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Wnt-mediated Down-regulation of Sp1 Target Genes by a Transcriptional Repressor Sp5*

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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)[2] 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, *Nes11Cre* mice were crossed to $Catnb^{lox(ex3)}$ mice. Mutant animals Nes11Cre/$Catnb^{lox(ex3)}$ 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/beta-catenin signaling by directly repressing Sp1 target genes.

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[2] The abbreviations used are: CNS, central nervous system; GST, glutathione *S*-transferase; EMSA, electrophoretic mobility shift assay; dnTCF4, N-terminally truncated TCF4; aa, amino acid(s); SID, mSin3a-interacting domain.

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*, $Catnb^{lox(ex3)}$, were kindly provided by Dr. M. M. Taketo (8).

Plasmids—The mouse *Sp5* promoter and truncated promoters were amplified by PCR using *C57BL/6J* 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 SmaI-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 6xHis-Sp5, the coding sequence of Sp5 was cloned into the procaryotic expression vector pETH2α. 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 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 p21ΔGC-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 ($Nes11\text{-}Cre/Catnb^{lox(ex3)}$ or $Catnb^{lox(ex3)}$) 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% CO_2. 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 $D6Cre/Catnb^{lox(ex3)}$ 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 M) 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 $NaHCO_3$, 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, CCTAGAGATAACAAAGACACT; Sp5D-H_R, AGTCAGAGGAAAGATTTATGG; Sp5-2kb_F, TGGCTGCTTAATTGCCTAAAGAG; Sp5-2kb_R, CAGGGGTTTGAGTGCTGTGGA; Sp5 + 6kb_F, AACGGAAGCTGAGTGTAAATTAG; and Sp5 + 6kb_R, GTAACTAAGACAGACGCCTAAAC.

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, ATTGAAGAAACAAAGTTTGATCT; Sp5B, CACTCATCAACAAAGGAAAGCCC; Sp5C, GGATACCTCTTTGAACTGACCCC; Sp5D, CTAGAGATAACAAAGACACTTTG; Sp5E, AAGGCCCCCTTTGATCAGGAAAA; Sp5F, TTTGTGGATTCAAAGGATTTGCT; Sp5G, CCGCTATTCTTTGATGATTGGGT; and Sp5H, CGGCAAACTTCAAAGCCATAAAT. The following double-stranded oligonucleotides derived from the p21 promoter were used: I + II, GAATTCTGAGGCGGGCCCGGGCGGGGCGGTTGGAATTC; III + IV, GAATTCCGAGCGCGGGTCCCGCCTCCGAATTC; and V + VI, GAATTCGGAGGGCGGTCCCGGGCGGCGCGAATTC. The following double-stranded oligonucleotides representing consensus (wt) and multiple versions of the Sp1 binding site were used: WT, ATTCGATCGGGGCGGGGCGAGC; M1, ATTCGATCGGTTCGGGGCGAGC; M2, ATTCGATCGGGGAGGGGCGAGC; M3, ATTCGATCGGGGTGGGGCGAGC; M4, ATTCGATCGAGGCGGGGCGAGC; M5, ATTCGATCGGGGCGGAGCGAGC; SA1, GTGCGGAGGCGTGGTTAGAG; AX2, CGGGCGGCGGGGGAGGCGGGGTC; XN2, CGGCGGGGAGGTGGGGCGAGGAGAG; and BTE, AGCTTGAGAAGGAGGCGTGGCCAACGCATG.

Double-stranded oligonucleotides containing TCF/LEF or Sp1/Sp5 binding sites were radioactively labeled at the 5′-ends with [γ-^{32}P]dATP using polynucleotide kinase (Roche Applied Science) and purified on microspin columns (Amersham Biosciences). The ^{32}P-labeled oligonucleotides were incubated with *in vitro* synthesized LEF1 (TnT Quick, Promega), bacterially purified 6xHis-Sp5 (Qiagen), or Sp1 (Promega) in binding buffer (10 mM HEPES at pH 7.7, 75 mM KCl, 2.5 mM $MgCl_2$, 0.1 mM EDTA, 1 mM dithiothreitol, 20% glycerol, 0.5% bovine serum albumin, and 0.1 mg/ml poly(dIdC)) on ice for 15 min. For supershifts, ^{32}P-labeled oligonucleotides were preincubated on ice for 10 min with 1 μg of anti-Lef antibody. Samples were analyzed by 6% polyacrylamide gel electrophoresis and autoradiography.

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 phenylmethylsulfonyl 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 ml 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 Bioscience) 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 $MgCl_2$, 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

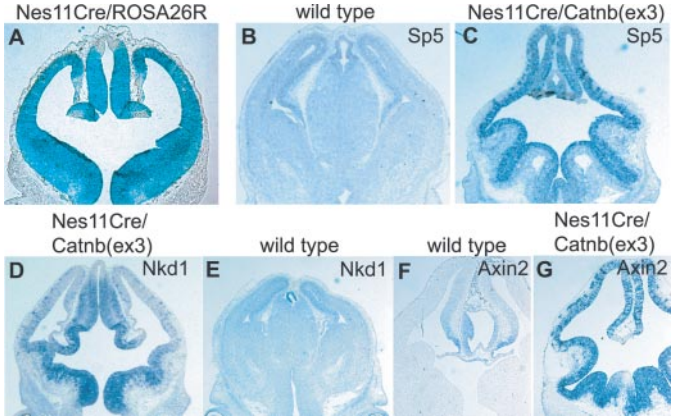


FIGURE 1. **Sp5 is regulated by Wnt/β-catenin signaling.** *A*, 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal) staining was performed on coronal brain sections of Nes11Cre/ROSA26R mice at E12.5. *B–G*, *in situ* hybridization was performed on coronal sections of wild-type and Nes11Cre/Catnb$^{lox(ex3)}$ mice using Sp5 (*B* and *C*)-, Nkd1 (*E* and *D*)-, or Axin2 (*F* and *G*)-specific probes.

mouse Sp5, Axin2, and Nkd1 cDNA were linearized with an appropriate restriction enzyme, and an antisense Riboprobe 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. *Catnb$^{lox(ex3)}$*, 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. 1*A*). To activate canonical Wnt signaling during CNS development, *Nes11Cre* mice were crossed to *Catnb$^{lox(ex3)}$* mice. Mutant animals Nes11Cre/Catnb$^{lox(ex3)}$ 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*/*Catnb$^{lox(ex3)}$* 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. 1*B*). In *Nes11Cre*/*Catnb$^{lox(ex3)}$* mice, *Sp5* is strongly expressed in the pallium and the subpallium, *i.e.* in the area of Cre-mediated recombination (Fig. 1*C*). 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. 2*A*).

