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The novel transmembrane aspartic protease BACE (for Beta-site APP Cleaving Enzyme) is the β-secretase that cleaves amyloid precursor protein to initiate β-amyloid formation. As such, BACE is a prime therapeutic target for the treatment of Alzheimer's disease. BACE, like other aspartic proteases, has a propeptide domain that is removed to form the mature enzyme. BACE propeptide cleavage occurs at the sequence RLPR ↓ E, a potential furin recognition motif. Here, we explore the role of furin in BACE propeptide domain processing. BACE propeptide cleavage in cells does not appear to be autocatalytic, since an inactive D93A mutant of BACE is still cleaved appropriately. BACE and furin co-localize within the Golgi apparatus, and propeptide cleavage is inhibited by brefeldin A and monensin, drugs that disrupt trafficking through the Golgi. Treatment of cells with the calcium ionophore A23187, leading to inhibition of calcium-dependent proteases including furin, or transfection with the α2-antitrypsin variant α2-PDX, a potent furin inhibitor, dramatically reduces cleavage of the BACE propeptide. Moreover, the BACE propeptide is not processed in the furin-deficient LoVo cell line; however, processing is restored upon furin transfection. Finally, in vitro digestion of recombinant soluble BACE with recombinant furin results in complete cleavage only at the established E46 site. Taken together, our results strongly suggest that furin, or a furin-like proprotein convertase, is responsible for cleaving the BACE propeptide domain to form the mature enzyme.

At the histopathological level, AD is characterized by neurofibrillary tangles and amyloid plaques throughout the parenchyma of the brain, as well as amyloid deposits in the cerebral vasculature (reviewed in Ref. 1). Amyloid plaques are composed of Aβ, a 40–42-amino acid peptide that varies in length at the C terminus (2). Many studies have suggested that Aβ, especially the more fibrillogenic Aβ42, is central to the pathogenesis of AD (3). Aβ is formed by the endoproteolysis of APP, a large type I transmembrane protein (4). Two proteolytic cleavages of APP are required to make Aβ. First, a protease termed β-secretase cleaves APP at the N terminus of the Aβ domain to generate the soluble ectodomain APPsβ and the membrane-bound fragment C99 (see Fig. 1 of Ref. 5). A second protease, γ-secretase, then cuts C99 within the transmembrane region to form Aβ, which is secreted from the cell. Inhibition of the β- and γ-secretases would limit the production of Aβ, widely considered to be a beneficial therapeutic goal for the treatment of AD.

The identities of the secretases have been elusive. Presenilins are required for γ-secretase activity (6, 7), and therefore it has not been directly shown that purified presenilins have γ-secretase activity. Recently, we (5) have identified the novel transmembrane aspartic protease BACE as the major β-secretase involved in APP processing, a conclusion subsequently confirmed by other groups (9–12).

Like other aspartic proteases, BACE is synthesized as a precursor protein containing an N-terminal propeptide domain that is removed during maturation of the enzyme (see Fig. 1A). Typically, removal of the propeptide domain is necessary for the activation of an aspartic protease (reviewed in Ref. 13). Mature BACE starts at residue Glu-46 as the result of propeptide domain processing (5, 10, 11), and cleavage appears to occur constitutively in a post-ER intracellular compartment, most likely the Golgi apparatus (14). Glu-46 is immediately preceded by the sequence RLPR, which appears to be a proprotein convertase (PC) recognition motif of the general sequence R/KXn(R/K), where n = 0, 2, 4, or 6. The PCs are a family of calcium-dependent serine proteases consisting of seven members as follows: furin, PC1/PC3, PC2, PC4, PACE4, PC5/PC6, and PC7 (reviewed in Ref. 15). Understanding the regulation of BACE propeptide cleavage and identifying the putative BACE propeptide cleaving enzyme may have important therapeutic implications.

Furin is a major PC in the constitutive secretory pathway and is involved in the processing of a wide variety of precursor proteins including serum proteins, growth factors, receptors, extracellular matrix proteins, bacterial toxins, and viral coat proteins (reviewed in Ref. 16). Like BACE, furin is ubiquitously expressed and has an intracellular localization within the Golgi, TGN, and endosomes. The sequence RLPR ↓ E at the propeptide cleavage site of BACE is a potential furin recognition motif; therefore, we have investigated whether furin may be the protease that cleaves the BACE propeptide domain. Here, we present evidence that furin, or a furin-like PC, mediates BACE propeptide cleavage.

