Presenilin-1 Interacts with Plakoglobin and Enhances Plakoglobin-Tcf-4 Association

IMPLICATIONS FOR THE REGULATION OF β-CATENIN/Tcf-4-DEPENDENT TRANSCRIPTION*

Received for publication, July 26, 2005, and in revised form, November 23, 2005 Published, JBC Papers in Press, November 23, 2005, DOI 10.1074/jbc.M508153200

Imma Raurell1, Julio Castaño1,2, Clara Franci1, Antonio García de Herreros3,3 and Mireia Duñach4,4

From the 1Unitat de Biofísica, Departament de Bioquímica i Biologia Molecular, Facultat de Medicina, Universitat Autònoma de Barcelona, E-08193 Bellaterra, Spain and 2Unitat de Biologia Cellular i Molecular, Institut Municipal d'Investigació Mèdica, Universitat Pompeu Fabra, E-08003 Barcelona, Spain

Alzheimer disease-linked Presenilin-1 (PS1) is a negative modulator of β-catenin/Tcf-4 activity. However, the mechanism underlying this effect is not well understood. We show here that the effects of PS1 on the activity of this complex in epithelial cells is independent of its γ-secretase activity and its interaction with β-catenin. As presented in this report PS1 also binds plakoglobin with similar affinity as β-catenin, involves equivalent residues in the two catenins. Moreover, PS1 associates with plakoglobin independently of its interaction with Tcf-4 and prevents its binding to DNA. These effects were observed with the unprocessed wild type and the truncated mutant PS1-H9252. In contrast, plakoglobin with other members of adherens junctions and desmosomes (2,7–9). In addition, plakoglobin is a component of desmosomes, somesis controlled by phosphorylation of specific Tyr residues in both plakoglobin and β-catenin (10, 11). Recent results from our laboratory show that in turn activate genes involved in embryonic development and tumorigenesis (11, 12). The translocation of β-catenin to the nucleus is tightly controlled by the activity of a complex involved in β-catenin degradation. This complex includes the product of the tumor suppressor adenomatous polyposis gene, axin, and the Thr/Ser protein kinases, CK1α and glycolen synthase kinase 3β (11, 13). As a result of the activity of this complex, the PS1 immunoblots in Fig. 6, the PS1 immunoblots in Fig. 4, and the PS1 and GST immunoblots in Fig. 6A, the PS1 immunoblot in Fig. 2B, and D, the PS1 and GST immunoblots in Fig. 4E, the Tcf-4 and β-catenin immunoblots in Fig. 6A, the PS1 immunoblot in Fig. 6C, and the GST immunoblots in Fig. 9A. The raw data are no longer available to validate the results. The authors have expressed the opinion that none of these errors affect the final conclusions of this article that, according to them, have been extensively validated.

This article has been withdrawn by the authors. Errors were identified in several figures. Evaluation by the Journal with image analysis software determined that images were reused to represent different experimental conditions in the β-catenin, PS1, and E-cadherin immunoblots in Fig. 2A, the PS1 immunoblots in Fig. 4, B and D, the PS1 and GST immunoblots in Fig. 4E, the Tcf-4 and β-catenin immunoblots in Fig. 6A, the PS1 immunoblot in Fig. 6C, and the GST immunoblots in Fig. 9A. The raw data are no longer available to validate the results. The authors have expressed the opinion that none of these errors affect the final conclusions of this article that, according to them, have been extensively validated.
dillo/β-catenin in cytoplasm (26). In transgenic mice, loss of PS1 in keratinocytes causes high β-catenin/Tcf-mediated signaling, epidermal hyperplasia, and tumors (27). Finally, PS1 deficiency in primary fibroblasts leads to increased activity of Tcf-4-dependent transcription of cyclinD1 gene (28).

