Current Opinion in Insect Science, Review manuscript

Endocrine regulation of female germline stem cells in the fruit fly *Drosophila melanogaster*

Short title: Endocrine regulation of female germline stem cells

Yuto Yoshinari[1], Yoshitomo Kurogi[2], Tomotsune Ameku[1,5], and Ryusuke Niwa[3,4]

[1] Graduate School of Life and Environmental Sciences, University of Tsukuba, Tennoudai 1-1-1, Tsukuba, Ibaraki 305-8572, Japan

[2] College of Biological Sciences, University of Tsukuba, Tennoudai 1-1-1, Tsukuba, Ibaraki 305-8572, Japan

[3] Faculty of Life and Environmental Sciences, University of Tsukuba, Tennoudai 1-1-1, Tsukuba, Ibaraki 305-8572, Japan

[4] AMED-CREST, Japan Agency for Medical Research and Development, 1-7-1 Otemachi, Chiyoda-ku, Tokyo 100-0004, Japan

[5] Present address: MRC Clinical Sciences Centre, Imperial College London, Hammersmith Campus, Du Cane Road, London W12 0NN, UK

Correspondence: Ryusuke Niwa (ryusuke-niwa@umin.ac.jp)

Abstract (100-120 words)

Germline stem cells (GSCs) are critical for the generation of sperms and eggs in most animals including the fruit fly *Drosophila melanogaster*. It is well known that self-renewal and differentiation of female *D. melanogaster* GSCs are regulated by local niche signals. However, little is known about whether and how the GSC number is regulated by paracrine signals. In the last decade, however, multiple humoral factors, including insulin and ecdysteroids, have been recognized as key regulators of female *D. melanogaster* GSCs. This review paper summarizes the role of humoral factors in female *D. melanogaster* GSC proliferation and maintenance in response to internal and external conditions, such as nutrients, mating stimuli, and aging.

Highlights (≤85 characters including space for each)

- *Drosophila* female germline stem cells (GSCs) are regulated by humoral factors
- Insulin and steroid hormones regulate proliferation and maintenance of female GSCs
- Nutrients, mating and aging affect female GSCs through multiple hormones

Introduction

Regulation of stem cell proliferation and maintenance is a key factor accompanying a number of developmental and physiological events in multicellular organisms [1,2]. Female germline stem cells (GSCs) in the fruit fly *Drosophila melanogaster* are one of the best understood stem cell types and provide an excellent model for study of the regulation of stem cell proliferation and maintenance *in vivo*. It is well known that *D. melanogaster* GSCs reside in their special microenvironment, called the stem cell niche. The niche produces some factors that regulate the balance between GSC self-renewal and differentiation. For example, the bone morphogenetic protein (BMP) ligands Decapentaplegic (Dpp) and Glass bottom boat (Gbb) produced from the niche cell directory activate BMP receptors in GSCs, leading to the repression of the differentiation inducer, *bag-of-marbles (bam)* [3••,4,5,6••]. GSCs that leave the niche cells can escape from BMP signaling, upregulate *bam* expression, and undergo the differentiation process. Many studies have reported that the niche and the paracrine signals from the niche described above have an important role in balancing the self-renewal and differentiation of GSCs. In contrast, how tissue-extrinsic hormonal signals systemically act on stem cells, particularly in response to environmental cues and changes in physiological status is not yet clearly understood in detail.

In the last decade, however, many studies have revealed that several insect hormones play indispensable roles in regulating GSC behavior. In this short review, we summarize the current knowledge of the roles of hormonal and systemic signals, such as steroid hormones and insulins, in GSC proliferation and maintenance in response to external and internal conditions, such as nutritional availability, mating stimuli, and aging.

Roles of insulin and other humoral signaling in diet-dependent GSC maintenance

Drosophila insulin-like peptides (DILPs) are the first example of the hormonal regulation involved in nutrient-dependent GSC maintenance [7]. On a nutrition-rich diet, each female lays an average of over 80 eggs per day. In contrast, egg production, as well as the division rate of GSCs are significantly suppressed when the female is fed a nutrition-deficient diet [8••]. Dr. Daniela Drummond-Barbosa's group clearly demonstrated that brain DILP-producing cells are required for the ovarian response to nutrition. Moreover, DILPs are received in the ovary via a single DILP receptor called the Insulin receptor (InR). InR-dependent signaling not only directly acts on GSCs to stimulate their proliferation and self-renewal, but also indirectly regulates GSCs by increasing the niche cell number and adhesion of GSC to the niche cells [7,8••,9•,10]. Since DILP secretion from brain insulin-producing cells is tightly regulated in response to sufficient nutrients [11] , it is quite likely that nutritional input can modify female GSC activity via the insulin signaling pathway. More recently, it is reported that diet reversibly controls escort cell-germ cell interaction via DILP-InR signaling [12].

