Senile plaques and neurofibrillary tangles constitute two of the neuropathological hallmarks of Alzheimer's disease. The predominant constituent of senile plaques is the 4-kDa \( \beta \)-amyloid protein derived from larger amyloid precursor proteins (APPs). APP is a member of a gene family, including amyloid precursor-like proteins APLP1 and APLP2.

Using interspecific mouse backcross mapping, we localized the mouse APLP2 gene to the proximal region of mouse chromosome 9, syntenic with a region of human 11q.

We cloned an 1.2-kilobase mouse genomic fragment containing the APLP2 gene promoter. The APLP2 promoter lacks a typical TATA box, is GC-rich, and contains several sequences for transcription factor binding. S1 nuclease protection analysis revealed the presence of multiple transcription start sites. The lack of a TATA box, the presence of a high GC content, and multiple transcription start sites place the APLP2 promoter in the class of promoters of "housekeeping genes."

Regulatory regions within the promoter were assessed by transfection of mouse N2a and Ltk\(^{-}\) cells with constructs containing progressive 5' deletions of the APLP2 promoter fused to the bacterial chloramphenicol acetyl transferase (CAT) reporter gene. A minimal region that includes sequences 99 bp upstream of the predominant transcription start site of the APLP2 promoter was sufficient to direct high levels of CAT expression.

Senile plaques and neurofibrillary tangles constitute two of the neuropathological hallmarks of Alzheimer's disease. The predominant constituent of senile plaques is the 4-kDa \( \beta \)-amyloid peptide, derived from larger amyloid precursor proteins (APPs)\(^1\) (1, 2). APP is a member of a larger gene family including amyloid precursor-like proteins APLP1 and APLP2 (3–8). Notably, APLP2 shares considerable sequence homology with APP with the exception of the \( \beta \)-amyloid domain (5, 7, 8). In earlier studies, we demonstrated that APLP2 matures through the same unusual secretory/trafficking pathway as APP. Furthermore, APLP2 pre-mRNAs are alternatively spliced to generate at least four alternatively spliced transcripts (9, 10). Using in situ hybridization and reverse transcriptase-polymerase chain reaction (RT-PCR) approaches, we and others have demonstrated that in most adult tissues, APLP2 and APP mRNAs were expressed at similar, if not identical, levels. There are several exceptions; notably, in liver APP mRNA is essentially undetectable, but APLP2 mRNA is fairly abundant (5, 7, 9, 11). In recent studies, we have also demonstrated that specific alternatively spliced APLP2 mRNAs are differentially expressed in the olfactory epithelium (12). Moreover, APLP2 is highly enriched in olfactory sensory axons and axon terminals in glomeruli. On the other hand, APP is expressed, albeit at lower levels, in olfactory sensory neurons and to a lesser extent in sensory axons. This suggests that APLP2 and APP are regulated differentially in selected neuronal populations.

In order to assess whether the differential levels of APLP2 and APP expression may be a reflection of differences in sequence elements contained within respective promoters, we cloned and characterized an 1–1.2-kb fragment of the mouse APLP2 gene promoter. The mouse APP promoter has been characterized previously (13). We show that the mouse APLP2 gene promoter contains several features characteristic of promoters of "housekeeping genes"; these include the lack of a typical TATA box, the presence of a high GC content, and multiple transcription start sites. These latter features of the APLP2 promoter are similar to features described for mouse, rat, and human APP promoter regions (13–17). We assessed whether the APLP2 promoter contained positive or negative regulatory elements by transfecting mouse neuroblastoma (N2a) cells and mouse fibroblast (Ltk\(^{-}\)) cells with constructs containing progressive 5'-truncated promoter fragments of the APLP2 gene fused with the reporter gene chloramphenicol acetyl transferase (CAT). We demonstrate that CAT expression remains fairly constant across different deletion constructs in both N2a and Ltk\(^{-}\) cells and that a fragment representing just 99 bp upstream of the predominant transcription start site is sufficient to direct high levels of transgene expression in both cell lines. Interestingly, 5'-deletion studies of the human, mouse, and rat promoters also revealed that 100 bp of the respective promoters can drive high levels of expression of reporter genes (13, 15, 18).
RESULTS AND DISCUSSION

