Is the deubiquitinase Usp5 required for cell cycle exit?

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Background

The coordination of cell proliferation and differentiation is crucial for proper development. Through a genetic screen, we previously identified *Usp5* as a gene that is potentially required for cell cycle exit after terminal differentiation. Our results indicated that cells lacking *Usp5* experience ectopic E2F activity, based on the expression of an E2F-responsive reporter gene in *Drosophila* eye cells. In addition, cells undergo ectopic cell divisions in the absence of *Usp5*. *Usp5* encodes a deubiquitinase (DUB) in the ubiquitin-specific protease (USP) subfamily. This is particularly interesting, as proteolysis and reversible ubiquitination are known to play important roles in cell cycle regulation.

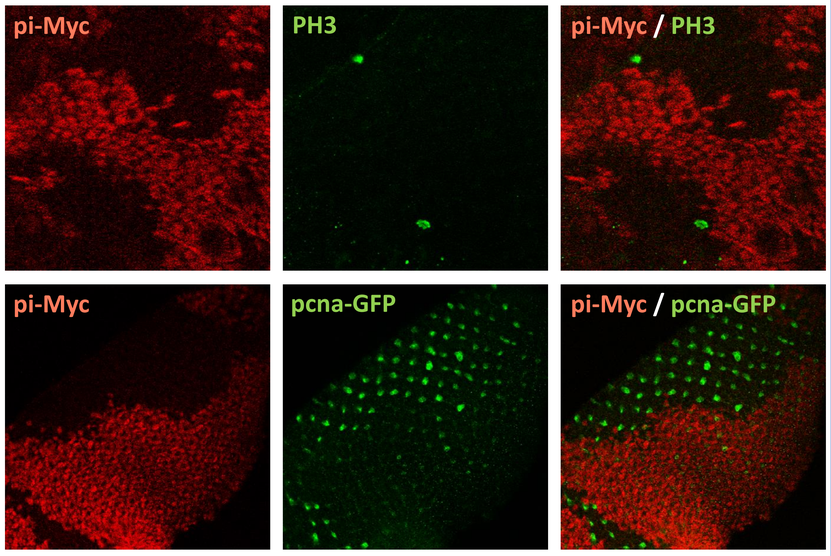

Figure 1. Preliminary data suggest the deubiquitinase Usp5 is required for cell cycle exit in *Drosophila*.

A mutant allele of *Usp5* was isolated in a genetic screen designed to identify genes required for cell cycle exit in *Drosophila* (Bandura 2013). (Top row) Homozygous mutant *Usp5* cells are marked by the absence of the red pi-Myc labeling. We dissected the pupal eyes later than 24 hours after puparium formation (APF), the time when all wild-type eye cells permanently stop dividing. At this timepoint, a small number of *Usp5* mutant cells are undergoing mitosis, as indicated by the green anti-phospho-Histone H3 (PH3) labeling. (Bottom row) Again, homozygous mutant *Usp5* cells are marked by the absence of the red pi-Myc labeling. The cells labeled green express a reporter for E2F activity, *pcna-GFP* (Thacker 2003). Many *Usp5* mutant cells in pupal eyes after 24 hours APF express high levels of *pcna-GFP*, while the wild-type cells express it at low levels or not at all.

Hypothesis

We hypothesize that further experiments analyzing cells in terminally differentiated tissues with reduced *Usp5* levels will provide confirmation that *Usp5* is required for cell cycle exit. In addition, it is possible that cells temporarily in a cell cycle arrest may undergo inappropriate cell division in the absence of *Usp5*. Based on the pattern of cells expressing *pcna-GFP* in *Usp5* mutant eye tissue, we further hypothesize the cell cycle exit defect is specific for the bristle precursor cells. Based on the similarity between the *Usp5* mutant phenotype and the *archipelago* (*ago*) mutant phenotype (Moberg 2001), it is possible that increased cyclin E activity is responsible for the *Usp5* cell cycle phenotype.

Experimental approach (Undergraduate student projects)

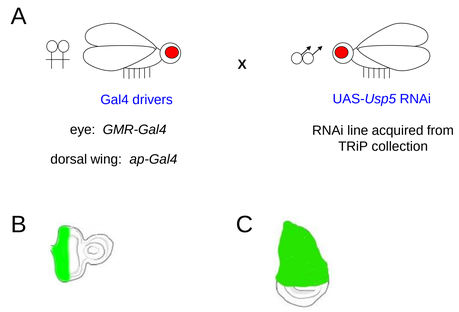

Figure 2. Using RNAi to knock down *Usp5* function in eye and wing tissues provides another method to test for a role in cell cycle exit and/or cell cycle arrest.

