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26. Transverse hippocampal slices (625 μm) were prepared from male Sprague-Dawley rats 22 to 32 days old and perfused at 31° to 33°C in a submerged recording chamber. ACSF was bubbled with a mixture of 95% O_2 and 5% CO_2 and contained 120 m*M* NaCl, 3.3 m*M* KCl, 1.23 m*M* NaH_2PO_4, 25 m*M* $NaHCO_3$, 0.9 m*M* $MgSO_4$, 1.3 m*M* $CaCl_2$ (Mg^{2+} and Ca^{2+} in accordance with values measured in normal rat CSF [G. G. Somjen, *J. Neurophysiol.* **44**, 617 (1980); J. G. Chutkow and S. Meyers, *Neurology* **18**, 963 (1968)]), and 10 m*M* dextrose. D-APV (Cambridge Research Biochemical, Cambridge, England) and MK-801 were dissolved in this ACSF and bath-applied. Extracellular field recordings were made from stratum pyramidale of area CA1 and CA3 with glass microelectrodes containing 2*M* or 0.15*M* NaCl (1 to 10 megohms). Stimulus trains (60 Hz, 2 s, twice the intensity of the single pulse that evoked the maximum orthodromic population spike in CA3) were delivered every 10 min to stratum radiatum of CA3 via monopolar tungsten electrodes. Between the first stimulus train and the establishment of stable EGSs (that is, at least three consecutive EGSs of constant pattern and duration), the number of individual discharges following a stimulus train increased by 21.1 ± 4.0 (mean ± SEM, paired *t* test, $P < 0.001$; $n = 20$). Although robust EGSs could be induced in slices from 22- to 32-day-old rats, minimal afterdischarges were elicited in those from more mature rats and virtually no afterdischarges in those from adult rats (older than 4 months).
27. Supported by the Veterans Administration Medical Center, Durham, North Carolina, and by NIH grant NS 17771. S.F.S. was supported by an NIH Medical Scientist Training grant and S.C. by an NIH Pharmacological Sciences Training grant. We thank Merck Sharpe and Dohme for a gift of MK-801. We also thank G. L. Collingridge and J. O. McNamara for helpful discussions.

2 February 1989; accepted 1 May 1989

A Novel mRNA of the A4 Amyloid Precursor Gene Coding for a Possibly Secreted Protein

FRÉDÉRIC DE SAUVAGE AND JEAN-NOËL OCTAVE*

The gene, encoding the A4 peptide found in the amyloid core of senile plaques isolated from the cerebral cortex of patients with Alzheimer's disease, produces at least three precursors that resemble cell surface receptors. A clone isolated from a human brain complementary DNA library contained the structural sequence for an A4 amyloid peptide precursor with a serine protease inhibitor domain in which 208 amino acids at the carboxyl terminal are replaced by 20 amino acids derived from nucleotide sequences with homology to the Alu repeat family. This protein devoid of the transmembrane domain most likely represents a secreted form of the A4 amyloid peptide precursor.

ALZHEIMER'S DISEASE IS A DEGENerative disorder characterized by neuronal loss and brain lesions such as senile plaques and neurofibrillary tangles (*1–4*). Senile plaques contain an amyloid core from which a 4.2-kD peptide was isolated as a major constituent (*5*). This peptide called the A4 peptide is identical to the β-amyloid protein found in the vascular amyloid deposits (*6*). From the amino acid sequence, several groups have synthesized oligonucleotides to isolate A4 amyloid peptide precursor (APP) cDNAs that hybridize with a 3.2- to 3.4-kb mRNA doublet expressed in the normal brain and other tissues (*7–10*).

Additional studies have shown that the gene encoding the A4 APP produces at least three different mRNAs referred to as APP_{695}, APP_{751}, and APP_{770} (*11–13*). APP_{751} and $_{770}$ are identical to the previously described APP_{695} (*7*), except for nucleotide inserts coding for a protease inhibitor domain. The three mRNAs appear to arise by alternative splicing of a single gene. In addition to the 3.2- to 3.4-kb mRNA doublet, a 2-kb RNA is also found in Northern blot analysis. We report that this mRNA can encode a secreted form of the A4 APP that is devoid of the A4 peptide and the transmembrane domain.

