The structural maintenance of chromosome protein SMC3 is a component of the cohesin complex that mediates sister chromatid cohesion and segregation in prokaryotes and eukaryotes. It is also present extracellularly in the form of a chondroitin sulfate proteoglycan known as bamacan. We have found previously that SMC3 expression is elevated in a large fraction of human colon carcinomas. The additional finding that the protein is significantly increased in the intestinal polyps of ApcMin/+ mice has led us to hypothesize that SMC3 expression is linked to activation of the APC/β-catenin/TCF4 pathway. The immunohistochemical analysis of colon adenocarcinomas from clinical specimens revealed that β-catenin and SMC3 antibodies co-localize with maximal stain intensity within the transformed areas. Cloning and sequencing of 1578 bp of the human SMC3 promoter unveiled the presence of seven putative consensus sequences for β-catenin/TCF4 binding, two of which are conserved in the mouse Smc3 promoter. Transient transfection experiments in HCT116 and SW480 human colon carcinoma cells using deletion and mutated promoter constructs in luciferase reporter vectors confirmed that the putative sites, the first located at −48 bp and the second located at −701 bp, are susceptible to β-catenin/TCF4 transactivation. Co-transfection with a β-catenin expression vector enhanced the promoter activity whereas E-cadherin had the opposite effect. Binding of β-catenin/TCF4 complexes from SW480 nuclear extracts to these sequences was confirmed by electrophoretic shift and supershift mobility assays. Altogether, these results are consistent with the idea that the β-catenin/TCF4 transactivation pathway contributes to SMC3 overexpression in intestinal tumorigenesis.

Mutations in the APC (adenomatous polyposis coli) gene are found in more than 70% of human intestinal adenomas (1). APC is regarded as the gatekeeper of the tumorigenesis process in the intestine, because the mutation and the loss of heterozygosity of APC occurs as the earliest event in hereditary and sporadic adenocarcinoma (1). The key role of APC in tumorigenesis has been attributed to its function in targeting β-catenin for degradation (2). The finding that β-catenin accumulates throughout the cell in the intestinal adenomas and that ectopic expression of APC in colon tumor cells lowers its level and inhibits tumor growth points to a link among APC mutation, β-catenin cytoplasmic level, and colon carcinoma (2–4). Further evidence for a key role of β-catenin in intestinal tumorigenesis has been provided by the findings that mutation in β-catenin glycogen synthase kinase β-dependent phosphorylation sites makes it resistant to proteolytic degradation, giving rise to a phenotype that is indistinguishable from that of APC null mutations (5, 6). Additionally, half of the sporadic colorectal cancer cells with wild-type APC have mutated β-catenin (4). β-Catenin acts as a transcriptional cofactor by migrating to the nucleus and associating with members of a family of DNA-binding proteins known as T cell factors, of which TCF4 is expressed in the intestinal epithelium (7, 8). The targets of β-catenin/TCF4 transcriptional activity include development-related genes activated through the Wingless/Wnt signaling pathway (9). More recently, a number of genes relevant for colorectal tumor formation and progression have been identified as being transcriptionally activated by the β-catenin/TCF4 complex. Some are relevant for growth control and cell cycling (c-Myc, cyclin D1, c-Jun, fra-1, gastrin, ITF2), some are implicated in cell survival (Ikd2, and MDR1), and some are implicated in tumor invasion and metastasis (matrilysin, VEGF) (10–19).

