Chromosomal Localization of the Human and Murine Orthologues of the *Drosophila smoothened* Gene

A. Chidambaram,* B. Gerrard,* M. Hanson,† and M. Dean†,[1]

*Intramural Research Support Program, SAIC Frederick, and †Laboratory of Genomic Diversity, NCI–FCRDC, Frederick, Maryland 21702

Received March 4, 1998; accepted August 13, 1998

Functional gene description: *Drosophila smoothened* is a segment polarity gene that encodes an integral membrane protein with seven transmembrane α helices and a long hydrophilic C-terminal tail. The Smoothened protein is a component of the evolutionarily conserved Hedgehog signaling pathway (2) and is of special interest in developmental biology. The receptor for vertebrate Sonic hedgehog (*SHH*) is Patched (*PTCH*), a putative 12-pass transmembrane protein, which forms a complex with Smoothened (*SMOH*). Mutations in the human *PTCH, SHH,* and downstream targets of *SHH* signaling such as *Gli3* and *Hoxd13* are associated with human developmental disorders. The precise function of *SMOH* in this pathway remains to be determined. Human and rat Smoothened are about 33% identical to the *Drosophila* protein. This identity improves to about 50% in the predicted transmembrane domains (4). We mapped *SMOH* and *Smoh,* the human and murine orthologues of *Smoothened,* to chromosomes 7 and 6, respectively.

Name of clone or DNA source: Monochromosomal human/rodent hybrid mapping panel DNA (Coriell, Camden, NJ) was used as source of human DNA. The Jackson Laboratory (Bar Harbor, ME) interspecific backcross mapping panel (C57BL/6Ei × *Mus spretus*) × C57BL/6J known as BSB was used as the source of murine DNA.

Description of clone or DNA: Primers were designed from the human *SMOH* coding region (GenBank Accession No. U84401). The mouse *Smoh* gene was mapped using primers designed from the 3′ untranslated region (UTR) sequences *smoh 7,* 5′ GGC TCT TCC TGA AAG CAC AC 3′, and *smoh 10,* 5′ GCC TGG AGC TGA ACC AGT AG 3′.

Method used to validate gene identity: Using the human/rat sequence information (2), the Dbest database was screened for similar sequences. Mouse expressed tag sequences that appeared to contain portions of *Smoothened* were identified. Considerable nucleotide identity was observed in the 3′ UTR between the human and the mouse sequences.

Flanking markers: No flanking markers were used.

Methods of mapping: A monochromosomal human/rodent hybrid mapping panel (Coriell) was screened by PCR using primers designed from the coding region of human SMOH as described earlier. We mapped the human *SMOH* gene to chromosome 7.

The Jackson Laboratory interspecific backcross mapping panel (C57BL/6JEi × *M. spretus*) × C57BL/6J known as Jackson BSB was used for mapping the murine *Smoh* locus. This was performed by SSCP analysis with PCR