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-terminally truncated β-catenin (β-cateninΔN), which is constitutively stabilized and able to bind with TCF/LEF transcription factors (18), stimulated reporter gene expression ~15-fold. Conversely, cotransfection with N-terminally truncated TCF4 (dnTCF4), which does not bind to β-catenin and acts as a potent inhibitor of the β-catenin/TCF complexes (19), repressed the activity of the promoter construct 4.9-fold (Fig. 2*B*). These results suggest that the *Sp5* promoter is directly responsive to Wnt/β-catenin signaling.

To identify functional TCF/LEF elements within the *Sp5* promoter, three reporter plasmids containing different regions of the promoter cloned upstream of the luciferase reporter gene were constructed (Fig. 2*B*). Luciferase reporter plasmids containing −206/+200, −27/+200, and −1536/+3 of the *Sp5* promoter were named D1, D2, and D3, respectively. Each plasmid was cotransfected in 293T cells with β-cateninΔN/Lef1 or dnTCF4. D1 and D2 were stimulated 10-fold and 6-fold by β-cateninΔN/Lef1, respectively, and repressed 2.9-fold and 9.4-fold by dnTCF4, respectively. In contrast, D3 was not affected by either β-cateninΔN/Lef 1 or dnTCF4 (Fig. 2*B*). 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. 2*C*). 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. 3*B*), EMSA was performed. Oligonucleotides containing sites A–H were incubated with *in vitro* translated LEF1 and were analyzed by electrophoresis. As shown in Fig. 3*A*, 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/Catnb*$^{lox(ex3)}$ 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. 3*C*). 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.

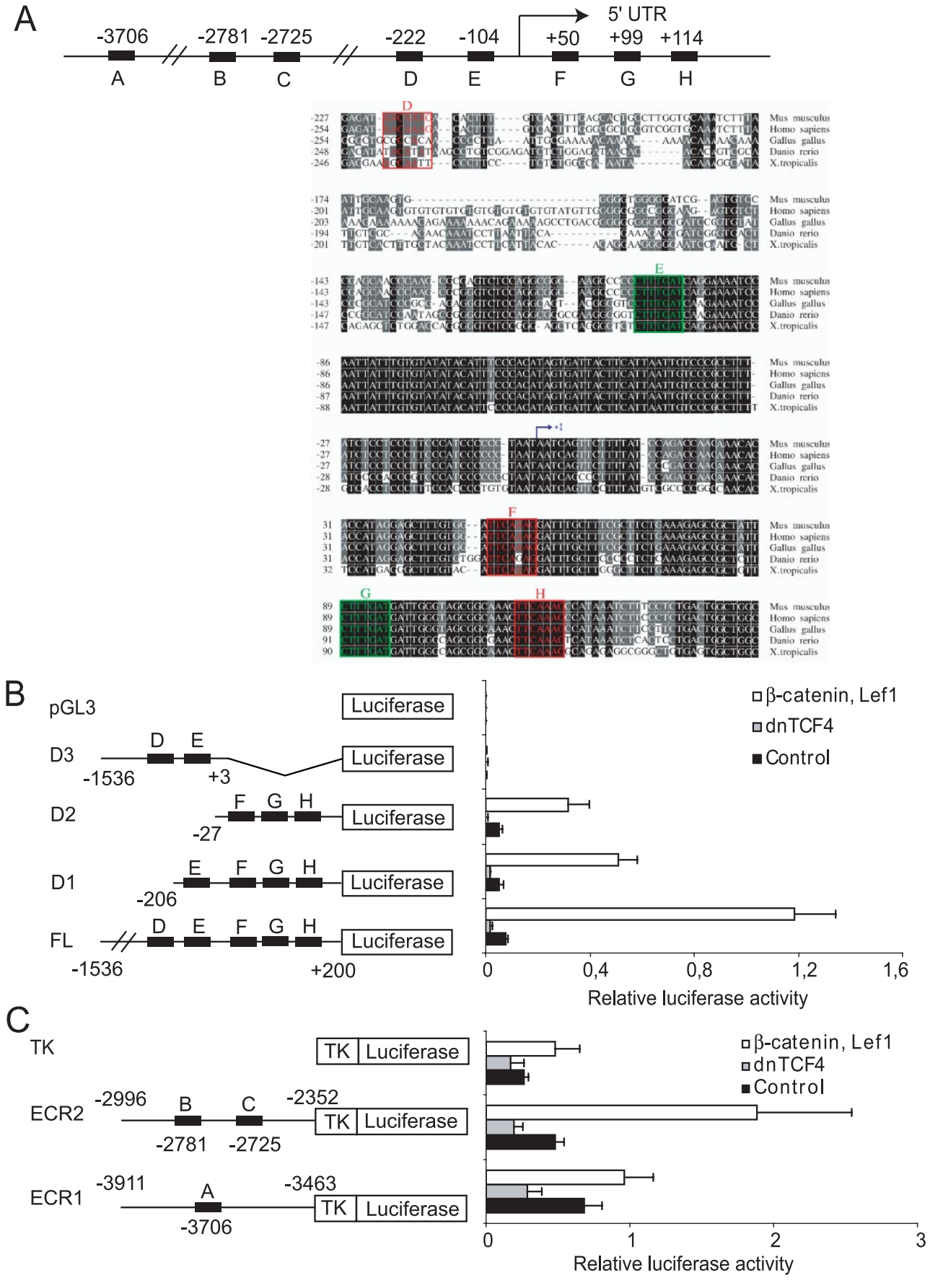
Sp5 Is a Potent Transcriptional Repressor—To our surprise, many genes were down-regulated in the telencephalon of *Nes11Cre/Catnb*$^{lox(ex3)}$ mice as compared with control mice. We hypothesized that down-regulation of at least some of the genes could be mediated by Sp5, because Sp5 itself is highly induced in *Nes11Cre/Catnb*$^{lox(ex3)}$ 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. 4*A*). 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. 4*B*). 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. 4*C*). Gal4 fusions with aa 77–222 and 77–151 activated transcription 21- and 15-fold, respectively (Fig. 4*D*). 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)*XX*L (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. 5*A*). In addition, another Gal4 fusion construct with the R1 domain lacking a SID (Gal4-Sp5R1Δ3–7) also worked as an activator.[3] 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. 5*B*). 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. 5*C*) 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.

