Evaluating the Effect of Extracellular Gaps on Border Cell Migration in Drosophila

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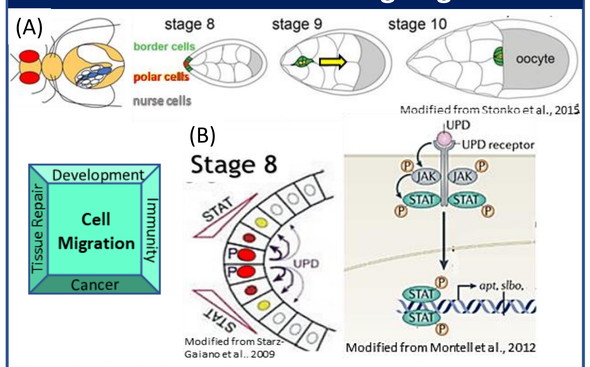
Abstract

Elucidating the underlying mechanisms governing collective cell migration is imperative, given their role in development, wound healing, and diseases like cancer. Many cell migration studies, however, are conducted in vitro, which neglects to consider the chemical and biophysical complexities of the in vivo tissue environment. Collectively migrating populations of cells traverse through the physically diverse architecture of a tissue, often along a concentration gradient of diffusible chemical attractants (chemoattractants). Using the border cells, which navigate through the three-dimensional cellular terrain of the Drosophila egg chamber during oogenesis, we can study the impact of physical architecture on collective cell migration in vivo. We observe extracellular gaps within the egg chamber and hypothesize that they directly affect the migration behaviors of the border cells, potentially by disrupting the local distributions of secreted chemoattractants. In silico, our lab has demonstrated these gaps affect the distribution of the morphogen that specifies border cell fate and preliminary results in vivo has shown that diffusible chemical signals concentrate between cells at potential gaps. Therefore, the underlying tissue architecture might affect chemical distribution. Additionally, genetically inducing a uniform concentration of the potent chemoattractant, Platelet Derived Growth Factor/Vascular Endothelial Growth Factor (PVF1), in the egg chamber stalled border cells at what may be an extracellular gap between cells. Furthermore, we are using mutant fly lines that perturb the egg chamber's innate physical architecture and lines that manipulate the chemoattractant gradient shape to evaluate the impact on border cell migration. This study identifies a role for surrounding tissue architecture in affecting collective cell migration and aims to explore how this can alter the distribution of chemical cues.

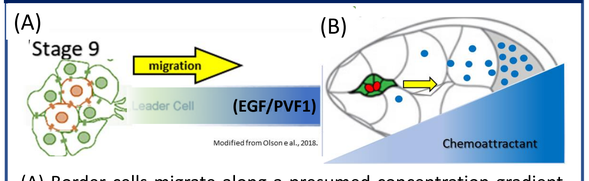
Figure 1: Drosophila border cells migrate in a collective cluster during oogenesis



Cell migration is imperative from early embryonic development to adult tissue maintenance. (A) Drosophila border cells enable study of collective cell migration in a 3-dimensional environment. Within the ovary, the border cells migrate posteriorly in the egg chamber over two stages. The cluster navigates between the germline nurse cells towards their destination. (Saadin and Starz-Gaiano, 2016). Arrow indicates direction of migration. (B) Anterior polar cells (red) secrete Unpaired (Upd) to activate Janus Kinase-Signal Transducer and Activator of Transcription (JAK/STAT) signaling in 6-8 fated border cells (Rorth et al., 2002).

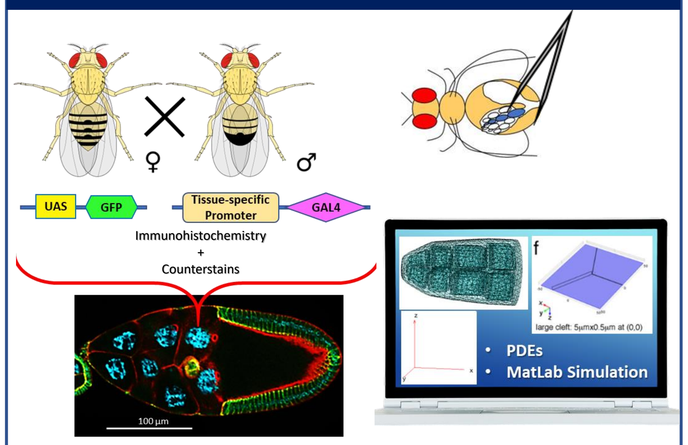
Research Question: How do cells navigate in vivo?

