

Mapping the APP/Presenilin (PS) Binding Domains: The Hydrophilic N-Terminus of PS2 Is Sufficient for Interaction with APP and Can Displace APP/PS1 Interaction

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Mutations in presenilin 1 and presenilin 2 (PS1 and PS2, respectively) genes cause the large majority of familial forms of early-onset Alzheimer's disease. The physical interaction between presenilins and APP has been recently described using coimmunoprecipitation. With a similar technique, we confirmed this interaction and have mapped the interaction domains on both PS2 and APP. Using several carboxy-terminal truncated forms of PS2, we demonstrated that the hydrophilic amino terminus of PS2 (residues 1 to 87, PS2NT) was sufficient for interaction with APP. Interestingly, only a construct with a leader peptide for secretion (SecPS2NT) and not its cytosolic counterpart was shown to interact with APP. For APP, we could demonstrate interaction of PS2 with the last 100 but not the last 45 amino acids of APP, including therefore the A β region. Accordingly, SecPS2NT is capable of binding to A β -immunoreactive species in conditioned medium. In addition, a second region in the extracellular domain of APP also interacted with PS2. Comparable results with PS1 indicate that the two presenilins share similar determinants of binding to APP. Confirming these results, SecPS2NT is able to inhibit PS1/APP interaction. Such a competition makes it unlikely that the PS/APP interaction results from nonspecific aggregation of PS in transfected cells. The physical interaction of presenilins with a region encompassing the A β sequence of APP could be causally related to the misprocessing of APP and the production of A β 1-42. © 1999 Academic Press

INTRODUCTION

The large majority of early-onset familial Alzheimer's disease (AD)² cases are linked to mutations on the two homologous presenilin genes, PS1 and PS2 (Levy-Lahad *et al.*, 1995; Rogaeu *et al.*, 1995; Sherrington *et al.*, 1995). Presenilins are transmembrane pro-

teins with at least six transmembrane regions (Doan *et al.*, 1996; Lehmann *et al.*, 1997; Li & Greenwald, 1996). All pathological mutations reported so far (over 40) are missense mutations except for one leading to deletion of one exon, which suggests a pathological gain of function (Hardy, 1997). However, the physiological function of PS is still largely uncharacterized. Both presenilins are expressed ubiquitously. In the central nervous system, presenilins are expressed essentially in neurons, with a large distribution throughout the brain (Elder *et al.*, 1996; Kovacs *et al.*, 1996; Moussaoui *et al.*, 1996). At the subcellular level, presenilins are localized in the endoplasmic reticulum (Cook *et al.*, 1996; Kovacs *et al.*, 1996; Moussaoui *et al.*, 1996), a cellular compartment where a large fraction of APP is

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²Abbreviations used: AD, Alzheimer's disease; PS, presenilin; APP, amyloid precursor proteins; sAPP, secreted form of APP; ER, endoplasmic reticulum.

³L. Pradier, unpublished observation.

also present. *In vivo*, the presenilin polypeptides are processed into amino- and carboxy-terminal fragments with no full-length PS detectable. This processing can be saturated by overexpression of PS in transfected cells or in PS1-transgenic animals (Czech et al., 1998; Thinakaran et al., 1996) although the physiological function of this cleavage is still unknown.

It has been demonstrated that PS mutations modify APP processing, leading to an increase in production of the long form of the amyloid peptide, A β 1-42 (42 amino acids), compared to A β 1-40 (40 amino acids) in carrier patients (Scheuner et al., 1996), in transfected cells (Borchelt et al., 1996; Tomita et al., 1997; Xia et al., 1997a), and in transgenic mice (Borchelt et al., 1996; Citron et al., 1997; Duff et al., 1996). Therefore, mutations of PS and APP result in the same phenotype (Hardy, 1997). The convergence of genetic evidence onto A β 42 would strongly suggest that this form of A β plays a critical role in AD pathology. Cellular mechanisms leading to A β 42 production could therefore represent a key target for therapeutical intervention. Recently, a physical interaction between APP and presenilins (PS1 and PS2) was demonstrated by coimmunoprecipitation (Weidemann et al., 1997; Xia et al., 1997b). Only the immature, N-glycosylated form of APP interacts with presenilins, consistent with the localization of both immature APP and presenilins in the endoplasmic reticulum. Interestingly, it is also within the endoplasmic reticulum that the production of intracellular A β 42 has been shown to occur in neurons (Cook et al., 1997; Hartmann et al., 1997). We report here on the characterization of APP/PS interaction and the fine-mapping of the interacting domains on PS2 and PS1 on the one hand and APP on the other. In particular, a secreted form of the hydrophilic N-terminus of PS2 (87 residues) is capable of binding to APP and of preventing its interaction with PS1. Furthermore, we present evidence that at least two domains of APP interact with PS1 and PS2, one of them encompassing the A β sequence.

MATERIALS AND METHODS

Expression Constructs

Expression vectors for APP and SP-C100, which consists of the last 100 carboxy-terminal residues of APP fused to APP signal peptide constructs, have been previously described (Dyrks et al., 1993). APP Myc-C100-Flag (MC100F) and APP Myc-C45-flag (MC45F, the last 45 carboxy-terminal residues of APP) were constructed by PCR amplification with addition of a