[3] N. Fujimura and Z. Kozmik, data not shown.



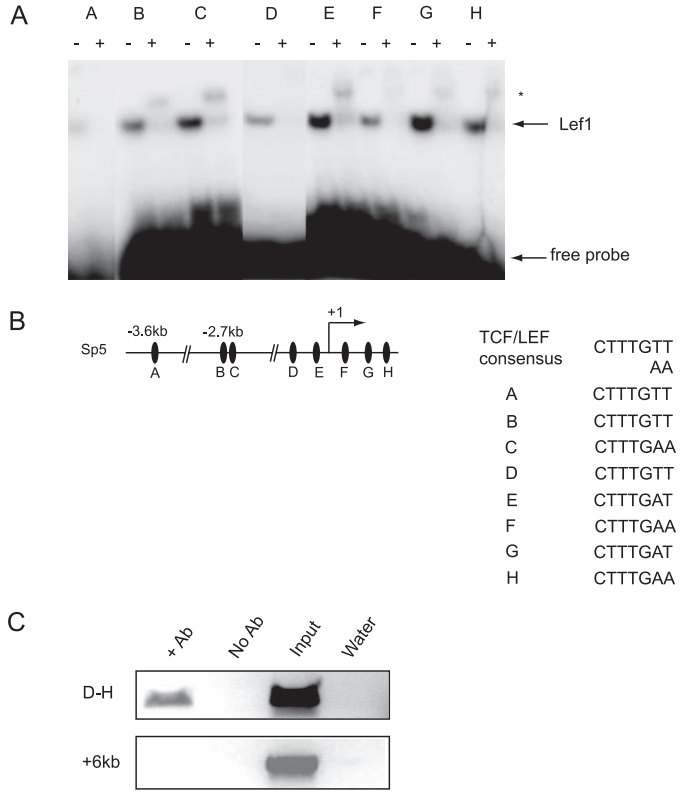


FIGURE 3. **TCF and LEF proteins bind to Sp5 regulatory sequences.** *A*, EMSAs demonstrated *in vitro* binding of LEF1 protein to putative binding sites *A–H* in the *Sp5* locus as depicted in *B*. LEF1 protein binds all of the sites (− *lanes*), and its binding specificity is demonstrated by the addition of anti-Lef1 antibody (+ *lanes*) that results in the formation of a supershifted complex (*asterisk*). *B*, the map of *Sp5* locus with putative TCF/LEF binding sites highlighted (*black circles*). *C*, chromatin immunoprecipitation assay was used to detect the presence of TCF/LEF/β-catenin complex on *Sp5* regulatory elements. TCF/LEF/β-catenin associated DNA in *D6Cre/*Catnb$^{lox(ex3)}$ cortical parts (E18.5) was analyzed by PCR with primers spanning sites *D–H*. The downstream (+6 kb) region was used as a negative control.

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 Cys_2-His_2 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 (GGGCGG) *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-βRI* 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. 6*A*, 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. 6*A*). 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. 6*B*, 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. 6*C*, both p21-Luc and p21GC-Luc were repressed by Sp5 (13- and 3.3-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 overexpressing 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

FIGURE 2. **Sp5 is a direct target of Wnt/β-catenin signaling.** *A*, localization of putative TCF/LEF binding sites, *A–H*, within the regulatory region of the mouse Sp5 gene. Sp5 promoter sequences from *M. musculus*, *H. sapiens*, *G. gallus*, *D. rerio*, and *X. tropicalis* were compared. Evolutionarily conserved, putative TCF/LEF binding sites are *boxed*. *B* and *C*, the indicated regions of the Sp5 promoter and enhancers were cloned into the pGL3 plasmid. The luciferase reporter plasmids (100 ng) were cotransfected with N-terminally truncated β-catenin (β-cateninΔN) and LEF1 (50 ng each) or N-terminally truncated TCF4 (dnTCF4, 100 ng) into 293T cells. β-Galactosidase expression plasmid (5 ng) was cotransfected to normalize for transfection efficiency. Luciferase reporter assay and β-galactosidase assay were performed as described under "Experimental Procedures."

