ZNF179, a RING finger protein encoding gene, has been mapped within the critical deletion region for Smith-Magenis syndrome (SMS), a disorder characterized by mental retardation and multiple congenital anomalies associated with del(17)(p11.2). Here we report the cloning of Znf179, the mouse homologue of ZNF179, and characterization of its gene structure. The 3028-bp cDNA has a 1.9-kb open reading frame that contains a RING finger domain at its N-terminus and an alanine-rich and glycine-rich domain at its C-terminus. Znf179 genomic sequence includes 15 introns and spans about 10 kb on mouse chromosome 11, which maintains conserved synteny with human 17p. Northern analysis indicates that Znf179 is predominantly expressed in brain and testis. Although contained within the SMS common deletion interval, FISH experiments show that ZNF179 is not deleted in two SMS patients with smaller deletions.

INTRODUCTION

The human RING finger protein gene ZNF179 was initially identified from a human genomic library by low-stringency screening for RING finger encoding genes (Matsuda et al., 1996). A partial cDNA sequence flanking the RING finger domain was isolated from the study (Matsuda et al., 1996). It maps to human chromosome 17p11.2 and mouse chromosome 11B1.3 (Matsuda et al., 1996). Recently, it was shown that ZNF179 maps within the Smith-Magenis syndrome (SMS) critical region (Chen et al., 1997) and is deleted in several SMS patients (Kimura et al., 1997).

SMS is a microdeletion syndrome due to a DNA rearrangement that results from homologous recombination between repeat gene clusters flanking the common deletion region (Chen et al., 1997). Although a consistent junction fragment band derived from the homologous recombination event is detected in the majority of SMS patients (Chen et al., 1997), smaller and larger deletions have been reported (Zori et al., 1993; Juyal et al., 1995a,b, 1996; Trask et al., 1996). SMS patients have a broad range of clinical features including mental retardation, delayed speech and motor development, craniofacial anomalies (brachycephaly, flat midface, prominent forehead, broad nasal bridge, ear anomalies), skeletal anomalies (brachydactyly, short stature), behavioral problems, sleep disturbance, and minor peripheral neuropathy (Chen et al., 1996; Greenberg et al., 1991, 1996). It is hypothesized that haploinsufficiency of several contiguous but functionally unrelated genes contributes to this wide spectrum of phenotypes in SMS patients. A number of genes have been mapped within this critical region, which spans about 4–6 Mb in most patients (Chen et al., 1997). It is tempting to speculate that the severity and complexity of the phenotype may be related to the deletion size. However, even patients with the common deletion have variable clinical manifestations. Characterization of patients with the smallest deletions will aid in identifying critical genes in which haploinsufficiency causes either common or unique clinical phenotypes.

To elucidate the possible role of ZNF179 in SMS phenotype, we report the cloning of mouse Znf179 and characterization of its genomic structure and expression in mouse. Furthermore, we show that ZNF179 is not deleted in two SMS patients with smaller deletions.

MATERIALS AND METHODS

Isolation of Znf179 cDNA. A postnatal day 20 mouse brain cDNA library (Stratagene) was screened with an [a-32P]dCTP-labeled 203-bp PCR fragment that contains the human RING finger domain sequence of ZNF179. Hybridization and washing were performed under high-stringency conditions. The cDNA inserts were rescued in vivo with helper phage R408 in pBluescript vector. Nine cDNA clones (pLZ3-1 to pLZ3-9) were obtained. After restriction digestion analysis, three cDNA clones, pLZ3-1, pLZ3-2, and pLZ3-7, were subsequently sequenced by automated DNA sequencing. The Znf179 cDNA sequence was constructed by double-strand coverage of these three clones. The full-length cDNA sequences were searched for ESTs in the NCBI database.

