

Single cell analysis in Myoblasts and Epithelial cells uncovers early events in fibre-type divergence in *Drosophila melanogaster*

Introduction

- Two types of adult thoracic muscles: Indirect Flight Muscles (IFM) and Direct Flight muscle (DFM) which rise from adult muscle precursor cells (AMPs) in the third instar larval wing disc (*Figure 1*), also known as myoblasts. The muscles have unique structure and function.
- Aim is to determine how the AMPs develop into two distinctly different muscles. Hypothesis: a transcriptional programme causing the divergence of myoblasts where genes are selectively transcribed in cells specific to the clusters that will become either the IFM or DFM.
- Methods: single cell RNA-sequencing (scRNA-seq), immunofluorescence staining and genetic screens- viability and flight tests.
- scRNA-seq of wildtype wing disc produced UMAP plot, and 26 clusters of myoblasts, epithelial and tracheal cells were identified.
- Dot plots produced showing expression levels of multiple genes in each cluster, and staining was undertaken to confirm the dot plot.

Myoblast markers

- Sc-RNA seq identified 7 clusters: 2 DFM, 5 IFM clusters by *ct*, *zfh1* and *vg*.
- Dot plot produced showing gene expression level in each cluster.
- Clusters mapped onto the wing disc by tracing the novel markers identified from the dot plot using immunofluorescence staining.
- Genes for IFM: *zfh1*, *nkd*, *E(spl)* genes, *Cg25C*, *vkg*, *edl* (*Figure 3 and 4*).
- *E(spl)* genes involved in Notch signalling- maintains undifferentiation state. Expressed in IFM_1-2 so undifferentiated cells at this stage.
- Genetic tracing of IFM markers using gTRACE-GAL4 driver- RFP shows where the gene is currently being expressed and GFP shows where the gene was previously expressed (*Figure 5*), confirms differentiation states of IFM_1-2.
- Genes for DFM: *ten-a*, *ct* (*Figure 6*).
- Identified role of new genes in muscle development using genetic screens- flight and viability test (*Figure 7 and 8*)- e.g. *mamo*, *LamC*, *chinmo*.

Results:

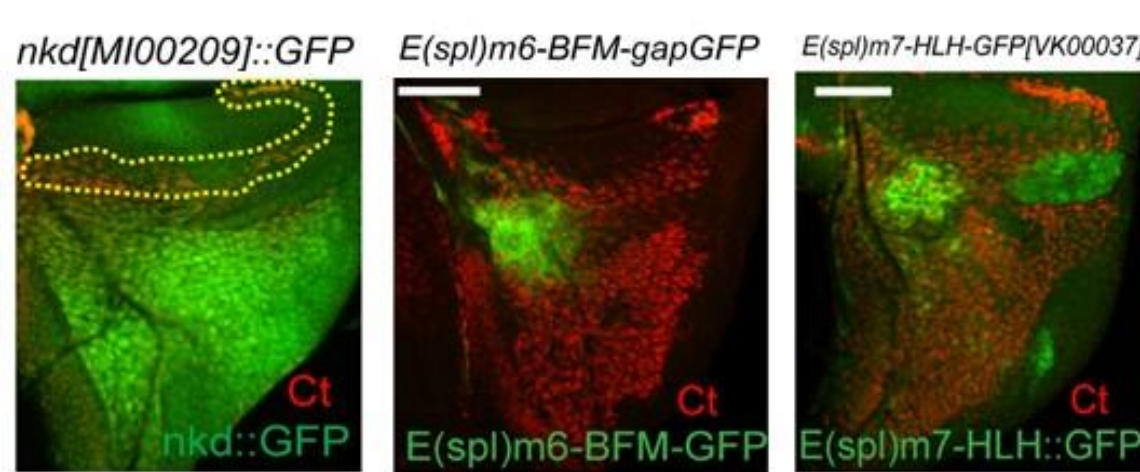


Figure 3: staining of wing disc stained anti-ct with A: nkd[M100209]:GFP, B: E(spl)m6-BFM-gapGFP and C: E(spl)m7-HLH-GFP

