Developmental Biology 456 (2019) 179–189

Contents lists available at ScienceDirect

Developmental Biology

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

The nuclear receptor seven up functions in adipocytes and oenocytes to control distinct steps of *Drosophila* oogenesis

Lesley N. Weaver, Daniela Drummond-Barbosa *

Department of Biochemistry and Molecular Biology, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, 21205, USA

The nuclear receptor Seven up is required in adipocytes and oenocytes to regulate distinct steps of oogenesis in adult Drosophila.

ARTICLE INFO

Keywords:
Seven up
Adipocytes
Oenocytes
Germline
Oogenesis
Drosophila

ABSTRACT

Reproduction is intimately linked to the physiology of an organism. Nuclear receptors are widely expressed transcription factors that mediate the effects of many circulating molecules on physiology and reproduction. While multiple studies have focused on the roles of nuclear receptors intrinsically in the ovary, it remains largely unknown how the actions of nuclear receptors in peripheral tissues influence oogenesis. We identified the nuclear receptor encoded by *svp* as a novel regulator of oogenesis in adult *Drosophila*. Global somatic knockdown of *svp* reduces egg production by increasing GSC loss, death of early germline cysts, and degeneration of vitellogenic follicles. Tissue-specific knockdown experiments revealed that *svp* remotely controls these different steps of oogenesis through separate mechanisms involving distinct tissues. Specifically, adipocyte-specific *svp* knockdown impairs GSC maintenance and early germline cyst survival, whereas oenocyte-specific *svp* knockdown increases the death of vitellogenic follicles without any effects on GSCs or early cysts. These results illustrate that nuclear receptors can control reproduction through a variety of mechanisms involving peripheral tissues.

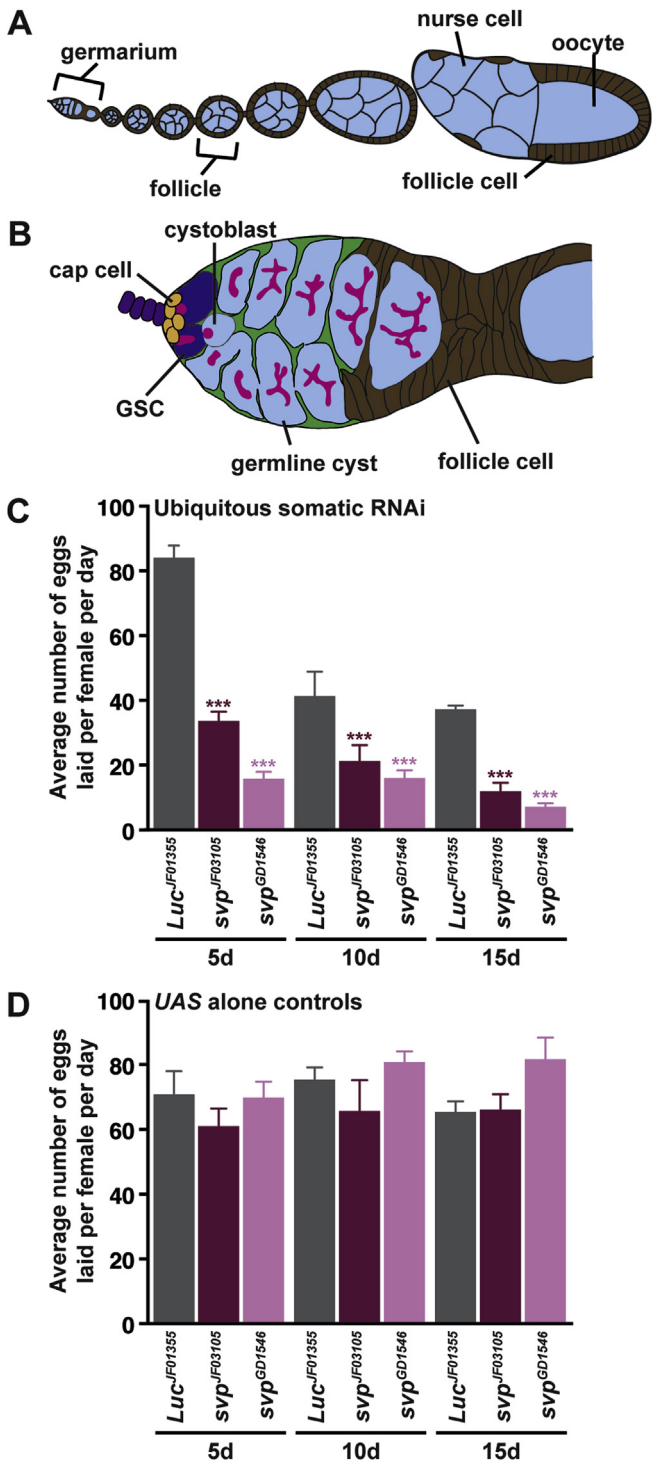
1. Introduction

Sexually reproducing species require proper oogenesis for their long-term survival. A variety of factors, including aging, infection, and diet, can influence reproductive fitness (Laws and Drummond-Barbosa, 2017). Many circulating molecules that tie our physiological status to specific cellular processes, including steroid hormones, fatty acids, and metals, serve as ligands for nuclear receptors. Nuclear receptors are ligand-dependent transcription factors that influence a wide range of developmental, metabolic, and reproductive processes (Francis et al., 2003; Thummel, 2005). Despite the large focus on the role of estrogen, progesterone and testosterone acting through their respective receptors (Aquila and De Amicis, 2014; Findlay et al., 2010; Jasienska et al., 2017), reproduction is also affected by other nuclear receptors, many of which function in the ovary itself to regulate oogenesis. Estrogen-related receptors (ERRs) and retinoic acid receptors (RARs) regulate many processes, including early germ cell development (Festuccia et al., 2017; Mouzat et al., 2013). Liver receptor homolog 1 (LRH-1) is required in somatic granulosa cells for ovulation (Duggavathi et al., 2008). Multiple NRs (e.g. SF-1, LXR, FXR, RXR, LRH-1) are involved in cholesterol homeostasis, thereby affecting steroid production (Maqdasy et al., 2013; Mouzat et al., 2013). Nevertheless, the potential reproductive roles of most nuclear receptors in adults remain largely unexplored, especially for those that function in peripheral tissues to control oogenesis.

The *Drosophila melanogaster* ovary is a powerful model system to understand fundamental aspects of how changes in physiology influence reproduction. Each ovary contains 16–20 ovarioles, each comprising an anterior germarium and progressively older follicles composed of a 16-cell germline cyst surrounded by somatic follicle cells (Fig. 1A). Each germarium houses two-to-three germline stem cells (GSCs) juxtaposed to cap cells, the major cellular components of the GSC niche (Fig. 1B). GSCs divide asymmetrically to self-renew and give rise to a cystoblast that undergoes four mitoses with incomplete cytokinesis, ultimately forming a 16-cell cyst composed of one oocyte and 15 supporting nurse cells. Follicle cells envelop the germline cysts to form follicles that bud from the germarium and develop through 14 recognizable stages. The oocyte begins yolk uptake, or vitellogenesis, at stage 8, and mature stage 14 oocytes are ready for ovulation and fertilization (Greenspan et al., 2015; Laws and Drummond-Barbosa, 2017). The *Drosophila* ovary responds to physiological changes by regulating GSC maintenance and proliferation, early germline cyst proliferation and survival, follicle growth, survival of vitellogenic follicles, and ovulation. Many of these steps are controlled by

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

https://doi.org/10.1016/j.ydbio.2019.08.015
Received 24 July 2019; Accepted 23 August 2019
Available online 27 August 2019



(caption on next column)

nuclear receptors (Ables and Drummond-Barbosa, 2017; Laws and Drummond-Barbosa, 2017).

The nuclear receptor superfamily is conserved from invertebrates to mammals. The *Drosophila* genome contains 18 nuclear receptors representing each of the major vertebrate subfamilies (King-Jones and Thummel, 2005). A heterodimer of the ecdysone receptor, EcR (liver X receptor and farnesoid X receptor homolog), and Ultraspiracle, Usp (retinoid X receptor homolog), cell-autonomously controls GSC maintenance and proliferation (Ables and Drummond-Barbosa, 2010). EcR and E75 (one of the REV-ERB homologs) act in escort cells to promote germline cyst differentiation (Konig and Shcherbata, 2015; Konig et al., 2011; Morris and Spradling, 2012) and also influence vitellogenesis (Buszczak et al., 1999; Sieber and Spradling, 2015). EcR also increases female appetite to support oogenesis (Sieber and Spradling, 2015). In addition, E78 (closely related to E75) is required for pre-vitellogenic follicle survival (Ables et al., 2015), whereas Hr39 (the LRH/SF1 homolog) is required for ovulation (Sun and Spradling, 2013). The roles of other nuclear receptors in adult oogenesis, however, remain largely unknown, and it is particularly unclear how nuclear receptor signaling in peripheral tissues outside of the ovary might regulate specific steps of oogenesis.

In this study, we identify the nuclear receptor Svp as a new non-autonomous, or extrinsic, regulator of oogenesis. We demonstrate that Svp activity is required in somatic tissues of the adult female to regulate GSC maintenance and survival of early germline cysts and vitellogenic follicles. Specifically, adult adipocyte-specific knockdown of *svp* increases GSC loss and early germline cyst death. By contrast, adult oenocyte-specific *svp* knockdown increases the death of vitellogenic follicles with no effects on GSCs or early germline cysts, revealing a novel role for oenocytes in regulating oogenesis. These findings illustrate that nuclear receptors can have complementary functions in distinct peripheral tissues to control oogenesis at specific stages.

