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Dimerization of presenilin-1 in vivo: suggestion of novel regulatory mechanisms leading to higher order complexes

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Abstract

A growing body of evidence indicates that presenilins could exist and be active as oligomeric complexes. Using yeast two-hybrid and cell culture analysis, we provide evidence that presenilin-1 (PS1) may self-oligomerize giving rise to specific full-length/full-length homodimers. When expressed in N2A and HEK239T cultured cells, full-length PS1-wt and 5′myc-PS1-wt form specific homodimers corresponding to twice their molecular weight. The Alzheimer's disease-associated PS1 mutations Y115H, M146L, L392V, ΔE10($PS1_{1–289/320–467}$), the γ-secretase dominant negative mutant D257A, and the PS1 polymorphism mutant E318G do not affect their ability to self-oligomerize. Under non-denaturing conditions, endogenous PS1 forms specific homo-oligomers in human cultured cells. The results obtained herein suggest that PS1 associates intramolecularly to form higher order complexes, which may be needed for endoproteolytic cleavage and/or γ-secretase-associated activity.

Keywords: Presenilin; γ-Secretase; Oligomerization; Protein dimerization; Yeast two-hybrid; Western blotting

Alzheimer's disease (AD) is, among other amyloidosis-related diseases, the most common cause of presenile death in the elderly. The pathological features of AD are neuronal loss, neurofibrillary tangles, and amyloid (senile) plaques. Most cases of early onset familial Alzheimer's disease (EOFAD) disease are associated with a point mutation in *presenilin* 1 (PS1). To date, over one hundred different mutations have already been found in this gene (for references, see www.alzforum.org). PS1 is a polytopic transmembrane protein located primarily in the endoplasmic reticulum (ER), the early Golgi and, to a lesser extent, at the cell surface and nuclear membrane [1–4]. The precise role of PS1 is not fully understood as it is associated with multiple biological functions such as protein folding and trafficking, intracellular adhesion, calcium homeostasis, GSK-3 and Tau metabolism, signal transduction, neuronal apoptosis, and γ-secretase cleavage [4].

The PS1 protein is approximately 45–50 kDa in size and undergoes a series of proteolytic cleavages by 'presenilinase' to generate N-terminal (NTF) and C-terminal (CTF) fragments of approximately 32–35 and 16–20 kDa, respectively. The stoichiometry of these fragments is maintained on a 1:1 ratio as artificial expression of PS1 results in only a modest increase in these fragments. It has also been reported that overexpression of PS1 may lead to the formation of aggresomes [5]. Endogenous PS1 forms low (~100–180 kDa) and high (>250 kDa) molecular weight complexes containing both full-length (fl) and heterodimeric NTF and CTF [6–9]. Recently, studies have identified PS1 key binding partners such as β-catenin and nicastrin which seem to act on or at least "help" PS1 to maintain these distinct oligomers [1,8,10]. However, even if a growing number of studies are underway to understand the enzymatic-related properties of PS1, little is known about the kinetics, the regulation, the contents, and the exact function(s) of these complexes, particularly the low molecular weight (LMW) species. Compiling evidences

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show that γ-secretase activity, which seems to be the 'key' function of these PS1 complexes, is associated to the highest (i.e., >250 kDa) molecular weight complex [8,11]. However, certain authors argue this concept [12]. Moreover, it has been speculated that a limited abundance of another unknown component of the PS1 complexes restrains the amount of PS1 that can be stabilized in complexes [13]. Since other numerous binding partners of PS1 have been elucidated [14] and their relation to presenilin function has not been fully understood, it is necessary to understand PS1 complex maturation and its link, if any, in the cellular mechanisms leading to the pathogenesis of Alzheimer's disease. To gain new insights into PS1 structure and function in vivo, we have studied its oligomerization properties. Here, we provide evidence that human wild-type (wt) or mutant PS1 may self-oligomerize giving rise to full-length/full-length homodimers. The AD-associated PS1 mutations Y115H, M146L, L392V, ΔE10 ($PS1_{1–289/320–467}$), the γ-secretase dominant negative mutant D257A, and the polymorphism mutant E318G [15–17] do not affect PS1 ability to self-oligomerize.

Materials and methods

Yeast two-hybrid assays. cDNA sequences encoding various parts of human PS1 were amplified by PCR using the Herculase Taq polymerase (Stratagene). The following PS1 constructs were prepared: fl-PS1-wt ($PS1_{1–467}$), fl-PS1-E318G, fl-PS1-D257A, fl-PS1-L392V, NTF ($PS1_{1–291}$), and CTF ($PS1_{292–467}$). Constructs were cloned both into pGBKT7 and pGADT7 vectors (Clontech) as a fusion to the Gal4-DNA binding domain and Gal4-DNA activating domain, respectively. Each construct was sequenced and shown to be free of autonomous Gal-4 activation.