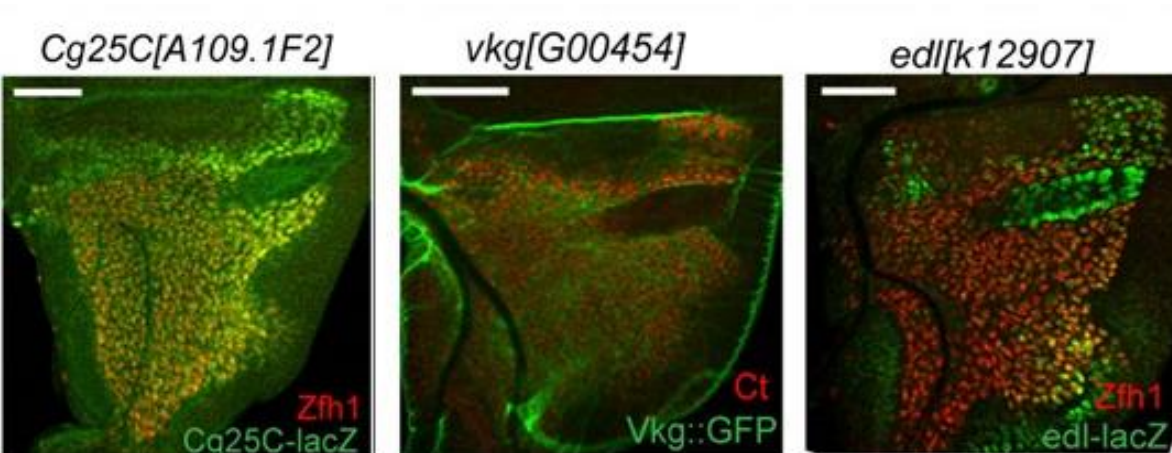


Figure 4: staining of wing disc stained with A: edl[k12907]-lacZ (green) and anti-zfh1 (red), B: Cg25C[A109.1F2]-lacZ (green) and anti-zfh1 (red), C: vkg[G00454]-GFP (green) and anti-ct (red)

