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Zinc Finger Protein Sall2 Is Not Essential for Embryonic and Kidney Development

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***SALL*/*Sall* is a mammalian homolog of the *Drosophila* region-specific homeotic gene *spalt* (*sal*), and heterozygous mutations in *SALL1* in humans lead to Townes-Brocks syndrome. We earlier reported that mice deficient in *Sall1* die in the perinatal period and that kidney agenesis or severe dysgenesis are present. We have now generated mice lacking *Sall2*, another *Sall* family gene. Although *Sall2* is expressed mostly in an overlapping fashion versus that of *Sall1*, *Sall2*-deficient mice show no apparent abnormal phenotypes. Morphology and gene expression patterns of the mutant kidney were not affected. Mice lacking both *Sall1* and *Sall2* show kidney phenotypes comparable to those of *Sall1* knockout, thereby demonstrating the dispensable roles of *Sall2* in embryonic and kidney development.**

Drosophila sal is the region-specific homeotic gene characterized by unique multiple double zinc finger motifs (10). *sal* was first identified by its capacity to promote terminal differentiation, and it is expressed in anterior and posterior compartments of *Drosophila* (5). Mutations in *sal* cause head and tail segments to develop trunk structures. *sal* also plays a critical role in wing development (4, 13). *sal* is expressed at the anterior-posterior boundary of wing imaginal disks, and its expression is controlled by *dpp* (BMP-4 ortholog), the expression of which is highest at the boundary and which is in turn controlled by *hedgehog* expressed in the posterior compartment.

Humans and mice have at least three *sal*-related genes, respectively (*SALL1*, *-2*, and *-3* for humans and *Sall1*, *-2*, and *-3* for mice) (2, 6–8, 15). *SALL1* is located on chromosome 16q12.1, and heterozygous mutations of *SALL1* lead to Townes-Brocks syndrome, an autosomal-dominant disease with features of dysplastic ears, preaxial polydactyly, imperforate anus and, less commonly, kidney and heart anomalies (9). Mice deficient in *Sall1* die in the perinatal period, and kidney agenesis or severe dysgenesis are present (14). *Sall1* is expressed in the metanephric mesenchyme surrounding ureteric bud, and homozygous deletion of *Sall1* results in an incomplete ureteric bud outgrowth and failure of tubule formation in the mesenchyme. Therefore, *Sall1* is essential for ureteric bud invasion, the initial key step for metanephros development.

Another *Sall* family gene, *SALL2* is located on human chromosome 14q12, possibly overlapping a region of loss of heterozygosity in ovarian cancers (1). Mouse *Sall2* binds to polyomavirus large T antigen and is proposed to be a potential tumor suppressor (11). Although mouse *Sall2* was reported to be expressed during development and abundantly in the adult brain (6), precise expression patterns and the physiological function of *Sall2* have remained unknown. We now report generation of *Sall2*-deficient mice, and in these animals we found that *Sall2* is dispensable for normal developmental processes. We also present phenotypes of mice lacking both *Sall1* and *Sall2*.

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MATERIALS AND METHODS

Cloning of *Sall2* genome. PCR was done by using as a template for fetal kidney cDNA obtained 14.5 days postcoitus (dpc) to clone *Sall2.* The resulting 188-bp product was used to screen the 129SvJ genomic library (Stratagene).

Generation of *Sall2*-deficient mice. The targeting vector was constructed by incorporating the 5′ *Bam*HI-*Eco*RI 6.0-kb fragment and the 3′ *Sma*I-*Bam*HI 1.7-kb fragment into a vector that contained the neomycin-resistant (Neo^r) gene (pMC1-NeopolyA) and a diphtheria toxin A subunit (pMC1-DTA) in tandem. The 5′ fragment was subcloned into a *Not*I-*Xho*I site 5′ of the Neo^r gene, and the 3′ fragment was cloned into an *Eco*RV site 3′ of the Neo^r gene. The construct was linearized with *Not*I.

E14.1 embryonic stem cells were plated on mitomycin C-treated primary embryonic fibroblasts and clones resistant to G418 (400 μg/ml) were screened by using Southern blots. The genomic DNA from clones was digested with *Eco*RI, electrophoresed through 0.7% agarose, transferred to nylon membrane (HybondN+; Amersham-Pharmacia), and hybridized to a radioactive probe. The probe used to screen the samples was a *Bam*HI-*Bam*HI 0.6-kb fragment downstream of the 3′ homology (probe B). The samples were also digested with *Spe*I and *Xho*I and then hybridized with the 5′ probe (probe A) to confirm the correct homologous recombination. A probe corresponding to the Neo^r sequence was also used to verify that only one copy of the vector was integrated into the genome. Of 120 clones, 6 were correctly targeted.

Recipient blastocysts were from C57BL/6J mice. Chimeric animals were bred with C57BL/6J females. Mutant animals studied were of F_2 and F_3 generations. Mice were genotyped by using Southern blots or genomic PCR. The primer sequences used for PCR were as follows: CACATTTCGTGGGTCACAAG, CTCAGAGCTGTTTTCCTGGG , and GCGTTGGCTACCCGTGATAT (188 bp for the wild-type *Sall2* allele and 380 bp for the mutated allele). To screen *Sall1* mutants, we used AGCTAAAGCTGCCAGAGTGC, CAACTTGCGATT GCCATAAA, and GCGTTGGCTACCCGTGATAT (288 bp for the wild-type *Sall1* allele,and 350 bp for the mutated allele).

