THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 278, No. 11, Issue of March 14, pp. 9290–9297, 2003
Printed in U.S.A.

Platelet-derived Growth Factor Induces the β-γ-Secretase-mediated Cleavage of Alzheimer's Amyloid Precursor Protein through a Src-Rac-dependent Pathway*

Received for publication, November 21, 2002, and in revised form, December 23, 2002
Published, JBC Papers in Press, January 6, 2003, DOI 10.1074/jbc.M211899200

Davide Gianni‡§, Nicola Zambrano‡§, Marida Bimonte‡, Giuseppina Minopoli‡¶, Luc Mercken‖, Fabio Talamo, Andrea Scaloni**, and Tommaso Russo‡‡§§**

*From the ‡Dipartimento di Biochimica e Biotecnologie Mediche, Università degli Studi di Napoli Federico II, Napoli 80131, Italy, ‡‡CEINGE biotecnologie avanzate, Napoli 80131, Italy ‖Neurodegenerative Disease Group, Aventis Pharma, Vitry-sur-Seine 94400, France, and the **Proteomics and Mass Spectrometry Laboratory, ISPAAM, National Research Council, Napoli 80147, Italy*

The β-amyloid peptide (Aβ) present in the senile plaques of Alzheimer's disease derives from the cleavage of a membrane protein, named APP, driven by two enzymes, known as β- and γ-secretases. The mechanisms regulating this cleavage are not understood. We have developed an experimental system to identify possible extracellular signals able to trigger the cleavage of an APP-Gal4 fusion protein, which is detected by measuring the expression of the CAT gene transcribed under the control of the Gal4 transcription factor, which is released from the membrane upon the cleavage of APP-Gal4. By using this assay, we purified a protein contained in the C6 cell-conditioned medium, which activates the cleavage of APP-Gal4 and which we demonstrated to be PDGF-BB. The APP-Gal4 processing induced by PDGF is dependent on the γ-secretase activity, being abolished by an inhibitor of this enzyme, and is the consequence of the activation of a pathway downstream of the PDGF-receptor, which includes the non-receptor tyrosine kinase Src and the small G-protein Rac1. These findings are confirmed by the observation that a constitutively active form of Src increases Aβ generation and that, in cells stably expressing APP, the generation of Aβ is strongly decreased by the Src tyrosine kinase inhibitor PP2.

β-Amyloid (Aβ)[1] deposition in the so-called amyloid plaques is one of the main features of Alzheimer's pathology. β-Amyloid consists of ~4-kDa peptides derived from the proteolytic processing of a membrane protein named amyloid precursor protein (APP). This amyloidogenic processing is driven by two enzyme activities, β-site APP cleaving enzyme (BACE) and γ-secretase. BACE cleaves APP at 28 residues from the boundary between the extracellular/intraluminal domain of APP and the transmembrane domain of the protein (for a review see Ref. 1), releasing a large soluble protein, including nearly all the extracellular/intraluminal part of APP, and a short transmembrane peptide, including the 99 C-terminal residues of APP. This transmembrane C99 stub is a substrate for the γ-secretase activity, which cleaves it, in a presenilin-dependent fashion, within the membrane α-helix, giving rise to the Aβ peptide 40–42 amino acids long and to a peptide named APP intracellular domain (AID), which includes the small C-terminal cytosolic domain of APP (for a review see Refs. 2 and 3).

The functions of APP and its proteolytic processing are still unknown. However, although the functions of the APP ectodomain remain elusive, there is increasing evidence that its cytodomain is the center of a complex network of interactions with several proteins, involved in vesicle transport and in signal transduction. In fact, it was demonstrated that APP cytoplasmic domain interacts with kinesin light chain and contributes to vesicles transport (4), thus suggesting that APP cleavage could regulate the transport of vesicles in the axons. On the other hand, the APP cytodomain binds several PTB domain-containing proteins, some of which are involved as adaptor proteins in signal transduction. Fe65 is the first protein that was found to form a stable complex with the cytosolic domain of APP, through one of the two PTB domains it possesses (5, 6). Fe65 is an adaptor protein that interacts with APP, Mena (7) (the mammalian orthologue of the product of the *enabled* gene of *Drosophila*), tyrosine kinase Abl (8) (through its WW domain), transcription factor LSF (9), the histone acetyltransferase Tip60 (10), and low density lipoprotein-receptor-related protein LRP (11) (through its second PTB domain). The involvement of Mena and Abl in the complexes, including APP and Fe65, is in agreement with the finding that APP cytodomain also interacts with mDab1 (12), the orthologue of the Drosophila *disabled* gene, and with the Abl TK, through its SH2 domain (8), given that genetic manipulation of the fly indicated that *enabled*, *disabled*, and DAbl are associated in the same pathway (13). Another interactor of APP is X11 (14), an adaptor protein that forms complexes with various proteins, including Munc18 and CASK (15). More recently, two adaptors involved in signal transduction, Shc and Jip1, have been demon-

* This work was supported in part by grants from the V Framework program (contract QLK6–1999-02238) EU, from the Italian Ministry of Health (Progetto Alzheimer), Miur-FIRB RBNE0IWY7B, from Biogem-Italy (to T. R.), and from MIUR-PRIN (to N. Z.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

§ Both authors contributed equally to this work.
¶ Recipient of a Biogem fellowship.
§§ To whom correspondence should be addressed: Dipartimento di Biochimica e Biotecnologie Mediche, Università degli Studi di Napoli Federico II, via S. Pansini 5, Napoli 80131, Italy. Tel.: 39-08-17-46-3131; Fax: 39-08-17-46-4359; E-mail: russot@dbbm.unina.it.

[1] The abbreviations used are: Aβ, beta-amyloid peptide; AID, APP intracellular domain; APP, amyloid precursor protein; AS, ammonium sulfate; BACE, β-site APP cleaving enzyme; CAT, chloramphenicol acetyltransferase; PTB, phosphotyrosine binding domain; TK, tyrosine kinase; PDGF, platelet-derived growth factor; PDGF-R, PDGF receptor; CMV, cytomegalovirus; DMEM, Dulbecco's modified Eagle's medium; CHO, Chinese hamster ovary; PBS, phosphate-buffered saline; FPLC, fast-protein liquid chromatography; MALDI-TOF, matrix-assisted laser desorption ionization time-of-flight; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; PI3K, phosphatidylinositol 3-kinase; AD, Alzheimer's disease; PDGF-B, PDGF B subunit; PDGF-BB, PDGF-B dimer; PP2, 4-amino-5-(4-chlorophenyl)-7-(*t*-butyl)pyrazolo(3,4-d)pyrimidine; PP3, 4-amino-7-phenylpyrazol(3,4-d)pyrimidine; sAPP, soluble APP.

strated to bind to APP and AID through their PTB domains (16–19).

The evident complexity of this protein-protein interaction network suggests that APP could be a multifunctional molecule that anchors several different oligomeric complexes close to the membrane, possibly in specific subdomains such as caveolae (20), and/or that APP regulates the availability of these complexes in their final destination, upon APP cleavage and detachment of the APP cytodomain from the membrane.

A crucial point that should be addressed to further study the possible interplay of APP and transduction pathways involving the above mentioned proteins concerns the molecular mechanisms that induce APP processing. In this report we show that PDGF-BB is a potent activator of APP β-γ cleavage, giving rise to an increased generation of Aβ through a pathway involving the non-receptor tyrosine kinase Src and the small G-protein Rac1.

MATERIALS AND METHODS

Generation of HeLaAG Clones—A vector driving the expression of a fusion protein, consisting of the human APP_{695} followed by a flexible hinge of ten glycines and by the entire yeast transcription factor Gal4, has been generated by cloning into RcCMV vector (Invitrogen) cDNA fragments amplified using as template the human APP_{695} cDNA and the yeast Gal4 cDNA. The following specific oligonucleotide primers (CEINGE) were used for PCR amplifications (94 °C, 1 min; 64 °C, 1 min; 72 °C, 7 min; for 40 cycles): forward $hAPP_{695}$ *Hin*dIII (5′-CCCAAGCTTACTAAGGCCATGCTGCCCGGTTTGGCACTGC-3′) and reverse hAPP695 *Apa*/*Not*I (5′-CATCGGGCCCCTACGCGGCCGCGTTCTGCATCTGCTCAAAGAACTTG-3′); forward yGAL4 *Not*/10Gly (5′-AAGGAAAAAAGCGGCCGCTGGTGGTGGTGGTGGTGGTGGTGGTGGTAAGCTACTGTCTTCTATCGAACAAGC-3′) and reverse yGAL4 *Apa*I (5′-CATCGGGCCCTTACTCTTTTTTTGGGTTTGGTGGGG-3′). Restriction sites are underlined, and the ten glycine codons are in italic. This APP-Gal4 expressing vector, containing the neomycin resistance gene, has been transfected into HeLa cells by calcium-phosphate method, and after a 14-day G418 selection (900 μg/ml final concentration), several G418-resistant clones have been isolated. Two pools of these clones have been used (HeLaAG).

