FEBS Letters 354 (1994) 274–278

FEBS 14750

Increased β-amyloid release and levels of amyloid precursor protein (APP) in fibroblast cell lines from family members with the Swedish Alzheimer's disease APP670/671 mutation

Janet A. Johnston[a,*], Richard F. Cowburn[a], Svante Norgren[b], Birgitta Wiehager[a], Nikolaos Venizelos[c], Bengt Winblad[a], Carmen Vigo-Pelfrey[d], Dale Schenk[d], Lars Lannfelt[a], Cora O'Neill[a,**]

[a]*Department of Geriatric Medicine, Karolinska Institute, Novum KFC, 141 86 Huddinge, Sweden*
[b]*Department of Clinical Genetics, Karolinska Hospital, 104 01 Stockholm, Sweden*
[c]*Department of Clinical Chemistry, Huddinge Hospital, 141 86 Huddinge, Sweden*
[d]*Athena Neurosciences Inc., 800F Gateway Boulevard, South San Francisco, CA 94080, USA*

Received 30 August 1994

Abstract Cell lines transfected with the Swedish Alzheimer's disease amyloid precursor protein APP670/671 mutation release significantly more β-amyloid than wild-type cells. Citron et al. [Proc. Natl. Acad. Sci. USA (1994) in press] have recently shown that fibroblasts carrying the APP670/671 mutation also release more β-amyloid than control cells [1]. The present study confirms a ca. threefold increase in β-amyloid release from mutation-bearing fibroblasts. APP mRNA levels did not differ between mutation-bearing and control cells, although mutation-bearing fibroblasts contained significantly more APP751/770 than controls. Mild stress decreased β-amyloid secretion and increased APP751/770 levels in all cell lines. In conclusion, the proportion of APP committed to amyloidogenic processing is increased in fibroblasts from family members with the APP670/671 mutation, and this mutation may also compromise the APP stress response.

Key words: Alzheimer's disease; Amyloid precursor protein; APP mRNA; β-Amyloid; Fibroblast; Foetal calf serum

1. Introduction

Alzheimer's disease is a progressive neurodegenerative disorder defined histopathologically by the excessive accumulation of extracellular proteinaceous deposits (amyloid plaques) and intraneuronal bundles of paired helical filaments (neurofibrillary tangles) throughout the hippocampus and neocortex. A 39–43 amino acid peptide, termed β-amyloid, has been identified as the major constituent of plaque and cerebrovascular amyloid [2]. This peptide is a metabolite of the amyloid precursor protein (APP), encoded by a gene on chromosome 21 [3]. The identification of pathogenic APP gene mutations in some Alzheimer's disease families has firmly established the importance of APP and β-amyloid in the aetiology of this disorder [4,5].

APP RNA is alternatively spliced in a tissue-specific manner, giving rise to at least ten different mRNA species. These include APP770 with domains showing homology to a Kunitz type protease inhibitor (KPI) and the MRC OX-2 antigen; APP751 which includes the KPI region only; and APP695 which lacks both the KPI and the MRC OX-2 domains [3,6–8].

Two alternative APP processing routes utilising the as yet unidentified enzymes, α- and β-secretase, have been identified. αSecretase cleaves APP at amino-acid 16 of the β-amyloid peptide, just outside the APP transmembrane domain [9,10]. Metabolism along this route results in the release of the amino terminal portion of APP and precludes the generation of full-length β-amyloid. The detection of soluble β-amyloid in the medium of normal cells in culture [11,12] and in the CSF of healthy individuals [13] revealed the presence of the β-secretase pathway operating in parallel to the non-amyloidogenic α-secretase processing. β-Secretase cleaves APP between Met-671 and Asp-672 (APP770 numbering) to generate a secreted amino terminal APP metabolite and leaving full-length β-amyloid in a membrane-associated carboxy-terminal fragment. The mechanisms of further processing of the carboxy-terminal fragment to produce extracellular β-amyloid are not clearly defined, but have been proposed to occur intracellularly in an acidic compartment, possibly in early endosomes [14,15].

We have identified a double mutation in the APP gene resulting in amino acid substitutions of Lys to Asn (codon 670) and Met to Leu (671) in a large Swedish family where Alzheimer's disease is inherited in an autosomal dominant manner [5,16]. Subsequently, it was shown that human kidney 293 and neuroblastoma M17 cell lines transfected with this mutation released approximately 7 times more β-amyloid into culture medium than their wild type counterparts [17,18]. Recently, Citron et al. have shown that β-amyloid release is increased in fibroblast cell lines from Swedish APP670/671 mutation carriers, compared to control family members [1].

