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Splice variants of the β-site APP-cleaving enzyme BACE1 in human brain and pancreas☆

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Abstract

BACE is the β-secretase responsible for the first step in amyloidogenic processing of the amyloid precursor protein APP. We have identified two BACE isoforms, BACE1B and BACE1C, lacking 25 and 44 amino acids, respectively. Whereas the BACE1B transcript is present in human pancreas and brain, the BACE1C transcript is found in pancreas only. In transfected cells both BACE1A, which encodes the originally described full-length BACE1 protein and the close homolog BACE2 localized mainly to post-Golgi membranes. In contrast, the two shorter isoforms were found in the endoplasmic reticulum only, and they did not display β-secretase activity. Using RNase protection we in addition show that the major pancreatic transcript is BACE1A. This suggests that the known absence of β-secretase activity in the pancreas is not due to a missing BACE1A transcript.

Keywords: Amyloid precursor protein; APP; Alzheimer; BACE isoforms; β-Secretase

A hallmark of Alzheimer's disease is the progressive formation in the brain of insoluble amyloid plaques containing β-amyloid (Aβ). Aβ is produced by proteolytic cleavage from the amyloid precursor protein (APP), a transmembrane protein with a large N-terminal ectodomain and a short C-terminal cytoplasmic tail [1]. During its intracellular transport APP can also be cleaved by an alternative pathway (α-cleavage) to release the nonamyloidogenic secreted ectodomain of APP (αAPPsec). Aβ generation occurs in two steps [2]. First, APP is cleaved in its luminal domain (β-cleavage) to leave behind a C-terminal 10 kDa fragment. This C-terminal stub subsequently is the substrate for γ-secretase, which cleaves within the transmembrane domain to release Aβ.

Two enzymes capable of β-cleavage, the so-called β-site APP-cleaving enzymes (BACE1, originally referred to as BACE; and BACE2), have been identified [3–9]. BACE1 mRNA is found at very high levels in pancreas, at moderate levels in brain, and at low levels in most peripheral tissues [5,6]. Interestingly, β-secretase activity is high in brain only, but almost undetectable in pancreas [4]. The homologous protein BACE2 has a more broad mRNA distribution. It is widely expressed in peripheral tissues, but only at low levels in brain [7,9,10]. While both enzymes are capable of promoting β-cleavage in vitro and in cell culture [3–6,8,11], data from BACE1 knockout mice indicate that BACE1 at least in the brain of mice is the enzyme responsible for β-cleavage of APP in vivo [12,13].

Recently, a shorter transcript of BACE1 (BACE457) has been found in pancreas, but not in brain [14]. However, the same transcript (BACE-I-457), together with two more (BACE-I-476 and BACE-I-432), now has also been cloned from human brain [15]. While the BACE457 product was shown to be localized to the endoplasmic reticulum (ER) and to be devoid of β-sec-

☆ *Abbreviations:* APP, amyloid precursor protein; αAPPsec, α-cleaved ectodomain of APP; Aβ, β-amyloid fragment of APP; BACE, β-site APP-cleaving enzyme; βAPPsec, β-cleaved ectodomain of APP; CFP, cyan color variant of GFP; ER, endoplasmic reticulum; GFP, green fluorescent protein; YFP, yellow color variant of GFP.

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retase activity, BACE-I-457 and BACE-I-476 were shown to have a detectable, but reduced β-secretase activity. Based on their observation Bodendorf and colleagues [14] suggested that tissue-specific splicing of the BACE1 mRNA might be responsible for the lack of β-secretase activity in the pancreas.

In this paper we report the independent identification of two of these shorter BACE1 isoforms. Their transcripts are present in brain and pancreas. The respective proteins localize to the ER and have no detectable β-secretase activity. Using RNase protection we show that the major pancreatic transcript is the full-length BACE1, suggesting that the absence of β-secretase activity in the pancreas is not due to the presence of shorter transcripts.

Materials and methods

cDNA cloning of BACE1 and BACE2. Full-length BACE1A, BACE1B, and BACE2 were amplified by PCR from a human hippocampal QUICK-Clone cDNA library (Clontech; catalogue number 7169-1), and transferred by TA-cloning into the vector pGEMT (Promega). *Sal*I and *Not*I restriction sites were present in the primers at the 5′- and 3′-ends, respectively. To allow the subsequent addition of a CFP-tag to the 3′-end of BACE1 and BACE2, *Afl*II and *Cla*I sites, respectively, were added upstream of the stop codon. These mutations are silent at the protein level. Oligonucleotides used for BACE1 were 5′-AAGTCGACGCCA CCATGGCCCAAGCCCTGCCCTGG-3′ and 5′-AAGCGGCCGC ATTGATCACTTAAGCAGGGAGATGTCATCAGC-3′ (*Afl*II site underlined), for BACE2 5′-AAGTCGACGCCACCATGGGCGCAC TGGCCCGG-3′ and 5′-AAGCGGCCGCATTGATCATTTCCATC GATGTCTGACC-3′ (*Cla*I site underlined). All products were confirmed by DNA sequencing. CFP-tags were added as follows. CFP containing a short N-terminal spacer was amplified by PCR from VSVG3-SP-CFP [16]. Oligonucleotides used for the BACE1 fusions were 5′-TGCTTAAGGGTCTTGGAAAGGATCTCCCC-3′ and 5′AG AGTCGCGGCCGCTTTA-3′, for the BACE2 fusion 5′-TGATCGAT CAGGTCTTGGAAAGGATCTCCCC-3′ and 5′-AGAGTCGCGGC GCTTTA-3′. Those products were transferred as *Afl*II–*Not*I and *Cla*I–*Not*I fragments to the 3′-ends of BACE1 and BACE2, respectively. BACE1-CFP and BACE2-CFP were subsequently transferred as *Sal*I–*Not*I fragments into the mammalian expression vector pShuttle-CMV [17].