(A) To evaluate the effects of reducing the function of *Drosophila Usp5* in eyes and wings at different developmental stages, we will express *UAS-Usp5* RNAi using two different Gal4 driver lines. *GMR-Gal4* will be used to express RNAi in differentiating cells of the eye, and *apterous-Gal4* (*ap-Gal4*) will be used for the dorsal portion of the wing. Multiple cell cycle markers (PH3, EdU, *pcna-GFP*, and *Fly-FUCCI*) will be used to determine if ectopic cell divisions or aberrant cell cycle phasing occurs. We will examine pupal tissues after 24 hours APF (when cell cycle exit normally occurs) and regions of larval imaginal discs where cells experience a cell cycle arrest (the morphogenetic furrow in the eye disc and the zone of non-proliferating cells in the wing disc). (B) Green indicates the expression pattern of *GMR-Gal4* in the eye-antennal imaginal disc. (C) Expression pattern of *ap-Gal4* in the wing imaginal disc.

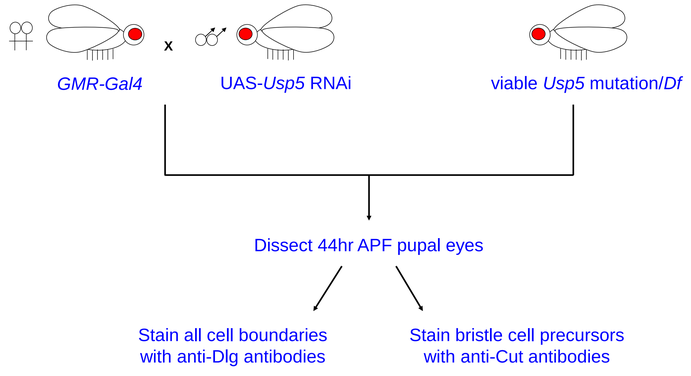

Figure 3. Staining cells in pupal eyes with reduced *Usp5* function will allow us to determine which cell type(s) experience extra divisions.

The pattern of *Usp5* mutant cells that express *pcna-GFP* (Figure 1) led us to hypothesize that bristle precursor cells are the cell type affected by loss of *Usp5*. To test our hypothesis, we will dissect 44 hour APF pupal eyes from two types of pupae that have reduced levels of *Usp5*: pupae where RNAi has been used to knock down *Usp5* levels in developing eyes (top left) and pupae that contain one viable mutant allele of *Usp5* and one deficiency allele from which the entire *Usp5* gene has been deleted (top right). Some eyes will be stained with anti-Discs large (Dlg) to stain the boundaries of all cells, so the number of each cell type per ommatidium can be quantified. Other eyes will be stained with anti-Cut, which stains bristle precursor cells in pupal eyes (Du 1996).

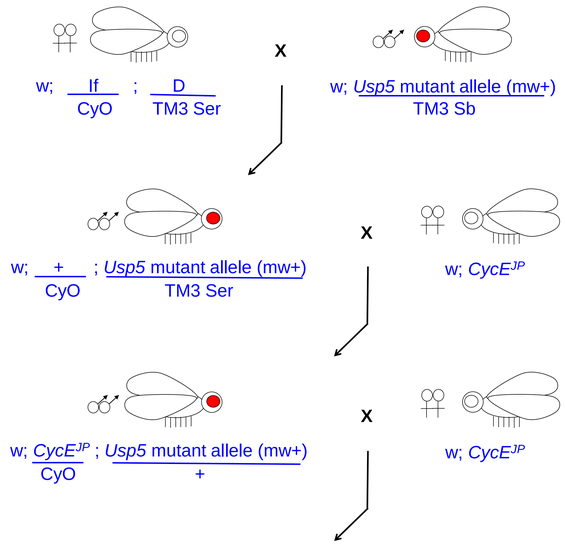

Figure 4. Is the *Usp5* loss-of-function defect due to an increase in Cyclin E?

Based on the similarity between the *pcna-GFP* phenotype in *Usp5* mutant eye clones and the phenotype of *archipelago* mutant eye clones (Moberg 2001), we hypothesize that reduction of *Usp5* may result in increased levels of Cyclin E protein. To begin to test this hypothesis, we are performing a genetic modification experiment. *Usp5* alleles of differing strengths are being crossed to $CycE^{JP}$, a hypomorphic *CycE* allele that results in rough eyes due to reduced cell division (Secombe 1998). We will compare the eyes of $CycE^{JP}$ homozygotes with $CycE^{JP}$ homozygotes that also carry one mutant allele of *Usp5*. If loss of *Usp5* results in increased Cyclin E, we would expect to see suppression of the $CycE^{JP}$ rough eye phenotype.

References

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