SMC3 (formerly called Bamacan, Cspg6, HCAP, SmcD, or Mmp1) is a member of the multimeric cohesin complex that plays a key role in establishing chromatid cohesion and is also involved in chromosomal DNA repair (20–22). Cohesins associate with the AT-rich sequences of chromosomal DNA near the centromeres and also along the arms with a periodicity of 9 to 15 kb. At the onset of anaphase, the complex is cleaved by the cysteine protease separin allowing chromosomal segregation (23). Interference with this process generates chromosomal instability (24). A post-translationally modified form of SMC3 carries chondroitin sulfate chains and is secreted as a proteoglycan known as bamacan (25). We have reported previously (26) that SMC3 mRNA level is elevated in about 70% of tumors from patients with colon carcinoma and that Smc3 protein is specifically increased in the intestinal polyps of ApcMin/+ mice in which loss of heterozygosity of the Apc gene causes a large increase of intracellular β-catenin (27). In addition, NIH-3T3 and BALB/c 3T3 murine fibroblasts stably transfected with Smc3 display a transformed phenotype, suggesting that this protein is involved in tumorigenesis (26). These findings have led us to hypothesize that SMC3 overexpression in intestinal carcinoma is linked to β-catenin/TCF4 transcriptional activation and that its subsequent overexpression is a relevant pathogenetic event.

In this paper we provide evidence in support of this hypothesis. Both the human and the mouse SMC3 promoters contain...
several conserved β-catenin/TCF4 binding consensus sequences that are responsible for the transactivation of the gene. We found that SMC3 promoter transcriptional activity is increased by elevated β-catenin levels and suppressed by a lowered β-catenin level. Immunohistochemical analysis of β-catenin and SMC3 in serial sections of colon from patients with invasive adenocarcinoma and of the intestinal adenomas from ApcMin/− mice revealed that two antigens co-localize with maximal stain intensity at the tumoral sites. The results are consistent with the idea that SMC3 is a target for the β-catenin/TCF4 transactivation pathway.

EXPERIMENTAL PROCEDURES

Materials—Goat anti-human SMC3 and goat anti-human SMCG1 antibodies were purchased from Santa Cruz Biotechnologies (Santa Cruz, CA). Anti-human β-catenin and anti-β-integrin monoclonal antibodies were obtained from BD Biosciences. Immunohistochemistry kits were from Dako (Carpenteria, CA) and Vector Laboratories (Burlingame, CA). Tri-Reagent, horseradish peroxidase-conjugated anti-rabbit and anti-mouse IgG antibodies, and anti-α-tubulin monoclonal antibody were purchased from Sigma. The West-Dura Supersignal ECL detection system, the NE-PER nuclear-cytoplasmic extraction kit, and the bichinonic acid-based protein assay kit were from Pierce (Rockford, IL). Porcine testicular and bovine testicular cDNA were purchased from Mediatech (Herndon, VA). BioMax x-ray films were from Eastman Kodak Co. (Rochester, NY). [γ-32P]dATP and [α-32P]dCTP were from ICN Biochemical (Irvine, CA). The Agt11 HeLa genomic library was from Clontech (Palo Alto, CA). Turbo-Pfu polymerase was from Stratagene (La Jolla, CA). The DNA restriction and modifying enzymes, pGL3-Vector luciferase empty vector, pRL-SV40 Renilla luciferase reporter vector, the Dual luciferase assay kit, and the Tkt cell transfection agent were from Promega (Madison, WI). Quick-spin oligo columns and proteinase inhibitors were from Roche Molecular Biochemicals. The reverse-transcriptase reagents were from Qiagen (Valencia, CA). The PCR reagents were from Takara (Madison, WI). All the chemicals were of ACS or biochemical purity and were purchased from Sigma or Fisher.

