Functional Characterization of Multiple Transactivating Elements in β-Catenin, Some of Which Interact with the TATA-binding Protein in Vitro*

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β-Catenin, a member of the family of Armadillo repeat proteins, plays a dual role in cadherin-mediated cell adhesion and in signaling by Wnt growth factors. Upon Wnt stimulation β-catenin undergoes nuclear translocation and serves as transcriptional coactivator of T cell factor DNA-binding proteins. Previously the transactivation potential of different portions of β-catenin has been demonstrated, but the precise location of transactivating elements has not been established. Also, the mechanism of transactivation by β-catenin and the molecular basis for functional differences between β-catenin and the closely related proteins Armadillo and Plakoglobin are poorly understood. Here we have used a yeast system for the detailed characterization of the transactivation properties of β-catenin. We show that its transactivation domains possess a modular structure, consist of multiple subelements that cover broad regions at its N and C termini, and extend considerably into the Armadillo repeat region. Compared with β-catenin the N termini of Plakoglobin and Armadillo have different transactivation capacities that may explain their distinct signaling properties. Furthermore, transactivating elements of β-catenin interact specifically and directly with the TATA-binding protein in vitro providing further evidence that a major function of β-catenin during Wnt signaling is to recruit the basal transcription machinery to promoter regions of Wnt target genes.

β-Catenin together with Plakoglobin and the more distantly related p120ctn (where ctn is catenin) belong to a large family of proteins that are involved in diverse cellular processes (1). The members of this protein family are characterized by the presence of multiple copies of a 42-amino acid motif, the so-called Armadillo (Arm)3 repeat, which was named after a founding member of the family, the product of the Drosophila Armadillo gene (1). In vertebrates, β-catenin and Plakoglobin were first described as components of cadherin-catenin cell adhesion complexes, where they link cadherin transmembrane proteins to α-catenin and the actin filaments of the cytoskeleton (2). Meanwhile, the dual involvement of β-catenin and its invertebrate homolog Armadillo in cell adhesion and cell-cell signaling is well established. These proteins are central components of the Wnt/wingless signal transduction cascade which for example functions to specify anterior-posterior segment polarity in Drosophila larvae or to determine the embryonic dorso-anterior body axes in Xenopus laevis (3–5).

Wnt/wingless growth factors are secreted glycoproteins that utilize members of the Frizzled family of seven transmembrane domain proteins as receptors (5, 6). Stimulation of Frizzled receptors by Wnt activates a signaling pathway which includes Dishevelled, GSK-3β, Axin, or Conductin and the adenomatous polyposis coli tumor suppressor protein. GSK-3β, Axin, and adenomatous polyposis coli are thought to form a multiprotein complex that regulates the stability of β-catenin and thereby its subcellular distribution (5, 7). In cells receiving a Wnt signal β-catenin is translocated into the nucleus where it interacts with transcription factors of the TCF family (5, 7–12). Formation of β-catenin-TCF complexes ultimately leads to the activation of specific target genes such as Ultrabithorax, siamois, twin, nodal-related-3 or c-MYC (13–17).

Whereas the importance of the β-catenin-TCF complex during activation of Wnt target genes is well established, the specific function of β-catenin and its mechanism of action are less clear. TCF factors are sequence-specific DNA-binding proteins with a high mobility group domain recognizing a common consensus motif but which lack classical transactivation domains (12, 18). Instead, LEF-1, for instance, performs architectural functions or relies on interactions with accessory factors such as TLE/groucho co-repressors or a transcriptional coactivator termed ALY to modulate gene expression (19–21). β-Catenin could therefore act either by inducing changes in promoter structure (8), by alleviating repression through displacement of the TLE/groucho factors, or by providing a TAD. In fact, a TAD has been identified at the C terminus of β-catenin (22). This domain is both necessary and sufficient for the signaling activity of β-catenin in early Xenopus development (23), and in the fly the absence of the corresponding region from Armadillo causes diverse developmental defects (24, 25). Thus, the currently prevailing view is that docking of β-catenin to TCF factors primarily serves to deliver the C-terminal TAD to promoter elements of Wnt target genes and thereby to attract the basal transcription machinery (5, 12, 22). Therefore, it is important to seek evidence for an interaction between β-catenin and the general transcription apparatus.

β-Catenin shares 68 and 71% sequence similarity with Plakoglobin and Armadillo, respectively (1). All three proteins can interact with TCFs (7, 9) and possess transactivation domains at their C termini (22, 26). Nonetheless neither β-catenin nor Plakoglobin can rescue the signaling defects of Armadillo mutant flies (27), even though β-catenin can interact with Pango-
lin4/LEF-1 and murine LEF-1 can function as a downstream effector of Armadillo in Drosophila (15, 28). Moreover, Plakoglobin cannot replace β-catenin in mouse embryonic development (29), and it is dispensable during early Xenopus development, whereas β-catenin is absolutely required to establish Spemann organizer activity (30, 31). Although the basis for these differences is unknown, it is likely that they are associated with β-catenin, Plakoglobin, and Armadillo themselves, and further characterization of their transactivation properties might provide insight into their distinct functions.

