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Characterization and Chromosomal Localization of a cDNA Encoding Brain Amyloid of Alzheimer's Disease

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Four clones were isolated from an adult human brain complementary DNA library with an oligonucleotide probe corresponding to the first 20 amino acids of the β peptide of brain amyloid from Alzheimer's disease. The open reading frame of the sequenced clone coded for 97 amino acids, including the known amino acid sequence of this polypeptide. The 3.5-kilobase messenger RNA was detected in mammalian brains and human thymus. The gene is highly conserved in evolution and has been mapped to human chromosome 21.

WE REPORT THE ISOLATION AND characterization of complementary DNA (cDNA) clones coding for the polypeptide that forms the brain amyloid of paired helical filaments of neurofibrillary tangles within neurons, the extracellular amyloid plaque cores, and the vascular wall amyloid deposits in Alzheimer's disease (AD) and adult Down syndrome (DS) (*1–3*). This is the same amyloid in the paired helical filaments in neurofibrillary tangles of Guamanian amyotrophic lateral sclerosis parkinsonism dementia and of the amyloid in the aging brain (*4*).

Computer analysis of the 28–amino acid sequence of the polypeptide (*1–3*) revealed that the first 20 amino acids included unique regions not found in known sequences deposited in computer banks of protein sequences. A 59-residue oligonucleotide probe corresponding to these first 20 amino acids was synthesized with deoxyinosine in every third position (Fig. 1) (*5–7*).

Four clones (λAm1, λAm2, λAm3, and λAm4) were isolated from a λgt11 cDNA library derived from human brain (*8, 9*) by screening with this synthesized oligonucleotide probe. Restriction maps of the four positive cDNA clones revealed that they were identical, except for λAm1, which was approximately 100 bp longer than the other three and contained an internal Eco RI site (Fig. 2).

```
   asp ala glu phe arg his asp ser gly tyr
5'-GAI GCI GAI TTI AGI CAI GAI TCI GGI TAI
                  C       AG

   glu val his his gln lys leu val phe phe
   GAI GTI CAI CAI CAI AAI CTI GTI TTI T[T]-3'
                           T

   ala glu asp val gly ser asn lys
```

Fig. 1. The 59-base oligonucleotide probe with the deoxyinosine (I) in every third position (first line), corresponding to the first 20 amino acids of the published 28–amino acid sequence (second line) for brain amyloid of Alzheimer's disease and adult Down syndrome (*2*).

Southern blot analysis of the λAm4 clone showed that the 59–amino acid probe hybridized to the Eco RI–Pvu II fragment at the 5′ end of the insert. The whole 1-kb Eco RI insert of clone λAm4 was subcloned in the plasmid pGEM-3 and its fragments were cloned in the plasmid pGEM blue (*10*) and sequenced by the chain termination method (Fig. 2) (*11*). The sequence contained an open reading frame, which has been translated into an amino acid sequence.

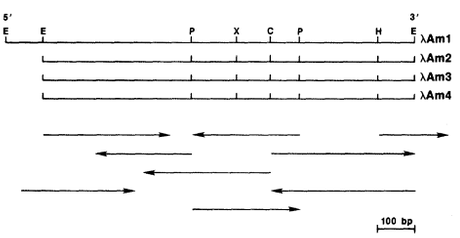

Fig. 2. Restriction maps of the four cDNA clones λAm1 through λAm4 isolated from adult human brain λgt11 cDNA library and the sequencing strategy of the λAm4 clone. The λAm2, λAm3, and λAm4 clones were about 1 kb; λAm1 was about 100 bp longer and contained an internal Eco RI site. Restriction enzyme sites: E, Eco RI; P, Pvu II; C, Cla I; X, Xmn I; and H, Hind III. The arrows represent pGEM blue subclones, direction of sequencing, and length of sequence derived by the chain termination ("dideoxy") method (*11*) with the use of unique restriction sites for subcloning and the sequencing protocol from Promega Biotec (*10*).

