Pontin52, an interaction partner of β-catenin, binds to the TATA box binding protein

(Transcription regulation)

Andreas Bauer*, Otmar Huber†, and Rolf Kemler‡

Department of Molecular Embryology, Max Planck Institute of Immunobiology, Stübeweg 51, D-79108 Freiburg, Germany

Communicated by Christiane Nüsslein-Volhard, Max Planck Institute for Developmental Biology, Tübingen, Germany, October 15, 1998 (received for review August 13, 1998)

ABSTRACT β-catenin, the vertebrate homolog of the Drosophila Armadillo protein, has been shown to have dual cellular functions, as a component of both the cadherin-catenin cell adhesion complex and the Wnt signaling pathway. At Wnt signaling, β-catenin becomes stabilized in the cytoplasm and subsequently available for interaction with transcription factors. In this pathway, a Wnt signal induces, via several intermediate steps from the Wnt receptor Frizzled over the cytoplasmic proteins Dishevelled and glycogen synthase kinase 3β, an increase in the cytoplasmic pool of β-catenin by inhibiting its ubiquitin-dependent degradation. Stabilized cytoplasmic β-catenin thereby becomes available to form a bipartite complex with transcription factors of the lymphocyte enhancer factor-1/T-cell factor (LEF-1/TCF) family and accumulates in the nucleus. In this pathway, a Wnt signal induces, via several intermediate steps from the Wnt receptor Frizzled over the cytoplasmic proteins Dishevelled and glycogen synthase kinase 3β, an increase in the cytoplasmic pool of β-catenin by inhibiting its ubiquitin-dependent degradation. Stabilized cytoplasmic β-catenin thereby becomes available to form a bipartite complex with transcription factors of the lymphocyte enhancer factor-1/T-cell factor (LEF-1/TCF) family and accumulates in the nucleus.

β-catenin originally was identified as a central component of the E-cadherin-catenin cell–cell adhesion complex, bridging the cytoplasmic domain of E-cadherin to α-catenin and thus connecting the adhesion complex to the actin cytoskeleton. β-catenin is also a member of the Armadillo-repeat protein family and like Armadillo plays an important role in the Wnt signaling pathway (reviewed in ref. 4).

In this pathway, a Wnt signal induces, via several intermediate steps from the Wnt receptor Frizzled over the cytoplasmic proteins Dishevelled and glycogen synthase kinase 3β, an increase in the cytoplasmic pool of β-catenin by inhibiting its ubiquitin-dependent degradation (5, 6). Stabilized cytoplasmic β-catenin thereby becomes available to form a bipartite complex with transcription factors of the lymphocyte enhancer factor-1/T-cell factor (LEF-1/TCF) family and accumulates in the nucleus (7–9).

The LEF-1/TCF transcription factors first were identified in lymphoid cells, but they exhibit a more complex expression pattern during embryonic development (10–12). They are characterized by their conserved mobile homology box DNA-binding domain, with members from different species sharing over 90% amino acid similarity. Alone, LEF-1/TCF transcription factors are able to bind their DNA target sequences but possess little or no transcription activation potential (9). In contrast, β-catenin has no DNA binding capacity but contains a transactivation domain in its carboxyl-terminal region (13). The DNA-binding domain of LEF-1/TCF and the transactivation domain of β-catenin are required for activation of target genes of Wnt/Wg signaling such as siamois, Twin, and Xnr3 in Xenopus or ultrabithorax (Ubx) in Drosophila (14–18).

Little is known about the molecular mechanism of β-catenin-dependent transcriptional activation. In particular, no direct interaction of β-catenin with components of the basic transcriptional machinery has been described so far. Here we report the identification of an interaction partner of β-catenin, Pontin52, a nuclear protein that may play a role in the nuclear function of β-catenin.

MATERIALS AND METHODS

Cell Lines, Reagents, and Antibodies. All cells were grown in DMEM supplemented with 10% fetal calf serum in a 10% CO2 atmosphere at 37°C. Cells were transfected by a modified calcium phosphate precipitation method with 10 μg of plasmid DNA (19), using 2× Hepes instead of 2× N,N-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid (BES)-buffered saline.

