

Nectin-1 α , an Immunoglobulin-like Receptor Involved in the Formation of Synapses, Is a Substrate for Presenilin/ γ -Secretase-like Cleavage*

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Nectin-1 is a member of the immunoglobulin superfamily and a Ca²⁺-independent adherens junction protein involved in synapse formation. Here we show that nectin-1 α undergoes intramembrane proteolytic processing analogous to that of the Alzheimer's disease amyloid precursor protein, mediated by a presenilin (PS)-dependent γ -secretase-like activity. 12-*O*-tetradecanoylphorbol-13-acetate (TPA) treatment of Chinese hamster ovary cells activated a first proteolytic event, resulting in ectodomain shedding of nectin-1 α . Subsequent cleavage of the remaining 26-kDa membrane-anchored C-terminal fragment (CTF) was inhibited independently by three specific γ -secretase inhibitors and by expression of the dominant negative form of PS1. The PS/ γ -secretase-like cleavage product was detected *in vivo* following proteasome inhibitor treatment of cells. An *in vitro* γ -secretase assay confirmed the generation of a 24-kDa nectin-1 α intracellular domain, peripherally associated with the membrane fraction. We also found nectin-1 α to interact with the N-terminal fragment of PS1. Finally, γ -secretase inhibition resulted in β -catenin release from cell junctions, concomitantly with the accumulation of the 26-kDa nectin-1 α CTF, suggesting that high levels of nectin-1 α CTF interfere with TPA-induced remodeling of cell-cell junctions. Our results are consistent with a previously reported role for PS/ γ -secretase in adherens junction function involving cleavage of cadherins. Similar to nectin-1, other members of the immunoglobulin superfamily involved in synapse formation may also serve as substrates for PS/ γ -secretase-like intramembrane proteolytic activity.

also been termed PRR, for poliovirus receptor-related protein, and HveC, for herpesvirus entry mediator C, given its role as a cellular receptor mediating α -herpes simplex virus entry (3, 4). At synapses, the nectin-afadin system co-localizes with the cadherin-catenin system, but it is mainly found in puncta adherentia-like junctions where different isoforms of nectin asymmetrically connect pre- and post-synaptic sides (5). Cadherin forms symmetrical homo-trans-dimers at the same junctions (6). Additionally, the distribution of the cadherin/catenin cell-cell junctions is less specific than that of the nectin/afadin system, which is enriched in the CA3 area of the hippocampus (7). An inhibitor of nectin-1 modulates synapse size and number (5). Mutations in the human nectin-1 gene are responsible for cleft lip/palate ectodermal dysplasia, a disease characterized by ectodermal dysplasia and mental retardation (8).

Presenilins (PS1 and PS2) are polytopic membrane proteins required for the intramembrane γ -secretase cleavage of the β -amyloid precursor protein (APP). The major γ -secretase cleavage sites in APP are at positions 40 and 42 of the A β region, generating A β ₄₀ and A β ₄₂, respectively. Early onset familial Alzheimer's disease mutations in the *APP*, *PSEN1*, and *PSEN2* genes lead to increases in A β ₄₂, relative to other A β species (reviewed in Ref. 9). PS1 is either the catalytic subunit or a necessary co-factor of a high molecular weight multisubunit aspartyl protease complex that exhibits γ -secretase activity (10, 11). The functional PS/ γ -secretase complex contains a stabilized PS1-N-terminal fragment (NTF) and CTF, in an equimolar ratio, and the fully glycosylated form of nicastrin. Recently, two additional components of the PS/ γ -secretase complex have been identified, aph-1 and pen-2 (12, 13). Based on the sequence of the γ -secretase-generated intracellular domain (ICD) of APP, a second intramembrane cleavage site of APP (ϵ -cleavage) has been identified (14–16). ϵ -cleavage of APP is also PS1-dependent, and the ϵ -site is homologous to the S3 site in Notch, also cleaved by the PS/ γ -secretase.

To date, six substrates have been reported to undergo PS/ γ -secretase-like proteolysis: APP, Notch, ErbB4, E-cadherin, the LDL receptor-related protein (LRP), and CD44 (17–23). All of these substrates are type I membrane proteins residing at or near the cell surface, which undergo ectodomain shedding prior to γ -secretase-like cleavage and release ICDs following prote-

The nectin-afadin cell adhesion system consists of nectin, a type I membrane protein that belongs to the immunoglobulin superfamily (IgCAM cell adhesion molecule), and afadin, a cytoplasmic actin filament-binding protein (reviewed in Ref. 1). Nectin-based cell-cell adhesion is Ca²⁺-independent and is recruited cooperatively to the cadherin-catenin system during the formation of adherens junctions (AJs)¹ (2). Nectin-1 has

