Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 2478–2482, April 1989
Neurobiology

A *Drosophila* gene encoding a protein resembling the human β-amyloid protein precursor

(neurogenetics/Alzheimer disease/membrane protein/invertebrate neurodevelopment/RNA expression)

DANIEL R. ROSEN*, LINDA MARTIN-MORRIS, LIQUN LUO, AND KALPANA WHITE†

Department of Biology, Brandeis University, Waltham, MA 02254

Communicated by Seymour Benzer, December 28, 1988 (received for review November 17, 1988)

ABSTRACT **We have isolated genomic and cDNA clones for a *Drosophila* gene resembling the human β-amyloid precursor protein (APP). This gene produces a nervous system-enriched 6.5-kilobase transcript. Sequencing of cDNAs derived from the 6.5-kilobase transcript predicts an 886-amino acid polypeptide. This polypeptide contains a putative transmembrane domain and exhibits strong sequence similarity to cytoplasmic and extracellular regions of the human β-amyloid precursor protein. There is a high probability that this *Drosophila* gene corresponds to the essential *Drosophila* locus *vnd*, a gene required for embryonic nervous system development.**

Development of the nervous system is dependent on coordinate input from many genes. In *Drosophila melanogaster*, previous studies have demonstrated that genomic region 1A1–1B10 contains several genes essential for normal neural development (1–8). Within this region, we have been investigating genes essential for neural development during embryogenesis (2–4, 8), including the ventral nervous system condensation defective (*vnd*) gene (2, 3). Mutations of the *vnd* gene cause severe structural derangement of the embryonic nervous system, ultimately resulting in embryonic lethality. Since all known mutations of *vnd* are recessive, the phenotype of mutant embryos is expected to result from loss of a factor crucial for proper assembly of the embryonic nervous system.

To extend our knowledge of the function of the *vnd* gene product in neurogenesis, molecular cloning of this gene was undertaken (9). The *vnd* gene has been delimited to a 30-kilobase (kb) genomic region by breakpoint analysis of deficiency chromosomes. Within this DNA, we have identified a 6.5-kb nervous system-enriched transcript, which we suggest is an excellent candidate for the *vnd* transcript. We have isolated and sequenced cDNAs corresponding to this transcript. Our analysis indicates that the protein encoded by this gene exhibits strong homology to a polypeptide from humans: β-amyloid precursor protein (APP) (10).

Recent cloning and sequencing of the human APP cDNAs predict a 695-amino acid polypeptide (APP^{695}) with a single transmembrane domain (10–13). The 42-amino acid β-amyloid protein, which is deposited in the amyloid plaques of Alzheimer disease, spans the membrane–extracellular junction of APP^{695} (10). Sequencing of different alternatively spliced APP cDNAs revealed a polypeptide containing 57 additional amino acids within the extracellular domain, APP^{752}, with homology to a class of serine protease inhibitors (14–16). At present, the normal function of the APP gene in humans is not understood, although the structure and biogenesis of the protein have prompted the suggestion that the APP is a cell-surface protein mediating cell–cell interactions (17, 18). Data suggesting that the APP is a heparan sulfate proteoglycan core protein have also been reported (19).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

In this paper, the predicted amino acid sequence encoded by the 6.5-kb *Drosophila* transcript is compared to the human APP, and evidence that this gene corresponds to the *vnd* gene is discussed.‡ The discovery of a *Drosophila* APP-like protein is expected to facilitate the experimental investigation of the role of this protein in normal development. Elucidation of APP's normal function will aid in understanding its role in the manifestation of Alzheimer disease in humans.

MATERIALS AND METHODS

Genomic Southern Analysis. Genomic DNA was isolated from flies of the appropriate genotype as described in Pirotta *et al.* (20). DNA (8 μg) was digested with either *Eco*RI, *Hin*dIII, or *Bam*HI according to the manufacturer's specifications (New England Biolabs). DNA gel electrophoresis, transfer, hybridization, and wash conditions were as described in Maniatis *et al.* (21). Nick-translated DNA probes from subclones from the *vnd* genomic walk were used to probe the Southern blots (9).