BACE1A, BACE1B, and BACE1C were also amplified from human pancreas by reverse transcription-PCR with the RNeasy and One Step Kits (Qiagen) according to manufacturer's instructions with minor modifications. The primer 5′-GTCTCCTACTTGTGACCA-3′ was used for reverse transcription, while 5′-AAGGCCGGGGCCCA CCATGGCCCAA-3′, and 5′-TCTGCCCATGGGCCTCCTCACTT CA-3′ were employed as sense and antisense amplification primers, respectively.

Cell culture and transient transfection. Potoroo kidney PtK_2 cells were grown in MEM, 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine, 1× nonessential amino acids, and 10% FCS (complete medium). To acquire fluorescent images cells were grown on 11 mm coverslips in complete medium without phenol red. Mouse neuroblastoma N2a cells were grown in DMEM, 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine, and 10% FCS. Transient transfections using calcium phosphate precipitation were performed as described [18].

Subcellular localization. PtK_2 cells double transfected with YFP- and CFP-tagged markers were fixed 16 h post-transfection using 4% paraformaldehyde. To label endocytic structures PtK_2 cells were incubated for 3 h with 0.5 mg/ml Texas Red-dextran (Molecular Probes) in complete dye-free medium. Cells were washed once in dye-free medium, fixed, and used for microscopy. Images were acquired on an Olympus IX70 inverted microscope equipped with a polychrome II monochromator (TILL Photonics, Graefelfing, Germany), a custom filter block for simultaneous visualization of YFP, CFP, and Texas Red (AHF Analysentechnik, Tübingen, Germany), a 100× oil-immersion lens (NA 1.35, Olympus), and a 12-bit CCD digital IMAGO camera (0.134 μm/pixel; 2 × 2 binning) (TILL Photonics), controlled by TILLvisION v3.3 software (TILL Photonics).

Processing of APP. Cells grown in 3 cm dishes and transfected with either wtAPP695 alone or together with equal amounts of the different BACE constructs were metabolically labeled for 3 h with 100 μCi [^{35}S]methionine (Amersham). Subsequently, βAPPsec and Aβ were immunoprecipitated from the conditioned medium using antibodies ANGU and 70JE, respectively. Full-length APP was recovered from the cell lysate with antibody IP60. ANGU was raised in rabbits against the peptide CISEVKM (amino acids 591–596 of APP695; coupled to KLH) and recognizes specifically the β-cleaved wtAPP ectodomain, but neither the α-cleaved ectodomain nor the full-length form of wtAPP. 70JE was raised in rabbits against the peptide DAEFRHDSGYEC (amino acids 1–11 of Aβ; coupled to KLH) and recognizes Aβ. IP60 was raised in rabbits against the peptide CNPTYKFFEQMQN (amino acids 684–695 of APP695; coupled to KLH) and recognizes full-length APP. After separation on 10% polyacrylamide [19] or 10–20% Tricine (Invitrogen) gels, individual bands were quantitated using a Fujifilm BAS 1800II image plate reader and Science Lab 99 Image Gauge v3.3 software (Raytest Isotopenmessgeräte, Berlin, Germany).

RNase protection assay. BACE1A obtained from pancreas and transferred by TA cloning into pGEMT was digested with *Bsr*GI and *Nde*I, blunted with mung bean nuclease and religated to remove most of the sequence on the 3′-side. The new plasmid obtained (pGEMT-BACE1A-DEL1) was linearized with *Sex*AI and used to prepare a riboprobe with the SP6 in vitro transcription system (Promega) according to manufacturer's instructions, and the riboprobe was purified over an RNeasy minispin column (Qiagen, RNA clean-up protocol). The RNase protection assay was performed with the RPA III kit (Ambion) according to manufacturer's instructions with minor modifications. Briefly, 10 μg of total RNA from human pancreas (extracted with the RNeasy midi kit, Qiagen) was hybridized with the probe (54,000 cpm) overnight at 42 °C, and then digested with a 1:100 dilution of the RNase mix. Protected fragments were separated in a 0.75 mm thick and 40 cm long 5% polyacrylamide gel, and revealed on an X-ray film after 15 days of exposure at −80 °C with intensifying screen.