Myc epitope (MEQKLISEEDL) to the N-terminus and a Flag epitope (DYKDDDDK, IBI-Kodak) to the C-terminus. MC100F and MC45F cDNAs were subcloned into pSV2 vector (Mercken et al., 1991). sAPP α and sAPP β cDNAs were generated by insertion of a stop codon at the α - and β -cleavage sites, respectively, of APP by PCR and subcloned into the expression vector pcDNA3 (Invitrogen) as well as all the following constructs. PS1 and PS2 cDNAs were flanked by the 5' and 3' noncoding sequences of the *Xenopus laevis* β -globine gene (Pradier et al., 1996). PS2 constructs with progressive deletions of the carboxy-terminus were obtained by restriction digestion using either the *Pst*I site (nucleotide 679, PS2 Δ C2 terminating after the fourth transmembrane domain at residue 228), the *Msc*I site (nucleotide 590, PS2 Δ C3 terminating after the third transmembrane domain at residue 198), or the *Nco*I site (nucleotide 504, PS2 Δ C4 terminating after the second transmembrane domain at residue 168), taking as position 1 the starting codon (see Fig. 2). The PS2 hydrophilic N-terminus (amino acids 1 to 87) was generated by PCR amplification and subcloned in-frame with an N-terminal Myc epitope (mycPS2NT) in pcDNA3 or in-frame with the I κ -chain leader sequence (SecPS2Nt) in pSecTagB vector (Invitrogen). Similar to PS2, a PS1 carboxy-terminus truncated construct was obtained by restriction digest at the *Pf*MI site (nucleotide 636, PS1 Δ C2 terminating after the fourth transmembrane domain at residue 213). All constructs obtained by PCR were fully sequenced using fluorescent dideoxynucleotides (ABI Prism). The human neurokin1 receptor (NK1) expression vector has been previously described (Pradier et al., 1995).

Cell Expression and Immunoprecipitation

COS1 cells were transfected using lipofectamine reagent (Gibco BRL) together with a synthetic peptide (KTPKKAKKPKTPKKAKKP) to enhance DNA compaction. The ratio of DNA/peptide and of DNA/lipofectamine was in both cases 1:8 (w/w). For cotransfections, the total amount of DNA transfected was kept constant by the addition of the empty pcDNA3 vector. Cells were lysed 48 h after transfection in 10 mM Tris, pH 7.5, 1 mM EDTA, 1% Triton X-100, 1% NP-40 with protease inhibitors (Complete, Boehringer Mannheim). To solubilize membrane proteins, lysates were sonicated and stirred overnight at 4°C. After centrifugation at 15,000g for 20 min at 4°C, supernatants were recovered as detergent-soluble fractions. For immunoprecipitation, 5 μ l of polyclonal antibodies directed to the N-terminus of PS2, 95041 (Weidemann et al., 1997),

or PS1, 1805 (Duff *et al.*, 1996), or to a non-PS2 related protein, anti-a, were incubated overnight with 100 μ g of the detergent-soluble lysates in modified RIPA (150 mM NaCl, 25 mM Tris, pH 7.4, 1% DOC, 1% NP-40, 0.1% SDS). SDS-PAGE analysis was carried out on Nu-PAGE gels (Novex). A β immunoprecipitation from conditioned medium was performed in a similar manner using a rabbit polyclonal A β antibody (L6) raised against A β 1-40 synthetic peptide (unpublished data). For Western blots, primary antibodies to PS2 (95041), APP holoprotein (22C11, Boehringer Mannheim), A β peptide (W0-2; Ida *et al.*, 1996), or the carboxy-terminal domain of APP (CT-43, Stephens & Austen, 1996) were used.

RESULTS

Characterization of APP/PS2 Interaction in Transfected Cells

As previously reported using the same polyclonal antiserum directed against amino acids 43–58 of PS2 (Weidemann *et al.*, 1997), APP can be readily identified in PS2 immunoprecipitates of COS1 cells cotransfected with APP and PS2 (Fig. 1A, lanes 3 and 4). APP could not be detected in immunoprecipitates if a non-PS2-related serum was used (Fig. 1B, lanes 3 and 4) or the preimmune serum was used (see Weidemann *et al.*, 1997). As a further control, APP was not detected in

immunoprecipitates if APP was coexpressed with PS1 (Fig. 1A, lane 5) or with another hydrophobic protein, NK1, a classical G-protein-coupled receptor (Fig. 2C, lane 2). Expression of the NK1 receptor in this system has been previously shown by us to reach 5 pmol/mg membrane proteins in radioligand binding experiments (Pradier *et al.*, 1995), with a significant fraction in the ER/Golgi apparatus compartment (Tarasova *et al.*, 1997) where presenilins are mainly localized. Together, these data indicate that the APP/PS2 interaction does not result from the nonspecific precipitation of APP, trapped in hydrophobic presenilin protein aggregates.

The fraction of APP coimmunoprecipitated with PS2 represents only a small percentage of total APP levels (Fig. 1A; the total amount of proteins in lane 1 represents one-fifth of proteins in the immunoprecipitation for lane 3). In cells transfected with PS2 only, the endogenous APP751 isoform could be detected at very low levels in PS2 immunoprecipitates (Fig. 1A, lane 6), indicating that APP/PS2 interaction can take place at normal APP levels. However, higher APP expression makes this interaction more readily detectable (see for instance Fig. 1B, lanes 6 and 7, in which interaction with endogenous APP cannot be detected).

Similar to other reports (Weidemann *et al.*, 1997; Xia *et al.*, 1997b), only the low-molecular-weight form of APP (immature N'-glycosylated form of the endoplasmic reticulum) was shown to coprecipitate with PS2

