Wnt/β-catenin signaling regulates many processes during vertebrate development. To study transcriptional targets of canonical Wnt signaling, we used the conditional Cre/loxP system in mouse to ectopically activate β-catenin during central nervous system development. We show that the activation of Wnt/β-catenin signaling in the embryonic mouse telencephalon results in the up-regulation of Sp5 gene, which encodes a member of the Sp1 transcription factor family. A proximal promoter of Sp5 gene is highly evolutionarily conserved and contains five TCF/LEF binding sites that mediate direct regulation of Sp5 expression by canonical Wnt signaling. We provide evidence that Sp5 works as a transcriptional repressor and has three independent repressor domains, called R1, R2, and R3, respectively. Furthermore, we show that the repression activity of R1 domain is mediated through direct interaction with a transcriptional corepressor mSin3a. Finally, our data strongly suggest that Sp5 has the same DNA binding specificity as Sp1 and represses Sp1 target genes such as p21. We conclude that Sp5 transcription factor mediates the downstream responses to Wnt/β-catenin signaling by directly repressing Sp1 target genes.

Wnt/β-catenin signaling plays important roles in multiple developmental processes and has a profound effect on cell proliferation, cell polarity, and cell fate determination (1). Wnt molecules are secreted glycoproteins that work as signaling molecules. Wnt molecules bind with Frizzled receptors and low density lipoprotein receptor-related protein coreceptors at the cell surface to initiate the signaling. In the absence of Wnt/β-catenin signaling, the level of cytoplasmic β-catenin, the key mediator of Wnt/β-catenin signaling, is kept low. β-Catenin is recruited to a destruction complex containing the tumor suppressors adenomatous polyposis coli, axin, casein kinase 1, and glycogen synthase kinase 3β, respectively, and is constitutively phosphorylated. The phosphorylated β-catenin protein is degraded by the ubiquitin pathway. Members of the TCF/LEF transcription factor family bind corepressor Groucho and repress Wnt target genes in the nucleus. The binding of Wnt molecules to the receptors and the coreceptors results in the inactivation of the kinase activity of the destruction complex. As a consequence, β-catenin protein is not phosphorylated, begins to accumulate in the cytoplasm, and is then translocated to the nucleus where it binds to TCF/LEF transcription factors. The binding converts TCF/LEF into an activator that initiates the transcription of Wnt target genes, including c-myc, Axin2, and Lef1 (2–4). During central nervous system (CNS) development, multiple Wnt genes are expressed, including Wnt3a, Wnt7a, Wnt7b, and Wnt8b (5). Transgenic mice, which express a stabilized form of β-catenin in neural progenitor cells, develop enlarged brains (6). In Wnt3a mutant mice as well as in Lef1 mutant mice, the hippocampus is missing (5, 7). These reports indicate critical roles of canonical Wnt signaling in CNS development.

To study targets of Wnt/β-catenin signaling, we used the conditional Cre/loxP system in mice to ectopically activate Wnt/β-catenin signaling during CNS development. Activation of Wnt/β-catenin signaling is achieved by a deletion of exon 3 of the β-catenin gene that encodes phosphorylation sites necessary for β-catenin degradation (8). To activate canonical Wnt signaling during CNS development, Nes1ICre mice were crossed to Catnblox(ex3) mice. Mutant animals Nes1ICre/Catnblox(ex3) display hyperplasia in the telencephalon that resembles the phenotype of the mouse mutants in which activated β-catenin is directly coupled to the nestin enhancer (6). We show that the constitutive activation of Wnt/β-catenin signaling results in the up-regulation of the Sp5 gene in the mouse telencephalon. The Sp5 gene encodes a member of Sp1 transcription factor family (9). The proximal promoter of the Sp5 gene is highly evolutionarily conserved and has five TCF/LEF binding sites that mediate direct regulation of Sp5 expression by Wnt/β-catenin signaling. Sp5 appears to work as a transcriptional repressor at least in part by directly interacting with a corepressor mSin3a. We show that Sp5 has the same DNA binding specificity as Sp1 and represses Sp1 target genes such as p21. In conclusion, our report suggests that the Sp5 transcription factor mediates the downstream responses to Wnt/β-catenin signaling by directly repressing Sp1 target genes.
**EXPERIMENTAL PROCEDURES**

**Mouse Lines**—Analysis of Cre-mediated recombination pattern in *Nes11-Cre* (10) was performed by mating to the ROSA26R reporter line as described previously (11). The ROSA26R mice (stock #003309) and *Nes11-Cre* mice (stock #003771) were purchased from Jackson Laboratory. D6Cre transgenic mice express Cre recombinase under the control of *Dach1* enhancer, which is active in the telencephalon (12). Mice with a conditional “floxed” allele of β-catenin, *Catnblox(ex3)*, were kindly provided by Dr. M. M. Taketo (8).

**Plasmids**—The mouse *Sp5* promoter and truncated promoters were amplified by PCR using *C57BL/6* mouse genomic DNA (kindly provided by J. Forejt) as a template. PCR products were cloned to pCR4-TOPO (Invitrogen) and sequenced. The resulting plasmids were digested with EcoRI (New England Biolabs), blunted with T4 DNA polymerase (New England Biolabs), and cloned into Smal-digested pGL3 basic (Promega) vector. Mouse *Sp5* enhancers were amplified by PCR using primers with XbaI recognition sites. PCR products were digested by XbaI and cloned into a NheI site upstream of the minimal TK promoter cloned in the pGL3 vector. For Gal4-Sp5, the full-length mouse *Sp5* cDNA was excised from *pBS-KX-Sp5* (kindly provided by D. Houzelstein) and cloned into a Gal4 expression plasmid. To generate Gal4 fusion constructs with individual domains of *Sp5*, the corresponding regions of mouse *Sp5* cDNA were amplified by PCR and cloned into the Gal4 expression plasmid. To generate 6×His-Sp5, the coding sequence of *Sp5* was cloned into the procaryotic expression vector pETHis2a. For Sp5-FLAG, the *Sp5* cDNA was amplified by PCR and cloned into pKw-FLAG in-frame with the FLAG coding sequence located at the N terminus. For retroviral infection of neurosphere cultures, *Sp5* cDNA was inserted into *pNIT* retroviral vector (provided by F. Gage). To generate GST fusions with a *Sp5* R1 domain, the corresponding region was amplified by PCR and cloned into the GST expression plasmid. To generate 6×His-GST-Sp5, the coding sequence of *Sp5* was cloned into the procaryotic expression vector pETHis2a. For Sp5-FLAG, the *Sp5* cDNA was amplified by PCR and cloned into pKw-FLAG in-frame with the FLAG coding sequence located at the N terminus. For retroviral infection of neurosphere cultures, *Sp5* cDNA was inserted into *pNIT* retroviral vector (provided by F. Gage). To generate GST fusions with a *Sp5* R1 domain, the corresponding region was amplified by PCR and cloned into the *pET42a* (+) (Novagen). For GST-Sp5R1A3P and Gal4-Sp5A3P, the R1 region was amplified by PCR using primers that contained the corresponding point mutation and cloned into *pET42a* (+) or Gal4 expression plasmid. All constructs were verified by sequencing. A luciferase reporter plasmid containing the p21 promoter (p21-Luc) was kindly provided by E. Sancho. For p21GC-Luc, the p21-Luc plasmid was digested with *PstI* and *BglII* (−198/+12), blunted, and cloned into pGL3 basic. For p21A6GC-Luc, a p21 promoter fragment (−2326/+197) was cut with *PstI/*HindIII and fused to the minimal p21 promoter (−30/+12) located in pGL3 basic.

