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Artemis, a Novel DNA Double-Strand Break Repair/V(D)J Recombination Protein, Is Mutated in Human Severe Combined Immune Deficiency

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Summary

The V(D)J recombination process insures the somatic diversification of immunoglobulin and antigen T cell receptor encoding genes. This reaction is initiated by a DNA double-strand break (dsb), which is resolved by the ubiquitously expressed DNA repair machinery. Human T-B-severe combined immunodeficiency associated with increased cellular radiosensitivity (RS-SCID) is characterized by a defect in the V(D)J recombination leading to an early arrest of both B and T cell maturation. We previously mapped the disease-related locus to the short arm of chromosome 10. We herein describe the cloning of the gene encoding a novel protein involved in V(D)J recombination/DNA repair, Artemis, whose mutations cause human RS-SCID. Protein sequence analysis strongly suggests that Artemis belongs to the metallo-β-lactamase superfamily.

Introduction

B and T lymphocytes recognize foreign antigen through specialized receptors: the immunoglobulins and the T cell receptor (TCR), respectively. The highly polymorphic antigen-recognition regions of these receptors are composed of variable (V), diversity (D), and joining (J) gene segments that undergo somatic rearrangement prior to their expression by a mechanism known as V(D)J recombination (Tonegawa, 1983). Each V, D, and J segment is flanked by recombination signal sequences (RSSs) composed of conserved heptamers and nonamers separated by random sequences of either 12 or 23 nucleotides. V(D)J recombination can be roughly divided into three steps. First, the RAG1 and RAG2 proteins initiate the rearrangement process through the recognition of the RSS and the introduction of a DNA double-strand break (dsb) at the border of the heptamer (Schatz et al., 1989; Oettinger et al., 1990). The subsequent step consists of recognition and signaling of the DNA damage to the ubiquitously expressed DNA repair machinery. The description of the murine scid anomaly, characterized by a lack of circulating mature B and T lymphocytes (Bosma et al., 1983), as a general DNA repair defect accompanied by an increased sensitivity to ionizing radiation or other agents causing DNA dsb, provided the link between V(D)J recombination and DNA dsb repair (Fulop and Phillips, 1990; Biedermann et al., 1991; Hendrickson et al., 1991). This was further confirmed by the analysis of Chinese ovary cell lines (CHO), initially selected on the basis of their defect in DNA repair (Taccioli et al., 1993). This led to the description of the Ku70/Ku80/DNA-PKcs complex as a DNA damage sensor (reviewed in Jackson and Jeggo, 1995). Briefly, DNA-PKcs is a DNA-dependent protein kinase that belongs to the phosphoinositol (PI) kinase family, which is recruited at the site of the DNA lesion through the interaction with the regulatory complex Ku70/80 that binds to DNA ends (Gottlieb and Jackson, 1993). Cells from scid mice lack DNA-PK activity owing to a mutation in the *DNA-PKcs* encoding gene (Blunt et al., 1996; Danska et al., 1996). In the final phase of the V(D)J rearrangement, the DNA repair machinery per se will ensure the re-ligation of the two chromosomal broken ends. This last step resembles the well-known DNA nonhomologous end joining (NHEJ) pathway in the yeast *Saccharomyces cerevisiae* (reviewed in Haber, 2000). It involves the XRCC4 (Li et al., 1995) and the DNA ligaseIV (Robins and Lindahl, 1996) factors. V(D)J recombination represents a critical checkpoint in the development of the immune system. Indeed, all the animal models carrying a defective gene of either one of the known V(D)J recombination factors, either natural (murine and equine scid) or engineered through homologous recombination, exhibit a profound defect in the lymphoid developmental program owing to an arrest of the B and T cell maturation at early stages (Mombaerts et al., 1992; Shinkai et al., 1992; Nussenzweig et al., 1996; Zhu et al., 1996; Jhappan et al., 1997; Shin et al., 1997; Barnes et al., 1998; Frank et al., 1998; Gao et al., 1998a, 1998b; Taccioli et al., 1998).

A fully defective T cell development program is observed in a number of rare human conditions denominated severe combined immune deficiency (SCID) (Fischer et al., 1997). In about 20% of SCID, the phenotype consists of a virtually complete absence of both circulating T and B lymphocytes, associated with a defect in the V(D)J recombination process, while natural killer (NK) cells are normally present and functional. This condition is autosomal and recessive. It is lethal within the first year of life because of the occurrence of multiple and

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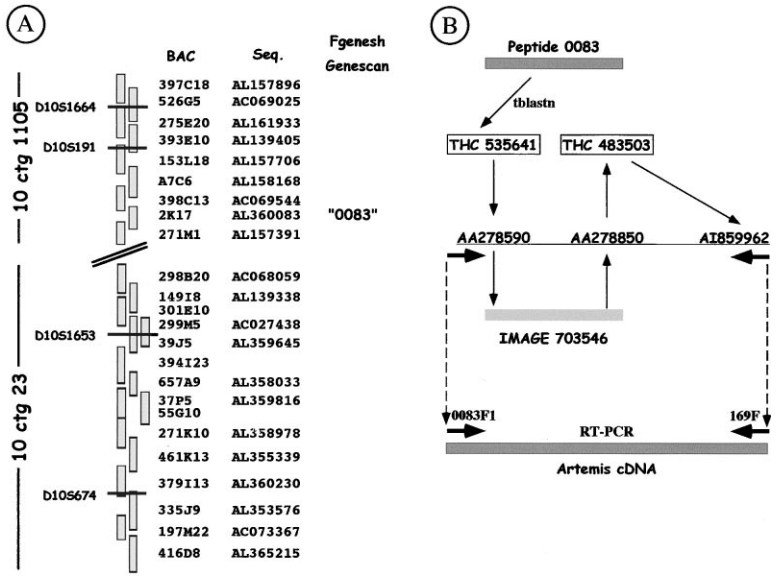

Figure 1. Cloning of the *Artemis* Gene

(A) Schematic representation of the two BAC contigs, 10ctg23 and 10ctg1105, that contained 15 and 9 BACs (thick bars), respectively. These contigs include the two most external STS markers defining the RS-SCID region, D10S1664 and D10S674. The sequence accession number corresponding to each BAC is mentioned on the right (seq.). The peptide 0083 (see text) was identified in the AL360083 sequence of clone 2K17 using both FGENESH and GENESCAN gene prediction softwares.

(B) Scheme of the strategy employed for cloning the *Artemis* cDNA. See text for explanations.

protracted infections, often caused by opportunistic micro-organisms. Allogeneic stem cell transplantation can cure this immunodeficiency. Mutations in either the *RAG1* or *RAG2* gene account for a subset of patients with T-B-SCID (Schwarz et al., 1996; Corneo et al., 2000; Villa et al., 2001). In some patients (RS-SCID), the defect is not caused by *RAG1* or *RAG2* mutations and is accompanied by an increased sensitivity to ionizing radiations of both bone marrow cells (CFU-GMs) and primary skin fibroblasts (Cavazzana-Calvo et al., 1993), as well as a defect in V(D)J recombination in fibroblasts (Nicolas et al., 1998). Although this condition suggests that RS-SCID could have a general DNA repair defect reminiscent of the murine scid situation, DNA-PK activity was found normal in these patients and the implication of the *DNA-PKcs* gene has been unequivocally ruled out by genetic means in several consanguineous families (Nicolas et al., 1996). A role for all the other known genes involved in V(D)J recombination/DNA repair was equally excluded as being responsible for RS-SCID condition (Nicolas et al., 1996, 1998). The gene defective in RS-SCID, therefore, encodes an as yet undescribed factor. We recently assigned the disease-related locus to the short arm of human chromosome 10 in a 6.5 cM region delimited by two polymorphic markers, D10S1664 and D10S674 (Moshous et al., 2000), a region in which the locus of a similar SCID condition found in Athabascan speaking American Indians (A-SCID) was also mapped (Hu et al., 1988; Li et al., 1998). Here we report the identification and cloning of the *Artemis* gene, localized in this region of chromosome 10. *Artemis* codes for a novel V(D)J recombination/DNA repair factor that belongs to the metallo-β-lactamase superfamily and whose mutations cause human RS-SCID condition.