Each of the pGBKT7-construct was co-transformed into Y187 or AH109 *Saccharomyces cerevisiae* strain (Clontech) together with each pGADT7-construct. Transformants were selected on yeast drop-out media (Clontech) that lacked both tryptophan and leucine (−TL) and assayed for β-galactosidase activity (β-gal) in the case of Y187 or histidine (His) and adenine (Ade) activity in the case of AH109. Negative control experiments were directed against empty vectors (either pGBKT7 or pGADT7), or Lamin C (Clontech). Positive controls included pGBKT7-p53/pGADT7-T antigen and pGBKT7-$PS1_{Loop6–7}$/pGADT7-hNPRAP as previously described [1].

Preparation of yeast cell lysates and immunoblotting. Transformed yeasts were harvested by centrifugation and washed twice in sterile H_2O. Yeast pellets were lysed in liquid nitrogen, and then thawed on ice. Acid washed glass beads (Sigma) (~250 μl) were added to the cells with 300 μl of yeast extraction buffer (Clontech), 1 mM PMSF, and mini-complete protease inhibitors (Roche-Diagnostic). The mixture was subjected to four cycles of vortexing at full-speed for 30 s and then placed on ice for another 30 s. Five percent v/v of β-mercaptoethanol was added to the samples. Samples were then boiled for 2 min and centrifuged at 14,000 rpm. Ten microliter of supernatants was loaded on a 4–20% gel and subjected to SDS–PAGE. Blots were probed with either the anti-c-myc (Roche Diagnostics), anti-PS1 Ab14 (gift from Dr. P. Fraser), PSN2, N-19 (Santa Cruz) or C-20 (Santa Cruz) antibodies.

Cell culture and transfection. Murine neuroblastoma 2A (N2A) and human embryonic kidney 293T (HEK293T) cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco) supplemented with 10% (v/v) fetal bovine serum (Biomedia) and Geneticin antibiotics (Invitrogen). Stock cultures were maintained at 37 °C in a humidified atmosphere consisting of 5% CO_2, 95% air. For overexpression studies, N2A or HEK293T cells were transiently transfected with cDNAs corresponding to: human PS1-wt, human PS1-wt tagged at the N-terminus with c-myc, EQKLISEEDLN, (PS1-wt-5′myc), a FAD-associated splicing mutation, exon 10 deletion, (PS1ΔE10), a FAD-associated mutant Y115H (PS1 Y115H), and an aspartate 257 mutant (PS1 D257A) expressed from pcDNA3 (Invitrogen). Constructs (10 μg) were transfected using LipofectAMINE according to the manufacturer protocol (Life Technologies). Cells were harvested and processed for immunoblot analyses 48 h after transfection.

Preparation of cell lysates and immunoblotting. For typical (i.e., denaturing and reducing) SDS–PAGE, HEK293T, N2A, and PS1 K/O native cells, as well as PS1-transfected cells, were washed twice with ice-cold phosphate buffer saline (PBS), and then lysed for 30 min on ice in lysis buffer A [25 mM Hepes, 150 mM NaCl, 1% Triton X-100, 0.1% SDS, 2 mM EDTA, 1 mM PMSF, and mini-complete protease inhibitors (Roche-Diagnostic)]. Insoluble material was removed by centrifugation at 13,000*g* for 15 min at 4 °C. Equal amounts of proteins were fractionated on Novex 4–20% Tris–Glycine gels (Invitrogen) by SDS–PAGE and transferred onto PVDF membrane (Amersham) using the XCell II Mini-Cell blot module (Invitrogen) according to the manufacturer's protocol. For native PAGE (i.e., non-denaturing), native PS1 K/O mice cells, as well as native and transfected HEK293T cells, were lysed under non-denaturing conditions using lysis buffer B [25 mM Hepes, 150 mM NaCl, 1% Triton X-100, 2 mM EDTA, 1 mM PMSF, and complete protease inhibitors (Roche-Diagnostic)]. Equal amounts of proteins were mixed with Novex 2× native sample buffer (0.5 M Tris–HCl, pH 8.8, glycerol 20%, and bromophenol blue 0.1%), incubated at 37 °C for 15 min, and then subjected to native PAGE as described by the manufacturer. For "revisited" native PAGE (i.e., partially denaturing), equal amounts of proteins were mixed with Novex 2× native sample buffer then subjected to SDS–PAGE (i.e., with SDS–PAGE running buffer). Western blots were probed with the appropriate antibodies, c-myc, Ab14, PSN2, N-19 or C-20 and detected using chemiluminescence detection system, ECL plus (Amersham).