In the present study, we quantified β-amyloid release from primary fibroblast cell lines established from heterozygous APP670/671 mutation carriers and control family members using a sensitive ELISA assay [13]. In addition, we examined the influence of this mutation on APP mRNA and protein levels in these cell lines. The effects of a mild stress, in the form of a 20 h serum deprivation, on cellular APP levels and β-amyloid release was also investigated.

*Corresponding author. Fax: (46) (8) 746 5235.

***Current address:* Department of Biochemistry, University College, Cork, Ireland. Fax: (353) (21) 274 034.

SSDI 0014-5793(94)01137-0

2. Materials and methods

2.1. Cell culture

Fibroblast cell lines were established from upper arm skin biopsies taken from 12 informed family members. Cells were grown in two groups with three mutation-bearing and three control lines in each group. The cell lines were age matched, mean age ± S.E.M. being 55 ± 3.7 for the family members with the mutation and 59 ± 2.6 for the controls. Three of the family members with the mutation had developed symptoms of Alzheimer's disease, whereas the other three were asymptomatic.

Fibroblasts were grown in minimum essential medium with Earle's salts containing 10% heat-inactivated foetal calf serum (FCS), 2 mM L-glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin and 60 μg/ml Tylocine (Anti-PPLO agent) (Gibco-BRL, Life Technologies, European Division). Cells were passaged between 6 and 9 times and grown to near confluency before harvesting one 75 cm^2 flask for APP immunoblotting and four 75 cm^2 flasks for RNA assay.

The cells for RNA assay were maintained in medium containing 10% FCS throughout, harvested by trypsination and centrifuged at 400 × *g* for 15 min at room temperature. The pellet was snap frozen to −135°C and stored at −70°C.

Prior to harvesting for APP immunoblotting, cells were washed in medium either with or without 10% FCS, and incubated in this medium for 20 h. The conditioned media was stored at −20°C prior to assay for β-amyloid. Fibroblasts were harvested by washing twice with ice-cold Ca^{2+} and Mg^{2+} free PBS (Gibco-BRL), and scraping with a rubber policeman in 1.5 ml of the same buffer. Cells were collected by centrifugation at 600 × *g* for 15 min at 4°C and lysates prepared by adding 200 μl of 40 mM Tris-HCl, 2% SDS, pH 6.8 to the pellet and sonicating for 12 s (amplitude 22 microns). The lysates were boiled for 3 min, and stored frozen at −20°C prior to APP immunoblotting. Protein concentration was determined using the Pierce BCA protein assay kit (Pierce, USA).

2.2. β-Amyloid assay

β-Amyloid concentrations in conditioned media (15 ml per 75 cm^2 flask) were determined by enzyme-linked immunoassay (ELISA) employing the 266 and 6C6 monoclonal antibodies directed against amino acids 13 to 28 and 1 to 16 of the β-amyloid peptide. The medium, which had been in contact with the cells for 20 h, was concentrated 10-fold by freeze-drying before assay as previously described [13].

2.3. RNA assay

RNA was quantified using a sensitive RNA–RNA solution hybridisation assay [19,20] adapted for APP RNA (Johnston et al, in preparation). Briefly, a *Bam*HI (position 99) to *Kpn*I (position 107) fragment spanning the exon 1/2 boundary was digested from the cDNA pUC12-APP695 and inserted into a pGEM3zf$^+$ vector (Promega). Radiolabelled ([^{35}S]CTP, Amersham) anti-sense APP RNA probe and sense APP RNA standard were transcribed in vitro using T7 or SP6 RNA polymerase (Riboprobe Gemini II system, Promega). This probe hybridises to all reported APP RNA species. Hybridisation of probe with sense RNA enabled construction of a standard curve. Total nucleic acid (TNA) was prepared from the fibroblasts. TNA and DNA concentrations were determined spectrophotometrically and using Hoechst 33258 fluorimetry, respectively [21]. Sense RNA or TNA extract were hybridised in solution with excess probe for 18 h at 68°C with 25% formamide, 0.75 mM DTT, 0.6 M NaCl, 20 mM Tris and 4 mM EDTA. Non-hybridised RNA was then degraded by the addition of RNase T1 (2 μg/ml), RNase A (40 μg/ml) and salmon sperm DNA (100 μg/ml). Ribonuclease-resistant hybrids were precipitated with 10% TCA, captured on Whatman GF/C filters, washed twice with 4% TCA in 1% sodium pyrophosphate, once with 95% ethanol and quantified by scintillation counting. The equivalent amount of standard APP RNA (pg of 166 nucleotide transcripts) was calculated by comparison with the standard curve, and results expressed as pg APP RNA per μg RNA in the sample.

