Cleavage of Amyloid-β Precursor Protein and Amyloid-β Precursor-like Protein by BACE 1*

Received for publication, September 9, 2003, and in revised form, December 23, 2003
Published, JBC Papers in Press, December 29, 2003, DOI 10.1074/jbc.M310001200

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Site-specific proteolysis of the amyloid-β precursor protein (APP) by BACE 1 and γ-secretase, a central event in Alzheimer disease, releases a large secreted extracellular fragment (called APPβ), peptides of 40–43 residues derived from extracellular and transmembrane sequences (Aβ), and a short intracellular fragment (APP intracellular domain) that may function as a transcriptional activator in a complex with the adaptor protein Fe65 and the nuclear protein Tip60. APP is closely related to APP-like protein (APLP) 1 and APLP2, but only APP is known to be cleaved by BACE 1 and to be involved in Alzheimer disease. We now demonstrate that similar to APP, APLP1 and APLP2 are also cleaved by BACE 1 but not by ADAM 9, another APP protease, and also transactivate nuclear Tip60 in a complex with Fe65. The existence of an APLP cleavage suggests a target gene encoding a tetraspanin) for the AICD-Fe65-Tip60 complex was identified in a study on how interleukin-1β activates transcription (21), thereby demonstrating the in vivo relevance of the original experiments using heterologous DNA binding domains. APP belongs to a gene family that includes two additional members, APLP1 and APLP2 (22–24). APP, APLP1, and APLP2 are closely related and exhibit the same domain structure (see Fig. 1A). Knock-out mice revealed that APP, APLP1, and APLP2 are functionally redundant (25). Similar to APP, APLPs appear to be cleaved by extracellular proteases followed by proteolytic processing by γ-secretase (26–28). Furthermore, Gal4 fusion proteins of APLPs also transactivate Gal4-dependent transcription in an Fe65-dependent manner (27, 28). These observations suggest that APLPs may be proteolytically processed by mechanisms similar to APP and may have similar functions. However, not all evidence is consistent with this hypothesis. It is noticeable that although the extracellular domains and the AICDs of APP and APLPs are highly homologous, the linker sequences connecting these domains exhibit no homology (Fig. 1B). Because this linker sequence in APP is the substrate for α- and β-secretases, it is unclear whether the same extracellular proteases digest APP and APLPs. This is a particularly important question for BACE 1, the primary β-secretase (29–33), because it is the major extracellular processing enzyme for APP in neurons, and because it initiates production of Aβ from APP (4–6). This question is not only...
important for understanding BACE 1 function but also for insight into the biological role of APP and APLPs, because a common cleavage of these proteins by BACE 1 would support the notion that proteolytic processing of these proteins by a common set of enzymes serves to regulate a physiological function such as transcriptional activation.

In the present study, we have investigated whether APLPs can be substrates for BACE 1 even though APLPs exhibit no sequence homology to the BACE 1 cleavage site in APP and whether BACE 1 cleavage has an effect on the putative transmembrane region (TMR). We demonstrate that APLP1 is cut by BACE 1 at a position that is within 13 residues of the TMR resulting in the secretion of essentially the entire extracellular sequences of APLP1. In the same assay, ADAM 9 (which is one of several candidate α-secretases for APP (34, 35)) specifically cleaved APP but not APLP1. Furthermore, we show that BACE 1 substrate recognition is specific because other cell surface proteins are not cleaved in the same assay and that a short sequence from APP (seven residues) is sufficient to confer BACE 1 cleavage onto a normally non-cleaved protein. These results indicate that BACE 1 may have a coordinate function in regulating APP and APLP processing in neurons.