Northern Analysis. RNA extractions were as described in Vincent *et al.* (22) for embryos, larvae, and pupae and as in Barnett *et al.* (23) for adults. Poly(A)$^+$ RNA fractionation was as in Aviv and Leder (24). RNA concentration was assessed by spectrophotometric analysis and confirmed by hybridization to a ribosomal protein gene *rp49* probe as described (25). RNA from central nervous tissue was prepared using hand-dissected third larval instar central nervous systems (CNSs). Four micrograms of poly(A)$^+$ RNA was loaded per lane (unless otherwise indicated) onto formaldehyde (2.2 M) gels. After electrophoresis, the RNA was transferred to nitrocellulose (Schleicher & Schuell). Hybridization with nick-translated DNA was carried out as described in Colot and Rosbash (26). Northern blots were washed as described by Campos *et al.* (8). The molecular weight of the transcript was determined by using single-stranded RNA markers (BRL 5620SA).

cDNA Isolation. Two *D. melanogaster* adult head cDNA libraries were screened. Twenty-three cDNA phage clones (c1–c23) were isolated from a library prepared by Y. Citri (Weizmann Institute), probed with probe III (see Fig. 1). Screening of a library from P. Salvaterra (City of Hope) with subclone a in Fig. 1 yielded six positive clones (s1–s6). The cDNA inserts of the phage clones were purified and cloned into Gemini (Promega) or Bluescript (Stratagene) vectors for restriction analysis, Southern hybridization, and sequence analysis.

Sequence Analysis. The nucleotide sequence of both strands was determined by the dideoxy-nucleotide-chain-

Abbreviations: APP, β-amyloid precursor protein; CNS, central nervous system; nt, nucleotide.

*Present address: Dana–Farber Cancer Institute, 44 Binney Street, Boston, MA 02115.
†To whom reprint requests should be addressed.

‡The sequence reported in this paper is being deposited in the EMBL/GenBank data base (accession no. J04516).

termination method of Sanger *et al.* (27), using a sequenase kit (United States Biochemicals), and dATP [^{35}S] (New England Nuclear). Sequence was analyzed using the DNA Inspector II$^+$ program (Textco, West Lebanon, NH). The Protein Identification Resource data bank was searched for homology using the program XFASTA. Amino acid alignment of the conserved regions shown in Fig. 5 was according to Needleman and Wunsch (28), and the conservative amino acid assignments were made based on the PAM 250 matrix (29).

RESULTS

Molecular Cloning of the Genomic Interval Including *vnd*. The *vnd* locus is flanked by the *elav* (embryonic lethal abnormal visual system) gene distally and the *l(1)1Bg* gene proximally within the salivary segment 1B5-9, at the tip of the X chromosome (8, 9). To clone DNA corresponding to the genomic interval proximal to the previously cloned *elav* gene (8), 90 kb of DNA was isolated by chromosomal walking techniques (8, 9). The progress of this *vnd* walk was monitored by defining the molecular locations of breakpoints for a series of terminally deficient X chromosomes that genetically flank the *vnd* gene (9).

Genomic Southern blots of DNA isolated from flies carrying the deficiency chromosomes were probed with appropriate DNA from the *vnd* chromosomal walk (9). Molecular breakpoints of seven terminal deficiencies—Df(1)RT107, Df(1)RT115, Df(1)RT208, Df(1)RT114, Df(1)RT129, Df(1)RT155, and Df(1)RT178—that retained *vnd* function but were deficient for *elav* function were defined in the 40-kb interval distal to the *vnd*-associated DNA. The most proximal of these breakpoints was that of Df(1)RT178, which occurred within the proximal 8 kb of a genomic 23-kb *Eco*RI fragment and defined the distal limit of the *vnd* gene (Fig. 1). Proximal to *vnd*, the breakpoints of eight terminal deficiency chromosomes—Df(1)RT184, Df(1)RT385, Df(1)RT409, Df(1)RT158, Df(1)RT124, Df(1)RT128, Df(1)RT143, and Df(1)RT530—were molecularly located. Also located proximal to *vnd* was the breakpoint for interstitial deficiency Dp(1;3)E2, which possesses *vnd* and more distal gene functions, but lacks *l(1)1Bg* and more proximal functions. The breakpoints for Dp(1;3)E2 and three of the terminal deficiencies [Df(1)RT184, Df(1)RT385, and Df(1)RT409] occurred within a genomic 8.0-kb *Eco*RI fragment, and the breakpoint of Dp(1;3)E2 defined the proximal limit of the *vnd* gene. Together, the breakpoints of Df(1)RT178 and Dp(1;3)E2 define a 30-kb interval of genomic DNA completely encompassing the *vnd* gene (Fig. 1).

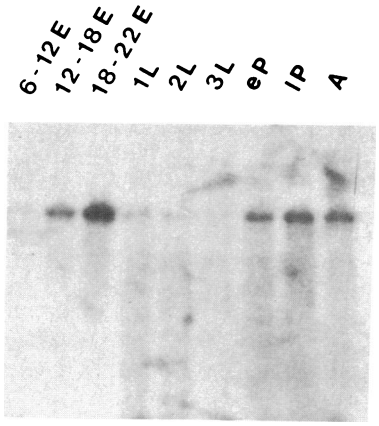


FIG. 2. A representative Northern blot demonstrating a 6.5-kb transcript from the *vnd* region. This Northern blot was probed with subclone I in Fig. 1. Poly(A)$^+$ RNA (≈4 μg) from three embryonic intervals (6–12 hr E, 12–18 hr E, and 18–22 hr E), first, second, and third instar larvae (1L, 2L, 3L), early and late pupae (eP and lP), and adults (A) was loaded.