2. Results

2.1. *svp* is required in adult somatic cells of *Drosophila* females for oogenesis

Nuclear receptors have known cell-autonomous, or intrinsic, roles in regulating a variety of cellular processes in the ovary (Ables and Drummond-Barbosa, 2017; Laws and Drummond-Barbosa, 2017). It is not clear, however, the extent to which nuclear receptors function in peripheral tissues to regulate oogenesis. To identify nuclear receptors

Fig. 1. *svp* is required in somatic cells of adult females for normal egg production. (A) The *Drosophila* ovary has 16–20 ovarioles, each comprising an anterior germarium followed by developing follicles arranged in chronological order. Each follicle consists of a germline cyst (15 nurse cells and one oocyte; light blue) surrounded by somatic follicle cells (brown). (B) The germarium contains 2–3 germline stem cells (GSCs; dark blue) residing in a niche composed primarily of cap cells (orange). Each asymmetric GSC division renews the GSC and gives rise to a cystoblast that further divides incompletely to form a 16-cell cyst. GSCs and germline cysts are identified based on their characteristic fusome morphology (pink), a germline-specific organelle. Follicle cells (brown) surround the developing cyst to bud a new follicle. (C) Females carrying *Gal80^{ts}; tub-Gal4* (*tub-Gal4^{ts}*) and *UAS-hairpin* transgenes against *Luc* (for control RNAi) or *svp* raised at 18 °C were switched to 29 °C (in the presence of *y w* males) for adult-specific ubiquitous somatic RNAi knockdown for five, 10, or 15 days. Knockdown of *svp* caused a significant decrease in the average number of eggs laid per female per day. (D) Control females carrying *UAS-hairpin* transgenes against *Luc* control or *svp* in the absence of *tub-Gal4^{ts}* were subjected to the same conditions as in (C), showing that the *UAS* alone does not alter egg-laying rates. Note that *UAS* alone controls were tested in a separate experiment than (C). Variations among experiments (e.g. batch of yeast paste) may lead to variations in egg counts; therefore, genotypes should be compared within the same experiment. Data shown as mean ± SEM. ***$P < 0.001$, two-tailed Student's *t*-test.

required in somatic cells (in the ovary or other organs) to control oogenesis, we used the *tub-Gal4* driver in combination with *tub-Gal80^{ts}* (*tubGal4^{ts}*) to ubiquitously knock down individual nuclear receptors in adult females, and measured egg production. [*tub-Gal4^{ts}* drives expression of *UAS* transgenes effectively in somatic cells, but not in the germline (Fig. S1)]. Our screen positively identified nuclear receptors that are known regulators of oogenesis, including EcR, E75, and E78. We also found that ubiquitous adult somatic knockdown of *seven up* (*svp*) significantly decreases the number of eggs laid by females compared to *Luciferase* (*Luc*) RNAi controls or *UAS-hairpin* alone (Fig. 1C and D). *svp* has known roles in regulating eye development (Mlodzik et al., 1990), fat cell differentiation in embryos (Hoshizaki et al., 1994), and neuroblast identity specification (Syed et al., 2017). Our results now indicate that *svp* has a new role in regulating oogenesis in adult females.

2.2. *svp is required in adult somatic cells for maintaining proper numbers of GSCs, but not for normal GSC proliferation or follicle growth*

Previous studies have shown that physiological factors can influence oogenesis at multiple steps in adult *Drosophila* females (Laws and Drummond-Barbosa, 2017). Thus, the decreased egg laying observed upon ubiquitous somatic knockdown of *svp* in adult females could result from defects in one or more processes in oogenesis. We first asked whether *svp* is required for maintaining normal numbers of GSCs. Ubiquitous somatic knockdown of *svp* in adult females increased the rate of GSC loss relative to *Luc* RNAi controls (Fig. 2A–C, Fig. S2). This increase in GSC loss was not due to leaky expression of *UAS* transgenes, as the *UAS-svp hairpin* alone controls did not show a change relative to *UAS-Luc hairpin* (Fig. S2D). In addition, there were no significant differences in cap cell number between control and experimental genotypes (Fig. 2D, Fig. S2C), ruling out a decrease in niche size as a potential cause of GSC loss (Hsu and Drummond-Barbosa, 2009).

We also measured rates of GSC proliferation and pre-vitellogenic follicle growth, which can both contribute to changes in egg production (Laws and Drummond-Barbosa, 2017). We compared GSC proliferation rates between control RNAi females and those with ubiquitous somatic knockdown of *svp* based on the frequencies of EdU incorporation and phospho-histone H3-positive staining in GSCs, and found no statistically significant differences (Figs. S3A and B). To compare pre-vitellogenic follicle growth, we used follicle cell proliferation during stages 4–6 as a proxy because follicle cells proliferate proportionately to the growth of follicles (LaFever and Drummond-Barbosa, 2005; Laws and Drummond-Barbosa, 2016; Maines et al., 2004). The frequencies of phospho-histone H3-positive follicle cells in pre-vitellogenic follicles was statistically similar between females with ubiquitous somatic RNAi against *Luc* control or *svp* (Fig. S3C). Taken together, these results indicate that Svp activity in somatic cells promotes GSC maintenance, but GSC proliferation and pre-vitellogenic follicle growth are not part of the mechanism by which Svp regulates egg production.

2.3. *svp is required in somatic cells for early germline cyst and vitellogenic follicle survival*

We reasoned that the rapid decline in egg production in females with ubiquitous somatic *svp* was not due solely to the observed increase in GSC loss. We also noticed that germaria of females with ubiquitous somatic *svp* knockdown were visibly shorter and appeared to have reduced numbers of germ cells based on Vasa immunostaining. Therefore, we asked whether *svp* is required for early germline cyst survival by labeling dying cysts using ApopTag, which detects DNA double-stranded breaks (Drummond-Barbosa and Spradling, 2001). Ubiquitous somatic knockdown of *svp* in adult females significantly increased the percentage of germaria containing ApopTag-positive germline cysts relative to *Luc* control knockdown females, and these dying cysts were predominantly observed in germarium Region 2 (which contains 16-cell cysts not yet fully surrounded by follicle cells) (Fig. 3).

Physiological inputs can also regulate germline survival during vitellogenic stages (Laws and Drummond-Barbosa, 2017), prompting us to examine these later follicles. Indeed, we found that females with ubiquitous adult somatic knockdown of *svp* displayed an increased frequency of ovarioles containing vitellogenic follicles with pyknotic nuclei (visualized by DAPI staining) relative to *Luc* control knockdown females (Fig. 4). These results demonstrate that *svp* is required in somatic tissues to regulate the survival of the germline at two major points of oogenesis regulation.

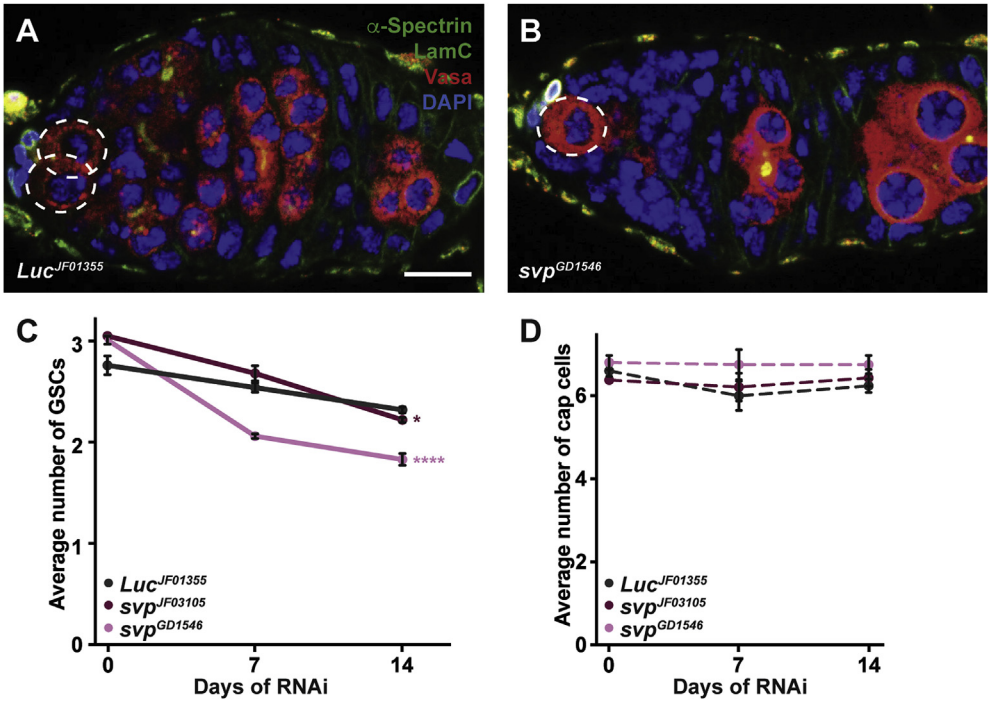


Fig. 2. Ubiquitous somatic knockdown of *svp* in adult females increases the rate of GSC loss. (A,B) Germaria from females at 14 days of adult-specific ubiquitous somatic RNAi knockdown of *Luc* (control) or *svp*. Vasa (red), germ cells; α-Spectrin (green), fusome; LamC (green), cap cell nuclear lamina; DAPI (blue), nuclei. GSCs are outlined. Scale bar, 10 μm. (C,D) Average number of GSCs (C) or cap cells (D) per germarium of females with *tub-Gal4^{ts}*-driven control or *svp* RNAi over time. Data shown as mean ± SEM. *$P < 0.05$; ****$P < 0.0001$, two-way ANOVA with interaction.