2.4. Immunoblotting

Three μg of protein from each sample of fibroblast cell lysate was separated by 8% SDS-PAGE [22]. Proteins were transferred electrophoretically (200 V, 2 h) to nitrocellulose membranes (Schleicher and Schuell, 0.45 μm) [23]. Non-specific binding sites were blocked by incubating blots for 1 h in 20 mM Tris-HCl (pH 7.6), 137 mM NaCl, and 0.1% Tween 20 (TBS-T) with 5% non-fat dried milk. Blots were rinsed in TBS-T prior to incubation for 3 h at room temperature with the monoclonal antibody 22C11 (1:10,000 dilution), raised against full-length APP695 [24]. Blots were rinsed again prior to the addition of the secondary antibody, HRP-linked anti-mouse IgG (1:2000 dilution) for 1 h, and rinsed thoroughly overnight before detection of immunoreactivity using the enhanced chemiluminescence (ECL) detection system (Amersham, UK), followed by exposure to Hyperfilm MP (Amersham, UK). The cell lysates were run in two different ways. Firstly, cell lysates from family members with and without the mutation were age matched and run in adjacent lanes. Secondly, the same cell line maintained in the presence or absence of serum was run in adjacent lanes. Each cell lysate was run at least three times. Levels of APP immunoreactivity were quantified by densitometric analysis of the autoradiograms using an IBAS image analysis system (Zeiss). The data was expressed as absolute mean grey density values obtained from the analysis of three separate autoradiograms.

3. Results

Fig. 1 shows the β-amyloid levels detected in conditioned media. Medium conditioned by mutation-bearing fibroblasts contained ca. 2.7 times more β-amyloid than medium conditioned by control cells in the presence of serum, and ca. 3.5 times more in the absence of serum. There was no difference in the β-amyloid levels in mutation-bearing cells derived from individuals who had developed symptoms of Alzheimer's disease and those who were under the age of disease onset. Interestingly, Fig. 1 also shows that the levels of β-amyloid detected in serum free medium were approximately 50% of those detected in the presence of serum.

There was no significant difference in the level of APP RNA in mutation-bearing and control fibroblasts. The level of APP RNA, expressed as equivalent pg standard APP RNA/μg total

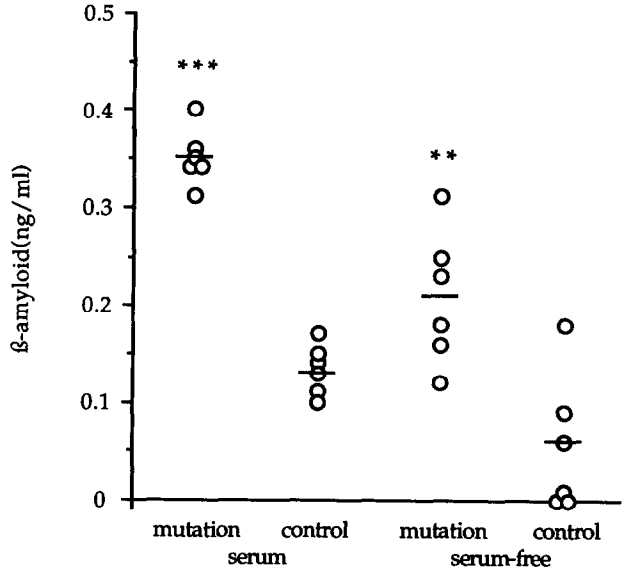

Fig. 1. β-Amyloid levels detected by ELISA of concentrated conditioned media from mutation-bearing and control cell lines maintained in the presence and absence of 10% foetal calf serum. Mean values are represented by a horizontal bar. Significance levels are shown for the comparison of mutation bearing and control cells, ***$P < 0.001$ **$P < 0.01$ (Student's *t*-test). The levels of β-amyloid were significantly lower in serum-free compared to serum-containing media for both mutation bearing and control cells, ($P < 0.05$, paired *t*-test).

