The *Drosophila* transcript encoded by the β-amyloid protein precursor-like gene is restricted to the nervous system

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Summary

We have molecularly delineated a *Drosophila* β-amyloid protein precursor-like (Appl) gene and analyzed its pattern of expression. Appl defines a new locus within the IB division of the X-chromosome, a region previously shown to be important for neural development. The genomic limits of the Appl gene were defined by mapping of the Appl cDNAs. The Appl transcript spans ~38 kb (1 kb = 10^3 base-pairs) of genomic DNA. Genomic regions surrounding the first two exons were sequenced. The first exon contains 78 nucleotides of the coding sequence and is separated from the second exon by a ~21 kb intron. The second exon is 171 nucleotides long and is separated from the third exon by a ~7 kb intron.

We present *in situ* RNA localization data that demonstrate that the Appl transcript is found in post-mitotic neurons in all developmental stages, in the central and peripheral nervous systems. Within the nervous system transcripts are not observed in neuroblasts, newly generated neurons and at least one class of presumed glial cells. The temporal and spatial specificity of Appl expression suggests that the gene product has a function that is common to most neurons. Appl cDNA predicts an 886-amino acid polypeptide that exhibits strong sequence similarity to the human β-amyloid protein precursor (APP) (Rosen et al. 1989). In this paper, we compare the Appl gene expression with the pattern of expression of the β-amyloid protein precursor (APP) gene in mammals. Furthermore, we suggest that during evolution, a neural-specific function encoded by the APP gene has been selectively maintained.

Key words: amyloid protein precursor, neural locus, neural development, *in situ* hybridization, *Drosophila melanogaster*, Alzheimers disease, promoter sequence.

Introduction

β-Amyloid, a 4.2×10^3 M_r peptide, is a principle component of the neuritic and cerebrovascular plaques associated with Alzheimers disease (Wong et al. 1985; Glenner and Wong, 1984). Molecular analyses have identified a gene encoding the β-peptide and have demonstrated that this peptide is derived from a large transmembrane protein, i.e. β-amyloid protein precursor (APP) (reviewed by Müller-Hill and Beyreuther, 1989; Selkoe, 1989). Alternatively spliced mRNAs encoded by the APP gene give rise to at least three different forms of the APP protein: a 695-amino acid (aa) isoform, APP_695, APP_770, which includes a putative protease inhibitor domain, and APP_770, which contains the putative protease inhibitor domain and an additional exon whose function is unknown (Ponte et al. 1988; Tanté et al. 1988; Kitaguchi et al. 1988). A fourth mRNA product from the APP locus was recently described (de Sauvage and Octave, 1989). This class of transcript may give rise to a secreted form of the protein that lacks the transmembrane and cytoplasmic domains.

The deduced amino acid sequence of APP is exceptionally well conserved among the species so far analyzed. In both mouse (Yamada et al. 1987) and rat (Shivers et al. 1988), there is 97 % identity at the protein level when each is compared with human APP_695. A transcript encoding an APP-like protein (APPL) has been described in *Drosophila* (Rosen et al. 1989). The *Drosophila* APPL protein possesses many features found in human APP (Kang et al. 1987). Both molecules have a signal sequence near the amino terminus and a membrane-spanning sequence toward the carboxy terminus of the protein resulting in a large putative extracellular domain (Rosen et al. 1989). Within this extracellular domain two regions of high homology are observed, E1 and E2. E1 (~190 aa) contains 11 conserved cysteine residues and is 38 % identical to human APP_695. *Drosophila* E2 (~160 aa) has 37 % identity when compared with the human molecule and includes one of two putative glycosylation sites. A small putative cytoplasmic domain of ~50 aa is even more highly conserved (47 % identity). The extent of similarity in both the amino acid sequence and organization of APP suggests that an ancestral gene encoding this
protein existed before the arthropod and vertebrate lineages diverged and, further, that an important function is provided by this molecule.