Recent studies have indicated that APP is a member of a larger gene family that includes APLP1 and APLP2. The phys-
Chromosomal Localization of APLP2—The chromosomal location of the mouse APLP2 gene was determined by interspecific backcross analysis using progeny derived from matings of (C57BL/6J × M. spreitus)F1 × C57BL/6J mice. This interspecific backcross mapping panel has been typed for over 1800 loci that are well distributed among all of the autosomes as well as the X chromosome (19). C57BL/6J and M. spreitus DNAs were digested with several enzymes and analyzed by Southern blot hybridization for informative restriction fragment length polymorphisms using a mouse cDNA APLP2 probe. The 6.6-, 4.2-, and 2.7-kb M. spreitus restriction fragment length polymorphisms (see "Materials and Methods") were used to follow the segregation of the APLP2 locus in backcross mice. The mapping results indicated that APLP2 is located in the proximal region of mouse chromosome 9 linked to Ldr, Penk, and Ets1. Although 152 mice were analyzed for every marker and are shown in the segregation analysis (Fig. 1), up to 185 mice were typed for some pairs of markers. Each locus was analyzed in pairwise combinations for recombination frequencies using the additional data. The ratios of the total number of mice exhibiting recombinant chromosomes to the total number of mice analyzed for each pair of loci and the most likely gene order are: centromere, Ldr (3/162) Penk (13/185) APLP2 (5/158) Ets1. The recombination frequencies (expressed as genetic distances in centimorgans ± the standard error) are as follows: Ldr (1.9 ± 1.1) Penk (7.0 ± 1.9) APLP2 (3.2 ± 1.4) Ets1.

We have compared our interspecific map of chromosome 9 with a composite mouse linkage map that reports the location of many uncloned mouse mutations (provided from the Mouse Genome database, a computerized database maintained at The Jackson Laboratory, Bar Harbor, ME). APLP2 maps in a region of the composite map that lacks mouse mutations with a phenotype that might be expected for an alteration in this locus (data not shown).

The proximal region of mouse chromosome 9 shares regions of homology with human chromosomes 19p, 8q, and 11q (summarized in Fig. 1). The recent assignment of APLP2 to 11q23-q25 (31) confirms and extends the synteny between mouse chromosome 9 and human 11q.

Transcription Initiation Site of Mouse APLP2 mRNA—RNA prepared from CHO cells and several mouse tissues was subjected to S1 nuclease protection analysis using a double-stranded DNA probe 5′-end-labeled 40 bp downstream of the translation start codon (Fig. 2A). Although the predominant start site is located −89 bp upstream of the translation start codon, there appeared to be variable levels of alternatively initiated APLP2 transcripts in mRNA isolated from CHO cells or from mouse thymus, heart, brain, liver, kidney, lung, testes, and spleen (Fig. 2B, lanes 1 and 2–9, respectively). Primer extension analysis also revealed the presence of multiple transcription start sites in mouse tissues (data not shown). Multiple transcription start sites have been identified for human (14) and rat (15) APP mRNA, with the predominant start sites located 146 and 156 bp upstream of the translation start codons, respectively. However, the transcription start site of mouse APP has not yet been reported.

Isolation of Genomic Sequences Containing the Mouse APLP2 Promoter—We screened ~800,000 independent phage-containing genomic DNA from a 129 SV embryonic stem cell library with a 67-bp fragment of the 5′-untranslated region of APLP2 (position −64 to +3 with respect to the translation start codon). We obtained two overlapping phage with the longest insert containing 2.8 kb of sequence upstream of the translation start codon, −1.2 kb of this promoter region was sequenced (Fig. 3).