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¹ The abbreviations used are: AJ, adherens junction; PS, presenilin;

PS1, presenilin 1; PS2, presenilin 2; APP, β -amyloid precursor protein; A β , amyloid β -peptide; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; LDL, low density lipoprotein; LRP, LDL receptor-related protein; ICD, intracellular domain; CTF, C-terminal fragment; NTF, N-terminal fragment; NE-ICD, nectin-1 α ICD; NE-CTF, nectin-1 α CTF; CHO, Chinese hamster ovary; PIPES, 1,4-piperazinediethanesulfonic acid; CHAPSO, 3-[[3-(cholamidopropyl)dimethylammonio]-2-hydroxy-1-propanesulfonic acid; MES, 4-morpholineethanesulfonic acid; DAPT, *N*-[*N*-(3,5-difluorophenacetyl)-*L*-alanyl]-*S*-phenylglycine-*t*-butyl ester; Ni-NTA, nickel-nitrilotriacetic acid; E, embryonic day.

olysis. Although the site of proteolysis varies among the six proteins (21), they all harbor a loose consensus sequence for PS/ γ -secretase recognition/cleavage at the membrane-cytosol interface. Here we report the identification of a novel substrate for PS/ γ -secretase-like cleavage, nectin-1 α . The important role of nectin in synaptic development and maintenance raises the possibility that familial Alzheimer's disease mutations in PS1 may directly perturb synaptic activity by modulating nectin processing.

EXPERIMENTAL PROCEDURES

Plasmids, Transfection, and Primary Neuronal Cultures—An expression construct encoding full-length nectin-1 α /HveC (herpesvirus entry mediator C) containing a C-terminal V5/His tag (in pcDNA3.1/GS) was purchased from Invitrogen. We produced stably transfected CHO cells with the nectin-1 α , wild-type PS1, and PS1(D385A) expression constructs by using Effectene transfection reagent (Qiagen). Individual zeocin-resistant colonies were isolated and screened for expression of the transfected proteins by Western blot analysis. Clones with similar expression levels were maintained in selection medium.

Mouse cortical neurons were prepared according to standard procedures (24). Cortices were dissected from E16 mouse fetal brains and dissociated by repeated passages through a fire-polished Pasteur pipette. The dissociated cells were plated on glass coverslips, treated with L-polylysine/laminin in phosphate-buffered saline for 1 h at 37 °C in a humidified atmosphere, and incubated in plating media consisting of minimal essential medium supplemented with 5% horse serum, 5% fetal bovine serum, 21 mM glucose, 26.5 mM bicarbonate, and 2 mM L-glutamine. Cultures were maintained at 37 °C in a humidified 5% CO₂ atmosphere for 1–5 days (DIV 1–5).

Western Blot Analysis, Antibodies, and Inhibitors—For immunoblotting, Triton-soluble cell extracts were prepared by directly lysing cells in a buffer containing 10 mM Tris-HCl, pH 6.8, 1 mM EDTA, 150 mM NaCl, 0.25% Nonidet P-40, 1% Triton X-100, and a protease inhibitor mixture (Roche Molecular Biochemicals) followed by a spin at 16,000 \times g. For Triton-insoluble extracts, the remaining pellet was re-extracted twice with lysis buffer to ensure that all detergent-soluble material was removed. The final pellet after a spin at 16,000 \times g was extracted with an SDS-containing buffer (10 mM Tris-HCl, pH 6.8, 2 mM EDTA, 150 mM NaCl, 1% SDS). The samples were quantitated using the BCA protein assay kit (Pierce). 20–100 μ g of protein were resolved on 4–12% gradient Bis-Tris gels (Novex) under reducing conditions. Primary antibodies were used at the following dilutions: anti-V5 antibody (1:5000 dilution, Invitrogen), anti-nectin-1 (1:1000, a gift of Dr. Y. Takai, Osaka University, Osaka, Japan), anti- β -catenin (1:1000 dilution, BD Transduction Laboratories), Ab14 (1:2000, PS1-NTF, a gift from Dr. S. E. Gandy, Thomas Jefferson University). The blots were visualized by enhanced chemiluminescence (ECL) using the SuperSignal substrate (Pierce) according to the manufacturer's instructions. The γ -secretase inhibitor L-685,458 was obtained from Calbiochem, and DAPT and WPE31C were a kind gift of Drs. R. E. Tanzi (Massachusetts General Hospital) and M. Wolfe (Brigham and Women's Hospital), respectively. The proteasome inhibitor clasto-lactacystin β -lactone was obtained from BioMol.