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EXPERIMENTAL PROCEDURES

Cell Culture and Transfections—293T cells were maintained in 10% fetal bovine serum in DMEM, high glucose media (Life Technologies, Inc.). LoVo cells were grown in 10% fetal bovine serum in DMEM/F-12 1:1 media (Life Technologies, Inc.). 293T cells were co-transfected with either pCMVi-Sol.BACE-HA or pCMVi-BACE-HA and pRSV-Puro by the calcium phosphate method (17). After selection in 5 \( \mu \)g/ml puromycin, clones for each cell line were selected and named Sol.BACE-HA/293 and BACE-HA/293, respectively. Transient transfections of 293T cells were performed using DMRIE-C (Life Technologies, Inc.) or Lipo-fectAMINE 2000 (Life Technologies, Inc.) for LoVo cells.

Plasmids—All cDNAs were cloned into the expression vector pCMVi (Cellular & Molecular Technologies Inc.). The BACE-HA construct has been previously described (5). A soluble form of BACE was made by truncating full-length BACE at Tyr-460 and fusing the 11-residue HA epitope tag (YPYDVPDYASG) in frame at the C terminus of the molecule to create Sol.BACE-HA.

\( \alpha \)-1-Antitrypsin was cloned by PCR from human liver cDNA using the primers CCATCGATGGACAGTGAATCGACAATG and GAAGATCTGGTTGAGGAGCGAGAGGCAG. The \( \alpha \)-1-antitrypsin PDX variant (18) was generated by PCR using the following primers: CCATCGATGGACAGTGAATCGACAATG and ACCTCGGGGGAGGACCTGGGTATGCGCTCTAAAAAC. The mutated fragment was then re-cloned into \( \alpha \)-1-antitrypsin as a ClaI-AvaI fragment to create \( \alpha \)-1-PDX.

BACE D93A Mutagenesis and Radiosequencing—The D93A BACE-HA mutant was generated by a single nucleotide change of A to G at position 278 of the cDNA creating an aspartic acid to alanine substitution at position 93 of the peptide sequence by two-step PCR using the following primers: GTGCCGATGTAGCGGGCTCCGGA, CTGCTGCTGTAGCCACCAGGATG, CATCCTGGTGGCTACAGGCCAGC, and CACCCGCACAATGATCACCTCATAA. The mutated fragment was then re-cloned into BACE-HA as a BamHI-BsrGI fragment to create D93A BACE-HA.

293T cells were transiently transfected with D93A BACE-HA. Two days post-transfection, cells were labeled with 5 mCi of \[^{3}H\]phenylalanine or \[^{3}H\]threonine for 2 h and then chased for 3 h. Cell extracts were prepared as described (19), pre-cleared with recombinant protein-A Sepharose beads, and immunoprecipitated with anti-HA affinity matrix beads (Covance). The samples were separated on a NuPAGE 7% Tris acetate gel (NOVEX), transferred to PVDF membrane, and exposed to film. The film was then used to locate the bands on the membrane, which were excised and radiosequenced as described previously (19).

Metabolic Labeling and Immunoprecipitations—24–48 h post-transfection, 293T or LoVo cells grown in 10-cm dishes (\( \sim 7 \times 10^6 \) cells) were incubated in DMEM minus methionine for 2 h at 37 °C. Cells were then labeled for 1 h with 1 mCi/10 cm dish of \[^{35}S\]methionine and chased in complete growth media supplemented with 2.5 mM methionine for designated times. For inhibitor experiments, \( \sim 7 \times 10^6 \) BACE-HA/293 cells were labeled for 1 h using the above protocol and subsequently chased for 5 h in complete growth media supplemented with 2.5 mM methionine plus or minus inhibitor. Immunoprecipitations were performed.

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**FIG. 1.** A, schematic structure of BACE-HA showing signal peptide (SP), propeptide domain (PD), active site motifs (DTG and DSG), transmembrane domain (TM), and hemagglutinin epitope tag (HA). Numbers refer to amino acid positions; asterisks denote N-linked glycosylation sites, and the arrow indicates the D93A active site mutation. The signal peptide and propeptide domain sequence is shown below in brackets, with the underlined RLPR representing the furin recognition motif, and the thin arrow indicating the propeptide domain cleavage site at Glu-46. B, appropriate propeptide cleavage of the active site mutant D93A BACE-HA. D93A BACE-HA from transfected 293 cells labeled with \[^{3}H\]threonine (left) or \[^{3}H\]phenylalanine (right) was radiosequenced. The figures show radioactivity in counts/min per sequencing cycle. The D93A BACE-HA radiosequences show a major peak of \[^{3}H\]threonine in cycle 2 and a major peak of \[^{3}H\]phenylalanine in cycle 15, consistent with mature BACE starting at Glu-46. Radiosequencing of wild-type BACE reveals the same Glu-46 start site (data not shown). The N-terminal sequence of mature BACE is shown below, with the cycle 2 threonine and the cycle 15 phenylalanine indicated by numbers.
A23187 (2 μM) BACE-HA/293 cells were pulse-labeled and bands were excised and sequenced as described (14).