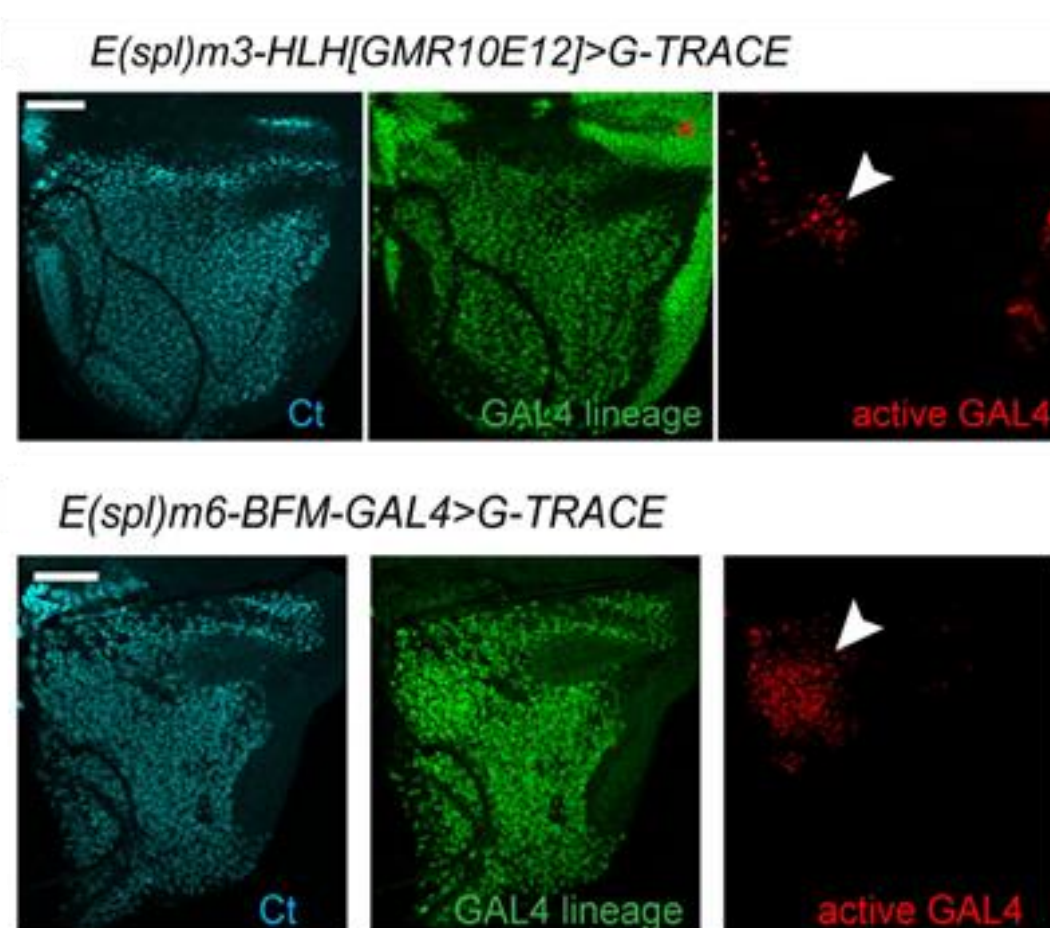


Figure 5: staining of wing disc of E(spl)m3-HLH >gTRACE (A), E(spl)m6-BFM >gTRACE (B), stained with anti-ct (blue).

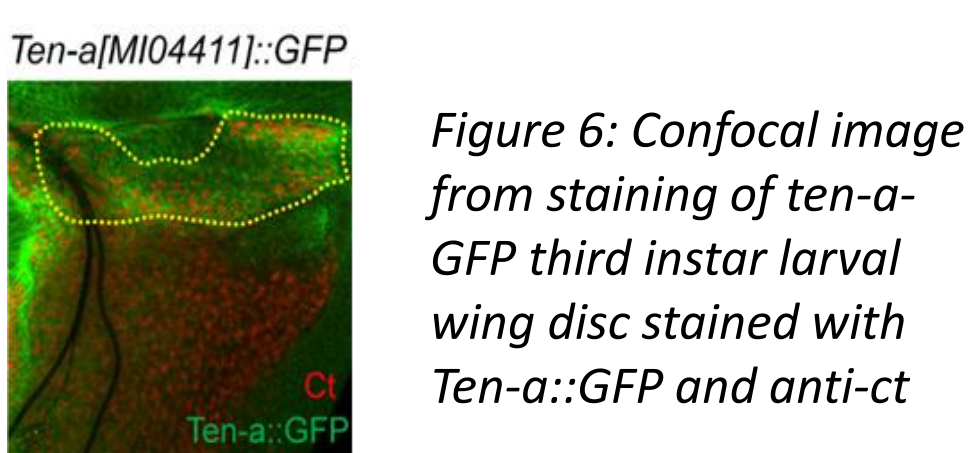


Figure 6: Confocal image from staining of ten-a-GFP third instar larval wing disc stained with Ten-a::GFP and anti-ct