Antibodies against three different parts of the Pontin52 sequence were produced in rabbits. Antibody anti-23 was generated against a synthetic peptide (amino acid positions 129–140) coupled to keyhole limpet hemocyanin. Antibodies anti-24 and anti-25 were raised against two recombinant proteins Dishevelled and glycogen synthase kinase 3β, respectively.

Pontin52, a nuclear protein that may play a role in the nuclear function of β-catenin. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. §1734 solely to indicate this fact.

© 1998 by The National Academy of Sciences

Abbreviations: LEF-1/TCF, lymphocyte enhancer factor-1/T-cell factor; GST, glutathione S-transferase; TBP, TATA box binding protein; HA, hemagglutinin.

Data deposition: The sequence reported in this paper has been deposited in the GenBank database (accession no. AF099084).

*Present address: Institut fuer klinische Chemie, Uniklinik Benjamin Franklin, Hindenburgdamm 30, D-12200 Berlin, Germany.

†To whom reprint requests should be addressed. e-mail: kemler@immunobi.mpimp.de.
**RESULTS**

**Isolation and Identification of Pontin52.** To identify new interaction proteins of β-catenin we looked for *in vitro* binding partners for the amino-terminal 284 aa of β-catenin expressed as a GST fusion protein (GST-β284). Three proteins from metabolically labeled human SW480 colon carcinoma cells, with relative molecular masses of 102, 52, and 44 kDa, were found to bind specifically to the GST-β284 fusion protein (Fig. 1, A). The 102-kDa protein, identified as α-catenin, was expected because GST-β284 harbors the binding site for α-catenin (amino acids 117–143) (23). The 52- and 44-kDa proteins were novel and also were detected as binding partners in other cell lines, including human AN3-CA and A431 carcinoma cells (not shown).

We used large-scale preparations (1.5 × 10⁶ SW480 cells) to purify the 52- and 44-kDa proteins in sufficient amounts for detection by Coomassie blue staining and amino acid sequence analysis of tryptic fragments (Fig. 1, CB). For the 52-kDa protein, sequences were obtained for three peptides (Fig. 2), and database searches revealed that all three peptides have strong matches with several expressed sequence tag (EST) cDNA clones of human and mouse origin. By using EST sequence information, specific oligonucleotides were designed as primers for reverse transcription–PCR, and from several overlapping clones isolated from a SW480 cell cDNA library the human full-length coding sequence was established. Nucleotide and amino acid sequence comparison revealed a high sequence homology of the 52-kDa protein to several known database sequences (Fig. 2). Among these were two inferred proteins from the yeast *Saccharomyces cerevisiae* genome with

**Affinity Precipitation and Immunological Procedures.** A recombinant GST-tagged Pontin52 was expressed in *E. coli* and affinity purified on GSH beads (Sigma); 2 μg of GST-Pontin52 was used for the affinity precipitation of β-catenin and TBP from cell lysates as described (21). Immunoprecipitations were carried out as described (22) except that the cell lysis buffer described above was used. For metabolic labeling, cells were grown in methionine- and cysteine-free DMEM and then cultured for 12 h in the presence of 50 μCi/ml of [35S] methionine/[35S] cysteine (3,000 Ci/mmol, Amersham). *In vitro* transcription and translation were performed according to the manufacturer’s descriptions (Promega).

**Immunofluorescence.** Cells were grown on collagen-coated coverslips, washed three times with PBS, fixed in 3% paraformaldehyde at room temperature for 20 min, treated with 1 M glycine in PBS, pH 8.5, for 5 min, washed in PBS, and permeabilized with 0.5% Triton X-100 for 5 min at room temperature. Alternatively, cells were fixed with methanol for 4 min at −20°C. Cells were incubated with anti-Pontin52 antibodies (anti-25) at 1:100 dilution or anti-MYC antibodies (2 μg/ml each) at 37°C for 1 h, washed, and treated with fluorescent dye (dichlorotriazinyl amino fluorescein, CY3)-conjugated secondary antibodies (Sigma) (1:200) in PBS for 1 h at 37°C. For control experiments, anti-25 antibodies were preincubated with 10 μg of a recombinant GST-Pontin52 fusion protein for 1 h. For nuclear staining, cells were treated with Hoechst 33342 (1 μg/ml in PBS) for 5 min at room temperature. Finally, cells were mounted in 50% glycerol/50% PBS/100 mg/ml of 1,4-diazabicyclo[2.2.2]octane and viewed under an Axioskop microscope (Zeiss).