However, the molecular basis of this inhibitory effect of PS1 on β-catenin-dependent transcription is not clearly understood. PS1 interacts with β-catenin (29, 30) and E-cadherin (31) in epithelial cells. According to some authors, PS1 might down-regulate β-catenin-mediated transcription by enhancing the stability of the E-cadherin-β-catenin complex (32). In parallel, loss or mutation of PS1 increases the stability of β-catenin (28, 33), whereas other authors have not detected this effect (34–36). Moreover, PS1 interacts with glycogen synthase kinase 3β (33–34), although the regulation of β-catenin-mediated transcription seems to be independent of the activity of this kinase (36–37). In this study, we analyzed the regulation of β-catenin signaling by PS1 in epithelial cell lines and report that the effect on β-catenin-mediated transcription is not dependent on the down-regulation of this protein. We show that PS1 interacts in these cell lines with plakoglobin and facilitates its interaction with Tcf-4, decreasing the capability to bind DNA of this essential element in β-catenin signaling.

**EXPERIMENTAL PROCEDURES**

*Expression of Recombinant Proteins and Protein Binding Assays—*

The preparation of all the plasmids encoding β-catenin, Tcf-4, and plakoglobin deletion and point mutants has been described elsewhere (7, 9, 38), except pGEX-β-catenin arm repeats 1–6. This plasmid was prepared inserting the BamH1/EcoRI fragment of β-catenin in the same sites of pGEX-6P (Amersham Biosciences). cDNAs inserted in pGEX-6P plasmid were expressed in *Escherichia coli* as glutathione S-transferase (GST) fusion proteins and purified by affinity chromatography on glutathione-Sepharose (7). When required, GST was removed by cleaving with Pre-Scission protease (Amersham Biosciences). Pull-down assays were performed using purified recombinant proteins fused to a GST tag and extracts from RWP1 cells as described (9). Glutathione-Sepharose-bound proteins were analyzed by Western blot with specific monoclonal antibodies (mAbs) against β-catenin, plakoglobin, α-catenin, E-cadherin, c-Myc, Rho (all from BD Biosciences) or Tcf-4.
PS1 Regulates Plakoglobin Binding to Tcf-4

FIGURE 2. PS1 does not down-regulate β-catenin. SW-480 cells were transfected with 5 μg of either empty vector or pcDNA3.1His-PS1 as a control. After 48 h, cell extracts were prepared as described under "Experimental Procedures." 20 μg of each cell extract were immunoprecipitated with anti-β-catenin or with anti-plakoglobin, and the associated proteins were analyzed with specific mAbs. Membrane was re-analyzed with a monoclonal antibody against presenilin-1, β-catenin, and plakoglobin. Western blotting; WB, immunoprecipitation; IP.

FIGURE 3. PS1 associates with β-catenin and plakoglobin in SW-480 cells. SW-480 and RWP1 cells were transfected with 15 μg of pcDNA3.1His-PS1 or with empty vector as a control. After 48 h, cell extracts were prepared, and 300 μg of each cell extract were immunoprecipitated with anti-β-catenin or with anti-plakoglobin, and the associated proteins were analyzed with specific mAbs. Membrane was re-analyzed with a monoclonal antibody against presenilin-1, β-catenin, and plakoglobin. WB, Western blotting; IP.

Transient Transfections—The cell lines RWP1, MiaPaca-2, SW-480, and SW-620 were used in this study. SW-480 and SW-620 were established from a primary colon adenocarcinoma and a metastasis from the same patient and express a mutant form of APC (40). RWP1 and MiaPaca-2 were established from pancreas tumors; RWP1 presents also a mutation in the same gene. MiaPaca-2 cells is unknown. Cells were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum. Transient expression plasmids and controls were cotransfected when indicated. Activity of the product of the Renilla luciferase gene under the control of a constitutive thymidine kinase promoter (Promega) was used as a control. Assays were always performed in triplicate; the average of the results of three-four independent transfections ± S.D. is given.

