The translational repressor Brat constrains regenerative growth to ensure proper patterning after tissue damage

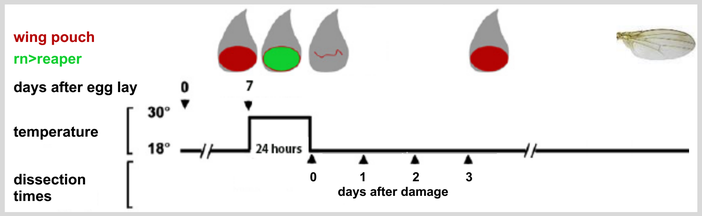
S. Nayab F. Abidi and **Rachel K. Smith-Bolton**
Department of Cell & Developmental Biology
University of Illinois at Urbana-Champaign

A key question in regeneration biology is how regenerating tissue undergoes repatterning and ensures replacement of the correct cell types. By using genetic tools to damage and induce regeneration in third-instar wing imaginal discs, we have identified mutants that have aberrant patterning in the regenerated structure. Through this screen we have shown that the translational repressor *brain tumor (brat)* is a regulator of both growth and patterning during regeneration. While *brat/+* wing discs regenerated better than controls, the resulting adult wings had disrupted wing margins. The enhanced regeneration in *brat/+* mutants was due to elevated expression of Wingless and Myc, which promote regenerative growth, as well as elevated expression of Dilp8, which delays pupariation. However, it was unclear why regenerating tissue would constrain expression of these pro-regeneration factors. Interestingly, overexpressing Myc after tissue damage to replicate the enhanced-regeneration phenotype also caused a disrupted margin phenotype. We determined that this aberrant patterning was not caused by enhanced growth itself, but rather by elevated expression of Myc targets such as the transcription factor Chinmo, which negatively regulates the margin cell fate gene *cut*. Thus, Brat constrains expression of the pro-regeneration factor Myc, and this constraint prevents aberrant patterning of the regenerated structure.

1. A genetic system for tissue ablation

In the *Drosophila* wing imaginal disc *rotund* is expressed in the wing primordium. We use the *rotund*-GAL4 driver to induce the pro-apoptotic gene *reaper.* GAL80^{ts} is used to provide temporal control for GAL4 induction.

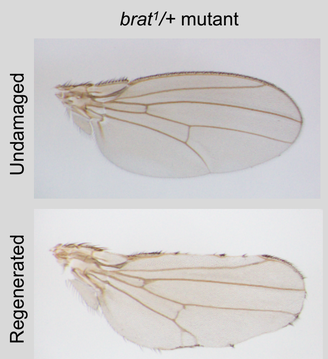
Tissue damage is induced in the wing pouch by shifting third instar larvae to 30°C on day 7 after egg lay, for 24 hours.[1]

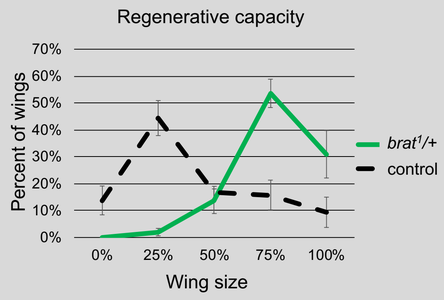


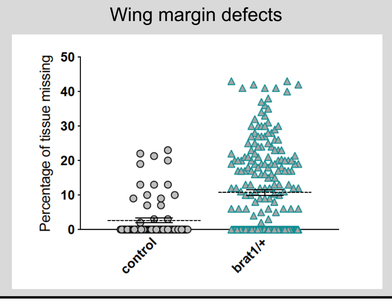
Regenerating wing discs are examined at different days after damage. Adult wing size is used as a measure of imaginal disc regeneration.

2. The gene *brain tumor* is a regulator of growth and patterning during regeneration

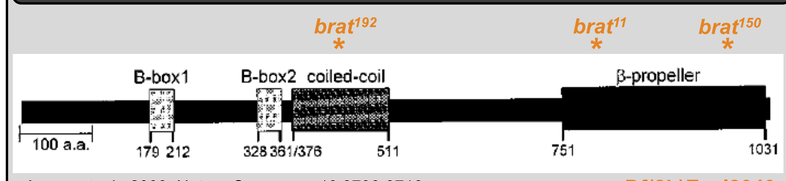
brain tumor (brat) heterozygous mutants display enhanced regenerative capacity and regeneration-specific patterning defects