While DILPs play an indispensable role in diet-mediated GSC proliferation in female *D. melanogaster*, there are other humoral signals regulating GSC number in response to nutrition [13•]. For example, DILPs specifically control the G2 phase of the GSC cell cycle, while the G1 phase appears to be regulated by a separate diet mediator [10]. Since a recent GFP-trap screen has found many uncharacterized candidate genes encoding diet-regulating proteins [14], future studies will reveal new candidates involved in diet-dependent GSC maintenance. Furthermore, a recent study shows that diet-regulated signaling and metabolic pathways in adipocytes have a significant role in GSC maintenance in a DILP-independent manner [15••,16,17]. Interestingly, enzymes catalyzing fatty acid oxidation and phosphatidylethanolamine synthesis in

adipocytes regulate GSC maintenance, whereas lipid and iron transport from adipocytes controls the GSC number [15••]. These findings suggest that not only peptides (such as DILPs), but also metabolic products, originating from the diet and produced by internal organs, affect GSC regulation in a product-specific manner. Since all metabolites circulating in the fly body are not produced in adipocytes, metabolites from other tissues may have other roles in GSC regulation.

Ecdysteroid signaling and its role in GSC maintenance

About 5 years after the discovery of DILP-mediated GSC maintenance [7], it was reported that a signaling pathway of the principal insect steroid hormones, ecdysteroids, are indispensable for GSC maintenance in female *D. melanogaster* [18••,19,20•]. While ecdysteroids are best known to control molting and metamorphosis during development [21], this steroid hormone also has a significant impact on adult physiology [22–24]. These studies [18••,19] have demonstrated that the active form of ecdysteroids, 20-hydroxyecdysone (20E), its two nuclear hormone receptors coded by *Ecdysone Receptor* (*EcR*) and *ultraspiracle* (*usp*), and downstream epigenetic machinery have a significant impact on GSCs, controlling GSC maintenance during aging by affecting BMP signaling in GSCs. The downstream targets of EcR/USP, including E74 and E78, are also involved in regulating GSC maintenance [18••,25]. In addition, a genetic mosaic screen has further identified a bunch of ecdysteroid-responsive genes regulating GSC maintenance and other oogenesis processes [26]. Moreover, our group demonstrates that the biosynthesis of ecdysteroids in the ovary is essential for GSC proliferation and maintenance. For example, knockdown of the ecdysteroidogenic enzyme gene *neverland* (*nvd*) impairs GSC proliferation and maintenance [27•,28].

Ecdysteroid signaling is also involved in the control of early germline differentiation. Ecdysteroids are necessary for the establishment of the identity of escort cells, which coordinate GSC differentiation via somatic-germ cell communication [19]. The EcR/USP complex and its soma-specific co-activator and co-repressor, Taiman and Abrupt, respectively, regulate the niche establishment [19]. These ecdysteroid-signaling components and *let-7* microRNA form a positive feedback loop to modify the Wnt/β-catenin signaling strength in the germline cells, leading to their normal development [29].

While the adult ovary in female *D. melanogaster* is well known to biosynthesize ecdysteroids [23], it is still unclear which tissue is responsible for ecdysteroid biosynthesis in adult male flies [22,30]. Nevertheless, a recent interesting study reveals that EcR/USP and its downstream genes *ftz-f1* and *E75* are required for stem cell maintenance in the testes [31]. Therefore, it is likely that both male and female GSCs share the similar ecdysteroid-dependent mechanisms to regulate their self-renewal and differentiation.

It is also noteworthy that neuronal ecdysteroid signaling controls female feeding rates and nutritional state of the whole body [32]. Thus, ecdysteroids not only directly act on the ovary to control GSC maintenance, but might also indirectly influence GSCs in a diet-dependent manner.

