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Regulatory region of human amyloid precursor protein (APP) gene promotes neuron-specific gene expression in the CNS of transgenic mice

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The accumulation of β-amyloid protein in specific brain regions is a central pathological feature of Alzheimer's disease (AD). The 4 kd β-amyloid protein derives from a larger amyloid precursor protein (APP) by as yet unknown mechanisms. In the absence of a laboratory animal model of AD, transgenic mice expressing various APP gene products may provide new insights into the relationship between APP and β-amyloid formation and the pathogenesis of AD. β-amyloid accumulation in AD brain may result from interactions between APP and other molecules. Such interactions are likely to be developmentally regulated and tissue-specific. A transgenic mouse model of AD, therefore, would aim for APP transgene expression that mimics the endogenous APP gene. As an initial step in developing an animal model, we have identified a 4.5 kb DNA fragment from the 5′ end of the human APP gene, which mediates neuron-specific gene expression in the CNS of transgenic mice, using *E.coli lacZ* as a reporter gene. Detectable levels of transgene expression are found in most neurons but not in glial and vascular endothelial cells. The expression pattern of this reporter gene closely resembles the distribution of endogenous APP mRNA in both the human and mouse CNS.

Key words: Alzheimer's disease/β-amyloid/neurons/transgenic mice

Introduction

Alzheimer's disease (AD) is the most prevalent cause of dementia (Alzheimer, 1907). Progressive neurological impairments in AD patients are associated with degenerating neurons in the basal forebrain, cerebral cortex, hippocampus, and other brain regions (for reviews see: Kemper, 1984; Katzman, 1986; Price, 1986). Post-mortem AD brains are characterized by several neuropathological changes, including the presence of neurofibrillary tangles (NFT), neuritic (senile) plaques, and amyloid accumulation inside and adjacent to some meningeal blood vessels (for review see: Selkoe, 1989; Muller-Hill and Beyreuther, 1989). A major component of amyloid plaques is a 4 kd protein (referred to as A4 or β-amyloid protein) which is derived from one or more forms of a larger amyloid precursor protein (APP). Although AD-related mutations in the APP gene have not been identified (Vitek *et al.*, 1988) and the familial form of AD (FAD) is not genetically linked to the APP gene (St.George-Hyslop *et al.*, 1987a,b; Van Broeckhoven *et al.*, 1987), deposition of A4 protein is a significant and early event in AD pathology (Mann and Esiri, 1988; Tagliavani *et al.*, 1988; Yamaguchi *et al.*, 1988; Joachim *et al.*, 1989).

At least four different isoforms of APP are encoded by a single gene on chromosome 21 and contain either 695, 714, 751, or 770 amino acid residues (Goldgaber *et al.*, 1987; Kang *et al.*, 1987; Robakis *et al.*, 1987; Tanzi *et al.*, 1987; Golde *et al.*, 1990). The APP's are glycosylated transmembrane proteins (Weidemann *et al.*, 1989)} and two isoforms, APP 751 and 770, contain a protease inhibitor domain which is homologous to the Kunitz type of serine protease inhibitors (Ponte *et al.*, 1988; Tanzi *et al.*, 1987; Kitaguchi *et al.*, 1988; Donnelly *et al.*, 1988). The physiological role(s) of the APP isoforms are not known. However, APP 751 and 770 share amino acid homology with protease inhibitor, protease nexin II (Oltersdorf *et al.*, 1989; Van Nostand *et al.*, 1989).

The mechanism by which A4 is derived from its precursor in AD patients is not known (Muller-Hill and Beyreuther, 1989; Selkoe, 1989; Price *et al.*, 1989). Proteolytic processing of APP appears to be a normal cellular event. A C-terminal truncated form of APP 695, 751 and 770 isoforms is secreted by a number of cell lines (Sisodia *et al.*, 1990; Weidemann *et al.*, 1989) and is found in brain and cerebrospinal fluid (Palmert *et al.*, 1989; Weidemann *et al.*, 1989). Constitutive processing and secretion results in proteolytic cleavage within the A4 peptide domain of APP (Esch *et al.*, 1990). Generation of A4, therefore, is most likely to occur by altered proteolytic processing of APP. A4 is unlikely to result from alternative RNA splicing because the A4 amyloid peptide is encoded by two separate exons (Lemaire *et al.*, 1989).

The sporadic and late onset of amyloid deposition has significantly impeded the elucidation of the role β-amyloid plays in the progression of AD. Furthermore, convenient laboratory animal models for AD are not available. Transgenic animals may provide new insights into the relationship between APP and β-amyloid formation and the pathogenesis of AD. For example, A4 amyloidosis may be induced by direct expression of the A4 amyloid peptide or other mutant forms of APP in the CNS of transgenic mice. Ideally, APP transgenes should be expressed with the same developmental and spatial distribution as the endogenous gene (Unterbeck *et al.*, 1990).

The purpose of this study was to determine whether 5′ regulatory sequences of the human APP gene confer the appropriate specificity to heterologous gene elements when introduced into transgenic mice. We have used the reporter gene *E.coli lacZ* (Hall *et al.*, 1983) to monitor the cellular expression of the human APP gene promoter (regulatory

region). Our results demonstrate that a 4.5 kb 5′-end sequence of the human APP gene contains sufficient information to target transgene expression in the CNS of mice with patterns consistent with endogenous mouse and human APP gene expression.

