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The Adenomatous Polyposis Coli-protein (APC) interacts with the protein tyrosine phosphatase PTP-BL via an alternatively spliced PDZ domain

Kai S Erdmann^{*.1}, Jürgen Kuhlmann², Volkmar Lessmann¹, Lutz Herrmann¹, Volker Eulenburg¹, Oliver Müller³ and Rolf Heumann¹

¹Department of Molecular Neurobiochemistry, Ruhr-University Bochum, 44780 Bochum, Germany; ²Arbeitsgruppe Biophysikalische Analytik, Max-Planck-Institut für molekulare Physiologie, 44227 Dortmund, Germany; ³Arbeitsgruppe Tumorgenetik, Max-Planck-Institut für molekulare Physiologie, 44227 Dortmund, Germany

Mutations of the tumor suppressor protein APC (Adenomatous Polyposis Coli) are linked to familial and sporadic human colon cancer. Here we describe a novel interaction between the APC protein and the protein tyrosine phosphatase PTP-BL carrying five PDZ protein-protein interaction domains. Exclusively, the second PDZ domain (PDZ2) of PTP-BL is binding to the extreme C-terminus of the APC protein, as determined by yeast two-hybrid studies. Using surface plasmon resonance analysis we established a dissociation constant (K_D) of 8.1×10^{-9} M. We find that a naturally occurring splice insertion of five amino acids (PDZ2b) abolishes its binding affinity to the APC protein. The *in vivo* interaction between PTP-BL and the APC protein was shown by coprecipitation experiments in transfected COS cells. Furthermore, in cultured epithelial Madine Carnine Kidney cells the subcellular colocalization was demonstrated for the nucleus and also for the tips of cellular extensions. The interaction of the APC protein with a protein tyrosine phosphatase may indirectly modulate the steady state levels of tyrosine phosphorylations of associated proteins, such as β -catenin playing a major role in the regulation of cell division, migration and cell adhesion.

Keywords: tumor suppressor; PDZ; protein tyrosine phosphatase

Introduction

The APC (Adenomatous Polyposis Coli) gene is a tumor suppressor gene, which was initially identified by its involvement in familial and sporadic forms of colorectal cancer. Germline mutations in the APC gene have been found in most cases of familial adenomatous polyposis coli, an inherited form of colorectal cancer (Grodin *et al.*, 1991; Joslyn *et al.*, 1991; Kinzler *et al.*, 1991; Nishisho *et al.*, 1991). The human APC gene is predicted to encode a protein of 2843 amino acids. It consists of an N-terminal oligomerization domain followed by seven *armadillo* repeats. The middle part of the protein contains three 15 amino acid repeats joined with seven related 20 amino acid repeats. The APC protein contains a tubulin-binding domain and a C-terminal consensus binding motif for PDZ (PSD-95/Discs large/ZO-1) protein-protein interaction domains

(Polakis, 1997). However, the function of this tumor suppressor protein is still unknown. Interestingly, overexpression of the APC protein leads to a decoration of the tubulin cytoskeleton (Munemitsu *et al.*, 1994) and biochemical analysis revealed, that the APC protein is able to nucleate tubulin polymerization *in vitro* (Deka *et al.*, 1998). In addition the protein EB1, which is associated with the tubulin cytoskeleton (Morrison *et al.*, 1998), was demonstrated to interact with the APC protein (Su *et al.*, 1995).

Besides the involvement of the APC protein in microtubule dynamics, it was demonstrated, that it also binds β -catenin (Rubinfeld *et al.*, 1993; Su *et al.*, 1993). This protein plays a major role in cell adhesion, where it is associated with the cadherins, a family of transmembrane cell adhesion molecules (Kemler, 1993). In addition, it has been shown recently, that β -catenin associates with the transcription factor lymphocyte enhancer binding factor/T-cell factor (LEF/TCF) and is translocated into the nucleus, where it is able to regulate gene transcription (Behrens *et al.*, 1996; He *et al.*, 1998). Recent evidence suggests that the SAMP repeats of APC directly bind to the protein axin/conductin (Behrens *et al.*, 1998; Ikeda *et al.*, 1998). The recruitment of glycogen synthase kinase-3 β (GSK-3 β) to this complex leads to phosphorylation of β -catenin, thereby promoting its degradation by proteasomes (Rubinfeld *et al.*, 1996; Hart *et al.*, 1998; Ikeda *et al.*, 1998; Sakanaka *et al.*, 1998).

Taken together, the APC protein seems to act as a scaffold for the assembly of a number of signaling molecules. However, the functional regulation of this complex is largely unknown, but regulation of the phosphorylation status of APC interacting proteins may play an important role for proper signaling of the APC complex. Thus, it could be demonstrated, that the APC protein interacts with the B56 subunit of the serine/threonine phosphatase 2A resulting in modulation of the β -catenin protein level (Seeling *et al.*, 1999). The APC protein itself is phosphorylated on serine and threonine by GSK-3 β and dephosphorylated by the protein phosphatase 2A (Ikeda *et al.*, 2000). Furthermore, the kinase activity of GSK-3 β as well as the binding of β -catenin to cadherins can be regulated by tyrosine phosphorylation (Hughes *et al.*, 1993; Roura *et al.*, 1999; Kim *et al.*, 1999).