Genomic characterization of Znf179. A mouse 129 SvEv genomic library (Stratagene) was screened with the [a-32P]dCTP-labeled full-length Znf179 cDNA probe from clone pLZ3-2. Screening was carried out using a high-stringency condition. After three rounds of screen-
MOUSE RING FINGER PROTEIN GENE Znf179

FIG. 1. Sequence of mouse Znf179. The complete nucleotide and deduced amino acid sequence is shown with the potential translation start site designated as nucleotide +1. The Amber stop codon TAG is marked by an asterisk. The RING finger domain is underlined with a solid line. The alanine- and glycine-rich domain is underlined with a dashed line. The putative polyadenylation recognition site located upstream of the polyadenosine is boxed.

ing, two phage clones were identified. Primers designed in the cDNA were used to sequence the genomic DNA.

Northern blot analysis. RNAs for Northern blots were extracted from tissues of adult mice. Total RNA was prepared with RNA zolB (Biotech Laboratories, Inc.). RNAs (15 μg) were loaded on each lane, and uniform loading of samples was monitored by ethidium bromide staining of ribosomal RNA. The probe used for Northern blot analysis was a specific 583-bp fragment at the 3'-end of Znf179 cDNA from 2381 to 2964 bp. Northern blotting was performed according to the standard protocol (Bio-Rad Zeta probe).

BAC library screening. A PCR-based screening approach was used to screen a human BAC library (Research Genetics). The primer
FIG. 2. Conservation of the RING finger domain of Znf179. (A) Comparison of the RING finger domain of mouse Znf179 (mZnf179) with that of human ZNF179 (hZNF179) and rat Znf179 (rZnf179). The amino acids that are identical to those of mZnf179 are indicated by a dash. (B) Comparison of the sequence of RING finger domain of Znf179 with that of other RING finger domain-containing proteins. Gaps to improve sequence alignment are marked by asterisks. The amino acids that are identical to those of mZnf179 are indicated by a dash. RPT1, an intracellular regulatory protein; ZNF173, a human RING finger protein, also called acid finger protein (AFP); RFP, ret-finger protein. The conserved cysteine and histidine residues are boxed in the C3HC4 domains.

RESULTS

Cloning of Mouse Znf179

A 203-bp PCR fragment that contains the entire human RING finger domain cDNA sequence was used to screen a postnatal day 20 mouse whole brain cDNA library. From \( 5.0 \times 10^5 \) phage clone pools, nine identical clones were isolated based on restriction mapping and sequencing. The 3028-bp cDNA contains a 1962-bp open reading frame that encodes 654 amino acid residues with digoxigenin by nick-translation and detected with anti-digoxigenin conjugated to rhodamine (fluoresces red); cosmid clone c70E2 (Fig. 1). However, no Kozak consensus translation initiation site is found at the first ATG start codon. Although two downstream methionines at amino acid positions 25 and 43 have the consensus ATG- motif, the exact translation initiation site for this gene is uncertain. The RING finger domain with a C3HC4 motif is located at...
was mapped to several mouse YAC clones that are (Fig. 2A). Using PCR and Southern analyses, Znf179 Expression of Znf179
et al., human acid finger protein ZNF173 (Chu et al., 1988), and human ret-proto-oncogene activating protein RFP (Takahashi et al., 1988) (Fig. 2B). Outside the RING finger domain, Znf179 does not share homology with other known proteins.

Genomic Structure of Znf179

The full-length mouse cDNA from clone pLZ3-2 was used as a probe to isolate two genomic phage clones, Znf 8 and Znf 9, from a mouse 129SvEv genomic library. Restriction mapping shows that these two clones overlap. A schematic view of the mouse Znf179 gene structure is shown in Fig. 3. The mouse Znf179 gene contains 15 introns and 16 exons. The entire RING finger domain is encoded by a single exon, exon 4. A summary of the exon/intron boundaries is listed in Table 1. Not all splice donor sites match the 5'-GT rule; however, all splice acceptor sites conform to the 3'-AG rule.

Expression of Znf179

Northern blot analysis with a 3'-end cDNA probe from 2381 to 2964 bp of Znf179 showed one predominant messenger RNA band in brain and testis in adult mice (Fig. 4). Although the exact transcriptional initiation site was not determined, the size of the single RNA band shown in the Northern blot is approximately 3.0 kb.