To understand better the signaling activities of β-catenin and its relatives, we have systematically investigated their transactivation properties. Since analyses of catenin-dependent transcriptional activation in vertebrate cells is frequently biased by the presence of endogenous β-catenin (26, 32, 33), we have used the yeast Saccharomyces cerevisiae as a model system that does not possess cadherins, catenins, TCFs, or homologs of any of the components of the Wnt signaling pathway except GSK-3β. Our results indicate that the overall activity of β-catenin stems from the functional cooperation of multiple transactivating elements distributed over broad regions at both its C terminus and N terminus, as well as parts of the Arm repeat region. Interestingly, β-catenin differs from Plakoglobin and Armadillo with respect to the transactivation capacities of its N terminus. This could, at least in part, explain their distinct signaling characteristics. We also show that transactivating elements of β-catenin can specifically interact with the TATA-binding protein in vitro. This further supports the model that a major function of β-catenin during Wnt signaling is to recruit components of the basal transcription machinery to promoter regions of β-catenin/TCF target genes.

**Experimental PROCEDURES**

**Yeast Strains**—The strains used in this study were EGY48 (MATa, his3, trpl, ura3-1, leu2-2,2α::LEU2-LexAop6) (34) and its derivative AYH50 that carries a single chromosomal copy of the LexAop8-Gal1-lacZ::URA3 cassette in the ura3 locus. Yeast were grown under standard conditions in rich or synthetic minimal media (35).

**Plasmids**—All plasmids were constructed and purified using standard procedures (36). Sequences of plasmids generated with polymerase chain reaction amplification of the desired fragments and insertions without the ScaI sites, whereas construction of YH50, which was made by cloning a EcoRI fragment carrying the ADH1 promoter/terminator cassette from pACT2 (CLONTECH) without the EcoRI sites, and EcoRI/EcoRl fragment or an EcoRI/NcoI fragment from pGEX4T1MMBC, a Neol/EcoRl sites, and site of p570.1, which was made by cloning a ScaI/SalI fragment carrying the ADH1 promoter/terminator cassette from pACT2 (CLONTECH) without the Gal4 activation domain, into pRS424 (39) cut with ScaI and XhoI. The parental plasmid for expression of the LexA fusion was p573.2, which is a derivative of pEG202 (34) based on the pRS423 backbone (39). To generate the LexA-LEF-1 expression vector, the entire coding region of murine LEF-1 (9) was cloned into p573.2. From this construct an EcoRI/EcoNl fragment or an EcoNl/XhoI fragment was deleted to obtain LexA-LEF-1. To make constructs coding for β-catenin, a LexA-β-catenin, LexA-P265α, LexA-Plakoglobin, and LexA-Armadillo, an EcoRI/BamHI fragment from pGEX4T1MMBC, a Neol/XhoI fragment from pGEX4T1HGP, an EcoRI fragment from pGEXP120, or a BamHI/NotI fragment from pGEXArmAdillo was inserted into p573.2. Mutants with N-terminal deletions of LexA-β-catenin, LexA-Plakoglobin, and LexA-Armadillo were generated by in-frame insertion of suitable restriction fragments into p573.2. C-terminal deletions of LexA-β-catenin, LexA-Plakoglobin, and LexA-Armadillo were obtained by cutting of the corresponding full-length constructs with appropriate restriction enzymes within coding regions and downstream thereof and religation of these restriction fragments into the expression vectors in β-catenin, Plakoglobin, and Armadillo were made by polymerase chain reaction amplification of the desired fragments and insertion into the EcoRI/BamHI sites of p573.2. To obtain plasmids for the expression of Gal4-β-catenin fusion proteins in mammalian cells, we excised EcoRI/NotI restriction fragments coding for the desired β-catenin portions from the appropriate yeast expression vectors and cloned them into the EcoRI/BamHI sites downstream of the GAL4 DBD in pCMVGal4 (40). The luciferase reporter construct pG5E1bLuc was made by inserting a PstI/BamHI fragment from pG5E1bCAT (41) into the SmaI/BglII sites of pGL3Basic (Promega).

**Preparation of Whole Cell Extracts from Yeast and Western Blot Analyses**—For preparation of whole cell extracts the same cultures were used as for determining β-galactosidase activity. From each culture aliquots with 2 A600 units of cells were pelleted, washed once in sterile water, and resuspended in 50 μl of 50 mM HEPES/NaOH, pH 7.4, 300 mM NaCl, 1 mM EDTA, 10% glycerol, 0.5% Nonidet P-40, and 0.4% “Complete” protease inhibitor mix (Roche Molecular Biochemicals). An equal volume of glass beads was added, and cell lysis was carried out by vortexing for 5 min on an IKA VibraMax VX mixer at 4 °C. After addition of SDS-PAGE loading buffer 0.2 A600 units were separated by SDS-polyacrylamide gel electrophoresis and transferred onto nitrocellulose. Western blotting experiments were performed as described (23) (Western blotting experiments were performed as described (23) using polyclonal antisera against LexA (Invitrogen) or mouse mononuclear antibodies against β-catenin, Plakoglobin, and p120ctn (Transduction Laboratories).