The cleavage of APP-Gal4 fusion protein has been assayed by transiently transfecting HeLaAG cells (5×10^5 cells/60-mm dishes) by calcium-phosphate method with G5BCAT vector (3 μg), in which the transcription of chloramphenicol acetyltransferase (CAT) gene is under the control of a Gal4-dependent promoter (21). CAT expression was measured by using colorimetric CAT enzyme-linked immunosorbent assay (Roche Molecular Biochemicals). Other transfections of HeLaAG cells were carried out by using the calcium-phosphate method; all the plasmids were used at 3 μg each, and the total amount of DNA in co-transfections was always brought to 10 μg with RcCMV vector.

Cell Culture Conditions—Wild type HeLa and HeLa AG cells were grown at 37 °C in the presence of 5% CO_2 in Dulbecco's modified Eagle's medium (DMEM, Invitrogen) supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin (all from HyClone). C6 and NIH3T3 cells and co-cultures were at 37 °C in the presence of 5% CO_2 in RPMI medium (Invitrogen) supplemented with 10% fetal bovine serum and antibiotics.

Wild type HeLa or HeLa AG cells, 24 h after transfection with G5BCAT vector, were treated for the indicated times with 40 ng/ml recombinant human PDGF-BB (Sigma), 5 or 10 μg/ml protein fraction precipitated with 40% AS or 200-μl fractions eluted from Sephadex G-75, diluted in DMEM without serum to a final volume of 2 ml. Inhibitors were added at the indicated times at the following concentrations: 10 μM PP2 (Calbiochem), 2 μM AG1296 (Calbiochem), 30 μM genistein (Calbiochem), 10 μM γ-secretase inhibitor compound X (Calbiochem), 100 μM PD098059 (Sigma), and 100 nM Wortmannin (Sigma).

HEK293 and CHO cells were grown in the same conditions as HeLaAG. HEK293 were transfected with 0.5 μg of human APP_{695} expression vector and 0.5 μg of SrcYF vector by LipofectAMINE 2000 (Invitrogen) in 35-mm plates. Total Aβ peptide was measured by sandwich enzyme-linked immunosorbent assay with 6E10 and 4G8 antibodies.

Purification of the Activity That Induces APP-Gal4 Cleavage—C6 cells were grown as described above in 100 dishes of 150-mm diameter (Falcon) to confluence; cell sheets in each dish have been washed twice with phosphate-buffered saline (PBS), and then cells were cultured in RPMI medium without serum. After 3 days of incubation, 3 liters of conditioned medium was harvested, centrifuged at 1000 rpm for 20 min to remove debris, and concentrated to 480 ml by using a Centriplus YM-3000 (Amicon). Then, 104 g of ammonium sulfate was added to the concentrated conditioned medium to obtain a 40% ammonium sulfate solution, which was stirred overnight at 4 °C and then centrifuged at 9000 rpm for 2 h. 70% AS saturation was reached by adding 87.4 g of ammonium sulfate to the 40% saturated solution, and 100% AS saturation was reached by adding 100.3 g of ammonium sulfate to the 70% saturated solution.

The precipitates were dissolved in 15 ml of PBS and dialyzed against $5 \times$ 2-liter changes of PBS. 1 mg of this sample was separated by FPLC onto a Sephadex G-75 column (30 g of swollen resin, pre-equilibrated in PBS) and run in this solvent at 0.25 ml/min while collecting 400-μl fractions every 1.6 min.

Bands from SDS-PAGE were excised from the gel, triturated, and washed with water. Proteins were reduced in-gel, *S*-alkylated with iodoacetamide, and digested with trypsin as previously reported (22). Digested aliquots were subjected to a desalting/concentration step on μZipTipC18 (Millipore Corp., Bedford, MA) before MALDI-TOF mass spectrometry analysis. Peptide mixtures were loaded on the instrument target, using the dried droplet technique and α-cyano-4-hydroxycinnamic as matrix, and analyzed by using a Voyager-DE PRO mass spectrometer (Applied Biosystems, Framingham, MA). The PROWL software package was used to identify proteins unambiguously from an independent non-redundant sequence data base (23).

Immunodepletion of 40% AS fraction was obtained by incubating 20 μg of the fraction diluted in 500 μl of PBS with 60 μg of anti-PDGF antibody (Sigma) or with 60 μg of mouse IgG (Sigma) for 2 h at 4 °C. Then the mixtures were chromatographed on 20 μl of Protein AG-Sepharose (Santa Cruz Biotechnology) for 30 min at 4 °C, and, after centrifugation, the supernatants were diluted in DMEM without serum to a final volume of 2 ml.

Preparation of Cell Extracts and Western Blotting Analyses—For CAT assay, transiently transfected HeLa AG cells were harvested in cold TEN (40 mM Tris-HCl, pH 7.5, 1 mM EDTA, 150 mM NaCl), frozen at −80 °C for 30 min, and resuspended in lysis buffer (10 mM Hepes, pH 7.9, 0.1 mM EGTA, 0.5 mM dithiothreitol, 5% glycerol, 0.2 mM phenylmethylsulfonyl fluoride, 400 mM NaCl). Total extracts were clarified by centrifugation at 14,000 rpm at 4 °C, and protein concentration was determined by Bio-Rad assay; for CAT concentration measurement, 150 μg of each protein extract was used.

For Western blotting analyses, HeLa AG cells were harvested in cold PBS, resuspended in lysis buffer (40 mM Tris-HCl, pH 7.2, 1% Triton X-100, 150 mM NaCl, 1 mM EDTA, 0.2 mM phenylmethylsulfonyl fluoride, 100 μg/ml aprotinin, 100 μg/ml leupeptin) and kept in ice for 15 min. Then total extracts were clarified by centrifugation at 14,000 rpm at 4 °C. 20 μg of each extract or 20 μl of pooled fractions eluted from the G-75 column was electrophoresed on 4–12% SDS-polyacrylamide gradient gel under reducing conditions and transferred to Immobilon-P membranes (Millipore). Filters were then blocked in 5% nonfat dry milk in T-PBS solution (PBS and 0.05% Tween) and incubated with appropriate dilutions of primary antibody, overnight at 4 °C. The excess antibody was removed by sequential washing of the membranes in T-PBS, and then a 1:5000 dilution of the appropriate secondary antibody (horseradish peroxidase-conjugated) was added to filters for 30 min, at room temperature. The excess was removed by sequential washing of the membranes in T-PBS, and the signals were detected by chemiluminescence, using the ECL system (Amersham Biosciences). The antibodies used and their dilutions were: anti-PDGF (Sigma), 1:750; anti-Gal4DBD (Calbiochem), 1:1000; anti-APP 6E10 (Sigma), 1:1000; anti-APP CT695 (Zymed Laboratories Inc.), 1:250; anti-phosphoERK (Santa Cruz Biotechnology), 1:1000; and anti-phosphoAkt (Santa Cruz), 1:1000.

