# Oligomerization of human presenilin-1 fragments

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**Abstract To gain insight into presenilin-1 (PS1) structural aspects, we explored the structure–function relationship of its N- and C-terminal (NTF and CTF, respectively) complexes. We demonstrated that both NTF and CTF act as independent but inter-changing binding units capable of binding each other (NTF/CTF) or their homologues (NTF/NTF; CTF/CTF). The Alzheimer's disease-associated PS1 mutations Y115H and M146L do not affect their ability to hetero- and/or homodimerize, thus conserving their basic integrity and function(s). These results suggest that PS1 associates intra-molecularly to form higher order complexes, which may be needed for endoproteolytic cleavage and/or γ-secretase-associated activity.**


*Key words:* Presenilin; γ-secretase; Homodimerization; Heterodimerization

## 1. Introduction

Mutations in the presenilin-1 (*PS1*) gene account for most cases of early onset familial Alzheimer's disease. In vivo, the PS1 protein is approximately 45–50 kDa in size and undergoes a proteolytic cleavage by 'presenilinase' to generate N- and C-terminal fragments (NTF and CTF, respectively) of approximately 32–35 and 16–20 kDa, respectively [1]. PS1 forms also low (~100–180 kDa) and high (>250 kDa) molecular weight complexes containing both full-length and heterodimeric PS1 NTF and CTF fragments [2–5]. Recent work indicates that the higher molecular weight complexes contain, among PS1 fragments, at least three other proteins, namely Nicastrin, Aph-1, and Pen-2 [6,7]. Accumulating evidences tend to demonstrate that the appearance of presenilin fragments in these complexes is intrinsic to PS1 and γ-secretase activity, is involved in the cleavage of integral membrane proteins such as amyloid-β precursor protein, Notch1, ErbB4, etc.

Using the yeast two-hybrid assay and GST pull-down experiments we provide evidence that human PS1 NTF and CTF moieties can associate directly to form specific heterodimers and that AD-associated PS1 mutations Y115H and M146L do not affect their ability to hetero-oligomerize. Using deletion mutants coding for truncated PS1, we demonstrate that multiple binding regions are implicated in PS1 heterodimerization and that both cytoplasmic and transmembrane (TM) regions are necessary. We also show that PS1 may self-oligomerize giving rise to NTF/NTF and CTF/CTF homodimers. Although inter-molecular heterodimers have previously been reported following in situ chemical cross-linking and co-immunoprecipitation [4,8] – which are indirect methods –, our data are the first to confirm a direct interaction between human NTFs and CTFs and identify potential regions of contact under in vivo conditions.

## 2. Materials and methods

### 2.1. Yeast two-hybrid assays

cDNA sequences coding for various parts of human PS1 were amplified by PCR using the Herculase Taq polymerase (Stratagene). The following wild-type (wt) PS1 cDNA were prepared: NTF ($PS1_{1-291}$), 1-132 ($PS1_{1-132}$), TM1-2 ($PS1_{101-153}$), Loop 1-2 ($PS1_{101-132}$), TM2 ($PS1_{131-153}$), Loop 6-7 ($PS1_{239-409}$), CTF ($PS1_{292-467}$), TM8 ($PS1_{406-426}$), CTF-short ($PS1_{427-467}$). AD-associated PS1 short version mutants, 1-132 Y115H and TM1-2 M146L were also generated. cDNAs were cloned both into pGBKT7 and pGADT7 vectors (Clontech) as a fusion to the Gal4-DNA binding domain (BD) and Gal4-DNA activating domain (AD), respectively. Each construct was sequenced and shown to be free of autonomous Gal-4 activation. Functional expression of fusion proteins in yeast was confirmed by Western blot.

