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DEVELOPMENTAL AND DIFFERENTIAL EXPRESSION OF BETA AMYLOID PROTEIN PRECURSOR mRNAs IN MOUSE BRAIN

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Summary: S1 nuclease analysis was used to determine the levels and patterns of three beta amyloid protein precursor (BPP) mRNAs in mouse developmental brain and in primary neuronal and glial cultures. BPP695 mRNA lacking the Kunitz proteinase inhibitor (KPI) domain was detected exclusively in neuronal cultures and increased considerably in late embryonic and early postnatal periods. On the other hand, BPP751 and 770 mRNAs with KPI domain were detected predominantly in astrocyte- and microglia-enriched cultures and increased slightly only in embryonic stages. These results suggest that the product of each BPP mRNA may play a different role in the brain.

Beta protein (BP) or A4 protein is the major component of the fibrillar amyloid deposit in Alzheimer's disease (AD)(1). Recent studies have revealed that BP is derived from a larger precursor protein (BPP) which displays the structure of a typical cell surface receptor molecule (2). In addition, the BPP gene generates at least three mRNAs (BPP695, BPP751 and BPP770) through alternative splicing (3-5) and the two larger forms, BPP751 and 770 mRNA, encode an additional domain with the Kunitz proteinase inhibitor (KPI) function (5).

Recently, several groups have reported that levels of certain BPP mRNAs differ significantly in brains of AD as compared to normal individuals (4,6-8). These findings suggest that the abnormal regulation of BPP gene expression may be

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responsible for amyloid accumulation or neuronal degeneration. Therefore, to investigate the regulation of BPP gene expression more precisely, we developed a method to determine the amount of each BPP mRNA species. Here we report the levels and patterns of BPP mRNAs in mouse primary neuronal and glial cultures, as well as those in mouse brain during development.

MATERIALS AND METHODS

Cell cultures: Neuronal cultures were prepared from embryonic day-15 BALB/c mouse brain by plating in the serum-free medium as described previously (9), and were harvested 2 days after. In this condition, the cultures were comprised almost entirely of neurons, as judged by immunocytochemical staining for neurofilament. Astrocyte- and microglia-enriched cultures were prepared from primary mixed brain cell cultures obtained on postnatal day 2 by utilizing the difference in their ability to adhere to the plastic dish (10,11). Purity of the culture cells was determined by morphological appearance and cell type-specific stainings for GFAP and MAC-1, respectively.

RNA preparation: Total cellular RNA was isolated from tissue by lysis in 8 M guanidine-HCl, followed by centrifugation through a layer of 5.7 M CsCl (12). For cell cultures, cytoplasmic RNA was prepared by lysing the cells with 0.5% Noidet P-40 as described previously (13).

RNA analysis: For S1 nuclease analysis, a uniformly labeled single-stranded probe was prepared on M13 template by a modification of a published procedure (14). Briefly, the template was constructed by ligation of the BanII-HincII fragment of mouse BPP770 cDNA containing KPI domain and 19 amino acids (19AA) domain into the SmaI site of M13 mp19 (Fig. 1A). The probe (427 nucleotides) was synthesized on the recombinant phage DNA using the Klenow pol I and the M13 universal primer in the presence of $\alpha[^{32}P]$dCTP and gel-purified after digestion with PstI. Conditions for S1 nuclease digestion were essentially similar to the method described (15), using 5×10^4 cpm of the probe and 20 µg of total RNA. The proportion of three BPP mRNAs was determined by the densitometrical scanning of the autoradiogram and then standardized by the number of cytosine residues. Northern blot hybridization was performed as previously described (16).

