Presenilin/γ-Secretase-mediated Cleavage Regulates Association of Leukocyte-Common Antigen-related (LAR) Receptor Tyrosine Phosphatase with β-Catenin

Received for publication, December 11, 2006. Published, JBC Papers in Press, January 26, 2007, DOI 10.1074/jbc.M611324200

Annakaisa Haapasalo†§, Doo Yeon Kim‡, Bryce W. Carey‡, Mari K. Turunen§, Warren H. Pettingell‡, and Dora M. Kovacs†§

From the †Neurobiology of Disease Laboratory, Genetics and Aging Research Unit, Massachusetts General Hospital and Harvard Medical School, Charlestown, Massachusetts 02129 and the §Department of Neuroscience and Neurology, University of Kuopio, 70211 Kuopio, Finland

Leukocyte-common antigen-related (LAR) receptor tyrosine phosphatase regulates cell adhesion and formation of functional synapses and neuronal networks. Here we report that LAR is sequentially cleaved by α- and presenilin (PS)/γ-secretases, which also affect signaling and/or degradation of type-I membrane proteins including the Alzheimer disease-related β-amyloid precursor protein. Similar to the previously characterized PS/γ-secretase substrates, inhibition of γ-secretase activity resulted in elevated LAR C-terminal fragment (LAR-CTF) levels in stably LAR-overexpressing Chinese hamster ovary (CHO) cells, human neuroglioma cells, and mouse cortical neurons endogenously expressing LAR. Furthermore, LAR-CTF levels increased in cells lacking functional PS, indicating that γ-secretase-mediated cleavage of LAR was PS-dependent. Inhibition of α-secretase activity by TAPI-1 treatment blocked LAR-CTF accumulation, demonstrating that prior ectodomain shedding was prerequisite for PS/γ-secretase-mediated cleavage of LAR. Moreover, we identified the product of PS/γ-secretase cleavage, LAR intracelluar domain (LICD), both in vitro and in cells overexpressing full-length (FL) LAR or LAR-CTFs. LAR localizes to cadherin-β-catenin-based cellular junctions. Assembly and disassembly of these junctions are regulated by tyrosine phosphorylation. We found that endogenous tyrosine-phosphorylated β-catenin coimmunoprecipitated with LAR in CHO cells. However, when PS/γ-secretase activity was inhibited, the association between LAR and β-catenin significantly diminished. In addition to cell adhesion, β-catenin is involved in transcriptional regulation. We observed that LICD significantly decreased transcription of cyclin D1, one of the β-catenin target genes. Thus, our results show that PS/γ-secretase-mediated cleavage of LAR controls LAR-β-catenin interaction, suggesting an essential role for PS/γ-secretase in the regulation of LAR signaling.

Presenilin (PS)2γ-secretase is a high molecular weight enzymatic complex composed of PS1 or PS2, nicastrin, Aph-1, and Pen-2 (1–5). PS/γ-secretase plays a key role in the pathogenesis of Alzheimer disease (AD) by cleaving β-amylloid precursor protein (APP) by regulated intramembrane processing (RIP) at two adjacent sites (6–8). Following ectodomain shedding of APP, the γ-cleavage generates the Aβ-peptide, the major constituent of amyloid plaques in AD brain, and a small p3 fragment. The product of the e-cleavage is the APP intracellular domain (AICD) (9–11). APP ectodomain shedding is mediated by either α-secretases of the ADAM family metalloproteases (12, 13) or BACE (β-site APP cleaving enzyme) (14–17), producing membrane-bound APP C-terminal fragments (CTFs). In addition to APP, other type-I membrane proteins are known to undergo PS/γ-secretase-mediated RIP (18). Among these are Notch (8), cadherins (19), nectin-1α (20), and voltage-gated sodium channel β2-subunit (21, 22). Similar to APP, these proteins undergo ectodomain shedding prior to γ-secretase-mediated cleavage and release intracellular domains (ICDs) as C-terminal products. Typically, loss of PS function or lack of PS/γ-secretase activity results in increased levels of membrane-associated CTFs (23–25).

LAR (leukocyte-common antigen-related) phosphatase (26, 27) is a type-I transmembrane protein and a member of the receptor protein tyrosine phosphatase (RPTP) family. The RPTP family phosphatases play pleiotropic roles in both developing and adult nervous system, including regulation of cell adhesion, formation and maintenance of synaptic connections, and learning and memory (28). The adhesion molecule-like LAR ectodomain contains immunoglobulin (IgG)-like and fibronectin type-III domains. The transmembrane domain is followed by two cytosolic phosphatase domains (D1 and D2), of which 1 domain is involved in transcriptional regulation.
**PS/γ-Secretase Regulates LAR-β-Catenin Signaling**

which the membrane-proximal D1 domain is catalytically active (26, 27, 29, 30).

Increasing evidence suggests that LAR is a central regulator of cell-cell and cell-matrix interactions. A developmentally expressed extracellular domain splice variant of LAR induces neurite outgrowth by homophilic binding to the ectodomain of full-length LAR (31–35). Heparan sulfate proteoglycans Syndecan and Dallylike as well as the extracellular matrix complex of laminin–nidogen have been identified as ligands for LAR, which regulate neurite outgrowth and synaptic development (36–38). LAR localizes to neurites and growth cones in neuronal cells and to adherens junctions in epithelial cells. Because its intracellular domain directly interacts with β- and γ-catenin and other proteins regulating actin cytoskeleton, LAR may regulate cell adhesion and neurite outgrowth (39–42). This potential role of LAR is substantiated by its ability to dephosphorylate β-catenin, an event increasing the association of β-catenin with cadherin (39, 42).

Studies in LAR function-deficient mice have revealed that LAR is required for the proper formation and maintenance of the cholinergic system. These mutant mice display decreased size and number of basal forebrain cholinergic neurons and deficient innervation of the hippocampal dentate gyrus (43, 44), show deficiencies in regenerative neurite outgrowth (45, 46), and develop spatial learning impairment and hyperactivity (47). LAR interacts with liprins, which regulate glutamate receptor clustering in dendritic spines and differentiation of presynaptic termini in neurons (48–52). In **Drosophila**, loss of LAR-homolog Dlar or α-liprin results in reduced synapse size and terminal branch complexity (50). Recently, LAR knockdown or expression of phosphate-inactive LAR in cultured hippocampal neurons was shown to result in the loss of functional excitatory synapses (53). Together, these data demonstrate that LAR is essential for development and maintenance of functional synapses and neural networks.

Here we show that LAR is a novel substrate for consequent proteolytic cleavages by α- and PS/γ-secretases, and that PS/γ-secretase activity regulates association of LAR with β-catenin. Furthermore, we show that LICD is involved in the regulation of β-catenin-mediated transcription of the cyclin D1 gene, suggesting that LAR proteolytic processing by PS/γ-secretase affects LAR signaling.