Immunoprecipitation—For co-immunoprecipitation of PS1-NTF with nectin-1 α , we used CHO cell lysates extracted with 1% CHAPSO (10). Briefly, cells were harvested and broken by homogenizer strokes followed by passage through a 27-gauge needle. After removing the nuclei and unbroken cells by centrifugation, the supernatant was centrifuged at 100,000 \times g for 1 h at 4 °C. Membrane pellets (P2–P3) were subsequently solubilized in 50 mM MES buffer (pH 6.0) containing 1% CHAPSO. After centrifugation (100,000 \times g, 1 h), the supernatant was diluted in PIPES buffer (50 mM PIPES, pH 7.0, containing 5 mM MgCl₂, 5 mM CaCl₂, 150 mM KCl, 0.25% CHAPSO, and complete protease inhibitor mixture). The diluted supernatant was incubated overnight with a polyclonal antibody against PS1-NTF (Ab14) and isolated using Biomagnetic beads conjugated with a goat anti-mouse antibody. For the purification of nectin-1 α and its fragments, 30 μ l of Ni-NTA agarose beads (Qiagen) were directly added to 1% CHAPSO-solubilized cell extracts and incubated for 1 h at 4 °C. The bound proteins were washed and eluted according to the manufacturer's instructions.

In Vitro Generation of Nectin-intracellular Domain (NE-ICD)—Membrane preparation and *in vitro* generation of nectin-1 α proteolytic products were performed as described in Ref. 14. The P2–P3 fractions (see "Immunoprecipitation") were resuspended in Buffer H (20 mM HEPES, 150 mM NaCl, 10% glycerol, 5 mM EDTA, pH 7.4) with protease inhib-

itors. *In vitro* cleavage experiments were performed by incubating the membrane fractions at 37 °C for 1 h in the presence or absence of the indicated amounts of DAPT. After incubation, the soluble and membrane-associated fragments were separated by centrifugation of the reaction mixture at 120,000 \times g for 45 min.

RESULTS AND DISCUSSION

Nectin-1 α Is a Novel Substrate for PS1-dependent γ -Secretase-like Proteolytic Cleavage—We used a BLAST search to identify novel substrates for PS/ γ -secretase-like cleavage based on the homology between the Notch S3 and APP ϵ -cleavage sites at the membrane-cytosol interface. Our searches yielded a large number of proteins containing homologous amino acid sequences, including E-cadherin (21) and LRP (22). We chose to study nectin-1 (Fig. 1A) because the nectin family of proteins is found in adherens junctions together with cadherin. Nectin-3 and -4, but not nectin-2, also harbor sequence homology to the APP- ϵ and Notch-S3 cleavage sites, but nectin-1 is the best-characterized member of the family. Interestingly, nectin-1 and -3 have been identified as asymmetric integral components of puncta adherentia-like junctions at synaptic sites (5). The nectin-1 mRNA undergoes alternative splicing, resulting in the expression of two isoforms, α and β , both harboring the APP- ϵ /Notch-S3-like cleavage site. The two isoforms of nectin-1 only differ in four amino acids in their cytoplasmic tails, EWYV at positions 514–517, that allow nectin-1 α to bind afadin via its PSD-95/DLG/20-1 (PDZ) domain (2).

Following transfection, Western blot analysis showed that full-length nectin-1 α migrated at \sim 87 kDa, as shown by staining with an antibody against its C-terminal V5 epitope tag (Fig. 1B). Overexposed images revealed an additional, higher band of full-length nectin-1 α , presumably a glycosylated form of the protein. 100 ng/ml TPA treatment of cells has been reported previously to induce ectodomain shedding of APP, erbB4, and LRP by activating tumor necrosis factor- α converting enzyme (TACE) or other metalloproteases (19, 22, 25). Similarly, TPA treatment of CHO cells resulted in the appearance of a nectin-1 α C-terminal cleavage product of \sim 26 kDa (Fig. 1B). DAPT, a γ -secretase inhibitor, further elevated cellular levels of the 26-kDa nectin-1 α C-terminal fragment (NE-CTF; Fig. 1B). The NE-CTF is anchored to membranes since it was undetectable in the cytosol and, additionally, a sodium carbonate wash failed to remove it from the membrane fraction (Fig. 1B).

To confirm that NE-CTF was processed by a PS/ γ -secretase-like activity, we treated transfected CHO cells with increasing concentrations of DAPT in the presence of TPA (Fig. 1C). NE-CTF levels increased in a concentration-dependent manner, depending on the amount of DAPT. Furthermore, two additional γ -secretase inhibitors, L-685,458 and WPE-III-31C, also independently elevated NE-CTF levels, when ectodomain shedding was stimulated by TPA (Fig. 1D). Similarly, the 26-kDa NE-CTF was specifically increased in CHO cells stably transfected with a construct expressing the dominant negative PS1(D385A) and was further increased by TPA treatment of these cells (Fig. 2). Similar results have been reported previously for APP (Fig. 2, lower panel). These data suggest that the 26-kDa NE-CTF is the precursor of a PS/ γ -secretase-like cleavage product that may be quickly degraded after its generation.