**Preparation of Nuclear Extracts—**Preparation of nuclear extracts was performed as described (23) (Preparation of Nuclear Extracts—Nuclear extracts from HELA cells grown to confluence in eight 15-cm tissue culture dishes were prepared as described (44) except that nuclei were extracted in 1 volume of elution buffer (20 mM HEPES/KOH, pH 7.5, 420 mM NaCl, 25% glycerol, 1.5 mM MgCl2, 0.2 mM EDTA, 0.5 mM dithiothreitol, and Complete protease inhibitor mix). After elution the nuclei were removed by centrifugation at 20,000 × g for 30 min, and the nuclear extract was stored in small aliquots at −80 °C without dialysis. For use in pull-down assays the crude nuclear extracts were diluted 1:5 in elution buffer without NaCl.

**Expression and Purification of Recombinant Protein**—GST fusion proteins were expressed in Escherichia coli BL21 (DE3) and purified with GST-Sepharose beads (Amerham Pharmacia Biotech) as described (9, 37). After elution from the GST-Sepharose fusion proteins were dialyzed against 50 mM Tris/HCl, pH 8.0, concentrated using ammonium sulfate precipitation and dialyzed further for 3 h at 4 °C against 20 mM Tris/HCl, pH 7.5, 0.5 mM dithiothreitol, and 2 mM MgCl2 (DE3), and applied to a fast protein liquid chromatography column where necessary, frozen in liquid nitrogen, and stored at −80 °C until use. To express TBP-His6 in E. coli the plasmid pQE60-TBP was transformed into E. coli M15/pREP4 (Qia- gen) and TBP-His6 was purified by ion exchange and metal affinity chromatography as described (42, 45). **GST Pull-down Assays—**Approximately 10 μl bed volume of GST-Sepharose beads (Amerham Pharmacia Biotech) was preincubated in

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200 μl of pull-down buffer (20 mM HEPES/KOH, pH 7.5, 100 mM KCl, 5 mM MgCl₂, 0.5 mM EDTA, 0.05% Nonidet P-40, 1 mM dithiothreitol, 0.02% bovine serum albumin, and Complete protease inhibitor) mix together with the various GST fusion proteins. For pull-down assays from nuclear extracts 5 μg GST or GST fusion protein was used; for pull-down assays from reticulocyte lysates we used 1 μg of GST or GST fusion proteins with C-terminal portions of β-catenin and 5 μg of GST or GST fusion proteins with N-terminal portions of β-catenin, respectively. After 30 min of preincubation binding reactions were supplemented with 20 μl of the Ni-NTA eluate containing approximately 500 ng of TBP-His, or with 1 μl of reticulocyte lysate containing human TBP that had been transcribed from pCS2+ TBP and translated in vitro in the presence of [35S]methionine as described by the manufacturer (SP6/TNT system, Promega). For pull-down experiments from nuclear extracts, the prebinding solution was removed and replaced with 500 μl of diluted nuclear extract (approximately 500 μg of protein) from HeLa cells. Binding reactions were done on a shaker platform for 2 h at 4 °C. The GSH-Sepharose beads were pelleted; the supernatant was removed, and the beads were washed extensively in pull-down buffer with 200 mM KCl. Bound proteins were eluted from the GSH beads with SDS-PAGE loading buffer, resolved by electrophoresis on 10% SDS-polyacrylamide gels, and analyzed by Western blotting with mouse monoclonal anti-TBP antibodies (Transduction Laboratories, Promega) or by fluorography after soaking the gels in Amplify solution (Amersham Pharmacia Biotech).

RESULTS

β-Catenin and Plakoglobin Can Function as Transcriptional Coactivators of LEF-1 in Yeast—To determine whether it was possible to analyze transactivation by β-catenin and other Arm family members in the yeast S. cerevisiae, we inserted the coding regions for β-catenin, Plakoglobin, and p120ctn into a multicopy yeast expression vector. Full-length LEF-1, the N-terminal CBD of LEF-1 (8, 9), or a C-terminal portion lacking the CBD (Fig. 1A) were fused to the LexA DNA-binding domain and also inserted into a multicopy yeast expression plasmid. Combinations of the resulting plasmids were transformed into the yeast strain EGY48 carrying a plasmid-borne lacZ reporter gene driven by the GAL1 minimal promoter and the LexA operator (34). Individual transformants were isolated and analyzed for expression of the LexA-LEF-1 fusion proteins, β-catenin, Plakoglobin, and p120ctn. All factors were properly expressed as shown by Western blotting experiments performed on whole cell extracts that were probed with anti-LexA antibodies to detect the different LexA-LEF-1 fusions (Fig. 1B, lanes 1–4) or monoclonal antibodies against β-catenin, Plakoglobin, and p120ctn (Fig. 1B, lanes 5–13).

Next, we analyzed expression of the lacZ gene (Fig. 1C). As expected, no activation of the lacZ reporter gene was seen in control experiments with the catenins alone (Fig. 1C, lanes 1–4) or with the LexA-LEF-1 fusion proteins in the absence of the catenins (Fig. 1C, lanes 5, 9, and 12). Also, the combination of LexA-LEF-1 and p120ctn had no effect on reporter gene activity (Fig. 1C, line 9). In contrast, coexpression of β-catenin or Plakoglobin and LexA-LEF-1 strongly activated the lacZ gene (Fig. 1C, lines 6 and 7). The addition of the LEF-1 CBD to the LexA DNA-binding domain was sufficient to allow activation of the lacZ gene by β-catenin or Plakoglobin, whereas no transactivation was seen with LexA-LEFAN lacking the CBD (Fig. 1C, lines 10, 11, 13, and 14). Thus, as in vertebrate cells, β-catenin and Plakoglobin can interact with LEF-1 through the CBD and function as transcriptional coactivators of LEF-1 when expressed in yeast.

