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Down syndrome-critical region contains a gene homologous to *Drosophila sim* expressed during rat and human central nervous system development

(trisomy 21/monosomy 21/holoprosencephaly/exon trapping)

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ABSTRACT **Many features of Down syndrome might result from the overdosage of only a few genes located in a critical region of chromosome 21. To search for these genes, cosmids mapping in this region were isolated and used for trapping exons. One of the trapped exons obtained has a sequence very similar to part of the *Drosophila* single-minded (*sim*) gene, a master regulator of the early development of the fly central nervous system midline. Mapping data indicated that this exonic sequence is only present in the Down syndrome-critical region in the human genome. Hybridization of this exonic sequence with human fetal kidney poly(A)$^+$ RNA revealed two transcripts of 6 and 4.3 kb. *In situ* hybridization of a probe derived from this exon with human and rat fetuses showed that the corresponding gene is expressed during early fetal life in the central nervous system and in other tissues, including the facial, skull, palate, and vertebra primordia. The expression pattern of this gene suggests that it might be involved in the pathogenesis of some of the morphological features and brain anomalies observed in Down syndrome.**

Down syndrome (trisomy 21) is the most frequent human birth defect, afflicting 1 in 700 live-born infants. It is characterized by a complex and specific phenotype with associated mental retardation. In most cases, it results from the presence in all cells of an extra copy of chromosome 21. The phenotypic and molecular analysis of rare patients with partial trisomy 21 has led to the definition of a region of chromosome 21, the Down syndrome chromosome region or DCR (1, 2), critical for the pathogenesis of Down syndrome. The duplication of this region is associated with the expression of many features of the disease, including short stature, joint hyperlaxity, hypotonia, and several morphological anomalies of the face, hand, and foot, and contributes significantly to the mental retardation. Thus, the complex phenotype that constitutes Down syndrome may in large part result from the overdosage of only a few genes within the DCR. The DCR is located around the 21q22.2 sub-band on the distal part of the long arm, contains probe *D21S55*, and is flanked by probes *D21S17* and *ERG* (1–3). To facilitate analysis of this region, a genomic and yeast artificial chromosome (YAC) physical map spanning 3.8 Mb of DNA was recently established (4, 5).

To search for genes within the DCR, we isolated and mapped cosmids corresponding to this region and used these cosmids for trapping exons. One of the exons obtained was found to have a strong sequence similarity with the *Drosophila* single-minded (*sim*) gene, which encodes a transcription factor that acts as a master regulator of central nervous system midline development of the fly (6–10).§ Mapping data suggested that there is only one copy of this exonic sequence in the human genome and that it is in the DCR. Northern blot analysis using this exon as a probe revealed two transcripts of 6 and 4.3 kb in human fetal kidney. *In situ* hybridization experiments on human and rat fetuses with an oligonucleotide probe derived from this human sequence showed that the corresponding gene is expressed during early fetal life in the central nervous system and in various other tissues, including the palate, the facial, the skull, and the vertebra primordia. This gene is, thus, a candidate for a pathogenic determinant of some morphological features and brain anomalies observed in Down syndrome.

MATERIALS AND METHODS

Cosmids. Sequence tagged sites (STSs) *D21S395* and *D21S396*, derived from *Not* I-linking clones (11), were used as probes to screen the Imperial Cancer Research Fund chromosome 21 cosmid library (12).

Fluorescent *in Situ* Hybridization with Metaphasic Chromosomes. Cosmid DNA (1 μg) was labeled with biotin-14-dATP (GIBCO/BRL) by nick translation and used as a probe on metaphasic chromosomes (3) isolated from lymphoblasts of patient IG (1, 2).

Exon-Trapping Experiments. The procedure was as described by Church *et al.* (13). Two pools containing equal amounts of *Bam*HI/*Bgl* II-digested DNA from four different cosmids were inserted into the pSPL3 vector (GIBCO/BRL). The DNA of the libraries was introduced into COS-7 cells by electroporation. The trapped products were amplified after reverse transcription of the cellular RNAs (Pharmacia) by two rounds of amplification with oligonucleotides corresponding to the vector. Exon-trapping products were then inserted into the pAMP10 vector (GIBCO/BRL).

