAC 943g4, which we map by FISH to chromosome 8q11.1-11.2. We conclude that D8S359 and CEBPδ map to 8q11 in the same region as p350.

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Absence of p350 Subunit of DNA-Activated Protein Kinase from a Radiosensitive Human Cell Line

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The radiosensitive rodent mutant cell line *xrs-5* is defective in DNA double-strand break repair and lacks the Ku component of the DNA-activated protein kinase, DNA-PK. Here radiosensitive human cell lines were analyzed for DNA-PK activity and for the presence of related proteins. The radiosensitive human malignant glioma M059J cell line was found to be defective in DNA double-strand break repair, but fails to express the p350 subunit of DNA-PK. These results suggest that DNA-PK kinase activity is involved in DNA double-strand break repair.

DNA double-strand breaks (dsb) are induced by x-rays and by oxidative metabolism, and their repair is essential to cell survival. Several radiosensitive mutant cell lines have been identified in mammalian cells that are defective both in DNA dsb repair and in V(D)J recombination (*1*). V(D)J recombination is required for the assembly of antigen receptor gene segments and requires both lymphocyte-specific genes and general DNA repair activities. However, little is known about the genes and gene products involved in the DNA dsb repair process itself. Ku, the DNA targeting subunit of DNA-activated protein kinase, DNA-PK, may function in both DNA dsb repair and V(D)J recombination.

DNA-PK is a serine-threonine protein kinase that requires double-stranded DNA for activity (*2*–*6*). DNA-PK phosphorylates many protein substrates in vitro including the 90-kD heat-shock protein (hsp90), Sp1, SV40 T antigen, the tumor suppressor protein p53, serum response factor (SRF), fos, jun, and the CTD of RNA polymerase II (*6*–*8*). DNA-PK has been purified and characterized from HeLa cells (2, 3, 6), Raji Burkitt's lymphoma cells (*5*), and human placenta (*9*), and consists of at least two protein components; a large polypeptide of approximately 350,000 daltons (p350) and the Ku autoantigen. The p350 component binds adenosine triphosphate (ATP) analogs (*2, 3*), and is thought to contain the kinase domain of DNA-PK. Ku antigen is a dimer of 70- and 80-kD subunits, which are recognized by antibodies from sera of patients with auto-immune disorders including scleroderma-polymyositis (*10*). Ku interacts with the termini of double-stranded DNA (*11*) and by this means is thought to provide the DNA targeting required by DNA-PK.

The biochemical properties of both Ku and DNA-PK suggest it has a role in DNA damage detection or in repair or in both processes (*12*). The radiosensitive rodent cell line, *xrs-5*, lacks a DNA end binding activity that is characteristic of Ku and is defective in DNA dsb repair and in V(D)J recombination pathways (*13*–*15*). Transfection of *xrs* cells with the human Ku p80 gene was shown to restore partial radio resistance to these cells (*15, 16*). The human XRCC5 was identified as Ku p80 (*15*). These data suggest a role for Ku in dsb repair and V(D)J recombination. However, although DNA end binding activity like that of Ku occurs in rodent cells (*13*–*16*), simian cell lines (*17*), *Drosophila* (*18*), and yeast (*19*), little is known about DNA-PK activity in these organisms. We previously did not detect DNA-PK activity in cell lines from nonprimates using either a synthetic peptide or the protein hsp90 as substrate (*4*).

To date, only few human cell lines with radiosensitivities similar to those of the mutant rodent *xrs-5* cells have been characterized. Among these are fibroblast strains belonging to several ataxia telangiectasia complementation groups. Whereas ataxia telangiectasia cells are extremely sensitive to ionizing radiation, the underlying basis of this radiosensitivity is thought to involve misrepair of dsb rather than a reduced ability to rejoin dsb (*20*). Another radiosensitive human cell line is M059J. The M059J and M059K cell lines were isolated from different portions of the same human malignant glioma biopsy specimen. Whereas M059K cells are relatively radioresistant, M059J cells are ~10-fold more sensitive, with a radiosensitivity similar to that of *xrs-5* cells (*21*). We have examined the

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