Here we describe an interaction of the APC protein with a protein tyrosine phosphatase, PTP-BL (Banville *et al.*, 1994; Maekawa *et al.*, 1994; Saras *et al.*, 1994; Hendriks *et al.*, 1995). PTP-BL is a nontransmembrane protein tyrosine phosphatase implicated in the modulation of the cytoskeleton. Binding of PTP-BL to proteins

*Correspondence: KS Erdmann

such as RhoGAP protein, RIL or the zyxin related protein ZRP-1 suggest an involvement in the dynamic changes of the actin cytoskeleton (Saras *et al.*, 1997; Cuppen *et al.*, 1998; Murthy *et al.*, 1999). However, a defined function of this protein tyrosine phosphatase is still missing. Here we characterize a novel interaction between the APC protein and PTP-BL implicating, that this protein tyrosine phosphatase may contribute to modulation of the function of the APC complex.

Results

The APC-protein interacts with the protein tyrosine phosphatase PTP-BL in the two-hybrid system

In order to find new interaction partners for the APC-protein, we compared consensus binding sequences of PDZ domains with the C-terminus of the APC protein. We noticed, that the consensus binding sequence of the PDZ2 domain of the human homolog of PTP-BL/PTP11 exactly matched the C-terminus of APC (Songyang *et al.*, 1997). Furthermore, this protein tyrosine phosphatase is expressed within epithelial cells and thus shows an overlapping expression pattern with the APC protein (Hendriks *et al.*, 1995; Midgley *et al.*, 1997). Using the two-hybrid system we checked all five PDZ domains of PTP-BL for interaction with the last 73 amino acids of the APC protein. Indeed, as predicted by the consensus binding sequences of this PDZ domain we found specific binding only with the PDZ2 domain (Figure 1a). By using different point mutations and deletions of the APC-C-terminus, we characterized the binding specificity of this interaction. Mutation of the valine in position 0 or the threonine in position -2 as well as deletion of the last three amino acids led to a complete abolishment of the interaction (Figure 1b).

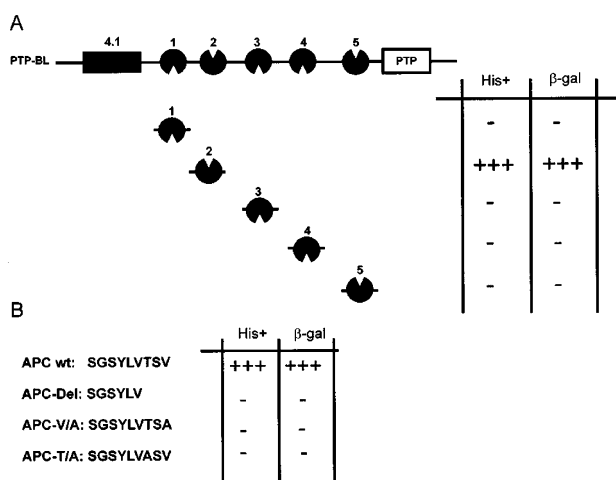


Figure 1 Yeast two-hybrid assay for interaction of APC and PTP-BL. (a) The APC protein specifically interacts with the PDZ2 domain of PTP-BL. PCR-amplified cDNAs encoding the corresponding PDZ domains were subcloned into pGADGH, a cDNA encoding the last C-terminal 73 amino acids of APC was subcloned into pGBT9. Interaction was tested by cotransformation of yeast HF7c and determination of histidine protrophine (+) and β -galactosidase activity (+). (b) Interaction of APC with PDZ2 domain depends on the last three amino acids of APC. Point mutations were introduced into the C-terminus of APC using site directed mutagenesis. Interaction was determined as described in (a)

The APC protein and PTP-BL can be coprecipitated from transfected COS cells

To prove the interaction between the APC protein and PTP-BL also in lysates of mammalian cells, we constructed APC/GST-fusion proteins corresponding to those used in the two-hybrid system. These GST/APC fusion constructs were used for precipitation from COS cell lysates transiently transfected with an expression construct for PTP-BL. In line with our two-hybrid data, subsequent Western-analysis of the precipitates revealed a band corresponding to full-length PTP-BL only with the GST/wildtype APC-fusion protein but not with the other mutant versions or with GST alone (Figure 2a). In addition there was a second band at 205 kd, which probably reflects a partial degradation of PTP-BL. Furthermore, using COS cell lysates transiently cotransfected with expression vectors for the APC protein and PTP-BL we were able to coprecipitate the full-length proteins using an antibody against PTP-BL, whereas there was no coprecipitation using the respective preimmune serum (Figure 2b).

The APC protein shows differential binding to alternative spliced PDZ2 variants

There are at least two splice variants of PDZ2 (PDZ2a and PDZ2b) differing by the insertion of five amino acids (Figure 3a) (Sato *et al.*, 1995). Interestingly, the insertion of five amino acids into PDZ2a domain (PDZ2b) leads to a complete abolishment of APC binding as determined by the two-hybrid system. Furthermore, we bacterially expressed and purified PDZ2a and PDZ2b protein. They were each incubated with streptavidin beads coupled with an N-terminal biotin labeled peptide derived from the last 19 amino acids of the APC protein. In line with our two-hybrid data PDZ2a was able to bind to the beads, but no binding was detected with PDZ2b (Figure 3b).

Differential expression of m-RNA coding for PDZ2a or PDZ2b

In order to determine the *in vivo* expression pattern of these two splice variants we analysed different tissues for the corresponding mRNA (Figure 3c). Reverse transcription polymerase chain reaction (RT-PCR) was used to amplify the PDZ2 splice variants from a number of tissues. Interestingly, we found different ratios of expression levels between both splice variants in various tissues. There was high expression of PDZ2b in the lung and to a lesser extent in the spleen and brain. Very low expression of PDZ2b was detected in kidney and heart. In contrast PDZ2a could be clearly detected in all tissues examined but not in muscle and liver.