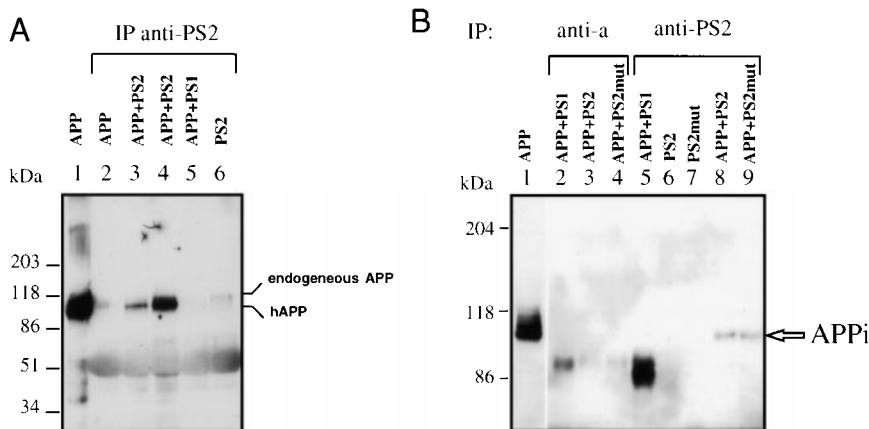


FIG. 1. Characterization of APP/PS2 interaction. COS1 cells were transfected and lysed as described under Materials and Methods. In both panels, lane 1 represents total protein lysate (five times less than for immunoprecipitates). (A) Lysates were immunoprecipitated with anti-PS2 antibody 95041 and immunoblotted with the APP antibody, 22C11. APP can be detected in PS2 immunoprecipitates from cells transfected with APP and PS2 (lanes 3 and 4) but not with APP and PS1 (lane 5). Endogenous APP, APP751, can also be weakly detected in immunoprecipitates of PS2 transfected cells. (B) Immunoprecipitation with a PS2 nonrelated serum (anti-a, lanes 2–4) does not reveal any APP signal in APP and PS2 transfected cells (lane 4). In immunoprecipitation of the same samples with anti-PS2 (lanes 5–9), only the low-molecular-weight immature form of APP (APPi) interacts with PS2 wild-type (lane 8) and PS2 mutant N141I (lane 9).

be more efficient for the full-length PS2. Additional regions on PS2 could therefore be involved in binding (but see Discussion). Alternatively, APP could be a limiting factor under these conditions. To further delineate the region of interaction on PS2, the hydrophilic N-terminus of PS2 (residues 1 to 87) was fused to either a myc epitope sequence for cytoplasmic localization (mycPS2NT) or to the Ig κ -chain signal peptide for targeting to the luminal compartment (SecPS2NT). Both mycPS2NT and SecPS2NT proteins were detected in cell lysates (Fig. 3A, bottom) without production of the multimeric forms detected for the membrane-anchored PS2 truncated forms. Only SecPS2NT was detected in cell medium (Fig. 3B, lanes 3 and 4, bottom). SecPS2NT migrated as a closely spaced triplet band in SDS-PAGE, possibly reflecting posttranslational modifications. The difference in molecular weight with mycPS2NT is most likely due to the extra myc epitope. In cell lysates, APP could be coimmunoprecipitated with the secreted form of PS2NT (Fig. 3A, lane 2, top), demonstrating that the first 87 residues of PS2NT were sufficient for interaction with APP. However, no interaction of the cytoplasmic mycPS2NT with APP could be detected (Fig. 3A, lane 1, top), indicating that the localization to the lumen of cellular organelles is

obligatory for interaction. In agreement with this finding, the secreted form of APP could also be detected in association with SecPS2NT in cell medium (Fig. 2B, lane 3); the APP/PS2NT interaction was therefore strong enough to persist through the different steps of the secretory pathway.

Mapping the Interacting Domain on APP

We used a similar approach with truncated forms of APP to map the interaction domain with PS2. Two forms of the 100 carboxy-terminal residues of APP with the APP signal peptide (SP-C100, initially named SPA4CT, see Dyrks *et al.* (1993)) and without the signal peptide (MC100F) were used (Fig. 4, bottom right). The signal peptide of SP-C100 is cleaved off after translation and the resulting fragment (C100) resembles APP cleaved at the β -secretase site (Dyrks *et al.*, 1993). SP-C100 and MC100F expression lead to A β secretion although more efficiently for SP-C100 (data not shown). Both forms were shown to coimmunoprecipitate with PS2 in cell lysates (Fig. 4A, lanes 3 and 4). The higher molecular weight of the MC100F peptide is due to the presence of myc and flag epitopes at the N- and C-termini of C100, respectively. SP-C100 gave rise to