Mapping of the Exon-Trapping Products. Total human (from blood), YAC, or cosmid DNA was digested with *Eco*RI, *Bam*HI, *Hin*dIII, or *Pvu* II and transferred to nylon membranes (Appligene, Strasbourg, France). Probes corresponding to cloned exons were generated by PCR amplification of the insert either with M13 primers or with internal primers derived from sequence analysis. Two sets of somatic cell hybrids were used for mapping exons in the human genome: a multichromosomal somatic cell hybrid panel (Biosys, Compiegne, France) and a monochromosomal somatic cell hybrid panel (Human Genome Mapping Project, Harrow, U.K.).

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Abbreviations: DCR, Down syndrome chromosome region; YAC, yeast artificial chromosome; G*n*, gestational day *n*.

§The sequence reported in this paper has been deposited in the GenBank data base (accession no. X84790).

Sequencing. DNA sequencing was performed by using an Automatic Laser Fluorescent (ALF) sequencer (Pharmacia). A total of 5–10 μg of plasmid DNA was used for the double-stranded DNA sequencing reaction with T7 DNA polymerase (Autoread kit, Pharmacia). Sequences of PCR fragments were also determined by using the SequiTherm autocycle kit (Epicentre Technologies, Madison, WI). Sequence data base analysis was performed with BLAST software.

mRNA Detection. Northern blot analysis of human fetal poly(A)$^+$ RNAs (Clontech) was performed by using the 256-bp exon-trap product as a probe. Tissue *in situ* hybridization was performed on rat and human tissues by using an ^{35}S-labeled oligonucleotide probe as described (14, 15). Rat tissues (whole embryos, embryo heads, and neonatal and adult brains) were fixed with 1% formaldehyde by either immersion or perfusion. Human tissues were frontal sections of cerebral hemispheres collected from fetuses obtained after abortion for morphological or chromosomal abnormalities, among which were sections from brains of fetuses with Down syndrome. Human tissues were obtained and processed as described in compliance with the recommendations of the Comité National d'Ethique (15). The probe, L1, was a 45-mer synthetic oligonucleotide (CACTGGGATAAGCCTAAATGGACAGAAGCGGTCTCGGATATATAC) derived from the 256-bp exon-trap product and included 42 bp of exon E16. Controls included the use of an oligonucleotide probe derived from a different gene (14, 15), and experiments with excess unlabeled L1 added to the probe.

RESULTS AND DISCUSSION

To facilitate the search for genes in the DCR, we isolated and mapped cosmids corresponding to this region. *Not* I DNA restriction sites are frequently associated with CpG islands, which are gene markers in the mammalian genome (16). We therefore first isolated cosmids around these sites in the *D21S17–ERG* region (4). Eight cosmids were isolated around *D21S395* and *D21S396* (Fig. 1*A*) and ordered in a contig 75 kb long by analysis of *Eco*RI digestion patterns. We verified that these cosmids belonged to the DCR, the proximal boundary of which was previously located between probes *D21S17* and *D21S55* from the analysis of patient IG. This patient has the Down syndrome phenotype and a *de novo* duplication of a chromosome 21 segment including probe *D21S55* to *MX1* gene on sub-band 21q22.3 (1, 2). Fluorescence *in situ* hybridization on metaphase chromosomes of patient IG by using cosmid E0669 gave a hybridization signal at one locus on the normal chromosome 21 and at two loci on the rearranged chromosome 21 (Fig. 1*B*). No hybridization signal was observed on other chromosomes. Similar data were obtained with cosmid F0869. Thus, these cosmids are chromosome 21 specific and duplicated in patient IG and therefore belong to the DCR.

The cosmids were used in two pools for exon-trapping experiments. From the pool containing cosmids E0669, G0321, D06112, and F0452, 25 presumptive exons were inserted into pAMP10 and systematically mapped back to the cosmids and the YACs of the region. One exon, E16, identified an 800-bp *Eco*RI restriction fragment in cosmids G0321, E0669, C0763, D06112, and G0592 and in YAC 745H11 (data not shown). Hybridization of this same exon on a Southern blot containing DNAs of the 25 presumptive exons showed that six other clones were redundant, carrying the same sequence.

