The B cell defect in –/– mice closely resembles that seen in IL-7 receptor–deficient mice (*4*) and, thus, is most likely due to a lack of IL-7 receptor signaling. Comparisons of γ_c deficiencies in mice and humans have suggested that IL-7 plays a greater role for mouse as compared with human B cell development, as X-linked severe combined immunodeficiency (XSCID) patients (γ_c-deficient) have normal numbers of B cells (*11*). This differential requirement for γ_c-dependent cytokine signaling during B cell development is further supported by the normal numbers of B cells found in a SCID Jak3-deficient patient (*12*).

The phenotype of –/– thymuses is comparable with that observed for thymuses in IL-7 receptor–deficient (*4*) and γ_c-deficient (*10*, *13*) mice. However, both the thymuses and the spleens of –/– mice show an increase in the ratio of $CD4^+$ cells to $CD8^+$ cells. This observation suggests that cytokine signaling may be more critical for the maturation or survival of CD8 lineage T cells than CD4 lineage T cells.

Previous biochemical studies of Jak3 indicated an association with IL-2 receptor signaling (*1*, *2*). Furthermore, inhibitors of IL-2 receptor signaling block T cell proliferative responses (*14*). Our results suggest that Jak3 is essential for IL-2 receptor signaling in primary T cells. However, Jak3 may not be the only tyrosine kinase required for IL-2 receptor signaling, because both Jak1 and $p56^{lck}$ associate with the IL-2R β chain (*2*, *15*).

Splenic T cells from –/– mice, when stimulated, produce much less IL-2 than wild-type splenic T cells. This reduction in IL-2 secretion may result from a nonresponsiveness, or anergy, induced in the –/– T cells as a result of prior T cell receptor stimulation in the absence of Jak3. This possibility is compatible with previous studies showing that activation of Jak3 is associated with the prevention of anergy (*16*). Consistent with this, we also observed increased expression of activation markers on the vast majority of –/– splenic T cells, suggesting a prior T cell receptor activation event. One possibility is that most T cells routinely encounter a T cell receptor stimulation signal, perhaps as a component of the mechanism inducing thymic emigration (*17*), and that –/– T cells are deficient in returning to the normal resting state. These unusual aspects of T cell development and differentiation in –/– mice indicate previously undescribed functions of cytokine receptor signaling pathways.

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7. D. C. Thomis, A. H. Sharpe, L. J. Berg, data not shown.
8. For flow cytometry, 5×10^5 cells were stained with antibodies to IgM, CD43, Mac1, CD8, CD3, CD69, CD44 (Pharmingen), CD45R (antibody B220), CD4, CD25 (Gibco-BRL), Thy1.2 (Caltag), or CD28 [J. A. Gross, E. Callas, J. P. Allison, *J. Immunol.* **149**, 380 (1992)] and analyzed on a FACScan (Becton Dickinson). All profiles shown were gated on viable cells by forward and side scatter parameters, unless otherwise noted.
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Mutation of Jak3 in a Patient with SCID: Essential Role of Jak3 in Lymphoid Development

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Males with X-linked severe combined immunodeficiency (XSCID) have defects in the common cytokine receptor γ chain (γ_c) gene that encodes a shared, essential component of the receptors for interleukin-2 (IL-2), IL-4, IL-7, IL-9, and IL-15. The Janus family tyrosine kinase Jak3 is the only signaling molecule known to be associated with γ_c, so it was hypothesized that defects in Jak3 might cause an XSCID-like phenotype. A girl with immunological features indistinguishable from those of XSCID was therefore selected for analysis. An Epstein-Barr virus (EBV)–transformed cell line derived from her lymphocytes had normal γ_c expression but lacked Jak3 protein and had greatly diminished Jak3 messenger RNA. Sequencing revealed a different mutation on each allele: a single nucleotide insertion resulting in a frame shift and premature termination in the Jak3 JH4 domain and a nonsense mutation in the Jak3 JH2 domain. The lack of Jak3 expression correlated with impaired B cell signaling, as demonstrated by the inability of IL-4 to activate Stat6 in the EBV-transformed cell line from the patient. These observations indicate that the functions of γ_c are dependent on Jak3 and that Jak3 is essential for lymphoid development and signaling.

