Regulation of β-catenin transformation by the p300 transcriptional coactivator

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The β-catenin protein plays a critical role in embryonic development and mature tissue homeostasis through its effects on E-cadherin-mediated cell adhesion and Wnt-dependent signal transduction. In colon and other cancers, mutations of β-catenin or the adenomatous polyposis coli (APC) tumor suppressor appear to stabilize β-catenin and enhance its interaction with T cell factor (TCF) or lymphoid enhancer factor (Lef) transcription factors. At present, a complete picture of the means by which β-catenin's interactions with TCF/Lef proteins contribute to neoplastic transformation is lacking. We report that the transcriptional coactivator p300 interacts with β-catenin *in vitro* and *in vivo* and is critical for β-catenin-mediated neoplastic transformation. p300 synergistically activates β-catenin/TCF transcription, and their biochemical association requires the CH1 domain of p300 and a region of β-catenin that includes its NH_2-terminal transactivation domain and the first two armadillo repeats. Lowering of cellular p300 levels by using a ribozyme directed against p300 reduced TCF transcriptional activity and inhibited the neoplastic growth properties of a β-catenin-transformed rat epithelial cell line and a human colon carcinoma line with a β-catenin mutation. These findings demonstrate a critical role for p300 in β-catenin/TCF transcription and in cancers arising from defects in β-catenin regulation.

The β-catenin protein and its *Drosophila* homologue armadillo have been established as critical downstream factors in the Wnt signaling pathway in vertebrates and the conserved *Drosophila* Wingless pathway. Binding of the Wnt protein to the Frizzled receptor activates the disheveled protein, which, in turn, inhibits the function of glycogen synthase kinase 3β (GSK3β) (1). GSK3β, when complexed with the adenomatous polyposis coli (APC) tumor suppressor protein and the axin or conductin proteins, phosphorylates specific serine and/or threonine residues in the NH_2 terminus of β-catenin. Phosphorylation of these NH_2-terminal sequences of β-catenin promotes its interaction with F-box proteins and its subsequent ubiquitination and rapid degradation by the proteasome. When it escapes degradation and accumulates in the nucleus, β-catenin binds to T cell factor (TCF) or lymphoid enhancer factor (Lef) proteins and stimulates transcription of TCF/Lef-target genes (2–4). As might be predicted from present models of β-catenin function in the Wnt pathway (reviewed in refs. 5–8), biallelic inactivation of the *APC* gene in colon cancers results in stabilization of β-catenin, its translocation to the nucleus, and constitutive activation of TCF/Lef transcription. In a subset of colon and other cancers, deregulation of TCF/Lef transcription results from missense mutations or deletions of the GSK3β phosphorylation sites in the NH_2-terminal domain of β-catenin.

Several aspects of the regulation of TCF/Lef transcription by β-catenin and other proteins have been clarified. Two independent transactivation domains in β-catenin have been identified (9, 10): one in the NH_2-terminal region (amino acids 1–132) and another in the COOH-terminal (amino acids 695–781) region. In addition to β-catenin, other TCF/Lef-binding proteins include the transcriptional corepressor Gro (human homologs TLE1–4) and a microtubule-associated protein kinase (MAPK)-related protein, termed NEMO-like kinase (NLK), that down-regulates TCF/β-catenin-mediated transcription by binding and phosphorylating TCF-4 (11). The p300 and related CREB-binding proteins (CBPs) serve as coactivators that also control cell cycle progression, and *Drosophila* CBP has been shown to functionally affect the Wingless pathway (12). We therefore asked whether the p300/CBP coactivators might interact with β-catenin in mammalian cells and affect the Wnt pathway of malignant transformation. Our results showed that p300 interacts with β-catenin and synergistically activates β-catenin/TCF transcription. Lowering of cellular p300 levels reduced β-catenin/TCF transcription activity and inhibited β-catenin-mediated transformation, demonstrating a critical role for p300 in β-catenin/TCF transcription and in tumorigenesis resulting from altered β-catenin regulation.