Ecdysoteroids and mating-induced GSC proliferation

While ecdysteoids are important for GSC regulation, it was still unclear what kind of external conditions were required for ecdysteroid signaling to regulate GSCs. In 2016, our group succeeded in partly answering this question, demonstrating that mating stimuli induce GSC proliferation in an ecdysteroid-dependent manner in female *D.*

melanogaster [27•]. We showed that the mated female retains more GSCs compared to the virgin female, and that mating enhances the division of GSCs. Mating increases the ovarian 20E titer due to upregulation of the transcription of some ecdysteroidgenic genes [27• ,28]. The post-mating GSC proliferation is disrupted by *EcR or nvd* knockdown in ovarian somatic cells, especially escort cells, but *EcR* knockdown in germ cells had no effect.

The mating-induced GSC proliferation is under the control of Sex peptide (SP), a male seminal fluid protein which is produced from the male accessory gland [33]. Sex peptide is received by its specific receptor, the Sex peptide receptor (SPR), in a small number of SPR-positive sensory neurons (SPSNs), which innervate the uterine lumen and send afferent processes into the tip of the abdominal ganglion [34•,35 •,36,37]. The SP-SPR signaling in the SPSNs is required for post-mating responses such as increasing rejection behaviors to further copulation, feeding, ovulation, and egg-laying.

Since SPSNs do not directly innervate into the ovary, a question to be addressed is how the SP-SPR signal eventually transmits the mating information to GSCs. Unknown neuronal and/or endocrine systems downstream of SPSNs would contribute to the upregulation of ovarian ecdysteroid biosynthesis to regulate GSC proliferation. Interestingly, some octopaminergic neurons, whose cell bodies are located at the tip of the abdominal ganglion, directly project to the ovary and regulate the oviposition [38]. It is intriguing to examine whether the mating signal from SPSNs is transmitted to the octopaminergic neurons and is required for mating-induced GSC proliferation.

Besides ecdysteroids, it is noteworthy that the hemolymph level of Juvenile hormone (JH), another important insect hormone, is triggered by mating [39].

166 Intriguingly, the mating-induced increase of JH titer is also under the control of SP
167 signaling, similar to mating-induced ecdysteroid elevation [39,40]. Moreover, JH
168 signaling promotes gut remodeling to sustain egg production and also suppresses the
169 immune system after mating [39,41,42]. However, disruption of the JH biosynthesis
170 enzyme (JH acid *O*-methyltransferase) in the corpus allatum or JH receptors
171 (Methoprene-tolerant and Germ cell-expressed) in ovarian somatic cells have no
172 effect on mating-induced GSC proliferation, suggesting that JH signaling is not
173 required for mating-induced GSC proliferation [27•]. Thus, while the increase in JH
174 titer is essential for the mating-induced morphological and physiological changes
175 after mating, it seems unlikely that JH has a major role in mating-induced GSC
176 proliferation in *D. melanogaster*.
177
178 **Aging and GSC maintenance**
179 In general, the proliferation and maintenance of stem cells are strongly influenced by
180 animal aging [43]. In female *D. melanogaster*, aging leads to a gradual loss of GSCs and
181 niche cells, and governs the decrease of the BMP level and E-cadherin-mediated GSC-
182 niche adhesion [44•]. As a consequence, GSCs in aged females exhibit a prolonged G2
183 phase and a delay at the S phase of the cell cycle [10,45•]. At the organismal level,
184 aged female flies exhibit decreases in feeding amounts [46] and insulin production [9
185 •], which also potentially affect GSC maintenance. In addition, aging may also
186 attenuate ecdysteroid synthesis, as *InR* mutants are defective in terms of ecdysteroid
187 synthesis [47]; conversely, ecdysteroid signaling affects the aging process [48].
188 Therefore, nutrition, DILPs, and ecdysteroids mutually interact with each other to
189 influence age-associated changes of GSC maintenance.
190 DNA damage and Reactive oxygen species (ROS), the by-products of

oxidative energy metabolism that have long been suggested as the main causes of aging, accumulate within aging GSCs. Aging GSCs show a decreased regenerative potential, which results in a defective ability to recover from DNA damage [45•]. The regenerative potential is regulated by two key components of the insulin signaling pathway: *foxo* and *Tor (Target of rapamycin)*. In young flies, Foxo represses Tor activity, pushing the GSCs into quiescent states in response to radiation stress. After the stress, Foxo is deactivated and then Tor overcomes the action of Foxo, followed by the resumption of GSC division and regeneration. On the other hand, in old flies, quiescent GSCs cannot reenter the cell cycle due to over activation of Foxo, leading to the loss of GSCs and differentiating cystoblasts [49••]. Aging-related GSC cell cycle collapse is regulated by the reduction of insulin signaling and ecdysteroid signaling, i.e. indeed due to DNA damage (ROS production). Furthermore, most surprisingly, the lack of cell cycle progression capacity in aging GSCs after ionizing radiation can indeed be rescued by loss of *foxo* [49••], indicating an unexpected "positive" role of insulin signaling in the youth of GSC maintenance.