The γ_c chain is an essential signaling component of receptors for IL-2 (*1*), IL-4 (*2*, *3*), IL-7 (*4*, *5*), IL-9 (*6*, *7*), and IL-15 (*8*). Defects in this chain cause XSCID (*9*, *10*), a disease characterized by impaired function of B cells and a complete or almost complete deficiency of T cells (*10*). Thus, XSCID occurs as a result of inactivation of numerous cytokine signaling pathways, an observation compatible with the severe phenotype of XSCID (*9*, *10*) and of γ_c-deficient mice (*11*). IL-2, IL-4, IL-7, IL-9, and IL-15 all activate the same Janus family kinases, Jak1 and Jak3 (*6*, *12*). In each case, Jak1 associates with the receptor chain that plays the major role in determining both the cytokine binding specificity (*6*, *13*) and the STAT (signal transducers and activators of transduction) proteins that are activated (*14*, *15*). Jak3 is primarily associated with γ_c, and as no other signaling molecules have been identified that associate with γ_c, we hypothesized that the phenotype resulting from defects in Jak3 might be indistinguishable from that resulting from defects in γ_c, and that the key role of γ_c may be to bring Jak3 into proximity with the primary binding chain and its associated signaling molecules (*6*).

Although true XSCID ($\gamma_c^{-/-}$) females

could be born [for example, to XSCID carrier ($\gamma_c^{+/-}$) females and healthy ($\gamma_c^{+/Y}$) fathers in whom a γ_c mutation occurred during spermatogenesis], such individuals would be expected to be quite rare. Because the gene for Jak3 is not on the X chromosome (*16*), we hypothesized that females with clinical and immunological features typical of XSCID might instead have defects in Jak3. A girl with features typical of XSCID was identified (Table 1, patient AP), and an EBV-transformed B cell line was generated from her peripheral blood lymphocytes (PBLs). We then examined protein lysates from these cells for γ_c expression (Fig. 1A). In contrast to EBV-transformed cells from a γ_c-deficient XSCID patient, AP had γ_c expression equivalent to the normal control EBV-transformed cell line from individual PN. AP also had normal expression of Jak1 (Fig. 1B); however, AP did not express detectable amounts of Jak3 protein (Fig. 1C). Protein immunoblot analysis of lysates from EBV-transformed cells derived from the parents (GP and JP, both of whom were healthy) showed substantial Jak3 expression (Fig. 1D). The lack of Jak3 in AP was explained by the observation that Jak3 mRNA was undetectable in AP cells by Northern (RNA) blot analysis (Fig. 1E) in contrast to γ_c mRNA, which was normal (Fig. 1F).

Despite the absence of Jak3 mRNA as detected by Northern blot, trace amounts of Jak3 RNA were identified by reverse transcriptase–polymerase chain reaction (RT-PCR) after 40 cycles of amplification, allowing us to sequence the entire coding region of Jak3 from both AP and normal controls. Consistent with this girl's form of SCID being an autosomal trait in which a different mutation was inherited from each parent, she had mutations at two positions (m1 and m2, Fig. 2A) (*17*). A single base insertion of a G nucleotide was found in the JH4 domain at nucleotide 1172 (amino acid 391) that resulted in a frame shift and subsequent stop codon at amino acid 408 (m1, Fig. 2B), and a point mutation was found at nucleotide 1695 (amino acid 565) in the JH2 domain that converts TGC (encoding Cys) to TGA (stop codon) (m2, Fig. 2C). These observations may explain why Jak3 RNA was not detectable by Northern analysis, as premature stop codons frequently cause reduced RNA expression [as is the case with the XSCID patient of Fig. 1, C and E (*18*)] through a number of mechanisms (*19*). Thus, it was assumed that the amount of RNA derived from the mutant allele would be much less than that derived from the wild-type allele in AP's parents, so genomic DNA from EBV-transformed cells from the mother (JP) and father (GP) was amplified. Direct sequencing indicated a homozygous TGC codon in PN and GP, whereas AP and JP were both heterozygous for TGC and TGA at this position, indicating that the stop codon mutation (m2) was inherited from the mother, and the insertion mutation (m1) was inherited from the father (*18*).

