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# Identification of members of the Wnt signaling pathway in the embryonic pituitary gland

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**Abstract.** *Prop1* is one of several transcription factors important for the development of the pituitary gland. Downstream targets of PROP1 and other critical pituitary transcription factors remain largely unknown. We have generated a partial expression profile of the developing pituitary gland containing over 350 transcripts, using cDNA subtractive hybridization between *Prop1*$^{df/df}$ and wild-type embryonic pituitary gland primordia. Numerous classes of genes including transcription factors, membrane associated molecules, and cell cycle regulators were identified in this study. Of the transcripts, 34% do not have sequence similarity to known genes, but are similar to ESTs, and 4% represent novel sequences. Pituitary gland expression of a number of clones was verified using in situ hybridization.

Several members of the Wnt signaling pathway were identified in the developing pituitary gland. The frizzled2 receptor, *Apc*, β-catenin, groucho, and a novel isoform of TCF4 (officially named *Tcf7l2*) were identified in developing pituitary libraries. Three N-terminal alternatively spliced *Tcf7l2* isoforms are reported here, each of which lacks a DNA-binding domain. Functional studies indicate that these isoforms can act as endogenous inhibitors of Wnt signaling in some contexts.

This is the first report of *Tcf7l2* and *Fzd2* expression in the developing pituitary. These molecules may be important in mediating Wnt signaling during pituitary ontogeny. We expect other transcripts from these libraries to be involved in pituitary gland development.

## Introduction

The rodent pituitary gland is composed of the anterior, intermediate, and posterior lobes. The anterior lobe contains five hormone-producing cell types that secrete hormones necessary for growth, fertility, lactation, response to physiological stress, and thyroid function. The major secretory product of the intermediate lobe is melanocyte-stimulating hormone; oxytocin and vasopressin are produced in the posterior lobe.

The anlage of the pituitary gland begins to develop at embryonic day 9.0 (e9.0) in response to inductive signals provided by surrounding tissues (Treier and Rosenfeld 1996; Burrows et al. 1999; Sheng and Westphal 1999). Over the next several days, signals from the ventral diencephalon and juxtaposed mesenchymal cells induce spatially restricted patterns of transcription factor expression in the nascent pituitary. Stratified transcription factor domains are translated into distinct regions of cell differentiation within the gland. Several critical genes regulating pituitary gland development have been identified through the characterization of hereditary mouse and human pituitary endocrine deficiencies. The pituitary transcription factors *Pitx2, Lhx3, Hesx1,* and *Prop1* are each essential for pituitary gland development; however, their downstream targets remain largely unknown.

Differential gene expression analysis is invaluable for gaining a better understanding of the molecular events regulating pituitary gland development. The RIKEN group (http://genome.rtc.riken.go.jp) released a large transcriptome from adult male pituitary (Aizawa et al. 2000). Although this is a rich resource for the identification of genes important for adult pituitary function, embryo-specific genes such as *Hesx1* and *Prop1* are not represented. We demonstrated the feasibility of establishing an expression profile of the developing organ using differential display to compare transcripts present at e12.5 and e14.5 in the pituitary primordium. This is a period of intense cell proliferation (Carbajo-Perez et al. 1989) preceding the differentiation of four of the five mature cell types and a time when the effects of mutations in *Pitx2, Lhx3, Hesx1,* and *Prop1* are evident. We reported a limited transcriptome, including two validated, novel, differential transcripts (Douglas and Camper 2000). Here, we describe a library of pituitary transcripts not previously reported that are expressed at e14.5. This library was generated by using cDNA subtractive hybridization comparing normal pituitary primodia with *Prop1*$^{df/df}$ mutants.

Analysis of both libraries reveals that numerous members of the Wnt signaling pathway are expressed in the developing pituitary gland. Several Wnt genes, including *Wnt4, Wnt5a,* and *Wnt10a,* are known to be expressed in or adjacent to the developing gland (Wang and Shackleford 1996; Treier et al. 1998), and *Wnt4* has been shown to have a functional role in pituitary gland development (Treier et al. 1998). We identified a novel TCF4 isoform (the *Tcf4* gene is officially named *Tcf7l2*), frizzled2, adenomatosis polyposis coli (*Apc*), beta-catenin, and groucho in the developing pituitary. The presence of several members of the Wnt signaling pathway in these pituitary libraries supports the importance of Wnt signaling in the development of the pituitary gland.

## Materials and methods

*Mice and genotyping.* Swiss Webster mice were purchased from Taconic (Germantown, N.Y.), CD1 stocks were obtained from Charles River Laboratories (Wilmington, Mass.), and *Prop1*$^{df/+}$ stocks were obtained from A. Bartke (Southern Illinois University at Carbondale, Ill).

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The nucleotide sequence data reported in this paper have been submitted to GenBank and have been assigned the accession numbers: BE692747–BE693109; BG487405; BG487404; AF363722–AF363726.

CD1 and *Prop1*$^{df/+}$ stocks were maintained at the University of Michigan according to NIH guidelines, and all experiments were approved by the University of Michigan Committee on Use and Care of Animals.

Embryonic material for the cDNA subtraction was generated from *Prop1*$^{df/+}$ heterozygous matings. Developing pituitary glands were dissected from embryonic day 14.5 (e14.5) embryos with the aid of a dissection microscope and were individually frozen at −80°C resting on approximately 50 μl Trizol (Gibco BRL, Gaithersburg, Md.). Embryos were genotyped, and pituitaries of the same genotype were pooled for RNA extraction.

A *Hinf*I restriction fragment length polymorphism (RFLP) was utilized to genotype embryos generated from *Prop1*$^{df/+}$ × *Prop1*$^{df/+}$ matings. The point mutation in the *Prop1*$^{df}$ allele eliminates a *Hinf*I restriction site. Genomic DNA for genotyping assays was extracted from embryonic tails, and approximately 200–500 ng genomic DNA was used as a template for PCR amplification with *Prop1*-specific primers (5′ GAGCTGGGGAGACCTAAGCTTTGCC and 5′ GCCCAGATGTCAGGATACTG) in a 25-μl reaction volume containing 1× Taq polymerase buffer, 0.5 mM each primer, 0.2 mM dNTPs, 0.2 μl BSA (20mg/ml stock), and 0.5 U Taq polymerase under the following cycling parameters: 94°C, 3 min, followed by 30 cycles of 94°C, 30 s; 56°C, 30 s; 72°C, 30 s; followed by a 10-min extension at 72°C. Following amplification, PCR products were incubated with 2U *Hinf*I (Roche, Indianapolis, Ind.) at 37°C for 2 h. Digested PCR products were resolved on 2% agarose gels. The *Prop1*$^{df}$ allele remains uncut (137 bp), whereas the wild-type allele is digested into 97-bp and 40-bp bands.

*cDNA subtraction.* Total RNA was extracted from pools of approximately 30 *Prop1*$^{+/+}$ or *Prop1*$^{df/df}$ embryonic pituitaries in a volume of 0.5 ml TRIZOL (Gibco, BRL) following manufacturer's instructions. RNA was treated with DNase I and purified via RNeasy columns (Qiagen, Valencia, Calif.). Typical RNA yields using this pooling method were 0.5 μg total RNA per *Prop1*$^{df/df}$ pituitary and 0.8 μg total RNA per *Prop1*$^{+/+}$ pituitary. PolyA$^+$ RNA was purified from approximately 17 μg mutant pituitary RNA and 27 μg wild-type pituitary RNA with the Oligotex mRNA Mini Kit (Qiagen). The entire quantity of extracted polyA$^+$ RNA was then used as a template to generate linkered cDNA libraries with the SMART PCR cDNA Synthesis Kit (Clontech, Palo Alto, Calif.). Briefly, full-length first-strand cDNA was generated with a modified oligo (dT) primer and the SMARTII oligonucleotide linker. The resulting *Prop1*$^{+/+}$ and *Prop1*$^{df/df}$ embryonic pituitary linkered libraries were linearly amplified and used as starting material for cDNA subtraction by using the PCR-Select cDNA Subtraction Kit (Clontech) as recommended by the supplier. *Prop1*$^{df/df}$ pituitary transcripts were used as the "driver" and were subtracted away from *Prop1*$^{+/+}$ transcripts, the "tester." Following two rounds of subtraction and the suppression PCR step of this protocol, the subtracted pool of transcripts was T-tail cloned into the pGEMT-easy vector (Promega, Madison, Wis.). Approximately 500 subtracted clones were randomly selected for further analysis.