These seven exons were sequenced, and BLASTX and BLASTN were used to screen nucleotide and peptide sequences in data bases for similarities. Exon E16, 90 bp long, was found to have a strong sequence similarity with the *Drosophila sim* gene: 72% at the nucleotide level and 93% at the protein level (Fig. 2) in the region corresponding to nt 202–291 of the *sim* cDNA, which corresponds to the PAS1 domain (region of amino acid sequence similarity common to PER, ARNT, and SIM proteins) of this protein (7). No significant similarity—i.e., >70%—was found between E16 and any other sequence

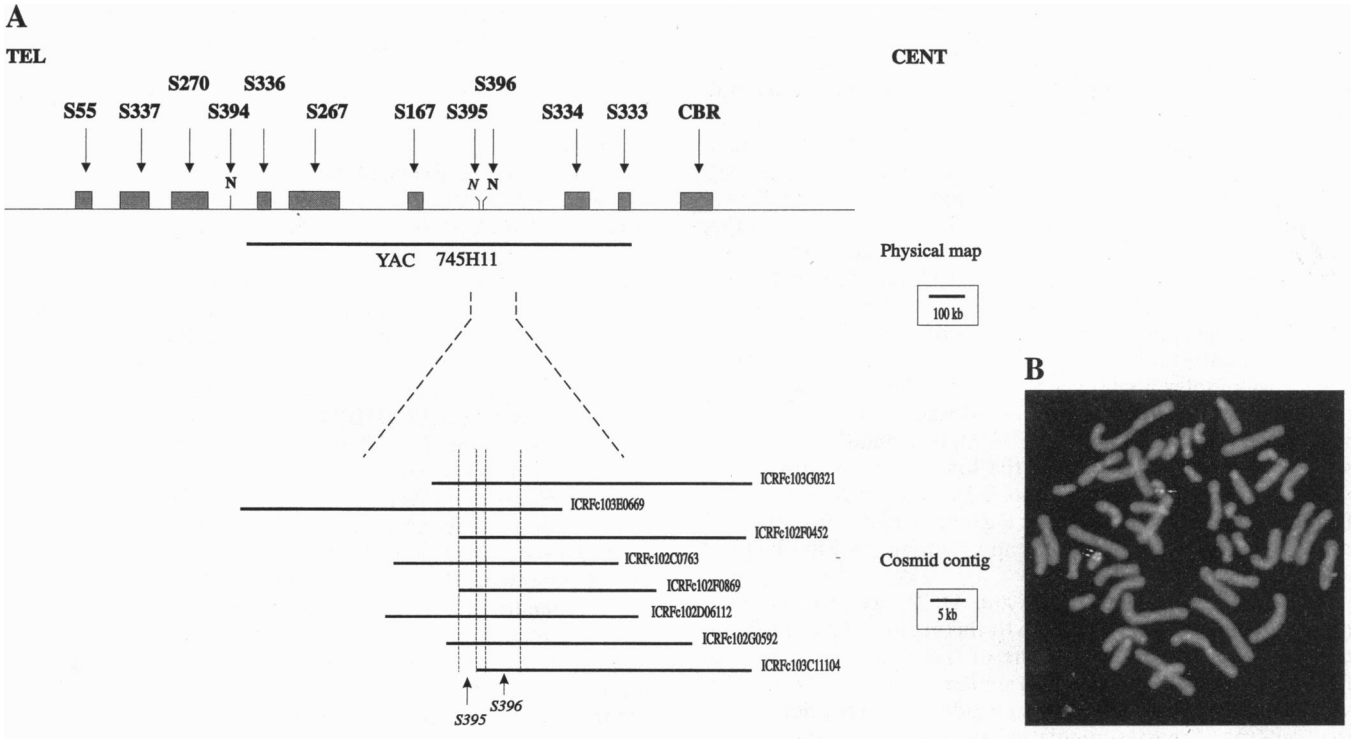

FIG. 1. Localization of the cosmid contig in the DCR. (*A*) (*Upper*) Location of DNA markers (boxes) on the physical map of chromosome 21 with the positions of *Not* I sites (N) and YAC 745H11 (1100 kb) (ref. 4) indicated. (*Lower*) Cosmid contig from the ICRF c102 library with the location of the DNA markers *D21S395* and *D21S396* indicated. (*B*) *In situ* hybridization on metaphasic chromosomes of patient IG with the cosmid E0669. White arrows indicate the signals on the normal and rearranged chromosomes.