**EXPERIMENTAL PROCEDURES**

**Plasmids**—Most of the plasmids used for the transactivation assays, such as pG5E1B-luc (Gal4 reporter plasmid), pCMV-LacZ (β-galactosidase control plasmid), pM-Tip60 (expression of Gal4-Tip60 fusion protein), pCMV-Fe65, and pCMV-APP, were reported previously (12). pCDNA3.1-APLP2 encoding APLP2 was constructed by inserting a pair of mismatched oligonucleotides (sequence QL0311, GCC CGA GAA GTG AAG ATG GAT GCA GAA AGC and QL0312, GCC GCC GCT TTC TGC ATC CAG GTT CAC TTC TC (Swedish mutation, the mismatched bases are underlined) into the NotI site of pCMV-Nrx1 containing the Swedish mutation as confirmed by sequencing. pCMV-NrxAPP was constructed by ligating a 0.6-kb fragment encoding residues 494–695 of APP695 into the NotI site of pCMV-Nrx1β. pCDNA3.1-APLP1 encoding APLP1 was acquired from ATCC (Image clone ID 3865417). The Aβ sequence in APP is shown in red and underlines; the α-, β-, γ-, and ε-cleavage sites are marked by arrows. The C-terminal sequences in APP and APLPs and the internal APLP1 sequence that were used to raise antipeptide antibodies are boxed.

These assays were performed essentially as described (12, 40). Briefly, HEK293, HeLa, and COS7 cells were transfected at 50–80% confluency in 6-well plates...
using FuGENE 6 reagent (Roche Diagnostics). For Gal4-Tip60 transactivation assays, HEK293 cells were co-transfected with five plasmids: 1) pG5E1B-luc, 0.15 μg; 2) pCMV-LacZ, 0.10 μg; 3) pM-Tip60, 0.5 μg; 4) pCMV-FE65, 0.5 μg, and 5) 0.05–1.0 μg of pCMV-APP, 0.025–0.5 μg of pCMV-Sport6-APLP1, or 0.05–1.0 μg of pCMV3.1-APLP2 as indicated. For the Fe65 titration experiment, HEK293 cells were co-transfected with five plasmids: 1) pG5E1B-luc, 0.15 μg; 2) pCMV-LacZ, 0.10 μg; 3) pM-Tip60, 0.5 μg; 4) 0.5 μg of APP, 0.25 μg of APLP1, or 0.5 μg of APLP2, and 5) 0–20 μg of pCMV-Fe65. γ-Secretase inhibition experiments were performed essentially the same way except that 1.0 μg of pCMV-FE65 and 0.5 μg of APP, APLP1, or APLP2 were used to achieve optimal transactivation for all three proteins. DAPT was added at 2 and 10 μM 1 h after transfection. For Gal4-Tip60 transactivation assays and the Fe65 titration experiment, cells were harvested 2 days after transfection in 0.25 ml/well reporter lysis buffer (Promega), and their luciferase and β-galactosidase activities were determined with the Promega luciferase assay kit using a chemiluminescence reader from Berthold (20). Lanes were loaded with ~20 μg of protein from whole cell extracts harvested in sample buffer and analyzed by immunoblotting after SDS-PAGE using antibody dilutions of 1:1000–2000 for primary antibodies and 1:10,000 horseradish peroxidase-conjugated goat anti-rabbit secondary antibodies (IGN). For immunoblotting analysis of secreted proteins, supernatants from transfected cells (30 μl with ~5 μg of protein) were loaded/lane.

RESULTS

Coordinate Transcriptional Activity of APP, APLP1, and APLP2—Gal4-Tip60, although part of a nuclear protein complex that binds to DNA (20), is unable to mediate Gal4-dependent transcription but requires both Fe65 and APP for transactivation (12). The Gal4-Tip60 assay is a more stringent test for transcriptional function for APP and APLPs, Tip60 is a common potential target for APLPs, we co-transfected into HEK293 cells increasing amounts of expression plasmids encoding APP, APLP1, or APLP2 and a constant amount of plasmids expressing Gal4-Tip60 and Fe65. We then measured Gal4-dependent transactivation using a co-transfected reporter plasmid and corrected all transactivation data for β-galactosidase activity produced by a co-transfected control expression vector to account for differences in transfection efficiency (Fig. 2).

For all three proteins (APP, APLP1, and APLP2), we observed a bell-shaped dose-response curve of transactivation that depended on APP or an APLP (Fig. 2A). We next performed a similar analysis with a constant amount of APP or APLP1 and APLP2 but with increasing amounts of Fe65 (Fig. 2B). Again, we detected dose-dependent, specific transactivation of Gal4-dependent transcription. The absolute amount of transactivation varied depending on the precise combination of plasmids that we co-transfected. The highest levels of transactivation (400-fold increase) were reached when maximal amounts of Fe65 were co-transfected with APLP1 (Fig. 2B). To test whether transactivation mediated by APP, APLP1, or APLP2 in these assays involves γ-secretase cleavage, we examined the effect of the γ-secretase inhibitory DAPT on transactivation (Fig. 2C). Two relatively low concentrations of DAPT (2 and 10 μM) were used. We observed significant inhibition of transactivation by DAPT for all three proteins (APP, APLP1, and APLP2). Inhibition was incomplete, possibly because the doses of DAPT do not fully inhibit γ-secretase, or because a DAPT-independent proteolytic pathway contributes to transactivation. Together these results document that APLP1 and -2 can efficiently substitute for APP in Tip60-dependent transactivation, suggesting that consistent with a common transcriptional function for APP and APLPs, Tip60 is a common nuclear target.