FISH Analysis

A human BAC clone, HBZNF, for Znf179 was isolated from screening of a human BAC library. Before it was used as a probe for FISH experiments, the BAC clone was confirmed to contain the ZNF179 locus by PCR and direct sequencing experiments. Cell lines from lymphoblasts of two SMS patients HOU142-540 and HOU202-641 (Zori et al., 1993; Juyal et al., 1995a,b, 1996) with smaller deletions were tested. FISH results show that ZNF179 is present on both chromosomes 17 in cell lines derived from these two SMS individuals, whereas FLI1, a control probe that is known to be deleted in all SMS patients, is present on only one chromosome 17 homologue (Fig. 5).

### TABLE 1
The Exon-Intron Boundaries of the Mouse Znf179 Genea

<table>
<thead>
<tr>
<th>Exon</th>
<th>Start (bp)</th>
<th>End (bp)</th>
<th>3' UTR</th>
<th>5' UTR</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>131</td>
<td>136</td>
<td>CTCCCAG/GAGGAC</td>
<td>142</td>
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<tr>
<td>2</td>
<td>172</td>
<td>122</td>
<td>CTTCAG/GGTCTTCT</td>
<td>183</td>
</tr>
<tr>
<td>3</td>
<td>241</td>
<td>516</td>
<td>CCCCAG/GTCCCA</td>
<td>252</td>
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<td>4</td>
<td>527</td>
<td>161</td>
<td>CACCAG/GAGACC</td>
<td>538</td>
</tr>
<tr>
<td>5</td>
<td>734</td>
<td>118</td>
<td>ACGTG/AGACTCT</td>
<td>745</td>
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<td>2377</td>
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<td>GGGGGG/GATGCCA</td>
<td>2388</td>
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</tbody>
</table>

a The numbers indicate the positions of the 5' and 3' nucleotides within the mouse cDNA sequence. Intron number and size are given. Splice acceptor and donor sites are in boldface letters. ND, not determined.

The 5'-end of the protein. In addition to the potential DNA binding RING finger domain, there is a glycine-rich (23/59) and alanine-rich (18/59) region of unknown function at the 3'-end of the protein (Fig. 1). A putative polyadenylation recognition site, AATAAA, is located 19 bp upstream from the poly(A) tail.

The cDNA matches two EST clones from the NCBI database: a fragment from bp 1619 to 1888 matches EST clone MDB0005R from mouse brain, and a fragment from bp 2799 to 3011 matches EST clone MDB0005 from mouse brain. A comparison of the putative mouse Znf179 to human ZNF179 in a 500-bp region that includes the RING finger domain and currently available surrounding sequences shows 83.8% nucleotide identity and 84.3% amino acid identity. In the RING finger domain, amino acid sequences are 97% identical or conserved between mouse and human (Fig. 2A). Using PCR and Southern analyses, Znf179 was mapped to several mouse YAC clones that are located in the SMS syntenic region (our unpublished data) on chromosome 11. Based on the conserved sequence homology and on its genomic localization in the mouse SMS syntenic region, we designate this mouse gene Znf179. The RING finger domains of Znf179 is also related to that of human interleukin 2 receptor regulatory protein RPT-1 (Patarca et al., 1988), human acid finger protein ZNF173 (Chu et al., 1995), and human ret-proto-oncogene activating protein RFP (Takahashi et al., 1988) (Fig. 2B). Outside the RING finger domain, Znf179 does not share homology with other known proteins.

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FIG. 5. Two-color FISH analysis using ZNF179 and FLI probes. The ZNF179 BAC DNA is detected with anti-digoxigenin conjugated to rhodamine (fluoresces red), and the FLI cosmid is detected with avidin conjugated to fluorescein isothiocyanate (fluoresces green). The interphase nuclei are counterstained with DAPI (fluoresces blue). Lymphoblast cell lines from SMS patient HOU202-641 (A) and HOU142-540 (B) with smaller deletions show two chromosomes 17, each hybridized with Znf179 (two red signals), but only one chromosome hybridized with FLI (one green signal), indicating a deletion of this locus in one chromosome 17. Del, deletion; nl, normal.