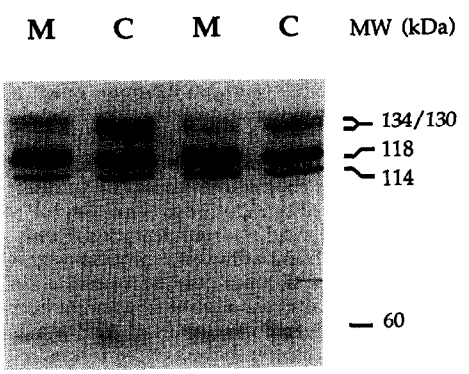

Fig. 2. Representative autoradiogram of cell lysates from fibroblasts maintained in the absence of serum for 20 h prior to harvesting. Mutation bearing (M) and control (C) cells are shown. 3 μg of protein from each cell line was separated by 8% SDS-PAGE and probed with the monoclonal antibody 22C11 as described in section 2. Molecular weights were determined by comparison with a pre-stained molecular weight standard mixture (Sigma SDS-7B).

RNA in the sample, was 0.92 ± 0.11 (mean ± S.E.M.) in the mutation-bearing fibroblasts and 0.81 ± 0.14 in the controls.

Figs. 2 and 3 show representative immunoblots of control and APP670/671 mutation-bearing cell lysates. The 22C11 antiserum recognised immunoreactive bands with relative mobilities corresponding to 134 kDa, 130 kDa, 118 kDa, 114 kDa, and a lower molecular weight band of 60 kDa. The most prominent bands were those of 118 and 114 kDa, which are considered to represent comigratory full-length N-glycosylated APP751/APP770 and APP695, respectively, with the fainter higher molecular weight bands representing the N- and O-glycosylated forms of these APP isoforms [24,25]. The 60 kDa band may be a shorter APP fragment. Densitometric analysis was performed on the 118 kDa and 114 kDa isoforms only, due to the lightness of the 134 and 130 kDa bands. Analysis of the autoradiogram shown in Fig. 2 revealed that the fibroblasts contained approximately three times more APP751/770 than APP695, although the APP751/770 band was dark, and at the limits of linearity for densitometry. Further results were therefore generated by analysing the bands on separate autoradiograms with different exposure times so that both would be within the linear range for densitometry. Fig. 3 shows an autoradiogram with a shorter exposure time, used to analyse the APP751/770 band.

The data obtained from the densitometric analyses is presented in Table 1. These results revealed that in the presence of serum, mutation bearing cell lines contained significantly (ca. 30%) higher levels of APP751/770 than controls ($P < 0.05$, Student's t-test). Following a 20 h serum deprivation, APP751/770 levels increased in both the mutation-bearing and control cell lines. This increase was statistically significant in the control cells where levels were ca. 30% higher in the absence of serum ($P < 0.05$, paired t-test). In contrast, the mutation-bearing cell lines had only 17% higher levels of APP751/770 after serum deprivation ($0.05 < P < 0.1$, paired t-test). Consequently, the mutation-related difference in APP751/770 levels was less pronounced (ca. 18%) in the absence of serum ($0.05 < P < 0.1$, Student's t-test).

APP695 levels in these fibroblasts were not significantly affected by the presence of the mutation, or serum deprivation (Table 1). However, some trends similar to those for APP751/770 were seen, as mutation-bearing cell lines contained ca. 15% more APP695 than controls in the presence of serum. Serum deprivation resulted in a ca. 16% increase in APP695 levels in control cell lines, and this response was absent in mutation-bearing cells.

4. Discussion

This study shows that fibroblasts derived from family members with the Swedish Alzheimer's disease APP670/671 mutation released approximately three times more β-amyloid into the culture medium than control fibroblasts. Recent cell transfection studies have shown that this mutation increases the proportion of APP committed to amyloidogenic processing [17,18]. The Met to Leu conversion at codon 671 was sufficient to increase β-amyloid release, strongly implying that the proximity of this mutation to the amino terminus of the β-amyloid peptide renders this cleavage site more susceptible to β-secretase activity [17]. A recent investigation of β-amyloid release from mutation-bearing fibroblast cell lines using metabolic labelling and immunoprecipitation techniques detected an increased β-amyloid release from these more physiological cell lines [1]. These findings, and those of the present study, provide strong evidence that increased β-amyloid release is the underlying disease mechanism in the Swedish APP670/671 family, as initially suggested by the transfection studies [17,18].