BACE 1 Cleavage of APLPs—APP and APLPs presumably can only mediate nuclear transactivation if they are cleaved and their AICDs are released into the cytoplasm. APLPs are...
metalloproteases are thought to cleave APP as cells, several proteases that belong to the ADAM family of against synthetic peptides. Epitope sequences are residues N-terminal to the TMR (APLP1x, APLP2c, or APLP2, APLP1, or APLP2 were analyzed by immunoblotting with various antibodies to the C-terminal sequences of APP, APLP1, and APLP2). Antibodies were raised against synthetic peptides. Epitope sequences are boxed in the sequence alignment shown in Fig. 1. Numbers on the left indicate positions of molecular weight markers.

known to be cut by extracellular proteases and a presenilin-dependent γ-secretase similar to APP (26–28). In non-neuronal cells, several proteases that belong to the ADAM family of metalloproteases are thought to cleave APP as α-secretases, whereas in neurons, APP is probably primarily cleaved by the β-secretase enzyme BACE 1 in which activity is responsible for production of the pathogenic Aβ peptides (4–6). Although APLPs are cleaved extracellularly in non-neuronal and neuronal cells, it is not known whether the same enzymes cleave APP and APLPs. This question is particularly important for BACE 1 for which only two other substrates, the sialyltransferase ST6Gal and P-selectin glycoprotein ligand 1, besides APP are currently known (44, 45). In preparation to examining APLP cleavage, we generated antibodies to the cytoplasmic C-terminal sequences of APP, APLP1, and APLP2. In addition, we produced an antibody to a peptide from the linker region of APLP1 that connects the TMR to the central domain (Fig. 1, boxed sequence). We then tested the specificity of these antibodies by the immunoblotting of HEK293 cells that had been transfected with APP, APLP1, or APLP2 expression vectors (Fig. 3). We found that the antibodies reacted specifically with their cognate proteins except for a small degree of cross-reactivity of the APLP2 antibody with APP and APLP1.

In the next set of experiments we employed the newly raised antibodies to probe for APP, APLP1, and APLP2 cleavage in cells that either expressed these proteins alone or in combination with BACE 1 (Fig. 4). As negative controls, we examined two other neuronal cell surface proteins, neurexin 1β (Nrx1β) and SynCAM (36, 37, 39). For each of the five proteins studied, six conditions were investigated: control cells that were transfected without or with BACE 1 (lanes 1 and 2) and test cells that expressed APP, APLP1, APLP2, neurexin 1β, or SynCAM either alone or together with BACE 1, which were additionally either left untreated or incubated with the γ-secretase inhibitor DAPT (lanes 3–6). The DAPT treatments were performed to stabilize the CTFs, proteolytic intermediates that are produced by extracellular α-/β-cleavage of a cell surface protein and are subsequently digested by γ-secretase (Fig. 1). We then analyzed the transfected proteins by immunoblotting to monitor the full-length proteins and their CTFs as a function of BACE 1 and DAPT treatment.