The deduced 97–amino acid sequence from the first 291 nucleotides included the sequence for amino acids 3 to 42 of the brain amyloid polypeptide of AD and DS (*1–3, 12, 13*). Beyond amino acid 42, which is the termination of the brain amyloid polypeptide from AD and DS patients, there are another 57 amino acids and a TAG termination codon (Fig. 3). The nucleotide sequence of the cDNA clone and the deduced amino acid sequences had no extensive homology to any previously known sequences (*14*).

Hydropathy analysis (*15*) of the deduced amino acid sequence showed a large hydrophobic domain and alternating hydrophobic and hydrophilic regions typical for membrane proteins (Fig. 4). This structure could possibly result in aggregate formation. Secondary structure predictions were made with the methods of Chou and Fasman and Garnier *et al.* (*16, 17*). There was a strong tendency for the polypeptide to form a β-pleated sheet configuration in amino acids 31 to 50 (Fig. 4A), which is consistent with the known structure of amyloid (*18, 19*). In conversion of the precursor protein to amyloid, cleavage should occur between amino acid 42 and amino acid 43, which lie within the main hydrophobic domain. There are two potential *N*-glycosylation sites (Asn-X-Thr/Ser) at positions 84 and 88. It is unknown whether this protein is glycosylated, but amino acid 88 is unlikely to be glycosylated because it has an adjacent proline residue (Fig. 3) (*20, 21*). A fusion protein of 126 kD was produced in *Escherichia coli* Y1089. The size of the recombinant part of this protein (12 kD) is consistent with the estimated size of the deduced polypeptide.

Northern blot analysis of polyadenylated RNA (*9*) was performed with the 1-kb Eco RI fragment of the λAm4 clone (the Am4 probe) under high stringency conditions. A single band of about 3.5 kb was detected in