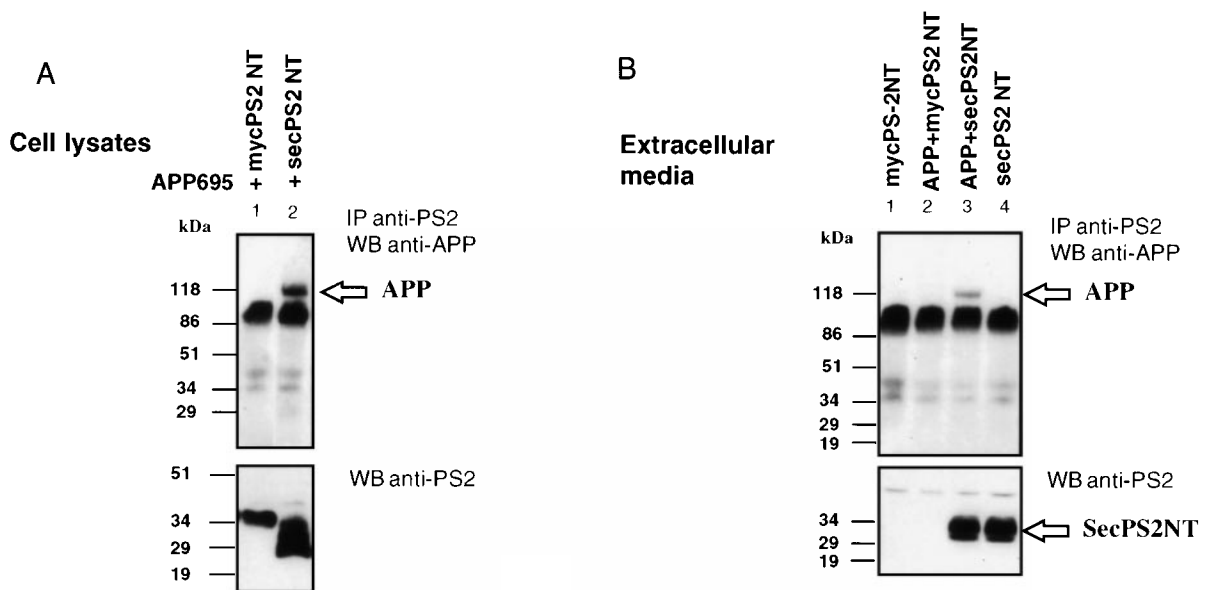


FIG. 3. APP interacts with the secreted but not the cytosolic PS2N-terminal construct in cell lysates and extracellular medium. Constructs of the hydrophilic PS2NT (residues 1–87) were generated without and with a signal peptide inducing secretion. Top panels represent immunoprecipitates of samples with the PS2 antibody and Western blots detected with the APP W0-2 antibody. Bottom panels represent direct immunoblot analysis of samples with PS2 antibody. (A) In cell lysates, only the form with signal peptide and not its cytosolic counterpart interacts with APP as detected by coimmunoprecipitation (compare lanes 1 and 2). (B) In conditioned medium, SecPS2NT is indeed secreted (lanes 3 and 4, bottom panel). SecPS2NT again interacts with secreted APP (lane 3, top panel), whereas mycPS2NT does not (lane 2).

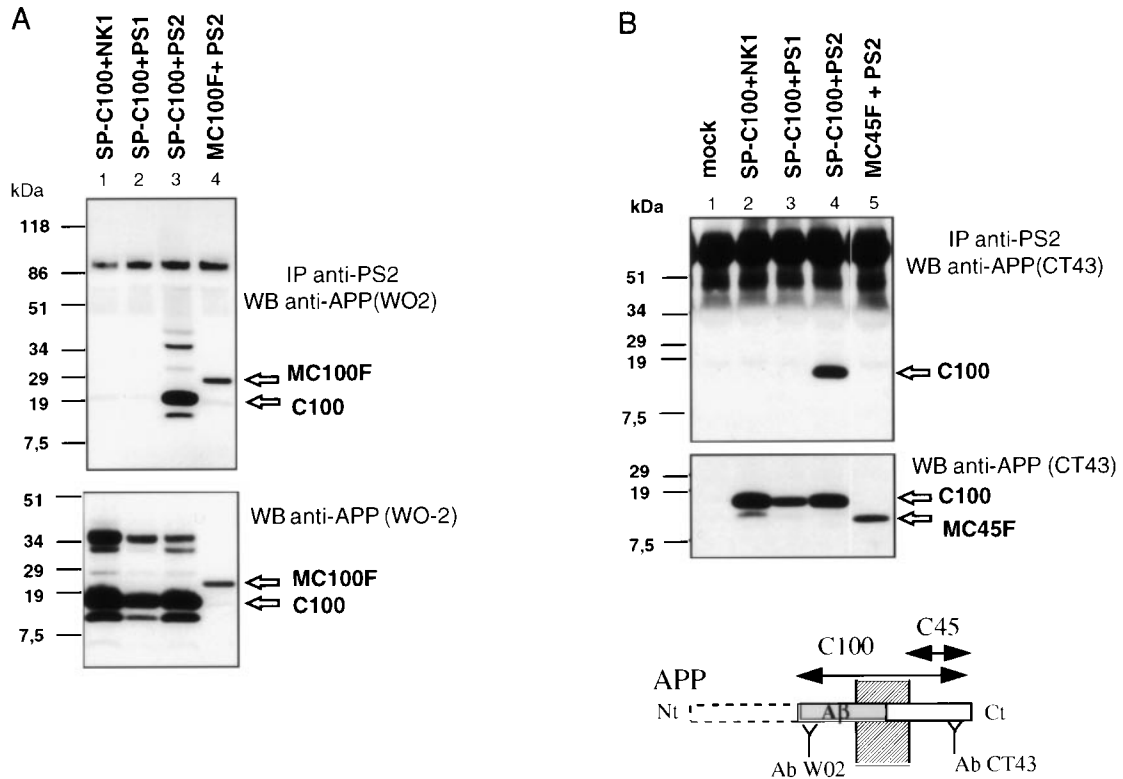


FIG. 4. PS2 interacts with the last 100 but not the last 45 C-terminal residues of APP. Transfected cell lysates were immunoprecipitated with the PS2 antibody (top panels) and Western blots detected with the APP antibodies W0-2 (A) or CT43 (B). Direct Western blot analysis of the same lysates with the same APP antibodies was also performed (bottom panels). The bottom right panel indicates the location of the 100 (C100) and 45 (C45) carboxy-terminal fragments of APP and of the W02 and CT43 epitopes. (A) In cell lysates, carboxy-terminal APP C100 fragments with (SP-C100) and without signal peptide (MC100F with a myc and flag epitopes) interact equally well with PS2 (lanes 3 and 4) as detected by immunoprecipitation with the PS2 antibody. C100 is not detected in immunoprecipitates if it is coexpressed with NK1 or PS1 although C100 is expressed at similar levels (bottom panel). (B) C100 interacts with PS2 (lane 4) but not with the cytoplasmic domain of APP (MC45F with myc and flag epitopes, lane 5) although both are expressed at comparable levels (bottom panel).

two additional peptides at 16 and 35 kDa when detected with the W02 antibody which recognizes residues 1–10 of the A β peptide (Fig. 4A, lane 3) but not with the APP carboxy-terminal CT43 antibody (Fig. 4B, lane 4). All three peptides derived from SP-C100 were shown to interact with PS2. In contrast, the cytoplasmic domains of APP (last 45 carboxy-terminal residues, MC45F) could not be immunoprecipitated with PS2 (Fig. 4B, lane 5). This was not due to the sensitivity of the detection with the CT43 antibody since C100 was readily detectable in PS2 immunoprecipitates (Fig. 4B, lane 4). The controls with PS1 and NK-1, two other multitransmembrane domain proteins, showed the specificity of this interaction (Fig. 4B, lanes 2 and 3). We consider it unlikely that the additional myc and flag epitopes on the APP cytoplasmic domain (MC45F) could hinder the interaction with

PS2 since the same epitopes on the 100 carboxy-terminal residues of APP (MC100F) did not prevent coimmunoprecipitation with PS2 (see above). These results show that PS2 interacts with the last 100 but not the last 45 C-terminal residues of APP, a region including the amyloid peptide A β sequence.

