Localization of a neural crest transcription factor, *Slug*, to mouse Chromosome 16 and human Chromosome 8

Hyangshuk Rhim,[1,*] Pierre Savagner,[2,3] Giselle Thibaudeau,[1,] Jean Paul Thiery,[3] William J. Pavan[1]**

[1]Laboratory of Genetic Disease Research, 49/4A82, National Human Genome Research Institute, 49 Convent Drive MSC4472, National Institutes of Health, Bethesda, Maryland 20892-4472, USA
[2]Craniofacial Developmental Biology and Regeneration Branch, National Institute of Dental Research, National Institutes of Health, Bethesda, Maryland 20892-4442, USA
[3]CNRS UMR 144, Institut Curie, 26 rue d'Ulm, 75248 Paris CEDEX 05, FRANCE

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Species: Mouse and human
Locus name: Slug
Locus symbol: *Slugh*
Map positions: Mouse (linkage): centromere–*D16Mit32*–1.1 ± 1.1–*Prm1*–4.3 ± 2.1–*Slugh/D16Bir4/D16Hun3/Gp1bb/Tbx1*–1.1 ± 1.1–*D16Mit1*–2.1 ± 1.5–*Dagk3/Siat1*–6.4 ± 2.5–*D16Bir5*–2.1 ± 1.5–*D16Mit3/D16Bir6*–telomere. Human (SCH): Chromosome (Chr) 8. Human (RH): SLUGH/D8S2090 Chr 8 at 66–69 cM.
Methods of mapping: Mouse linkage analysis with 94 N_2 progeny from The Jackson Laboratory interspecific backcross panel [C57BL/6JEi × SPRET/Ei]F_1 × SPRET/Ei (Jackson BSS, Jackson Laboratory) [1] (Fig. 1). Human chromosome assignment by somatic cell hybrid analysis (BIOS) (Fig. 2). Human mapping with 83 human–rodent G3 radiation hybrid cell lines (Research Genetics).
Molecular reagents used for mapping: For Southern and Northern blot hybridization, a *Slugh* probe, including bp 45 to 432 from mouse *Slugh* cDNA sequence was excised from PCR II constructs previously described [2]. For radiation hybrid mapping, a human ortholog of *Slugh* was identified from the expressed sequence tag (dbEST) database using (BLASTN). Several ESTs were identified (Clone identification numbers 261671, 270049, 268993, 269377, 272919, 292023) that were overlapping clones (98% nucleic acid identity over 300 bases) and shared 62–82% nucleic acid sequence identity over different portions of the mouse *Slugh* cDNA. Consistent with this finding, these ESTs also exhibit 100% nucleic acid identity to a partial human ortholog of *Slug* [2].
Allele detection: Mouse linkage analysis: *Apa*I digestion resulted in different-sized restriction fragments (14.5 kb in C57BL/6J and 13 kb in *Mus spretus/ei*). Somatic cell hybrid analysis: *Eco*RI digestion resulted in different-sized restriction enzyme fragments (10 kb in human, 6 kb in mouse, and 11 kb in hamster). For radiation hybrid mapping, the 3′ sequence of human SLUGH EST clone 292023 was used to design human-specific PCR oligonucleotides: 5′SLUG atgggaataagtgcaaaagag and 3′SLUG agacaacatctcagtttcata. The presence or absence of a human SLUGH-specific, 210-bp PCR product was determined for the 83 human-rodent RH cell lines. Raw scoring data: 0000000000 0100111010 0000010000000000001 0001001000 0101011101 1011001000 000(1: presence, 0: absence of 210-bp PCR product). SLUGH was closely linked to marker D8S2090 on human Chr 8 (LOD 15).
Previously identified homologs: *Gallus* sp. GSSLUG (X77572)
Expression: Northern blot analysis (Clontech MOUSE EMBRYO and MOUSE MTN) in hybridization solution (5 × SSC, 5 × Denhardt's, 50% formamide, 10% dextran sulfate, 50 μg/ml salmon sperm DNA and 0.4% SDS). Washed twice for 10 min at 42°C in 0.25 × SSC, then 0.1% SDS. Quantitation was performed with a PhosphorImager (Molecular Dynamics). A 1.95 kb *Slugh* signal was observed at all embryonic ages examined. Relative intensities