The probes used for Northern blots were as follows: the Sall1-*Xho*I *Sall1* fragment (2.5 kb), the *Eco*RI-*Sma*I N-terminal *Sall2* fragment (2.0 kb), the *Sma*I-*Hin*dIII C-terminal *Sall2* fragment (1.4 kb), and the N-terminal *Sall3* fragment (2.0 kb).

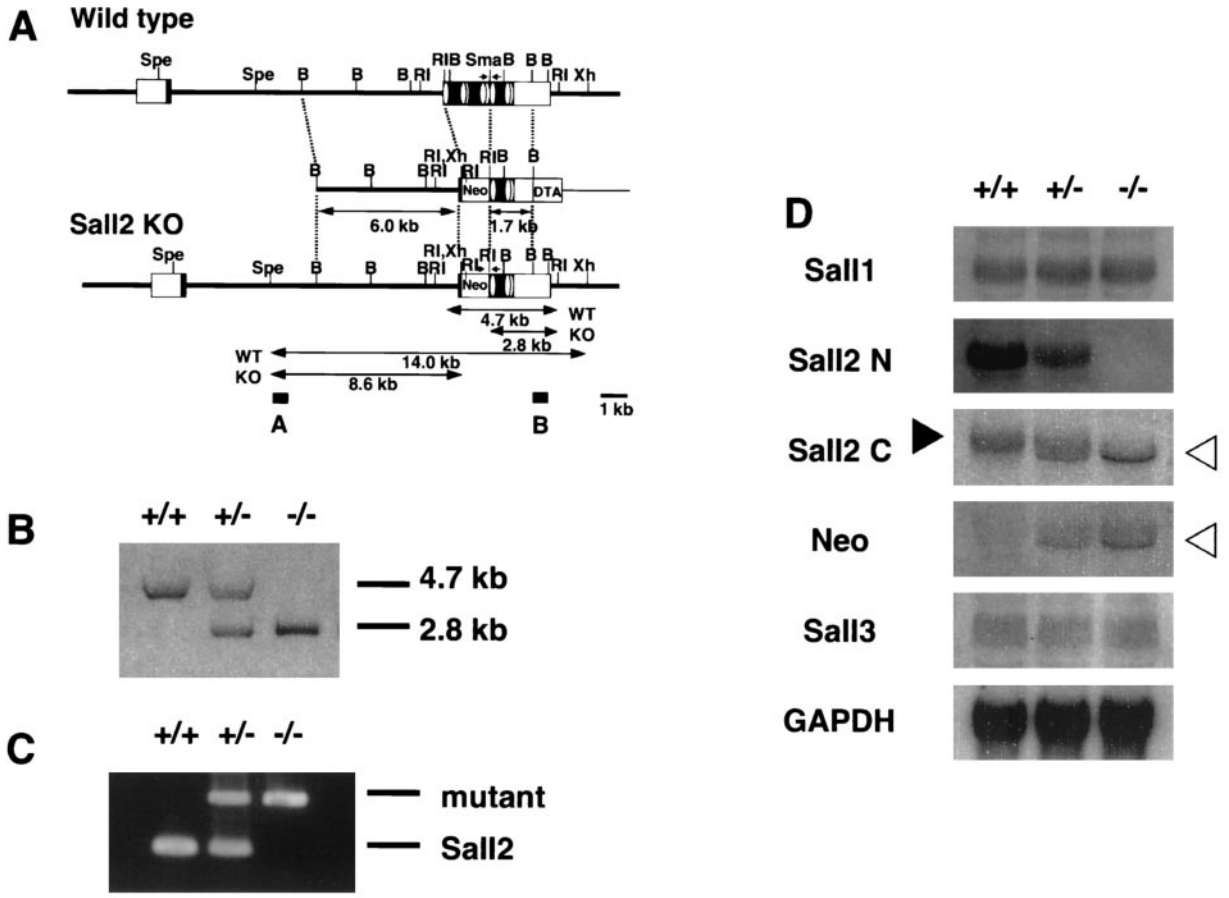

FIG. 1. Generation of *Sall2*-deficient mice. (A) Targeting strategy of *Sall12* locus. Positions of the zinc finger motifs are indicated by ovals. Restriction sites: B, *Bam*HI; RI, *Eco*RI; Spe, *Spe*I; Sma, *Sma*I; Xh, *Xho*I. (B) Southern blot analysis of wild-type (+/+), heterozygous (+/−), and homozygous (−/−) *Sall2*-deficient mice. Tail DNA was digested with *Eco*RI and hybridized with probe B. (C) Genomic PCR of wild-type (+/+), heterozygous (+/−), and homozygous (−/−) *Sall2*-deficient mice. The 388-bp band was amplified from the mutant allele, and the 188-bp band was amplified from the wild-type *Sall2* genome. The positions of the PCR primers are indicated by arrows in panel A. (D) Northern blotting analysis of *Sall* genes in *Sall2*-deficient embryos at 13.5 dpc. Note that N-terminal *Sall2* probe gave no signal in *Sall2*-deficient mice. In the case of the C-terminal *Sall2* probe, the *Sall2* band was absent in *Sall2*-deficient mice (solid arrowhead), but a shorter band appeared that also hybridized with Neo^r probe (open arrowheads).

Histological examination and in situ hybridization. Samples were fixed in 10% formalin and processed for paraffin-embedded sections (6-μm thick), followed by double staining with hematoxylin and eosin.

In situ hybridization was done with digoxigenin-labeled antisense riboprobes as described previously (14). A 1-kb fragment of *Sall2* cDNA corresponding C-terminal three zinc fingers was amplified by using PCR, subcloned into pCRII (Invitrogen), and sequenced. Antisense transcript was generated with SP6 polymerase. Other probes were as described previously (14). None of the sense probes yielded signals.