Aging gradually reduces the number and division ability of GSCs. However, it is still unclear whether aged females can undergo post-mating GSC proliferation. Post-mating increase in GSC number is observed in a 3-week-old female that experienced at least-2 time-mating-induced GSC proliferation [27•]. The rate of increase in GSC number gradually reduced along with aging, suggesting that the female lost post-mating GSC proliferation ability with aging. Aging, diet, and hormones are highly interactive and closely related; next-generation studies will shed light on how these components change adult physiology and fertility.

Conclusion

As described above, the accumulated evidence has shown that DILPs and ecdysteroids coordinately regulate GSC proliferation and maintenance by acting on both ovarian somatic cells and germ cells dependently, in the context of nutrition, mating, and aging (Fig. 1). However, many interesting questions still remain to be answered. Firstly, we need to investigate whether and how humoral factors other than DILPs and ecdysteroids influence GSC proliferation and maintenance. We recently found that a gut-derived peptide hormone directly acts on the ovary to positively regulate mating-induced GSC proliferation in female *D. melanogaster* (T.A., Y.Y., R.N., unpublished). Our transgenic RNAi screen also revealed the existence of additional secreted factors and their receptors, which seem to be involved in mating-induced GSC proliferation (Y.Y., Y.K., T.A., R.N., unpublished). We expect that a complex of humoral factor networks must exist and possibly reflect some external environmental conditions to properly regulate GSC activity. Secondly, which cell types of the niche cells, such as cap cells and escort cells, receive each of the many types of humoral factors, and how multiple signals are integrated to regulate GSC maintenance have not yet been fully understood. Elucidation of the cross-talk among multiple signaling pathways in GSCs and niche cells would be very important for future studies. Finally, whether a part of the identified humoral mechanisms in female *D. melanogaster* is functionally conserved and plays essential roles in regulating GSC maintenance and fertility in other animals including mammals must be examined.

Acknowledgements

Y.Y and T.A. were recipients of the fellowship from the Japan Society for the Promotion of Science. R.N. is supported by a grant from AMED-CREST, AMED.

References

1. Fuchs E, Tumbar T, Guasch G: **Socializing with the Neighbors: Stem Cells and Their Niche**. *Cell* 2004, **116**:769–778.
2. Daley GQ: **Stem cells and the evolving notion of cellular identity.** *Philos Trans R Soc Lond B Biol Sci* 2015, **370**:20140376.
3. Xie T, Spradling AC: ***decapentaplegic* Is Essential for the Maintenance and Division of Germline Stem Cells in the *Drosophila* Ovary**. *Cell* 1998, **94**:251–260.

•• This epoch-making article provides the first evidence showing that Dpp directly regulates the proliferation and maintenance of the adjacent GSCs.

4. Spradling A, Drummond-Barbosa D, Kai T: **Stem cells find their niche**. *Nat 2001 4146859* 2001,
5. Chen D, McKearin D: **Dpp Signaling Silences *bam* Transcription Directly to Establish Asymmetric Divisions of Germline Stem Cells**. *Curr Biol* 2003, **13**:1786–1791.
6. Xie T, Spradling AC: **A niche maintaining germ line stem cells in the *Drosophila* ovary.** *Science* 2000, **290**:328–30.

•• This is the first study identifying the GSC niche. The authors have revealed that cap cells and terminal filament cells acts as a GSC niche, as a loss of these cells direct the loss of GSCs. This paper is also the first proof of the concept of the stem cell niche.

7. LaFever L, Drummond-Barbosa D: **Direct control of germline stem cell division and cyst growth by neural insulin in *Drosophila*.** *Science* 2005, **309**:1071–3.

8. Drummond-Barbosa D, Spradling AC: **Stem Cells and Their Progeny Respond to Nutritional Changes during *Drosophila* Oogenesis**. *Dev Biol* 2001, **231**:265–278.

