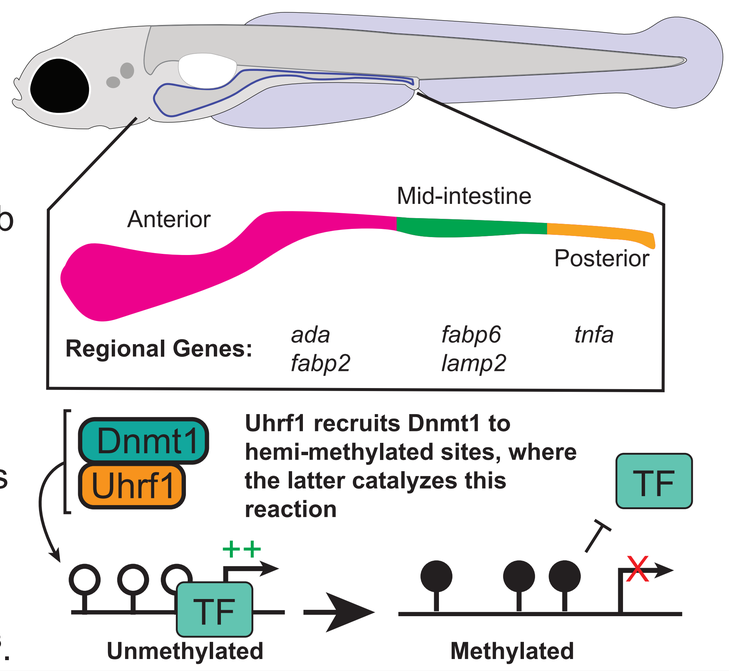
DNA methylation is required for regional gene expression signatures in the zebrafish intestine

Gilberto Padilla Mercado[1], Colin R. Lickwar[1], Jia Wen[1], Lindsey Marjoram[2], Michel Bagnat[2], John F. Rawls[1]
[1]Department of Molecular Genetics and Microbiology, Duke Microbiome Center, Duke University School of Medicine.
[2]Department of Cell Biology, Duke University.

Regional gene expression is critical to intestinal function

- Anterior and mid-intestinal regions absorb the majority of nutrients and the latter can function to re-absorb bile acids/salts.
- The posterior intestine is involved osmoregulation.
- Patterns of gene expression along the zebrafish gut are also conserved in humans and mice and correlate with duodenal/jejunal, ileal, and colonic regional expression, respectively[1].
- Regionality is perturbed in human IBDs[2,3].



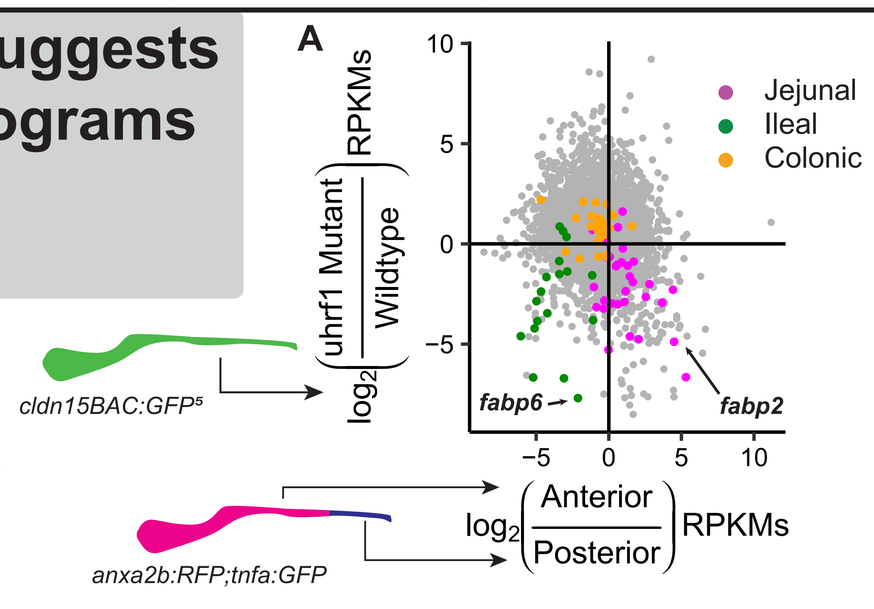
DNA methylation regulates gene expression via transcriptional control and can perturb intestinal physiology

- DNA methyltransferases add methyl groups to the 5-position in the cytidine ring.
- Methylation of DNA can hinder transcription factor (TF) binding
- Previous work identified zebrafish mutants with disrupted intestinal barrier function and expansion of *tnfa:GFP* expression to the anterior[4].
  - *uhrf1*^{pd1092} is a non-sense mutation which abrogates Uhrf1 protein expression
  - *dnmt1*^{s872} is a loss of function point mutation
- Both these mutants result in DNA hypomethylation and intestinal pheynotypes

1. Transcriptomic data suggests that anterior regional programs are perturbed in DNA methylation mutants

Using bulk RNA Seq data from fluorescence-sorted IECs in either *uhrf1* mutant or wild-type IECs, we notice that small intestinal programs are downregulated.