Transcription from the 30-kb Region. Northern blots containing adult poly(A)$^+$ RNA were probed with subclones a–n spanning the 30-kb interval (Fig. 1). A 6.5-kb RNA species, which spanned ≈18 kb of the genomic DNA, was observed (Fig. 2). To assess transcriptional activity at all stages, Northern blots containing poly(A)$^+$ RNA prepared from embryonic, larval, pupal, and adult stage were analyzed. The 6.5-kb transcript is weakly expressed in 6- to 12-hr embryos but becomes more highly transcribed with increasing embryonic age. In larvae, the transcript is again weakly expressed during first and second instar stages and is undetectable during the third instar stage using this assay, but it is detected at later developmental stages in pupae and adults (Fig. 2). The direction of transcription for the 6.5-kb message was determined to be distal to proximal.

The test whether the transcript is enriched in nervous tissue, poly(A)$^+$ RNA was prepared from fly heads and bodies, as well as whole adults. Fig. 3*A* shows a Northern blot with equal loading of these poly(A)$^+$ RNAs. The head lane shows a strong signal, indicating that the transcript is preferentially expressed in the head, which is largely composed of neuronal tissue. Signal in the body lane was

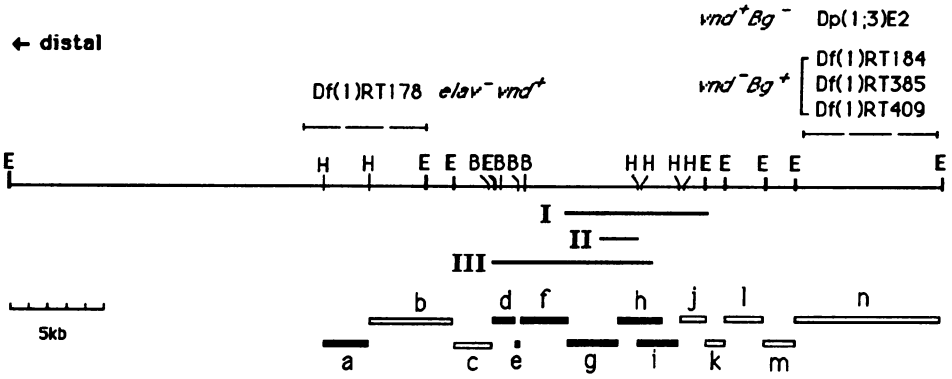


FIG. 1. Genomic map of the *vnd* region and characterization of the 6.5-kb transcript. The distal border of the *vnd* gene is defined by the Df(1)RT178 (*elav*$^-$ *vnd*$^+$) breakpoint within the proximal 8 kb of the distal 23-kb *Eco*RI fragment (9). The proximal limit of the *vnd* gene is defined by the Dp(1;3)E2 [*vnd*$^+$ *l(1)1Bg*$^-$] breakpoint within the genomic 8-kb *Eco*RI fragment; breakpoints of Df(1)RT184, Df(1)RT385, and Df(1)RT409, all of which are *vnd*$^-$ and *l(1)1Bg*$^+$, map within the same 8-kb *Eco*RI fragment n(9). Sites for *Eco*RI (E), *Bam*HI (B), and *Hin*dIII (H) are indicated. The genomic subclones a–n were used to map the transcriptionally active regions; subclones represented as solid bars detected the 6.5-kb transcript; others represent transcriptionally silent regions. I, II, and III represent the genomic subclones used for probing cDNA library and Northern blots.

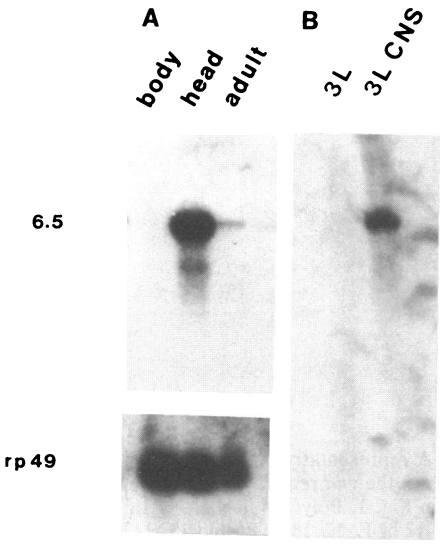


FIG. 3. Northern blots demonstrating that the 6.5-kb transcript is preferentially expressed in the nervous system. (*A*) Poly(A)$^+$ RNA (≈4 μg) prepared from adult bodies, adult heads, and whole adults was loaded and probed with subclone II shown in Fig. 1. (*Lower*) The head and body lanes were equally loaded as assessed by subsequent probing with a ribosomal protein gene (*rp49*) probe. (*B*) Total RNA prepared from hand dissected CNSs of third instar larvae (3L CNS), and poly(A)$^+$ RNA from third larvae (3L) were compared. Total RNA (10 μg) from larval CNSs is compared with poly(A)$^+$ RNA (10 μg) from whole larvae when probed with subclone I shown in Fig. 1.