The transcriptional pattern of Appi is similar, but not identical, to that of the mammalian APP gene. Mammalian APP transcripts are abundant in fetal and in adult stages (Kitaguchi et al. 1988; Tanzi et al. 1988). Similarly, RNA blot analysis of Appi mRNA suggests that transcription begins in the Drosophila embryo and continues throughout development (Rosen et al. 1989). The mammalian APP transcripts are found in many tissues including the nervous system. However, transcript-specific probes show that the transcript encoding APP695 is exclusively expressed in the neural tissues, whereas the transcripts encoding other isoforms are expressed in neural as well as non-neural tissues (Ponte et al. 1988; Tanzi et al. 1988). Further support for this difference in APP695 expression comes from protein studies on neural and non-neural cell lines (Weidemann et al. 1989). Collectively, these studies imply a neural-specific function for APP695 and a more general requirement for the function provided by the other isoforms. By Northern analysis, the Appi transcripts are found predominantly in the neural tissue in larval and adult stages (Rosen et al. 1989). The observed enrichment of Appi transcripts in the nervous system raises the possibility that, unlike mammalian APP transcripts encoding the APP695 and APP710, the Appi gene is expressed exclusively in the nervous system and provides a neural-specific function. To resolve this issue, and to gain further insight into the neural function provided by this gene, spatial and temporal aspects of Appi transcription were studied by in situ RNA hybridization. Specifically, we address the following questions: (1) is the transcript expressed in non-neural tissues or is it restricted to the nervous system? (2) What is the distribution of transcripts within the nervous system? (3) When during neuronal differentiation does the gene become activated?

The discovery of the Drosophila Appi gene was fortuitous. We had been examining two neighboring genes, elav (embryonic lethal abnormal visual system) and vnd (ventral nervous system condensation defective), that are necessary for embryonic neural development (White et al. 1983; Campos et al. 1987; Rosen et al. 1989). The vnd gene was delimited to a 30-kb (1 kb=10^3 base-pairs) genomic region and within this region the 6.5-kb nervous system enriched Appi transcript was identified (Rosen et al. 1989). On the basis of several lines of circumstantial evidence we proposed that the Appi transcript was encoded by the vnd gene. In this study, the mapping of the 5' end of the Appi transcript was undertaken to determine the genomic limits of the Appi transcriptional unit, with the hope that it would help clarify whether Appi and vnd are distinct loci.

Materials and methods

Stocks
All tissue sections were made from Canton-S Drosophila melanogaster raised on cornmeal/agar/molasses food at 25°C. All embryonic staging is according to the method of Campos-Ortega and Hartenstein (1985). Larvae aged 48–72 h post-fertilization (second instar), and climbing third instar larvae were used. For pupae, white prepupae were collected and aged for 72 h. Two- to four-day-old flies served as adult material.

Preparation of tissue
Staged animals were prepared essentially as described by Robinow and White (1988), with the following exceptions. Embryos were devitellinized by pipeting embryos forcefully into 90 % methanol:50 mM EGTA. Both anterior and posterior portions of larvae were removed before fixation for better fixative penetration, but the anterior cut was made leaving the mouthhooks intact. 72 h pupae were gently teased out of the pupal case, and the posterior aspect of their abdomens was cut away before fixation.

Probe synthesis
Linearized cDNA, c1, cloned into Bluescript (Stratagene) vector SK+, was used to generate antisense and sense probes. [3H]UTP (NEN) was incorporated into c1 DNA at specific activity of ~2×10^7 cts min^-1/μg^-1. Alkaline hydrolysis (Cox et al. 1984) to ~80 nucleotides was found to be helpful in reducing background.

Preparation, hybridization and washing of tissue sections
Paraffin sections (6 μm) were pretreated as described by Ingham et al. (1985). Hybridization was performed in: 50 % formamide, 0.6 M NaCl, 10 mM Tris–HCl, pH 7.5, 1 mM EDTA, 1 % SDS (ultra-pure), 1×Denhardt’s, 10 mM dithiothreitol (DTT), 0.25 mg ml^-1 tRNA (phenol-extracted), 10 % PEG 8000, 0.1 μg ml^-1 kb^-1 probe, for 6 h at 55°C. Unbound [3H]RNA was removed by washing in: 50 % formamide, 0.3 M NaCl, 10 mM Tris–HCl, pH 7.5, 5 mM EDTA, 10 mM sodium phosphate, pH 8, 2 % SDS, 10 mM DTT, for 4 h at 55°C. RNase treatment, washing and dehydration were performed as described by Ingham et al. (1985). Slides were dipped in NTB-2 nuclear track emulsion (Kodak) diluted 1:1 with water, dried and stored desiccated at 4°C for 4-7 weeks. Development and staining was performed as described by Robinow and White (1988).