*Sequence analysis.* All DNA sequencing was performed on ABI Models 377XL, 373XL, or 373A automated DNA sequencers at the University of Michigan sequencing core. Sequences obtained were compared with known genes via BLASTn (Altschul et al. 1997) searches of the nonredundant (nr) database through the NCBI BLAST website (http://www.ncbi.nlm.nih.gov/BLAST/). After single-pass sequencing from the T7 promoter of the pGEMT-easy vector, selected clones were sequenced from the opposite end of the insert to obtain the complete insert sequence. Multiple DNA sequences were aligned by using the ClustalW alignment option of MacVector 6.5.3 (Oxford Molecular Group, Inc, Campbell, Calif.)

*Mapping.* The differential display clone SAC183 and the *Tcf7l2* gene were mapped on the T31 mouse/hamster radiation hybrid panel (Research Genetics, Huntsville, Als.) as previously described (Douglas and Camper 2000). PCR primers from the divergent region of SAC183 [5′GTGAGTCGCTGTGACTTCTTG (primer 2, Fig. 2) and 5′TTATACCCGCACATGTCCAC (primer 3, Fig. 2)] and from the 3′UTR of *Tcf7l2* (5′CCTGTCCATGATGCCTCC and 5′ACACTTCAATCAAGCAGGGG) were used for mapping. *Tcf7l2* 3′ UTR sequences were designed to the *Tcf7l2B* isoform described by Korinek et al. (1998).

*RT-PCR and PCR.* Two independent RNA samples from each tissue examined were analyzed for expression of transcripts as previously described (Douglas and Camper 2000). RT-PCR was performed with primers designed to the divergent region of SAC183 (listed in mapping methods) and to SAC265 (5′AGTCCGGACCTGGGAGAG and 5′AATACCTCATGACGCTCATCG). Embryonic heads, bodies, and pituitaries from e12.5 and e14.5 embryos were surveyed for expression along with adult lung, spleen, kidney, brain, skeletal muscle, liver, heart, testis, and eye. Pituitary cell lines representing *Pit1* precursor (GHFT1; Lew et al. 1993), corticotrope-like (G7, an AtT20 derivative; Gumbiner and Kelly 1982), gonadotrope-like (αT3; Windle et al. 1990), thyrotrope-like (αTSH; Akerblom et al. 1990), and somatomammotrope-like (GH3; Tashjian et al. 1968) cells were surveyed for expression.

The genomic structure of *Tcf7l2* in the region of the SAC183 divergent exon was determined by comparing C57BL/6J genomic DNA to the SAC183 differential display clone. Expand Taq polymerase (Roche) was used to amplify a 4.2-kb C57BL/6J genomic PCR product by using the following primers: 5′GAAATCCACCTCCGCACTTA and 5′TTATACCCGCACATGTCCAC (primers 1 and 3, Fig. 2). The genomic product was cloned into pGEMT-easy (Promega) and sequenced.

Longer cDNAs representing SAC183 containing transcripts were amplified from e12.5 and e14.5 pituitary cDNA by using an SAC183-specific primer and an oligonucleotide designed to the 5′UTR of *Tcf7l2B* described by Korinek et al. (1998): 5′TTATACCCGCACATGTCCAC (primer 3, Fig. 2) and 5′GGGGGGACTCGCAAAACT, respectively.

An adult pituitary cDNA library was screened by PCR (Gage and Camper 1997) by using SAC265 specific primers to identify the partial frizzled2 cDNA reported here.

*In situ hybridization.* In situ hybridization was performed on 6-μ paraffin sections of e12.5 embryos or 18–20 μ cryosections of e14.5 wild-type embryos. Antisense and sense probes were labeled with 10× DIG RNA labeling mix (Roche). For cryosectioned tissue, slides were warmed to room temperature for 30 min, then fixed in 4% paraformaldehyde/1× PBS for 30 min. Paraffin-embedded sections were deparaffinized by soaking in xylene 2× 10 min and rehydrated. After extensive 1× PBS washes, slides were treated with proteinase K: 0.1 μg/ml for 5 min (cryosections) or 10 μg/ml for 15 min (paraffin sections) at 37°C in prewarmed proteinase K buffer (100 mM Tris, pH 8.2; 50 mM EDTA) and subsequently washed in RNase-free water followed by 1× PBS. Sections were acetylated by equilibrating slides in 0.1 M triethanolamine (TEA; Sigma, St. Louis, Mo.) for 5–10 min followed by a 10-min incubation in 0.1 M TEA/0.25% acetic anhydride (Sigma) and washed three times in 1× PBS. Sections were prehybridized in a 5× SSC humidified chamber at 57°C for a minimum of 1 h in the following solution: 50% formamide, 5× SSC, 2% Roche blocking reagent, 0.1% Triton X-100, 0.5% CHAPS (Sigma), 1mg/ml yeast RNA, 5 mM EDTA, and 50 μg/ml heparin. Following prehybridization, labeled probe was added to the prehybridization solution at a concentration of 0.1mg/ml and hybridized to sections overnight at 57°C. Slides were washed in the following solutions: 5× SSC prewarmed to 57°C for 5 min, 0.5× SSC/50% formamide prewarmed to 57°C for 1 h, and 0.5× SSC (room temperature) for 5 min. Sections were then blocked for 1 h in the following blocking solution: 10% sheep serum; 2% BSA; 0.02% sodium azide in 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.1% Triton X-100. Sections were then incubated with an alkaline phosphatase-conjugated anti-DIG antibody (Roche) diluted 1:2000 in blocking solution (1:500 for paraffin sections) for 1 h. Excess antibody was removed through two 10-min washes in 0.05 M Tris (pH 7.5), 0.15 M NaCl, and the pH was re-equilibrated through three 10-min washes in 0.1 M Tris (pH 9.5), 0.05 M MgCl, and 0.1 M NaCl. Slides were incubated overnight with substrates for alkaline phosphate activity, NBT and BCIP (Roche). Following color development, slides were fixed in 4% paraformaldehyde/1× PBS and mounted by using Vectashield (Vector Labs, Burlingame, Calif.).

*Functional analysis of Tcf isoforms.* Full-length cDNAs representing SAC183 containing transcripts were amplified by PCR, and a fragment consisting of the entire coding region was subcloned into the pcDNA3.1+ expression vector (Invitrogen, Carlsbad, Calif.). To assess the ability of novel *Tcf7l2* isoforms to alter TCF-responsive reporter gene expression, 293T cells in 6-well plates were transfected by calcium-phosphate coprecipitation, as described (Erickson et al. 2001). The reporter plasmids used were pTOPFLASH or pFOPFLASH (25 ng; Upstate Biotech, Lake Placid, N.Y.) containing the luciferase reporter gene under the control of

multimeric consensus- or mutant inactive-TCF binding sites, respectively. Expression constructs for β-catenin (10 ng), β-galactosidase (100 ng) and novel *Tcf7l2* (corresponding to Fig. 3A, isoform G) were cotransfected. The total mass of DNA per well was kept constant by adding pcDNA3.1+. Cells were lysed after 48 h, and luciferase and β-galactosidase activities were assayed as described (Erickson et al. 2001). β-galactosidase activity was used to normalize for efficiency of transfection. Normalized luciferase activities were compared with values obtained with reporter gene alone and are reported as fold activation.

## Results

Morphological differences between wild-type and *Prop1*$^{df/df}$ mutant pituitaries are detectable at e14.5 (Gage et al. 1996), 2 days after peak levels of expression of *Prop1* in wild-type mice (Sornson et al. 1996). Thus, expression of PROP1 target genes involved in expansion and cell specification in the pituitary is expected to peak between e12.5 and e14.5. To identify genes expressed during this critical window, embryonic day e14.5 developing pituitary glands were dissected from *Prop1*$^{+/+}$ and *Prop1*$^{df/df}$ embryos, RNA was extracted, and cDNA subtractive hybridization was performed.