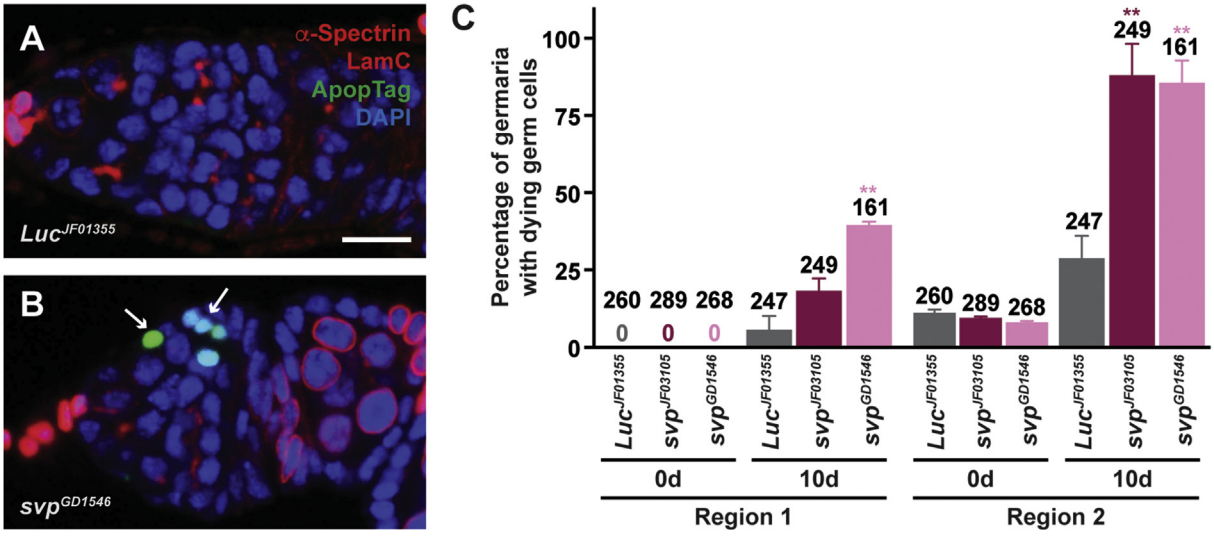


Fig. 3. *svp* functions in somatic cells of adult females to promote survival of early germline cysts. (A,B) Germaria from females at 10 days of adult-specific ubiquitous somatic RNAi against *Luc* (control) (A) or *svp* (B). α-Spectrin (red), fusome; LamC (red), cap cell nuclear lamina; ApopTag (green), dying cells; DAPI (blue), nuclei. Scale bar, 10 μm. (C) Average percentage of germaria containing ApopTag-positive dying cysts in Regions 1 or 2 from adult females at zero and 10 days of ubiquitous somatic RNAi against *Luc* (control) or *svp*. Data shown as mean ± SEM. $^{**}P < 0.01$. The numbers of germaria analyzed are shown above bars.

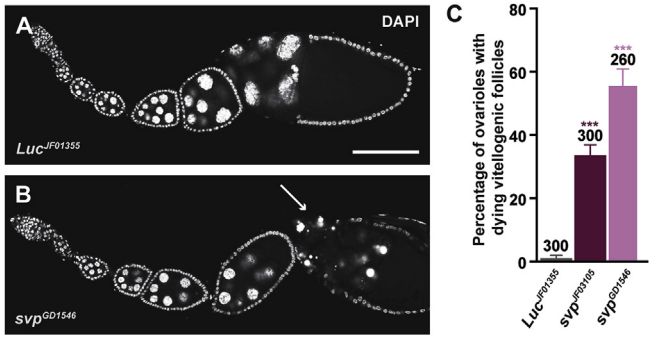


Fig. 4. Ubiquitous somatic knockdown of *svp* causes death of vitellogenic follicles. (A,B) Ovarioles at 10 days of adult specific ubiquitous *Luc* RNAi (A) or *svp* RNAi (B) stained with DAPI (white, nuclei). Scale bar, 50 μm. Arrow points to a dying vitellogenic follicle. (C) Average percentage of dying ovarioles observed by pyknotic nuclei. Data shown as mean ± SEM. $^{***}P < 0.001$. The numbers of ovarioles analyzed are shown above bars.

2.4. svp is expressed in multiple somatic tissues and its activity does not appear to be required in the germline for oogenesis

The defects in oogenesis observed in females with ubiquitous adult somatic *svp* knockdown could reflect a requirement for *svp* in one or more specific tissues. To determine in what tissues *svp* functions to control oogenesis, we performed tissue-specific RT-PCR analysis of adult *Drosophila* females to detect *svp* transcripts (Fig. S4A). *svp* transcripts were robustly expressed in most of the major organs of the body, except for adult female ovaries, which had nearly undetectable *svp* transcript levels. To further rule out a possible role of very low *svp* levels in the germline, we generated genetic mosaic females and analyzed GSC loss events in homozygous *svp* null GSC clones (recognized by the absence of a GFP marker). Relative to control mosaic germaria, we did not detect any increase in GSC loss events in either svp^1 or svp^2 null mosaic germaria (Fig. S4B). We also did not observe any other obvious oogenesis phenotypes in *svp* mutant mosaic ovarioles, suggesting that *svp* does not have a major function in the germline to control ovarian function.

2.5. svp is required in adipocytes, but not oenocytes, for GSC maintenance independently of E-Cadherin or bone morphogenetic protein signaling

Our RT-PCR analysis suggested multiple tissues where *svp* might be acting to regulate oogenesis. We therefore used a panel of tissue-specific Gal4 drivers (in combination with *tub-Gal80^{ts}*) to knock down *svp* and quantified egg production by counting the number of eggs laid per female per day (Fig. S5). Knockdown of *svp* specifically in adult adipocytes (*3.1Lsp2-Gal4*), gut (*myo31D-Gal4*), muscle (*MHC-Gal4*), or neurons (*nSyb-Gal4*) did not significantly affect egg production (Fig. S5A,C-E). In contrast, females with adult oenocyte-specific *svp* knockdown driven by *PromE(800)-Gal4* showed a significant decrease in the number of eggs laid compared to control *Luc* knockdown females (Fig. S5B). These results reveal a previously unknown role for oenocytes in the regulation of oogenesis.

We next examined the requirement for *svp* activity not only in oenocytes, but also in adipocytes, for several reasons. First, hepatocyte-like oenocytes function closely together with adipocytes in an organ called the fat body (Arrese and Soulages, 2010; Gutierrez et al., 2007; Makki et al., 2014). Second, *svp* is required for fat cell differentiation during *Drosophila* embryogenesis (Hoshizaki et al., 1994) and the mammalian Svp homolog COUP-TFII has roles in adipocytes. Specifically, COUP-TFII heterozygous mice have less white adipose tissue and decreased expression of genes required for adipogenesis (Li et al., 2009). Third, our previous proteomics analysis identified *svp* as a diet-regulated factor in the adult female fat body (Matsuoka et al., 2017). Finally, the egg count assay is not sufficiently sensitive to detect relatively small changes in specific processes in oogenesis, which may nonetheless contribute to the fine-tuning of the ovarian response to changes in physiology. We thus tested whether *svp* is required in adult female adipocytes and/or oenocytes for the specific oogenesis processes that were altered upon ubiquitous adult somatic *svp* knockdown.

We first examined *svp* requirement in adult adipocytes and oenocytes for GSC maintenance (Fig. 5A–D). For adult adipocyte-specific knockdown, we used the *3.1Lsp2-Gal4* driver with *tub-Gal80^{ts}* that is exclusively expressed in adult female adipocytes (Armstrong et al., 2014) (Fig. S1B). To target adult oenocytes, we combined *tubGal80^{ts}* with the previously described *PromE(800)-Gal4* driver (Billeter et al., 2009), which we confirmed is specifically expressed in oenocytes and not in other tissues (Fig. S1C). Knockdown of *svp* specifically in adult female

