ENTEROBLAST CELLS AFFECTED BY INSULIN SIGNALING MODULATE LONGEVITY, STRESS RESISTANCE AND METABOLISM IN *DROSOPHILA*

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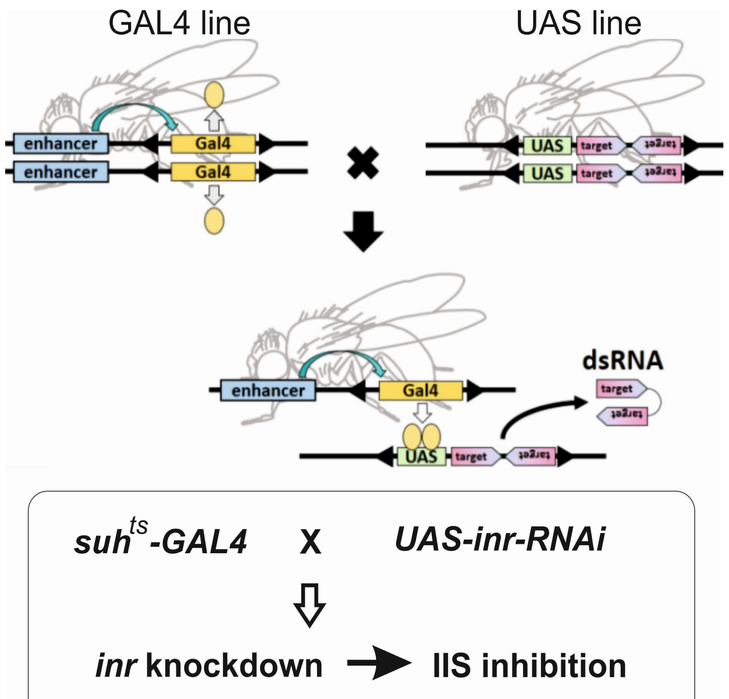
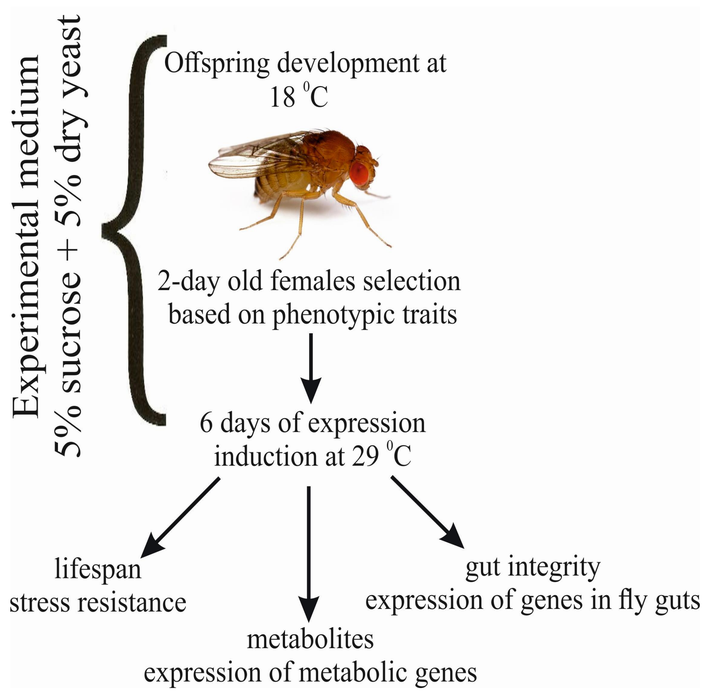
Introduction

Midgut homeostasis is regulated by multipotent intestinal stem cells (ISCs) which divide and give rise to immature enteroblasts (EBs) or become new stem cells. Conserved metabolic pathways are involved in midgut homeostasis including Insulin/IGF signaling (IIS). In the present work, we asked if the manipulation of IIS in EBs – a small group of gut cells may have global effects on fly physiology and metabolism and effects localized to a single tissue.