Sequence analysis
Double-stranded deletions prepared as described by Henikoff (1987) of genomic Appi DNA cloned into Bluescript vectors (Stratagene) were used for single-stranded sequencing of both strands. Chain termination (Sanger et al. 1977) reactions using Sequenase (U.S. Biochemical Corp.) were carried out according to manufacturer’s specifications using [35S]dATP (NEN). Sequence data were entered into and analyzed by DNA Inspector (Textco).

Results

Organization of the Appi transcript
A 2.5-kb genomic HindIII fragment was the most distal fragment within the genetically defined vnd region that detected the 6.5-kb transcript on Northern blots (Rosen et al. 1989). Therefore, we sequenced the 2.5-kb HindIII fragment to determine if it included the 5' end of the cDNA s1. cDNA s1 includes the presumed trans-
lational start site of the Appl open reading frame, and 152 bp of 5' untranslated sequence (Rosen et al. 1989). Within this genomic sequence 171 nucleotides correspond to part of the cDNA sequence, but it does not contain the most 5' cDNA sequence, including 78 nucleotides of the open reading frame. This indicates the presence of an intron upstream from the exon located within the 2.5-kb HindIII fragment (2 in Fig. 1A). A 3 kb length of genomic DNA located 5' of this exon was sequenced and found to be devoid of sequences corresponding to the 5' end of the cDNA, implying that this upstream intron extended beyond the 3 kb.

To locate the 5' exon(s), Southern blots of genomic DNA were probed with oligonucleotide probes derived from the 5' end of the cDNA (data not shown). An exon was localized to a 1.5-kb PstI-Xhol fragment (1 in Fig. 1A). Sequencing of this fragment allowed precise mapping of the remaining 5' cDNA sequence. All of the remaining 5' cDNA 1 nucleotides were located in one exon within this subclone. Fig. 1A depicts a partial restriction map of the genomic region from which the

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**Fig. 1.** Organization of Appl transcription unit. The genomic DNA from which the Appl transcript is derived is shown in A. The telomere is located to the left of the diagram. Hatch marks indicate the location of EcoRI restriction fragments. 1 and 2 indicate the first two exons, which will be described further. RT178 and Dp(1;3)E2 define the vnd interval (Rosen et al. 1989). RT178 is a terminal deficiency that retains vnd function while eliminating the next most distal lethal complementation group, and Dp(1;3)E2 is a duplication of a portion of the X chromosome including vnd but not the next-most proximal lethal complementation group. Thus the boxed regions indicate DNA that is present in the individual aberrations, h, HindIII site; p, PstI site; and x, Xhol site. In B the sequence corresponding to exon 1 and untranscribed leader sequence is shown. Numbering is arbitrarily started with the PstI site used to clone the DNA. The stars indicate the sequence that shows homology to the DDC TATA; filled circles define the sequence corresponding to the RNA start point consensus. The sequence that corresponds to that from the cDNA clone (Rosen et al. 1989) is underlined. The exon contains 78 nucleotides of the coding sequence, starting with a methionine start codon at position 709-711 (circled). In C, the sequence corresponding to the exon 2 is given. Underlined sequences indicate correspondence with cDNA. The second exon is 171 nucleotides long and is separated from the third exon by a ~7-kb intron. Numbering is arbitrarily begun at the start of the 3' splice site consensus.
Appl transcript is derived and shows its overall exon–intron organization. Fig. 1B gives a partial genomic sequence of the 1.5-kb Pstl–Xhol fragment; this includes the first exon and 563 nucleotides upstream from the start of the cDNA sequence (underlined). Fig. 1C gives a partial sequence of the 2.5-kb HindIII fragment that includes the second exon and flanking intronic sequence at the splice junctions. Exon 1 is separated from exon 2 by a 21-kb intron, which localizes exon 1 well outside the genetically defined vnd interval (Fig. 1A). At exon–intron boundaries, consensus splice site sequences are observed (5' splice site consensus: CAG/GT/G/GAGT, exon 1 – CAG/GTGAGT, exon 2 – AAG/GTGAAAGT; 3' splice site consensus: (T/C)nN C/TAG/G, exon 2 – TTTTCAG/G (Breathnach et al. 1978; Mount, 1982)).

