Genetic Dissection of Developmental Heterogeneity in the Testis Stem Cell Niche

Jeeun Song, Katie Conlon, Erika Matunis
PhD Programme in Biochemistry, Cellular & Molecular Biology, Department of Cell Biology
Johns Hopkins University School of Medicine, Baltimore, MD 21205

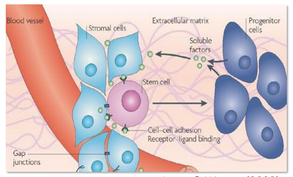
Abstract

Adult stem cells require a specialised microenvironment to maintain their pluripotent state and capacity for self-renewal. The Drosophila testis is a well-established model system for the in vivo analysis of stem cell niche function. In this tissue, niche cells (hub cells) maintain two stem cell populations that respectively replenish the germline gametes and the somatic stroma. Intriguingly, the somatic mesenchymal cells that coalesce to form the niche initially arise from multiple locations in the Drosophila embryo. Whether the cellular components of a stem cell niche are uniform or heterogenous in nature is an open question. Little definitive work has explored the possibility of cellular heterogeneity or its developmental origins across stem cell niches.

Here, we use the Split Gal4-UAS ternary system to target patterns of developmental gene expression during gonadogenesis in the Drosophila embryo. Preliminary results using fixed immunostaining and live imaging in vivo and ex vivo show that these constructs are capable of expressing transgenes in somatic gonadal precursors in the embryo. Furthermore, this approach may enable lineage tracing that persists beyond embryogenesis. Ultimately, these genetic drivers may enable the labelling, lineage tracing, and manipulation of potentially heterogenous cells within the Drosophila testis stem cell niche.

What is the Stem Cell Niche?

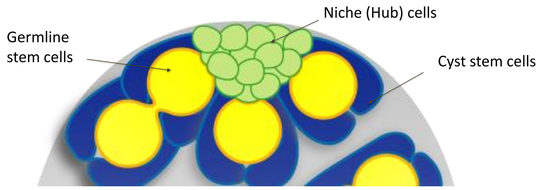
- ❑ Specific specialised tissue microenvironment
- ❑ Maintains stem cell self-renewal and regulates cell fate
- ❑ Crucial for tissue homeostasis & regeneration



Jones & Wagers (2008)

The Drosophila Testis Hub as a Model Stem Cell Niche

- ❑ Genetically, anatomically, developmentally accessible model
- ❑ Stem cell niche of the *Drosophila* testis
  - 10 -15 non-mitotic somatic cells form niche
  - Polarised architecture
  - Maintains two stem cell populations:
    - Germline stem cells → gametes
    - Somatic cyst stem cells → stromal support cells
- ❑ Niche cells may not be created equal: Pathways that exhibit heterogenous activity:
  - BMP
  - TOR
  - Notch



Drosophila Testis Niche Cell Precursors in Embryonic Development

- ❑ Testis niche function & architecture established during embryogenesis
- ❑ Coalescence of two progenitor populations form the embryonic gonad
  - Germline: Primordial germ cells → Germline stem cells
  - Somatic: Somatic gonadal precursors (SGP) → Niche cells & Cyst stem cells
- ❑ SGPs that form the niche arise from mesoderm of parasegments 10 -12

References

1. Anllo, L., Plasschaert, L.W., Sui, J., DiNardo, S. (2019). Live imaging reveals hub cell assembly and compaction dynamics during morphogenesis of the Drosophila testis niche. Dev. Biol. 446(1): 102--118.
2. de Cuevas, M., Matunis, E. L. 2011. The stem cell niche: Lessons from the Drosophila testis. Development 14:2861-2969
3. Greenspan LJ, Matunis EL. 2017. Live Imaging of the Drosophila Testis Stem Cell Niche. Methods Mol Biol. 1463:63-74.
4. Ferraro F, Celso CL, Scadden D. Adult stem cells and their niches. Adv Exp Med Biol. 2010;695:155–168. doi:10.1007/978-1-4419-7037-4_11
5. Pfeiffer B. D., Ngo T.-T. B., Hibbard K. L., Murphy C., Jenett A., et al., 2010 Refinement of tools for targeted gene expression in Drosophila. Genetics 186: 735

Questions and Aims

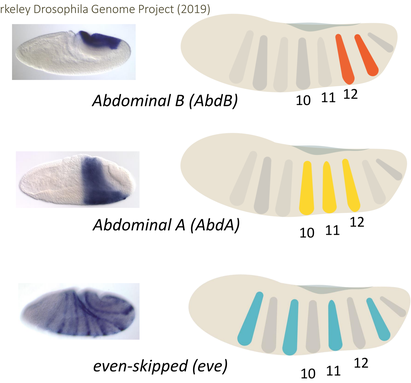
- How is the testis niche organised from cells from different regions of the embryo?
- How do niche cells differ according to their developmental origin?
- What are the functional implications for the stem cell niche?