To detect endogenous PS/ γ -secretase-dependent processing of nectin-1, we cultured mouse cortical neurons from E16 embryos since neurons have been reported to express high levels of nectin-1 (2). Primary neurons were cultured *in vitro* for 5 days (DIV 5) and then treated with DAPT (Fig. 3). A C-terminal antibody against endogenous nectin-1 (from Dr. Y. Takai, Osaka University) detected a 23-kDa nectin CTF (lacking the V5/His epitope tag) induced by DAPT, indicating that PS/ γ -secretase-like activity is required for the processing of this protein. Interestingly, at DIV 5, TPA-induced ectodomain shed-

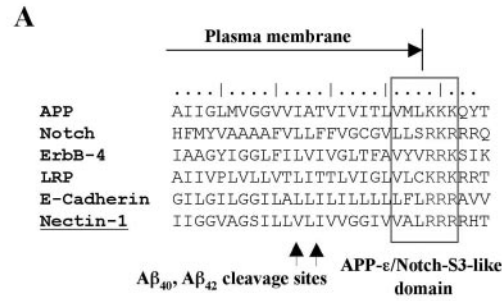


FIG. 1. Nectin-1 α is cleaved by a PS1-dependent γ -secretase-like activity after TPA-induced ectodomain cleavage. *A*, sequence comparison of the APP- ϵ /Notch-S3 domains in five of the six known human PS/ γ -secretase-like substrates and in nectin-1. *B*, Western blot analysis of CHO cells expressing nectin-1 α . 3 h of TPA treatment results in the accumulation of a 26-kDa NE-CTF (*arrowhead*) potentiated by the γ -secretase inhibitor DAPT. Sodium carbonate wash (*S.C.*) of the membrane fraction does not repartition the 26-kDa NE-CTF to the soluble cytoplasmic fraction (*Cyto.*). As shown in *C*, the accumulation of NE-CTF in the presence of TPA is dependent on the concentration of DAPT. As shown in *D*, NE-CTF levels are increased by three different γ -secretase inhibitor treatments, DAPT and two additional compounds, L-685,458 and WPE-III-31C, in the presence of TPA.