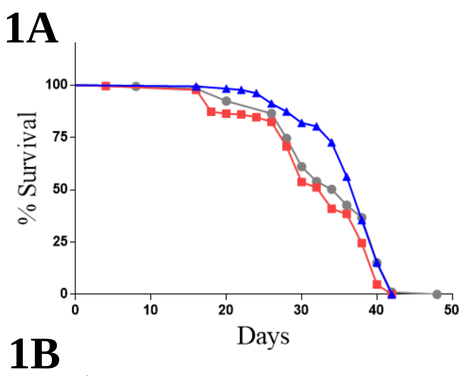
Materials and methods





Experimental flies were used to estimate lifespan and resistance to malnutrition, starvation and oxidative stress. Hemolymph glucose, whole body glucose and glycogen were measured. Gut integrity was evaluated using blue food dye E133. "Smurf" flies were defined by visible blue food dye throughout the body, which suggest disruption of gut integrity. The steady state levels of mRNA in guts (*upd2*, *upd3*, *soc36*, *spi*, *krn*, *vn*), heads (*dilp2*, *3*, *5*) and bodies (*dilp6*, *akh*, *tobi*, *pepck*) were measured using an ABI Prism 7000 instrument (Applied Biosystems) and a QuantiTect SYBR Green PCR Kit (Qiagen).

Results



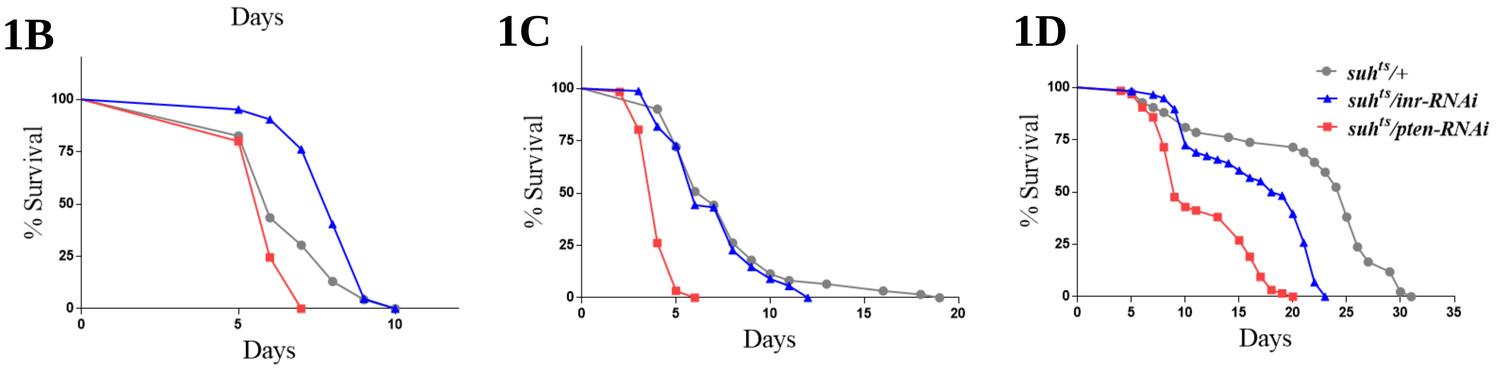


Figure 1. Survival under standard conditions (5%S + 5%Y) **(A)** and malnutrition (1%S – **(B)**, 1%AY – **(C)**, 0.5%S + 0.5%AY – **(D)**).

We found that activation of IIS via *pten-RNAi* expression in the EBs shortens lifespan (Fig. 1A) and decrease resistance to nutritive stress. Fly survival under various dietary regimens was not affected by IIS inhibition, however, *inr* knockdown in conjunction with 1% sucrose consumption prolonged *Drosophila* lifespan (Fig. 1B). Lower survival was detected under IIS activation in EBs (Fig. 1B-D).