3. Brain tumor protein



Arama et. al., 2000. Nature Oncogene, 19:3706-3716

X Df(2L)Exel8040 Df(2L)TE37C-7

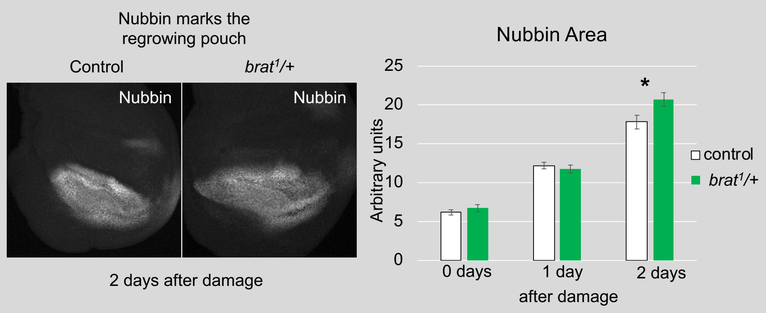
- Brat is a member of the conserved NHL family of proteins, which control post-transcriptional gene expression.
- Brat acts as a translational repressor by either directly binding to its target RNAs or suppressing them by interacting with other repressors.
- Brat regulates cell differentiation and growth by acting as a translational repressor.
- TRIM32 and TRIM3 are vertebrate orthologs of Brat.

4. *brat* mutants delay pupariation

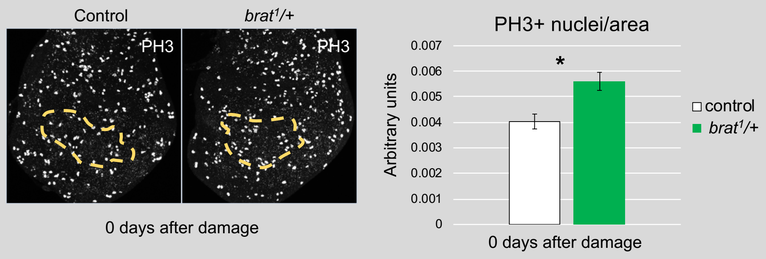
Imaginal discs are able to regenerate during the larval stages up until the onset of metamorphosis. *brat/+* mutants show a delay in metamorphosis after tissue damage, which gives them more time for regeneration, contributing to their improved regenerative capacity.

5. Regenerative growth is enhanced in *brat* mutants

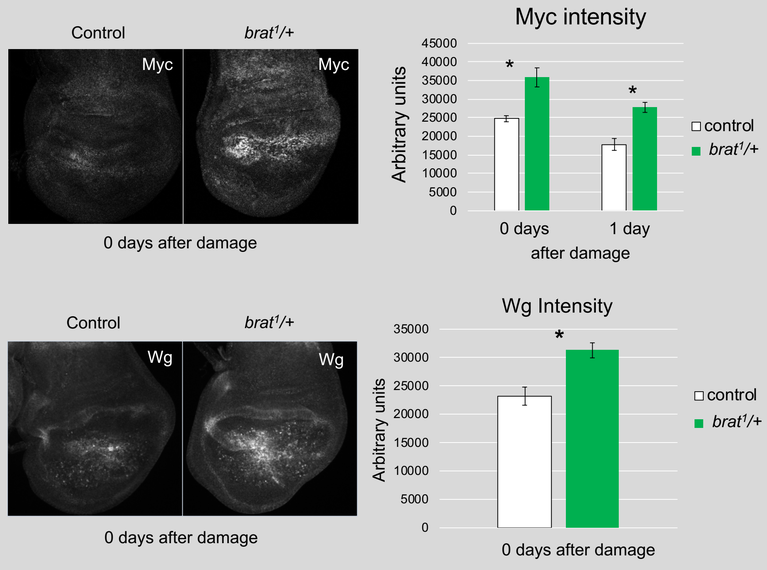
The regenerating tissue in *brat/+* mutants grows faster



The *brat/+* mutants have a proliferative advantage

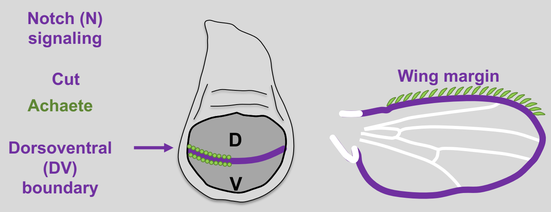


Myc and Wg are both regulators of regenerative growth [1]. *brat/+* mutants experience elevated Myc and Wg expression early in regeneration.

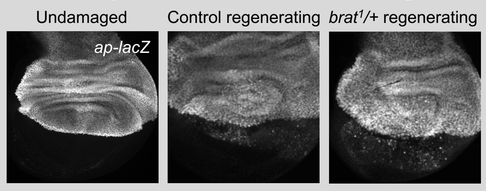


Faster growth (Myc and Wg) + more time = better regeneration

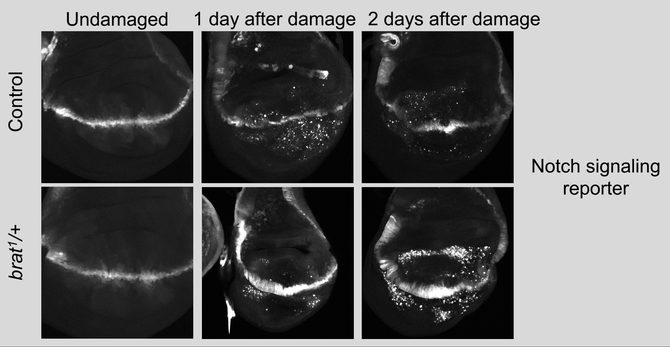
6. Margin signaling is perturbed in regenerating *brat* mutants