•• This article is the first study showing that oogenesis and stem cell behavior are regulated by environmental factors. Authors have shown the importance of insulin signaling in oogenesis and proposed the similarity between insulin-dependent growth regulation in *D. melanogaster* and vertebrates.

9. Hsu H-J, Drummond-Barbosa D: **Insulin levels control female germline stem cell maintenance via the niche in *Drosophila*.** *Proc Natl Acad Sci U S A* 2009, **106**:1117–21.

• This study demonstrates that systemic insulin signal and aging regulate GSC maintenance via the niche. In this article, the authors have shown that insulin signaling and aging affect niche cell number and niche-GSC adhesion. In addition, they have proposed that the proliferation decline of GSCs in aged females results from reduced feeding and insulin signaling.

10. Hsu H-J, LaFever L, Drummond-Barbosa D: **Diet controls normal and tumorous germline stem cells via insulin-dependent and -independent mechanisms in *Drosophila***. *Dev Biol* 2008, **313**:700–712.

11. Ikeya T, Galic M, Belawat P, Nairz K, Hafen E: **Nutrient-dependent expression of insulin-like peptides from neuroendocrine cells in the CNS contributes to growth regulation in *Drosophila*.** *Curr Biol* 2002, **12**:1293–300.

12. Su Y-H, Rastegri E, Kao S-H, Lai C-M, Lin K-Y, Liao H-Y, Wang M-H, Hsu H-J: **Diet regulates membrane extension and survival of niche escort cells for**

germline homeostasis via insulin signaling. *Development* 2018, **145**:dev159186.

13. Laws KM, Drummond-Barbosa D: **Control of Germline Stem Cell Lineages by Diet and Physiology**. In *Results and problems in cell differentiation*. . 2017:67–99.

• Comprehensive review of diet-dependent GSC regulation in *D. melanogaster*, *Caenorhabditis elegans*, and mammals. This review illustrates that GSC lineages are controlled by highly conserved diet-dependent pathways.

14. Hsu HJ, Drummond-Barbosa D: **A visual screen for diet-regulated proteins in the *Drosophila* ovary using GFP protein trap lines**. *Gene Expr Patterns* 2017, **23–24**:13–21.

15. Matsuoka S, Armstrong AR, Sampson LL, Laws KM, Drummond-Barbosa D: **Adipocyte Metabolic Pathways Regulated by Diet Control the Female Germline Stem Cell Lineage in *Drosophila melanogaster*.** *Genetics* 2017, **206**:953–971.

•• The authors have utilized an unbiased proteomic approach and successfully identified a number of metabolic pathways altered by dietary-changes in the female fat body. Surprisingly, they have shown that metabolites from the fat body are necessary for GSC maintenance. These data suggest that nutrient transport from the organs other than the ovary controls GSC proliferation.

16. Armstrong AR, Drummond-Barbosa D: **Insulin signaling acts in adult adipocytes via GSK-3β and independently of FOXO to control *Drosophila* female germline stem cell numbers**. *Dev Biol* 2018,

doi:10.1016/J.YDBIO.2018.04.028.

17. Laws KM, Drummond-Barbosa D: **AMP-activated protein kinase has diet-dependent and -independent roles in *Drosophila* oogenesis**. *Dev Biol* 2016, **420**:90–99.

18. Ables ET, Drummond-Barbosa D: **The Steroid Hormone Ecdysone Functions with Intrinsic Chromatin Remodeling Factors to Control Female Germline Stem Cells in *Drosophila***. *Cell Stem Cell* 2010, **7**:581–592.

•• This is the first study reporting that ecdysteroids play essential roles in adult female GSCs. The authors provide evidence showing that ecdysteroids promote GSC self-renewal independently from insulin signaling and interact with the NURF chromatin-remodeling complex to maintain GSCs.

19. König A, Yatsenko AS, Weiss M, Shcherbata HR: **Ecdysteroids affect *Drosophila* ovarian stem cell niche formation and early germline differentiation.** *EMBO J* 2011, **30**:1549–62.

20. Ables ET, Drummond-Barbosa D: **Steroid Hormones and the Physiological Regulation of Tissue-Resident Stem Cells: Lessons from the *Drosophila* Ovary**. *Curr Stem Cell Reports* 2017, **3**:9–18.