Immunohistochemistry and in Situ Hybridization Analysis—Polyacrylamide and normal tissue from the intestines of seven 6-month-old ApcMin/− mice were dissected under a stereo microscope, fixed in 3.7% buffered formalin, and embedded in paraffin. Archival paraffin blocks of tumoral and matched normal colon tissue from 10 patients that had undergone surgery for colon carcinoma were obtained from the Surgical Pathology service of the Jefferson Hospital. For the immunohistochemistry, serial tumor and normal tissue sections of 5 μm were subjected to antigen retrieval by microwaving in 0.1 M citrate solution, pH 6.0, for 10 min, and the immunostaining was performed on a Biogenex Optimax Autostainer. Sections were then incubated for 1 h at room temperature with a 1:50 dilution of the primary β-catenin antibody (1:100 to 1:5000) or goat anti-human SMC3 antibody (1:50 to 1:150). SMC3 and β-catenin immunocomplexes were visualized in the clinical specimens using Vectastain ABC kits and a multilink horseradish peroxidase-conjugated secondary antibody. Mouse Smc3 was detected using a biotinylated rabbit anti-goat antibody, whereas β-catenin was detected using a DAKO ARK kit designed for the immunohistochemical staining with mouse primary antibodies of mouse tissue sections. Mouse and human stained specimens were examined under a light microscope. For the statistical analysis of the results we tested the hypothesis of coincidence of staining. Adenocarcinoma and normal specimens from the same subject were analyzed separately. We computed a 95% lower confidence bound (one-tailed test) and the coincidence of the staining was evaluated. We tested the hypothesis of coincidence of the transactivation of the same gene (statistical analysis we tested the hypothesis of coincidence of staining. Adenocarcinoma and normal specimens from the same subject were analyzed separately. We computed a 95% lower confidence bound (one-tailed test) and the coincidence of the staining was evaluated. We tested the hypothesis of coincidence of the transactivation of the same gene (statistical analysis).

β-Catenin/TCF4 Activity Assay—Cells cultivated in 12-well plates were seeded in all the experiments. The transfection mixture containing 100 ng of plasmid DNA and 0.5 μg of pRL-SV40 plasmid was added to correct for transfection efficiency and 1 μg/ml of the designed plasmids. Plasmids were mixed in medium 199, followed by the addition of 36 μg/ml DNA of TRκ-50 transfection agent according to the manufacturer’s directions. Mixtures were incubated for 15 min at room tempera-
ture prior to addition to the cultures. Three hundred µl of the transfection mix was added to each plate well. After 1 h of incubation at 37 °C in a humidified incubator, the cells were supplemented with 1 ml of growth medium. Twenty-four hours later the cell medium was discarded, and the cultures were washed with ice-cold phosphate-buffered saline. After cell solubilization in 200 µl of lysis buffer, a 20-µl aliquot was assayed using a dual-luciferase kit. Sample luminescence was read on a Zylux Sirius luminometer (Oak Ridge, TN). Firefly luciferase activity readings were corrected for transfection efficiency using the Renilla luciferase readings. All experiments were carried out with triplicate samples. The statistical differences between groups of data were analyzed by Student’s t test.

Western Immunoblotting and Semi-quantitative RT-PCR—For immunoblotting, cells were collected in 150 mM NaCl, 1% Nonidet P-40, 0.5% sodium deoxycholate, 50 mM Tris-HCl, pH 7.4, lysis buffer supplemented with 50 µg/ml antipain, 40 µg/ml bestatin, 5 µg/ml E-64, 0.5 µg/ml leupeptin, 0.7 µg/ml pepstatin, 1 µg/ml aproptin, 1 mM Na2EDTA. The cell lysate protein (50 µg) was electrophoresed on a 10% SDS-PAGE slab gel. Separated proteins were transferred to a nitrocellulose by electrophotrob, and the filters were blocked in 5% dry milk followed by incubation for 1 h with goat anti-human SMC3 (1:1000). After washing, the filters were incubated with an anti-goat IgG horse-radish peroxidase-conjugated (1:10,000) secondary antibody, and the immunocomplexes were identified using a West-Dura ECL kit followed by autoradiography. To confirm that equal amount of proteins had been loaded and to examine β-catenin levels, the immunocomplexes were stripped by incubating the membranes in 2% SDS, 100 mM 2-mercaptoethanol, 62.5 mM Tris-HCl, pH 6.7, at 56 °C for 30 min. After blocking in 5% dry milk, the filters were cut at the 90-kDa molecular mass standard mark, and the bottom section was incubated with anti-human α-tubulin monoclonal antibody (1:1000). The top portion of the filter was instead incubated with anti-human β-catenin monoclonal antibody (1:2000). After reaction with anti-mouse IgG horseradish peroxidase (1:10,000), the immunocomplexes were detected by ECL followed by autoradiography and densitometric scanning. SMC3 and SMC1 transcript levels were assessed by semi-quantitative RT-PCR. For this purpose, the transfected cells were extracted in Tri-Reagent, and 1 µg of RNA was reverse-transcribed using the Qiagen Senscript kit and oligo(T) primers. PCR cycling was stopped after completion of 20 and 30 cycles to ensure that products were quantitated during the non-saturating phase of the reaction. SMC3 and SMC1 cDNA levels were assessed by agarose electrophoresis. Gels were photographed, and the propidium iodine stained bands were quantitated by densitometric scanning.