Materials and Methods

Plasmids. The constructs 12S E1A, Δp300 E1A (12S pm 563) were a kind gift from E. Harlow (13). CMV-p300 was constructed as described (14). β-Catenin/pCDNA3 was constructed as described (15). Glutathione *S*-transferase (GST)-p300 (amino acids 302–530) and GST-p300 (amino acids 565-1065) were constructed by PCR cloning. The primers for GST-p300 (amino acids 302–530) are as follows: 5′ primer, 5′-ATGGGTTCCATGGGTCAACAGCCAGCCCCG-3′, and 3′ primer, 5′-TCTCTTAAGAGTCAGTCTTTCTTCTGAGCCGCAAAC-3′; the PCR product from using these two primers was then cloned in-frame into the pGEX-6p vector at *Bam*HI and *Eco*RI sites. The 5′ primer for GST-p300 (amino acids 565-1065) is 5′-GCTAGTCGACTCGGAATTCGGAAACAGTGGCAC-3′ and the 3′ primer is 5′-AGTCAGTCGCGGCCGCTCAGGATTCTGGATCCTGACGGTA-3′; the PCR product from using these two primers was cloned at the *Sal*I and *Not*I sites of the pGEX-6p vector. The GST-p300 (amino acids 1240–2412) was subcloned from pBS3′p300 (16). Wild-type and mutant TCF reporters were constructed by cloning the double-stranded oligonucleotide containing three copies of wild-type or mutant TCF-binding sites into pGL2 (Promega) at *Kpn*I and *Hin*dIII restriction sites. The sequence of the oligonucleotide is 5′-GATCGGTACCCCTTTGATCCCTTTGATCCCTTTGATCGAGCTCAGGGTATATAATGAAGCTTGATC-3′ for

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Abbreviations: APC, adenomatous polyposis coli; TCF, T cell factor; Lef, lymphoid enhancer factor; CBP, CREB-binding protein; GST, glutathione *S*-transferase; β-gal, β-galactosidase.

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wild-type reporter and 5′-GATCGGTACCCCAATCGCCCC-AATCGCCCCAATCGCCGAGCTCAGGGTATATAAT-GAAGCTTGATC-3′ for the mutant reporter. Wild-type or mutant p300 ribozymes were cloned into the murine leukemia virus retroviral vector pRNVL-neo (based on pLZR-CMV-Rev M10) (17) at the *Nhe*I site. The sequence of the oligonucleotide is 5′-TATGCCAACTGATGAGGACCGAAAGGTCGAAA-CTTGTCTG-3′ for the wild-type p300 ribozyme, and 5′-TAGTACAACTGATGAGGACCGAAAGGTCGAAAC-TGAGCTG-3′ for the scrambled arm mutant p300 ribozyme.

Cell Lines and Tissue Culture. Human kidney cell line 293 (ATCC CRL-1573), RK3E/S33Y and RK3E/Kras cells (15), CT26 (18), Jurkat (ATCC T1B-152), HCT116 (ATCC CCL-247), and SW480 (ATCC CCL-228) were cultured in Dulbecco's modified Eagle's medium (DMEM, GIBCO/BRL) containing 10% fetal bovine serum (FBS) at 37°C in a 5% CO_2/95% air atmosphere. RKO was cultured in McCoy's medium containing 10% FBS.

Immunoprecipitation and Western Blotting. Immunoprecipitation of the whole cell lysates from several types of cells, SW480 (human colon carcinoma), HCT116 (human colon carcinoma), CT26 (mouse colon carcinoma), or Jurkat (human T leukemia) was performed as described (14).

GST Precipitations. GST p300-fusion proteins containing residues 302–530, 565-1065, or 1240–2412 were expressed and purified as described (14). β-Catenin and deletion mutants ΔN47, ΔN218, S33Y/Δ48–217, S33Y/Δ218–467, S33Y/ΔC468, and S33Y/ΔC695, constructed as described (15), were translated *in vitro* and ^{35}S-labeled by using the T7 TNT reticulocyte transcription/translation system (Promega). The deletion mutant of β-catenin containing residues 1–88 was produced by using the same transcription/translation system with truncated DNA template generated by restriction digestion of the β-catenin/pcDNA3 with restriction enzyme *Xho*I. Twenty microliters of *in vitro* translated β-catenin and GST beads containing approximately 200 ng of GST-p300 (amino acids 302–530) were incubated in 200 μl of IP buffer [20 mM Hepes, pH 7.9/75 mM KCl/2.5 mM $MgCl_2$/0.1% Nonidet P-40/1 mM DTT and complete protease inhibitors mixture (Boehringer Mannheim, catalog no. 1836145)] at 4°C for 1 h. The GST beads were washed three times with 1× IP buffer and resolved by electrophoresis on a Tris·HCl Ready Gel (4–15% gradient gel, Bio-Rad). The gel was then dried and subjected to autoradiography.

Retroviruses and Transduction. To produce retrovirus containing the wild-type or mutant p300 ribozyme, plasmids expressing gag/pol, env, and p300 ribozyme were transfected into 293T cells (17) by using the calcium phosphate method. Viral supernatant was collected 48 h after transfection and used for transduction. RK3E/S33Y-D cells were plated onto 6-well plates (2×10^6 cells per well) 1 day before transduction. One milliliter of viral supernatant composed of retroviruses expressing either wild-type or mutant p300 ribozyme was added to the cells. After 6 h, 2 ml of complete DMEM containing 10% FBS was added. The