Results

Distribution of APP mRNA in the mouse

The APP gene and its expression pattern is highly conserved across species (Yamada *et al.*, 1987; Manning *et al.*, 1988; Shivers *et al.*, 1988; Rosen *et al.*, 1989). The distribution of APP mRNA within the CNS of humans, primates, rats, and mice has been investigated by northern blot and *in situ* hybridization studies and the majority of APP mRNA in the CNS has been localized to neuronal cytoplasm (Bahmanyar *et al.*, 1987; Goedert, 1987; Bendotti *et al.*, 1988; Shivers *et al.*, 1988).

Whereas the general distribution of APP mRNA has been described in mouse CNS (Bendotti *et al.*, 1988), the precise location of these gene products has not. Therefore, the distribution of APP mRNA within normal mice (4−5 weeks old) was determined by hybridizing 8 μm thick paraffin sections with ^{35}S-labeled DNA complementary (cDNA) to the APP mRNA (Figure 1). Silver grains, representing APP mRNA, occurred in clusters that reflected the general distribution of neurons in all brain regions studied. For example, neuronal perikarya present in the pyramidal layer of the hippocampus and granular layer of the dentate gyrus were labeled intensely (Figure 1A). Significantly less

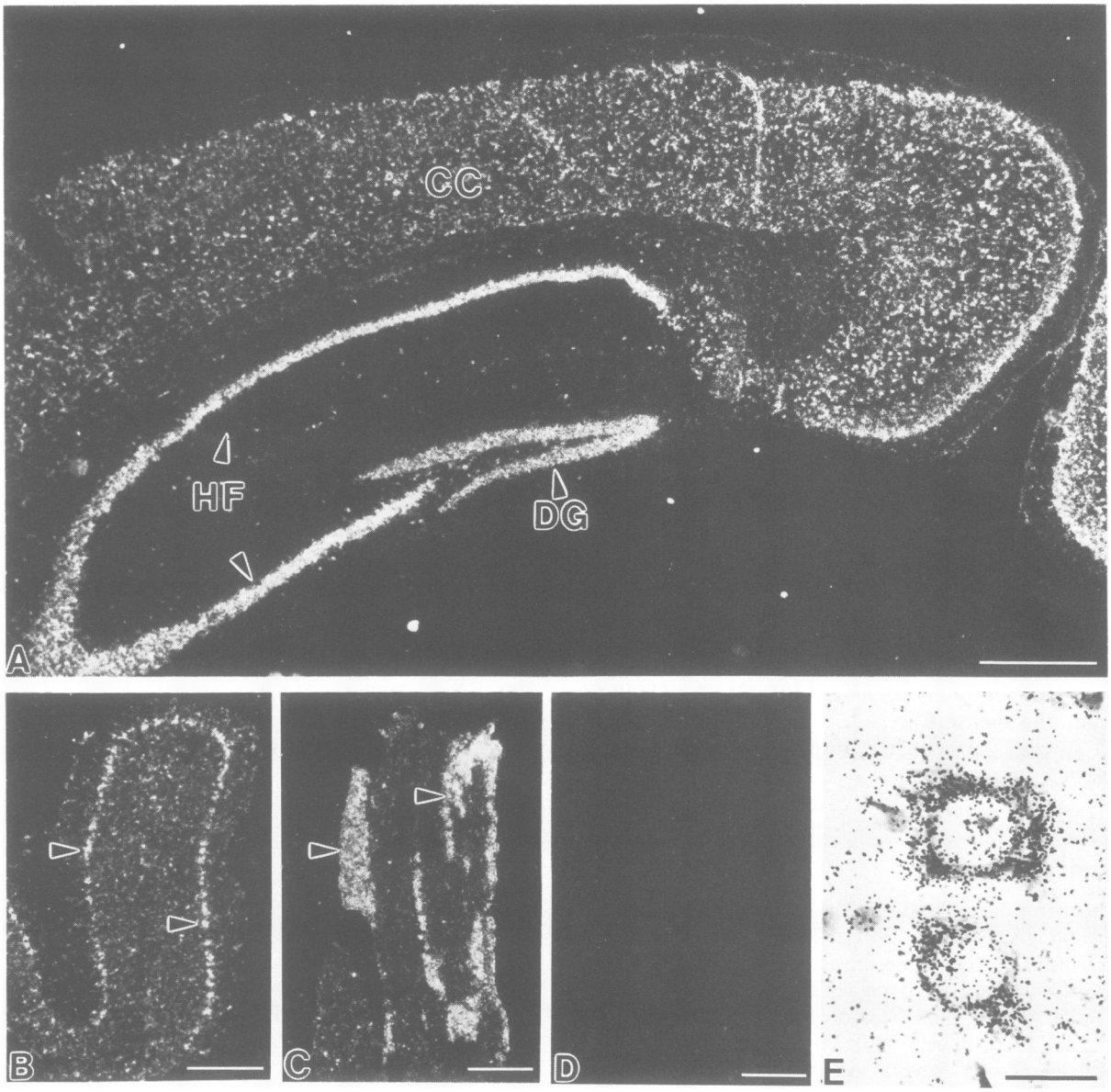

Fig. 1. Distribution of endogenous APP mRNA in paraffin section (6−8 μm thick) of normal mouse. In sections of brain (**A**), APP mRNA occurs in clusters that reflect the general distribution of neurons in the cerebral cortex (CC), hippocampal formation (HF), and dentate gyrus (DG). Purkinje cells in the cerebellum (**B**, arrowheads) and neuronal perikarya in the trigeminal ganglia (**C**, arrowheads) were also labeled by APP cDNA. White matter tracts in the CNS (A) and PNS (C) and sections of liver (**D**) contained little hybridization signal. Viewed at higher magnification, silver grains were found primarily in neuronal perinuclear cytoplasm (E). Parts A−D are viewed in dark-field optics. Part E is viewed in bright-field optics. Scale bars: A = 500 μm, B−D = 200 μm, E = 50 μm.

hybridization signal was detected in other layers of the hippocampal formation and in subcortical white matter. The cerebral cortex contained significant levels of APP mRNA (Figure 1A); and the labeling pattern in various cortical areas (i.e., occipital, temporal and frontal) reflected the distribution of neuronal perikarya. For example, Layer I, which contains few neurons, had the lowest hybridization signal in all cortical regions, and variations in labeling intensity in Layer II correlated with known changes in neuronal cell densities that were confirmed by analysis with bright-field optics.

