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Insulin signaling acts in adult adipocytes via GSK-3β and independently of FOXO to control *Drosophila* female germline stem cell numbers

Alissa R. Armstrong[a,b], Daniela Drummond-Barbosa[a,*]

[a] Department of Biochemistry and Molecular Biology, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD 21205, USA
[b] Department of Biological Sciences, University of South Carolina, Columbia, SC 29208, USA

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ABSTRACT

Tissue-specific stem cells are tied to the nutritional and physiological environment of adult organisms. Adipocytes have key endocrine and nutrient-sensing roles and have emerged as major players in relaying dietary information to regulate other organs. For example, previous studies in *Drosophila melanogaster* revealed that amino acid sensing as well as diet-dependent metabolic pathways function in adipocytes to influence the maintenance of female germline stem cells (GSCs). How nutrient-sensing pathways acting within adipocytes influence adult stem cell lineages, however, is just beginning to be elucidated. Here, we report that insulin/insulin-like growth factor signaling in adipocytes promotes GSC maintenance, early germline cyst survival, and vitellogenesis. Further, adipocytes use distinct mechanisms downstream of insulin receptor activation to control these aspects of oogenesis, all of which are independent of FOXO. We find that GSC maintenance is modulated by Akt1 through GSK-3β, early germline cyst survival is downstream of adipocyte Akt1 but independent of GSK-3β, and vitellogenesis is regulated through an Akt1-independent pathway in adipocytes. These results indicate that, in addition to employing different types of nutrient sensing, adipocytes can use distinct axes of a single nutrient-sensing pathway to regulate multiple stages of the GSC lineage in the ovary.

1. Introduction

Adult tissue homeostasis relies heavily on stem cells, especially in high turnover organs such as the intestine, skin, or blood (Barker, 2014; Goodell et al., 2015). Tissue stem cell lineages are physiologically regulated according to diet, stress, injury, and age, through mechanisms involving extensive inter-organ communication (Ables et al., 2012). Understanding the role of adipocytes in the regulation of stem cells is particularly relevant given their crucial roles in regulating whole-body physiology and the link between malfunctioning adipocytes and a number of pathologies, including wound healing defects and cancers (Fasshauer and Bluher, 2015; Rosen and Spiegelman, 2014).

Germline stem cells (GSCs) in the adult *Drosophila* ovary are a useful system for investigating how stem cell lineages are shaped by physiological inputs (Laws and Drummond-Barbosa, 2017). Each ovary contains 16–20 ovarioles, and the anterior region of each ovariole, the germarium, contains two to three GSCs in a somatic niche composed primarily of cap cells (Fig. 1A–C) (Laws and Drummond-Barbosa, 2017). Each GSC divides asymmetrically to self-renew and generate a cystoblast that forms two-, four-, eight-, and 16-cell germline cysts through successive, synchronized mitotic divisions with incomplete cytokinesis. One of the cyst cells becomes the oocyte, which is supported by the remaining 15 nurse cells. Follicle cells surround each germline cyst to form an individual egg chamber, or follicle. The follicle develops through morphologically distinct stages, including vitellogenesis, to give rise to a stage 14 mature oocyte that is ovulated, fertilized and laid (Laws and Drummond-Barbosa, 2017). Oogenesis is markedly responsive to diet, resulting in up to 60-fold changes in egg laying rates on yeast-rich versus -free diets (Drummond-Barbosa and Spradling, 2001).

Many steps of oogenesis are highly regulated by diet to ensure that egg production is precisely coordinated with the physiology of the organism as a whole (Laws and Drummond-Barbosa, 2017). In well-fed females, robust GSC proliferation and development of their progeny provide a steady supply of new eggs. Upon removal of dietary yeast, GSCs and their progeny divide more slowly, early germline cysts die at higher rates, follicle growth is reduced, the vast majority of early vitellogenic follicles degenerate, and a partial block in ovulation causes prolonged retention of mature eggs in ovarioles (Drummond-Barbosa and Spradling, 2001). In addition, pre-vitellogenic follicles accumulate enlarged P bodies and undergo microtubule rearrangement (Shimada et al., 2011). Prolonged starvation also leads to increased rates of GSC

* Corresponding author.
E-mail address: dbarbosa@jhu.edu (D. Drummond-Barbosa).