Peripheral blood count and renal parameter measurement. Cardiac puncture was done in 12-week-old mice, and samples were processed with Celltac α (Nihon Koden, Japan) for peripheral blood counts.

Next, 10- to 14-month-old mice were used to measure blood urea nitrogen and creatinine in serum with an automatic analyzer 7150 (Hitachi, Tokyo, Japan). Urinary protein was measured by using Pretest (Wako, Tokyo, Japan) containing tetrabromophenol blue.

RESULTS AND DISCUSSION

Generation of *Sall2*-deficient mice. To examine developmental functions, we inactivated *Sall2* in the mouse by using embryonic stem cells (Fig. 1A). The *Sall2* gene consists of two exons, the intron being ca. 12 kb. All eight zinc finger domains are located in exon 2. We generated a targeting construct, which deleted the N-terminal five zinc fingers. Chimeras from two independent homologous recombinants transmitted the mutations through the germ line. Mice were genotyped by using Southern blots and genomic PCR (Fig. 1B and C). Northern blots confirmed that full-length *Sall2* transcript was indeed absent in *Sall2*-deficient mice, with either the N-terminal region or the C-terminal region of *Sall2* as a probe (Fig. 1D). Probing with the C-terminal region of *Sall2*, however, showed a slightly shorter transcript in heterozygous and homozygous mice and was expressed more abundantly in the latter. This transcript was also evident with a Neo^r probe, indicating that it may be a transcript that read through the poly(A) addition signal of Neo^r and fused to C-terminal *Sall2* (Fig. 1D). We isolated this cDNA from *Sall2*-deficient mice and found that this was indeed the case. There were several stop codons in the junction and the *Sall2* region was out of frame (data not shown). There were no other irregular transcripts indicative of aberrant *Sall2* molecules. Therefore, it is unlikely that the functional C-terminal protein of Sall2 was expressed in the mutant mice. Expression of *Sall1* and *Sall3*

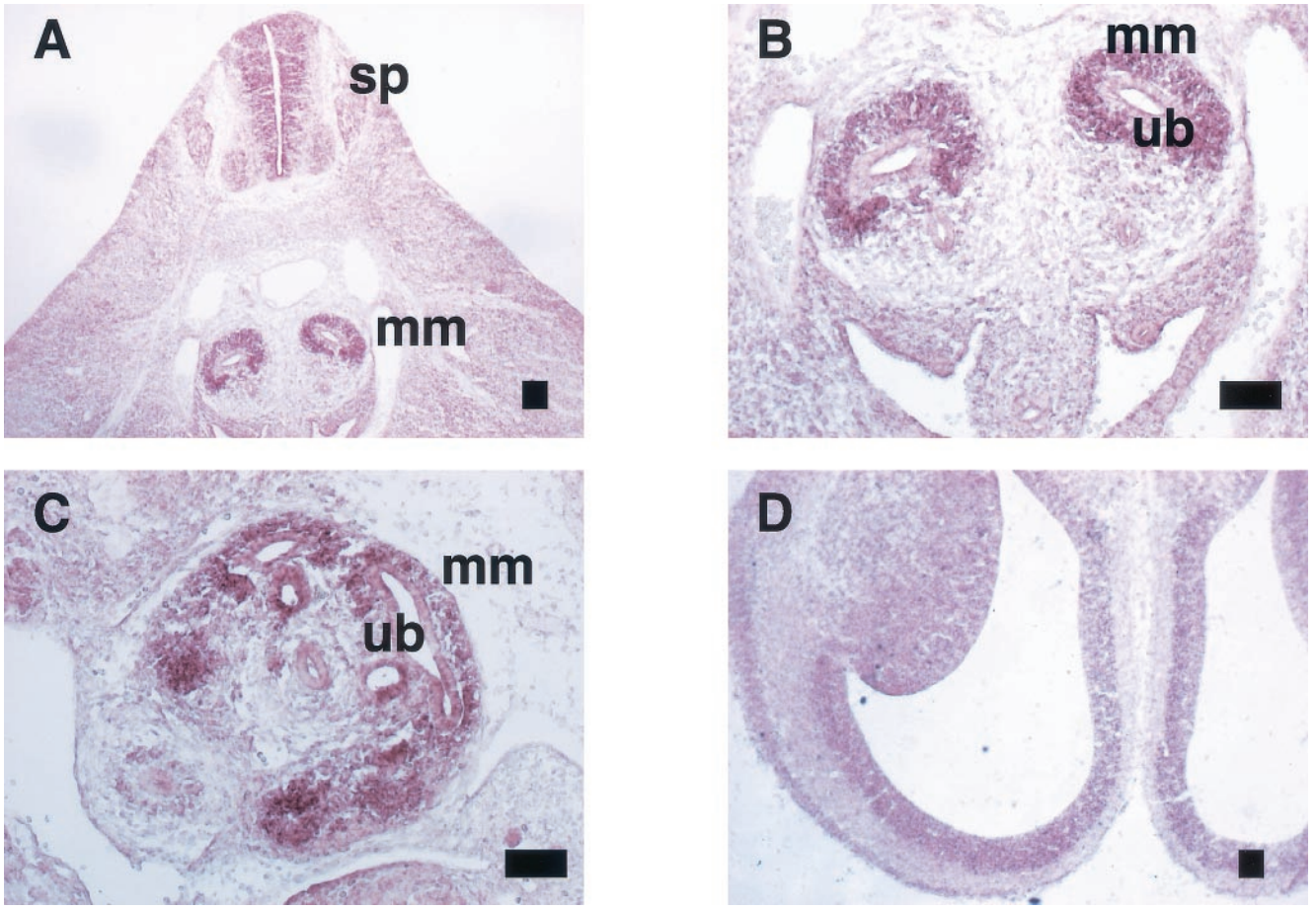

FIG. 2. Expression of *Sall2* in developing embryos. (A) Metanephros and spinal cord at 11.5 dpc (sp, spinal cord; mm, metanephric mesenchyme); (B) metanephros at 11.5 dpc (ub, ureteric bud; mm, metanephric mesenchyme); (C) metanephros at 13.5 dpc; (D) brain at 14.5 dpc. Scale bar, 100 μm.

was not altered in the absence of *Sall2*, as determined by using Northern blots (Fig. 1D).