RESULTS

C6 Cell-conditioned Medium Induces Gal4-dependent-CAT Gene Transcription in HeLa Cells Expressing APP-Gal4 Fusion Protein—We examined the possibility that extracellular signals could induce the β-γ-secretase-mediated cleavage of APP. To address this point we developed an experimental system based on a recombinant protein in which the yeast Gal4 transcription factor is fused to the cytosolic C-terminal end of APP_{695}. This system is based on the prediction that, in cells expressing APP-Gal4, upon the cleavage of this molecule by β-γ-secretase activities, AID-Gal4 is released from the membrane and should become available to activate the transcription of the chloramphenicol acetyltransferase (CAT) gene cloned under the control of five Gal4 cis-elements in the

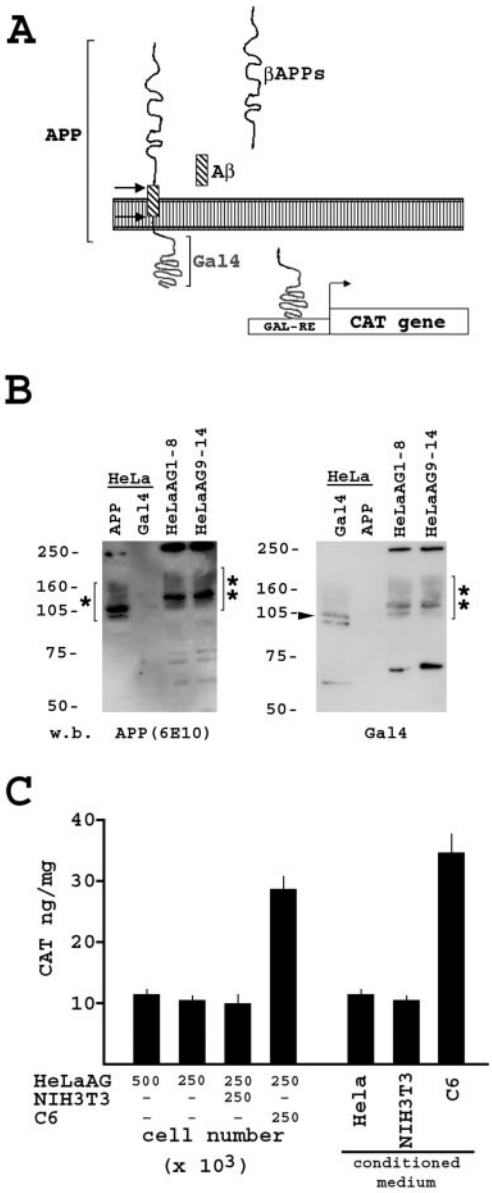

FIG. 1. **Isolation and characterization of HeLa cell clones stably expressing APP-Gal4 fusion protein.** *A*, schematic representation of the experimental system: APP-Gal4 fusion protein is cleaved by β- and γ-secretases, and this results in the release from the membrane of Gal4 protein fused to the intracellular domain of APP (AID-Gal4). This protein then activates the transcription of the CAT gene cloned under the control of five Gal4 cis-elements. *B*, Western blot analyses of lysates from HeLa cells transfected with human APP_{695} or with Gal4 expression vectors and from two pools of HeLa clones (HeLaAG1–8, HeLaAG9–14) stably expressing APP-Gal4 fusion protein. The two Western blots are with APP 6E10 antibody, recognizing the extracellular domain of APP, and with Gal4 antibody, respectively. *One asterisk* indicates wild type APP bands, *two asterisks* indicate APP-Gal4 bands, and the *arrowhead* indicates wild type Gal4 bands. C, HeLaAG cells transfected with G5BCAT vector were co-cultured with either NIH3T3 or C6 cells or cultured in the presence of the conditioned medium of these cell lines. For co-cultures, cells were plated at the indicated cell numbers, harvested 72 h after plating and their extracts were assayed for CAT concentration. Conditioned media from 72 h cultures of the indicated cells were added to 2.5×10^5 HeLaAG cells. Extracts from cells harvested 48 h after the exposure to conditioned medium were assayed for CAT concentration. Standard deviations of triplicate experiments are reported.

G5BCAT vector (21) (see Fig. 1*A*). Based on this experimental design, HeLa cells were transfected with a vector driving the expression of APP-Gal4 fusion protein and G418 resistance gene, and several clones stably expressing APP-Gal4 have been isolated. Fig. 1*B* shows a Western blot of the extracts from several HeLa clones challenged with either APP or Gal4 antibodies and demonstrates the expression of a protein recognized by both antibodies. The experiments reported below were conducted by using two pools of these clones, HeLaAG1–8 and HeLaAG9–14, thereafter indicated as HeLaAG.

To evaluate both cell-anchored and secreted factors that could activate APP-Gal4 proteolytic processing, the first experimental approach we used consisted of 1) a co-culture of HeLaAG cells, transiently transfected with G5BCAT plasmid, with various cell lines of different origin and 2) an assay of CAT accumulation in HeLaAG cultures pure or co-cultured with these cells. Fig. 1*C* shows that co-culturing of HeLaAG with C6 cells resulted in a significant increase of the CAT expressed by HeLaAG, whereas no change was observed in the co-cultures with other cell lines, such as NIH3T3 fibroblasts. C6 cells are derived from a rat glioma and are known to secrete several growth factors (24). Therefore, we examined whether the conditioned medium from C6 cells mimics the effect of CAT accumulation observed in the co-cultures. As shown in Fig. 1*C*, HeLaAG cells, grown in the presence of C6-conditioned medium, express higher levels of CAT compared with the cells grown in the conditioned medium from HeLa cells or from NIH3T3 cultures.

Purification of the APP-Gal4 Cleavage-inducing Activity—A large-scale preparation of C6-conditioned medium was used as a source for the purification of the one or more molecules that induce the CAT accumulation in HeLaAG cells transfected with G5BCAT vector. Fig. 2 shows the steps of this purification based on ammonium sulfate (AS) precipitation, size-exclusion chromatography, and SDS-PAGE. The activity is restricted to the 40% AS fraction (Fig. 2*A*), which was applied on FPLC equipped with a Sephadex G-75. Eluted fractions from the chromatography were assayed for their ability to induce CAT accumulation in HeLaAG cells, and the results allowed us to identify two peaks of activity of about 70 and 30 kDa, respectively (Fig. 2*B*). SDS-PAGE of the proteins present in the relevant fractions, compared with fractions devoid of activity, suggested that one band of about 15 kDa could be a good candidate (see Fig. 2*C*). This band, separately excised from the lanes of the corresponding active fractions, was digested with trypsin and analyzed by MALDI-TOF mass spectrometry. Peptide mass fingerprint analysis and non-redundant sequence data base matching in both cases allowed its unambiguous identification as PDGF-B. To confirm the identification, the relevant fractions were electrophoresed and blotted with a PDGF antibody. This blot demonstrated that PDGF is present only in the fractions that activate the accumulation of CAT in HeLaAG cells (Fig. 2*D*).

PDGF Induces a γ-Secretase-dependent Cleavage of APP-Gal4 Protein—HeLaAG were exposed to 40 ng/ml of purified human PDGF-BB for 12 h, and this resulted in a dramatic induction of CAT expression (Fig. 3). This phenomenon depends on the activation of the PDGF receptor (PDGF-R), considering that the treatment of HeLaAG cells with a TK-nonspecific inhibitor such as genistein or with PDGF-R inhibitor AG1296 resulted in a significant decrease of CAT accumulation, following the treatment with PDGF. Although, unexpectedly, PDGF is present also in the ~70-kDa fraction of the size exclusion chromatography (see Fig. 2, *C* and *D*), it cannot be excluded that other molecules with an activity similar to that of PDGF could be also present in the C6-conditioned medium. To address this point, HeLaAG cells were treated with the 40% ammonium sulfate fraction and with the PDGF-R inhibitor. Also in this case, the accumulation of CAT was prevented by the PDGF-R inhibitor (see Fig. 3), thus strongly supporting that PDGF is the only fac-