Both β-catenin and Plakoglobin harbor transactivation domains that can act independently from LEF-1 when added to a heterologous DNA binding moiety (22, 26). To test whether these TADs also functioned in yeast, we fused the entire coding region of β-catenin or Plakoglobin directly to the LexA DNA-binding domain. We also constructed a LexA-p120ctn fusion since we could not rule out that lack of transactivation by coexpressing LexA-LEF-1 and p120ctn was due to a lack of interaction between the two factors. The different LexA fusions were expressed in EGY48 (Fig. 1B), and stimulation of reporter gene expression was determined. The LexA-p120ctn fusion protein did not increase expression of the lacZ reporter (Fig. 1C line 18) even though its expression was clearly detectable (Fig. 1B, lane 13). Apparently, p120ctn has no transactivation capacity. In contrast, high levels of β-galactosidase activity were measured in cell lysates from transformants expressing LexA-
β-catenin and LexA-Plakoglobin (Fig. 1C, lines 16 and 17). Thus, the transactivation domains present in β-catenin and Plakoglobin can act independently from LEF-1 also in yeast cells. In summary, transcriptional stimulation by the LexA-β-catenin and LexA-Plakoglobin fusions or the LexA-LEF-1-β-catenin and LexA-LEF-1-Plakoglobin complexes in yeast appears very similar to their activities in vertebrate or insect cells. This indicates that the mechanism of transactivation by the catenins is conserved throughout eukaryotic organisms and that S. cerevisiae can be used as a model system to characterize further the transactivation properties of β-catenin and related proteins.

β-Catenin Contains Multiple Transactivating Elements within Its N and C Termini—A systematic analysis of the regions within β-catenin that contribute to its activity as transcriptional activator has not previously been undertaken. Therefore, we generated a series of C-terminal and N-terminal deletion mutants of β-catenin which were fused to the LexA DNA-binding domain. To assess their transactivation capacities, the various deletion mutants were introduced into the yeast strain AYH50 which contains a single, chromosomal copy of the LexA-operator-GAL1-driven lacZ gene. Thus, variations in expression levels of the LexA-β-catenin fusions are less likely to influence the evaluation of their transactivation potential.

First, the N-terminal TAD was analyzed (Fig. 2A). A construct with residues 1–159 was chosen as reference, because preliminary tests had revealed that the highest transactivating capacity was associated with this region. The presence of additional sequences from the Arm repeat region in some constructs reduced or even abolished the activity of the N-terminal TAD (data not shown). Expression of the various LexA fusions was examined by Western blotting (Fig. 2C). LexA fusions with β-catenin residues 1–159 and 47–159 were equally potent in reporter gene stimulation and achieved about 50% of the activity of β-catenin (Fig. 2A, lines 2, 3, and 5). The fusion with residues 47–117 also transactivated but to a lesser extent (30% activity of β-catenin) (Fig. 2A, line 6). No transactivation potential was associated with residues 1–52 and 108–159 (Fig. 2A, lines 4 and 7). Comparison of reporter gene activation by constructs with residues 47–159, 47–117, and 108–159, however, indicates that residues 108–159 augment transactivation by elements within residues 47–117. Thus, it appears that the full activity of the N-terminal TAD is conferred by the cooperation of multiple elements present within residues 47–159.

Next, we examined the activity of LexA-β-catenin fusions with residues from the Arm repeat region, all of which were properly expressed when introduced into AYH50 (Fig. 2D). A construct with β-catenin residues 108–683 reached approximately 42% of the activity of β-catenin (Fig. 2B, line 3). All additional deletion mutants lacking portions from the C-terminal end of the Arm repeat region were unable to stimulate reporter gene expression (Fig. 2B, lines 4–7). Analyses of the complementary set of mutants showed that the transactivation capacity of the remaining β-catenin sequences was abolished upon removal of residues 159–281 (Fig. 2B, lines 8 and 9). However, even shorter LexA-β-catenin fusions containing residues 400–683 or 536–683 regained some activity, reaching about 10.5 and 6.5% of the activity of full-length β-catenin (Fig. 2B, lines 10 and 11). From this we conclude that aside from the elements between residues 47–117 and 108–159, additional transactivating regions of β-catenin reside between residues 159–281 and 536–683. Although the elements between residues 159–281 and 536–683 have no or only little capacity to stimulate expression of the lacZ reporter gene by themselves, when present together they can achieve considerable activity.

Again, this implies that overall activity of β-catenin results from the combined action of multiple transactivating elements.

Finally, we analyzed the C-terminal TAD. A LexA fusion with β-catenin residues 536–781 was nearly as active as β-catenin (Fig. 3A, lines 2 and 3). Further removal of residues up to positions 630, 662, and 695 reduced the activity of the resulting LexA fusions to 68, 30, and 32% of maximum levels (Fig. 3A, lines 4–6). Slightly higher activity was seen with residues 728–781 (50% activity of β-catenin) (Fig. 3A, line 7). Weak, but significant, reporter gene activation was mediated even by a fusion construct with only the C-terminal 31 residues. In conclusion, the C-terminal transactivating elements are largely contained within residues 662–781 and that there is probably only a minor contribution of residues 536–661 to the overall activity of the C-terminal TAD.