Results

Cloning of the *Artemis* cDNA

Among all the chromosome 10 bacterial artificial chromosome (BAC) contigs derived at the Sanger center, 10ctg1105 and 10ctg23 (Figure 1A) were of particular interest to us as they included several BACs bearing the RS-SCID region flanking markers D10S1664 and D10S674, as well as D10S191 and D10S1653, that showed the maximum pairwise LOD scores in A-SCID populations (Li et al., 1998). We initiated a systematic survey of the nucleotide sequences, covering the 24 BACs present in these two contigs. The gene analysis software FGENESH predicted six peptides in the 260 Mb AL360083 nucleotide sequence (07/15/2000 draft release), derived from the 2K17 BAC. In one of them (subsequently named "0083" peptide), 121 aa turned out to be 35% and 31% identical to the MuSNM1 (AAF64472) and Yeast PSO2 (P30620) proteins, respectively. The same peptide prediction was obtained using GENESCAN. SNM1 and PSO2 are two proteins involved in the reparation of DNA damages caused by DNA interstrand cross-linking agents (ICL), including cisplatin, mitomycin C, or cyclophosphamide (Henriques and Moustacchi, 1980; Dronkert et al., 2000; and references therein). Mouse and yeast mutants for SNM1/PSO2 are hypersensitive to several agents that cause ICLs, but not to γ rays, in contrast to RS-SCID patients. Despite this important discrepancy in the radiosensitivity phenotype, the putative "0083" peptide-encoding gene represented a good candidate owing to its chromosomal localization and its similarity with DNA repair proteins. A search for RNA transcripts encoding this peptide in the TIGR Human Gene Indices database (Figure 1B) returned the THC535641 index within which the AA278590 represented the most 5′ sequence and corresponded to the I.M.A.G.E. clone 703546. The nucleotide sequence of this clone's opposite end (AA278850) matched the THC483503 index. The AI859962 nucleotide sequence in this second index provided the most 3′ extension of the cDNA coding for the "0083" peptide. A complete cDNA (Figures 1B and 2) was obtained by RT-PCR amplification of fibroblast RNA using 0083F1 and 169F primers chosen within the AA278590 and AI859962 nucleotide sequences, respectively. The entire cDNA sequence of 2354 bp (Figure 2) contains an open reading frame (ORF) of 2058 bp. The ATG at position 60 is as-

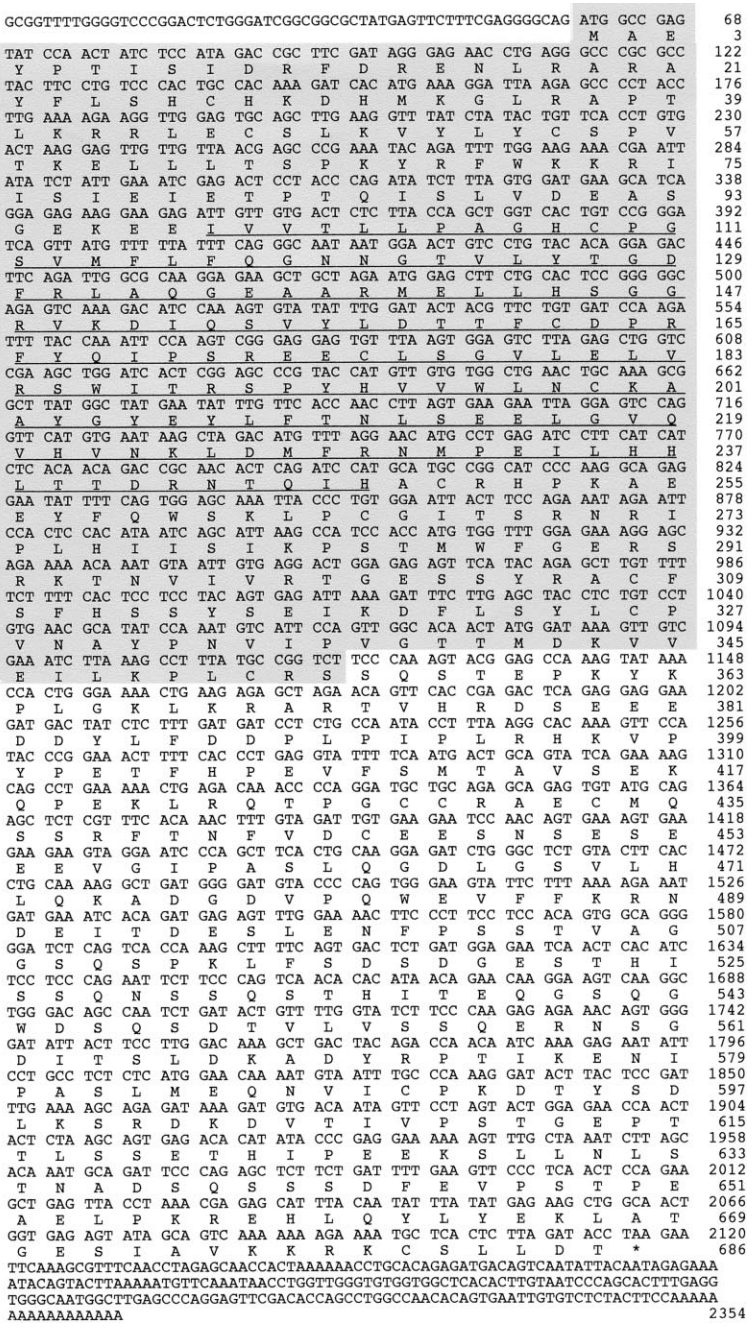

Figure 2. *Artemis* cDNA Sequence

The sequence of peptide 0083 is underlined. It is part of the gray shaded SNM1 homology domain.

sumed to represent the translational initiation site based on the analysis of surrounding nucleotides matching the Kozack consensus for translation initiation, as well as sequence comparison with a murine cDNA homolog (not shown). The encoded protein of 685 aa, which we name Artemis, has a predicted molecular weight of 77.6 kDa. The "0083" peptide (underlined in Figure 2) corresponds to I99-H247 and is part of a larger region (gray box) that shows similarity to scPSO2 and muSNM1. We cannot fully ascertain that this cDNA represents the full-length sequence even though repeated attempts to further extend the 5′ sequence failed. The polyA tail in the 3′ untranslated region of the sequence presented in Figure 2 is encoded by the genomic sequence and is not the consequence of RNA processing, suggesting that this cDNA may extend further downstream, in accordance with the transcript size detected on Northern blot (see below). However, because of this polyA tract, we were unable to further extend the 3′ sequence. Functional complementation studies (see below) strongly suggest, however, that we have indeed cloned the full Artemis ORF. The *Artemis* gene structure (Figure 3) was deduced by comparison of the cDNA sequence to the genomic (AL360083) sequence. *Artemis* is composed of 14 exons with sizes ranging from 52 bp to 1160 bp. The exact length of the *Artemis* gene is presently unknown as the AL360083 draft sequence is not yet fully contiguous. Database search with the Artemis protein sequence did