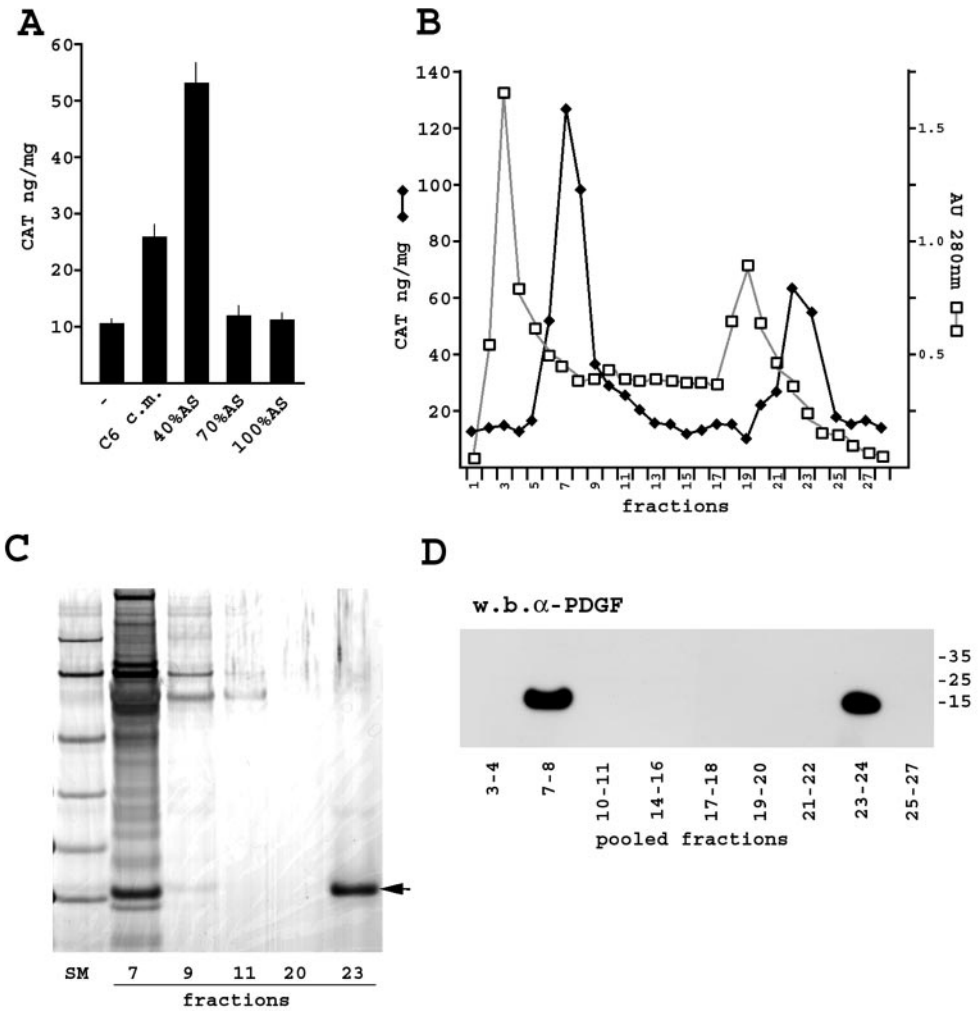

FIG. 2. **Purification of the activity present in the C6-conditioned medium, which induces CAT expression in HeLaAG cells.** A, 3 liters of C6-conditioned medium was concentrated and fractionated by ammonium sulfate precipitation. The fractions obtained with a salt saturation of 40, 70, or 100% were dialyzed and assayed for their ability to induce CAT accumulation in HeLaAG cells transfected with G5BCAT vector. The concentrations of assayed samples were: 40% AS, 5 μg/ml; 70% AS, 200 μg/ml; 100% AS, 200 μg/ml. Standard deviations of triplicate experiments are reported. *B*, 40% AS fraction was applied on a Sephadex G-75 column and chromatographed by FPLC. Fractions of 400 μl were collected and assayed for their ability to induce CAT accumulation in HeLaAG cells. The calibration of the column with pure standards of known molecular mass indicated that the two peaks of activity were eluted as ~70 and 30 kDa proteins, respectively. *C*, fractions of the Sephadex chromatography containing (*7* and *23*) and not containing (*9*, *11*, and *20*) the activity were concentrated and electrophoresed on a reducing SDS-PAGE and silver-stained. The *arrow* indicates a band of about 15 kDa present in fractions containing the activity. *D*, pooled fractions containing (7–8 and 23–24) or not containing (3–4, 10–11, 14–16, 17–18, 19–20, 21–22, 25–27) the activity were electrophoresed on a reducing SDS-PAGE and blotted with a PDGF antibody, thus confirming the identification made by MALDI-TOF mass spectrometry.

tor, present in C6-conditioned medium, that activates APP-Gal4 cleavage. Furthermore, immunodepletion of the 40% AS fraction with anti-PDGF antibody resulted in the abolishment of the CAT accumulation observed upon exposure of HeLaAG cells to pure 40% AS fraction, whereas the depletion with mouse IgG was completely ineffective (see Fig. 3). Therefore, the activity present in the 70-kDa fraction could be a multimer of the PDGF-B subunit.

To rule out the possibility that PDGF treatment induces CAT accumulation through a mechanism independent from the cleavage of APP-Gal4, we exposed wild type HeLa cells mock transfected or transfected with Gal4 to 40 ng/ml PDGF-BB. As shown in Fig. 4*A*, PDGF-BB treatment did not modify the accumulation of CAT in these experimental conditions. Therefore, the increase of CAT concentration observed in HeLaAG cells exposed to PDGF could be due to an activation of the CAT gene transcription by GAL4 released upon the cleavage of APP-Gal4. To address this point, extracts from HeLaAG cells exposed to PDGF or AS 40% fraction were analyzed by Western blot with anti-APP or anti-Gal4 antibodies. These experiments showed the presence, in extracts from HeLaAG cells exposed to PDGF or 40% AS fraction, of a band of a size very similar to that of Gal4, with both the Gal4 antibody and the CT695 antibody, recognizing the APP C-terminal domain (see Fig. 4*B*). A similar blot was challenged with the 6E10 antibody, which was directed against the N-terminal sequence of the β-amyloid peptide. This antibody recognizes the uncleaved APP-Gal4 but not the cleaved molecule. This indicates that the cleaved molecule contains the C-terminal domain of APP (AID-Gal4) and not the N-terminal sequence of Aβ.

To evaluate whether the observed cleavage of APP-Gal4 requires the γ-secretase activity, we treated HeLaAG cells exposed to PDGF or to the 40% AS fraction with the γ-secretase inhibitor compound X (25). As shown in Fig. 4*C*, the treatment of HeLaAG cells exposed to either PDGF or partially purified fraction with 10 μM of the γ-secretase inhibitor resulted in an almost complete abolishment of the effects on CAT accumulation. These results indicate that PDGF, through the activation

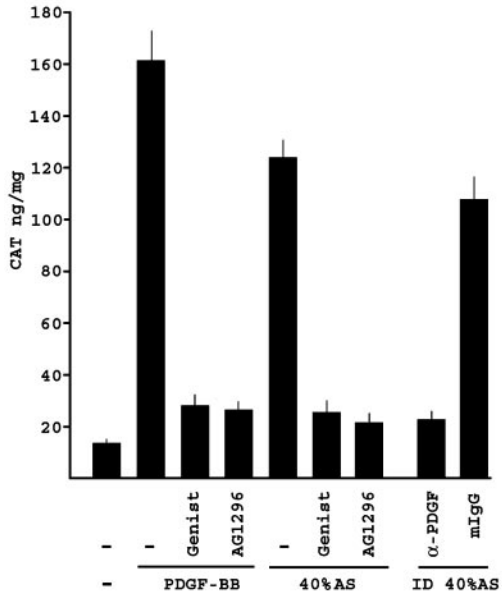

FIG. 3. **PDGF-BB contained in C6 cell-conditioned medium induces CAT accumulation in HeLaAG cells.** HeLaAG cells transfected with G5BCAT vector were exposed to 40 ng/ml recombinant PDGF-BB or to 10 μg/ml 40% AS fraction for 24 h before harvesting. In the same conditions the cells were also exposed, as indicated, to 30 μM genistein or to 2 μM AG1296, which are a general TK inhibitor and a PDGF-R TK inhibitor, respectively. To ascertain whether the 40% AS fraction also contains factors, other than PDGF-BB, activating CAT expression, the 40% AS fraction was immunodepleted either with anti-PDGF antibody (α-PDGF) or with mouse IgG (mIgG), and HeLaAG cells were exposed to these mixtures (ID 40%AS). Standard deviations of triplicate experiments are reported.

of its receptor, induces a proteolytic cleavage of APP-Gal4, which requires the γ-secretase activity.

PDGF-induced APP-Gal4 Cleavage Functions through an Src-dependent Pathway—There are many pathways activated following PDGF-R interaction with its cognate growth factor. Tyrosine-phosphorylated PDGF-R activates the Ras-MAPK pathway through Grb2/SOS and Shc/Grb2/SOS. The possible involvement of this pathway in the APP-Gal4 processing was explored by treating HeLaAG cells exposed to PDGF with the inhibitor of ERKs, PD098059; this inhibitor does not modify the effects of both PDGF and 40% AS fraction (Fig. 5). Another pathway that mediates the effects of PDGF-R activation is that of PI3K-Akt. Also, Fig. 5 shows that the PI3K inhibitor wortmannin does not affect the CAT accumulation induced by both PDGF and 40% AS fraction.

