Characterization and Analysis of Sim1 GENSAT BAC transgenic mice

GENSAT is NIH funded project that was initiated to generate BAC/EGFP transgenic lines with the intention to provide genetic tools that would facilitate the study of the central nervous system (CNS). We have take advantage of the availability of GENSAT transgenic mice to address whether any of the transgenic lines that have been generated would be appropriate to study renal development. The analysis here provides the kidney research community with basic information as to the utility of GENSAT transgenic strains in furthering the study of kidney development. As part of the GUDMAP consortium, we have tested several strains from GENSAT at a single appropriate time point (E15.5) and screened the mice for their ability to aid in the isolation of specific components from the developing kidney for gene expression profiling. Here we report the pattern of EGFP expression in the embryonic day 15.5 kidney of the *Sim1-EGFP*strain. **Our analysis suggests that the *Sim1-EGFP* transgenic mice may be useful in studying the development of the distal tubule and cells that contribute to the development of the ureteric bud.**

Sim1 Gene Notes

This gene is a member of the basic helix-loop-helix PAS family of transcription factors and has been shown to play essential roles in the formation of the supraoptic and paraventricular (PVN) nuclei of the hypothalamus. Disruptions in *Sim1* have been associated with developmental abnormalities and obesity. Although northern blot analysis has shown Sim1 expression in the kidney, the precise pattern of expression has not been described (Holder *et al*).

Strain Information

Strain Name: STOCK Tg(Sim1-EGFP)1Gsat/Mmmh
Stock Number: 000306-MU/H
Gene Details:
Promoter: Sim1
Name: single-minded homolog 1 (Drosophila)
Alteration at locus: Transgenic
Reporter: EGFP (Jelly Fish)
Name: Enhanced Green Fluorescent Protein
Alteration at locus: Transgenic

For further information and strain distribution please use the following URL:
http://www.mmrrc.org/strains/306/0306.html

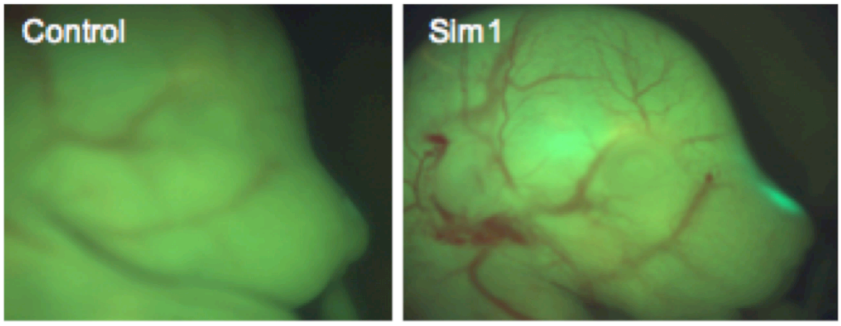


Figure 1. Analysis of *Sim1*-EGFP expression in whole-embryos. Fluorescent image detailing expression of EGFP in E15.5 embryos. The embryo on the left is a non-transgenic littermate, while the embryo on the left is a *Sim1*-EGFP BAC transgenic. This analysis only detected weak GFP expression in the developing facial region of the embryo.