Mutant transcripts were subtracted from wild-type transcripts, resulting in a library of clones enriched for genes dependent on PROP1, either directly or indirectly. These transcripts are activated as the pituitary primodium proliferates, doubling in size over a period of 2 days, and the final cell fate decisions necessary for differentiation of somatotropes, thyrotropes, lactotropes, and gonadotropes occur (Carbajo-Perez et al. 1989; Burrows et al. 1999). The library of transcripts was cloned, and 480 clones were randomly picked for further analysis.

DNA sequence analysis of each of the clones revealed an average insert size of 600 bp, validated the subtraction method, and showed a low level of redundancy within the subtracted library. Both *Pit1* and neuronatin are expressed at lower levels in *Prop1*$^{df/df}$ mutant pituitaries relative to wild-type (Gage et al. 1996; Sornson et al. 1996), and both of these genes were identified in the subtraction products (SAC207 and SAC234, respectively). The majority of clone inserts were sequenced in their entirety, and sequence comparisons demonstrate a low level of redundancy in the library. 70% of the clones are represented one time in the library, 13% are represented two times, and one clone is represented six times in the library. Each clone sequence was compared with known genes and expressed sequence tags (ESTs) via BLASTn searches of the non-redundant (nr) and EST (dbest) databases (Altschul et al. 1997). Clones exhibiting sequence similarity to many types of genes were identified (Table 1). Thirty-four percent of the clones, do not have sequence similarity to known genes, but are similar to ESTs (data not shown), and 4% represent novel sequences (GenBank accession numbers BE692930–BE693109).

Expression of a subset of the clones was assessed by using RT-PCR and in situ hybridization. Almost all of the clones tested by RT-PCR were shown to be expressed in the pituitary gland (23/24), confirming the integrity of the dissection. The frizzled2 transcript (SAC265) is present in the developing anterior, intermediate, and posterior lobes (Fig. 1A). A second transcript, SAC324, is identical to a follistatin-like TGF-β inducible gene (Shibanuma et al. 1993) and is expressed in the mesenchymal cells surrounding the developing pituitary gland (Fig. 1C). Transcripts expressed in and adjacent to the developing pituitary have been identified in this screen.

We have utilized two approaches to identify differentially expressed transcripts in the developing pituitary gland. Approximately 100 expressed sequence tags (ESTs) were generated in a differential display screen (Douglas and Camper 2000), and approximately 500 ESTs were generated in the cDNA subtraction experiment described here. There is no sequence overlap between

**Table 1.** Embryonic pituitary transcripts identified have sequence similarity to many types of known genes[a]

| Clone name | Accession number | | Gene name | |
|---|---|---|---|---|
| **Transcription factors/nuclear proteins:** | | | | |
| SAC200* | BE692747 | X | Polybromo | C |
| SAC201 | BE692748 | | Transcription factor IID | H |
| SAC202 | BE692749 | | Thyroid hormone receptor coactivation protein | H |
| SAC203* | BE692750 | X | Zinc finger protein 281 | H |
| SAC204 | BE692751 | X | Nonamer binding protein | M |
| SAC205* | BE692752 | | Dyskerin | M |
| SAC206 | BE692865 | | Sox9 | H |
| SAC207 | BE692866 | X | Pitl | M |
| SAC208 | BE692867 | | Nuclear protein gene | H |
| SAC209 | BE692868 | X | Zinc finger protein 106 | M |
| SAC210 | BE692869 | | CREB binding protein 16 | H |
| SAC211 | BE692870 | | KIAA0164 cDNA | H |
| SAC212 | BE692871 | | SWI/SNF related protein | H |
| SAC213 | BE692872 | X | Nucleolar protein N038 | M |
| SAC214 | BE692873 | | MMSET type I (HMG box) | H |
| SAC215 | BE692874 | X | Mismatch repair protein (MSH6) | M |
| SAC216 | BE692875 | X | Chromatin structural protein (supt4h) | M |
| **Zinc finger proteins:** | | | | |
| SAC217 | BE692876 | | Zinc finger 198 | H |
| SAC218 | BE692877 | X | Zinc finger RNA binding protein | M |
| SAC219 | BE692878 | | C2H2 type Zinc finger | H |
| SAC220 | BE692879 | X | Zinc finger 95 | M |
| SAC221 | BE692880 | X | Zinc finger 54 | M |
| **Membrane associated:** | | | | |
| SAC222 | BE692881 | X | EF hand calcium binding protein p22 | R |
| SAC223 | BE692882 | X | E-selectin ligand-1 (FGFR) variant | M |
| SAC224 | BE692883 | | Na+/K+ transport molecule | M |
| SAC225* | BE692884 | X | Orphan G-protein coupled receptor | M |
| SAC226 | BE692885 | X | S182 protein | M |
| SAC227 | BE692886 | X | Vacuolar proton pump | R |
| SAC228 | BE692887 | X | Vacuolar sorting protein (Mem3) | M |
| SAC229 | BE692888 | X | Integral membrane protein | M |
| SAC230 | BE692889 | X | Vesicle-associated membrane protein 8 | M |
| SAC231 | BE692890 | X | Sigma receptor | M |
| SAC232 | BE692891 | X | Epsilon-sarcoglycan | M |
| SAC233 | BE692892 | X | Cytokine receptor gamma chain | M |
| SAC234* | BE692893 | X | Neuronatin | M |
| SAC235 | BE692894 | | Calnexin | D |
| **Cell cycle control:** | | | | |
| SAC236 | BE692895 | X | Cyclin C | M |
| SAC237 | BE692896 | | Rb binding protein | R |
| **Extracellular matrix molecules:** | | | | |
| SAC238 | BE692897 | X | PGM3 (proteoglycan) | M |
| SAC239 | BE692898 | | N-Cadherin | H |
| SAC240 | BE692899 | X | Neurophilin | M |
| SAC241 | BE692900 | X | Fibrillin-1 | M |
| **Kinases/phosphates:** | | | | |
| SAC242 | BE692901 | X | Protein phosphatase V | R |
| SAC243 | BE692902 | | Phosphatase 2A B56-alpha | H |
| SAC244 | BE692903 | X | Astrocytic phosphoprotein PEA-15 | M |
| SAC245 | BE692904 | X | P21 activated kinase-3 (mPAK-3) | M |
| SAC246 | BE692905 | | cAMP dependent protein kinase type 1 regulatory subunit | R |
| SAC247 | BE692906 | X | PRP4 protein kinase homolog | M |
| SAC248 | BE692907 | X | AMP-activated protein kinase beta subunit | M |
| SAC249 | BE692908 | | Ca2+/calmodulin dependent kinase II alpha subunit | M |
| SAC250 | BE692909 | | Leukocyte common antigen-related phosphatase | M |
| **Structural/motor transport:** | | | | |
| SAC251 | BE692910 | X | Cytoplasmic dynein intermediate chain 2 | M |
| SAC252 | BE692911 | X | Beta-tubulin | R |
| SAC253 | BE692912 | | Scaffold attachment factor | H |
| SAC254 | BE692753 | | Cynein light intermediate chain 53/55 | R |
| SAC255 | BE692754 | | Beta-spectrin | D |
| SAC256 | BE692755 | | Alpha-tubulin II | H |
| SAC257 | BE692756 | X | Unconventional myosin VI | M |
| SAC258 | BE692757 | X | A-X actin | M |
| SAC259 | BE692758 | X | Matrin 3 | R |
| SAC260 | BE692759 | | Tcp-t complex polypeptide | M |
| SAC261 | BE692760 | X | Tctex-1 | M |

