Wesleyan University

Lord of the fl-Eyes:

Effects of Cytoskeletal Untethering on Nuclear Position, Shape, and Cell Morphology in the *Drosophila melanogaster* Eye

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Introduction

- The fly eye is made of repeating structures called **ommatidia.**
- Each cell in the ommatidium has a unique shape and position, which we hypothesize is influenced by the nucleus.

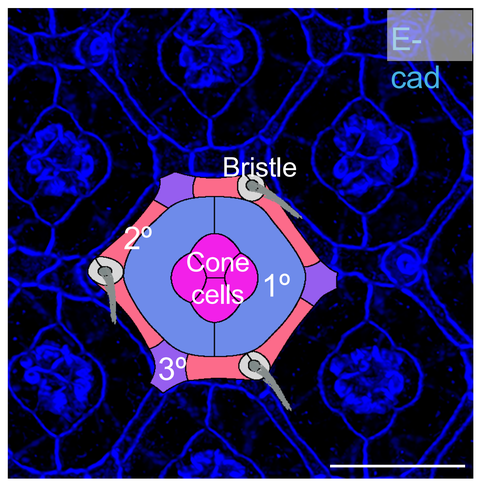


Fig 1: *Cartoon depiction of ommatidia with labels for cone cells, primary (1º), secondary (2º), and tertiary (3º) pigment cells, and bristle groups. Cartoon overlays tissue at 40 hours after puparium formation (APF) where cell membranes are labeled with E-cad via antibody staining.*
All scalebars are 10μm.

- The nucleus is tethered to the cell's cytoskeleton via the **LINC protein complex**, which attaches to nuclear Lamins on the inner nuclear membrane.

How does detaching the nucleus from the cytoskeleton affect nuclear position, structure, and cell shape in primary pigment cells?

Results and Discussion

Methods:

- Expression of the LINC protein Klarsicht was decreased using RNAi, untethering the nuclei from the cytoskeleton.
- Cell and nuclear membranes were detected via antibody staining for **E-cad** and **Lamin**.
- ImageJ was used to obtain a variety of measurements of 20-30 1° cells of 18 eyes.

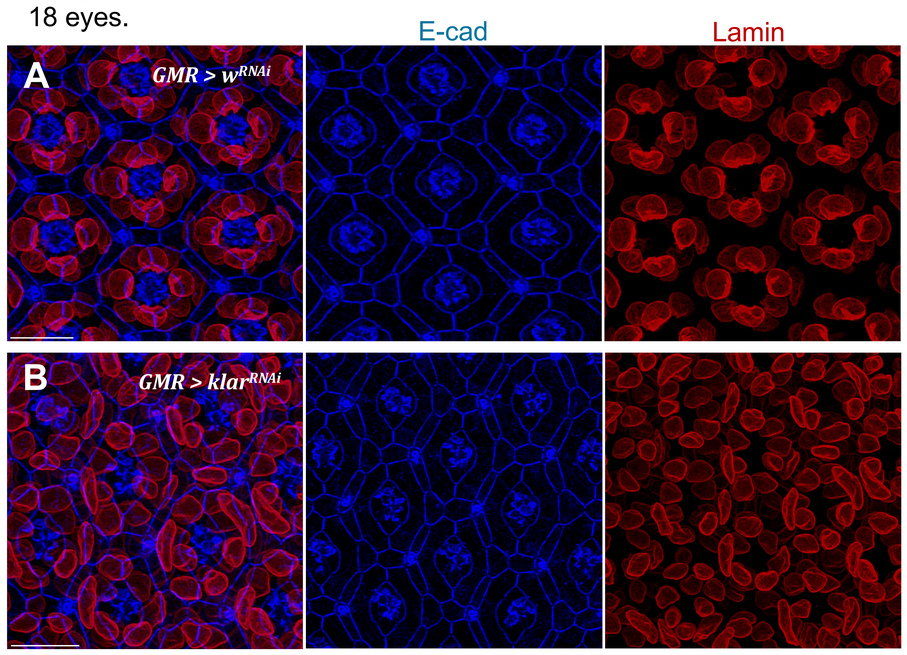
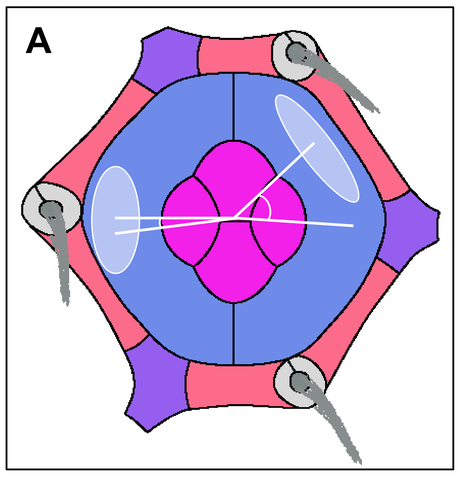


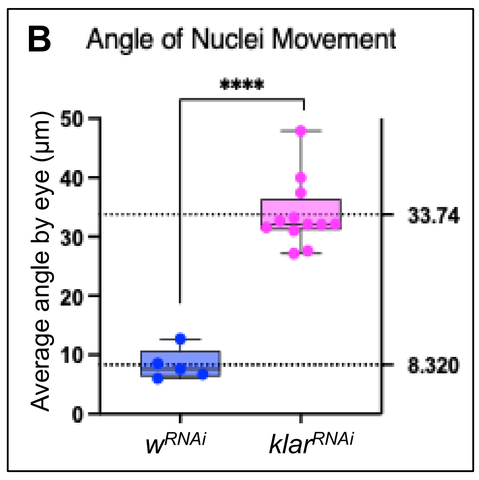
Fig 2: Control (A) vs Klarsicht deficient (B) pupal eyes at 40h APF. E-cad and Lamin outline cell and nuclei membranes. First image shows overlay of both channels. Scale bar is 10 μm.