The correlation between the lack of Jak3 expression and greatly diminished T cells in this patient indicates a role for Jak3 in T cell development. This diminished immunoglobulin synthesis makes sense in view of the diminished T cell help in the patient. To determine whether there was also an intrinsic B cell defect, we investigated whether Stat6 could be activated by IL-4 in the EBV-transformed B cell line from AP. Stat6 was activated by IL-4 in the PN but not in the AP EBV line (Fig. 3A), demonstrating an apparent defect in B cell signaling and that Jak3 was required for Stat6 activation in these cells. Stat6 is ubiquitously expressed (*14*), and the lack of Stat6 activation by IL-4 was not due to a lack of Stat6 protein in AP cells (Fig. 3B). We also observed the same defect in Stat6 activation by IL-4 in XSCID cell lines (*18*). Thus, the defects in B cell function seen in the patient AP and in XSCID patients are probably due to deficiencies in B cell signaling as well as to deficiencies in T cell help. It is interesting that B cell numbers are normal or increased in humans with XSCID and Jak3 deficiency, but are essentially absent in mice with γ_c deficiency (*11*) and in young (4- to 6-week-old) mice with Jak3 deficiency (*20*). This is probably because IL-7 is an essential pre–B cell growth factor in mice but not in humans (*21*).

Table 1. Comparison of clinical and immunological parameters of patient AP and normal control individuals. AP was healthy until age 4 months and then developed recurrent otitis media, frequent diarrhea, poor weight gain, and two episodes of monilial dermatitis. At age 9.5 months, she developed a right upper lobe pneumonia and was found to have hypogammaglobulinemia, lymphopenia, and a lack of visible or palpable lymphoid tissue. At 10 months of age, she was referred to Duke University Medical Center for possible bone marrow transplantation. Immunologic evaluation revealed normal serum immunoglobulin M (IgM) but no detectable serum IgG or IgA. Her total lymphocyte count was 2,912/mm^3 (normal >4,500/mm^3), of which 89% were CD20+ B cells and 7% were CD3$^+$ T cells. Her lymphocytes exhibited low responses to mitogens and allogenic cells in [^{3}H]thymidine incorporation assays. Although 1% of her peripheral blood lymphocytes was CD16$^+$, natural killer (NK) cell functional studies revealed no killing of K562 erythroleukemia cells, analogous to the absence of NK cells in XSCID. For all assays, n = 167 for normal controls. N.d., not detected; PHA, phytohemagglutinin; Con A, concanavalin A; PWM, pokeweed mitogen.

	Concentration of serum Ig	
Serum Ig	Patient	Controls
IgG	n.d.	192–515 mg/dl
IgA	n.d.	12–31 mg/dl
IgM	50 mg/dl	39–92 mg/dl
IgE	2 IU/ml	0–222 IU/ml
	Lymphocyte subtype abundance (cells/mm^3)	
Lymphocyte subtype	Patient	Controls
CD3	204	1,014–5,784
CD4	204	567–3,725
CD8	58	390–2,673
CD16	29	87–1,189
CD20	2,592	121–1,072
	Lymphocyte proliferation (cpm)	
Treatment	Patient	Controls*
Medium	632	693 ± 895
PHA	8,221	222,330 ± 52,592
Con A	12,357	208,534 ± 53,751
PWM	2,621	135,520 ± 35,077
Autologous cells	11,191	3,163 ± 2,874
Allogenic cells	15,337	59,818 ± 38,445

*The values are given as mean ± SD, n = 167.

