Characterization and Analysis of Sema3b GENSAT BAC transgenic

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 *Sema3b-EGFP*strain. **Our analysis suggests that the *Sema3b-EGFP* transgenic mice may be useful in studying the development of the collecting ducts and tubules in the embryonic kidney.**

Sema3b Gene Notes

The semaphorin/collapsin family of molecules plays a critical role in the guidance of growth cones during neuronal development. In addition, semaphorins have been implicated in angiogenesis and vascular development. The secreted protein encoded by this gene family member is important in axonal guidance and has been shown to act as a tumor suppressor by inducing apoptosis (Tran *et al*.).

Strain Information

Strain Name: STOCK Tg(Sema3b-EGFP)S52Gsat/Mmmh
Stock Number: 000253-MU

Gene Details:
Promoter: Sema3b
Name: Sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3B
Alteration at locus: Transgenic Reporter: EGFP (Jelly Fish)
Name: Enhanced Green Fluorescent Protein
Alteration at locus: Transgenic Transgene: Tg(Sema3b-EGFP)S52Gsat
Name: transgene insertion S52, GENSAT Project at Rockefeller University
Alteration at locus: Transgenic

For further information and strain distribution please use the following URL:
http://www.mmrrc.org/strains/253/0253.html

Characterization of Sema3b expression in the developing kidney

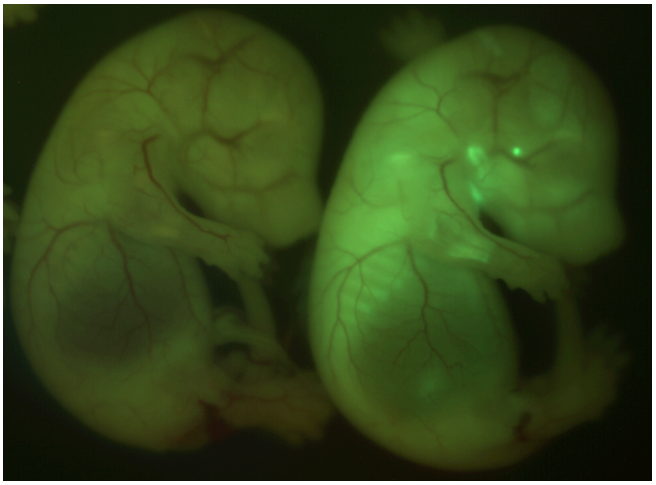

Figure 1. Analysis of *Sema3b*-EGFP expression in whole-embryos. Fluorescent image detailing expression of EGFP in E15.5 embryos. The embryo on the left is a non-transgenic littermate, while the embryo on the left is a *Sema3b*-EGFP BAC transgenic. This analysis only detected GFP expression in the developing skeletal elements of the embryo.

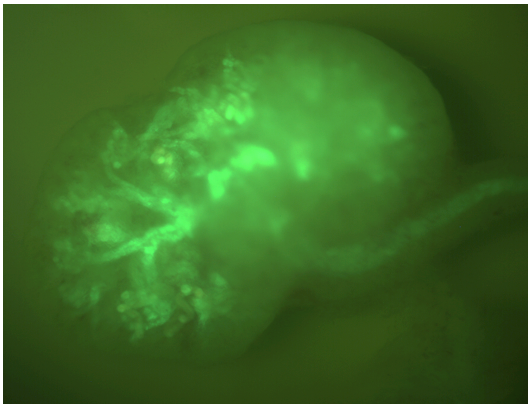

Figure 2. Expression of EGFP in the kidneys of *Sema3b*-EGFP BAC transgenic mice at E15.5. This fluorescent microscopic image shows *Sema3b*-EGFP expression in the kidney in E15.5 embryos. Note EGFP in the developing collecting ducts, tubules as well as the ureter (arrow).

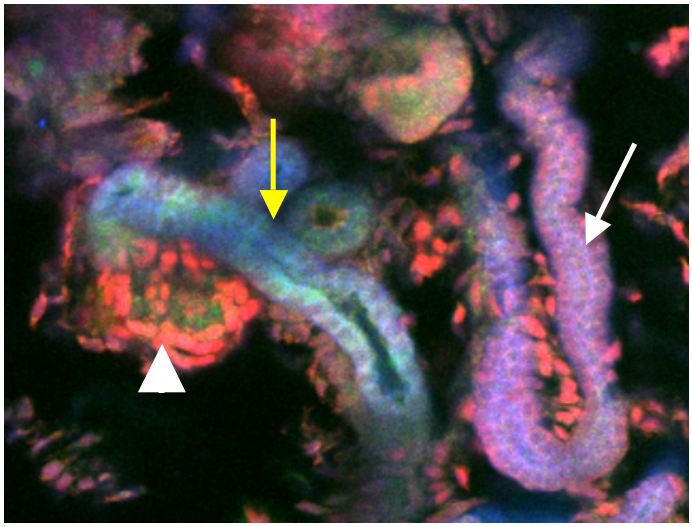

Figure 3. Confocal analysis of *Sema3b*-EGFP expression in the developing kidney. To further analyze expression from the *Sema3b*-EGFP transgenic, a kidney from a E15.5 *Sema3b*-EGFP transgenic mouse was isolated, bisected and subjected to confocal analysis. This image details the expression of *Sema3b*-EGFP, which was detected in the developing glomerulus (arrowhead), proximal tubule (yellow arrow) and loop of Henle (white arrow) The tubules of the kidney were labeled with E-cadherin, and the mesenchyme and developing glomeruli labeled by WT-1 expression. Sema3b (green), E-cadherin (blue), WT-1 (red).

Characterization of Sema3b expression in the developing kidney

Confocal movie showing expression of *Sema3b*-EGFP in the developing kidney of E15.5 embryos. To further visualize *Sema3b*-EGFP expression, a file containing a movie that details the expression of *Sema3b*-EGFP is provided. *Sema3b*-EGFP expression can be detected in the in the developing tubules, glomeruli, loop of Henle, and collecting ducts. The tubules of the kidney were labeled with E-cadherin, and the mesenchyme and developing glomeruli labeled by WT-1 expression. Sema3b (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

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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)."

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