Images were taken with a charge-coupled device C4880 digital camera (Hamamatsu Photonics, Hamamatsu City, Japan). Optical sections were taken at 0.2-μm intervals from the bottom to the top of the cell layers, and out-of-focus information was removed by using a deconvolution algorithm. Camera and microscope were controlled by the computer program OPENLAB (Improvision, Coventry, U.K.).

**Fig. 1.** Affinity binding experiments with recombinant GST-β-catenin284 (GST-β284). Cell lysates from 1 × 10⁹ metabolically labeled and 1.5 × 10⁹ nonlabeled SW480 cells were affinity precipitated with a recombinant GST-β284 fusion protein. Bound proteins were eluted, separated by SDS/PAGE and analyzed by autoradiography (A) and Coomassie blue staining (CB).
approximately 70% and 42% sequence identity. A similar high degree of homology was found with two inferred proteins of unknown function from the *Caenorhabditis elegans* genome (not shown). Of particular interest was the high homology to the recently described rat protein TIP49 (rTIP49), which was identified as a binding partner of the TBP, a component of the basic transcription machinery (24). This finding suggested that the 52-kDa protein also could bind to TBP and thus bridge β-catenin to TBP. The 52-kDa protein therefore was named Pontin52 (pons, bridge in Latin).

**In Vivo Interaction of β-catenin and Pontin52.** To identify Pontin52 unambiguously in subsequent biochemical analysis, three independent polyclonal antibodies were raised against different parts of the protein (Fig. 2). Each antibody specifically recognized Pontin52, as shown here for the anti-23 and anti-24 antibodies in immunoblots on SW480 cell lysates (Fig. 3A). Immunoprecipitation with Pontin52-specific anti-23 and anti-24 antibodies on cell lysates from SW480 cells and subsequent analysis of the obtained immunocomplex by Western blotting with anti-β-catenin antibodies demonstrated an association of endogenous β-catenin and Pontin52 in vivo (Fig. 3A).

For a reciprocal binding experiment Pontin52 was fused to six myc-epitope tags to increase the molecular weight of the fusion protein, which was necessary because endogenous Pontin52 migrates at a similar position in SDS-PAGE to that of the antibody heavy chain. On transfection Pontin52-MYC was well expressed in SW480 cells as monitored by immunoblot with anti-myc antibodies (Fig. 3B, lysate). More importantly, immunoprecipitations with anti-β-catenin revealed an association of Pontin52-MYC and β-catenin in SW480 cells transfected with Pontin52-MYC but not in the untransfected cells used as a control [Fig. 3B, compare β-catenin immunoprecipitations of nontransfected (n) and transfected (t) cells]. Several additional controls were included in these experiments. For example, when cell lysates from SW480 cells transfected with Pontin52-MYC were immunoprecipitated with antibodies against E-cadherin, glycogen synthase kinase 3β, or p120ctn, no Pontin52-MYC was coimmunoprecipitated (Fig. 3B). The association of Pontin52 and β-catenin also was observed when both components were overexpressed in Neuro2A cells (not shown).

**Direct Binding of Pontin52 to β-Catenin and TBP.** The results reported so far did not exclude the possibility of an indirect association between β-catenin and Pontin52 mediated by another cellular component. To test for a direct interaction of the two proteins *in vitro*, binding of recombinant GST-
Pontin52 to His<sub>6</sub>-tagged β-catenin was investigated. The results clearly demonstrated a direct interaction of the two proteins (Fig. 4A, Left).

