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Evidence That β-Amyloid Protein in Alzheimer's Disease Is Not Derived by Normal Processing

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The β-amyloid protein (β/A4), derived from a larger amyloid precursor protein (APP), is the principal component of senile plaques in Alzheimer's disease. APP is an integral membrane glycoprotein and is secreted as a carboxyl-terminal truncated molecule. APP cleavage, which is a membrane-associated event, occurred at a site located within the β/A4 region. This suggests that an intact amyloidogenic β/A4 fragment is not generated during normal APP catabolism. Therefore, an early event in amyloid formation may involve altered APP processing that results in the release and subsequent deposition of intact β/A4.

ALZHEIMER'S DISEASE (AD), THE most common form of dementia in the elderly, affects >10% of the population over the age of 65 years. The major neuropathological features of AD are (i) senile plaques, composed of amyloid surrounded by neurites (abnormal neuronal processes including nerve terminals), (ii) amyloid deposits around blood vessels, and (iii) neurofibrillary tangles (NFT), composed of filamentous aggregates in cell bodies of certain populations of neurons (*1, 2*). Plaques are particularly common in certain regions of the brain, including the cerebral cortex, and the severity of these lesions is correlated with the presence of dementia (*3*). The principal component of amyloid in plaques is an ~4-kD peptide, β/A4 (*4*), derived from APP (*5, 6*). Three APP mRNAs derived by alternative splicing of APP pre-mRNA transcripts have been identified that encode integral membrane glycoproteins of 695, 751, and 770 amino acids (*6, 7*). A fourth alternatively spliced transcript has also been reported that encodes a 563–amino acid polypeptide lacking the β/A4 region, APP transmembrane, and cytoplasmic domains (*8*). The 563–amino acid polypeptide, as well as the APP-751 and APP-770 isoforms, contains a domain homologous to the Kunitz serine protease inhibitors (KPI) (*7*). The ~4-kD β/A4 peptide includes between 12 and 14 amino acids of the transmembrane domain and between 24 and 28 amino acids of the adjacent extracellular domain of APP (*4*). Molecular and cellular events responsible for generating the amyloidogenic β/A4 peptide from the proteolysis of APP have not yet been elucidated (*2*).

Studies of cultured cells have documented that APPs have a short intracellular half-life (~20 to 30 min) and are tyrosine-sulfated, N- and O-glycosylated membrane proteins (*9*). APP appears transiently on the cell surface, and proteins lacking the cytoplasmic domain are secreted into the medium. Such COOH-terminal truncated molecules also appear in human cerebrospinal fluid (CSF), suggesting that similar events occur in vivo (*9, 10*). Although the precise sites of APP cleavage in vivo are unknown, knowledge of the normal biology of this cleavage process is crucial for understanding mechanisms that lead to amyloid formation in the brain.

To identify sequences required for the initial cleavage of APP, we transfected genes encoding authentic or hybrid APPs into cultured mammalian cells. Transcription of these genes (which included the SV40 small t antigen's splice and polyadenylation signals at the 3′ end) was controlled by the SV40 promoter/enhancer region (*11*). COS-1 cells, an African green monkey kidney line that constitutively synthesizes SV40 T antigen, were chosen for transfection for two reasons: (i) T antigen expression allows