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O. W. McBride, Laboratory of Biochemistry, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892.

```
  1                                  10                                  20
        GAA TTC CGA CAT GAC TCA GGA TAT GAA GTT CAT CAT CAA AAA TTG GTG TTC TTT   60
        Glu Phe Arg His Asp Ser Gly Tyr Glu Val His His Gln Lys Leu Val Phe Phe
Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val His His Gln Lys Leu Val Phe Phe

                                28      30                                  40
GCA GAA GAT GTG GGT TCA AAC AAA GGT GCA ATC ATT GGA CTC ATG GTG GGC GGT GTT GTC  120
Ala Glu Asp Val Gly Ser Asn Lys Gly Ala Ile Ile Gly Leu Met Val Gly Gly Val Val
Ala Glu Asp Val Gly Ser Asn Lys Gly Ala Ile Ile Gly Leu Met Val Gly Gly Val Val

     42                                 50                                  60
ATA GCG ACA GTG ATC GTC ATC ACC TTG GTG ATG CTG AAG AAG AAA CAG TAC ACA TCC ATT  180
Ile Ala Thr Val Ile Val Ile Thr Leu Val Met Leu Lys Lys Lys Gln Tyr Thr Ser Ile
Ile  Ala

                                   70                                  80
CAT CAT GGT GTG GTG GAG GTT GAC GCC GCT GTC ACC CCA GAG GAG CGC CAC CTG TCC AAG  240
His His Gly Val Val Glu Val Asp Ala Ala Val Thr Pro Glu Glu Arg His Leu Ser Lys

                                   90                                 100
ATG CAG CAG AAC GGC TCA GAA AAT CCA ACC TAC AAG TTC TTT GAG CAG ATG CAG AAC TAG  300
Met Gln Gln Asn Gly Ser Glu Asn Pro Thr Try Lys Phe Phe Glu Gln Met Gln Asn End

ACCCCCGCCACAGCAGCCTCTGAAGTTGGACAGCAAAACCATTGCTTCACTACCCATCGGTGTCCATTTATAGAATAAT  379

GTGGGAAGAAACAAACCCGTTTTATGATTTACTCATTATCGCCTTTTGACAGCTGTGCTGTAACACAAGTAGATGCCTG  458

AACTTGAATTAATCCACACATCAGTAATGTATTCTATCTCTCTTTACATTTTGGTCTCTATACTACATTATTAATGGGT  537

TTTGTGTACTGTAAAGAATTTAGCTGTATCAAACTAGTGCATGAATAGATTCTCTCCTGATTATTTATCACATAGCCCC  616

TTAGCCAGTTGTATATTATTCTTGTGGTTTGTGACCCAATTAAGTCCTACTTTACATATGCTTTAAGAATCGATGGGGG  695

ATGCTTCATGTGAACGTGGGGAGTTCAGCTGCTTCTCTTGCCTAAGTATTCCTTTCCTGATCACTATGCATTTTAAAGT  774

TAAACATTTTAAGTATTTCAGATGCTTTAGAGAGATTTTTTTTCCATGACTGCATTTTACTGTACAGATTGCTGCTTCT  853

GCTATATTTGTGATATAGGAATTAAGAGGATACACACGTTTGTTTCTTCGTGCCTGTTTTATGTGCACACATTAGGCAT  932

TGAGACTTCAAGCTTTTCTTTTTTTGTCCACGTATCTTTGGGTCTTTGATAAAGAAAAGAATCCCTGTTCATTGTAAGC 1011

ACTTTTACGGGGCGGGTGGGGAGGGGTGCTCTGCTGGTCTTCAATTACCAAG                          1063
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Fig. 3. Nucleotide sequence of the cDNA clone λAm4 (first line) and deduced protein sequence (second line) compared with amino acid sequence for the isolated amyloid polypeptide from patients with Alzheimer's disease and Down syndrome (third line). Numbers above lines indicate amino acid position; numbers on the right-hand margin indicate nucleotide positions beginning with nucleotide 7. End, stop codon; box, potential *N*-glycosylation sites at positions 84 and 88; broken line, the main hydrophobic domain.

RNA preparations from mouse, rat, and bovine brains and from human thymus, indicating that our 1-kb cDNA corresponds to a portion of the messenger RNA (mRNA) (Fig. 5). No bands were detected in RNA preparations from the hippocampus of a 70-year-old man, human placenta, epithelium, or liver; however, the amount of mRNA may have been below the sensitivity limit of the Northern blot method (Fig. 5). Thus, the gene encoding the protein that forms amyloid in Alzheimer's disease (AD-AP) is transcribed in normal tissues and encodes a single mRNA species. We now know that the gene is expressed in certain neurons and some glial cells (*22*). The presence of this mRNA in brains of distantly related species suggests that the gene is conserved.