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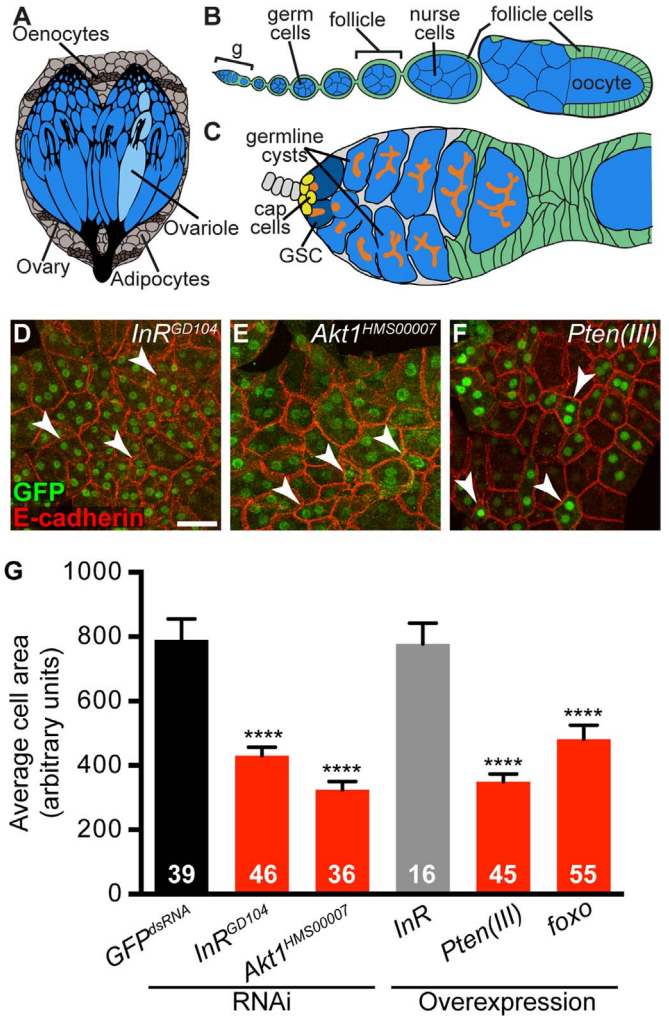

Fig. 1. Insulin signaling in adult adipocytes cell autonomously controls cell size. (A) Diagram illustrating that adipocytes and oenocytes constitute the *Drosophila* fat body, which underlies the cuticle and surrounds the ovaries (composed of ovarioles). (B) Diagram of ovariole showing the anterior germarium (g) and developing egg chambers (or follicles), each composed of a germline cyst (one oocyte and 15 nurse cells) surrounded by follicle cells. (C) Diagram of germarium showing GSCs housed in direct contact with cap cells. GSCs generate cystoblasts, which in turn incompletely divide four times to form germline cysts containing 16 interconnected cells. The position and morphology of a special organelle, the fusome (orange), allows for the identification of GSCs, cystoblasts, two-, four-, eight-, and 16-cell germline cysts. (D–F) Adipocytes from females subjected to adipocyte-specific manipulation of insulin signaling. *Gal80^{ts}; Lsp2-Gal4* drove expression of a *UAS-nuclear GFP* reporter in combination with other *UAS* transgenes (see Table S1), and females were raised at 18°C and switched to 29°C four days after eclosion for 10 days to induce transgene expression. Nuclear GFP intensity varies between adipocytes indicating variable driver expression. Adipocytes with relatively robust nuclear GFP expression (arrowheads indicate some examples) were used for quantification shown in (G). GFP (green); E-cadherin (red), cell membranes. Scale bar, 25 µm. (G) Average cell area of adipocytes upon adult adipocyte-specific expression of various transgenes at 29°C for 10 days. *GFP^{dsRNA}* was used as RNAi control. Mean ± s.e.m. Number of adipocytes quantified are shown inside bars. ****P < 0.0001; ordinary one-way ANOVA. See Supplementary material Fig. S1 for additional transgenes tested.

loss (Hsu and Drummond-Barbosa, 2009). Many diet-dependent pathways, including insulin, Target of Rapamycin (TOR), AMP-dependent kinase, and nuclear hormone signaling, function in the ovary to mediate these effects of diet (Laws and Drummond-Barbosa, 2017). Adipocytes, which in *Drosophila* co-exist with hepatocyte-like oenocytes in an organ called the fat body (Hoshizaki et al., 1995), also communicate their dietary status to the GSC lineage through as yet unknown systemic signals. Reduced amino acid transport into adipocytes causes increased GSC loss and a partial block in ovulation on a yeast-rich diet (Armstrong et al., 2014), whereas a number of diet-regulated metabolic pathways in adipocytes have specific effects on GSC number and early cyst survival (Matsuoka et al., 2017).