undetectable under these conditions even after longer exposures. To test whether the transcript is present in the nervous system during the larval stage, RNA was prepared from dissected third instar larval CNSs. Fig. 3*B* shows a Northern blot in which the CNS lane is loaded with 10 μg of total RNA, and, for comparison, the adjacent lane is loaded with 10 μg of larval poly(A)$^+$ RNA. A strong signal is present in the CNS lane, but one was not detected in the whole larval poly(A)$^+$ RNA lane. We believe that the absence of signal in the total larval poly(A)$^+$ RNA in the third instar stage, the weak expression in RNA from earlier larval stages, and the absence of signal in the body are due to dilution of the nervous system RNA with the total organismal RNA in these preparations. In preliminary *in situ* RNA hybridization analyses, the 6.5-kb transcript appears to be expressed in the embryonic neural tissue; the pattern of hybridization suggests a uniform distribution of the transcript throughout the cell body-containing cortical layer of the nervous system (L.M.M., unpublished observations).

Several cDNAs that correspond to the 6.5-kb transcript were isolated. After genomic mapping, cDNA s1 (3.1 kb) and cDNA c4 (3.25 kb) were chosen for further analysis. cDNA s1 has homology to the most distal subclone a, and subclones d, e, f, and g; the homology with g is weak. cDNA c4 lacks homology to the distal subclone a but demonstrates strong homology to the subclones d, e, f, and g and thus potentially extended further in the 3′ direction than s1. Neither cDNA exhibits homology to subclone h or i.

Predicted Protein Sequence Encoded by the 6.5-kb Transcript. The nucleotide (nt) sequence of the 5′ ends of cDNA s1 (1400 nt) and cDNA c4 (2468 nt) was determined. From these sequences, a 3525-nt composite sequence (s1/c4), with an overlap of 343 nt, was derived (Fig. 4). Analysis of this sequence revealed a single open reading frame of 2658 nt, starting with an ATG methionine codon at position 1 and extending to a termination codon at position 2659. Following the termination codon, stop codons were present in all three frames. The ATG methionine is preceded by a sequence that is in good agreement with the consensus defined for the *Drosophila* translation start (30). Assuming that this ATG does encode the initiator methionine, the s1/c4 polypeptide has 886 amino acid residues and a predicted molecular weight of 98,152. Near the C terminus, there is a stretch of 23 hydrophobic amino acids, characteristic of membrane-spanning domains (31). If this hydrophobic domain is indeed a membrane-spanning domain, the s1/c4 polypeptide is expected to possess a cytoplasmic domain of 53 amino acids at its C terminus and an extracellular domain of 810 amino acids (Fig. 4).

The s1/c4 Polypeptide Is Homologous to the Human APP Protein. A search of the Protein Identification Resource data bank for protein homology revealed that the s1/c4 polypeptide was similar to the human APP, a protein associated with the amyloid plaques of Alzheimer disease. The comparison in this paper is with the human APP^{695}, a 695-amino acid polypeptide, which has a 24-amino acid membrane-spanning domain and a predicted 47-amino acid cytoplasmic domain at its C terminus (10). As depicted in Fig. 5, three regions of strong amino acid homology were observed between the s1/c4 polypeptide and the APP: (*i*) an extracellular N-terminal domain, designated E1 (s1/c4, 1–198; APP, 1–188); (*ii*) a second extracellular domain, designated E2 (s1/c4, 417–576; APP, 322–469); and (*iii*) the C-terminal cytoplasmic domain, designated C (s1/c4, 834–886; APP, 649–695). These three domains, E1, E2, and C, when aligned, yield amino acid identity values of 38%, 37%, and 47%, respectively; corresponding values for percent conservative changes are 35%, 37%, and 20%.

Noteworthy aspects of the conserved regions are as follows: (*i*) Within E1, 12 cysteine residues are present in both proteins, and a highly conserved region of 19 amino acids with 15 identities is present, spanning s1/c4 residues 180–198. (*ii*) Within E2, a highly conserved 71-amino acid region spanning residues 462–532 of s1/c4 contained 31 identities and 27 conservative substitutions. In addition, a putative s1/c4 *N*-glycosylation site (residues 574–576) corresponding to one of the two possible *N*-glycosylation sites found in the APP (residues 467–469) was conserved. (*iii*) Domain C was the most conserved. Within the cytoplasmic region, close to the C terminus, a nonapeptide, Asn-Gly-Tyr-Glu-Asn-Pro-Thr-Tyr-Lys, is present in both sequences. This nonapeptide is embedded in a 22-amino acid stretch containing 15 identities, 5 conservative changes, and only 2 mismatches.