Immunoblotting of control cells showed that transfected BACE 1 by itself did not produce immunoreactivity with any of the antibodies (Fig. 4, lanes 1 and 2). In transfected test cells incubated without DAPT, CTFs were observed only for APP and the APLPs but not for neurexins 1β or SynCAM (Fig. 4, lanes 3 and 4). Under these conditions, co-transfection of BACE 1 had little effect on full-length APP or APLPs but altered their
CTFs. For APP and APLP2, the size of the CTF was shifted up, whereas for APLP1 a distinctive CTF was first induced by BACE 1 expression (Fig. 4, asterisks in lane 4). When DAPT was added, the CTFs for APP, APLP1, and APLP2 became very abundant, independent of BACE 1 expression, making it difficult to detect an effect of BACE 1 (Fig. 4, lanes 5 and 6). Surprisingly, CTFs were also observed for neurexin 1β upon DAPT treatment (Fig. 4). The neurexin 1β CTFs could reflect a physiological extracellular cleavage of neurexin 1β by an enzyme different from BACE 1, because BACE 1 had no effect on the CTFs under any conditions. However, studies of primary cultures of neurons incubated with DAPT failed to yield evidence for a physiological extracellular cleavage of β-neurexins (data not shown), and at present it is unclear whether or not neurexin 1β is normally cleaved. Together these data suggest that in the absence of BACE 1, APP and APLPs are efficiently cleaved in transfected cells by secretases, likely endogenous α-secretases, but that BACE 1 expression alters cleavage of all three proteins consistent with the notion that all three proteins are BACE 1 substrates.

To confirm that BACE 1 cleaves APLPs, we examined the effect of BACE 1 co-transfection on Gal4-Tip60-dependent transactivation by APLP1 and Fe65 similar to the assay described in Fig. 2. Co-transfecting a small amount of APLP1 with Fe65 and Gal4-Tip60 resulted in a small degree of transactivation (3-fold). However, adding increasing amounts of BACE 1 expression plasmid to the co-transfection mix caused a proportional increase in transactivation (Fig. 5), indicating that BACE 1 cleavage of APLP1 contributes to Gal4-Tip60-dependent transcription. Control experiments without APLP1 showed no such increase demonstrating that BACE 1 alone does not transactivate Gal4-Tip60 by inducing cleavage of endogenous APP.

How Specific Is BACE 1 Cleavage Measured by Immunoblotting in Transfected Cells?—The possible cleavage of APLP1 and -2 by BACE 1 is surprising considering the lack of sequence homology in the APP cleavage site for BACE 1 (Fig. 1B). Although the co-transfection/cleavage assay for BACE 1 cleavage has been used previously to confirm BACE 1 enzyme activity (46), the specificity of BACE 1-mediated cleavage under these conditions has not been investigated, and the substrate specificity of BACE 1 and of other secretases has been studied primarily with short synthetic peptides (47). The data in Fig. 4 demonstrate that BACE 1 does not randomly cleave overexpressed cell surface proteins, because neurexin 1β and SynCAM were not digested. However, the data raise the question about what determines the cleavage specificity of BACE 1. Is cleavage a property of a specific sequence in APP, possibly in combination with the proximity of that sequence to the membrane, or are the extra- or intracellular domains of APP involved in directed BACE 1 cleavage? To address these questions, we constructed a hybrid neurexin 1β/APP protein in which the extracellular sequence of neurexin 1β is fused to the C-terminal sequences of APP at a position just N-terminal to the normal BACE 1 cleavage site (Fig. 6A). Comparison of the cleavage of APP and of the neurexin/APP hybrid protein in transfected cells revealed that they were almost identically processed, with a similar production of CTFs that were significantly shifted to larger sizes by co-expression of BACE 1 (Fig. 6B, lanes 3, 5, 7, and 9). For both APP and the APP/neurexin hybrid, DAPT induced a massive accumulation of CTFs consistent with normal digestion of the CTFs by γ-secretase (Fig. 6B, lanes 4, 6, 8, and 10). Thus the extracellular domains of APP N-terminal to the β-secretase cleavage site are dispensable for BACE 1 cleavage.