• This is the best comprehensive review describing the roles of ecdysteroid signaling in the *D. melanogaster* GSCs.

21. Niwa YS, Niwa R: **Transcriptional regulation of insect steroid hormone biosynthesis and its role in controlling timing of molting and metamorphosis**. *Dev Growth Differ* 2016, **58**:94–105.

22. Ishimoto H, Kitamoto T: **Beyond molting--roles of the steroid molting**

hormone ecdysone in regulation of memory and sleep in adult *Drosophila*. *Fly (Austin)* 2011, **5**:215–220.

23. Uryu O, Ameku T, Niwa R: **Recent progress in understanding the role of ecdysteroids in adult insects: Germline development and circadian clock in the fruit fly *Drosophila melanogaster***. *Zool Lett* 2015, **1**:32.

24. Belles X, Piulachs MD: **Ecdysone signalling and ovarian development in insects: from stem cells to ovarian follicle formation**. *Biochim Biophys Acta - Gene Regul Mech* 2014, **1849**:181–6.

25. Ables ET, Bois KE, Garcia CA, Drummond-Barbosa D: **Ecdysone response gene *E78* controls ovarian germline stem cell niche formation and follicle survival in *Drosophila***. *Dev Biol* 2015, **400**:33–42.

26. Ables ET, Hwang GH, Finger DS, Hinnant TD, Drummond-Barbosa D: **A Genetic Mosaic Screen Reveals Ecdysone-Responsive Genes Regulating *Drosophila* Oogenesis**. *G3: Genes|Genomes|Genetics* 2016, **6**:2629–2642.

27. Ameku T, Niwa R: **Mating-Induced Increase in Germline Stem Cells via the Neuroendocrine System in Female *Drosophila***. *PLOS Genet* 2016, **12**:e1006123.

• We have revealed that mating stimuli promotes GSC proliferation by pushing the GSC cell cycle. Moreover, we have shown that ecdysteroid signaling is necessary for post-mating GSC proliferation and provides evidence that mating input promotes ovarian ecdysteroiod biosynthesis via neuronal signaling.

28. Ameku T, Yoshinari Y, Fukuda R, Niwa R: **Ovarian ecdysteroid biosynthesis and female germline stem cells.** *Fly* 2017, **11**:185–193.

29. König A, Shcherbata HR: **Soma influences GSC progeny differentiation via**

the cell adhesion-mediated steroid-*let-7*-Wingless signaling cascade that regulates chromatin dynamics. *Biol Open* 2015, **4**:285–300.

30. Ishimoto H, Sakai T, Kitamoto T: **Ecdysone signaling regulates the formation of long-term courtship memory in adult *Drosophila melanogaster*.** *Proc Natl Acad Sci U S A* 2009, **106**:6381–6.

31. Li Y, Ma Q, Cherry CM, Matunis EL: **Steroid signaling promotes stem cell maintenance in the *Drosophila* testis.** *Dev Biol* 2014, **394**:129–141.

32. Sieber MH, Spradling AC: **Steroid Signaling Establishes a Female Metabolic State and Regulates SREBP to Control Oocyte Lipid Accumulation**. *Curr Biol* 2015, **25**:993–1004.

33. Chen PS, Stumm-Zollinger E, Aigaki T, Balmer J, Bienz M, Böhlen P: **A male accessory gland peptide that regulates reproductive behavior of female *D. melanogaster***. *Cell* 1988, **54**:291–298.

34. Yapici N, Kim Y-J, Ribeiro C, Dickson BJ: **A receptor that mediates the post-mating switch in *Drosophila* reproductive behaviour.** *Nature* 2008, **451**:33–37.

• The authors have identified that the SPR is responsible for the female post-mating response. This article has long influenced many other following studies of neuronal circuits that regulate several post-mating responses.

35. Krupp JJ, Levine JD: **Neural Circuits: Anatomy of a Sexual Behavior**. *Curr Biol* 2014, **24**:R327–R329.

• A comprehensive review of the neuronal circuit of the post-mating response in female *D. melanogaster*.

36. Yang C, Rumpf S, Xiang Y, Gordon MD, Song W, Jan LY, Jan Y-N: **Control of**

the Postmating Behavioral Switch in *Drosophila* Females by Internal Sensory Neurons. *Neuron* 2009, **61**:519–526.

