Btk29A Plays a Role in Early Wing Imaginal Disc Regeneration

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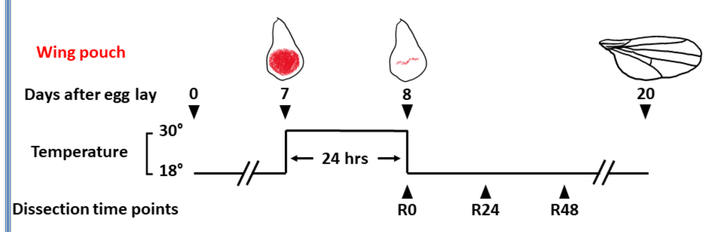
1. Background

After sustaining an injury, an organism relies on the complex biological process of regeneration to repair damaged tissue. We study the regeneration of imaginal wing disc tissue following a genetic ablation procedure. These discs are found in the larval stage of development and contain the necessary information for adult wing formation and patterning.

2. Tissue Ablation System

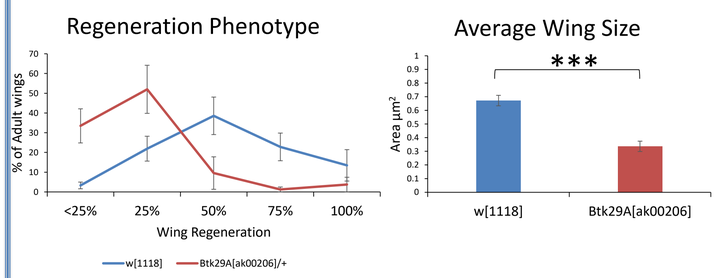
w1118; rnGal4, UAS-reaper, tubGal80^{ts} x Mutant
TM6B, tubGal80



To study regenerative phenotypes, we use an *in situ* genetic ablation system to induce apoptosis. We use a GAL4 enhancer trap in the *rotund* locus to drive the expression of UAS*reaper*. This activates cell death within the imaginal disc wing pouch specifically[1]. To temporally control this ablation, temperature sensitive GAL80 was used to repress expression of the rnGAL4 at 18°. Seven day old larvae are shifted from 18° to 30° for 24 hours to induce ablation at the beginning of the third instar. After 24 hours, the ablation is stopped, and the wing disc is allowed to regenerate. After eclosion, the wings of the flies are scored by size. The level of regeneration is determined by the percentage of wing regenerated as shown below.

0% **25%** **50%** **75%** **100%**

3. Btk29A is Required for Regeneration

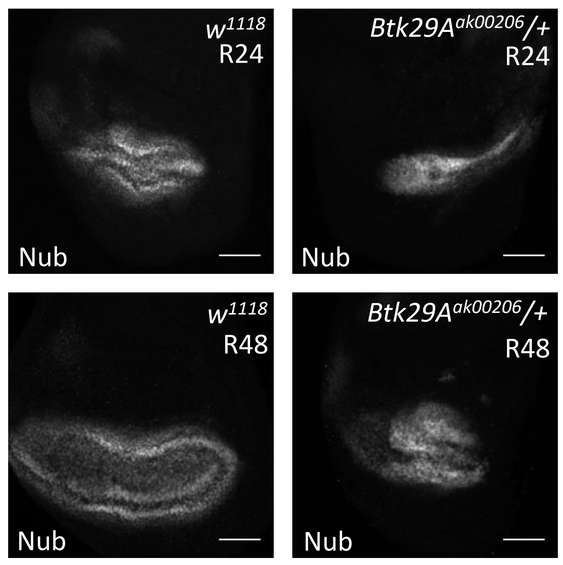


A pilot genetic screen of known actin regulators revealed that a loss Btk29A resulted in lower levels of regeneration. Btk29A is a non-receptor tyrosine kinase, which plays a part in a number of aspects of *Drosophila* development, including blastoderm cellularization, male genital formation, and oogenesis. Further wing scoring and quantification experiments confirmed the Btk29A mutant's poor regeneration phenotype.

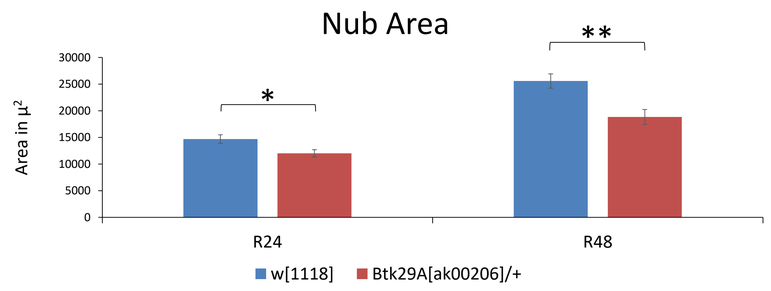
References

1. Smith-Bolton, R.K. et al. *Dev Cell* 2009; 16: 797-809.
2. Hamada-Kawaguchi N, et al. PLoS ONE10(3): e0121484.
3. Tsikala, G. et al. Dev Biology 2014; 396: 42-56.