Electrophoretic Mobility Shift Assay—The cell nuclear extracts were prepared using the NE-PER extraction kit. Before use, the nuclear extracts were dialyzed at 4 °C overnight against 20 mM HEPES, pH 7.9, 75 mM KCl, 0.1% Na2EDTA, and protein content was assayed. The cell nuclear extracts—

FIG. 1. Detection of β-catenin and SMC3 in colon carcinomas. β-Catenin and SMC3 distribution in human (A–J) and mouse (J and K) colonic adenomas were detected by immunohistochemistry. A–C, serial sections (×20) of formalin-fixed and paraffin-embedded tissue were immunostained with antibodies against β-catenin (A), SMC3 (B), or SMC3 antibodies plus competing SMC3 polypeptide (C). Immunoreactivity was visualized with a peroxidase-conjugated secondary antibody and using 3,3′-diaminobenzidine as substrate. D, F, and H, β-catenin immunostained sections from the adenomatous specimens from another patient. D and F illustrate two different areas of the same specimen (×80). The inset in F is shown enlarged in H (×500). E, G, and I, tissue sections immunostained with SMC3 and corresponding to those shown in D–H. Note the intense immunostaining for both β-catenin and SMC3 in the transformed areas associated to the intracellular accumulation of the antigen. J and K, consecutive sections (×40) of a polyp from Apc–/+ mouse immunostained with β-catenin (J) or SMC3 (K) as described for the human specimens.

cell interface. On the contrary, adenoma and adenocarcinoma cells had diffuse β-catenin staining suggestive of an increase of the cytoplasmic antigen level. Adenomatous areas that displayed intense staining for β-catenin also had enhanced anti-SMC3 antibody reactivity. Neither β-catenin nor SMC3 were found at high levels in the surrounding normal tissue. This finding was invariably observed in all the specimens examined. As for β-catenin, the strongest staining for SMC3 was detected intracellularly. Incubation of the tissue sections with a blocking peptide of sequence corresponding to the epitope recognized by the SMC3 antibody effectively neutralized the tissue immunoreactivity. Furthermore, no immunoreactivity was observed by incubation with the secondary antibody alone. With all the specimens displaying an identical SMC3 and β-catenin distri-
bution pattern, the positivity rate, i.e. the proportion of samples displaying identical β-catenin and SMC3 staining pattern, was 100% (10/10) with a 95% confidence bound of 74.1%. These results are in line with our previous results (26) showing a statistically significant higher expression of SMC3 in the tumoral colon specimens compared with matched normal tissues. Mouse intestinal sections displayed the same pattern of staining as the human tissues. In particular, the greater β-catenin staining intensity in the transformed areas was matched by that of Smc3 (Fig. 1, J and K). Polyps from the intestine of seven mice were examined. Given the coincidence of SMC3 and β-catenin staining in the mouse samples examined, the positivity rate had a 95% confidence bound of 65.3%.