Protein extraction from SDS–PAGE gel. Migration of protein extracts under partial (i.e., "revisited") denaturing conditions was performed as described above. For each sample, approximately 1 mg of total protein (60 mm confluent dish) was deposited on the gel. After a preliminary electrophoretic run, the gel was cut at the molecular weight range corresponding to the PS1 dimer (approximately 3–4 mm wide). Excised polyacrylamide gel bands were crushed, resuspended in 100 μl lysis buffer A, and incubated at 37 °C for 1 h. Following incubation, 100 μl Novex 2× reducing sample buffer was added to each sample. Samples were boiled for 2 min and then 20 μl of the recuperated proteins (surnageant) was resubjected to SDS–PAGE.

Results and discussion

Human presenilin-1 interacts with itself

Yeast two-hybrid assay, an in vivo system lacking endogenous PS1, was used to investigate potential homodimerization between PS1 proteins. This initiative was incited by recent literature showing that overexpression of fl-PS1 leads to low (approximately 100–180 kDa) molecular weight complexes [6,8,12] suggesting possible in vivo homodimerization. We assayed numerous wt and mutant PS1 constructs for intramolecular interactions. Interaction between two PS1 holo-

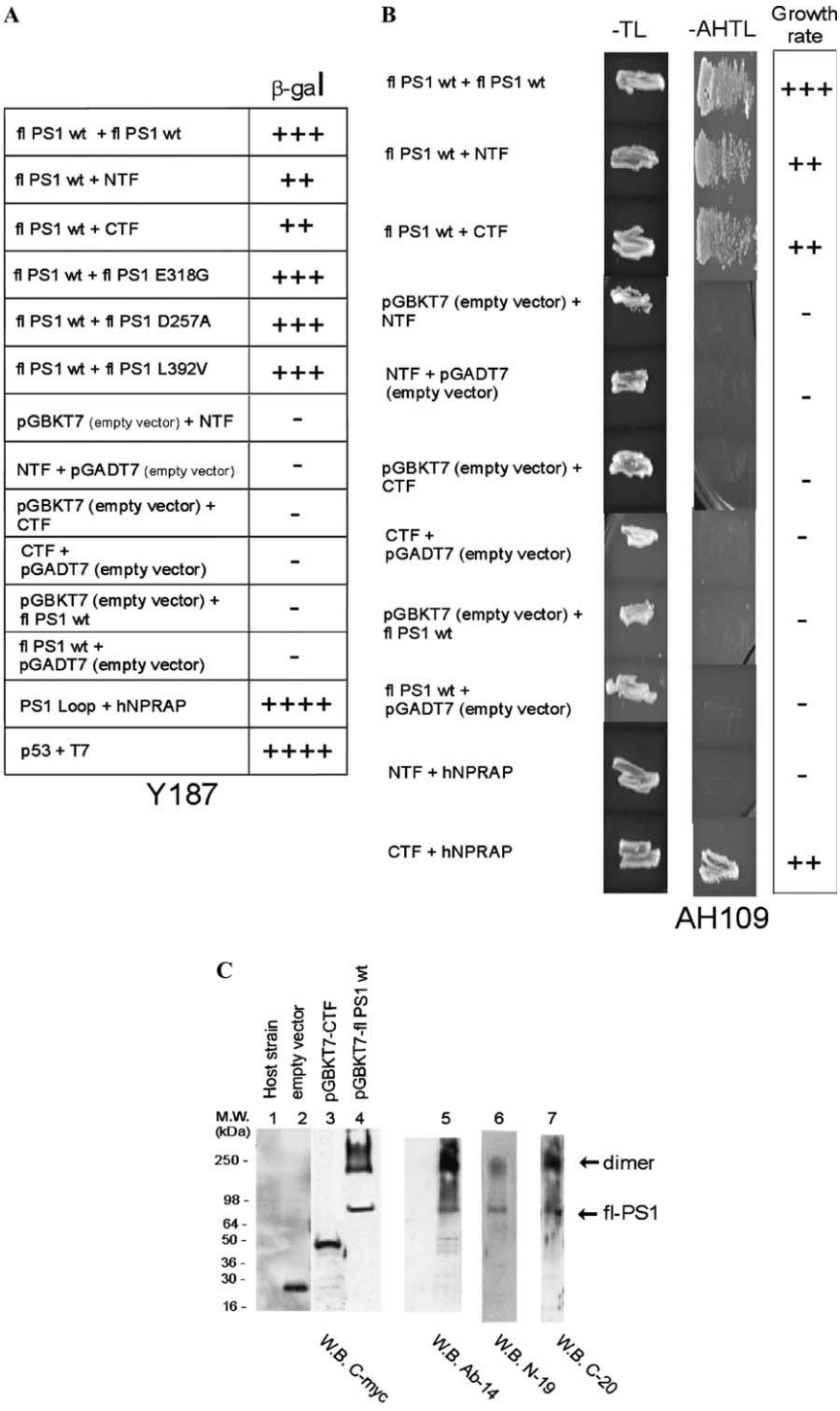

Fig. 1. Two-hybrid assay demonstrating PS1 self-association. (A) Y187 yeast co-transformed colonies were assayed for colony lift-galactosidase activity according to Clontech procedures. Positive interactions were measured by the blue coloration intensity (++++ represented the highest level and (−) represented absence of coloration). Positive controls include pGBKT7-p53/pGADT7-T antigen and pGBKT7-PS1$_{Loop6-7}$ loop/pGAD-hNPRAP. (B) AH109 yeast co-transformed colonies were assayed for His^+ and Ade^+ activity according to Clontech procedures. Colony growing on SD/−AHTL indicates positive interactions between two partners. Growth rate is indicated by positive (+) signs, whereas +++ represent the fastest (i.e., early growth observed between 2 and 4 days). Negative (−) sign indicates absence of colony growth after 10 days of incubation. Negative controls include pGBKT7-fl-PS1/pGAD-Lamin and pGBKT7-Lamin/pGAD-fl-PS1. Results shown represent at least three independent experiences. (C) Western blot of yeast protein extract demonstrating fl-PS1 expression and dimerization in yeast strain AH109. Lane 1 represents an extract from a non-transformed AH109 strain. In