Expression patterns of Sall2. Transverse sections obtained 11.5 dpc showed *Sall2* expression in the metanephric mesenchyme surrounding the ureteric bud and subventricular region of the spinal cord (Fig. 2A,B). At 13.5 dpc, *Sall2* expression was observed in the mesenchyme around the ureteric buds in the cortical regions of the developing kidney (Fig. 2C). *Sall2* was also expressed in the subventricular zone of the brain at 14.5 dpc (Fig. 2D). This expression pattern partly overlaps that of mouse *Sall1* (3, 14, 16).

Normal phenotypes in Sall2-deficient mice. No obvious phenotype was observed in the heterozygous mutants. When heterozyotes were intercrossed, the homozygous mice were of Mendelian frequency (Table 1), they had a normal appearance, and both male and female homozygotes were fertile. We found no abnormalities despite extensive anatomical examinations. Figure 3A to F show an almost-normal histology of the *Sall2* mutant kidney, heart, and ears at 13.5 dpc. The hematological parameters of peripheral blood samples were also normal (Table 2).

The expression patterns of well-characterized molecular markers of either metanephric mesenchyme or ureteric bud-derived cells were also examined.

Sall1 is expressed in the metanephric mesenchyme, and the expression of *Sall1* was not altered in the absence of *Sall2*, findings consistent with the data in Fig. 1D (Fig. 4A and B).

Pax2-deficient mice do not develop mesonephric tubules and lack ureteric buds (21). In metanephros at 13.5 dpc, *Pax2* is expressed both in the ureteric bud and in the condensed mesenchyme surrounding the ureteric bud (Fig. 4C). In *Sall2* mutant mice, the expression of *Pax2* was unaltered in both these locations (Fig. 4D).

Wnt4 is required for epithelialization of the induced mesenchyme but not for the initial induction by the ureter (20). *Wnt4* is expressed in mesenchymal cells on the sides of the ureteric bud and correlates to the site where the first pretubular aggre-

TABLE 1. Genotype analysis of mice from *Sall2* heterozygous intercrosses[a]

Mouse	*n*	% of total
+/+	26	27.7
+/−	46	48.9
−/−	22	23.4

[a] DNA was extracted from the tails of 3-week-old mice and analyzed by using PCR as for Fig. 1C.