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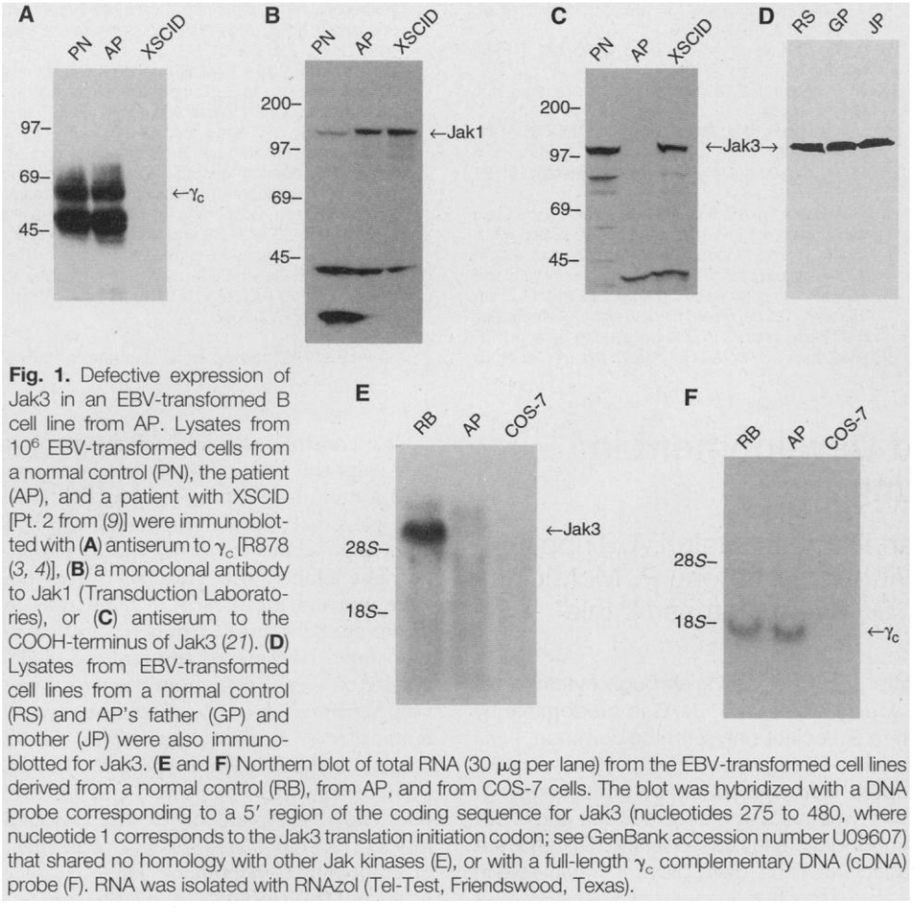

Fig. 1. Defective expression of Jak3 in an EBV-transformed B cell line from AP. Lysates from 10^6 EBV-transformed cells from a normal control (PN), the patient (AP), and a patient with XSCID [Pt. 2 from (*9*)] were immunoblotted with (**A**) antiserum to γ_c [R878 (*3*, *4*)], (**B**) a monoclonal antibody to Jak1 (Transduction Laboratories), or (**C**) antiserum to the COOH-terminus of Jak3 (*21*). (**D**) Lysates from EBV-transformed cell lines from a normal control (RS) and AP's father (GP) and mother (JP) were also immunoblotted for Jak3. (**E** and **F**) Northern blot of total RNA (30 μg per lane) from the EBV-transformed cell lines derived from a normal control (RB), from AP, and from COS-7 cells. The blot was hybridized with a DNA probe corresponding to a 5′ region of the coding sequence for Jak3 (nucleotides 275 to 480, where nucleotide 1 corresponds to the Jak3 translation initiation codon; see GenBank accession number U09607) that shared no homology with other Jak kinases (E), or with a full-length γ_c complementary DNA (cDNA) probe (F). RNA was isolated with RNAzol (Tel-Test, Friendswood, Texas).