**Microarray Experiment**—RNA was isolated from the dissected telencephalon of E13.5 mouse embryos (*Nes11-Cre/Catnblox(ex3)*, or *Catnblox(ex3)*) using an Ambion kit and subjected to hybridization on Affymetrix MOE 430A GeneChip. Neurospheres were cultured in neurobasal-A medium with B27 supplement (both Invitrogen) and with epidermal growth factor (20 ng/ml) and basic fibroblast growth factor (8 ng/ml, both R&D Systems). Cells were passaged every 3 days. The *Sp5* retrovirus was produced in a Phoenix packaging cell line (provided by G. Nollan) by transient transfection of pNIT-*Sp5*, and neurosphere cells were infected as described in a previous study (13). Three days after infection, selection with G418 antibiotics was started (250 μg/ml), and pools of cell clones were maintained in the selection media. RNA was isolated from three separate plates of *Sp5* virus or mock infected neurospheres using an Ambion kit and used for hybridization on Affymetrix MOE 430A. Microarray data were analyzed by Affymetrix Suite 5.1 software.

**Cell Culture, Transient Transfection, and Luciferase Reporter Assay**—293T cells were cultured in Dulbecco’s modified Eagle’s medium (Sigma) supplemented with 10% fetal bovine serum (PAA Laboratories), 2 mM L-glutamine, 100 units/ml penicillin, and 0.1 mg/ml streptomycin (Sigma). Neurospheres were cultured in neurobasal-A medium with B27 supplement (both Invitrogen), epidermal growth factor (20 ng/ml), and basic fibroblast growth factor (8 ng/ml, both R&D Systems). Cells were passaged every 3 days and maintained at 37 °C in an atmosphere of humidified air with 5% CO₂. Transient transfection of 293T cells was performed using FuGENE 6 (Roche Applied Science) according to the manufacturer’s protocol. Cells were plated in 24-well plates 24 h prior to transfection. Typically, the total amount of DNA transfected per well was 300 ng and was adjusted with pUC18 when necessary. A β-galactosidase expression plasmid was cotransfected to normalize the transfection efficiency. Triplicate assays were performed to obtain standard deviations. Two days after transfection, the cells were lysed in 100 μl of 1× passive lysis buffer (Promega, Madison, WI). Luciferase reporter assays were performed using Luciferase Reporter assay kit (Promega). β-Galactosidase was detected with Galacto-Star system (Applied Biosystems, Foster City, CA).

**Chromatin Immunoprecipitation Assay**—A chromatin immunoprecipitation assay was performed according to the manufacturer’s protocol (Upstate Biotech) with modifications. The cortical parts of *D6Cre/Catnblox(ex3)* brains were harvested at E18.5, homogenized in 1% formaldehyde in phosphate-buffered saline and cross-linked at 37 °C for 15 min. Cross-linking was stopped by adding glycine (0.125 mM) and incubating at room temperature for 5 min. Cross-linked cells were washed twice with cold phosphate-buffered saline containing fresh protease inhibitors, pelleted, and resuspended in 2 ml of SDS lysis buffer (1% SDS, 10 mM EDTA, 50 mM Tris-HCl, pH 8.0) with protease inhibitors. Samples were incubated on ice for 10 min, and lysates were sonicated on an ice water bath to produce 150–500 bp of DNA fragments. Cell debris was removed by centrifugation for 10 min at 14,000 rpm at 4 °C, and the supernatant was diluted ten times with dilution buffer (0.01% SDS, 1.1% Triton X-100, 1.2 mM EDTA, 16.7 mM Tris-HCl, pH 8.0, 167 mM NaCl) containing protease inhibitors. 30 μg of sonicated chromatin was precleared with 50 μl of protein A(G)/agarose slurry (Upstate Biotech) for 1 h at 4 °C. Beads were pelleted by centrifugation for 5 min at 3,000 rpm at 4 °C. The supernatant was incubated either with 5 μg of antibody or with no antibody (no antibody control) overnight at 4 °C. The following antibodies were used: anti-β-catenin (E-5, sc-7963, Santa Cruz Biotechnology), anti-Lef1 (N-17, sc-8591, Santa Cruz Biotechnology), and anti-Tcf4 (a gift from V. Korinek). 30 μl of protein A(G)/agarose slurry (Upstate Biotech) was added, and samples were rocked at 4 °C for 1 h. After washing for 5 min at 4 °C twice in low salt buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM...
Tris-HCl, pH 8.0, 150 mM NaCl), twice in high salt buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl, pH 8.0, 500 mM NaCl), four times in LiCl buffer (0.25 M LiCl, 1% Nonidet P-40, 1% deoxycholate, 1 mM EDTA, 10 mM Tris-HCl, pH 8.0), and twice in TE buffer (10 mM Tris- HCl, 1 mM EDTA, pH 8.0), immunocomplexes were eluted twice with 100 µl of elution buffer (0.1 M NaHCO3, 1% SDS) for 15 min at room temperature. Immunoprecipitated DNA was de-cross-linked overnight at 65 °C in the presence of proteinase K (0.06 unit/µl, Roche Applied Science) and 250 mM NaCl. Samples were purified using a MinElute reaction cleanup kit (Qiagen), and 1/20th of eluate was used for PCR. PCR was performed as follows: 95 °C 2 min for 1 cycle; then 95 °C 30 s, 60 °C 30 s, and 72 °C 30 s for 40 cycles; and finally 72 °C 5 min. The primers used were as follows: Sp5D-H_F, CTA-GAGATAACAAAGAGACTT; Sp5D-H_R, AGTCGAGAGAGATTTATG; Sp5-2kb_F, TGGCTGCTTAATTGGC- TAAAGAG; and Sp5-2kb_R, CAGGGGTTCAGTGTCGTGGGA; Sp5 + 6kb_F, AACGGGAAGCTGAGTGTAAATTAG; and Sp5 + 6kb_R, GTAAGCTAGACAGCCTAAAC.

Electrophoretic Mobility Shift Assay—The following double-stranded oligonucleotides derived from the Sp5 promoter were used in EMSA (only the top strand is shown for simplicity): Sp5A, ATTTAGAAGACAACTTGATCTC; Sp5C, GGAATCTTTTGGATCACTT; Sp5D, TTTGTTGATTAAGGGTGTTG; and Sp5, CGCTTTTCTTTGATTTGCTG. The following double-stranded oligonucleotides derived from the p21 promoter were used: I, ATTCGATCGGGGCGGGGCGAGC; II, ATTCGATCGGTCGGGGCGAGC; III, ATTCGATCGGGGCGGGGCGAGC; IV, GAATTCCGAGCGCGGGTCCCGCCTCCGAATTC; and V, GTGCGGAGGCGTGGCCAACGCATG. The following double-stranded oligonucleotides representing consensus (wt) and multiple versions of the Sp1 binding site were used: WT, ATTCGATCGGGGCGGGGCGAGC; M1, ATTCGATCGGTCGGGGCGAGC; M2, ATTCGATCGGTCGGGGCGAGC; M3, ATTCGATCGGGGCGGGGCGAGC; M4, ATTCGATCGGTCGGGGCGAGC; M5, ATTCGATCGGGGCGGGGCGAGC; M6, ATTCGATCGGGGCGGGGCGAGC; SA1, GTGCCGAGGGCTT- GGGTGGA; AX2, CGGGCGGCGGGGGAGGCGGGGTC; AX3, CGGGCGGCGGGGGAGGCGGGGTC; and BTE, CGGGCGGCGGGGGAGGCGGGGTC.