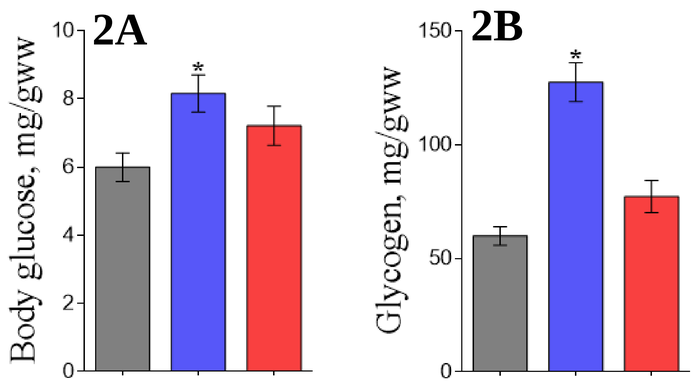


Figure 2. Body glucose content **(A)** and glycogen amount **(B)** in flies with IS modulated in stem and progenitor cells.

The *inr-RNAi* expression enhances the level of whole body glucose by 1.3-fold (Fig. 2A) and glycogen (Fig. 2B) by 2-fold as compared to control.

The manipulations of IIS in EBs in this study affected the relative expression of metabolic genes (Fig. 3).

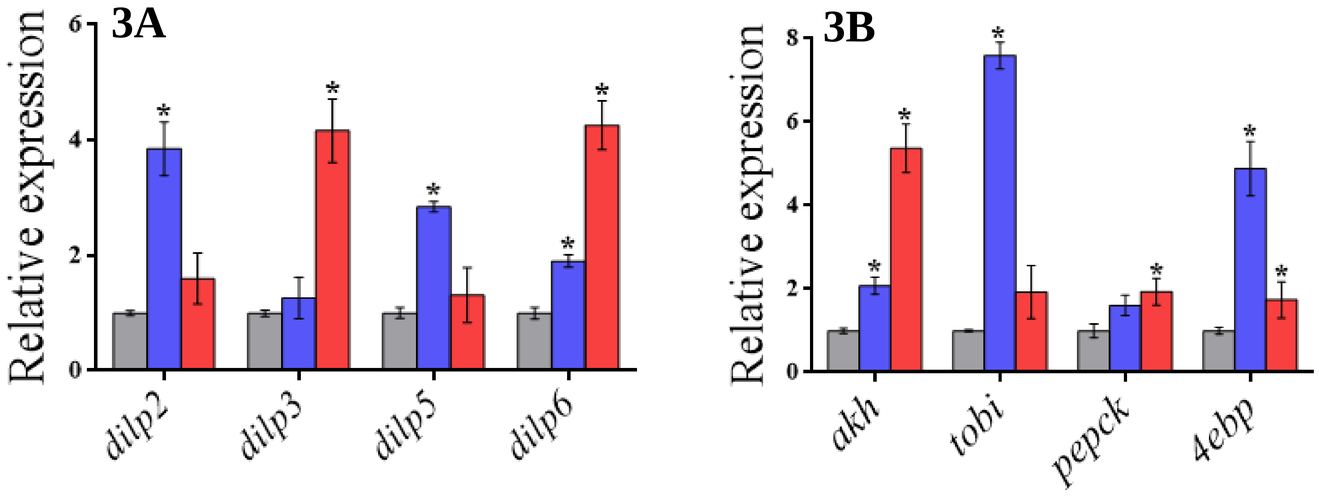
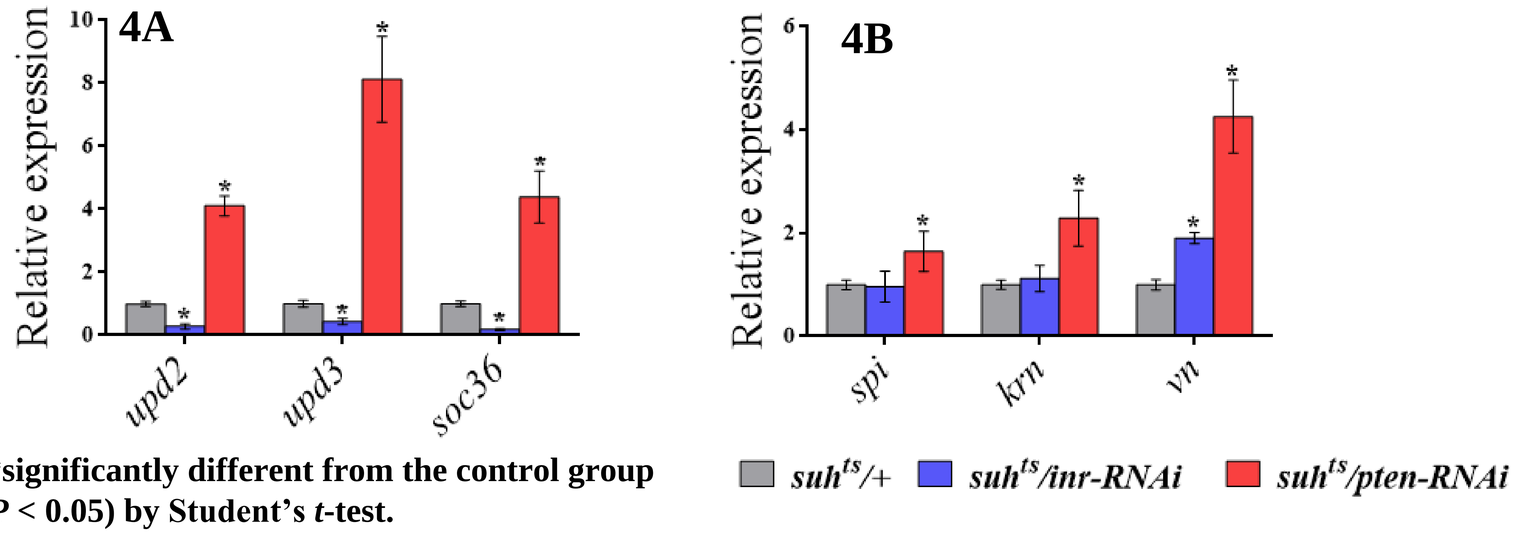