Interestingly, we also found that the β-amyloid release from both mutation-bearing and control cells was reduced by ca. 50% when cells were grown in serum free medium. Decreased β-amyloid release from M17 neuroblastoma and human kidney 293 cells following serum deprivation has recently been noted by other investigators [26,27].

The present study also identified a difference between APP751/770, but not APP695, levels in cell lysates of mutation-bearing, compared to control cell lines. Mutation bearing cells contained modestly (ca. 30%), but nonetheless significantly higher APP751/770 levels than control cells in the presence of serum. However, quantification of total APP RNA under these conditions did not reveal a corresponding increase in mutation bearing cells. Although we cannot disregard the possibility of altered RNA splicing in favour of the APP751/770 isoforms, this seems unlikely since no corresponding decrease in the level of APP695 (as detected by immunoblotting) was evident. The RNA data thus suggest that the difference in protein levels is due to altered protein translation or processing. The mutation-

Table 1
Levels of APP751/770 and APP695 in mutation-bearing and control cell lines maintained in the presence and absence of 10% foetal calf serum

	Mutation bearing cells		Control cell lines	
	Serum	Serum-free	Serum	Serum free
APP 751/770	83.3 ± 1.5	97.3 ± 6.1^{+}	63.5 ± 7.0	82.3 ± 4.6*
APP 695	28.2 ± 4.5	26.7 ± 3.3	24.5 ± 3.7	28.3 ± 2.9

Data is expressed as mean optical density values ± S.E.M. from 3 individual experiments performed as described in section 2. Significance levels are shown for the comparison of APP levels in cells maintained in serum-containing and serum-free media, $^{*}P < 0.05$, $^{+}0.1 > P > 0.05$ (paired t-test). A significant difference was also seen for the comparison of APP751/770 levels in mutation-bearing and control cell lines maintained in serum-containing medium ($P < 0.05$, Student's t-test).

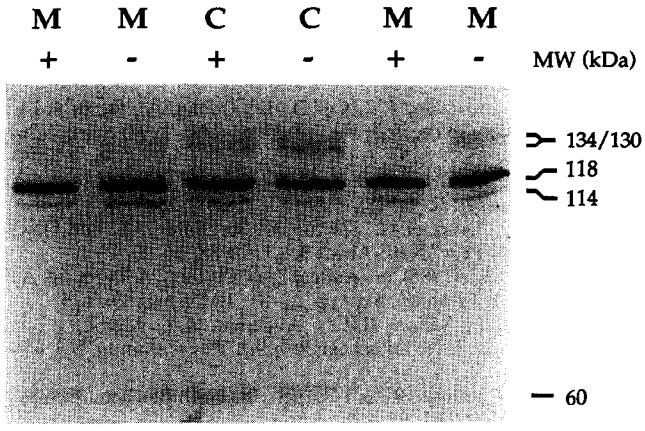

Fig. 3. Representative autoradiograms of fibroblast lysates prepared from mutation-bearing (M) and control (C) cells maintained in the presence (+) or absence (−) of serum for 20 h prior to harvesting. 3 μg protein from each cell line was separated by 8% SDS-PAGE and probed with the monoclonal antibody 22C11 as described in section 2. The molecular weights were determined by comparison with a pre-stained molecular weight standard mixture (Sigma SDS-7B).

related increase in β-amyloid release may result in up-regulated APP translation in order to maintain physiological levels of full-length APP.

We were also interested in investigating the effect of stress on full-length APP levels in these cells, since the promoter region of APP contains a stress response element [28]. A mild stress in the form of 20 h serum deprivation resulted in increased APP751/770 levels in both the mutation bearing and control cell lines, whilst APP695 levels were not significantly affected. A modest 17% increase in the level of APP751/770 was seen in mutation bearing cell lines, but the response was more marked and statistically significant in the control fibroblasts, where a ca. 30% increase was observed. Thus in the absence of serum, the difference in APP751/770 levels between mutation-bearing and control cell lines was reduced to ca. 18% (compared to 30% in the presence of serum) and lost statistical significance. This physiological response to stress may therefore be compromised in mutation-bearing cells, perhaps due to the slightly elevated basal levels of APP.