Results

While cloning BACE1 from a human hippocampal cDNA library and human exocrine pancreatic tissue we consistently observed two bands of similar size on agarose gels. Sequencing of a number of individually cloned PCR products revealed, in addition to the previously published BACE1 sequence [3–6,8], one and two shorter isoforms in hippocampus and pancreas, respectively (Fig. 1). We renamed BACE1 to BACE1A and called the two new isoforms BACE1B (GenBank AF338816) and BACE1C (GenBank AF338817). The BACE1B transcript was found both in the hippocampal library and in the pancreas, lacks at the protein level a stretch of 25 amino acids, and is identical with the recently found

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A  MAQALPWLLL WMGAGVLPAH GTQHGIRLPL RSGLGGAPLG LRLPRETDEE
B  MAQALPWLLL WMGAGVLPAH GTQHGIRLPL RSGLGGAPLG LRLPRETDEE
C  MAQALPWLLL WMGAGVLPAH GTQHGIRLPL RSGLGGAPLG LRLPRETDEE

A  PEEPGRRGSF VEMVDNLRGK SGQGYYVEMT VGSPPQTLNI LVDTGSSNFA
B  PEEPGRRGSF VEMVDNLRGK SGQGYYVEMT VGSPPQTLNI LVDTGSSNFA
C  PEEPGRRGSF VEMVDNLRGK SGQGYYVEMT VGSPPQTLNI LVDTGSSNFA

A  VGAAPHPFLH RYYQRQLSST YRDLRKGVYV PYTQGKWEGE LGTDLVSIPH
B  VGAAPHPFLH RYYQRQLSST YRDLRKGVYV PYTQGKWEGE LGTDLVSIPH
C  VGAAPHPFLH RYYQRQLSST YRDLRKGVYV PYTQGKWEGE LGTDL-----

A  GPNVTVRANI AAITESDKFF INGSNWEGIL GLAYAEIARP DDSLEPFFDS
B  GPNVTVRANI AAITESDKFF INGSNWEGIL GLAYAEIAR- ----------
C  ---------- ---------- ---------- ---------P DDSLEPFFDS

A  LVKQTHVPNL FSLQLCGAGF PLNQSEVLAS VGGSMIIGGI DHSLYTGSLW
B  ---------- ----LCGAGF PLNQSEVLAS VGGSMIIGGI DHSLYTGSLW
C  LVKQTHVPNL FSLQLCGAGF PLNQSEVLAS VGGSMIIGGI DHSLYTGSLW

A  YTPIRREWYY EVIIVRVEIN GQDLKMDCKE YNYDKSIVDS GTTNLRLPKK
B  YTPIRREWYY EVIIVRVEIN GQDLKMDCKE YNYDKSIVDS GTTNLRLPKK
C  YTPIRREWYY EVIIVRVEIN GQDLKMDCKE YNYDKSIVDS GTTNLRLPKK

A  VFEAAVKSIK AASSTEKFPD GFWLGEQLVC WQAGTTPWNI FPVISLYLMG
B  VFEAAVKSIK AASSTEKFPD GFWLGEQLVC WQAGTTPWNI FPVISLYLMG
C  VFEAAVKSIK AASSTEKFPD GFWLGEQLVC WQAGTTPWNI FPVISLYLMG

A  EVTNQSFRIT ILPQQYLRPV EDVATSQDDC YKFAISQSST GTVMGAVIME
B  EVTNQSFRIT ILPQQYLRPV EDVATSQDDC YKFAISQSST GTVMGAVIME
C  EVTNQSFRIT ILPQQYLRPV EDVATSQDDC YKFAISQSST GTVMGAVIME

A  GFYVVFDRAR KRIGFAVSAC HVHDEFRTAA VEGPFVTLDM EDCGYNIPQT
B  GFYVVFDRAR KRIGFAVSAC HVHDEFRTAA VEGPFVTLDM EDCGYNIPQT
C  GFYVVFDRAR KRIGFAVSAC HVHDEFRTAA VEGPFVTLDM EDCGYNIPQT

A  DESTLMTIAY VMAAICALFM LPLCLMVCQW RCLRCLRQQH DDFADDISLLK
B  DESTLMTIAY VMAAICALFM LPLCLMVCQW RCLRCLRQQH DDFADDISLLK
C  DESTLMTIAY VMAAICALFM LPLCLMVCQW RCLRCLRQQH DDFADDISLLK
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Fig. 1. Sequence comparison of BACE1 isoforms. Protein sequences were deduced from the corresponding cDNA clones. Stretches lacking in the individual isoforms are indicated by gaps (–). GenBank accession numbers for BACE1B and BACE1C are AF338816 and AF338817, respectively.

BACE-I-476 [15]. The BACE1C transcript was found in pancreas only. It is identical with the meanwhile published BACE457 [14] and BACE-I-457 [15], and lacks 44 amino acids including two *N*-glycosylation sites. Both deletions occur at the boundary between exon 3 and exon 4 and are immediately adjacent to each other. Since the flanking regions correspond to consensus splice sites, these two isoforms probably reflect alternative splice variants [15].