Does the Intracellular AICD or the TMR of APP Direct BACE 1-dependent Cleavage?—We tested this question by inserting into full-length neurexin 1β a seven-residue sequence from APP that encompasses its normal BACE 1 cleavage site (sequence EVKMDAE) or the corresponding sequence from the Swedish APP mutant that is preferentially cleaved by BACE 1 (sequence EVNLDAS (48)). The insertion was placed just outside of the TMR, corresponding to the normal position of this sequence in APP, but no other APP-related sequences were present in the neurexin 1β derivatives (referred to as Nrx1βWt or Nrx1βSw). We then tested the effect of the insertions on the cleavage of Nrx1β by BACE 1 (Fig. 6C). An analysis of co-transfected cells revealed that BACE 1 did not induce a decrease in the levels of full-length Nrx1β that did not contain an insertion (Fig. 6C, lanes 3–6). BACE 1 caused the appearance of low levels of a CTF from Nrx1β that was almost undetectable and could only be seen as a faint band in the presence of DAPT (Fig. 6C, asterisk in lane 6). Thus overexpressed BACE 1 cleaves co-expressed Nrx1β at extremely low rates. However, insertion of the seven-residue sequence was sufficient to convert neurexin 1β into a full-fledged BACE 1 substrate (Fig. 6C, lanes 7–14). With both Nrx1β constructs, BACE 1 co-expression resulted in a dramatic loss of full-length Nrx1β*. This loss was because of the complete digestion of Nrx1β* by BACE 1, because the addition of DAPT induced the accumulation of high levels of CTFs (Fig. 6C, lanes 10 and 14). Interestingly, Nrx1βSw was cleaved at the β-secretase cleav-
Transfection of BACE 1 or ADAM 9 alone into HEK293 cells had no significant effect on the processing of endogenous APP probably because the majority of the endogenous APP is from non-transfected cells (Fig. 7, lanes 1–3). Without BACE 1 or ADAM 9, a single major CTF was observed from transfected APP at steady-state (Fig. 7, lanes 4 and 7). Co-transfection of APP with BACE 1 at two concentrations (Fig. 7, lanes 5 and 6) or ADAM 9 at two concentrations (lanes 8 and 9) increased the abundance of the CTF from APP, consistent with cleavage of APP by both transfected BACE 1 and ADAM 9. In parallel, APP<sub>S</sub>, the secreted extracellular fragment of APP that results from α/β-cleavage, was monitored with antibodies to the extracellular APP sequences in the medium from the transfected cells. A dramatic increase in APP<sub>S</sub> production was observed both after BACE 1 and after ADAM 9 co-transfection (Fig. 7). The CTF that was produced by ADAM 9 in the transfected cells appeared to be larger than expected for regular α-secretase cleavage of APP, suggesting that ADAM 9 may cleave APP at a site that is not the normal α-secretase site. However, ADAM 9 unequivocally enhanced production of CTFs in these experiments, suggesting that even if ADAM 9 is not the physiological α-secretase, it can still serve as a control for BACE 1 because it is another enzyme that cleaves APP in the extracellular domain. Thus together these results demonstrate that in co-

**Fig. 6. Determinants of BACE 1 cleavage of APP.** A, diagrams of the structures of APP, the neurexin 1β/APP hybrid protein (Nrx1β/APP), and the modified neurexin 1β* (Nrx1β*). Nrx1β*/Sw contains a seven-residue insertion from the Swedish mutant of APP (sequence EVKLDAES), whereas Nrx1β*/Wt contains the corresponding insertion from wild-type APP (sequence EVKMDAE). Insertions were placed into neur

**Fig. 7. Analysis of APP cleavage by BACE 1 and ADAM 9.** Panels show immunoblots from HEK293 control cells (lane 1), cells transfected with the indicated amount of BACE 1 or ADAM 9 alone (lanes 2 and 3, respectively), cells transfected either with APP alone (lanes 4 and 7) or together with two concentrations of BACE 1 plasmid (lanes 5 and 6), or ADAM 9 plasmid (lanes 8 and 9). Blots show the analysis of cellular proteins to detect full-length APP (FL) separated on regular SDS-gels (top) and CTFs separated on Tricine gels (middle panel). In addition, the media of the cells were collected and analyzed for the secreted proteolytic APP fragment (APP<sub>S</sub>, bottom panel). The top two blots were obtained with antibodies to the extracellular C terminus of APP, and the bottom blot was obtained with antibodies to extracellular APP sequences.

**Where Is APLP1 Cleaved by BACE 1?**—It is puzzling that BACE 1 cleavage is relatively specific in that, for example, neurexin 1β is not normally a substrate but is cut by BACE 1 when the seven-residue substrate sequence is inserted. At the same time, BACE 1 cleavage appears to be relatively nonspecific because APLP1 and -2 are also cleaved by BACE 1 at a site that exhibits no sequence similarity with the APP cleavage site for BACE 1. Do all extracellular proteases that cleave APP also digest APLPs, and do these proteases cleave APLPs at a position comparable with the APP cleavage position? We addressed these questions by a more detailed study of the cleavage of APLP1 (chosen because it is less similar to APP than APLP2) and by comparing the activity of BACE 1 with that of ADAM 9, a disintegrin metalloprotease that may be one of several α-secretase enzymes (43, 46). To validate the activity of BACE 1 and ADAM 9, we first examined APP (Fig. 7).
transfected cells, both BACE 1 and ADAM 9 cleave APP and increase secretion of APPS.