To determine the chromosomal location of the AD-AP gene, we examined the DNA of 53 human-mouse and 32 human-hamster somatic cell hybrids that retained varying groups of human chromosomes after segregation. Strongly hybridizing 3.2-kb and polymorphic 9.6- or 11.0-kb bands (*23*) in Eco RI digest of human DNA were widely separated from 2.9-kb and 5.4-kb homologous mouse sequences when tested with the Am4 probe (Fig. 6). The Eco RI 9.6-kb human polymorphic band was less well resolved from a single 8.1-kb homologous sequence of hamster DNA digested with the same endonuclease. Hence, Southern blot analyses of both Hind III and Eco RI digests of these hybrid cell DNAs were performed with the same probe. These results were in complete agreement and permitted unambiguous assignment of all detectable human sequences to chromosome 21 (Table 1). This assignment is based upon the fact that at least 34% discordancy of the AD-AP gene with all other human chromosomes was observed. The single discordancy with chromosome 21 represents a hybrid

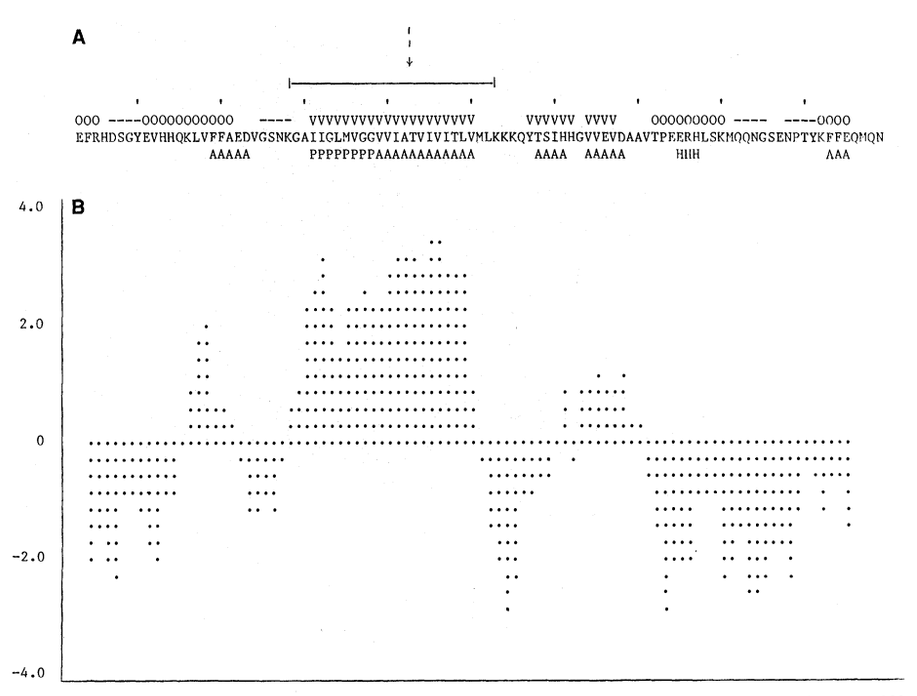

Fig. 4. (**A**) Predicted secondary structure of the deduced protein. The first line is the secondary structure predicted by the method of Chou and Fasman (*16*). O, alpha helix; V, β sheet; −, turn. The second line is the deduced amino acid sequence in one letter code (amino acids 3 through 99). The third line is the secondary structure predicted by the method of Garnier *et al.* (*17*). A, antiparallel β sheet; P, parallel β sheet; H, α helix. (**B**) Hydropathy analysis (*15*) of the deduced protein. The numbers on the *x* axis indicate the position of amino acid residues. The numbers on the *y* axis are values of hydrophobic index. The main hydrophobic domain of the deduced amino acid sequence (amino acids 29 through 52) is indicated by the bar at the top of the figure. The putative cleavage site between amino acids 42 and 43 is shown with a broken arrow. Abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

that had undetectable human superoxide dismutase (*SOD1*) activity, but that retained the human AD-AP gene. This single discordancy probably reflects differing sensitivities for the detection of these two markers, but a chromosomal break between these two loci cannot be excluded. Although all human sequences detected by the Am4 probe were located on chromosome 21, we have not excluded the presence of more than one gene with homology to our clone on this chromosome. Hybridizing bands, unexplained by recognition sites within the Am4 probe (Fig. 2), were observed in digests of human DNA with Eco RI, Hind III (Fig. 6), and six other (*24*) restriction enzymes. These results indicate either the presence of restriction sites within introns of the gene, or the presence of more than one gene detected by the Am4 probe on chromosome 21, or both. No additional hybridizing bands were observed in the human genome when the stringency of hybridization and washing was reduced to allow detection of sequences diverging by 30% from the Am4 probe sequence. This indicates that the probe does not detect a large family of related genes. The evolutionary conservation of part or all of the AD-AP gene in mammals was demonstrated by the detection of one or more strongly hybridizing bands with the probe on Southern blots after digesting DNAs from nonhuman primates (*25*), rat, mouse, and Chinese hamster with any of several different restriction endonucleases (Fig. 6).