Immunoprecipitation—Cell extracts were prepared from cultured cells suspended in lysis buffer (25 mM Tris-HCl, pH 7.6, 150 mM NaCl, 1 mM EDTA, 1% digitonin, 10 mM NaF, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin, and 10 μg/ml aprotinin) on ice for 30 min. Lysates were cleared at 12,000 × g for 15 min at 4°C. 300 μg of cell extracts were incubated with 4 μg/ml of antibody for 5 h at 4°C. Precipitated material was removed by centrifugation at 12,000 × g and the resulting supernatant was incubated for 90 min with 30 μl of Protein A-agarose (Sigma). Immunoprecipitates were washed three times with lysis buffer; bound proteins were directly eluted with electrophoresis sample buffer and either analyzed by Western blot or used for kinase assays. Alternatively, ectopically expressed proteins were purified with nickel-nitrilotriacetic acid-agarose (Qiagen). 250 μg of cell extracts were incubated in a final volume of 300 μl with 20 μl of a 50% (w/v) suspension of nickel-agarose for 1 h at 4°C. Proteins present in the complex were analyzed by Western blot using specific mAbs. Immunofluorescence—Cells were transfected with pcDNA3-APC, pcDNA3-Tcf-4, and pcDNA3-plakoglobin when indicated. After transfection, cells were plated on glass coverslips, fixed with 4% paraformaldehyde (clone 6H5–3; Upstate Biotechnology, Lake Placid, NY). PS1 was analyzed with a polyclonal antibody from Calbiochem raised against the carboxyl-terminal fragment. As shown, this antibody mostly recognizes a band of 50 kDa after PS1 transfection to cell lines, although other bands of smaller molecular mass were eventually detected. These bands correspond to partially proteolized forms and were also detected with another polyclonal antibody from Santa Cruz Biotechnology. In any case, the data considered in this work only refer to the 50-kDa molecular mass band. Other polyclonal antibodies used were phosphorylated Ser-33, Ser-37, and Thr-41 in β-catenin (Cell Signaling, Beverly, MA), Tcf-4 (amino-terminal; Santa Cruz Biotechnology), and GST (Amersham Biosciences). The blots were re-analyzed with a different antibody after the membranes were stripped as described (39).

Transient Transfections—The cell lines RWP1, MiaPaca-2, SW-480, and SW-620 were used in this study. SW-480 and SW-620 were established from a primary colon adenocarcinoma and a metastasis from the same patient and express a mutant form of APC (40). RWP1 and MiaPaca-2 were established from pancreas tumors; RWP1 presents also a mutation in the same gene. MiaPaca-2 cells is unknown. Cells were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum. Transient expression plasmids and controls were cotransfected when indicated. Activity of the product of the Renilla luciferase gene under the control of a constitutive thymidine kinase promoter (Promega) was used as a control. Assays were always performed in triplicate; the average of the results of three-four independent transfections ± S.D. is given.

Immunoprecipitation—Cell extracts were prepared from cultured cells suspended in lysis buffer (25 mM Tris-HCl, pH 7.6, 150 mM NaCl, 1 mM EDTA, 1% digitonin, 10 mM NaF, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin, and 10 μg/ml aprotinin) on ice for 30 min. Lysates were cleared at 12,000 × g for 15 min at 4°C. 300 μg of cell extracts were incubated with 4 μg/ml of antibody for 5 h at 4°C. Precipitated material was removed by centrifugation at 12,000 × g and the resulting supernatant was incubated for 90 min with 30 μl of protein A-agarose (Sigma). Immunoprecipitates were washed three times with lysis buffer; bound proteins were directly eluted with electrophoresis sample buffer and either analyzed by Western blot or used for kinase assays. Alternatively, ectopically expressed proteins were purified with nickel-nitrilotriacetic acid-agarose (Qiagen). 250 μg of cell extracts were incubated in a final volume of 300 μl with 20 μl of a 50% (w/v) suspension of nickel-agarose for 1 h at 4°C. Proteins present in the complex were analyzed by Western blot using specific mAbs.

Immunofluorescence—Cells were transfected with pcDNA3-APC, pcDNA3-Tcf-4, and pcDNA3-plakoglobin when indicated. After transfection, cells were plated on glass coverslips, fixed with 4% paraformaldehyde.