37. Häsemeyer M, Yapici N, Heberlein U, Dickson BJ: **Sensory Neurons in the *Drosophila* Genital Tract Regulate Female Reproductive Behavior**. *Neuron* 2009, **61**:511–518.

38. Rezával C, Nojima T, Neville MC, Lin AC, Goodwin SF: **Sexually dimorphic octopaminergic neurons modulate female postmating behaviors in *Drosophila*.** *Curr Biol* 2014, **24**:725–30.

39. Schwenke RA, Lazzaro BP: **Juvenile Hormone Suppresses Resistance to Infection in Mated Female *Drosophila melanogaster***. *Curr Biol* 2017, **27**:596–601.

40. Sugime Y, Watanabe D, Yasuno Y, Shinada T, Miura T, Tanaka NK: **Upregulation of Juvenile Hormone Titers in Female *Drosophila melanogaster* Through Mating Experiences and Host Food Occupied by Eggs and Larvae**. *Zoolog Sci* 2017, **34**:52–57.

41. Rus F, Flatt T, Tong M, Aggarwal K, Okuda K, Kleino A, Yates E, Tatar M, Silverman N: **Ecdysone triggered PGRP-LC expression controls *Drosophila* innate immunity.** *EMBO J* 2013, **32**:1626–38.

42. Reiff T, Jacobson J, Cognigni P, Antonello Z, Ballesta E, Tan KJ, Yew JY, Dominguez M, Miguel-Aliaga I: **Endocrine remodelling of the adult intestine sustains reproduction in *Drosophila***. *Elife* 2015, **4**:e06930.

43. Ahmed ASI, Sheng MHC, Wasnik S, Baylink DJ, Kin-Hing William Lau KHW: **Effect of aging on stem cells**. *World J Exp Med* 2017, **7**:1–10.

44. Pan L, Chen S, Weng C, Call G, Zhu D, Tang H, Zhang N, Xie T: **Stem Cell Aging Is Controlled Both Intrinsically and Extrinsically in the *Drosophila* Ovary**. *Cell*

Stem Cell 2007, **1**:458–469.

• This article shows that aging affects the physiology of both the niche and GSCs, resulting in a gradual loss of GSCs. Especially, reduction of ROS in aged GSCs could rescue the loss of GSCs, suggesting that aging accumulates ROS in GSCs and affects their maintenance.

45. Kao S-H, Tseng C-Y, Wan C-L, Su Y-H, Hsieh C-C, Pi H, Hsu H-J: **Aging and insulin signaling differentially control normal and tumorous germline stem cells**. *Aging Cell* 2015, **14**:25–34.

• The authors have elucidated how the aging alters the cell cycle in GSCs. According to this article, aged GSCs showed a delay in the S phase, but not in the M or G2 phase. While deprivation of Insulin signaling in GSCs causes the delay in the G2 phase, they have suggested that aging influences the GSC cell cycle in an insulin-independent manner.

46. Guarente L, Kenyon C: **Genetic pathways that regulate ageing in model organisms**. *Nature* 2000, **408**:255–262.

47. Tu M-P, Yin C-M, Tatar M: **Impaired ovarian ecdysone synthesis of *Drosophila melanogaster insulin receptor* mutants**. *Aging Cell* 2002, **1**:158–160.

48. Gáliková M, Klepsatel P, Senti G, Flatt T: **Steroid hormone regulation of *C. elegans* and *Drosophila* aging and life history**. *Exp Gerontol* 2011, **46**:141–147.

49. Artoni F, Kreipke RE, Palmeira O, Dixon C, Goldberg Z, Ruohola-Baker H: **Loss of foxo rescues stem cell aging in *Drosophila* germ line**. *Elife* 2017, **6**:e27842.

•• The authors have used ionizing radiation to injure GSCs and have revealed that aged GSCs cannot re-enter the cell cycle after these injuries. Surprisingly, the aged

441 GSC phenotype is not due to DNA damage, but caused by the overactivation of Foxo.
442 The authors have proposed that the balance between Foxo and Tor is crucial for the
443 regeneration of the damaged GSCs.
444

Figure Legend

Figure 1. A cartoon illustrating the mechanisms that regulate the proliferation and maintenance of germline stem cells (GSCs) in female *D. melanogaster*. GSCs are affected by insulin, ecdysteroids, and several metabolites from the fat body and the gut. These hormones and metabolites respond to internal and external conditions, such as nutrition, mating stimuli, and aging. While mating activates juvenile hormone (JH) biosynthesis and there are interactions between ecdysteroids and JHs, it is unclear whether JHs play a direct role in regulating GSC proliferation and/or maintenance.