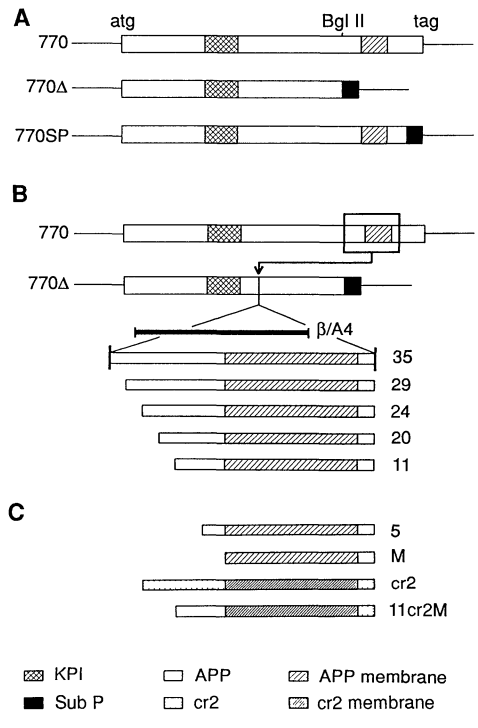

Fig. 1. Cleavage and secretion of APP. (**A**) Construction of authentic, hybrid, and COOH-terminal–deleted APP-770 cDNAs. Plasmids were constructed as described (*16, 17*). Regions labeled atg and tag signify translation initiation and termination sites, respectively. (**B**) Construction of modified APP-770Δ cDNAs. The number at the end of each construct signifies the length (in residues) of the inserted APP extracellular domain. This strategy for construction of plasmids is described (*18, 19*). (**C**) Construction of plasmids p770Δ5, p770ΔM, p770Δcr2, and p770Δ11cr2M. The cr2 regions in p770Δcr2 and p770Δ11cr2M include amino acids 955 to 1005 and 976 to 1005 of the type-2 complement receptor, respectively. The strategy for construction of these plasmids is described (*18, 19*).

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replication of transfected DNA, and (ii) synthesis and secretion of endogenous APP-751 and APP-770 occur constitutively in these cells. The process of APP cleavage was assessed by analysis of APP-derived molecules secreted into conditioned media.

To define an approximate region where proteolytic cleavage occurs, we transfected a hybrid APP gene, resulting in the synthesis of a polypeptide that comigrates with the secreted form of authentic APP (Fig. 1). COS-1 cells were transfected with two plasmids: one encoded authentic APP-770 (p770), and the other (p770Δ) encoded APP-770 in which the last 105 amino acids (containing 34 amino acids of the extracellular domain, transmembrane region, and entire cytoplasmic domain) were replaced with a 13–amino acid sequence from the COOH-terminus of the neuropeptide substance P (*12*). Thus, the hybrid 770Δ molecule was expected to mature as a soluble (nonmembrane-associated) molecule through the secretory pathway. After short-term labeling, authentic cellular APP-770 migrated at ~100 kD, a position appropriate for the N-glycosylated precursor (Fig. 2A, lane 1) (*9*). Subsequently, both the ~100-kD species and higher molecular size precursors (ranging from ~120 to 135 kD), which result from the addition of O-linked carbohydrates to the ~100-kD precursor, were observed in cells (Fig. 2A, lane 4) (*9*). Only a ~110-kD species was secreted into the media (Fig. 2A, lane 7). After short-term labeling, newly synthesized 770Δ precursor migrated as an ~90-kD species, a finding consistent with the deletion of APP transmembrane and COOH-terminal residues (Fig. 2A, lane 2). With a longer duration of labeling, both the ~90-kD and higher molecular size forms were detected (Fig. 2A, lane 5), suggesting that these molecules mature through a similar intracellular pathway as authentic APP-770. A secreted product from 770Δ (Fig. 2A, lane 8) migrated indistinguishably from the ~110-kD product derived from the wild-type APP-770; this finding was confirmed in a cotransfection experiment (Fig. 2A, lane 9). These results suggested that truncation of APP-770 occurred approximately 13 amino acids COOH-terminal to the site at which the substance P region had been inserted, that is, within the β/A4 region. After chemical deglycosylation of secreted 770 and 770Δ molecules, secreted 770 migrated more slowly (by ~1 to 2 kD) than 770Δ (Fig. 2B, compare lanes 1 and 2), implying that the cleavage site was likely to reside several residues downstream of the COOH-terminus of substance P, that is, closer to the interface between the extracellular and transmembrane domains.

Immunoblotting with a monoclonal antibody that detected the COOH-terminal methionine residue of substance P (*12*) verified that secreted 770Δ was indeed full length (Fig. 2C, lane 1). In parallel studies, we also transfected plasmid p770SP, in which substance P sequences replaced the COOH-terminal residues of APP-770. This molecule behaved identically to authentic APP-770 with respect to both maturation and secretion (Fig. 2D). Moreover, the absence of substance P immunoreactivity in the secreted form of 770SP (Fig. 2C, lane 2) provided additional verification that COOH-terminal residues had been deleted in secreted 770SP molecules. Additional immunoblots (Fig. 2C, lanes 4 and 5), with a monoclonal antibody (22C11) that recognized an epitope residing between residues 60 and 100 of the APP molecule, confirmed the presence of NH_2-terminal APP-derived molecules in the medium.