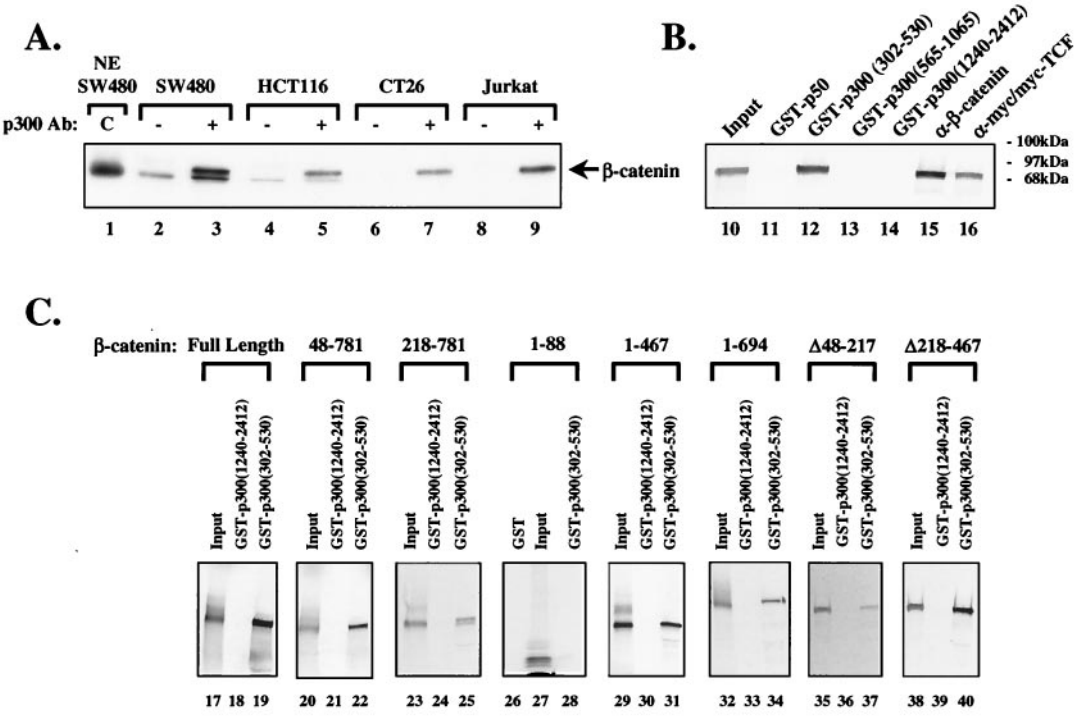

Fig. 1. p300 interacts with β-catenin. (*A*) β-Catenin coimmunoprecipitates with p300. Nuclear extracts from the indicated cell lines were immunoprecipitated without (−) or with (+) anti-p300 antibody, and immune complexes were resolved by SDS/PAGE, followed by Western blotting with anti-β-catenin antibodies. The arrow indicates the position of β-catenin. NE SW480 represents nuclear extract of SW480 human colon carcinoma cells. Ten percent of the extract used for immunoprecipitation was loaded in the control lane. (*B*) β-Catenin interacts with the CH1 domain of p300. Different fragments of p300 were fused to GST and used to pull down *in vitro* translated ^{35}S-labeled β-catenin. GST-p300 (amino acids 302–530), GST-p300 (amino acids 565-1065), and GST-p300 (amino acids 1240–2412) contain residues 302–530, 565-1065, and 1240–2412 of p300, respectively. GST-p300 (amino acids 302–530) contains the CH1 domain of p300. Anti-β-catenin and anti-myc antibodies complexed with myc-tagged TCF were used to bind β-catenin to evaluate the binding strength of GST-p300 (amino acids 302–530). Ten percent of the *in vitro* translated β-catenin extract used in the pull-down assays was loaded as input. (*C*) p300 interacts with the NH_2- or COOH-terminal regions of β-catenin. The indicated deletion mutants of β-catenin were generated by *in vitro* transcription and translation and incubated with GST-p300 (amino acids 302–530) as in *B*. A COOH-terminal fragment of GST-p300 (amino acids 1240–2412) was used as a negative control. Ten percent of the *in vitro* translated β-catenin extract used in the pull-down assays was loaded as input.

next day the medium was replaced with complete DMEM containing 1 mg/ml G418. After 24 h, the medium was replaced with the complete medium containing 0.7 mg/ml G418 and the cells were cultured for 7–10 days. The cells were then used for Western blotting, reporter assays, or colony formation assays.

Soft Agar Colony Formation Assay. The colony formation assay was performed as described (15). Cells (10^4) were plated in 0.3% agar medium containing DMEM and 20% FBS over a bottom layer of 0.6% agar medium and incubated for 3 weeks. The cells were fixed with glutaraldehyde and stained with methylene blue, and colony formation was determined from three independent experiments.