G. Capellà and F. X. Real, personal communication.
dehydefor30min,andpermeabilizedbyincubationwith1%SDSfor10min. Blocking was performed for 1 h with phosphate buffered-saline containing 0.1% saponin and 1% bovine serum albumin. Mouse mAb anti-Tcf-4 or rabbit polyclonal antisera anti-PS1 were used to analyze the distribution of these proteins. After washing, binding of primary antibodies was detected by anti-mouse and anti-rabbit antibodies conjugated to fluorescein isothiocyanate (Dako) and Alexa 488 (Molecular Probes), respectively. Finally, fluorescence was viewed through a TCS-SP2 Leica confocal microscope.

Tcf-4-DNA Binding Assays—Tcf-4-DNA binding assays were performed by incubation of 200 ng of two oligonucleotides corresponding to the Tcf-4 binding site TBE2 (5′-CTCTTGATCAAAGC GCGG-3′) within the myc promoter and labeled with biotin, with 200 μg of transfected total cell extract for 45 min at 4°C (18). Incubations were performed in the presence of 20 mM HEPES, pH 7.6, 150 mM KCl, 3 mM MgCl₂, 10% glycerol, and 0.3 mg/ml bovine serum albumin in a final volume of 200 μl. 20 μg of poly(dI-dC) were used as a nonspecific competitor. Protein complexes were isolated by incubation with 40 μl of a 50% (w/v) suspension of streptavidin-agarose (Sigma) for 30 min at 4°C. Beads were collected by spinning in a microcentrifuge and washed three times with binding buffer. Samples were separated by SDS-PAGE, and the presence of bound proteins in the complex was analyzed by Western blot with specific mAbs against β-catenin, plakoglobin, or Tcf-4 (amino terminus).

RESULTS
As mentioned above (see Introduction), PS1 inhibits the Wnt/β-catenin signaling pathway. We checked whether this effect occurred in epi-
PS1 Regulates Plakoglobin Binding to Tcf-4

FIGURE 5. Repression of β-catenin-dependent transcription by PS1 is not dependent on γ-secretase activity or interaction with β-catenin. A, SW-480 cells were transfected with pcDNA3.1Myc-His-PS1 (300 ng), TOP-FLASH (50 ng), and pTK-Renilla (50 ng) luciferase plasmids. 24 h after transfection, 5 μM of γ-secretase inhibitor LS-685,458 (Calbiochem) was added to the medium for 24 h. Relative luciferase activity was detected 48 h after transfection. B, RWP1 cells were cotransfected with 50 ng TOP-FLASH, 150 ng pcDNA3.1His, 50 ng pTK-Renilla, and 50 ng PC1-β-catenin mutants inserted into pcDNA3.1His luciferase activity was determined using the Renilla luciferase plasmid Renilla Mivel (Fig. 2).

reinforcement by the fact that mutation of the plakoglobin equivalent residue of PS1 does not modify this interaction (Fig. 4).

The effect of PS1 and β-catenin was also detected by pull-down assays, using GST-β-catenin as bait (Fig. 4B). A similar amount of PS1 also bound by GST-plakoglobin (Fig. 4B), indicating that the affinities of both proteins for PS1 are similar. The interaction sites of PS1 in both proteins were determined. PS1 had a slightly greater affinity for the armadillo repeat domain than for the entire β-catenin, similar to what has been described for other β-catenin-binding proteins (39). PS1 associated with armadillo repeats 7–12 to the same extent as the complete central domain and with repeats 10–12 only slightly less, indicating that these repeats are the main binding site for PS1 (Fig. 4C). On the other hand, armadillo repeats 1–6 barely interact with PS1. Deletion of the β-catenin amino-tail, which strengthens the interaction of the carboxyl-tail with the armadillo domain and reduces the interaction of this element with other β-catenin-binding proteins (41), decreases the affinity for PS1 (Fig. 4C). The effects of β-catenin point mutants that modify the interaction with other proteins were also studied. Tyr-654 → Glu (Y654E), a mutant that mimics the phosphorylation of this residue by epidermal growth factor receptor or other tyrosine kinases, decreases 4-fold the association between β-catenin and PS1, but Tyr-142 → Glu did not modify this interaction (Fig. 4D). In this regard, it is worth mentioning that Tyr-654 is placed in armadillo repeat 12 (Fig. 4A).