**EXPERIMENTAL PROCEDURES**

**Plasmids, Transfection, and Cell Lines—**Expression construct encoding full-length (FL) human LAR was kindly provided by Drs. A. Dunah and M. Sheng (MIND, Harvard Medical School and Massachusetts General Hospital, Charlestown, MA, and Massachusetts Institute of Technology, Cambridge, MA). The FL-LAR cDNA and the nucleotides encoding the LAR-CTF were subcloned into pcDNA 3.1/GS to produce C-terminally V5-His-tagged FL-LAR and LAR-CTF constructs. This tag adds ~5 kDa to the molecular mass of the expressed LAR proteins.

Parental Chinese hamster ovary (CHO), H4 human neuroglioma, or N2a mouse neuroblastoma cells were cultured in DMEM containing 10% fetal bovine serum, 2 mM l-glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin (DMEM-C). Stable CHO cell lines overexpressing FL-LAR-V5-His (G418) and LAR-CTF-V5-His (Hygromycin B) were produced by transfection with Effectene transfection reagent (Qiagen) according to the manufacturer’s instructions, and individual antibiotic-resistant colonies were isolated. Cell clones overexpressing FL-LAR and LAR-CTF with relatively high levels, as determined by Western blot analysis, were maintained in selection medium (DMEM-C with 200 μg/ml G418 or 150 μg/ml Hygromycin B, respectively). CHO cells overexpressing either wild-type (WT) PS1 or PS1/D385A dominant negative mutant were produced as described earlier (20) and cultured in DMEM-C containing 240 μg/ml zeocin. Mouse embryonic stem cell (ES) lines from WT mice (WT ES) or PS1 and PS2 double knock-out mice (PS1/2 dKO ES) were a generous gift from Dr. S. Sisodia (University of Chicago, IL), and cultured in Knock-out MEM media supplemented with 10% fetal bovine serum for ES cells, 1-glutamine and penicillin-streptomycin as above, 1000 units of ESGRO (Calbiochem), 110 μM β-mercaptoethanol, and 100 μM nonessential amino acids (all Invitrogen). All cell lines were cultured in a humidified cell culture incubator in 5% CO2 atmosphere and at +37 °C.

**Western Blot Analysis, Antibodies, and Inhibitors—**The cells were treated with TPA (Sigma), γ-secretase inhibitors DAPT, L-685,458 (both from Calbiochem), or WPE-31C (a gift from Dr. M. Wolfe, Brigham and Women’s Hospital, Boston, MA), α-secretase inhibitor TAPI-1 (TNF-α processing inhibitor-1, IC2; BIOMOL), and proteasome inhibitor clasto-lactacystin β-lactone (Calbiochem) alone or in combinations at indicated concentrations for indicated times. Control cells were treated with the vehicle Me2SO. Total protein lysates were prepared by lysing the cells in a lysis buffer (100 mm Tris, pH 7.6, 2 mm EDTA, 150 mm NaCl, 1% Triton X-100, 0.25% Nonidet P-40) supplemented with a protease inhibitor mixture (Roche Applied Science) for 30 min on ice and centrifuging at 13,000 × g for 10 min. Protein concentrations were determined using the BCA protein assay kit (Pierce). 10–50 μg of total proteins were separated on 3–8% Tris acetate or 4–12% bis-Tris gels (Novex) and separated on 3–8% Tris acetate or 4–12% bis-Tris gels (Novex) and blotted onto polyvinylidene fluoride membranes. The blots were stained with V5 antibody (1:5000, Invitrogen) to detect LAR overexpression, and anti-transferrin receptor (1:1000) antibody was from BD Transduction Laboratories, antibody (1:1000) and anti-phosphotyrosine antibody (PY20; Chemicon; 1:1000), and PS1 by an antibody recognizing the APP C terminus using a polyclonal antibody recognizing the APP C terminus (Zymed Laboratories Inc.). APP and APP-CTFs were visualized and anti-β-secretase antibody (1:5000, Invitrogen) to detect LAR overexpression, and the bands were visualized by enhanced chemiluminescence (ECL) using the ECL Plus substrate (Amersham Biosciences). Rabbit polyclonal anti-LAR antibody (D5013) was a gift from Drs. A. Dunah and M. Sheng and used at 1:500 dilution for detecting the endogenously expressed LAR. Anti-β-catenin antibody (1:1000) and anti-phosphotyrosine antibody (PY20; 1:1000) were purchased from BD Transduction Laboratories, and anti-transferrin receptor (1:1000) antibody was from Zymed Laboratories Inc. APP and APP-CTFs were visualized using a polyclonal antibody recognizing the APP C terminus (Chemicon; 1:1000), and PS1 by an antibody recognizing the PS1 loop region (MAB5232, Chemicon, 1:1000).

**Coinmunoprecipitation—**Total proteins were extracted as described above. Sodium orthovanadate (1 mM) was added to the lysis buffer to prevent protein dephosphorylation. 500 μg of total proteins were precleared with protein G-agarose beads (Pierce) in a total volume of 500 μl by overnight rotation at +4 °C. The V5 antibody (1 μl/reaction) was prebound to pro-
tein G beads in a similar way. Next day, the precleared samples were added to the antibody-bound beads and rotated at +4 °C for 4 h. The beads were washed three times in washing buffer I (10 mM Tris, 1 mM EDTA, and 1% Nonidet P-40), twice in washing buffer II (10 mM Tris, 1 mM EDTA, 0.5 mM NaCl, and 0.1% Nonidet P-40), and once in washing buffer III (50 mM Tris, 1 mM EDTA). Immunoprecipitated proteins were detached from the beads by heating at +70 °C in 1× LDS gel-loading buffer (Novex) and resolved by SDS-PAGE. Immunoprecipitation reactions of LAR-overexpressing cells without antibodies (beads only) or parental CHO cells with antibody-bound beads were used as negative controls. Total protein lysates were used as positive controls for staining.

In Vitro γ-Secretase Assay—P2 + P3 membrane fractions from LAR-CHO cells were prepared as described earlier (20). The membranes were then incubated at +37 °C in the absence or presence of the γ-secretase inhibitor L-685,458 at indicated concentrations for 2 h.