lane 2, the AH109 strain was transformed with pGBKT7 empty vector to demonstrate the presence of the c-myc tagged Gal-4 binding domain expression. Both pGBKT7-PS1-CTF and pGBKT7-fl-PS1 constructs were transformed in AH109 strain and tested for expression using anti-c-myc probe (lanes 3 and 4, respectively). To confirm the specificity of pGBKT7-fl-PS1 expression and dimerization, the same protein extract used in lane 4 was probed using either anti-PS1 antibody Ab14 (lane 5), N-19 (lane 6) or C-20 (lane 7).

proteins is observed following a β-gal assay using the yeast strain Y187, which is very sensitive for this assay (Fig. 1A).

In order to verify if these two-hybrid interactions were specific, and not due to non-specific β-gal reporter activation from Y187 strain, we tested other reporter genes by co-transforming the constructs in the AH109 strain. This AH109 strain virtually eliminates false positives by using three reporters, Ade2, His3, and Mel1 (or lacZ), under the control of distinct Gal4 responsive promotors. These promoters yield strong and specific responses to Gal4. After plating the co-transformed AH109 strain on selective medium lacking Ade, His, Trp, and Leu (−AHTL), colony growth is observed (Fig. 1B), confirming all β-gal results obtained using the yeast strain Y187.

Taken together, these results suggested possible homo- (fl/fl) dimerization of human PS1 in vivo. Western blot studies of yeast protein extracts confirmed both PS1 construct expression and fl-PS1 dimerization in vivo (Fig. 1C, lanes 3 and 4, respectively). To confirm that the dimer observed using anti-c-myc probe (Fig. 1C, lane 4) is not an artifact, we reprobed this extract with three different anti-PS1 antibodies. As expected, the dimer is observed using specific antibodies directed against both N-terminal (Ab14 and N-20) and C-terminal (C-20) regions of PS1 (Fig. 1C, lanes 5–7). The apparent increase in molecular weight for both CTF and fl-PS1 reflects their fusion with the Gal-4 DNA binding domain and the c-myc tagged from the pGBKT7 vector. This binding domain alone is observed in the extract from empty vector transformed yeast (Fig. 1C, lane 2). Interestingly, no endoproteolytic processing of PS1 could be observed with fusion protein in *S. cerevisiae* Y187 and AH109 (personal observation and Fig. 1C, lanes 4–7), implying that homo-oligomerization was not due to intermolecular associations between endoproteolyticaly cleaved PS1 fragments in yeast.