Each of the pGBKT7-construct was co-transformed into Y187 or AH109 *Saccharomyces cerevisiae* (Clontech) together with each pGADT7-construct. Transformants were selected on yeast drop-out media (Clontech) that lacked both tryptophan (Trp) and leucine (Leu) ($TL^-$) and assayed for β-galactosidase $(\beta\text{-gal}^+)^+$ in the case of Y187 and $His^+$ and $Ade^+$ activity in the case of AH109. Positive controls included pGBKT7-p53/pGADT7-T antigen and pGBKT7-PS1-Loop 6-7/pGADT7-hNPRAP as previously described [9].

### 2.2. Preparation of yeast cell lysates and immunoblotting

Transformed yeasts were harvested by centrifugation and washed twice in sterile $H_2O$. Pellets were resuspended in ~300 μl of yeast extraction buffer [Tris 50 mM pH 7.5, 150 mM NaCl, 1% CHAPS, 1 mM EDTA, 1 mM PMSF and mini-complete protease inhibitors (Roche-Diagnostic, Indianapolis, IN, USA)]. Proteins were extracted using acid washed glass beads (Sigma, St. Louis, MO, USA) as described by the manufacturer (Clontech, Palo Alto, CA, USA) and loaded on a 4–20% NuPAGE gel (Invitrogen) as described above. Blots were probed with either the anti-*c*-myc (Roche Diagnostics) or anti-PS1 PSN2 (gift from Dr. Hiroshi Mori) antibodies.

### 2.3. GST pull-down assays

PS1-CTF ($PS1_{292-467}$) cDNA was cloned into the pGEX-4T1 vector and fixed on glutathione–Sepharose beads as previously described [10]. Proteins were eluted from the beads, mixed with 2× sample buffer and ~20 μl was loaded on gel for SDS–PAGE. Blots were probed with anti-*c*-myc antibodies (Roche Diagnostics).

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*Abbreviations:* PS1, presenilin-1; NTF, N-terminal fragment; CTF, C-terminal fragment; β-gal, β-galactosidase; His, histidine; Ade, adenine; Trp, tryptophan; Leu, leucine; TM, transmembrane; wt, wild-type

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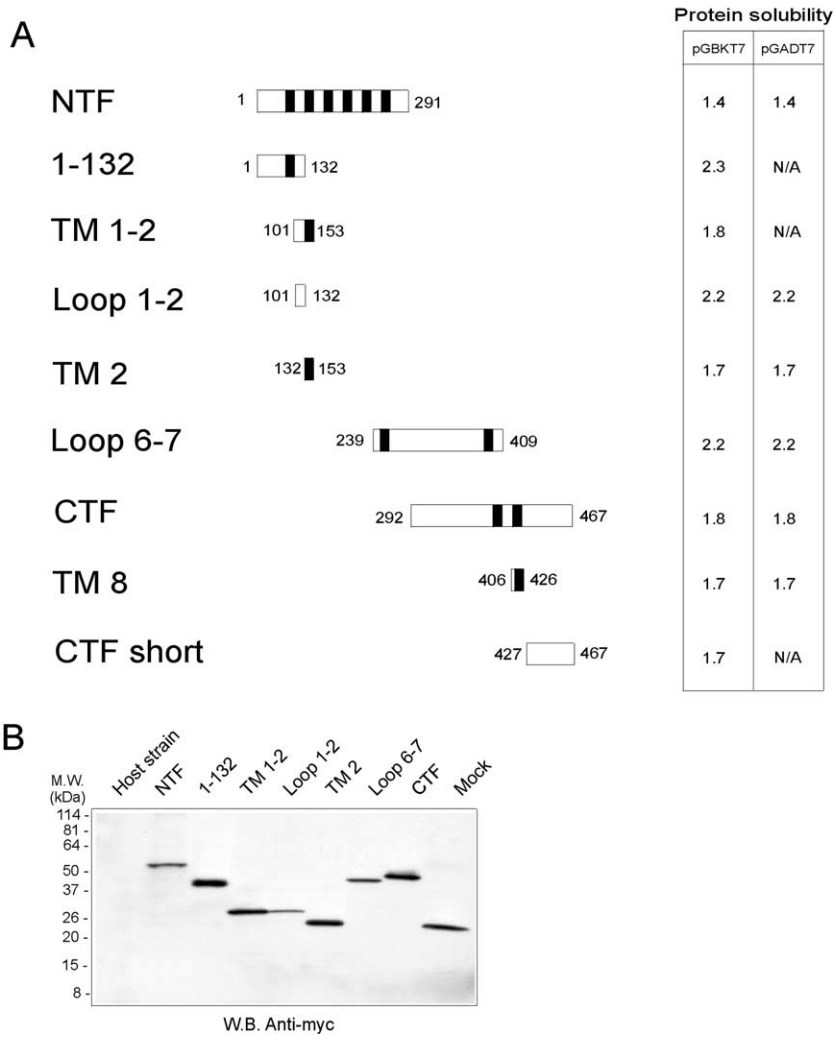