The precise site of initiation of transcription has not been confirmed, but preliminary primer extension analysis indicates the presence of start sites located within 50 nucleotides of the underlined exon sequence. Within the DNA between the proposed start sites and the homology to cDNA, no candidate splice acceptor sites are observed, implying that this clone contains the first exon and 5' untranscribed leader sequence. Within the sequence shown in Fig. 1B, a TATA box that bears a resemblance to the TATA associated with the Drosophila gene encoding dopa decarboxylase (DDC – GCTTTAAAAGCA, Appl – GTGTTAAACGAA) (Scholnick et al. 1986) is observed, as is a sequence showing homology to the RNA start point consensus of a number of Drosophila genes (consensus: AT(N)CA0/AT1C/AT, Appl – ATACAAAT (Hultmark et al. 1986). However, no indication that this start is commonly used has been found by primer extension.

In situ hybridization analysis
To study Appl transcription, antisense RNA probes were prepared from the 1.3-kb cDNA clone, c1, which corresponds to the carboxyl half of the open reading frame and ~240 nucleotides of the 3' untranslated region. On Northern blots c1 recognizes only a 6.5-kb transcript associated with this gene, and no transcript was ever observed with the sense strand (data not shown). Single-stranded RNA probes corresponding to the sense strand were used as controls for nonspecific tissue-probe affinity. Nervous system-specific signal was not observed with this control probe.

Transcript localization in embryos
Appl transcripts are not observed during early embryogenesis, up to and including stage 11 (data not shown). Before this stage, neural precursors, neuroblasts, have segregated from the ventral ectoderm, and have begun dividing asymmetrically to produce ganglion mother cells. In addition, the older ganglion mother cells have divided to produce post-mitotic neurons (Poulson, 1950; Hartenstein et al. 1987). Beginning late in stage 12 (roughly 9h after fertilization at 25°C), sparse grains are observed over the contracting germ band (data not shown). During this stage, the ventral nerve cord separates from the epidermis and the first neural processes appear. In stage 13 embryos, the ventral cord is well differentiated and the sub- and supra-esophageal ganglia have formed (Campos-Ortega and Hartenstein, 1985). In situ hybridization shows transcripts exclusively in the nervous system of stage 13 embryos (Fig. 2A and B). Fig. 2C and D shows a 15 h embryo (stage 16) whose nerve cord is in the process of condensation. The transcript appears to be uniformly distributed in the central nervous system (CNS) cortex.

In the embryo, in addition to the CNS expression of the Appl gene, its transcript is routinely observed in a segmental manner reminiscent of the localization of peripheral nervous system (PNS) cell clusters (Campos-Ortega and Hartenstein, 1985). Fig. 2E and F shows a lateral section through a stage 14 embryo, in which grains are observed over presumed PNS cells.

Transcript localization in larvae
In all larval instars, Appl transcripts are restricted to the nervous system. During the larval period, the nervous system is undergoing dramatic growth by virtue of neuroblast divisions that occur throughout larval life (Bodenstein, 1950; White and Kankel, 1978; Truman and Bate, 1988). The pattern of hybridization in second instar larvae is very similar to that in late embryos. Fig. 3 shows a second instar larva, and demonstrates the nervous system-specific localization of transcripts. While the signal is intense over the cortex of the CNS, no grains are observed over non-neural tissue including larval muscle, salivary glands, fat bodies and gut.

