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Chapter 2

The Regulation of Germline Stem Cells and Their Neighbouring Somatic Cells in the Fruit Fly (*Drosophila melanogaster*)

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Additional information is available at the end of the chapter

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Abstract

The *Drosophila* germline stem cells (GSCs) remain as one of the most well-understood adult stem cells. The number of stem cells that self-renews and differentiates must be tightly controlled to maintain tissue homeostasis. The *Drosophila* GSCs are maintained by local signals emanated from the niche, which is composed of the surrounding somatic cells. Notably, GSC homeostasis is also known to be influenced by systemic signals and external stimuli. The *Drosophila* hormone ecdysone and its signalling cascade were found to regulate GSC homeostasis. The insulin signalling pathway as well as nutrient availability can also regulate GSC number. Furthermore, neuronal sex peptide signalling induced in female flies after mating was shown to increase GSC number. Hence, the *Drosophila* GSC system serves as a useful model towards understanding the mammalian stem cells. Compared with the mammalian stem cell models, the *Drosophila* GSC system is anatomically simpler where stem cells can be easily identified, imaged and manipulated genetically. Nevertheless, recent findings have facilitated our understanding into how GSCs and their neighbouring somatic cells sense and respond to changes in a variety of local, systemic and external stimuli.

Keywords: *Drosophila*, germline stem cells (GSCs), stem cell niche, nutrients, insulin signalling, insulin-like peptides (Ilps), ecdysone, sex peptide (SP), mating

1. Introduction

Germline stem cells (GSCs) are adult stem cells that give rise to gametes. Sperm and egg production is an important process, whereby genetic information is transferred to the next

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generation by GSCs. Hence, GSC self-renewal and differentiation must be tightly regulated to ensure a homeostasis for a healthy egg and sperm production. The GSCs in both female and male *Drosophila* are one of the best-understood adult stem cells by far. The *Drosophila* is a useful in vivo model to study how GSCs and their surrounding somatic cells are co-regulated. Short-range signals from GSC niche, systemic signals and external stimuli aid to determine the fate of GSCs. Upon these signals, GSCs undergo asymmetric divisions, whereby they self-renew to produce one cell that remains as a stem cell and another daughter cell that is displaced away from the niche and is fated to differentiate. The daughter cell maintains its stemness because it stays in direct contact to and receives immediate signals from the niche, whereas the other daughter cell receives low/no signals because it is further away from the niche and, hence, is programmed to differentiate. Under certain circumstances such as genetic mutation or impaired internal or external signals, GSCs become poorly regulated, leading to over-proliferation of GSCs (GSC tumours) or precocious differentiation of the GSCs (GSC loss). Both conditions are unfavourable for the organism as they can cause infertility and hence impaired reproduction and endangering the species population. In this chapter, a brief description on the *Drosophila*'s ovary and testis will be covered. In addition, the molecular mechanisms underlying GSC maintenance by short-range signals produced from the niche and long-range signals such as hormones and insulin-like peptides produced from the brain or external stimuli such as nutrient availability and mating will be discussed.

2. The *Drosophila* ovary and testis germ cell system

2.1. The *Drosophila* ovary system

The female *Drosophila* has a pair of ovaries each of which consists of about 17 repeated units called the ovarioles. The ovarioles are further subdivided into two main parts with the anterior region being the germarium, and a series of gradually differentiated egg chambers are positioned at the posterior end. The germarium is where all the stem cell activity takes place, and there are two types of stem cells present in the germarium: GSCs which eventually generate gametes and somatic stem cells (SSCs, also referred to as follicle stem cells (FSCs)). The apical tip of the germarium consists of approximately 8–10 terminal filament (TF) cells followed by 5–7 cap cells (CCs) at the base of TF cells which are directly in contact with 2–3 GSCs [1–3]. The cap, TF cells and ECs (escort cells that line at the surface of the anterior half of the germarium) provide the stem cell niche for the regulation of GSCs by short-range signals. The GSCs divide asymmetrically to produce one daughter cell that stays in contact with the CCs and hence maintains its stem cell identity and another daughter cell that moves away from the niche to differentiate called a cystoblast (CB) [4, 5]. Loss of GSCs can signal the neighbouring GSCs to go through symmetric division, producing two daughter cells that both retain GSC fate and stay in contact with CCs; hence, this mechanism replaces the unoccupied niche space [6]. The GSCs stay connected to the CCs through adherens junction and loss of *adherens junction* can lead to GSCs moving away from the niche to differentiate [7]. GSCs and its differentiated progeny can be recognized by the presence of fusome, which