A 1.4-kb cDNA fragment (*14*) was used for the screening of λgt11 cDNA libraries constructed from the cerebral cortex or the cerebellum of a 54-year-old individual with Alzheimer's disease. Of several hybridizing clones, one clone from each library showed a nucleotide sequence that diverged at position 1630 from the sequence described by Kang *et al.* (*7*). The clone isolated from the cerebral cortex library was shown by DNA sequence analysis to contain a serine protease inhibitor domain at position 865 (Fig. 1). Nucleotide sequence homology was analyzed by the ALIGN program (IntelliGenetics). From position 1630 to position 1900, alignment of the sequence with the human consensus Alu repeat unit showed 70% homology. The 3′ end of the cDNA molecule, which is similar to the consensus Alu sequence, encodes 20 amino acids that lack both the A4 peptide and the hydrophobic carboxyl terminus corresponding to the transmembrane domain of the receptor. The previous potential N-glycosylation site at positions 496 to 498 of the Kang sequence (*7*) is replaced by another one at positions 551 to 553 of the new clone reported here.

The 1.4-kb cDNA probe used for the screening detected several RNAs in Northern blot analysis. In addition to the strong 3.2- to 3.4-kb bands, a band was present at 2 kb (Fig. 2B). The same band was recognized by another cDNA probe from the divergent sequence (Fig. 2A). The existence of the novel mRNA was confirmed by enzymatic amplification of the cDNA region where divergence occurred. Whereas no am-

Laboratoire de Neurochimie, Université Catholique de Louvain, UCL 1352, Avenue Hippocrate 10, B-1200 Bruxelles, Belgium.

* To whom correspondence should be addressed.