Figure 2: A chemoattractant gradient guides the border cells



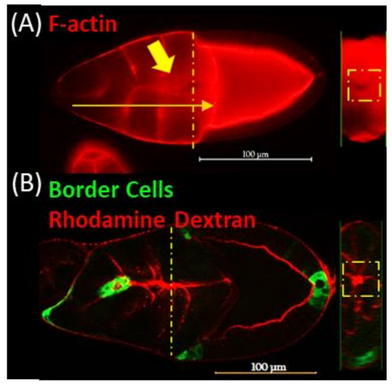
(A) Border cells migrate along a presumed concentration gradient. The Receptor Tyrosine Kinase (RTK) ligands, epidermal growth factor (EGF) and Platelet Derived Growth Factor/Vascular Endothelial Growth Factor 1 (PVF1), are necessary and sufficient to direct migration. (Duchek et al., 2001; Duchek et al., 2001; McDonald et al., 2006, McDonald et al., 2003). (B) While chemical signals are hypothesized to be graded, the physical 3-dimensional tissue architecture could theoretically perturb the formation of a continuous gradient.

Figure 3: Experimental overview



Egg chambers are dissected from the ovaries of transgenic flies and either fixed and immunostained or live-imaged and treated with relevant live stains. Imaging is conducted on a microscope equipped with Zeiss Apotome for optical sectioning. To supplement biological data, the egg chamber is modeled in 3-dimensions. Using partial differential equations, a reaction-diffusion system mimicking the secretion of chemical signals is established in the context of a simulated egg chamber complete with extracellular geometry.

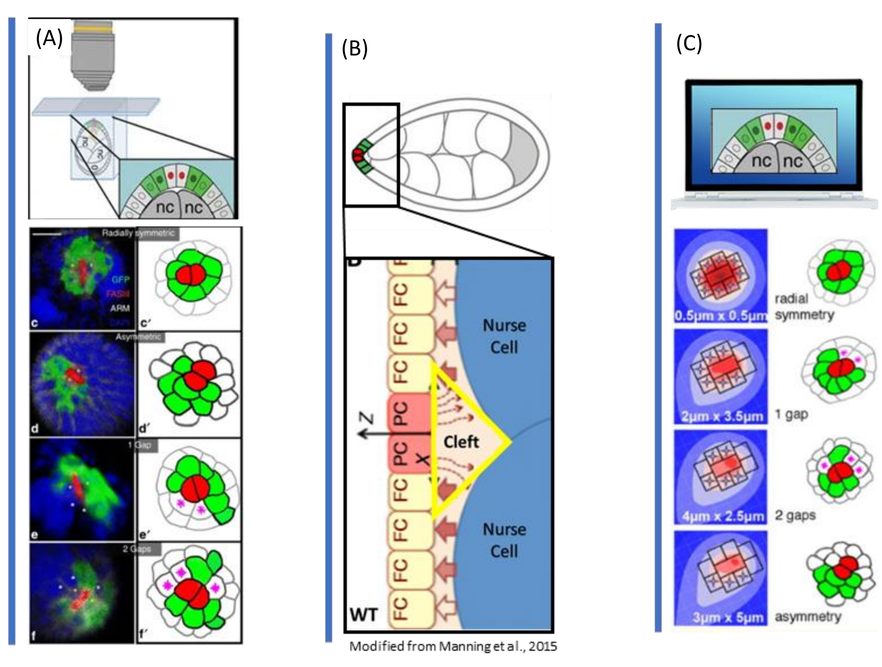
Figure 4: Extracellular gaps exist within the egg chamber



Maximum intensity projections in the X-Y plane from Z-stack images. Yellow dashed lines in the X-Y images shows the X-Z cross-section that corresponds with the inset image. (A) Wild type egg chambers stained with phalloidin reveals gaps between nurse cells along the border cell navigation route. The large arrow points to the border cell cluster and the thin arrow indicates direction of migration (B) Slbo-GAL4, UAS-GFP egg chamber incubated in diffusible Dextran (red). Dextran concentrated between nurse cells at presumptive gaps.