Effects of mutations on self-association capacities of PS1

AD-associated PS1 mutations were shown to increase the production of the β-amyloid fragments probably by increasing the γ-secretase complex activity. We then tested the effect of different mutations on binding properties of PS1. We first investigated if AD-associated PS1 mutants could affect self-association of fl-PS1. Since AD-associated mutations are dominant [18], we investigated if mutant fl-PS1 is able to associate with its wild-type counterpart. Our results show that AD-associated PS1-Y115H, L392V, and ΔE10 mutants, as well as the non-AD-associated PS1 polymorphism E318G, could dimerize with fl-PS1-wt (Fig. 2A). We also verified and confirmed that these mutants could self-associate with each other (Fig. 2B). Interestingly, the ΔE10 deletion mutant was still able to homodimerize and to heterodimerize with fl-PS1-wt. This observation suggests that the PS1 endoproteolytic cleavage region, which is absent in the ΔE10, is not implicated in PS1 self-association. However, endoproteolytic cleavage of PS1 may play an important role elsewhere as, for example, in complex stability [9,19,20]. Finally, the non-AD-associated PS1 D257A mutant, inhibiting both PS1 endoproteolytic cleavage and γ-secretase-associated activity [15], was also able to self-oligomerize (Figs. 1A and 2B). These results suggest that all mutants tested here may self-oligomerize in vivo, giving rise to full-length homodimers. Mutants' oligomerization capabilities appeared similar to their wild-type counterparts.

Formation of PS1 dimers in cultured cells

Expression of fl-PS1 in cultured cells leads mainly to the production of monomeric species of approximately 45–50 kDa with immuno-specific aggregation at higher molecular ranges (>250 kDa) when analyzed under normal SDS–PAGE denaturing and reducing conditions

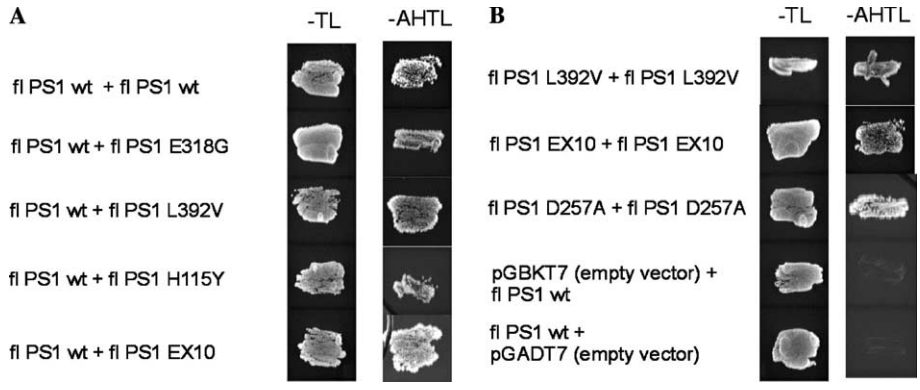

Fig. 2. Dimerization of mutant presenilins in yeast. (A) AH109 strain co-transformed with PS1-wt or mutant constructs were assayed for His^+ and Ade^+ activity according to Clontech procedures. Colony growth is observed with all mutants tested herein. Results shown represent at least three independent experiences.

