Hedgehog signaling between the wing-disc epithelium and muscle precursors revealed by single-cell analysis in *Drosophila*

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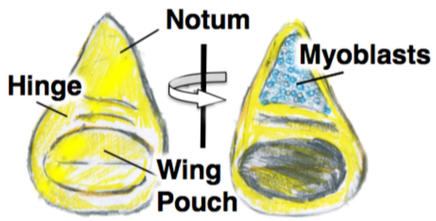
Abstract and background

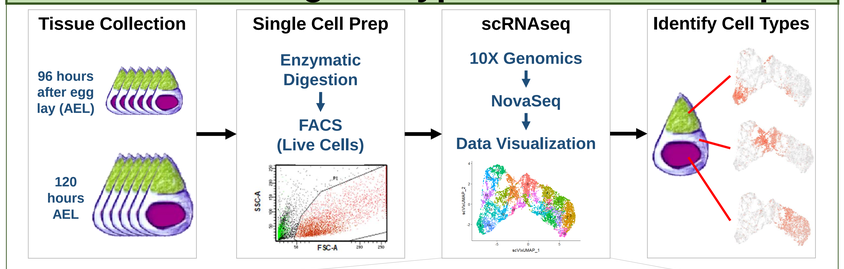
Organs are composed of distinct cell types that need to coordinate growth and patterning for proper development. The mechanisms of how this is achieved are not fully understood. We address questions related to these topics with the wing-imaginal disc of *Drosophila melanogaster*, a relatively simple structure composed of both epithelial and mesodermal cells that develop into the adult wing blade, thorax, and flight muscles. In this study, we:

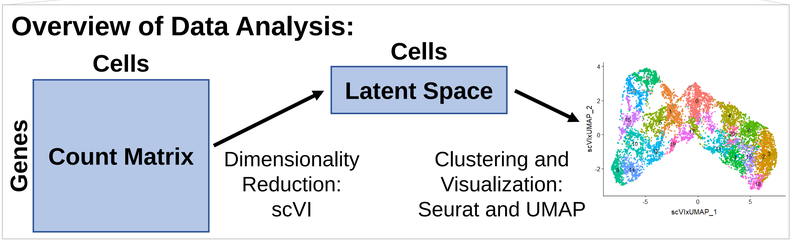
- Collected and harmonized single-cell RNA sequencing data from wing disc cells at two developmental time points
- Constructed single-cell RNA atlases that define the primary domains within the wing-imaginal disc and the disc-associated adult flight muscle precursor cells (AMPs)
- Found that a subset of AMPs – those that underlie the posterior compartment of the disc epithelium – transduce Hedgehog (Hh) signaling
- Identified that the genes Neurotactin and Midline are AMP-specific targets of Hh signaling



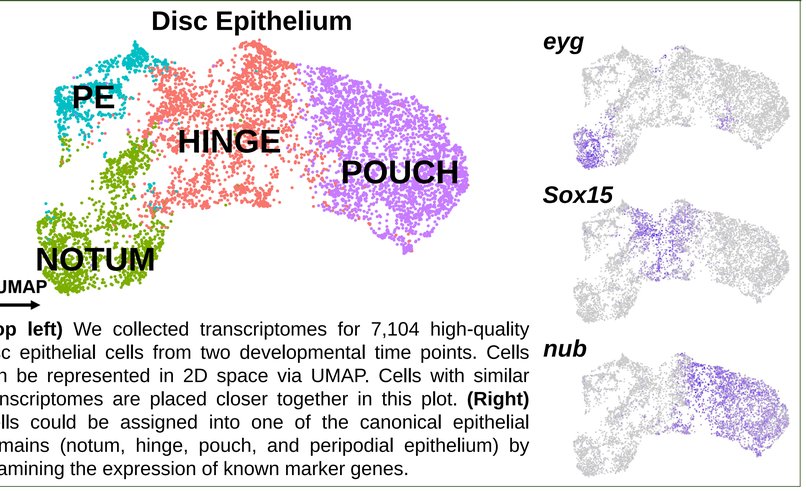
(Left) The wing disc epithelium is composed of four major domains: the pouch, hinge, notum, and peripodial epithelium (PE; not shown). The disc-associated myoblasts or adult muscle precursors (AMPs) are precursors of direct and indirect flight muscles. The indirect flight muscles generate the mechanistic force required for flight. The direct flight muscles control flight steering by fine-tuning the position of the adult wing blades.