The overall organization of the two proteins appears to be similar, although the s1/c4 polypeptide of *Drosophila* is 191 residues longer than the human APP. At the N terminus, a putative signal sequence is found in the s1/c4 polypeptide, as is the case for the APP. At least 11 of the 12 cysteine residues in E1 are in conserved positions. The region between the E1 and E2 domains consists of 218 and 133 residues in the s1/c4 polypeptide and the APP, respectively. The region of 100 amino acids immediately after the E1 domain of the s1/c4 polypeptide is rich in glutamic and aspartic acids (28%), similar to the APP, where these acidic residues account for 42% of the amino acids. However, the seven uninterrupted threonine residues found in the APP were not observed in the s1/c4 polypeptide.

The region between the E2 and C domains consists of 257 and 179 residues in the s1/c4 polypeptide and the APP, respectively. The putative transmembrane domains occupy the same relative positions, with respect to the C domain, of both proteins. Interestingly, homology to the β-amyloid protein, which consists of 28 residues of the extracellular domain and 14 or 15 residues of the transmembrane domain of the APP, was not observed. Amino acid sequence homology between the transmembrane domains was also not observed.

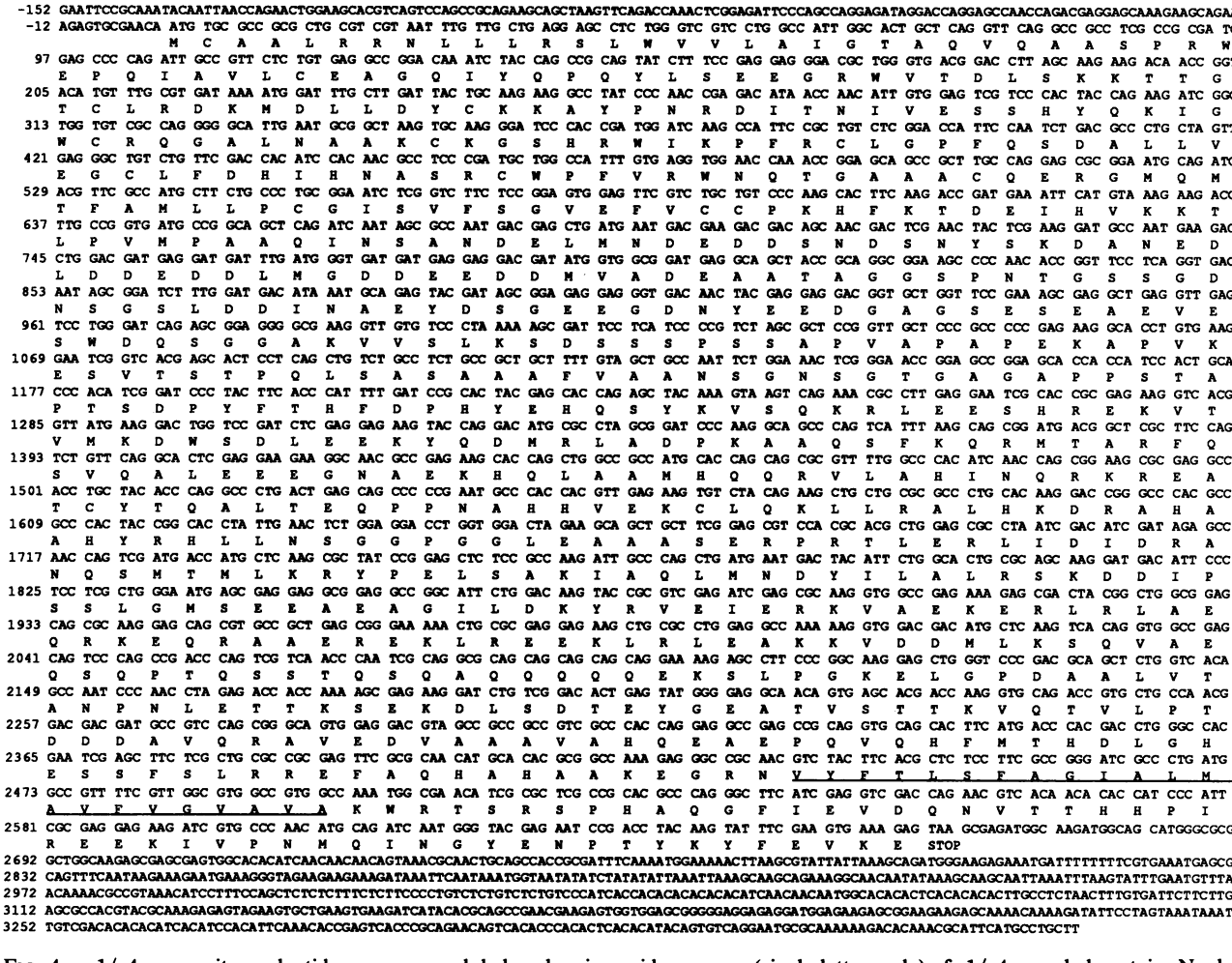


FIG. 4. s1/c4 composite nucleotide sequence and deduced amino acid sequence (single-letter code) of s1/c4-encoded protein. Nucleotide residues are numbered in the 5′ to 3′ direction beginning with the first nucleotide of the presumed translation initiation codon AUG. The s1/c4 open reading frame consists of 2658 nt beginning at nucleotide position 1 and extending to position 2658. The predicted s1/c4 polypeptide encodes an 886-amino acid polypeptide. The putative membrane-spanning region of the s1/c4 polypeptide, residues 811–833, is underlined. To generate this composite sequence, 1400 nt of the 5′ end of cDNA s1 and 2468 nt of cDNA c4 were sequenced in both directions. The region of overlap was between nt 905 and 1248.

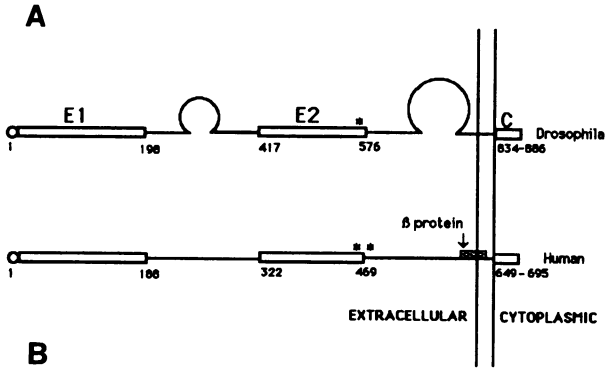
DISCUSSION