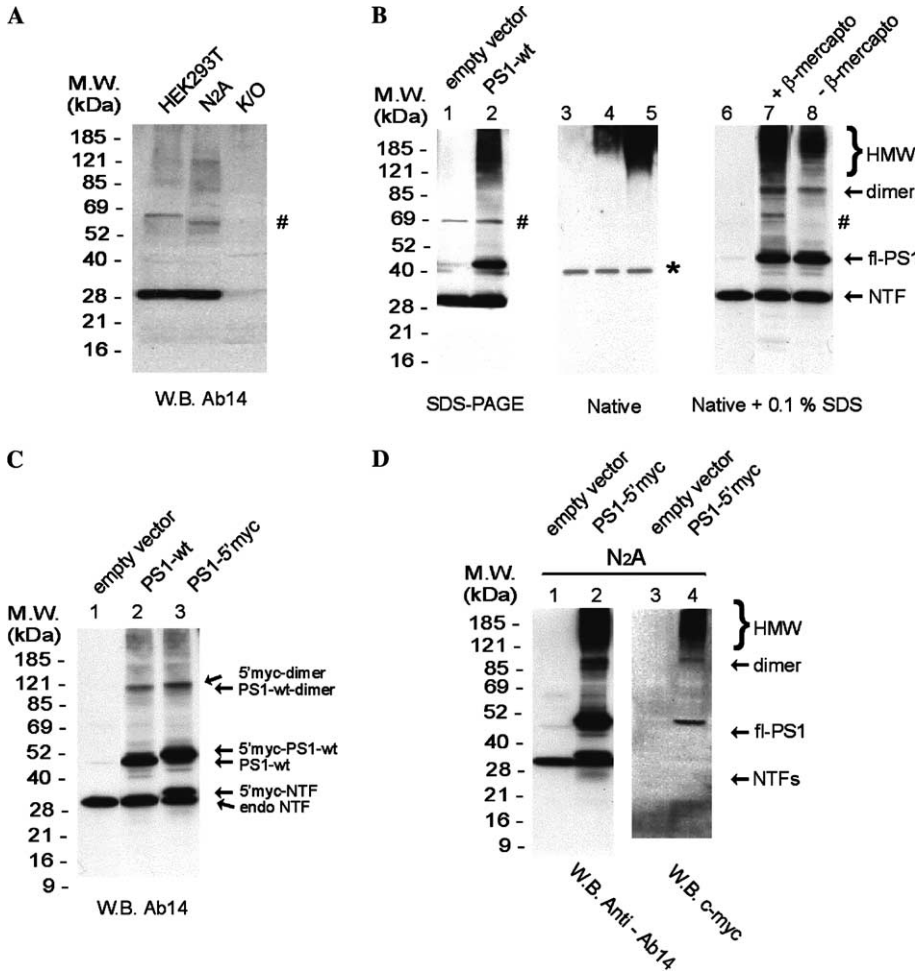

Fig. 3. Dimerization of PS1 in cultured cells. (A) Western blot demonstrating anti-PS1 Ab14 antibody specificity in different cell types. Equal amounts of proteins extracted from native HEK293T, N2A or PS1 K/O cells were mixed with reducing SDS–PAGE sample buffer, boiled for 2 min, then fractionated on SDS–PAGE, and transferred to PVDF membrane. The membrane was probed using Ab14 antibody. (B) Western blot following SDS–PAGE of proteins extracted from non-transfected (lane 1) and PS1-wt transfected (lane 2) HEK293T cells. Cells were mixed with SDS–PAGE reducing sample buffer and fractionated on SDS–PAGE. Lanes 3–5 represent a Western blot following native gel electrophoresis conditions. Proteins were extracted from both non-transfected PS1 K/O (lane 3) and HEK293T cells (lane 4), and from fl-PS1-wt transfected HEK293T cells (lane 5). In this case, the cells were lysed in extraction buffer B, mixed with native sample buffer, incubated at 37 °C for 15 min, and then subjected to native-PAGE. Lanes 6–8 represent a Western blot of protein extracts from both non-transfected HEK293T cells (lane 6) and from fl-PS1-wt transfected HEK293T cells (lanes 7 and 8). As above, the cells were lysed using buffer B, mixed with reducing or non-reducing native sample buffer, and incubated at room temperature for 15 min. Following incubation, SDS–PAGE was carried out using SDS–PAGE running buffer (containing 0.1% SDS). The effect of a reducing agent, β-mercaptoethanol, on PS1 dimerization was evaluated (lane 7 vs. 8). (C) Analysis of proteins extracted from either, empty vector (lane 1), fl-PS1-wt (lane 2), and fl-PS1-5′myc (lane 3) transfected HEK293T cells and submitted to partial non-denaturing SDS–PAGE. (D) Equal amounts of proteins extracted from both empty vector (lanes 1 and 3) and fl-PS1-5′myc (lanes 2 and 4) transfected N2A cells were mixed with native sample buffer (without reducing agents), incubated at 37 °C for 15 min, and then fractionated on SDS–PAGE. Blots were probed with either Ab14 antibody (lanes 1 and 2) or c-myc antibody (lanes 3 and 4). Sign (#) represents non-specific immunoreactive bands due to reducing agents. Sign (*) represents non-specific immunoreactive bands due to native electrophoretic conditions.