Results

p300 and β-Catenin Interact in Cells and *in Vitro*. To determine whether p300 was complexed with β-catenin in cells, p300 was immunoprecipitated from various cell lines, including SW480 and HCT116, two colon cancer lines known to have alterations in β-catenin regulation (3), as well as lines displaying no evidence of defects in β-catenin regulation, such as Jurkat. Immune complexes were subsequently resolved by SDS/PAGE, followed by Western blotting with antibodies against β-catenin. The p300-precipitated complexes contained detectable levels of β-catenin (Fig. 1*A*, lanes 3, 5, 7, and 9 vs. 2, 4, 6, and 8), demonstrating the *in vivo* interaction between p300 and β-catenin.

To define the region of p300 that interacts with β-catenin, different fragments of p300 were expressed as recombinant GST fusion proteins and incubated with *in vitro* translated β-catenin. The CH1 domain of p300 (amino acids 302–530) was shown to bind β-catenin (Fig. 1*B*, lane 12), in a fashion similar to that of an anti-β-catenin antibody or TCF-4 (Fig. 1*B*, lanes 15 and 16). In contrast, GST-p50 or two other GST-p300 fusion proteins lacking the CH1 domain, GST-p300 (amino acids 565-1065) and GST-p300 (amino acids 1240–2412), did not bind β-catenin (Fig. 1*B*, lanes 11, 13, and 14). The regions of β-catenin required for binding to p300 were defined by incubation of GST-p300 (amino acids 302–530) with various β-catenin mutant proteins, generated by *in vitro* translation. The p300 CH1 fragment bound strongly to full-length β-catenin and an NH_2-terminal deletion mutant lacking the first 47 aa (Fig. 1*C*, lanes 17–19 and 20–22). In contrast, binding of p300 was significantly reduced to β-catenin mutants lacking amino acids 1–217 or 48–217 (Fig. 1*C*, lanes 23–25 and 35–37). Binding of p300 to β-catenin proteins lacking the COOH-terminal transactivation domain (TAD) (amino acids 1–694), or armadillo repeats 9–13 and the COOH-terminal TAD (amino acids 1–467) remained readily detectable (Fig. 1C; lanes 32–34 and 29–31, respectively). However, the strength of the interaction of p300 with β-catenin mutants lacking the COOH-terminal region was somewhat decreased compared with the binding seen with full-length β-catenin. The p300 CH1 fragment also bound to a β-catenin protein lacking armadillo repeats 3–8 (Fig. 1*C*, lanes 38–40), a region required for β-catenin's binding to TCF, APC, conductin/axin, E-cadherin, and pontin 52. A β-catenin protein with extensive deletion of all sequences COOH-terminal to amino acid 88 abrogated binding to p300 (CH1) (Fig. 1*C*, lane 28). Taken together, the results suggest that sequences in the NH_2-terminal region of β-catenin, located between amino acids 48 and 217 and containing the transactivation domain and the first two armadillo repeats, are important for p300 binding. It is interesting to note that two transactivation domains of β-catenin have been previously localized (9, 10). In independent studies, deletion of either domain substantially inhibited the ability of a cancer-derived mutant