Quantification of APC protein-PDZ2 interaction using surface plasmon resonance analysis

We applied surface plasmon resonance analysis to quantify the binding affinity of PDZ2a or PDZ2b to an immobilized APC-peptide corresponding to the last 19 amino acids of the APC protein. Biotinylated peptide

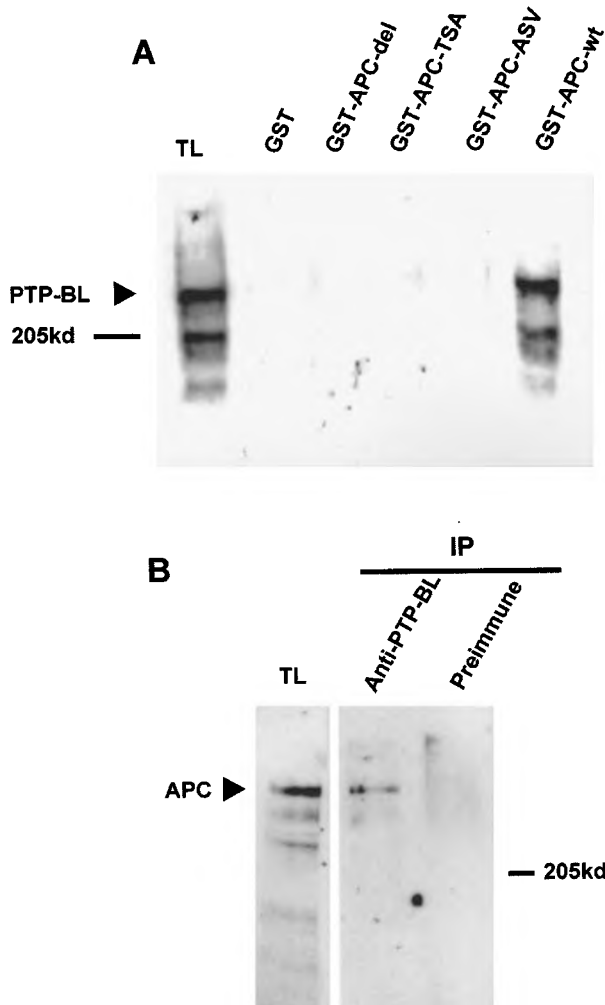


Figure 2 APC and PTP-BL interact independent from the two-hybrid system. (a) COS cells were transiently transfected with an expression construct for PTP-BL, 48 h after transfection cells were lysed and equal amounts of protein were incubated with the indicated fusion proteins immobilized on glutathione-sepharose beads. Precipitates were separated on a 3%-agarose-gel and proteins were transferred to nitrocellulose. The blot was developed using an anti-PTP-BL antibody. (b) Full-length APC and PTP-BL can be coprecipitated. COS cells were transiently transfected with expression constructs for APC and PTP-BL, 48 h after transfection cells were lysed and incubated with anti-PTP-BL or preimmune serum. The antibody complexes were collected using protein-A sepharose beads and precipitates were separated on a 5% SDS polyacrylamid gel, proteins were transferred to nitrocellulose and the blot was developed using anti-APC antibody (Ab-5)

was immobilized on the streptavidin modified dextran matrix of the biosensor and incubated with the GST-PDZ2 fusion proteins. The binding of PDZ2a and PDZ2b to the streptavidin surface alone was negligible. We observed specific binding to the immobilized APC-peptide for PDZ2a but not for PDZ2b (Figure 4a).

Kinetic analysis was performed by incubating an APC-peptide surface with GST-PDZ2a, concentrations varying from 20–800 nM (Figure 4b). The main amplitudes of the curves (>80% of the association curves) were fitted by a monoexponential function and resulted in a linear relationship between the observed rate constants and protein concentration resulting in a slope of $1.6 \times 10^5 / (\text{M} \times \text{s})$ for the association rate constant k_{on} . Dissociation of a preformed APC-PDZ2a complex by rinsing the surface with buffer