Immunocytochemistry—Cells were cultured on glass coverslips or chamber slides (Nunc) coated with poly-D-lysine (0.5 mg/ml) and laminin (10 μg/ml) on 12-well plates. The cells were fixed in 4% paraformaldehyde for 15 min, and blocked and permeabilized in PBS containing 1.5% goat IgG (PBS-IgG) and 0.1% Triton X-100 for 30 min. The cells were incubated with V5 antibody (1:5000) in PBS-IgG for 1.5 h and with anti-mouse-Alexa-488 (1:500; Molecular Probes) for 1 h. The nuclei were stained with Hoechst-33342 stain (1:5000; Molecular Probes) for 5 min. The coverslips were mounted onto glass slides with Fluoromount (Southern Biotechnology Associates Inc.), and localization of LICD was observed under a confocal microscope system (Olympus DSU with IX70 microscope and Hamamatsu EM CCD camera).

Extraction of Cytosplasmic, Nuclear, Membrane, and Cytoskeletal Fractions—The subcellular fractions were extracted using Compartamental Protein Extraction kit (Chemicon) according to the kit instructions. Cells were cultured in the absence of serum for 24 h (54), and treated with lactacystin and/or TPA and DAPT for 3 h. For Western blots, GAPDH (Abcam, 1:5000) was used as a cytosolic marker, Lamin A/C (BD Biosciences; 1:1000) as a nuclear marker, transferrin receptor (Zymed Laboratories Inc., 1:1000) as a membrane marker protein, and α-tubulin (Sigma, 1:8000) as a cytoskeletal marker. Different forms of LAR were detected with the V5 antibody (1:5000).

Quantitative PCR (qPCR) for Cyclin D1—Total RNA was extracted using the TRIzol reagent (Invitrogen) from N2a mouse neuroblastoma cells, which were transiently transfected with cDNA encoding LICD-V5-His. Nontransfected cells served as a control. Effectene transfection reagent was used according to the kit instructions. N2a cells were used in this experiment instead of CHO cells, because of the availability of mouse, but not hamster, cyclin D1 mRNA sequence (accession number NM_007631) in GenBank™. PCR primers, producing a single PCR product of 248 bp, were as follows: Forward primer (20-mer), 5′-TGGTGAAACAAGCTCAAGTTGG-3′; and reverse primer (20-mer), 5′-GCAGGAGGAAAGTGTGG-3′. 1 μg of total RNA was used as a template in cDNA synthesis in SuperScript III First-Strand Synthesis System for RT-PCR (Invitrogen). qPCR reactions were performed using SybrGreen PCR mix (Applied Biosystems), 15 pmol of each primer, and 1 μl of cDNA as a template and run with ABI Prism 7500 (Applied Biosystems). The same cDNA samples were used in qPCR reactions with TaqMan Rodent GAPDH Control Reagents (Applied Biosystems) for normalization of cyclin D1 mRNA levels. Results were analyzed using the standard curve method. Results shown are expressed as % of control (nontransfected cells) of GAPDH-normalized cyclin D1 mRNA levels.

RESULTS

Inhibition of γ-Secretase Activity Results in the Accumulation of LAR-CTFs—The currently known PS/γ-secretase substrate proteins share lysine- and/or arginine-rich sequence homology characteristic of the γ-secretase cleavage site at the membrane-cytosol boundary. Using a BLAST search based on the homology at the APP e-cleavage site and the S3 cleavage site of Notch, we have identified a number of type-I membrane proteins with homologous amino acid sequences at their membrane-cytosol interface, including nectin-1α (20) and β2-subunit of the voltage-gated sodium channel (VGSC) (21). Our homology search suggested that the RPTP LAR, a type-I transmembrane protein involved in cell adhesion and synapse formation, could also be a novel PS/γ-secretase substrate. LAR is expressed as a full-length protein of ~235 kDa, consisting of two noncovalently associated subunits, the ~150-kDa extracellular E-subunit and ~85-kDa membrane-bound P-subunit (26, 27, 30) (see Fig. 5C). LAR has previously been shown to undergo ectodomain cleavage, which results in the release of the E-subunit and proteolytic cleavage of the P-subunit (41, 55). We hypothesized that the P-subunit is enzymatically cleaved to form a membrane-bound LAR-CTF that can function as a substrate for PS/γ-secretase-mediated cleavage. We first treated CHO cells stably expressing FL-LAR with three well characterized pharmacological γ-secretase inhibitors, DAPT, L-685,458, and WPE-31C. Accumulation of LAR-CTFs was then investigated by Western blotting. As shown in Fig. 1A, treatment of cells with the γ-secretase inhibitors alone resulted only in slightly increased levels of LAR-CTFs when compared with the control cells. However, studies with other γ-secretase substrates indicate that γ-secretase-mediated cleavage requires preceding ectodomain shedding. Therefore, we treated the cells with the phorbol ester TPA, a known inducer of ectodomain shedding, alone or in combination with each of the three γ-secretase inhibitors. Indeed, treatment of the cells together with TPA and any of the three γ-secretase inhibitors resulted in a prominent accumulation of LAR-CTFs of ~72 kDa in size (Fig. 1A). We also observed that LAR-CTFs accumulated in a dose- and time-dependent manner upon DAPT treatment (Fig. 1, B and C). In addition, it should be noted that we always observed small amounts of LAR-CTFs present in the control cells, indicating some constitutive ectodomain shedding. These results not only suggest that LAR-CTFs undergo γ-secretase-mediated cleavage, but that ectodomain shedding is prerequisite for the γ-secretase-mediated cleavage of LAR.

Recently, monoubiquitination of the lysine at the interface of transmembrane and cytosolic domains was reported to control
endocytosis and subsequent γ-secretase-mediated cleavage of Notch (56). Interestingly, LAR harbors two lysine residues at this site. We transiently overexpressed FL-LAR containing mutations at the first (LAR/K1275R) or both (LAR/K1275R + K1277R) of these lysine residues in CHO cells, and studied LAR-CTF accumulation after inhibition of γ-secretase activity. We observed that LAR-CTFs accumulated in cells overexpressing either of these mutants in a similar manner to the WT LAR after TPA and DAPT treatment (supplemental Fig. S1). These results suggest that, unlike Notch, LAR proteolytic processing by γ-secretase is not controlled by monoubiquitination.

LAR-CTFs Accumulate in Cells Deficient in PS Function and Neuronal Cells—APP-CTFs are known to accumulate in cell systems deficient in PS activity (23–25). In the next two experiments, we asked whether functional PS is required for the γ-secretase-mediated cleavage of LAR. First, CHO cells overexpressing either WT PS1 or PS1/D385A dominant negative mutant were transiently transfected with FL-LAR cDNA and treated with TPA and/or DAPT. In WT PS1-expressing cells, treatment of the cells together with TPA and DAPT resulted in the accumulation of LAR-CTFs, as expected (Fig. 2A). Treatment of the PS1/D385A cells with TPA alone was sufficient to cause accumulation of LAR-CTFs when compared with the control cells (Fig. 2A). In the second experiment, ES cells were transiently transfected with LAR. Similar to WT PS1-overexpressing CHO cells, LAR-CTFs accumulated strongly in WT ES cells after treatment of the cells with TPA and DAPT (Fig. 2B). Interestingly, control PS1/2 dKO ES cells demonstrated already higher steady-state levels of LAR-CTFs than did control WT ES cells, and we did not observe any further increase in the CTF levels after TPA treatment (Fig. 2B). Together, these results demonstrate that functional PS is required for the γ-secretase-mediated cleavage of LAR. Control Western blots from TPA-treated WT and PS1/2 dKO cells indicated that DAPT had no effect on APP processing in PS1/2 dKO ES cells (supplemental Fig. S2).