PAGE on a 4–20% Tris glycine gel, transferred to Problot PVDF matrix beads (Covance). Samples were then subjected to SDS–CHAPS, and immunoprecipitated overnight at 4 °C with anti-HA antibody. Radiolabeled D93A BACE-HA accumulated upon A23187 treatment has a molecular mass of ~70 kDa (Fig. 2), indicating that A23187 inhibits propeptide processing without substantially affecting intracellular trafficking. Immunoprecipitation with anti-HA antibody, which recognizes all forms of BACE-HA, was performed as described previously (19) using either anti-HA affinity matrix beads (Covance) or propeptide antiseraum (14).

Immunocytochemistry—Immunocytochemistry of paraformaldehyde-fixed, Triton X-100-permeabilized cells was performed as described previously (5). Primary antibodies and dilutions were as follows: anti-HA monoclonal antibody (Covance) 1:100, anti-furin affinity purified polyclonal antibody (Affinity Bioreagents, Inc.) 1:400, Alexa 488 (green) and Alexa 594 (red) secondary antibodies (Molecular Probes) were used at 1:200 dilution.

In Vitro Digestion of Recombinant Soluble BACE with Recombinant Furin and N-terminal Sequencing—Conditioned medium from the stable cell line Sol.BACE-HA/293 was concentrated and buffer-exchanged into 200 mM MES, pH 6.2, 0.1 mM MgCl₂, 0.1 mM EDTA, 0.05% Tween 20, and 0.5 mM NaCl (Buffer C). The sample was mixed with anti-HA affinity matrix beads (Covance) and incubated overnight at 4 °C. Beads were washed 4 times with Buffer C, and Sol.BACE-HA was released from the matrix with HA peptide (Covance) at a concentration of 0.4 mg/ml in Buffer C at 30 °C for 30 min. The purified sample was then dialyzed overnight against 50 mM Tris-HCl, pH 7.5, 1 mM CaCl₂, 0.5% Triton X-100. The digestion was performed in the same buffer in the presence or absence of 10 or 50 units of recombinant furin (Affinity Bioreagents, Inc.) for 24 h at room temperature. For Western blot analysis the reactions were stopped by the addition of 4X sample buffer, directly loaded onto a NuPAGE 7% Tris acetate gel, and visualized by enhanced chemiluminescence (Amersham Pharmacia Biotech). For N-terminal sequence analysis reactions were diluted with 1 ml of IP buffer (25 mM Heps, pH 7.2, 150 mM NaCl, 2 mM EDTA, 2% CHAPS), and immunoprecipitated overnight at 4 °C with anti-HA affinity matrix beads (Covance). Samples were then subjected to SDS-PAGE on a 4–20% Tris glycine gel, transferred to Problot PVDF membrane (Applied Bioystems), and stained with Coomassie Blue, and bands were excised and sequenced as described (14).

RESULTS

BACE Propeptide Cleavage Does Not Appear to be Autocatalytic—It is well established that the activation of pepsin, as well as other aspartic proteases, requires proteolytic processing of the inactive proprotein to a mature enzyme (13). This process involves the autocatalytic removal of the propeptide domain. To determine whether BACE propeptide cleavage may be autocatalytic, we generated an inactive form of BACE by mutating the aspartic acid at position 93 to alanine in the N-terminal active site motif (see Fig. 1A). To facilitate immunological detection, the HA epitope tag was fused to the C terminus of D93A BACE (D93A BACE-HA). When transfected into 293 cells that stably express APP with the Swedish familial AD mutation (APPsw (20)), D93A BACE-HA did not significantly increase the production of the β-secretase-cleaved APPβ ectodomain (APPβsw) in conditioned media as compared with cells transfected with vector alone (not shown). In contrast, cells transfected with wild-type BACE-HA generate approximately 2–5-fold more APPβsw in conditioned media, verifying that the D93A mutant is inactive (data not shown). To determine whether this inactive mutant was processed to the mature form, we radiolabeled D93A BACE-HA transfected 293 cells with [3H]thymidine or [3H]phenylalanine (Fig. 1B) and performed radiosequencing of D93A BACE-HA immunoprecipitated from cell lysates with anti-HA antibody. Radiosequencing established that the propeptide domain of D93A BACE-HA is still cleaved appropriately at the known Glu-46 site. Thus, BACE propeptide cleavage does not appear to be autocatalytic, but instead may occur through intermolecular cleavage by the action of another protease.

