Developmental Biology 424 (2017) 10–17

Contents lists available at ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/locate/developmentalbiology

Short Communication

Intestinal stem cell ablation reveals differential requirements for survival in response to chemical challenge

Luís Pedro F. Resende[a,1], Melissa E. Truong[a], Adam Gomez[a,b], D. Leanne Jones[a,b,c,*]

[a] Department of Molecular, Cell, and Developmental Biology, University of California-Los Angeles, Los Angeles, CA 90095, United States
[b] Molecular Biology Institute, University of California-Los Angeles, Los Angeles, CA 90095, United States
[c] Eli and Edythe Broad Center of Regenerative Medicine and Stem Cell Research, University of California-Los Angeles, Los Angeles, CA 90095, United States

ARTICLE INFO

Keywords:
Stem cell
Niche
Intestine
Drosophila
Headcase

ABSTRACT

The *Drosophila* intestine is maintained by multipotent intestinal stem cells (ISCs). Although increased intestinal stem cell (ISC) proliferation has been correlated with a decrease in longevity, there is some discrepancy regarding whether a decrease or block in proliferation also has negative consequences. Here we identify *headcase (hdc)* as a novel marker of ISCs and enteroblasts (EBs) and demonstrate that Hdc function is required to prevent ISC/EB loss through apoptosis. Hdc depletion was used as a strategy to ablate ISCs and EBs in order to test the ability of flies to survive without ISC function. While flies lacking ISCs showed no major decrease in survival under unchallenged conditions, flies depleted of ISCs and EBs exhibited decreased survival rates in response to damage to mature enterocytes (EC) that line the intestinal lumen. Our findings indicate that constant renewal of the intestinal epithelium is not absolutely necessary under normal laboratory conditions, but it is important in the context of widespread chemical-induced damage when significant repair is necessary.

1. Introduction

The long-term maintenance of adult tissues is facilitated by stem cells that are capable of integrating systemic and local signals to maintain homeostasis or to respond to altered tissue demands, when necessary (Fuchs et al., 2004). Many adult tissue stem cell populations are relatively quiescent under homeostatic conditions but can be activated to divide in response to damage or wounding in order to increase the number of stem and progenitor cells to facilitate tissue repair (Fuchs and Chen, 2013). Therefore, tight regulation of stem cell behavior (proliferation, survival, and differentiation) is necessary to maintain tissue function under homeostatic conditions as well as in response to damage (Voog and Jones, 2010; Resende and Jones, 2012).

The *Drosophila* intestine shows remarkable similarity, on a cellular and molecular level, to the human intestine, including the regulation of stem cell activity via highly conserved pathways, such as the Wnt, Notch, Hippo, and Epidermal Growth Factor Receptor signaling pathways (Li and Jasper, 2016). *Drosophila* intestinal stem cells (ISCs) are located basally, immediately adjacent to the basement membrane and in close proximity to the visceral muscle (Fig. 1A–C). The majority of multipotent ISCs divide asymmetrically to generate a new ISC and a transient enteroblast (EB) that differentiates into an EC through activation of the Notch pathway (Ohlstein and Spradling, 2006; Micchelli and Perrimon, 2005). A smaller subset of ISCs, on the other hand, gives rise to enteroendocrine (EE) cells through asymmetric divisions (ISC+EE) or direct differentiation (Zeng and Hou, 2015; Biteau and Jasper, 2014; Amcheslavsky et al., 2014; Guo and Ohlstein, 2015).

In *Drosophila*, maintenance of a functional intestinal epithelium has been shown to be an important determinant of health and viability at the organismal level (Biteau et al., 2010; Rera et al., 2011, 2013, 2012). The ISCs are the only proliferative cells in the intestine, and in the absence of challenges, the female posterior midgut epithelium is renewed roughly every 12 days (Jiang et al., 2009). However, ISC division increases significantly in response to a variety of stimuli and/or damage, including loss of ECs, infection with pathogenic bacteria, and oxidative stress (Amcheslavsky et al., 2009; Biteau et al., 2008; Buchon et al., 2009). Importantly, it has

Abbreviations: arm, armadillo; EB, enteroblast; EC, enterocyte; EE, enteroendocrine; Esg, escargot; GFP, green fluorescent protein; Hdc, headcase; ISC, intestinal stem cell; pH3, phospo histone H3; pros, prospero; Su(H), Suppressor of Hairless