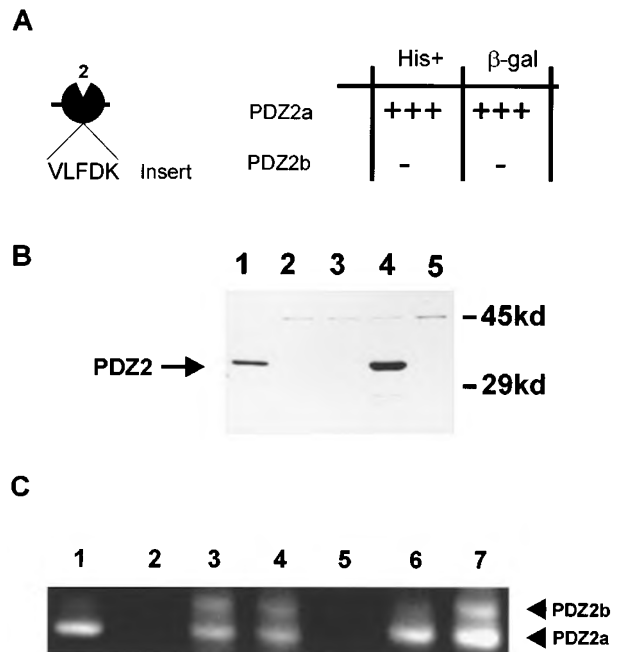


Figure 3 APC shows different binding to alternatively spliced PDZ2 domains. (a) cDNAs encoding PDZ2a or PDZ2b were subcloned into pGADGH and tested for interaction with APC in the two-hybrid system (see also Figure 1). (b) An N-terminal biotinylated peptide APC-19 SGSGQSPKRHSYLVTSV corresponding to the last 19 amino acids of APC was immobilized using streptavidin-sepharose beads. 5 μg recombinant PDZ2a (lanes 4 and 5) or PDZ2b (lanes 2 and 3) diluted in PBS/1% BSA were incubated with immobilized peptide APC-19 (lanes 2 and 4) or streptavidin-sepharose beads alone (lanes 3 and 5), beads were washed and loaded on a 15% SDS polyacrylamid gel. The gel was stained with Coomassie blue, 0.5 μg PDZ2a was loaded for reference (lane 1). (c) Expression profile of PDZa- and PDZb-mRNA, RNA isolated from different tissues of an adult mouse was reverse transcribed and amplified using specific primer for PDZ2, position of PDZa and PDZ2b is indicated by arrowheads, 1, kidney; 2, muscle; 3, heart; 4, brain; 5, liver; 6, spleen; 7, lung

yielded a rate constant for the dissociation reaction k_{diss} of $1.3 \times 10^{-3} / \text{s}$ and a resulting K_{D} constant of $8.1 \times 10^{-9} \text{ M}$.

Colocalization of the APC protein and PTP-BL in epithelial MDCK-cells

To characterize cellular colocalization of the APC protein and PTP-BL we performed immunocytochemistry on cultured epithelial Madine Carnine Kidney (MDCK) cells. As described previously we found a prominent localization of the APC protein in the nucleus as indicated by costaining with 4,6-Diamidino-2-phenylindole (DAPI). Likewise we observed nuclear localization of PTP-BL. The specificity of the PTP-BL immunoreactivity was shown by the lack of staining upon preadsorption of the antibody with the peptide used for immunization (Figure 5). In addition in about 5% of the cells examined we found dot like colocalization of the APC protein and PTP-BL at the tips of cell extensions (Figure 6a).

β -catenin is an in vitro substrate for PTP-BL

The APC protein is colocalized with β -catenin at the tips of extending membrane extensions in MDCK cells (Näthke *et al.*, 1996, Figure 6b). Since we describe here

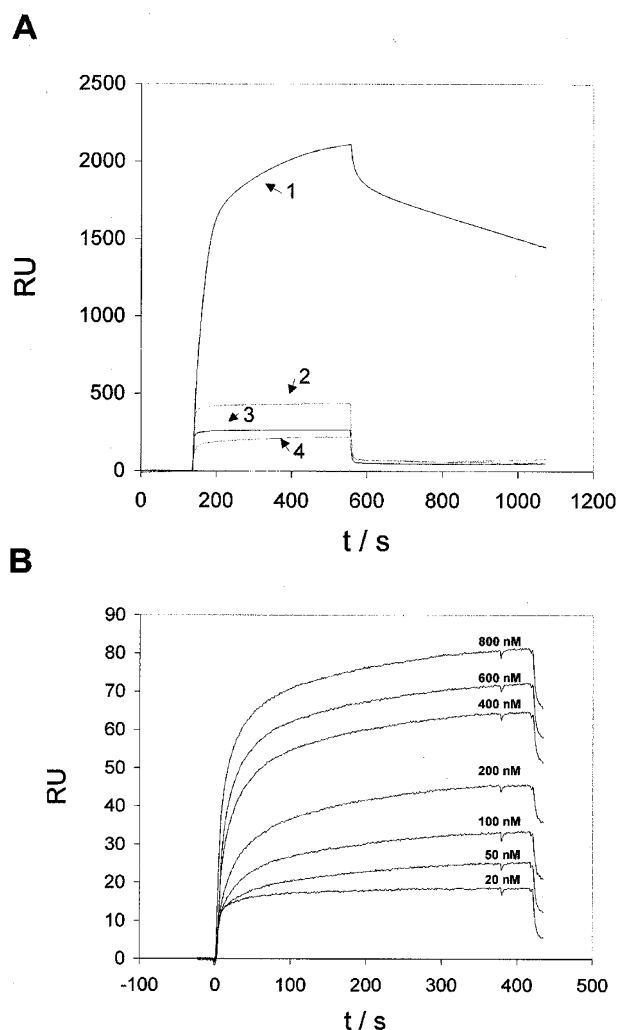


Figure 4 PDZ2a but not PDZ2b interacts specifically with a peptide representing the C-terminal 19 amino acids of the APC protein. (a) Incubation of the dextran matrix of a CM5 BIAcore sensor surface coupled with streptavidine alone (2 and 3) or after binding of biotinylated APC-19 peptide (1 and 4) with GST-PDZ2 fusion proteins (1 μ M). 1 and 3 show signal change during incubation with GST-PDZ2a, 2 and 4 experiments with GST-PDZ2b. (b) GST-PDZ2a associates with biotinylated APC-19 peptide in a concentration dependent manner. 20, 50, 100, 200, 400, 600 and 800 nM GST-PDZ2a were injected and the resulting sensograms are shown

colocalization of the APC protein with PTP-BL, we investigated whether β -catenin might serve as a substrate for PTP-BL. Recombinant PTP-BL/glutathione-S-transferase fusion proteins were produced in *E. coli* and purified using glutathione-agarose (Figure 6c). Tyrosine phosphorylated β -catenin was prepared by stimulation of MDCK-cells with pervanadate and immunoprecipitation using β -catenin specific antibodies. Incubation of tyrosine phosphorylated β -catenin with the recombinant tyrosine phosphatase domain led to a complete dephosphorylation, whereas no significant decrease in tyrosine phosphorylation could be observed after incubation with the catalytically inactive tyrosine phosphatase, which carried a cysteine 2374 to serine mutation (Figure 6d). Equal loading was verified by reprobating the Western blot with an antibody against β -catenin, taking into account, that the antibody used here recognizes tyrosine phosphorylated β -catenin less efficient than