BACE Propeptide Cleavage Occurs in the Golgi Apparatus and Involves a Calcium-dependent Protease—BACE undergoes N-linked glycosylation and is converted from immature (~60 kDa) to mature (~70 kDa) glycosylated forms during transit through the ER and Golgi apparatus (14). Moreover, the BACE propeptide domain is processed following a time course that parallels the maturation of BACE glycosylation in the Golgi (Fig. 5B (14)). The glycosylation of BACE obscures the reduction in molecular weight due to propeptide cleavage. Thus, in order to follow BACE propeptide processing, we used an antiserum raised against the propeptide domain (designated anti-pro (14)). We were interested in determining the subcellular compartment in which BACE propeptide cleavage occurs, and for these studies we used a stable 293 cell line expressing BACE-HA (BACE-HA/293 (5)).

We performed pulse-labeling experiments on BACE-HA/293 cells treated with either brefeldin A (BFA; an agent that fuses Golgi with ER (21)), monensin (a Golgi-disturbing agent (21)), or A23187 (a calcium ionophore (22)) (Fig. 2). Cell lysates immunoprecipitated with anti-pro and analyzed by SDS-PAGE show that the ~60-kDa BACE-HA unprocessed form accumulates upon BFA and monensin treatment (lanes 2 and 4, respectively, Fig. 2). In contrast, the ~60-kDa species is undetectable in lysates from untreated cells (lanes 1 and 3, Fig. 2). The accumulation of the ~60-kDa species is also observed upon BFA and monensin treatment in cell lysates immunoprecipitated with anti-HA antibody, which recognizes all forms of BACE-HA (lanes 8 and 10, Fig. 2). These results demonstrate that treatment with BFA or monensin decreases BACE propeptide processing by inhibiting intracellular trafficking through the ER and the Golgi apparatus. Since monensin disrupts traffic within the Golgi (21), it is likely that BACE propeptide cleavage occurs in the Golgi apparatus.

In contrast to treatment with BFA and monensin, A23187 treatment causes an accumulation of the ~70-kDa form of BACE-HA containing the propeptide region (lane 6, Fig. 2), indicating that A23187 inhibits propeptide processing without substantially affecting intracellular trafficking. Immunoprecipitation with anti-HA antibody confirms that the BACE-HA species accumulating upon A23187 treatment has a molecular mass of ~70 kDa (lane 12, Fig. 2), suggesting a mature glycosylation pattern consistent with localization in the trans-Golgi. Taken together, our inhibitor data suggest that the BACE propeptide may be cleaved by a calcium-dependent protease in the Golgi apparatus. Furin is a ubiquitous calcium-dependent protease of the constitutive secretory pathway (16). Moreover, antibody staining patterns for BACE-HA and furin largely overlap within shared intracellular compartments (Fig. 3), including the Golgi apparatus, TGN, and endosomes (5). Although the intracellular localization of endogenous BACE has yet to be determined, the BACE-HA subcellular localization together
with our inhibitor results are consistent with a role for furin in BACE propeptide processing.

Furin Is Required for BACE Propeptide Processing in Cells—The Portland variant of \( \alpha_1 \)-antitrypsin (\( \alpha_1 \)-PDX) is a potent and selective inhibitor of furin (18). To investigate initially whether furin may mediate BACE propeptide cleavage, we transfected BACE-HA/293 cells with \( \alpha_1 \)-PDX, wild-type \( \alpha_1 \)-antitrypsin (\( \alpha_1 \)-WT), which does not inhibit furin, or vector alone, then pulse-labeled cells, and finally immunoprecipitated cell lysates with either anti-pro or anti-HA antibodies (Fig. 4). SDS-PAGE of BACE-HA immunoprecipitated with anti-pro reveals that \( \alpha_1 \)-PDX inhibits propeptide cleavage as compared with transfection with either \( \alpha_1 \)-WT or vector alone (compare lane 1 with lanes 2 and 3, Fig. 4). Interestingly, the propeptide-containing BACE-HA species (proBACE-HA) has a molecular mass of \( 70 \) kDa, indicating that it has a mature glycosylation pattern consistent with localization in the trans-Golgi. As a control, immunoprecipitation with anti-HA shows that the levels of BACE-HA remain unchanged in all transfections (lanes 4–6, Fig. 4).