RESULTS AND DISCUSSION

As previously reported (6,17), all three forms of BPP mRNA are detected in mouse and human brains. To define the neural cell types producing each BPP mRNA, we performed S1 nuclease analysis using cytoplasmic RNA from mouse primary neuronal and glial cultures and an antisense probe prepared from BPP770 cDNA (Fig. 1A). In this system, BPP695, 751 and 770 mRNAs gave

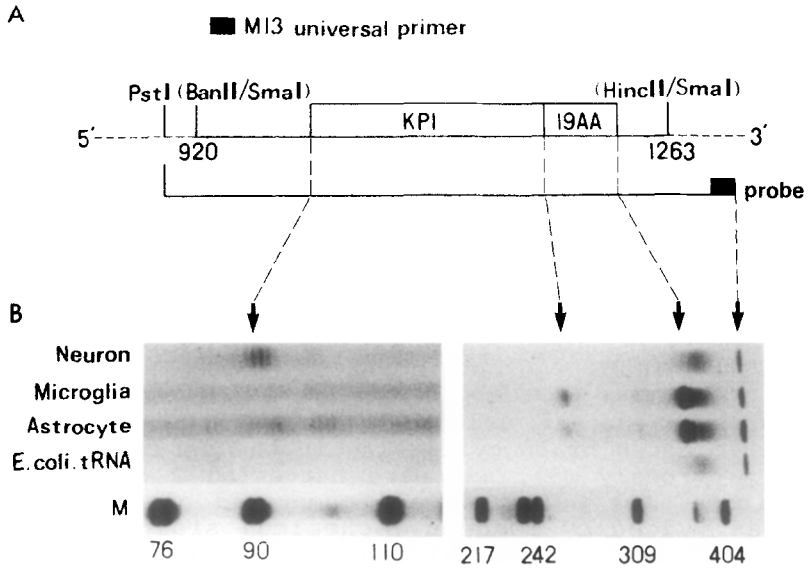

Fig. 1. Expression of three BPP mRNAs in neuronal cultures, astrocyte- and microglia-enriched cultures.
A) Schematic representation of the recombinant phage template using the preparation of the antisense probe.
B) S1 nuclease analysis of cytoplasmic RNA from primary neural cultures. The panels show the relevant area of an autoradiogram on BPP751 and 770 mRNAs (right), and on BPP695 mRNA (left), respectively. Autoradiographic exposure was 3 days (right) and 6 days (left).

specific protected fragments with the length of 89, 257 and 349 nucleotides, respectively. As shown in Fig. 1B, bands corresponding to BPP695 mRNA were detected only in neuronal cultures, indicating that this species is specific for neuronal cells. The densitometrical determination revealed that more than 90% of neuronal BPP RNA was 695 species. While two bands corresponding to BPP751 and 770 mRNAs were observed in all cultures, intensities of the bands predominated in astrocyte- and microglia-enriched cultures, BPP751 mRNA could be detected in neuronal cultures after a longer exposure (data not shown). We also compared the total amount of BPP RNA in neural cultures by Northern blot hybridization probed with BPP695 cDNA. Both astrocyte and microglia cultures contained slightly higher levels of total BPP RNA than neuronal cultures (data not shown). On the

contrary, in situ hybridization experiments performed recently (6,8,18,19) resulted in undetectable levels of expression of BPP RNA in astrocytes and microglial cells at their normal state. It is therefore likely that transcription of BPP mRNA might be stimulated in these cells during the preparation of cell cultures. Recently, Siman et al. (20) have proved immunocytochemically that a large amount of BPP is accumulated in reactive astrocytes of the injured rat brain. Conceivably, the production of BPP may be related to proliferation and differentiation of glial cells.

It has been reported that the expression of BPP mRNA is regulated developmentally in hamster (21) and mouse brain (17). To further characterize the levels and patterns of three BPP