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BLASTN
Score = 226 (62.4 bits), Expect = 2.9e-11, P = 2.9e-11
Identities = 62/83 (72%), Strand = Plus
                                                                              L1
                                                           [-------------------------------------------]
E16  :    1 ACTTTGGATGGATTTGTTTTTGTGGTAGCATCTGATGGCAAAATCATGTATATATCCGAGACCGCTTCTGTCCATTTAGGCTTATCCCAG
            ||  |||| |||||  | || ||||| ||  | ||||||||||||||||| || || || || || || || ||||| ||| |    |||
Sim  :  202 ACGCTGGACGGATTCATCTTCGTGGTGGCTCCGGATGGCAAAATCATGTACATCTCGGAAACGGCCTCCGTGCATTTGGGCCTCAGTCAG

BLASTX
 Score = 141 (64.9 bits), Expect = 8.8e-14, P = 8.8e-14
 Identities = 28/30 (93%), Positives = 29/30 (96%), Frame = +1

E16  :    1 TLDGFVFVVASDGKIMYISETASVHLGLSQ 90
            TLDGF+FVVA DGKIMYISETASVHLGLSQ
Sim  :   86 TLDGFIFVVAPDGKIMYISETASVHLGLSQ 115
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FIG. 2. Sequence similarities between E16 and *Drosophila sim*.

present in data bases, including the genes for human aryl hydrocarbon receptor (AHR) (17) and aryl hydrocarbon receptor nuclear translocator (ARNT) (18), both of which contain PAS domains. One of the other trapped products, 256 bp long, was sequenced and found to contain E16. Its sequence was used to generate primers for PCR amplification. A 450-bp product was amplified from human genomic DNA and from DNA isolated from the WA17 somatic cell hybrid, which contains chromosome 21 as its only human chromosome. This 450-bp fragment was cloned, and its sequence was determined. It contains an intron flanked by two exons: E16 (90 bp) and an exon of 166 bp. The boundaries of the intron obey the GT/AG rule. The 256-bp product, which presents an uninterrupted open reading frame, was shown in COS cells to be derived from the splicing of these two adjacent exons in the 450-bp genomic fragment. PCR amplification of DNA from two panels of somatic hybrids containing human chromosomes only detected the 450-bp band in hybrids containing chromosome 21 (data not shown). The 256-bp exon-trapping product was used to probe Southern blots containing human genomic DNA digested with different restriction enzymes. Only one fragment was revealed after digestion with *Hin*dIII or *Bam*HI (Fig. 3). Similarly, single *Eco*RI (800 bp) and *Pvu* II (5.4 kb) restriction fragments hybridized with this probe (data not shown). The sizes of these fragments were identical to those observed after digestions with the same enzymes of DNAs from YAC 745H11 or cosmids covering this region. These data suggest that there is only one locus for exon E16 in the human genome. Chen *et al.* (19), using exon trapping from cosmids isolated from another library (LLNL) and randomly distributed along chromosome 21, have recently reported the isolation of exons with sequence similarity to the *Drosophila sim* gene. It is likely that these exons belong to the same chromosome 21 gene, which is homologous to *Drosophila sim*.

In *Drosophila*, the *sim* gene is expressed early in neurogenesis at the beginning of central nervous system midline development and is required for all known developmental events of the midline lineage, including neuronal and nonneuronal cells (6–10). No homologous gene in vertebrates has been described. To determine if there was a transcript corresponding to the human homolog of *sim*, we used the 256-bp exon-trapping product to probe a Northern blot containing poly(A)$^+$ RNA from human fetal (22 weeks gestation) tissues (brain, lung, liver, and kidney). This probe hybridized two transcripts in the kidney: a major one at 6 kb and a minor one at 4.3 kb (Fig. 4).

To investigate the potential role of this *sim* homolog in vertebrate development, we performed *in situ* hybridization experiments on rat and human fetal tissues by using an oligonucleotide probe, L1, derived from exon E16 and homologous to *Drosophila sim*. This probe gave positive signals on rat tissues, indicating that a sequence homologous to *sim* is also present in the rat. Hybridization with sections of rat fetuses demonstrated specific labeling in various areas of the body, especially inside the facial and vertebra primordia and the central nervous system from gestational day 12 (G12) (Figs. 5–7). Studies at later stages (G13–G15) showed that the most prominent signals were present in various areas of the skull and facial primordia, particularly the sphenoidal anlage (Fig. 5), the palatal shelves (Figs. 6 *B* and *D* and 7), the mandibular primordium (Fig. 7 *D* and *F*), and other structures in the laryngeal region that may include several laryngeal cartilages. Other formations, such as the vertebral body anlages and ribs were also strongly reactive (Fig. 5 *C* and *D*). The palate septum was weakly labeled (Fig. 6 *C* and *F*). Analysis at the microscopic level (Fig. 7) confirmed heavy labeling of mesenchymal cells at the early stages, especially in the palatal shelves and maxillary bud, and discrete labeling of the cells located at the periphery of the cartilage primordium in the mandibula, vertebral bodies, larynx, and septum palate. The mature cartilage cells themselves were poorly reactive. The tongue epithelium was strongly labeled (Fig. 6*C*). The central nervous system displayed a moderate and homogenous signal that predominated in the neuroepithelium region close to the ventricles (Figs. 5*C* and 6*C*).