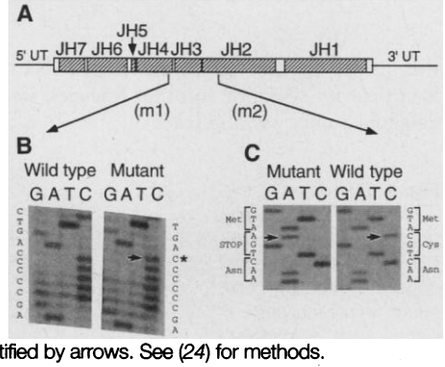

Fig. 2. Analysis of the Jak3 defects in AP. (**A**) Schematic of the coding region for Jak3 (boxed) and 5′ and 3′ untranslated (UT) regions. The seven Jak homology (JH) domains are indicated by hatched boxes, and the positions of the two mutations (m1, insertion at nucleotide 1172; m2, nonsense mutation at nucleotide 1695) identified in AP are shown. (**B**) Sequencing of the m1 insertion (sequenced on the reverse strand). Compare the AP mutation on the right [the band corresponding to the sixth C (asterisk) is identified by an arrow] to the wild type on the left. (**C**) Sequencing of the m2 nonsense mutation that resulted from a C-to-A transversion. The bands corresponding to the normal (wild-type, at right) and mutated (AP, at left) nucleotides are identified by arrows. See (*24*) for methods.

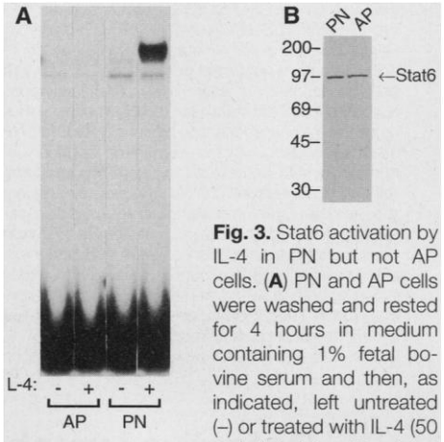

Fig. 3. Stat6 activation by IL-4 in PN but not AP cells. (**A**) PN and AP cells were washed and rested for 4 hours in medium containing 1% fetal bovine serum and then, as indicated, left untreated (–) or treated with IL-4 (50 ng/ml) (+) for 15 min at 37°C. Total cell extracts were analyzed by electrophoretic mobility shift assays with a ^{32}P-labeled Fc interferon-γ–activated site (GAS) probe derived from the FcγRI promoter as described (*15*). The IL-4–induced complex was supershifted by antiserum to Stat6, indicating that the complex contained Stat6 (*19*). (**B**) Protein lysates from PN and AP cells were immunoblotted with antiserum to Stat6 (Santa Cruz, Santa Cruz, California) to show equivalent expression of Stat6 in these cells.

The identification of defective Jak3 expression in a case of SCID allows a number of conclusions to be drawn. First, the diminished number of T and natural killer (NK) cells indicates the important role played by Jak3 in the development of these lineages. Second, because the phenotype of Jak3-deficient SCID is indistinguishable from that of XSCID, most if not all γ_c-dependent signals may be dependent on Jak3. Third, the observation that the Jak3 deficiency was not more severe than XSCID suggests that Jak3 may not be essential for signaling from cytokines other than those whose receptors contain γ_c. The cell type–restricted expression of Jak3 (*22*), and the fact that Jak3 appears only to be required for signaling through γ_c-containing receptors, is in contrast to other Jak family kinases, which are ubiquitously expressed and associate with multiple cytokine receptors. Fourth, the lack of Stat6 activation by IL-4 in AP cells indicates that Jak3 plays an important role in STAT protein activation; indeed, it is possible that the loss of STAT protein activation significantly contributes to the phenotype in XSCID and Jak3-deficient SCID. Because STAT activation is only one type of signal induced by IL-2, IL-4, IL-7, IL-9, and IL-15, it will be interesting to determine the role of Jak3 in activating the other signaling pathways.