are germ-specific organelles rich in membrane skeletal proteins like the α-spectrin and hu-li tai shao (Hts) [8, 9]. The fusome appears round in shape (also referred to as spectrosome) in GSCs and cystoblasts (CBs) but is branched in the CB progenies. In the female, the fusome degenerates shortly after the formation of 16-cell cysts [8]. The CBs will undergo synchronous division with incomplete cytokinesis to produce 2-, 4-, 8- and 16-cell cysts. The early cyst cells are encased by long cytoplasmic extension from escort cells (ECs), whereas the late-stage cysts are encased by follicle cells (FCs) produced by the FSCs [10]. The 16-cell cysts surrounded by FCs will bud off from the germarium to produce individual egg chambers [10]. The female GSCs are dominantly regulated by the bone morphogenic protein (BMP) signalling from the niche. In *Drosophila*, the *decapentaplegic* (*dpp*) and *glass-bottom boat* (*gbb*) are ligands for BMP and are mainly expressed in the TF cells and CCs. Loss of *dpp* and *gbb* caused GSCs to dive into differentiation mode, whereas too much of *dpp* leads to over-proliferation of GSCs and forms GSC tumours [11, 12]. The *bag of marbles* (*bam*) and *benign gonial cell neoplasm* (*bgcn*) are essential for CB differentiation [13–15]. CBs lost the ability to differentiate with *bam* loss of function and eventually form germline tumour [11, 14]. On the other hand, overexpression of *bam* caused loss of GSCs as seen in *dpp* loss of function [11, 16]. BMP signalling promotes GSC self-renewal by the mechanism, whereby mothers against *dpp* (Mad) is phosphorylated leading to the formation of Mad and Med complex, which subsequently translocates into the nucleus to bind to *bam* promoter and, hence, represses the transcription of *bam* in GSCs [12]. The GSC niche formed by TF cells, CCs and ECs also expresses P-element-induced wimpy testis (*piwi*), *fs*(1)*Yb* (also known as *Yb*) and *hedgehog* (*hh*), which are required for GSC maintenance [17–19]. *Piwi* and *hh* expressions in the GSC niche require *Yb*. Loss of function of *piwi* and *Yb* in the GSC niche causes GSC exhaustion as seen in *BMP* signalling mutants, whereas overexpression of *piwi* or *Yb* expands GSC number to 2.5-folds, although the increase was not as dramatic as *dpp* overexpression which led to GSC tumour [17, 19]. *Hh* mutation in the GSCs niche affects the GSC population at a lower rate which may suggest that it has a minor role in GSC maintenance [19]. Additionally, Hh signalling activation in ECs promotes germline differentiation [20–22]. Besides that, the Notch signalling controls the formation of GSC niche, whereby elevated *Notch* signalling resulted in increased niche size (CC number) and hence more GSCs; reduced *Notch* signalling resulted in decreased CC number and niche size which in turn reduced the number of GSCs (**Figure 1**) [23].

2.2. The *Drosophila* testis system

The male *Drosophila* has a pair of testes and at the apical tip is where the stem cell niche is housed. The stem cell niche consists of postmitotic hub cells, GSCs and cyst stem cells (CySCs). In the male *Drosophila*, about 6–12 GSCs are arranged in a rosette pattern around a tightly packed cluster of hub cells by adherens junction rich in E-cadherin [24, 25]. Each GSC is encased by a pair of somatic CySCs, which are also in contact with the hub cells by their cytoplasmic extensions such that the distance of the CySC nuclei is further from the hub cells compared to the GSC nuclei [26]. The female and male GSCs have many processes in common; one of them is the asymmetric division of GSCs to produce one daughter cell that self-renews and another that differentiates. By doing so, the male GSCs generate one daughter cell that stays in contact with the hub cells and retains its stem cell identity and another daughter cell that is further away from the hub cells, called gonialblast (GB). The GB is fated for differentiation due to the lack of local signals it receives from the