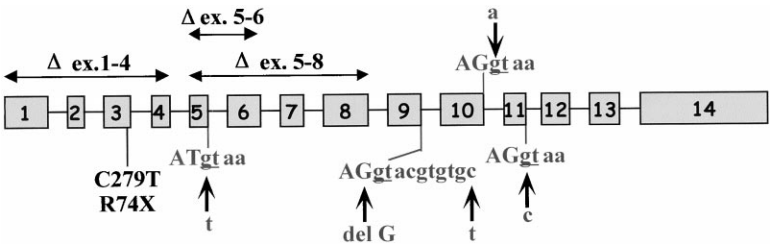

Figure 3. Genomic Organization of the *Artemis* Gene with the Mutations Identified in RS-SCID Patients

not uncover orthologs in any of the fully sequenced organisms. However, cDNA cloning of the murine Artemis revealed a 78% protein identity over the whole sequence (data not shown), which suggests a high degree of conservation among higher eukaryotes.

Expression of *Artemis*

Increased radiosensitivity of RS-SCID to γ rays is not restricted to the cells of the immune system, but is also a characteristic of fibroblasts, suggesting that *Artemis* is ubiquitously expressed. Northern blot analysis on a panel of eight different tissues (Figure 4A) confirmed the broad spectrum of *Artemis* expression. As discussed above, the smaller size of the Artermis cDNA compared to the main transcript size (4.0 kb) is likely caused by the reverse transcriptase priming on the genomic encoded polyA tract present in the 3′ untranslated region. Ubiquitous *Artemis* expression was further confirmed by PCR analysis on a panel of 15 cDNAs representing a wide range of hematopoietic and nonhematopoietic tissues (Figure 4B). The level of *Artemis* expression is weak and required 30 PCR cycles (38 cycles for the skeletal muscle) to get an appropriate signal with an internal P32-labeled oligonucleotide, compared to the strong ethidium bromide staining obtained for the control gene GAPDH. Low level expression of *Artemis* could reflect a general property of the SNM1 protein family as the basal expression of mSNM1 in ES cells was found very low as well (Dronkert et al., 2000). Of note, compared to other tissues, *Artemis* RNA level is not increased in thymus or bone marrow, the sites of V(D)J recombination.

The *Artemis* Gene Is Mutated in Human RS-SCIDs

We next analyzed the structure and the sequence of the *Artemis* gene in a series of 11 RS-SCID families including 13 patients (Table 1; Figure 3). Three patients (P6, P15, and P40) were characterized by a complete absence of the *Artemis* transcript caused by a genomic deletion extended from exon 1 to exon 4. This mutation can be considered as a complete null allele. The same genomic deletion was present on one allele in P1, who carried a C279T nucleotide change on the other allele that led to the formation of a nonsense codon at R74. Homozygous C279T mutation was also present in P2 and found heterozygous in P4. Two other genomic deletions were characteristic of this series of RS-SCID patients. A homozygous deletion spanning exons 5 to 8 in P47 led to the formation of a cDNA in which exon 4 was spliced in-frame to exon 9, resulting in a putative protein lacking K96 to Q219. In P3, a heterozygous genomic deletion of exons 5 and 6 resulted in the out of frame splicing of exons 4 to 7, leading to a frameshift at K96. The second allele in this patient carried a heterozygous G to C nucleotide change in the exon 11 canonical splice donor sequence, which caused the out of frame splicing of exons 10 to 12, leading to a frameshift at T300. Lastly, three other splice donor sequence mutations were identified in six patients. A heterozygous G to A nucleotide change in the exon 10 splice donor site in P4 gave rise to the production of a cDNA where the fusion of exons 9 to 12 preserved the open reading frame and potentially led to the production of a protein lacking A261 to E317. Homozygous G to T mutation in the exon 5 donor site was found in the siblings P5, P11, and P12, as well as

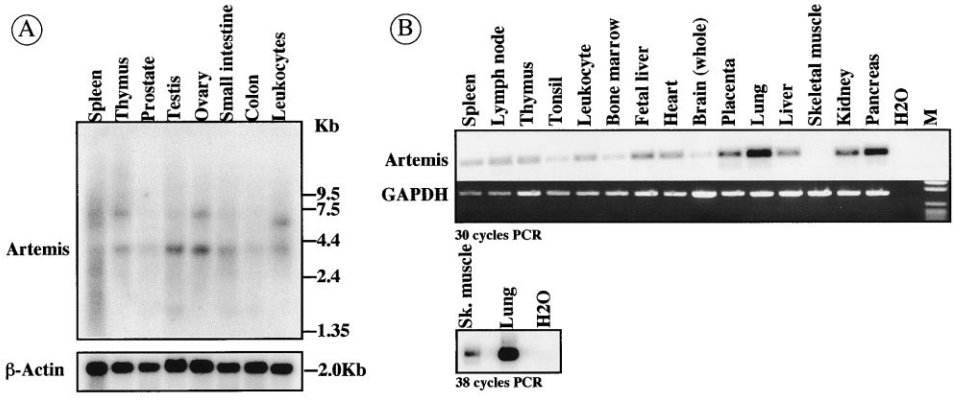

Figure 4. *Artemis* cDNA Expression

(A) Northern blot analysis. the membrane was successively hybridized with an *Artemis* and a β-actin probe.

(B) RT-PCR analysis using a commercial panel of cDNA from hematopoietic and nonhematopoietic tissues. GAPDH-specific primers were used as control.

Table 1. Mutations of the *Artemis* Gene in RS-SCID Patients

Patients	Mutation	Effect	Status
P1	Genomic deletion (exons 1–4)	No RNA	Heteroz.
	C279T	R74X[a]	Heteroz
P2	C279T	R74X	Homoz.
P3	Genomic deletion (exons 5–6)	K96 frameshift	Heteroz.
	Exon 11 splice donor (G→C)	T300 frameshift	Heteroz.
P4	C279T	R74X	Heteroz.
	Exon 10 splice donor (G→A)	Del A254–E317	Heteroz
P5/P11/P12/P38	Exon 5 splice donor (G→T)	K96 frameshift	Homoz.
P6/P15/P40	Genomic deletion (exons 1–4)	No RNA	Homoz.
P16	Del G818	A254 frameshift	Homoz.
P47	Genomic deletion (exons 5–8)	Del K96–Q219	Homoz.

[a] Stop codon.

in P38, creating the out of frame splicing of exons 4 to 6 and the formation of a frameshift at K96. Although this form of cDNA lacking exon 5 as a result of an alternative splicing event was also detected at a low level in RNA from normal cells (data not shown), it accounted for all the cDNAs in P5 and P38. In patient P16, a homozygous deletion of G818 in exon 9, together with a homozygous C to T change nine nucleotides downstream in the intron, caused the formation of a frameshift at A254. Whenever samples of the patients' parents were available, we tested them for the presence of the mutations. We could confirm the inheritance of the mutations in P5, P11, and P12, as well as P38 and P16, by direct sequencing of the exon-specific genomic PCR obtained from parents' DNA, which concurs with the autosomal recessive inheritance. In summary, all of the 13 RS-SCID patients tested in this series carried homozygous or heterozygous mutations in the *Artemis* gene. None of these mutations were simple missense, and one of them (genomic deletion of exons 1 to 4) can be considered as a true null allele given the complete lack of *Artemis* transcript in P6, P15, and P40.