Fig. 1. Schematic representation of PS1 fusion proteins. A: Illustration (not to scale) of fusion proteins coding for PS1 generated fragments. Black squares represent hydrophobic regions corresponding to putative TM domains. Numbers represent the amino acid positions. Kyte–Doolittle hydrophilicity plots for each construct cloned in fusion with Gal-4 binding and/or activating domains are presented. Value smaller than 1.1 indicates an insoluble protein. B: PS1 engineered fragments cloned in fusion with Gal-4 binding domain were expressed in yeast strain Y187. Equal amount of 1% CHAPS protein extracts were run on 4–20% NuPAGE, blotted, and probed with anti-myc antibodies. Note: pGBKT7 empty vector (mock) containing the DNA binding domain (DNA-BD) tagged with *c*-myc epitope runs at approximately 25 kDa. Similar results were obtained with PS1 engineered fragments cloned in fusion with Gal-4 activating domain (data not shown).

## 3. Results

### *3.1. Direct association between PS1 NTF and CTF*

Partial PS1 cDNA constructs were produced in order to represent putative functional domains and sites of multiple protein interactions. Western blot analysis confirmed correct protein expression for all constructs (Fig. 1B). Although certain PS1 constructs tested herein contain hydrophobic TM domains, its fusion to the Gal-4 binding and/or activating domain in the pGBKT7/pGADT7 vectors reveals over-all protein solubility (Fig. 1A and [5]). These constructs were shown to be functional in yeast two-hybrid assays [9–11]. First, we investigated if our NTF (aa 1–291) of human PS1 could form a heterodimer with its remaining CTF (aa 292–467). Specific interaction between NTF (expressed in human HEK293T cells) and CTF is observed following GST pull-down using GST-tagged CTF (Fig. 2A). This result corroborates with previous reports demonstrating NTF/CTF heterodimerization [4,8]. After this initial result, we tested if NTF/CTF heterodimerization could be reproduced in vivo in yeast, a system lacking both endogenous PS1 and γ-secretase activity [10,12]. As shown in Fig. 2B, interaction between the NTF and CTF is observed following a β-gal assay using the yeast strain Y187.

To further verify if these two-hybrid interactions were specific, and not due to non-specific LacZ reporter activation from strain Y187, we cotransformed all constructs in the AH109 yeast strain (Clontech). As shown in Fig. 2C, colony growth on selective medium lacking Ade, His, Trp and Leu ($AHTL^-$) is observed, confirming all β-gal assays. Negative and positive control experiments were done using empty vectors (either pGBKT7 or pGADT7) and hNPRAP, a known CTF PS1-interacting protein [9]. Taken together, these results confirm that PS1 NTF and CTF associate directly to form a stable complex in vivo.