In late third instar larvae, the imaginal discs are well formed and the CNS is undergoing continued development (Kankel et al. 1980; Poodry, 1980). Here, the transcript is present in most cells within the CNS. Fig. 4A and B shows the ventral ganglion of a late third instar larva. Although the signal is relatively uniform over the cortical regions in which cell nuclei reside, midline neurons are clearly unlabeled. Large cells located at the cortex–neuropil boundary, which fit morphological descriptions of glial cells in other insects (Wigglesworth, 1960; Meyer et al. 1987), also show no detectable transcript (Fig. 4C and D). Cortical neuroblasts, recognizable by their large size, in the superficial cortical areas of the ventral ganglion are also unlabeled (Fig. 4E and F). Additional cell types can be observed over which no grains are witnessed; these could be glial cells, ganglion mother cells, or neurons that are not expressing Appl transcripts at the time of preparation (Fig. 4F).

In the brain lobes of a late third instar larva (Fig. 5), hybridization is also restricted to post-mitotic neurons. The proliferation centers, outer (OPC) and inner (IPC), consist of neuroblasts that give rise to the neurons of the optic lobes of adult flies. These proliferation centers are recognizable due to their position, as well as their staining properties. In this larva, the proliferation centers are reduced in size and the optic lobe primordia are now evident. Neuroblasts in the IPC and OPC are unlabeled. Post-mitotic cells of the medulla cortex arise prior to those in the lamina (White and Kankel, 1978). Neurons in the developing medulla
Fig. 2. Embryonic expression of Appl. Embryonic tissue sections were probed with antisense c1 riboprobe to analyze transcript localization. (A,B) Sagittal section of a stage 13 embryo; bright-field and dark-field, respectively. Grains are sparsely distributed over the contracting germ band. (C) Sagittal section of a stage 16 embryo in bright-field; and D dark-field, note hybridization over brain lobes (br) and ventral ganglion (vg). (E,F) Parasagittal section of a stage 14 embryo showing grain clusters, bright-field and dark-field, respectively. Arrowheads point to the grains seen in each segment, which possibly represent the peripheral neurons. In all panels, anterior is left and ventral is down. Bar, 50 μm.

express the 6.5-kb transcript whereas cells in the early developing lamina have no detectable Appl transcript (Fig. 5).

As with embryos, the transcript is also found in the peripheral nervous system. Grains are observed over the eye–antenna disc (Fig. 6). Transcripts are detected only in the eye portion of this disc and, within it, grains are restricted to cells posterior to the morphogenic furrow. Eye disc neurons differentiate as a wave that progresses from posterior to anterior. The morphogenic furrow is a visible demarkation that allows one to distinguish differentiated (posterior) from undifferentiated (anterior) regions (for review, see Tomlinson, 1988; Ready, 1989). At least three cell columns separate the furrow and the onset of hybridization (4 columns are unlabeled in Fig. 6C). It is at the four–five column boundary that the second mitotic division occurs leading to the fully formed eight-cell cluster associated with each ommatidia (Ready et al., 1976). Following this division, post-mitotic nuclei move toward the basal surface of the disc. Transcripts are detected only in the apical two-thirds of the disc.

Pupal and adult transcription
24 and 72 h pupae have been examined for Appl transcript localization. During the pupal period, some continued cell divisions are believed to occur (White and Kankel, 1978; Truman and Bate, 1988) and the central nervous system is rearranged into the adult configuration (Kankel et al., 1980). Fig. 7 shows a 72 h pupa after hybridization with the c1 probe. Intense signal is observed over all cortical areas of the CNS. This includes the optic lobes and sub-esophageal ganglion in the head, and the thoracic and abdominal ganglia cortices. In addition, the expression of Appl is detected in the retina and antenna. No labeling of non-neuronal tissues, including muscle, gut and gonads, is evident.