In sections of cerebellar cortex, Purkinje and granular cells were labeled by APP cDNA (Figure 1B) and the distribution of silver grain clusters in sections of brain stem and spinal cord was consistent with the known distribution of neuronal perikarya (data not shown). To investigate whether neurons in the peripheral nervous system express the APP gene, sections of trigeminal ganglia were hybridized with APP cDNA (Figure 1C). Neuronal perikarya, which occur in clusters, were labeled intensely but little hybridization signal was found in myelinated fiber tracts in the PNS.

The distribution of silver grains in all sections hybridized were analyzed at higher magnification under bright-field optics. In all sections, silver grains were concentrated within perinuclear cytoplasm of neurons (Figure 1E). Few silver grains were present over neuronal nuclei or scattered throughout the neuropil. APP mRNA was not detected in sections of liver (Figure 1D), a finding consistent with northern blot analysis (Yamada *et al.*, 1989).

Human APP promoter–lacZ reporter gene expression in transgenic mice

The reporter gene, pMTI-2402, was constructed by inserting sequences encoding a *lacZ* fusion protein and SV40 polyadenylation signal into a 4.5 kb genomic fragment encompassing the 5′-end of the human APP gene (Figure 2A). The genomic fragment contains 2835 bp of sequences 5′ to the primary transcriptional start site, exon I (Lemaire *et al.*, 1989), and ~1.7 kb of the first intron. Three lines of transgenic mice were identified which carry multiple head-to-tail integrations of the intact reporter gene (data not shown). The distribution of β-galactosidase expression within the central nervous system of adult mice from each transgenic line was determined by histochemical staining of 0.5 cm thick tissue slices. One line, BE803, exhibited intense β-galactosidase expression throughout the CNS and is described in this report. The expression pattern of an APP promoter–*lacZ* reporter gene in adult transgenic mouse brain was determined at the tissue and cellular levels and was strikingly similar to the distribution of endogenous APP mRNA in mouse and human brain. The other lines (BE1805 and BE3002) exhibited a similar pattern but lower levels of transgene expression (data not shown).

In 0.5 cm thick slices of adult BE803 brain, stained histochemically for β-galactosidase, blue reaction product was concentrated in brain regions with high densities of neuronal perikarya (Figure 3A–C). For example, the cerebral cortex, dentate gyrus, basal ganglia, thalamus, and regions of the hippocampus were stained intensely. Prominent white matter tracts such as the corpus callosum and internal and external capsule were not stained. β-galactosidase was also present in slices of brain stem and spinal cord (data not shown) in patterns similar to endogenous mouse APP mRNA. β-galactosidase was not detected in slices of normal mouse brain (Figure 3D).

Regions of cerebellar cortex that contain high concentrations of neuronal perikarya were positive for β-