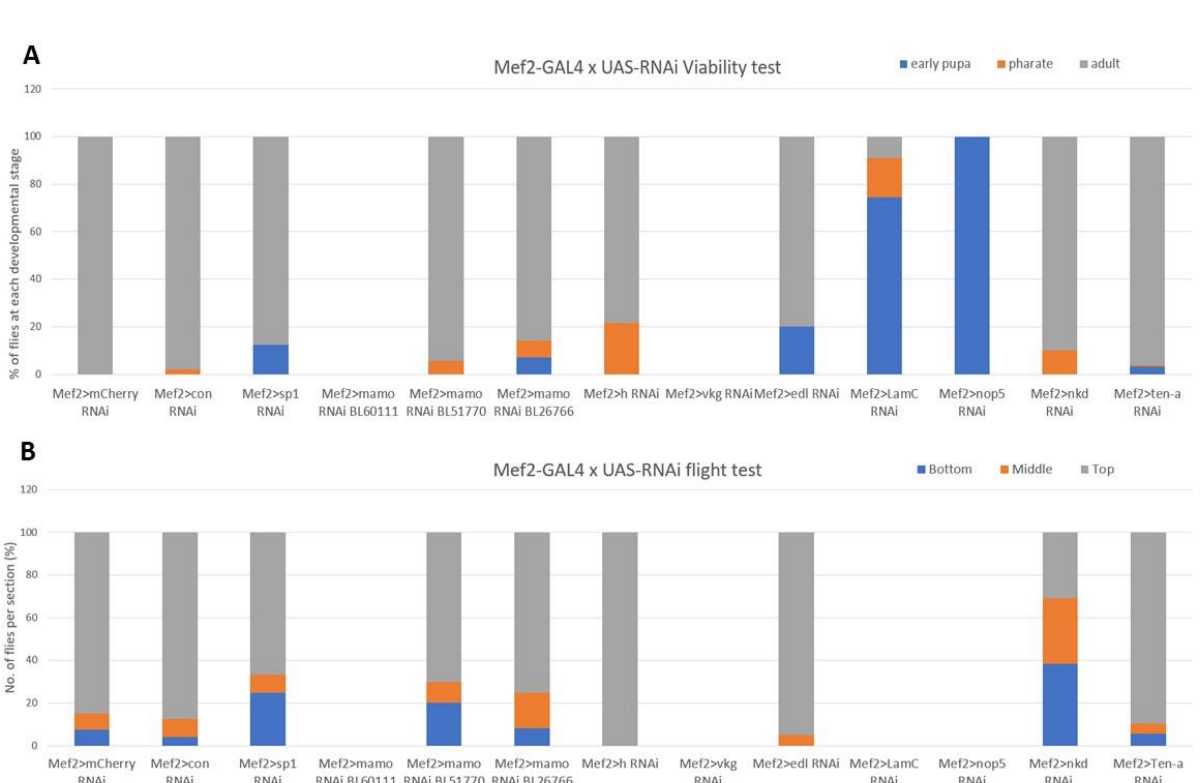


Figure 7: RNAi screens of markers using Mef2-GAL4 driver, A: viability test scoring % of flies at each stage of development. B: flight test scoring % of flies at each section of the column; bottom (blue), middle (orange) and top (grey)