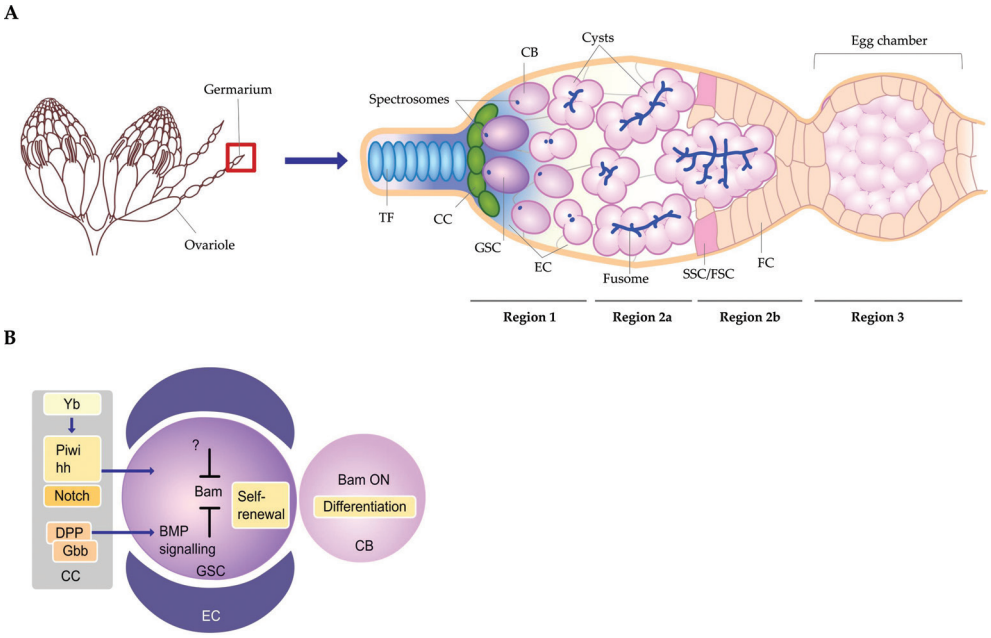


Figure 1. A schematic diagram of the *Drosophila* ovary. (**A**) A pair of *Drosophila* ovaries consisting of several ovarioles each and the zoom in section of the germarium. (**B**) The stem niche of the *Drosophila* ovary consisting of TF, CC and GSC and the signalling pathways that maintain the niche.

niche and is programmed to advance into four rounds of transit-amplifying divisions to generate 2-, 4-, 8- and 16-spermatogonial cells. These 16-spermatogonial cells will then enter the premeiotic S-phase followed by spermatocyte growth and spermatogenesis to produce spermatids and eventually mature sperms [27]. A pair of cyst cells which are progenies of CySCs continue to completely wrap around each GB and its progenies of differentiated spermatogonial cells; cyst cells do not increase in number but only grow to accommodate the expanding spermatogonial cells. CySCs and cyst cells provide protective layer which also isolate germ cells from each other, and only ring canals can connect the spermatogonial cells together [27]. The spectrosomes also appear spherical in the GSCs and GBs but are branched fusome in the differentiated spermatogonial cells. However, unlike the fusome in the ovaries which perishes after mitosis, fusome in the testis continues to develop further through meiosis and spermatid elongation [28].

The male GSCs are also regulated by local signals from the niche to ensure a balanced population of germ cells. The Janus kinase-signal transducers and activators of transcription (JAK-STAT) signalling pathway was the first to be discovered to regulate GSCs in the fly testis. The ligand of the pathway called *Unpaired* (*Upd*) is expressed in the hub cells, whereby it activates the JAK-STAT signalling in the adjacent GSCs and CySCs. When the transcription factor STAT is exhausted from the testis, GSCs and CySCs are lost, whereas misexpression of *Upd* led to GSCs and CySCs that can self-renew without close proximity to the niche [29, 30]. Activation of *STAT* in somatic cells outside the niche is adequate to initiate CySC and GSC self-renewal but, *STAT* activation in GSCs was inadequate to activate GSC self-renewal outside the niche. This suggests that

CySCs with activated JAK-STAT signalling may provide signals which support the self-renewal of adjacent GSCs and that CySC loss might have directly caused the loss of GSCs in the previous *STAT* depletion study [31]. The zinc-finger homeodomain protein 1 (zfh1) is expressed in the CySCs and is required for its maintenance. Zfh1 is a target of JAK-STAT, whereby it is likely that the activation of BMP ligands in the CySCs is through zfh1 [31]. The expression of *zfh1* in the cyst cells outside the niche caused self-renewal of CySCs and GSCs outside the niche as seen in previous similar study with *STAT* [32]. Chronologically inappropriate morphogenesis (chinmo) is another target of JAK-STAT signalling, and it is required for CySC maintenance [33]. Just like in the female *Drosophila*, the BMP signalling is likewise involved in the regulation of GSCs in the *Drosophila* testis. *Dpp* and *gbb* are expressed in both the hub cells and CySCs, and they activate the self-renewal of GSCs while repressing the transcription of *bam* (**Figure 2**) [34–37].