* Correspondence to: University of California, Los Angeles, Department of Molecular, Cell, and Developmental Biology, 5139 Terasaki Life Sciences Building, Los Angeles, CA 90095, United States.
E-mail address: leannejones@ucla.edu (D.L. Jones).
[1] Current address: Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal and IBMC, Instituto de Biologia Molecular e Celular, Universidade do Porto, Portugal.

http://dx.doi.org/10.1016/j.ydbio.2017.01.004
Received 14 May 2016; Received in revised form 8 August 2016; Accepted 5 January 2017
Available online 17 January 2017

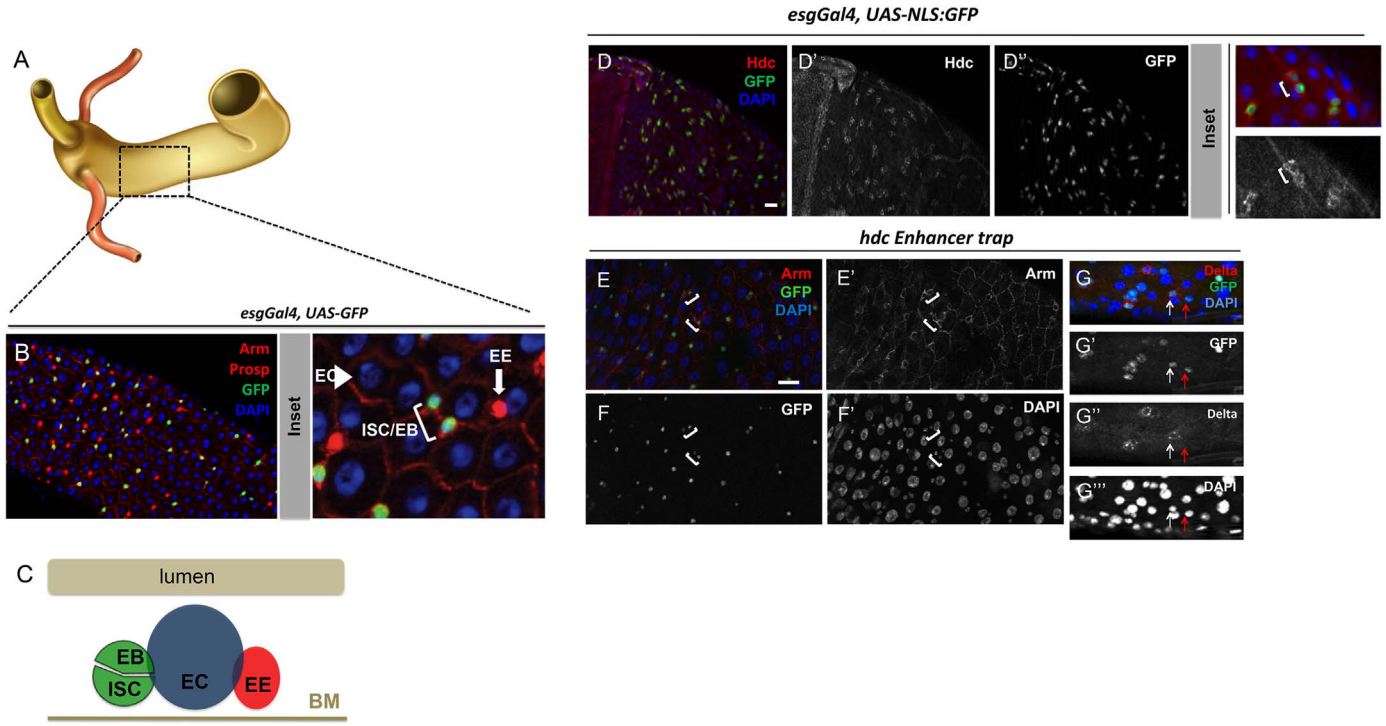

Fig. 1. Headcase is a novel marker of ISCs/EBs in the *Drosophila* intestine. A) Anatomical organization of the adult *Drosophila* intestine. B) Immunofluorescence image of the posterior midgut showing the four different cell types found in this epithelium: ISCs and EBs are generally found in nests, and express *escargot* (reflected by UAS-GFP expression under the control of an *esgGal4* driver, white bracket, green cells), secretory enteroendocrine cells (EE, Prospero positive, red, nuclear signal, white arrow) and absorptive enterocytes (large polyploid cells, white arrowhead); Armadillo (red) marks the membrane of all cells; C) Schematic of the posterior midgut epithelium; ISCs and EBs are found in close association with the basement membrane (BM); ISCs (green) can either divide symmetrically generating more ISC or asymmetrically giving rise to one ISC and one daughter cell that will differentiate either as an EE (red) or an EC (Blue); D) Staining with a Hdc antibody shows co-localization with *esg* expression, which marks ISCs and EBs within the posterior midgut; inset shows GFP signal (Hdc) in ISC/EB nests (white brackets); E) and F) *hdc* reporter line (enhancer trap) shows the same expression pattern as detected with Hdc antibody; G) Within the ISC/EB nests, generally one GFP positive cell is positive for Delta (white arrow, ISC marker) and one GFP positive cell is negative for this marker (red arrow, EBs); Scale bars 20 μm.

been demonstrated that significantly increased or decreased ISC proliferation, achieved through genetic manipulation of the insulin/IGF signaling (IIS) pathway, resulted in shortened lifespan (Biteau et al., 2010). In addition, data indicate that impairment of intestinal regeneration results in lower survival rates when flies are infected with enteropathogenic bacteria (Buchon et al., 2009, 2010; Osman et al., 2012). In contrast, Petkau et al. demonstrated that disrupting the proliferative program of ISCs and ECs causes damage to the intestinal epithelium without significantly affecting lifespan, even after microbial infection (Petkau et al., 2014). Damage caused by chemical agents, such as the DNA damaging agent bleomycin or dextran sulfate sodium (DSS), also induces a regenerative response in the intestine (Amcheslavsky et al., 2009); however, it has not been determined whether ISC activity is important for organismal survival in these models. A recent study described an approach to ablate ISCs/EBs, but the impact on lifespan was not addressed (Lu and Li, 2015).