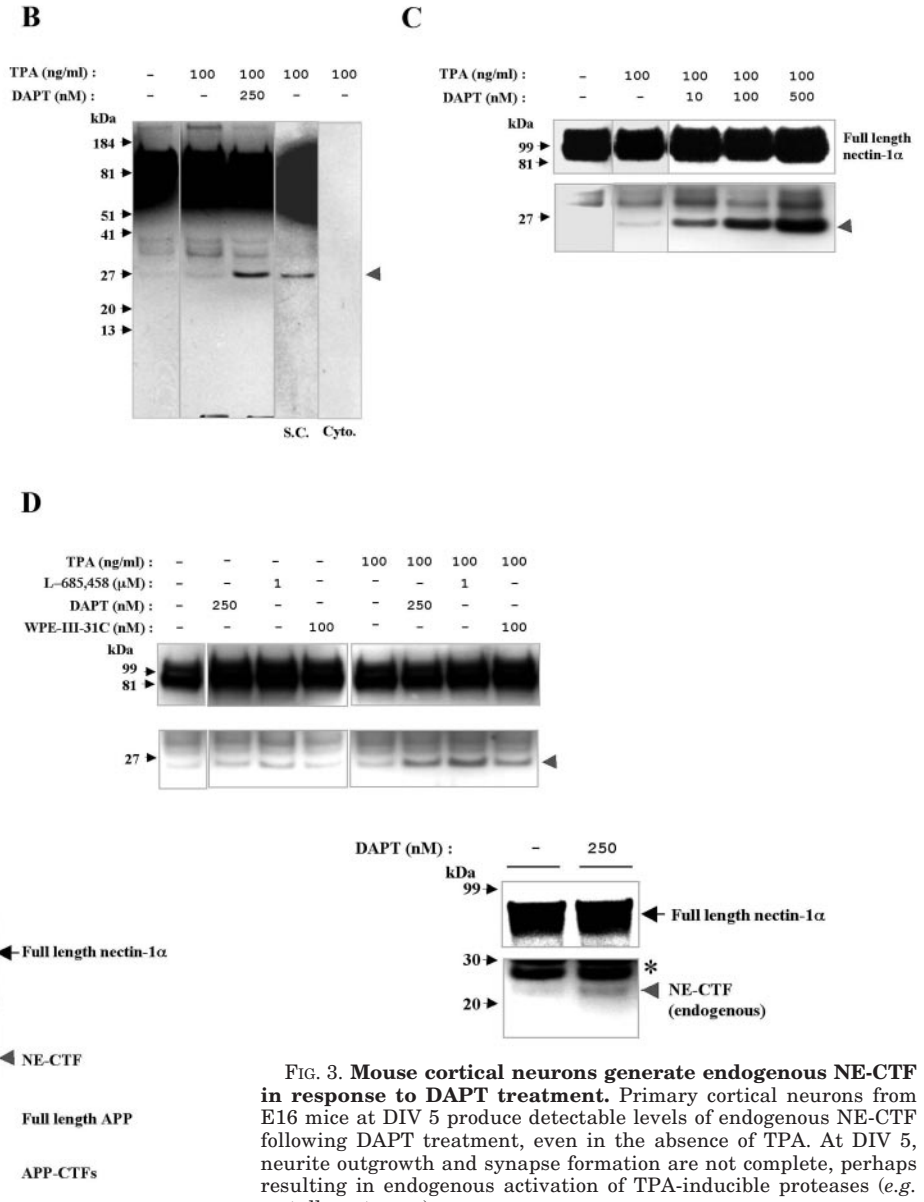


FIG. 2. The dominant negative PS1(D385A) also elevates NE-CTF levels. Expression of the dominant negative PS1(D385A) also results in the accumulation of the 26-kDa NE-CTF (*arrowhead*), further intensified by TPA treatment. A similar effect of PS1(D385A) has been reported for APP-CTFs, shown in the *lower panel*. *WtPS1*, wild-type PS1.

ding of nectin-1 was not required, and DAPT alone could cause accumulation of NE-CTF. Considering that neurite outgrowth and synapse formation are not yet complete at this time point,

this result may imply that ectodomain shedding occurs endogenously during synaptogenesis without the need of an additional stimulus, such as TPA.

The PS1-dependent Cleavage Product of Nectin-1 α Is a Peripheral Membrane Protein—To detect the PS/ γ -secretase-dependent cleavage product of nectin-1 α , we developed an *in vitro* γ -secretase assay. Nectin-overexpressing CHO cell membranes (P2–P3 fractions, see “Experimental Procedures”) were incu-