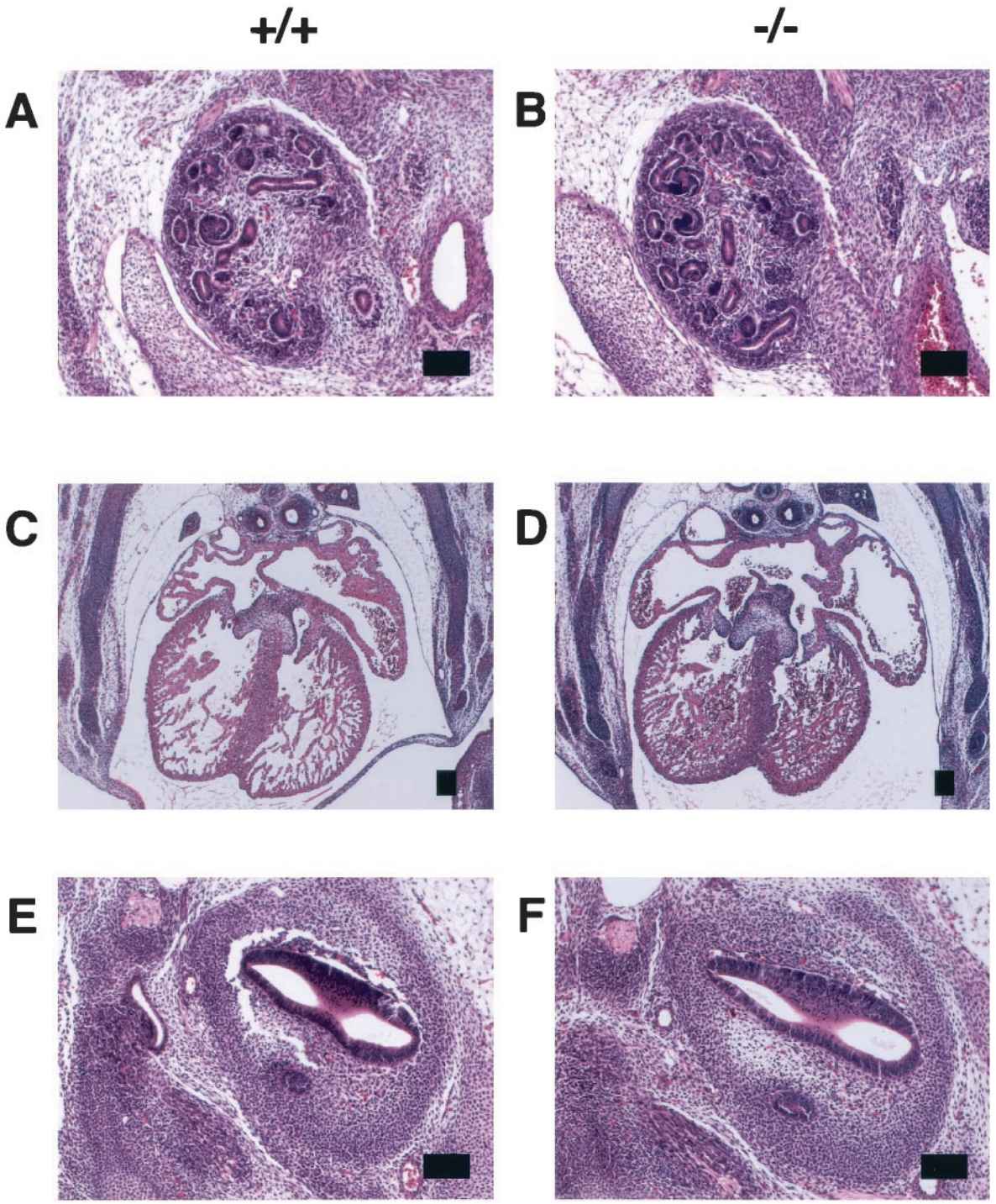

FIG. 3. Histology in *Sall2*-deficient mice at 13.5 dpc. (A and B) Kidneys in wild-type (A) and *Sall2*-deficient mice (B); (C and D) hearts in wild-type (C) and *Sall2*-deficient mice (D); (E and F) inner ears in wild-type (E) and *Sall2*-deficient mice (F). Scale bars, 100 μm.

gates form (Fig. 4E). *Sall2*-deficient mice showed unaltered *Wnt4* expression (Fig. 4F).

Mice deficient in the tyrosine-kinase type receptor, *Ret*, show a failure of ureteric bud invasion and subsequent failure of mesenchymal differentiation (12, 17–19). *Ret* was expressed in the ureteric bud in the wild type, and its expression in *Sall2* mutant mice was unaltered (Fig. 4G and H). These results

TABLE 2. Peripheral blood counts of *Sall2*-deficient mice

Mouse (*n*)	Mean amt (SD)				
	Leukocytes (10^3/μl)	Erythrocytes (10^6/μl)	Hemoglobin (g/dl)	Hematocrit (%)	Platelets (10^3/μl)
+/+ (9)	8.0 (2.4)	8.7 (2.9)	16.1 (1.6)	52.9 (6.8)	920 (248)
+/− (6)	10.6 (5.5)	9.5 (1.4)	16.6 (1.5)	51.7 (7.0)	821 (86)
−/− (8)	9.9 (3.8)	10.1 (0.6)	17.1 (0.8)	54.9 (2.7)	819 (117)