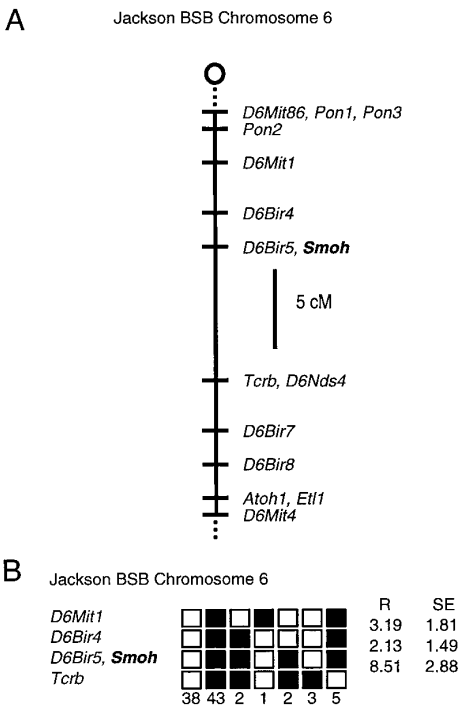

FIG. 1. (**A**) Map figure from The Jackson BSB backcross showing part of chromosome 6. This map is depicted with the centromere toward the top.A 5-cM scale bar is shown to the right of the figure. Loci mapping to the same position are listed in alphabetical order. Missing typings were inferred from surrounding data where assignment was unambiguous. Raw data from The Jackson Laboratory were obtained from the World Wide Web address http://www.jax.org/resources/documents/cmdata. (**B**) Haplotype figure from The Jackson BSB backcross showing part of chromosome 6 with loci linked to *Smoh*. Loci are listed in order with the most proximal at the top. The black boxes represent the C57BL6/JEi allele, and the white boxes represent the SPRET/Ei allele. The number of animals with each haplotype is given at the bottom of each column of boxes. The percentage recombination (*R*) between adjacent loci is given to the right of the figure, with the standard error (SE) for each *R*.

We thank Stan Cevario for oligonucleotide synthesis and assistance with DNA sequencing, the Frederick Biomedical Supercomputing Center for assistance with sequence analysis, and L. Rowe and M. Barter of The Jackson Laboratory for assistance with mouse mapping.

The nucleotide sequence data reported in this paper have been deposited with the GenBank Data Library under Accession Nos. AF089720 and AF089721.

[1] To whom correspondence should be addressed. Telephone: (301) 846-5931. Fax: (301) 846-1909. E-mail: dean@mail.ncifcrf.gov.

GENOMICS **53,** 416–417 (1998)
ARTICLE NO. GE985531
0888-7543/98 $25.00

protocols and SSCP acrylamide gel electrophoresis conditions as follows: 20-ng mouse genomic DNA samples were amplified with *Taq* polymerase in 1× reaction buffer (15 mM $MgCl_2$) with [^{32}P]dCTP. Samples were heated to 95°C for 2 min and amplified for 35 cycles of 96°C for 20 s; 57°C for 30 s; and 72°C for 30 s. Products were diluted in 1:3 stop solution, denatured at 95°C for 5 min, chilled on ice for 5 min, and loaded on gels. The gel formulation consisted of 6% acrylamide, Bis (2.6% cross-linking), 1× TBE, was run at 4°C for 2–4 h at 60 W, dried, and exposed to X-ray film for 5 h prior to genotyping. The allelic pattern of the mouse *Smoh* sequence was compared to the 770 loci mapped in the Jackson BSB cross (http://www.jax.org).

Results: Our mapping results of SMOH to human chromosome 7 were confirmed by radiation hybrid mapping, localizing the gene to chromosome 7q31 (www.ncbi.nlm.nih.gov/unigene, UNIGENE STSG4099). Figure 1 shows the localization of murine *Smoh*, which cosegregates with the marker D6Bir5 located on mouse chromosome 6. Homology data indicate that this region of the mouse genome is syntenic to human 7q31 (http://www3.ncbi.nlm.nih.gov:80/homology/).

Additional comments: The observations that *Smoh* is critical in Patched/Hedgehog communication (2) and that Patched and Smoothened are coexpressed in multiple tissues including cancers such as basal cell carcinomas (3, 5), as well as the expression pattern of *Smoh* during chondrogenesis and cartilage differentiation (1), make Smoothened an intriguing entity in developmental processes involving the hedgehog signaling pathway. The conservation of the 3′ UTR of the human and mouse *Smoh* suggests that these regions may contain important regulatory elements.