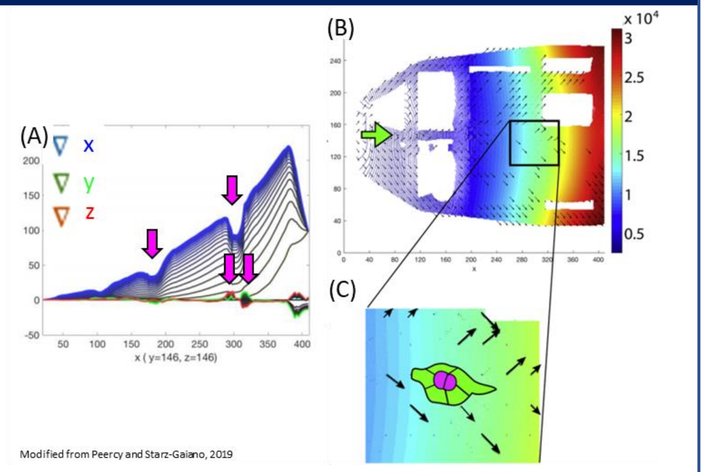
Hypothesis: extracellular gaps affect local concentrations of extracellular chemical signals, which in turn, affects the migration of border cells

Figure 5: Extracellular gaps affect morphogen distribution determining border cell specification



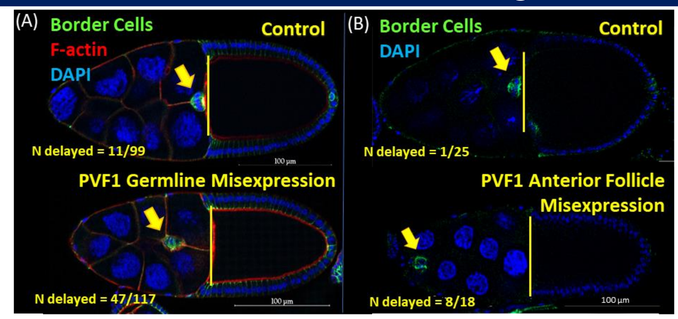
(A) Upright imaging the anterior of Slbo-GAL4, UAS-GFP egg chambers demonstrates that STAT activation can be asymmetrically patterned. Polar cells, red; border cells, GFP-positive green (B) Graphic model of how underlying tissue clefts or gaps in the anterior egg chamber could affect distribution of the secreted Upd. (D) In silico experiments manipulating the cleft dimensions and estimating Upd distribution yielded STAT-activation patterns comparable to patterns observed in vivo. (Manning et al., 2015)

Figure 6: Models suggest extracellular gaps disrupt a chemoattractant gradient



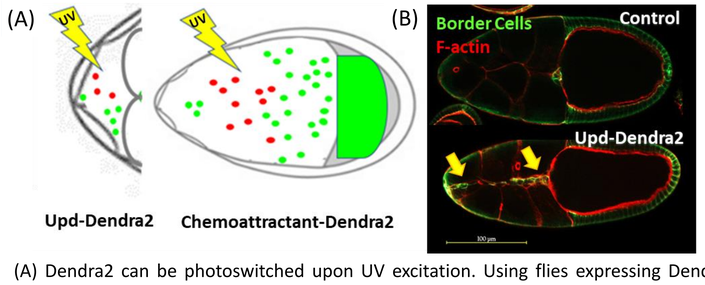
The 3-dimensional architecture of the egg chamber is modeled such that an oocyte face secretes chemoattractants, which diffuse throughout the egg chamber. (A) Concentration gradient profile of x, y, and z planes. Purple arrows represent shifts in the magnitude of the gradient near nurse-cell junctions, at putative gaps. (B) Heat map and vector field map showing the chemoattractant concentration and local gradient vectors in the X-Y dimensions. (C) Inset shows changes in gradient direction at junctions. (Mekus et al., 2018)

Figure 7: Flooding the extracellular gaps with chemoattractant results in border cell migration defects



(A) Misexpressing PVF1 from all germline cells (nurse cells and oocyte) causes a slight delay in border cell migration. Yellow arrows indicate position of border cell cluster and yellow lines indicate the oocyte face. (B) Similarly, misexpressing PVF1 from anterior follicle cells (polar cells and border cells) caused a delay in border cell migration.

Figure 8: Dendra2 enables live tracking of chemical distribution



(A) Dendra2 can be photoswitched upon UV excitation. Using flies expressing Dendra2 fused to Upd or chemoattractant under the control of UAS-GAL4, we can track "new" vs "old" chemical signal diffusing throughout the egg chamber. This will allow us to build a map of how the chemical gradients are formed. (B) Phenotypic validation of egg chambers expressing Upd-Dendra2 from anterior follicle cells. Yellow arrows point to formation of excess border cell clusters consistent with STAT overactivation.

Conclusions

- Extracellular gaps exist in the anterior end of the egg chamber and along the border cell's migration route
- The geometry of extracellular gaps likely affect the distribution of the Upd morphogen
- Computational modeling suggests that extracellular gaps affect local chemoattractant concentrations to create a discontinuous gradient
- Flooding gaps with chemoattractant in the egg chamber likely disrupts its gradient and delays border cell migration

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