### *3.2. Mapping of PS1-interacting domains*

Using various PS1 cDNA deletion constructs (as shown in Fig. 1A), we tested which domain(s) was implicated in PS1

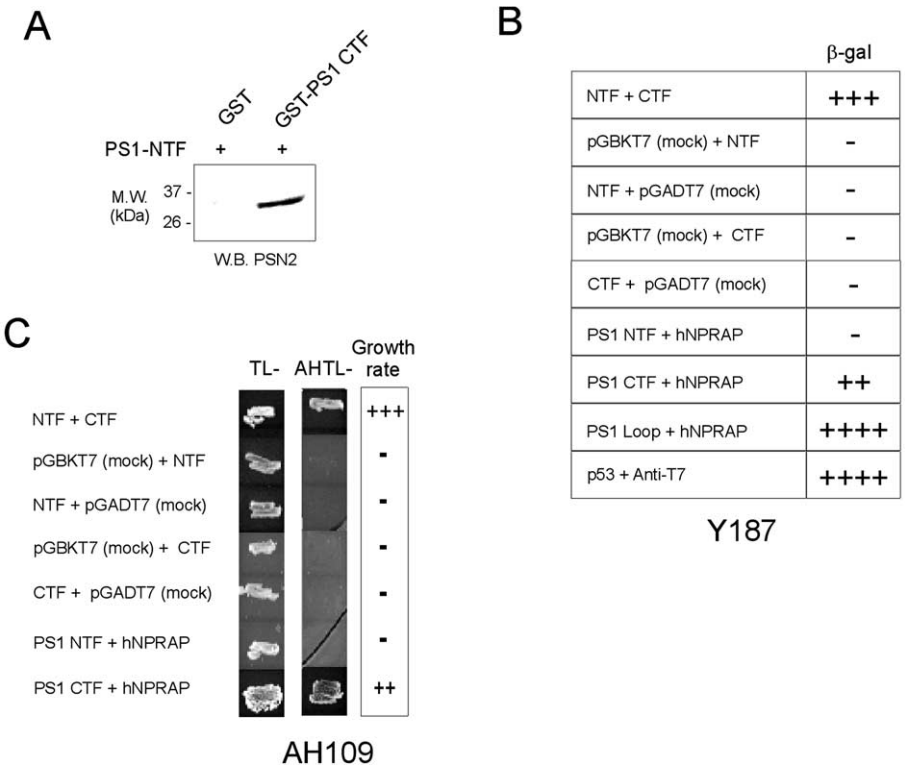


Fig. 2. PS1 NTF/CTF heterodimerization. A: 5′-HA-PS1-NTF was expressed in HEK293T cells and equal amounts of 1% CHAPS protein extracts were incubated with either GST-PS1 CTF or GST alone. Bound material was separated on 4–20% NuPAGE, blotted, and probed with anti-PSN2. B: Y187 yeast co-transformed colonies were assayed for β-gal activity according to Clontech procedures. Positive interactions were measured by the blue coloration intensity (++++ represented the highest level and (−) represented absence of coloration). Positive controls include pGBKT7-p53/pGADT7-T antigen and pGBKT7-PS1-Loop 6-7/pGADT7-hNPRAP. C: AH109 yeast co-transformed colonies were assayed for $His^+$ and $Ade^+$ activity according to Clontech procedures. Growth on SD/AHTL$^-$ indicates positive interactions between fusion partners. Growth is indicated by positive (+) signs, where +++ represent the fastest (i.e. early growth observed between 2 and 4 days) rate. Negative (−) sign indicate absence of growth after 10 days incubation. Results shown represent at least three independent experiences.

NTF/CTF heterodimerization. As summarized in Table 1, both the 1-132 and the TM1-2 fragments showed interaction with the PS1-CTF. These observations suggested that PS1 aa 101–132, corresponding to the first lumenal loop (Loop 1-2), are implicated in heterodimerization with the CTF. However, studies using solely the PS1 Loop 1-2 as bait or as target did not result in positive interactions. As suspected, TM2 did bind CTF, but weakly. These observations were confirm by GST pull-down assays showing that both TM1-2 and TM2, but not Loop 1-2 alone or mock (pGBKT7), bind GST-PS1-CTF (Fig. 3A). We suspect that heterodimerization of PS1 fragments must implicate large intermolecular binding domains thus possibly 'taken as a whole'.