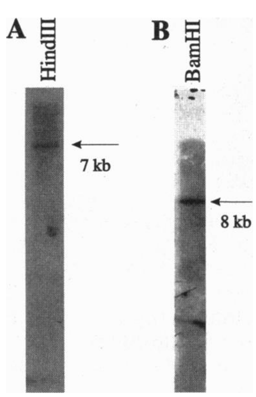

FIG. 3. Genomic Southern blot analysis. Two Southern blots containing human genomic DNA digested with *Hin*dIII (*A*) or *Bam*HI (*B*) and hybridized with the 256-bp exon-trap product.

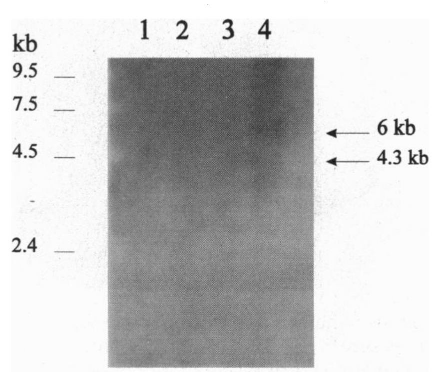

FIG. 4. Northern blot analysis. Human fetal (22 weeks gestation) poly(A)$^+$ RNA from brain (lane 1), lung (lane 2), liver (lane 3), and kidney (lane 4) were probed with the 256-bp exon-trap product. The positions of molecular size markers are indicated on the left.

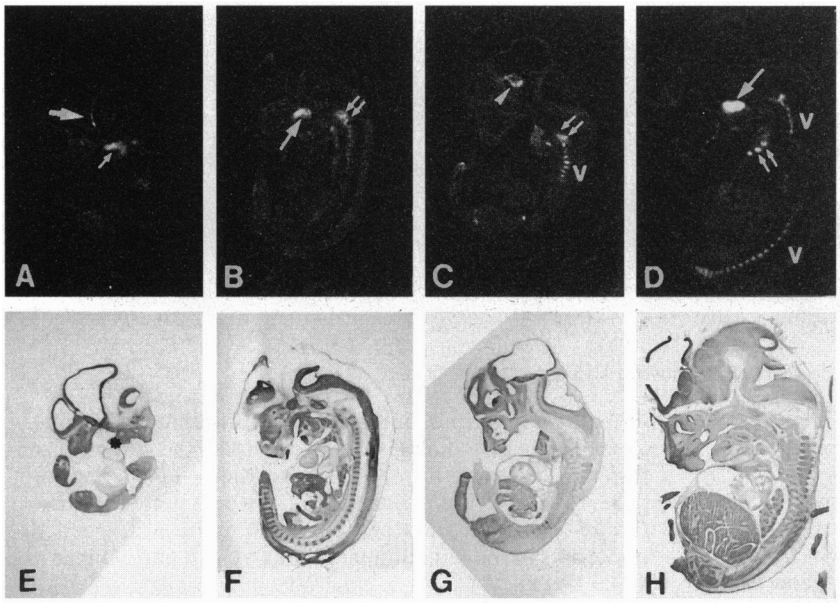

FIG. 5. Sagittal sections of rat embryos after hybridization with ^{35}S-labeled L1 oligonucleotide probe (see Fig. 2 for L1 sequence). (*A–D*) Negative prints of x-ray film (15-day exposure); (*E–H*) corresponding sections stained with toluidine blue. *A* and *E*, G13; *B*, *C*, *F*, and *G*, G14; *D* and *H*, G15. (*A–H*, ×3.) (*A*) Moderate labeling in the neuroepithelium in a cerebral vesicle (large arrow), intense labeling of the sphenoidal anlage (small arrow). * in *E*, tongue. (*B* and *C*) Labeling of the sphenoidal anlage (single arrow in *B*), several formations associated with the posterior part of the skull anlage (double arrows in *B* and *C*), the neuroepithelium (arrowhead in *C*), and the vertebral anlages (V). (*D*) Intense labeling of the sphenoidal anlage (single arrow), the ribs (double arrows), and vertebral anlages (V).