Immunoprecipitation and Western Blotting—293T cells were plated in 10-cm dishes 24 h prior to transfection. Myc-mSin3a plasmid (2 µg, kindly provided by C. Laherty) was cotransfected with FLAG-Sp5 expression plasmid (3 µg) or empty FLAG expression plasmid (3 µg) into 293T cells. Two days after transfection, 293T cells were washed with phosphate-buffered saline and lysed in lysis buffer (50 mM Tris HCl, pH 7.4, with 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, and 0.1 mM phenylmethysulfonyl fluoride) for 30 min on ice. Cell debris was pelleted by centrifugation at 12,000 × g for 10 min. An aliquot of this whole cell lysate was boiled with 2× SDS sample buffer for 5 min. For immunoprecipitation, 500 µl of the whole cell lysate was incubated with 40 µl of anti-FLAG M2 affinity beads (Sigma) overnight at 4 °C. The beads were washed with 1× Wash buffer (50 mM Tris HCl, pH 7.4, with 150 mM NaCl, 1 mM EDTA) five times and boiled with 2× SDS sample buffer for 5 min.

Samples were separated by 8% or 12% SDS-PAGE and transferred to nitrocellulose membranes for Western blotting. Myc-tagged mSin3a was detected by anti-Myc antibody (Roche Applied Science), and FLAG-tagged Sp5 was detected by anti-FLAG M2 (Sigma). Detection was performed using polyclonal rabbit anti-mouse immunoglobulins/horseradish peroxidase (DakoCytomation) and SuperSignal West Pico Chemiluminescent Substrate (Pierce).

GST pull-down Assay—Myc-tagged mSin3a was prepared by Tnt Quick Coupled Transcription/Translation Systems according to the manufacturer’s protocol (Promega). GST fusion expression plasmids were transformed into BL21 CodonPlus (DE3)-RIPL cells (Stratagene). A single colony from the transformation was cultured in 2 ml of LB medium containing 50 µg/ml chloramphenicol and 30 ng/ml kanamycin overnight at 37 °C. The cultures were transferred to 100 ml of LB without antibiotics. The expression of the fusion construct was induced by adding isopropyl-1-thio-β-D-galactopyranoside to a final concentration of 2 mM for 2 h. The cells were harvested by centrifugation and resuspended in 5 ml of NETN buffer (20 mM Tris, pH 8.0, 100 mM NaCl, 1 mM EDTA, 0.5% Nonidet P-40). Lysozyme was added to a final concentration of 0.1 mg/ml. The lysates were incubated on ice for 20 min, sonicated, and centrifuged to remove the cell debris. The supernatant was incubated with 200 µl of glutathione-Sepharose slurry beads (BD Bio-science) for 1 h at 4 °C. The beads were washed three times with 5 ml of Binding buffer (20 mM Tris, pH 8.0, 100 mM KCl, 5 mM MgCl2, 0.1 mM EDTA, 20% glycerol) containing 0.1% Nonidet P-40. GST fusion proteins bound to the beads were checked by SDS-PAGE. Beads containing normalized amounts of fusion proteins were blocked by Binding buffer containing 0.05% of Nonidet P-40 and 5 mg/ml bovine serum albumin for 2 h at 4 °C and resuspended in 150 µl of Binding buffer containing 0.05% Nonidet P-40, 1 mg/ml bovine serum albumin, and 100 µg/ml ethidium bromide. The beads were incubated overnight at 4 °C with 3 µl of Myc-tagged mSin3a produced by Tnt. The beads were washed three times with 500 µl of Binding buffer containing 0.05% Nonidet P-40 and boiled with SDS sample buffer. Myc-tagged mSin3a was detected by Western blotting using an anti-Myc antibody.

In Situ Hybridization—In situ hybridization on cryosections was carried out as described previously (14). Plasmids carrying...
mouse Sp5, Axin2, and Nkd1 cDNA were linearized with an appropriate restriction enzyme, and an antisense Riboprobes was synthesized using the DIG RNA labeling kit (Roche Applied Science).

RESULTS

Sp5 Is a Target Gene of Wnt/β-Catenin Signaling—To identify target genes of Wnt/β-catenin signaling during CNS development, two lines of mice were interbred to activate Wnt/β-catenin signaling. Catnblox(ex3), in which exon 3 of β-catenin gene is floxed by loxP sites (8), was mated to Nes11Cre, a transgenic mouse line expressing Cre recombinase under the control of nestin regulatory elements in neural progenitor cells (10). Exon 3 of β-catenin gene encodes phosphorylation sites necessary for β-catenin degradation (15). Cre recombinase-mediated deletion of exon 3 of β-catenin gene results in the expression of a stabilized form of β-catenin, which leads to the constitutive activation of Wnt/β-catenin signaling. To map the area in which Cre recombinase is active in the Nes11Cre mice, Nes11Cre mice were crossed with a reporter mouse line, ROSA26R (R26R) (11). Within the telencephalon, Cre recombinase activity was detected in the neural progenitor cells of the pallium and the subpallium (Fig. 1A). To activate canonical Wnt signaling during CNS development, Nes11Cre mice were crossed to Catnblox(ex3) mice. Mutant animals Nes11Cre/Catnblox(ex3) displayed hyperplasia in the telencephalon that resembles the phenotype of the mouse mutants in which activated β-catenin is directly coupled to the nestin enhancer (6). Further, the dorso-ventral patterning in the mutant telencephalon is impaired such that genes normally expressed in the dorsal pallium expand into the ventral areas, whereas ventrally expressed genes are down-regulated (69). To identify target genes of Wnt/β-catenin signaling, RNA was isolated from the telencephalon at E13.5, and overall gene expression was analyzed by Affymetrix microarray. We noticed that the expression levels of several known targets of Wnt/β-catenin were up-regulated, as follows: Axin2 (3.4×), Nkd1 (9.5×), Dkk1 (5×), and Pitx2 (7×) (3, 16, 17). On the other hand, ventrally expressed genes such as Dlx2, Dlx1, Lhx6, or Mash1 were down-regulated 10.5-, 5.1-, 15.1-, and 7.2-fold, respectively. The expression of several genes was verified by in situ hybridization on coronal sections of Nes11Cre/Catnblox(ex3) mice and wild-type mice at E13.5 (Fig. 1, D–G, see also Ref. 69). Interestingly, we found that Sp5, a member of Sp1 family, was up-regulated 32-fold in the Affymetrix data, and strong gene activation was confirmed by in situ hybridization (Fig. 1, B and C). In wild-type mice, Sp5 is expressed weakly in the hippocampal primordium (Fig. 1B). In Nes11Cre/Catnblox(ex3) mice, Sp5 is strongly expressed in the pallium and the subpallium, i.e. in the area of Cre-mediated recombination (Fig. 1C). These results suggest that Wnt/β-catenin signaling positively regulates Sp5.