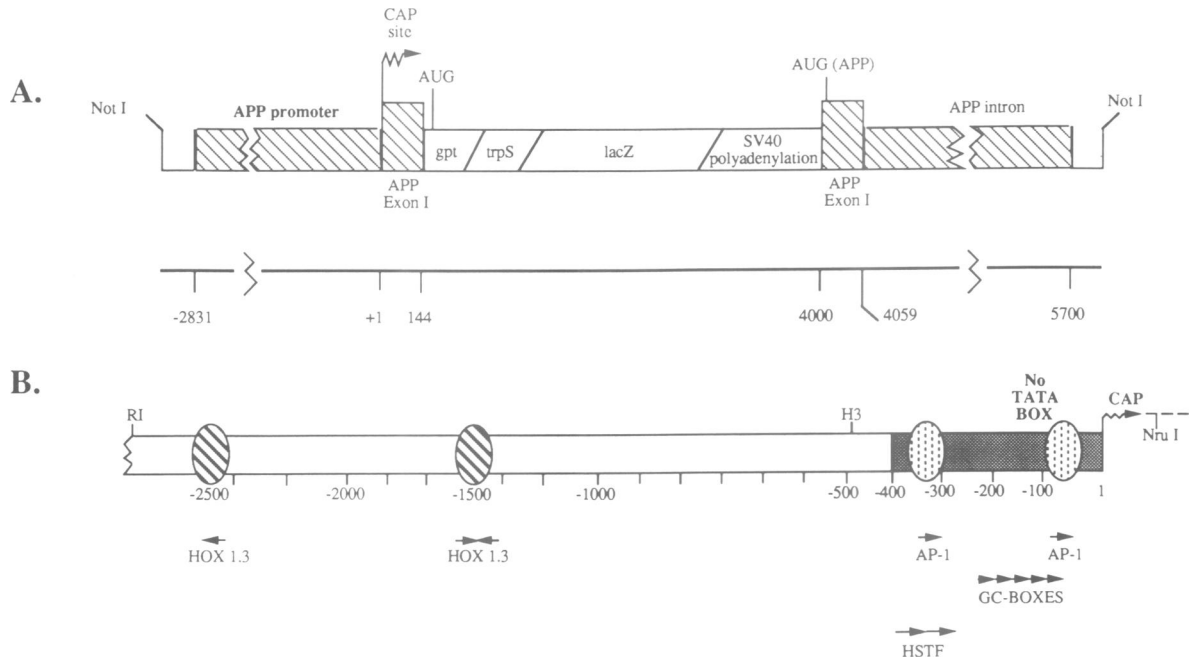

Fig 2. A. Schematic representation of the ~8.6 kb *Not*I restriction fragment from pMTI-2402 used to generate transgenic mouse lines BE803, BE1805, and BE3002. **B.** Schematic representation of the 5′ regulatory region of the APP gene. The DNA segment 400 bp upstream (shaded segment) of the CAP sites contains typical promoter elements such as binding sites for the transcriptional factor AP-1, which is analogous to the product of the proto-oncogene *v-jun* and associated with the product of *c-fos* (see (Salbaum et al., 1988)). Multiple GC-boxes are located in this region, two of which participate in sequence-specific protein building (Salbaum et al., 1988). At least one consensus sequence for heat shock transcription factors (HSTF) is present in this region(Salbaum et al., 1988). Consensus sequences for the homeobox protein Hox-1.3 (Odenwald et al., 1989) are located further upstream between positions −1400 bp and −2600 bp (see text).

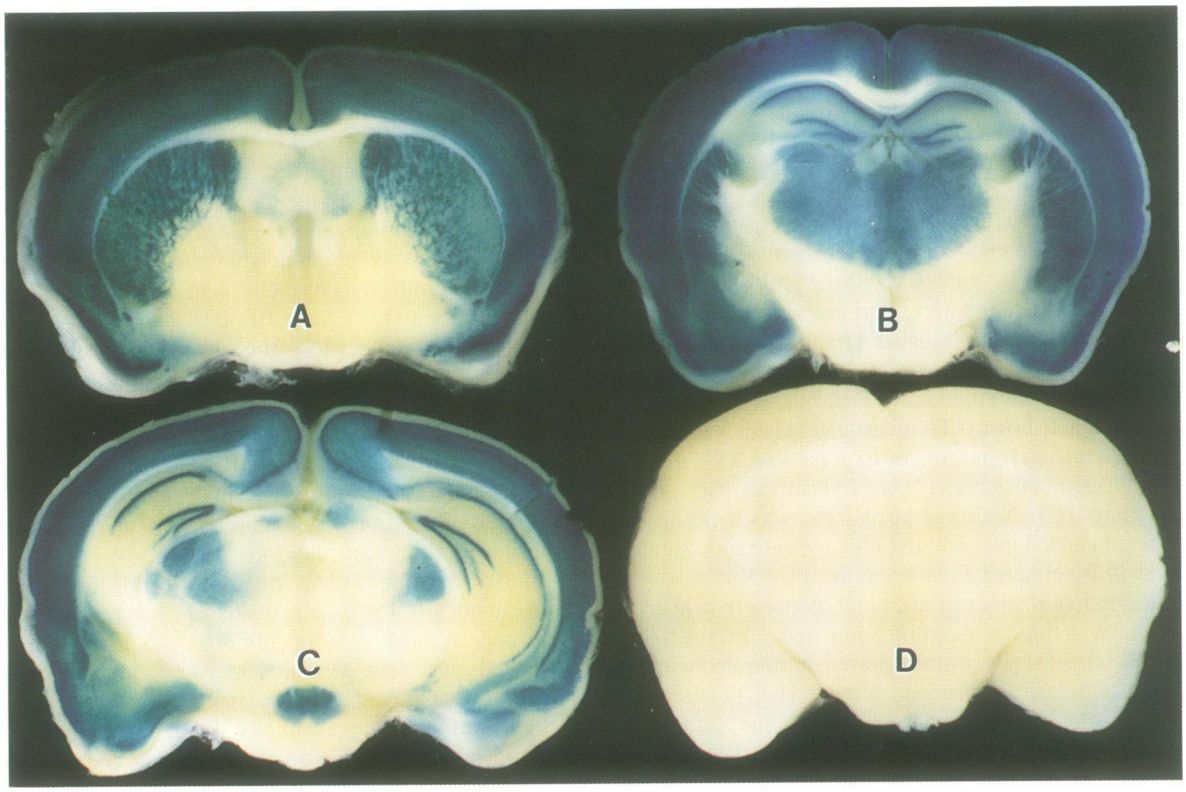

Fig. 3. Distribution of β-galactosidase in serial brain slices (0.5 cm thick) of BE803 transgenic (**A**–**C**) and control (**D**) mice. Blue reaction product indicates that β-galactosidase is expressed predominantly by neuronal perikarya in transgenic mice. β-galactosidase reaction product is not detected in brain slices from normal mice (D) nor in white matter tracts of transgenic brain.

galactosidase (Figure 4A), as were neuronal perikarya in the trigeminal ganglion (Figure 4C). In 20 μm thick vibratome sections from the cerebellar cortex, β-galactosidase was localized in perinuclear regions of Purkinje cells and granule cells (Figure 4B). White matter tracts in the cerebellum (Figure 4A) and trigeminal nerve (Figure 4C), and slices of liver (Figure 4D) from BE803 mice were negative for β-galactosidase activity. Identical β-galactosidase staining patterns have been observed in tissue slices from several BE803 transgenic mice (data not shown).