(Fig. 3B, lane 2 and [2,21,22]). Since little is known about PS1 properties under non-denaturing and non-reducing conditions, we performed analysis of PS1 dimerization under native electrophoresis conditions (i.e., native-PAGE). First, we tested and confirmed the specificity of the anti-PS1, Ab14, against endogenous PS1 in different cell lines (Fig. 3A). As expected with polytopic transmembrane proteins, electrophoresis of endogenous HEK293T protein extracts under native conditions resulted in specific, high (>250 kDa) PS1 immunoreactive aggregates when probed with the Ab14 antibody (Fig. 3B, lane 4). Overexpression of PS1 accentuated accumulation of aggregates (Fig. 3B, lane 5). Milder extraction detergents, such as CHAPS (0.5–1%) or Digitonin (0.5–1%), did not substantially affect PS1 aggregation under native conditions (data not shown). We next tested if different non-denaturing and non-reducing variables could "detach" potential PS1 dimers from these high molecular weight complexes. We observed that addition of 0.1% SDS in the electrophoresis

running buffer is sufficient to partially denature PS1 electrophoretic aggregates (Fig. 3B, lanes 7 and 8). Previous work has shown that certain LMW PS1-immunoreactive aggregates are SDS resistant [3], implying possible PS1 homo-oligomers. Moreover, other authors have shown that low amounts of SDS do not interfere with certain protein's homodimerization abilities [23–25], including GPCRs—which are structurally similar to presenilins. Of particular importance, addition of reducing agents, such as β-mercaptoethanol or DTT (data not shown), did not significantly interfere with PS1 dimerization as observed by Western blotting (Fig. 3B, lanes 7 and 8), thus suggesting that homo-oligomerization is not thiol-dependent. Further experiments are underway in our laboratory to address this issue.

We next tested if PS1-wt-5′myc could homodimerize. Results show that a major band of approximately 53 kDa corresponding to the expected PS1-wt-5′myc monomer was detected (Fig. 3C, lane 3). In addition, a higher molecular weight band of approximately 108 kDa was observed. As suspected, this ~108 kDa band corresponded in size to a putative PS1-wt-5′myc dimer. The non-tagged PS1 monomer or dimer migrates slightly lower than its 5′myc-tagged version, hence demonstrating the immunoreactive specificity of this high molecular weight species (Fig. 3C, lanes 2 and 3, respectively). To rule out the possibility that the ~108 kDa band represented HEK293T cell-specific artifacts, N2A cells overexpressing PS1-wt-5′myc were subjected to the same treatment. Both the polyclonal anti-PS1 antibody, Ab14, and the anti-c-myc antibody detected a similar set of PS1 immunoreactive species (Fig. 3D, lanes 2 and 4, respectively). Taken together, these observations suggest the existence of non-disulfide-linked full-length PS1 dimers or oligomers (in the case of the >250 kDa immunoreactive species) that preexist at least in HEK293T and neuronal type, N2A, cells.

The PS1-Y115H, ΔE10, and D257A mutants dimerize in cultured cells

Using SDS–PAGE under non-reducing and "revisited" non-denaturing conditions, we investigated the dimerization of different presenilin mutants in N2A cells. The N2A cells were transfected with different PS1 mutant constructs and the analysis showed that PS1-Y115H, ΔE10, and D257A mutants as well as PS1-wt formed putative dimers corresponding, in size, to twice their full-length moieties (Fig. 4A). In order to investigate the PS1 immunospecific constituents of this dimer, the band migrating at approximately 105 kDa was excised from the gel after a first electrophoretic run and subjected to a second electrophoretic run under reducing and denaturing conditions. This second run revealed the full-length monomer PS1 implying that the original ~105 kDa band contains full-length presenilins

