Wounding and Altered Cell Cycles

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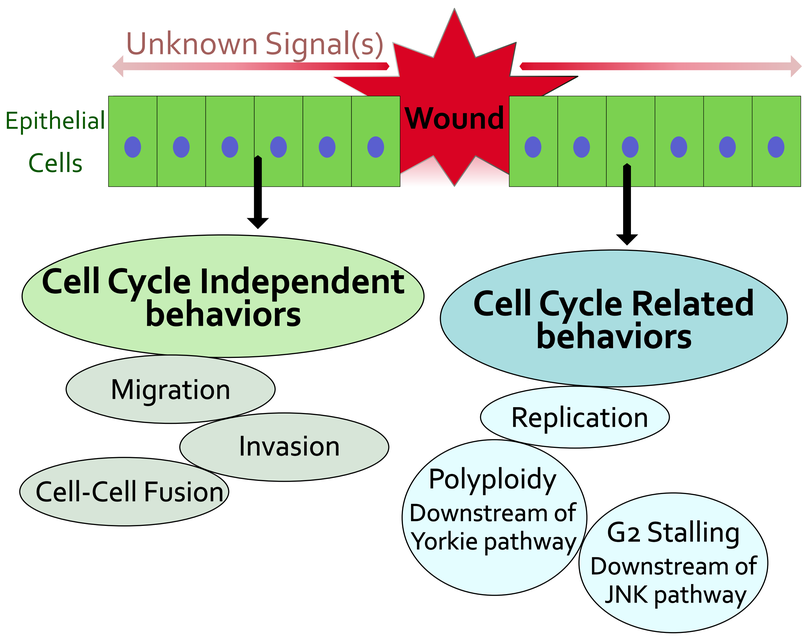
Cell and Dev Bio[1], Program in Dev Bio[2], Dep Physics and Astronomy[3]

Overview



Enlarged Nuclei and Apparent G2 Stalling Post Wounding

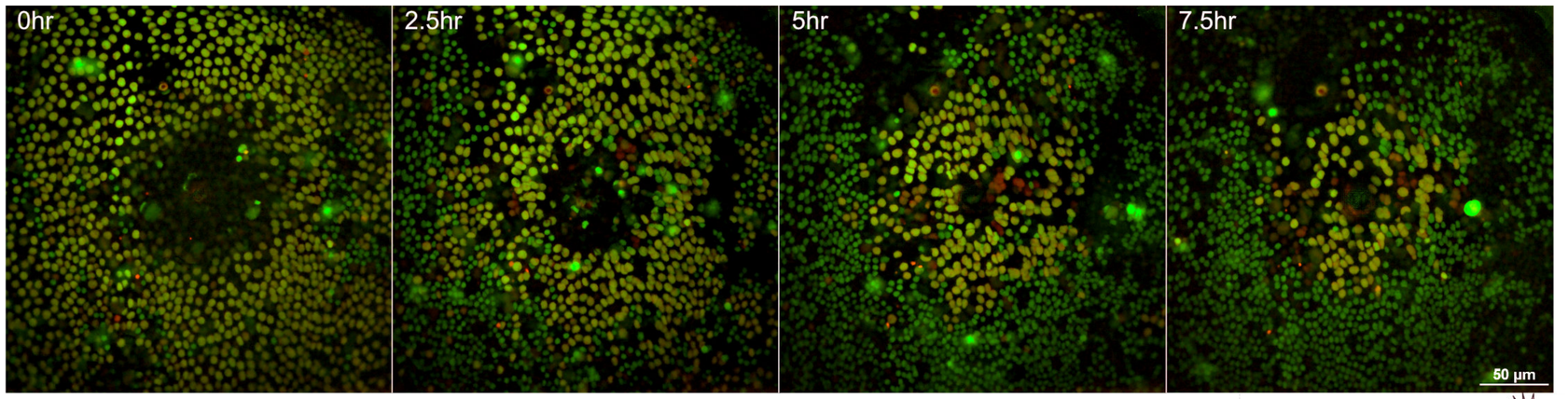
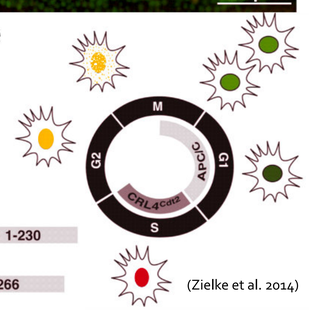
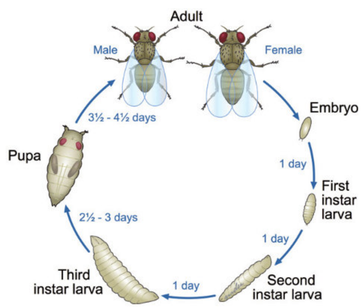


Fig. 1: Live imaging of the FUCCI cell cycle indicator following pulse laser ablation. Max intensity projections in the 488 (E2F1-GFP) and 561 (CycB-RFP) channels shows repair of the fly notum over time.