The possibility of using pharmacological agents that block γ_c-Jak3 association as immunosuppressants has been suggested (*6*). The current study further suggests that any agents that inactivate Jak3 function may be potent immunosuppressants. Moreover, the identification of Jak3 deficiency as the molecular basis for some autosomal recessive cases of SCID will allow the development of diagnostic procedures for identification of Jak3-deficient SCID patients and carriers and investigation into the possibility of gene therapy for Jak3-deficient SCID patients, analogous to that being developed for XSCID (*23*).

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17. As compared to the published Jak3 sequence, in normal controls we found the following differences: Ala^{34}-Pro^{35} (GCC CCG) → Gly-Pro (GGC CCC); Ala^{212} (GCT) → Arg (CGT); Pro^{222} (CCG) → Arg (CGC); Ala^{573} (GCG) → Glu (GAG); Ala^{845}-His^{846}

(GCC CAC) → Gly-Asp (GGC GAC); and Arg^{895}-Pro^{896}-Glu^{897} (CCG CCA GAG) → Arg-Gln-Ser (CGC CAG AGC). It should be noted that some transcripts from the patient contained insertions (67, 78, or 114 nucleotides) after nucleotide 1441. These insertions appeared to arise by alternative splicing because they represent intronic sequences beginning 230, 194, or 183 nucleotides 3′ of the exon-intron boundary at nucleotide 1441 and extend to a common endpoint. Although the insertions contain in-frame stop codons, the alternative splicing does not represent a disease-causing difference in AP as no mutations in the intron or surrounding exon sequence were found, and these splice variants were also found in other RNA samples including both of AP's parents and PBLs from a normal donor. The physiological effects, if any, of this alternative splicing are unknown.

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24. RNA was prepared with RNAzol, reverse-transcribed with Superscript II reverse transcriptase (Gibco BRL), and PCR-amplified in overlapping regions of 400 to 1200 base pairs from AP and normal controls (PN and RB) with Taq polymerase (Gibco BRL). Direct PCR sequencing was performed on both strands with the Applied Biosystems 373A DNA Sequencing System. PCR products were also subcloned with the TA Cloning kit (Invitrogen), and multiple clones were sequenced with Sequenase (USB, Amersham) to generate the gels shown in Fig. 2, B and C.

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Defective Lymphoid Development in Mice Lacking Jak3

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The Janus tyrosine kinases (Jaks) play a central role in signaling through cytokine receptors. Although Jak1, Jak2, and Tyk2 are widely expressed, Jak3 is predominantly expressed in hematopoietic cells and is known to associate only with the common γ (γ_c) chain of the interleukin (IL)–2, IL-4, IL-7, IL-9, and IL-15 receptors. Homozygous mutant mice in which the *Jak3* gene had been disrupted were generated by gene targeting. Jak3-deficient mice had profound reductions in thymocytes and severe B cell and T cell lymphopenia similar to severe combined immunodeficiency disease (SCID), and the residual T cells and B cells were functionally deficient. Thus, Jak3 plays a critical role in γ_c signaling and lymphoid development.

The Jaks have been implicated in the function of receptors of the cytokine receptor superfamily (*1*). After ligand binding, the Jaks are activated by tyrosine phosphorylation, and in turn they phosphorylate one or more of the receptor chains as well as cellular substrates. Jak3 is predominantly expressed in hematopoietic cells, associates with the γ_c chain, and is activated by the cytokines IL-2, IL-4, IL-7, IL-9, and IL-15 that use the γ_c chain (*1–3*). These cytokines control lymphoid differentiation and functions. Mutations of the γ_c chain are associated with human X-linked SCID (*4*) and account for approximately half of the cases of human SCID (*5*). The γ_c mutations affect receptor-mediated ligand activation of Jak3 (*3*), although it is unknown whether this effect is critical for the SCID phenotype. To address this question and to determine whether Jak3 is critical for signaling in other lineages, we developed mice lacking Jak3.