4. Pouch Size is Reduced in Regenerating Btk29A Mutants

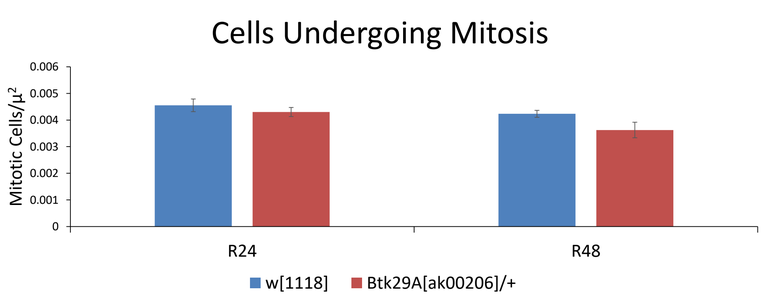


To characterize Btk29A's role in regeneration, we wanted to observe the effects on the blastema in mutants lacking the kinase. We examined the wing pouch containing the blastema across different regeneration time points using an α-Nubbin (Nub) antibody. Nubbin is a protein with expression localized to the wing primordium and therefore, the location of this protein can be used to determine the area of the wing pouch. The results showed that the wing pouch in Btk29A/+ mutant discs was significantly smaller both 24 and 48 hours after damage. These data suggest that the blastema is much smaller in the regenerating mutant discs.



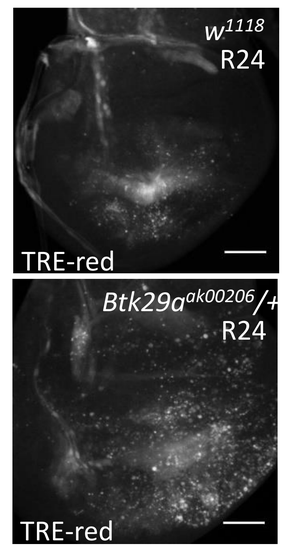
5. Btk29A Does Not Have a Significant Effect on Proliferation

To test the amount of cellular proliferation in the pouch, we stained the regenerating discs with fluorescent antibodies targeting Phospho-Histone H3 (PH3). Since histone 3 is phosphorylated during mitosis, PH3 can be used to mark cells undergoing the process. We found that Btk29A mutants did not exhibit a significant difference in levels of proliferation in their regenerating pouches.



6. Btk29A is Not Involved in JNK signaling

To investigate Btk29A's poor regeneration phenotype, we used a TRE-red transcriptional reporter of the Jun-N-terminal kinase (JNK) pathway. We found that there was not a significant difference in average JNK activity between the mutant and control discs, indicating that the poor regeneration phenotype exhibited by Btk29A/+ mutants is not due to an impairment in JNK signaling at R24.

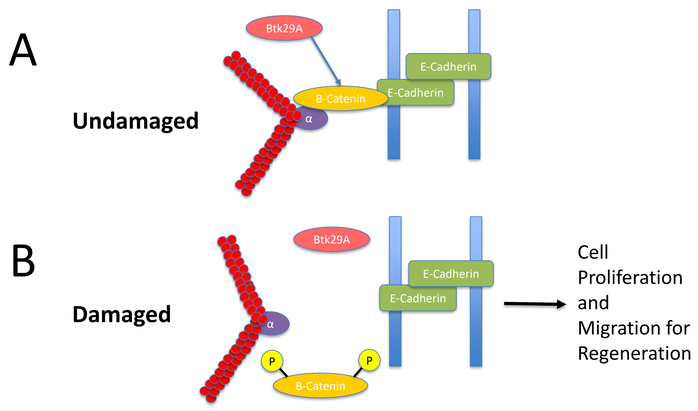


TRE-red Expression

7. The Role of β-Catenin

Btk29A has been shown to phosphorylate two conserved tyrosine residues on the Drosophila ortholog of β-catenin, Armadillo[2]. β-catenin regulates cell-cell adhesion by altering the actin organization found in structures between neighboring cells. We hypothesize that Btk29A regulates tissue regeneration by regulating the function of β-catenin.

Hypothetical Model



This is a hypothetical model of how we think Btk29A may affect regeneration. We hypothesize that this dissociation of β-catenin facilitates cellular proliferation and migration during regeneration.

8. Conclusions

From our experiments, we have concluded that the Btk29A protein plays a role in the regeneration process. The decreased size in the wing pouches of Btk29A/+ mutants at R24 suggests that the kinase may be required early in regeneration. Despite the smaller wing pouch area, there seems to be little effect on proliferation. These data suggest that the poor regeneration phenotype may be a result of impaired cell-cell interactions. Our future plans include repeating the above experiments at R0 to look into early regeneration. In addition to this, we plan explore possible disruptions in required cell junctions by visualizing β-catenin, actin, and possible indicators of tensions such as Ajuba and Yorkie.

Acknowledgements

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