Appl expression in adults is also restricted to the nervous system. Fig. 8 represents a typical adult-head
The abdomens of both female and male flies are ment (Jimenez and Campos-Ortega, 1979; White, 1980). Two vital loci: ventral nervous system condensation defective (vnd) (White, 1980) and embryonic lethal abnormal visual system (elav) (Campos et al. 1985; Campos et al. 1987) map within this chromosomal region. The vnd mutant phenotype includes a defect in condensation of the embryonic nervous system and disarray of the neuropil region evidenced in the transverse and longitudinal commissures (White et al. 1983; Jiménez and Campos-Ortega, 1987). elav mutant embryos also show defects in the ventral nerve cord late in embryogenesis, as well as optic lobe defects in adults (Jiménez and Campos-Ortega, 1987; Campos et al. 1985). The data presented in this paper unambiguously separate the Appl gene from the genetically defined vnd locus. At least one exon at the 5' end of the Appl transcription unit, which encodes the first 78 nucleotides of the open reading frame, is located in DNA known to be dispensable for vnd function (Rosen et al. 1989). Moreover, exon 2, within the 2.5-kb HindIII fragment, is located in the distal region of the fragment, thus its inclusion within the vnd region is also questionable. Although Northern blots show only one size of Appl transcript, alternatively spliced RNAs that co-migrate with the 6.5-kb form cannot be ruled out. However, we believe that the existence of additional exon(s) that replace the DNA eliminated in RT778 is unlikely. The DNA within the 7-kb intron fails to detect any transcript on Northern blots. Additionally, germ-line transformation of either (1) an 11-kb EcoRI fragment that contains exon 3 and more 3' genomic DNA including 3' untranscribed sequences, or (2) a 13.5-kb HindIII fragment that includes the 7-kb intron and extends to the 3' limit of the transcribed region, fails to rescue vnd lethal mutations (L.M.-M., unpublished observations). Our current interpretation of molecular-genetic mapping experiments is that the Appl transcript is encoded by a previously undefined locus that resides between exon and vnd (see Fig. 1). The three loci, elav, Appl and vnd, are contained within ~80 kb of genomic DNA.

Information about the 5' intron–exon organization and promoter of the Appl gene was obtained by sequencing the genomic DNA from which the first two exons are derived (Fig. 1). Consensus splice sites are observed at exon–intron boundaries. In addition, approximately 500 nucleotides of untranscribed leader sequence is shown. Motifs found in the human APP promoter region were not observed (Salbaum et al. 1988). Whether or not this 500 bp region is sufficient to direct the correct pattern of Appl transcription has not been ascertained.

We also show that in all developmental stages, Appl transcripts are localized to the cortical regions of the nervous system and that transcripts are not observed in any other tissues. The neural-specific nature of Appl gene expression is consistent with the neural enrichment previously observed by Northern analysis (Rosen et al. 1989). In contrast to the situation in Drosophila, the mammalian gene is transcribed in many tissues in addition to the nervous system. In rat, transcripts are detected in both neurons and muscles, with a higher

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**Discussion**

The Appl gene is located in a chromosomal region previously shown to be important for neuronal development (Jiménez and Campos-Ortega, 1979; White, 1980). Two vital loci: ventral nervous system condensation defective (vnd) (White, 1980) and embryonic lethal abnormal visual system (elav) (Campos et al. 1985; Campos et al. 1987) map within this chromosomal region. The vnd mutant phenotype includes a defect in condensation of the embryonic nervous system and disarray of the neuropil region evidenced in the transverse and longitudinal commissures (White et al. 1983; Jiménez and Campos-Ortega, 1987). elav mutant embryos also show defects in the ventral nerve cord late in embryogenesis, as well as optic lobe defects in adults (Jiménez and Campos-Ortega, 1987; Campos et al. 1985). The data presented in this paper unambiguously separate the Appl gene from the genetically defined vnd locus. At least one exon at the 5' end of the Appl transcription unit, which encodes the first 78 nucleotides of the open reading frame, is located in DNA known to be dispensable for vnd function (Rosen et al. 1989). Moreover, exon 2, within the 2.5-kb HindIII fragment, is located in the distal region of the fragment, thus its inclusion within the vnd region is also questionable. Although Northern blots show only one size of Appl transcript, alternatively spliced RNAs that co-migrate with the 6.5-kb form cannot be ruled out. However, we believe that the existence of additional exon(s) that replace the DNA eliminated in RT778 is unlikely. The DNA within the 7-kb intron fails to detect any transcript on Northern blots. Additionally, germ-line transformation of either (1) an 11-kb EcoRI fragment that contains exon 3 and more 3' genomic DNA including 3' untranscribed sequences, or (2) a 13.5-kb HindIII fragment that includes the 7-kb intron and extends to the 3' limit of the transcribed region, fails to rescue vnd lethal mutations (L.M.-M., unpublished observations). Our current interpretation of molecular-genetic mapping experiments is that the Appl transcript is encoded by a previously undefined locus that resides between exon and vnd (see Fig. 1). The three loci, elav, Appl and vnd, are contained within ~80 kb of genomic DNA.