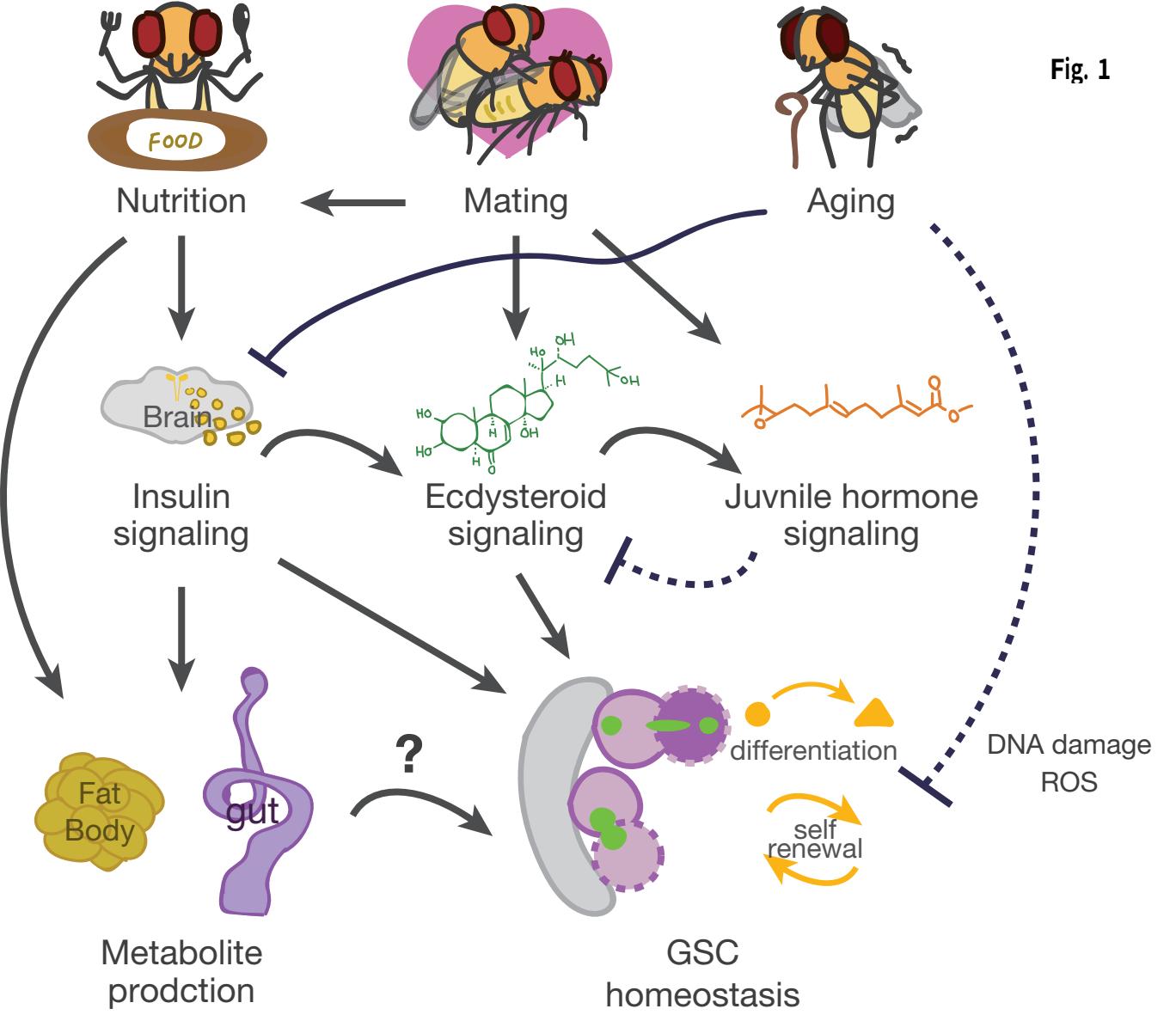
FOOD
Nutrition
Mating
Aging
Brain
Insulin signaling
Ecdysteroid signaling
Juvnile hormone signaling
Fat Body
gut
?
differentiation
self renewal
DNA damage ROS
Metabolite prodction
GSC homeostasis

Fig. 1