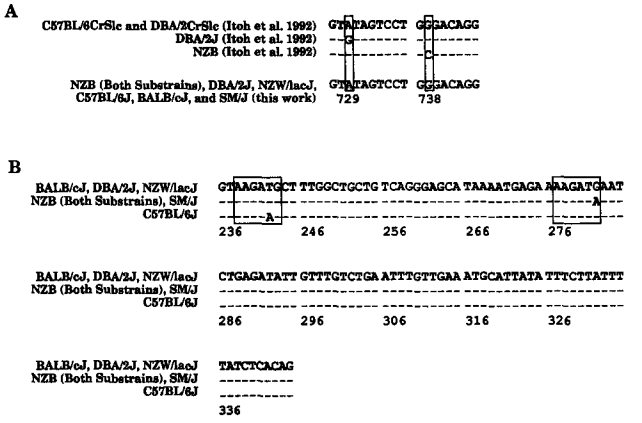

Fig. 1. **(A)** Shows the region between base pairs 727 and 745, which is the region that was previously reported to show NZB and DBA/2J polymorphisms [1]. In the current study, sequence analysis showed no polymorphism among the different strains in this region or in any other part of the coding sequence. **(B)** shows intronic sequence (residues 236 to 345). A number of forward and reverse sequencing primers were used to verify these results.

A correlation with different backcross analyses indicated that these intronic polymorphisms, even though they appeared to correlate with mRNA expression and serum SAP protein levels [1], could not account for the NZB disease contribution on distal Chr 1. Thus, the NZB contribution to lupus-like disease previously mapped near *Sap* in (NZB × SM/J)F_1 × NZW backcross mice cannot be due to an intronic SAP polymorphism since there were no differences between NZB and SM/J. Overall, our studies in mice confirm previous studies in humans that show the absence of structural polymorphism in human SAP [2], and suggest that this gene is unlikely to make a genetic contribution to murine lupus.

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References

1. Itoh, Y., Takeuchi, S., Shigemoto, K., Kubo, S., Handa, S., Ishikawa, N., Maruyama, N. (1992). Biochim. Biophys. Acta 1131, 261–269.
2. Pepys, M.B., Baltz, M.L. (1983). Adv. Immunol. 34, 141–212.
3. Drake, C.G., Rozzo, S.J., Hirschfeld, H.F., Smarnworawong, N.P., Palmer, E., Kotzin, B.L. (1995). J. Immunol. 154, 2441–2447.
4. Morel, L., Rudofsky, U.H., Longmate, J.A., Schiffenbauer, J., Wakeland, E.K. (1994). Immunity 1, 219–229.

LAF4 maps to mouse Chromosome 1 and human Chromosome 2q11.2–q12

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Species: Mouse and human

Locus name: *LAF4* (*lymphoid nuclear protein related to AF4*) [1] is related to *MLLT2* (also referred to as *AF4*) [2], the gene that is fused to the *MLL* gene (also termed *ALL-1, HRX,* and *HTRX*) in t(4;11)(q21;q23) acute lymphoblastic leukemia (ALL). *LAF4* encodes a 135-kDa tissue-restricted nuclear transcriptional activator preferentially expressed in lymphoid tissues [1].

Locus symbol: *Laf4* (mouse); *LAF4* (human)

Map position: *Laf4* is localized to the proximal region of mouse chromosome (Chr) 1: centromere–*Dst* (previously referred to as *Bpag1*)–(1.5 ± 1.0)–*Laf4*–(0.7 ± 0.7)–*Il1rl* (Fig. 1a). *LAF4* is localized on human Chr 2q11.2–q12 (Fig. 1b).

Methods of mapping: The mouse chromosomal location of *Laf4* was determined by interspecific backcross analysis, with 205 (C57BL/6J × *Mus spretus*) F_1 × C57BL/6J backcross mice [3]. The human chromosomal localization of *LAF4* was determined by fluorescence *in situ* hybridization (FISH) [4]. The biotin-labeled probe used for FISH analysis was detected with avidin-FITC and located relative to simultaneously visible fluorescent R-bands on normal human metaphases. Ten metaphases were analyzed for each clone.

Database deposit information: Genbank Accession Numbers, *LAF4* (U34360); *Laf4* (U34361).