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**Fig. 3.** Nervous system-specific Appl expression in the second larval instar. Sagittal section of a second instar larva is shown, showing CNS and other tissues. A shows a bright-field photomicrograph and in dark-field (B) grains are restricted to the cortex of the CNS, and are not observed over body wall muscle (m) or fat body cells (fb). Anterior is left, ventral is down. The grains observed anterior to the CNS are due to the probe sticking to a tissue edge. n, Neuropil; c, cellular cortex. Bar, 50 µm.

horizontal section in which the antennal lobes, ventrolateral protocerebrum, sub-esophageal ganglion, optic lobes and retina are all present. This section was hybridized at the same time, with the same probe, and exposed for the same length of time, as the pupal section shown in Fig. 7. The transcript is uniformly exposed for the same length of time, as the pupal unlabeled (data not shown). Some variable hybridization in gut tissue (crop and rectum) is observed but can also be observed with sense (control) probes.
Drosophila Appl transcript localization

Fig. 4. Third instar larval ventral ganglion hybridized with Appl probe. All sections are from the same horizontally oriented ventral ganglion. (A,B) Bright- and dark-field images of a section through the middle of the ventral ganglion. Grains are observed over the cortex. No hybridization is detected over midline cell nuclei (mc). C shows a more dorsal section of the same ventral ganglion. In this section several large nuclei that reside on the boundary between cortex and neuropil are observed. g, glial cells. (D) Boxed area in C is enlarged showing the absence of grains over these nuclei, which are presumed glial cells (g). In E, a ventral section through the superficial cortical region containing neuroblasts (nb) is shown; sn, salivary gland nuclei. (F) Boxed area in E is enlarged showing these neuroblasts and the paucity of grains over their nuclei, despite high grain density over neighboring cells. Anterior is left. Bar, 25 μm.

abundance in muscles (Shivers et al. 1988). In mouse, hybridization to brain and kidney RNA is high, while hybridization to other tissues is also observed to a lesser degree (Yamada et al. 1987). Current data suggest that, although human APP is transcribed in many tissues, APP695-encoding messages are present only in the brain (Ponte et al. 1988; Tanzi et al. 1988). The neural-specific expression pattern of the Drosophila gene may indicate that, during evolution, APP695-related function in the nervous system has been selectively maintained, and that the other mammalian gene products have no detectable Drosophila counterparts.

Since Appl transcripts are observed throughout development in all cortical areas of the nervous system, there is likely to be a general and continual requirement for this gene function. In the developing nervous system, Appl transcripts are not found in neuroblasts. In fact, the onset of gene expression during embryogenesis is well after the first ganglion mother cells have divided, suggesting that the gene is not expressed in ganglion mother cells, which are the immediate precursors to neurons. The pattern of expression observed with the Appl gene suggests that it is not one of the earliest genes activated in a post-mitotic neuron. Two other examples of genes expressed specifically and ubiquitously in the nervous system during embryogenesis are embryonic lethal abnormal visual system (elav) and zipper (zip). Contrary to Appl expression, in situ hybridization indicates that elav is expressed very early in post-mitotic neurons (Robinow and White, 1988).
Fig. 5. The brain lobe of a late third instar larva hybridized with Appl probes. (A) Schematic drawing of the section shown in B and C, indicating domains occupied by distinct cell groups. B and C show bright- and dark-field photomicrographs of a brain section that includes developing optic lobes. The central brain (hatched) nuclei are less tightly packed than those in the developing medulla (M). Within the optic lobe, medulla, lamina (L), inner and outer proliferation centers (IPC and OPC, respectively) can be readily delineated (proliferation centers are black in A). Note that the central brain is most heavily labeled, followed in intensity by medulla. The neuroblasts in the IPC and OPC are unlabeled. Grains in the right-hand corner are over the eye disc epithelium. n, Neuropil. Anterior is up, lateral is left. Bar, 50 μm.