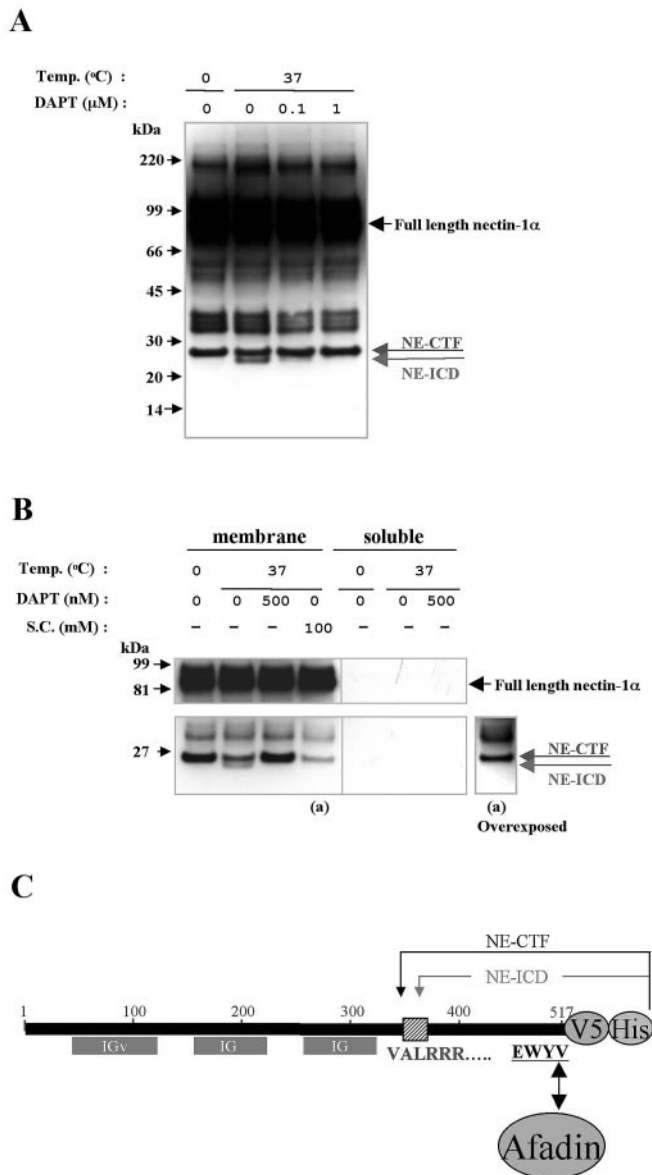


FIG. 4. *In vitro* generation of NE-ICD is PS/ γ -secretase-dependent. *A*, *in vitro* γ -secretase assay of nectin-1 α . A 24-kDa NE-ICD is only detectable when membrane fractions from nectin-1 α -transfected CHO cells are incubated at 37 °C and disappears following incubation with the γ -secretase inhibitor DAPT in a concentration-dependent manner. *B*, Western blot analysis of membrane and soluble fractions from an *in vitro* γ -secretase assay of nectin-1 α . The NE-ICD does not appear in the soluble fraction, and it remains loosely associated with membranes as shown by its dissociation following a sodium carbonate wash (S.C.). Lane (a) is shown on the right after overexposure to illustrate the absence of NE-ICD. *C*, nectin-1 α sequence containing the V5/His epitope tag used in our studies. The approximate positions of the 26-kDa NE-CTF and the 24-kDa NE-ICD are indicated.

bated for 1 h in the presence and absence of the γ -secretase inhibitor DAPT. These membrane fractions, as opposed to total cell extracts (Fig. 1B), contained easily detectable levels of the 26-kDa NE-CTF, even in the absence of TPA treatment (Fig. 4A). Further incubation at 37 °C resulted in the generation of an additional band at ~24 kDa, whereas the intensity of the NE-CTF diminished. The generation of the 24-kDa band was inhibited by DAPT in a concentration-dependent manner (Fig. 4A). Another γ -secretase inhibitor, L-685,458, also inhibited the generation of the 24-kDa band (data not shown). The 24-kDa fragment, as opposed to the 26-kDa NE-CTF, could be dissociated from the membranes when washed with sodium

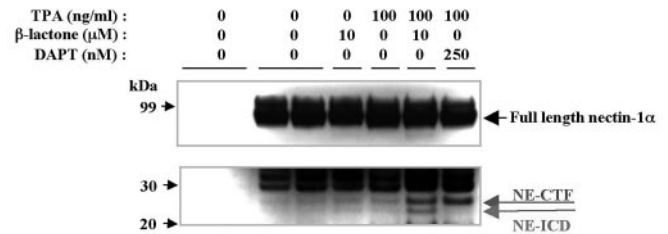


FIG. 5. Proteasome inhibitor treatment prevents degradation of the NE-ICD *in vivo*. 10 nM clasto lactacystin β -lactone treatment of CHO cells transfected with nectin-1 α and treated with TPA allows for detection of NE-ICD *in vivo*. DAPT prevents the generation of NE-ICD.

carbonate after the hour-long reaction (Fig. 4B). Although the 24-kDa fragment was not detectable in the soluble fraction under normal reaction conditions, we termed it NE-ICD (for nectin-intracellular domain) because it could be dissociated from the membranes by the sodium carbonate wash (Fig. 4B). These data indicate that the 24-kDa NE-ICD is generated by a PS1-dependent γ -secretase-like cleavage and remains associated peripherally to the membrane presumably via protein-protein interaction. A schematic representation of nectin-1 α containing the V5/His tag, as well as the approximate positions of the TPA-induced and PS1-dependent proteolytic cleavages, are shown in Fig. 4C.

To ascertain that the NE-ICD is also generated *in vivo*, we treated transfected CHO cells with various protease and proteasome inhibitors, while NE-ICD generation was induced by TPA (data not shown). The NE-ICD was detectable in cell lysates following treatment with the proteasome inhibitor clasto lactacystin β -lactone, indicating that this fragment is produced by a PS/ γ -secretase-like activity *in vivo* and subsequently undergoes proteasomal degradation (Fig. 5). These results, however, cannot exclude that a fraction of the NE-ICD is released and enters the nucleus or that afadin-interacting proteins with reported transcriptional activity *e.g.* Zona occludens protein (ZO-1) and calmodulin-dependent serine protein kinase (CASK), are released upon NE-ICD generation, similarly to β -catenin following PS/ γ -secretase-like cleavage of cadherin (21).