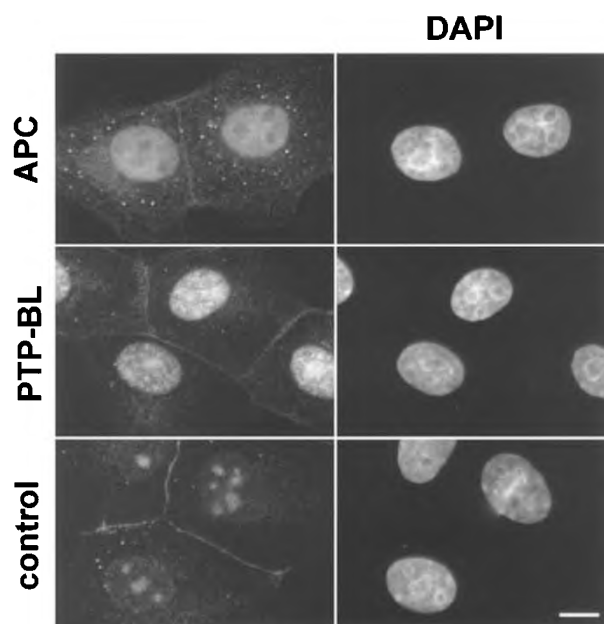


Figure 5 Coexpression of APC and PTP-BL within the nucleus. MDCK cells cultured on coverslips were stained with anti-APC-C-20 (APC) or anti-PTP-BL-P (PTP-BL) and DAPI. To prove the specificity of anti-PTP-BL-P nuclear staining, anti-PTP-BL-P was preadsorbed with the corresponding peptide used for immunization (control), bar = 10 μ M

unphosphorylated β -catenin (Müller *et al.*, 1999). We conclude, that β -catenin may serve as a substrate for PTP-BL.

Discussion

The results presented here show that the APC protein interacts with the protein tyrosine phosphatase PTP-BL. This interaction is mediated by the extreme C-terminus of the APC protein and the PDZ2 domain of PTP-BL (Figure 1). The C-terminus of the APC protein contains a PDZ domain consensus binding motif VTSV. We demonstrate by site directed mutagenesis, that the last three amino acids are necessary to mediate the interaction between the APC protein and the PTP-BL (Figures 1 and 2). Since the PDZ2 domain of the human disc large protein (hdlg) has previously been shown to interact with the APC protein (Matsumine *et al.*, 1996), we have to assume, that it is able to bind at least two different PDZ domain containing proteins. There are other examples showing, that more than one PDZ domain containing protein is binding to the same C-terminal target. For example the proteins PSD-95, SAP97, SAP102 are all binding to the C-terminus of the N-methyl-D-aspartate-receptor subtype 2 (NR2) (Kornau *et al.*, 1995; Niethammer *et al.*, 1996; Müller *et al.*, 1996).

The functional implications of this multiplicity of PDZ domain binding remains to be investigated. However, PDZ binding by the extreme C-terminus of APC is probably not essential for the prevention of tumor formation because mutations in APC predicted to eliminate selectively PDZ binding to the C-terminus are extremely rare.

Two splicing variants for the PDZ2 domain (here referred as PDZ2a and PDZ2b) have been described