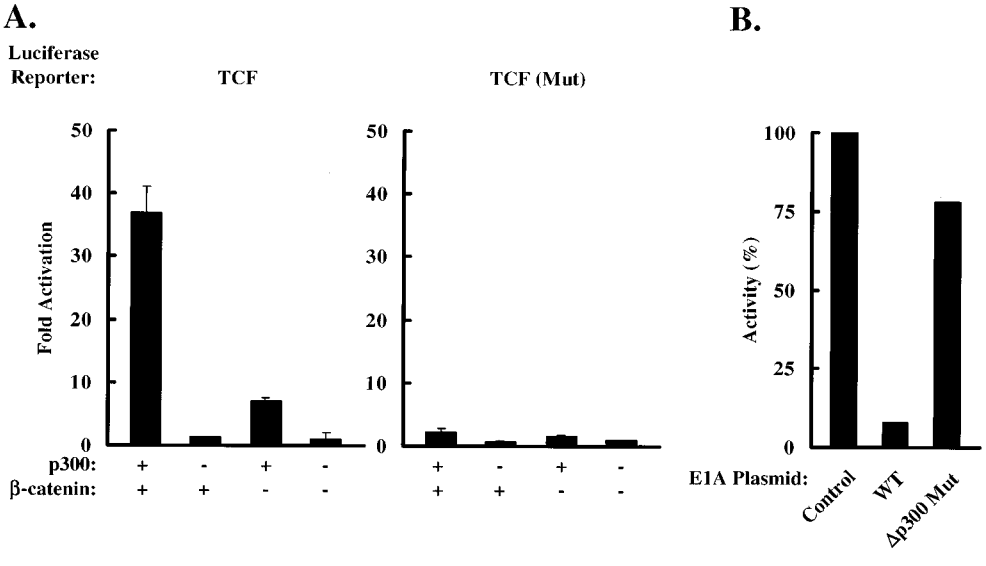

Fig. 2. p300 activates β-catenin/TCF-mediated transcription. (*A*) p300 and β-catenin synergistically activate the transcription of the TCF reporter. 293 cells were transfected with wild-type (*Left*) or mutant (*Right*) TCF luciferase reporter and the plasmid expressing p300 and/or the plasmid expressing β-catenin as indicated. An RSV-β-gal construct (β-galactosidase gene with a Rous sarcoma virus enhancer/promoter) was included in all transfections as a control for transfection efficiency. Twenty-four to 48 h after transfection, the cells were harvested and the luciferase activity was measured. All luciferase activity (relative light units, RLU) was normalized against β-gal activity. The value of the RLU from cells transfected with reporter alone was arbitrarily set as 1, and the fold activation was calculated accordingly. 293 cells were transfected by using the calcium phosphate method as described (31). One-half microgram of TCF or mutant TCF reporter, 1 μg of β-catenin/pCDNA3, or 3 μg of pCMVp300 was used as indicated. pCDNA3 was used as filler DNA to ensure equal amount of DNA in each transfection. RK3E/S33Y cells were transfected by using Lipofectamine Plus transfection reagents (BRL) according to the manufacturer's protocol. RSV-β-gal (0.2 μg) was included in all transfections for standardization/normalization. Twenty-four to 48 h after transfection, the cells were lysed by using 1× reporter buffer (Promega) and the luciferase and β-gal activities were measured. (*B*) The transcriptional activation of the TCF reporter was inhibited by 12S E1A, but not by mutant E1A that does not bind p300. Wild-type (WT) TCF reporter was transfected into 293 cells with plasmids expressing 12S E1A or mutant E1A [Δp300 E1A (125 pm 563) (13), unable to bind p300]. Cells were harvested and their luciferase activity was measured as in *A*. The luciferase activity of cells (control) transfected with reporter alone is arbitrarily set to 100, and the percent inhibition was calculated accordingly.