A 72 h serum deprivation has previously been shown to increase expression of APP751/770 and 695 in fibroblasts [29]. Our results confirm that a shorter period of serum deprivation is adequate to elicit a similar APP751/770 response. APP levels may increase either as a general response to the stress presented by serum starvation, or as a more specific response to the removal of a component of serum. Foetal calf serum is a complex mixture of proteins, polypeptides and growth factors, hormones, lipids, amino acids and minerals [30]. Several growth factors such as nerve, epidermal, and fibroblast growth factor are capable of altering APP expression and release [31,32]. Further experiments involving isolation of the potential active ingredients in serum and investigation of their individual effects on these cells are required in order to explore more fully the mechanism behind the increased APP751/770 levels and decreased β-amyloid release in response to serum deprivation.

In summary, this study of primary fibroblast cell lines derived from individuals carrying the APP670/671 mutation provides strong evidence that increased β-amyloid release leads to the development of Alzheimer's disease in the Swedish APP670/671 family. Mutation-bearing fibroblasts derived from family members who were 5 to 10 years younger than the predicted age of onset released as much β-amyloid as cell lines from individuals who had developed symptoms of the disease, showing that this is an early event in the disease process. These fibroblast cell lines provide a useful model system to investigate mechanisms promoting accelerated amyloidosis and to screen for compounds capable of reducing β-amyloid production. These results also raise some crucial questions concerning the molecular basis of the selectivity of the disease symptoms, since peripheral cells are clearly also over-producing β-amyloid. The observation that levels of full-length APP and the response of these fibroblasts to stress may be altered by the presence of the APP670/671 mutation is also worthy of further investigation.

Acknowledgements: We thank Professor K. Beyreuther, Center for Molecular Biology, Heidelberg, for generously providing us with the mAb 22C11 and Professor N. Robakis, Mount Sinai Medical Center, New York, for the APP695 cDNA. We thank Karin Axelman for her geneological research and the family members for their co-operation. We are indebted to the Swedish Medical Research Council and the Gamla Tjänarinnor, Åke Wiberg, Goljes and Swedish Match Foundations for their generous financial support.

References

[1] Citron, M., Vigo-Pelfrey, C., Teplow, D.B., Miller, C., Schenk, D., Johnston, J., Winblad, B., Venizelos, N., Lannfelt, L. and Selkoe, D.J. (1994) Proc. Natl. Acad. Sci. USA, in press.
[2] Glenner, G.G. and Wong, C.W. (1984) Biochem. Biophys. Res. Commun. 122, 1131–1135.
[3] Kang, J., Lemaire, H.-G., Unterbeck, A., Salbaum, J.M., Masters, C.L., Grzeschik, K.-H., Multhaup, G., Beyreuther, K. and Müller-Hill, B. (1987) Nature 325, 733–736.
[4] Goate, A., Chartier-Harlin, M.-C., Mullan, M., Brown, J., Crawford, F., Fidani, L., Giuffra, L., Haynes, A., Irving, N., James, L., Mant, R., Newton, P., Rooke, K., Roques, P., Talbot, C., Pericak-Vance, M., Roses, A., Williamson, R., Rossor, M., Owen, M. and Hardy, J. (1991) Nature 349, 704–706.
[5] Mullan, M., Crawford, F., Axelman, K., Houlden, H., Lilius, L., Winblad, B. and Lannfelt, L. (1992) Nature Genet. 1, 345–347.
[6] Ponte, P., Gonzalez-DeWhitt, P., Schilling, J., Miller, J., Hsu, D., Greenberg, B., Davis, K., Wallace, W., Lieberburg, I., Fuller, F. and Cordell, B. (1988) Nature 331, 525–527.
[7] Tanzi, R.E., McClatchey, A.I., Lamperti, E.D., Villa-Komaroff, L., Gusella, J.F. and Neve, R.L. (1988) Nature 331, 528–530.
[8] Kitaguchi, N., Takahashi, Y., Tokushima, Y., Shiojiri, S. and Ito, H. (1988) Nature 331, 530–532.
[9] Esch, F.S., Keim, P.S., Beattie, E.C., Blacher, R.W., Culwell, A.R., Oltersdorf, T., McClure, D. and Ward, P.J. (1990) Science 248, 1122–1124.
[10] Anderson, J.P., Esch, F.S., Keim, P.S., Sambamurti, K., Lieberburg, I. and Robakis, N.K. (1991) Neurosci. Lett. 128, 126–128.
[11] Haass, C., Schlossmacher, M.G., Hung, A.Y., Vigo-Pelfrey, C., Mellon, A., Ostaszewski, B.L., Lieberburg, L., Koo, E.H., Schenk, D., Teplow, D. and Selkoe, D. (1992) Nature 359, 322–325.
[12] Shoji, M., Golde, T.E., Ghiso, J., Cheung, T.T., Estus, S., Shaffer, L.M., Cai, X.-D., McKay, D.M., Tintner, R., Frangione, B. and Younkin, S.G. (1992) Science 258, 126–129.
[13] Seubert, P., Vigo-Pelfrey, C., Esch, F., Lee, M., Dovey, H., Davis, D., Sinha, S., Schlossmacher, M., Whaley, J., Swindlehurst, C., McCormach, R., Wolfert, R., Selkoe, D., Lieberburg, I. and Schenk, D. (1992) Nature 359, 325–327.
[14] Haass, C., Hung, A., Schlossmacher, M.G., Teplow, D.B. and Selkoe, D. (1993) J. Biol. Chem. 268, 3021–3024.
[15] Seubert, P., Oltersdorf, T., Lee, M.G., Barbour, R., Blomquist, C., Davis, D.L., Bryant, K., Fritz, L.C., Galasko, D., Thal, L.J., Lieberburg, I. and Schenk, D.B. (1993) Nature 361, 260–263.