DISCUSSION

We have described the cloning and characterization of the mouse RING finger protein gene Znf179, the homologue of human ZNF179 that maps within the SMS critical region. Znf179 has been physically mapped to mouse chromosome 11 at 32 cM from the centromere (data not shown), the region syntenic to human chromosome 17p11.2. The RING finger domain is very conserved among mouse, rat, and human, suggesting that its molecular function is conserved in mammalian development. The putative DNA binding and/or protein–protein interaction RING finger motif in the protein has led to speculation that Znf179 is a transcription factor.

In recent years, zinc finger protein genes have been isolated from various species and in various tissue types. The common zinc finger domain contains pairs...
of cysteine and histidine residues and forms a finger-like DNA binding motif. RING finger proteins are a special type of zinc finger protein with a C3HC4 zinc binding motif (Freemont et al., 1991). Like other zinc finger proteins, RING finger proteins can bind Zn$^{2+}$ and then interact with DNAs and other proteins in vitro (Barlow et al., 1994), although the natural target sites of these proteins are yet to be discovered. Recent experiments have demonstrated that RING finger proteins participate in many important molecular and developmental events. RAD5 from yeast is involved in DNA repair (Johanson et al., 1992; Xnf-7, whose expression is turned on in Xenopus oocytes, is implicated in early embryogenesis (Reedy et al., 1991). Studies of RFP, which activates the ret proto-oncogene, show that it can bind double-stranded DNA and activate transcription (Isomura et al., 1992). Moreover, mutations of BRCA1 lead to predisposition and early onset of breast and ovarian cancers (Hall et al., 1990; Miki et al., 1994; Couch and Weber, 1996). Molecular and genetics studies in mouse have provided additional evidence for the involvement of BRCA1 in cell proliferation and differentiation (Marquis et al., 1995; Gowen et al., 1996; Hakem et al., 1996). In addition to the RING finger domain, there is an alanine- and glycine-rich domain located at the C-terminus of Znf179. Alanine-rich and glycine-rich domains have been found in many transcription modulators. These examples indicate that RING finger proteins are involved in diverse and important biological processes across many species.

The deletion in the majority of SMS patients is mediated by homologous recombination between repeat gene clusters flanking the critical region, which results in a common deletion (Chen et al., 1997); however, there are both larger and smaller deletions observed. SMS is considered a contiguous gene syndrome. A number of genes mapped within the deleted region could contribute to the complex phenotype of SMS patients. However, to date, none of the candidate genes has been proven to be related to the phenotype. Haploinsufficiency of a transcription factor gene has been recently shown to be responsible for Rubinstein–Taybi syndrome, another microdeletion syndrome (Petrij et al., 1995). Recently, it was reported that ZNF179 is deleted in six SMS patients (Kimura et al., 1997). Therefore, ZNF179 could be an interesting candidate gene. However, in this study, we observed that ZNF179 is not deleted in two SMS patients with smaller deletions. Patients HOU142-540 and HOU202-641 were shown previously to have a smaller deletion size by FISH, marker analysis, and flow cytometry (Juyal et al., 1995a,b; Trask et al., 1996). They share many clinical features with other SMS patients, including mental retardation, speech delay, craniofacial abnormalities, hearing impairment, and some ophthalmic abnormalities. Results from our study indicate that haploinsufficiency of Znf179 is most likely not associated with mental retardation, speech delay, hearing impairment, or renal and eye manifestations of SMS. Northern blot analysis shows that Znf179 is expressed extensively in brain and may indicate that haploinsufficiency of ZNF179 could contribute to neurological abnormalities.

Human chromosome 17p11–p12 has a high gene density and is genetically unstable. Primitive neuroectodermal tumor (PNET) breakpoints (Wilgenbus et al., 1997; Scheurien et al., 1997) and the nonsyndromic autosomal recessive deafness gene (DFNB3) (Liang et al., 1997) have been mapped within the Smith–Magenis critical region. Since there do not appear to be repeat gene clusters in the syntenic region of the mouse genome (our unpublished results), construction of a complete physical map and cloning genes in the syntenic region in mouse will certainly help to elicit a dosage-sensitive gene involved in these diseases.

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