Artemis Complements the RS-SCID V(D)J Recombination Defect

We previously demonstrated the absence of V(D)J recombination-derived coding joint formation in RS-SCID patients' fibroblasts upon transfection of RAG1 and RAG2 expression constructs together with extrachromosomal V(D)J recombination substrates specific for the analysis of the coding (pHRecCJ) joint (Nicolas et al., 1998). In contrast, signal joint formation was always found normal in RS-SCID fibroblasts (Nicolas et al., 1998; Table 2). We cloned the Artemis cDNA in the mammalian expression vector pIres-EGFP and assessed its functional complementation activity in the V(D)J recombination assay in fibroblasts from seven RS-SCID patients using the pHRecCJ substrate (Table 2). In all cases, bacterial blue colonies were repeatedly recovered following transfections in the presence of wt Artemis (+), attesting for the RAG1/2 driven recombination of the substrate, while virtually no such colonies were obtained in the absence of exogenous wt Artemis (−). Sequence analysis of the recovered pHRecCJ plasmids forming blue colonies in this assay in fibroblast lines from P1 and P40 demonstrated that the junctions were bona fide V(D)J coding joints with limited trimming of the coding ends similar to those obtained in control fibroblasts (data not shown). Altogether, these results indicate that the V(D)J recombination defect in RS-SCID is directly related to the described mutations in the *Artemis* gene and can be complemented by the introduction of a wt *Artemis* cDNA in the patients' fibroblasts. Although transient high level expression of Artemis did not seem to be toxic, we could not derive stable transfectants to analyze the complementation of the hypersensitivity to ionizing radiation. This could reflect a toxicity of long-term high level expression of wt Artemis in the transfected fibroblasts. An analogous cellular toxicity was previously noted upon overexpression of other human or murine homologs of SNM1 in vitro and may be a characteristic of this family of proteins (Dronkert et al., 2000). This is also in agreement with the physiological low level RNA expression of these genes (see above).

Artemis Belongs to the Metallo-β-Lactamase Superfamily

Database searches using Artemis protein sequence revealed significant similarities to several proteins, including the yeast PSO2 and murine SNM1 proteins, over the first 360 amino acids of Artemis (Figure 5 and data not shown). Subsequent iterations with the PSI-BLAST program highlighted significant similarities of the first 150 amino acids to well-established members of the metallo-β-lactamase superfamily (Figures 5A and 5B). The metallo-β-lactamase fold, first described for the *Bacillus cereus* β-lactamase (Carfi et al., 1995), is adopted by various metallo-enzymes with a widespread distribution and substrate specificity (Aravind, 1997). It consists of a four-layered β sandwich with two mixed β sheets flanked by α helices, with the metal binding sites located at one edge of the β sandwich (Figure 5C). Sequence analysis as well as secondary structure prediction for Artemis clearly indicated the conservation of motifs typical of the metallo-β-lactamase fold. This is true in particular for the amino acids D10, [HXHKDH]26–31, H108, and D129 (underlined with a star in Figures 5A and 5B and represented as ball-and-stick in Figure 5C) participating in the metal binding pocket and representing the catalytic site of the metallo-β-lactamases. The last metal binding residue of the metallo-β-lactamases, H225 in *Tenotrophomonas maltophilia* metallo-β-lactamase (1SML), which is located at the end of a β strand (strand β12), is appar-

Table 2. Complementation of V(D)J Coding Joint Formation in RS-SCID Fibroblasts Transfected with wt Artemis

Cell Line	wt Artemis Plasmid	Coding (pHRecCJ)			Signal (pHRecSJ)		
		Blue Col.	Total	R[a]	Blue Col.	Total	R[b]
		50	42,000	3.5	27	10,840	2.5
Control	−						
	+	16	15,000	3.2			
		0	34,800	<0.03	34	45,600	0.7
P1	−						
	+	23	40,000	1.7			
P4	−	2	36,300	0.16	336	98,200	3.4
	+	23	54,000	1.3			
P5	−	0	21,400	<0.05	17	3,000	5.7
	+	52	61,600	2.5			
	+	65	16,300	11.9			
	+	48	10,800	13.3			
	+	48	20,000	7.2			
P15	−	2	11,160	0.53	192	30,000	6.4
	+	16	31,200	1.5			
P16	−	0	13,000	<0.08	51	33,200	1.5
	+	30	13,680	6.6			
P40	−	0	40,250	<0.02	nd	nd	nd
	+	90	160,000	1.7			
	+	65	58,800	3.3			
	+	100	42,840	7.0			
	+	76	29,736	7.6			
P47	−	0	28,200	<0.003	25	66,800	0.4
	+	48	50,000	2.9			

[a] R (coding joints) = 3 × (Blue col.)/(Total) × 1,000.
[b] R (signal joints) = (Blue col.)/(Total) × 1,000.

ently absent in Artemis/SNM1/PSO2, but could be substituted for by the aspartic acid D158 of Artemis, also conserved in SNM1/PSO2. The later residue is located at the end of a predicted β strand, separated by an α helix from the strand bearing the preceding metal binding residue. However, it is also possible that the last metal binding residue occurs much farther (H312, Figure 5D), the intervening sequences representing, then, a large insertion within the metallo-β-lactamase domain (Aravind, 1997). Altogether, this analysis not only indicates that Artemis probably adopted the β-lactamase fold, but may also have conserved an associated catalytic activity, in contrast to several other proteins having lost many of the catalytic residues (Aravind, 1997).

Discussion

Identification of the *Artemis* Gene

Genetic studies in humans have changed gear since the recent availability of the near complete whole genome DNA sequence together with the policy of several centers, such as the Sanger center, to freely distribute, on a real time basis, their data to the scientific community. The second major contribution to the identification of the *Artemis* gene was the development of very powerful programs (and again their free availability to the scientific community) capable of hunting genes embedded within megabases of genomic DNA sequences. As an example of their robustness, the 0083 peptide sequence, which was recovered from the AL360083 nucleotide sequence, is encoded by five different exons, the splicing of which was correctly predicted by both GENESCAN and FGENESH programs. The RS-SCID was previously assigned to a 6.5 cM chromosomal region flanked by the D10S1664 and D10S674 polymorphic markers, a region too large for classical cDNA selection studies (Li et al., 1998; Moshous et al., 2000). Systematic in silico annotation of draft genomic sequences covering this region led us to the identification of the *Artemis* gene. As expected, given the generalized increased radiosensitivity in RS-SCID patients' cells (Cavazzana-Calvo et al., 1993), *Artemis* demonstrated a pleiotropic expression pattern.