Sp5 Is a Direct Target Gene of Wnt/β-Catenin Signaling—We next examined whether Sp5 is regulated by Wnt/β-catenin signaling directly. To find important transcriptional regulatory elements, we compared the upstream sequences of Sp5 of Mus musculus, Homo sapiens, Gallus gallus, Danio rerio, and Xenopus tropicalis, because the important transcriptional regulatory elements are often evolutionarily conserved. We found three evolutionarily conserved regions containing TCF/LEF consensus sites located at positions −200 bp/+200 bp, −2.9 kbp/−2.7 kbp, and −3.9 kbp/−3.4 kbp, referred to as proximal promoter, ECR2, and ECR1, respectively. ECR2 contains two conserved TCF/LEF consensus sites named B and C. ECR1 contains one conserved TCF/LEF consensus site named A. The Sp5 proximal promoter contains five TCF/LEF consensus sites, named D, E, F, G, and H, respectively. Sites E, G, and H in the Sp5 promoter were evolutionarily conserved among all five vertebrate species. Site F was not conserved in D. rerio, and site D was conserved only between M. musculus and H. sapiens (Fig. 2A).

To examine if the Sp5 promoter is responsive to Wnt/β-catenin signaling, a mouse Sp5 promoter (−1536/+200) was cloned into the luciferase reporter plasmid and transiently transfected into 293T cells. Cotransfection of the promoter with Lef1 and N/Lef1, respectively, and repressed 2.9-fold and 2.9 kbp, and 1536/27/1536/200, and 1536/+3 of the Sp5 promoter were named D1, D2, and D3, respectively. Each plasmid was cotransfected in 293T cells with β-cateninN/Leff or dnTCF4. D1 and D2 were stimulated 10-fold and 6-fold by β-cateninN/Lef1, respectively, and repressed 2.9-fold and 9.4-fold by dnTCF4, respectively. In contrast, D3 was not affected by either β-cateninN/Leff or dnTCF4 (Fig. 2B). These results suggest that sites F, G, and H play a critical role in mediating Wnt/β-catenin signaling and site E supports site F, G, and H to give further activation.
To examine whether ECR1 and ECR2 are also responsive to Wnt/β-catenin signaling, ECR1 and ECR2 were cloned upstream of the minimal TK promoter driving luciferase reporter gene expression. Each of the constructs was cotransfected in 293T cells with β-cateninΔN/LEF1 or dnTCF4. ECR1, ECR2, and TK were stimulated 1.4-, 4.0-, and 1.8-fold by β-cateninΔN/LEF1, respectively, and repressed 2.4-, 2.3-, and 1.5-fold by dnTCF4 (Fig. 2C). These results suggest that ECR2 is an additional Wnt-responsive regulatory element.

To examine whether TCF/LEF binds putative A–H binding sites within the Sp5 proximal promoter, ECR1, and ECR2 (Fig. 3B), EMSA was performed. Oligonucleotides containing sites A–H were incubated with in vitro translated LEF1 and were analyzed by electrophoresis. As shown in Fig. 3A, all sites were bound by LEF1. The identity of the LEF1 protein in the complex was verified by supershifts using LEF1 antibody. This result suggests that TCF/LEF can bind with TCF/LEF binding sites within the Sp5 proximal promoter, ECR1, and ECR2.

We next examined whether LEF/TCF transcription factors and β-catenin are associated with the Sp5 promoter in vivo. Chromatin immunoprecipitation was performed using antibodies against LEF1, TCF4, and β-catenin using cortical part of brain from D6Cre/Catnblox<sup>lox(ex3)</sup> mice at E18.5. D6Cre is a transgenic line expressing Cre recombinase in the telencephalon using Dach1 enhancer (12). Chromatin immunoprecipitation data show that LEF/TCF/β-catenin complexes are present on the proximal Sp5 promoter (Fig. 3C). This result suggests that LEF/TCF/β-catenin complex binds the Sp5 promoter in vivo to regulate transcription. We therefore conclude that Sp5 is a direct target gene of Wnt/β-catenin signaling.

**Sp5 Is a Potent Transcriptional Repressor**—To our surprise, many genes were down-regulated in the telencephalon of Nes11Cre/Catnblox<sup>lox(ex3)</sup> mice as compared with control mice. We hypothesized that down-regulation of at least some of the genes could be mediated by Sp5, because Sp5 itself is highly induced in Nes11Cre/Catnblox<sup>lox(ex3)</sup> mice, and several Sp1 family members are known to act as repressors (20). To examine the transcriptional properties of Sp5, a Gal4 reporter assay was employed. Plasmids encoding Gal4, Gal4 fusion with Sp5 (Gal4-Sp5), or Gal4 fusion with Dach1 (Gal4-Dach1), a known repressor (21), were cotransfected with a Gal4-dependent reporter plasmid driving luciferase gene expression. Both Gal4-Sp5 and Gal4-Dach1 repressed transcription 7.8- and 1.9-fold, respectively (Fig. 4A). This result indicates that Sp5 acts as a transcriptional repressor.

To identify functional domains within Sp5 that mediate transcriptional activity, Gal4 fusion constructs with different regions of Sp5 were cotransfected together with the Gal4 reporter plasmid. The Gal4 fusion proteins containing amino acids (aa) 1–76, 1–297, and 1–297 plus 379–398 and 379–398 of Sp5 repressed transcription 5.5-, 11-, 16-, and 7.1-fold, respectively. However, the Gal4 fusion proteins containing aa 1–151 and 1–222 of Sp5 did not exert any significant effect on transcription (Fig. 4B). These results suggest that aa 1–76, 223–297, and 379–398 of Sp5 contain repressor domains and that the region between aa 77 and 222 of Sp5 might contain an activation domain. To examine our hypothesis, Gal4 fusions with aa 223–297, 379–398, 77–222, or 152–222 were cotransfected with a Gal4 reporter plasmid. Gal4 fusions with aa 223–297 and 379–398 repressed transcription 10- and 7.1-fold, respectively (Fig. 4C). Gal4 fusions with aa 77–222 and 77–151 activated transcription 21- and 15-fold, respectively (Fig. 4D). These results suggest that, overall, Sp5 acts as a repressor and has three separable and independent repressor domains located within aa 1–76, 223–297, and 379–398. In addition, there is a potential transcriptional activation domain located within residues 152–222 of Sp5. In the following text, we refer to the repressor domains located within aa 1–76, 223–297, and 379–398 as R1, R2, and R3, respectively.

