Analysis of *Drosophila* Sox gene expression in the intestinal stem cell lineage

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Introduction

The capacity of embryonic stem (ES) cells to self-renew and differentiate into diverse cell types has resulted in the emergence of stem cell therapy as a promising treatment for several diseases. Recent studies have revealed that co-expression of four transcription factors—Oct3/4, Klf4, Sox2, and c-Myc—can transform differentiated mammalian somatic cells into induced pluripotent stem cells (iPScs).[1] Sox2 is a member of the Sox protein family, all of which contain a conserved DNA-binding High Mobility Group (HMG) domain and regulate diverse developmental processes. In *Drosophila melanogaster*, the homologue of Sox2 is Dichaete, one of four related fly Group B Sox proteins; the others being SoxN, Sox21a, and Sox21b.[2] Recently, several populations of intestinal stem cells were identified in adult *Drosophila* (Figure 1). These cells are concentrated at the anterior hindgut, in the hindgut proliferation zone (HPZ), and at the basement membrane of the posterior midgut; stem cells are also scattered in other areas of the midgut.[3,4,5] Since Sox2 is essential for the generation of iPScs, **we hypothesize that Dichaete and the other three Group B Sox proteins are expressed within *Drosophila* intestinal stem cells and regulate their stem cell properties. *In the current study, we demonstrate that each of the four fly Group B Sox genes is transcribed in the digestive system of the adult fly and that at least SoxN and Dichaete are expressed within intestinal regions known to contain stem cells.***

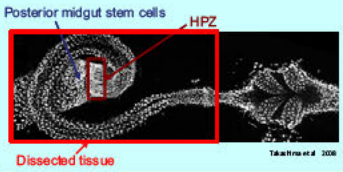


Figure 1: Section of the intestine from the rectum (on the right) to the posterior midgut, with the stem cells labeled. The portion of the digestive tract dissected includes the section outlined in the red box and an additional section of the midgut adjacent to that pictured.

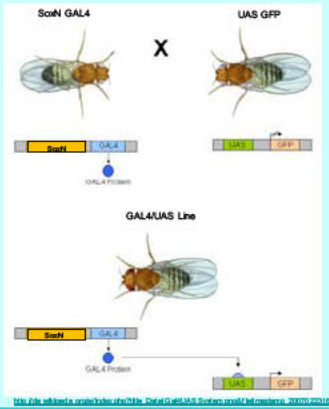
Characterize expression of Group B Sox genes in the adult digestive tract

Approach 1

Isolate adult digestive tract mRNA → Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) for D, SoxN, Sox21a, Sox21b → Agarose gel electrophoresis

Approach 2

GAL4/UAS System: Yeast transcription factor GAL4 is expressed in cells that transcribe the gene of interest; GAL4 drives expression of a detectable marker (i.e. GFP).



Set up genetic crosses

SoxN	Gal4 X UAS GFP
Dichaete	Gal4 X UAS GFP
Sox21a	Gal4 X UAS GFP
Sox21b	Gal4 X UAS GFP

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Dissect digestive tracts and analyze via fluorescence microscopy

Results

mRNA expression of all 4 Sox Group B genes in adult intestines

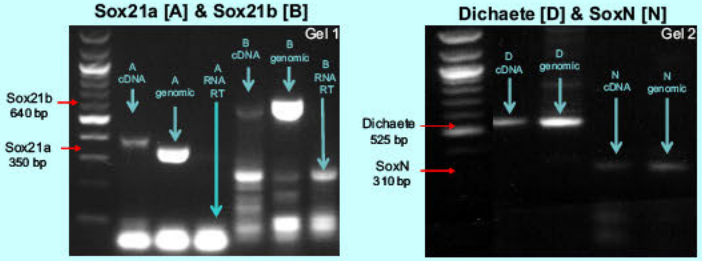


Figure 2: Agarose gel electrophoresis reveals generation of Group B Sox gene cDNAs from adult intestinal mRNAs. The sample is cDNA obtained from intestinal RNA; genomic (or full-body) DNA serves as the positive control, and RNA–RT (RNA without reverse transcriptase) serves as the negative control. **Gel 1**: bands are present at expected sizes for both *Sox21a* and *Sox21b* with cDNA and genomic DNA (lanes 1, 2, 4, and 5 to the right of the ladder); this indicates expression of both genes in the intestine. No band for either gene appears at the expected size in lanes 3 and 6, suggesting no DNA is present in the RNA. **Gel 2**: bands are present at expected sizes for *Dichaete* and *SoxNeuro* with both cDNA and genomic DNA (lanes 1-4 to the right of the ladder); this indicates expression of both genes in the intestine.