### FIGURE 1. Inhibition of PS/γ-secretase activity results in the accumulation of LAR-CTFs. A, Western blot analysis of CHO cells stably overexpressing FL-LAR, showing strongly increased levels of LAR-CTFs after TPA and γ-secretase inhibitor-treatment. The cells were treated with TPA and/or γ-secretase inhibitors, DAPT, L-685,458, or WPE-31C for 3 h. Western blot analysis of CHO cells stably overexpressing FL-LAR treated with TPA and different concentrations of DAPT for 3 h, showing a dose-dependent increase in LAR-CTF levels. Western blot analysis of CHO cells stably overexpressing FL-LAR treated with 250 nM DAPT for different times, showing that DAPT alone increases LAR-CTF levels after 24 h of treatment, but not at earlier time points. The blots were stained with V5 antibody (1:5000) to visualize the expression of LAR followed by staining with transferrin receptor (Tfr) antibody (1:1000) to confirm equal loading of the lanes. Arrows on the right indicate the FL-LAR (225 kDa), LAR P-subunit (85 kDa), or LAR-CTF (72 kDa). The C-terminal V5-His tag adds ~5 kDa to these molecular masses.

### FIGURE 2. LAR-CTF levels are increased in cells lacking functional PS. A, Western blot analysis of transfected LAR in CHO cells stably overexpressing either WT PS1 or PS1/D385A dominant negative mutant. The cells were treated with TPA and/or DAPT for 3 h. In WT PS1-overexpressing cells, LAR-CTF levels slightly increased after treatment with DAPT alone. Treatment of the WT PS1 cells with TPA and DAPT resulted in a more pronounced accumulation of LAR-CTFs. In PS1/D385A cells, TPA alone induced accumulation of LAR-CTFs. B, Western blot analysis of CHO cells stably overexpressing FL-LAR, showing strongly increased levels of LAR-CTFs after TPA treatment (Fig. 2B). We transiently transfected the cells with FL-LAR cDNA and treated with TPA and/or DAPT. In WT PS1-expressing cells, treatment of the cells together with TPA and DAPT resulted in the accumulation of LAR-CTFs, as expected (Fig. 2A). Treatment of the PS1/D385A cells with TPA alone was sufficient to cause accumulation of LAR-CTFs when compared with the control cells (Fig. 2A). In the second experiment, ES cells were transiently transfected with LAR. Similar to WT PS1-overexpressing CHO cells, LAR-CTFs accumulated strongly in WT ES cells after treatment of the cells with TPA and DAPT (Fig. 2B). Interestingly, control PS1/2 dKO ES cells demonstrated already higher steady-state levels of LAR-CTFs than did control WT ES cells, and we did not observe any further increase in the CTF levels after TPA treatment (Fig. 2B). Together, these results demonstrate that functional PS is required for the γ-secretase-mediated cleavage of LAR. Control Western blots from TPA-treated WT and PS1/2 dKO cells indicated that DAPT had no effect on APP processing in PS1/2 dKO ES cells (supplemental Fig. S2).
inhibited LICD generation and resulted in the accumulation of LAR-CTFs (Fig. 4B). Treatment with the proteasome inhibitor lactacystin stabilized the levels of both LICD and LAR-CTFs. As expected, cotreatment of the lactacystin-treated cells with DAPT specifically inhibited the generation of LICD and resulted in the accumulation of the LAR-CTFs (Fig. 4B). Together, these results indicate that LICD is generated in both in vitro and in cells overexpressing LAR-CTFs by γ-secretase activity.

To visualize subcellular localization of LICD, we transiently transfected naïve CHO cells with a LICD-V5-His cDNA construct and stained the cells with V5 (1:500) antibody. Confocal microscopy revealed that LICD localized both to the cytoplasm and the nucleus of the cells (Fig. 4C). The nuclear localization of LICD was indicated by the overlap of the V5 and Hoechst-33342 staining.

Finally, we extracted cytoplasmic, nuclear, membrane, and cytoskeletal fractions from CHO cells stably overexpressing FL-LAR treated with lactacystin alone or in combination with TPA and/or DAPT to show subcellular localization of different LAR forms. As expected, the majority of FL-LAR, the P-subunit, and LAR-CTFs resided in the membrane fraction. Some P-subunits and LAR-CTFs were also detected in the nuclear and cytoskeletal fractions, which may be caused by their interaction with other proteins present in these fractions. LICD was detected in the cytoplasmic and nuclear fractions, confirming the immunocytochemical data from CHO cells transfected with the LICD construct (Fig. 4, C and D). The fact that lactacystin was required to stabilize the levels of LICD before it could be detected in FL-LAR-overexpressing cells supports the notion that LICD is generated only at low levels in these cells. Staining of the fractions with antibodies against different marker proteins indicated that there was no detectable cross-contamination between the fractions (Fig. 4D). These results suggest that LICDs are released after γ-secretase cleavage from the plasma membrane to the cytoplasm from where some LICDs may translocate to the nucleus.

**LAR Ectodomain Is Shed by α-Secretase Activities**—It has been reported previously that LAR ectodomain shedding can be induced by calcium ionophore, phorbol esters, or high cell density (41). Recently, activation of epidermal growth factor receptor signaling was shown to induce LAR ectodomain shedding by ADAM-17/TACE (tumor necrosis factor-α converting enzyme) (55). The major α-secretase responsible for the ectodomain shedding of APP is ADAM-10, whereas the α-secretase predominantly cleaving Notch ectodomain is ADAM-17/TACE (12, 57–60). We asked whether LAR-CTF accumulation was dependent on the activity of α-secretase, and treated LAR-overexpressing CHO cells with a metalloprotease
**PS/γ-Secretase Regulates LAR-β-Catenin Signaling**