Characterizing cell types with scRNAseq



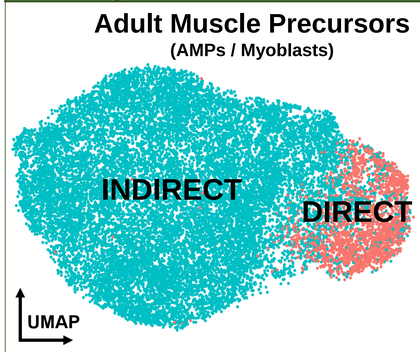


Single-cell atlas of disc epithelium

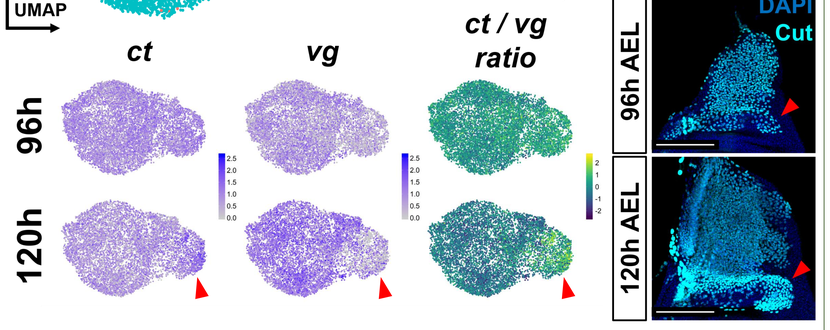


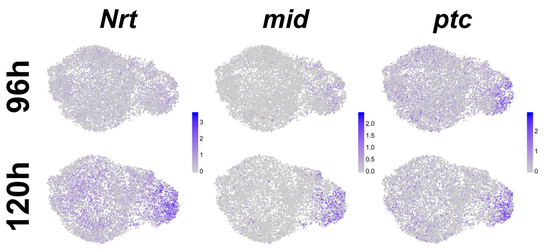
(Top left) We collected transcriptomes for 7,104 high-quality disc epithelial cells from two developmental time points. Cells can be represented in 2D space via UMAP. Cells with similar transcriptomes are placed closer together in this plot. **(Right)** Cells could be assigned into one of the canonical epithelial domains (notum, hinge, pouch, and peripodial epithelium) by examining the expression of known marker genes.

Single-cell atlas of adult muscle precursors



(Left) UMAP plot of 20,730 high-quality AMP transcriptomes. **(Bottom left)** AMPs can be classified into indirect and direct cell types via the expression of canonical markers *vestigial (vg)* and *cut (ct)*. We observe uniform expression of both genes in all AMPs at 96h, but by 120h, these genes become differentially expressed between direct and indirect AMPs. **(Bottom right)** We confirm that Cut is expressed at higher levels in direct AMPs (red arrow) only at 120h via antibody staining; at 96h, most AMPs are stained uniformly.



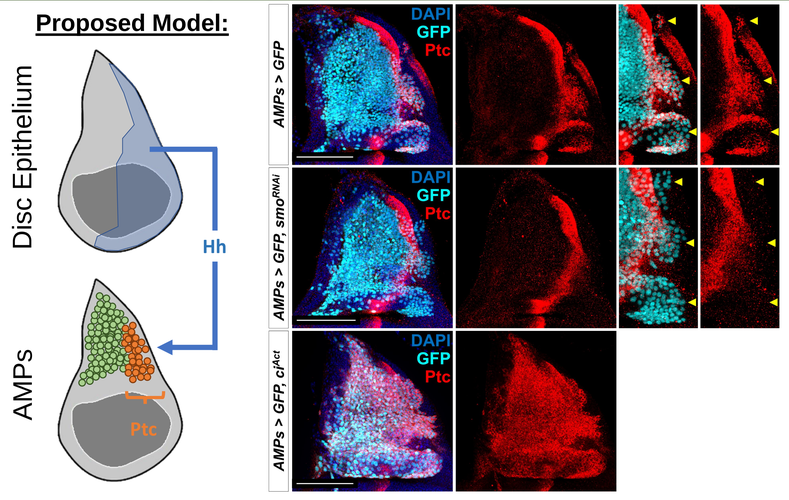


(Left) Transcripts for *Neurotactin (Nrt)*, *midline (mid)*, and *patched (ptc)* mark a large subset of direct AMPs. Interestingly, both *Nrt* and *mid* show a dramatic increase in expression from 96h to 120h, while *ptc* has similar expression levels at both time points.