The human colon carcinoma cell line LoVo has mutations in both furin gene alleles and therefore does not express functional furin (23). We used the LoVo cell line to determine if furin was required for BACE propeptide cleavage (Fig. 5). LoVo cells transfected with BACE-HA were subjected to pulse-chase labeling, and cell lysates were immunoprecipitated with either anti-pro or anti-HA antibodies, followed by SDS-PAGE (Fig. 5A). Immunoprecipitation with anti-pro shows that BACE-HA transfection alone in LoVo cells results in the accumulation of unprocessed \( 70 \)-kDa proBACE-HA exhibiting mature glyco- sylation (Fig. 5A, lanes 1–4). In contrast, co-transfection with furin results in complete processing of the \( 70 \)-kDa species (Fig. 5A, lanes 5–8), indicating that BACE propeptide cleavage is rescued by furin transfection in LoVo cells. As a control, immunoprecipitation of lysates from the furin-transfected cells with anti-HA shows the appearance of the processed \( 70 \)-kDa BACE-HA species (Fig. 5A, lanes 9–12), demonstrating that BACE-HA is not degraded upon furin transfection. For comparison, when expressed in 293 cells, proBACE-HA is predominant at 1 h and is then completely processed to the mature form by \( 3 \) h (Fig. 5B). Qualitatively, the conversion of proBACE-HA from \( 60 \) to \( 70 \) kDa appears much more rapid in furin-transfected LoVo cells, presumably due to overexpression of furin. Taken together, our results in furin-deficient LoVo cells and in \( \alpha_1 \)-PDX-transfected BACE-HA/293 cells demonstrate that furin is required for BACE propeptide cleavage, at least in the cells studied.

Furin Cleaves BACE Propeptide at the Known Glu-46 Site in Vitro—Previously, we have shown that secreted BACE derivatives are only partially processed to the mature form (14). To demonstrate directly that furin cleaves the BACE propeptide domain in vitro, we generated a soluble, truncated form of BACE-HA lacking the transmembrane domain and C-terminal tail (Sol.BACE-HA). A stable 293 cell line expressing Sol.BACE-HA was made (Sol.BACE-HA/293), and Sol.BACE-HA was purified to homogeneity from conditioned media using anti-HA affinity chromatography. We then digested varying amounts of Sol.BACE-HA with recombinant furin in vitro, and we performed Western blot analysis with anti-pro and an anti-serum raised against the BACE luminal domain (anti-BACE; Fig. 6). Western analysis with anti-pro shows that furin cleaves the BACE propeptide in vitro, as indicated by the dramatically reduced intensity of anti-pro-labeled Sol.BACE-HA that has been digested with furin as compared with mock digestion (lanes 4–6 versus lanes 1–3, Fig. 6). As a control, the blot was stripped and re-incubated with anti-BACE to show that equivalent amounts of Sol.BACE-HA were present in the respective reactions with or without furin (Fig. 6, lanes 7–12).

Next we determined the N-terminal sequence of Sol.BACE-HA generated upon furin cleavage in vitro. Purified Sol.BACE-HA following digestion with or without furin was immunoprecipitated with anti-HA, separated by SDS-PAGE, and blotted onto PVDF membrane. Sol.BACE-HA bands were excised, and the N-terminal sequences were determined. Mock-digested Sol.BACE-HA exhibits two N-terminal sequences, TQHGIRLPLR (0.78 pmol; 45%) and ETDEEPEEPG (0.83 pmol; 55%). These two sequences derive from BACE cleaved at the signal peptide (Thr-22) and the propeptide domain (Glu-46), respectively. The propeptide domain is removed from approximately...
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, 10 units/reaction; vitro.

The indicated amounts of purified recombinant Sol.BACE-HA
lanes 7–12), and then was stripped and re-probed with anti-BACE
run on a NuPAGE 7% Tris acetate gel and visualized by PhosphorImager analysis. B, time course of BACE propeptide cleavage in BACE-HA/293 cells. BACE-HA/293 cells were pulse-chase-labeled, and immunoprecipitates were analyzed following the same strategy as for LoVo cells in (A). Molecular mass markers (left) and the ~60-kDa and ~70-kDa BACE species (right) are indicated.

FIG. 5. A, BACE propeptide cleavage is absent in LoVo cells but is rescued by furin transfection. LoVo cells, either transiently transfected with BACE-HA cDNA (-; lanes 1–4) or co-transfected with BACE-HA and furin cDNAs (+; lanes 5–12), were pulse-labeled for 30 min with [35S]methionine and then chased for the indicated times. Cell lysates were prepared, and equal amounts were immunoprecipitated with either anti-pro (- and + furin transfections; lanes 1–8) or anti-HA antibodies (+ furin transfections; lanes 9–12). Immunoprecipitates were run on NuPAGE 7% Tris acetate gels and visualized by PhosphorImager analysis.

BACE propeptide cleavage is absent in LoVo cells but is rescued by furin transfection. LoVo cells, either transiently transfected with BACE-HA cDNA (-; lanes 1–4) or co-transfected with BACE-HA and furin cDNAs (+; lanes 5–12), were pulse-labeled for 30 min with [35S]methionine and then chased for the indicated times. Cell lysates were prepared, and equal amounts were immunoprecipitated with either anti-pro (- and + furin transfections; lanes 1–8) or anti-HA antibodies (+ furin transfections; lanes 9–12). Immunoprecipitates were run on NuPAGE 7% Tris acetate gels and visualized by PhosphorImager analysis. B, time course of BACE propeptide cleavage in BACE-HA/293 cells. BACE-HA/293 cells were pulse-chase-labeled, and immunoprecipitates were analyzed following the same strategy as for LoVo cells in (A). Molecular mass markers (left) and the ~60-kDa and ~70-kDa BACE species (right) are indicated.