To define a domain that was both necessary and sufficient for the cleavage of APP-770, we constructed p770Δ35 by subcloning a region of APP that includes five amino acids of the cytoplasmic domain, the transmembrane region, and 35 residues of the extracellular domain into plasmid p770Δ (Fig. 1B). The subcloned fragment contains a sequence that encodes the entire β/A4 peptide. Although 770Δ was secreted as an ~110-kD molecule (Fig. 3A, lane 3, also shown in Fig. 1A), a cleaved polypeptide of ~68 kD was observed in the medium from cells transfected with p770Δ35 (Fig. 3A, lane 4). Immunoblotting with the APP-specific monoclonal antibody (*9*) confirmed that the ~68-kD molecule contained the NH_2-terminal domain of APP (Fig. 3B, lane 3).

To define a minimal sequence essential for APP cleavage, we tested additional p770Δ plasmids (Fig. 1B) that contained APP regions encompassing the cytoplasmic COOH-terminal anchor; the transmembrane domain; and either 29 (p770Δ29), 24 (p770Δ24), 20 (p770Δ20), or 11 (p770Δ11) amino acids of the extracellular domain. In all cases, molecules (~65 to 68 kD) were secreted whose sizes were consistent with cleavage at a site adjacent to or within the transmembrane domain (Fig. 3A, lanes 5 to 8, arrows). We conclude that a domain that includes 11 amino acids NH_2-terminal to the APP transmembrane region is sufficient to direct APP cleavage.

However, although 770Δ35 and 770Δ11 were cleaved efficiently (Fig. 4A, lanes 2 and

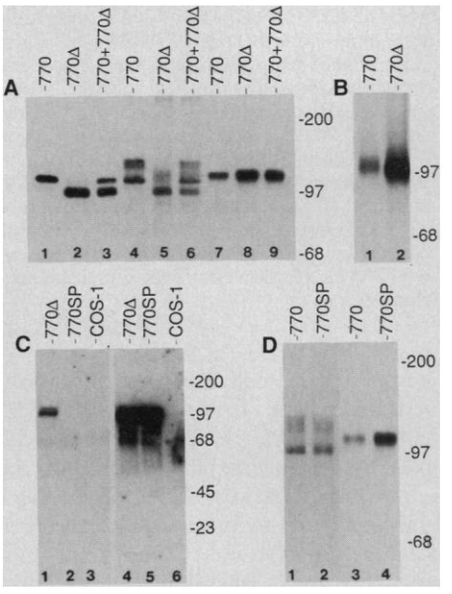

Fig. 2. Analysis of authentic, hybrid, and COOH-terminal–deleted APPs in transfected COS-1 cells. (**A**) COS-1 cells were transfected with plasmid DNA and were then labeled with [^{35}S]methionine 36 hours after transfection either for 10 min (short term) (lanes 1 to 3) or 4 hours (long term) (lanes 4 to 9) (*20*). Immunoprecipitation was performed as described (*21*). Lanes 1 to 3 represent immunoprecipitable APP-related products after short-term labeling of cells transfected with p770 and p770Δ and after cotransfection of p770 and p770Δ, respectively. Lanes 4 to 6 represent immunoprecipitable APP-related products after long-term labeling of cells transfected with p770 and p770Δ and after cotransfection of p770 and p770Δ, respectively. Lanes 7 to 9 represent immunoprecipitates from conditioned media of cells transfected with p770 and p770Δ and after cotransfection of p770 and p770Δ, respectively. (**B**) Analysis of secreted APP-770 and APP-770Δ molecules after chemical deglycosylation. Secreted APP-related 770 and 770Δ molecules were immunoprecipitated from conditioned media and then deglycosylated with trifluoromethane sulfonic acid (TFMS), which removed O-linked and peripheral N-linked sugars (*22*). Samples were analyzed after SDS-PAGE, fluorography, and autoradiography. (**C**) Confirmation of molecular features of APP-770Δ by immunoblot analysis. Plasmids p770Δ and p770SP were transfected into COS-1 cells as described (*20*). Conditions for preparation of conditioned medium from transfected cells and immunoblotting are described (*23*). Lanes 1 to 3 document substance P immunoreactivity in conditioned media prepared from cells transfected with p770Δ, p770SP, or untransfected cells, respectively. Lanes 4 to 6 represent 22C11 immunoreactivity with APP-derived molecules in conditioned media from cells transfected with p770Δ, p770SP, and untransfected cells, respectively. The heterogeneity is due to a long exposure of the autoradiogram that shows degradation products of the secreted molecule that are further enhanced because samples were fractionated on a gradient gel (compare to the 7% gel in Fig. 3B). (**D**) Confirmation that 770SP matures as authentic APP-770. Plasmids p770 and p770SP were transfected into COS-1 cells as described (*20*). Cell labeling (*20*) and immunoprecipitation of APP-related molecules were performed as described (*21*). Lanes 1 and 3 represent cellular and secreted forms of APP-770, respectively, whereas lanes 2 and 4 represent cellular and secreted forms of 770SP, respectively. Size markers are indicated in kilodaltons.