We constructed a *Jak3* targeting vector to disrupt the first coding exon, which created a null allele for expression (Fig. 1A). The construct was electroporated into E14 embryonic stem (ES) cells, and four independent clones with normal karyotypes were injected into C57BL/6 blastocysts to create chimeric mice. Chimeric mice from two clones transmitted the targeted allele, and heterozygous mice from the two clones were separately bred to create homozygous mutant (−/−) mice. Genotyping of 59 progeny yielded 16 wild-type (+/+) mice, 30 heterozygous (+/−) mice, and 13 −/− mice; this correspondence to the expected ratios (1:2:1) indicated the lack of an effect on embryonic development. The −/− mice were indistinguishable from their littermates and thrived comparably under specific pathogen–free conditions. No Jak3 protein was detectable in splenic, thymic, or bone marrow extracts from the −/− mice (*6*).

Morphologically, the lymphoid tissues of the −/− mice were markedly different from

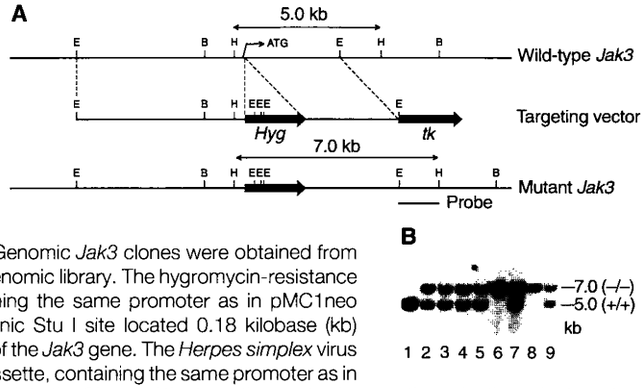

Fig. 1. Disruption of the *Jak3* gene by homologous recombination. (**A**) Maps of the *Jak3* locus (top), the targeting construct in pBluescript II (Stratagene) (middle), and the targeted locus (bottom). Restriction enzymes were Eco RI (E), Bam HI (B), and Hind III (H). Genomic *Jak3* clones were obtained from a 129-derived CCE ES cell genomic library. The hygromycin-resistance gene (*Hyg*) cassette, containing the same promoter as in pMC1neo (*13*), was inserted in an exonic Stu I site located 0.18 kilobase (kb) downstream of the first ATG of the *Jak3* gene. The *Herpes simplex* virus thymidine kinase (*tk*) gene cassette, containing the same promoter as in the *Hyg* cassette, was inserted into the 3′ end of the targeting vector for negative selection. The targeting vector contained 5.8- and 3.2-kb *Jak3* fragments. Electroporation of the linearized plasmid into 129-derived E14 ES cells and screening by Southern (DNA) blot analysis for homologous recombination were as described (*14*); the 3′ flanking probe used in Southern blot analysis is shown as a bar. The efficiency of homologous recombination was 96%. (**B**) Southern blot analysis of mouse tail DNA. Genomic DNA from +/+ mice (lane 1), +/− mice (lanes 2 through 5, 7, and 9), and −/− mice (lanes 6 and 8) was digested with Hind III and probed with a 1.4-kb Eco RI–Hind III fragment. The 5.0- and 7.0-kb bands represent the wild-type and mutated alleles, respectively. When the blot was rehybridized with a *Hyg* probe, only the 7.0-kb band was detected (*15*). Bam HI digests probed with another 3′ probe and Eco RI digests probed with a 0.85-kb Sst I complementary DNA probe containing the first ATG further confirmed appropriate homologous recombination (*15*).

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