The binding site in β-catenin for Pontin52 was mapped more precisely by testing for binding of a series of carboxyl-terminal-truncated, GST-tagged β-catenin fusion proteins with in vitro-translated Pontin52. The results indicate that the region of amino acids 187–284 of β-catenin, corresponding roughly to Armadillo-repeat motifs 2–5 of the protein, harbors a binding site for Pontin52, although weak binding also was observed in the amino-terminal region of β-catenin (Fig. 4B).

As indicated above, Pontin52 is highly homologous to rTIP49, which was identified as an interaction partner of the TBP, although no direct interaction of these two proteins could be demonstrated (24). Therefore, similar in vitro reconstitution experiments were performed with recombinant GST-Pontin52 and His<sub>6</sub>-tagged TBP, and again a direct interaction between Pontin52 and TBP was found (Fig. 4A, Right). In analogous experiments, recombinant LEF-1 did not interact with Pontin52 (not shown).

**Nuclear Localization of Pontin52.** Pontin52 is predominantly localized in the nucleus, as visualized by immunostaining of endogenous Pontin52 in COS cells (Fig. 5A). At higher magnification, Pontin52 is seen distributed over the nucleus in small dot-like structures, but with no staining of nucleoli. The nuclear localization also was observed with anti-23 and anti-24 antibodies (not shown), and the staining was eliminated when antibodies were preincubated with a recombinant GST-Pontin52 fusion protein (Fig. 5C). Nuclear localization of Pontin52 also was seen in human SW480 and HCT116 cells when using the same antibodies or when a myc-tagged Pontin52 was transiently expressed in COS cells and the cells were stained with anti-myc antibodies (not shown).

Pontin52 appears to be rather ubiquitously expressed as monitored by immunoblot analysis of cell lysates from various human and mouse cell lines (Fig. 6A) and by Northern blot analysis of RNA from different human tissues (Fig. 6B). The notion that Pontin52 might be a constitutive cellular component is further supported by the fact that the expressed sequence tag clones were isolated from cDNA libraries of various human and mouse tissues, including several that were not tested here, such as liver, testis, breast, spleen, and bone marrow.

**Triple Complex Formation with LEF-1.** The results presented so far demonstrate that Pontin52 is a nuclear protein and can bind directly to β-catenin and TBP. To obtain further information about the protein–protein interactions of nuclear β-catenin, Neuro2A cells were transiently transfected with cDNAs coding for myc-tagged Pontin52, HA-tagged LEF-1, and β-catenin-nuclear localization signal. Cell lysates were immunoprecipitated with anti-myc antibodies, and the immunoprecipitates were subjected to immunoblot analysis with anti-HA antibodies. A complex composed of LEF-1, β-catenin, and Pontin52 could be detected when all three proteins were expressed in combination, demonstrating that Pontin52 is associated with the LEF-1-β-catenin complex (Fig. 7). No direct interaction of Pontin52 with LEF-1 in the absence of β-catenin was observed in these experiments, in agreement
with the in vitro binding studies using recombinant proteins mentioned above. The faster migrating band recognized by the anti-HA antibody (Fig. 7, *) is likely a degradation product of LEF-1, because both the larger and smaller proteins were recognized by two independent anti-LEF-1 antibodies in parallel experiments (not shown). Attempts to demonstrate an even higher order complex composed of LEF-1, β-catenin, Pontin52, and TBP by including TBP cDNA in this kind of experiment have so far been inconclusive. But the triple complex formation by LEF-1, β-catenin, and Pontin52 provides good evidence that Pontin52 bridges the LEF-1-β-catenin complex to TBP and thus to the basic transcriptional machinery.

**DISCUSSION**

We report here the identification of Pontin52 as an interacting partner of β-catenin, which also binds directly to the TBP. Pontin52 is a nuclear protein that is evolutionarily highly conserved even in lower eukaryotes (e.g., yeast) that do not have β-catenin. We show here expression of Pontin52 in several human tissues and cell lines, and it is represented by a large number of expressed sequence tag clones in mouse and several human tissues and cell lines, and it is represented by a small number of expressed sequence tag clones in mouse and human from a variety of different tissues. These data indicate that Pontin52 is rather ubiquitously expressed and may exhibit a constitutive cellular function. Such a view is supported by gene disruption of the Pontin52 homolog in yeast, which resulted in a lethal phenotype (unpublished observation). The interaction of Pontin52 with β-catenin could reflect a nuclear function of β-catenin that is independent of its interaction with