*Continued on next page*

**Table 1.** Continued

| Clone name | Accession number | | Gene name | |
|---|---|---|---|---|
| SAC262 | BE692761 | X | Sid23p (actin depolymerizing factor) | M |
| SAC263 | BE692762 | | Alpha-tubulin isotype m-alpha-1 | M |
| SAC264 | BE692763 | | Actin depolymerizing factor | H |
| **Wnt signaling molecules:** | | | | |
| SAC265* | BE692764 | X | Frizzled 2 receptor | M |
| SAC266* | BE692765 | X | Groucho | M |
| SAC267* | BG487405 | X | Beta-catenin | M |
| **Heat shock proteins:** | | | | |
| SAC268 | BE692766 | X | Heat shock protein hsp84 | M |
| SAC269 | BE692767 | X | Heat shock protein hsp86 | M |
| SAC270 | BE692768 | X | DNAJ like protein | M |
| SAC271 | BE692769 | | Heat shock protein HSPCO14 | H |
| SAC272 | BE692770 | X | Heat shock protein hsp60 | M |
| SAC273 | BE692771 | X | Heat shock protein hsp70 | M |
| SAC274 | BE692772 | | Heat shock factor binding protein 1 | H |
| SAC275 | BE692773 | | 70kd Heat shock protein | C |
| **RNA/DNA processing/translational regulation:** | | | | |
| SAC276 | BE692774 | | U5 snRNP protein | H |
| SAC277 | BE692775 | X | DNA topoisomerase | M |
| SAC278 | BE692776 | X | Eifebp1 translation initiation factor 4E binding protein | M |
| SAC279 | BE692777 | | Splicing factor CC1.4 | H |
| SAC280 | BE692778 | | Splicing factor 9G8 | H |
| SAC281 | BE692779 | X | CDC46 (DNA replication) | M |
| SAC282 | BE692780 | X | C11-4 protein (polypeptide release factor) | M |
| SAC283 | BE692781 | | Translation initiation factor 3, subunit 5 | H |
| SAC284 | BE692782 | | U6 snRNA-associated protein | H |
| SAC285 | BE692783 | X | CArG-binding factor-A | M |
| SAC286 | BE692784 | X | p68 RNA helicase | M |
| SAC287 | BE692785 | X | Protein synthesis elongation factor Tu | M |
| SAC288 | BE692786 | | KIAA0332 | H |
| SAC289 | BE692787 | | Origin recognition complex ORC3L subunit | H |
| SAC290 | BE692788 | X | DNA repair protein RAD50 | |
| SAC291 | BE692789 | X | RNPS1 RNA/DNA binding protein | M |
| **Unknown function:** | | | | |
| SAC292 | BE692790 | | Estrogen induced LIV-1 | H |
| SAC293 | BE692791 | | KIAA0663 protein | H |
| SAC294 | BE692792 | X | Coiled-coil protein | M |
| SAC295 | BE692793 | | RANP-1 protein | R |
| SAC296 | BE692794 | X | Small acidic protein (sid2057p) | M |
| SAC297 | BE692795 | | cDNA mapping to HSA 22q13 | H |
| SAC298 | BE692796 | | KIAA0217 protein | H |
| SAC299 | BE692797 | | KIAA0383 protein | H |
| SAC300 | BE692798 | X | B61 protein | M |
| SAC301* | BE692799 | | Set protein | R |
| SAC302 | BE692800 | | DEAD box protein p72 | H |
| SAC303 | BE692801 | | KIAA0966 protein | H |
| SAC304 | BE692802 | X | WSB-1 protein | M |
| SAC305 | BE692803 | | KIAA0235 protein | H |
| SAC306 | BE692804 | X | SH3 containing protein SH3P7 | M |
| SAC307 | BE692805 | | KIAA0077 protein | H |
| SAC308 | BE692806 | X | Scq protein | M |
| SAC309 | BE692807 | X | von Hippel-Lindau binding protein | M |
| SAC310 | BE692808 | X | Mtprd protein | M |
| SAC311* | BE692809 | X | QM protein | M |
| SAC312 | BE692810 | | CGI-114 protein | H |
| SAC313 | BE692811 | | MAGE tumor antigen D1 | H |
| SAC314 | BE692812 | | CGI-29 protein | H |
| SAC315 | BE692813 | X | Alpha 4 protein | M |
| SAC316 | BE692814 | | Myristolyled alanine-rich C kinase substrate | M |
| SAC317 | BE692815 | | Breast cancer resistance protein | H |
| SAC318 | BE692816 | X | GEG-154 protein | M |
| SAC319 | BE692817 | | KIAA0637 protein | |
| SAC320 | BE692818 | | Thymus expressed acidic protein | M |
| SAC321 | BE692819 | X | E25 protein | M |
| SAC322 | BE692820 | | CGI-39 protein | H |
| SAC323 | BE692821 | X | Impact protein | M |
| **Miscellaneous:** | | | | |
| SAC324* | BE692822 | X | Follistatin-like TGF-beta inducible gene | M |
| SAC325* | BE692823 | X | Prothymosin alpha | M |
| SAC326 | BE692824 | X | Retinal short-chain dehydrogenase/reductase | M |
| SAC327 | BE692825 | | Rod-1 RNA binding protein | R |
| SAC328* | BE692826 | X | BMP6 | M |
| SAC329 | BE692827 | X | Alpha-catenin | M |
| SAC330 | BE692828 | X | Nip21 (associates with Bcl-2) | M |
| SAC331 | BE692829 | X | Prion protein | M |
| SAC332 | BE692830 | X | Complement component C5 protein | M |
| SAC333 | BE692831 | | Histone protein | M |
| SAC334 | BE692832 | | Clathrin-associated adaptor protein | R |
| SAC335 | BE692833 | | Adapter related protein complex 2-beta subunit | H |
| SAC336 | BE692834 | | Clathrin-associated/assembly/adapter protein (CLAPB1) | H |
| SAC337 | BE692835 | X | L14 lectin | M |
| SAC338* | BE692836 | X | Thrombospondin 3 | M |
| SAC339 | BE692837 | | RAB8 | H |
| **Metabolic/housekeeping:** | | | | |
| SAC340 | BE692838 | X | GM3 synthase | M |
| SAC341 | BE692839 | | Oxoglutarate carrier protein | H |
| SAC342 | BE692840 | X | Ubiquitin activating enzyme | M |
| SAC343 | BE692841 | X | Ubiquitin | M |
| SAC344 | BE692842 | | N-actylglucosamine galactosyltransferase | M |
| SAC345 | BE692843 | | Ribophorin MARib | M |
| SAC346 | BE692844 | | High glucose regulated protein 8 | H |
| SAC347 | BE692845 | X | Ubiquitin-conjugating enzyme | M |
| SAC348 | BE692846 | | Arginine methyltransferase | H |
| SAC349 | BE692847 | | NAD(H)-specific isocitrated dehydrogenase alpha subunit | H |
| SAC350 | BE692848 | X | Housekeeping type protein (MER5) | M |
| SAC351 | BE692849 | X | Aldolase A | M |
| SAC352 | BE692850 | | P5 protein | M |
| SAC353 | BE692851 | X | Ubiquitin specific protease 14 | H |
| SAC354 | BE692852 | X | Lysophosphatidic acid acyltransferase | M |
| SAC355 | BE692853 | | Aromatic-L-aa decarboxylase | M |
| SAC356 | BE692854 | X | Alpha globin | M |
| SAC357 | BE692855 | X | Cathepsin B | M |
| SAC358 | BE692856 | X | Cytochrome b5 | R |
| SAC359 | BE692857 | | Proteasome subunit, non-ATPase 12 | H |
| SAC360 | BE692858 | X | Cyclophilin | M |
| SAC361 | BE692859 | | Serine palmitoyl transferase subunit II | M |
| SAC362 | BE692860 | X | Protective protein (Mo54) | M |
| SAC363 | BE692861 | X | COP9 complex subunit 3 | M |
| SAC364 | BE692862 | X | Histone deacetylase | M |
| SAC365 | BE692863 | | Golgi stacking protein | R |
| SAC366 | BE692864 | | Calpain-like protease (Capa6) | R |
| SAC367 | BE692913 | X | Epoxide hydrolase | M |
| SAC368 | BE692914 | X | Golgi protein p22 | M |
| SAC369 | BE692915 | | Arp2/3 protein complex subunit p34Arc | H |
| SAC370 | BE692916 | X | NRD convertase | M |
| SAC371 | BE692917 | X | Phospholy-cerate kinase | M |
| SAC372 | BE692918 | X | Carbon catabolite repression 4 protein | M |
| SAC373 | BE692919 | | EPS glutamyl-prolyl tRNA synthetase | H |
| SAC374 | BE692920 | X | 54K subunit of signal recognition particle | M |
| SAC375 | BE692921 | X | cGMP phosphodiesterase | M |
| SAC376 | BE692922 | X | Ubiquitin-conjugating enzyme E214K | H |
| SAC377 | BE692933 | | Monamine oxidase A | R |
| SAC378 | BE692924 | | Signal recognition particle 72 | H |
| SAC379 | BE692925 | X | Macrophage ferritin heavy subunit | M |
| SAC380 | BE692926 | X | Glutathione S-transferase | M |
| SAC381 | BE692927 | X | Oligosaccharyltransferase | M |
| SAC382 | BE692928 | X | Transcobalmin II | M |
| SAC383 | BE692929 | X | Alpha-enolase X5 | M |

[a] Clone names are presented in the far left column. An X to the left of the gene name indicates >95% sequence identity between the subtracted clone and the gene listed. The far right column indicates the species from which the database gene sequence was derived. M, mouse; H, human; R, rat; D, dog; C, chicken. Clones with validated embryonic pituitary expression are indicated with an asterisk to the right of the clone name.

the differential display EST set and the sequences presented here. The partial transcripts represented in the differential display EST set are biased toward the 3′ end of the transcript, while partial transcripts from cDNA subtractive hybridization can come from any region of the transcripts. Future comparisons of full-length transcripts identified from both techniques may reveal overlap between the two sets of embryonic pituitary transcripts.