Additional analyses of LexA fusions with β-catenin residues 662–756 and 662–729 revealed that both constructs reached...
approximately 36% of maximum activity (Fig. 3B, lines 11 and 12). The finding that each of the LexA fusions with β-catenin residues 662–729 and 728–781 strongly activated the lacZ reporter gene identifies at least two independent transactivating subdomains at the C terminus of β-catenin. To define further the transactivating element(s) within residues 662–729, we generated constructs containing residues 662–698 and 681–729. Despite extensive overlap both fusions had little or no transactivation capacity (Fig. 3B, lines 6 and 7). Most likely, this region, too, contains two or more elements that together generate the transactivating properties of the parental fragment. Interestingly, residues 662–683 on their own do not transactivate (Fig. 3A, lines 9, 10, and 13), yet their presence confers some activity on the LexA fusion with residues 536–683. This result implies that a transactivating element is located around position 662 and therefore may have been destroyed in constructs with this end point. Alternatively, an additional transactivating element, without autonomous activity but with the ability to synergize with other elements, may be located more N-terminally of position 662. At present, we cannot distinguish between these two possibilities. Nonetheless, it appears that the C-terminal TAD of β-catenin is a complicated assembly of multiple subdomains that are distributed over the entire region encompassing at least residues 662–781 and that functionally cooperate to generate its full transactivation capacity.

Functional Testing of β-Catenin Transactivating Elements in Human Cells—To determine whether the transactivating elements identified in yeast were also functional in vertebrate cells, we fused various portions of β-catenin to the GAL4 DNA-binding domain. The resulting constructs were transfected into human 293 kidney cells together with a luciferase reporter gene by the various GAL4-β-catenin fusions in 293 cells is shown in Fig. 4. For comparison, the transactivation potential of the corresponding LexA fusions in yeast is included. Qualitatively, the results obtained in yeast and in 293 cells are very similar, although quantitatively we observed differences. Compared with full-length β-catenin the N-terminal fragment with residues 1–159 is considerably more active in 293 cells. However, as in yeast cells, the only autonomous transactivating activity is associated with residues 47–117 (20–30% of full-length β-catenin). As before, residues 1–52 and 108–159 have no transactivation potential above control levels. Apparently, the function of the N-terminal TAD depends on cooperation of its subelements even more in 293 cells than in yeast. Although the C-terminal fragment with residues 536–781 displayed approximately the same relative activity in yeast and 293 cells, fragments with residues 695–781 and 728–781 turned out to be much more potent in the human cell line and exceeded the activity of full-length β-catenin by 15- and 2.5-fold, respectively. Residues 662–729, on the other hand, reached only 16% of the activity of full-length β-catenin in 293 cells compared with 36% in yeast. The reduced activity of this fragment indicates that at least one of the elements, which is active in yeast, is not functional in 293 cells. In accordance with this assumption we found that residues 536–683, which weakly transactivated in yeast (see Fig. 2B), are completely inactive in the human cell line. Given that in the absence of this portion of β-catenin the activity of residues 695–781 vastly decreases, it is even possible that a negative regulatory function resides within residues 536–683. The lack of transactivation by this region of β-catenin also explains why residues 159–683 failed to activate the reporter gene in 293 cells since transcriptional activation by this domain in yeast required the simultaneous activity of transactivating elements at both of its ends. In summary, with the exception of the transactivating element located between residues 536 and 683, all other ele-
results obtained with the corresponding LexA-b and expression levels of the respective constructs (Fig. 5) exhibited somewhat lower activities concomitant with a drop in fusions that consisted primarily of the C-terminal extension conserved C-terminal extension stimulated gene. LexA-Plakoglobin or LexA-Armadillo fusions including (Fig. 5) expression at levels even higher than the full-length proteins determined as before.

properties, we constructed LexA-Plakoglobin and LexA-Armadillo N-terminal regions in Plakoglobin and Armadillo. A. full-length Plakoglobin (light gray bars) or Armadillo (dotted bars) and N-terminal or C-terminal fragments as shown in left part of the figure were fused to the LexA DNA-binding domain and expressed in AY1550. The Arm repeat regions are highlighted (dark boxes), and end points of Plakoglobin and Armadillo sequences are given. lacZ reporter gene activity was determined as before. B and C, expression of the LexA fusion proteins was analyzed by Western blotting experiments performed on whole cell extracts with antibodies against LexA. M, molecular weight markers. B, LexA-Plakoglobin fusion proteins. C, LexA-Armadillo fusion proteins. Lane 7 shows a longer exposure of the Western blot to visualize weakly expressed full-length LexA-Armadillo.

ments identified in yeast are also functional in 293 cells. Although some of these elements appear to operate more efficiently in human cells, this result further validates the use of the yeast system to analyze transactivation by β-catenin.