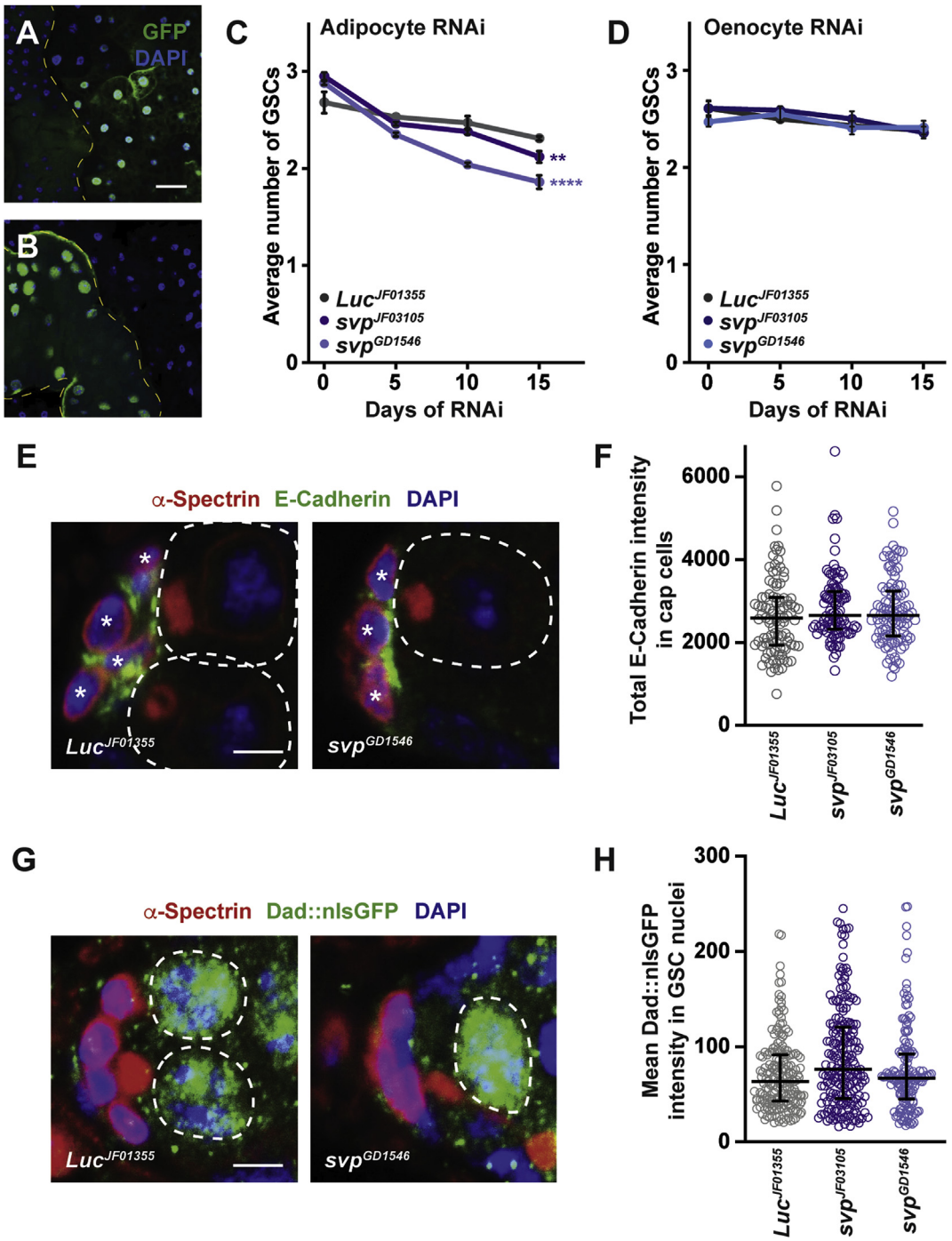


Fig. 5. *svp* is required in adult female adipocytes, but not oenocytes, to regulate GSC maintenance independently of niche E-cadherin or BMP signaling. (A,B) Fat bodies from adult females labeled with DAPI (blue, nuclei) and nuclear GFP (nucGFP, green) driven by adipocyte-specific *3.1Lps2-Gal4* (A) or oenocyte-specific *PromE(800)-Gal4* (B) in combination with *tub-Gal80^{ts}* for adult-specific expression. The dotted line separates the adipocytes (right) from oenocytes (left, identified by their autofluorescence in the green channel). Scale bar, 25 μm. (C,D) Average number of GSCs per germarium over time for females with adult adipocyte-specific (C) or oenocyte-specific (D) RNAi against *Luc* (control) or *svp*. Data shown as mean ± SEM. $^{**}P < 0.01$; $^{****}P < 0.0001$, two-way ANOVA with interaction. (E) Germaria from females at 10 days of adult adipocyte-specific *Luc* or *svp* RNAi. α-Spectrin (red), fusome; LamC (red), cap cell nuclear lamina; E-Cadherin (green). GSCs are outlined. (F) Dot plot of total cap cell E-Cadherin intensity per germarium for experiment in (E). (G) Germaria from females as described in E. α-Spectrin (red), fusome; LamC (red), cap cell nuclear lamina; Dad:nlsGFP (green), reporter of BMP signaling. GSC nuclei are outlined. Scale bars in (E) and (G), 5 μm. (H) Dot plot of mean Dad:nlsGFP intensity per germarium for experiment in (G). Black lines in (F) and (H) indicate mean ± SEM for each experiment. No statistically significant differences, Mann Whitney *U* test.

adipocytes significantly increased the rate of GSC loss, whereas adult oenocyte *svp* knockdown had no effect on GSC numbers (Fig. 4C–D, Figs. S6A–B). *svp* knockdown in either adipocytes or oenocytes did not affect fat body morphology, suggesting that oogenesis phenotypes are not simply a secondary consequence of gross adipocyte dysfunction (Fig. S7).

The GSC loss phenotype observed when *svp* is knocked down in adult adipocytes could be due to compromised E-Cadherin-mediated niche adhesion (Song and Xie, 2002) or to reduced levels of bone morphogenetic protein (BMP) signaling, which is required for GSC maintenance (Xie and Spradling, 1998). We knocked down *svp* specifically in adult

female adipocytes, measured the intensity of E-Cadherin in the niche, and found no difference in E-Cadherin levels compared to *Luc* control RNAi (Fig. 5E and F). We also measured the nuclear intensity of the BMP signaling reporter Dad:nlsGFP in GSCs when *svp* was knocked down in adult adipocytes, and found that Dad:nlsGFP levels were comparable to those of control *Luc* knockdown females (Fig. 5G and H). These results show that the GSC loss caused by knockdown of *svp* specifically in adipocytes does not involve niche E-Cadherin or BMP signaling.

2.6. Loss of svp in adult female adipocytes, but not oenocytes, increases germline cyst death

We next examined whether early germline cyst survival was dependent on *svp* activity in adipocytes and/or oenocytes. Knockdown of *svp* specifically in adult female adipocytes increased the percentage of germaria with dying germline cysts relative to control females (Fig. 6A). By contrast, there was no difference in cyst survival when *svp* was knocked down in adult oenocytes (Fig. 6A). To determine when developing early germline cysts were dying, we counted the number of cystoblasts, two-, four-, eight-, and 16-cell cysts (normalized per GSC) within the germaria of females with adult adipocyte-specific RNAi knockdown of *svp* compared to control *Luc* knockdown. Adipocyte *svp* knockdown did not alter the number of cystoblasts, two-cell, four-cell, or eight-cell cysts in germaria (Fig. 6B–E), but significantly reduced the number of 16-cell cysts (Fig. 6F), indicating that *svp* functions in adult adipocytes to regulate early germline cyst survival at or near the transition between eight- and 16-cell cyst stages.

2.7. svp is required in adult oenocytes, but not adipocytes, for vitellogenesis

Ubiquitous somatic knockdown of *svp* in adult females also decreases the survival of vitellogenic follicles. Knockdown of *svp* specifically in adult adipocytes did not increase the percentage of ovarioles with dying vitellogenic egg chambers relative to control (Fig. 7), demonstrating a distinct cellular requirement compared to GSC maintenance and early germline cyst survival. Instead, we found that *svp* knockdown in adult oenocytes increased the occurrence of dying vitellogenic follicles (Fig. 7). These results, combined with the reduced egg laying rates observed in oenocyte *svp* knockdown females (Fig. S5B), suggest that the function of *svp* in oenocytes has a relatively large and novel contribution to the overall role of *svp* in egg production.

3. Discussion

Nuclear receptors mediate the effects of many circulating factors throughout the body, including on reproductive tissues. Germline cyst breakdown and follicle formation require a drop in progesterone levels (McGinnis et al., 2013). Transzonal projections (which allow gap junctions to form between the oocyte and granulosa cells) are regulated by hormones, including estrogen (Allworth and Albertini, 1993). Several nuclear receptors also control spermatogenesis (Maqdasy et al., 2013). As widely expressed transcriptional factors modulated by ligands, nuclear receptors can have direct effects by regulating transcriptional targets in a given tissue, indirect effects through intermediate organs/tissues, or a combination thereof. ERRs indirectly affect processes in various organs by regulating energy metabolism and ion channel expression (Festuccia et al., 2017). Estrogens have effects on most organs (e.g. brain, heart, bones, ovaries) by binding to widely distributed estrogen receptors (Rettberg et al., 2014). Much remains to be understood, however, regarding the roles of this large family of nuclear receptors during oogenesis, and their direct and indirect modes of action. In this study, we uncovered a novel, non-ovary-autonomous role for Svp/COUP-TFII in oogenesis. Interestingly, we found that *svp* is required in adipocytes for the control of GSC number and early germline cyst survival, whereas *svp* functions in oenocytes to support vitellogenic follicles (Fig. 8). These results suggest that a single nuclear receptor can regulate distinct sets of downstream factors in different peripheral tissues to influence oogenesis at particular steps.

3.1. A novel role for Svp/COUP-TFs in oogenesis

The homologs of Svp, COUP-TFs, control many processes; however, our study suggests a new role for this family of factors in the control of oogenesis. COUP-TFs are involved in tumorigenesis, angiogenesis, eye and nervous system development, and control of luteinizing hormone expression (Tang et al., 2015; Xu et al., 2015; Zheng et al., 2010). COUP-TFII also antagonizes androgen receptor function in prostate cancer cells (Song et al., 2012). COUP-TFI and COUP-TFII regulate expression of luteinizing hormone in the pituitary gland (Zheng et al., 2010), and COUP-TFII might also act in ovarian theca cells to control steroid production (Murayama et al., 2008). COUP-TFII is also required in the uterine muscle and stromal cells for proper placenta formation and embryo implantation (Petit et al., 2007). Our findings suggest that it would be worthwhile asking whether COUP-TFII might act in mammalian adipocytes and/or hepatocytes to control early mammalian female germ cell development and/or adult oogenesis.

3.2. Non-ovary-autonomous roles for nuclear receptors in the control of the female germline

Previous studies have identified multiple nuclear receptors that function in the germline or somatic reproductive tissues to influence *Drosophila* oogenesis, including EcR, Usp, E75, E78, and Hr39 (Ables et al., 2015; Ables and Drummond-Barbosa, 2010; Buszczak et al., 1999; Konig and Shcherbata, 2015; Konig et al., 2011; Sieber and Spradling, 2015; Sun and Spradling, 2013). By contrast, our RT-PCR and clonal analysis indicated there is no intrinsic role for *svp* in the germline. Instead, Svp functions in peripheral somatic tissues to control oogenesis, which implies that its targets are either secreted themselves or regulate other circulating factors. Identifying different sets of Svp targets in adipocytes and oenocytes that act in complementary ways to regulate oogenesis will help elucidate the enormous complexity of the mechanisms tying physiology to oogenesis. Our findings also underscore the importance of considering non-ovary-autonomous roles of other nuclear receptors.