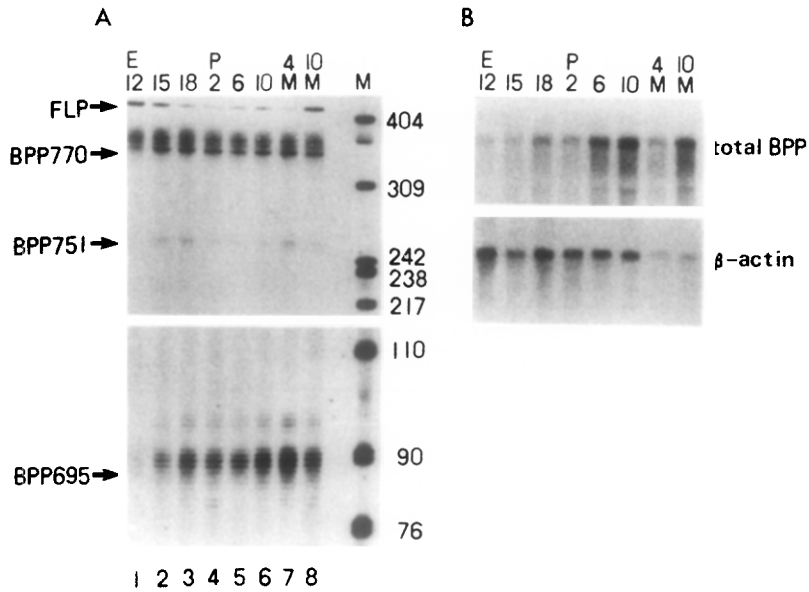

Fig. 2. Developmental expression of three BPP mRNAs in mouse brain.
A) S1 nuclease analysis. Total RNA was isolated from whole brain at periods indicated above. FLP represents a full length of probe. Autoradiographic exposure was 3 days (upper) and 6 days (lower).
B) Northern blot hybridization. The same RNA used in S1 nuclease analysis was determined by Northern blot hybridization probed with BPP695 cDNA (upper) and β-actin cDNA (lower). Autoradiographic exposure was 2 days.

mRNAs in the developing brain, we analyzed total RNA samples from mouse prenatal and postnatal brains by S1 nuclease analysis and Northern blot hybridization (Fig. 2, A, B). The results were quantified by densitometrical scanning (Table 1). Total BPP RNA, as indicated by Northern blot hybridization (Fig. 2B), was first detected at embryonic day 12 (E12), the earliest time point examined. At this stage, all BPP mRNAs were observed and 770 species made up the largest proportion of BPP RNA (78%)(Fig. 2A). The amount of total BPP RNA increased by about 15-fold until postnatal day 10 (P10), and the levels were retained thereafter. This developmental increment of total BPP RNA was primarily accounted for by the increased expression of BPP695 mRNA, i.e. the proportion of BPP695 mRNA rose from 14% at E12 to 74% at P10. In contrast, proportions of BPP751 and 770 mRNAs decreased steadily throughout brain development, although actual levels of these mRNAs increased significantly between E12 and E18 as judged by Northern blot analysis probed with the inhibitor domain (data not shown). These results indicate that BPP RNA, especially 695 species,

Table 1. Developmental profile of BPP mRNAs in the mouse brain

	Proportion of each BPP mRNA (%)			Relative amount
Developmental age	695	751	770	of total BPP
E12	16	6	78	1.0
E15	27	12	62	2.5
E18	34	8	58	4.3
P2	45	7	48	7.9
P6	61	5	34	13.1
P10	74	3	23	15.2
4M	70	2	27	14.6
10M	71	3	26	15.0

The proportion of each type of BPP mRNAs was calculated by densitometrical scanning of S1 nuclease analysis as pictured in Fig. 2. Each value is expressed as a percentage. The relative amount of total BPP RNA was obtained by densitometry of Northern blot hybridization and normalized by the β-actin signal. Each value is expressed as a ratio to E12.

has a developmental path closely related to neuronal growth. Mobley et al. have reported that BPP RNA in hamster septum is induced rapidly between P9 and P16 (21). Thus, timing of the appearance of BPP RNA may vary regionally in the developing brain.

BPP resides perhaps on or near neuronal surface membranes in the rat brain (22). Our observations of both developmental and neuron-specific expression of BPP695 mRNA suggest that this protein may play a role in establishing the mature neuron network. In addition, the persistence of high levels of BPP695 mRNA in adult brain may imply the importance of 695 species in maintaining normal neuronal functions. On the other hand, Schubert et al. have reported that BPP751 has a mitogenic activity to fibroblast, while BPP695 does not (23), indicating that BPP with the proteinase inhibitor domain may exert a distinct role, such as growth modulation, in neuronal differentiation.

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