The DNA sequence upstream of the predominant transcription start site contains a CAAT box (−135 in antisense orientation) but lacks a typical TATA box (Fig. 3). The promoter has a high GC content, specifically between positions −1 and −300 (68%) and −500 and −700 (69%). Multiple consensus sequences for transcription factor binding sites are present in the entire region, including one AP-1, two AP-2s, five GC boxes, one GC element, two GC factors, and seven SP-1 sites. Similar putative transcription factor binding sites are found in the APP promoter, however, at different locations with respect to the transcription start site (13, 14, 16, 17). Furthermore, the APP promoter contains sites for transcription factors not present in the APLP2 promoter, including a potential heat shock element and an overlapping AP-1/AP-4 site (14, 16, 18), suggesting that the transcriptional regulation of APLP2 and APP genes may be dissimilar. The presence of multiple transcription start sites, the absence of a typical TATA box, the high GC content, and the presence of GC-rich boxes places the APLP2 promoter in the class of promoters of housekeeping genes; these include the human, rat, and mouse APP genes (13, 14, 16, 17), the adenine deaminase gene (32), the dihydrofolate reductase gene (33), and the hamster prion gene (34).

Recently, the upstream AP-1 site (position −350 with respect
the predominant transcription start site) in the APP promoter has been implicated in protein kinase C mediated up-regulation of APP gene expression (35). The AP-1 binding activity is thought to be composed of Jun-Jun homodimers. Interleukin-1, nerve growth factor, and retinoic acid, agents known to increase APP gene expression, have been shown to induce c-jun and c-fos expression and cause transcriptional activation of target genes through AP-1 sites (36–39). Furthermore, interleukin-1 effects are thought to involve protein kinase C activation (40). It remains to be determined if APLP2 gene expression is also regulated by interleukin-1, nerve growth factor, and retinoic acid, particularly in view of the presence of a potential AP-1 site located at position 299.

99 bp of the Mouse APLP2 Promoter Is Sufficient to Direct High Levels of CAT Expression in N2a and Ltk- Cells—To identify regulatory sequences responsible for the expression of the mouse APLP2 gene, we constructed plasmids containing progressive 5' deletions of the APLP2 promoter fused upstream of the bacterial reporter gene CAT, as diagrammed in Fig. 4B. Equimolar amounts of each construct were transfected into mouse neuroblastoma (N2a) (Fig. 4C) and mouse fibroblast (Ltk-) (Fig. 4D) cells. RT-PCR analysis of cytoplasmic RNA from mouse N2a and mouse Ltk- cells with degenerate primers which hybridize to both APLP2 and APP mRNA revealed that these two cell lines express moderate levels of endogenous APLP2 mRNA (Fig. 4A, lanes 1 and 2). Hence, we concluded that these cell lines would be appropriate for analysis of the APLP2 promoter.

Progressive 5'-deletions from position -971 to position -99, with respect to the predominant transcription start site, had no significant effect on promoter activity in either of the two cell lines tested. These findings suggest that in N2a and Ltk- cells, 99 bp of the APLP2 promoter are sufficient for directing high

levels of promoter activity. Similarly, studies that analyzed progressive 5'-deletions of the APP promoter from human, mouse, and rat have shown that reporter gene expression levels remained fairly constant up to approximately 100 bp upstream of the predominant transcription start site (13, 15, 18).

In summary, we have localized APLP2 to the proximal region of mouse chromosome 9, characterized as 1.2 kb of the APLP2 promoter, and shown it to contain features characteristic of promoters in the class of housekeeping genes. We further showed that 99 bp upstream of the predominant transcription start site are sufficient to direct high levels of promoter activity.

Given the similarities in overall structure of the APLP2 and APP promoters and the minimal sequence requirements for transcription initiation, it is highly likely that additional sequence elements distal to the regions analyzed here are responsible for differential expression of APLP2/APP in specific neuronal populations or systemic organs (i.e., liver). Further studies will be directed toward using transgenic strategies with larger genomic fragments to clarify these issues with the eventual goal of identifying transcription factors responsible for mediating basal level of APLP2 gene expression.

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