Further investigation revealed that 1-132, as contrary to the TM1-2 fragment, can bind to the cytoplasmic Loop 6-7 (Table 1). This suggests that the first hydrophilic N-terminus stretch before TM1 (aa 1–80) may interact with the large cytoplasmic loop, as they are both oriented to the cytoplasm. Moreover, since both the TM1-2 and 1-132 bind to CTF but not to CTF-short our results, altogether, suggest that PS1-NTF/CTF heterodimerization is dependent of at least four distant binding regions: (i) the first 80 aa portion of PS1-NTF, (ii) the second lumenal loop combined with the second TM domain (TM1-2), (iii) the large cytoplasmic loop (Loop 6-7) and (iv) a 61 aa portion of PS1-CTF (aa 406–467).

### 3.3. Homodimerization between PS1 NTF and CTF fragments

We have previously reported PS1 full-length/full-length homodimerization in yeast as well as in mammalian cells [5]. Interestingly, we also observe NTF/NTF and CTF/CTF homodimerization (Table 1). We used GST pull-down assays to confirm specific fragment homodimerization using, as example, GST-tagged PS1-CTF against expressed PS1-CTF (Fig. 3B).

Crude domain mapping of NTF/NTF homodimerization led us to demonstrate that both the 1-132 and the TM2 region, but not Loop 1-2, could bind PS1-NTF (Table 1). Mapping of CTF/CTF homodimerization shows that the last 61 aa portion (aa 406–467) of PS1-CTF, implicated in NTF/CTF heterodimerization, is required for binding. Since TM8/TM8 (aa 406–426) do not bind to each other and CTF (aa 292–467) do not bind to CTF-short (aa 427–467) or Loop 6-7 (aa 239–409), this suggest that the last 61 aa portion of CTF is required as a whole for binding (Table 1 and Fig. 3B). Taken together, these results suggest that binding regions implicated in NTF/CTF heterodimerization are equally important in PS1 fragments homodimerization.

### 3.4. Effects of mutations on hetero- and homodimerization capacities of PS1-NTF and CTF fragments

We next investigated if mutated PS1-NTF was able to heterodimerize with the wt PS1-CTF. We constructed two PS1-NTFs containing either the Y115H or M146L mutation ($PS1_{1-132}$-Y115H and $PS1_{TM1-2}$-M146L). When assayed for interaction against the PS1-CTF, both mutants showed interaction (Table 1). Finally, we tested if these NTF-mutants

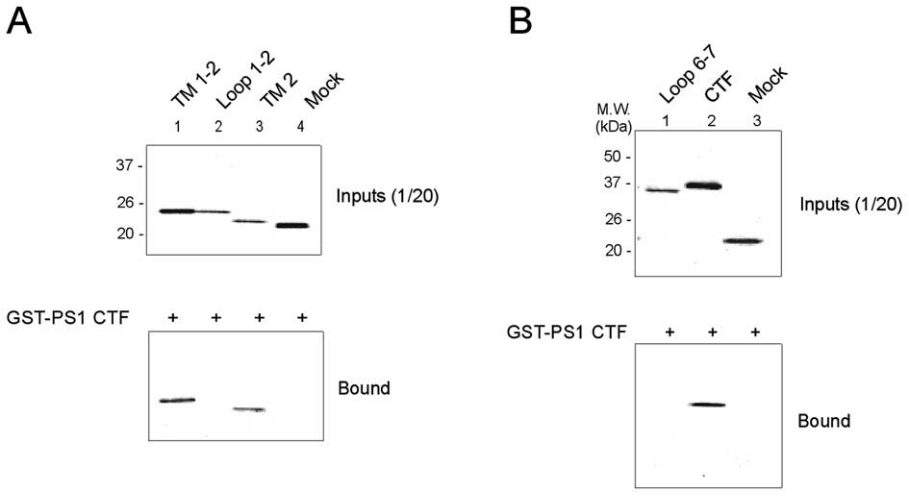


Fig. 3. GST pull-down analysis. A: pGBKT7-PS1-TM1-2, pGBKT7-PS1-Loop 1-2, pGBKT7-PS1-TM2 and Mock (pGBKT7) were expressed in strain Y187, and equal amounts of 1% CHAPS yeast protein extracts were incubated with GST-PS1-CTF. Bound material was separated on 4-20% NuPAGE, blotted, and probed with anti-myc. B: pGBKT7-PS1-Loop 6-7, pGBKT7-PS1-CTF and Mock (pGBKT7) were expressed in strain Y187, and equal amounts of 1% CHAPS yeast protein extracts were incubated with GST-PS1 CTF. Bound material was separated on 4–20% NuPAGE, blotted, and probed with anti-myc.