Several discrete regions, including the basal hypothalamus, were also strongly reactive (Fig. 7 *B* and *C*). The spinal cord seemed unreactive. Intensity and aspect of the signals changed with age. Thus, in the neonate and adult, signals in the central nervous system were weak and only found in limited areas, including the cerebellum (data not shown). The heart and mesonephros were unreactive. At later stages (G15 and G16), the metanephros was clearly labeled. The liver displayed a light heterogenous signal. Sections from hemispheres from human nontrisomic 21 brains at 16 weeks of gestation were similarly studied (Fig. 6*A*). The so-called neuroepithelium ganglionic eminence was heavily labeled, and weaker and more diffuse labeling was observed in certain cortical layers. Microscopic examination did not allow us to distinguish which cell population was labeled in the cortex. Strong signals were also seen in various areas of the face similar to those observed in rat (data not shown). Limited investigations in the two trisomic brains at 16 weeks of gestation did not reveal any evident special feature.

Thus, the expression pattern of the *sim* homolog in rat and human seems to present similarities to that of *Drosophila sim*. The *sim* homolog is expressed during early development; it is also expressed in the central nervous system neuroepithelium, which contains neuronal and glial precursor cells, like the midline in insects. In addition, the *sim* homolog is expressed in mesoderm derivatives, particularly in the mesoblastic cells of the cartilage primordia of the face, skull, and vertebra. Preliminary data from human tissues indicate that the pattern of *sim* homolog expression in rat and human is very similar. In the central nervous system, there is a strong signal in the neuroepithelium region close to the ventricle and a weaker signal in other defined areas. We also observed in early human embryos a strong signal in the palatal shelves and various areas of the face that constitute cartilage and bone anlages.

This expression pattern suggests that the *sim* homolog may have pleiotropic effects during early development. Thus, when there are three copies, it might contribute to the pathogenesis of several features of the Down syndrome phenotype. Consistent with this view, the *sim* homolog is expressed in areas that are abnormal in patients with Down syndrome. For example, it might be involved in the facial and skull features characteristic of the syndrome, such as the highly arched palate, the protruding tongue, the hypoplasia of the midface, the hypertelorism, and the flat occiput. The second phase of the cortical development, the emergence of lamination, is both delayed and disorganized in trisomy 21 (20). The *sim* homolog might also, therefore, be involved in this brain anomaly and consequently contribute to the pathogenesis of mental retardation.

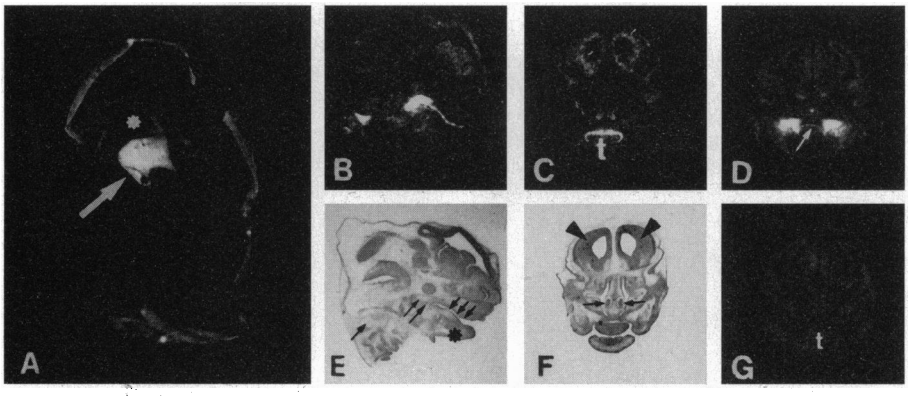

FIG. 6. Sections of human (*A*) and rat (*B–G*) tissues hybridized with ^{35}S-labeled L1 oligonucleotide probe. (*A–D* and *G*) Negative prints of x-ray film (15-day exposure); (*E* and *F*) toluidine blue staining. (*A*) Hemisphere from a 16-week-old human fetus. Strong labeling in the so-called ganglionic eminence (14) (arrow) composed of neuroepithelial cells close to the ventricle (*); fainter labeling in the cortex. (×1.8.) (*B–D*) Frontal sections of heads from G14 (*B*) and G15 (*C* and *D*) rat embryos. (*E* and *F*) Corresponding sections of *B* and *C* stained with toluidine blue. (*B*) Intense signal in the occipital (single arrow in *E*) and sphenoïd (double arrows in *E*) bone anlages, and in palate primordium (triple arrows in *E*). *, Tongue. (*C*) Moderate signal in the neuroepithelium in the vicinity of the ventricle (arrowheads in *F*), in the septum palate (single arrows in *F*), and in the tongue epithelium (t). (*D*) Heavy signal in the palatal shelves on each side of the tongue. The tongue epithelium is also reactive (arrow). (*G*) Adjacent control section hybridized with 0.1 ng of ^{35}S-labeled L1 plus excess unlabeled L1. t, Tongue. (*B–G*, ×3.6.)