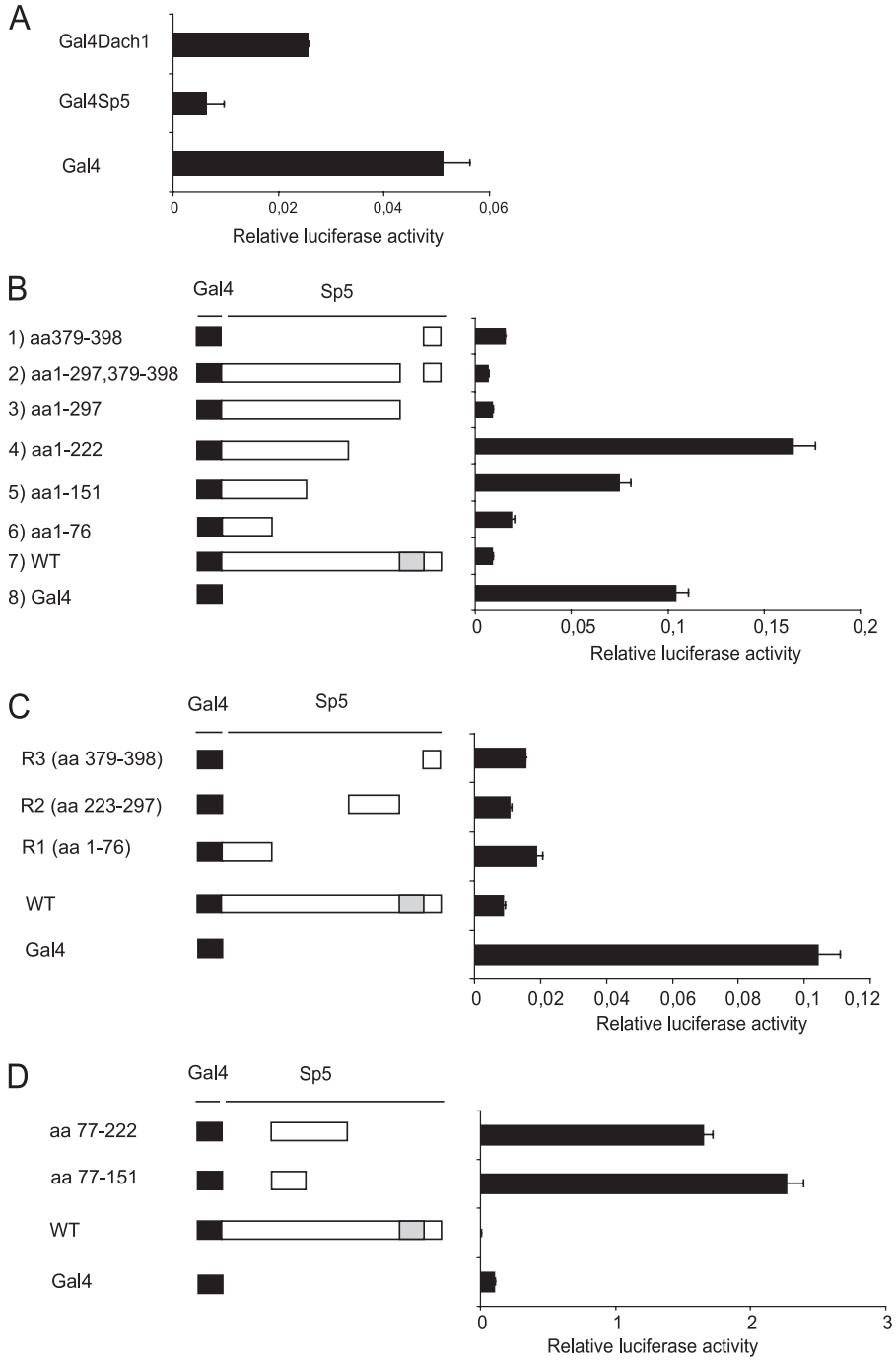


FIGURE 4. **Mapping of transcriptional regulatory domains within Sp5.** *A*, the expression plasmids encoding Gal4, Gal4-Sp5, or Gal4-Dach1 (100 ng) were cotransfected with the Gal4 reporter plasmid (100 ng) into 293T cells. A β-galactosidase expression plasmid (5 ng) was cotransfected to normalize for transfection efficiency. Luciferase reporter assays were performed as described under "Experimental Procedures." *B–D*, the expression plasmids encoding Gal4 fusions with various regions of Sp5 (100 ng) were cotransfected with the Gal4 reporter plasmid (100 ng) into 293T cells. The β-galactosidase expression plasmid (5 ng) was cotransfected to normalize for transfection efficiency.

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

We have shown that *Sp5* is regulated by Wnt/β-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/β-catenin signaling is constitutively active (57). Previous reports and results presented here suggest that *Sp5* is directly regulated by Wnt/β-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/β-catenin signaling in our cell transfection assays, we cannot rule out the possibility that ECR1 and ECR2 represent genuine regulatory elements under Wnt/β-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

[4] T. Vacik, data not shown.

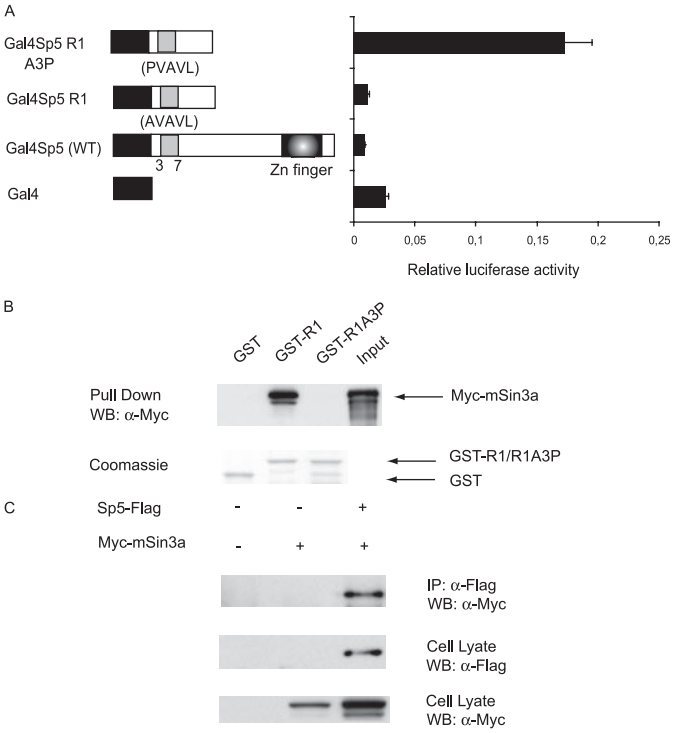


FIGURE 5. **Sp5 interacts with corepressor mSin3a.** *A*, the expression plasmids encoding Gal4 fusion with the R1 domain (Gal4Sp5R1) or mutated R1 domains (Gal4Sp5R1A3P) (100 ng) were cotransfected with the Gal4 reporter plasmid (100 ng). The β-galactosidase expression plasmid (5 ng) was cotransfected 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. Further, 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 green fluorescent protein reporter despite extremely high sequence conservation between mouse and fish, suggesting a requirement for additional regulatory elements.[5]