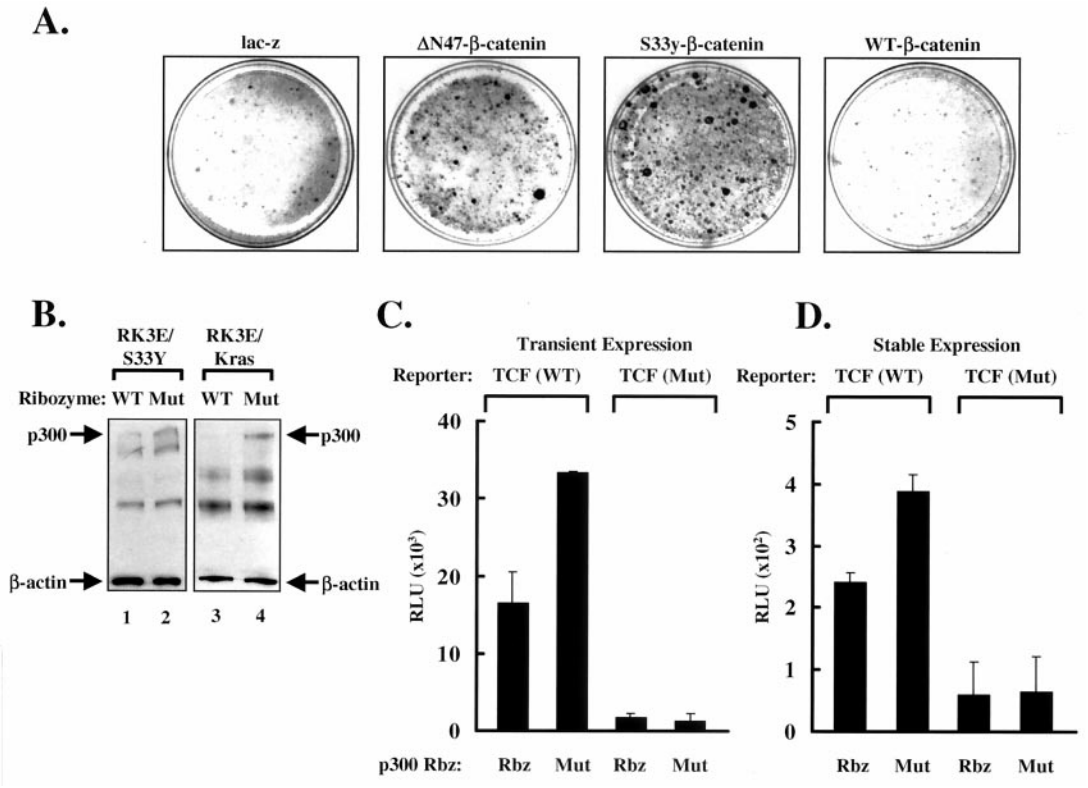

Fig. 3. Reduction of the endogenous p300 by the p300 ribozyme and its effect on β-catenin/TCF-mediated transcription. (*A*) Focus-formation assays. Immortalized neonatal rat kidney cells RK3E were transduced with retroviruses expressing the *lacZ* gene (as negative control), ΔN47 (47 residues from the NH_2 terminus of β-catenin were deleted), S33Y (point mutation at residue 33 on β-catenin), and wild-type β-catenin. (*B*) Wild-type p300 ribozyme reduces the endogenous levels of p300. Immortalized neonatal rat kidney cells transformed with a mutant β-catenin (RK3E/S33Y) or K-ras (RK3E/Kras) were transduced with retroviruses expressing wild-type or mutant p300 ribozyme. After 7 days of G418 selection, the cells were harvested and resolved by SDS/PAGE followed by Western blotting. Anti-p300 antibody was used to detect the endogenous p300 protein and anti-β-actin antibody was used to detect endogenous β-actin, which serves as an internal control. The arrows indicate the position of p300 or β-actin. (*C*) Reduction of transcription of the TCF reporter by transient expression of the p300 ribozyme (Rbz). RK3E/S33Y cells were transfected with wild-type or mutant TCF reporter and a plasmid expressing wild-type or mutant p300 ribozyme. The RSV-β-gal construct was included in all transfections as a control for transfection efficiency. The cells were harvested 24 h after transfection and assayed for the luciferase activity (relative light units, RLU). Luciferase activity was normalized against β-gal activity. (*D*) Reduction of transcription of the TCF reporter by stably expressing p300 ribozyme. The RK3E/S33Y cells were transduced with retroviruses expressing wild-type or mutant p300 ribozyme. After 7 days of G418 selection, the ribozyme-transduced cells were transfected with the wild-type or mutant TCF reporter. The cells were harvested and the luciferase activity (RLU) was measured and calculated as in *B*.

β-catenin protein to promote neoplastic transformation of the RK3E immortalized epithelial cell line, although deletion of the COOH-terminal domain of β-catenin had a somewhat greater effect than deletion of the NH_2-terminal region (15).

p300 Function in β-Catenin–TCF Transcription. Previous studies have shown that β-catenin activates TCF-mediated transcription in various cell lines (4). To further assess the functional significance of the interaction between β-catenin and p300, we carried out studies in 293 cells to determine whether p300 enhanced the ability of β-catenin to activate TCF transcription. TCF activity in 293 cells transfected with p300 was increased approximately 7-fold compared with cells transfected with a control plasmid (Fig. 2*A Left*), indicating that p300 enhances the level of endogenous TCF transcription in these cells and that endogenous p300 may be limiting. Whereas transfection of β-catenin plasmids stimulated TCF transcription ≈2-fold, cotransfection of β-catenin and p300 vectors stimulated luciferase activity more than 37-fold, revealing strong cooperativity between β-catenin and p300 in TCF transcription (Fig. 2*A Left*). A reporter plasmid with mutated TCF binding sites showed minimal changes, confirming the specificity of this effect (Fig. 2*A Right*). Furthermore, transcription of the TCF reporter was inhibited by 12S E1A, but not by a mutant E1A protein that does not bind p300 (Fig. 2*B*), further supporting the role of p300 as a coactivator for β-catenin/TCF-mediated transcription.

Role of p300 in β-Catenin-Mediated Neoplastic Transformation. β-Catenin proteins harboring missense mutations or restricted deletions of the NH_2-terminal phosphorylation sites of glycogen synthase kinase 3β have been found to induce neoplastic transformation of the RK3E cell line (15). Dense foci of morphologically transformed cells are induced roughly 3–4 weeks after infection of RK3E with retroviruses encoding mutated forms of β-catenin, but not by retroviruses encoding wild-type β-catenin or a control β-gal (*lacZ*) gene (Fig. 3*A*). Prior work has also indicated that TCF plays a critical role in neoplastic transformation of RK3E by mutated β-catenin. This role of TCF in β-catenin transformation is specific, as inhibition of TCF function in RK3E cells does not affect K-ras transformation (15). A clonal tumorigenic cell line established from a focus of RK3E cells transformed by the S33Y mutated form of β-catenin, termed RK3E/S33Y, was used in our subsequent studies to further define the role of p300 in β-catenin/TCF transcription and neoplastic transformation.