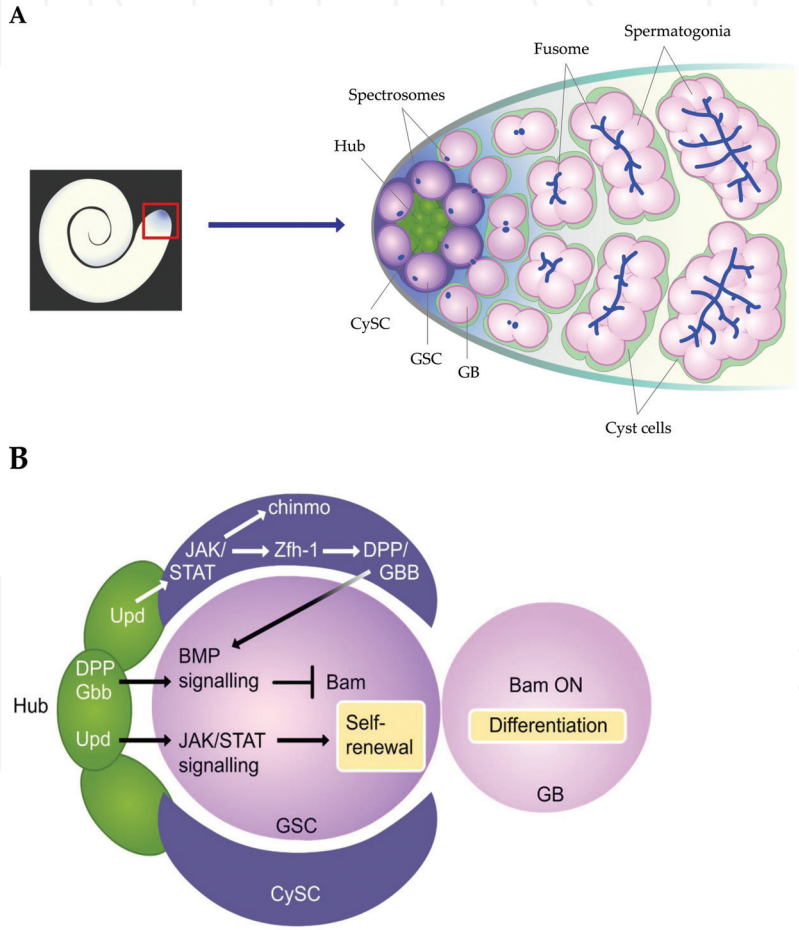


Figure 2. A schematic diagram of the *Drosophila* testis. (**A**) A *Drosophila* testis and the zoom in section of the apical tip of the testis. (**B**) The stem niche of the *Drosophila* testis consisting of the Hub, GSC and CySC and the signalling pathways that maintain the niche.

3. Nutrients and the insulin signalling pathway regulate the germ cell system in both the ovary and testis of *Drosophila*

3.1. Nutrition plays a big role in the development of *Drosophila* ovary

Besides the local signals from the niche, stem cells can respond to external signals such as changes in nutrient availability. Under life-threatening environment such as starvation, organisms often respond by compromising their developmental and/or reproductive programmes. When female flies were fed with diet lacking protein (poor diet), egg-laying was greatly affected with 60-fold difference compared to flies fed on a yeast-rich diet. The ovaries were also greatly reduced in size under poor diet. These effects can be seen within 1 day of switching the flies from normal to poor food, and it takes 2 days for these flies to recover from the effect of poor food to normal egg production and ovary size. Such rapid reproductive changes suggest that egg production is highly dependent on changes in nutrition. Switching female flies from normal to poor food caused a reduction in proliferation rates in both germline and somatic stem cells as well as their progenies to two to fourfold. This is to a lesser extent when compared to female flies raised entirely on poor diet. Although the proliferation rates were reduced, the number of active stem cells remained the same. On the other hand, a checkpoint mechanism was identified at the region 2a and 2b of the germarium. Under poor nutrient condition, apoptosis of the cyst cells was detected at the region where FCs first begin to surround the germline cysts. Cysts moving through the 2a region are preparing for meiosis, and nutrient limitation might have activated cell death programme of both the cysts and somatic cells. Lacking somatic cells to envelope the cysts, this programmed cell death upon nutrient deprivation can prevent insufficient somatic cells from encasing the cyst and cause developmental lapse. The dramatic decline in egg production under poor nutrition might have been due to a slower proliferation programme of the germ line and FSCs and its progenies as well as apoptosis that occurred in the 2a and 2b region [38].