Despite its close similarity to β-catenin, plakoglobin interacted differently with PS1. The plakoglobin armadillo domain showed slightly less affinity for PS1 than the full-length protein (Fig. 4E). The effect of removing the carboxyl- or amino-tails was also small. The armadillo repeats involved in PS1 binding were identified as 1–6, suggesting that the interaction of PS1 with plakoglobin requires a more amino-terminal part of this domain than in the case of β-catenin. This conclusion was reinforced by the fact that mutation of the plakoglobin equivalent resi-
FIGURE 6. Plakoglobin mediates PS1 interaction with Tcf-4. A, RWP1 and SW-480 cells were transfected with 7.5 μg of pcDNA3.1His-Tcf-4, in the absence or presence of 8.5 μg of pcDNA3.1Myc-His-PS1 or empty vector. B, GST-Tcf-4-(1–53) or GST-Tcf-4-(S1–110) (30 pmol) were preincubated with 60 pmol β-catenin or plakoglobin, when indicated. Afterward, pulldown assays were performed by incubating the GST proteins with 500 μg of total cell extracts from RWP1 transfected with pcDNA3.1Myc-His-PS1. C, SW-480 cells were transfected with 7.5 μg of pcDNA3.1His-Tcf-4-(1–80), either wild-type or the S60E mutant, and the presence or absence of 8.5 μg of pcDNA3.1His-PS1 or empty vector. After 48 h, cell extracts were prepared, 500 (A) or 350 (C) μg of whole-cell extracts were immunoprecipitated with anti-Tcf-4 antibody, and the complexes were analyzed by immunoblotting with indicated antibodies. D, GST-plakoglobin proteins containing the full-length or armadillo repeats domain (11 pmol) were preincubated with 23 pmol Tcf-4-(1–80) and pulldown assays performed as above with 500 μg of whole-cell extract transfected with pcDNA3.1Myc-His-PS1. In the Input lane, a sample corresponding to 5% of the total cell extract used for the assay was loaded.
due to β-catenin Tyr-654, Tyr-643, and Ser-60, but, to the contrary, increased Ser-60 phosphorylation.

These results indicate that PS1-binding inactivates plakoglobin, although they suggest that Ser-60 is not directly involved in the interaction with PS1. In addition, phosphorylation of Thr-653 weakens the interaction of plakoglobin with PS1.

It has been suggested that PS1 stimulates β-catenin-mediated transcription through its direct interaction with β-catenin or by increasing the binding of this protein to the cytoplasmic domain of E-cadherin, generated by the PS1-dependent γ-secretase activity. Both possibilities were ruled out in our cell lines. First, blocking γ-secretase activity with 1-685,458, a widely used specific inhibitor (45, 46), did not prevent the effect of PS1 on β-catenin-dependent transcription in epithelial cell lines (Fig. 5A). Moreover, PS1 repression of β-catenin-mediated transcription in RWP1 cells was increased by ectopic transfection of β-catenin. As Fig. 5B shows, β-catenin forms containing Y654E mutant were more active than wild-type form in stimulating the activity of the TOP promoter. Although this mutant has less interaction with PS-1 and with E-cadherin, the activity induced by Y654E and Y664E,Y142E β-catenin forms was sensitive to inhibition by P53 (Fig. 5B), indicating that PS1 affects β-catenin-mediated transcription by a mechanism independent of its direct interaction with β-catenin.

