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Analysis of the *Drosophila* Sox gene *Dichaete* in the adult olfactory circuit

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Olfactory circuit formation in Drosophila starts from antennal lobes. Antennal lobes consist of glomeruli where the olfactory receptor neurons synapse with the Projectile Neurons and local interneurons. Previous studies from our lab has demonstrated that *Dichaete*, a conserved SOX gene play an important role in embryonic nervous system development and is expressed in several clusters of neurons in the brain, including intermingled olfactory local interneurons and central complex neurons. (Melnattur et al.). Dichaete protein is localized to neuronal nuclei and the anti-Dichaete immunostaining does not permit observation of neuronal processes and sites of projections. This study focus on two different aims first is the generation of P(acman) strains(Venken et al.) to rescue the Dichaete mutant phenotype. If the P(acman) construct rescues mutant phenotype, it will be evident that it contains all the regulatory elements needed for normal Dichaete expression. Second use of P(acman) clone to express a tagged Dichaete protein in native fashion. This will allow us to label the projections of Dichaete expressing neurons in olfactory circuit formation.

Analysis of Drosophila *Dichaete* gene in the adult olfactory system

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Introduction

The glomeruli of the antennal lobes are central sites for integration of olfactory sensory input in *Drosophila*. Here olfactory receptor neurons synapse with the projection neurons and local interneurons. Previous studies demonstrated that *Dichaete*, a conserved SOX HMG domain gene, is expressed in many local interneurons but not in olfactory receptor neurons or projection neurons, and that *Dichaete* mutants exhibit disruptions in projection neurons (1).

Dichaete protein is localized to neuronal nuclei and anti-Dichaete immunostaining does not permit examination of neuronal processes and projection sites. In this study, we describe a preliminary studies to generate different *Dichaete* P(acman) strains useful for genetic rescue experiments and characterization of *Dichaete* neuron projections.

Dichaete Expression **Schematic of the olfactory system**

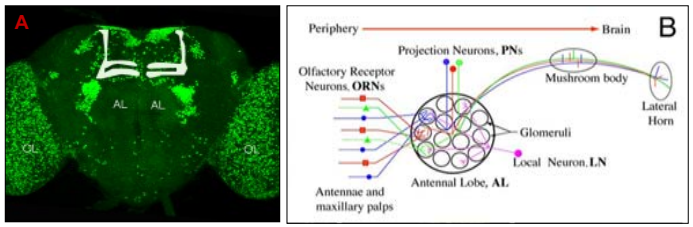

Figure1: (A). Dichaete protein expression in adult brain: antennal lobe (AL), optic lobe (OL), mushroom bodies (white) are indicated. (B). Olfactory receptor neurons expressing the same receptor (colour) send their axons to the same glomerulus within the antennal lobe. Here, these axons synapse with projection neurons (same colour as ORN) and local interneurons. Projection neurons project axons to mushroom body and lateral horn. (from Komiyama et. al 2003)

Background to *Dichaete* gene

The *Drosophila* transcription factor protein Dichaete (D) is a member of the Sox family. The function of *Dichaete* is well known in the developing embryos (2). Similar to mammalian Sox proteins, Dichaete has been shown to induce an 85° bend in DNA giving it the ability to modulate chromatin structure and directly activate transcription. Like other Sox proteins, it is known to recruit other proteins to aid in this process. *Dichaete* has a strong role in development, affecting processes that include differentiation of specific neuronal and glial cells, segmentation, hindgut development, the differentiation of imaginal discs, and elaboration of the adult olfactory system (3).

Goals

1. To generate *Dichaete* P(acman) strains that genetically rescue *Dichaete* mutant phenotypes.
2. To generate recombineered P(acman) strains for labeling the projections of Dichaete expressing neurons in the adult olfactory system.