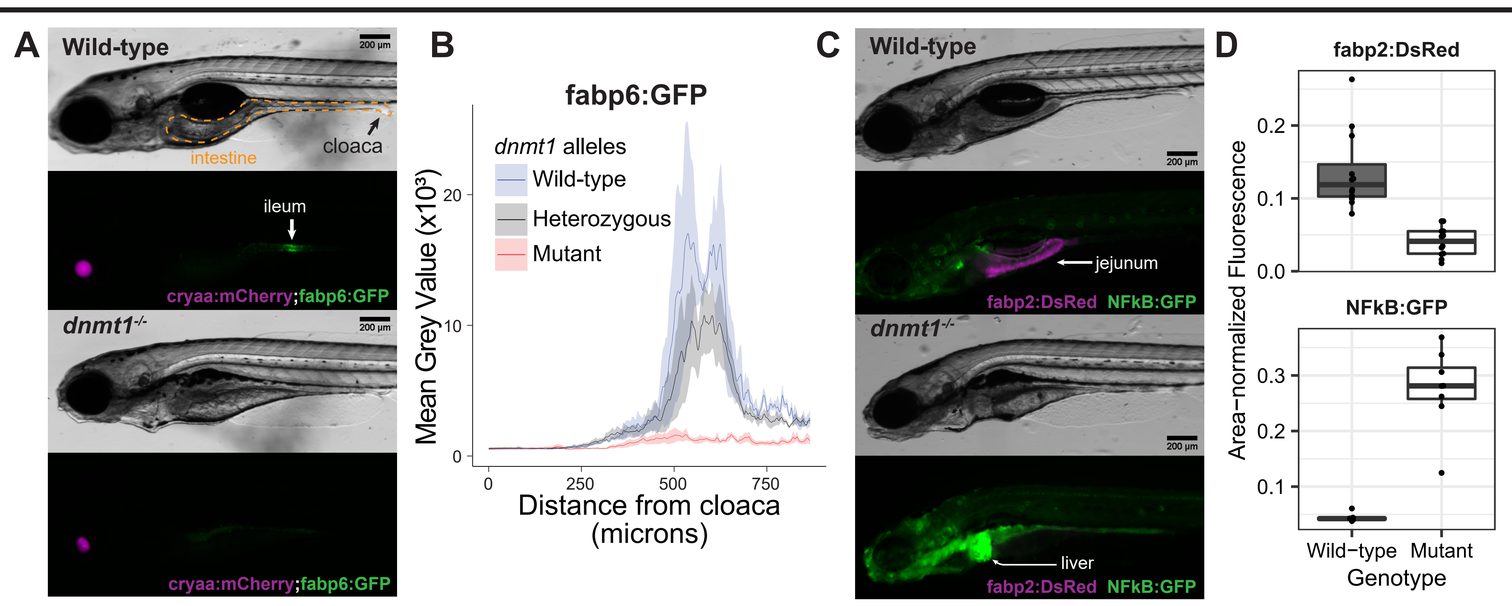
Does DNA methylation have an effect on small intestinal enterocyte gene expression?



Integrating single-cell RNA-Seq data from sorted IECs reveals that marker genes for anterior enterocyte clusters are downregulated in DNA methylation mutants

2. Transgenic zebrafish confirm the concurrent impact of DNA methylation defects on intestinal inflammation and anterior regional gene programs