The highly conserved insulin/insulin-like growth factor pathway is a prominent and multifaceted actor in the regulation of oogenesis. In *Drosophila*, insulin-like peptides bind and activate a single receptor tyrosine kinase, the insulin receptor (InR) (Nassel et al., 2015). In response to InR activation, phosphoinositide-3-kinase (PI3K) phosphorylates phosphatidylinositol-4-5-diphosphate (PIP2) to produce phosphatidylinositol-triphosphate (PIP3), which recruits the serine/threonine kinase Akt1 to the membrane, allowing its phosphorylation and activation. Akt1 has many substrates that regulate a variety of cellular processes. For example, glycogen synthase kinase-3 β (GSK-3β) and the transcription factor Forkhead Box O (FOXO) are inhibited by Akt1, whereas phosphorylation of the tuberous sclerosis complex (TSC) by Akt1 leads to activation of the TOR-containing protein kinase complex mTORC1 (Manning and Toker, 2017; Nassel et al., 2015). Brain-derived insulin-like peptides act directly on the germline to control GSC proliferation, follicle growth and vitellogenesis (LaFever and Drummond-Barbosa, 2005). Distinct downstream effectors, however, are involved in the control of the GSC division cycle (PI3K/FOXO) versus growth of their progeny at later stages (PI3K/TOR) (Hsu et al., 2008; LaFever et al., 2010). Insulin signaling acts through FOXO in somatic niche cells to promote GSC maintenance by regulating the number of cap cells and adhesion of GSCs to cap cells (Hsu and Drummond-Barbosa, 2009, 2011; Yang et al., 2013). Insulin signaling is also required in follicle cells for P body and microtubule responses in the underlying germline of previtellogenic follicles (Burn et al., 2015) and for the mitotic-to-endocycle switch (Jouandin et al., 2014). The reproductive function of the insulin pathway is conserved from *C. elegans* to mammals, where insulin signaling also plays important roles in oocyte growth, development and maturation (Das and Arur, 2017).

Insulin signaling in adult adipocytes has been shown to control fat storage and lifespan in *Drosophila* (DiAngelo and Birnbaum, 2009; Giannakou et al., 2004; Hwangbo et al., 2004); however, it has remained unknown how insulin signaling in adipocytes might modulate the adipocyte-ovary communication axis. In this study, we find that insulin signaling within adipocytes promotes GSC maintenance, survival of early germline cysts, and vitellogenesis. Akt1 and GSK-3β mediate the effect of adipocyte insulin signaling on GSCs independently of FOXO. By contrast, early germline cyst survival and vitellogenesis do not require either GSK-3β or FOXO in adipocytes. These results indicate that insulin signaling in adult adipocytes employs distinct downstream effectors to regulate different steps of GSC lineage development in the ovary.

2. Materials and methods

2.1. Drosophila strains and culture conditions

Fly stocks were maintained at 22–25°C on standard medium containing cornmeal, molasses, yeast, and agar. Standard medium supplemented with wet yeast paste was used for all experiments. The adipocyte-specific Gal4 line, *3.1Lsp2-Gal4* (Lazareva et al., 2007), and temperature-sensitive *tub-Gal80^{ts}* (McGuire et al., 2003) transgenes were used as previously described (Armstrong et al., 2014). *UAS-RNA hairpin* lines obtained from the Vienna *Drosophila* RNAi stock center (http://stockcenter.vdrc.at), and the Transgenic RNAi Project (http://www.flyrnai.org) collection at the Bloomington Drosophila Stock Center (http://flystocks.bio.indiana.edu) for manipulation of insulin signaling components are listed in Table S1. Other transgenic lines used were: *UAS-InR* (Brogiolo et al., 2001; Huang et al., 1999), *UAS-Pten (II)* (Potter et al., 2001), *UAS-Pten (III)* (Gao et al., 2000), *UAS-Pten.ORF* (FlyORF), *UAS-foxo* (Hwangbo et al., 2004), *UAS-foxo (III)* (Junger et al., 2003), *UAS-sgg^{S9A}* (Bourouis, 2002), *ilp5-lacZ* (Ikeya et al., 2002), and *tGPH* (Britton et al., 2002). Other genetic elements used are described in FlyBase (http://www.flybase.org).