Src and other members of the Src non-receptor TK family interact with and are activated by PDGF-R (26). To explore this pathway, HeLaAG cells have been treated with a specific inhibitor of Src TK, PP2, and with a related compound unable to inhibit Src (PP3). The treatment with PP2 of HeLaAG cells almost completely abolished the accumulation of CAT observed upon the exposure to either PDGF or 40% ammonium sulfate fraction, whereas the treatment with PP3 was completely ineffective (see Fig. 5).

To further explore this finding, HeLaAG cells were transiently transfected with SrcYF vector expressing a constitutively active Src mutant (27). As shown in Fig. 6*A*, the expression of active Src resulted in the accumulation of CAT in the absence of stimulation by either PDGF or purified fractions. Accordingly, HeLaAG cells transfected with a dominant negative mutant of Src (SrcY-FKM) (28) and exposed to PDGF or to 40% AS fraction showed a significantly decreased accumulation of CAT, compared with mock transfected cells exposed to PDGF.

The possible effectors downstream of Src are not completely understood. One of these downstream factors is the non-receptor TK Abl (29). The possible role of Abl TK in APP-Gal4 cleavage was explored by transfecting HeLaAG cells with a constitutively active mutant of Abl (Abl-PP) (30). Under these conditions no induction of APP-Gal4 cleavage was observed, thus indicating that this kinase is not involved in this phenomenon. On the contrary, another molecule that has been recently observed to be activated by PDGF and Src is Rac1, which belongs to the family of Rho G-proteins. The transfection of HeLaAG cells with a vector driving the expression of a constitutively active form of Rac (RacQL) (31) resulted in an increase of CAT comparable to that observed upon the transfection with SrcYF, and the co-transfection of SrcYF with a dominant negative mutant of Rac (RacN17) strongly decreased the amount of CAT compared with that accumulated in the cells transfected only with SrcYF (see Fig. 6*A*). Furthermore, a similar inhibition of CAT accumulation, following the exposure to either PDGF or 40% ammonium sulfate fraction, was observed in the cells transfected with RacN17.

To ascertain whether Src and Rac1, like PDGF, also activate APP-Gal4 processing through a γ-secretase-dependent pathway, HeLaAG cells were transfected with SrcYF or with RacQL, the constitutively active mutants of these two proteins, and treated with the γ-secretase inhibitor compound X. As shown in Fig. 6*B*, the γ-secretase inhibitor almost completely abolished the effects of SrcYF and RacQL transfections.

PDGF Induces the Generation of Aβ from Wild Type APP through an Src-dependent Pathway—The above reported results indicate a clear dependence of the PDGF-Src-induced cleavage of APP upon the γ-secretase activity, but they don't allow us to distinguish between α-secretase and BACE activities, whose actions are known to precede the γ-secretase-induced cleavage. To address this point, we examined the effects of the PDGF-Src pathway on the processing of APP, by measuring the accumulation of Aβ in cultured cells, in which this pathway is activated or blocked. To do this, HEK293 cells were transfected with APP_{695} alone or with APP_{695} plus SrcYF. As shown in Fig. 7*A*, there is a significantly increased accumulation of Aβ in the medium of cells expressing the constitutively active form of Src. Furthermore, CHO cells stably expressing APP_{695}, which generate high levels of Aβ, were treated with two concentrations of the inhibitor of Src TK, PP2. In these conditions, Aβ generation is significantly decreased, whereas the analogous molecule PP3, not affecting Src TK activity, was completely ineffective (see Fig. 7*B*).

DISCUSSION

The proteolytic processing of APP leading to the generation of Aβ peptide is an extensively studied phenomenon, due to its implication in the pathogenesis of Alzheimer's disease (AD). The great effort to understand the machineries involved in the various types of cleavages of APP resulted in the identification and in the molecular characterization of two out three of the secretases, *i.e.* α- and β-secretases, and many preliminary results indicate that, despite its complexity, also γ-secretase is near to be understood. On the contrary, the mechanisms regulating this proteolytic processing are not completely understood. Here, we report experiments demonstrating that the β-γ processing of APP is under a positive control by PDGF through a pathway involving Src and Rac1.

Most of the available data on the regulation of APP processing concerns with sAPP secretion (for a review see Ref. 32). It is well demonstrated that the activation of muscarinic receptor induces an increased secretion of sAPP (33), and a similar phenomenon has been reported also for metabotropic glutamate receptor (34) and for serotonin receptors (35). These effects are regulated through a PKC-dependent pathway (33),

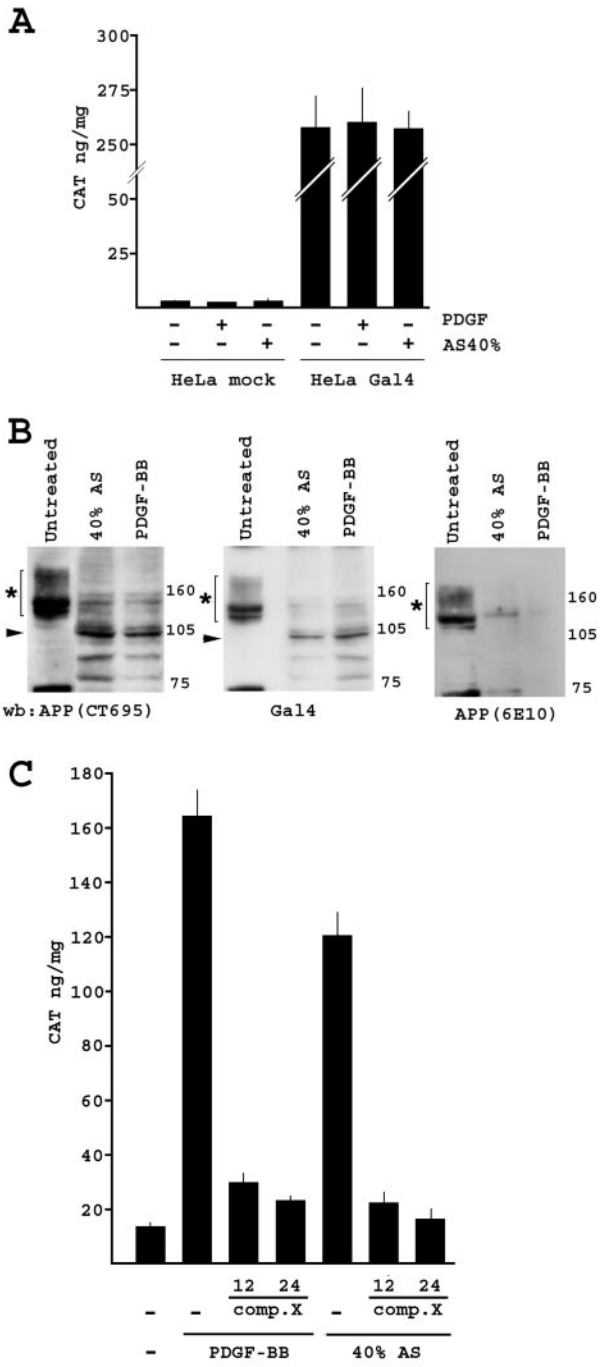

FIG. 4. **PDGF-induced CAT accumulations depends on APP-Gal4 cleavage by γ-secretase activity.** *A*, wild type HeLa cells were transfected with G5BCAT vector alone (mock) or with both G5BCAT vector and Gal4 expression vector (Gal4) and treated with 40 ng/ml recombinant PDGF-BB or with 10 μg/ml of the 40% AS fraction for 24 h before harvesting. The amount of CAT was measured in triplicate experiments, and standard deviations are reported. *B*, cell extracts from HeLaAG cells exposed or not for 48 h to 40% AS fraction or to PDGF-BB were electrophoresed on SDS-PAGE and analyzed by Western blot with CT-695 or 6E10 APP antibodies or Gal4 antibody, as indicated. This demonstrated that the exposure to 40% AS fraction or PDGF-BB results in a change of the size of the APP-Gal4 bands, toward a major band of about 100 kDa, similar to that of wild type Gal4 (indicated by an *arrowhead*), and recognized by both Gal4 antibody and CT-695 antibody directed against the C-terminal domain of APP. On the contrary, 6E10 antibody failed to recognize the cleaved protein, thus demonstrating that it does not contain the N-terminal β-amyloid epitope. The *asterisk* indicates the APP-Gal4 bands. *C*, HeLaAG cells transfected with G5BCAT vector and exposed to PDGF-BB or 40% AS fraction, as in Fig. 3, were treated with 10 μM γ-secretase inhibitor compound X for 12 or 24 h, as indicated. Standard deviations of triplicate experiments are reported.