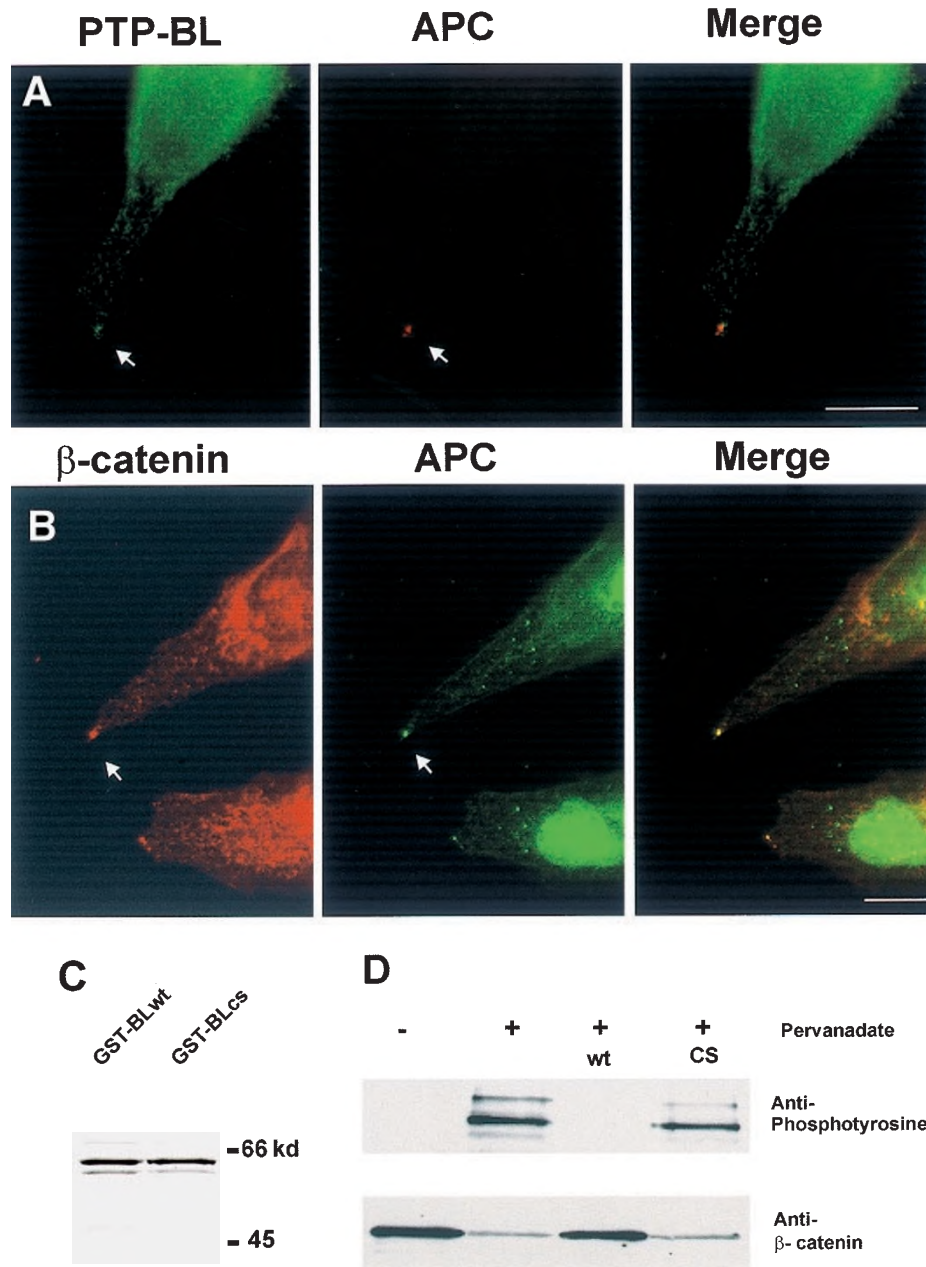


Figure 6 APC can be found colocalized with PTP-BL and β -catenin on membrane extensions (white arrows). β -catenin is an *in vitro* substrate for PTP-BL. MDCK cells cultured on coverslips were stained with anti-PTP-BL and anti-Ab-7 (a), or with anti-APC-C-20 and anti- β -catenin (b), bars = 10 μ m. (c) Recombinant expression of protein tyrosine phosphatase domain of PTP-BL. 1 μ g of GST fusion proteins of protein tyrosine phosphatase domain of PTP-BL and the catalytically inactive version were loaded on a 9% SDS-PAGE and stained with coomassie blue. β -catenin was phosphorylated on tyrosine by stimulation of MDCK cells as indicated with pervanadate. β -catenin was immunoprecipitated using β -catenin specific antibodies and incubated with the indicated fusion proteins: wt, wildtype protein, cs, catalytically inactive protein. (d) Immunoprecipitates were separated by 8% SDS-PAGE and probed with an anti-phosphotyrosine specific antibody. Equal loading was verified by reprobing with an anti- β -catenin antibody (notice, the anti- β -catenin antibody used here recognizes tyrosine phosphorylated β -catenin less efficient than unphosphorylated β -catenin)

previously, which differ by the insertion of five amino acids (Sato *et al.*, 1995). This insertion takes place exactly behind the second β -sheet of the PDZ2a domain at the beginning of a long structured loop, which has been proposed to be involved in binding of the C-terminal target sequence (Kozlov *et al.*, 2000). Using the yeast two-hybrid system and coprecipitation assays, we were able to show, that PDZ2a but not PDZ2b was binding to the APC protein (Figure 3). The apparent dissociation constant $K_D = 8.1 \times 10^{-9}$ M between the PDZ2a domain and the C-terminus of the APC protein as

determined using surface plasmon analysis, is comparable to other PDZ domain protein-protein interactions i.e. in the micromolar to nanomolar range (Marfatia *et al.*, 1997; Kim *et al.*, 1998). We were not able to detect specific binding of the PDZ2b domain to the APC protein. Previously a regulation of PDZ domain binding was shown by phosphorylation of the target sequence (Cohen *et al.*, 1996) or by competition with other PDZ domain carrying proteins (Jaffrey *et al.*, 1998). Our results suggest, that alternative splicing may also regulate PDZ domain target interactions.

The *in vivo* expression pattern of these splice variants was analysed using reverse transcription polymerase chain reaction (RT-PCR). Interestingly, we found different patterns for the ratio of the expression levels between both variants. PDZ2a could be well detected in all PTP-BL expressing tissues, yet the relative expression level of PDZ2b was very low in kidney.

Next, we analysed the subcellular localization of the APC protein and PTP-BL within MDCK-cells and found prominent coexpression of both proteins within the nucleus (Figure 5). In addition we found colocalization of both proteins at the tips of membrane extensions (Figure 6a). This result is in line with previous reports localizing the APC protein at the tips of extending membrane protrusions (Näthke *et al.*, 1996; Pollack *et al.*, 1997) and within the nucleus (Miyashiro *et al.*, 1995; Neufeld and White, 1997). Our analysis of the protein sequence of PTP-BL reveals three putative nuclear localization signals in the amino-terminal part of the protein. This fits with our observation of nuclear localization of PTP-BL. Moreover, it was described recently, that PTP-BL is able to interact with another nuclear protein called BP75 (Cuppen *et al.*, 1999). Due to its modular organization, PTP-BL may build up a multiprotein complex recruiting the APC protein within the nucleus, which may be involved in cell cycle regulation. Thus, overexpression of the APC protein blocks the progression from G0-G1 to the S phase of the cell cycle (Baeg *et al.*, 1995; Ishidate *et al.*, 2000) and the capability of the APC protein to interact directly with DNA was demonstrated recently (Deka *et al.*, 1999). Although these data suggest an involvement of the APC protein in cell division, the intracellular signaling cascades involved in APC function remain elusive.