PS1-NTF Interacts with Full-length Nectin-1 α and with NE-CTF—PS1 has previously been reported to co-immunoprecipitate with Notch and E-cadherin (26, 27). To detect a possible association between nectin-1 α and PS1, we prepared cell lysates from CHO cells stably transfected with nectin-1 α by using 1% CHAPSO. Utilizing an antibody against PS1-NTF, we were able to specifically co-immunoprecipitate PS1-NTF and both full-length nectin-1 α and NE-CTF but not the control protein transferrin receptor (Fig. 6A). NE-CTF only appeared in cells treated with TPA and DAPT, as expected from the previous experiments. In a reverse experiment, 1% CHAPSO-extracted proteins were incubated with Ni-NTA agarose beads (Qiagen). The Ni-NTA column bound His-tagged full-length nectin-1 α and also co-purified PS1-NTFs (Fig. 6B). C-terminal fragments of PS1 were not detected in these preparations, but we cannot exclude that PS1-CTF may also co-purify with nectin-1 α . These data demonstrate the interaction of the PS1-NTF with nectin-1 α . Additional evidence suggests a possible connection among PS1, APP, and the nectin-afadin system. mLin-7 (MALS/Velis), which is localized at cell-cell junctions via the nectin-afadin system (28), forms a ternary complex with mLin-2 (CASK) and mLin-10 (Mint1/X11) (29). X11 mediates the formation of complexes between PS1 and APP by directly interacting with both proteins (30). Therefore, the preferential localization of the nectin-afadin system to puncta adherentia junctions in neurons may also gather PS1 and APP to these sites through protein-protein interaction.

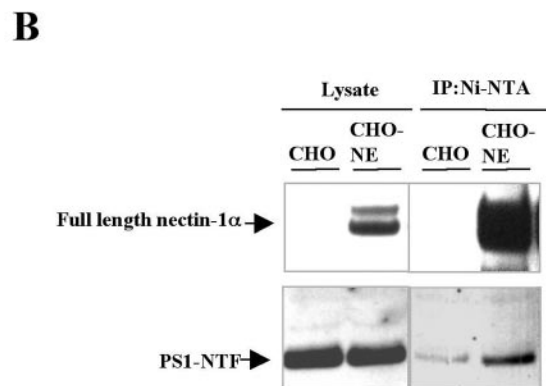
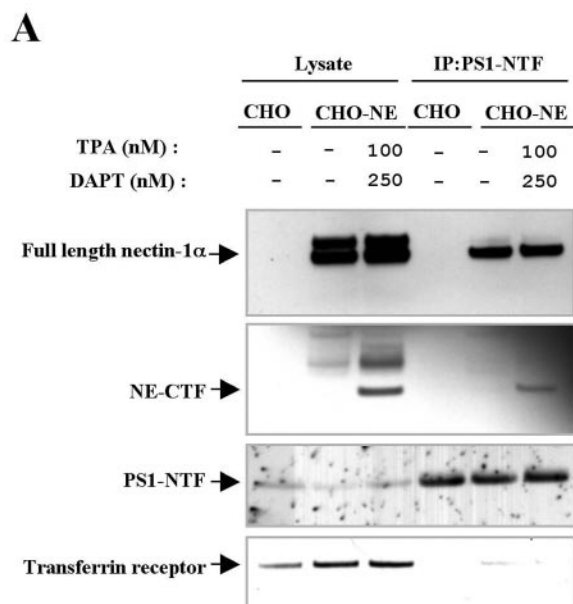


FIG. 6. Co-immunoprecipitation of PS1-NTF with nectin-1 α . As shown in *A*, 1% CHAPSO-soluble lysates from untransfected CHO cells and cells stably expressing nectin-1 α (*CHO-NE*) were immunoprecipitated with an antibody against PS1-NTF (Ab14). Full-length nectin-1 α co-immunoprecipitated with PS1-NTF independently of TPA and DAPT treatments, whereas NE-CTF only associated with PS1-NTF in the presence of the drugs. For stably transfected cells, the concentration of TPA was reduced from 100 ng/ml (or 162 nM) to 100 nM. As shown in *B*, Ni-NTA agarose beads retained His-tagged full-length nectin-1 α and co-purified PS1-NTFs.

PS/ γ -Secretase-like Activity Removes NE-CTFs from Cytoskeletal Fractions—To begin investigating the role of the PS1-dependent γ -secretase-like cleavage of nectin-1 α in cell-cell junction function, we studied levels of nectin-1 α and β -catenin in the Triton X-100-insoluble fraction from TPA- and DAPT-treated cells. Triton X-100-insoluble fractions have been shown previously to contain functional AJ complexes and β -catenin, anchored to the actin cytoskeleton (31). These experiments were carried out in stably transfected cells with the nectin-1 α -V5/His cDNA construct. Confocal immunofluorescence analysis revealed the expected distribution of nectin-1 α to cell-cell contacts, as detected with an anti-V5 epitope tag antiserum (Fig. 7*A*) (2). Full-length nectin-1 α fractionated to both the Triton X-100-soluble (membrane) and -insoluble (cytoskeletal) fractions, similarly to the previously reported E-cadherin (Fig. 7*B*) (21). Interestingly, the Triton X-100-insoluble fraction only contained the full-length nectin-1 α of lower molecular weight, which is the major form of the protein appearing upon nectin-1 α cDNA transfection and co-immunopre-