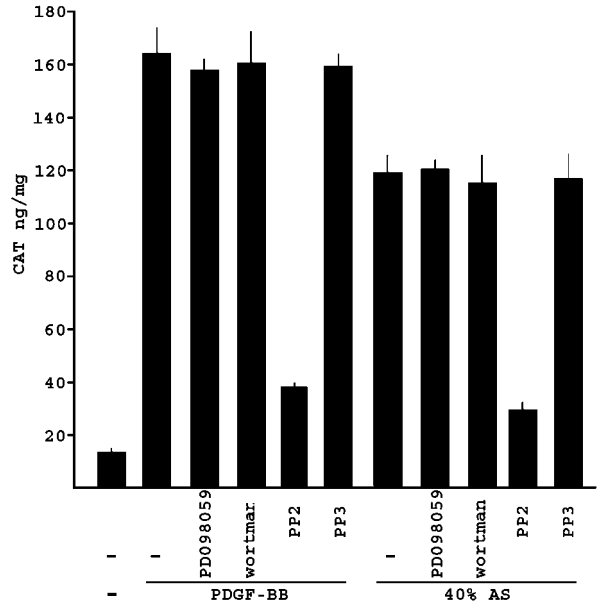

FIG. 5. **Inhibition of Src TK activity prevents APP-Gal4 cleavage in HeLaAG cells exposed to either PDGF-BB or partially purified C6-conditioned medium.** HeLaAG cells transiently transfected with G5BCAT vector were exposed to 40 ng/ml recombinant PDGF-BB or to 10 μg/ml of the 40% AS fraction, as reported in Fig. 4. These cells were also treated for 24 h before harvesting with either 100 μM ERK inhibitor PD098059, 100 nM PI3K inhibitor wortmannin, 10 μM Src inhibitor PP2, or with a PP2-like molecule, PP3, devoid of Src TK inhibiting activity. Standard deviations of triplicate experiments are reported.

and, accordingly, it is well known that activated PKC induces sAPP secretion and inhibits Aβ generation (36, 37). On the contrary, very little is known of the possible effects of the activation of tyrosine kinase receptors on APP processing.

The relevance of the reported results for neuronal APP functions and for the pathogenesis of AD should be addressed through further work. However, PDGF-R, Src, and Rac, although widely expressed in many different cell types, are known to play significant roles in the nervous system. In fact, it was clearly documented that PDGF α-receptor is expressed in neurons of various districts of mouse and rat CNS. This expression, detected as early as postnatal day 1, is observed during all the postnatal life, whereas the expression of PDGF α-receptor in oligodendrocytes is abundant during development, but is restricted in the adult to few precursor cells (38). These results are in agreement with several observations indicating a protective role for PDGF in several neuronal cells (39–41). PDGF-A and PDGF-B are constitutively expressed by neurons *in vivo* (42), and this suggests further that these growth factors, which regulate proliferation and differentiation of oligodendrocytes (42), could also regulate the functions of the neurons themselves (38). Src, and the related non-receptor TK Fyn, are expressed in the neurons, are enriched in growth cones (43), and are involved in several neuronal functions, such as for example Ig CAM-mediated neurite growth and guidance (44). The three members of the Rho family of small GTPases, Rho, Rac1, and Cdc42, are ubiquitously involved in actin cytoskeleton regulation, affecting cell attachment and contraction, lamellipodia formation, and filopodia formation, respectively (45). Their involvement in the regulation of neuronal functions is well documented. In particular, Rac has been implicated in neurite outgrowth and axonal pathfinding (46). In addition to numerous *in vitro* results, this is demonstrated by the expression of a constitutively active form of Rac1 in Purkinje cells, which resulted in an ataxic phenotype of mice that

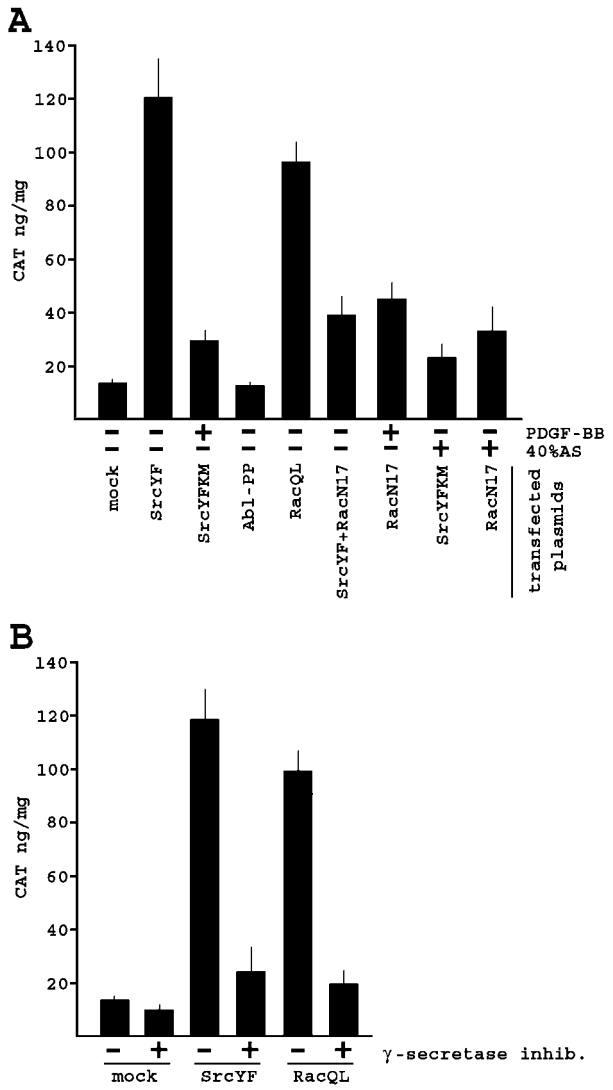

FIG. 6. **The non-receptor Src TK and the small G-protein Rac1 are responsible for the PDGF-induced cleavage of APP-Gal4.** *A*, HeLaAG cells transiently co-transfected with G5BCAT vector and with vectors driving the expression of constitutively active mutants of Src (SrcYF), Abl (Abl-PP), and Rac1 (RacQL) or with SrcYF plus a dominant negative mutant of Rac1 (RacN17). When indicated, HeLaAG cells were transiently transfected with the dominant negative mutants of Src (SrcYFKM) or of Rac (RacN17) and treated for 24 h before harvesting with PDGF-BB or 40% AS fraction. *B*, HeLaAG cells transiently co-transfected with G5BCAT vector and with SrcYF or RacQL expression vectors and treated or not with 10 μM γ-secretase inhibitor, compound X. Standard deviations of triplicate experiments are reported.

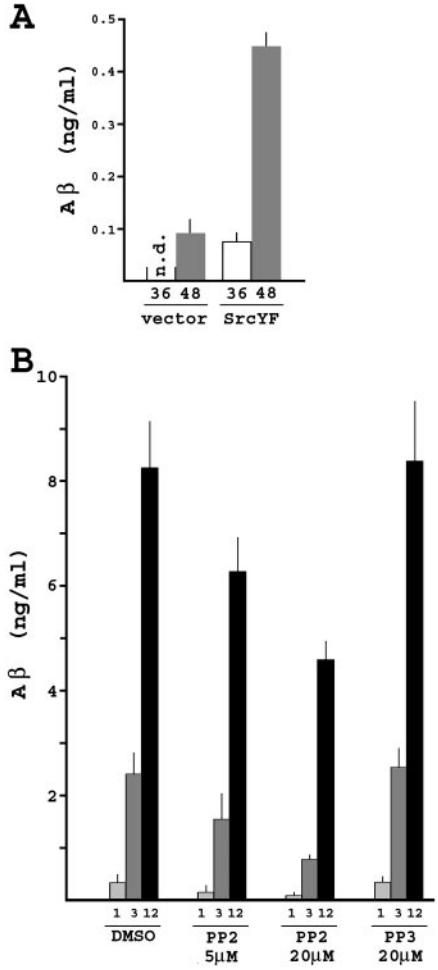

FIG. 7. **Src TK activity regulates Aβ generation from wild type APP_{695}.** *A*, HEK293 cells were transiently transfected with the vector encoding APP_{695} with or without the vector driving the expression of the constitutively active SrcYF mutant. 36 and 48 h after transfections, total Aβ peptide present in the culture medium was measured by enzyme-linked immunosorbent assay. In the case of the 36-h point of the cells transfected with APP alone, some measurements were below the detection limit (*n.d.*, non-detectable). *B*, CHO cells stably expressing APP_{695} were exposed to 5 or 20 μM concentrations of the Src TK inhibitor PP2 or to 20 μM PP3. The *bars* indicate the amount of total Aβ present in the medium after 1, 3, and 12 h. The values are means of at least triplicate experiments, and standard deviations are reported.

is accompanied by alterations of dendritic spines (47). Accordingly, the phenotypes induced by combined mutations of the three Rac GTPases of *Drosophila* are characterized by defects of branching, guidance, and growth of axons (48).