FIG. 6. Furin cleaves Sol.BACE-HA propeptide domain in vitro. The indicated amounts of purified recombinant Sol.BACE-HA were either digested with recombinant furin (+, 10 units/reaction; lanes 4–6 and 10–12) or mock-digested without furin (-; lanes 1–3 and 7–9), run on a NuPAGE 7% Tris acetate gel, and subjected to Western analysis. The immunoblot was first incubated with anti-pro-pro antibody (lanes 1–6), and then was stripped and re-probed with anti-BACE antiserum (lanes 7–12). Molecular mass markers are indicated on the left.

50–70% of Sol.BACE-HA secreted from the Sol.BACE-HA/293 stable cell line due to endogenous processing within the cell (not shown). When Sol.BACE-HA was incubated with furin in vitro, only one sequence was observed, ETDEEPEEPG (1.89 pmol; 100%), directly demonstrating that furin cleaves the propeptide domain only at the known start site of mature BACE.

DISCUSSION

Eukaryotic aspartic proteases are synthesized as inactive precursor proteins containing an N-terminal signal peptide and a propeptide domain. Subsequent to signal peptide removal in the ER and glycosylation in the ER and Golgi, the propeptide domain is cleaved to form the mature enzyme. In general, activation of the aspartic protease requires the removal of the propeptide domain. For many aspartic proteases in the peptidase family, such as pepsin, cathepsin D, and cathepsin E, cleavage of the propeptide domain is autocatalytic and is induced by acidic conditions (13, 24, 25). However, in the case of renin, propeptide cleavage may not be autocatalytic but appears to require endoproteolysis by another protease, possibly cathepsin B, PC1, or PC5 (26–28). Our data suggest that BACE propeptide cleavage also may not be autocatalytic, since the enzymatically inactive D93A BACE mutant is still cleaved at the known Glu-46 site (Fig. 1B). This indicates that another protease may mediate intermolecular cleavage of the BACE propeptide domain. Recently, it has been reported that enriched preparations of soluble BACE generated by bacterial expression exhibit cleavage at two sites within the BACE propeptide domain upon extended incubation under acidic conditions (12). However, neither of the cleavages corresponds to the known Glu-46 start of mature BACE observed in cultured cells or in human brain. Therefore, it is unlikely that these propeptide cleavages of BACE in vitro have physiological relevance in vivo.

We have determined that BACE propeptide cleavage is inhibited by BFA, monensin, and A23187 (Fig. 2). BFA causes fusion of the Golgi apparatus with the ER, and monensin disrupts intra-Golgi traffic, especially in trans-Golgi and acidic post-Golgi compartments (21). The calcium ionophore A23187 depletes intracellular stores of calcium and therefore is an indirect inhibitor of calcium-dependent proteases (22). Thus, based on our inhibitor results, we conclude that the propeptide domain of BACE is likely to be cleaved by a calcium-dependent protease in the Golgi apparatus.

The known BACE propeptide cleavage site RLPR ↓ E is a PC recognition motif of the general sequence (R/K)(X,R/K) ↓, where n = 0, 2, 4, or 6 (15), and therefore suggests that a member of this family of calcium-dependent serine proteases may cleave the BACE propeptide. Since BACE is expressed ubiquitously, the putative PC that cleaves the BACE propeptide would also be expected to have a wide tissue distribution. In addition, BACE is localized within compartments of the constitutive secretory pathway including Golgi, TGN, and endosomes, and thus PCs localized within these same compartments should be considered prime candidates for BACE propeptide cleaving enzymes. The PCs furin and PC7 have ubiquitous tissue distributions, whereas PACE4 and PC5B (an alternatively spliced isoform of PC5 containing a C-terminal transmembrane domain (29)) have more restricted expression patterns (15, 30). All four of these PCs localize within the constitutive secretory pathway, and therefore any one of them could potentially cleave the BACE propeptide. We currently favor furin as the most likely PC to cleave the BACE propeptide for the following reasons. The known cleavage site RLPR ↓ E is a potential furin recognition motif, and furin and BACE-HA largely co-localize within shared intracellular compartments (Fig. 3). In addition, transfection of α1-PDX into BACE-HA/293 cells inhibits BACE propeptide cleavage (Fig. 4). α1-PDX is a potent inhibitor of furin, and also inhibits PC5B to a lesser extent, but has little effect on PACE4 and PC7 (31). Cleavage of the BACE propeptide domain is blocked in furin-deficient LoVo cells, and furin transfection rescues cleavage (Fig. 5). Finally, in vitro, recombinant furin cleaves purified, soluble BACE-HA exactly and only at Glu-46 (Fig. 6), the known start of mature BACE (5, 10, 11, 14).
LoVo cells express PACE4 and PC7 but not PC5B (30, 32), leaving open the possibility that PC5B may also process the BACE propeptide domain. However, PC5B is predominantly expressed in intestine, adrenal gland, and lung but does not appear to be expressed significantly in brain or other tissues (29). Given the more restricted tissue distribution of PC5B relative to furin, it appears that furin may be the most likely candidate enzyme to mediate BACE propeptide cleavage. However, we cannot exclude the possibility that PC5B or another PC may participate with furin to cleave the BACE propeptide in specific tissues.