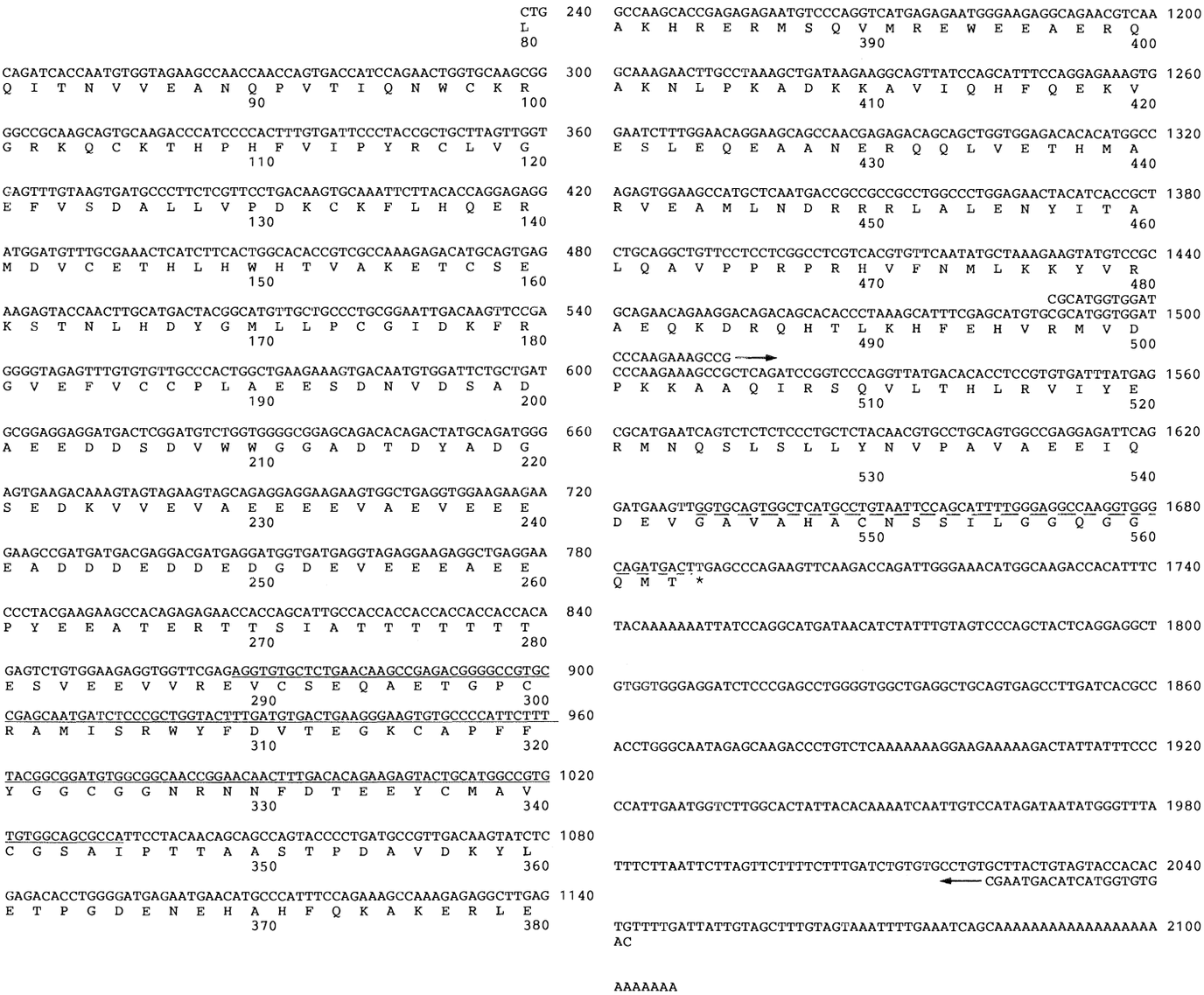

Fig. 1. Nucleotide and deduced amino acid sequences of the cDNA coding for a secreted form of the A4 APP. The nucleotide sequence proceeds in a 5′ to 3′ direction for a total of 1870 bases. The sequence starts at position 238 of the Kang sequence (7). The 168 inserted nucleotides corresponding to the serine protease inhibitor domain are underlined. The sequence contributing to a nonhydrophobic carboxyl terminal is underlined by a broken line. The oligonucleotides used for the enzymatic amplification reaction are indicated. If we assume that the initiation codon reported by Kang *et al.* (7) and Ponte *et al.* (*11*) is used by the novel RNA, the amino acid composition of the new precursor is A(52), C(19), D(36), E(71), F(14), G(29), H(19), I(16), K(28), L(37), M(17), N(21), P(23), Q(27), R(30), S(22), T(35), V(45), W(8), and Y(14), resulting in a calculated M_r of 63,515. Complementary DNAs were isolated from libraries constructed from the cerebral cortex or the cerebellum mRNA of an individual with Alzheimer's disease. Oligo(dT)-primed double-stranded cDNA was ligated with linkers into the Eco RI site of the λgt11 vector. About 2×10^6 recombinant phages from each library were screened with a cDNA fragment (positions 363 to 1795 in the Kang sequence), labeled by multi-priming nick translation. Among 18 hybridizing clones, 10 were subcloned in M13 vector for sequence analysis. Eight had a sequence identical to the previously reported and two showed nucleotide sequence diverging at position 1630. Both strands of each cDNA were sequenced by the dideoxynucleotide termination method (*22*).

plification was observed on the DNA, a fragment of 0.5-kb was amplified with cDNA templates from normal mRNA and mRNA from an individual with Alzheimer's disease (Fig. 3). This fragment was recognized in a DNA hybridization blot by two cDNA probes specific for the 5′ and 3′ ends of the targeted sequence. The sequence of the amplified fragment confirmed the existence of the novel mRNA, and the absence of amplification on the DNA ruled out the possibility that the region homologous to the Alu sequences at the 3′ end of the APP represents an unspliced intron.

The contribution of Alu sequences at the 3′ end of cDNA molecules has been reported (*15, 16*). In the mRNA coding for the decay accelerating factor, Alu sequences are involved in a splicing event that causes a coding frameshift near the carboxyl terminus. Two proteins were therefore possible, having divergent carboxyl-terminal domains that differ in their hydrophobicity (*15*). They represent the membrane-bound and the secreted forms. The novel mRNA described here, devoid of the A4 peptide and the transmembrane sequences, most likely corresponds to a secreted form of the APP_{751}.