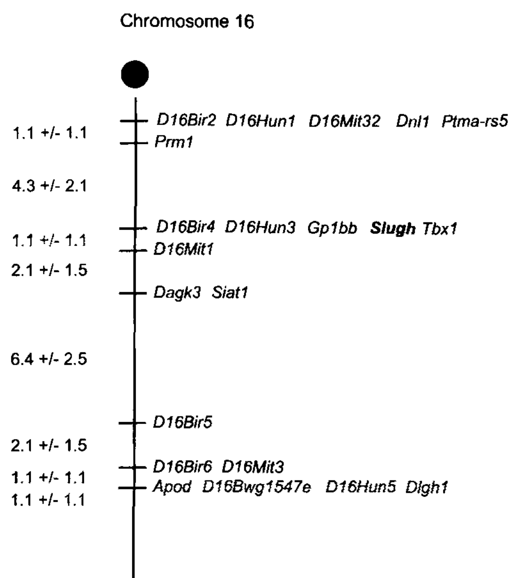

Fig. 1. Localization of *Slug* in the mouse genome by linkage analysis with The Jackson laboratory BSS mapping panel. Map figure from The Jackson BSS backcross showing Chr 16. The map is depicted with the centromere toward the top. Loci mapping to the same position are listed in alphabetical order. Raw data from The Jackson Laboratory were obtained from the World Wide Web address http://www/jax.org/resources/documents/cmdata.

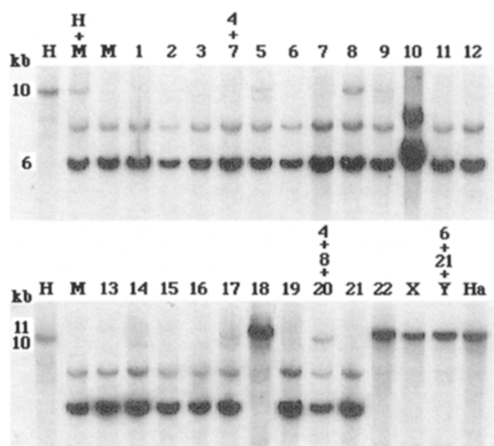

Fig. 2. Localization of SLUG in the human genome with a human–rodent somatic cell hybrid panel of cell lines. To identify chromosomal localization of the SLUG gene in human, somatic cell hybrid (SCH) panel DNA samples (BIOS) were analyzed with the SLUGH cDNA probe by Southern blot analysis. (H, total human; M, total murine; Ha, total hamster; +, indicates more than one human chromosome in the SCH line; 1–22, human autosomes; X and Y, human sex chromosomes) Lanes denoted 18, 22, X and Y + 21 + 6 are human × hamster SCH lines. The remaining lanes are human × murine SCH lines.

Correspondence to: W.J. Pavan

* Current address: Research Institute of Molecular Genetics, Catholic Research Institutes of Medical Science, Catholic University, Seoul, Korea

** Current address: Department of Biological Sciences, Mississippi State University, Mississippi State, MS 39762-3120

compared with 11 days p.c. are: 7 days p.c. 80%, 11 days p.c. 100%, 15 days p.c. 89%, 17 days p.c. 29%. Expression of a 1.95-kb signal was also detected in adult lung and testes but not heart, brain, spleen, skeletal muscle, or testes.

Discussion: The molecular events involved in conversion of pleuripotent epithelial derivatives into various neural crest derivatives require complex cellular and environmental interactions modulated by lineage-specific transcription factors. One important event in the development of neural crest-derived cells is the transition of epithelial to mesenchymal characteristics during emigration from the neural tube. *Slug,* a zinc finger protein, is one gene believed to play an important role in this transition [2,3]. *Slug* is a neurogenic, transcription factor belonging to the Snail family in *Drosophila melanogaster.* Embryological studies in chick and frog demonstrated that *Slug* mRNA is expressed in the developing neural crest and in mesodermal cells emerging from the primitive streak [3–5]. Indirect functional analyses with antisense oligonucleotides to the *Slug* mRNA showed specific and transient developmental failures at the early embryonic stages. These failures resulted in defects in neural tube closure between the midbrain and cervical regions, block of the epithelial-mesenchymal transition in the neural crest, and in the emergence of mesoderm from the primitive streak. These anomalies suggest that SLUG is required for the genetic control of cell activity during early stages of neural tube and neural crest development. Consistent with a role for SLUGH function in mouse embryonic development, our Northern blot analyses demonstrated that *Slugh* is expressed at 7 days p.c., and the signal intensity decreases subsequent to 11 days p.c. Further experiments with in situ hybridization or immunohistochemistry will be necessary to determine the specific sites of *Slug* expression during mouse embryogenesis.

Identification of known mutations caused by alterations of specific genes can provide essential clues for understanding the normal function of those genes in mammalian development. To determine whether *Slugh* is a candidate gene for a disease locus, we identified the chromosomal localization of *Slugh* in the mouse and human genomes. Segregation analysis of a *Slugh* RFLP in The Jackson Laboratory F_1(C57BL/6J × *Mus spretus*) × *Mus spretus* (BSS) interspecific backcross panel [1] determined that the mouse *Slugh* gene is located on the proximal end of Chr 16 and cosegregated with four previously mapped loci: *Tbx1* (T/omb homologous domain containing gene 1), *D16Bir4, D16Hun3,* and *Gp1bb* (Fig. 1).