Experimental Approach

1.Selections of Clones from the Bellen library

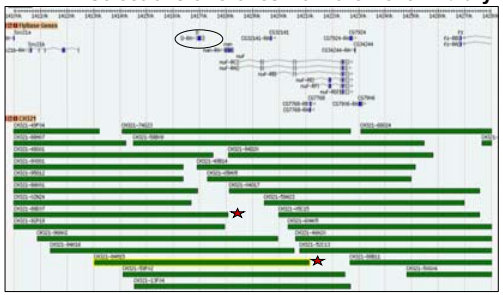

Figure 2: P(acman) clones CH321-84M15 and CH321-88E07 (4) were selected for transgenesis. *Dichaete* is shown in circle and clones are marked by stars.

2. Rescue Experiment

Figure 3: Rescue of *Dichaete* mutation: Transgenic lines of CH321-84M15 containing *Dichaete* region were generated and crossed with *Dichaete89* mutants (mostly lethal, escapers exhibit PN disruptions).

3. Recombineering Experiment

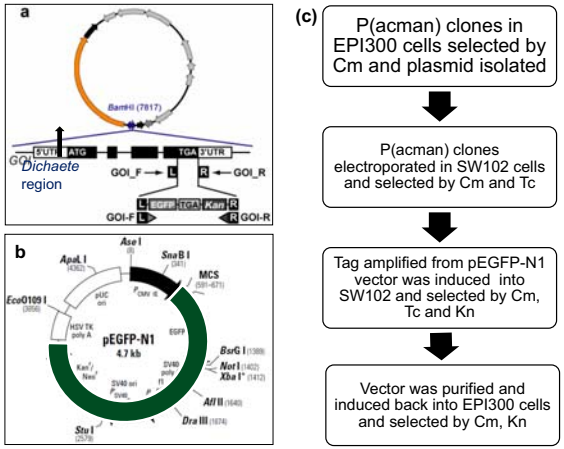

Figure 4: Recombineering strategy: (a) P(acman) BAC clone containing *Dichaete* with fused EGFP cassette amplified (green) from pEGFP-N1 vector (b). Fused EGFP cassette will introduce a novel stop codon replacing the endogenous stop codon. Recombineering homology regions are indicated on the left (L) and right (R). (c) Flow diagram showing the steps of recombineering (4).

Results

1. Obtained 3 transgenic strains from BestGene (Chino Hills, CA) for CH321-84M15 P(acman). Obtained 0 transgenic strains for CH321-88E07
2. CH321-84M15 was used for rescue experiment. Screen of progeny via PCR for D89 allele from final rescue cross is in process.
3. P(acman) construct CH321-88E07 has been recombineered, verified, and send it out to BestGene for transgensis.

mEGFP cassette amplified **Verification of CH321-88E07**

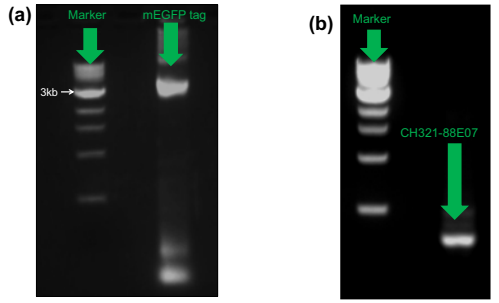

Figure 6: (a) Cassette amplified from mEGFP-N1 vector containing EGFP and Kanamycin used for tagging P(acman) vector CH321-88E07. (b) Verification of correct recombineering using primers made along left and right homology arms. The construct has been sent out for transgenesis.

Conclusion/ Future directions

1. If a P(acman) construct rescues *Dichaete* mutant phenotypes, then it is clear that all the necessary regulatory elements for normal *Dichaete* expression are present on the genomic DNA fragment.
2. To define the specific projection patterns of LNs clusters expressing D into discrete glomeruli.
3. Identify components of the *Dichaete*-dependent intercellular signaling pathway important for proper elaboration of PNs and organization of glomeruli.

References:

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