Surprisingly, the **2D shape of 1° cells was not completely distorted,** despite obvious positional and structural changes of the nuclei.

How Does the Nucleus Move?

- **83.15% of experimental** nuclei migrated towards one pole, compared to **3.5% of control** nuclei.
- Nuclei were **equally likely to move up as down** and neighboring nuclei **did not have correlated behavior.**





- To quantify movement, we calculated the angle between the nucleus, cone cells, and 1° cell (fig 3A). **The larger the angle, the more the nucleus has moved**.
- The average angle for control nuclei was **8.32°**, compared with **33.74°** for experimental (fig 3B).

Fig 3: (A) Cartoon portrayal of ommatidia with depiction of angle calculation for a nucleus with little movement (left) vs a nucleus with significant movement (right). (B) Angle of movement for control vs Klarsicht deficient nuclei. **** = p < 0.0001, results of unpaired t-test taken on the average angle for each eye. n = 18 eyes.

How was Cell and Nuclei Shape Effected?

- Untethered nuclei became **less round**, with **larger perimeters,** a **larger aspect ratio** (length/width), and thus a **larger overall area** (not shown).
  - **Area and perimeter** of corresponding 1° cells **decreased.**
    - Decrease could be due to a developmental delay.
  - **Nuclei grew with respect to the cell**, for a **larger** nuclei to cell **area ratio**.
  - Significance changes based on statistical approach.
    - When comparing variables by eye, only four variables remained significant: **Area ratio, Nucleus perimeter, Aspect Ratio,** and **Roundness.**
  - Phenotype caused by reduced Klarsicht was variable, with some eyes more severely effected.

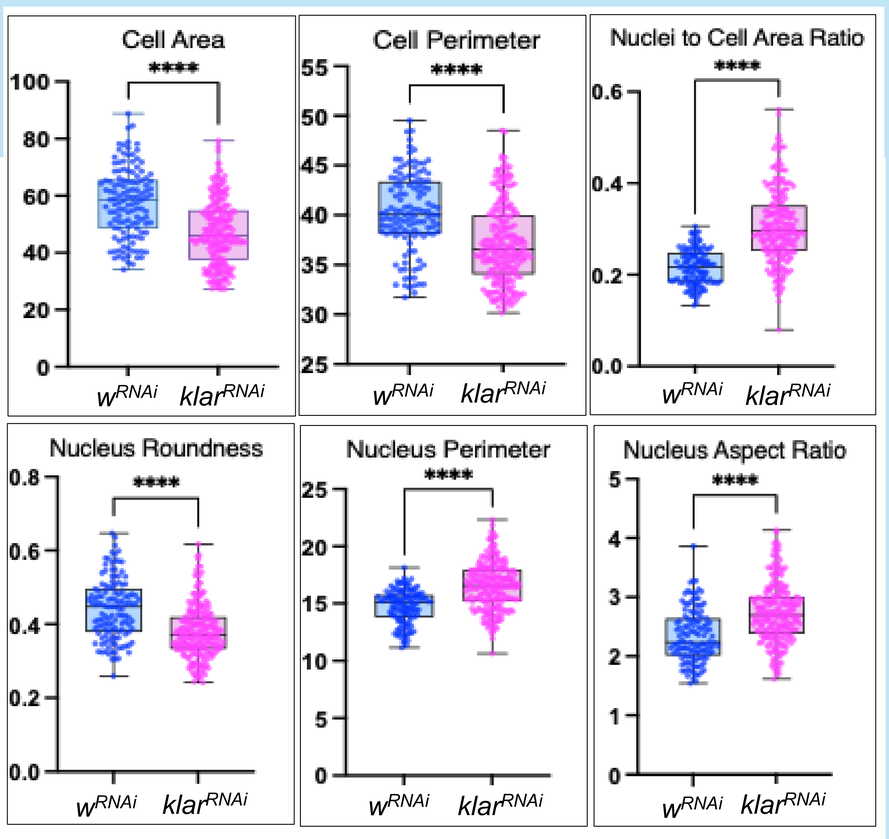


Fig 4: Significant shape changes of control vs Klarsicht deficient cells. **** = p < 0.0001, results of unpaired t-test. n = 350 cells from 18 eyes. Units on y axis are μm. Area ratio, nucleus perimeter, nucleus roundness, and nucleus aspect ratio remained significant when performing linear mixed effects model controlling for eye (p=0.009, p = 0.018, p=0.013, p=0.012 respectively).

Conclusion:

- Untethering the nucleus from the cytoskeleton caused the nucleus to **migrate significantly** in the cell**,** seemingly **at random**.
- Untethered nuclei also adopted a **crescent shape**, growing longer, thinner, and larger.
- Despite **profound differences in nuclei shape and position**, 2D cell shape is mainly intact.
- The **cell** is able to **compensate for a larger, misshapen, and displaced nucleus**, and still retain its structure.

Future directions:

- 3D structure of cells:
  - Are there shape changes not visible in 2D?
  - Does the nucleus move deeper or shallower within the cell?
- Is there a linear relationship between nuclear shape and movement?
  - Do larger, more "messed up" nuclei migrate more?
- What happens at later time points?
  - Do changes become more extreme or are they corrected?

References And Acknowledgements