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.[3] 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.[3] 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.[3] 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/Catnb$^{lox(ex3)}$ 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/Catnb$^{lox(ex3)}$ 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 neurospheres by microarray and real-time reverse transcription-PCR.[6] 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.

We attempted to correlate a profound CNS phenotype observed *in vivo* (Ref. 6 and this study) with any discernable phenotype in neurospheres cultured *in vitro*. To this end, we have isolated neurospheres from Nes11Cre/Catnb$^{lox(ex3)}$ telencephalon and compared them to Sp5-infected and mock-infected neurospheres. However, neurospheres overexpressing Sp5 or activated β-catenin (isolated from Nes11Cre/Catnb$^{lox(ex3)}$ telencephalons) did not show any significant

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

[6] O. Machon and T. Vacik, data not shown.

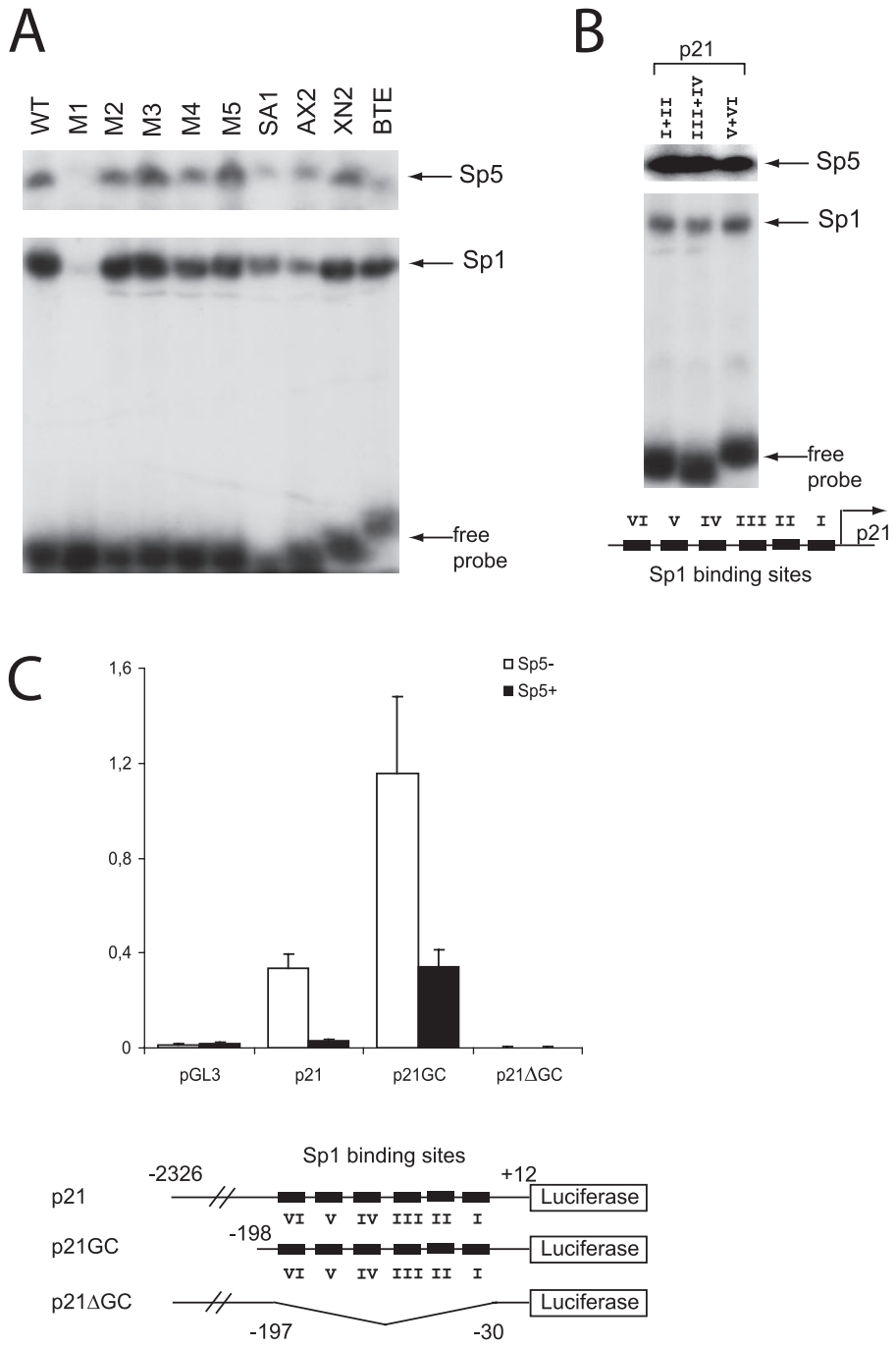


FIGURE 6. **Sp5 binds the Sp1 recognition sequences and regulates the Sp1 target gene, *p21*.** *A*, EMSA was performed using a consensus Sp1 binding site (*WT*) and various modifications (mutants *M1–M5*). *SA1*, *AX2*, and *XN2* represent Sp1 regulatory elements from the *TGF-βRI* gene (25). The BTE binding site is a target sequence of the closely related transcription factor BTEB3 (26). Sequences of the oligonucleotides are described under "Experimental Procedures." *B*, Sp5 binds Sp1 regulatory elements from the human *p21* gene promoter in EMSA. *C*, mapping of the Sp5-responsive elements in *p21* promoter. An expression plasmid encoding Sp5 or an empty expression plasmid (100 ng) were cotransfected with the indicated luciferase reporter plasmids (100 ng) into 293T cells. β-Galactosidase expression plasmid (5 ng) was cotransfected to normalize for transfection efficiency.