Recent studies indicate that the A4 APP can be detected in two forms by the use of immunoblotting: a membrane-bound form, which can be stained with both the antibodies to the amino-terminal and carboxyl-terminal portions of the A4 APP, and a soluble form detected only with the amino-terminal antibodies (*17–19*). Cells, including fibroblasts (*20*), express the A4 APP at their surface but also secrete carboxyl-terminal

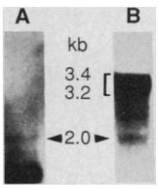

Fig. 2. Analysis of human brain total RNA hybridized with (**A**) a probe corresponding to the positions 1670 to 2042 of the divergent sequence or (**B**) with a 1.4-kb cDNA probe used for the screening of the cDNA library (Fig. 1). Glyoxal-denaturated total RNA (10 μg) was separated by electrophoresis on 1% agarose gel, in 10 m*M* sodium phosphate (*p*H 7) (*23*), and transferred to a nylon membrane (Amersham). The ^{32}P labeling of the probe was carried out with the Klenow fragment of the *Escherichia coli* DNA polymerase I. The filters were hybridized in 3.5× SSC (1× SSC = 0.15*M* NaCl and 0.015*M* sodium citrate, *p*H 7.0), 1× Denhardt's solution, and salmon sperm DNA (200 μg/ml) at 60°C. The filters were washed at 65°C, three times for 30 min each time in 2× SSC and 0.5% SDS, then for 30 min in 0.2× SSC and 0.1% SDS.

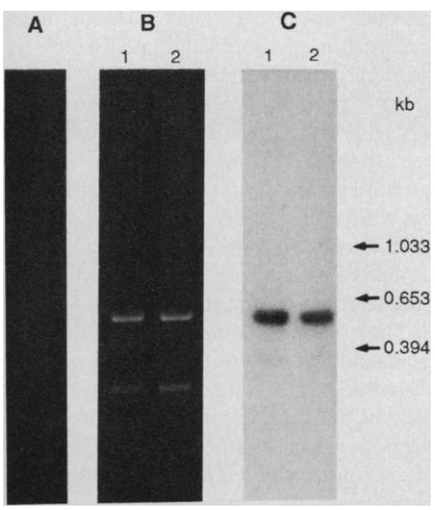

Fig. 3. Enzymatic amplification with oligonucleotide primers. (**A**) Ethidium bromide–stained gel showing the total amplification products of the DNA. (**B**) Total amplification products of cDNA synthesized with mRNA templates from (lane 1) normal brain or (lane 2) the brain of an individual with Alzheimer's disease. (**C**) DNA blot showing specific amplified products. Two probes specific for the 5′ (not shown) and 3′ ends of the target sequence give the same signal on (lane 1) normal cDNA and (lane 2) cDNA amplified from the brain of an individual with Alzheimer's disease. DNA was amplified by mixing 1 μg of DNA or 50 ng of oligo(dT)-primed single-stranded cDNA in polymerase buffer with 200 ng of each primer (Fig. 1). Samples were subjected to 60 cycles of polymerase chain reaction (*24*), each consisting of 1 min of denaturation at 90°C and 5 min of polymerization at 60°C in the presence of 1 unit of *Thermus aquaticus* DNA polymerase per 30 cycles. After amplification, one-fifth of the total reaction was subjected to agarose gel electrophoresis. The gel was photographed and the DNA was transferred to nylon membranes and hybridized to two labeled probes specific for the 5′ (positions 1602 to 1629) and 3′ (positions 1843 to 2107) ends of the target sequence. The blot was then washed and autoradiographed.

truncated proteins into the medium (*19*). It was recently reported that the conditioned medium from transfected cells overexpressing the A4 APP potentiated neurite outgrowth when added to untreated cells (*21*). A proteolytic cleavage of the amyloid precursor that generates a soluble peptide has been proposed (*19*). We report that a modified APP_{751} is devoid of the transmembrane domain. Therefore, the proteolytic cleavage of the transmembrane precursor is not the only way to obtain a soluble precursor.

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25. Abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

26. We thank A. Burny for interesting discussion of the results and manuscript preparation; E. C. Laterre, T. de Barsy, and J. M. Maloteaux for clinical data and discussions; J. M. Brucher for anatomopathological examination, and B. Tasiaux-Doumont for excellent technical assistance. Supported by Institut pour l'Encouragement de la Recherche Scientifique dans l'Industrie et l'Agriculture (IRSIA), Celltarg, Fonds de la Recherche Scientifique Médicale (FRSM), and Fondation Médicale Reine Elisabeth.

19 January 1989; accepted 15 May 1989