Subcellular distribution of β-galactosidase in transgenic mice

The cellular and subcellular distribution of β-galactosidase was determined in several brain regions by light and electron microscopic procedures. For light microscopic studies, β-galactosidase was localized histochemically in 20 μm thick vibratome sections. In these sections, β-galactosidase reaction product occurred as small dots that were restricted to regions of the CNS that contained neuronal perikarya. Reaction product was detected in all layers of the cerebral cortex (Figure 5A), including occasional deposits in Layer I. When examined at higher magnification with Nomarski optics, β-galactosidase reaction product was restricted to perinuclear regions of neurons (Figure 5B and C). β-Galactosidase was not detectable in endothelial cells and cellular perikarya within white matter tracts.

To characterize the subcellular distributions of β-galactosidase in cerebral cortex, β-galactosidase was localized immunocytochemically in ultrathin cryosections by immunogold procedures (Figure 5D). The majority of gold particles in electron micrographs were found within neuronal perinuclear cytoplasm. Some particles were distributed diffusely while most were confined to secondary lysosomes. Glial cells and endothelial cells were not labeled by β-galactosidase antibodies.

Analysis of vibratome sections also detected the presence of β-galactosidase in regions of the CNS that were not labeled intensely in the brain slices. For example, consistent but weak staining of some but not all neurons in CA-3 region of the hippocampus was found (Figure 6). Occasional neurons that did not contain β-galactosidase reaction product were also found in other regions of the CNS, including some Purkinje cells.

Discussion

The primary defect(s) leading to AD has not been determined, although genetic loci on chromosome 21 appear to be associated with some cases of Alzheimer's disease (St.George-Hyslop *et al.*, 1987a,b; Van Broeckhoven *et al.*, 1987). Amyloid plaques are one of the characteristic and predominant pathological markers associated with the disease. A major constituent of amyloid plaques is a 42–43 amino acid peptide, which is derived from a larger amyloid precursor protein, APP (Goldgaber *et al.*, 1987; Kang *et al.*, 1987; Robakis *et al.*, 1987; Tanzi *et al.*, 1987). Although plaques are observed in brains of non-diseased individuals, their number is much higher in the brains of AD patients, and are found specifically in those regions of the brain most severely affected by cellular degeneration (Price, 1986). Since the amyloid peptide is encoded at a genetic locus on chromosome 21 that is distinct from the gene(s) associated with familial cases, it is apparent that the A4 peptide itself

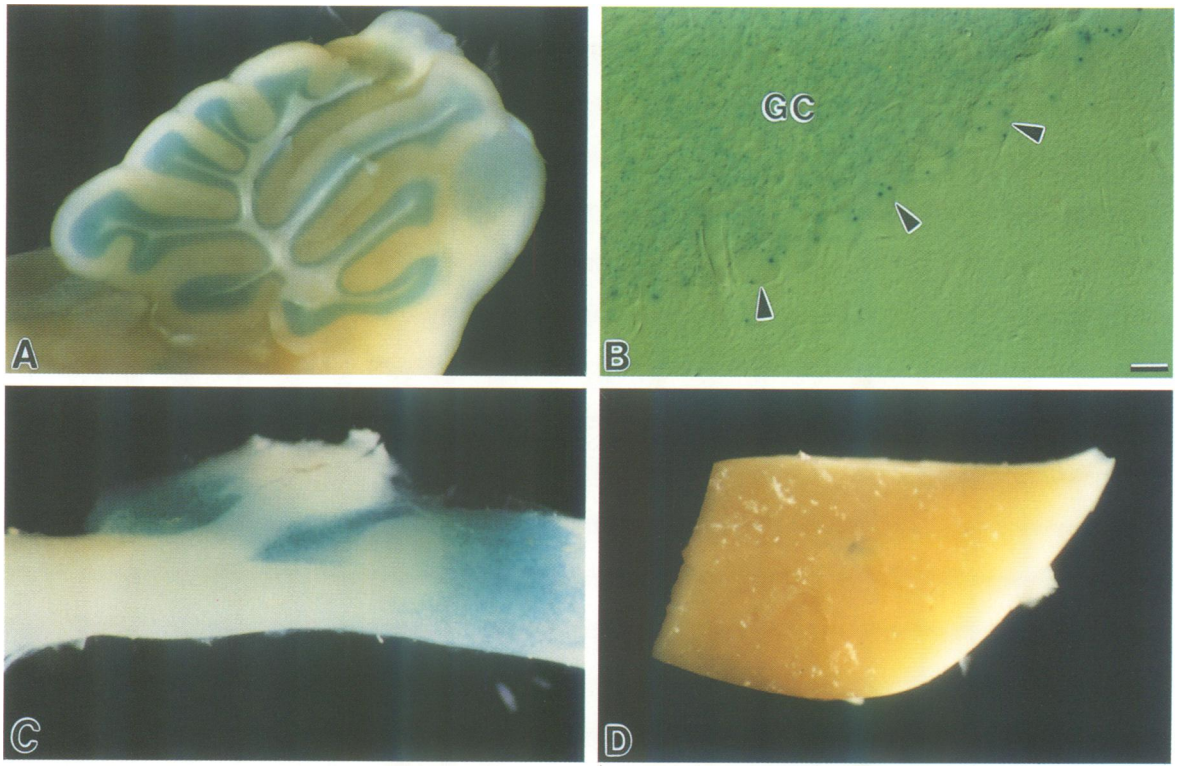

Fig. 4. Histochemical distribution of β-galactosidase in tissue slices of cerebellum (**A**,**B**), trigeminal ganglia (**C**), and liver (**D**) from BE803 transgenic mice. Blue reaction product in slices of cerebellum (A) is located predominantly in Purkinje cells (B, arrowheads) and granule cells (B, GC). Similar to endogenous APP mRNA, β-galactosidase is detected in neuronal perikarya of trigeminal ganglia (C) and is not detected in sections of liver (D). Parts A, C and B are 0.5 cm slices; Part B is a 20 μm thick vibratome section viewed in Nomarski optics. Scale bar in Part B = 25 μm.

or a mutation in the APP gene is not the primary cause of familial AD. Nevertheless, an understanding of the mechanisms by which the peptide is processed from a larger precursor, and a determination of the extent to which plaque formation contributes to neuronal cell death and disease progression remain central and important questions in our understanding of Alzheimer's disease.