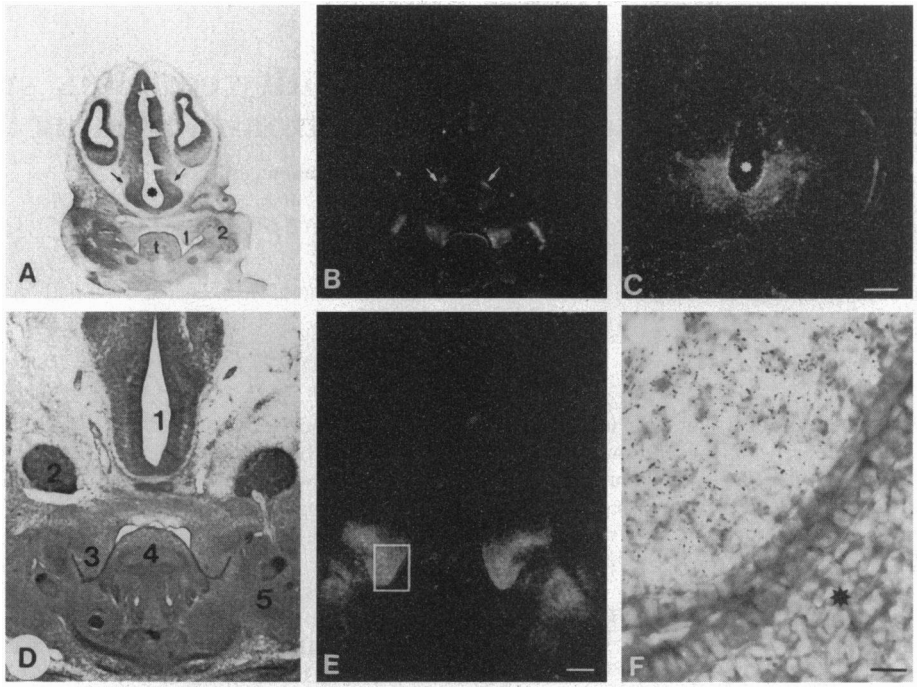

FIG. 7. Sections of rat tissues hybridized with ^{35}S-labeled L1 oligonucleotide probe, examined under bright- (*A*, *D*, and *F*) or dark- (*B*, *C*, and *E*) field illumination. (*A* and *B*) Same frontal section of the head of a G14 embryo (×2.7). (*A*) Toluidine blue staining. t, Tongue. *, Third ventricle. (*B*) Labeling of the palate shelf (1 in *A*), the maxillary bud (2 in *A*), and unidentified cells in the diencephalon (arrows). (*C*) Basal diencephalon (G15) with heavy labeling in the ventral hypothalamus. *, Third ventricle. (Bar = 50 μm.) (*D* and *E*) Same section of the ventral diencephalon and mouth region from a G15 rat embryo. (Bars = 100 μm). (*D*) Toluidine blue staining. 1, Third ventricle; 2, nerve ganglion; 3, palate shelve; 4, tongue; and 5, mandibular bud. (*E*) Intense labeling of the palate shelves and the mandibular bud. (*F*) Enlarged view of the squared area in *E* with intense labeling in the palate shelf anlage, while the adjacent tongue muscle (*) is unreactive. (Bar = 10 μm.)

Given the role of *sim* in *Drosophila* development, it was also proposed that a homolog of this gene in human might be involved in holoprosencephaly (21, 22), which results in fusion of both lateral brain hemispheres and facial structures. Interestingly the *sim* homolog is expressed in the neuroepithelium of telencephalic vesicles and, therefore, may be involved in the morphogenesis of the cerebral hemispheres. Studies at early fetal stages would demonstrate whether the *sim* homolog is expressed in the primary prosencephalic vesicle. Holoprosencephaly has been associated to various chromosomic defects (21). Among these are several cases of deletion of the distal part of chromosome 21 (23, 24) and it would be interesting to test whether this gene is deleted in such cases.

Note Added in Proof: After this paper was submitted, the work presented as an abstract in ref. 19 appeared in full form (25) and describes six exons, one of which is identical to E16.