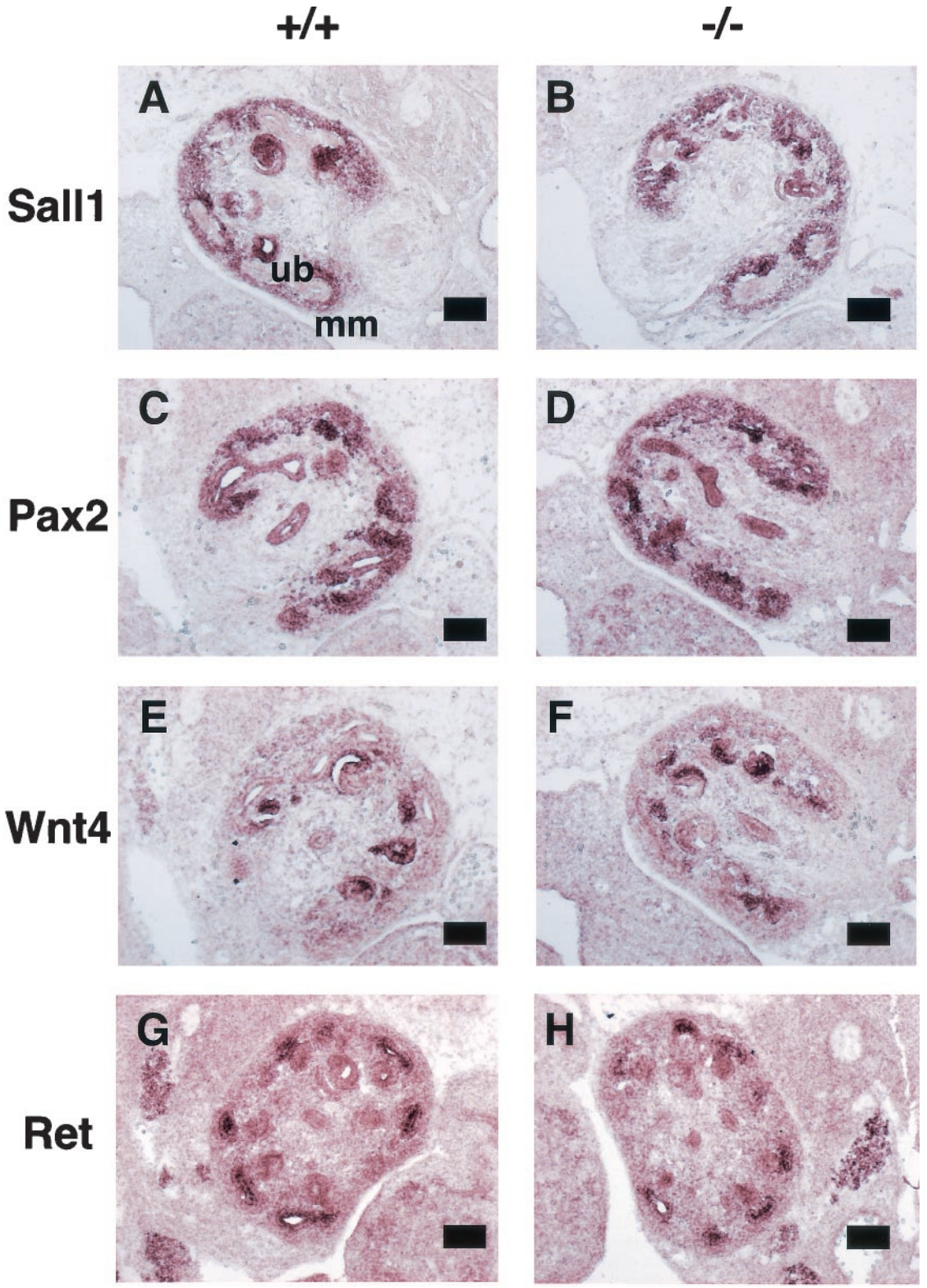

FIG. 4. In situ hybridization of molecular markers in 13.5-dpc metanephros of wild-type (left panels [A, C, E, and G]) and *Sall2*-deficient mice (right columns [B, D, F, and H]). Scale bars, 100 μm. (A and B) *Sall1*; (C and D) *Pax2*; (E and F) *Wnt4*; (G and H) *Ret*.

TABLE 3. Genotype analysis of mice from *Sall1*$^{+/-}$ *Sall2*$^{-/-}$ intercrosses[a]

Sall1	*Sall2*	*n*	% of total
+/+	−/−	12	23.1
+/−	−/−	28	53.8
−/−	−/−	12	23.1

[a] DNA was extracted from the tails of newborn mice and analyzed by using PCR.

indicate that markers of metanephric mesenchyme and ureteric bud were not affected in the absence of *Sall2* and that *Sall2* is not required for normal kidney development.

There was no limb deformity, anorectal anomaly, or ear anomaly, all of which are characteristic of Townes-Brocks syndrome, which is caused by *SALL1* mutation. We suggest that