Understanding BACE propeptide processing may provide insight into the regulation of BACE enzyme activity and therefore may reveal novel therapeutic approaches for the treatment of AD. The motivation for this study was to identify the enzyme responsible for BACE propeptide processing and to evaluate it as a potential therapeutic target. Here we have shown that furin, or a furin-like proprotein convertase, mediates BACE propeptide cleavage. Given the large number and diversity of furin substrates with important biological functions in vivo (reviewed in Ref. 16), long term inhibition of furin, as would be required for the treatment of AD, may have untoward side effects, and therefore furin is not a viable therapeutic target.

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REFERENCES
1. Selkoe, D. J. (1999) Nature 399, A23–A31
2. Glenner, G. G. & Wong, C. W. (1984) Biochem. Cell Biol. 120, 885–890
3. Younkin, S. G. (1998) J. Physiol. (Paris) 92, 289–292
4. Kang, J., Lemaire, H.-G., Unterbeck, A., Salbaum, J. M., Masters, C. L., Graeber, K.-H., Multhaup, G., Beyreuther, K. & Muller-Hill, B. (1987) Nature 325, 733–736
5. Vassar, R., Bennett, B. D., Babu-Khan, S., Kahn, S., Mendiaz, E. A., Denis, P., Teplow, D. B., Ross, S., Amurante, F., Loeloff, R., Loo, Y., Fisher, S., Fuller, J., Edenson, S., Lile, J., Jarosinski, M. A., Bieri, A. L., Curran, E., Burgess, T., Louis, J.-C., Collins, F., Treanor, J., Rogers, G. & Citron, M. (1999) Science 268, 735–741
6. De Strooper, B., Saftig, P., Craessaerts, K., Vanderstichele, H., Guhe, G., Annaert, W., Figura, K. V. & Leuven, F. V. (1998) Nature 391, 387–390
7. De Strooper, B., Annaert, W., Cuppers, P., Saftig, P., Craessaerts, K., Mumm, J. S., Schroeter, E. H., Schrijvers, V., Wolfe, M. S., Ray, W. J., Goate, A. & Kops, R. (1999) Nature 398, 518–522
8. Wolfe, M. S., Xia, W., Ostaszewski, B. L., Diehl, T. S., Kimberly, W. T. & Selkoe, D. J. (1999) Nature 398, 513–517
9. Hussain, I., Powell, D., Howlett, D. R., Tew, D. G., Meeke, T. D., Chapman, C., Gloger, I. S., Murphy, K. E., Southan, C. D., Ryan, D. M., Smith, T. S., Simmons, D. L., Walsh, F. S., Dingwall, C. & Christie, G. (1999) Mol. Cell. Neurosci. 14, 419–427
10. Yan, R., Bienkowski, M. J., Shuck, M. E., Miao, H., Tiry, M. C., Pauley, A. M., Braslialer, J. R., Stratman, N. C., Mathews, W. R., Buhl, A. E., Carter, D. B., Tomasselli, A. A., Parodi, L. A., Heinrikson, R. L. & Gurney, M. E. (1999) Nature 402, 533–537
11. Sinha, S., Anderson, J. P., Barbour, B. G., Cacciavello, R., Davis, D., Doan, M., Doey, H. F., Frigon, N., Hong, J., Jacobson-Crauk, K., Jewett, N., Keim, P., Krogs, J., Lieberburg, I., Power, M., Tan, H., Tatsuno, G., Tung, J., Schenk, D., Seubert, P., Suomensaari, S. M., Wang, S., Walker, D., Zhao, J., McConlogue, L. & John, V. (1999) Nature 402, 537–540
12. Lin, X., Koelsch, G., Wu, S., Downs, D., Dashi, A. & Tang, J. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 1456–1460
13. Tang, J. & Wong, R. N. S. (1987) J. Cell Biochem. 33, 53–63
14. Haniu, M., Denis, P., Young, Y., Mendiaz, E. A., Fuller, J., Hui, J. O., Bennett, B. D., Kahn, S., Ross, S., Burgess, T., Katta, V., Rogers, G., Vassar, R. & Citron, M. (2000) J. Biol. Chem. 275, 21099–21106
15. Seidah, N. G. & Chretien, M. (1997) Curr. Opin. Biotechnol. 8, 602–607
16. Molloy, S. S., Anderson, E. D., Jean, F. & Thomas, G. (1999) Trends Cell Biol. 9, 28–35