The deposition of β-amyloid in plaques may involve a complex interaction of different cell types and various protein factors. A crucial step in understanding these processes is the development of an experimental animal model which would facilitate the analysis and manifestation of plaque formation in the brain. For this purpose, we have initiated a series of experiments to generate transgenic animals that express various forms of human APP. Ideally, we would like the APP transgenes to be expressed with the same developmental and spatial distribution as the endogenous gene. The purpose of this study was to determine whether 5′ regulatory sequences of the human APP gene confer the appropriate specificity to heterologous sequences when introduced into transgenic mice.

We have demonstrated that a 4.5 kb genomic fragment encompassing the 5′-end of the human APP gene (Figure 2) has sufficient sequence information to direct cell and tissue-specific expression of a reporter gene, *E.coli lacZ*, in transgenic mice (Figure 3). The expression pattern of the reporter gene in the CNS was strikingly similar to the expression pattern of the endogenous mouse APP gene in the central nervous system, the peripheral nervous system, and liver. β-galactosidase in transgenic CNS and APP mRNA in normal mouse CNS were expressed predominantly by neurons (Figure 1 and 3). The majority of β-galactosidase fusion protein was localized in secondary lysosomes within neuronal perikarya. In most cases, the β-galactosidase staining in the CNS of BE803 transgenic mice was consistent with *in situ* hybridization patterns of mouse APP mRNA. One exception was the CA3 region of the hippocampus (Figure 6) where the β-galactosidase staining was not as intense as would be expected from the observed levels of endogenous mouse APP mRNA (Figure 1). This difference may be due to a lowered expression level of the reporter gene in this region, to altered stability of the β-galactosidase fusion protein or to regulatory elements not included in the 4.5 kb genomic fragment.

Although our analyses, in this report, describe one line of transgenic mice, two additional lines showed patterns of expression that were qualitatively similar, although the level of expression was lower in these lines. The quantitative differences probably reflect a position effect, which has been seen with other genes in transgenic animals (Palmiter and Brinster, 1986). Genomic elements which circumvent these position effects have been identified for a number of mammalian gene loci, including the human β-globin, human CD2, rat growth hormone, and mouse metallothionein genes (Grosveld *et al.*, 1987; Brinster *et al.*, 1988; Greaves *et al.*, 1989). The likely interpretation of our data is that the human APP gene elements included on the chimeric construct are sufficient to confer spatial and temporal patterns of expression which are very similar to the endogenous gene. However, additional sequences may be necessary for quantitatively correct regulation.

While APP gene expression is prominent in brain, transcripts can be identified in many tissues (Yamada *et al.*, 1989). The 5′-end of the human APP gene contains