changes in cell growth and differentiation into various neural lineages when compared with wild-type neurospheres.[7] This is most likely due to 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 telencephalon 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. In addition, Sp5 expression was determined across the whole neurosphere population at the mRNA level using quantitative reverse transcription-PCR. To achieve a widespread overexpression of Sp5, the infected cells carrying the Sp5 retrovirus were selected using G418. Nevertheless, the selected cell pools might contain some proportion of G418-resistent cells not expressing Sp5 protein. No commercial Sp5 antibodies are currently available to allow analysis of Sp5 protein expression at the single-cell level.

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	Fold change (Sp5+ / Sp5-)	reference
Rpl14	ribosomal protein L14	0,38	
Gpiap1	GPI-anchored membrane protein 1	0,42	
Ccnd1	cyclin D1	0,47	43
Sc4mol	sterol-C4-methyl oxidase-like	0,5	
2610209M04Rik	RIKEN cDNA 2610209M04 gene	0,33	
Cd24a	CD24a antigen	0,43	51
Poldip3	polymerase (DNA-directed), delta interacting protein 3	0,45	
Trim27	tripartite motif protein 27	0,44	39
Rab8a	RAB8A, member RAS oncogene family	0,46	
Mtap2	Microtubule-associated protein 2	0,37	
Msn	moesin	0,26	52
Ranbp9	RAN binding protein 9	0,45	
Hnrpab	heterogeneous nuclear ribonucleoprotein A/B	0,46	
Tde1	Tumor differentially expressed 1	0,48	
Hip1	huntingtin interacting protein 1	0,44	
Atp1b2	ATPase, Na+/K+ transporting, beta 2 polypeptide	0,35	31
Klf3	Kruppel-like factor 3 (basic)	0,32	
Mtx1	metaxin 1	0,5	36
1110001A07Rik	RIKEN cDNA 1110001A07 gene	0,5	
Tfrc	transferrin receptor	0,46	42
Fscn1	fascin homolog 1, actin bundling protein (Strongylocentrotus) purpuratus)	0,4	34
Eif2s3x	eukaryotic translation initiation factor 2, subunit 3, structural gene X-linked	0,48	
Pdlim5	PDZ and LIM domain 5	0,41	
Vps35	vacuolar protein sorting 35	0,48	
Git2	G protein-coupled receptor kinase-interactor 2	0,47	
Ptprs	protein tyrosine phosphatase, receptor type, S	0,23	
Stard4	StAR-related lipid transfer (START) domain containing 4	0,48	47
Nes	nestin	0,38	35
Pafah1b2	platelet-activating factor acetylhydrolase, isoform 1b, alpha2 subunit	0,46	
Cbx5	chromobox homolog 5 (Drosophila HP1a)	0,45	
Tagln2	transgelin 2	0,47	
Scd1	stearoyl-Coenzyme A desaturase 1	0,32	45
Kif2a	kinesin family member 2A	0,36	
Myo10	myosin X	0,22	
Slc41a3	solute carrier family 41, member 3	0,35	
Slc12a2	solute carrier family 12, member 2	0,29	46
Ctcf	CCCTC-binding factor	0,37	40
Epb4.1l4a	erythrocyte protein band 4.1-like 4a	0,48	
Ctdsp2	CTD (carboxy-terminal domain, RNA polymerase II, polypeptide A) small phosphatase 2	0,45	
Kif11	kinesin family member 11	0,39	
Coro1c	coronin, actin binding protein 1C	0,5	
Skp2	S-phase kinase-associated protein 2 (p45)	0,38	38
Ndn	necdin	0,48	49
Map2k4	mitogen activated protein kinase kinase 4	0,32	
Eif4g1	eukaryotic translation initiation factor 4, gamma 1	0,15	
D2Ertd435e	DNA segment, Chr 2, ERATO Doi 435, expressed	0,43	
Syncrip	synaptotagmin binding, cytoplasmic RNA interacting protein	0,37	
Fzd2	frizzled homolog 2 (Drosophila)	0,47	
Trip4	thyroid hormone receptor interactor 4	0,3	
Ddx6	DEAD (Asp-Glu-Ala-Asp) box polypeptide 6	0,4	
Mthfd2	methylenetetrahydrofolate dehydrogenase (NAD+ dependent), methenyltetrahydrofolate cyclohydrolase	0,39	32
Smarcc1	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily c, member 1	0,45	29
Trp53inp1	transformation related protein 53 inducible nuclear protein 1	0,4	
Clcn6	chloride channel 6	0,49	
AW557805	expressed sequence AW557805	0,41	
Mdm2	transformed mouse 3T3 cell double minute 2	0,48	33
Brd3	bromodomain containing 3	0,46	
Wnt7b	wingless-related MMTV integration site 7B	0,49	50
Spry4	sprouty homolog 4 (Drosophila)	0,38	37
Pik3r1	phosphatidylinositol 3-kinase, regulatory subunit, polypeptide 1 (p85 alpha)	0,34	
Csen	calsenilin, presenilin binding protein, EF hand transcription factor	0,49	
Baz1b	bromodomain adjacent to zinc finger domain, 1B	0,42	
Usp1	ubiquitin specific protease 1	0,45	
Sort1	sortilin 1	0,44	
D030056L22	hypothetical protein D030056L22	0,3	
Shoc2	soc-2 (suppressor of clear) homolog (C. elegans)	0,44	
Lman1	lectin, mannose-binding, 1	0,44	48
Kif1b	kinesin family member 1B	0,41	
Ccnd2	cyclin D2	0,4	41
Rab3b	RAB3B, member RAS oncogene family	0,42	
Adcyap1r1	adenylate cyclase activating polypeptide 1 receptor 1	0,34	30
Rrbp1	Ribosome binding protein 1	0,49	
Arhgef1	Rho guanine nucleotide exchange factor (GEF) 1	0,48	
2610507B11Rik	RIKEN cDNA 2610507B11 gene	0,44	
Ugcg	UDP-glucose ceramide glucosyltransferase	0,36	
Adcyap1r1	adenylate cyclase activating polypeptide 1 receptor 1	0,4	
Xpr1	xenotropic and polytropic retrovirus receptor 1	0,42	
BC010304	cDNA sequence BC010304	0,23	
Smad5	MAD homolog 5 (Drosophila)	0,33	
Tbl1xr1	transducin (beta)-like 1X-linked receptor 1	0,46	
Kif1a	kinesin family member 1A	0,48	
Narg1	NMDA receptor-regulated gene 1	0,5	
Mbtps1	membrane-bound transcription factor protease, site 1	0,48	44
C1galt1	core 1 UDP-galactose:N-acetylgalactosamine-alpha-R beta 1,3-galactosyltransferase	0,4	
2610005L07Rik	RIKEN cDNA 2610005L07 gene	0,2	
Dia1	diaphorase 1 (NADH)	0,17	
Rev3l	REV3-like, catalytic subunit of DNA polymerase zeta RAD54 like (S. cerevisiae)	0,34	
Fmr1	fragile X mental retardation syndrome 1 homolog	0,33	
Smc1l1	SMC (structural maintenance of chromosomes 1)-like 1 (S. cerevisiae)	0,37	