We next performed similar experiments for APLP1 (Fig. 8), probing the intracellular sequence with the antibody to the C terminus used above and the secreted extracellular domain sequences (APLP1S) with a new polyclonal antibody that we raised to a short synthetic peptide derived from the extracellular sequence of APLP1 that is located 13 residues N-terminal to the TMR (boxed in Fig. 1). Numbers on the left indicate positions of molecular weight markers.

FIG. 8. Analysis of APLP1 cleavage reveals lack of an effect by ADAM 9 and site of BACE 1 cleavage. Panels show immunoblots from control cells (lane 1), cells transfected with the indicated amount of BACE 1 or ADAM 9 alone (lanes 2 and 3, respectively), or cells transfected either with APLP1 alone (lanes 4 and 7) or together with two concentrations of BACE 1 plasmid (lanes 5 and 6) or ADAM 9 plasmid (lanes 8 and 9). Three different cell lines were analyzed to control for possible cell-type-specific effects on cleavage. Blots show analysis of cellular proteins to detect full-length APLP1 (FL) separated on regular SDS gels (top) and CTFs separated on Tricine gels (middle panel). In addition, the cell media were collected and analyzed for the secreted proteolytic APLP1 fragment (APLP1S) in the bottom panel. The top two blots were obtained with antibodies to the cytoplasmic C terminus of APLP1, and the bottom blot was obtained with antibodies to a short extracellular peptide sequence of APLP1 that is located 13 residues N-terminal to the TMR (boxed in Fig. 1). Numbers on the left indicate positions of molecular weight markers.

demonstrates that BACE 1 cleaves APLP1 in the 13 residues that are located between the epitope of the antibody and the TMR (Fig. 1B).

DISCUSSION

Our data support the notion that APP is functionally redundant with APLP1 and -2 as described in the survival of knock-out mice (25) and the similar transactivation obtained with APP- and APLP-Gal4 fusion proteins (27) and extended here with the demonstration that APLPs similar to APP act via nuclear Tip60 (Fig. 2). Furthermore, we show that all three proteins are cleaved by BACE 1, the enzyme that is responsible for the β-secretase activity of APP cleavage and is the most abundant neuronal APP cleavage protein. The evidence that BACE 1 cleaves APLP1 and -2 can be summarized as follows. Co-transfection of BACE 1 with APLP1 or -2 results in the production of a new CTF (Fig. 4). Increasing amounts of BACE 1 enhance transactivation of Gal4-Tip60 mediated by APLP1 (Fig. 5). Co-expression of BACE 1 with APLP1 results in a large and specific increase in the secretion of APLP1S (Fig. 8). A newly generated antibody against a short extracellular sequence of APLP1 that is adjacent to the TMR only recognizes a secreted APLP1S proteolytic fragment when BACE 1 is co-transfected with APLP1, localizing the BACE 1 cleavage site to a position next to the TMR (Fig. 8). The majority of this evidence rests on the use of a transfection assay whereby BACE 1 is co-transfected with APP or an APLP, and the effect of BACE 1 on the production of proteolytic fragments from the co-transfected protein is examined. This assay was validated by demonstrating that two other neuronal cell surface proteins, neurexin 1β and SynCAM (34–36), are not cleaved by BACE 1 (Fig. 4), and by the finding that a seven-residue sequence from APP, when inserted into neurexin 1β next to the TMR, is sufficient to convert neurexin 1β into a BACE 1 substrate (Fig. 6). The fact that APLPs are BACE 1 substrates similar to APP, that BACE 1 is the major extracellular neuronal secretase for APP, and that APLPs and APP are functionally redundant suggests that BACE 1 cleavage is an integral component of the functions of APP and APLPs in neurons.