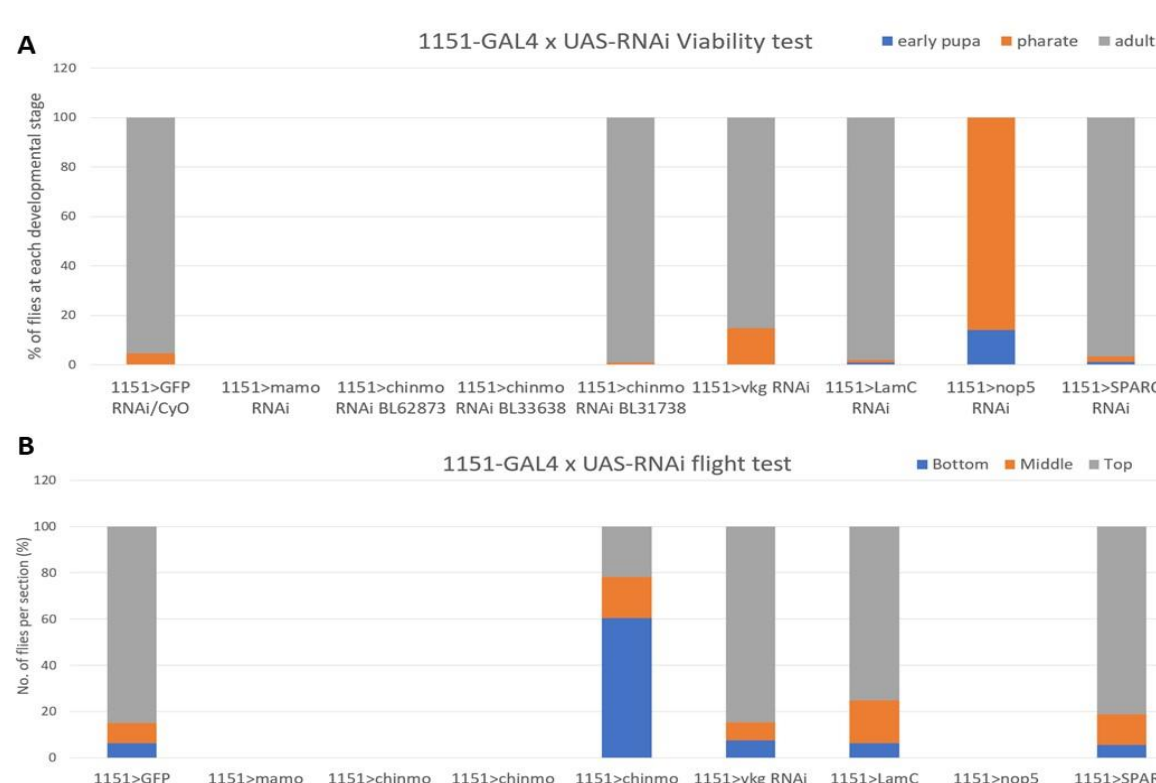


Figure 8: RNAi screens of markers using 1151-GAL4 driver, A: viability test scoring % of flies at each stage of development. B: flight test scoring % of flies at each section of the column; bottom (blue), middle (orange) and top (grey)