To combine the results on mapping the interaction sites on APP and PS2, we next demonstrated that APP C100 could be immunoprecipitated with the secreted form of the PS2 N-terminus, residues 1–87, in cell lysates (Fig. 5A, lane 1). This confirmed that these two limited segments of APP and PS2 were sufficient for interaction. The SP-C100 form of APP has been previously shown to generate high levels of A β peptide in cell medium (Dyrks *et al.*, 1993). In conditioned medium of cells expressing SP-C100 and SecPS2NT, A β can be detected in PS2 immunoprecipitates (Fig. 5B,

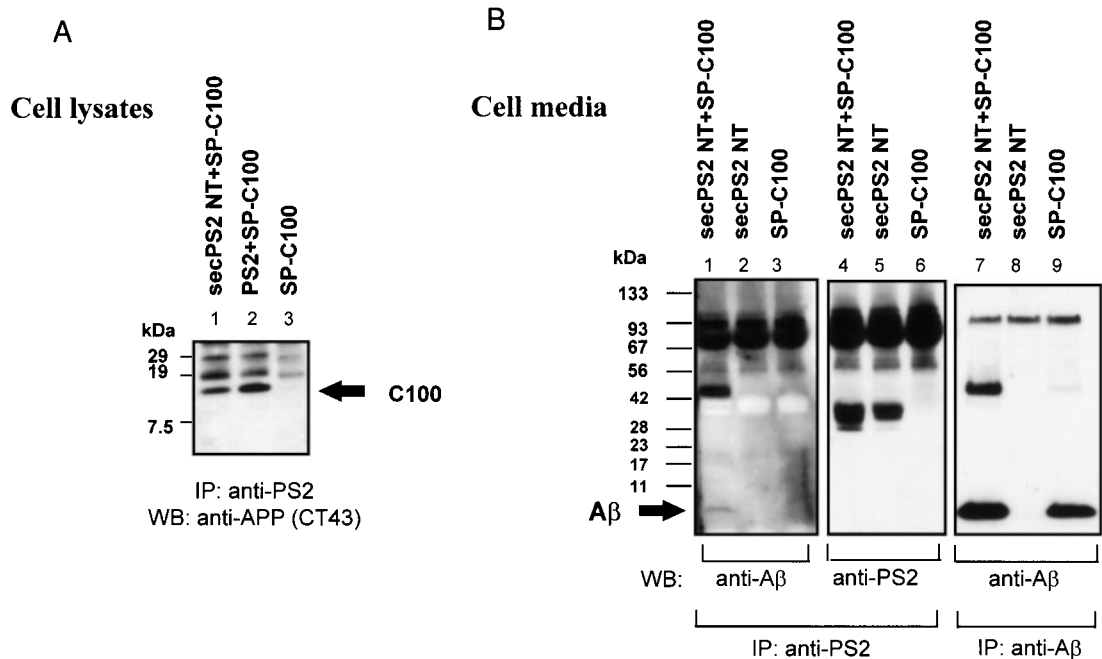


FIG. 5. SecPS2NT interacts with APP C100 in lysates and with A β in cell medium. Transfected cell lysates were immunoprecipitated with the PS2 antibody and Western blots detected with several antibodies as described. (A) The APP C100 domain can be immunoprecipitated together with SecPS2NT (lane 1) and full-length PS2 (lane 2) in cell lysates. (B) In conditioned medium, the A β 4-kDa band can be detected with W0-2 in PS2 immunoprecipitates (lane 1). In addition, a 42-kDa band immunoreactive for W0-2 can also be detected. As a control, the same blot was stripped and revealed with PS2 antibody, indicating similar levels of immunoprecipitated PS2NT in double transfectants (lanes 4 and 5). Total levels of A β in conditioned medium were also analyzed by immunoprecipitation with L6, a polyclonal A β antibody, and immunoblotting with W0-2 (lanes 7 to 9). A β levels were similar in single and double transfectants (lanes 7 and 9) but the SP-C100-related 42-kDa species was dramatically increased upon coexpression with SecPS2NT.

lane 1), indicating a direct interaction between A β and PS2NT. This interaction cannot be detected if SecPS2NT or SP-C100 is expressed individually (Fig. 5B, lanes 2 and 3). As controls, levels of SecPS2NT protein (Fig. 5B, lanes 4 and 5) or of total secreted A β (Fig. 5B, lanes 7 and 9) were not modified in double transfections. Additionally, a second A β -immunoreactive band at approximately 42 kDa is also detected in PS2 immunoprecipitates of cotransfected cells (Fig. 5B, lane 1). This 42-kDa species could also be identified using a second A β antibody (4G8) directed against a different epitope on A β (data not shown). By immunoprecipitation of conditioned medium with a third antibody against A β (polyclonal L6), the SP-C100-related 42-kDa species could also be detected and its presence is dramatically increased upon coexpression with SecPS2NT (Fig. 5B, lanes 7 and 9). The exact identity of the 42-kDa species remains to be determined but it contains part of the A β sequence (as exemplified with three different antibodies) and could represent either an APP C100 multimer or a SDS-stable protein complex.