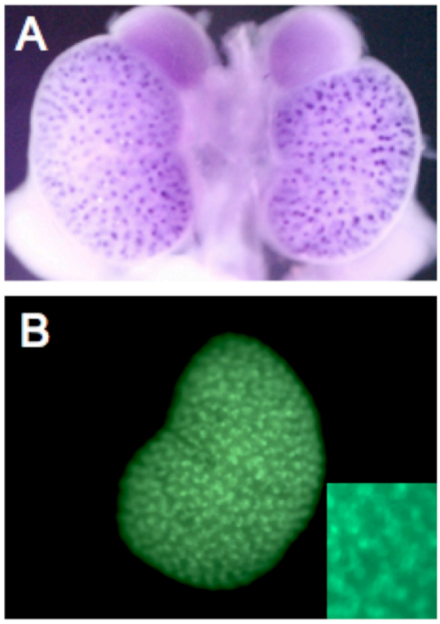
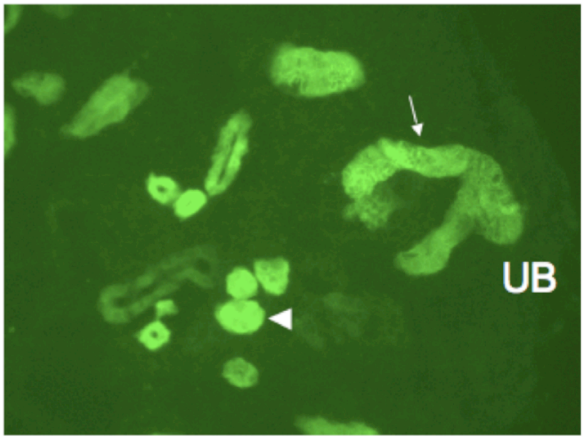


Figure 2. Expression of EGFP in the kidneys of *Sim1*-EGFP BAC transgenic mice. A) Bright field microscopy image detailing the expression of *Sim1* using whole-mount *in situ* hybridization in the kidneys of E15.5 embryos (Image courtesy of GUDMAP, McMahon Group). **B)** Fluorescent microscopy image showing *Sim1*-EGFP expression in the kidney from E15.5 embryos. Note the punctate GFP expression recapitulates the endogenous *Sim1* expression. Inset represents a close-up image detailing GFP expression in the developing ureteric buds.

Figure 3. Expression pattern *Sim1*-EGFP transgenic mice. Fluorescent microscopy image showing *Sim1*-EGFP expression in the developing kidney of an E15.5 embryo. Note the expression of GFP in the developing ureteric bud. In addition, EGFP expression can be detected in the developing distal tubule (arrow) as well as the more mature distal tubule structures (arrowhead)