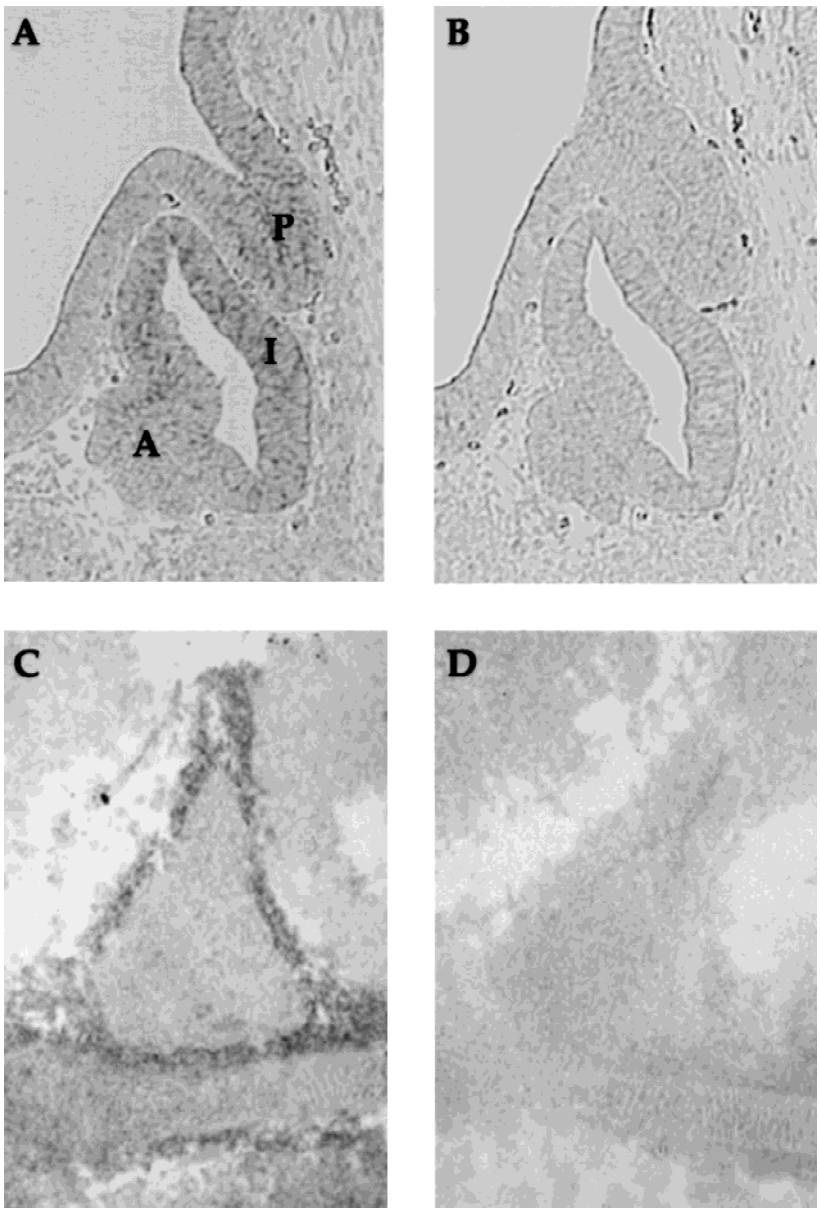


**Fig. 1.** cDNA subtraction products are expressed in the developing pituitary gland. **A–B.** Frizzled2 expression in e12.5 wild-type pituitary; A. Anti-sense probe, B. Sense probe. **C–D.** SAC324 expression in e14.5 wild-type pituitary; C. Anti-sense probe, C. Sense probe. P, posterior lobe; A, anterior lobe; I, intermediate lobe.

Comparisons of embryonic pituitary EST sequences to known genes via BLAST searches revealed that a number of genes involved in the Wingless (Wg)/Wnt signaling pathway are present in the libraries we generated. A novel isoform of the transcription factor, TCF4 (T-cell factor 7 like-2, *Tcf7l2*), and *Apc* were identified by using differential display. Partial cDNAs encoding frizzled2 (*Fzd2*), β-catenin, and groucho were identified in the subtraction experiment.

*Novel isoforms of Tcf7l2.* Clone SAC183 (GenBank accession BG487404) is a 365-bp EST identified through differential display analysis of genes expressed at e12.5 and e14.5 in the developing pituitary gland (Douglas and Camper 2000). BLASTn analysis of the differential display clone revealed 100% identity over a 136-nucleotide region to *Tcf7l2*. The last 129 bp of SAC183 shows only 36% identity to known *Tcf7l2* isoforms (Fig. 2). Furthermore, the divergent sequence of SAC183 encodes a stop codon that would terminate the peptide prior to the DNA binding domain. Oligonucleotides designed to the sequence spanning the breakpoint in conservation with other *Tcf7l2* isoforms (Fig. 2, primers 1 and 3) amplified a product of the predicted size and sequence from adult brain cDNA, indicating that SAC183 is a bonafide transcript (data not shown).

Oligonucleotides designed to the divergent region of SAC183 (Fig. 2, primers 2 and 3) and the 3′UTR of *Tcf7l2* were used for mapping on the T31 mouse/hamster radiation hybrid panel. Both SAC183 and *Tcf7l2* map to distal MMU 19 between *D19Mit104* and *D19Mit50* (data not shown). The human ortholog of *Tcf7l2* maps to a region of HSA10q (Duval et al. 2000) that exhibits synteny homology to this region of MMU 19. The mapping data are consistent with the hypothesis that SAC183 is a novel isoform of *Tcf7l2*.

Both human and mouse *Tcf7l2* transcripts are extensively alternatively spliced, and numerous *Tcf7l2* mRNA isoforms generating proteins with different C-termini have been previously reported in mouse (summarized in Fig. 3, A–E; Cho and Dressler 1998; Korinek et al. 1998; Lee et al. 1999; Duval et al. 2000). To determine the origin of the divergent sequence in clone SAC183,