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*.

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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* **281,** 1509–1512
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. Hovanes, K., Li, T. W., Munguia, 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. Chenn, 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
10. Tronche, F., Kellendonk, C., Kretz, O., Gass, P., Anlag, K., Orban, P. C., Bock, R., Klein, R., and Schutz, G. (1999) *Nat. Genet.* **23,** 99–103
11. Soriano, P. (1999) *Nat. Genet.* **21,** 70–71
12. van den Bout, C. J., Machon, O., Rosok, O., Backman, M., and Krauss, S. (2002) *Mech. Dev.* **110,** 179–182
13. Rappa, G., Kunke, D., Holter, J., Diep, D. B., Meyer, J., Baum, C., Fodstad, O., Krauss, S., and Lorico, A. (2004) *Neuroscience* **124,** 823–830
14. Machon, O., van den Bout, C. J., Backman, M., Rosok, O., Caubit, X., Fromm, S. H., Geronimo, B., and Krauss, S. (2002) *Neuroscience* **112,** 951–966
15. Liu, C., Li, Y., Semenov, M., Han, C., Baeg, G. H., Tan, Y., Zhang, Z., Lin, X., and He, X. (2002) *Cell* **108,** 837–847
16. Kioussi, C., Briata, P., Baek, S. H., Rose, D. W., Hamblet, N. S., Herman, T., Ohgi, K. A., Lin, C., Gleiberman, A., Wang, J., Brault, V., Ruiz-Lozano, P., Nguyen, H. D., Kemler, R., Glass, C. K., Wynshaw-Boris, A., and Rosenfeld, M. G. (2002) *Cell* **111,** 673–685
17. Niida, A., Hiroko, T., Kasai, M., Furukawa, Y., Nakamura, Y., Suzuki, Y., Sugano, S., and Akiyama, T. (2004) *Oncogene* **23,** 8520–8526
18. Gat, U., DasGupta, R., Degenstein, L., and Fuchs, E. (1998) *Cell* **95,** 605–614
19. van de Wetering, M., Sancho, E., Verweij, C., de Lau, W., Oving, I., Hurlstone, A., van der Horn, K., Batlle, E., Coudreuse, D., Haramis, A. P., Tjon-Pon-Fong, M., Moerer, P., van den Born, M., Soete, G., Pals, S., Eilers, M., Medema, R., and Clevers, H. (2002) *Cell* **111,** 241–250
20. Kaczynski, J., Cook, T., and Urrutia, R. (2003) *Genome Biology* http://genomebiology.com/2003/4/2/206
21. Li, X., Perissi, V., Liu, F., Rose, D. W., and Rosenfeld, M. G. (2002) *Science* **297,** 1180–1183
22. Zhang, J. S., Moncrieffe, M. C., Kaczynski, J., Ellenrieder, V., Prendergast, F. G., and Urrutia, R. (2001) *Mol. Cell. Biol.* **21,** 5041–5049
23. Knoepfler, P. S., and Eisenman, R. N. (1999) *Cell* **99,** 447–450
24. Cook, T., Gebelein, B., Belal, M., Mesa, K., and Urrutia, R. (1999) *J. Biol. Chem.* **274,** 29500–29504
25. Ji, C., Casinghino, S., McCarthy, T. L., and Centrella, M. (1997) *J. Biol. Chem.* **272,** 21260–21267
26. Kaczynski, J., Zhang, J. S., Ellenrieder, V., Conley, A., Duenes, T., Kester, H., van Der Burg, B., and Urrutia, R. (2001) *J. Biol. Chem.* **276,** 36749–36756
27. Baker, D. L., Dave, V., Reed, T., and Periasamy, M. (1996) *J. Biol. Chem.* **271,** 5921–5928
28. Koutsodontis, G., Moustakas, A., and Kardassis, D. (2002) *Biochemistry* **41,** 12771–12784
29. Ahn, J., Ko, M., Lee, K., Oh, J., Jeon, S. H., and Seong, R. H. (2005) *Biochem. Biophys. Res. Commun.* **338,** 1435–1446
30. Aino, H., Hashimoto, H., Ogawa, N., Nishino, A., Yamamoto, K., Nogi, H., Nagata, S., and Baba, A. (1995) *Gene* (*Amst.*) **164,** 301–304
31. Avila, J., Alvarez de la Rosa, D., Gonzalez-Martinez, L. M., Lecuona, E., and Martin-Vasallo, P. (1998) *Gene* (*Amst.*) **208,** 221–227
32. Belanger, C., Peri, K. G., and MacKenzie, R. E. (1991) *Nucleic Acids Res.* **19,** 4341–4345
33. Bond, G. L., Hu, W., Bond, E. E., Robins, H., Lutzker, S. G., Arva, N. C., Bargonetti, J., Bartel, F., Taubert, H., Wuerl, P., Onel, K., Yip, L., Hwang, S. J., Strong, L. C., Lozano, G., and Levine, A. J. (2004) *Cell* **119,** 591–602
34. Bros, M., Ross, X. L., Pautz, A., Reske-Kunz, A. B., and Ross, R. (2003) *J. Immunol.* **171,** 1825–1834
35. Cheng, L., Jin, Z., Liu, L., Yan, Y., Li, T., Zhu, X., and Jing, N. (2004) *FEBS Lett.* **565,** 195–202
36. Collins, M., and Bornstein, P. (1996) *Nucleic Acids Res.* **24,** 3661–3669
37. Ding, W., Bellusci, S., Shi, W., and Warburton, D. (2004) *Am. J. Physiol.* **287,** L52–L59
38. Imaki, H., Nakayama, K., Delehouzee, S., Handa, H., Kitagawa, M., Kamura, T., and Nakayama, K. I. (2003) *Cancer Res.* **63,** 4607–4613
39. Iwata, Y., Nakayama, A., Murakami, H., Iida, K., Iwashita, T., Asai, N., and Takahashi, M. (1999) *Biochem. Biophys. Res. Commun.* **261,** 381–384
40. Klenova, E. M., Fagerlie, S., Filippova, G. N., Kretzner, L., Goodwin, G. H., Loring, G., Neiman, P. E., and Lobanenkov, V. V. (1998) *J. Biol. Chem.* **273,** 26571–26579
41. Martino, A., Holmes IV, J. H., Lord, J. D., Moon, J. J., and Nelson, B. H.