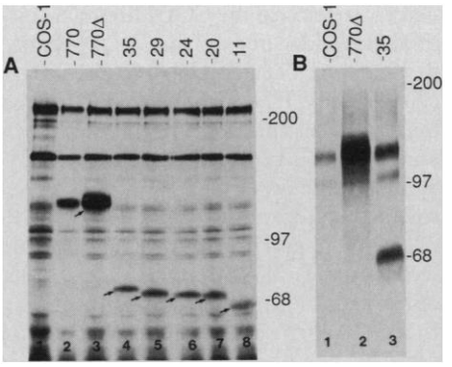

Fig. 3. Identification of a minimal region in APP necessary and sufficient for APP cleavage. (**A**) Analysis of modified APP-770Δ molecules after transfection. Transfection and cell labeling were performed as described (*20*). Shown are the total labeled products in conditioned media from untransfected cells (lane 1) or cells labeled for 4 hours after transfection of plasmids p770, p770Δ, p770Δ35, p770Δ29, p770Δ24, p770Δ20, or p770Δ11 (lanes 2 to 8, respectively). The compromised signal intensity of cleaved 770Δ molecules relative to the secreted parental 770Δ molecule is a reflection of unequal distribution of methionine residues in the NH_2-terminal part of the molecule. Arrows indicate secreted 770Δ molecules. (**B**) Verification that cleaved molecules contain the APP NH_2-terminal domain. Plasmids pSV770Δ and pSV770Δ35 were transfected, and serum-free conditioned medium was prepared (*23*). APP-specific immunoreactivity in these preparations was detected after immunoblot analysis with 22C11, which recognizes the NH_2-terminus of APP. Lanes 1 to 3 represent 22C11 immunoreactivity with polypeptides in conditioned media from untransfected cells or from cells transfected with plasmids p770Δ or p770Δ35, respectively. Lanes 1 and 3 document secretion of APP-751 and APP-770–related forms from COS-1 cells. The observation that media from 770Δ35-transfected cells seem to contain higher levels of endogenously secreted molecules is an artifact of protein loading. Generally, we loaded a constant volume rather than equal amounts of total protein. Restaining of the blot with Ponceau S confirmed that the predominant albumin signal (residual from the transfection) is reduced in lane 1 (*24*). Size markers are indicated in kilodaltons.

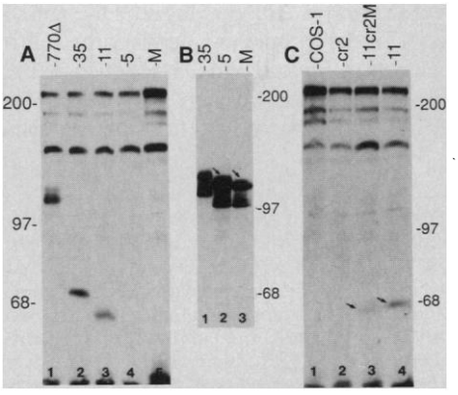

Fig. 4. Examination of cleavage sequence specificity of hybrid APP molecules. (**A**) Analysis of hybrid 770Δ35, 770Δ11, 770Δ5, and 770ΔM molecules in conditioned media. Conditions for transfections and cell labeling are described (*20*). Lanes 1 to 5 represent total labeled products in conditioned media from COS-1 cells transfected with p770Δ, p770Δ35, p770Δ11, p770Δ5, or p770ΔM, respectively. (**B**) Cellular synthesis of hybrid 770Δ35, 770Δ5, and 770ΔM molecules. After long-term labeling, p770Δ35-, p770Δ5-, and p770ΔM-transfected cells were lysed and immunoprecipitated with APP-specific antisera (*21*). Lanes 1 to 3 represent cellular APP-related molecules after transfection with p770Δ35, p770Δ5, and p770ΔM. Arrows indicate accumulated high molecular size 770Δ5 and 770ΔM molecules. (**C**) Analysis of chimeric APP-cr2 molecules. Chimeric plasmids were prepared as described (*18, 19*). Transfection and cell labeling are described (*20*). Lanes 1 to 4 represent total labeled products in conditioned media from untransfected cells or from cells transfected with p770Δcr2, p770Δ11cr2M, or p770Δ11, respectively. Arrows indicate secreted 770Δ molecules. Size markers are indicated in kilodaltons.