Molecular reagents: The mouse chromosomal localization of *Laf4* was determined with a mouse *Laf4* cDNA probe. The probe contains 39 bp of the 5′ untranslated region and the first 2004 bp of the

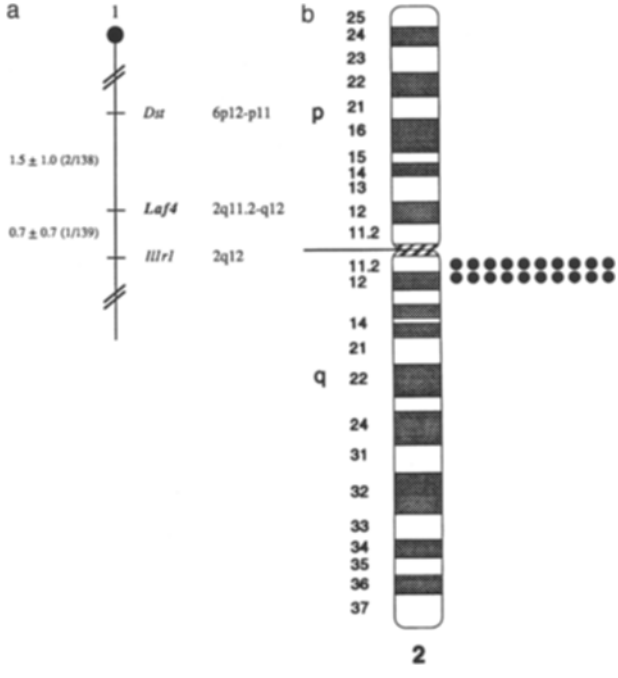

Fig. 1a. *Laf4* maps to the proximal region of mouse Chr 1. *Laf4* was mapped by interspecific backcross analysis. The linkage map shows the location of *Laf4* in relation to the linked loci. The positions of human homologous loci are shown on the right. Recombination distances in cM (± one standard error), shown on left, were calculated as described [8]. The numbers in parentheses indicate the ratios of the total number of mice exhibiting recombinant chromosomes to the total number of mice analyzed for each pair of loci. **(b)** An idiogram of human Chr 2 showing the location of signals after FISH with a lambda genomic clone positive for *LAF4*. Each dot represents signals observed on both chromatids. Ten metaphases were analyzed; thus, hybridization efficiency was 100%. All signals were observed in the region 2q11.2–q12. No specific signals were seen on other chromosomes.

Laf4 coding sequence subcloned into the *Eco*RI site of pBluescript vector SK- (Stratagene) [1]. *Laf4*-linked loci, *Dst* and *Il1rl*, were typed previously [5,6]. The DNA probes used for FISH were isolated from a human genomic library cloned in the FIXII vector (Stratagene) by screening with a human *LAF4* cDNA clone (E41) [1], which contains a 1.9-kb insert including the 5′ end of the gene. Two independent clones (with 17-kbp and 18-kbp inserts, respectively) were biotin labeled by nick translation.

Allele detection: The mouse *Laf4* cDNA probe detected major fragments of 7.2 kb and 5.9 kb in *Eco*RI-digested C57BL/6J DNA and major fragments of 18.0 kb and 4.4 kb in *Eco*RI-digested *M. spretus* DNA. The presence or absence of the *M. spretus*-specific fragments, which cosegregated, was followed in backcross mice.

Previously identified homologs: None

Discussion: The *MLL* gene is translocated to more than ten different fusion loci in human leukemias resulting in chimeric proteins with virtually identical *MLL* amino termini (for review, [7]). The various *MLL* fusion partners are all novel proteins. It is important to identify and characterize the molecular nature of the *MLL* fusion partners, since they seem to correlate with the lineage restriction of the leukemia [7]. The *MLL/MLLT2* translocation is found in approximately 50% of ALL in children under one year of age [7]. *MLL* is a human homolog of the *Drosophila trithorax* gene [2]; *MLLT2* shows no strong similarity to previously characterized genes. *LAF4* encodes a lymphoid nuclear protein of 1227 amino acids with transactivation potential [1]. *LAF4* is similar to *MLLT2* throughout its entire coding region; the greatest similarity is within an amino terminal region (75% homology over 69 amino acids) and a carboxy terminal region (62% homology over 244 amino acids). The isolation of *LAF4* has defined a highly conserved *LAF4/MLLT2* gene family of nuclear transcription factors that may function in lymphoid development and oncogenesis. The mapping of *LAF4* provides a foundation for further characterization of the gene and isolation of other members of this novel *LAF4/MLLT2* gene family.