3.3. Adipocytes as major modulators of oogenesis

Our previous studies have highlighted adipocytes as major regulators of specific steps in *Drosophila* oogenesis. Specifically, we discovered that adipocyte-specific disruption of amino acid transport, TOR, or insulin signaling causes distinct GSC lineage phenotypes, and that diet controls multiple metabolic pathways within adipocytes that can influence specific processes in the GSC lineage, including GSC maintenance and survival of early germline cysts and vitellogenic follicles (Armstrong and Drummond-Barbosa, 2018; Armstrong et al., 2014; Matsuoka et al., 2017). In addition, we also found that collagen IV produced in adipocytes is transported to the GSC niche to regulate GSC numbers (Weaver and Drummond-Barbosa, 2018). Our current findings that *svp* is required in adipocytes to influence GSC number and early germline cyst death further underscore the complex roles of adipocytes in oogenesis. Further, they are potentially relevant to the known link between adipocyte dysfunction (e.g. caused by obesity or lipodystrophy) and infertility in humans (Silvestris et al., 2018).

Of particular interest in *Drosophila*, the maintenance of GSCs appears to be highly sensitive to physiological perturbations in adipocytes. Disruption of specific metabolic pathways (Matsuoka et al., 2017), amino acid sensing (Armstrong et al., 2014), insulin signaling (Armstrong and Drummond-Barbosa, 2018), collagen IV production (Weaver and Drummond-Barbosa, 2018), and *svp* (this study) all lead to higher rates of GSC loss. It is unlikely that Svp and collagen IV function within a common pathway because adipocyte collagen IV controls GSC numbers

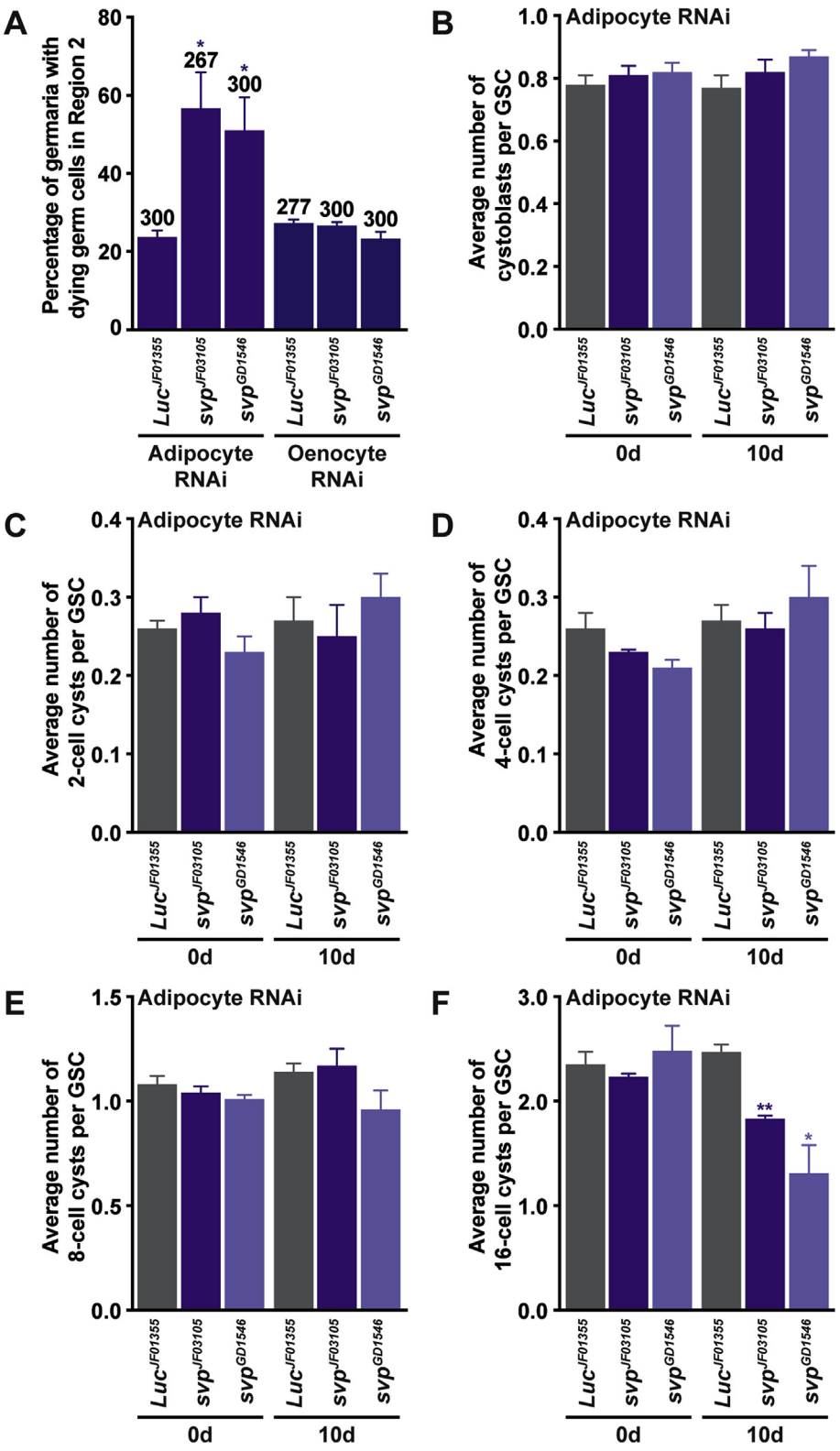


Fig. 6. Knockdown of *svp* in adult female adipocytes, but not oenocytes, increases 16-cell cyst death. (A) Average percentages of germaria containing dying cysts in Region 2 in females with adipocyte- or oenocyte-specific RNAi knockdown of *Luc* (control) or *svp* at 10 days of transgene expression. The numbers above each bar represent the number of analyzed germaria. (B–F) Average number of cystoblasts (B), two-cell cysts (C), four-cell cysts (D), eight-cell cysts (E), and 16-cell cysts per GSC per germarium of females at zero or 10 days of adult adipocyte-specific RNAi against *Luc* (control) or *svp*. Data shown as mean ± SEM. $^{*}P < 0.05$; $^{**}P < 0.01$, Student's *t*-test. 75 germaria were analyzed for each condition in (B–E).

through E-cadherin, whereas Svp does not. Future studies will address whether and how *svp* and/or its downstream targets functionally interact with metabolic pathways, amino acid sensing, or insulin signaling to control GSC numbers.

In addition to GSC loss, we observed that adipocyte knockdown of *svp* increases cyst death in Region 2 of the germarium, resulting in a significant reduction of 16-cell cyst numbers. Key stages of meiosis (assembly of the synaptonemal complex and specification of the oocyte), occur in 16-cell cysts (Hughes et al., 2018), as well as the handover of developing cysts from escort cells to follicle cells (Laws and Drummond-Barbosa, 2017). It is possible that downstream adipocyte-specific *svp* targets could influence signaling from escort cells or early follicle cells to influence cyst survival. For example, compromised EcR signaling in escort cells specifically reduces 16-cell cyst number and prevents entry into

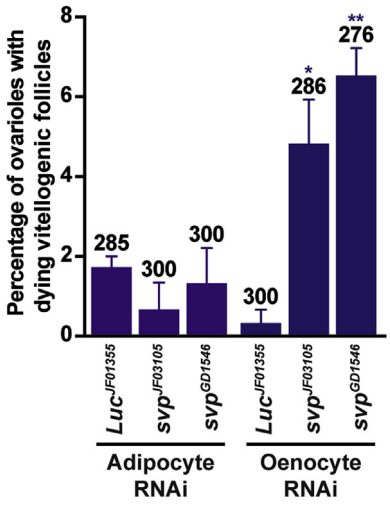


Fig. 7. Knockdown of *svp* in adult female oenocytes, but not adipocytes, increases vitellogenic follicle death. Average percentages of ovarioles containing dying follicles in females with adult adipocyte- or oenocyte-specific knockdown of *Luc* (control) or *svp* at 10 days of transgene expression. Data shown as mean ± SEM. $*P < 0.05$; $**P < 0.01$, Student's *t*-test. The numbers above each bar represent the number of analyzed ovarioles.

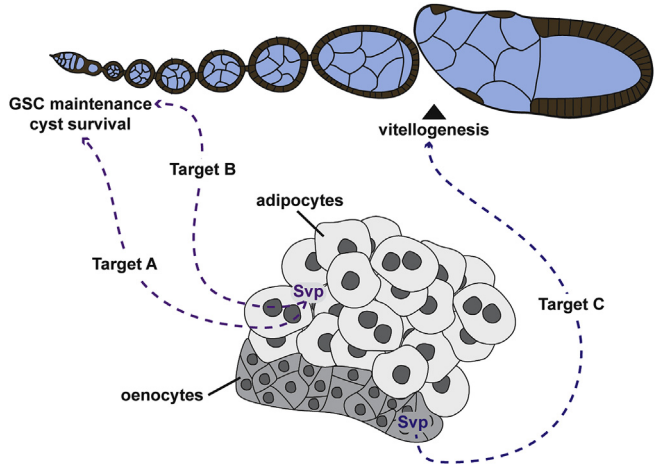


Fig. 8. Model for how Svp signaling in different somatic tissues regulates specific steps of oogenesis. This study shows that *svp* is required in adult female adipocytes to regulate GSC number and germline cyst survival at the 16-cell cyst stage. In addition, Svp activity is required specifically in adult oenocytes for survival of vitellogenic follicles. Future studies should identify the specific downstream effectors of Svp in adipocytes (Target A and B) versus oenocytes (Target C) that function in a complementary way to regulate distinct processes in oogenesis.

meiosis (Morris and Spradling, 2012), but factors upstream and downstream of EcR regulating these processes remain unknown.