We then set out to study the subcellular localization of the three BACE1 isoforms as well as of BACE2. To this end we generated fusions of the BACE1 isoforms and BACE2 with the cyan color variant (CFP) of the green fluorescent protein GFP [20]. Since BACE1C has previously been reported to be localized to the ER [14], we first cotransfected the CFP-tagged BACEs with the ER marker sec61-YFP [21]. To get the best possible resolution we decided to use potoroo kidney PtK_2 cells because they are very flat (about 0.5 μm in the cell periphery) and therefore particularly easy to image at high resolution. Using these cells we have previously been able to do multicolor imaging of post-Golgi sorting and trafficking in live cells [16]. BACE1A-CFP did not colocalize with sec61-YFP, and instead was enriched perinuclearly, but was also found in vesicular structures throughout the cytoplasm and on the cell surface (Fig. 2A). BACE1B-CFP and BACE1C-CFP almost perfectly overlapped with sec61-YFP (Figs. 2B and C), suggesting they do not leave the ER. BACE2-CFP had a similar distribution as BACE1A-CFP, however, was more prominent on the cell surface (Fig. 2D and H). Next we

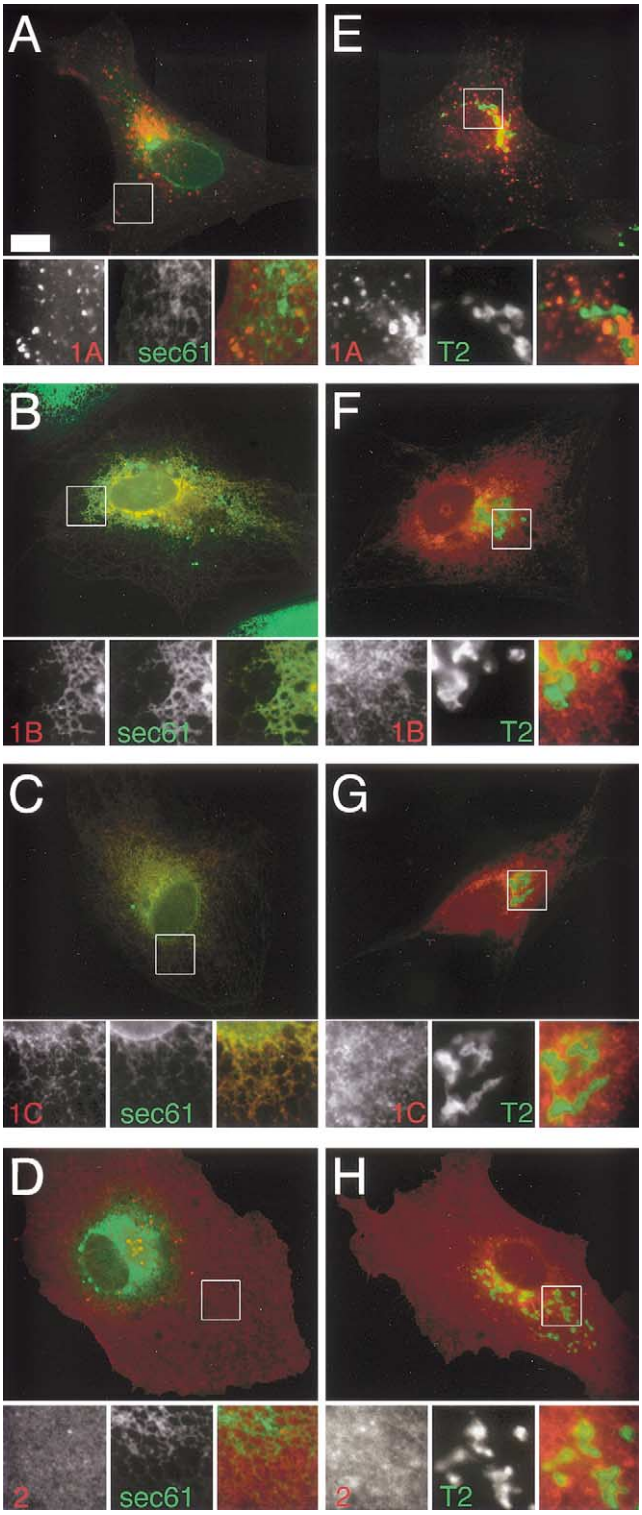

Fig. 2. BACE1B and BACE1C are retained in the endoplasmic reticulum. The subcellular localization of the different BACE1-CFP isoforms as well as of BACE2-CFP was analyzed in PtK_2 cells cotransfected with YFP-tagged ER (A–D; sec61-YFP) and Golgi markers (E–H; T2-YFP), respectively. (A, E) BACE1A. (B, F) BACE1B. (C, G) BACE1C. (D, H) BACE2. The boxed areas are enlarged 2.5-fold and show the individual channels as well as the merged images. Overlap is visible as yellow. Scale bar: 10 μm.

compared the CFP-tagged BACEs with the Golgi stack marker GalNAc-T2 (T2-YFP) [22]. As expected BACE1B-CFP and BACE1C-CFP did not colocalize (Fig. 2F and G), however, also the overlap with BACE1A-CFP and BACE2-CFP was minimal (Fig. 2E and H), suggesting that BACE1A and BACE2 were mainly present in post-Golgi structures. The patterns we observed were independent of the FP-tag because constructs containing a VSVG-tag behaved the same (data not shown).