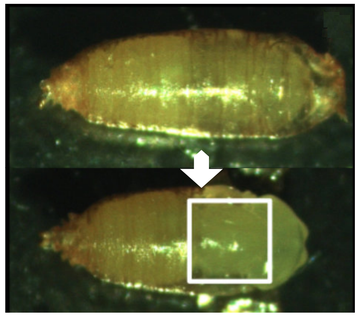
- 5hr post wounding, enlarged apparently G2 stalled (yellow) nuclei are evident compared to distal G1 (green) nuclei.
- JNK has been implicated in G2 stalling, however, the wound signal(s) inducing it are unknown. Knockdown of upstream signaling components may allow us to assign a damage associated molecular pattern (DAMP, eg. cell lysate, ATP, ROS) to reveal drivers of G2 stalling.
- These experiments may provide insight into the earliest factors driving tissue re-pattering following wounding.



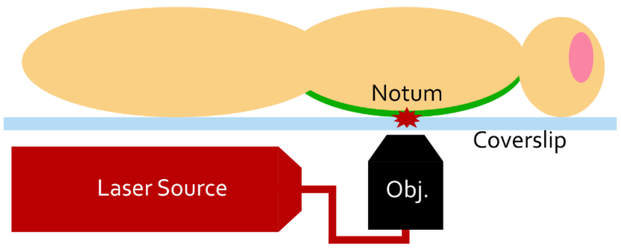
Drosophila as a Wound Model



A 10 day generation time and large litter size make flies easy to use. (Cynthia Ong, 2015)



A simple dissection exposes the notum, an ideal tissue for wounding and imaging.

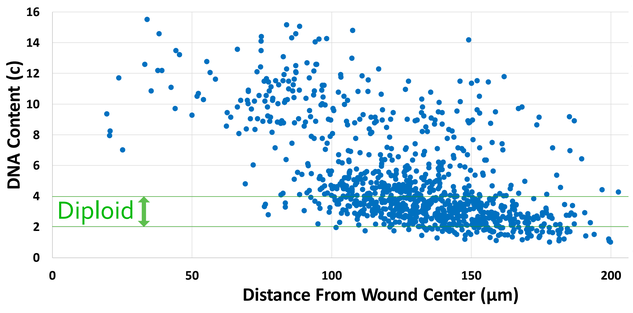


Our system lets us image from immediately after wounding through the duration of pupation.

DNA Content Increase

Fig.2: Dissected, formaldehyde fixed, and DAPI stained pupal notum.

- Nuclei segmented with (*pnr>mCherry.NLS*) and epithelial specific cells defined by (*E-cadherin:GFP*).
- DAPI intensity summed within segmented regions, and normalized to spermatid intensity.
- Future experiments will explore pathways responsible for the observed ploidy increase near the wound margin, as well as distally from the wound.



Syncytial Cell Formation

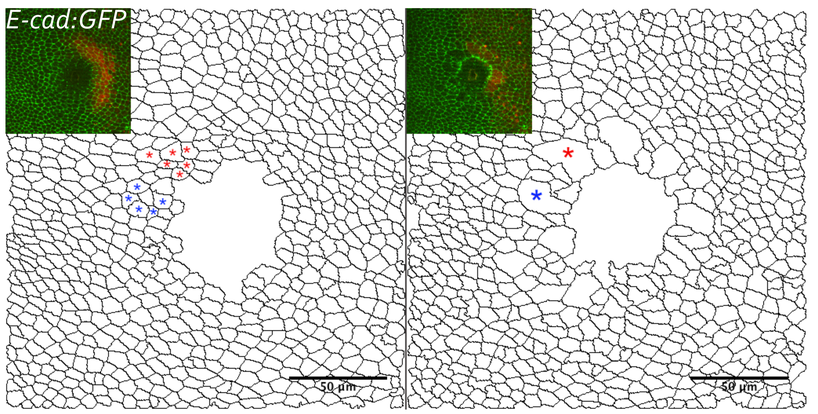


Fig. 3: SeedWater segmentation. We can investigate non-cell cycle related behaviors such as cell-cell fusions following wounding by tracking E-cad:GFP. (Asterisks represent pre/post fused cells)

Acknowledgments

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