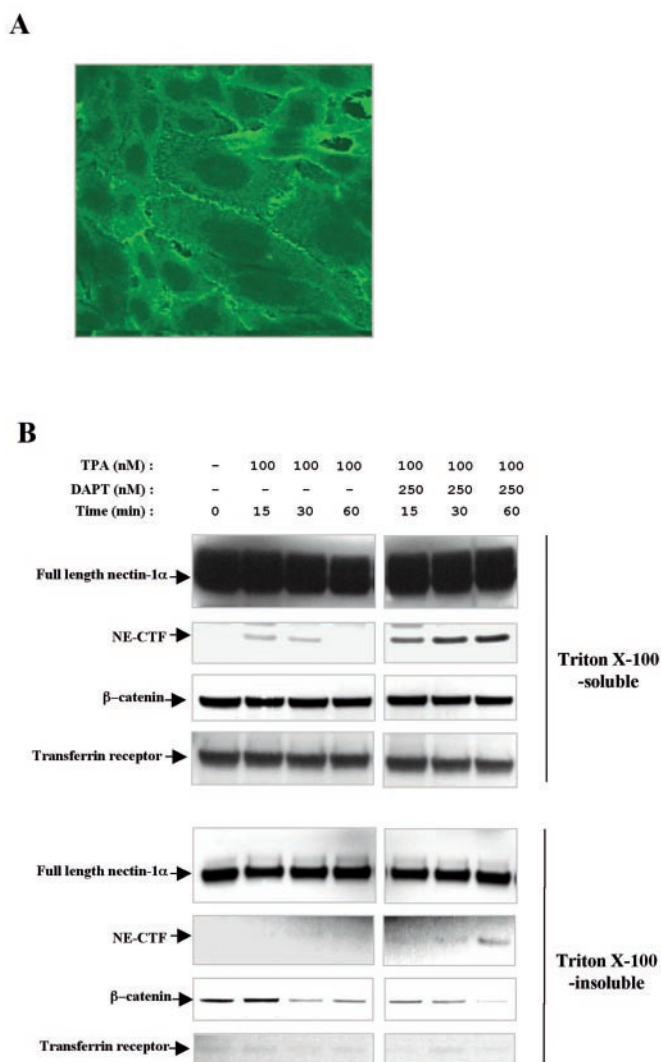


FIG. 7. Inhibition of PS1-dependent γ -secretase-like cleavage of nectin-1 α elevates NE-CTF and decreases β -catenin levels in the cytoskeletal fraction. As shown in *A*, V5 immunoreactivity detecting nectin-1 α -V5/His localizes to cell-cell contacts of stably transfected CHO cells. As shown in *B*, stably transfected CHO cells were treated with TPA or TPA and DAPT for increasing amounts of time and extracted to separate Triton X-100-soluble and -insoluble fractions (as described under “Experimental Procedures”). Absence of transferrin receptor staining in the Triton-insoluble fraction shows that the extraction of the soluble fraction was complete. The blots shown here were exposed to illustrate differences in nectin-1 α levels and do not reflect the much higher amount of full-length nectin-1 α as compared with the 26-kDa NE-CTF.

cipitating with PS1-NTFs. The 26-kDa NE-CTF mainly localized to the membrane fraction, where its levels increased in a time-dependent manner following γ -secretase inhibition by DAPT in the presence of TPA (Fig. 7*B*). At 1 h of DAPT treatment, the 26-kDa NE-CTF was also found in the Triton-insoluble fraction, suggesting that its generation may occur in AJs. Interestingly, γ -secretase inhibition by DAPT had the opposite effect on Triton X-100-insoluble (cytoskeletal) β -catenin levels (Fig. 7*B*). Partition of β -catenin to this fraction decreased over time, indicating that inhibition of the PS/ γ -secretase-like activity destabilizes the AJ complex or interferes with new AJ formation in dividing cells, releasing β -catenin to the cytosol.

In this study, we present evidence that nectin-1 α , and probably nectin-3 and -4, belong to the growing family of PS/ γ -secretase-like substrates. The specific role of recognition molecules such as nectin or Notch in the adult nervous system

remains to be investigated. The involvement of a PS/ γ -secretase-like activity in regulating nectin- and/or E-cadherin-based AJs implicates an analogous role for this complex in synapse function, perhaps in the rearrangement of existing cell-cell contacts that determine synaptic plasticity and regeneration after trauma. The potential relevance of the PS/ γ -secretase-like proteolytic cleavage in nectin-1 for A β generation, Alzheimer's disease pathology, and formation of nectin-based synapses will be an important topic for further studies.

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