Figure 3. Steady state mRNA level for *dilp2*, *dilp3*, *dilp5* in fly heads and *dilp6* from whole fly bodies **(A)**. Expression of genes related to glucagon-like signaling and metabolism – *akh*, *tobi*, *pep*ck and *4ebp* **(B)**.

Smurf assay showed, that perturbation of IIS did not affect tissue integrity. Relative expression of genes *upd2*, *upd3* (ligands for JAK/STAT) and *soc36* (target JAK/STAT gene) was significantly lower when IIS was inhibited in EBs, and was higher under IIS activation (Fig. 4A). Furthermore, *pten* knockdown also led to higher mRNA level of *spi*, *krn* and *vn*, which encode ligands to EGFR pathway (Fig. 4B).



***significantly different from the control group ($P < 0.05$) by Student's *t*-test.**

Figure 4. Expression of genes related to JAK/STAT **(A)** and EGFR **(B)** signaling pathways in the gut.

Conclusions

EB cells play a critical role in maintaining tissue homeostasis which is necessary for organismal survival. IIS inhibition/activation in EBs affect energy metabolism. Increased expression of the *dilp2*, *dilp5* and *dilp6* under IIS inhibition may reflect increased IIS to peripheral tissues that is supported by up-regulation of the target of brain insulin gene (*tobi*). Identification of changes in the expression of specific genes encoding signal transduction proteins involved in proliferation and differentiation suggest that IIS in EBs is critical for maintaining gut homeostasis. The study of signaling pathway regulation only in EBs could shed light on potential aging mechanisms in *Drosophila*.