[8] N. Fujimura and Z. Kozmik, unpublished data.

(2001) *J. Immunol.* **166,** 1723–1729
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 Morrisey, E. E. (2002) *J. Biol. Chem.* **277,** 21061–21070
51. Wenger, R. H., Rochelle, J. M., Seldin, M. F., Kohler, G., and Nielsen, P. J. (1993) *J. Biol. Chem.* **268,** 23345–23352
52. Wilgenbus, K. K., Hsieh, C. L., Lankes, W. T., Milatovich, A., Francke, U., and Furthmayr, H. (1994) *Genomics* **19,** 326–333
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. Okubo, T., and Hogan, B. L. (2004) *J. Biol.* **3,** 11
56. Morkel, M., Huelsken, J., Wakamiya, M., Ding, J., van de Wetering, M., Clevers, H., Taketo, M. M., Behringer, R. R., Shen, M. M., and Birchmeier, W. (2003) *Development* **130,** 6283–6294
57. Takahashi, M., Nakamura, Y., Obama, K., and Furukawa, Y. (2005) *Int. J. Oncol.* **27,** 1483–1487
58. Weidinger, G., Thorpe, C. J., Wuennenberg-Stapleton, K., Ngai, J., and Moon, R. T. (2005) *Curr. Biol.* **15,** 489–500
59. Tan, Y., Rouse, J., Zhang, A., Cariati, S., Cohen, P., and Comb, M. J. (1996) *EMBO J.* **15,** 4629–4642
60. Yang, S. H., Galanis, A., and Sharrocks, A. D. (1999) *Mol. Cell. Biol.* **19,** 4028–4038
61. Tallafuss, A., Wilm, T. P., Crozatier, M., Pfeffer, P., Wassef, M., and Bally-Cuif, L. (2001) *Development* **128,** 4021–4034
62. Hanna-Rose, W., and Hansen, U. (1996) *Trends Genet.* **12,** 229–234
63. Verger, A., Perdomo, J., and Crossley, M. (2003) *EMBO Rep.* **4,** 137–142
64. Perdomo, J., Verger, A., Turner, J., and Crossley, M. (2005) *Mol. Cell. Biol.* **25,** 1549–1559
65. Ross, S., Best, J. L., Zon, L. I., and Gill, G. (2002) *Mol. Cell.* **10,** 831–842
66. Gill, G. (2005) *Curr. Opin. Genet. Dev.* **15,** 536–541
67. Aronson, B. D., Fisher, A. L., Blechman, K., Caudy, M., and Gergen, J. P. (1997) *Mol. Cell. Biol.* **17,** 5581–5587
68. Turner, J., and Crossley, M. (1998) *EMBO J.* **17,** 5129–5140
69. Backman, M., Machon, O., Mygland, L., van den Bout, C. J., Zhong, W., Taketo, M. M., and Krauss, S. (2005) *Dev. Biol.* **279,** 155–168
70. Li, C., Ling, X., Yuan, B., and Minoo, P. (2000) *Biochim. Biophys. Acta* **1490,** 213–224
71. Tetsu, O., and McCormick, F. (1999) *Nature* **398,** 422–426
72. 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
73. Ellenrieder, V., Zhang, J. S., Kaczynski, J., and Urrutia, R. (2002) *EMBO J.* **21,** 2451–2460
74. Davis, R. J. (1993) *J. Biol. Chem.* **268,** 14553–14556
75. Kawakami, Y., Esteban, C. R., Matsui, T., Rodriguez-Leon, J., Kato, S., and Belmonte, J. C. (2004) *Development* **131,** 4763–4774
76. Treichel, D., Schock, F., Jackle, H., Gruss, P., and Mansouri, A. (2003) *Genes Dev.* **17,** 2630–2635
77. 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

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