The most known effectors of Rac1 are the PAK serine/threonine kinases, which are activated through the binding of Rac-GTP or Cdc42-GTP to their N-terminal autoinhibitory domain (for a review see Ref. 49). A second known effector downstream of Rac1 is the kinase Cdk5 (50), and the observation that APP β-γ processing is activated by Rac1 and the well demonstrated function of Rac1 in the activation of p35/Cdk5 suggest a possible crucial role for this small G-protein in the generation of the pathological signs of AD. In fact, the two histologic hallmarks of the disease are Aβ accumulation in senile plaques and the organization of hyperphosphorylated tau protein in fibrillary tangles. It is well demonstrated that one of the two kinases involved in anomalous tau phosphorylation is p35/Cdk5 (51), and therefore, activation of Rac1 could, at the same time, increase Aβ generation and cause tau hyperphosphorylation, leading to conditions that favor plaque and tangle formation.

The results reported here support the hypothesis that other extracellular signals, different from PDGF and known to induce Src and/or Rac1, could trigger the processing of APP. In fact, numerous signaling pathways converge on Src: (i) several other tyrosine kinase receptors, such as nerve growth factor receptor, epidermal growth factor receptor, and fibroblast growth factor receptor, are able to activate Src (52–54); (ii) Src is activated by engagement of integrins during cell interaction with extracellular matrix (55); (iii) G-protein-coupled receptors activate Src, as in the case of thrombin (56, 57); and (iv) voltage-dependent and ligand-gated channels have been demonstrated to interact with Src such as, for example, the *N*-methyl-D-aspartic acid channel (58).

Among the possible targets of the pathway described above are secretases and APP. The structure of γ-secretase is not completely known, and little information is available on the regulation of BACE activity; on this basis, it is hard to hypothesize mechanisms through which these machineries could be

activated. On the other hand, there are experimental results suggesting that phosphorylation of APP does not affect its processing. In fact, it was well documented that APP is phosphorylated on Ser and Thr *in vitro* and *in vivo* (59), but these post-translational modifications are not involved in the regulation of APP cleavage (60). APP is also phosphorylated at the level of Tyr-682 (APP_{695} isoform numbering) of its intracellular domain (8). One of the kinases that is able to phosphorylate APP on Tyr-682 is the non-receptor tyrosine kinase Abl. However, we showed here that the expression of a constitutively active form of Abl does not affect APP-Gal4 processing (see Fig. 6) and that, in cells expressing a mutant form of APP in which Tyr-682 is substituted with a Phe residue, SrcYF induces an increase of Aβ accumulation similar to that observed in cells expressing wild type APP (data not shown).

There are several results indicating that the processing of APP by γ-secretase could have a role in signal transduction (61). In fact, we and others (10, 62) demonstrated that Fe65, one of the ligands of APP cytodomain, is a nuclear protein and that APP functions as an anchor that restricts Fe65 outside of the nucleus. Following APP processing by γ-secretase, the cytodomain of APP (AID) together with Fe65 is translocated into the nucleus (10, 62–63). Fe65 and/or AID·Fe65 complex, through the interaction with the transcription factor LSF (9) or with the histone acetyltransferase Tip60 (10), could regulate the transcription. In support to this hypothesis, we found that Fe65 overexpression in the nucleus regulates the transcription of the thymidylate synthase gene driven by LSF (64). These findings suggest that PDGF, or other molecules activating the Src-Rac1 cascade, could be signals that trigger the cleavage of APP and, in turn, nuclear translocation of Fe65 and/or Fe65-AID, which regulate gene expression.

Taken together these data suggest the possibility that the activation of PDGF-R, Src, and Rac1 could be relevant for the generation of Aβ by neurons and that new possible targets for therapeutic interventions in Alzheimer's disease could be found in this pathway. Furthermore, the experimental system described in this report could be used to find molecules that inhibit the PDGF-Src-Rac-induced processing of APP and that, in turn, could be useful for the development of anti-AD drugs.

Acknowledgments—We thank J. Silvio Gutkind and Mario Chiariello for the Src and Rac vectors, Daniela Barilà and Giulio Superti-Furga for the Abl-PP vector, and Eddie Koo for the CHO cells expressing APP. Véronique Hubert and Thierry Canton are greatly acknowledged for Aβ quantification.