**A.** In vitro γ-secretase assay of isolated membranes from CHO cells stably overexpressing FL-LAR. P2 + P3 membrane fractions were incubated at 0°C or at +37°C in the absence or presence of L-685,485. LICD (~70 kDa) was generated at +37°C, but not in the presence of L-685,485. LICD generation was accompanied with a concomitant decrease in LAR-CTF levels. **B.** Western blot analysis of CHO cells stably overexpressing LAR-CTFs showing that LICDs are generated from overexpressed LAR-CTF. Proteasome inhibitor Clasto-lactacystin β-lactone (lactacystin; 10 μM) prevented degradation of LICDs. LICDs were not generated in the presence of the DAPT even after lactacystin treatment. The blots were stained with V5 antibody (1:5000) and reprobed with TfR antibody (1:1000). Arrows on the right indicate FL-LAR, the P-subunit, LAR-CTF, and LICD (75 kDa with the V5-His tag). The cells were stained with V5 antibody (1:500) and anti-mouse-Alexa-488 (1:500) showing that LICD localizes to the cytoplasm and the nucleus (green). The nuclear localization of LICD is indicated by the overlapping V5 and Hoechst-33342 (blue) staining. The images were taken at ×60 magnification using an Olympus IX70 microscope and DSU confocal unit. **C.** Western blot analysis of cytoplasmic, nuclear, membrane, and cytoskeletal fractions extracted from FL-LAR-overexpressing CHO cells treated with lactacystin and/or TPA and DAPT. FL-LAR, P-subunit, and LAR-CTFs are mainly localized in the membrane protein fraction, whereas LICD localizes to the cytoplasmic and nuclear fractions. Some P-subunits and CTFs are also present in the nuclear and cytoskeletal fractions. It should be noted that because of the high amounts of FL-LAR, P-subunit, and LAR-CTFs in the membrane fraction, the exposure time of the membrane fraction blot probed with V5 antibody was only 5 s compared with 4 min with the other fractions (indicated by *). The blots were stained with V5 antibody (1:5000) to visualize LAR, and reprobed with antibodies against GAPDH (1:5000 cytoplasmic marker, ~36 kDa), Lamin A/C (1:1000, nuclear marker, ~74 and 60 kDa), TR (1:1000, membrane protein marker, ~90 kDa), α-tubulin (1:8000, cytoskeletal marker, ~40 kDa). Arrows on the right indicate different LAR forms and each of the marker proteins.

**D.** Western blot analysis of cytoplasmic, nuclear, membrane, and cytoskeletal fractions extracted from FL-LAR-overexpressing CHO cells stably overexpressing LAR by utilizing a V5 antibody against the C-terminal tag in our LAR construct. We observed that LAR coimmunoprecipitated with endogenous β-catenin, confirming previously published data dependent on α-secretase activity. In a similar manner, TAPI-1 also inhibited accumulation of APP-CTFs by TPA and DAPT, as detected with an antibody against the APP C terminus (Fig. 5B). These data confirm that the LAR ectodomain is cleaved by α-secretase activities, and thus are in agreement with the idea of ADAM-17 mediating LAR ectodomain shedding (55). Furthermore, these results indicate that ectodomain shedding of LAR is prerequisite for the subsequent γ-secretase cleavage.

Summarizing our and previously published data, we propose the following model for LAR proteolytic cleavage (Fig. 5C). First, FL-LAR undergoes proprotein processing by a furin-type enzyme. This cleavage yields the E- and P-subunits, which remain noncovalently bound at the plasma membrane. An activating stimulus activates α-secretase, which proteolytically cleaves the P-subunit to generate the membrane-bound CTF and releases the E-subunit and the N-terminal stub of the P-subunit to the extracellular space. Finally, our results show that LAR-CTF undergoes PS/γ-secretase-mediated processing to release the LICD.

**PS/γ-Secretase Activity Regulates Association of LAR with β-Catenin and β-Catenin-mediated Transcription**—To clarify the physiological significance of the PS/γ-secretase-mediated proteolytic processing of LAR, we asked whether PS/γ-secretase activity affected LAR signaling. Previously, it has been shown that β-catenin directly binds LAR C terminus, and that this interaction results in β-catenin dephosphorylation (39, 42). β-Catenin tyrosine dephosphorylation, in turn, is suggested to result in increased cell adhesion. To assess whether γ-secretase activity affected association between LAR and β-catenin, we performed coimmunoprecipitation studies in CHO cells stably overexpressing LAR by inhibitor, TAPI-1. As shown in Fig. 5A, TAPI-1 inhibited LAR-CTF accumulation induced by TPA and DAPT, demonstrating that LAR ectodomain shedding and LAR-CTF generation are
PS/γ-Secretase Regulates LAR-β-Catenin Signaling

FIGURE 5. LAR ectodomain is shed by an α-secretase-like activity. A. Western blot analysis of CHO cells stably overexpressing LAR treated with TPA and/or DAPT and/or the metalloprotease inhibitor TAPI-1 for 3 h. LAR-CTFs accumulated in the presence of TPA and DAPT, and this accumulation was inhibited by TAPI-1. The blot was stained with V5 antibody (1:5000) and reprobed with TR antibody (1:1000). Arrows on the right indicate FL-LAR, the P-subunit and LAR-CTF. B. Western blot analysis of endogenous APP from the same samples as in A. APP-CTFs accumulated in response to TPA and DAPT treatment, and the accumulation was inhibited in the presence of TAPI-1. The blot was stained with APP C-terminal antibody (1:10000), followed by reprobing with TR antibody. Arrows on the right indicate mature (mat) and immature (imm) FL-APP and APP-CTFs. C, schematic drawing of proposed LAR proteolytic processing. The FL-LAR is first cleaved by furin to produce the E- and P-subunits, which remain noncovalently associated on the plasma membrane. An activating signal results in the α-secretase-mediated shedding and release of the E-subunit to the extracellular space, and the cleavage of the membrane-bound P-subunit to form the LAR-CTFs. LAR-CTFs are further cleaved by PS/γ-secretase to generate the LICDs, some of which translocate to the nucleus.

Because LICD partially resides in the nucleus, we next asked whether LICD regulates transcription of one of the β-catenin-TCF/LEF target genes, cyclin D1. Total RNA extracted from N2a mouse neuroblastoma cells was reverse-transcribed to cDNA and used as a template in qPCR to examine the levels of endogenously expressed cyclin D1 mRNA in nontransfected control cells and cells transiently transfected with LICD. The GAPDH-normalized data indicated that LICD decreased cyclin D1 mRNA levels by 28% as compared with the control cells (Fig. 6C), suggesting that PS/γ-secretase-mediated cleavage of LAR plays a role in the regulation of β-catenin-mediated transcription. Fig. 6D shows a Western blot of of overexpressed LICD in CHO cells transiently transfected with LICD-V5-His. Protein lysates from CHO cells stably expressing FL-LAR (without and with DAPT) treatment were used as controls for size comparison.

Taken together, our data show that PS/γ-secretase activity regulates the interaction between LAR and tyrosine-phosphorylated β-catenin. Previous evidence indicates that association of β-catenin with the cytoplasmic domain of LAR regulates its tyrosine phosphorylation status. Therefore, our results suggest an important role for PS/γ-secretase in the regulation of LAR signaling through β-catenin.