[16] Lannfelt, L., Bogdanovic, N., Appelgren, H., Axelman, K., Lilius, L., Hansson, G., Schenk, D., Hardy, J. and Winblad, B. (1994) Neurosci. Lett. 168, 254–256.
[17] Citron, M., Oltersdorf, T., Haass, C., McConlogue, L., Hung, A.Y., Seubert, P., Vigo-Pelfrey, C., Lieberburg, I. and Selkoe, D.J. (1992) Nature 360, 672–674.
[18] Cai, X.-D., Golde, T.E. and Younkin, S.G. (1993) Science 259, 514–516.
[19] Durnam, D.M. and Palmiter, R.D. (1983) Anal. Biochem. 131, 385–393.
[20] Norgren, S., Arner, P. and Luthman, H. (1994) J. Clin. Endocrinol. Metab. 78, 757–762.
[21] Labarca, C. and Paigen, K. (1980) Annal. Biochem. 102, 344–352.
[22] Laemmli, U.K. (1970) Nature 227, 680–685.
[23] Towbin, H., Staehlin, T. and Gordon, J. (1979) Proc. Natl. Acad. Sci. USA 76, 4350–4354.
[24] Weidemann, A., König, G., Bunke, D., Fischer, P., Salbaum, J.M., Masters, C.L. and Beyreuther, K. (1989) Cell 57, 115–126.
[25] Podlisny, M.B., Tolan, D.R. and Selkoe, D.J. (1991) Am. J. Pathol. 138, 1423–1435.
[26] Querfurth, H.W., Geiger, J.D. and Selkoe, D.J. (1994) Neurobiol. Aging 15 (Suppl.1), S53.
[27] Suzuki, N., Cheung, T.T., Cai, X.-D., Odaka, A., Otvos Jr., L., Eckman, C., Golde, T.E. and Younkin, S.G. (1994) Science 264, 1336–1340.
[28] Salbaum, J.M., Weidermann, A., Lemaire, H.-G., Masters, C.L. and Beyreuther, K. (1988) EMBO J. 7, 2807–2813.
[29] Adler, M.J., Coronel, C., Shelton, E., Seegmiller, J.E. and Dewji, N.N. (1991) Proc. Natl. Acad. Sci. USA 88, 16–20.
[30] Maurer, H.R. (1986) in: Animal Cell Culture, A Practical Approach (R.I. Freshney, Ed.) pp. 13–31, IRL Press, Oxford.
[31] Refolo, L.M., Salton, S.R.J., Anderson, J.P., Mehta, P. and Robakis, N.K. (1989) Biochem. Biophys. Res. Commun. 164, 664–670.
[32] Quon, D., Catalono, R. and Cordell, B. (1990) Biochem. Biophys. Res. Commun. 167, 96–102.