**LEF-1/TCF.** It has been reported recently that β-catenin can be imported into the nucleus without being complexed with LEF-1/TCF proteins (25), which suggests that β-catenin may exhibit additional nuclear activities, as reflected in particular by its association with Pontin52. Alternatively, in terms of the current model of molecular interactions that transduce Wnt/Wg signaling, it is tempting to speculate that Pontin52 is involved in linking LEF-1/TCF-β-catenin complexes to the basic transcriptional machinery. Thus Pontin52 could function as a coactivator in regulating the expression of target genes on Wnt/Wg signaling. We show that a triple complex composed of LEF-1-β-catenin-Pontin52 can be formed in a cell, demonstrating that the binding sites of LEF-1 and Pontin52 in β-catenin are not mutually exclusive. Furthermore, a second potential transactivation domain recently was identified in the amino-terminal region of β-catenin (26), and as described here this amino-terminal transactivation domain is very close to or may even overlap with the binding site for Pontin52. Pursuing this attractive hypothesis, we have directly tested whether Pontin52 could enhance the transactivating activity of β-catenin in reporter assays, but this was only marginally the case (unpublished observation). More work is needed to elucidate the physiological role of Pontin52 for the nuclear function of β-catenin. Further hints for future experiments come from the structural features of Pontin52. It contains a P-loop motif responsible for ATP/GTP binding and exhibits multiple conserved regions, indicating that it is putatively an ATP-dependent DNA helicase. Thus, in the biological context studied here, i.e., Wg/Wnt-dependent formation of the LEF-1/TCF-β-catenin complex resulting in target gene expression, the role of Pontin52 might be to unwind nucleic acid duplexes, a step known to be essential for initiation of transcription. Members of the high mobility group-box transcription factor family, such as LEF-1 and TCFs, are known to bind with high affinity to four-way DNA junctions (27, 28). LEF-1 was reported to function as an architectural transcription factor by making a sharp bend in the DNA helix (29), whereas the additional binding of β-catenin to LEF-1 decreases the angle

---

**Fig. 6.** Pontin52 exhibits a widespread expression pattern. (A) Western blot analysis of cell lysates from human and mouse cell lines with Pontin52-specific antibody (anti-24). An equal number of cells for each cell line was taken for comparing the relative amount of Pontin52 with Pontin52-specific antibody (anti-24). An equal number of cells for each cell line was taken for comparing the relative amount of Pontin52 with Pontin52-specific antibody (anti-24). (B) Northern blot analysis on human tissues probed with a 0.69-kb Bgl–EcoRV fragment of the Pontin52 cDNA. A REAL blot was purchased from Invitrogen, which as indicated by the manufacturer had been loaded with 2 μg of total RNA per lane. A band expected for the size of the Pontin52 mRNA was detected in each of the tissues. GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

**Fig. 7.** In vivo association of Pontin52 with the LEF-1-β-catenin complex. Several combinations of plasmids were transfected into N2A cells as indicated. Pontin52-myc was precipitated from cell lysates with myc-specific antibodies, and the immunoprecipitates were analyzed by Western blotting of HA-tagged LEF-1 with anti-HA specific antibodies. The expression of transfected β-catenin and Pontin52-MYC was monitored by Western blotting of cell lysates with anti-β-catenin and anti-myc antibodies. Arrowhead indicates LEF-1-HA; * indicates degradation products of LEF-1-HA as monitored in parallel experiments with anti-LEF-1 antibodies (not shown).
of the bent DNA (7). Thus, a possible role for Pontin52 in the LEF-1/TCF-β-catenin complex might be to modulate the specific DNA structure of target gene promoters.

We thank Dr. R. Deutzmann (University of Regensburg, Germany) for the peptide sequence analysis, Dr. Randy Cassada for critically reading the manuscript, and Rosemary Schneider for secretarial work. This work was supported by the Max-Planck Society and the German-Israeli Foundation.