Structure of the Human SMC3 Promoter—The transcription start site was determined by primer extension (Fig. 2A) and by comparison of the published sequences of dEST cDNAs. Based on our calculations, the site would be located 76 bp upstream of the translational start site. The size of the human gene 5′-untranslated region is therefore similar to that identified previously for the mouse Smc3 gene and calculated to be 95 bp in size. The mouse and human promoter sequences were examined for the presence of conserved putative transcriptional binding sites utilizing the TFSEARCH data base of published recognition sequences. As in the mureine gene, a TATA box was also absent in the human sequence (see Fig. 2B). Both promoters, however, had a conserved region of sequence 5′-GCGCCG-3′ beginning at −56 in the human gene and −55 in the murine gene. In addition to this highly conserved sequence, a series of putative transcriptional factor binding sites were identified whose location was conserved in the initial 40 bp flanking the transcriptional start site. Recognition sites for the ubiquitously expressed Sp1 transcriptional factor are situated in the −20- to −250-bp region. Binding sites for c-Myc and E2F are found within the −270- to −380-bp range. Finally, a conserved binding site for the tumor suppressor Cdx is located in the −670-bp region and is flanked by a CREB-P binding sequence in the −700-bp neighborhood. This region also harbors one of the two conserved β-catenin/TCF4 binding sites located at position −701 bp in the human promoter and at position −763 bp in the mouse sequence. The human binding site 5′-CTTTTGTG-3′ is a perfect consensus sequence (30), whereas the mouse binding site (5′-ATCAAG-3′) had sequence complementary to that identified in the human Id2 promoter (10). Both the human and the mouse promoters contained an imperfect TCF4 binding sequence within the highly conserved promoter initiation site (5′-TCTTTTGTG-3′) diverging one nucleotide from the canonical T cell factor consensus as indicated by the nucleotide in lowercase. In addition to the consensus sequence starting at −701, the human promoter harbored a second canonical β-catenin/TCF4 binding site at −504 bp (5′-AACAAGG-3′), which had no match within the mouse promoter. Finally, both the human and mouse promoters harbored four additional putative β-catenin/TCF4 binding sequences located upstream to the conserved distal β-catenin/TCF4 binding site.

Functional Characterization of the SMC3 Promoter—To assess the potential role of β-catenin in the regulation of the human SMC3 promoter, a series of terminal and nested deletions of the promoter were cloned into a Firefly luciferase reporter vector (pGL3), and their activity was assayed in cells following transient transfection. We first determined whether the full-length promoter was affected by intracellular β-catenin and/or TCF4 levels. For these experiments we selected the human colon carcinoma HCT116 cell line. HCT116 cells have a low β-catenin/TCF4 pool (11), and alteration of its level should result in changes of gene transcription. We transfected these cells with a luciferase reporter vector (Fig. 3A) harboring the full-length SMC3 promoter (SMC3 (−1578/+56 bp)), together with an increasing concentration of β-catenin expression vector. A dose-dependent increase in gene transactivation could be evidenced with a 10-fold maximal activation (Fig. 3B). Higher concentration of β-catenin negatively affected cell survival at 24 h post-transfection (data not shown) consistent with the report that overexpression of β-catenin may initiate apoptosis (31). Co-transfection of the promoter construct with the TCF4 expression vector enhanced the promoter activity and potentiated the effect of β-catenin (Fig. 3C). On the contrary, transfection with murine E-cadherin negatively affected the basal promoter activity (Fig. 3D) in line with the idea that this maneuver lowers the available endogenous β-catenin pool by sequestering the protein at the cell membrane (32). We next examined whether the conserved 5′-TCTTTTGTG-3′-site proximal to the transcriptional start site was functionally active. The β-catenin/TCF4 responsive elements of the matrix metalloproteinase Matrilysin (14), Id2 (10), cyclin D1 (11, 19), and gastrin (17) genes have been mapped in close proximity to the transcriptional start site. The putative binding sequence identified diverges by one base from the canonical β-catenin/TCF4 binding site, but it has been found previously (11) to be relevant for the transactivation of the cyclin D1 promoter. The sequence is located within a perfectly conserved short region in the human and murine promoters. A 263-bp promoter segment (−207/+56 bp) harboring this sequence (Fig. 4A) was tested in the luciferase reporter assay in HCT116 and SW480 cell lines. The human carcinoma SW480 cells were selected, because this cell line has a high constitutive level of the β-catenin/TCF4 transactivation complex (11). As observed for the full promoter, β-catenin greatly enhanced luciferase activity in HCT116 cells, and the effect rapidly reached plateau (Fig. 4B). The activity of the truncated promoter was 5-fold greater in SW480 cells as it would be expected if a β-catenin/TCF4 responsive element were present within its sequence. In these cells, however, co-transfection with β-catenin reduced the luciferase activity by about 55%. As we also observed in HCT116 cells, exceedingly high levels of β-catenin appear to have an inhibitory effect on the transcription of SMC3. To confirm the functional significance of this conserved β-catenin/TCF4 transactivation site, a two-base substitution mutation was introduced within the putative binding sequence. The new construct, MT-pGL3-SMC3(−207/+56), displayed the same basal luciferase activity in HCT116 and in SW480 cells (Fig. 4C). In addition, contrary to that observed with the construct harboring the intact promoter sequence, co-transfection with β-catenin did not result in enhanced luciferase activity in HCT116 cells. The luciferase reporter construct SMC3(−438/+56) was tested, because the proximal 450 bp of the human promoter contains binding sites for transcriptional factors that are conserved in the murine promoter and that are likely to be relevant for the constitutive SMC3 promoter activity. Basal activity of the reporter was 4-fold higher (Fig. 4D) compared with that of SMC3(−207/+56) in HCT116 cells consistent with idea that the additional 231-bp promoter sequence harboring the recognition sequences for the ubiquitous Sp1 transcriptional factor and for c-Myc and E2F are likely responsible for the constitutive transcription of the SMC3 gene. β-Catenin further enhanced in a dose-dependent fashion the activity of the truncated promoter, corroborating the idea of a major role of the β-catenin/TCF4 transactivation pathway in the regulation of SMC3 expression. Accordingly, when tested in SW480 cells, the construct displayed higher activity than in HCT116 cells, and co-transfection with β-cate-
FIG. 2. Structural and functional analysis of the SMC3 promoter. A, 5’-extension experiments. Total RNA was extracted from subconfluent human skin fibroblasts and HCT116 and HeLa cells. 5’-Extension of labeled primers was initiated by annealing at 55 °C for 30 min with 32P-labeled 21-bp primer complementary to the cDNA sequence 5’ to the translational start codon of SMC3 gene based on the information from the dEST data base. Reverse transcription of SMC3 RNA was initiated by the addition of Superscript reverse-transcriptase and was allowed to proceed for 30 min at 40 °C. The samples were subjected to electrophoresis on a 6% polyacrylamide sequencing gel. After drying, the gel was subjected to autoradiography. [32P]-Labeled standards were used to establish the size of the primer extension products.