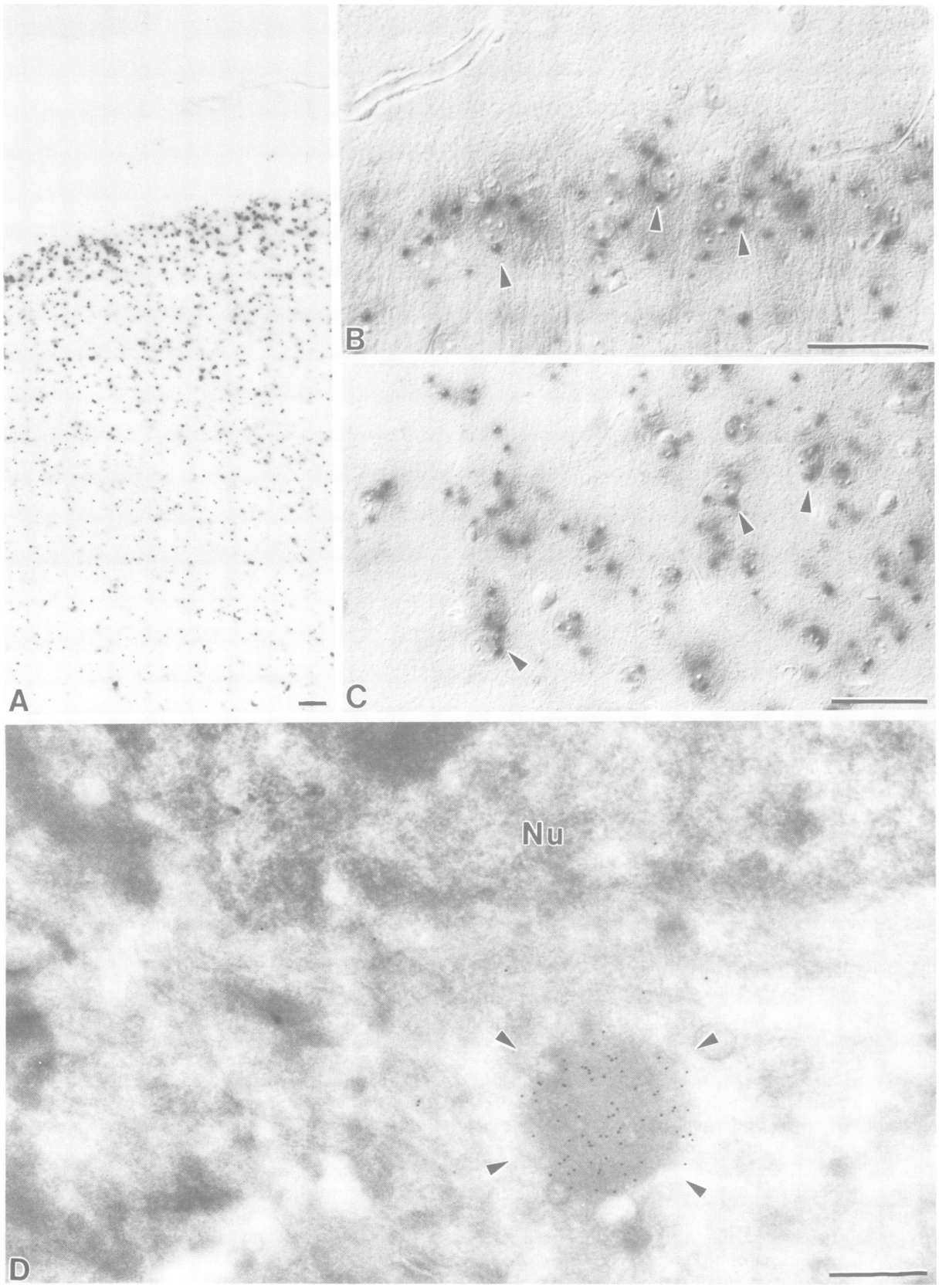

Fig. 5. Cellular and subcellular distribution of β-galactosidase reaction product in BE803 cerebral cortex. In 20 μm thick vibratome sections, reaction product occurs as small dots (**A**) that are located exclusively in perinuclear regions of neurons (**B**,**C**). In ultrathin cryosections of cerebral neurons stained with β-galactosidase antibodies and immunogold procedures, β-galactosidase was located predominantly in lysosomes (**D**, arrowheads). NU = nucleus. Part A is viewed in bright-field optics; Parts B and C are viewed in Nomarski optics. Scale bars: A–C = 50 μm, D = 0.5 μm.

sequences suggestive of several gene regulatory elements and displays characteristics of a 'housekeeping' gene promoter. Within the 2.8 kb of DNA sequences upstream of the strongest RNA start site, there are no typical TATA and CAAT elements (Salbaum *et al.*, 1988; La Fauci *et al.*, 1989). The regulatory region contained within 400 bp upstream of the strongest RNA start site confers promoter activity to a reporter gene in cell transfection experiments (Salbaum *et al.*, 1988) and shows a similarity to a variety of typical promoter-binding elements (Figure 2b). These include two AP-1 consensus sites (Lee *et al.*, 1987), a heat shock recognition consensus sequence (Wu *et al.*, 1987), and several copies of a 9 bp long GC-rich consensus sequence (Salbaum *et al.*, 1988).

Regulation of the APP gene in mammalian development is of considerable interest. Although a detailed study is still in progress, preliminary analyses demonstrate clear spatial and temporal patterns of expression that resemble previously determined patterns for some homeobox genes (for review, see Gehring, 1987; Holland and Hogan, 1988). The APP gene regulatory region contains at least five homeobox consensus binding sequences (CPyNATTAT/GPy) (Odenwald *et al.*, 1989) and preliminary experiments have shown that the mouse homeobox protein Hox 1.3 binds at least two of these sites (W.F.Odenwald and D.Goldgaber, personal communication). Work is in progress to study expression patterns of the APP promoter–*lacZ* reporter gene during embryogenesis of transgenic mice. These transgenic mice provide a unique opportunity to investigate the regulation of the APP gene during development, including the potential involvement of homeobox proteins. Studies of these transgenic mice, and of mouse lines carrying various modified forms of the APP gene, will provide additional insights into the normal function and regulation of this gene, and into its role in the pathogenesis of Alzheimer's disease.

Materials and methods

Construction of recombinant minigenes and production of transgenic mice

Clone pMTI-2307 contains an ~4.5 kb *Eco*RI fragment encompassing the 5′-end of the human APP gene inserted (blunt-end) into the *Hin*dIII site of pMTI-2301. The APP genomic fragment was isolated from a human chromosome 21 cell-sorted genomic library (obtained from the American Type Culture Association; ATCC no. LA21NS01), using probe generated from an *Apa*I–*Xho*I fragment of APP cDNA. The cloning vector, pMTI-2301, contains a unique cloning site, *Hin*dIII, flanked by *Not*I restriction sites; and was generated by replacing the multiple cloning sites (*Eco*RI–*Hin*dIII restriction fragment) of pUC19 with the multiple cloning sites (the *Eco*RI–*Eco*RI restriction fragment) of pWE16 (from Stratagene; the *Bam*HI site was converted to a *Hin*dIII site using adapters). Plasmid pMTI-2402 was constructed by inserting a *Hin*dIII–*Bam*HI fragment from pCH126 (~3.9 kb in size and containing the lacZ fusion protein and a SV40 polyadenylation signal) into the *Nru*I site of pMTI-2307 by blunt-end ligation. The plasmid, pCH126, is identical to pCH110 (Hall *et al.*, 1983; Goring *et al.*, 1987) except that the SV40 promoter (*Pvu*II–*Hin*dIII fragment) has been deleted (the *Hin*dIII site remains).