The striking degree of homology in amino acid sequences and similarity of overall structure between the *Drosophila* and human proteins indicate that this molecule is of a design predating the arthropod–vertebrate divergence. The highly conserved stretches of sequences within the E1, E2, and C domains indicate a strong evolutionary constraint on function for portions of this molecule. The normal function of either of these molecules is presently unknown. However, the high level of conservation suggests that both the *Drosophila* and human proteins are engaged in similar roles in their respective organisms.

In humans, the APP gene is actively transcribed in embryonic tissues and continues to be transcribed at later stages (e.g., see refs. 12 and 32). In this context, it is interesting to note that the *Drosophila* transcript is also expressed in the embryonic period, and also continues to be expressed in postembryonic and adult stages, implying a need for this function throughout the life of the organism. Our data show that the *Drosophila* transcript is enriched in neural tissue. Whether it is also present in nonneural tissues, as is the human APP transcript, is not yet known. If the *Drosophila* APP-like protein is encoded by the *vnd* gene, as we believe, then a role in the development of the nervous system and, possibly, in postdevelopmental brain structure and function would be implicated, and a similar function for the human APP gene implied.

Because of the manipulability of *Drosophila* as an experimental system (33), our discovery will accelerate elucidation of the function of APP-like protein. The ability to insert altered genes in *Drosophila* can aid in structure–function analyses (33). For example, the importance of the region in the s1/c4 polypeptide corresponding to the β-amyloid protein could be directly tested in *Drosophila* by constructing chimeric genes with either altered domains or human APP sequences, and observing the effects of the altered genes *in vivo*. This is of interest since the sequence of the β-amyloid protein, the principal component of the amyloid deposits (34, 35), is not conserved in *Drosophila*, suggesting that the β-amyloid protein by itself may not be functionally important. One caveat to this statement is that there might be another gene in *Drosophila* that encodes a protein with a conserved β-amyloid protein domain.