3, respectively), molecules with similar deletions that retained the cytoplasmic COOH-terminal anchor, the transmembrane domain, and only five amino acids of the extracellular domain (770Δ5) or the cytoplasmic COOH-terminal anchor and transmembrane domain alone (770ΔM) were not cleaved (Fig. 4A, lanes 4 and 5, respectively). Immunoprecipitation of labeled precursors in cell pellets (Fig. 4B) showed the presence of the 770Δ5 and 770ΔM precursors. Moreover, the accumulation of high molecular size precursor forms of 770Δ5 and 770ΔM (Fig. 4B, lanes 2 and 3, respectively, arrows) suggests that these polypeptides represented uncleaved, cell-surface molecules.

To characterize the specificity of cleavage reactions, we transfected two additional plasmids. In p770Δcr2, we subcloned a region from the type-2 complement receptor (cr2) (*13*), a nonsecreted integral membrane glycoprotein, into p770Δ (Fig. 1C). This region of cr2 included six amino acids of the cytoplasmic domain, the transmembrane region (with <10% sequence identity to the APP transmembrane region), and 20 residues of the extracellular domain. Second, we constructed plasmid p770Δ11cr2M (Fig. 1C) in which the cr2 extracellular domain in 770Δcr2 was replaced with 11 amino acids of the APP extracellular domain, the minimal region sufficient for cleavage if placed NH_2-terminal to the APP transmembrane region. As expected, a cleaved 770Δcr2 polypeptide was not observed (Fig. 4C, lane 2), whereas cleavage of 770Δ11cr2M was apparent, that is, a ~68-kD molecule (lane 3, arrow) that migrated indistinguishably from the 770Δ11 cleaved product (lane 4, arrow). Therefore, we suggest that cleavage of APP is a sequence-specific reaction independent of the identity of transmembrane sequences.

Cleavage reactions are not specific to COS-1 cells. For example, when mouse fibroblast (Ltk$^-$), Chinese hamster ovary (CHO), and human embryonic kidney (293) cell lines were transfected with p770 and p770Δ35, cleaved and secreted molecules derived from both the full-length 770 and 770Δ35 polypeptides were observed (*14*), confirming that the essential domain for cleavage in COS-1 cells is conserved across species and cell lineage. We have also shown that APP-695 is processed in a similar manner (*14*).

In conclusion, these investigations suggest that normal cleavage of APP is a membrane-associated event that occurs within the extracellular domain of β/A4. These results provide evidence that in cultured mammalian cells the secreted form of APP contains part of the β/A4 peptide. Although the processing of APP in transfected cells may not precisely mimic the in vivo condition, studies showing β/A4 immunoreactivity in APP-related molecules in human CSF are consistent with this interpretation (*15*). Nevertheless, the precise cleavage site within β/A4 awaits direct sequencing of the COOH-terminus of the secreted form of APP or NH_2-terminus of the retained membrane-associated fragment. Our results are consistent with the concept that amyloidogenesis in brain parenchyma of individuals with AD is the result of altered APP processing, resulting in the release of an intact β/A4 domain and its subsequent self-assembly into amyloid fibrils.