could homodimerize with its wild-type counterpart. Mutants' oligomerization capabilities appeared similar to their wild-type counterparts (Table 1). As expected, these PS1 AD-associated mutants do not disturb the dimerization suggesting that PS1 fundamental physiological function is conserved.

## 4. Discussion

The structure–function analysis of PS1 N- and C-terminal complexes are important if we consider that both fragments share putative catalytic elements (within separate TM domains) needed for PS1 enzymatic-related activity(ies) [13]. In this report, we clearly show direct heterodimerization between NTF and CTF fragments of PS1. Our results demonstrate that these two fragments can form heterodimers in vivo. We also demonstrated that NTF and CTF are capable of self-association, thus forming homodimers.

Previous studies aimed at understanding PS1 structure and/or maturation have proposed a model in which PS1 molecules are first stabilized then subsequently cleaved to generate the NTF and CTF [14,15]. Our results support this model and are also in accordance with the hypothetical 'tetramer' model proposed by Haass and Steiner [16]. Hence, we propose a new model (Fig. 4) in which PS1 homodimerization precedes endoproteolytic cleavage by 'presenilinase'. Following endoproteolytic cleavage, formation of a tetramer would be a cru-

Table 1
Summary of regional mapping of PS1 hetero- and homodimerization by yeast two-hybrid assay

| pGBKT7+pGADT7 | AHTL- | pGBKT7+pGADT7 | AHTL- |
|---|---|---|---|
| NTF+CTF[a] | +++ | Loop 1-2+CTF[a] | − |
| NTF+CF short | − | Loop 1-2+CTF short | − |
| NTF+TM8[a] | − | Loop 1-2+TM8[a] | − |
| NTF+Loop 6-7[b] | − | Loop 1-2+Loop 6-7 | − |
| NTF+NTF | ++ | Loop 1-2+NTF[a] | − |
| 1-132+CTF | ++ | Loop 1-2+TM2[a] | − |
| 1-132 H115Y+CTF | ++ | Loop 1-2+Loop 1-2 | − |
| 1-132+CTF short | − | TM2+CTF[a] | + |
| 1-132+TM8 | − | TM2+CTF short | − |
| 1-132+Loop 6-7 | + | TM2+ TM8[a] | − |
| 1-132+NTF | + | TM2+Loop 6-7 | − |
| 1-132 H115Y+NTF | + | TM2+NTF[a] | ++ |
| 1-132+Loop 1-2 | | TM2+TM2 | − |
| 1-132+TM2 | − | Loop 6-7+CTF | − |
| TM1-2+CTF | +++ | Loop 6-7+CTF short | − |
| TM1-2 M146L+CTF | +++ | Loop 6-7+TM8 | − |
| TM1-2+CTF short | − | Loop 6-7+Loop 6-7 | + |
| TM1-2+TM8 | − | Loop 6-7+hNPRAP | +++ |
| TM1-2+Loop 6-7 | − | CTF+CTF short | − |
| TM1-2+NTF | + | CTF+TM8 | − |
| TM1-2+M146L+NTF | + | TM8+TM8 | − |
| TM1-2+Loop 1-2 | − | CTF+CTF | ++ |
| TM1-2+TM2 | − | | |

Growth activity of selective SD/AHTL-medium is shown. Constructs cloned in DNA-BD (pGBKT7) and GAL4-AD (pGADT7) vectors are positioned left and right, respectively.
[a]Inverse complement experiences confirmed interactions.
[b]Inverse complement experiments gave inconsistent results.