→

- ❑ Create origin-specific genetic driver lines
- ❑ Characterise somatic gonadal progenitors by origin
  - Lineage tracing
  - Genetic ablation
  - Cell dynamics & migration
- ❑ Live imaging of embryonic gonadogenesis & niche development

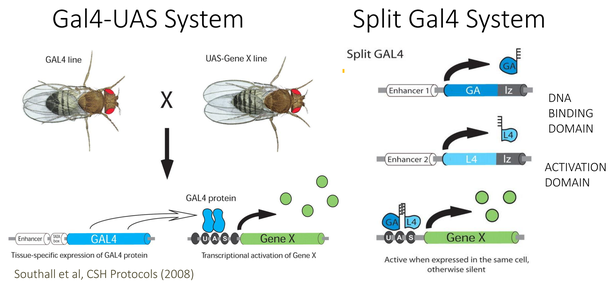
Specific Patterns of Homeobox Gene Expression during Development

Berkeley Drosophila Genome Project (2019)



Generating Drivers

To study the effects of multiple developmental genes, we use the Split Gal4 system in Drosophila, a three-component variation of the Gal4-UAS system that comprises a Gal4-AD, Gal4-DBD, and UAS.



Southall et al, CSH Protocols (2008)

Gal4 DNA-Binding Domain ; *Gal4 Activation Domain* x

UAS-effectors for:
- Genetic Ablation
- Lineage Tracing
- Migration
- Morphology
- Cellular Dynamics

Many thanks to the JHUSOM BCMB programme and members of the Erika Matunis Lab, especially Dr. M. de Cuevas for invaluable guidance in the art of fly pushing.Special thanks are due to Dr. Jihoon Kim (JHUSOM), members of the Steven DiNardo Lab (UPenn), and the JHU SOM Microscope Facility for their expertise and instruction on dissecting and visualizing embryos.

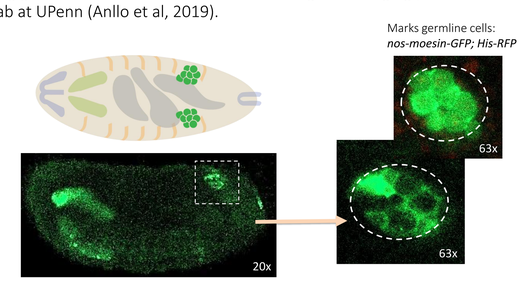
Dissection & Imaging of the Embryonic Drosophila Gonad

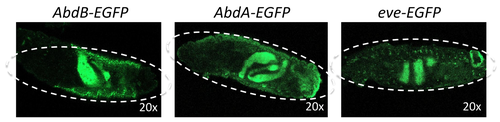
Visualisation of the developing gonad involved adaptation of an *ex vivo* gonad imaging protocol developed by the DiNardo Lab at UPenn (Anllo et al, 2019).

- Embryo collection on apple-juice agar
- Removal of chorion in 50% bleach
- Age to appropriate stage on humidified agar plate
- Mount on glued cover slip
- Removal of vitelline membrane by hand
- Select GFP+ embryos

→ Fixation & Immunostaining → Imaging with confocal microscopy

→ Dissection of the embryonic gonad with microneedles in glass-bottom dish → Live imaging of isolated gonads in *ex vivo* culture

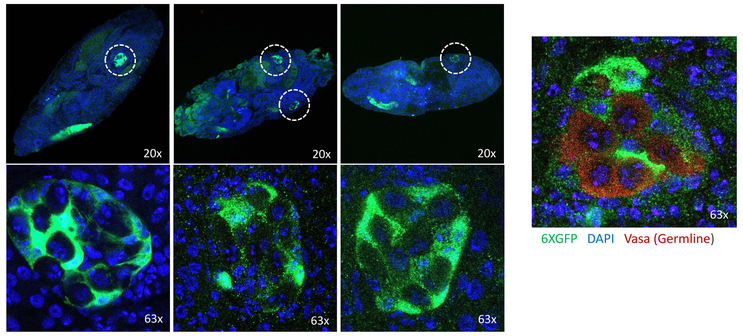




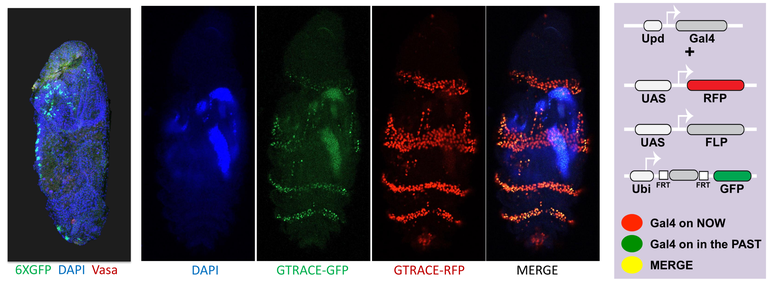
Gene Expression Charaterisation & Lineage Tracing

Crosses to UAS-hexameric GFP can enable fixed and live imaging of expression patterns of the final driver constructs. Drivers with UAS- GTRACE opens up the possibility of lineage tracing.

UAS-6XGFP



x UAS-6XGFP *UAS-GTRACE*



Implications & Future Directions

- ❑ Generation of *Drosophila* drivers for spatially specific transgene expression during development
- ❑ Specificity attained by overlapping patterns of gene expression
- ❑ Develop tools to study the developmental basis of heterogeneity in the testis stem cell niche
- ❑ Will further our understanding of stem cell function in tissue regeneration, reproduction & fertility