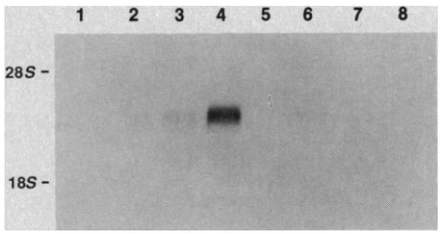

Fig. 5. Northern blot analysis of polyadenylated RNA isolated from mammalian brains and various human tissues. Lane 1, human hippocampus (male, 70 years of age); lane 2, mouse brain; lane 3, rat brain; lane 4, bovine brain; lane 5, human placenta; lane 6, human thymus; lane 7, human epithelium; lane 8, human liver. RNA (5 μg) (*9*) was electrophoresed through a 0.8% formaldehyde-agarose gel. RNA was transferred to nitrocellulose and hybridized with the ^{32}P-labeled 1-kb Eco RI fragment from the λAm4 clone (specific activity, 3×10^8 cpm/μg) (*27*).

There are several groups of genes known to be highly conserved in evolution—those coding for structural proteins, growth factors and their receptors, and stress proteins. The human AD-AP gene may belong to one

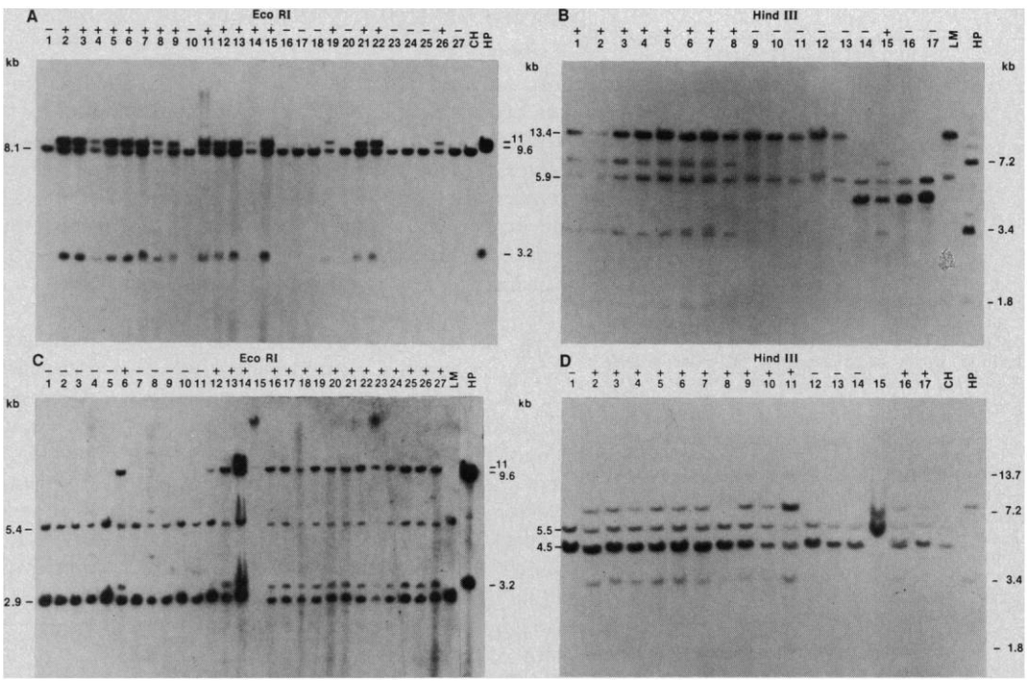

Fig. 6. Southern blot analysis for detection of the AD-AP gene in a representative group of human-rodent somatic cell hybrid DNAs. Hybridizing human sequences (+) were readily distinguished from rodent homologs in the hybrid cell DNAs. DNAs isolated from individual human-hamster hybrids (**A**; **B**, lanes 14–17; and **D**) or human-mouse hybrids (**C** and **B**, lanes 1–13) are depicted in the numbered lanes. Sizes of hybridizing human sequences and homologous rodent sequences in each panel are shown (in kilobases) at the right and left margins, respectively. Incomplete digestion of DNA was observed in lane 15 in (C) and (D). Control DNA: LM, mouse; CH, Chinese hamster; HP, human placenta. DNA (10 μg) was cleaved with Eco RI or Hind III, size fractionated by electrophoresis in 0.7% agarose gels, transferred to nylon membranes (*28, 29*) and hybridized with a ^{32}P-labeled (*30*) 1-kb Eco RI fragment of the λAm4 clone under high stringency (that is, less than 10% divergence allowed) (*31*). After hybridization, the blots were washed in 0.3*M* NaCl, 0.03*M* sodium citrate, and 0.2% SDS at room temperature, and then in 0.015*M* NaCl, 0.015*M* sodium citrate, 0.2% SDS at 55°C. Hybridization was detected by autoradiography.

Table 1. Segregation of the AD-AP gene in human-rodent hybrids. The AD-AP gene was detected as 3.2- and 9.6- or 11-kb polymorphic bands in Eco RI digests, or as 1.8-, 3.4-, 7.2-, and 13.7-kb bands in Hind III digests of somatic cell hybrid DNAs after hybridization with the 1-kb Eco RI fragment of the λAm4 clone. Detection of the human AD-AP sequences is correlated with the presence or absence of each human chromosome in the somatic cell hybrids. Discordancy indicates presence of the chromosome when the gene is absent or absence of the chromosome despite the presence of the gene, and the sum of these numbers divided by total hybrids examined multiplied by 100 is percent discordancy. The human-hamster hybrids consisted of 20 primary clones and 12 subclones (25 positive of 32 total) and human-mouse hybrids consisted of 15 primary clones and 38 subclones (35 positive of 53 total). The human, mouse, and Chinese hamster cells, the cell fusion procedure, and the isolation and characterization of the hybrids have been described (*31–33*).

Human chromosome	Percent discordancy	Human chromosome	Percent discordancy
1	53	13	47
2	61	14	40
3	36	15	38
4	38	16	48
5	52	17	45
6	40	18	34
7	39	19	45
