Investigating the sex-specific, tissue-specific, and segment-specific regulation of the *branchless* gene in *Drosophila*

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Background

One of the more interesting challenges in developmental biology is determining how diverse cues are integrated to determine where and under what conditions a specific gene is expressed. A particularly fascinating example of such a gene regulated by multiple factors is *branchless* (*bnl*), which encodes a fibroblast growth factor in the fruit fly *Drosophila melanogaster*. In the genital imaginal disc of the *Drosophila* larva, *bnl* is expressed in a:

- **Sex-specific manner** – *bnl* is expressed in the male genital disc, but not in the female genital disc (Fig. 1).
- **Segment-specific manner** – The genital disc is comprised of primordia derived from three segments, A8, A9, and A10. *bnl* is expressed in the male genital disc in only the A9-derived segment (Fig. 1).
- **Tissue-specific manner** – *bnl* is expressed in only two subsets of the A9-derived segment of the male genital disc, and not along the entire A9-derived primordium, indicating that it must also be regulated by tissue-specific factors (Fig. 1).

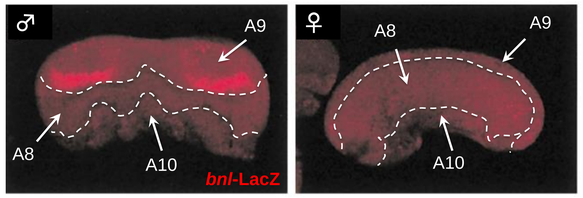

Figure 1. *bnl* (red) is expressed in two subsets of only the A9-derived primordium of the male genital disc (Ahmad and Baker, 2002).

Multiple factors are expected to be involved in regulating the sex-specific, segment-specific, and tissue-specific expression of *bnl*:

- Sex-specific expression in somatic tissues is regulated by the terminal transcription factors (TFs) of the *Drosophila* sex determination hierarchy, the male and female isoforms of the Doublesex (DSX) proteins (Burtis and Baker, 1989). Thus, male specific expression of *bnl* is most likely to be regulated by the DSX proteins.
- The identities of the A8, A9, and A10 segments are mediated by the Abdominal-A (ABD-A), Abdominal-B (ABD-B), and Caudal (CAD) homeotic TFs. The specific expression of ABD-A and the ABD-B m isoform in the A8 primordium, the ABD-B isoform r in the A9 primordium, and CAD in the A10 primordium specify their identity (Casares et al., 1997). Thus, one or more of these homeotic transcription factors are expected to regulate the A9-specific expression of *bnl*.
- *bnl* is expressed in a subset of the expression domain of the Drop (DR) TF in the male genital disc (Fig. 2), and local inactivation of DR eliminates *bnl* expression (Chatterjee et al., 2011). This data indicates than DR regulates the tissue-specific expression of *bnl*.

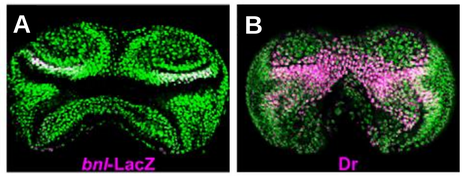

Figure 2. *bnl* is expressed in a subset of the DR expression domain (Chatterjee et al., 2011).

Objectives

A research goal of Dr. Ahmad's laboratory is to functionally dissect and analyze the sex-specific, segment-specific, and tissue-specific regulation of *bnl*. A critical initial step in achieving this goal, and thus our **primary objective**, is identifying one or more **enhancers** that determine the sex-, segment-, and tissue-specific regulation of *bnl*.

Enhancers are regions of the genome to which specific TFs bind to determine where, when, and under what conditions a nearby gene is expressed. Therefore the most parsimonious hypothesis is that the *bnl* enhancer or enhancers will integrate sex-, segment-, and tissue-specific inputs by binding TFs specific to these cues. Hence, we should be able to predict candidates for the *bnl* enhancer(s) by identifying regions near the endogenous gene where the binding sites of these TFs overlap or are closely clustered (Fig. 3).