3.4. The emerging role of oenocytes in oogenesis

In addition to adipocytes, there is increasing evidence that other tissues such as skeletal muscle, brain, gut, and the liver (Droujinine and Perrimon, 2016) participate in inter-organ communication to influence physiology. Our results show that *svp* is required in oenocytes [specialized cells that have been proposed to share functions with hepatocytes (Gutierrez et al., 2007)] to regulate survival of vitellogenic egg chambers. Interestingly, Svp is a known oenocyte-specific modulator of the cuticle lipid profile, as knockdown of *svp* specifically in adult female oenocytes modifies the 7,11-pentacosadine lipid species (Chiang et al., 2016), a low abundance 25-C hydrocarbon specifically found in females (Chertemps et al., 2007). Our results suggest that adult female oenocytes have a previously unknown systemic role in regulating vitellogenic follicle survival, raising the possibility that such role might be mediated through the production of hydrocarbons. The mechanistic link between oenocytes and vitellogenic follicles, however, remains to be explored in the future. Nonetheless, our results strongly suggest that the adipocyte-oenocyte axis functions not only to regulate mobilization of lipids under nutrient-deprived conditions during larval stages (Gutierrez et al., 2007), but also to coordinately regulate different steps of oogenesis.

In summary, our results identify a novel role of *svp* in oogenesis through a dual cellular requirement in adipocytes and oenocytes to regulate complementary steps of oogenesis and thus influence final egg output. The key next steps will be to determine the relevant transcriptional targets of Svp - which could include metabolic enzymes, extracellular matrix proteins, secreted signaling molecules, or transcriptional factors upstream of other effectors - in adipocytes and oenocytes and their mechanisms of action in the control oogenesis, as well as to identify upstream regulators of Svp and the physiological context in which they function. Although the effects of *svp* knockdown in adipocytes and oenocytes individually result in relatively modest effects in oogenesis, that is to be expected when it comes to the regulation of physiological processes. Specifically, physiology encompasses a great number of factors with individually small effects that work together to finely tune the function of each organ to the demands and constraints of the organism as a whole.

4. Materials and methods

4.1. Drosophila strains and culture conditions

Drosophila stocks were maintained on standard medium containing cornmeal, molasses, agar, and yeast at 22–25 °C. Unless otherwise noted, standard medium was supplemented with wet yeast paste for all experiments. Previously described Gal4 lines were used, including *tub-Gal4* (Nabel-Rosen et al., 2002), *3.1Lsp2-Gal4* (Lazareva et al., 2007), *PromE800-Gal4* (Billeter et al., 2009), *nSyb-Gal4* (Liu et al., 2012), *MHC-Gal4* (Schuster et al., 1996), and *w; Myo31DF^{NP0001}; UAS-3xFLAG-dCas9 tub-Gal80^{ts}* (Regan et al., 2016). The temperature-sensitive *tub-Gal80^{ts}* transgene, *vsg::GFP* reporter, and *dad::nlsGFP* reporter have been previously described (McGuire et al., 2003; Ayyaz et al., 2015; Buszczak et al., 2007). The *svp^2* allele (Mlodzik et al., 1990) and *UAS-GFP.nls* were obtained from the Bloomington *Drosophila* Stock Center (BDSC; bdsc.indiana.edu/). *UAS-luc^{JF01355}* (Matsuoka et al., 2017) and *UAS-svp^{JF03105}* (Transgenic RNAi Project; fgr.hms.harvard.edu/) lines were obtained from the BDSC. The *UAS-svp^{GD1546}* RNAi line was obtained from the Vienna *Drosophila* RNAi Stock Center (VDRC; stockcenter.vdrc.at/). Lines carrying multiple genetic elements were generated by standard crosses. Balancer chromosomes, *FRT* and *FLP* strains, and other genetic elements are described in Flybase (www.flybase.com).

4.2. Quantification of egg laying

To measure egg production, five females of appropriate genotype and 5 *y w* males were maintained at 29 °C in inverted perforated plastic bottles lidded by molasses/agar plates covered by a thin layer of wet yeast paste. Plates were changed twice daily, and eggs laid within the preceding 24 h were counted on specific days throughout experiments. Five replicates per genotype were analyzed and statistical significance was determined by a Student's *t*-test.

4.3. Tissue- and cell type-specific RNA interference (RNAi)

Females of genotypes *y w; tub-Gal80^{ts}/ + ; tub-Gal4/UAS-hairpin* (for adult ubiquitous somatic RNAi), *y w; tub-Gal80^{ts}/ + ; 3.1Lsp2-Gal4/UAS-hairpin* (for adult adipocyte-specific RNAi), *y w; PromE(800)-Gal4 tub-*

Gal80^{ts}/ + ; UAS-hairpin/ + (for adult oenocyte-specific RNAi), *y w; tub-Gal80^{ts}/ + ; nSyb-Gal4/UAS-hairpin* (for adult neuron-specific RNAi), *y w; tub-Gal80^{ts}/ + ; MHC-Gal4/UAS-hairpin* (for adult muscle-specific RNAi) and *w; Myo31DF^{NP0001}/ + ; UAS-3xFLAG-dCas9 tub-Gal80^{ts}/UAS-hairpin* (for adult gut-specific RNAi) were raised at 18 °C [the permissive temperature for Gal80^{ts} (McGuire et al., 2003)] to prevent RNAi induction during development. Zero-to 2-day-old females were maintained at 18 °C for 3 days with *y w* males, and then switched to 29 °C (the restrictive temperature for Gal80^{ts}) for various lengths of time to induce RNAi in specific adult tissues. *UAS-luc^{JF01355}* was used as an RNAi control. To control for "leaky" *UAS* expression, females of similar genotypes but without *Gal4* and *Gal80^{ts}* transgenes were raised and maintained under the same conditions for analysis at 5–15 days after switching to 29 °C.

4.4. Genetic mosaic analysis

Females of genotype *y w hs-FLP; FRT82B ubi-GFP/FRT82B svp** were generated through standard crosses. (*svp** represents null or wildtype alleles of *svp*). Zero-to 3-day-old females were maintained on standard food with dry yeast and heat shocked twice daily at 37 °C for 3 days to induce flippase (FLP)/*FLP recognition target* (*FRT*)-mediated mitotic recombination, as described (Laws and Drummond-Barbosa, 2015). After the final heat shock, females were kept on standard medium supplemented with wet yeast paste for 10 days prior to dissection. *svp** homozygous clones were recognized by the absence of GFP, and GSCs were identified based on their anterior location and typical fusome morphology (Laws and Drummond-Barbosa, 2015). To quantify GSC loss, germaria containing GFP-negative cystoblasts and/or cysts were analyzed, and the percentage of germaria that no longer contained the GFP-negative GSC that gave rise to those GFP-negative progeny (i.e. "GSC loss events") was calculated (Laws and Drummond-Barbosa, 2015). Three independent experiments were performed, and statistical significance was calculated using the Student's *t*-test.

4.5. Adult female tissue immunostaining and fluorescence microscopy

Ovaries were dissected (and ovarioles were teased apart) in Grace's Insect Medium (Bio Whittaker) and fixed for 13 min in 5.3% formaldehyde (Ted Pella) in Grace's medium at room temperature. Abdominal carcasses without ovaries or guts but with the fat body still attached, brains, and ripped thoracic muscles were also dissected and fixed for 20 min, whereas dissected guts were fixed for 1 h. Samples were subsequently rinsed and washed three times for 15 min each in phosphate-buffered saline (PBS; 10 mM NaH_2PO_4/$NaHPO_4$, 175 mM NaCl, pH 7.4) containing 0.1% Triton X-100 (PBST). Samples were blocked for at least 3 h at room temperature in 5% normal goat serum (NGS; Jackson ImmunoResearch) plus 5% bovine serum albumin (BSA, Sigma) in PBST, and then incubated overnight at 4 °C in the following primary antibodies diluted in blocking solution: mouse monoclonal anti-α-Spectrin (3A9) (DSHB; Developmental Studies Hybridoma Bank, 1:25); mouse monoclonal anti-Lamin C (LC28.26) (DSHB, 1:100); chicken polyclonal anti-GFP (Abcam, 1:1000); rat monoclonal anti-E-Cadherin (DCAD2) (DSHB, 1:3); rat monoclonal anti-Vasa (DSHB, 1:20); and rabbit polyclonal anti-phospho-Histone H3 (ser10) (Millipore Sigma, 1:200). Samples were washed in PBST and incubated for 2 h at room temperature in 1:400 Alexa Fluor 488- or 568-conjugated goat species-specific secondary antibodies (Molecular Probes) in blocking solution. Samples were washed and mounted in Vectashield containing 1.5 μg/ml 4′,6-diamidino-2-phenylindole (DAPI) (Vector Laboratories). Fat bodies were scraped off the abdominal carcass and muscle fibers removed from cuticle before mounting and imaged using a Zeiss LSM700 confocal microscope.

For lipid droplet visualization, fixed and washed fat bodies attached to abdominal carcasses were incubated with 1:200 Alexa Fluor 488-conjugated phalloidin (Molecular Probes) in PBST for 20 min, rinsed, and washed three times for 15 min each in PBST. Samples were stored in Vectashield plus DAPI (Vector Laboratories) containing 25 ng/ml Nile Red dye, and fat bodies were scraped off the abdominal carcass before mounting and imaging on a Zeiss LSM700 confocal microscope.

For Dad:nlsGFP quantification, the densitometric mean of individual GSC nuclei was measured from optical sections containing the largest nuclear diameter (visualized by DAPI) using ImageJ (Armstrong et al., 2014). For E-Cadherin quantification, the total densitometric value from maximum intensity projections around the cap cells (identified using LamC staining) was measured with ImageJ (Weaver and Drummond-Barbosa, 2018). Quantification of E-Cadherin levels by immunofluorescence is a well-established method to determine whether adherence of GSCs to the niche is compromised (Hsu and Drummond-Barbosa, 2011). Densitometric data were subjected to the Mann-Whitney *U* test. To determine adipocyte area, the largest cell area of each adipocyte (based on phalloidin staining) was measured using ImageJ. Three independent experiments were performed, and statistical analysis was performed using a Student's *t*-test.

