New backward-compatible, drug-controllable misexpression tools to investigate intestinal stem cell activation in vivo

Supervisory team:
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Project description:

This project aims at developing a new method for genetic manipulation in Drosophila, and applying it to understand the baseline activity of the Drosophila adult intestinal stem cells (ISCs) – a sophisticated model for the regulation of intestinal homeostasis. The activity of ISCs of the Drosophila adult is monitored in vivo through lineage tracing by genetic labelling – the gold standard to analyse tissue stem cell activity. Methods for genetic labelling in Drosophila require the controlled expression of the recombinase FLP, which is generally attained by either a heat shock or a wide temperature switch. These methods (1) preclude analysis of the effect of heat stress on stem cell activity and (2) slow down the pace of research, as rearing flies at low temperature extend the life cycle 3-fold. The latter affects not only stem cell biology but also other fields such as aging or adult metabolism and immunity. You will implement two independent, complementary approaches aimed at turning the Gal4/UAS misexpression system into a drug-inducible system. This will require engineering repression-release control mechanisms based on the Gal80 protein, the natural repressor of the Gal4 transcriptional activator. Such control mechanism will turn the >20,000 Gal4 transgenes available in Drosophila into drug-inducible with just a simple cross, and is likely to impact the broader Drosophila community. This part of the project will involve molecular cloning and generation of transgenic strains, as well as Drosophila husbandry and expression analysis methods (qRT-PCR, western blot, immunohistochemistry, microscopy). Next you will apply the new method to the analysis of adult ISC proliferative activity through lineage tracing and genetic manipulation, and evaluate the impact of heat stress on the regenerative properties of the ISCs. At this stage you will work in close collaboration with a physicist, extracting quantitative data from the lineage tracing experiments to support the mathematical modelling of ISC activity. This part of the project will help develop expertise with lineage tracing methods and familiarity with their mathematical analysis, sound Drosophila genetics (using mutants or RNAi), and quantitation of microscopy images using custom scripts in Python (adapting and extending pre-existing tools from the lab).