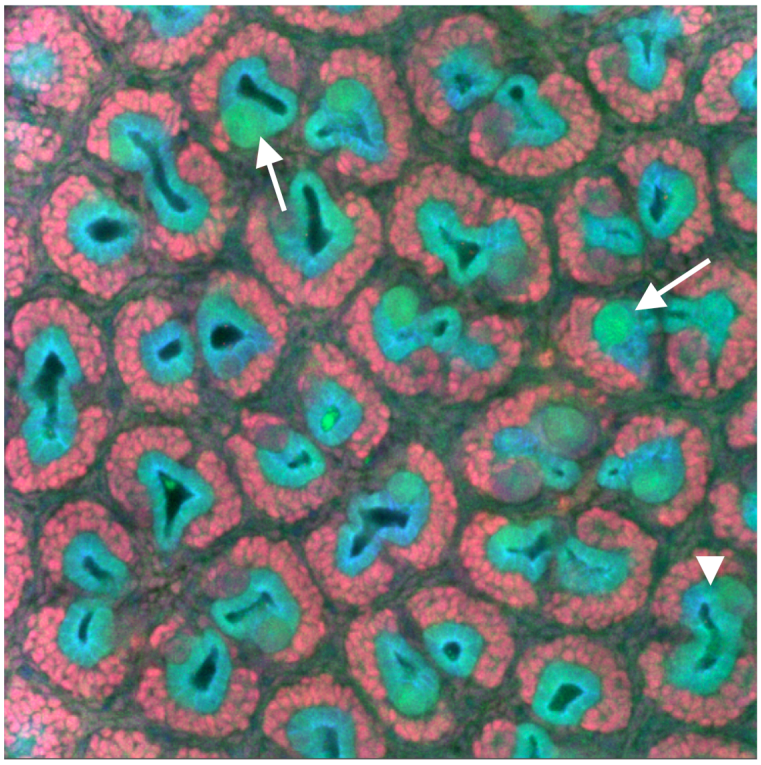


Figure 4. Confocal analysis of *Sim1*-EGFP expression in the developing kidney. To further delineate and localize the expression pattern of *Sim1*-EGFP in the kidney of E15.5 embryos, we performed confocal analysis. This image details the expression of *Sim1*-EGFP, which can be seen in the connecting segment of the developing distal tubule (arrows) as well as in the ureteric bud (arrow head). The tubules of the kidney were labeled with E-cadherin, and the mesenchyme and developing glomeruli labeled by WT-1 expression. Sim1 (green), E-cadherin (blue), WT-1 (red).

Confocal movie showing expression of *Sim1*-EGFP in the developing kidney of E15.5 embryos. To further visualize *Sim1*-EGFP expression, a file containing a movie that details the expression of *Sim1*-EGFP is provided. Strong *Sim1*-EGFP expression can be detected in the in the developing distal tubules and ureteric bud. The tubules of the kidney were labeled with E-cadherin, and the mesenchyme and developing glomeruli labeled by WT-1 expression. Sim1 (green), E-cadherin (blue), WT-1 (red). The confocal images are available as movies and can be downloaded from http://www.gudmap.org/Resources/MouseStrains/index.html.

Methods

Tissue processing for confocal microscopy

Kidneys were dissected in phosphate buffered saline (PBS). The kidneys or the organ explants were rocked for 1–2 h in 2% paraformaldehyde in PBS, washed twice with PBS, and then rocked for 1–2 h in 100% methanol. The tissues were washed twice with cold PBS containing 0.05% Tween-20 (PBT). Kidneys were bisected. Primary antibodies, diluted to 1:250 to 1:400, were added to the tissues in 400 μL of PBT containing 2% goat serum and incubated overnight with rocking. Tissues were washed with 5 exchanges of PBT over 8 h with rocking. The secondary antibodies, diluted to 1:400 in PBT containing 2% goat serum, were added and incubated overnight. The tissues were again washed with 5 exchanges of PBT over 8 h. The tissue was washed for 5–10 min and mounted in a depression slide in PBT before they were examined by confocal microscopy. The entire procedure was performed at 4 °C with pre- cooled reagents.

The following primary antibodies were utilized: anti-WT1 (c-19, Santa Cruz), anti-Uvomorulin (E-cadherin, Sigma). The secondary antibodies were Alexa 555-conjugated anti-rabbit and Alexa 633-conjugated anti-rat secondary antibodies (Molecular Probes).

Confocal imaging

The tissues were imaged with a Zeiss LSM510 equipped with an Argon (488 nm) and two HeNe lasers (543 nm and 633 nm). We used a multi-track configuration, refractive index correction, and automatic gain control. Approximately 2 μm thick optical sections were obtained every 5 μm to a depth of at least 80 μm. The sections began at the surface of the kidney and were on a plane tangential to it.

References

Gong S, Zheng C, Doughty ML, Losos K, Didkovsky N, Schambra UB, Nowak NJ, Joyner A, Leblanc G, Hatten ME, Heintz N. A gene expression atlas of the central nervous system based on bacterial artificial chromosomes Nature. 2003 Oct 30;425(6961):917-25.

GENSAT Project, Howard Hughes Medical Institute, The Rockefeller University, 1230 York Avenue, Box 260, New York 10021, USA."The Gene Expression Nervous System Atlas (GENSAT) Project, NINDS Contracts N01NS02331 & HHSN271200723701C to The Rockefeller University (New York, NY)."

Hartman HA, Lai, HL, Patterson LT. Cessation of renal morphogenesis in mice. Dev Biology. 2007 310:379-387

Holder JL, Butte NF, Zinn AR. Profound obesity associated with a balanced translocation that disrupts the SIM1 gene. Hum Mol Genet. 2000 Jan 1;9(1):101-8.