B, sequence of the human SMC3 gene promoter. The putative β-catenin/TCF4 recognition sequences discussed in the text are underlined. Numbering is based on the position of the transcriptional start site. C, comparative analysis of the human and the murine SMC3 gene promoters was performed with the TFSEARCH program (150.82.196.184/research/db/TFSEARCH.html). The mapping of the conserved transcriptional binding sites is shown. MZF1 transcriptional factor belongs to the Kruppel family of zinc finger proteins. Cdx is a homeobox-containing gene product that is involved in axial patterning in development, and loss of one allele causes intestinal tumors. The position of the binding sites for the β-catenin/TCF4 transactivation complex is indicated with arrowheads. Only the transcriptional binding sites present in both the human and the mouse promoter are shown.
E-cadherin expression vector without (B) or together with (C) human TCF4 expression vector (1 μg/ml) were also co-transfected. The effect of E-cadherin (1 μg/ml) in cells transfected with 2.5 μg/ml β-catenin is illustrated in D. Luciferase activity was assayed as described under "Experimental Procedures" and corrected for the transfection efficiency based on the Renilla luciferase activity. Values were normalized to the luciferase activity of cells transfected with the reporter vector alone. The bars represent the mean ± S.D. of samples run in triplicate. Transfected cells expressed luciferase activity at a significant different level (p < 0.05) than did untransfected cells.