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16. We constructed p770 by the insertion of the 2700-bp APP-770 cDNA insert into a puc 19–based vector that contained the SV40 promoter/enhancer upstream and SV40 small t splice and polyadenylation signals downstream of the cloning site. This insert included 150 bp of the 5′ untranslated region, the entire APP-770 coding region, and 280 bp of the 3′ untranslated sequence. p770Δ was constructed by insertion of a 65-bp fragment that encoded the six COOH-terminal amino acids of substance P with additional spacer sequences into the Bgl II site upstream of sequences that encode the APP transmembrane domain. p770SP, a recombinant molecule that replaced the COOH-terminal 17 amino acids of APP with 16 amino acids of substance P sequence, was prepared as follows. A full-length cDNA encoding APP-770 cloned in the Okayama-Berg vector, pCDv-1 [H. Okayama and P. Berg, *Mol. Cell. Biol.* **3**, 280 (1982)], was digested at the Spe I site in the 3′ untranslated region and then digested for varying periods of time with Exonuclease III. After blunt ending with S1 nuclease and secondary digestion with Hind III, molecules with lengths commensurate with deletions extending into the APP COOH-terminus were selected. The Hind III site delimits the 5′ end of the SV40 promoter/enhancer region of pCDv-1. These molecules were ligated with a vector molecule containing a Hind III site 5′ and a 3′ blunt-ended site directly upstream of substance P and SV40 small t splice and polyadenylation sequences. Recombinants were verified to be in-frame after double-stranded sequencing of plasmid templates (*17*) with a primer complementary to the substance P sequences.
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18. Subcloning of various APP regions was facilitated by polymerase chain reaction (PCR) techniques. Twenty-eight base "sense" primers, which encode regions NH_2-terminal to the APP transmembrane region or the transmembrane domain alone (in p770ΔM) and which contain a sequence CCGCTCGAG at the 5′ end, were synthesized and purified by gel electrophoresis. A 28-base "antisense" primer complementary to the sequence, which encodes five residues of the APP cytoplasmic domain and proximal transmembrane region and which contains the sequence CCGCTCGAG at its 5′ end, was also synthesized. The insert in plasmid p770Δcr2 was generated with a 28-base sense primer, which encodes a region upstream of the cr2 transmembrane domain, and a 28-base antisense primer, complementary to six amino acids of the cr2 cytoplasmic domain and the transmembrane region. These primers were used in a PCR in the presence of plasmid pBSCR2.1 [C. A. Lowell *et al.*, *J. Exp. Med.* **170**, 1931 (1989)], which encodes the full-length cr2 molecule. These primers also contained the sequence CCGCTCGAG at the 5′ end. To generate the insert in plasmid p770Δ11cr2M, we used a 60-base sense primer, which encoded 11 amino acids NH_2-terminal to the APP transmembrane domain and 6 amino acids of the cr2 transmembrane domain, in combination with the cr2 antisense primer in a PCR in the presence of plasmid pBSCR2.1. PCRs were carried out using a Thermal Cycler (Perkin-Elmer Cetus) with each of the sense oligonucleotides in conjunction with the antisense oligonucleotide in the presence of plasmid p770. PCR products were digested with Xho I and fractionated by polyacrylamide gel electrophoresis (PAGE). Fragments were electroeluted and ligated with plasmid p770Δ previously digested with Xho I and dephosphorylated with alkaline phosphatase (*19*). Test plasmids were verified by double-stranded sequencing of plasmid templates (*17*) with primers used in the PCR.
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20. COS-1 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS). Plasmids were purified by double banding in CsCl-EtBr gradients (*19*). Cells were transfected by the $CaPO_4$ coprecipitation technique with minor modifications [F. L. Graham and A. J. Van der Eb, *Virology* **52**, 456 (1973); S. S. Sisodia, B. Sollner-Webb, D. W. Cleveland, *Mol. Cell. Biol.* **7**, 3602 (1987)] and were metabolically labeled with [^{35}S]methionine 36 hours after transfection. For short-term labeling, cells were washed and then incubated for 15 min with DMEM lacking methionine. Cells were labeled with 150 μCi [^{35}S]methionine (>800 Ci/mmol, Du Pont Biotechnology Systems) for 10 min in 1.6 ml of DMEM lacking methionine and containing 1% dialyzed FBS (DFBS). For long-term labeling studies, cells were washed with DMEM lacking methionine and then incubated for 4 hours with 150 μCi [^{35}S]methionine in DMEM lacking methionine and containing 1% DFBS.