An interspecies somatic-cell hybrid (SCH) panel was used to determine the chromosomal localization of SLUGH in the human genome. The mouse *Slugh* cDNA hybridized to a human-specific 10 kb band in two SCH lanes: one SCH cell line containing only human Chr 8; the other SCH cell line containing three human chromosomes, 4, 8, and 20 (Fig. 2). This result indicated that SLUGH was located on human Chr 8. Only one other gene, a CCAAT/enhancer binding protein, C/EBP-delta (CRP3/CELF), has been localized to this portion of human Chr 8 and mouse Chr 16 ([6], mouse human homology map http://www3.ncbi.nlm.nih.gov/Homology/mouse16.html). Therefore, to confirm and further refine the localization of Slugh in the human genome, we identified a human SLUGH EST and determined its human map location with the Stanford G3 radiation hybrid (RH) mapping panel (http://shgc.stanford.edu/RH/index.html). Comparison of RH mapping data with previously scored markers determined that SLUGH was closely linked to marker *D8S2090* (LOD 15) on human Chr 8 at cM 66–69 (http://www/ncbi.nlm.nih.gov/cgi-bin/SCIENCE96/loc?WI-8188, http://www.ncbi.nlm.nih.gov/cgi-bin/Schuler/clust2html?Homo+sapiens+8760). These data are consistent with and further define a region of conserved linkage between mouse Chr 16 and human Chr 8q11. Survey of the biomedical literature did not indicate any genetically linked, mammalian disease loci that would suggest a defect in *Slugh.* Therefore, additional molecular and embryonic studies of SLUGH function are required to determine its role in mammalian development.

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Mapping of six germ cell-specific genes to mouse chromosomes

M. Matsui,[1] H. Ichihara,[1] S. Kobayashi,[1] H. Tanaka,[2] J. Tsuchida,[2] M. Nozaki,[3] Y. Yoshimura,[2] H. Nojima,[4] J.M. Rochelle,[5] Y. Nishimune,[2] M.M. Taketo,[1] M.F. Seldin[5]

[1]Laboratory of Biomedical Genetics, Graduate School of Pharmaceutical Sciences, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113, Japan
[2]Department of Science for Laboratory Animal Experimentation, Osaka University, Yamadaoka, Suita City, Osaka 556, Japan
[3]Department of Developmental Genetics, Osaka University, Yamadaoka, Suita City, Osaka 556, Japan
[4]Department of Molecular Genetics, Research Institute for Microbial Diseases, Osaka University, Yamadaoka, Suita City, Osaka 556, Japan
[5]Department of Medicine, Duke University Medical Center, Durham, North Carolina 27710, USA

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Species: Mouse
Locus names: Germ cell-specific protein 1, Germ cell-specific protein 2, Germ cell-specific protein 3, Protamine 1, Protamine 2, Outer dense fiber protein 1
Locus symbols: *Gsg1, Gsg2, Gsg3, Prm1, Prm2, Odf1*
Map positions: Chromosome (Chr) 6: Centromere–*Cd4*–6.1 ± 2.3–*Gsg1*–2.6 ± 1.5–*Recql*–0.9 ± 0.9–*Gsg3*–1.8 ± 1.2–*Kras2.* Chr 11: Centromere–*Trp53*–2.6 ± 1.5–*Gsg2/Nos2*–2.6 ± 1.5–*Thra/Erbb2.* Chr 15: Centromere–*Ptgerep2*–3.5 ± 1.7–*Odf1*–0.9 ± 0.9–*Dhfr-rs1.* Chr 16: Centromere–*Prm1*–0.9 ± 0.9–*Prm2*–0.9 ± 0.9–*Igl* (Fig. 1)
Method of mapping: [(C3H/HeJ-*gld* × *M. spretus*) F_1 × C3H/HeJ-*gld*] interspecific backcross mapping panel [1].
Database deposit information: The GenBank/EMBL/DDBJ DNA databases accession numbers for *Gsg1, Gsg2,* and *Gsg3* are D87325, D87326, and D87471, respectively. The accession numbers for the gene mapping database at The Jackson Laboratory are MGD-CREX-698 (*Gsg1* and *Gsg3*), MGD-CREX-699 (*Gsg2*), MGD-CREX-700 (*Odf1*), and MGD-CREX-701 (*Prm1* and *Prm2*).

Correspondence to: M.M. Taketo

* *Present address:* Rowe Program in Genetics, Departments of Biological Chemistry and Medicine, University of California at Davis, Davis, California 95616, USA

The terminology for *Gsg1, Gsg2,* and *Gsg3* has been approved by the International Committee on Standard Genetic Nomenclature for Mice (The Jackson Laboratory, Bar Harbor, Me).