The DV boundary is normal in the *brat/+* regenerating tissue

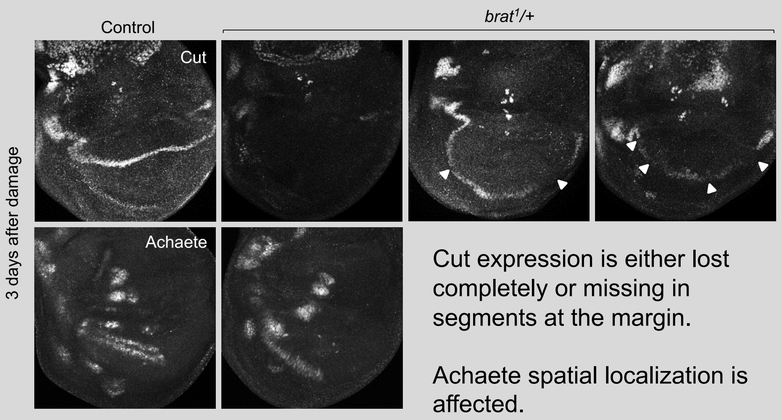


N signaling is upregulated at the margin in *brat* mutants after damage but reducing N activity does not rescue the *brat* mutant phenotype



7. Margin cell fate specification is disrupted in regenerating *brat* mutants

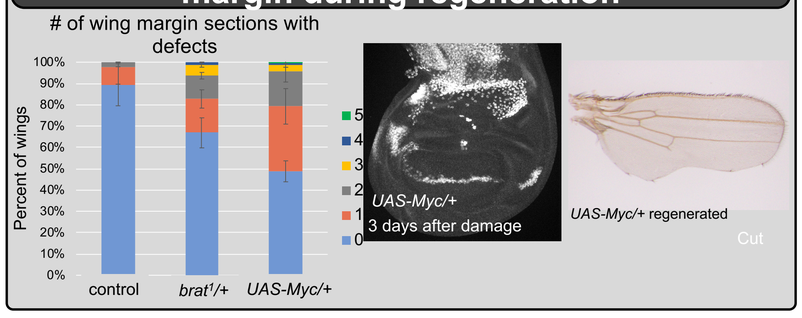
Cell fate gene expression is affected in *brat/+* mutants after damage



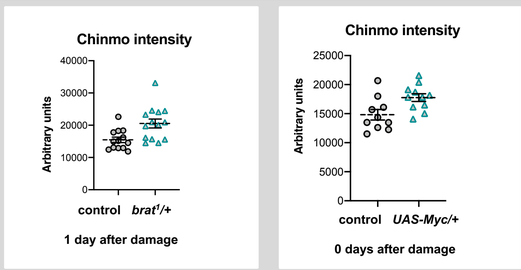
Cut expression is either lost completely or missing in segments at the margin.

Achaete spatial localization is affected.

8. Myc overexpression also causes loss of margin during regeneration

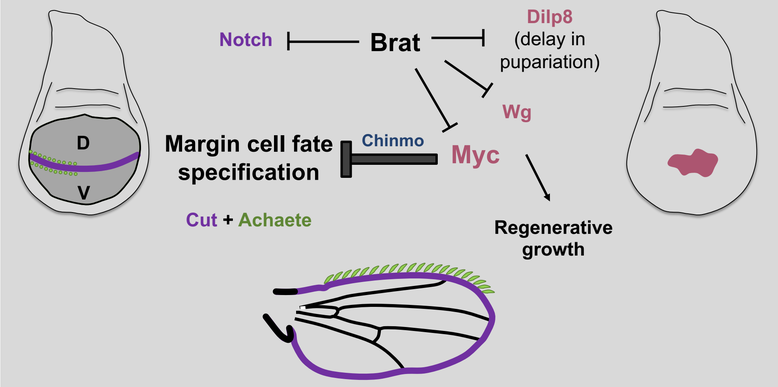


9. *Brat* mutations and Myc overexpression upregulate Chinmo



Chinmo inhibits margin fate genes such as Cut.[3] Thus, upregulation of Myc targets such as Chinmo can account for disruption of cell fates during regeneration.

Pro-growth factors must be restricted to ensure proper patterning and cell fate during regeneration



References

1. Smith-Bolton R. K. et. al., Dev Cell, 2009, 16(6):797-809
2. Arama et. al., Nature Oncogene, 2000, 19:3706-3716
3. Narbonne-Reveau and Maurange, PLOS Biology, 2019, 17(2) e3000149

Acknowledgements

We would like to thank the University of Illinois, the Roy J. Carver Foundation and NIH NIGMS for funding, the Bloomington Drosophila Stock Center, the Developmental Studies Hybridoma Bank, Dr. Cheng-Yu Lee, Dr. Jurgen Knoblich, Dr. Michael Kim, Dr. Gerard Campbell and Dr. Robin Wharton for reagents.