4.6. Quantification of cap cells, GSCs, and GSC progeny

Cap cells were identified based on their ovoid shape and LamC-positive staining, whereas GSCs were identified based on their juxtaposition to cap cells and typical fusome morphology, as described (de Cuevas, 1998). Two-way ANOVA with interaction (GraphPad Prism) was used to calculate the statistical significance of any differences among genotypes in how much cap cell or GSC numbers change over time (i.e. the rate of cap cell or GSC loss, respectively) from at least three independent experiments, as described (Armstrong et al., 2014). The number of cystoblasts and two-, four-, eight-, and 16-cell germline cysts present in germaria were identified based on fusome morphology, and normalized to the number of GSCs, as described (Laws et al., 2015). The average number of cystoblasts and cysts per GSC was calculated from at least three independent experiments. The means of all experiments were averaged and subjected to the Student's *t*-test.

4.7. Analysis of proliferation, early dying cysts and degenerating vitellogenic follicles

For EdU incorporation analysis, intact dissected ovaries were incubated for 1 h at room temperature in 100 μM EdU (Molecular Probes) diluted in Grace's, washed, and fixed as described above. Following primary antibody incubation, samples were subjected to the Click-iT reaction according to the manufacturer's instructions (Life Technologies) for 30 min at room temperature. Relative GSC proliferation rates were measured by calculating the fraction of EdU- or phospho-histone H3-positive GSCs as a percentage of the total number of GSCs analyzed per genotype. To measure follicle cell proliferation, single confocal plane images at the top and bottom of flatly mounted ovarioles were acquired, and the average percentage of phospho-histone H3-positive follicle cells was calculated for each field. This analysis included follicle cells in stage 4–6 follicles, as previously described (Laws and Drummond-Barbosa, 2016).

To determine the percentage of germaria containing dying germline cysts, the ApopTag Indirect *In Situ* Apoptosis Detection Kit (Millipore Sigma) was used according to the manufacturer's instructions as previously described. Briefly, fixed and teased ovaries were rinsed in equilibration buffer twice for 5 min each at room temperature. Samples were incubated in 100 μl TdT solution at 37 °C for 1 h with mixing at 15-min intervals. Ovaries were washed three times in 1X PBS followed by incubation in anti-digoxigenin conjugate for 30 min at room temperature protected from light. Samples were washed four times in 1X PBS and processed for immunofluorescence as described above. Cyst death in the germarium was separately measured in Region 1 (which contains the GSCs, cystoblasts, and proliferating germline cysts) versus Region 2 (populated by 16-cell cysts not yet completely enveloped by follicle cells). Regions 1 and 2 were distinguished based on fusome morphology,

and cyst size and shape. Progression through vitellogenesis was assessed using DAPI staining. The percentage of ovarioles containing one or more dying vitellogenic follicles (recognized by the presence of pyknotic nuclei), as opposed to exclusively healthy vitellogenic follicles (which had no obvious defects in nuclei morphology or yolk accumulation), was determined for each genotype. Three independent experiments were performed and subjected to a Student's *t*-test for statistical analysis.

4.8. Analysis of glycogen levels in the fat body

For analysis of glycogen levels in the fat body, 10 abdominal carcasses (without the guts or ovaries) were dissected and fixed as described above. Fat bodies attached to abdominal carcasses were rinsed and washed three times in water for 10 min each at room temperature and processed using the Periodic Acid-Schiff (PAS) Staining System (Sigma Aldrich). Carcasses were then incubated in 300 µl Periodic Acid solution for 15 min and washed four times with water for 10 min each. Carcasses were stained with Schiff's Reagent for 20 min followed by three washes in water for 10 min each. Carcasses were stored in 50% glycerol and fat bodies were scraped off the abdominal carcass before mounting and imaged using a Zeiss Cell Observer Z1 linked to an ORCA-FLASH 4.0 CMOS camera (Hamamatsu).

4.9. Reverse transcriptase-polymerase chain reaction

For RNA extraction, 5 whole females, 5 pairs of ovaries, 10 abdominal carcasses (including adipocytes and oenocytes, but without ovaries and gut), 10 guts, 10 thoraces, or 10 heads were incubated in RNAlater (Ambion) for 30 min according to the manufacturer's instructions to stabilize RNA. Samples were then incubated in 250 µl lysis buffer from the RNAqueous-4PCR DNA-free RNA isolation for RT-PCR kit (Ambion). RNA was extracted from all samples using a motorized pestle and following the manufacturer's instructions. The primers used for the PCR reactions are listed in Supplemental Material, Table S1. *Rp49* primers were used as a control. Net band intensity for each sample was quantified using ImageJ by subtracting background pixels from band pixels in a fixed-size box, and normalized to the band intensity for the corresponding *Rp49* band. Controls were set to one and experimental sample intensities were calculated relative to control.

Declaration of interests

The authors declare no conflict of interests.

Funding

This work was supported by National Institutes of Health R01 GM069875. L.N.W. was supported by National Institutes of Health T32 CA009110, F32 GM119199, and K99 GM127605.

Data availability

Drosophila strains are available upon request. All data generated for this study are included in the main text and figures or in the supplemental files provided.

Acknowledgements

L.N.W. and D.D.-B. designed experiments, analyzed and interpreted data, and wrote the manuscript; L.N.W. performed all experiments. We thank the Developmental Studies Hybridoma Bank for antibodies and the Bloomington Stock Center (National Institutes of Health P400D018537), Vienna *Drosophila* Stock Center, Heinrich Jasper, and Mark Wu for *Drosophila* stocks. We are grateful to Tianlu Ma and Rodrigo Dutra Nunes for critical reading of the manuscript. The data and analyses reported in this paper are described in the main figures and Supplementary Materials.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ydbio.2019.08.015.

References

Ables, E.T., Bois, K.E., Garcia, C.A., Drummond-Barbosa, D., 2015. Ecdysone response gene E78 controls ovarian germline stem cell niche formation and follicle survival in Drosophila. Dev. Biol. 400 (1), 33–42. https://doi.org/10.1016/j.ydbio.2015.01.013.
Ables, E.T., Drummond-Barbosa, D., 2010. The steroid hormone ecdysone functions with intrinsic chromatin remodeling factors to control female germline stem cells in Drosophila. Cell Stem Cell 7, 581–592.
Ables, E.T., Drummond-Barbosa, D., 2017. Steroid hormones and the physiological regulation of tissue-resident stem cells: lessons from the Drosophila ovary. Curr. Stem Cell Rep. 3, 9–18.
Allworth, A.E., Albertini, D.F., 1993. Meiotic maturation in cultured bovine oocytes is accompanied by remodeling of the cumulus cell cytoskeleton. Dev. Biol. 158, 101–112.
Aquila, S., De Amicis, F., 2014. Steroid receptors and their ligands: effects on male gamete functions. Exp. Cell Res. 328, 303–313.
Armstrong, A.R., Drummond-Barbosa, D., 2018. Insulin signaling acts in adult adipocytes via GSK-3beta and independently of FOXO to control Drosophila female germline stem cell numbers. Dev. Biol. 440, 31–39.
Armstrong, A.R., Laws, K.M., Drummond-Barbosa, D., 2014. Adipocyte amino acid sensing controls adult germline stem cell number via the amino acid response pathway and independently of Target of Rapamycin signaling in Drosophila. Development 141, 4479–4488.
Arrese, E.L., Soulages, J.L., 2010. Insect fat body: energy, metabolism, and regulation. Annu. Rev. Entomol. 55, 207–225.
Ayyaz, A., Li, H., Jasper, H., 2015. Hemocytes control stem cell activity in the Drosophila intestine. Nat. Cell Biol. 17, 736–748.
Billeter, J.C., Atallah, J., Krupp, J.J., Millar, J.G., Levine, J.D., 2009. Specialized cells tag sexual and species identity in Drosophila melanogaster. Nature 461, 987–991.
Buszczak, M., Freeman, M.R., Carlson, J.R., Bender, M., Cooley, L., Segraves, W.A., 1999. Ecdysone response genes govern egg chamber development during mid-oogenesis in Drosophila. Development 126, 4581–4589.
Buszczak, M., Paterno, S., Lighthouse, D., Bachman, J., Planck, J., Owen, S., Skora, A.D., Nystul, T.G., Ohlstein, B., Allen, A., et al., 2007. The carnegie protein trap library: a versatile tool for Drosophila developmental studies. Genetics 175, 1505–1531.
Chertemps, T., Duportets, L., Labeur, C., Ueda, R., Takahashi, K., Saigo, K., Wicker-Thomas, C., 2007. A female-biased expressed elongase involved in long-chain hydrocarbon biosynthesis and courtship behavior in Drosophila melanogaster. Proc. Natl. Acad. Sci. U.S.A. 104, 4273–4278.
Chiang, Y.N., Tan, K.J., Chung, H., Lavrynenko, O., Shevchenko, A., Yew, J.Y., 2016. Steroid hormone signaling is essential for pheromone production and oenocyte survival. PLoS Genet. 12, e1006126.
de Cuevas, M.,S.A., 1998. Morphogenesis of the Drosophila fusome and its implications for oocyte specification. Development 125, 2781–2789.
Droujinine, I.A., Perrimon, N., 2016. Interorgan communication pathways in physiology: focus on Drosophila. Annu. Rev. Genet. 50, 539–570.
Drummond-Barbosa, D., Spradling, A.C., 2001. Stem cells and their progeny respond to nutritional changes during Drosophila oogenesis. Dev. Biol. 231, 265–278.
Duggavathi, R., Volle, D.H., Mataki, C., Antal, M.C., Messaddeq, N., Auwerx, J., Murphy, B.D., Schoonjans, K., 2008. Liver receptor homolog 1 is essential for ovulation. Genes Dev. 22, 1871–1876.
Festuccia, N., Owens, N., Navarro, P., 2017. Esrrb, an estrogen-related receptor involved in early development, pluripotency, and reprogramming. FEBS Lett. 592 (6), 852–877. https://doi.org/10.1002/1873-3468.12826.
Findlay, J.K., Liew, S.H., Simpson, E.R., Korach, K.S., 2010. Estrogen signaling in the regulation of female reproductive functions. Handb. Exp. Pharmacol. 29–35.
Francis, G.A., Fayard, E., Picard, F., Auwerx, J., 2003. Nuclear receptors and the control of metabolism. Annu. Rev. Physiol. 65, 261–311.
Greenspan, L.J., de Cuevas, M., Matunis, E., 2015. Genetics of gonadal stem cell renewal. Annu. Rev. Cell Dev. Biol. 31, 291–315.
Gutierrez, E., Wiggins, D., Fielding, B., Gould, A.P., 2007. Specialized hepatocyte-like cells regulate Drosophila lipid metabolism. Nature 445, 275–280.
Hoshizaki, D.K., Blackburn, T., Price, C., Ghosh, M., Miles, K., Ragucci, M., Sweis, R., 1994. Embryonic fat-cell lineage in Drosophila melanogaster. Development 120, 2489–2499.
Hsu, H.J., Drummond-Barbosa, D., 2009. Insulin levels control female germline stem cell maintenance via the niche in Drosophila. Proc. Natl. Acad. Sci. U.S.A. 106, 1117–1121.
Hsu, H.J., Drummond-Barbosa, D., 2011. Insulin signals control the competence of the Drosophila female germline stem cell niche to respond to Notch ligands. Dev. Biol. 350, 290–300.
Hughes, S.E., Miller, D.E., Miller, A.L., Hawley, R.S., 2018. Female meiosis: synapsis, recombination, and segregation in Drosophila melanogaster. Genetics 208, 875–908.
Jasienska, G., Bribiescas, R.G., Furberg, A.S., Helle, S., Nunez-de la Mora, A., 2017. Human reproduction and health: an evolutionary perspective. Lancet 390, 510–520.
King-Jones, K., Thummel, C.S., 2005. Nuclear receptors–a perspective from Drosophila. Nat. Rev. Genet. 6, 311–323.
Konig, A., Shcherbata, H.R., 2015. Soma influences GSC progeny differentiation via the cell adhesion-mediated steroid-let-7-Wingless signaling cascade that regulates chromatin dynamics. Biol. Open 4, 285–300.