At present we do not know whether the *Drosophila* gene is capable of producing a polypeptide with the additional protease inhibitor domain similar to APP^{752} (14–16). Further analyses of genomic DNA and cDNA sequences associated with the 6.5-kb transcript species will reveal whether the protease inhibitor domain is also conserved. Alternatively, there might be a distinct gene that encodes the protease




```
Extracellular 1
 Dr   1 McaaLrrnLLLrslWvvlAigtaqvqaAsprwEPQIAvlCeaGqiyqpqylse
 Hu   1 MlpgL  aLLLlaaWtarAlevptdgnAgllaEPQIAmfC  G rlnmhmnvq
                                                 Δ
 Dr  54 eGrWvtDlSkktTgpTClrdKmdlLdYCkkaYPnrdITNiVEsshyqkIggWC
 Hu  49 nGkWdsDpS  gT kTCidtKegiLqYCqevYPelqITNvVEanqpvtIqnWC
                  Δ        Δ                             Δ
 Dr 107 rqGalnaaKckgsHrwIkPfRCL GpFqSDALLVPegClFdHihnasrCwpfv
 Hu  99 krG rkqcK thpHfvI PyRCLvGeFvSDALLVPdkCkFlHqermdvCethl
                        Δ          Δ        Δ
 Dr 159 rWnqtgaaaCqErgmqmrtfaMLLPCGIsvFsGVEFVCCP
 Hu 149 hWhtvaketCsEkstnlhdygMLLPCGIdkFrGVEFVCCP
               Δ          Δ          ΔΔ
Extracellular 2
 Dr 417 RLEesHREkvtrVMkdWsdlEekyQdmrLadPKaAqsfKqrmtarFQtsVqaL
 Hu 322 RLEakHRErmsqVMreW  eEaerQaknL  PK A  dKkaviqhFQekVesL

 Dr 470 EeEgnaEkhQLaamHqqRVlAhiNqRkReAmtcYtqALteqPPnahHVekcLq
 Hu 368 EqEaanErqQLvetHmaRVeAmlNdRrRlAlenYitALqavPPrprHVfnmLk

 Dr 523 KllRAlhKDRaHaLaHyrHllnsggPggleaAAseRprtlerLidIdravNQS
 Hu 421 KyvRAeqKDRqHtLkHfeH vrmvdP   kkAAqiRsqvmthLrvIyermNQS

Cytoplasmic
 Dr 834 KwrtsrSpHaqGfiEVDqnVTthhPivrEEkivpnMQiNGYENPTYKyFEvke
 Hu 649 KkkqytSiH hGvvEVDaaVT   P  EErhlskMQqNGYENPTYKfFEqmqn
```

FIG. 5. Comparison of s1/c4 polypeptide and the human APP. (*A*) The model of APP is adapted from Kang *et al.* (10) and the analogous s1/c4 polypeptide model is presented. The N terminus is at left; the circle at the N terminus represents the putative signal sequence. The regions of homology E1, E2, and C between s1/c4 polypeptide (*Drosophila*) and APP (human) are drawn as wide blocks; the loops in the *Drosophila* polypeptide represent the additional amino acids in the regions between the conserved domains. Asterisks indicate putative *N*-glycosylation sites. (*B*) Amino acid alignment (single-letter code) in the regions of strong homology. s1/c4 polypeptide (top line; Dr), APP (bottom line; Hu), vertical line indicates amino acid identity, dot indicates conservative change. Amino acid number is given preceding each row of sequence. In the comparison of the extracellular 1 region, conserved cysteine residues are indicated by Δ below the residue; the 19-amino acid stretch showing a high degree of homology is underlined. In the extracellular 2 region, the putative *N*-glycosylation site is highlighted by a dotted line. In the cytoplasmic comparison, 12 residues, including the conserved nonapeptide, are underlined.

inhibitor domain containing protein in *Drosophila*, or such a function may not be required in *Drosophila*.

We postulate that the *Drosophila* APP-like protein produced from the 6.5-kb transcript is encoded by the *vnd* gene. The 6.5-kb transcript is the only one detected within the genomic region containing the *vnd* gene, and only one essential function (*vnd*) genetically maps to this interval. The *vnd* function has been previously shown to be important for the formation of the embryonic nervous system, as evidenced by the failure to complete ventral cord condensation in the mutant embryos (2, 3). In addition, the *vnd* mutant embryonic neuropil appears highly disorganized, with lesions in the axonal tracts along the transverse and longitudinal commissures (3, 5). The correspondence between the neural phenotype displayed by *vnd* mutations and the nervous system-enriched expression of the 6.5-kb transcript supports our hypothesis. This hypothesis will be validated if transduced DNA encoding the 6.5-kb transcript is capable of rescuing the phenotype of *vnd* mutations.

We appreciate comments on the manuscript from Dr. Jeffrey C. Hall. We thank Drs. Y. Citri and P. Salvaterra for cDNA libraries and Drs. J. Mason and R. Voelker for genetic deletion stocks. This work was supported by National Institutes of Health Grant GM-33205. L.M.-M. was supported by Predoctoral Training Grant NS-07292.