Since PtK_2 cells are new for APP-related studies we have biochemically analyzed APP processing in these cells (Fig. 3B). PtK_2 cells express only very low amounts of APP (visible as two bands, which probably reflect different glycosylation forms), and when overexpressed APP is barely cleaved at the β-site. Upon longer exposure the β-cleaved APP ectodomain (βAPPsec) became apparent (data not shown), suggesting that PtK_2 cells possess very low levels of endogenous β-secretase. If APP, however, was coexpressed with BACE1A, then βAPPsec was easily detectable. This clearly shows that BACE1A is active when expressed in PtK_2 cells.

Fig. 3 also provides data on our newly generated antibodies. The localization of the epitopes is depicted in the scheme (Fig. 3A). Antibody IP60 specifically recognizes full-length APP (Fig. 3C), and shows the same specificity as the well-characterized antibody B10/4 [23]. Antibody 70JE pulls down a single band (Fig. 3D) that comigrates with Aβ immunoprecipitated by antibody B7/7 [23]. Antibody ANGU has been generated against the five amino acids immediately upstream of the β-cleavage site. It does not recognize full-length APP, but recognizes the neo-epitope generated in βAPPsec by β-cleavage (Fig. 3E). While antibody ANGU is specific for βAPPsec, the monoclonal antibody 6E10 pulls down only αAPPsec (Fig. 3F).

The acidic pH optimum of BACE1A and BACE2 [5,8,11] suggests that they are active in endosomes. We therefore looked for colocalization of the CFP-tagged BACEs with Texas Red-dextran taken up by endocytosis (Fig. 4). Perinuclear BACE1A-CFP only partially colocalized with Texas Red-dextran (Fig. 4A). Peripheral elements containing BACE1A-CFP more extensively overlapped with dextran, as reported previously for endocytosed transferrin [24]. This overlap may not be obvious from the large image since the ratio of the two probes varied strongly among these structures. The extent of overlap, however, becomes apparent when comparing the individual markers side by side (enlarged inset in Fig. 4A). BACE2-CFP colocalized particularly well with Texas Red-dextran in the perinuclear region (see, e.g., the inset in Fig. 4B), the overlap however was not complete. Overall, the majority of BACE1A appear to be intracellular (perinuclear plus vesicular elements throughout the cytoplasm) and only a minor fraction is present on the cell surface, consistent with published