**Corepressor mSin3a Interacts with the R1 Domain of Sp5 and Regulates Its Transcriptional Activity**—We next examined the mechanism(s) that control the transcriptional properties of the repressor domains. We found a core mSin3a-interacting domain (SID), A(A/V)XXL (22), within the R1 domain of Sp5. Corepressor mSin3a is known to interact with Class I histone deacetylases and a number of transcription factors containing α-helical structure harboring SID (23, 24). We found that the R1 domain is predicted to form α-helical structure. We therefore examined whether the putative SID within Sp5 is responsible for the transcriptional repression function of the R1 domain. The Gal4 fusion constructs with the R1 domain containing wild-type (Gal4-Sp5R1) or a mutated SID (Gal4-Sp5R1A3P), in which alanine is changed to proline to disrupt the formation of α-helical structure, were cotransfected with the Gal4 reporter plasmid. Interestingly, in contrast to the wild-type Gal4-Sp5R1, which acts as a potent repressor, the Gal4-Sp5R1A3P acted as an activator (Fig. 5A). In addition, another Gal4 fusion construct with the R1 domain lacking a SID (Gal4-Sp5R1Δ3–7) also worked as an activator. Combined, these results suggest that the SID is crucial for the repressive activity of the R1 domain.

To examine whether Sp5 interacts with mSin3a directly through a SID, GST–pull-down assays were performed with the wild-type Sp5 R1 domain (GST–R1) and the SID mutated R1 domain (GST–R1A3P). GST–R1 pulled down in vitro translated mSin3a. In contrast, neither GST nor GST–R1A3P were able to interact with mSin3a (Fig. 5B). In accordance with the fact that we have not been able to detect any potential SID motifs within R2 and R2, GST–Sp5R2 and GST–Sp5R3 domain fusions did not pull down in vitro translated mSin3a (data not shown). These results suggest that mSin3a interacts with Sp5 directly through the SID located within R1. To provide further evidence that mSin3a interacts with Sp5 in vivo, coimmunoprecipitation was performed. FLAG-tagged Sp5 expression plasmid (Sp5–FLAG) was cotransfected with Myc–tagged mSin3a expression plasmid (Myc–mSin3a) into 293T cells, and the total cell lysate was precipitated using FLAG antibody beads. We found that Sp5–FLAG was immunoprecipitated with Myc–mSin3a (Fig. 5C) providing evidence that Sp5 can interact with mSin3a in vivo. In summary, our results suggest that the transcriptional repression activity of R1 domain is mediated through the interaction with mSin3a corepressor.

---

2 N. Fujimura and Z. Kozmik, data not shown.
Wnt-mediated Down-regulation of Sp1 Target Genes by Sp5

A

B

C

pGL3

D3

D2

D1

FL

TK

ECR2

ECR1

Luciferase

Luciferase

Luciferase

Luciferase

Luciferase

Luciferase

Luciferase

Luciferase

Relative luciferase activity

Relative luciferase activity

β-catenin, Lef1

dnTCF4

Control

β-catenin, Lef1
dnTCF4
Control
Wnt-mediated Down-regulation of Sp1 Target Genes by Sp5

Sp5 Binds Sp1 Target Sequences and Attenuates Sp1-regulated Transcription—Sp5 belongs to the large family of Sp1-like transcription factors. Intrigued by the fact that the founding member, Sp1, acts as an activator, whereas Sp5 acts as a repressor, we next examined whether Sp5 down-regulates Sp1 target genes. The zinc finger domain of Sp1 family members conforms to the Cys2-His2 zinc finger consensus sequence. The similarity of the zinc finger between Sp1 and Sp5 is 92.6% (20). The amino acids predicted to make contact with the DNA are conserved between Sp5 and Sp1. Furthermore, it is shown that Sp5 binds to a canonical Sp1 consensus site (GGGGCGGG) in vitro by EMSA (9). To examine whether Sp5 has the same DNA binding specificity as Sp1, EMSA was performed using bacterially purified Sp5 and Sp1 proteins on a large panel of binding sites. Oligonucleotides containing the canonical Sp1 binding site (WT), mutated Sp1 binding sites (M1, M2, M3, M4, and M5), Sp1 binding regions within the proximal promoter of TGF-βRII gene (SA1, AX2, and XN2), or the BTE (basic transcription element) site (BTE), a well characterized GC-rich element (25, 26), were tested. Binding site M1 has a mutation that abolishes Sp1 binding (27). As shown in Fig. 6A, Sp5 and Sp1 bound to WT, M2, M3, M4, M5, XN2, SA1, AX2, and BTE with similar affinities. Consistent with Sp1 data, Sp5 did not bind to M1 (Fig. 6A). This result suggests that Sp5 has a very similar if not identical DNA binding specificity as Sp1. We next examined whether Sp5 represses Sp1 target genes. First of all, we have investigated p21 as a well characterized Sp1 target gene. It was shown previously that the proximal promoter of p21 gene contains six Sp1 binding sites (I–VI) and that it is positively regulated by Sp1 through these binding sites (28). To examine whether Sp5 binds to the Sp1 binding sites within the p21 promoter, EMSA was performed using bacterially purified Sp5 or Sp1 and oligonucleotides containing sites I + II, III + IV, and V + VI. As shown in Fig. 6B, Sp5 bound strongly with the oligonucleotides in the same manner as did Sp1. To examine whether Sp5 has the ability to repress p21 gene promoter, the luciferase reporter assay was performed. Reporter genes containing 2.3 kb of the p21 promoter (p21-Luc), the proximal p21 promoter (p21GC-Luc), or the promoter lacking the six Sp1 binding sites (p21ΔGC-Luc) were cotransfected with or without the Sp5 expression plasmid into 293T cells. As shown in Fig. 6C, both p21-Luc and p21GC-Luc were repressed by Sp5 (3.3- and 3.5-fold, respectively). We were unable to see any effect of Sp5 on p21ΔGC-Luc, because the basal level of p21ΔGC-Luc was even lower than that of the parental plasmid pGL3. Our results suggest that Sp5 can repress p21 promoter, most likely due to its ability to compete with Sp1 (or with related activator, Sp3) for promoter binding. Because full-length p21 promoter and truncated p21 promoter were repressed 13- and 3.3-fold, respectively, there may be additional Sp5-responsive elements upstream of the proximal p21 promoter. These results suggest that Sp5 binds p21 gene regulatory elements and represses its promoter.

To obtain further evidence that Sp5 represses Sp1 target genes in vivo, and to identify additional Sp5 targets in neural cells, we have established primary neurosphere cultures over-expressing Sp5. Neurospheres represent cultured neural stem cells that divide in vitro and yield major neural lineages upon differentiation. pNIT retroviral vector carrying Sp5 coding sequence as well as G418 resistance was used to infect neurosphere cells isolated from the mouse telencephalon at E12.5. Neurospheres were grown in medium containing G418, and the pool of G418-resistant clones was used for isolation of RNA. Real-time reverse transcription-PCR revealed that neurosphere cells infected with the Sp5 retrovirus manifested 107.6-fold...
induction of Sp5 mRNA as compared with mock infected cells.\(^4\) We then profiled gene expression of Sp5-overexpressing neurospheres by Affymetrix microarray analysis. We found that 107 genes were down-regulated \(>2\)-fold in Sp5-infected neurospheres. Notably, 90 genes were Sp1 target genes or genes that contain canonical Sp1 binding sites in the proximal promoter \((-500)/+1\) and 5'-untranslated region (Table 1) (29–52). Of a special interest is the gene encoding solute carrier family 12, member 2 (scl12a2, NKCC1), which contains canonical Sp1 binding sites and becomes down-regulated by Wnt/\(\beta\)-catenin signaling (53). In conclusion, our results show that Sp5 represses Sp1 target genes.