To explore the possibility that the transcriptional coactivator p300 regulates transformation by β-catenin, we sought to develop a strategy to inhibit p300 function and to assess the effects

on TCF transcription. A previously described p300 mRNA-specific ribozyme (19) was inserted into a retroviral vector (pRVNL3-neo). As a control, a ribozyme was prepared in which the sequence of each arm flanking the active catalytic site was scrambled, rendering the RNA unable to bind and/or degrade p300 mRNA. Retroviruses expressing either the p300-specific or control ribozyme were used to infect RK3E/S33Y or RK3E/Kras cells. After infection with ribozyme retroviruses, cells were cultured in the presence of G418 for 7–10 days and harvested, and p300 levels were assessed by Western blotting. The p300 level in the transduced cells expressing the wild-type p300 ribozyme was reduced to roughly 30% of the p300 protein level in cells transduced with the mutant p300 ribozyme construct (Fig. 3*B*, lanes 1 vs. 2 and 3 vs. 4). Because p300 plays a central role in many cellular processes, from general transcription to specific interaction with regulatory factors and involvement in signaling pathways, it is likely that complete inhibition of p300 expression by the ribozyme or other approaches is not compatible with cell survival. Haploinsufficiency of p300 has profound effects on cell viability *in vitro* and embryo survival in knockout mice (20).

We assessed the effect of the p300 ribozyme constructs on TCF reporter activity in RK3E/S33Y cells. Transient or stable expression of the p300 ribozyme reduced activity of a TCF reporter but had no significant effect on a similar reporter with mutant TCF-binding sites (Fig. 3 *C* and *D*). To determine whether reduction of endogenous p300 affected β-catenin-mediated transformation, RK3E/S33Y cells were infected with retroviruses encoding a p300-inactivating or a control ribozyme. The number of colonies from transduced cells expressing the wild-type p300 ribozyme was substantially reduced in soft agar compared with cells expressing the control scrambled-arm ribozyme (Fig. 4 *A* and *B*), suggesting that p300 had an important role in β-catenin-mediated transformation. Analysis of the effects of the p300-specific ribozyme in several independent β-catenin-transformed RK3E cell lines yielded similar results (data not shown). A similar reduction in neoplastic growth was observed when the p300 ribozyme was introduced into the HCT116 human colon carcinoma line, which contains a mutant β-catenin allele (Fig. 4 *A Lower*). In contrast, the p300 ribozyme had no effect on the soft agar colony-forming ability of the RKO colon cancer cell line, a line that has no APC or β-catenin mutation and no evidence of TCF/β-catenin deregulation (data not shown).

To assess the specificity of p300 in β-catenin transformation vs. neoplastic transformation by other oncogenes, K-ras-transformed RK3E cells (RK3E/Kras) were infected with the identical ribozyme retroviruses. Although p300 protein was similarly reduced in the RK3E/Kras cells (Fig. 3*B*), no substantial difference was observed in soft agar colony formation for cells expressing the p300-specific or control ribozymes. Taken together, these results establish that p300 interacts biochemically and functionally with β-catenin to activate TCF transcription and β-catenin-mediated transformation.

Discussion

In this study, the CH1 domain of p300 has been shown to interact directly with sequences in the NH_2-terminal region of β-catenin (amino acids 48–217). Furthermore, p300 was found to enhance the ability of β-catenin to activate TCF transcription and to be critical for β-catenin-mediated neoplastic transformation. Recently, two groups independently showed that the COOH terminus of β-catenin interacts directly with the CH3 region of p300/CBP, demonstrating that p300 interacts with β-catenin and activates β-catenin/TCF transcription (21, 22). Our data on the specific regions of the p300 and β-catenin proteins that are critical for their interaction differ from the results of Hecht *et al.* (21) and Takemaru and Moon (22). A potential explanation for the apparently discrepant findings may be the differing strategies that have been used to localize the interaction regions. The different methods of analysis, including the study of *in vitro*-synthesized p300 and β-catenin mutant proteins with various truncating mutations, may have contributed to the discordant data. Alternatively, because two independent transactivation domains have been identified in β-catenin, one in the NH_2-terminal region (amino acids 1–132) that overlaps with the p300 binding domain (amino acids 48–217) we identified in β-catenin and the other in the COOH-terminal region that overlaps with