Konig, A., Yatsenko, A.S., Weiss, M., Shcherbata, H.R., 2011. Ecdysteroids affect Drosophila ovarian stem cell niche formation and early germline differentiation. EMBO J. 30, 1549–1562.
LaFever, L., Drummond-Barbosa, D., 2005. Direct control of germline stem cell division and cyst growth by neural insulin in Drosophila. Science 309, 1071–1073.
Laws, K.M., Drummond-Barbosa, D., 2015. Genetic mosaic analysis of stem cell lineages in the Drosophila ovary. Methods Mol. Biol. 1328, 57–72. https://doi.org/10.1007/978-1-4939-2851-4_4.
Laws, K.M., Drummond-Barbosa, D., 2016. AMP-activated protein kinase has diet-dependent and -independent roles in Drosophila oogenesis. Dev. Biol. 420, 90–99.
Laws, K.M., Drummond-Barbosa, D., 2017. Control of germline stem cell lineages by diet and physiology. Results Probl. Cell Differ. 59, 67–99.
Laws, K.M., Sampson, L.L., Drummond-Barbosa, D., 2015. Insulin-independent role of adiponectin receptor signaling in Drosophila germline stem cell maintenance. Dev. Biol.
Lazareva, A.A., Roman, G., Mattox, W., Hardin, P.E., Dauwalder, B., 2007. A role for the adult fat body in Drosophila male courtship behavior. PLoS Genet. 3, e16.
Li, L., Xie, X., Qin, J., Jeha, G.S., Saha, P.K., Yan, J., Haueter, C.M., Chan, L., Tsai, S.Y., Tsai, M.J., 2009. The nuclear orphan receptor COUP-TFII plays an essential role in adipogenesis, glucose homeostasis, and energy metabolism. Cell Metabol. 9, 77–87.
Liu, Q., Liu, S., Kodama, L., Driscoll, M.R., Wu, M.N., 2012. Two dopaminergic neurons signal to the dorsal fan-shaped body to promote wakefulness in Drosophila. Curr. Biol. : CB 22, 2114–2123.
Maines, J.Z., Stevens, L.M., Tong, X., Stein, D., 2004. Drosophila dMyc is required for ovary cell growth and endoreplication. Development 131, 775–786.
Makki, R., Cinnamon, E., Gould, A.P., 2014. The development and functions of oenocytes. Annu. Rev. Entomol. 59, 405–425.
Maqdasy, S., Baptissart, M., Vega, A., Baron, S., Lobaccaro, J.M., Volle, D.H., 2013. Cholesterol and male fertility: what about orphans and adopted? Mol. Cell. Endocrinol. 368, 30–46.
Matsuoka, S., Armstrong, A.R., Sampson, L.L., Laws, K.M., Drummond-Barbosa, D., 2017. Adipocyte metabolic pathways regulated by diet control the female germline stem cell lineage in Drosophila melanogaster. Genetics 206, 953–971.
McGinnis, L.K., Limback, S.D., Albertini, D.F., 2013. Signaling modalities during oogenesis in mammals. Curr. Top. Dev. Biol. 102, 227–242.
McGuire, S.E., Le, P.T., Osborn, A.J., Matsumoto, K., Davis, R.L., 2003. Spatiotemporal rescue of memory dysfunction in Drosophila. Science 302, 1765–1768.
Mlodzik, M., Hiromi, Y., Weber, U., Goodman, C.S., Rubin, G.M., 1990. The Drosophila seven-up gene, a member of the steroid receptor gene superfamily, controls photoreceptor cell fates. Cell 60, 211–224.
Morris, L.X., Spradling, A.C., 2012. Steroid signaling within Drosophila ovarian epithelial cells sex-specifically modulates early germ cell development and meiotic entry. PLoS One 7, e46109.
Mouzat, K., Baron, S., Marceau, G., Caira, F., Sapin, V., Volle, D.H., Lumbroso, S., Lobaccaro, J.M., 2013. Emerging roles for LXRs and LRH-1 in female reproduction. Mol. Cell. Endocrinol. 368, 47–58.
Murayama, C., Miyazaki, H., Miyamoto, A., Shimizu, T., 2008. Involvement of Ad4BP/SF-1, DAX-1, and COUP-TFII transcription factor on steroid production and luteinization in ovarian theca cells. Mol. Cell. Biochem. 314, 51–58.
Nabel-Rosen, H., Volohonsky, G., Reuveny, A., Zaidel-Bar, R., Volk, T., 2002. Two isoforms of the Drosophila RNA binding protein, how, act in opposing directions to regulate tendon cell differentiation. Dev. Cell 2, 183–193.
Petit, F.G., Jamin, S.P., Kurihara, I., Behringer, R.R., DeMayo, F.J., Tsai, M.J., Tsai, S.Y., 2007. Deletion of the orphan nuclear receptor COUP-TFII in uterus leads to placental deficiency. Proc. Natl. Acad. Sci. U.S.A. 104, 6293–6298.
Regan, J.C., Khericha, M., Dobson, A.J., Bolukbasi, E., Rattanavirotkul, N., Partridge, L., 2016. Sex difference in pathology of the ageing gut mediates the greater response of female lifespan to dietary restriction. eLife 5, e10956.
Rettberg, J.R., Yao, J., Brinton, R.D., 2014. Estrogen: a master regulator of bioenergetic systems in the brain and body. Front. Neuroendocrinol. 35, 8–30.
Schuster, C.M., Davis, G.W., Fetter, R.D., Goodman, C.S., 1996. Genetic dissection of structural and functional components of synaptic plasticity. I. Fasciclin II controls synaptic stabilization and growth. Neuron 17, 641–654.
Sieber, M.H., Spradling, A.C., 2015. Steroid signaling establishes a female metabolic state and regulates SREBP to control oocyte lipid accumulation. Curr. Biol. : CB 25, 993–1004.
Silvestris, E., de Pergola, G., Rosania, R., Loverro, G., 2018. Obesity as disruptor of the female fertility. Reprod. Biol. Endocrinol. 16, 22.
Song, C.H., Lee, H.J., Park, E., Lee, K., 2012. The chicken ovalbumin upstream promoter-transcription factor II negatively regulates the transactivation of androgen receptor in prostate cancer cells. PLoS One 7, e49026.
Song, X., Xie, T., 2002. DE-cadherin-mediated cell adhesion is essential for maintaining somatic stem cells in the Drosophila ovary. Proc. Natl. Acad. Sci. U.S.A. 99, 14813–14818.
Sun, J., Spradling, A.C., 2013. Ovulation in Drosophila is controlled by secretory cells of the female reproductive tract. eLife 2, e00415.
Syed, M.H., Mark, B., Doe, C.Q., 2017. Steroid hormone induction of temporal gene expression in Drosophila brain neuroblasts generates neuronal and glial diversity. eLife 6.
Tang, K., Tsai, S.Y., Tsai, M.J., 2015. COUP-TFs and eye development. Biochim. Biophys. Acta 1849, 201–209.
Thummel, C.S., 2005. Powered by gas–a ligand for a fruit fly nuclear receptor. Cell 122, 151–153.
Weaver, L.N., Drummond-Barbosa, D., 2018. Maintenance of Proper Germline Stem Cell Number Requires Adipocyte Collagen in Adult Drosophila Females. Genetics.
Xie, T., Spradling, A.C., 1998. Decapentaplegic is essential for the maintenance and division of germline stem cells in the Drosophila ovary. Cell 94, 251–260.
Xu, M., Qin, J., Tsai, S.Y., Tsai, M.J., 2015. The role of the orphan nuclear receptor COUP-TFII in tumorigenesis. Acta Pharmacol. Sin. 36, 32–36.
Zheng, W., Horton, C.D., Kim, J., Halvorson, L.M., 2010. The orphan nuclear receptors COUP-TFI and COUP-TFII regulate expression of the gonadotropin LHbeta gene. Mol. Cell. Endocrinol. 330, 59–71.