Our finding that the APC protein interacts with a protein tyrosine phosphatase, implicates the modulation of tyrosine phosphorylation in APC function. Although we were not able to detect tyrosine phosphorylation of APC itself, we nevertheless demonstrate, that tyrosine phosphorylated β -catenin may serve as a substrate for PTP-BL at least *in vitro* (Figure 6d). Apart from catenins, there are several other possible substrates for the APC associated tyrosine phosphatase PTP-BL such as glycogen synthase kinase-3, GSK-3 β , whose activity may be regulated by tyrosine phosphorylation (Hughes *et al.*, 1993; Kim *et al.*, 1999). GSK-3 β activity in turn is implicated in the regulation of microtubuli dynamics (Goold *et al.*, 1999; Tseng *et al.*, 1999).

In conclusion, we were able to demonstrate a direct interaction between the tumor suppressor protein APC and the protein tyrosine phosphatase PTP-BL. This interaction is restricted to one alternatively spliced variant of the PDZ2 domain. The new interaction described here implicates that the APC protein modulates tyrosine phosphorylations on interacting proteins playing a major role in regulation of cell migration, cell adhesion as well as in cell cycle progression.

Materials and methods

Yeast two-hybrid system

The PDZ domains of PTP-BL were subcloned into expression vector pGADGH, amino acids 2770–2843 of rat APC were

amplified using rat brain cDNA and the primer pairs APC1 and APC2 and subcloned into pGBT9 (pGBT9-APC). Yeast strain HF7c was cotransformed with the plasmid pGBT-APC and one of the expression constructs of the PDZ domains of PTP-BL. Transformants were selected on minimal agar plates lacking leucine and tryptophane. Interaction was determined by replating on minimal plates lacking leucine, tryptophane and histidine and monitoring the growth for a period of 3 days. For β -galactosidase assays cotransformants were plated on plates lacking leucine and tryptophane, filter-lifts were performed and incubated with 40 μ g/ml X-Gal in buffer Z.

Transient expression, cell lysis and immunoprecipitation

Transfection of COS cells was performed using the calcium phosphate method (Chen and Okayama 1987). Forty eight hours after transfection cells were lysed in NP-40 buffer (0.5% NP-40, 50 mM Tris pH 7.4, 150 mM NaCl, 1 mM phenylmethylsulfonylfluoride, 1 μ g/ml leupeptin, 1 μ g/ml aprotinin). The lysate was precleared by centrifugation at 20 000 g for 30 min, 4°C. For the pull-down experiment, equal amounts of lysate were incubated with 5 μ g glutathione-S-transferase or GST-fusion proteins coupled to glutathione-sepharose beads (Amersham Pharmacia Biotech). Lysates were incubated with beads for 2 h at 4°C. The beads were washed extensively with NP-40 lysis buffer and eluted with 1 \times Laemmli buffer. Precipitates were separated by a 3% agarose gel and transferred to nitrocellulose membrane by electroblotting. After incubation with the respective antibody, blots were developed using the ECL-system (Amersham Pharmacia Biotech). Anti-PTP-BL was diluted 1:10 000, anti-Ab-5 was diluted 1:200 for immunoblotting. For coimmunoprecipitation lysates transfected with expression vector pcDNA3PTP-BLS and pcDNA3APC were lysed as described above. After centrifugation the lysate was precleared a second time by incubating with 150 μ l protein-A-sepharose for 2 h, 4°C. Equal amounts of lysate were incubated with 10 μ l anti-PTP-BL antiserum or preimmune serum for 2 h, to collect the antibody complex, 30 μ l protein-A-sepharose were added and incubation was prolonged for an additional hour. Immunoprecipitates were washed extensively with NP-40 lysis buffer and separated on a 6% SDS-polyacrylamid gel, proteins were transferred to nitrocellulose membrane and detected as described above.

Expression studies of PDZ2

Tissues of adult mice were transfected and total RNA was isolated using the NucleoSpin RNA II Kit (Machery-Nagel). cDNA synthesis was performed using total RNA and superscript II, (Gibco-BRL), according to the instructions of the manufacturer. For cDNA synthesis primer PDZ2-C (attctgcatctgactctgc) was used, subsequent PCR was performed using primer PDZ2-N1 (tgagctgctaaaactgatg) and PDZ2-C, the following conditions were used for PCR, 94°C denaturation 1 min, 55°C annealing 30 s, 72°C elongation 30 s, 30 cycles. DNA fragments were separated on a 3% agarose gel and stained with ethidium bromide.