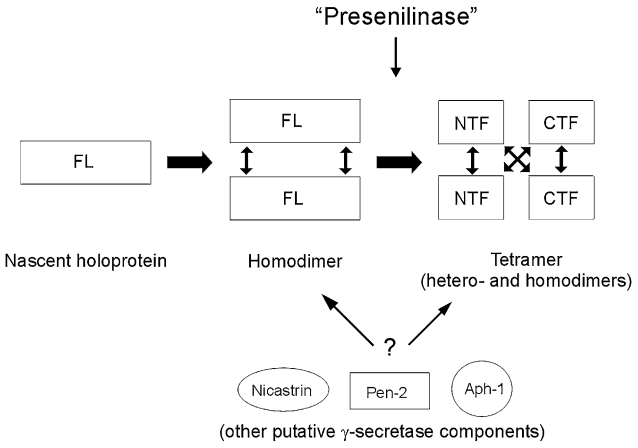


Fig. 4. Hypothetical model of PS1 maturation. Nascent PS1 is expressed as a 45–50 kDa holoprotein. Following full-length/full-length homodimerization, PS1 is cleaved by 'presenilinase' to generate stable fragment hetero- and homodimers (tetramers). Either pre- or post-PS1 endoproteolytic cleavage, PS1 fragments associate with Nicastrin, Aph-1 and Pen-2 to form a functionally active high molecular weight γ-secretase complex.

cial step for the assembly, stability and/or substrate specificity of the high molecular weight presenilin complex necessary for γ-secretase activity [16]. According to our data, AD-associated PS1-NTF Y115H and M146L mutants failed to demonstrate a disrupted interaction with the PS1-CTF. This observation is in accordance with in vivo studies suggesting that PS1 mutations lead to a gain of function [17] and not to an abrogated protein function, as seen in PS1 knock-out mice [18].

Nicastrin, Aph-1 and Pen-2 (presenilin partners in the γ-secretase complex), the long sought 'limiting factors' responsible for stabilization of PS1 endogenous levels, do not seem to be required for PS1 hetero- and homodimerization, since no homologues are found in yeast [12]. However, these proteins may be required to saturate, stabilize, generate and/or limit endogenous levels of mature PS1 in high molecular weight complexes [12,19].

In an attempt to map domains implicated in NTF/CTF oligomerization we have demonstrated that both NTF and CTF act as independent but inter-changing binding units capable of binding each other (NTF/CTF) or their homologues (NTF/NTF; CTF/CTF). We propose that each PS1 fragment acts as pseudo-independent structural units, namely domain A for the NTF and domain B for the CTF. Other polytopic TM proteins, such as G-protein coupled receptors, which are structurally similar to presenilins, do possess two distinct binding domains [20,21]. À priori, hetero-oligomerization of PS1 domain A seems dependent of its first 80 aa plus its first lumenal loop associated with TM2. However, our results demonstrate that most, if not all, of the domain A is necessary, perhaps at different levels, for its intramolecular and possibly intermolecular protein–protein binding properties. Recent findings showing that the first 1–132 portion of the NTF, and most importantly but not exclusively, the first TM domain are needed to bind APP and/or telencephanin [22] are in accordance with this hypothesis. Of particular importance, other reports have associated the first two TM domains as crucial for PS1 stability, endoproteolysis and cellular maturation [1,23].

Like its N-terminal counterpart, most of the PS1-CTF fragment (domain B) seems implicated in heterodimerization and/or homodimerization. Our results are in agreement with previous reports which, in sum, pinpoint different portions located at the C-terminus of PS1-CTF implicated in PS1 maturation and/or γ-secretase-associated activity [1,24,25].

Taken together, our results clearly demonstrate that PS1 and its endoproteolytic fragments may undergo self-dimerization in vivo. Because PS1 NTF and CTF generated fragments represent important AD therapeutic targets, their physiological existence as homo- or hetero-oligomers could have important implications for the development and screening of new drugs.

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