17. Gorman, C. M., Gies, D. R. & McCray, G. (1990) DNA Protein Eng. Technol. 2, 3–10
18. Anderson, E. D., Thomas, L., Hayflick, J. S. & Thomas, G. (1993) J. Biol. Chem. 268, 24887–24891
19. Haass, C., Schlossmacher, M. G., Hung, A. Y., Vigo-Pelfrey, C., Mellon, A., Ostaszewski, B. L., Lieberburg, I., Koo, E. H., Schenk, D., Teplow, D. B. & Selkoe, D. J. (1999) Nature 399, 223–225
20. Mullan, M., Crawford, F., Hundeh, D., Axelman, K., Lilis, L., Winblad, B. & Lannfelt, L. (1992) Nat. Genet. 1, 345–347
21. Duncia, A. & Bergesi, A. (1995) Histochem. Cell Biol. 100, 571–591
22. Logeat, F., Bessia, C., Brou, C., Lebail, O., Jarriault, S., Seidah, N. G. & Israel, B. (1999) J. Biol. Chem. 274, 11417–11423
23. Takeuchi, S., Essai, K., Hatsuza, K., Katamura, N., Misumi, Y., Ikehara, Y., Murakami, K. & Nakayama, K. (1993) Biochem. Cell Biol. 195, 1019–1026
24. Khan, A. R. & James, M. N. G. (1996) Protein Sci. 7, 815–836
25. Takeda-Ezaki, M. & Yamamoto, K. (1993) Arch. Biochem. Biophys. 304, 352–358
26. Wang, P. H., Do, Y. S., Macaulay, L., Shinagawa, T., Anderson, P. W., Baxter, J. D. & Housh, W. A. (1991) J. Biol. Chem. 266, 12633–12638
27. Benjannet, S., Reudelhuber, T., Mercure, C., Rondeau, N., Chretien, M. & Seidah, N. G. (1992) J. Biol. Chem. 267, 11417–11423
28. Mercure, C., Jatras, I., Day, R., Seidah, N. & Reudelhuber, T. (1996) Hyper-
tension 28, 840–846
29. Nakagawa, T., Murakami, K. & Nakayama, K. (1993) FEBS Lett. 327, 165–171
30. Seidah, N. G., Chretien, M. & Day, R. (1994) Biochimie (Paris) 76, 197–209
31. Jean, F., Stella, K., Thomas, L., Liu, G., Xiang, Y., Reason, A. J. & Thomas, G. (1999) Proc. Natl. Acad. Sci. U. S. A. 95, 533–540
32. Seidah, N. G., Hamelin, J., Mamarbachi, M., Dong, W., Tardos, H., Mbiak, M., Chretien, M. & Day, R. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 3388–3393
Additions and Corrections

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A furin-like convertase mediates propeptide cleavage of BACE, the Alzheimer’s β-secretase.

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Page 37713: Fig. 1, A and B, was incomplete. The corrected figure is shown below.

Fig. 1. A, schematic structure of BACE-HA showing signal peptide (SP), propeptide domain (PD), active site motifs (DTG and DSG), transmembrane domain (TM), and hemagglutinin epitope tag (HA). Numbers refer to amino acid positions; asterisks denote N-linked glycosylation sites; and the arrow indicates the D93A active site mutation. The signal peptide and propeptide domain sequence is shown below in brackets, with the underlined RLPR representing the furin recognition motif, and the thin arrow indicating the propeptide domain cleavage site at Glu-46. B, appropriate propeptide cleavage of the active site mutant D93A BACE-HA. D93A BACE-HA from transfected 293 cells labeled with [3H]threonine (left) or [3H]phenylalanine (right) was radiosequenced. The figures show radioactivity in counts/min per sequencing cycle. The D93A BACE-HA radiosequences show a major peak of [3H]threonine in cycle 2 and a major peak of [3H]phenylalanine in cycle 15, consistent with mature BACE starting at Glu-46. Radiosequencing of wild-type BACE reveals the same Glu-46 start site (data not shown). The N-terminal sequence of mature BACE is shown below, with the cycle 2 threonine and the cycle 15 phenylalanine indicated by numbers.
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