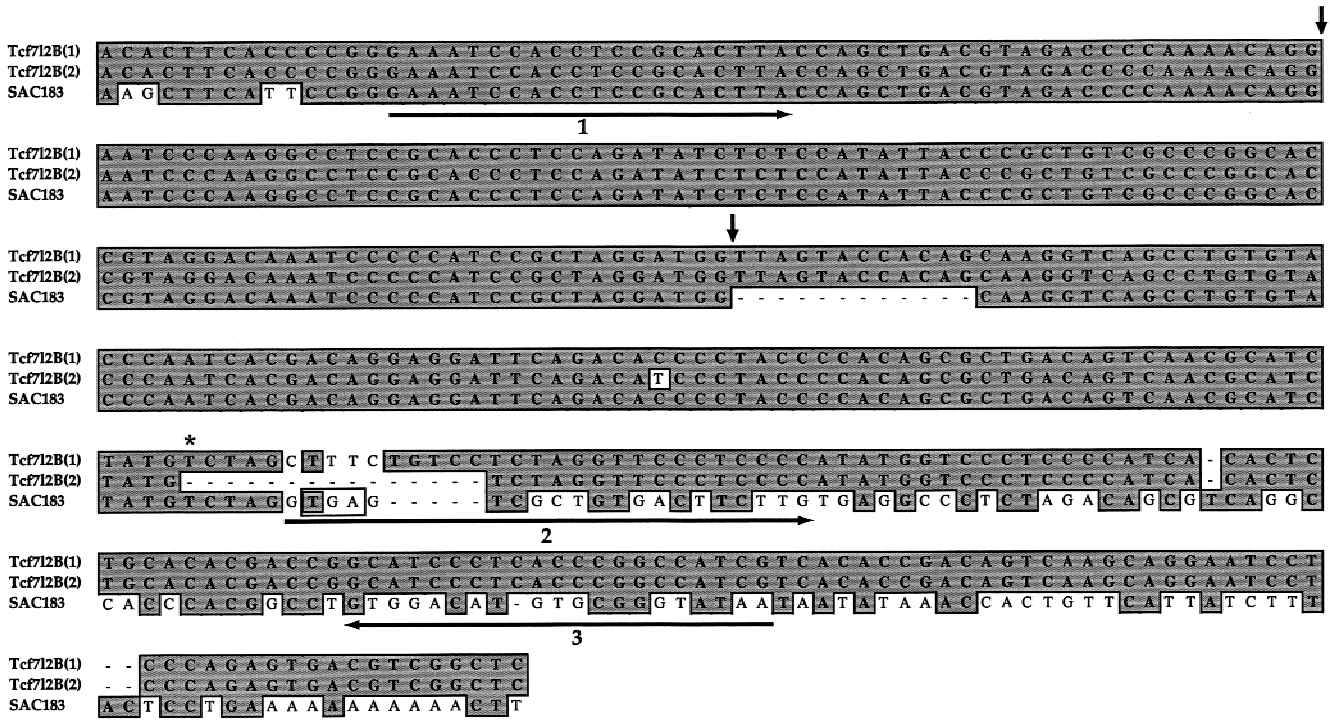


**Fig. 2.** Nucleotide sequence alignment of differential display clone, SAC183, to known *Tcf7l2* isoforms. Nucleotides corresponding to 834-1211 of GenBank entry AF107298 [represented as Tcf7l2B(1)] and 568-930 of GenBank entry NM 009333 [represented as Tcf7l2B(2)] are shown aligned to the SAC183 nucleotide sequence. The Tcf7l2B(1) isoform is identical the the Tcf7l2B(2) isoform except for the addition of 15 nucleotides (marked with an asterisk). This is the site where the sequence of SAC183 significantly diverges from other *Tcf7l2* isoforms. A termination codon is present shortly after the break in conservation of sequence (indicated by box); thus, the SAC183 transcript is predicted to lack a DNA binding domain. Long arrows indicate oligonucleotides used as PCR primers. Vertical arrows indicate exon/exon boundaries.

primers flanking the break in conservation between SAC183 and known *Tcf7l2* isoforms were used to amplify C57BL/6J genomic DNA (primers 1 and 3, Fig. 2). The resulting 4.2-kb product was cloned and sequenced (GenBank accession AF363722). Clone SAC183 cDNA sequence was compared with genomic sequence to determine intron/exon boundaries. Interestingly, there is no exon/exon boundary at the breakpoint in sequence conservation (marked by an asterisk in Fig. 2). This implies that intra-exon splicing occurs in the post-transcriptional processing of the *Tcf7l2* mRNA.

Clone SAC183 and the *Tcf7l2B* isoform reported by Lee and colleagues (1999) (represented as schematic C, Fig. 3) diverge from the amino terminal consensus sequence at the same residue, indicating a variation of exon/exon boundaries generated by alternative splicing. The amino acid sequence of each previously reported mouse *Tcf7l2* isoform (Fig. 3, A–E) and the putative protein sequence of a heart EST (Figure 3, I) from the *Tcf7l2* UniGene cluster (Mm.10712) were aligned. This analysis revealed six isoforms of *Tcf7l2*. Three different amino terminal and four different carboxy terminal sequences were identified (Fig. 3).

SAC183 represents a partial transcript; however, the deduced amino acid sequence of SAC183 predicts a truncated protein without a DNA binding domain. Longer cDNAs of the SAC183 isoform were amplified with an oligonucleotide designed to the 5′UTR of *Tcf7l2* and primer 3 (Fig. 2). Three different isoforms were identified from both e12.5 and e14.5 pituitary samples (Fig. 3, F–H). Similar *Tcf7l2* isoforms were independently cloned from 3T3-L1 preadipocytes and adipocytes. Two novel, alternatively spliced exons were identified (Fig. 3, black box in F and C-terminal black box in H). The N-terminal alternatively spliced exon depicted in schematic H has similarity to a human exon and is also present in a heart EST (Fig. 3, I, Duval et al. 2000). The three alternatively spliced isoforms of *Tcf7l2* identified in this study indicate that N-terminal alternative splicing occurs in mouse *Tcf7l2*. RNase protection assays with SAC183 as a probe show that these novel *Tcf7l2* isoforms are present in low amounts relative to previously described *Tcf7l2* isoforms (data not shown).

The expression pattern of SAC183 was analyzed by RT-PCR and in situ hybridization. A panel of embryonic tissues, adult tissues, and pituitary cell lines was examined for SAC183 expression by RT-PCR by using oligonucleotides designed to the divergent region of SAC183 (See Materials and methods for tissues examined). SAC183 is widely expressed and was detected in all tissues examined except the eye (data not shown). Interestingly, SAC183 is detected in the *Pit1* lineage precursor cell line (GHFT1) but not in corticotrope-like, gonadotrope-like, or thyrotrope-like cells. This suggests that this isoform of *Tcf7l2* is not ubiquitously expressed among specialized pituitary cells, leaving open the possibility that it could influence cell differentiation in the pituitary.

In situ hybridization was performed to compare the spatial patterns of expression of *Tcf7l2* and SAC183 in e12.5 and e14.5 embryos. No obvious differences were detected (data not shown). Expression was detected at high levels in the thalamus and the roof of the midbrain, and lower levels were detected in the floor of the diencephalon, the anterior hypothalamus, and the pontine flexure. A low but consistent level of expression was detected in the pituitary gland, mesenchymal cells underlying the pituitary, the tongue, and intestinal tissue. This confirms and extends known *Tcf7l2* expression sites (Cho and Dressler 1998; Korinek et al. 1998; Lee et al. 1999).

We hypothesized that the novel *Tcf7l2* isoforms would act as endogenous inhibitors of Wnt signaling, because they are predicted to bind β-catenin, but not DNA. To test this hypothesis, we performed reporter gene assays, using plasmids containing three copies of consensus (pTOPFLASH) or mutated (pFOPFLASH) TCF-binding sites upstream of the thymidine kinase (TK) minimal promoter and luciferase open reading frame (van de Wetering et al. 1997). In the presence of increasing amounts of the *Tcf7l2* isoform G, diagrammed in Fig. 3, the activation of TCF-responsive