**DISCUSSION**

In this study we have shown that the Sp5 gene is a direct target of Wnt/\(\beta\)-catenin signaling and that Sp5 acts as a transcriptional repressor and represses Sp1-regulated target genes. Because the induction of Sp5 by Wnt/\(\beta\)-catenin signaling is very high, Sp5 might be useful as a new marker for Wnt/\(\beta\)-catenin signaling. It is known that Wnt/\(\beta\)-catenin signaling represses the transcription of several genes (54–56). Our report may give some insight into Wnt/\(\beta\)-catenin signaling-dependent repression.

We have shown that Sp5 is regulated by Wnt/\(\beta\)-catenin signaling directly and predominantly through the proximal promoter, which has evolutionarily conserved TCF/LEF binding sites. We have shown that Sp5 promoter and ECRs are evolutionarily conserved between mouse and zebrafish. A recent report has shown elevated expression of Sp5 in colon cancer tissues in which Wnt/\(\beta\)-catenin signaling is constitutively active (57). Previous reports and results presented here suggest that Sp5 is directly regulated by Wnt/\(\beta\)-catenin signaling. In zebrafish embryos, Sp5 expression is induced by Wnt8 and repressed by dominant-negative TCF (58). Both ECR1 and ECR2 identified in our study are highly evolutionarily conserved and have TCF/LEF binding sites. Although neither ECR1 nor ECR2 mediated a strong response to Wnt/\(\beta\)-catenin signaling in our cell transfection assays, we cannot rule out the possibility that ECR1 and ECR2 represent genuine regulatory elements under Wnt/\(\beta\)-catenin control. Interestingly, FGF8, which activates MEF2 and ATF1 transcription factor (59, 60), can also induce Sp5 expression in zebrafish (61). Interestingly, ECR1 and ECR2 contain putative sites.

\(^4\) T. Vacik, data not shown.
We show here that Sp5 is a potent transcriptional repressor and has three autonomous repressor domains. Because mSin3a interacts with Sp5, and deletion and mutation of SID made R1 domain an activator, the transcriptional activity of the R1 domain is regulated by the interaction with corepressor mSin3a. We also examined the mechanisms that control the transcriptional activity of R2 and R3 domains. R2 domain has a polyalanine tract that is often found associated with repressor domains (62). However, a deletion of the polyalanine tract did not change the transcriptional activity of the R2 domain. The R3 domain has an evolutionarily conserved sumoylation consensus ΨKXE (63). Sumoylation consensus is often found in repressor domains and inhibitory domains of activators, and sumoylation facilitates transcriptional repression activity (64, 65). One of the consequences of sumoylation is to promote the interaction of transcription factors with corepressors (66). Mutation in sumoylation consensus within the R3 domain of Sp5 had only a modest effect on the repression activity of R3 in our transient reporter assays in 293T cells. However, we cannot exclude the possibility that the transcriptional activity of R3 might be affected by sumoylation in vivo. To identify corepressors mediating the activity of R2 and R3 domain, we also tested the effect of common corepressors, CtBP and Groucho, on the transcriptional activity of Sp5. Examining Sp5 protein sequence did not reveal a well defined binding motif for either CtBP or Groucho (67, 68). In accordance with this, cotransfection with either CtBP or Groucho did not have any effect on the transcriptional property of R2 and R3. Combined, our results indicate that the transcriptional property of R1 is mediated by mSin3a, whereas the repressor domains R2 and R3 interact with an, as yet, unidentified corepressor.

Wnt/β-catenin signaling is active in the pallium and is important for dorso-ventral specification of the telencephalon (69). In the telencephalon of Nes11Cre/Catnblox(ex3) mice, the expression level of subpallial markers, Nkx2.1, Mash1, Gsh2, Olig2, and Dlx2 were significantly decreased. Interestingly Nkx2.1 is regulated by Sp1 and Sp3 directly (70). In the telencephalon of Nes11Cre/Catnblox(ex3) mice, the expression level of Nkx2.1 is significantly reduced in the subpallium where Sp5 is ectopically expressed (69). In addition, other subpallial markers have putative canonical Sp1 binding sites and its related sequence in their proximal promoters. Furthermore we noticed that Mash1 was down-regulated in Sp5-infected neuropheres by microarray and real-time reverse transcription-PCR. Our results and previous reports suggest that Wnt/β-catenin signaling induces Sp5 and represses subpallial markers to establish dorso-ventral specification. Wnt/β-catenin signaling is also essential for the maintenance of proliferation of neural progenitors (6). Wnt/β-catenin signaling induces Cyclin D1 and c-myc, which affect cell proliferation (2, 71). It has been shown that Sp1 and other Sp1 family members have an effect on proliferation and apoptosis (20). In addition, the expression level of several genes that affect cell proliferation, differentiation, and apoptosis were changed in Sp5-infected neurosphere culture and in MCF7-transformed cell line (Table 1) (72). Because Sp5 gene is induced by Wnt/β-catenin signaling, the maintenance of proliferation of neural precursors might be partially regulated by Sp5.

Wnt-mediated Down-regulation of Sp1 Target Genes by Sp5

FIGURE 5. Sp5 interacts with corepressor mSin3a. A, the expression plasmids encoding Gal4 fusion with the R1 domain (Gal4Sp5R1) or mutated R1 domains (Gal4Sp5R1A3P) (100 ng) were cotransfected with the Gal4 reporter plasmid (100 ng). The β-galactosidase expression plasmid (5 ng) was cotransfected to normalize for transfection efficiency. B, GST-pull-down assays were performed with GST, GST-Sp5R1, and GST-Sp5R1A3P. An in vitro translated, Myc-tagged mSin3a was incubated with the indicated GST fusions bound to the glutathione-Sepharose beads. Western blotting was performed with an anti-Myc antibody to detect Myc-tagged mSin3a (upper panel). The normalized amounts of the GST proteins used in the pull-down assay are shown by Coomassie-stained gel (bottom panel). C, Myc-tagged mSin3a expression plasmid (Myc-mSin3a) was cotransfected with FLAG-tagged Sp5 expression plasmid (Sp5-FLAG) or empty expression plasmid into 293T cells. Cells were harvested 2 days later. Immunoprecipitation was performed with an anti-FLAG M2 affinity beads, and Western blotting was performed using anti-FLAG or anti-Myc antibodies.

AFT1 binding sites and ECR2 contains a putative MEF2 binding site. ECR1 and ECR2 may thus be responsive to FGF8. Furthermore, we have been unable to fully recapitulate the expression pattern of endogenous Sp5 in medaka using a transgene containing the mouse proximal Sp5 promoter fused to enhanced β-galactosidase expression plasmid. ECR1 and ECR2 may thus be responsive to FGF8.

5 J. Ruzickova and Z. Kozmik, unpublished data.

6 O. Machon and T. Vacik, data not shown.
changes in cell growth and differentiation into various neuronal lineages when compared with wild-type neurospheres. The dominant effect of epidermal growth factor/basic fibroblast growth factor growth factors necessary to propagate neurospheres in vitro. We have recently shown that the original dorsal telen-cephalon cell fate is lost in neurosphere cultures grown in the presence of epidermal growth factor/basic fibroblast growth factor and that the expression profile is specifically changed in cultured cells in just three passages (77).