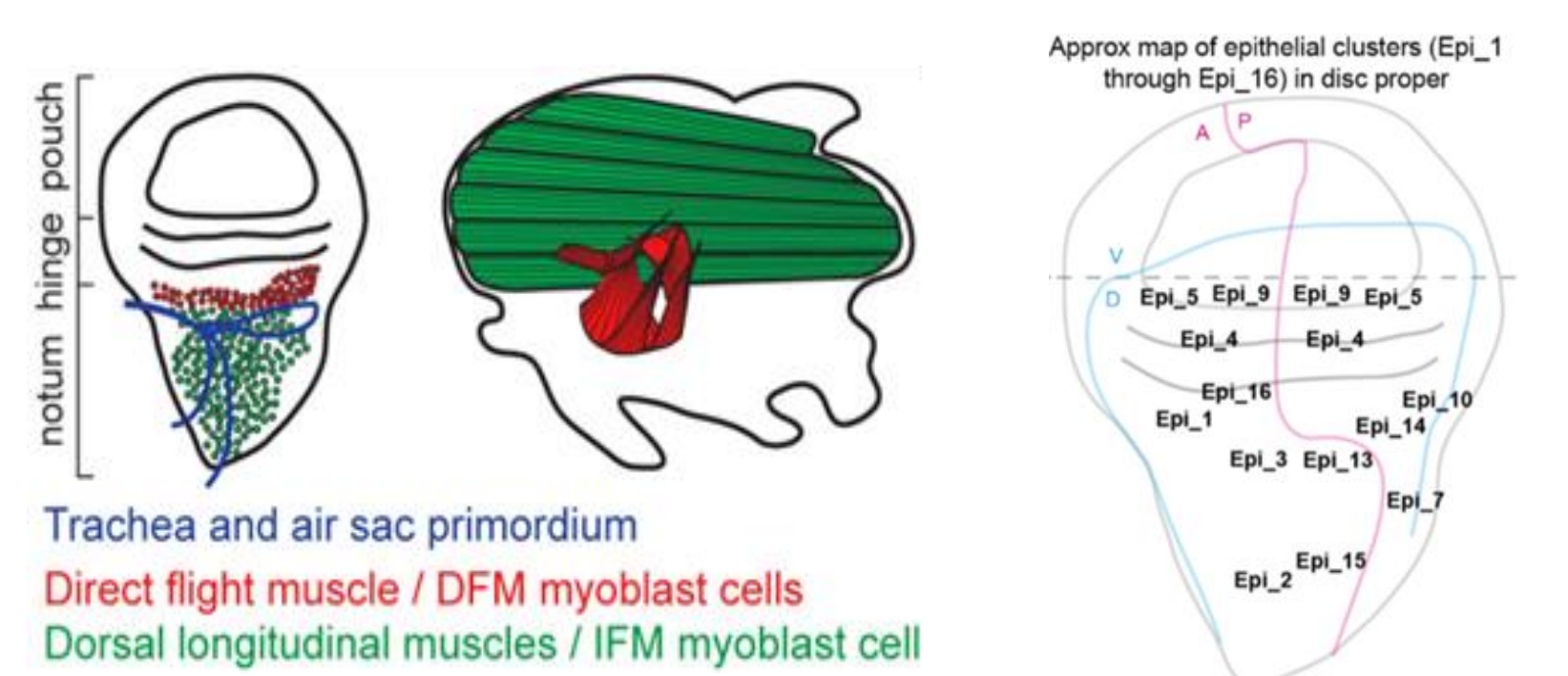


Figure 1: origin of the flight muscle- left is the wing imaginal disc, right is cross section of adult thorax (Zappia et al., 2020)

Figure 2: epithelial clusters mapped on to wing disc (Bryant 1975)

Epithelial markers

- 17 epithelial clusters identified by sc-RNA seq.
- Looking at the difference in gene expression between myoblast and epithelial cells.
- Dot plot produced showing gene expression level in each cluster.
- Known location of the clusters on wing disc (*Figure 2*).
- Clusters mapped onto wing disc by tracing novel markers for each cluster using immunofluorescence staining (*Figure 9*).

Results:

- Epi_5 and 9: *nub*, *wg*- the wing blade
- Epi_15: *dpp*- anterior to the A/P boundary in the medial notum
- Epi_16: *Dad*- central area of hinge along A/P boundary
- Epi_13: *hairy*, *svp*- presumptive scutum in the lateral heminotum

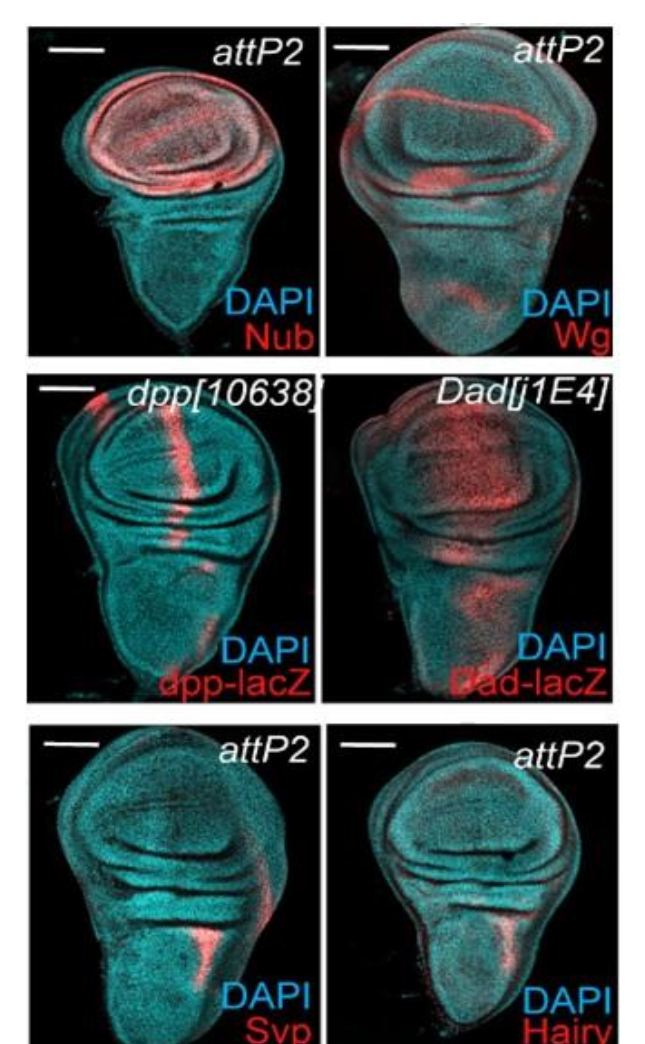


Figure 9: images of staining of wing disc of genes (red) and counter stained with DAPI (blue)

Conclusion

- Distinct transcriptional programme causes divergence in the AMPs in the wing disc to produce the two muscle types
- New genes identified specific to each muscle type
- Next: identify more genes involved, apply technique to other developmental processes, e.g. eye development