DISCUSSION

Here, we show that RPTP LAR undergoes sequential proteolytic processing by α- and γ-secretases, and that γ-secretase-mediated cleavage regulates association of LAR with β-catenin. In addition, we provide evidence that LICD, released by γ-secretase activity, regulates β-catenin-mediated transcription. Similar to the currently known PS/γ-secretase substrates, LAR is a type-I membrane protein containing adhesion molecule-like extracellular domains and regulating cell adhesion, synapse function, and neurite outgrowth (28). We found that LAR harbors homology to the APP-ɛ/Notch S3-cleavage sites in its membrane-cytosol interface. Both overexpressed and endogenous LAR-CTFs accumulated after γ-secretase inhibition in several different cell types in addition to cells expressing mutant PS1 or lacking both PS1 and PS2, indicating that LAR is cleaved by γ-secretase in a PS-dependent manner. In addition, we were able to visualize generation of LICD, the direct product of γ-secretase cleavage, in a PS/γ-secretase-dependent manner both in vitro and in cells overexpressing FL-LAR or LAR-CTFs. The finding that LICD localized to the cytoplasm and nucleus indicated that LICD can be released from the plasma membrane by γ-secretase cleavage. Concisely, our results demonstrate that, in addition to RPTPα (73), LAR is another RPTP undergoing α- and PS/γ-secretase-mediated cleavages.
Previously, monoubiquitination of the lysine at the plasma membrane-cytosol boundary and subsequent endocytosis were shown to be required for γ-secretase-mediated cleavage of Notch (56). Interestingly, APP contains three and LAR two lysines at this site. Our data showed that LAR-containing mutation(s) at these lysines was processed by γ-secretase in a similar manner to the WT LAR. To our knowledge, there are no reports on the mutational analysis of these lysines in APP or any other γ-secretase substrates other than Notch. Thus, it remains to be elucidated whether regulation of γ-secretase cleavage by monoubiquitination is specific to Notch, or whether processing of other substrates is also regulated in this manner.

Increasing evidence suggests a role for PS/γ-secretase in the regulation of cell adhesion dynamics and migration. We have previously shown that PS/γ-secretase-mediated cleavage of nectin-1α and voltage-gated sodium channel β2-subunit regulates their function in cell adhesion and migration (20, 21). PS/γ-secretase-mediated release of E-cadherin ICD has been shown to result in the disassembly of adherens junctions (36–42, 66). The tyrosine phosphorylation status of β-catenin is known to directly affect epithelial cell adhesion and migration (42). Decreased β-catenin tyrosine phosphorylation enhances β-catenin-E-cadherin association and cell adhesion. In contrast, increased β-catenin tyrosine phosphorylation results in increased levels of free β-catenin and disruption of adherens junctions (67). Our coimmunoprecipitation studies indicated that LAR associates with endogenous β-catenin, and regulates the transport of β-catenin-cadherin complexes to synaptic sites, suggesting that LAR is involved in the formation and maintenance of cellular junctions (39, 41, 42, 53). Activation of LAR by its ligands enhances neurite outgrowth and formation of synapses, further substantiating the idea that LAR regulates cell-cell and cell-matrix interactions (36–42, 66). The tyrosine phosphorylation status of β-catenin is known to directly affect epithelial cell adhesion and migration (42). Decreased β-catenin tyrosine phosphorylation enhances β-catenin-E-cadherin association and cell adhesion. In contrast, increased β-catenin tyrosine phosphorylation results in increased levels of free β-catenin and disruption of adherens junctions (67). Our coimmunoprecipitation studies indicated that LAR associates with endogenously expressed tyrosine-phosphorylated β-catenin, confirming the previous findings on the interaction of these proteins (39, 41). However, inhibition of γ-secretase activity resulted in diminished association of LAR and β-catenin, suggesting that PS/γ-secretase activity directly or indirectly regulates their interaction. The fact that β-catenin directly interacts with the D1 phosphatase domain of LAR suggests that the ICDs are important for the interaction, and that PS/γ-secretase-mediated cleavage of LAR affects β-catenin tyrosine phosphorylation status and thereby cell adhesion (41).
Ruhe et al. (55) reported that induction of LAR ectodomain shedding results in the proteolytic processing of the P-subunit, which decreases the overall phosphatase activity of LAR under serum starvation conditions. On the other hand, studies with recombinant LICDs have indicated that the ICDs maintain phosphatase activity (33). Interestingly, it has been shown previously that γ-secretase-mediated cleavage of the tyrosine kinase receptor ErbB4 results in the release of kinase-active ErbB4-ICD, which localizes to the cytoplasm and the nucleus and phosphorylates target proteins in these compartments (68–71). Therefore, PS/γ-secretase-mediated cleavage of LAR could result in the release of catalytically phosphate-active LICDs. We observed that LICD is released by γ-secretase activity to the cytoplasm from where some LICDs translocate to the nucleus. Therefore, the presence of LICD in the nucleus raises an intriguing possibility that LICD could dephosphorylate β-catenin and regulate its function also in the nucleus. Wnt signaling has been shown to induce association of free β-catenin with the transcription factor complex of TCF/LEF and to the cytoplasm from where some LICDs translocate to the nucleus. Therefore, the presence of LICD in the nucleus raises an intriguing possibility that LICD could dephosphorylate β-catenin and regulate its function also in the nucleus. Wnt signaling has been shown to induce association of free β-catenin with the transcription factor complex of TCF/LEF and to

Acknowledgments—We thank Drs. A. Dunah and M. Sheng (MIND, Massachusetts General Hospital and Harvard Medical School and Massachusetts Institute of Technology) for generously providing the LAR cDNA construct and antibody; Dr. M. Wolfe (Brigham and Women’s Hospital) for the gift of the γ-secretase inhibitor WPE-31C; Dr. S. S. Sisodia (University of Chicago) for the BD1 and BD6 ES cells; and Dr. M. Hiltunen (University of Kuopio) for kind help with qPCR.