8	46	20	44
9	60	21	1
10	59	22	56
11	55	X	38
12	58		

of these categories and the amyloid in AD and DS may be formed from a precursor synthesized in neurons as well as in other cells such as microglia and brain macrophages. Overexpression of this gene or altered posttranslational modification of its product in response to environmental stress, as a result of a genetic defect or increased gene dosage (*26*), or a combination of the above might lead to accumulation of the truncated amyloid polypeptide in the brain and to the observed neuropathological changes in AD, DS, Guamanian amyotrophic lateral sclerosis parkinsonism dementia, and aging. Thus a protein synthesized in neurons produces neurofibrillary tangles; that synthesized in microglial cells and brain macrophages is extruded from the cells and forms the extracellular amyloid of amyloid plaques and vascular amyloid deposits. Dying neurons and glial cells could also contribute to the extracellular deposits.

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Amyloid β Protein Gene: cDNA, mRNA Distribution, and Genetic Linkage Near the Alzheimer Locus

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The amyloid β protein has been identified as an important component of both cerebrovascular amyloid and amyloid plaques of Alzheimer's disease and Down syndrome. A complementary DNA for the β protein suggests that it derives from a larger protein expressed in a variety of tissues. Overexpression of the gene in brain tissue from fetuses with Down syndrome (trisomy 21) can be explained by dosage since the locus encoding the β protein maps to chromosome 21. Regional localization of this gene by both physical and genetic mapping places it in the vicinity of the genetic defect causing the inherited form of Alzheimer's disease.

ALZHEIMER'S DISEASE (AD) IS A progressive neurodegenerative disorder characterized by gradual loss of memory, reasoning, orientation, and judgment (*1*). AD generally occurs as a sporadic disorder of unknown cause. A proportion of cases, which have been termed familial Alzheimer's disease (FAD), are caused by a genetic defect that is transmitted in an autosomal dominant fashion (*2*). Although the nature of this defect is not known, it has been mapped to chromosome 21 by genetic linkage analysis (*3*). Aside from a generally earlier age of onset, the symptoms and pathology of FAD are similar to that of the apparently noninherited form of AD (*2*).

One of the hallmarks of AD is the presence of numerous neuritic plaques in postmortem brain tissue that are revealed by neuropathological examination (*4*). The degree of intellectual impairment in patients appears to be correlated with the frequency of neuritic plaques in the cortex (*5*). The mature neuritic plaque consists of degenerating axons and neurites surrounding an amyloid plaque core (APC) composed of 5- to 10-nm filaments (*6*) that stain with Congo red (*7*). Similar filaments are also found outside of plaques as independent bundles in the cortical neuropil and in the walls of meningeal and intracortical blood vessels [termed cerebrovascular amyloid (CA)] (*8*). A 4.2-kD polypeptide, called the β protein (due to its partial β-pleated sheet structure), has been isolated from both CA and APC. An identical 28–amino acid sequence (with the exception of a Glu to Gln substitution) has been obtained from the CA and APC β proteins, suggesting a possible common ori-

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