Additional Interaction Sites on APP

We had demonstrated the interaction of SecPS2NT with APP C100. However, our initial results also indicated that SecPS2NT bound to secreted APP (sAPP) in cell medium, a form of APP for the most part processed at the α -cleavage site and containing only the first 15 residues of APP C100 and A β peptide. Therefore, additional domains on sAPP could be involved in PS2 binding. To test this hypothesis, two truncated forms of APP terminating at the endogenous β - and α -cleavage sites of APP, respectively, were constructed to generate sAPP β and sAPP α .

These proteins were expressed and detected with 22C11, an antibody recognizing the common N-terminal region of APP. In coexpression studies, both sAPP α and sAPP β could be coimmunoprecipitated with full-length PS2 (Fig. 6, lanes 3 and 4) and with SecPS2NT (Fig. 6, lanes 5 and 6). Therefore, apart from A β and the transmembrane domain of APP, at least one other domain within the extracellular region of

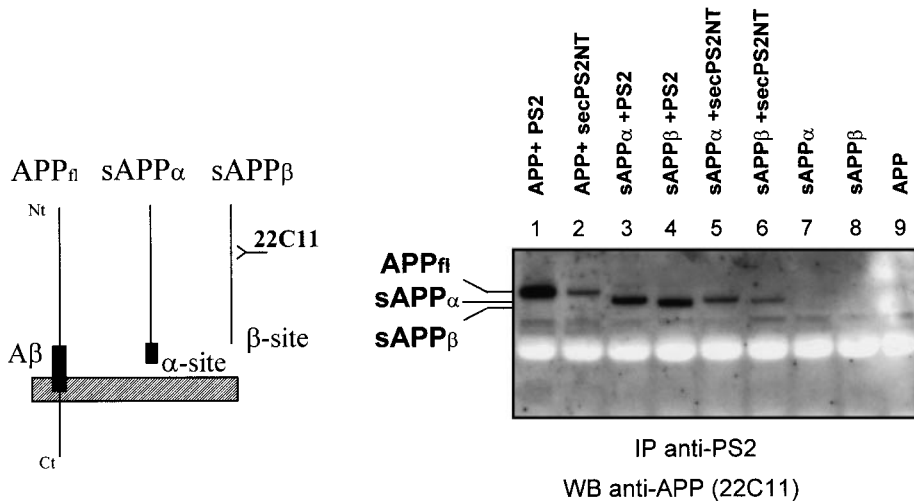


FIG. 6. A second region on secreted APP (sAPP) also interacts with PS2 and SecPS2NT. Artificial soluble sAPP α and sAPP β cDNAs were constructed by insertion of a stop codon at the respective α - and β -cleavage sites (left). Transfected cell lysates were immunoprecipitated with the PS2 antibody and the Western blot detected with the APP N-terminal antibody (22C11) recognizing all forms. Both constructs are expressed and interact with PS2 and SecPS2NT in cell lysates (lanes 3 and 4 and lanes 5 and 6, respectively). Therefore, another domain of sAPP can interact with PS2 and SecPS2NT apart from the A β segment.

APP is also involved in the interaction with the N-terminus of PS2 and further experiments will be required to map this second site.

APP/PS1 Interaction

PS1 and PS2 present an extensive homology and recently, PS1 was also shown to coimmunoprecipitate with APP in transfected cells (Xia *et al.*, 1997b). It is likely that PS1 and PS2 would share similar determinants of binding to APP. Mapping of the APP/PS1 interacting domains was therefore performed according to our previous results with PS2. Indeed, the APP C100 fragment can be selectively coimmunoprecipitated with PS1 (Fig. 7A, lane 4). By analogy to PS2, we constructed a truncated version of PS1 extending to the fourth transmembrane domain, residue 213, PS1 Δ C2 (see Fig. 2A). Again, full-length APP was shown to interact with PS1 Δ C2 (Fig. 7B, lane 4) by coimmunoprecipitation. These two results combined suggest that PS1 could interact with APP through essentially the same binding region as PS2.

Inhibition of APP/PS1 Interaction by SecPS2NT

The similarity of PS1 and PS2 binding domains to APP raised the possibility that the two presenilins could compete for interaction with APP. SP-C100 was expressed with PS1 wt or with mutant PS1-M146L.

C100 interacts with both wt and mutant forms of PS1 (Fig. 8A, lanes 1 and 2). If SecPS2NT, the shortest PS2 sequence that interacts with APP, is cotransfected with SP-C100 and PS1, C100 is no longer detectable in the PS1 immunoprecipitates (Fig. 8A, lanes 3 and 4) but becomes apparent in the subsequent PS2 immunoprecipitation (Fig. 8B, lanes 3 and 4). Therefore, SecPS2NT is capable of preventing the interaction of PS1 with C100 by binding itself to C100. Similar competition results have been obtained using full-length APP instead of C100 (data not shown). These data further confirm the similarity in the binding determinants of PS1 and PS2 to APP and the possibility of a heterologous competition between the two presenilins. Additionally, the displacement by the hydrophilic SecPS2NT confirms that the interaction of APP and PS is not due to a nonspecific aggregation of the multitransmembrane PS proteins trapping APP.