REFERENCES

1. Wolfe, M. S., Xia, W., Ostaszewski, B. L., Diehl, T. S., Kimberly, W. T., and Selkoe, D. J. (1999) Nature 398, 513–517.
2. Yu, G., Nishimura, M., Arawaka, S., Levitan, D., Zhang, L., Tandon, A., Song, Y. Q., Rogaeva, E., Chen, F., Kawarai, T., Supala, A., Levesque, L., Yu, H., Yang, D. S., Holmes, E., Milman, P., Liang, Y., Zhang, D. M., Xu, D. H., Sato, C., Rogaev, E., Smith, M., Janus, C., Zhang, Y., Aebersold, R., Farrer, L. S., Sorbi, S., Bruni, A., Fraser, P., and St. George-Hyslop, P. (2000) Nature 407, 48–54.
3. Francis, R. S., McGraith, G., Zhang, I., Ruddy, D. A., Sym, M., Apfeld, J., Nicoll, M., Maxwell, M., Bai, H., Ellis, M. C., Parks, A. L., Xu, W., Li, Gurney, M., Myers, R. L., Himes, C. S., Hiebsch, R., Ruble, C., Nye, J. S., and Curtis, D. (2002) Dev. Cell 3, 85–97.
4. De Strooper, B. (2003) Neuron 38, 9–12.
5. Ebubu, D., Winkler, E., Regula, J. T., Pesold, B., Steiner, H., and Haass, C. (2003) Nat. Cell Biol. 5, 486–488.
6. Selkoe, D. J. (2004) Ann. Intern. Med. 140, 627–638.
7. Mattson, M. P. (2004) Nature 430, 631–639.
8. De Strooper, B., Aancka, W., Cuipers, P., Saftig, P., Cressaerts, K., Mumm, J. S., Schroeter, E. H., Schrijvers, V., Wolfe, M. S., Ray, W. J., Goate, A., and Kolan, R. (1999) Nature 398, 518–522.
9. Kimberly, W. T., Zheng, J. B., Guenette, S. Y., and Selkoe, D. J. (2001) J. Biol. Chem. 276, 40288–40292.
10. Cao, X., and Sudhof, T. C. (2001) Science 293, 115–120.
11. Von Rotz, R. C., Kohli, B. M., Bosset, J., Meier, M., Suzuki, T., Nitsch, R. M., and Konieczko, U. (2004) J. Cell Sci. 117, 4435–4448.
12. Buxbaum, J. D., Liu, K. N., Luo, Y., Slack, J. L., Stocking, K. L., Peschon, J. J., Johnson, R. S., Castner, B. J., Corr, D. P., and Black, R. A. (1998) J. Biol. Chem. 273, 27765–27776.
13. Lopez-Perez, E., Zhang, Y., Frank, S. J., Creemers, J., Seidah, N., and Chequer, F. (2001) J. Neurochem. 76, 1532–1539.
14. Hussain, I., Powell, D., Howlett, D. R., Tew, D. G., Meek, T. D., Chapman, C., Gloger, I. S., Murphy, K. E., Southan, C. D., Ryan, D. M., Smith, T. S., Simmons, D. L., Walsh, F. S., Dingwall, C., and Christie, G. (1999) Mol. Cell. Neurosci. 14, 419–427.
15. Sinha, S., Anderson, J. P., Barbou, R., Basi, G. S., Caccavello, R., Davis, D., Doan, M., Dovey, H. F., Frigon, N., Hong, J., Jacobson-Croak, K., Jewett, N., Keim, P., Knops, J., Lieberburg, I., Power, M., Tan, H., Tatsuuno, G., Tung, J., Schenck, D., Seubert, P., Suhaimi, S., Wang, S., Walker, D., Zhao, J., McConlogue, L., and John, V. (1999) Nature 402, 537–540.
16. Vassar, R., Bennett, B. D., Babu-Khan, S., Kain, S., Mendiaz, E. A., Denis, P., Teplow, D. B., Ross, A., Amante, E., Leofl, R., Luo, Y., Fisher, S., Fuller, J., Edson, S., Lile, J., Jarosinski, M. A., Biere, A. L., Curran, E., Burgess, T., Louis, J. C., Collins, F., Trenor, J., Rogers, G., and Citron, M. (1999) Science 286, 735–741.
17. Yan, R., Bienkowski, M. J., Shuck, M. E., Miao, H., Tory, M. C., Pauley, A. M., Brasher, I. R., Stratman, N. M., Mathews, W. R., Buhl, A. E., Carter, D. B., Tomasselli, A. G., Parodi, L. A., Heinrikson, R. L., and Gurney, M. E. (1999) Nature 402, 533–537.
18. Kopan, R., and Ilagan, M. X. (2004) Nat. Rev. Mol. Cell. Biol. 5, 499–504.
19. Marambaud, P., Shioi, J., Serban, G., Georgakopoulous, A., Sarner, S., Nagy, V., Baki, L., Wen, P., Efthimiopoulos, S., Shao, Z., Wiesniewski, T., and Robakis, N. K. (2002) EMBO J. 21, 1948–1956.
20. Kim, D. Y., Ingano, L. A., and Kovacs, D. M. (2002) J. Biol. Chem. 277, 49976–49981.
21. Kim, D. Y., Ingano, L. A., Carey, B. W., Pettingell, W. H., and Kovacs, D. M. (2005) J. Biol. Chem. 280, 23251–23261.
22. Wong, H. K., Sakurai, T., Oyama, F., Kaneko, K., Wada, K., Miyazaki, H., Kurosawa, M., De Strooper, B., Saftig, P., and Nukina, N. (2005) J. Biol. Chem. 280, 23099–23017.
23. De Strooper, B., Saftig, P., Cressaerts, K., Vanderstichele, H., Gudhe, G., Annaert, W., Von Figura, K., and Van Leuven, F. (1998) Nature 391, 387–390.
24. Xia, W., Zhang, J., Ostaszewski, B. L., Kimberly, W. T., Seubert, P., Koo, E. H., Shen, J., and Selkoe, D. J. (1998) Biochemistry 37, 16465–16471.
25. Chen, F., Yang, S. D., Petanekas, S., Yang, A., Tandon, A., Yu, G., Rozmahel, R., Ghiso, J., Nishimura, M., Zhang, D. M., Kawarai, T., Levesque, G., Mills, J., Levesque, L., Song, Y. Q., Rogaeva, E., Westaway, D., Mount, H., Gandy, S., St George-Hyslop, P., and Fraser, P. E. (2000) J. Biol. Chem. 275, 36794–36802.
26. Pot, D. A., Woodford, T. A., Remboutsika, E., Hau, R. S., and Dixon, J. E. (1991) J. Biol. Chem. 266, 19688–19696.
27. Streuli, M. and Saito, H. (1989) EMBO J. 8, 787–796.
28. Paul, S., and Lombroso, P. J. (2003) Cell Mol. Life Sci. 60, 2465–2482.
29. Streuli, M., Krueger, N. X., Thai, T., Tang, M., and Saito, H. (1990) EMBO J. 9, 2399–2407.
30. Yu, Q., Lenardo, T., and Weinberg, R. A. (1992) Oncogene 7, 1051–1057.
PS/γ-Secretase Regulates LAR-β-Catenin Signaling