21. For immunoprecipitation from cell pellets, cells were lysed in immunoprecipitation buffer (IPB) [1× IPB: 150 m*M* NaCl, 50 m*M* tris-HCl, *p*H 6.8, 0.5% NP-40, 0.5% sodium deoxycholate, 5 m*M* EDTA, pepstatin (50 μg/ml), leupeptin (50 μg/ml), aprotinin (10 μg/ml), and phenylmethylsulfonyl fluoride (PMSF) (0.25 m*M*)]. Nuclei and residual cytoskeleton were removed by centrifugation, and the soluble extract was made 0.35% SDS and then boiled for 3 min. For analysis of secreted products, 400 μl of conditioned media from cells labeled for 4 hours were made 1× in IPB after the addition of one-fifth volume of 5× IPB and then boiled in the presence of 0.35% SDS. Cell extract equivalent to ~10^5 cells or conditioned media was cleared for 15 min with 50 μl of a 10% suspension of Pansorbin (Calbiochem) and then incubated at 4°C for 3 to 4 hours with 1 to 2 μl of APP-specific polyclonal sera elicited against bacterially synthesized APP (*9*). After a short incubation with 40 μl of Pansorbin, immunocomplexes were layered onto a sucrose cushion (1*M*) prepared in IPB and then centrifuged for 2 to 3 min at 10,000*g*. The cushion was aspirated and the pellet resuspended in 40 μl of Laemmli sample buffer and then boiled for 5 min. After centrifugation, soluble materials were subject to SDS-PAGE. Gels were fixed, impregnated with 2,5-diphenyloxazole–dimethyl sulfoxide [W. Bonner and R. Laskey, *Eur. J. Biochem.* **46**, 83 (1974)], and exposed to Kodak X-Omat film at −70°C.
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23. At 24 hours after transfection, media was replaced with a serum-free defined medium (MCDB 302, Sigma). Conditioned serum-free media was removed 72 hours later, briefly centrifuged to remove particulate material, and concentrated by ultrafiltration in Centricon 10 chambers (Millipore). The resulting material was fractionated by SDS-PAGE and electrophoretically transferred to nitrocellulose filter membranes. To detect substance P–containing polypeptides, we first modified blots by incubation in a solution (1*M*) of 1-ethyl-3-(3-aminopropyl) carbodiimide (EDC) (Sigma) in 0.5*M* ammonium chloride (*12*) to amidate the substance P COOH-terminal methionine residue. This modification is absolutely required for recognition by the monoclonal antibody to substance P, NCI/34. Substance P immunoreactivity on the blot was visualized after incubation for 16 hours with NCI/34 (Accurate Chemical & Scientific Corp), followed by incubation with rabbit antibody to rat H and L chain–specific immunoglobulin G (IgG) (Cappell Labs) and finally with ^{125}I-labeled protein A. To detect NH_2-terminal APP immunoreactivity, we incubated blots with an APP-specific monoclonal antibody, 22C11 (*9*), that recognizes an APP epitope residing between amino acids 60 and 100.
24. S. S. Sisodia, data not shown.
25. We thank D. Cleveland and G. Hart for helpful discussion and F. Davenport for excellent technical assistance. We are grateful to R. Ramabhadran and R. Bayney for providing the full-length APP cDNAs, A. Weidemann for providing APP-specific monoclonal and polyclonal antibodies, and J. Ahearn for providing a full-length type-2 complement receptor cDNA. Supported by grants from the USPHS (NIH AG 03359 and AG 05146), the American Health Assistance Foundation, and The Robert L. & Clara G. Patterson Trust. D.L.P. and E.H.K. are recipients of a Leadership and Excellence in Alzheimer's Disease (LEAD) award (NIA AG 07914). D.L.P. is the recipient of a Javits Neuroscience Investigator Award (NIH NS 10580).

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Two Gap Genes Mediate Maternal Terminal Pattern Information in *Drosophila*

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In *Drosophila* three maternal pattern organizing activities, the anterior, the posterior, and the terminal, establish the anterior-posterior body pattern of the embryo by initiating the spatially restricted activities of the gap class of zygotic segmentation genes. The activities of *tailless* (*tll*) and the newly identified gap gene *huckebein* (*hkb*) are specifically involved in mediating the maternal terminal information at the posterior end of the blastoderm embryo.

THE *Drosophila* GENE *torso* (*tor*) (*1–3*) is a key component of the terminal organizer system. It encodes a putative tyrosine kinase receptor (*4*). In embryos without maternal *tor* activity, all structures posterior to the seventh abdominal segment including the structures derived from the most posterior part of the blastoderm anlagenplan, hindgut and posterior midgut, fail to develop (*5, 6*). These mutant embryos deviate from wild-type development as early as gastrulation (*5*). Because the anlagen of hindgut and posterior midgut are absent, no corresponding invagination is formed at the posterior end of the embryo, and the germ band does not extend along the dorsal side of the embryo (Fig. 1). Genetic and phenotypic evidence indicates that one target of *tor*

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