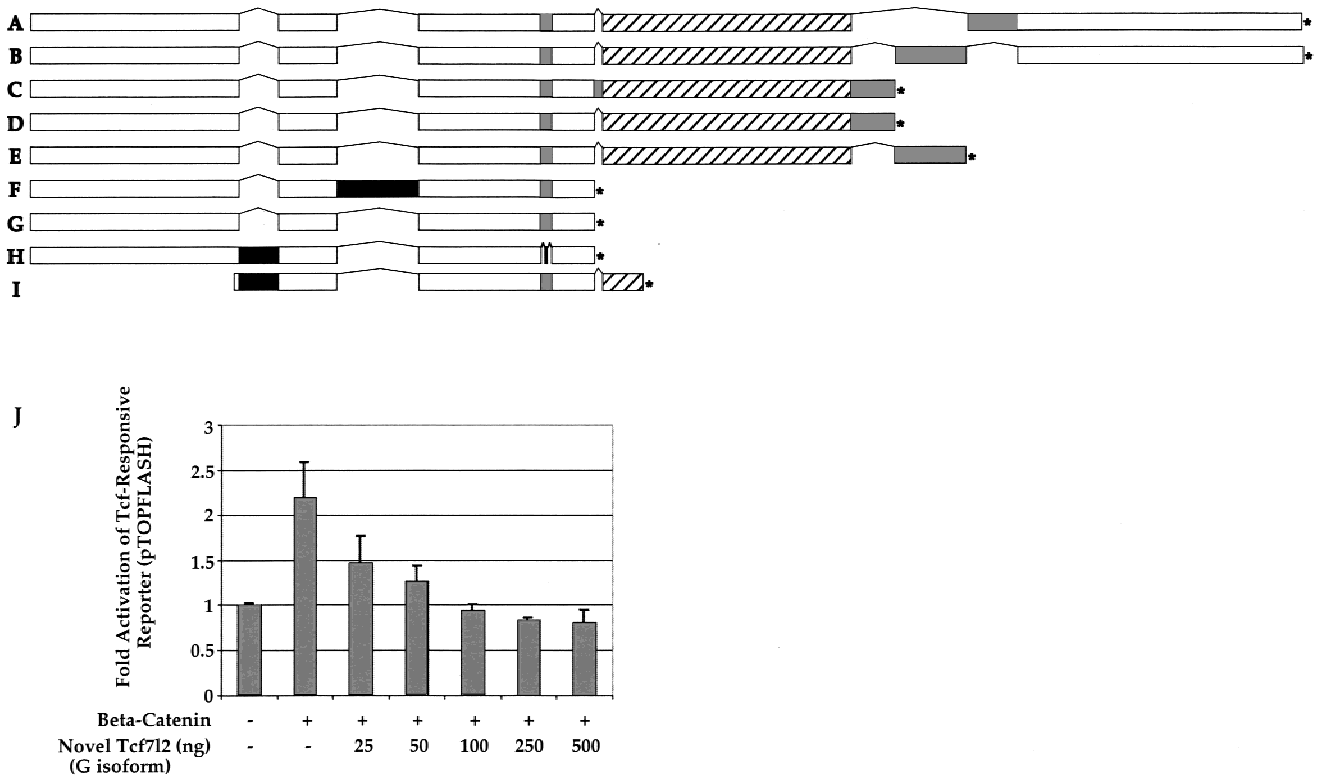


**Fig. 3.** Schematic diagram depicting TCF7L2 isoforms generated by alternative splicing. A–I. The amino acid sequences of each previously reported TCF7l2 isoform were aligned with the deduced amino acid sequences of the novel SAC183-containing transcripts identified in this study. Schematic representations of the alignments are shown. Non-alternatively spliced exons are shown as white boxes, alternatively spliced exons are shown as gray boxes, novel exons reported here are shown as black boxes, and the DNA binding domain is indicated as a hatched box. Asterisks indicate the positions of stop codons. Five alternatively spliced isoforms have previously been described (A–E). Three N-terminal alternatively spliced isoforms were identified in this study (F–H). The N-terminal novel exon in H (black box) is identical to an exon found in a heart EST (schematized in I). This heart EST represents a partial cDNA, but likely contains a DNA binding domain indicating that N-terminal alternative splicing can occur in TCF7L2 isoforms containing or lacking a DNA binding domain. Amino acid sequences were identified from the following sources: A. TCF7L2E (GenBank Accession AAD16968; Lee et al. 1999); B. TCF7L2E (this sequence is not in GenBank; Cho and Dressler 1998); C. TCF7L2B (GenBank accession AAD16967; Lee et al. 1999); D. TCF7L2B (this sequence is not in GenBank; Cho and Dressler 1998); E. TCF7L2B (GenBank Accession CAA11071; Korinek et al. 1998); F. Longer SAC183 cDNA (GenBank Accession AF363724); G. Longer SAC183 cDNA (GenBank Accession AF363725); H. Longer SAC183 cDNA (GenBank Accession AF363726); I. Heart EST (GenBank Accession AA671377). J. Inhibition of Tcf-responsive reporter (pTOPFLASH) activation by a novel *Tcf7l2* isoform in transiently transfected 293T cells. To correct for transfection efficiency, luciferase activity was normalized to β-galactosidase activity. Fold activation was determined by comparing luciferase activity to pTOPFLASH alone values. Results are reported as mean luciferase activity ± range (n = 2) and are representative of three independent experiments.

pTOP-FLASH by exogenous β-catenin decreased to levels at or below background (Fig. 3J). Similar results were observed for the two other novel *Tcf7l2* isoforms (data not shown). Total transfected DNA was kept constant by the addition of pcDNA3.1+, which minimizes the possibility of promoter squelching or other nonspecific effects. In addition, β-catenin and increasing amounts of the novel *Tcf7l2* had no effect on the activity of the negative control, pFOPFLASH (data not shown). This suggests that these novel isoforms may act as endogenous inhibitors by decreasing the ability of β-catenin to activate TCF-responsive target genes. However, the mechanism for novel *Tcf7l2* isoform action may be more complicated, because these isoforms appear to potentiate β-catenin activation of the Cyclin-D1 promoter (data not shown). This suggests the importance of promoter context and functional interactions with transcription factors other than TCF/LEFs.

*Parital mouse frizzled2 cDNA.* A 560-bp EST (clone SAC265) identified through cDNA subtraction shows sequence similarity to rat, human, and Xenopus orthologs of frizzled2. Each member of the large family of frizzled cell surface receptors has a similar protein structure composed of extracellular cysteine-rich ligand binding and linker domains, seven transmembrane domains, and an intracellular C-terminus (Wodarz and Nusse 1998). To obtain a longer cDNA of the mouse frizzled2 ortholog identified by clone SAC265, an adult pituitary cDNA library was screened by PCR with SAC265 specific primers, and a 940-bp partial cDNA was identified. Amino acid sequence alignments of the mouse FZD2 sequence identified here (GenBank Accession AF363723), human FZD2 (GenBank Accession NP 001457), rat FZD2 (GenBank Accession AAA41172), and frog FZD2 (GenBank Accession AA06359) revealed complete amino acid identity with human FZD2 and only four amino acid changes compared with either rat or frog FZD2. Thus, we identified the mouse ortholog of FZD2. The recently reported sequence of mouse frizzled10 (Malik and Shivdasani 2000) is 100% identical to mouse frizzled2, and the gene has been officially renamed *Fzd2.*

The expression pattern of *Fzd2* was determined by RT-PCR in a panel of embryonic and adult tissues. *Fzd2* expression was detected in embryonic tissues at e12.5 and e14.5 and in adult brain, heart, lung, skeletal muscle, kidney, and pituitary. *Fzd2* was not detected in the liver, spleen, testis, or eye. Our expression data are largely consistent with previous reports for mouse, human, and rat *Fzd2* expression (Chan et al. 1992; Sagara et al. 1998; Malik and Shivdasani 2000).

Expression was detected in the embryonic pituitary gland at e12.5 and e14.5 and in the adult pituitary gland by RT-PCR. *Fzd2*

expression was detected in the G7, GHFT1, αTSH, and αT3 pituitary cell lines, suggesting broad expression within the adult organ (data not shown). Furthermore, the partial frizzled2 cDNA isolated from the adult pituitary cDNA library was used as an in situ hybridization probe. *Fzd2* expression is detectable in the developing anterior, intermediate, and posterior lobes of the pituitary gland (Fig. 1A).

## Discussion

PROP1 mutations cause hypoplasia of the pituitary gland and deficiencies in most of the pituitary hormones. No direct targets of PROP1 are known. We have applied the cDNA subtractive hybridization approach to compare transcripts expressed in wild-type and *Prop1*$^{df/df}$ embryonic pituitaries at e14.5. The library we report here contains over 350 transcripts, none of which were identified by a differential display screen of embryonic pituitary transcripts (Douglas and Camper 2000). Ten novel transcripts were identified; thus, this partial expression profile represents a significant advance for the field.

Two genes known to be reduced in *Prop1*$^{df/df}$ pituitaries, *Pit1* and neuronatin, were identified in the subtracted library, indicating that this library is enriched for PROP1-dependent genes. The library also contains transcripts that encode transcription factors, cell surface molecules, and cell cycle regulators, as well as ESTs not previously known to be expressed in the developing pituitary gland and several novel clones. This library is a resource for identification of targets for genes such as *Pitx2*, *Lhx3*, and *Hesx1*, in addition to *Prop1*, because each of these genes influences early pituitary development. Validation of differential expression of individual transcripts is necessary and is currently under way.