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1. Rahmani, Z., Blouin, J. L., Créau-Goldberg, N., Watkins, P. C., Mattei, J. F., Poissonnier, M., Prieur, M., Chettouh, Z., Nicole, A., Aurias, A., Sinet, P. M. & Delabar, J. M. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 5958–5962.
2. Delabar, J. M., Théophile, D., Rahmani, Z., Chettouh, Z., Blouin, J. L., Prieur, M., Noel, B. & Sinet, P. M. (1993) *Eur. J. Hum. Genet.* **1,** 114–124.
3. Crété, N., Gosset, P., Théophile, D., Duterque-Coquillaud, M., Blouin, J. L., Vayssettes, C., Sinet, P. M. & Créau-Goldberg, N. (1993) *Eur. J. Hum. Genet.* **1,** 51–63.
4. Dufresne-Zacharia, M. C., Dahmane, N., Théophile, D., Orti, R., Chettouh, Z., Sinet, P. M. & Delabar, J. M. (1994) *Genomics* **19,** 462–469.
5. Gosset, P., Crété, N., Ait Ghezala, G., Théophile, D., Van Broeckhoven, C., Vayssettes, C., Sinet, P. M. & Créau, N. (1995) *Mamm. Genome* **6,** 127–130.
6. Thomas, J. B., Crews, S. T. & Goodman, C. S. (1988) *Cell* **52,** 133–141.
7. Crews, S. T., Thomas, J. B. & Goodman, C. S. (1988) *Cell* **52,** 143–151.
8. Nambu, J. R., Lewis, J. O., Wharton, K. A. & Crews, S. T. (1991) *Cell* **67,** 1157–1167.
9. Muralidhar, M. G., Callahan, C. A. & Thomas, J. B. (1993) *Mech. Dev.* **41,** 129–138.
10. Lewis, J. O. & Crews, S. T. (1994) *Mech. Dev.* **48,** 81–91.
11. Ichikawa, H., Hosoda, F., Arai, Y., Shimizu, K., Ohira, M. & Ohki, M. (1993) *Nat. Genet.* **4,** 361–365.
12. Nizetic, D., Zehetner, G., Monaco, A. P., Gellen, L., Young, B. D. & Lehrach, H. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 3233–3237.
13. Church, D. M., Stotler, C. J., Rutter, J. L., Murrell, J. R., Trofatter, J. A. & Buckler, A. J. (1994) *Nat. Genet.* **6,** 98–105.
14. Bloch, B., Normand, E., Kovesdi, I. & Böhlen, P. (1992) *Dev. Brain Res.* **70,** 267–278.
15. Brana, C., Charron, G., Aubert, I., Carles, D., Martin-Negrier, M. L., Trouette, H., Fournier, M. C., Vital, C. & Bloch, B. (1995) *J. Comp. Neurol.*, in press.
16. Lindsay, S. & Bird, A. P. (1987) *Nature (London)* **327,** 336–338.
17. Itoh, S. & Kamataki, T. (1993) *Nucleic Acids Res.* **21,** 3578.
18. Reisz-Porszasz, S., Probst, M. R., Fukunaga, B. N. & Hankinson, O. (1994) *Mol. Cell. Biol.* **14,** 6075–6086.
19. Chen, H. M., Chrast, R., Lalioti, M. D., Samec, S. N., Rossier, C., Blouin, J. L., Morris, M. A., Gos, A. & Antonarakis, S. E. (1995) *Cytogenet.-Cell Genet.* **70,** 179 (abstr.).
20. Golden, J. A. & Hyman, B. T. (1994) *J. Neuropathol. Exp. Neurol.* **53,** 513–520.
21. Münke, M. (1989) *Am. J. Med. Genet.* **34,** 237–245.
22. Nambu, J. R., Lewis, J. O. & Crews, S. T. (1993) *Comp. Biochem. Physiol.* **104,** 399–409.
23. Estabrooks, L. L., Rao, K. W., Donahue, R. P. & Aylsworth, A. S. (1990) *Am. J. Med. Genet.* **36,** 306–309.
24. Aronson, D. C., Janweijer, M. C. E., Hoovers, J. M. N. & Barth, P. G. (1987) *Clin. Genet.* **31,** 48–52.
25. Chen, H., Chrast, R., Rossier, C., Gos, A., Antonarakis, S. E., Kudoh, J., Shindoh, N., Maeda, H., Minoshima, S. & Shimizu, N. (1995) *Nat. Genet.* **10,** 9–10.