Mutations in the *Artemis* Gene Cause RS-SCID

The first indication that Artemis was indeed the gene involved in the RS-SCID came from the identification of mutations in several patients. Altogether, eight different alterations of the gene were found in 11 families. Although some of the mutations were recurrent, it was not possible to draw any clear correlation with the geographical origins of the patients. Several interesting features arise from the analysis of these mutations. First, three of the identified modifications involve genomic deletions spanning several exons, leading to frameshift and appearance of premature terminations in two cases and in-frame deletion of 216 aa in one case. This indicates that the *Artemis* gene may represent a hot spot for gene deletion. Second, none of the mutations consists of simple nucleotide substitutions generating amino acid changes, and only one, the C279T modification, creates a nonsense mutation. The other nucleotide changes affect splice donor sequences leading to either frameshifts in three cases or to in-frame deletion of part of the protein in one case. Third, in three patients (P6, P15, and P40), the genomic deletion comprises exons 1 to 4 and results in a complete absence of *Artemis*-encoded cDNA. This deletion, which can be considered

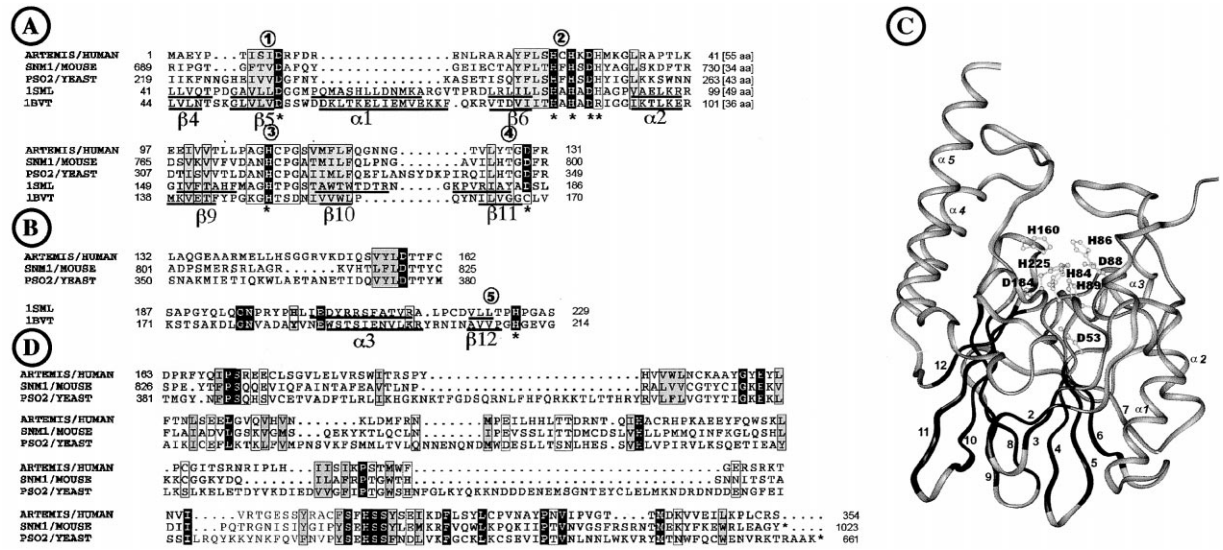

Figure 5. Artemis Belongs to the Metallo-β-Lactamase Superfamily

(A) Alignment of human Artemis, mouse SNM1, and yeast PSO2 amino acid sequences with two metallo-β-lactamases whose three-dimensional structures were solved (metallo-β-lactamase l1 from *Stenotrophomonas maltophilia* [Protein Data Bank identifier 1SML; Swiss-Prot identifier BLA1_XANMA], metallo-β-lactamase from *Bacillus cereus* [Protein Data Bank identifier 1BVT; Swiss-Prot identifier BLA2_BACCE]). Secondary structures, as experimentally determined, are reported below the two metallo-β-lactamase sequences and are labeled according to the representation of the three-dimensional structure of *Stenotrophomonas maltophilia* metallo-β-lactamase shown in (C). Identical amino acids appear white on a black background, similar amino acids are boxed (shaded when hydrophobic). Amino acids that participate in metal binding sites are represented with a star and depicted in a ball-and-stick representation in (C). Motifs 1 and 2 (encircled), typified by a conserved aspartate ending strand β5 (1SML Asp53) and by the HxHxDH signature (1SML His84, His86, Asp88, and His89), respectively, belong to the first β sheet of the metallo-β-lactamase fold. Motifs 3 and 4 (encircled) lie in the second β sheet and are characterized by conserved histidine (His160, preceded by a small residue) and aspartate (or cysteine) (Asp184) residues at the end of strands β9 and β11, respectively.

(B) No significant alignment can be obtained for 1SML helix α3 and strand β12, although a similar succession of secondary structures (α helix followed by β strand) is predicted for Artemis/SNM1/PSO2. It is worth noting that a conserved aspartate is observed after the predicted β strand, suggesting that this residue could be present in the active site. The last metal binding histidine (motif 5 in 1SML) could be provided for by downstream sequences.

(C) Three-dimensional representation of the *Stenotrophomonas maltophilia* metallo-β-lactamase (Protein Data Bank 1SML), highlighting conserved residues (ball-and-sticks) depicted in (A) and (B).

(D) A conserved region (the βCASP domain) accompanies the metallo-β-lactamase domain in members of the Artemis/SNM1/PSO2 subfamily.

as resulting in a null allele, therefore demonstrates that Artemis is not an essential protein for viability, in contrast to XRCC4 and DNA ligaseIV for example (Barnes et al., 1998; Frank et al., 1998; Gao et al., 1998b), or that it is partly redundant. This information is of particular interest in the setting of a murine knockout counterpart to the human RS-SCID condition. Lastly, the implication of Artemis in the RS-SCID condition was unequivocally established by complementation of the V(D)J recombination defect in patients' fibroblasts upon transfection of a wt *Artemis* cDNA.

Artemis Function

Although we can infer from the phenotype of RS-SCID patients that Artemis is part of the ubiquitous machinery involved in DNA dsb repair that is shared by the V(D)J recombination process, the precise function for this new factor remains to be defined. The repeated search for a global ortholog of both human and murine Artemis in other species in protein databases failed to provide a strong candidate, and we are left with the initial similarity of the 0083 peptide, which includes the whole N terminus moiety of Artemis, to the various members of the SNM1 family (Figure 5). However, Artemis is clearly not the human ortholog of either murine SNM1 or yeast PSO2 for at least two reasons. First, despite their SNM1 similarity regions, the three proteins differ in their associated domains. In particular, the 331 amino acids composing the C-terminal region of Artemis are not present in SNM1/PSO2 and do not show any obvious similarities with any other known protein. Second, while murine and yeast SNM1/PSO2 mutants demonstrate a strong defect in the repair of DNA damages caused by DNA interstrand cross-linking agents (Henriques and Moustacchi, 1980; Dronkert et al., 2000), they do not display elevated sensitivity to ionizing radiations, indicating that these two proteins are probably not directly involved in the repair of DNA dsb. This is in sharp contrast to the phenotype of RS-SCID patients whose primary molecular defect is indeed the absence of DNA dsb repair, illustrated by the lack of coding joint formation in the course of V(D)J recombination and the increased sensitivity of bone marrow and fibroblast cells to γ rays (Cavazzana-Calvo et al., 1993; Nicolas et al., 1998). Interestingly, Artemis, murine SNM1, and yeast PSO2 share a domain adopting a metallo-β-lactamase fold (Aravind, 1997) and possibly its associated enzymatic activity, given the presence of nearly all the critical catalytic residues (or conserved residues which could substitute for them for function). However, there is no obvious consensus with regard to

the nature of the various metallo-β-lactamase substrates, outside of a general negatively charged composition. Sequence analysis revealed the existence of a conserved region that accompanies the metallo-β-lactamase domain in members of the Artemis/SNM1/PSO2 subfamily (Figure 5D and data not shown), including various other sequences related to nucleic acid metabolism, such as two subunits of the cleavage and polyadenylation specificity factor (CPSF). We propose to name this domain, which we will describe in detail elsewhere, βCASP for metallo-β-lactamase associated *C*PSF *A*rtemis *S*NM1/*P*SO2 domain. This domain, although highly divergent and tolerating multiple insertions (e.g., within yeast PSO2 in Figure 5D), harbors several conserved residues that could play a role in the reaction catalyzed by members of this subfamily. It is tempting to speculate that this domain could contribute to substrate binding, in a similar way as the α-helical domain of glyoxalase, another member of the β-lactamase family (Cameron et al., 1999).