References

1. Ma, C., Staudt, L.M. (1996). *LAF4* encodes a lymphoid nuclear protein with transactivation potential that is homologous to *AF-4*, the gene fused to *MLL* in t(4;11) leukemia. Blood, *in press.*
2. Gu, Y., Nakamura, T., Alder, H., Prasad, R., Canaani, O., Cimino, G., Croce, C.M., Canaani, E. (1992). The t(4;11) chromosome translocation of human acute leukemias fuses the *ALL-1* gene, related to Drosophila trithorax, to the *AF4* gene. Cell 71, 701–708.
3. Copeland, N.G., Jenkins, N.A. (1991). Development and applications of a molecular genetic linkage map of the mouse genome. Trends Genet. 7, 113–118.
4. Trask, B.J. (1996). Fluorescence in situ hybridization: a review of the role of FISH in genome analysis, supporting background information, and detailed protocols. In *Genome Analysis: A Laboratory Manual,* B. Birren, E. Green, P. Hieter, R. Myers, eds. (Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory Press), *in press.*
5. Copeland, N.G., Gilbert, D.J., Li, K., Sawamura, D., Giudice, G.J., Chu, M.L., Jenkins, N.A., Uitto, J. (1993). Chromosomal localization of mouse bullous pemphigoid antigens BPAG1 and BPAG2: identification of a new region of homology between mouse and human chromosome. Genomics 15, 180–181.
6. Copeland, N.G., Silan, C.M., Kinsley, D.M., Jenkins, N.A., Cannizzaro, L.A., Croce, C.M., Huebner, K., Sims, J. (1991). Chromosomal location of murine and human IL-1 receptor genes. Genomics 9, 44–50.
7. Rowley, J.D. (1993). Rearrangements involving chromosome band 11q23 in acute leukemia. Semin. Cancer Biol. 4, 377–387.
8. Green, E.L. (1981). Linkage, recombination and mapping. In *Genetics and Probability in Animal Breeding Experiments,* New York: Oxford Univ. Press, pp. 77–113.

Comparative mapping of the reeler gene on human Chromosome 7q22, rat Chromosome 4q11.2, and mouse Chromosome 5 A3-B1

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Species: Human, rat, mouse
Locus name: reeler
Locus symbol: *rl*
Map position: Human Chromosome (Chr) 7q22
Rat Chr 4q11.2
Mouse Chr 5A3-B1

Method of mapping: For physical mapping, R-banded chromosomes of human, rat, and mouse and FISH were prepared as reported elsewhere [1,2]. The chromosome slides were hardened at 65°C for 2 h, denatured at 70°C in 70% formamide in 2 × SSC, and dehydrated in 70–85–100% ethanol series at 4°C. Biotin-labeled mouse 5-kb cDNA fragments, 250 ng, were denatured and hybridized to the chromosome slides at 37°C overnight in 20 μl of 50% formamide, 2 × SSC, 10% dextran sulfate, and 1 mg/ml BSA (Sigma). The slides were washed, then treated with anti-biotin antibody (Vector) at 1:500 dilution in 1% BSA/ 4 × SSC for 1 h at 37°C, and then stained with fluorescein-antigoat IgG (Nordic Immunology) at 1:500 dilution for 1 h at 37°C. After washing, they were stained with 0.75 μg/ml propidium iodide and examined under excitation wavelength of 450–490 nm (Nikon filter set B-2A), 510–560 nm (G-2A), and near 365 nm (UV-2A).

For genetic mapping, PCR-SSCP analysis was performed with BXD recombinant inbred strains. PCR amplification of genomic DNA with primers DIF1-DIR1 and DIF2-DIR2 generates 2.3-kbp and 2.0-kbp fragments, respectively, which correspond to the introns flanking the exon (nucleotides 3314–3533 of the published gene sequence [4]). These fragments were digested by *Hae*III, followed by kination reaction for end labeling. The labeled fragments were subjected to SSCP analysis with 3.5% polyacrylamide gel without-glycerol at 20°C. After 1.5 h electrophoresis, the strain distributionpattern was analyzed with the computer software Map Manager [3] and compared with the *D5Mit61* polymorphic pattern.

Database deposit information: GenBank Accession Number D63520

Molecular reagents: The plasmid clone containing a 5-kbp insert of downstream reeler cDNA [4] was used as a FISH probe. PCR primers are as follows: DIF1, 5′-GGCCGTCTGCATCTGCGATGAA-3′; DIR1, 5′-TGAAGACAGAGCAGTCGTCAC-3′; DIF2, 5′-CACTGGACCTCACTCGAGCAA-3′; DIR2, 5′-GGCTGGGCTC-CCAATTTGCAA-3′.

Discussion: Reeler gene is expressed by pioneer neurons on mouse brain at the developmental stage, and its sequence is well conserved in other mammals according to zoo-blot analysis [4]. Dysfunction of the reeler gene is thought to cause loss of neuronal migration. In situ hybridization of the mouse reeler cDNA probe gave fluorescent signals to 5A3-B1 of mouse chromosome (Fig. 1), and genetic mapping of PCR-SSCP with BXD recombinant inbred strains showed that the reeler gene was closely linked to *D5Mit61*. These findings agreed with our genetic mapping of 340 F_1 (*Mus spretus* x rl^{Or}) x rl^{Or} mice

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