Here, we identify *headcase* (*hdc*) as a novel marker of ISCs and EBs and demonstrate that loss of *hdc* function results in progressive loss of these progenitor cells via apoptosis. As targeted depletion of *hdc* in ISCs and EBs resulted in elimination of these cell types, this strategy was used to successfully ablate ISCs/EBs from young flies to determine the effects of ISC loss on intestinal homeostasis and survival. Loss of ISCs did not compromise survival of the flies under homeostatic conditions; however, flies depleted of ISCs had lower survival rates than flies capable of intestinal regeneration after bleomycin-induced EC damage. Our results confirm that renewal of the intestinal epithelium is not absolutely required if flies are maintained under unchallenged conditions. In contrast, ISC activity and intestinal regeneration promotes survival in response to damage.

2. Material and methods

2.1. Fly husbandry and stocks

Flies were raised on standard cornmeal-molasses-agar medium. Female progeny from experimental crosses were collected and maintained with less than 30 flies per vial. Flies were turned onto fresh food vials every two days. The following fly stocks used were from the Bloomington *Drosophila* Stock Center (BDSC), Vienna Drosophila Stock Center (VDRC), or generous gifts from the fly community as indicated: *Gal80^{ts}*; *UAS-lacZ^{NLS}*; *UAS-P35* (Bloomington stock center #7018, #3956 and #5073); *esgGal4,UASGFP* (gift from Norbert Perrimon); *esgGal4,2xYFP; Su(H)Gal80, tub-Gal80ts* (gift from Steven Hou) *hdc*RNAi lines used were from Vienna *Drosophila* stock center and labeled as *UAS-HdcRNAi^{1}* (VDRC#45069) and *UAS-HdcRNAi^{2}* (VDRC#104322). Wild-type flies were *Oregon R*. More detailed information about these stocks can be found at Flybase (http://flybase.bio.indiana.edu).

2.2. Antibodies

Intestines were stained with: mouse anti-Hdc (1:3) (gift from R. White); mouse anti-Armadillo (1:20) and mouse anti-Prospero (1:100) (Developmental Studies Hybridoma Bank, developed under the auspices of the National Institute of Child Health and Human Development and maintained by the University of Iowa, Department of Biological Sciences); rabbit anti-phospo-histone H3 (1:200) (Millipore); rabbit anti-GFP (1:5000) (Molecular Probes). Secondary antibodies were diluted 1:500 (Molecular Probes).