A more than 100-fold up-regulation of Sp5 expression in Sp5-infected neurospheres lead to only a 2- to 3-fold down-regulation of most known Sp1 target genes. There are at least two potential reasons to explain this apparent discrepancy. First of all, the level of Sp5 protein as a repressor has to reach the level at which it can overcome the activator function of ubiquitously expressed Sp1-family members such as Sp1 and Sp3. Therefore, even 100-fold up-regulation of Sp5 mRNA may not represent a sufficient amount of Sp5 protein to observe stronger repression of known Sp1 target genes in our experimental system.

Intriguingly, it was argued that zebrafish Sp5 might work as a transcriptional activator. First of all, Sp5 partially rescued Drosophila embryos mutated in buttonhead (Btd), one of Drosophila Sp1 homologues known to act as an activator (61). Furthermore, zebrafish Sp5 induced pax2.1 expression in the midbrain-hindbrain boundary (61). However, this latter result could be explained by an indirect effect: by Sp5 repressing a repressor of pax2.1. In fact all three repressor domains R1, R2, and R3 are highly conserved between mouse and zebrafish, 71%, 63 and 56%, respectively. In addition mSin3a core consensus site and small ubiquitin-

7 O. Machon, S. Krauss, and Z. Kozmik, unpublished data.
### TABLE 1

List of genes down-regulated in Sp5-overexpressing primary neurospheres

From a total of 107 genes downregulated > 2-fold, only those containing Sp1 binding sites in their regulatory regions (−500/+1 and 5' UTR) are shown. References indicate previous studies of the genes with respect to Sp1 regulation.

| Gene Symbol | Gene | F-value change (Sp5+/Sp5-) | reference |
|-------------|------|---------------------------|-----------|
| Braf1       | Braf 1 | 0.38                      | 43        |
| Gap4p       | 68k-membrane protein 1 | 0.42 |          |
| Ccd1        | Ccd1 | 0.47                      | 51        |
| Sirt1       | Sirt1 | 0.45                      | 52        |
| 261029M04Rk | 261029M04 gene | 0.33 |          |
| Ccd2       | Ccd2 | 0.43                      | 53        |
| Ptdk3a      | Ptdk3a | 0.43                      | 54        |
| Trim27      | Trim27 | 0.45                      | 55        |
| Rab11a      | Rab11a | 0.46                      | 56        |
| Mtap2       | Mtap2 | 0.37                      | 57        |
| Man         | Man 1 | 0.26                      | 58        |
| Ranbp2      | Ranbp 2 | 0.45                      | 59        |
| Hnbp        | Hnbp | 0.46                      | 60        |
| Tce1        | Tce1 | 0.48                      | 61        |
| Hsp         | Hsp | 0.44                      | 62        |
| Ab212p2     | Ab212p2 | 0.35                      | 63        |
| Kif3        | Kif3 | 0.32                      | 64        |
| Vn11        | Vn11 | 0.36                      | 65        |
| 111001A07Rk | 111001A07 gene | 0.5 |          |
| Tnf        | Tnf | 0.46                      | 66        |
| Egf1        | Egf1 | 0.48                      | 67        |
| Ecf2a       | Ecf2a | 0.48                      | 68        |
| Pdms6      | Pdms6 | 0.41                      | 69        |
| Fosb46      | Fosb46 | 0.48                      | 70        |
| Gcl2       | Gcl2 | 0.47                      | 71        |
| Ppsr        | Ppsr | 0.23                      | 72        |
| Sapro1     | Sapro1 | 0.48                      | 73        |
| Nesp       | Nesp | 0.38                      | 74        |
| Pa2f5       | Pa2f5 | 0.46                      | 75        |
| Cdx5        | Cdx5 | 0.45                      | 76        |
| Cdx12a      | Cdx12a | 0.47                      | 77        |
| Cdk2α       | Cdk2α | 0.32                      | 78        |
| Myo10       | Myo10 | 0.36                      | 79        |
| Sdc14a      | Sdc14a | 0.35                      | 80        |
| Cdk2a       | Cdk2a | 0.36                      | 81        |
| Nbs1        | Nbs1 | 0.32                      | 82        |
| PNA2       | PNA2 | 0.36                      | 83        |
| Map2k4     | Map2k4 | 0.32                      | 84        |
| Ef1g1       | Ef1g1 | 0.15                      | 85        |
| D22S4305R5 | D22S4305 | 0.45                      | 86        |
| Syn1       | Syn1 | 0.37                      | 87        |
| Fzd1        | Fzd1 | 0.47                      | 88        |
| Trp4       | Trp4 | 0.33                      | 89        |
| Ddx6       | Ddx6 | 0.45                      | 90        |
| Msd1       | Msd1 | 0.39                      | 91        |
| Cbn1c       | Cbn1c | 0.39                      | 92        |
| Sph2       | Sph2 | 0.38                      | 93        |
| Nes         | Nes | 0.49                      | 94        |
| Nrn1       | Nrn1 | 0.32                      | 95        |
| Vn11       | Vn11 | 0.39                      | 96        |
| Chx16      | Chx16 | 0.41                      | 97        |
| Mik6278B5  | Mik6278B5 | 0.41                      | 98        |
| Mt1n2      | Mt1n2 | 0.48                      | 99        |
| Ikzf3      | Ikzf3 | 0.48                      | 100       |
| Mx59b      | Mx59b | 0.49                      | 101       |
| Soro4      | Soro4 | 0.38                      | 102       |
| Pksr1      | Pksr1 | 0.34                      | 103       |
| Caspin     | Caspin | 0.34                      | 104       |
| Bact1b     | Bact1b | 0.42                      | 105       |
| Gpl10      | Gpl10 | 0.38                      | 106       |
| Sort1      | Sort1 | 0.44                      | 107       |
| D3D0505L22 | D3D0505L22 | 0.3 | 108       |
| Snoc2      | Snoc2 | 0.44                      | 109       |
| Lma1n1     | Lma1n1 | 0.44                      | 110       |
| Kif1b      | Kif1b | 0.44                      | 111       |
| Ccn2       | Ccn2 | 0.41                      | 112       |
| Rab3b      | Rab3b | 0.42                      | 113       |
| Adcyap1r1 | Adcyap1r1 | 0.34                      | 114       |
| Ribp1      | Ribp1 | 0.49                      | 115       |
| Arph51     | Arph51 | 0.48                      | 116       |
| Smd5      | Smd5 | 0.33                      | 117       |
| Ntfl1      | Ntfl1 | 0.49                      | 118       |
| Kif1a      | Kif1a | 0.49                      | 119       |
| Nang1      | Nang1 | 0.52                      | 120       |
| Mtap2     | Mtap2 | 0.52                      | 121       |
| C1gal1    | C1gal1 | 0.48                      | 122       |
| D0100505L07Rk | D0100505L07 gene | 0.3 | 123       |
| Dna1      | Dna1 | 0.17                      | 124       |
| Fmr1      | Fmr1 | 0.34                      | 125       |
| Emc311 | Emc311 | 0.37                      | 126       |
Wnt-mediated Down-regulation of Sp1 Target Genes by Sp5

related modifier (SUMO) modification site are evolutionarily conserved. According to the high sequence similarity within the repressor domains, zebrafish Sp5 has the potential to act as a repressor. Although in the full-length context Sp5 acts as a potent repressor, Sp5 has a cryptic transactivation domain between amino acids 77 and 151. Furthermore, the R1 domain with a mutation in the SID that abrogates mSin3a binding acted as transactivation domain. The phosphorylation of TIEG2 at Thr/Ser adjacent to SID by Erk2 results in the disruption of TIEG2-mSin3a interaction (73). Sp5 also contains an Erk2 consensus site, (S/T)P (74), adjacent to the SID. Previous reports and our results indicate that Sp5 may act as an activator in some contexts.