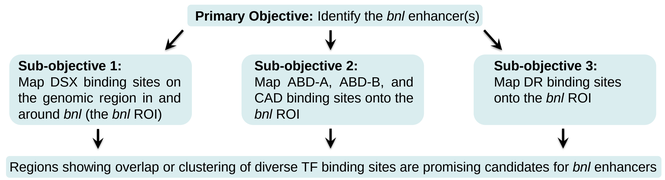

Figure 3. Our approach for predicting *bnl* enhancers

The stepwise procedure we are pursuing is described below. Completed steps are marked accordingly.

Step 1:

- ✓ Utilize FlyBase to identify and extract the coordinates of the *bnl* Region of Interest (ROI) where we expect to locate one or more *bnl* enhancers from the *Drosophila melanogaster* genome Release 6.
- ✓ The *bnl* ROI will be defined as the genomic interval spanning the region from the ends of each gene (*CG11453* and *CG31459*) immediately adjacent to the *bnl* gene.

Step 2:

- ✓ Extract the coordinates of the *in vivo* DSX (TF for sex-specific regulation) binding sites on the *Drosophila* genome from published DamID and ChIP-seq experimental data (Luo et al., 2011; Clough et al., 2014).
- ✓ Convert coordinates of DSX binding sites to Release 6 of the genome.
- ✓ Identify the subset of DSX binding sites on the *bnl* ROI.
- ✓ Map and visualize the DSX binding sites on the *bnl* ROI using the UCSC Genome Browser.

Step 3:

- Map and visualize the *in vivo* ABD-B and CAD (TFs for segment-specific regulation) binding sites on the *bnl* ROI using ChIP-seq experimental data (Slattery et al. from modEncode).
- Map and visualize the putative binding sites of ABD-A (another TF for segment-specific regulation) on the *bnl* ROI using *in vitro* position weight matrix (PWM) data from JASPAR and TRANSFAC.

Step 4:

- Map and visualize the putative binding sites of DR (TF for tissue-specific regulation) on the *bnl* ROI with the UCSC Genome Browser using *in vitro* protein binding microarray (PBM) data (Berger and Bulyk, 2009).

Results

Step 1: Determining the coordinates of the *bnl* ROI

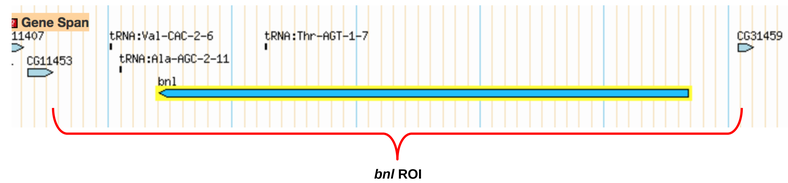

Figure 4. Schematic illustrating how the *bnl* ROI was defined

Chromosome location: 3R
Region of Interest (ROI): 3´end of CG11453 to 5´end of CG31459
Base pair coordinates: 19,785,519 to 19,840,845
Length: 55.326 kbp

Step 2: Mapping of DSX *in vivo* binding sites on the *bnl* ROI

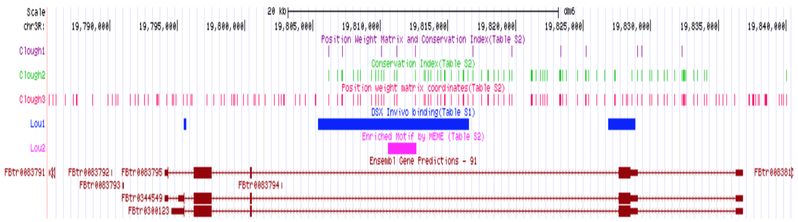

Figure 5. Visualization of a subset of the DSX *in vivo* DamID and ChIP-seq binding data on the *bnl* ROI

Future work

- Map the *in vivo* binding sites of ABD-B and CAD and the putative binding sites of ABD-A on the *bnl* ROI (Step 3).
- Map the putative binding sites of DR onto the *bnl* ROI (Step 4).

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