We demonstrated that a follistatin-like TGF-β-inducible gene, SAC324, is expressed in the mesenchymal cells surrounding the pituitary gland. This expression pattern is intriguing because several signaling molecules of the TGFβ superfamily are expressed in the region of the pituitary gland at this time during development (Ericson et al. 1998; Treier et al. 1998) and could induce expression of this gene. Furthermore, ventral mesenchymal cells, themselves, secrete signaling molecules important for pitutiary development (Ericson et al. 1998; Treier et al. 1998).

Comparisons between transcripts identified in a differential display screen (Douglas and Camper 2000) and the subtracted library described here reveal that several members of the Wnt signaling cascade are expressed in the developing pituitary gland. We identified a novel isoform of *Tcf7l2*, the *Fzd2* receptor, *Apc*, β-catenin, and groucho in the developing pituitary expression libraries. This is the first report identifying members of the Wnt signaling cascade in the developing pituitary and provides further support for the importance of Wnt signaling in its ontogeny. Specifically, FZD2 and the novel TCF7L2 isoforms might regulate Wnt-mediated developmental cues responsible for specification and expansion of anterior lobe cell types.

The novel *Tcf7l2* isoforms we identified reveal two previously unknown alternatively spliced exons. This alternative splicing introduces a novel stop codon that leads to truncation prior to the DNA binding domain. These isoforms decrease activation of a TCF-responsive reporter gene, suggesting that they can act as inhibitors of TCF-responsive gene expression. The SAC183 isoform of *Tcf7l2* is expressed in a *Pit1* precursor cell line, but is not expressed in cell lines representing differentiated pituitary cell types. This could mean that SAC183 has a role in undifferentiated pituitary cells that is similar to the role of TCF7L2 in the maintenance of intestinal stem cells (Korinek et al. 1998).

We have identified the mouse ortholog of frizzled2 in this screen and show that it is expressed throughout the developing pituitary. Wnt molecules transmit their signal via frizzled receptors. This identification of *Fzd2* in the developing pituitary is the first report of a possible receptor for Wnt molecules expressed in the region.

Many human patients with multiple pituitary hormone deficiencies do not have mutations in HESX1, LHX3, PROP1, or POU1F1 (the human ortholog of *Pit1*), indicating that other genes regulating the differentiation of these cell types exist. A subset of the transcripts described here may be targets of these transcription factors and could explain pituitary hormone deficiencies with unknown etiology.

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## References

Aizawa K, Akahira S, Akimura T, Arai A, Arakawa T et al. (2000) RIKEN Mouse ESTs. Unpublished

Akerblom IE, Ridgway EC, Mellon PL (1990) A α-subunit-secreting cell line derived from a mouse thyrotrope tumor. Mol Endocrinol 4, 589–596

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, et al. (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25, 3389–3402

Burrows HL, Douglas KR, Seasholtz AF, Camper SA (1999) Genealogy of the anterior pituitary gland: tracing a family tree. Trends Endocrinol Metab 10, 343–352

Carbajo-Perez E, Motegi M, Watanabe YG (1989) Cell proliferation in the anterior pituitary of mice during growth. Biomed Res 10, 275–281

Chan SDH, Karpf DB, Fowlkes ME, Hooks M, Bradley MS et al. (1992) Two homologs of the Drosophila polarity gene *frizzled* (*fz*) are widely expressed in mammalian tissues. J Biol Chem 267, 25202–25207

Cho EA, Dressler GR (1998) TCF-4 binds beta-catenin and is expressed in distinct regions of the embryonic brain and limbs. Mech Dev 77, 9–18

Douglas K and Campter S (2000) Partial transcriptome of the developing pituitary gland. Genomics 70, 335–346

Duval A, Rolland S, Tubacher E, Bui H, Thomas G, Hamelin R (2000) The human T-cell transcription factor-4 gene: structure, extensive characterization of alternative splicings, and mutational analysis in colorectal cancer cell lines. Cancer Res 60, 3872–3879

Erickson R, Hemati N, Ross S, MacDougald O (2001) p300 coactivates the adipogenic transcription factor CCAAT/enhancer-binding protein α. J Biol Chem 276, 16348–16355

Ericson J, Norlin S, Jessell TM and Edlund T (1998) Integrated FGF and BMP signaling controls the progression of progenitor cell differentiation and the emergence of pattern in the embryonic anterior pituitary. Development 125, 1005–1015

Gage PJ, Camper SA (1997) Pituitary homeobox 2, a novel member of the *bicoid*-related family of homeobox genes, is a potential regulator of anterior structure formation. Hum Mol Genet 6, 457–464

Gage PJ, Brinkmeier ML, Scarlett LM, Knapp LT, Camper SA, Mahon KA (1996) The Ames dwarf gene, *df*, is required early in pituitary ontogeny for the extinction of *Rpx* transcription and initiation of lineage specific cell proliferation. Mol Endocrinol 10, 1570–1581

Gumbiner B, Kelly RB (1982) Two distinct intracellular pathways transport secretory and membrane glycoproteins to the surface of pituitary tumor cells. Cell 28, 51–59

Korinek V, Barker N, Moerer P, van Donselaar E, Huls G et al. (1998a) Depletion of epithelial stem-cell compartments in the small intestine of mice lacking Tcf-4. Nat Genet 19, 379–83

Korinek V, Barker N, Willert K, Molenaar M, Roose J et al. (1998b) Two members of the Tcf family implicated in Wnt/beta-catenin signaling during embryogenesis in the mouse. Mol Cell Biol 18, 1248–1256

Lee YJ, Swencki B, Shoichet S, Shivdasani RA (1999) A possible role for the high mobility group box transcription factor Tcf-4 in vertebrate gut epithelial cell differentiation. J Biol Chem 274, 1566–1572

Lew D, Brady H, Klausing K, Yaginuma K, Theill LE et al. (1993) GHF-1

promoter-targeted immortalization of a somatotropic progenitor cell results in dwarfism in transgenic mice. Genes Dev 7, 683–693

Malik TH, Shivdasani RA (2000) Structure and expression of a novel frizzled gene isolated from the developing mouse gut. Biochem J 349, 829–834

Sagara N, Toda G, Hirai M, Terada M, Katoh M (1998) Molecular cloning, differential expression, and chromosomal localization of human frizzled-1, frizzled-2, and frizzled-7. Biochem Biophys Res Commun 252, 117–122

Sheng HZ, Westphal H (1999) Early steps in pituitary organogenesis. Trends Genet 15, 236–240

Shibanuma M, Mashimo J, Mita A, Kuroki T, Nose K (1993) Cloning from a mouse osteoblastic cell line of a set of transforming-growth-factor-beta 1-regulated genes, one of which seems to encode a follistatin-related polypeptide. Eur J Biochem 217, 13–19

Sornson MW, Wu W, Dasen JS, Flynn SE, Norman DJ et al. (1996) Pituitary lineage determination by the *Prophet* of *Pit-1* homeodomain factor defective in Ames dwarfism. Nature 384, 327–333

Tashjian AH, Yasumura Y, Levine L, Sato GH, Parker ML (1968) Establishment of clonal strains of rat pituitary tumor cells that secrete growth hormone. Endocrinology 82, 342–352

Treier M, Rosenfeld MG (1996) The hypothalamic-pituitary axis: co-development of two organs. Curr Opin Cell Biol 8, 833–843

Treier M, Gleiberman AS, O'Connell SM, Szeto DP, McMahon JA et al. (1998) Multistep signaling requirements for pituitary organogenesis in vivo. Genes Dev 12, 1691–1704

van de Wetering M, Cavallo R, Dooijes D, van Beest M, van Es J et al. (1997) Armadillo coactivates transcription driven by the product of the Drosophila segment polarity gene dTCF. Cell 88, 789–799

Wang J, Shackleford GM (1996) Murine Wnt10a and Wnt10b: cloning and expression in developing limbs, face and skin of embryos and in adults. Oncogene 13, 1537–1544

Windle JJ, Weiner RI, Mellon PL (1990) Cell lines of the pituitary gonadotrope lineage derived by targeted oncogenesis in transgenic mice. Mol Endocrinol 4, 597–603

Wodarz A, Nusse R (1998) Mechanisms of Wnt signaling in development. Annu Rev Cell Dev Biol 14, 59–88