1. Jiménez, F. & Campos-Ortega, J. A. (1979) *Nature (London)* **282,** 310–312.
2. White, K. (1980) *Dev. Biol.* **80,** 322–344.
3. White, K., DeCelles, N. L. & Enlow, T. C. (1983) *Genetics* **104,** 433–448.
4. Campos, A. R., Grossman, D. & White, K. (1985) *Neurogenetics* **2,** 197–218.
5. Jiménez, F. & Campos-Ortega, J. A. (1987) *Neurogenetics* **4,** 179–200.
6. Garcia-Bellido, A. & Santamaria, P. (1978) *Genetics* **88,** 469–486.
7. Dambly-Chaudiere, C. & Ghysan, A. (1987) *Genes Dev.* **1,** 297–306.
8. Campos, A. R., Rosen, D. R., Robinow, S. N. & White, K. (1987) *EMBO J.* **6,** 425–431.
9. Rosen, D. R. (1989) Ph.D. Thesis (Brandeis Univ., Waltham, MA).
10. Kang, J., Lemaire, H.-G., Unterbeck, A., Salbaum, J. M., Masters, C. L., Grzeschik, K.-H., Multhaup, G., Beyreuther, K. & Müller-Hill, B. (1987) *Nature (London)* **325,** 733–736.
11. Goldgaber, D., Lerman, M. I., McBride, O. W., Saffiotti, U. & Gajdusek, D. C. (1987) *Science* **235,** 877–880.
12. Tanzi, R. E., Gusella, J. F., Watkins, P. C., Bruns, G. A., St. George-Hyslop, P., Van Keuren, M. L., Patterson, D., Pagan, S., Kurnit, D. M. & Neve, R. L. (1987) *Science* **235,** 880–884.
13. Robakis, N. K., Ramakrishna, N., Wolfe, G. & Wisniewski, H. M. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 4190–4194.
14. Ponte, P., Gonzalez-DeWhitt, P., Schilling, J., Miller, J., Hsu, D., Greenberg, B., Davis, K., Wallace, W., Lieberburg, I., Fuller, F. & Cordell, B. (1988) *Nature (London)* **331,** 525–527.
15. Tanzi, R. E., McClatchey, A. I., Lamperti, E. D., Villa-Komaroff, L., Gusella, J. F. & Neve, R. L. (1988) *Nature (London)* **331,** 528–530.
16. Kitaguchi, N., Takahashi, Y., Tokushima, Y., Shiojiri, S. & Ito, H. (1988) *Nature (London)* **331,** 530–532.
17. Dyrks, T., Weidemann, A., Multhaup, G., Salbaum, J. M., Lemaire, H. G., Kang, J., Müller-Hill, B., Masters, C. L. & Beyreuther, K. (1988) *EMBO J.* **7,** 949–957.
18. Shivers, B. D., Hilbich, C., Multhaup, G., Salbaum, M., Beyreuther, K. & Seeburg, P. H. (1988) *EMBO J.* **7,** 1365–1370.
19. Schubert, D., Schroeder, R., LaCorbiere, M., Saitoh, T. & Cole, G. (1988) *Science* **241,** 223–226.
20. Pirrotta, V., Hadfield, C. & Pretorius, G. H. J. (1983) *EMBO J.* **2,** 927–934.
21. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
22. Vincent, A., O'Connell, P., Gray, M. R. & Rosbash, M. (1984) *EMBO J.* **3,** 1003–1013.
23. Barnett, T., Pachl, C., Gergen, J. P. & Wensink, P. (1980) *Cell* **21,** 729–738.
24. Aviv, H. & Leder, P. (1972) *Proc. Natl. Acad. Sci. USA* **69,** 1408–1412.
25. James, A. A., Ewer, J., Reddy, P., Hall, J. C. & Rosbash, M. (1986) *EMBO J.* **5,** 2313–2320.
26. Colot, H. V. & Rosbash, M. (1982) *Dev. Biol.* **94,** 78–84.
27. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467.
28. Needleman, S. & Wunsch, C. (1970) *J. Mol. Biol.* **48,** 444–453.
29. Dayhoff, M. & Schwartz, R. M. (1978) *Atlas of Protein Structure and Function,* ed. Dayhoff, M. (Natl. Biomed. Res. Found., Silver Spring, MD), Vol. 5, Suppl. 3, pp. 345–352.
30. Cavener, D. R. (1987) *Nucleic Acids Res.* **15,** 1353–1361.
31. Kyte, J. & Doolittle, R. F. (1982) *J. Mol. Biol.* **157,** 105–132.
32. Bahmanyar, S., Higgins, G. A., Goldgaber, D., Lewis, D. A., Morrison, J. H., Wilson, M. C., Shankar, S. K. & Gajdusek, D. C. (1987) *Science* **237,** 77–80.
33. Rubin, G. M. (1988) *Science* **240,** 1453–1459.
34. Glenner, G. G. & Wong, C. W. (1984) *Biochem. Biophys. Res. Commun.* **120,** 885–890.
35. Masters, C. L., Multhaup, G., Simms, G., Pottgiesser, J., Martins, R. N. & Beyreuther, K. (1985) *EMBO J.* **4,** 2757–2763.