As mentioned in the Introduction, β-catenin and plakoglobin bind to different subdomains in Tcf-4 and promote opposite effects. Whereas β-catenin increases the recruitment of positive transcriptional cofactors necessary for gene activation, plakoglobin inhibits DNA binding of Tcf-4. We checked whether PS1 binding interfered with the interaction of β-catenin or plakoglobin with Tcf-4 or whether, on the contrary, PS1 and Tcf-4 were present in the same complex. As shown in Fig. 6A, PS1 and Tcf-4 co-immunoprecipitated in both SW-480 and RWP1 cells. PS1 consistently increased the amount of plakoglobin in Tcf-4 immunocomplexes in SW-480 and only very slightly in RWP1 cells. β-catenin was also immunoprecipitated with Tcf-4; PS1 also increases the association between these two proteins although slightly less than in the case of plakoglobin, and only in SW-480 cells.

Pulldown assays using fragments of Tcf-4 fused to GST indicated that PS1 interacted through the same regions used by β-catenin and plakoglobin, amino acids 1–50 and 51–80, respectively (Fig. 6B). Preincubation with either β-catenin or plakoglobin increased the amount of PS1 retained by glutathione-Sepharose, suggesting that these proteins mediate the interaction of PS1 and Tcf-4. However, the effect was much stronger when plakoglobin was used (Fig. 6B), suggesting that binding of Tcf-4 to PS1 is mainly mediated by this protein. Moreover, plakoglobin also associated better with Tcf-4 when PS1 was present, reinforcing the conclusion of the experiment shown in Fig. 6A.

We also analyzed the effects of PS1 binding on the association of β-catenin and plakoglobin with wild-type Tcf-4 and with a mutant in which Ser-60 was replaced by a Glu. It has been reported that this mutation selectively decreases binding of Tcf-4 to plakoglobin but does not decrease it to β-catenin (18). Transfection of PS1 increased the amount of β-catenin that could be precipitated with wild-type Tcf-4, but the increase was only very modest with Tcf-4 S60E (Fig. 6C). Better PS1 stimulation was observed when binding of Tcf-4 to plakoglobin was analyzed. Surprisingly, PS1 also up-regulated binding of plakoglobin to Tcf-4 S60E, indicating that PS1 modifies plakoglobin-Tcf-4 association and becomes insensitive to Ser-60 phosphorylation.

The mechanism involved in stimulation of plakoglobin-Tcf-4 association by PS1 was further investigated. As shown in Fig. 6D, the armadillo repeat domain binds to Tcf-4 with a higher affinity than the full-length protein, suggesting that the two tails prevent binding of this element.
**PS1 Regulates Plakoglobin Binding to Tcf-4**

Similar effects of the tails have been reported for the association of other cofactors with the armadillo repeats of β-catenin or plakoglobin (21, 38, 39). PS1 increased 3.5-fold (as determined after densitometry of Fig. 6D) the amount of Tcf-4 bound to plakoglobin but did not affect the interaction of this factor with the armadillo repeat domain, suggesting that the effect of PS1 consisted in the elimination of the restriction imposed by the tails. Binding of PS1 to plakoglobin was only slightly affected by elimination of the tails, as reported above (Fig. 4E).

We examined whether PS1-Tcf-4 binding was accompanied by a lower association of Tcf-4 with DNA. SW-480 cell extracts from control or PS1-transfected cells were incubated with an oligonucleotide containing the Tcf-4 binding site present in the c-myc promoter. As shown in Fig. 7A, PS1 prevented Tcf-4 interacting with DNA. As expected, β-catenin was detected in the DNA-bound complexes, but plakoglobin was not.