Ptc encodes the receptor and downstream target of Hedgehog (Hh) signaling, and its expression is indicative of active Hh signaling within cells.

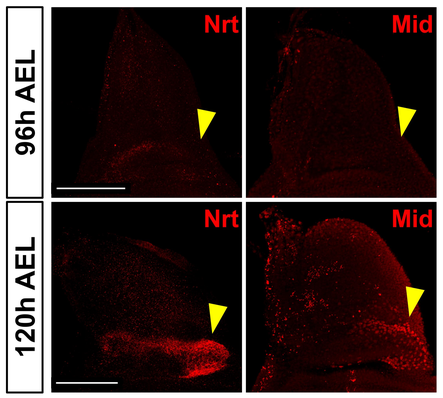
Could Hh signaling define a population of AMPs? And are Nrt and Mid downstream targets of Hh signaling?

Hh signaling defines AMP subset

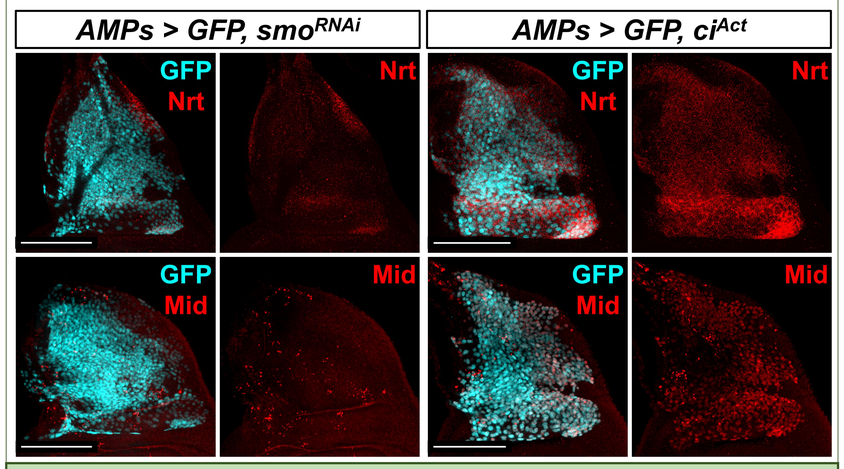


(Above) AMPs that overlay the Hh-producing posterior compartment of the disc epithelium are marked by active Hh signaling (higher levels of Ptc staining). Hedgehog signaling can be eliminated or activated in all AMPs via expression of *smo*^{RNAi} or *ci*^{Act}, respectively. Note that the persistent stripe of Ptc staining under *smo*^{RNAi} conditions is from the disc epithelium.

Nrt and Mid are targets of Hh signaling in AMPs



(Left) Staining for Nrt and Mid reveals expression of these genes in a similar location as Ptc expression. As suggested by our scRNAseq data, the expression of both genes increases from 96h to 120h. Notable, the expression pattern of Nrt extends farther into the domain of the anterior-localized AMPs. **(Bottom left)** Knocking down Hh signaling via *smo*^{RNAi} eliminates the expression of both Nrt and Mid in AMPs. Interestingly, this knock down of Hh signaling affects Nrt expression in both anterior- and posterior-localized AMPs. **(Bottom right)** Activating Hh signaling via *ci*^{Act} induces expression of Nrt and Mid in all AMPs, including ectopic expression in indirect AMPs.



Related work

Is Hh signaling important for proper muscle formation?

Check out Riku Yasutomi's poster on how perturbing Hh signaling within AMPs disrupts proper adult muscle fiber structure!

What genetic responses are activated in a regenerating wing disc?

Check out Melanie Worley's talk on how we used single-cell RNAseq to study regeneration within the wing-imaginal disc! Date: April 24th @ 12:00-12:15 pm.

And keep an eye out for our upcoming BioRxiv preprint on the work described in this poster (and more)!

Acknowledgements

Interested in more *Drosophila* organ development?

Check out posters by lab mates Maya Emmons-Bell, Sophia Friesen, and Jamie Lahvic, and listen to Jamie's talk too (April 23rd @ 3:45 pm)!

TAGC 2020 online

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Hariharan Lab @ UC Berkeley