Transactivating Properties of the Plakoglobin and Armadillo N Termini Differ from Their Counterpart in β-catenin—To test whether the signaling differences between β-catenin, Plakoglobin, and Armadillo might be related to their transactivating properties, we constructed LexA-Plakoglobin and LexA-Armadillo fusions containing amino acid sequences that correspond to the N-terminal and C-terminal transactivation domains in β-catenin. As expected, full-length LexA-Plakoglobin and LexA-Armadillo fusions strongly activated the lacZ reporter gene. LexA-Plakoglobin or LexA-Armadillo fusions including the C-terminal one and a half-Arm repeat as well as the nonconserved C-terminal extension stimulated β-galactosidase expression at levels even higher than the full-length proteins (Fig. 5A, lines 2 and 5 and lines 8 and 11). Slightly shorter LexA fusions that consisted primarily of the C-terminal extension exhibited somewhat lower activities concomitant with a drop in expression levels of the respective constructs (Fig. 5A, lines 6 and 12, and Fig. 5, B and C). This is highly reminiscent of the results obtained with the corresponding LexA-β-catenin fusions and suggests that functional differences between Armadillo, β-catenin, and Plakoglobin probably are not based on particular properties of their C-terminal transactivating regions.

In contrast, a striking difference between β-catenin, Armadillo, and Plakoglobin was revealed when we examined LexA fusions with N-terminal portions of the factors. Plakoglobin residues 1–149 (equivalent to residues 1–159 in β-catenin) possess no transactivation capacity, and a construct with Plakoglobin residues 39–108 (equivalent to β-catenin 47–117) was also completely inactive for reporter gene stimulation (Fig. 5A, lines 3 and 4). On the other hand, we found that LexA fusions with Armadillo residues 1–167 strongly activated the lacZ gene, as did their counterpart in β-catenin (residues 1–147) (Fig. 5A, lines 9). However, the relative importance or distribution of transactivating elements at the Armadillo N terminus seems to differ because Armadillo residues 58–125, which correspond to the main activating element at the β-catenin N terminus, confer only 4.5% of the maximum activity of the Armadillo N-terminal TAD as opposed to 56% of the same region in β-catenin (compare Figs. 2A and 5A, line 10). These results show that there are functional differences between the N termini of β-catenin, Plakoglobin, and Armadillo and suggest that the distinct signaling properties of these factors partly arise from the presence or absence of specific transactivating elements at their N termini.

Transactivating Elements of β-Catenin Interact with the TATA-binding Protein—The fact that the transactivation domains of β-catenin function in yeast and vertebrate cells suggests that an evolutionarily conserved component of the transcription apparatus may be one of the targets of β-catenin during the process of gene activation. TBP is highly conserved among various organisms and is known to interact with a variety of transcription factors (46). We therefore wished to determine whether β-catenin could interact with TBP. Full-length β-catenin, a C-terminal portion with residues 536–781, and a N-terminal fragment with residues 1–284 were expressed as GST fusion proteins in E. coli and used in pull-down assays with nuclear extracts from HeLa cells. Proteins bound to the GST fusions were recovered from the binding reactions and analyzed for the presence or absence of TBP by Western blotting experiments with specific antibodies. As shown in Fig. 6, TBP specifically associates with the fusion proteins containing β-catenin sequences as shown in Fig. 6, center panel. We also tested the interaction between the GST fusion proteins and recombinant, histidine-tagged TBP-His6. In these experiments β-catenin residues 536–781 again exhibited the highest level of TBP binding, whereas the N-terminal fragment showed an increased ability to interact with TBP compared with the full-length β-catenin fusion (Fig. 6, right panel). Although the basis for the differences in relative binding between TBP from nuclear extracts and recombinant TBP from E. coli is not clear at the moment, these results show that β-catenin and fragments harboring the transactivating elements of β-catenin can directly interact with TBP in vitro.
β-Catenin Transactivation Domains Interact with TBP

Fig. 7. Mapping of TBP interaction domains in β-catenin. A, GST or GST fusions with N-terminal portions of β-catenin (5 µg) as indicated were used in pull-down assays with bacterially expressed TBP-His6. For comparison, GST fusions with residues 536–781 from the C terminus of β-catenin (1 µg) (lane 3) or with residues 39–108 of Plakoglobin (lane 14) equivalent to residues 47–117 of β-catenin were also used. In lane 1 (Input) an aliquot corresponding to 10% of the total amount of TBP-His6 used for the assay was loaded. B, mapping of TBP interaction domains at the C terminus of β-catenin. GST or GST fusions with C-terminal portions of β-catenin (1 µg) as indicated were used in pull-down assays with bacterially expressed TBP-His6. In lane 1 (Input) an aliquot corresponding to 10% of the total amount of TBP-His6 was loaded. C, summary of the results from the binding experiments shown in A and B and the transactivation studies. Elements in β-catenin that transactivate autonomously are denoted by hatched bars, and elements that transactivate only when combined with autonomous elements or with each other are marked lightly hatched. Regions in β-catenin which can interact with TBP are shown above β-catenin.