Artemis in the NHEJ Pathway of DNA Repair

DNA dsb can be repaired either by homologous recombination (HR) or by the nonhomologous end joining pathway (NHEJ) (reviewed in Haber, 2000). While HR is the predominant repair pathway in yeast, NHEJ is mostly utilized in higher eukaryotes and represents the DNA repair pathway followed during V(D)J recombination. At least two protein complexes are thought to act in concert or sequentially at the site of the RAG1/2-derived dsb. The Ku70/80 complex is probably recruited first at the site of the lesion, followed by the addition of the DNA-PKcs subunit. This initial complex is considered as the primary DNA damage sensor that will activate the DNA repair machinery. The XRCC4/DNA ligaseIV represents the best candidate to actually repair the gap. One would like to know of course how Artemis intervenes in this cascade. At this point, we can only provide hypotheses based upon the analogy of phenotypes between the various V(D)J recombination-deficient models including the RS-SCID. Two major differences exist between the RS-SCID condition and the XRCC4 and DNA ligaseIV KO mice. First, a complete null allele of Artemis (such as in P6, P15, and P40) does not lead to embryonic lethality in humans in contrast to the murine situation. This observation therefore does not support an implication of Artemis in this phase of NHEJ. Second, the rejoining of linearized DNA constructs introduced in RS-SCID fibroblasts is not altered (unpublished results) while this assay, when defective, is highly diagnostic of abnormal NHEJ in yeast (Teo and Jackson, 1997; Wilson et al., 1997). Perhaps the most evident link between Artemis and NHEJ is found in regard to the Ku/DNA-PK complex. Indeed, human RS-SCID patients and scid mice, which harbor a mutation in the DNA-PKcs encoding gene, are the only two known conditions where a V(D)J recombination-associated DNA repair defect affects uniquely the formation of the coding joints, leaving signal joint formation unaltered, which is in striking contrast with all the other known V(D)J recombination/DNA repair deficiency settings. This raises the interesting possibility that Artemis, through its possible hydrolase activity, may participate in opening the hairpin at the coding ends.

In conclusion, we described the identification and cloning of the gene coding for Artemis, a novel actor of the V(D)J recombination. Mutations of Artemis are causing T-B-SCID defects in humans owing to an absence of repair of the RAG1/2-mediated DNA double-strand break. Artemis belongs to a large family of molecules that adopted the metallo-β-lactamase fold as part of their putative catalytic site. One branch of this family, which also includes yeast SNM1 and murine PSO2, has appended another domain, which we name βCASP, that may target the activity of this subgroup of proteins toward nucleic acids and, thus, serves the purpose of DNA repair. Finally, other domains, yet to be defined, are probably responsible for directing the various Artemis/SNM1/PSO2 proteins to their specific DNA repair pathways, the dsb repair for Artemis or the DNA-ICL repair for SNM1/PSO2.

Experimental Procedures

Patients and Cells

13 RS-SCID patients from 11 families were analyzed in this study having been selected for their typical phenotype of autosomal recessive SCID with complete absence of peripheral T and B lymphocytes, but presence of natural killer cells (Fischer et al., 1997). All the patients showed an impaired V(D)J recombination assay in fibroblasts, and for all RS-SCID families except P16, P40, and P47, radiosensitivity status could be determined on bone marrow cells and/or fibroblasts (Cavazzana-Calvo et al., 1993; Nicolas et al., 1998; Moshous et al., 2000; and our unpublished results). Genotyping of the consanguineous families using polymorphic microsatellite markers, as reported elsewhere (Moshous et al., 2000), concurred in every case with our previously described localization of the RS-SCID gene on chromosome 10p. We studied four patients of French origin (P1, P3, P6, and P15), one of which (P15) was born to related parents, one was of Italian origin (P16), one Greek (P4), and one African (P2). The remaining patients originate from four consanguineous Turkish families, two of them being related (P38, P5, P11, and P12, respectively). Informed consent was obtained from the families prior to this study. Primary fibroblast cell lines were derived from skin biopsies and pseudo-immortalized with SV40 as described elsewhere (Nicolas et al., 1998) and cultured in RPMI 1640 (GIBCO BRL) supplemented with 15% fetal calf serum.

Artemis cDNA Cloning and Genomic Amplification

Chromosome 10 BAC contigs were established by the "Chr10 mapping group" at Sanger center (http://www.sanger.ac.uk/HGP/Chr10). The Chr10 sequences were released by the "Chr10 sequencing group" at Sanger center (http://webace.sanger.ac.uk/cgi-bin/ace/simple/10ace).

Our cloning strategy was based on in silico nucleotide sequence annotation with GENESCAN (Burge and Karlin, 1997) and FGENESH (Salamov and Solovyev, 2000) softwares, two programs aimed at searching for putative peptide encoding genes in large genomic DNA sequences. *Artemis* full-length coding sequence was amplified by polymerase chain reaction (PCR) on cDNA, obtained from reverse transcribed fibroblast total RNA, using the Advantage-GC cDNA PCR Kit (Clontech) according to the manufacturers' recommendations and 0083F1 (5′-GATCGGCGGCGCTATGAGTT-3′) and 169F (5′-TGTCATCTCTGTGCAGGTTT-3′) primers designed from the AA278590 and AI859962 EST sequences (see Results section). The PCR products were sequenced directly on an ABI377 sequencer (Perkin-Elmer) using BigDyeTerminator Cycle Sequencing Ready Reaction (Applied Biosystems) with a series of internal oligonucleotides. Artemis cDNA was subcloned into pIRES-EGFP (Clontech) for subsequent use in transfection experiments. Genomic structure of the *Artemis* gene was deduced by alignment of the cDNA sequence to the draft sequence of the Bac 2K17 (AL360083). Series of oligonucleotide primer pairs were designed for the specific amplification of each exon: Ex1F1 5′-CTCCGGACTCCTCTGATTGG-3′; Ex1R1 5′-GGG