REFERENCES

1. Vassar, R., (2001) *J. Mol. Neurosci.* **17,** 157–170
2. Selkoe, D. J. (1999) *Nature* **399,** (suppl.) A23–A31
3. Annaert, W., and De Strooper, B. (2002) *Annu. Rev. Cell Dev. Biol.* **18,** 25–51
4. Kamal, A., Almenar-Queralt, A., LeBlanc, J. F., Roberts, E. A., and Goldstein, L. S. (2001) *Nature* **414,** 643–648
5. Fiore, F., Zambrano, N., Minopoli, G., Donini, V., Duilio, A., and Russo, T. (1995) *J. Biol. Chem.* **270,** 30853–30856
6. Zambrano, N., Buxbaum, J. D., Minopoli, G., Fiore, F., De Candia, P., De Renzis, S., Faraonio, R., Sabo, S., Cheetham, J., Sudol, M., and Russo, T. (1997) *J. Biol. Chem.* **272,** 6399–6405
7. Ermekova, K. S., Zambrano, N., Linn, H., Minopoli, G., Gertler, F., Russo, T., and Sudol, M. (1997) *J. Biol. Chem.* **272,** 32869–32877
8. Zambrano, N., Bruni, P., Minopoli, G., Mosca, R., Molino, D., Russo, C., Schettini, G., Sudol, M., and Russo, T. (2001) *J. Biol. Chem.* **276,** 19787–19792
9. Zambrano, N., Minopoli, G., de Candia, P., and Russo, T. (1998) *J. Biol. Chem.* **273,** 20128–20133
10. Cao, X., and Sudhof, T. C. (2001) *Science* **293,** 115–120
11. Trommsdorff, M., Borg, J. P., Margolis, B., and Herz, J. (1998) *J. Biol. Chem.* **273,** 33556–33560
12. Howell, B. W., Lanier, L. M., Frank, R., Gertler, F. B., and Cooper, J. A. (1999) *Mol. Cell. Biol.* **19,** 5179–5188
13. Gertler, F. B., Comer, A. R., Juang, J. L., Ahern, S. M., Clark, M. J., Liebl, E. C., and Hoffmann, F. M. (1995) *Genes Dev.* **9,** 521–533
14. Borg, J. P., Ooi, J., Levy, E., and Margolis, B. (1996) *Mol. Cell Biol.* **16,** 6229–6241
15. Borg, J. P., Lopez-Figueroa, M. O., de Taddeo-Borg, M., Kroon, D. E., Turner, R. S., Watson, S. J., and Margolis, B. (1999) *J. Neurosci.* **19,** 1307–1316
16. Russo, C., Dolcini, V., Salis, S., Venezia, V., Zambrano, N., Russo, T., and Schettini, G. (2002) *J. Biol. Chem.* **277,** 35282–35288
17. Tarr, P. E., Roncarati, R., Pelicci, G., Pelicci, P. G., and D'Adamio, L. (2002) *J. Biol. Chem.* **277,** 16798–16804
18. Matsuda, S., Yasukawa, T., Homma, Y., Ito, Y., Niikura, T., Hiraki, T., Hirai, S., Ohno, S., Kita, Y., Kawasumi, M., Kouyama, K., Yamamoto, T., Kyriakis, J. M., and Nishimoto, I. (2001) *J. Neurosci.* **21,** 6597–6607
19. Scheinfeld, M. H., Roncarati, R., Vito, P., Lopez, P. A., Abdallah, M., and D'Adamio, L. (2002) *J. Biol. Chem.* **277,** 3767–3775
20. Lee, S. J., Liyanage, U., Bickel, P. E., Xia, W., Lansbury, P. T., Jr., and Kosik, K. S. (1998) *Nat. Med.* **4,** 730–734
21. Lillie, J. W., and Green, M. R. (1989) *Nature* **338,** 39–44
22. Allegrini, S., Scaloni, A., Ferrara, L., Pesi, R., Pinna, P., Sgarrella, F., Camici, M., Eriksson, S., and Tozzi, M. G. (2001) *J. Biol. Chem.* **276,** 33526–33532
23. Zhang, W., and Chait, B. T. (2000) *Anal. Chem.* **72,** 2482–2489
24. Westermann, R., Hardung, M., Meyer, D. K., Ehrhard, P., Otten, U., and Unsicker, K. (1988) *J. Neurochem.* **50,** 1747–1758
25. Beher, D., Wrigley, J. D., Nadin, A., Evin, G., Masters, C. L., Harrison, T., Castro, J. L., and Shearman, M. S. (2001) *J. Biol. Chem.*, **276,** 45394–45402
26. Kypta, R. M., Goldberg, Y., Ulug, E. T., and Courtneidge, S. A. (1990) *Cell* **62,** 481–492
27. Hirai, H., and Varmus, H. E. (1990) *Proc. Natl. Acad. Sci. U. S. A.* **87,** 8592–8596
28. Snyder, M. A., Bishop, J. M., McGrath, J. P., and Levinson, A. D. (1985) *Mol. Cell. Biol.* **5,** 1772–1779
29. Furtoss, O., Dorey, K., Simon, V., Barilà, D., Superti-Furga, G., and Roche, S. (2002) *EMBO J.* **21,** 514–524
30. Barilà, D., and Superti-Furga, G. (1998) *Nat. Genet.* **18,** 280–282
31. Chiariello, M., Marinissen, M. J., and Gutkind, J. S. (2001) *Nat. Cell Biol.* **3,** 580–586
32. Mills, J., and Reiner, P. B. (1999) *J. Neurochem.* **72,** 443–460
33. Nitsch, R. M., Slack, B. E., Wurtman, R. J., and Growdon, J. H. (1992) *Science* **258,** 304–307
34. Lee, R. K. K., Wurtman, R. J., Cox, A. J., and Nitsch, R. M. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92,** 8083–8087
35. Nitsch, R. M., Deng, M., Growdon, J. H., and Wurtman, R. J. (1996) *J. Biol. Chem.* **271,** 4188–4194
36. Buxbaum, J. D., Gandy, S. E., Cicchetti, P., Ehrlich, M. E., Czernik, A. J., Fracasso, R. P., Ramabhadran, T. V., Unterbeck, A. J., and Greengard, P. (1990) *Proc. Natl. Acad. Sci. U. S. A.* **87,** 6003–6006
37. Hung, A. Y., Haass, C., Nitsch, R. M., Qiu, W. Q., Citron, M., Wurtman, R. J., Growdon, J. H., and Selkoe, D. J. (1993) *J. Biol. Chem.* **268,** 22959–22962
38. Oumesmar, B. N., Vignalis, L., and Baron-Van Evercooren, A. (1997) *J. Neurosci.* **17,** 125–139
39. Cheng, B., and Mattson, M. (1995) *J. Neurosci.* **15,** 7095–7104
40. Nikkhah, G., Odin, P., Smits, A., Tingstrom, A., Othberg, A., Brundin, P., Funa, K., and Lindvall, O. (1993) *Exp. Brain Res.* **92,** 516–523
41. Smits, A., Ballagi, E., and Funa, K. (1993) *Eur. J. Neurosci.* **5,** 986–994
42. Yeh, H. J., Ruit, K. G., Wang, Y. X., Parks, W. C., Snider, W. D., and Deuel, T. F. (1991) *Cell* **64,** 209–216
43. Maness, P. F., Aubry, M., Shores, C. G., Frame, L., and Pfenniger, K. H. (1988) *Proc. Natl. Acad. Sci. U. S. A.* **85,** 5001–5005
44. Suter, D. M., and Forscher, P. (2001) *J. Cell Biol.* **155,** 427–438
45. Hall, A., and Nobes, C. D. (2000) *Philos. Trans. R. Soc. London B. Biol. Sci.* **355,** 965–970
46. Nikolic, M. (2002) *Int. J. Biochem. Cell Biol.* **34,** 731–745
47. Luo, L., Hensch, T. K., Ackerman, L., Barbel, S., Jan, L. Y., and Jan, Y. N. (1996) *Nature* **379,** 837–840
48. Ng, J., Nardine, T., Harms, M., Tzu, J., Goldstein, A., Sun, Y., Dietzl, G., Dickson, B. J., and Luo, L. (2002) *Nature* **416,** 442–447
49. Sells, M. A., and Chernoff, J. (1997) *Trends Cell Biol.* **7,** 162–167
50. Nikolic, M., Chou, M. M., Lu, W., Mayer, B. J., and Tsai, L. H. (1998) *Nature* **395,** 194–198
51. Maccioni, R. B., Otth, C., Concha, I. I., and Munoz, J. P. (2001) *Eur. J. Biochem.* **268,** 1518–1527
52. Kremer, N. E., D'Arcangelo, G., Thomas, S. M., De-Marco, M., Brugge, J. S., and Halegoua, S. (1991) *J. Cell Biol.* **115,** 809–818
53. Weernink, P. A., and Rijksen, G. (1995) *J. Biol. Chem.* **270,** 2264–2267
54. Zhan, X., Plourde, C., Ku, X. G., Friesel, R., and Maciag, T. (1994) *J. Biol. Chem.* **269,** 20221–20224
55. Kaplan, K. B., Swedlow, J. R., Morgan, D. O., and Varmus, H. E. (1995) *Genes Dev.* **9,** 1505–1517
56. Luttrell, L. M., Della Rocca, G. J., van Biesen, T., Luttrell, D. K., and Lefkowitz, R. J. (1997) *J. Biol. Chem.* **272,** 4637–4644
57. Chen, Y., Pouyssegur, J., Courtneidge, S. A., and Van Obberghen-Schilling, E. (1994) *J. Biol. Chem.* **269,** 27372–27377
58. Yu, X. M, Askalan, R., Keil, G. J., and Salter, M. W. (1997) *Science* **275,** 674–678
59. Suzuki, T., Oishi, M., Marshak, D. R., Czernik, A. J., Nairn, A. C., and Greengard, P. (1994) *EMBO J.* **13,** 1114–1122
60. da Cruz e Silva, O. A., Iverfeldt, K., Oltersdorf, T., Sinha, S., Lieberburg, I., Ramabhadran, T. V., Suzuki, T., Sisodia, S. S., Gandy, S., and Greengard, P. (1993) *Neuroscience* **57,** 873–877
61. Ebinu, J. O., and Yankner, B. A. (2002) *Neuron* **34,** 499–502
62. Minopoli, G., de Candia, P., Bonetti, A., Faraonio, R., Zambrano, N., and Russo, T. (2001) *J. Biol. Chem.* **276,** 6545–6550
63. Kimberly, W. T., Zheng, J. B., Guenette, S. Y., and Selkoe, D. J. (2001) *J. Biol. Chem.* **276,** 40288–40292
64. Bruni, P., Minopoli, G., Brancaccio, T., Napolitano, M., Faraonio, R., Zambrano, N., Hansen, U., and Russo, T. (2002) *J. Biol. Chem.* **277,** 35481–35488

Platelet-derived Growth Factor Induces the β-γ-Secretase-mediated Cleavage of Alzheimer's Amyloid Precursor Protein through a Src-Rac-dependent Pathway

Davide Gianni, Nicola Zambrano, Marida Bimonte, Giuseppina Minopoli, Luc Mercken, Fabio Talamo, Andrea Scaloni and Tommaso Russo

J. Biol. Chem. 2003, 278:9290-9297.
doi: 10.1074/jbc.M211899200 originally published online January 6, 2003