To gain information about the importance of the TBP interaction for transactivation by β-catenin, we localized the TBP-binding elements at the β-catenin N and C termini. GST fusions with various subfragments derived from β-catenin residues 1–284 or 536–781 were prepared and used for binding experiments with TBP-His6 (Fig. 7). Fusion proteins with β-catenin residues 1–183, 1–119, and 120–302 all bound TBP but with reduced efficiency compared with β-catenin residues 1–284 (Fig. 6A, compare lanes 4–6 and 12). These findings indicate that at least two binding sites for TBP are present at the N terminus of β-catenin. One of these binding regions most likely is contained within residues 183–284. The other, which appears to localize more N-terminally, was examined more closely. Analyses of complementary sets of β-catenin fragments show that the presence of residues 47–107 is absolutely required for the interaction with TBP (Fig. 7A, lanes 5–7 and 9–11), even though residues 47–117 by themselves bound TBP only weakly and preferentially associated with a particular degradation product of TBP-His6 (Fig. 7A, lane 13). Yet, a fragment with residues 39–108 from Plakoglobin corresponding to β-catenin residues 47–117 did not interact with TBP at all (Fig. 7A, lane 14).

Analyses of fusion proteins with residues derived from the C terminus of β-catenin demonstrated that constructs with residues 536–781, 630–781, 536–755, and 536–729 all bound TBP with equal efficiency (Fig. 7B, lanes 3 and 4 and 8 and 9), whereas a fusion with residues 536–675 displayed a much reduced TBP binding activity (Fig. 7B, lane 10). GST fusions with β-catenin residues 683–781, 728–781, 750–781, and 536–630 were not able to interact with TBP (Fig. 7B, lanes 5–7 and 11). Also, a β-catenin fragment with residues 662–729 did not interact with TBP (data not shown). From this we conclude that the interaction between TBP and the C terminus of β-catenin is mediated by residues 630–729 and that residues 630–675 critically contribute to this binding activity.

A comparison of TBP-binding elements and transactivating elements in β-catenin reveals that of the three TBP-binding regions TI, TII, and TIII, which we have identified, TII located within Arm repeats 2–4 correlates with a domain that was unable to transactivate on its own but that was found to play an auxiliary role during transactivation by β-catenin or β-catenin derivatives (Fig. 7C). TIII partially overlaps with an autonomous transactivating element at the C terminus of β-catenin but also requires residues located more N-terminally in a region that does not transactivate by itself. TI between residues 47 and 107 coincides with the main stimulatory element in the N terminus of β-catenin. Taken together, our results suggest that the interaction with the TATA-binding protein may be involved in various aspects of β-catenin function and is likely to be of particular importance for the activity of the N-terminal transactivation domain.

DISCUSSION

Functional Differences among Arm Family Members—The importance of β-catenin and Armadillo as transcriptional coactivators of TCF family members is well documented, and there is considerable evidence that transactivation is the primary function that is required for the signaling activity of β-catenin (22, 23, 47). Yet the molecular basis for the functional differences among the otherwise highly related catenins and their precise mode of action are poorly understood. Here we have used the yeast S. cerevisiae as a model system to analyze catenin-mediated transcriptional activation. As p120ctn shares certain features with β-catenin, Plakoglobin, and Armadillo, including the presence of multiple Arm repeats, binding to cadherins, and being a target for tyrosine kinases (48), one might have expected that p120ctn also acts as transcriptional activator. However, the absence of any transactivation capacity of p120ctn in our test system is in good agreement with its apparent lack of signaling activity upon overexpression in Xenopus embryos (38). On the other hand this underscores the significance of transactivation by β-catenin, Armadillo, and specifically Plakoglobin. Although to date there is no evidence that Plakoglobin can have signal transducing activity, its ability to associate with LEF-1 and its transactivation potential nonetheless argue that Plakoglobin, too, may perform a function similar to β-catenin and Armadillo. A possible signaling activity of Plakoglobin may become important later in development or in the adult organism, when an involvement of Plakoglobin in transcriptional regulation is more difficult to investigate and might be obscured by the presence of β-catenin (49).

Previous studies have established that β-catenin, Armadillo, and Plakoglobin can activate reporter gene constructs when fused to a heterologous DNA-binding domain (22, 26) but hitherto mainly the activities of their C termini have been considered, and here the three factors do not appear to differ (26). However, this does not explain why Plakoglobin and β-catenin cannot substitute for each other or complement Arm mutations in Drosophila. The presence of transactivating elements at the N termini of Armadillo and β-catenin raises the possibility that the N-terminal TADs and possibly other portions of β-catenin or Armadillo contribute to efficient target gene induction or to the activation of a particular set of target genes. Cooperativity of multiple transactivation domains in a single transcription factor as well as cell type-specific and promoter-specific activity of distinct transactivation domains is also found among nuclear hormone receptors.
(50, 51). In support of this idea the Armadillo mutant Arm\textsuperscript{S10D} with a complete deletion of the N terminus is less potent at rescuing armadillo mutant flies than the Arm\textsuperscript{S10D} allele carrying only a short deletion of the GSK-3β phosphorylation sites (52). In addition, we have observed here that LexA fusions with the β-catenin Arm repeat region are able to activate a reporter gene. This activity is probably specific since p120\textsuperscript{ctn}, despite the presence of multiple Arm repeats, lacks any transactivation potential. In earlier experiments expression of the Arm repeat region of β-catenin induced ectopic body axes in X. laevis (53). Although this effect was recently assumed to be mediated by endogenous β-catenin (33), our findings imply that indeed the Arm repeats themselves may have functioned as signal transducers. Moreover, whereas either the C-terminal TAD of β-catenin or a strong heterologous transactivation domain can elicit the formation of ectopic dorso-ventral body axes in Xenopus embryos (23), it appears that efficient transformation of fibroblasts involves a β-catenin specific activity and is less related to the potency of the transactivator used (47). Thus, although transactivation per se is clearly critical for the signaling functions of catenins, some of their activities may be context-dependent, and β-catenin, Armadillo, and Plakoglobin may have evolved to perform specific tasks possibly through distinct properties of their N termini.