The inhibition of Tcf-4 binding to DNA by PS1 may be due to an intrinsic inhibitory effect of PS1 or plakoglobin in the complex that precludes interaction to the DNA or to an altered subcellular distribution of Tcf-4. Therefore, the localization of Tcf-4 and PS1 was studied by immunofluorescence. As observed in Fig. 7B, PS1 immunoreactivity was detected in the cytosol, with a mainly perinuclear distribution, and it was completely excluded from the nucleus. On the other hand, Tcf-4 was present in both cellular compartments. Tcf-4 reactivity was not substantially altered by ectopic expression of PS1 unless plakoglobin was also simultaneously transfected; in these cells a reproducible decrease in Tcf-4 nuclear immunoreactivity was observed. These results suggest that PS1 interacts with Tcf-4 in the cytosolic compartment only in the presence of plakoglobin, which retains Tcf-4 outside the nucleus and prevents its association with promoters.

These results indicate that PS1 prevents Tcf-4 binding to DNA in both β-catenin-dependent and β-catenin-independent promoters. These results go in line with the use of cellular compartments transfected with Tcf-4, TOP-FLASH, and TOP-FLASH plasmids. In these conditions, TOP-FLASH plasmid alone was not able to repress TOP activity (Fig. 8). Moreover, cotransfection of plakoglobin returned sensitivity to PS1. In this case, the PS1 repression was much higher than in the control cells, an effect that is probably due to the stimulation of TOP activity observed after addition of plakoglobin.

As observed in our assays, PS1 migrated in our SDS gels mainly as a band of ~50 kDa, suggesting that it was not being processed in our cell lines. To determine whether this modification might alter PS1 binding to Tcf-4 and plakoglobin, we analyzed cellular extracts from cell lines in which PS1 was predominantly present in the processed form. As shown in Fig. 9, when binding of processed and unprocessed forms of PS1 to GST fusion proteins was analyzed, the PS1 carboxyl-terminal fragment bound with lower affinity to β-catenin (~5-fold lower, Fig. 9B) and plakoglobin (~3-fold) than the 50-kDa form. Binding of Tcf-4 was barely detectable for the processed PS1 (Fig. 9A). Similar results were obtained when a mixed extract containing both forms was used as source of PS1. Although the most abundant band in this extract corresponded to the 20-kDa band, the 50-kDa form was much more represented in the fraction bound to GST-β-catenin or GST-plakoglobin (Fig. 9A). In these conditions the 50-kDa associated 6- and 5-fold better, approximately, to β-catenin and plakoglobin, respectively. Therefore, these results suggest that interaction of Tcf-4 is restricted to the unprocessed form of PS1 because of its greater affinity for plakoglobin.

**DISCUSSION**

Because PS1 was identified as a negative modifier of Wnt/wingless pathway in epithelial cells (27), several alternative mechanisms to explain this result have been put forward. Initial studies ruled out the possibility of this inhibitory effect being associated with its function as a component of the γ-secretase complex (28, 37). However, because PS1 interacts with β-catenin and E-cadherin (31, 32) it might enhance the phosphorylation of β-catenin by protein kinase A and subsequently by glycogen synthase kinase 3β. The mechanism of this paired phosphorylation is independent of the presence of axin, a protein normally required for coupling protein kinase CK1 to the complex (33). Protein kinase A, in the case of PS1, and CK1, for axin, are responsible for the initial priming that allows the subsequent phosphorylation by glycogen synthase kinase 3β that leads to protein destabilization. However, although we detected decreases in β-catenin-mediated transcription in our epithelial cell lines, these changes were not accompanied by down-regulation of β-catenin. Moreover, use of β-catenin mutants deficient in PS1 binding reinforced our conclusion that effects of PS1 on β-catenin transcriptional activity were independent of β-catenin binding. The
reasons for this discrepancy are unknown, although several explanations are possible. First, protein kinase A activity in our cell lines may be low and unable to phosphorylate β-catenin Ser-45, even in the presence of PS1. The second reason is due to the lack of functional APC gene product in our cell lines (40). Although the requirement of this protein for PS1-induced β-catenin down-regulation has not been established, it is likely that, as in the case of axin, PS1 permits β-catenin degradation only when it is in a complex with APC. Finally, the effects of PS1 on β-catenin degradation might depend on the processing of PS1 and might only be detected when the protein has been proteolyzed in the two amino- and carboxyl-terminal fragments. We considered this last possibility unlikely, because unprocessed PS1 has a greater affinity for
PS1 Regulates Plakoglobin Binding to Tcf-4

β-catenin than the processed protein, but it is still possible that only the processed protein has the capability to anchor protein kinase A to β-catenin.