Fig. 6. Localization of Appl transcripts in the eye disc. A section through the eye portion of an eye-antenna disc of a late third instar larva is shown. (A) Bright-field photomicrograph of the eye disc, in which the morphogenic furrow is indicated by an arrow. (B) A dark-field image of A. Grains are localized to cells posterior to the furrow. In addition, expression appears to be restricted to the apical 2/3 of the disc. Brackets indicate depth of the disc epithelium. In C the region in which hybridization appears is enlarged. One can count four columns of cells separating the furrow from the onset of hybridization. Apical is up, posterior is right. Bar, 25 μm.

elav transcripts are detected over neurons as early as stage 9. zip transcripts are restricted to the central nervous system during embryogenesis, and are observed just before the onset of germ band condensation (Côté et al. 1987). Thus neurons that have begun to differentiate and express some neural antigens do not immediately express the Appl gene. This is readily evident in the developing photoreceptor cells where the genetic program is best characterized (Tomlinson and Ready, 1987). Appl transcripts are restricted to cells several columns posterior to the morphogenic furrow.

Screens for lethal mutations in our laboratory and by
Fig. 7. Seventy-two-h pupal section demonstrating neural-specific transcript localization. A and B show bright- and dark-field images of a horizontal section through a late pupa. Grains are observed over retina (r), lamina (l), and nuclei of the medulla, lobula and lobula plate (region designated by ol, optic lobes). In the subesophageal, thoracic and abdominal ganglia, signal is present over the cellular cortex. No labeling of cells in muscle (m), or other tissues is observed. n, Neuropil; t2, second thoracic ganglion; t3, third thoracic ganglion; a, abdominal ganglion. Anterior is up. Bar, 50μm.

Fig. 8. Localization of Appl transcript in the adult head. A and B represent bright- and dark-field photomicrographs of a head section hybridized in parallel with the Fig. 6 pupa. Cells in the retina (r), lamina (l), and optic lobes (ol) all have grains above background levels. The signal intensity is reduced in comparison to pupal structures, especially in the retina (compare with Fig. 6); n, Neuropil. Anterior is up. Bar, 50μm.

others have defined 11 lethally mutable loci in the 1B region (see Lindsley and Zimm, 1986). None of these map between elav and vnd. It is reasonable to assume that these screens have saturated the region, as many of the complementation groups have over a dozen independently isolated alleles. Therefore we consider it unlikely that Appl function is important for viability. To generate mutations at the Appl locus, other strategies will have to be used. It is worth noting that Appl transcript specificity is similar to findings for the elav transcript (Robinow and White, 1988). About 14 kb of genomic DNA separates the two loci, which are transcribed divergently.

As human APP is expressed in fetal tissue at a level comparable to or higher than that found in adults (Tanzi
et al. 1988; Kitaguchi et al. 1988), a developmental role for mammalian APP is likely. Several recent studies have attempted to understand the function of the APP gene and the mechanism by which the β-peptide accumulation occurs in amyloid deposits. Two suggestions about the putative function of this molecule are: (1) a receptor with a role in cell–cell recognition (Shivers et al. 1988; Dyrrk et al. 1988), and (2) a peptide that promotes neuronal survival (Whiton et al. 1989). A fragment of APP has also been shown to be toxic to neurons in culture (Yankner et al. 1989). Using *Drosophila* as a model system will permit enormous flexibility in studying this protein in normal and mutant forms. Further characterization of the mechanism of *App* function will advance our understanding of its biological role.

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