When recruited to a promoter by LexA-LEF-1 fusion proteins, Plakoglobin was somewhat less efficient as a transcriptional coactivator than β-catenin. This could reflect the absence of the N-terminal TAD. Yet, Plakoglobin is a potent coactivator of LEF-1 in yeast. This is in contrast to the situation in human 293 embryonic kidney cells where it was suggested that β-catenin was the preferred interaction partner for LEF-1 (26). The differential behavior of Plakoglobin in yeast and in 293 cells implies that the interaction of Plakoglobin with LEF-1, and possibly of catenins with TCF family members in general, is differentially controlled in a cell type-specific manner, e.g. by posttranslational modification. A candidate for such a modifying activity is the CBP acetylase, which was recently identified as a regulator of Wnt signaling in Drosophila (54) and which is absent from yeast. In fact, our observations that WT β-catenin and Plakoglobin readily coactivate transcription in yeast, while in vertebrate cells vast overexpression of WT proteins or the use of mutant β-catenin is required, also indicate that in S. cerevisiae the catenins are not subject to the negative control mechanisms that govern their activities in other eucaryotic cells. Therefore, yeast may be useful for the reconstitution of these control mechanisms and their detailed mutational analysis.

Structural and Functional Characteristics of Transactivating Elements in β-Catenin—The architectural function of β-catenin in cell adhesion raised the intriguing possibility that β-catenin performed its task as a transcription factor by an unusual mechanism (8, 18, 55). However, there is now increasing evidence that β-catenin functions primarily as a conventional transactivator. As is the case for other well characterized transcription factors, the transactivation domains of β-catenin possess a modular structure, function in combination with different types of DNA-binding domains at natural and artificial target gene promoters (22, 23, 26, 47, 55), and work in mammalian as well as yeast cells. Sequence examination of the transactivating elements in β-catenin reveals that they are highly enriched for acidic residues and in particular the C-terminal TAD harbors multiple amino acid sequence motifs with neighboring asparagine and leucine residues similar to the minimal transactivating modules of the VP16 and RelA (56, 57). A close relationship between β-catenin and VP16 is further implied by the fact that the VP16 transactivation domain can be substituted for β-catenin both in signaling and oncogenic transformation (23, 47). On the basis of these structural and functional similarities, we propose that β-catenin, like VP16, p53, and RelA, belongs to the class of acidic activators.

Interactions between β-Catenin and TBP—The rate-limiting step for gene activation appears to be the recruitment of general transcription factors to the promoter region which can be facilitated through interactions between GTFs and transactivators (46). By using an in vitro approach, we have shown that three distinct regions in β-catenin can directly bind to recombinant TBP or to endogenous TBP in nuclear extracts. Thus, it is possible that β-catenin indeed stimulates target gene expression by contacting the basal transcription apparatus. Although our results suggest that this may occur directly, additional indirect contacts between β-catenin and TBP may be established through the recently discovered bridging factor Pontin52 (42). At present, however, it is not clear whether Pontin52 has a stimulatory function or whether it rather serves to destroy β-catenin-containing transcription factor complexes similarly to the MOT1 ATPase, which disrupts TBP-DNA complexes (58). Further analysis is needed to distinguish between these possibilities.

By comparing transactivating and TBP-binding elements, we have attempted to gain information about the physiological relevance of the different TBP/β-catenin interactions. We have obtained a good match for the N-terminal core TAD, which we found to both bind to TBP and to transactivate. In contrast, the corresponding domain in Plakoglobin failed to interact with TBP and has no transactivation potential. This correlation suggests that TBP may well be a relevant target for the N-terminal transactivating element. The other two TBP-binding domains, TII and TIII, coincide with auxiliary transactivating elements and may be involved in transactivation by the Arm repeat domain. However, unlike TII, TII and TIII have no autonomous transactivation potential. Multiple, functionally distinct TBP-binding domains have also been described for the VP16 TAD (59, 60). In this case and also for the p53 tumor suppressor protein, certain mutations that disrupted the interaction with TBP in vitro turned out to have no impact on the activity of the VP16 or p53 transactivation domains in vivo (60, 61). TBP binding itself may not be sufficient to confer transactivation potential, and possibly additional contacts with the basal transcription machinery must be made. In fact, acidic activators are known to have multiple binding partners among the GTFs (59, 61–66). The functional difference between the three TBP-binding regions in β-catenin therefore could arise from the presence of additional GTF-binding sites in the vicinity of the N-terminal autonomously transactivating element. That TBP is not the only target for β-catenin is also suggested by the observation that C-terminal transactivating elements, which are functional in vivo, do not bind to TBP in vitro. Therefore, it appears likely that additional cofactors exist which assist β-catenin during gene activation. Experiments are under way to identify these factors.

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