The identity of the conserved β-catenin/TCF4 sequences was confirmed by performing electrophoresis mobility shift and supershift analysis. Double-stranded 19–29-bp oligomers containing the putative core sequences and their flanking sequences identified within the human SMC3 promoter were used. The complex formation could be prevented by adding an excess (50-fold) of unlabeled probe. The addition of β-catenin monoclonal antibody but not of an irrelevant monoclonal antibody (anti-β-integrin) resulted in a specific supershift of the band confirming that β-catenin was part of the complex. Identical results were obtained when the labeled 706/687 oligo-
DISCUSSION

SMC3 is a component of the cohesin multisubunit complex that holds together sister chromatids during mitosis and enables their segregation to the opposite poles of the cell prior to cytokinesis (22, 33, 34). In the multimeric complex, SMC3 combines with another SMC cohesin, SMC1, and two non-SMC proteins, named Scc1 and Scc3, after their discovery in budding yeast *Saccharomyces cerevisiae* (20). SMC proteins share a unique structural motif with globular N- and C-terminal ATPase-like domains separated by a long coiled-coil segment in the center of which is a globular hinge domain (35). Haering et al. (36) have recently proposed a molecular model whereby Scc1 links two heads of a single SMC1/SMC3 heterodimer. This proteinaceous ring holds together sister chromatids until Scc1 is cleaved by the cysteine protease separin. The function of the latter is modulated by securin (37). There is evidence in mammalian cells that interference with the securin/separin/Scc1 system causes chromosomal instability. For example, the homozygous deletion in HCT116 colon carcinoma cells of the *hSecurin* gene leads to retardation of sister chromatid separation and a high rate of chromosomal loss because of defective Scc1 cleavage (24). On the other hand, overexpression of the murine orthologue of Sec1 (PW29 protein) in mouse fibroblasts leads to inhibition of proliferation, implicating this protein and its complex with SMC proteins in the control of mitotic cell cycle progression (21). We have shown previously (26) that overexpression of Smc3 in 3T3 fibroblasts causes cell transformation and enhances cell proliferation. Furthermore, an increased expression of SMC3 in human colon carcinomas and in the tumoral tissue from the intestine of *ApcMin/+* mice was observed. Because in *ApcMin/+* mice loss of heterozygosity amplifies β-catenin activity, we considered the possibility that Smc3 and β-catenin overexpression be linked. By functionally analyzing the organization of the human gene promoter, we have now identified the β-catenin/TCF4 pathway as a main transcriptional regulator of SMC3. In support of this conclusion, we have also found that in colon carcinoma cells the SMC3 transcript and protein levels correlate positively with the intracellular β-catenin concentration. This finding is mirrored in colon adenocarcinomas by the increased expression of SMC3 at the sites where β-catenin is overexpressed. Two conserved transcriptional binding sites for β-catenin/TCF4 are present in the human and mouse SMC3 promoters. The first site is located proximal to the transcriptional start site. The promoters of the human *cyclin D1, Id2, matrilysin, MDR1,* and *gastrin* genes also harbor an active TCF4 transcriptional binding site.

![Diagram](image_url)
Cdx-4. Cdx2-null embryos die before gastrulation, but interestingly, the heterozygous animals develop intestinal tumors (39). Furthermore CDX2 has been found mutated in a colorectal cancer cell line with normal APC/β-catenin signaling, suggesting that the intact protein is necessary for normal cell behavior (40). A recognition sequence for the oncogene c-MYC is located apart from the binding sequence for the E2F transcriptional factor. c-MYC and E2F transcription factors share a number of functional properties including the ability to induce quiescent cells to enter the cell cycle and progress into S phase and to control cell fate by activating the p53-dependent apoptotic pathway (41). c-MYC is also a downstream target for the β-catenin/TCF4 transactivation pathway, raising the possibility of an amplifying effect of c-MYC and β-catenin on SMC3 expression in colon carcinoma. The presence in the promoter of a conserved CREB protein binding site may be of particular significance. As in the cyclin D1 promoter (11), in the human SMC3 promoter this site is located in close proximity (less than 20 bp) to a conserved β-catenin/TCF4 locus. The CREB-binding protein has intrinsic acetyltransferase activity and acetylates TCF4 and β-catenin (42–44). Acetylation of β-catenin decreases its transcriptional activity (44) whereas mutation at the acetylation site increases its transcriptional activity (45). 