3.2. Nutrition regulates GSCs and CySCs in the *Drosophila* testis

Just like the female flies, the male germ cells are also affected by poor nutrition. When male flies were switched from standard food to poor food for 20 days, their testes become much thinner overtime. The GSCs of these testes declined in numbers to about 35% and nearly 50% for CySCs and early cyst cells. The number of proliferating GSCs measured by cells in the S-phase of mitotic division also reduced greatly from 28 to 17% on 20 days of poor diet. No apoptosis of germ cells was detected in starving flies suggesting that apoptosis did not cause the loss of GSCs but direct differentiation. As seen in the fly ovaries, such phenotypes caused by nutrient deprivation are reversible. Upon switching flies back to normal diet after poor diet, testis development improved, and their testes returned to normal size. The proliferation of GSCs resumed leading to healthy GSC number, and spermatogonia repopulated the testis tip [39]. The ovaries and testes of the flies prove to be not the only organs affected by poor nutrition. The fly intestinal stem cells (ISCs) and its daughter cell called the enteroblast (EB) showed the same effect. The intestine became much smaller, and both the ISCs and EB

reduced in numbers when switched from rich to poor diet. When rich food was available again after starving, the intestine regained its original size. ISCs proliferated at a normal rate, and both ISCs and EB increased in numbers [39].

3.3. The insulin signalling pathway as the nutrient sensor which regulates the development of *Drosophila* ovary

The *Drosophila* insulin signalling pathway has been known for its role in regulating the body, organ and cell size of the animal as well as ageing and lifespan. In *Drosophila*, the insulin signalling consists of insulin-like peptides (Ilps), insulin receptor (InR) and insulin-like substrate or chico which are mediated by the PI3K, phosphoinositide-dependent kinase 1 (Pdk1) and Akt pathway. Genetic defects in components of the pathway caused developmental delays in *Drosophila* giving rise to smaller body and cell size, less cells, increased fat and sterile females. These effects are not caused by insulin signalling alone but can be seen in flies that were starved during development which suggests a link between the insulin signalling pathway and nutrient availability [40, 41]. In fact, research done on the endoreplicating tissues (ERTs) which constitute majority of the *Drosophila* larva showed that inhibition of *PI3K* can suppress cell growth as seen in starved animal; introducing *InR* or *PI3K* can rescue cell loss in starved animal; and *PI3K* activity is activated when nutrition is available [41].

There are seven *Drosophila* insulin-like peptides (Ilps) in total with three (*Ilp2*, *Ilp3 and Ilp5*) being produced in two clusters of the medial neurosecretory cells in both larvae and adult *Drosophila* brain [42, 43]. However, among the three Ilps produced in the brain, only *Ilp3* and *Ilp5* are regulated by nutrient availability, whereas *Ilp2* remain stable during starvation. The Ilp5 is also found in the FCs of the female adult ovaries [44]. The remaining *Ilps* are expressed in other parts of the animal such as imaginal discs, gut and ventral nerve cord [43]. When neurosecretory cells were ablated in the third instar larvae, female adult flies which eclosed later on showed a severe reduction in ovary size and vitellogenic oocytes leading to decreased fecundity [44]. The ability of FCs to proliferate was severely affected in female flies with ablated neurosecretory cells even when rich food was available [45]. A partial decline in proliferation of follicle cells can likewise be seen in female flies with homozygous mutation for *chico* and fed on rich food, reminiscence of flies on poor food. *Chico* mutant caused a serious impairment of egg chambers to develop beyond vitellogenesis in spite of the availability of rich food [38]. Besides that, loss of neurosecretory cells can cause a reduction in body weight and wing area of eclosed flies and reduced length of larvae to half the normal size, and development was slowed down by double [44, 46]. The developmental delays caused by fewer neurosecretory cells can be rescued by expression of *Ilp2* during larvae stage but not the proliferation rate of the FCs [45]. These larvae also have higher levels of glucose and trehalose compared to wild type, and this can be rescued when *Ilp2* was expressed which suggests that insulin signalling pathway can regulate energy metabolism in the animal [46]. Unlike in mammals where Ilps are produced in the pancreas, expression of Ilps in the brain is common in insects [47–49]. For instance, the *Bombyx mori* secretes Ilps from the neurosecretory cells in response to nutrients [50]. Taken together, this shows that the brain is the main organ that produces Ilps to control oogenesis, development and energy metabolism.