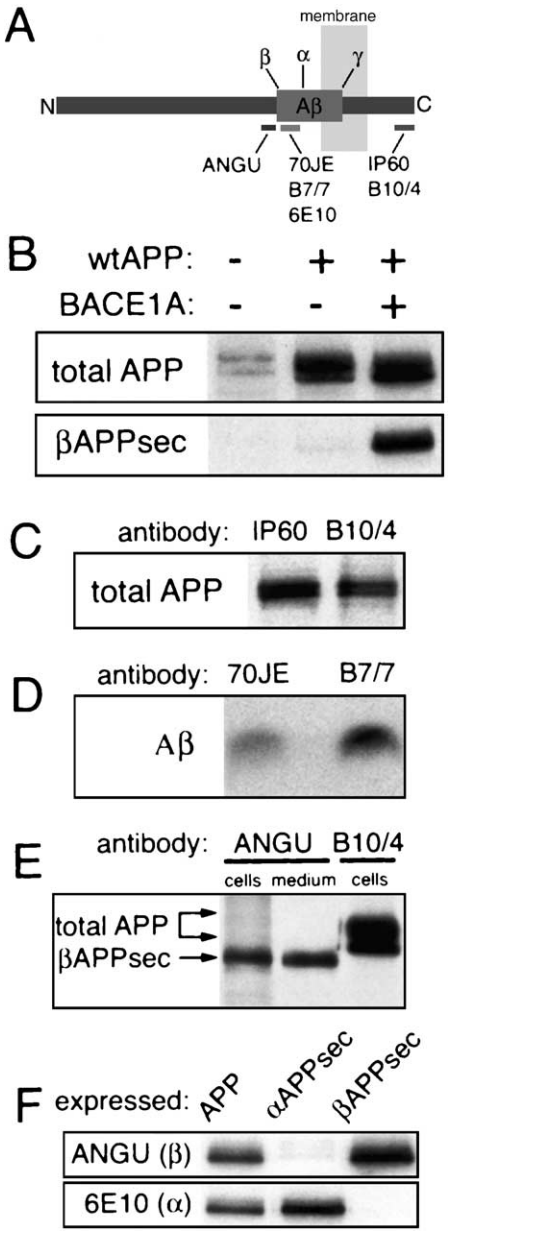

Fig. 3. Expression and processing of wtAPP in PtK_2 cells and characterization of antibodies. (A) The localization of the epitopes is depicted in the scheme. (B) PtK_2 cells expressing wtAPP only or wtAPP together with BACE1A were continuously labeled for 4 h. Full-length wtAPP and βAPPsec were immunoprecipitated from cell lysate and conditioned medium, respectively. (C–F) Antibody characterization. (C): IP60 recognizes the same bands as the published antibody B10/4, raised against the C-terminal 20 amino acids of APP [23]. (D) 70JE recognizes the same band as the published antibody B7/7, raised against synthetic human Aβ1-40 [23]. (E) ANGU does not recognize full-length wtAPP (B10/4), but precipitates a smaller band corresponding to βAPPsec from the conditioned medium. (F) Immunoprecipitation from N2a cells expressing full-length wtAPP or the secreted α- and β-cleaved wtAPP ectodomains (αAPPsec, βAPPsec). α- and β-cleaved ectodomains were recovered from the conditioned medium with antibodies 6E10 (Signet Laboratories, Dedham, MA) and ANGU, respectively.

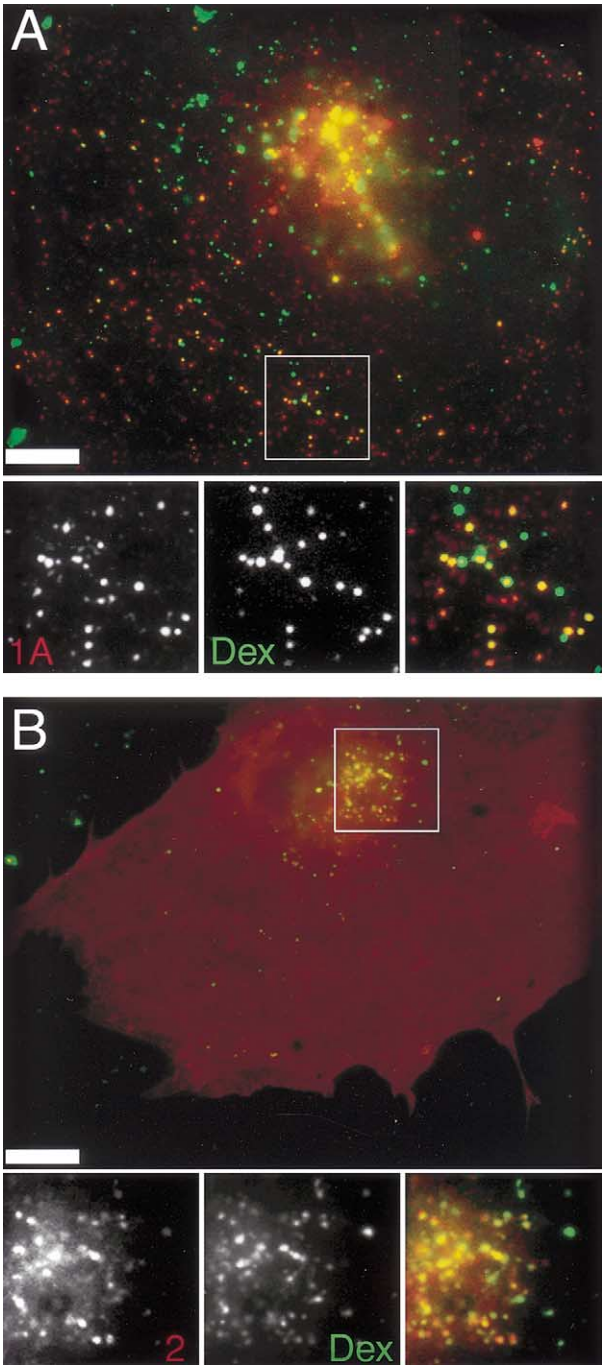

Fig. 4. BACE1A and BACE2 partially overlap with the endocytic marker Texas Red-dextran. PtK_2 cells expressing BACE1A-CFP (A) and BACE2-CFP (B), respectively, were continuously labeled for 3 h with Texas Red-dextran (Dex). After a brief wash, the cells were fixed and images were acquired. The boxed areas are enlarged 2-fold and show the individual channels as well as the merged images. Overlap is visible as yellow. *Scale bar*: 10 μm.

data [24]. BACE2 on the other hand is more prominent on the surface, and intracellular BACE2 is predominately perinuclear.

While BACE1C was reported to be inactive by Bodendorf et al. [14], it was found to have reduced but detectable activity by Tanahashi and Tabira [15]. The latter also showed BACE1B to be partially active. To address this issue we expressed human wild-type APP in mouse N2a cells either alone or together with the various CFP-tagged BACEs and checked for β-secretase activity (Fig. 5A). While BACE1A-CFP and BACE2-CFP led to a 5.4 and 2.7-fold increase of βAPPsec, respectively, the other two BACE1 isoforms had no significant effect on β-cleavage (Fig. 5B). BACE1A, but not BACE1B and BACE1C, also greatly increased the production of Aβ (Fig. 5C). Interestingly, overexpression of BACE2 led to a strong decrease of Aβ secretion in N2a cells (Fig. 5C). Since APP can be extensively processed at an alternative cleavage site in the middle of Aβ [11], the most likely