BACE 1 does not seem to have a stringent substrate recognition sequence. In APP, BACE 1 has two cleavage sites in which the sequences suggest that BACE 1 prefers a hydrophobic residue preceding the cleavage site and an acidic residue following the cleavage site (47). However, the β-cleavage sequences are not conserved in APLP1 or -2, and no similar sequence motif can be readily identified in APLP1 or -2. The 13-residue sequence in APLP1 that must be cleaved by BACE 1, based on the antibody data shown in Fig. 8, best resembles the second C-terminal BACE cleavage site of APP. Because of overall sequence similarity between the cleaved sequences in APP and APLPs, the cleavage of both APP and APLPs by BACE 1 was unexpected, leading us to hypothesize initially that additional specificity for BACE 1 cleavage may be provided by other parts of APP and APLPs, especially the extracellular cysteine-rich domain (CRD) and the central APP domain, which are highly conserved among APP and APLPs (Fig. 1). However, replacement of a large part of APP with neurexin 1β had little effect on the cleavage by BACE 1, whereas insertion of only seven amino acids from the β-secretase cleavage site of APP conferred BACE 1 cleavage onto neurexin 1β. Therefore, as long as a BACE 1 site is accessible to the enzyme, BACE 1 cleavage does not require any additional sequence information in vivo. If so, how is the BACE 1 cleavage regulated? One possibility is that the accessibility of the BACE 1 site could be regulated by a ligand or a protein that interacts with APP. Binding may physically block the access of BACE 1 directly or induce a conformational change so that the β-site is
such changes could lead to the accumulation of Aβ-proteins that may provide feedback regulation of cleavage ligands that influence cleavage and of the intracellular regulation to regulate APP cleavage (55). At present, the nature of the ligand for APP in the brain and the structural and functional similarity of APP and APLPs produce very similar secreted large extracellular proteins (APPs, APLP1α, and APLP2α) and soluble intracellular fragments (AICDs) but distinct small secreted peptides that are composed of non-homologous sequences. It remains to be established whether these diverse peptides execute distinct functions in addition to a common function of APP and APLPs mediated by their conserved domains. Another implication of our findings is that if APP and APLP cleavage is as physiologically important as suggested by transgenic experiments (50), then inhibition of BACE 1 in the brain will interfere with APP and APLP processing and may thus inhibit their common functions. As the list of substrates of γ-secretase grows, the therapeutic strategy for Alzheimer disease has shifted from inhibiting γ-secretase to modulating β- and α-secretases. BACE 1 inhibitors have been suggested as a promising cure for Alzheimer disease because knock-out mice with BACE 1 have no detectable abnormalities, and the level of Aβ in these mice is reduced (51, 52). However, inhibition of BACE 1 may abolish the functions of APP and APLPs as suggested in our study, and thus drugs that target BACE 1 may produce side effects when applied in treatment of Alzheimer patients.

The similarities between APP and APLPs make it likely that the function of APP is not directly involved in Alzheimer disease, because APLPs have not been linked to this disease. Nevertheless, their functions are probably important for Alzheimer disease. The abundance of the protofibrillogenic fragments that are produced by APP and APLP cleavage will depend on the regulation of cleavage. If cleavage by β-secretase is up-regulated or cleavage of α-secretase down-regulated, Aβ production will increase. The example of other transcription pathways suggests that the cleavage of APP and APLPs and the putative transcriptional signal caused by the cleavage may be controlled by extracellular ligands and be components of a regulatory loop (e.g. see the Notch paradigm; Refs. 53 and 54). Indeed, heparan sulfate has been proposed as a possible ligand to regulate APP cleavage (55). At present, the nature of the ligands that influence cleavage and of the intracellular regulatory proteins that may provide feedback regulation of cleavage are not well studied. However, if the regulation of cleavage and transcription is changed only slightly over long time periods such changes could lead to the accumulation of Aβ to cause Alzheimer disease. Thus identification of the signals and mechanisms involved in regulating the function of APP and APLP is of prime importance, because one possible mechanism by which one could interfere with the pathogenesis of Alzheimer disease would be to alter these signals and mechanisms.

Acknowledgments—We thank J. Kornblum and E. Borowiak for excellent technical assistance and Drs. C. Blobel (Memorial Sloan-Kettering Cancer Center), G. Yu (University of Texas Southwestern), E. Koo (San Diego), W. Wasco (Boston), A. Ho, X. Cao, and T. Biederer (all from University of Texas Southwestern) for advice and reagents.
Cleavage Specificity of β-Secretase

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J. Biol. Chem. 2004, 279:10542-10550. doi: 10.1074/jbc.M310001200 originally published online December 29, 2003

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