ACAAGGCGTGTGCT-3′; Ex2F1 5′-ACTTGCAGAAGAAAGAG-3′; Ex2R1 5′-AGTGTATTTGGTAGGATTAT-3′; Ex3F1 5′-ATTTTGTGC CAGCGTAA-3′; Ex3R1 5′-ATGAACTCCTGACCTCAAGT-3′; Ex4F1 5′-ACTTTACAATTCTTTCTGTT-3′; Ex4R1 5′-TGAGCATTCAAAA TCA-3′;Ex5F1 5′-AAGGATTCCACTTGTTTCTA-3′; Ex5R1 5′-TGCAG CCTCCAACTC-3′; Ex6F1 5′-CGAGGGAAGAGGTGACAG-3′; Ex6R1 5′-AAACCCCATCTCCACTAAAA-3′; Ex7F1 5′-ATAGTTGGGAGG CTGAGGTA-3′; Ex7R2 5′-CACCTACGGGGACAGTT-3′; Ex8F2 5′-ATATCCTAACTGTCCCCGTAG-3′; Ex8R1 5′-GGCCAACATGG TGAAATG-3′; Ex9F1 5′-GCACTGAATAGCCAAAAACT-3′; Ex9R1 5′-GCCTAGATCGCACCACT-3′; Ex10F1 5′-TTGAGACTCTGCCTA CAACA-3′; Ex10R1 5′-TAGCATCCCCTCCATTTA-3′; Ex11F1 5′-CAC ACGCGGTCTACAA-3′; Ex11R1 5′-GGGGACTACCTGTCAACTAC-3′; Ex12F1 5′-GGAGATCTTTGGGAGTGAG-3′; Ex12R1 5′-ACCTCT CAATTCTGCCACAC-3′; Ex13F1 5′-ATTTGCCACTTTATTACATC-3′; Ex13R1 5′-GCAAAGTACTTCCTGAGAC-3′; Ex14F1 5′-AGATTGGC CTCCCTATTCT-3′; Ex14R1 5′-CCAACCAGGTTATTTGAACA-3′.

Mutation Analysis

Artemis mutation sequence analyses were performed either on cDNA following RT-PCR amplification or on genomic DNA following exon-specific PCR amplification. All PCR products were directly sequenced using BigDyeTerminator Cycle Sequencing Ready Reaction.

RNA Expression Analysis

Human multiple tissue Northern blot (Clontech) was hybridized with P^{32}-labeled Artemis cDNA under manufacturer recommendations. PCR-ready cDNA from several tissues were purchased from Clontech and amplified with Artemis-specific primers 0083F4 (5′-AGC CAAAGTATAAACCACTG-3′) and 169F or manufacturer provided GAPDH primers as control and run onto 1% agarose gels. Artemis-specific PCR products were blotted onto nylon membrane and hybridized with an internal P^{32}-labeled oligonucleotide, whereas the GAPDH PCR control was revealed by ethidium bromide staining.

V(D)J Recombination Assay

V(D)J recombination assay was performed as previously described (Nicolas et al., 1998). Briefly, 5×10^6 exponentially growing SV40-transformed skin fibroblasts were electroporated in 400 μl of culture medium (RPMI1640, 10% FCS) with 6 μg of RAG1 and 4.8 μg of RAG2 encoding expression plasmid together with 2.5 μg of either pHRecCJ (coding joint) or pHRecSJ (signal joint) V(D)J extrachromosomal substrates. These plasmids carry a LacZ gene interrupted by a DNA stuffer flanked by V(D)J recombination signal sequences. Upon recombination, the stuffer DNA is excised and the LacZ gene reassembled, giving rise to blue bacteria colonies when plated onto Xgal/IPTG medium. pARTE-ires-EGFP (2.5μg) was added for complementation analysis. Transfected constructs were recovered after 48 hr, reintroduced into DH10B bacteria, and plated on Xgal/IPTG containing plates. Percentage of recombination was determined by counting blue and white colonies and calculating the ratio. Blue colonies were randomly picked and the plasmid DNA sequenced to analyze the quality of the V(D)J junctions.

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References

Aravind, L. (1997). An evolutionary classification of the metallo-β-lactamase fold. In Silico Biology 1, 0005, http://www.bioinfo.de/isb/1998/01/008.

Barnes, D.E., Stamp, G., Rosewell, I., Denzel, A., and Lindahl, T. (1998). Targeted disruption of the gene encoding DNA ligase IV leads to lethality in embryonic mice. Curr. Biol. *31*, 1395–1398.

Biedermann, K.A., Sum, J.R., Giaccia, A.J., Tosto, L.M., and Brown, J.M. (1991). Scid mutation in mice confers hypersensitivity to ionizing radiation and a deficiency in DNA double-strand break repair. Proc. Natl. Acad. Sci. USA *88*, 1394–1397.

Blunt, T., Gell, D., Fox, M., Taccioli, G.E., Lehmann, A.R., Jackson, S.P., and Jeggo, P.A. (1996). Identification of a nonsense mutation in the carboxyl-terminal region of DNA-dependant protein kinase catalytic subunit in the scid mouse. Proc. Natl. Acad. Sci. USA *93*, 10285–10290.

Bosma, G.C., Custer, R.P., and Bosma, M.J. (1983). A severe combined immunodeficiency mutation in the mouse. Nature *301*, 527–530.

Burge, C., and Karlin, S. (1997). Prediction of complete gene structures in human genomic DNA. J. Mol. Biol. *268*, 78–94.

Cameron, A.D., Ridderstrom, M., Olin, B., and Mannervik, B. (1999). Crystal structure of human glyoxalase II and its complex with a glutathione thiolester substrate analogue. Structure Fold Des. *7*, 1067–1078.

Carfi, A., Pares, S., Duee, E., Galleni, M., Duez, C., Frere, J.M., and Dideberg, O. (1995). The 3-D structure of a zinc metallo-beta-lactamase from Bacillus cereus reveals a new type of protein fold. EMBO J. *14*, 4914–4921.

Cavazzana-Calvo, M., Le Deist, F., de Saint Basile, G., Papadopoulo, D., de Villartay, J.P., and Fischer, A. (1993). Increased radiosensitivity of granulocyte macrophage colony-forming units and skin fibroblasts in human autosomal recessive severe combined immunodeficiency. J. Clin. Invest. *91*, 1214–1218.

Corneo, B., Moshous, D., Callebaut, I., de Chasseval, R., Fischer, A., and de Villartay, J.P. (2000). 3D clustering of human RAG2 gene mutations in severe combined immune deficiency (SCID). J. Biol. Chem. *275*, 12672–12675.

Danska, J.S., Holland, D.P., Mariathasan, S., Williams, K.M., and Guidos, C.J. (1996). Biochemical and genetic defects in the DNA-dependent protein kinase in murine scid lymphocytes. Mol. Cell. Biol. *16*, 5507–5517.

Dronkert, M.L., de Wit, J., Boeve, M., Vasconcelos, M.L., van Steeg, H., Tan, T.L., Hoeijmakers, J.H., and Kanaar, R. (2000). Disruption of mouse SNM1 causes increased sensitivity to the DNA interstrand cross-linking agent mitomycin C. Mol. Cell. Biol. *20*, 4553–4561.

Fischer, A., Cavazzana-Calvo, M., de Saint Basile, G., de Villartay, J.P., Di Santo, J.P., Hivroz, C., Rieux-Laucat, F., and Le Deist, F. (1997). Naturally occurring primary deficiencies of the immune system. Annu. Rev. Immunol. *15*, 93–124.

Frank, K.M., Sekiguchi, J.M., Seidl, K.J., Swat, W., Rathbun, G.A., Cheng, H.L., Davidson, L., Kangaloo, L., and Alt, F.W. (1998). Late embryonic lethality and impaired V(D)J recombination in mice lacking DNA ligase IV. Nature *396*, 173–177.

Fulop, G.M., and Phillips, R.A. (1990). The scid mutation in mice causes a general defect in DNA repair. Nature *347*, 479–482.

Gao, Y., Chaudhuri, J., Zhu, C., Davidson, L., Weaver, D.T., and Alt, F.W. (1998a). A targeted DNA-PKcs-null mutation reveals DNA-PK-independent functions for KU in V(D)J recombination. Immunity *9*, 367–376.