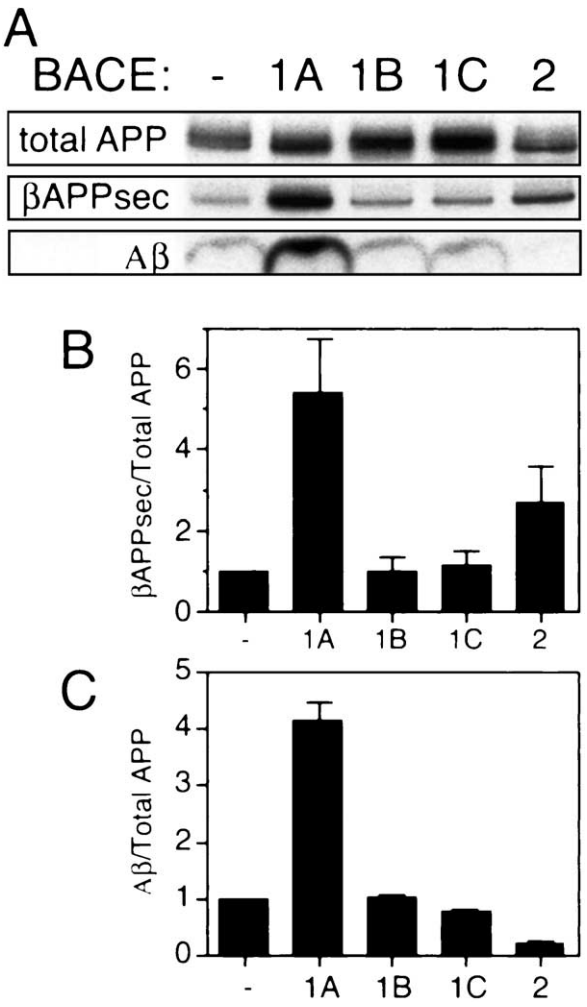

Fig. 5. Only BACE1A and BACE2, but not BACE1B and BACE1C, have β-secretase activity. N2a cells transfected with wtAPP alone or wtAPP together with the different BACE forms were metabolically labeled for 3 h. (A) Full-length APP, βAPPsec, and Aβ immunoprecipitated from cell lysate and conditioned medium, respectively. (B, C) Quantitation of βAPPsec (five independent experiments) and Aβ (two independent experiments), respectively. The values are normalized to the total amount of APP synthesized. The ratio has been arbitrarily set to one in cells transfected with APP only.

explanation for our observation is that this alternative cleavage site is prevalent in N2a cells. Although our antibody would pull down this Aβ1-19/20 fragment [11], we were not be able to detect it by autoradiography because of the absence of methionine residues in it.

The previously published data on BACE1 transcripts in tissue samples did not distinguish between the different splice variants. We therefore performed an RNase protection assay on total RNA from human pancreas (Fig. 6). The probe (Fig. 6A, 339 nucleotides) was designed to protect a fragment of 295 nucleotides in the BACE1A transcript. The presence of BACE1B was revealed by two fragments of 131 and 89 nucleotides, and BACE1C by a single fragment of 164 nucleotides (Fig. 6B). An additional BACE1 isoform [tentatively called BACE1D; identical to BACE-I-432 of Tanahashi and Tabira [15]] lacking both alternatively spliced segments would produce a single fragment of 89 nucleotides and would not be distinguishable from BACE1B. Qualitative intensity analysis of the protected fragments clearly showed that the BACE1A mRNA was present in human pancreas (Fig. 6C). Its amount was significantly higher as compared with the other isoforms.

Discussion

In this paper we document the identification and further characterization of two splice variants of the β-secretase BACE1. During the preparation of this manuscript two other groups independently reported the identification of the same BACE1 isoforms [14,15]. Although all sequences were identical we found a number of discrepancies between the recently published data and our results concerning tissue distribution of the transcripts and β-secretase activity of the respective proteins.

Do BACE1B and BACE1C have β-secretase activity? From our data it seems that they are inactive under physiological conditions. These results are supported by Bodendorf et al. [14], but are contradicted by Tanahashi and Tabira [15] who found significant activity especially in the case of BACE1C. We are currently not able to explain these differences. Although we transfected a different cell line (N2a) to measure the activity this clearly cannot explain the discrepancy, because both other groups used HEK 293 cells. The pH within the lumen of the ER, where BACE1B and BACE1C were