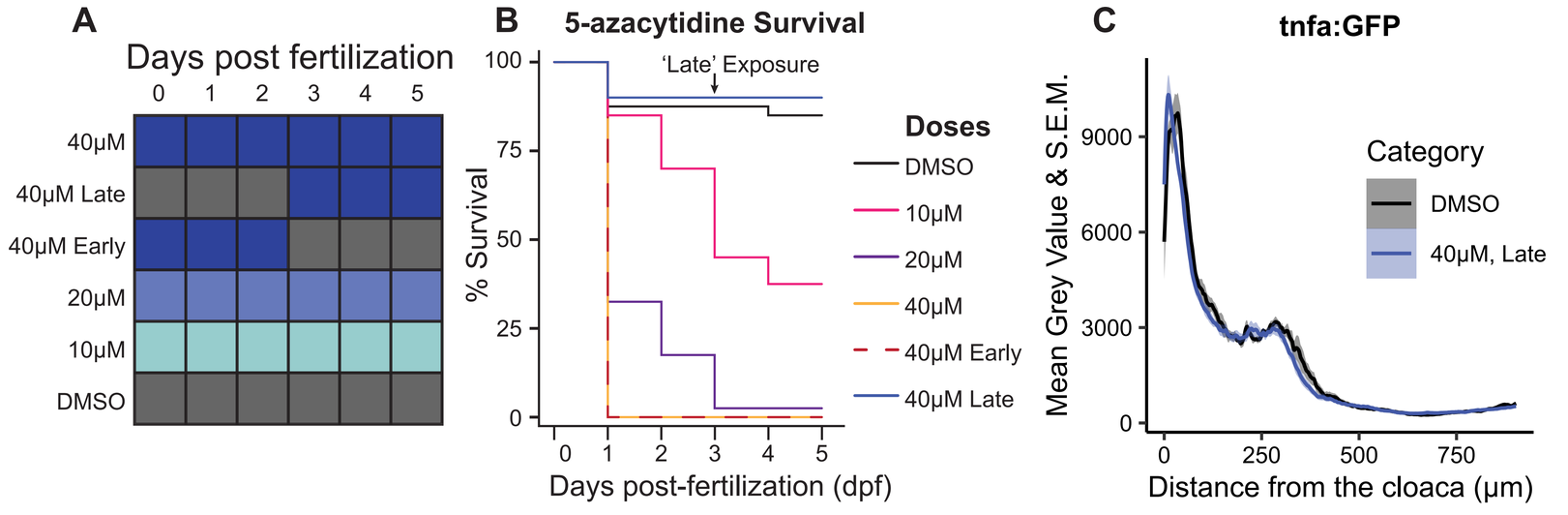
- I utilized previously generated transgenic lines that label the duodenum/jejunum (*fabp2:DsRed*)[6] and ileum (*fabp6:GFP*) regions of the zebrafish intestine and these showed decreased fluorescence in DNA methylation mutant *dnmt1.*
- An *in vivo* reporter of NFkB activity (*NFkB:GFP*)[6] shows increased fluorescence in *dnmt1* mutants.



3. Is DNA methylation temporally required for development of the zebrafish

I use 5-azacytidine, a cytidine analog that inhibits DNA methylation by stalling DNA methyltransferases.

For the exposure range I've selected (10-40uM), there seems to be a gradient of toxicity, but no recapitulation of *tnfa:GFP* expansion to the anterior intestine in surviving 5dpf larvae.



Conclusions

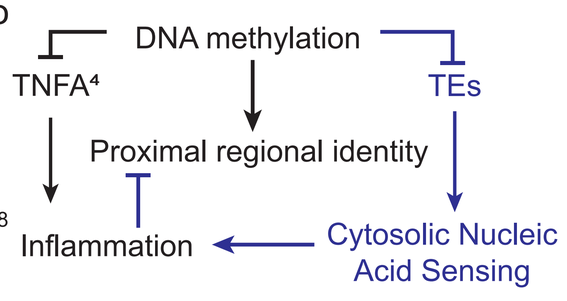
DNA methylation maintenance plays a key role in the development of small intestinal programs within the zebrafish gut.

This is also accompanied by intestine-associated inflammation and other detrimental changes to intestinal physiology.

Future Directions

- Optimize pharmacological inhibition of DNA methylation to test for temporal requirements.
- Explore the possiblity that de-repression of transposable elements (TEs) may trigger cytosolic nucleic acid sensing[7,8] and propagate inflammation in the intestine.

Working Model in Progress



Acknowledgements

Dan Levic and Esther Park *at Duke*
Mary Goll and Srivarsha Rajshekar *at UGA*
Duke Light Microscopy Core, Duke Zebrafish Core, Duke Molecular Genomics Core
Funding: P01-DK094779 and R01-DK113123

References

1. Lickwar et al. 2017, PLoS Biology
2. Weiser et al. 2018, Gut.
3. Howell et al. 2018, Gastroenterology.
4. Marjoram et al. 2015, PNAS.
5. Alvers et al. 2011, Development.
6. Kanther et al. 2015, Gastroenterology
7. Chernevskaya et al. 2017, Development.
8. Rajshekar et al. 2019, eLife.