Sp8 is another member of Sp1 family (75). The sequence similarity of the zinc finger domain between Sp5 and Sp8 is 93.8%. The expression pattern of Sp8 and Sp5 in mouse is quite similar, for example during CNS development (76). Sp8 knock-out mice die at birth and manifest severe phenotypes in the CNS (76). On the other hand, Sp5 knock-out mice show no obvious phenotype (9). Furthermore, Wnt/β-catenin signaling induces Sp8, although it is not known whether Sp8 is directly regulated by TCF/LEF/β-catenin transcription complex (75, 76). Sp8 acts as an activator during limb development (75). However, Sp8 has the potential to act as a repressor, because Sp8 has several mSin3a core consensus sequences and polyalanine tracts.8 Sp8 is likely to bind to most Sp1 binding sites due to the high sequence similarity of its zinc finger domain to that of Sp1. It may be Sp8 that compensates for Sp5 in the Sp5 knock-out mice. Our results and previous reports suggest that Wnt/β-catenin signaling regulates Sp1 target genes by inducing Sp5 and potentially Sp8.

Acknowledgments—We thank Drs. F. Gage, R. Eisenman, E. Sancho, D. Houzelstein, C. Laherty, G. Nollan, J. Forejt, and V. Korinek for reagents and M. M. Taketo for Catnblox<Δmice. We thank Janine Davis (NEI, National Institutes of Health) for valuable comments on the manuscript.

REFERENCES

1. Moon, R. T., Kohn, A. D., De Ferrari, G. V., and Kaykas, A. (2004) Nat. Rev. Genet. 5, 691–701
2. He, T. C., Sparks, A. B., Rago, C., Hermeking, H., Zawel, L., da Costa, L. T., Morin, P. J., Vogelstein, B., and Kinzler, K. W. (1998) Science 272, 1126–1129
3. Jho, E. H., Zhang, T., Domon, C., Joo, C. K., Freund, J. N., and Costantini, F. (2002) Mol. Cell. Biol. 22, 1172–1183
4. Dovvan, K., Li, T. W., Mungua, J. E., Truong, T., Milovanovic, T., Lawrence Marsh, J., Holcombe, R. F., and Waterman, M. L. (2001) Nat. Genet. 28, 53–57
5. Lee, S. M., Tole, S., Grove, E., and McMahon, A. P. (2000) Development 127, 457–467
6. Chen, A. and Walsh, C. A. (2002) Science 297, 365–369
7. Galceran, J., Miyashita-Lin, E. M., Devaney, E., Rubenstein, J. L., and Grosschedl, R. (2000) Development 127, 469–482
8. Harada, N., Tamai, Y., Ishikawa, T., Sauer, B., Takaku, K., Oshima, M., and Taketo, M. M. (1999) EMBO J. 18, 5931–5942
9. Harrison, S. M., Houzelstein, D., Dunwoodie, S. L., and Beddington, R. S. (2000) Dev. Biol. 227, 358–372

8 N. Fujimura and Z. Kozmik, unpublished data.
Wnt-mediated Down-regulation of Sp1 Target Genes by Sp5

42. Miskimins, W. K., McClelland, A., Roberts, M. P., and Ruddle, F. H. (1986) J. Cell Biol. 103, 1781–1788
43. Nagata, D., Suzuki, E., Nishimatsu, H., Satonaka, H., Goto, A., Omata, M., and Hirata, Y. (2001) J. Biol. Chem. 276, 662–669
44. Nakajima, T., Iwaki, K., Kodama, T., Inazawa, J., and Emi, M. (2000) J. Hum. Genet. 45, 212–217
45. Ntambi, J. M., Buhrow, S. A., Kaestner, K. H., Christy, R. J., Sibley, E., Kelly, T. J., Jr., and Lane, M. D. (1988) J. Biol. Chem. 263, 17291–17300
46. Randall, J., Thorne, T., and Delpire, E. (1997) Am. J. Physiol. 273, C1267–C1277
47. Soccio, R. E., Adams, R. M., Maxwell, K. N., and Breslow, J. L. (2005) J. Biol. Chem. 280, 19410–19418
48. Spatuzza, C., Renna, M., Faraonio, R., Cardinali, G., Martire, G., Bonatti, S., and Remondelli, P. (2004) J. Biol. Chem. 279, 42535–42544
49. Uetsuki, T., Takagi, K., Sugiura, H., and Yoshikawa, K. (1996) J. Biol. Chem. 271, 918–924
50. Weidenfeld, J., Shu, W., Zhang, L., Millar, S. E., and Morrissey, E. E. (2002) J. Biol. Chem. 277, 21061–21070
51. Weidinger, G., Thorpe, C. J., Wuennenberg-Stapleton, K., Ngai, J., and Moon, R. T. (2005) Curr. Biol. 15, 489–500
52. Yang, S. H., Galanis, A., and Sharrocks, A. D. (1999) Mol. Cell. Biol. 19, 4028–4038
53. Bierie, B., Nozawa, M., Renou, J. P., Shillingford, J. M., Morgan, F., Oka, T., Taketo, M. M., Cardiff, R. D., Miyoshi, K., Wagner, K. U., Robinson, G. W., and Hennighausen, L. (2003) Oncogene 22, 3875–3887
54. Ziegler, S., Rohrs, S., Tickenbrock, L., Moroy, T., Klein-Hitpass, L., Vetter, I. R., and Muller, O. (2005) FEBS J. 272, 1600–1615
55. Aronson, B. D., Fisher, A. L., Blechman, K., Caudy, M., and Gergen, J. P. (1997) Mol. Cell. Biol. 17, 5581–5587
56. Tetsu, O., and McCormick, F. (1999) Nature 398, 422–426
57. Chen, Y., Guo, Y., Ge, X., Itoh, H., Watanabe, A., Fujiwara, T., Kodama, T., and Aburatani, H. (2006) Biochem. Biophys. Res. Commun. 340, 758–766
58. Treichel, D., Schock, F., Jackle, H., Gruss, P., and Mansouri, A. (2003) Genes Dev. 17, 2630–2635
59. Machon, O., Backman, M., Krauss, S., and Kozmik, Z. (2005) Mol. Cell. Neurosci. 30, 388–397
Wnt-mediated Down-regulation of Sp1 Target Genes by a Transcriptional Repressor Sp5

Naoko Fujimura, Tomas Vacik, Ondrej Machon, Cestmir Vlcek, Simone Scalabrin, Martin Speth, Dzung Diep, Stefan Krauss and Zbynek Kozmik

J. Biol. Chem. 2007, 282:1225-1237.
doi: 10.1074/jbc.M605851200 originally published online November 6, 2006

Access the most updated version of this article at doi: 10.1074/jbc.M605851200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 74 references, 36 of which can be accessed free at http://www.jbc.org/content/282/2/1225.full.html#ref-list-1