DISCUSSION

Pathological mutations on either APP or presenilins lead to a similar phenotype, an increase in A β 42 production. The recently demonstrated physical interaction between these proteins in transfected cells could, therefore, represent a key step in the formation of the amyloid peptide, especially the A β 42 form. In the present report, we have analyzed the different

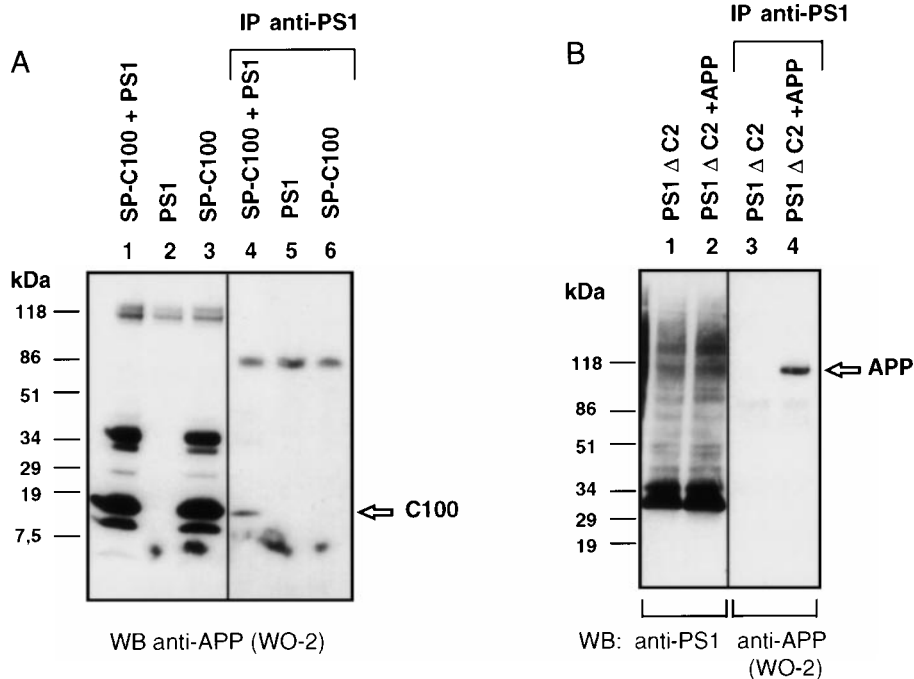


FIG. 7. Domains involved in APP/PS1 interaction are similar to APP/PS2. Transfected cell lysates were immunoprecipitated with the PS1 antibody 1805 and subsequent analysis with the A β antibody WO-2. (A) Similar to PS2, the APP C100 fragment is detectable in PS1 immunoprecipitates (lane 4). (B) Furthermore, a C-terminal truncated form of PS1 (PS1 Δ C2, see Fig. 1A) also binds to APP (lane 4). Altogether, these data suggest that the interactions between APP/PS1 and APP/PS2 involve similar protein domains.

protein domains involved in the APP/PS2 interaction and extended these data to PS1. Using truncated forms of PS2, the N-terminal part of the protein was shown to be sufficient for interaction with APP. The hydrophilic N-terminal domain of PS2 (residues 1–87) was sufficient for binding to APP when targeted to the lumen of the endoplasmic reticulum by addition of a signal peptide but not when expressed in the cytosol. On APP, two extracytosolic (i.e., transmembrane or luminal) domains were shown to interact with full-length PS2 and the secreted form of PS2NT. First, the region encompassing A β and the transmembrane region of APP (last 100 but not 45 C-terminal residues) interacted with PS2 and SecPS2NT. Moreover, A β itself could be coimmunoprecipitated with SecPS2NT from conditioned medium. Second, two APP constructs representing physiologically secreted forms of APP (sAPP α and sAPP β), devoid of cytoplasmic or transmembrane regions, were shown to interact with PS2 in cell lysates and with SecPS2NT in cell lysates and conditioned medium. This strongly suggests that the interaction with APP takes place in the luminal compartment of the cell even for full-length PS2. These mapping data could be partially extended to PS1. Most

importantly, the secreted but not the cytoplasmic form of PS2NT was shown to displace the interaction between APP and full-length PS1 efficiently. Overall, our data indicate that in the APP/PS2 (or PS1) complex, the PS2 N-terminus is localized, like A β and secreted APP, to the luminal side of the ER membrane. This is definitely at odds with several studies of the topology of the highly related PS1 protein (De Strooper *et al.*, 1997; Doan *et al.*, 1996; Lehmann *et al.*, 1997; Li & Greenwald, 1996). By antibody labeling under differential permeabilization conditions and chimeric constructions with a reporter protein, the N-terminus of PS1 and of its *Caenorhabditis elegans* homologue Sel12 was localized to the cytoplasm although a different team favored an extracellular localization of the PS1 and PS2 N-terminus (Dewji & Singer, 1996, 1997a, 1997b). However, the present results on PS2 N-terminus localization apply only to the small fraction of PS2 molecules interacting with APP and not necessarily to the native PS conformation. In such APP/PS2 complexes, PS2 could potentially display an altered topology with a translocation of the N-terminus to the lumen. It is well documented that a multitransmembrane protein like MDR can exist in at least two alternative topologies,