References

1. Akiyama, H., Shigeno, C., Hiraki, Y., Shukunami, C., Kohno, H., Akagi, M., Konishi, J., and Nakamura, T. (1997). Cloning of a mouse smoothened cDNA and expression patterns of hedgehog signaling molecules during chondrogenesis and cartilage differentiation in clonal mouse EC cells, ATDC5. *Biochem. Biophys. Res. Commun.* **235:** 142–147.
2. Alcedo, J., Ayzenzon, M., Von Ohlen, T., Noll, M., and Hooper, J. E. (1996). The Drosophila smoothened gene encodes a seven-pass membrane protein, a putative receptor for the hedgehog signal. *Cell* **86:** 221–232.
3. Kallasy, M., Toftgard, R., Ueda, M., Nakazawa, K., Vorechovsky, I., Yamasaki, H., and Nakazawa, H. (1997). Patched (ptch)—Associated preferential expression of smoothened (smoh) in human basal cell carcinoma of the skin. *Cancer Res.* **57:** 4731–4735.
4. Stone, D. M., Hynes, M., Armanini, M., Swanson, T. A., Qimin, G., Johnson, R. L., Scott, M. P., Pennica, D., Goddard, A., Phillips, H., Noll, M., Hooper, J. E., de Sauvage, F., and Rosenthal, A. (1996). The tumor-suppresser gene patched encodes a candidate receptor for Sonic hedgehog. *Nature* **384:** 129–134.
5. Xie, J., Murone, M., Luoh, S. M., Ryan, A., Gu, Q., Zhang, C., Bonifas, J. M., Lam, C. W., Hynes, M., Goddard, A., Rosenthal, A., Epstein, E. H., Jr., and de Sauvage, F. J. (1998). Activating Smoothened mutations in sporadic basal-cell carcinoma. *Nature* **391:** 90–92.

The Novel Skin-Specific Cytochrome P450 *Cyp2b19* Maps to Proximal Chromosome 7 in the Mouse, near a Cluster of *Cyp2* Family Genes

Diane S. Keeney[1]

Department of Biochemistry and Department of Medicine (Dermatology), Vanderbilt University, Nashville, Tennessee 37232

Received May 4, 1998; accepted August 13, 1998

Functional gene description: Cytochrome P450 genes are members of the very large *CYP* gene superfamily found in organisms as diverse as bacteria and humans. The general reaction mechanism of these mixed function monooxygenases is to fix molecular oxygen to their substrates. Mammalian cytochrome P450 enzymes metabolize endogenous (e.g., sterols, steroids, fatty acids, bile acids, and fat-soluble vitamins) and exogenous (e.g., drugs, natural substances, and environmental toxins) compounds. The novel cytochrome P450 gene *Cyp2b19* was discovered while screening mouse embryo RNAs for *Cyp* genes expressed during organogenesis (1). The *Cyp2b19* gene is expressed in suprabasal cells of fetal mouse epidermis and, postnatally, in the differentiated keratinocytes of the epidermis, hair follicles and sebaceous glands. Cytochrome P450 2B19 metabolizes arachidonic acid, generating epoxyeicosatrienoic and hydroxyeicosatetraenoic acids. It is proposed that these eicosanoids have yet undefined functions in the terminal differentiation of skin keratinocytes. These mapping studies were undertaken to determine whether *Cyp2b19* maps near loci known to be associated with skin abnormalities.

Name of clone or DNA source: The following DNAs were used: murine *Cyp2b19* cDNA clone 2-1B-1A; murine *Cyp2b10* cDNA clone pf3/46; and The Jackson Laboratory BSS interspecific backcross genomic DNA panel.

Description of clone or DNA: A *Cyp2b19* cDNA (2.7 kb) was isolated from a whole mouse skin cDNA library (11-week-old female C57BL/6, Stratagene) (1). A *Cyp2b10* cDNA, obtained from M. Negishi, was originally isolated from a BALB/cJ liver cDNA library (3). The BSS interspecific back-

This work was supported in part by U.S. Public Health Service Grants P30 AR41943, P30 ES00267, P30 DK26657, IBN-9306922, and AR02002. Support and assistance from the Skin Diseases Research Center at Vanderbilt (P30 AR41943); Drs. Brigid Hogan and Patricia Labosky; and Mary Barter, Lucy Rowe, and The Jackson Laboratory Backcross DNA Panel Mapping Resource are gratefully acknowledged.

Sequence data from this article have been deposited with the GenBank Data Library under Accession No. AF047529 and with the Mouse Genome Database under Accession No. J45291.

[1] To whom correspondence should be addressed. Telephone: (615) 322-3318. Fax: (615) 322-4349. E-mail: diane.keeney@vanderbilt.edu.

GENOMICS **53,** 417–419 (1998)
ARTICLE NO. GE985533
0888-7543/98 $25.00