Fluorescence microscopy reveals expression of Dichaete-GFP in stem cell regions

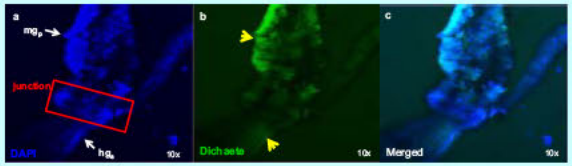


Figure 3: Reporter gene constructs indicate expression of Dichaete in the posterior midgut and slight expression in the hindgut. **a—c** midgut/hindgut junction of intestine from a Dichaete-GFP strain; **mg_p** : posterior midgut; **hg_a**: anterior hindgut **(a)** DAPI staining labeling the nuclei of the mg, hg, and junction **(b)** yellow arrowheads: GFP expression in the cells found at the end of the posterior midgut adjacent to the junction; there is slight expression of cells in the hindgut **(c)** overlay of DAPI staining and fluorescence indicating Dichaete expression in bona fide cells.

Fluorescence microscopy reveals expression of SoxNeuro-GFP in stem cell regions

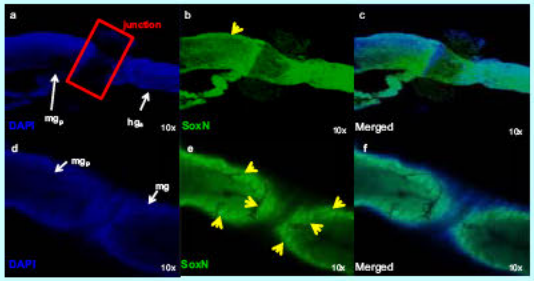
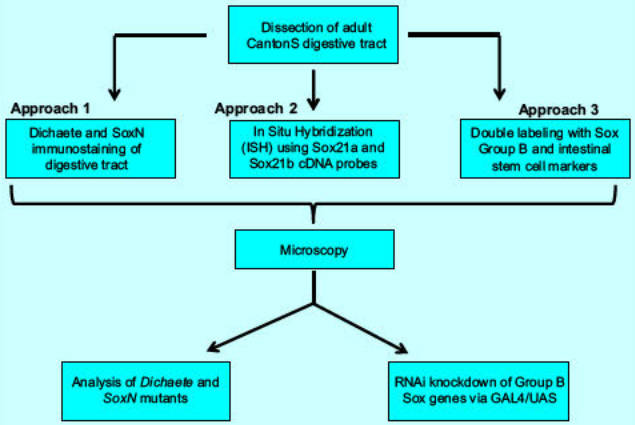


Figure 4: Reporter gene constructs indicate expression of SoxN in the posterior midgut. **a—c** midgut/hindgut junction of sample 1; **d—f** midgut of sample 2; **mg_p**: posterior midgut; **mg**: midgut; **hg_a**: anterior hindgut. **(a,d)** DAPI staining labeling nuclei of mg, hg, and junction **(b,e)** yellow arrowhead: expression of GFP along the basement membrane of mg_p and mg, with some expression in the middle of the mg **(c,f)** overlay of DAPI staining and fluorescence indicating SoxN expression in bona fide cells.

Discussion

- mRNA expression of all four Group B Sox genes is found within the dissected adult intestines
- Reporter gene constructs reveal expression of Dichaete and SoxN in the posterior midgut membrane known to contain stem cells. Dichaete is slightly expressed in the hindgut; SoxN shows no expression in the hindgut.
- This study provides an important step towards developing *Drosophila* as a model to study Sox gene functions in stem cell biology.

Future Work



- Immunostaining for Dichaete and SoxN along with ISH for Sox21a and Sox21b will be done to confirm expression in stem cell containing regions of the intestine
- To determine Sox gene expression specific to stem cells, Sox genes will be labeled with immunostaining/ISH while simultaneously labeling for stem cell markers (*esg*, *Su(H)GBE-lacZ*, Delta, Wg, Jak/Stat, and Hh) via reporter gene constructs.
- Functions of the Sox factors in stem cell development will be analyzed via *Dichaete* and *SoxN* mutants and RNAi knockdown using the GAL4/UAS system.

Literature Cited

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