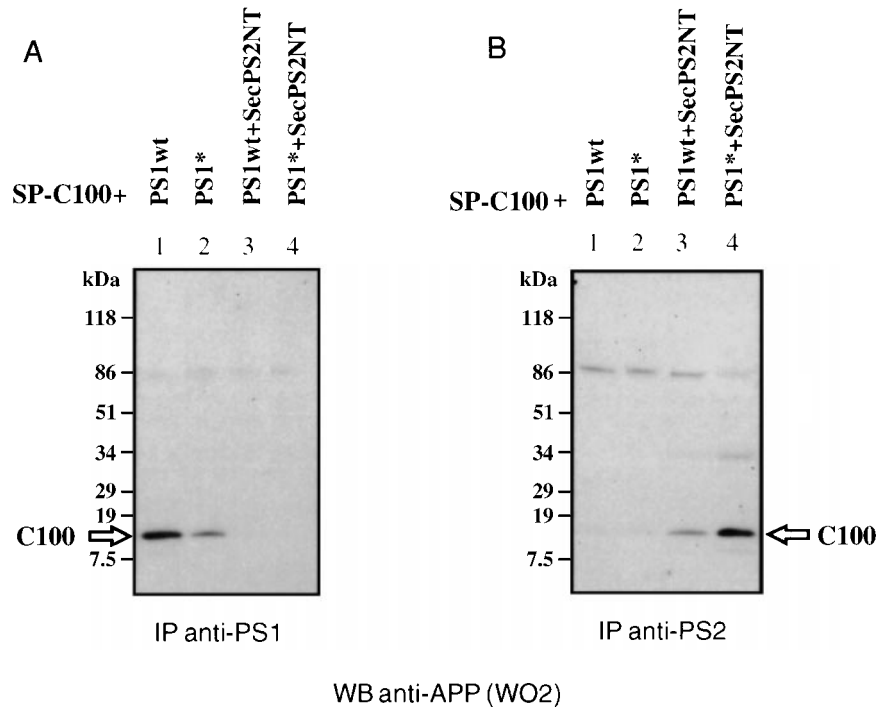


FIG. 8. SecPS2NT can displace PS1/APP C100 interaction. COS1 cells were cotransfected with SP-C100 and PS1wt or PS1M146L mutant (PS1*) without or with SecPS2NT. (A) PS1wt and PS1M146L mutant interact with C100 (lanes 1 and 2), whereas in the presence of SecPS2NT, no C100 can be detected in PS1 immunoprecipitates (lanes 3 and 4). (B) The supernatants of the previous precipitations were further immunoprecipitated with the PS2 antibody. Clearly, C100 interacts with SecPS2NT (lanes 3 and 4). Therefore, SecPS2NT can displace the interaction of PS1 and APP by binding to APP. This strongly suggests that the APP binding sites are similar for PS1 and PS2.

including a change in the orientation of the C-terminus. It has been proposed that the different topologies might subserve different functions (Zhang *et al.*, 1993; reviewed in Levy, 1996). APP/presenilin interaction takes place in the endoplasmic reticulum where nascent proteins are inserted in the membrane (Hegde & Lingappa, 1997). Binding of the N-terminus of PS2 to nascent APP during its transfer through the translocon could potentially lead to translocation of this domain of PS2 with APP. Alternatively, APP degradation in the ER has been proposed to contribute to A β production (Zhong *et al.*, 1994) and APP/PS interaction could participate in this process (Weidemann *et al.*, 1997). ER degradation of misfolded luminal and transmembrane proteins is mediated by a retrograde export to the cytoplasm by the translocon (Kopito, 1997; Plemper *et al.*, 1997) for targeting to the proteasome. Retrograde transport would necessarily involve major alterations in protein topology/structure, allowing the interaction between luminal epitopes of APP and the PS2 N-terminus. Further studies will be required to elucidate this issue.

We have demonstrated that the N-terminus of PS2 is

sufficient for APP binding. Other PS2 domains could potentially participate in the interaction but are likely to have a much weaker contribution since SecPS2NT can displace the interaction between full-length PS1 and APP. The mapping of the interaction to a short hydrophilic sequence on PS2 (87 residues) as well as the competition experiments demonstrates that this interaction is not an artifact of the well-known aggregation properties of the hydrophobic presenilins, which was our initial concern. The key role of the first 87 residues of PS2 might also explain why wild-type PS2 and mutant (N141I) PS2 interact equally well with APP although subtle quantitative differences would not be detectable in our assay. Our results on PS1 suggest that the determinants of binding to APP are very similar to PS2, which is further confirmed by the displacement of APP/PS1 interaction by SecPS2NT.

At least two domains of APP interact with the N-terminus of PS2. The A β sequence and the adjoining transmembrane domain of APP can interact with PS2 but with no involvement of the APP cytoplasmic extremity. The lack of requirement for an APP cytoplasmic domain in the interaction had also been previously

reported (Xia *et al.*, 1997b). It is interesting to note that the A β sequence within the APP holoprotein has been shown to be critical for proper intracellular routing of APP both in epithelial cells (De Strooper *et al.*, 1995) and in neurons infected with a recombinant Semliki Forest virus (Tienari *et al.*, 1996). The A β sequence could therefore serve as a binding epitope for several proteins involved in the correct sorting of the holoprotein. A second domain on the secreted APP, N-terminal from the A β sequence, also interacts with PS2, which is consistent with the finding that the APP-related protein APLP2 (which does not contain an A β -like sequence) interacts with PS2 (Weidemann *et al.*, 1997). We are currently mapping this second domain on APP.

Several lines of evidence point toward a critical role of APP/PS interaction in the production of A β 42, the commonly presented culprit in AD. The pathological mutations on APP and on the two presenilins lead to a common phenotype: increased production of A β 42. The physical interaction between the two proteins (wild type or mutants) in the endoplasmic reticulum is the most straightforward substrate for this identical phenotype. Indeed, in neurons, A β 42 appears to be specifically produced in the endoplasmic reticulum, the subcellular site of interaction of APP and PS (Cook *et al.*, 1997; Hartmann *et al.*, 1997). Additionally, in neurons of PS1-KO mice, production of A β is drastically reduced (De Strooper *et al.*, 1998). Finally, coexpression of PS2 and APP leads to a marked decrease in cellular APP levels (not in synthesis rates), suggesting that APP could be subject to a PS2-dependent degradation in transfected cells.³ Our present results demonstrating a direct interaction of PS2 with a short domain of APP encompassing the A β sequence would further suggest a role for presenilins in the production of the amyloid peptide.

The mapping of the domains of interaction between APP and PS has been conducted in the artificial context of overexpressing cells. Such an approach has allowed us to identify a dominant SecPS2NT construct which can effectively compete with the APP/presenilin interaction. It represents a key tool to further test the functional relevance of this interaction in terms of A β production in transfected cells and in more physiological settings such as neurons with endogenous levels of APP and presenilins.

The present fine-mapping of the interaction domains between presenilins and APP should allow the development of molecules disrupting this interaction and potentially blocking the production of A β 42, a key step toward the treatment of AD. As this interaction is also detectable with wild-type APP and presenilins

present in the large majority of AD cases, this new therapeutic approach should be, in principle, applicable to AD patients in general and not just familial cases.

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