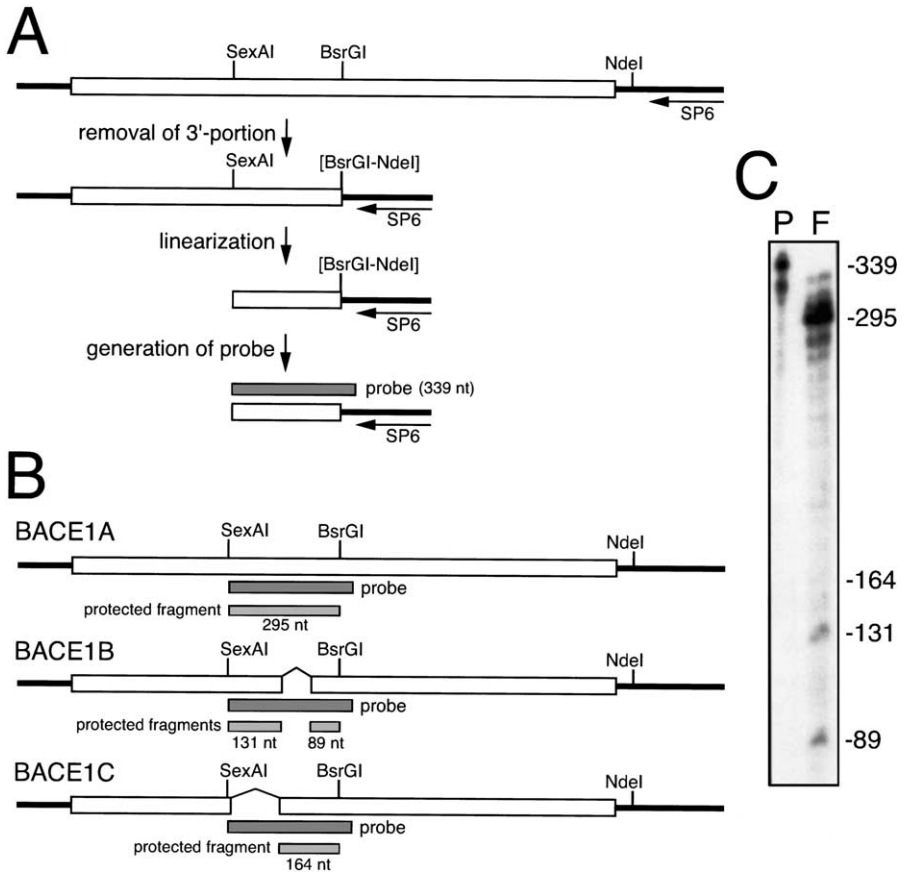

Fig. 6. BACE1A is the major transcript in human pancreas. (A) Scheme of how the riboprobe was generated (see also Materials and methods). (B) Fragments expected to arise from BACE1A, BACE1B, and BACE1C in the RNase protection assay. (C) Lane P shows the probe used (diluted 1:20) and lane F the protected fragments after RNase digestion. The 295 nucleotide fragment arises from the BACE1A transcript. The presence of the BACE1B transcript is revealed by the 131 and 89 nucleotide fragments, and BACE1C by the 164 nucleotide fragment.

found, is far from the in vitro determined acidic pH optimum of BACE1A [5,8,11]. At the moment we cannot exclude residual activity of BACE1B and BACE1C under such acidic conditions. In vitro experiments will have to be performed to give a definite answer to that question. Since the fluorogenic peptide substrates available [25] are also cleaved by a number of other proteases present in mammalian cells (our own unpublished observation) purification of the different BACE1 isoforms will be necessary. This then should also allow to address whether BACE1B and BACE1C have a pathophysiological relevance. At present this is unclear but, in case they have residual activity, they might contribute to the reported production of Aβ42 in the ER [26–28].

We have extended the previous reports [14,15] by a detailed analysis of the subcellular localization of BACE1B and BACE1C. Using FP-tagged fusion proteins we have shown that BACE1B and BACE1C localized to the ER, while BACE1A and BACE2 were found in post-Golgi compartments. Why do they localize to the ER? It is possible that the short deletions of 25 and 44 amino acids, respectively, cause misfolding and thus retention. On the other hand they might be properly folded ER resident BACE1 isoforms. During the course of this study we have observed retention in the ER of BACE1A containing a number of single point mutations caused by PCR errors. A construct containing the mutations P54H, A183T, Y184H, A333T, L346P, and L455F (numbering of pre-pro-BACE1A) was efficiently retained in the ER. This suggests that BACE1A might be very sensitive to small structural changes. While P54H and L455F probably represent true PCR errors all the other point mutations were also found when we sequenced the partial human brain BACE1 cDNA clone KIAA1149 (GenBank AB032975; note that the deposited sequence does not report these mutations) generously provided by the Kazusa DNA Research Institute. The latter therefore may represent mutations naturally occurring in the human population.

Finally, we also found discrepancies with respect to the tissue distribution of the various transcripts. As reported by Bodendorf and colleagues [14] we found the BACE1C transcript in the pancreas only. Tanahashi and Tabira [15] on the other hand also found it in the brain. Human pancreas has previously been shown to contain large amounts of BACE1 mRNA, but only very low β-secretase activity [4]. Being unable to detect the BACE1A transcript in the pancreas, and showing BACE1C expression both at the mRNA and protein levels, Bodendorf et al. [14] hypothesized that the lack of β-secretase activity might be due to BACE1C being the major form expressed in the pancreas. Our data on RNase protection however clearly show that BACE1A mRNA is the predominant transcript in the pancreas. Tanahashi and Tabira [15], who by their reverse transcription-PCR approach clearly could detect the BACE1A transcript in the pancreas, support these data. Our findings thus suggest that the regulation of β-secretase activity in the pancreas takes place at a post-transcriptional level. Where exactly this happens is currently explored in our laboratories.

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