In any case, our results have identified plakoglobin as a protein with high affinity for PS1. As mentioned in the Introduction, plakoglobin, also called γ-catenin, is a protein very similar to β-catenin, especially in the central armadillo repeat domain, which is the binding site for PS1. However, the two proteins do not associate with PS1 through equivalent residues in their molecules; whereas β-catenin uses the last six armadillo repeats, PS1 binds to the first six repeats in plakoglobin. Moreover, the dependence of this binding on phosphorylation in specific tyrosine residues is also different. PS1-β-catenin interaction is reduced by modification of Tyr-654, a residue placed in the last armadillo repeat of β-catenin that also controls the interaction with E-cadherin (7). However, phosphorylation of the equivalent residue in plakoglobin, Tyr-643, does not repress PS1 binding. Therefore, growth factors that modulate phosphorylation of these tyrosine residues in the two catenins may modulate PS1 binding to one or the other protein. Preliminary results indicate that addition of epidermal growth factor to RWP1 cells transfected with PS1 increases its interaction with plakoglobin and evidence that association of PS1 to catenins can be modulated by extracellular signals. This dependence on tyrosine phosphorylation of PS1-catenin binding also helps explain why effects of PS1 on β-catenin-dependent transcription might be different in distinct cell lines.

Association of PS1 with plakoglobin and β-catenin also presents several interesting features. First, it enhances the interaction of plakoglobin and Tcf-4. Plakoglobin binds to this transcriptional factor through its interaction with repeats 1–6 of plakoglobin, potentiating the interaction caused by the tails. We have previously reported that binding of factors to the armadillo domain of plakoglobin renders the binding site for the second factor (18) and that some of the positive effects reported so far for plakoglobin on β-catenin-dependent signaling, such as those shown in Fig. 8, are due to the displacement of β-catenin from adherens junctional complexes, thus facilitating its transport to the nucleus and increasing the activity of the β-catenin-Tcf-4 complex (48). Therefore, the role of plakoglobin as a positive or negative effector of β-catenin-dependent signaling depends on its binding to Tcf-4, an interaction modulated by PS1 and possibly by post-translational modifications of plakoglobin.

Finally, it must be remembered that these results have been obtained using epithelial cell lines. Moreover, as mentioned above, the interaction of PS1 and β-catenin and plakoglobin depends on the post-translational modification of these two proteins and on the processing of PS1, two parameters that may vary in different cell lines and circumstances. Therefore, the relative contribution of plakoglobin to the modulation of β-catenin-mediated transcription may differ in cell lines from distinct origins and would not be the same in neurons, fibroblasts, or epithelial cells. In any case, the data given here provide evidence for a new mechanism controlling the activity of this transcriptional complex and help explain the function of plakoglobin as a repressor of this pathway.

Acknowledgments—We thank R. Killick and J. Baulida for providing reagents. We also thank Dr. C. Saura for helpful comments on this work. The technical assistance of Neus Ontiveros is appreciated.

7 I. Raurell, A. García de Herreros, and M. Dunach, unpublished observations.

REFERENCES

Presenilin-1 Interacts with Plakoglobin and Enhances Plakoglobin-Tcf-4 Association: IMPLICATIONS FOR THE REGULATION OF β-CATENIN/Tcf-4-DEPENDENT TRANSCRIPTION

Imma Raurell, Julio Castaño, Clara Francí, Antonio García de Herreros and Mireia Duñach

doi: 10.1074/jbc.M508153200 originally published online November 23, 2005

Access the most updated version of this article at doi: 10.1074/jbc.M508153200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 48 references, 25 of which can be accessed free at http://www.jbc.org/content/281/3/1401.full.html#ref-list-1