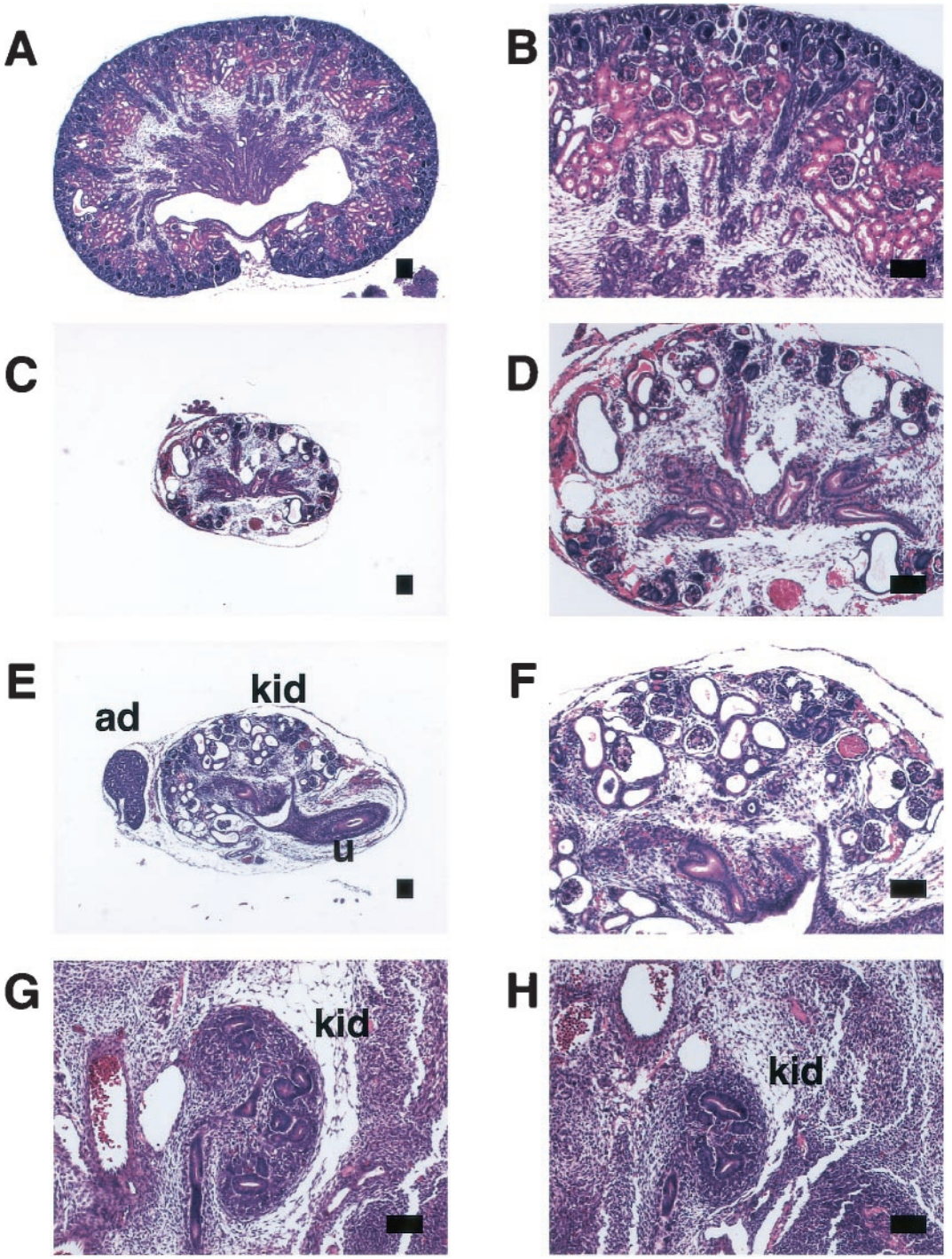

FIG. 5. Kidney development in *Sall1/2* double deficient mice. (A and B) Kidney of wild-type newborn. (C and D) Kidney of *Sall1/2* double deficient mice. The kidney is small and contains multiple cysts. (E and F) Kidney of *Sall1*-deficient mice, which shows a similar histology to *Sall1/2* doubly deficient mice. (G) Metanephros in wild-type mice at 12.5 dpc. Branching is evident. (H) Metanephros in *Sall1/2* doubly deficient mice at 12.5 dpc. Kidney size and ureteric branching are reduced. kid, kidney; ad, adrenal gland; u, ureter. Scale bars, 100 μm.

TABLE 4. Renal function in adult animals[a]

Sall1	*Sall2*	*n*	Mean level (SD)	
			BUN[b] (mg/dl)	Creatinine (mg/dl)
+/+	+/+	5	27.3 (2.4)	0.30 (0.03)
+/−	+/−	8	28.7 (3.4)	0.29 (0.04)
+/+	−/−	8	24.5 (4.3)	0.31 (0.04)
+/−	−/−	7	24.0 (4.1)	0.31 (0.03)

[a] Ten- to fourteen-month-old mice were used.
[b] BUN, blood urea nitrogen.

Sall2 is not essential for development and that *Sall2* absence may be compensated for by other *Sall* genes, the expression of which overlaps with that of *Sall2*.

Human *SALL2* is located on chromosome 14q12, possibly overlapping a region of loss of heterozygosity in ovarian cancers (1). In addition, mouse *Sall2* binds to polyomavirus large T antigen and was proposed to be a potential tumor suppressor (11). *Sall2* mutant mice, however, did not show spontaneous tumor formation for more than 1 year after birth. Tumor formation upon virus inoculation will be required to test the hypothesis that *Sall2* is a tumor suppressor.

Kidney defects in mice lacking both *Sall1* and *Sall2*. Mouse *Sall1* is essential for the initial step for metanephros formation: ureteric bud attraction. Hence, kidney agenesis or severe dysgenesis was present in *Sall1*-deficient mice. Other organs, however, were not affected, although heterozygous mutations of human *SALL1* lead to Townes-Brocks syndrome, with features of dysplastic ears, preaxial polydactyly, imperforate anus, and heart anomalies in addition to kidney anomalies. The relative importance of *SALL1* over *SALL2* and *-3* may be higher in humans than in mice, and *Sall1* deficiency may be compensated for by *Sall2* and *-3* in mice.

To address this question, we crossed *Sall1* and *Sall2* mutants and generated mice lacking both genes. Some pups from a double heterozygous cross were dead perinatally and had kidney abnormalities; most were *Sall1* single mutants, but we did find some double homozygotes. To further confirm the phenotypes, we set up pairs of $Sall1^{+/-}$ $Sall2^{-/-}$ mice and found that double mutants were born at a Mendelian frequency (Table 3). All of the double mutants, as well as the *Sall1* mutants, were dead perinatally, and they all had kidney abnormalities. Of 12 double mutants (25.0%), 3 had no kidneys or ureters bilaterally. Four mice (33.3%) had unilateral kidney agenesis and hypoplasia on the other side. Five mice (41.7%) had two small remnant kidneys. Histological examination of all of the residual kidneys in the double mutant newborn showed size reduction and multiple cysts, which are comparable to the *Sall1* mutants (Fig. 5A to F). At 12.5 dpc, size reduction and impaired ureteric branching were observed in *Sall1*/*2*-null mutants, which is also comparable to findings in the *Sall1* mutants (Fig. 5G and H and data not shown). Thus, the severity of the kidney impairment of the double mutants was comparable to that of *Sall1* single mutants reported earlier (14), indicating that *Sall2* absence does not exacerbate the kidney defects caused by *Sall1* mutation.

To address the role of *Sall* genes in maintaining renal function in adults, several parameters were examined in aged $Sall1^{+/-}$ $Sall2^{-/-}$ mutants, as well as in $Sall1^{+/-}$ $Sall2^{+/-}$ mutants, *Sall2* single mutants, and wild-type control (Table 4). Blood urea nitrogen and creatinine levels in serum were not significantly different among the four groups. Urinary protein was undetectable in all animals tested. Although these parameters are not sensitive enough for detecting minor renal malfunction, the data do suggest that the absence of *Sall2* or reducing *Sall1* dosage upon *Sall2* mutant background does not lead to overt kidney diseases in adult mice.

Furthermore, the double mutants showed no phenotypes of Townes-Brocks syndrome, such as dysplastic ears, preaxial polydactyly, and imperforate anus. These data suggest that the discrepancy of the mutant phenotypes of human *SALL1* and mouse *Sall1* cannot be explained by compensation by *Sall2* in mice. Generation of mice lacking all of the *Sall* genes will be necessary in order to address the developmental roles of *Sall* genes.