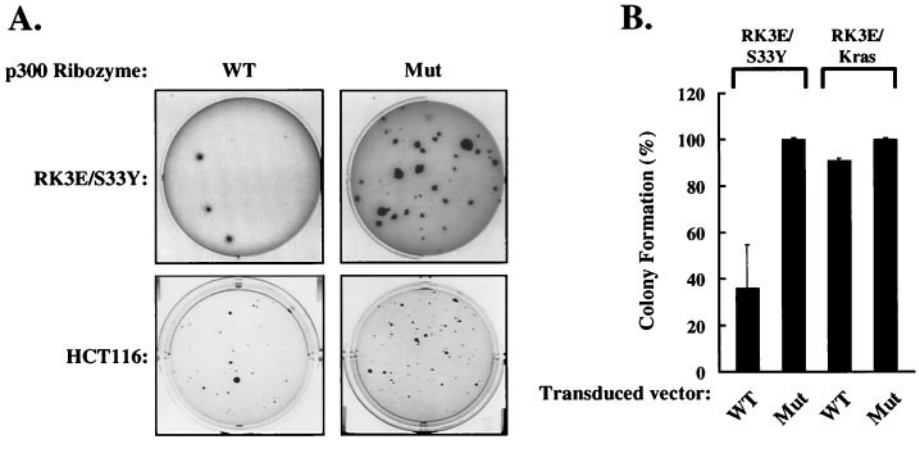

Fig. 4. p300 ribozyme suppresses β-catenin- but not K-ras-associated transformation. (*A*) Colony formation assay of β-catenin-transformed cells (RK3E/S33Y) or human colon cancer cell line HCT116 (containing a β-catenin mutation) stably transduced with the retroviral vector encoding the active p300 ribozyme (WT, *Left*) or the scrambled-arm mutant (Mut, *Right*) that served as its negative control. Similar suppression was observed in HCT116 cells for active p300 ribozyme (69 ± 14 colonies) compared with mutant, inactive p300 ribozyme (139 ± 37 colonies; $P > 0.037$). In RKO, a human colon cancer cell line containing wild-type APC and β-catenin with no evidence of TCF deregulation; active vs. inactive p300 ribozyme yielded similar numbers of colonies (67 ± 37 vs. 74 ± 42; $P > 0.853$). (*B*) p300 ribozyme suppresses colony formation of β-catenin-transformed cells (RK3E/S33Y), but not K-ras-transformed cells (RK3E/Kras), in a soft agar assay. The RK3E/S33Y and RK3E/Kras cells were transduced with retroviruses expressing wild-type or mutant p300 ribozyme as in *A*. For the colony-formation assay, 10^4 cells were used. After incubation for 3 weeks, the cells were fixed with glutaraldehyde and stained with methylene blue. The colony number of the cells transduced with mutant p300 ribozyme was arbitrarily set as 100, and the percentage of reduction of the colony number was calculated accordingly. The mean numbers of colonies from three independent experiments ±SD are shown.

the p300/CBP-binding domain identified by Hecht *et al.* and Takemaru and Moon, it is entirely possible that there may be two different p300/CBP-binding domains in β-catenin and two distinct β-catenin-binding domains in p300.

The interaction between β-catenin and p300 in mammalian cells and its resultant effect on enhancing TCF transcription contrast with the suggested role of CBP in suppressing TCF transcription in *Drosophila*. Specifically, in prior studies in *Drosophila*, a distinct region of the p300-related protein CBP, namely the COOH terminus (residues 2240–2507), interacted with TCF to suppress the transcription of the TCF-responsive *Ubx* gene during midgut development (12). A potential mechanism by which CBP repressed TCF-mediated transcription of *Ubx* was that CBP-mediated acetylation of TCF decreased the affinity of TCF's binding to Armadillo. Notwithstanding these findings and the potential mechanism offered to explain the results, CBP appears likely to regulate TCF transcription and the wingless pathway in a complex fashion. In certain tissues, *Drosophila* CBP represses TCF transcription, thus antagonizing Wingless signaling. Yet, in other settings, CBP appears to have a positive role in the Wingless signaling pathway, via its role as coactivator of transcription factors critical in wingless gene expression.

Taken together, the results of the *Drosophila* and mammalian studies imply that there may be quite complicated regulation of β-catenin/TCF-mediated transcription by the p300 and CBP coactivators. Perhaps, the apparently disparate effects might reflect important differences between p300 and CBP (19, 23–26). For instance, different phosphorylation events may regulate these proteins and their functions. Phosphorylation of CBP may increase its histone acetyltransferase activity, which may play an important role in repression of TCF. Other cellular factors could also be involved in regulating the role of p300 and CBP in activation or repression of TCF transcription. NF-κB, Stat2, CREB, ATF-1, c-Jun, and c-Myb all have been shown to interact with the CH1 region of p300/CBP (14, 23, 27–34). As a result, these factors could interfere with the ability of β-catenin to bind to p300 in certain settings. Similarly, p53, E1A, c-Fos, c-Jun, MyoD, and TFIIB all interact with the CH3 region of p300/CBP, and these factors would likely interfere with the TCF binding to p300/CBP. Therefore, one would expect that the transcription of β-catenin/TCF-targeted genes is activated or suppressed in response to different signals or during different stages of cell growth. However, because transcriptional activation of β-catenin/TCF-targeted genes appears to play an important role in the Wnt signaling pathway and cell transformation, and a dominant-negative TCF that neither binds β-catenin nor activates TCF-mediated transcription reduces transformation by β-catenin (15), the bulk of the evidence indicates that p300 most likely functions in enhancing TCF transcription in cancers arising from defects in β-catenin regulation.

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