Creb binding site also present in the promoter plays a minor role (45). 

Furthermore, CREB binding site may act as the docking site. Interestingly, the acetylation site increases its transcriptional activity (44) whereas mutation at the acetylation site increases its transcriptional activity (45). 

**FIG. 5. Effect of ectopic β-catenin overexpression on the SMC3 transcript and protein levels.** A, cells were co-transfected with the indicated concentrations of β-catenin expression vector, together with human TCF4 expression vector (1 μg/ml). After 24 h total RNA was extracted in Tri-Reagent. One μg of RNA was reverse-transcribed using oligo(dT) primers, and the generated cDNA was amplified by PCR using SMC3- and SMC1-specific primers. The product generated after 20 reaction cycles was analyzed on a 0.7% agarose gel by electrophoresis. B, after transfection with the β-catenin expression vector, the cells were lysed in buffer containing 0.1% Tween 20 and a mixture of anti-protease inhibitors. Fifty μg of proteins were electrophoresed on a 10% SDS-PAGE slab gel and then transferred to nitrocellulose membrane and immunoreacted with a goat anti-human SMC3 antibody. The top portion of the filter was probed for -catenin antibody. The results shown are representative of the three experiments performed. 

The comparative analysis of the mouse and the human promoters has enabled us to identify several conserved features that are therefore likely to be relevant for the regulation of the gene and indicative of the functional role of SMC3. In addition to the conserved β-catenin/TCF4 binding sites, a number of putative binding sites for tumor suppressors and oncoproteins were also identified, emphasizing the concept that the SMC3 gene is targeted in tumorigenesis. A binding site for MZF1 maps to the −38/−31 bp region in both the human and the mouse promoter. MZF1 is a transcription factor belonging to the Kruppel family of zinc finger proteins. Mzf1(−/−) knockout mice develop lethal neoplasia characterized by the infiltration and complete disruption of the liver architecture by a monomorphic population of myeloid cells (38). A putative recognition sequence for a second tumor suppressor, CDX, maps to the same distal region as one of the conserved β-catenin/TCF4 binding sites. CDX is a homeobox-containing gene. The mouse expresses three related genes named CdX-1, CdX-2/3, and CdX-4. CDX2 has been found mutated in a colorectal cancer cell line with normal APC/β-catenin signaling, suggesting that the intact protein is necessary for normal cell behavior (40). A recognition sequence for the oncogene c-MYC is located apart from the binding sequence for the E2F transcriptional factor. c-MYC and E2F transcription factors share a number of functional properties including the ability to induce quiescent cells to enter the cell cycle and progress into S phase and to control cell fate by activating the p53-dependent apoptotic pathway (41). c-MYC is also a downstream target for the β-catenin/TCF4 transactivation pathway, raising the possibility of an amplifying effect of c-MYC and β-catenin on SMC3 expression in colon carcinoma. The presence in the promoter of a conserved CREB protein binding site may be of particular significance. As in the cyclin D1 promoter (11), in the human SMC3 promoter this site is located in close proximity (less than 20 bp) to a conserved β-catenin/TCF4 locus. The CREB-binding protein has intrinsic acetyltransferase activity and acetylates TCF4 and β-catenin (42–44). Acetylation of β-catenin decreases its transcriptional activity (44) whereas mutation at the acetylation site increases β-catenin ability to specifically activate the c-myc promoter. It has been hypothesized (42) that the CREB-binding protein might participate in the recruitment of the basal transcriptional machinery, and the region of the c-myc and SMC3 promoters harboring the β-catenin/TCF4 and CREB binding sites may act as the docking site. Interestingly, the Wnt-1- and β-catenin-mediated transactivation of the WISP-1 growth factor (Wnt-1 induced secreted protein 1) gene specifically involves a CREB binding site whereas a T cell factor binding site also present in the promoter plays a minor role (45).
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The Cohesin SMC3 Is a Target for β-Catenin/TCF4 Transactivation Pathway
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