Plasmid pMTI-2402 was double purified in CsCl/ethidium bromide equilibrium density gradients. The 8.5 kb linear DNA fragment, encompassing the APP–*lacZ* reporter gene, was excised from vector sequences using *Not*I and isolated from an agarose gel using NA45 paper (Schleicher and Schuell Inc., Keene, NH). The DNA was precipitated in ethanol–ammonium acetate three times and resuspended, at a concentration of 6 μg/ml, in filtered (0.2 μm) injection buffer (10 mM Tris, pH 7.5, and 0.3 mM EDTA; (Brinster *et al.*, 1985)). Purified DNA fragments were microinjected into 1-cell embryos of Hsd:(ICR)BR females and

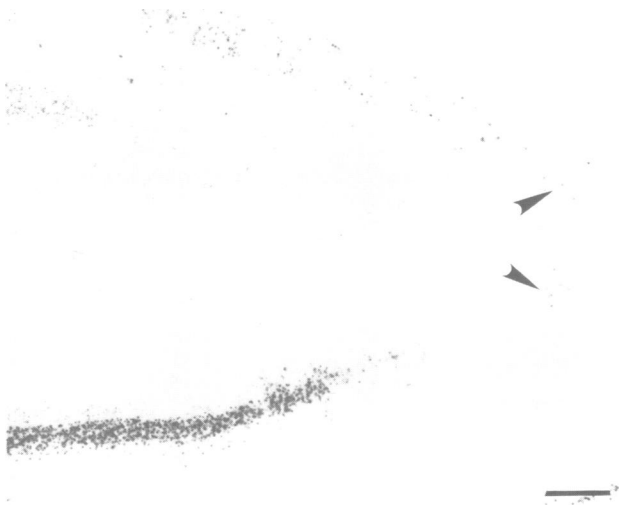

Fig. 6. Histochemical distribution of β-galactosidase in 20 μm thick vibratome sections of B-803 hippocampus. Some, but not all, neurons in CA3 regions are labeled (arrowheads). Bright-field optics. Scale bar = 100 μm.

$B6D2F_1$Hsd BR males and reimplanted into Hsd:(ICR)BR females as described (DePamphilis *et al.*, 1988). Transgenic founder mice were identified by PCR analysis of tail biopsy DNA using 30 bp oligonucleotides complementary to the *E.coli lacZ* gene. DNA from transgenic mice was further analyzed by restriction enzyme digestion and Southern blot analysis (Wirak *et al.*, 1985).

Histochemical detection of β-galactosidase

Transgenic and normal control mice, 4–5 weeks of age, were anesthetized and perfused with 4% paraformaldehyde and 0.08 M phosphate buffer, pH 7.6. The CNS, trigeminal nerve, and liver were removed and placed in the fixative overnight at 4°C. These tissues were cut into 0.5 cm thick slices that were either stained histochemically for β-galactosidase or sectioned at a thickness of 20 μm on a vibrating microtome prior to staining. β-galactosidase activity was detected histochemically by the method of Sanes *et al.* (1986), using 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-Gal) as a substrate. This reaction results in the deposition of a blue substrate. Vibratome sections were infiltrated with 100% glycerol, mounted on glass slides, and then photographed with a Zeiss Axiophot microscope using bright-field or Nomarski optics.

EM immunocytochemistry

Transgenic mice, 4–5 weeks of age, were perfused with 2.5% glutaraldehyde and 4% paraformaldehyde in 0.08 M phosphate buffer. The brain was removed and placed in the fixative overnight at 4°C. Segments of the cerebral cortex (2 mm^2) were cryoprotected with 2.3 M sucrose and 30% polyvinyl pyrrolidon, placed on specimen stubs, and frozen in liquid nitrogen. Ultrathin frozen sections (~120 nm thick) were cut in a Reichert Ultracut-FC4 ultracryomicrotome maintained at approximately −110°C. The sections were transferred to formvar and carbon-coated hexagonal mesh grids and stained by immunogold procedures using described modifications (Trapp *et al.*, 1989a,b) of standard methods (Slot and Geuze, 1984; Tokuyasu, 1986). After the immunostaining, the grids were placed in 2.5% glutaraldehyde in PBS for 15 min and rinsed. The sections were then stained with neutral uranyl acetate followed by embedding in 1.3% methylcellulose containing 0.3% uranyl acetate. Grids were examined in an Hitachi H600 electron microscope.

In situ hybridization

Four- to five-week-old mice were anesthetized and perfused with 4% paraformaldehyde in 0.08 M phosphate buffer, pH 7.6. The following tissues were removed and processed to paraffin by standard procedures: cerebral hemispheres plus diencephalon, pons, medulla, cervical and lumbar spinal cord, trigeminal nerve and liver. All tissues from individual mice were embedded in a single block. Sections were cut at a thickness of 6 μm and hybridized according to published procedures (Trapp *et al.*, 1987; Trapp *et al.*, 1988). Briefly, pre-hybridization treatment consisted of 0.2 N HCl for 20 min and 25 μg/ml protease K for 15 min at 37°C. Slides were

hybridized at room temperature for 16 h in a standard buffer containing 0.2 ng/μl of single-stranded, full-length APP cDNA, labeled with ^{35}S by the Klenow procedure (specific activity, 2.3×10^9 c.p.m./μg). Stringency washes included 50% formamide containing 0.3 M NaCl, 1 mM EDTA, and 5 mM Tris (pH 8) for 30 min at room temperature and 2× SSC (1× SSC = 0.3 M NaCl, 0.03 M sodium citrate, pH 7.4) in 1 mM EDTA for 1 h at 55°C. Slides were then dehydrated, air dried, dipped in emulsion (Kodak, NTB-3), exposed for 7 days, developed for autoradiography, and counterstained with hematoxylin. Sections were photographed with a Zeiss Axiophot using dark-field and bright-field optics.

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