Gao, Y., Sun, Y., Frank, K.M., Dikkes, P., Fujiwara, Y., Seidl, K.J., Sekiguchi, J.M., Rathbun, G.A., Swat, W., Wang, J., et al. (1998b). A critical role for DNA end-joining proteins in both lymphogenesis and neurogenesis. Cell *95*, 891–902.

Gottlieb, T.M., and Jackson, S.P. (1993). The DNA-dependant protein kinase: requirement for DNA ends and association with Ku antigen. Cell *72*, 132–142.

Haber, J.E. (2000). Partners and pathways repairing a double-strand break. Trends Genet. *16*, 259–264.

Hendrickson, E.A., Qin, X.Q., Bump, E.A., Schatz, D.G., Oettinger, M., and Weaver, D.T. (1991). A link between double-strand break related repair and V(D)J recombination: the scid mutation. Proc. Natl. Acad. Sci. USA *88*, 4061–4065.

Henriques, J.A., and Moustacchi, E. (1980). Isolation and characterization of pso mutants sensitive to photo-addition of psoralen derivatives in Saccharomyces cerevisiae. Genetics *95*, 273–288.

Hu, D.C., Gahagan, S., Wara, D.W., Hayward, A., and Cowan, M.J. (1988). Congenital severe combined immunodeficiency disease (SCID) in American Indians. Pediatr. Res. *24*, 239.

Jackson, S.P., and Jeggo, P.A. (1995). DNA double-strand break repair and V(D)J recombination: involvement of DNA-PK. Trends Biochem. Sci. *20*, 412–415.

Jhappan, C., Morse, H.C., Fleischmann, R.D., Gottesman, M.M., and Merlino, G. (1997). DNA-PKcs: a T-cell tumour suppressor encoded at the mouse scid locus. Nat. Genet. *17*, 483–486.

Li, Z.Y., Otevrel, T., Gao, Y.J., Cheng, H.L., Seed, B., Stamato, T.D., Taccioli, G.E., and Alt, F.W. (1995). The Xrcc4 gene encodes a novel protein involved in DNA double-strand break repair and V(D)J recombination. Cell *83*, 1079–1089.

Li, L., Drayna, D., Hu, D., Hayward, A., Gahagan, S., Pabst, H., and Cowan, M.J. (1998). The gene for severe combined immunodeficiency disease in Athabascan-speaking Native Americans is located on chromosome 10p. Am. J. Hum. Genet. *62*, 136–144.

Mombaerts, P., Iacomini, J., Johnson, R.S., Herrup, K., Tonegawa, S., and Papaionnou, V.E. (1992). RAG-1 deficient mice have no mature B and T lymphocytes. Cell *68*, 869–877.

Moshous, D., Li, L., de Chasseval, R., Philippe, N., Jabado, N., Cowan, M.J., Fischer, A., and de Villartay, J.P. (2000). A new gene involved in DNA double-strand break repair and V(D)J recombination is located on human chromosome 10p. Hum. Mol. Genet. *9*, 583–588.

Nicolas, N., Finnie, N.J., Cavazzana-Calvo, M., Papadopoulo, D., Le Deist, F., Fischer, A., Jackson, S.P., and de Villartay, J.P. (1996). Lack of detectable defect in DNA double-strand break repair and DNA-dependant protein kinase activity in radiosensitive human severe combined immunodeficiency fibroblasts. Eur. J. Immunol. *26*, 1118–1122.

Nicolas, N., Moshous, D., Papadopoulo, D., Cavazzana-Calvo, M., de Chasseval, R., Le Deist, F., Fischer, A., and de Villartay, J.P. (1998). A human SCID condition with increased sensitivity to ionizing radiations and impaired V(D)J rearrangements defines a new DNA recombination/repair deficiency. J. Exp. Med. *188*, 627–634.

Nussenzweig, A., Chen, C., da Costa Soares, V., Sanchez, M., Sokol, K., Nussenzweig, M.C., and Li, G.C. (1996). Requirement for Ku80 in growth and immunoglobulin V(D)J recombination. Nature *382*, 551–555.

Oettinger, M.A., Schatz, D.G., Gorka, C., and Baltimore, D. (1990). RAG-1 and RAG-2, adjacent genes that synergistically activate V(D)J recombination. Science *248*, 1517–1523.

Robins, P., and Lindahl, T. (1996). DNA ligase IV from HeLa cell nuclei. J. Biol. Chem. *271*, 24257–24261.

Salamov, A.A., and Solovyev, V.V. (2000). Ab initio gene finding in Drosophila genomic DNA. Genome Res. *10*, 516–522.

Schatz, D.G., Oettiger, M.A., and Baltimore, D. (1989). The V(D)J recombination activating gene, RAG-1. Cell *59*, 1035–1048.

Schwarz, K., Gauss, G.H., Ludwig, L., Pannicke, U., Li, Z., Linder, D., Friedrich, W., Seger, R.A., Hansen-Hagge, T.E., Desiderio, S., Lieber, M.R., and Bartram, C.R. (1996). RAG mutations in human B cell-negative SCID. Science *274*, 97–99.

Shin, E.K., Perryman, L.E., and Meek, K. (1997). A kinase-negative mutation Of DNA-Pkcs in equine Scid results in defective coding and signal joint formation. J. Immunol. *158*, 3565–3569.

Shinkai, Y., Rathbun, G., Lam, K.P., Oltz, E.M., Stewart, V., Mendelsohn, M., Charron, J., Datta, M., Young, F., Stall, A.M., and Alt, F.W. (1992). RAG-2 deficient mice lack mature lymphocytes owing to inability to initiate V(D)J rearrangement. Cell *68*, 855–867.

Taccioli, G.E., Rathbun, G., Oltz, E., Stamato, T., Jeggo, P.A., and Alt, F.W. (1993). Impairement of V(D)J recombination in double-strand break repair mutants. Science *260*, 207–210.

Taccioli, G.E., Amatucci, A.G., Beamish, H.J., Gell, D., Xiang, X.H., Torres Arzayus, M.I., Priestley, A., Jackson, S.P., Marshak Rothstein, A., Jeggo, P.A., and Herrera, V.L. (1998). Targeted disruption of the catalytic subunit of the DNA-PK gene in mice confers severe combined immunodeficiency and radiosensitivity. Immunity *9*, 355–366.

Teo, S.H., and Jackson, S.P. (1997). Identification of Saccharomyces cerevisiae Dna ligase .4. Involvement in Dna double-strand break repair. EMBO J. *16*, 4788–4795.

Tonegawa, S. (1983). Somatic generation of antibody diversity. Nature *302*, 575–581.

Villa, A., Sobacchi, C., Notarangelo, L.D., Bozzi, F., Abinun, M., Abrahamsen, T.G., Arkwright, P.D., Baniyash, M., Brooks, E.G., Conley, M.E., et al. (2001). V(D)J recombination defects in lymphocytes due to RAG mutations: severe immunodeficiency with a spectrum of clinical presentations. Blood *97*, 81–88.

Wilson, T.E., Grawunder, U., and Lieber, M.R. (1997). Yeast DNA ligase IV mediates non-homologous DNA end joining. Nature *388*, 495–498.

Zhu, C., Bogue, M.A., Lim, D.S., Hasty, P., and Roth, D.B. (1996). Ku86-deficient mice exhibit severe combined immunodeficiency and defective processing of V(D)J recombination intermediates. Cell *86*, 379–389.

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