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REFERENCES

1. **Bandera, C. A., H. Takahashi, K. Behbakht, P. C. Liu, V. A. LiVolsi, I. Benjamin, M. A. Morgan, S. A. King, S. C. Rubin, and J. Boyd.** 1997. Deletion mapping of two potential chromosome 14 tumor suppressor gene loci in ovarian carcinoma. Cancer Res. **57:**513–515.
2. **Buck, A., L. Archangelo, C. Dixkens, and J. Kohlhase.** 2000. Molecular cloning, chromosomal localization, and expression of the murine *SALL1* ortholog *Sall1*. Cytogenet. Cell Genet. **89:**150–153.
3. **Buck, A., A. Kispert, and J. Kohlhase.** 2001. Embryonic expression of the murine homologue of SALL1, the gene mutated in Townes-Brocks syndrome. Mech. Dev. **104:**143–146.
4. **de Celis, J. F., R. Barrio, and F. C. Kafatos.** 1996. A gene complex acting downstream of *dpp* in *Drosophila* wing morphogenesis. Nature **381:**421–424.
5. **Jurgens, G.** 1988. Head and tail development of the *Drosophila* embryos involves *spalt*, a novel homeotic gene. EMBO J. **7:**189–196.
6. **Kohlhase, J., M. Altmann, L. Archangelo, C. Dixkens, and W. Engel.** 2000. Genomic cloning, chromosomal mapping, and expression analysis of *msal-2*. Mamm. Genome **11:**64–68.
7. **Kohlhase, J., S. Hausmann, G. Stojmenovic, C. Dixkens, K. Bink, W. Schulz-Schaeffer, M. Altmann, and W. Engel.** 1999. *SALL3*, a new member of the human *spalt*-like gene family, maps to 18q23. Genomics **62:**216–222.
8. **Kohlhase, J., R. Schuh, G. Dowe, R. P. Kuhnlein, H. Jackle, B. Schroeder, W. Schulz-Schaeffer, H. A. Kretzschmar, A. Kohler, U. Muller, M. Raab-Vetter, E. Burkhardt, W. Engel, and R. Stick.** 1996. Isolation, characterization, and organ-specific expression of two novel human zinc finger genes related to the *Drosophila* gene *spalt*. Genomics **38:**291–298.
9. **Kohlhase, J., A. Wischermann, H. Reichenbach, U. Froster, and W. Engel.** 1998. Mutations in the *SALL1* putative transcription factor gene cause Townes-Brocks syndrome. Nat. Genet. **18:**81–83.
10. **Kuhnlein, R. P., G. Frommer, M. Friedrich, M. Gonzalez-Gaitan, A. Weber, J. F. Wagner-Bernholz, W. J. Gehring, H. Jackle, and R. Schuh.** 1994. *spalt* encodes an evolutionarily conserved zinc finger protein of novel structure which provides homeotic gene function in the head and tail region of the *Drosophila* embryo. EMBO J. **13:**168–179.
11. **Li, D., K. Dower, Y. Ma, Y. Tian, and L. T. Benjamin.** 2001. A tumor host range selection procedure identifies $p150^{sal2}$ as a target of polyoma virus large T antigen. Proc. Natl. Acad. Sci. USA **98:**14619–14624.
12. **Moore, M. W., R. D. Klein, I. Farinas, H. Sauer, M. Armanini, H. Phillips, L. F. Reichardt, A. M. Ryan, K. Carver-Moore, and A. Rosenthal.** 1996. Renal and neuronal abnormalities in mice lacking GDNF. Nature **382:**76–79.
13. **Nellen, D., R. Burke, G. Struhl, and K. Basler.** 1996. Direct and long-range action of a DPP morphogen gradient. Cell **85:**357–368.
14. **Nishinakamura, R., Y. Matsumoto, K. Nakao, K. Nakamura, A. Sato, G. N. Copeland, J. D. Gilbert, A. N. Jenkins, S. Scully, L. D. Lacey, M. Katsuki, M. Asashima, and T. Yokota.** 2001. Murine homolog of *Sall1* is essential for ureteric bud invasion in kidney development. Development **128:**3105–3115.
15. **Ott, T., K. H. Kaestner, A. P. Monaghan, and G. Schutz.** 1996. The mouse homolog of the region specific homeotic gene *spalt* of *Drosophila* is expressed in the developing nervous system and in mesoderm-derived structures. Mech. Dev. **56:**117–128.
16. **Ott, T., M. Parrish, K. Bond, A. Schwaeger-Nickolenko, and A. P. Monaghan.** 2001. A new member of the spalt like zinc finger protein family, Msal3, is expressed in the CNS and sites of epithelial/mesenchymal interaction. Mech. Dev. **101:**203–207.

17. **Pichel, J. G., L. Shen, H. Z. Sheng, A. C. Granholm, J. Drago, A. Grinberg, E. J. Lee, S. P. Huang, M. Saarma, B. J. Hoffer, H. Sariola, and H. Westphal.** 1996. Defects in enteric innervation and kidney development in mice lacking GDNF. Nature **382:**73–76.
18. **Sanchez, M. P., I. Silos-Santiago, J. Frisen, B. He, S. A. Lira, and M. Barbacid.** 1996. Renal agenesis and the absence of enteric neurons in mice lacking GDNF. Nature **382:**70–73.
19. **Schuchardt, A., V. D'Agati, L. Larsson-Blomberg, F. Costantini, and V. Pachnis.** 1994. Defects in the kidney and enteric nervous system of mice lacking the tyrosine kinase receptor Ret. Nature **367:**380–383.
20. **Stark, K., S. Vainio, G. Vassileva, and A. P. McMahon.** 1994. Epithelial transformation of metanephric mesenchyme in the developing kidney regulated by Wnt-4. Nature **372:**679–683.
21. **Torres, M., E. Gomez-Pardo, G. R. Dressler, and P. Gruss.** 1995. Pax-2 controls multiple steps of urogenital development. Development **121:**4057–4065.