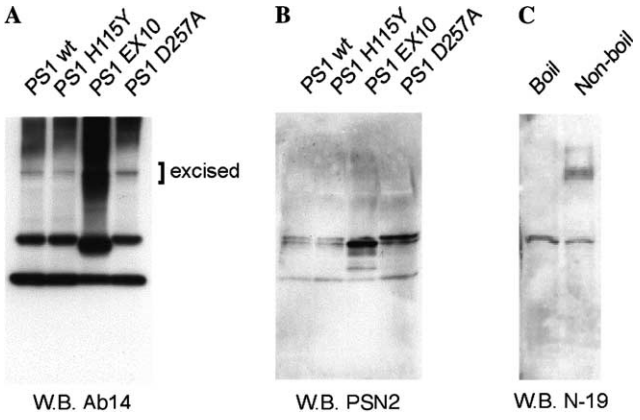

Fig. 4. Overexpression of wild-type or mutant PS1 as well as endogenous PS1 shows dimerization in cultured cells. (A) Equal amounts of proteins extracted from fl-PS1-wt or mutants transfected N2A cells were submitted to partial non-denaturing (and non-reducing) SDS–PAGE and transferred to PVDF membranes. Blot was probed with the Ab14 antibody. (B) Analysis of the PS1 dimer fragment. The ~105 kDa bands were excised from a first electrophoretic run as in (A), resuspended in lysis buffer A, and incubated at 37 ° C for 1 h. Following incubation, SDS–PAGE sample buffer was added to the lysates. Lysates were boiled for 2 min and 20 μl of the suspension was subjected to SDS–PAGE and transferred to PVDF membranes. To confirm the presence of PS1, blots were analyzed with PSN2 monoclonal antibody. (C) Endogenous PS1 dimerization in cultured cells. Equal amounts of proteins extracted from HEK293T cells were mixed with SDS–PAGE sample buffer (without reducing agents), boiled for 2 min (lanes 1) or incubated at 37 °C for 15 min (lanes 2), then fractionated on SDS–PAGE and transferred to PVDF membranes. Blots were probed with the N-19 antibody.

(Fig. 4B). Similar results were observed in HEK293T cells (data not shown). Our results demonstrate that PS1 mutants can form homodimers in vivo.

Evidence for endogenous PS1 self-association

It has previously been shown that, as a consequence of overexpression, the high molecular weight PS1 complexes are highly unstable and could either be ubiquitinated and then degraded [9,13,25] and/or mature to form aggresomes [5]. Therefore, it cannot be excluded that the dimerization of full-length PS1 is a trigger to aggregation and breakdown rather than a step prior to complex maturation. Hence, in order to evaluate if these oligomers are functional derivatives of PS1 or simply artifacts caused by overexpression, we next tested if endogenous PS1 could homodimerize. A commercially available anti-PS1 antibody, N-19, directed against the N-terminal portion of the protein, recognizes specifically full-length PS1 in endogenous HEK293T cells following Western blotting (Fig. 4C, lane 1). This distinctive immunoreactivity for full-length PS1 has been reported elsewhere with other non-commercial antibodies [26–28]. Interestingly, when endogenous HEK293T non-denaturing and non-reducing protein extracts (i.e., no addition of reducing agents and no boiling) are sub-

jected to typical SDS–PAGE, an immunoreactive band corresponding to the putative PS1 dimer is also observed with the N-19 antibody (Fig. 4C, lane 2). Similar results were observed with the C-20 antibody and confirmed in P19 (embryonal carcinoma) cells (data not shown). Taken together, these results are consistent in showing that endogenous PS1 oligomerizes in vivo to form specific homodimers.

The oligomerization properties of presenilins observed in vivo [22,29–31] and in vitro [29] have not been clearly understood. In this report, we clearly show PS1 homodimerization. Our results are consistent at demonstrating that presenilins can form low molecular weight complexes in vivo. Similar results were observed by Cervantes et al. [32] using the *Drosophilia* PS1 when expressed in yeast. We confirmed in vivo PS1 dimerization in HEK293T and N2A cultured cells overexpressing PS1-wt or mutants. Most importantly, we demonstrated endogenous PS1 dimerization under non-reducing and non-denaturing conditions in human cultured cells (HEK293T).

The functional role of PS1 dimerization is not clear. However, observations of the PS1 maturation process may give some clues on this role. It is known that PS1 physiological maturation leads to an endoproteolytic cleavage by an unknown 'presenilinase' resulting in PS1-NTF and CTF that are the major constituents found in vivo. The mechanisms of this maturation leading to the PS1 complexes are actually unknown. Different studies have proposed a model in which PS1 molecules are first stabilized and then subsequently cleaved to generate the NTF and CTF [19,33]. Based on this model, it is tempting to speculate that PS1 dimerization may well be a limiting and/or necessary step in PS1 complex maturation.

We have demonstrated that AD-associated and γ-secretase dominant negative PS1 mutations failed to inhibit self-association of PS1. These observations are in accordance with in vivo studies suggesting that PS1 mutations lead to a gain of function [34,35], and not to an abrogated protein function, as seen in PS1 and/or PS2 knock-out mice [36,37]. Indeed, our results demonstrate that PS1 mutations do not prevent complex oligomerization, thus presumably conserving its integrity and basic function(s). However, AD-associated mutations may lead to distinct and/or perturbed high molecular weight complexes, hence having new properties. Further studies will be needed to evaluate this possibility.

Altogether, our results clearly demonstrate that presenilin 1 may undergo self-dimerization in vivo. Because fl-PS1 as well as NTF and CTF represent important AD therapeutic targets [38], their physiological existence as homo- or hetero-oligomers could have important implications for the development and screening of new drugs.

Acknowledgments

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