

Microarray analysis of migratory cells in the Drosophila ovary

Experiment 1: Expression profile of border cells in the Drosophila ovary.

Fly stocks:

- 1) slbo-Gal4/Cyo.
- 2) c522-Gal4
- 3) UAS-mCD8-GFP.
- 4) UAS-mCD8-GFP / Cyo; tub-Gal4/TM6B.

Reagents: Cell dissociation buffer (Sigma, C-1544); Elastase (Sigma, E-0258); mouse CD8a (Ly-2) microbeads (494-01, Miltenyi Biotec); reagents for RNA purification, cRNA probe labeling and hybridization for microarray experiment as suggested by Affymetrix GeneChip Expression Analysis Manual and Small Sample Target Labeling Assay Version II. (<http://www.affymetrix.com/index.affx>).

Protocol:

1. Fly crosses and virgin collection.
UAS-mCD8-GFP females were crossed in bottles to either slbo-Gal4/Cyo (for marking border cells and centripetal cells), or c522 (for marking border cells only). slbo-Gal4/UAS-mCD8-GFP and UAS-mCD8-GFP /+;c522/+ virgins were collected and aged at 18°C for 2-7 days, then mated with the males of the same genotype and fattened overnight at 18°C and 25°C respectively before dissection. w1118 females were crossed to UAS-mCD8-GFP / Cyo; tub-Gal4/TM6B. UAS-mCD8-GFP /+; tub-Gal4/+ virgins were collected and aged at 18°C for 2-7 days, then mated with the males of the same genotype and fattened overnight at 18°C (for the comparison with slbo-Gal4/UAS-mCD8-GFP) or 25°C (for the comparison with UAS-mCD8-GFP /+;c522/+) before dissection.

2. Dissection.

Ovaries were dissected in Grace's medium with 10% normal goat serum. After dissecting about 10 ovaries, the ovaries were transferred to a tube on ice. The total time for dissection was controlled to less than two hours.

3. Cell dissociation.

About 100 ovary pairs were collected to an eppendorf tube, washed with cell dissociation buffer three times and digested with elastase at 4mg/ml in cell dissociation buffer for 5 minutes with stirring and inverting. 0.5mls of supplemented Grace's medium were added, and the supernatant was transferred to a new tube (2 times). Dissociated cells were spin at 1000g for 5 minutes at 4°. Pelleted cells were resuspended in supplemented Grace's media or buffer for sorting.

4. Cell purification.

The dissociated cells were subjected to magnetic cell purification with magnetic beads coupled with anti-mouse CD8 antibody (Miltenyi Biotec, <http://www.miltenyibiotec.com>) according to the manufacturer's manual. CD8+ and CD8- cells were kept for RNA preparation.

5. cRNA probe labeling and hybridization.

Total RNA was prepared from purified cells. For each experiment (genotype), RNA was independently isolated in triplicate. 5µg of each RNA from slbo-Gal4/UAS-mCD8-GFP and UAS-mCD8-GFP / +; tub-Gal4/+ flies was used to make cRNA probes following Affymetrix probe labeling instruction (<http://www.affymetrix.com/index.affx>).

100ng of each RNA from UAS-mCD8-GFP /+;c522/+ and slbo-Gal4/UAS-mCD8-GFP and UAS-mCD8-GFP / +; tub-Gal4/+ flies was used to make cRNA probes following Small Sample

Target Labeling Assay Version II

(<http://www.affymetrix.com/index.affx>). cRNA probes were hybridized to Affymetrix Drosophila Genome Array, washed and signal detected as suggested by Affymetrix. Each experiment was repeated three times using independent biological samples.

Experiment 2: Downstream targets of slow border cell (*slbo*) in border cells in the Drosophila ovary.

Fly stocks:

- 1) *slbo*-Gal4/Cyo.
- 2) c522.
- 3) UAS-mCD8-GFP.
- 4) *slbo*-Gal4, *slbo*^{e7b}/Cyo.
- 5) UAS-mCD8-GFP, *slbo*^{ly6}/Cyo.
- 6) c522, *slbo*^{e7b}/T(2;3)SM6a-TM6B, Tb(1).

Protocol:

This protocol is basically the same as the one used in Experiment 1, except the following additions.

1. Fly crosses and virgin collection.

UAS-mCD8-GFP, *slbo*^{ly6}/Cyo females were crossed in bottles to either *slbo*-Gal4, *slbo*^{e7b}/Cyo (for marking border cells and centripetal cells), or c522, *slbo*^{e7b}/T(2;3)SM6a-TM6B, Tb(1) (for marking border cells only). *slbo*-Gal4, *slbo*^{e7b}/UAS-mCD8-GFP, *slbo*^{ly6} and c522, *slbo*^{e7b}/UAS-mCD8-GFP, *slbo*^{ly6} virgins were collected and aged at 18°C for 2-7 days, then mated with the males of the same genotype and fattened overnight at 18°C and 25°C respectively before dissection.

5. cRNA probe labeling and hybridization.

5µg of each RNA from *slbo*-Gal4, *slbo*^{e7b}/UAS-mCD8-GFP, *slbo*^{ly6} flies was used to make cRNA probes following

Affymetrix probe labeling instruction
(<http://www.affymetrix.com/index.affx>).

100ng of each RNA from c522, *slbo*^{e7b} / UAS-mCD8-GFP, *slbo*^{ly6} flies was used to make cRNA probes following Small Sample Target Labeling Assay Version II
(<http://www.affymetrix.com/index.affx>).

Experiment 3: Downstream targets of unpaired (*upd*) in border cells in the *Drosophila* ovary.

Fly stocks:

- 1) *slbo*-Gal4/Cyo.
- 2) UAS-mCD8-GFP.
- 3) UAS-mCD8-GFP / Cyo; *tub*-Gal4/TM6B.
- 4) *slbo*-Gal4, UAS-mCD8-GFP /Cyo.
- 5) UAS-UPD/Cyo.

Protocol:

This protocol is basically the same as the one used in Experiment 1, except the following additions.

1. Fly crosses and virgin collection.
slbo-Gal4, UAS-mCD8-GFP /Cyo females were crossed in bottles to UAS-UPD/Cyo. *slbo*-Gal4, UAS-mCD8-GFP / UAS-UPD virgins were collected and aged at 18°C for 2-7 days, then mated with the males of the same genotype and fattened overnight at 25°C before dissection.

5. cRNA probe labeling and hybridization.

100ng of each RNA from *slbo*-Gal4, UAS-mCD8-GFP / UAS-UPD flies was used to make cRNA probes following Small Sample Target Labeling Assay Version II
(<http://www.affymetrix.com/index.affx>).