31. Pulido, R., Serra-Pages, C., Tang, M., and Streuli, M. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 11686–11690
32. Zhang, J. S., and Longo, F. M. (1995) J. Cell Biol. 128, 415–431
33. Honkaniemi, J., Zhang, J. S., Yang, T., Zhang, C., Tisi, M. A., and Longo, F. M. (1998) Brain Res. Mol. Brain. Res. 60, 1–12
34. Yang, T., Bernabeu, R., Xie, Y., Zhang, J. S., Massa, S. M., Rempel, H. C., and Longo, F. M. (2003) J. Neurosci. 23, 3353–3363
35. Yang, T., Yin, W., Derevyanny, V. D., Moore, I. A., and Longo, F. M. (2005) Eur. J. Neurosci. 22, 2159–2170
36. O’Grady, P., Thai, T. C., and Saito, H. (1998) J. Cell Biol. 141, 1675–1684
37. Fox, A. N., and Zinn, K. (2005) J. Cell Biol. 170, 1–11
38. Johnson, K. G., Tenney, A. P., Ghose, A., Duckworth, A. M., Higashi, M. E., and Longo, F. M. (1998) J. Biol. Chem. 273, 10173–10183
39. Yeo, T. T., Yang, T., Massa, S. M., Zhang, J. S., Honkaniemi, J., Butcher, L. L., and Longo, F. M. (1997) J. Neurosci. Res. 47, 348–360
40. Van Lieshout, E. M., Van der Heijden, I., Hendriks, W. J., and Van der Zee, C. E. (2001) Neuroscience 102, 833–841
41. Xie, Y., Ye, T. T., Zhang, C., Yang, T., Tisi, M. A., Massa, S. M., and Longo, F. M. (2001) J. Neurosci. 21, 5130–5138
42. Van der Zee, C. E., Man, T. Y., Van Lieshout, E. M., Van der Heijden, I., Van Bree, M., and Hendriks, W. J. (2003) Eur. J. Neurosci. 17, 991–1005
43. Kolkim, M. J., Streiger, F., Linkels, M., Moemen, M., Heeren, B. J., Hendriks, W. J., and Van der Zee, C. E. (2004) Behav. Brain. Res. 154, 171–182
44. Serra-Pages, C., Medley, Q. G., Tang, M., Hart, A., and Streuli, M. (1998) J. Biol. Chem. 273, 15611–15620
45. Zhen, M., and Jin, Y. (1999) Nature 401, 371–375
46. Kaufmann, N., DeProto, J., Ranjan, R., Wun, H., and Van Vactor, D. (2002) Neuron 34, 27–38
47. Wyszynski, M., Kim, E., Dunah, A. W., Passafaro, M., Valschonoff, J. G., Serra-Pages, C., Streuli, M., Weinberg, R. J., and Longo, F. M. (2002) Neuron 34, 39–52
48. Ko, J., Kim, S., Valschonoff, J. G., Shin, H., Lee, J. R., Sheng, M., Premont, R. T., Weinberg, R. J., and Kim, E. (2003) J. Neurosci. 23, 1667–1677
49. Dunah, A. W., Hueske, E., Wyszynski, M., Hoogenraad, C. C., Jaworski, J., Pak, D. T., Simonetta, A., Liu, G., and Longo, F. M. (2005) Nat. Neurosci. 8, 458–467
50. Frade, J. M. (2005) J. Neurosci. 25, 1407–1411
51. Ruhe, J. E., Streit, S., Hart, S., and Ullrich, A. (2006) Cell Signal. 18, 1515–1527
52. Gupta-Rossi, N., Six, E., LeBail, O., Logeat, F., Chastagner, P., Olyr, A., Israel, A., and Brou, C. (2004) J. Cell Biol. 166, 73–83
53. Lammich, S., Kojro, E., Postina, R., Gilbert, S., Peffer, R., Jasionowski, M., Haass, C., and Fahrenholz, F. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 3922–3927
54. Brou, C., Logeat, F., Gupta, N., Bessia, C., LeBail, O., Doedens, I. R., Cuman, A., Roux, P., Black, R. A., and Israel, A. (2000) Mol. Cell 5, 207–216
55. Mumm, J. S., Schroeter, E. H., Saxena, M. T., Griesemer, A., Tian, X., Pan, D. J., Ray, W. J., and Kopan, R. (2000) Mol. Cell 5, 197–206
56. Lieber, T., Kidd, S., and Young, M. W. (2002) Genes Dev. 16, 209–221
57. Geoghapoulos, A., Marambaud, P., Ethemioopoulos, S., Shioli, C., Cui, Q., Li, H. C., Schutte, M., Gordon, R., Holstein, G. R., Martinelli, G., Mehta, P., Friedrich, V. L., Jr., and Robakis, N. K. (1999) Mol. Cell 4, 893–902
58. Uemura, K., Kikugawa, K., Kohno, R., Kuzuya, A., Kageyama, T., Chonabashishi, K., Shiibashi, H., and Shimohama, S. (2003) J. Neurosci. Res. 74, 184–191
59. Kulas, D. T., Goldstein, B. J., and Mooney, R. A. (1996) J. Biol. Chem. 271, 748–754
60. Wang, X., Weng, L. P., and Yu, Q. (2000) Oncogene 19, 2346–2353
61. Tsujikawa, K., Ichijo, T., Mori, K., Tadotsu, N., Sakamoto, K., Sakane, N., Fukuda, S., Furukawa, T., Saito, H., and Yamamoto, H. (2002) Mol. Cancer Res. 1, 155–163
62. Serra-Pages, C., Kedersha, N. L., Fazikas, L., Medley, Q., Debant, A., and Streuli, M. (1995) EMBO J. 14, 2827–2838
63. Kinch, M. S., Clark, G. J., Der, C. J., and Burridge, K. (1995) J. Cell Biol. 130, 461–471
64. Lee, H. J., Jung, K. M., Huang, Y. Z., Bennett, L. B., Lee, J. S., Mei, L., and Kim, T. W. (2002) J. Biol. Chem. 277, 6318–6323
65. Omerovic, J., Puggioni, E. M., Napoletano, S., Visco, V., Fraioli, R., Frati, L., Fukuda, S., and Saftig, P. (2004) Exp. Cell Res. 294, 469–479
66. Arasada, R. R., and Carpenter, G. (2005) J. Biol. Chem. 280, 30783–30787
67. Vidal, G. A., Naresh, A., Marrero, L., and Jones, F. E. (2005) J. Biol. Chem. 280, 19777–19783
68. Nelson, W. J., and Nusse, R. (2004) Science 303, 1483–1487
69. Anders, L., Mertins, P., Lammich, S., Murgia, M., Hartmann, D., Saftig, P., Haass, C., and Ullrich, A. (2006) Mol. Cell. Biol. 26, 3917–3934