Antibodies, expression constructs

Three different rabbit antisera (anti-PTP-BL) to PTP-BL were produced by immunizing rabbits with a purified GST-PDZ1 (amino acids 1066–1285 of PTP-BL) fusion protein. Two different rabbit antisera (anti-PTP-BL-P) to human PTP-BL/PTP11 were produced against the peptide LLIEPT-RQENWTPKNDLEN. Anti-APC monoclonal antibodies Ab-5 and Ab-7 were purchased from Oncogene Science, polyclonal rabbit antibody APC-C20 was purchased from Santa Cruz. Anti-phosphotyrosine antibody 4G10 was purchased from Upstate Biotechnology, monoclonal anti- β -

catenin antibody was purchased from Transduction Laboratories and polyclonal anti- β -catenin antibody C-18 from Santa Cruz. Full-length PTP-BL was cloned by amplifying its entire coding region by six PCR-fragments, the primer pairs used for the different fragments were: fragment I: BL1 up (gccattcccttagggggtc), BL2 down (gttgcggccgccgataatgcatgtgtcactgg), fragment II: BL2 up (cccagatcttgccatcggtacaag), BL2 down (caaagcttccatgctcagatc), fragment III: BL3 up (gccagcacagatcacctttg), BL3 down (ccttgtagccgatggcaagatc), fragment IV: BL4 SOE up (ccgagggaggtaaaacatggcac), BL4 down (gatggcagaattcacaaggtg), fragment V: BL4 up (cttgagatcatcatagatgcatc), BL4 SOE down (ccatgtgcatgtttacctcc), fragment VI: BL5 up (gacctcgagcaggagtcggtcgatgacag), BL5 down (gcaggcctgactgaaccaaag). Fragment II was subcloned into pBS II SK+ containing fragment III using *XbaI/BglII*, fragment II and III were then subcloned using *XbaI/EcoRI* site into pBS II SK+ containing fragment IV, subsequently fragment I was subcloned into this construct using *NotI/XbaI* sites. At last fragment V was added using *SmaI/XhoI* sites. Full-length PTP-BL was then subcloned into *NotI/XhoI* linearized pcDNA3 (Invitrogen). The multiple cloning site of this construct was shortened by digestion with *Acc65I/NotI*, filled in with Klenow fragment and religated to generated pcDNA-PTP-BL.

The eucaryotic expression construct pcDNA-APC was generated by linearizing pGBR APC0-87 with *NcoI* and filled in with Klenow fragment, full-length APC cDNA was excised by *BamHI* and the fragment was subcloned blunt end/*BamHI* into pcDNA3.

Cell cultures and immunocytochemistry

MDCK-cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum. For immunofluorescence studies MDCK cells were cultured in low densities, 50 000 cells/3.5 cm dish, on polyornithine coated coverslips and fixed with 4% paraformaldehyde for 15 min on ice. Anti-PTP-BL was diluted 1:50, anti-PTP-BL-P 1:100, monoclonal Ab-7 was diluted 1:20, polyclonal anti-APC-C-20 1:50, anti- β -catenin 1:2000.

Surface plasmon resonance

Association and dissociation reactions involving PDZa and PDZ2b and a peptide with the C-terminal sequence of APC were studied by Surface Plasmon Resonance (SPR) in a BIAcore[®] system (BIAcore AB, Uppsala). The carboxylated dextran surface of a CM5 research grade sensorship was activated by carbodiimide and succinimide chemistry as described before (O'Shannessy *et al.*, 1992) and incubated

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with 20 μ g/ml minimal streptavidin (IBA, Göttingen) in 10 mM acetic acid (pH 4.6) for 7 min. Covalently coupled streptavidin was then utilized to bind biotinylated APC-peptides with high affinity.

PDZ-domains were applied in 10 mM HEPES, pH 7.4, 5 mM MgCl₂, 150 mM NaCl and 0.005% (w/v) Igepal CA-630 (Sigma). Due to a strong loss in binding capacity of the surface by treatment with chaotropic dyes regeneration of the surface was achieved by a long washing step with buffer (approx. 90 min). All experiments were performed at 20°C. Association kinetic were fitted according to a A+B=AB model with an additional drift (Karlsson and Falt, 1997) by using either the BIAevaluation software (version 3.0, BIAcore AB) or the software package GraFit (version 3.0, Erithacus Software Ltd.). Dissociation phases were analysed according to a monoexponential decay + drift.

In vitro dephosphorylation assay of β -catenin

We expressed the protein tyrosine phosphatase domain and the catalytically inactive version (corresponding to amino acids: 2108–2460, (Hendriks *et al.*, 1995)) in fusion with glutathione-S-transferase. Catalytically inactive phosphatase domain was generated using PCR-based site directed mutagenesis by replacing cysteine 2374 by serine using primers: CS up: GCTGGAGTGTGTGATGACTGGGCCCG, CS down: CCCAGTCATCACACTCCAGCGCTGGCATTGGACGCTC. Recombinant fusion proteins were produced in *E. coli*. and purified using glutathione-agarose (Amersham Pharmacia Biotech). Tyrosine phosphorylated β -catenin was prepared by stimulation of MDCK-cells with 1 mM pervanadate. Pervanadate stock was generated by incubating 10 mM orthovanadate with 100 mM H₂O₂ in a ratio 1:5 for 10 min, 24°C, excess H₂O₂ was degraded by incubation with catalase in 30 mM HEPES pH 7.5, 150 mM NaCl. β -catenin was immunoprecipitated using C-18 antibody (Santa Cruz) and immunoprecipitates were incubated with 500 ng GST-fusion proteins in 25 mM HEPES, pH 7.5, 5 mM EDTA, 10 mM DTT. Immunoprecipitates were separated by 8% SDS-PAGE. Phosphotyrosine content of the immunoprecipitates was determined using an anti-phosphotyrosine antibody 4G10 (Upstate Biotechnology).

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