When the *Drosophila insulin receptor* (*InR*) was mutated in the germ cells of female flies, the development and size of the germline cysts were greatly reduced. Germ cells with homozygous mutant for *InR* led to cysts which fail to complete vitellogenesis and degenerate. A complete loss of function of *InR* in the germ line resulted in a comprehensive hindrance in vitellogenesis. However, such hindrance was only partial when the neurosecretory cells were ablated. Therefore, Ilp5 produced in the follicle cells most likely works together with the brain Ilps to control vitellogenesis. On the other hand, *InR* mutation in the GSCs of the female flies divides at a much slower rate, and their division rate is dependent on *InR* activity, suggesting that GSCs receive Ilp signal directly and not through the niche to regulate its division [45]. There were less GSCs and CCs in *InR* mutant compared to control, and such loss of GSCs and CCs was quicker with increased age which can be suppressed by overexpression of *Ilp2* in somatic cells. CCs with *InR* mutation showed decreased ability to attach to GSCs due to changes in E-cadherin levels [51]. Loss of *chico* in the female GSCs caused a decline in GSCs and its division rate which can be rescued when wild-type *chico* transgene was introduced or *PI3K* was activated in the GSCs [45, 51].

3.4. The insulin signalling pathway regulates the GSCs in *Drosophila* testis

In the male flies, the *InR* is expressed in the hub cells, GSCs and early germline and somatic cells. Loss of GSCs was seen when *InR* mutation was activated for 10 days in the GSCs. Constitutive expression of *InR* in early germ cells resulted in partial rescue of GSC loss in starved flies, whereas expression of *InR* in both the GSCs and hub cells led to a significant suppression of GSC loss. Similar trend was seen when the activated form of *Drosophila PI3K* was expressed in both the GSCs and hub cells. These results indicate that *InR* can regulate GSC maintenance and constitutive activation of *InR* in the GSCs and hub cells can effectively overturn the loss of GSCs under poor nutrition [39]. A combination of *InR* mutation can cause sterility in both male and female flies. In male sterile flies, their testis showed a reduction of germ cells or sperms. Further investigations showed a decrease in GSCs with increased age in these *InR* mutant male flies. A severe decrease in spermatocyte cysts was also found in testes with *InR* or *chico* mutation even in newly eclosed males. Ablation of neurosecretory cells can also cause a decline in spermatocyte cysts. *InR* mutant testes also showed a decrease in phosphorylated Akt compared to control suggesting that loss of GSCs and spermatocyte cysts can be caused by inactive insulin signalling through the PI3K/Akt pathway. Besides that, *InR* mutation in the testes affected the cell cycle progression of GSCs. There were less GSCs in the S-phase and G2/M phase of the mitotic cycle in the *InR* mutant testes. The spermatocytes undergo a dramatic increase in size before meiosis takes place. These results suggest that InR plays a role in the asymmetric division of the male GSCs, the cell cycle progression of GSCs and the cell growth of spermatocytes [52]. The CySCs of the male *Drosophila* requires the PI3K/target of rapamycin (Tor) activity to differentiate, and lack of which directs the CySCs into a proliferative state [53].

In another study, centrosome misorientation was found to be the culprit of GSC loss or GSC proliferation delay caused by reduced insulin signalling or poor nutrition in male flies. The cell cycle of GSCs is halted in the event of centrosome misorientation and will resume once the orientation is back to normal [54–56]. Male flies under poor nutrition had higher percentage of GSCs with misoriented centrosome compared with flies grown on rich food. However, the spindle orientation remained normal which advocates that the centrosome orientation

checkpoint was intact. This means that the GSCs had a slower cycling rate under poor nutrition. The impaired centrosome orientation was reversible and restored within 3–5 days when flies under poor diet were transferred to rich diet. To investigate if centrosome misorientation can be affected by insulin signalling, a dominant-negative form of *InR* or hypomorphic *InR* mutant was expressed in the germ cells, and the result was a significant increase in centrosome misorientation occurrence regardless of nutrient condition. When the active form of *InR* was expressed, centrosome misorientation reduced significantly even in poor diet. Another component of the insulin signalling pathway, Akt, also regulates centrosome orientation. Knockdown of *Akt* led to high frequency of centrosome misorientation, and the opposite effect can be seen in overexpression of *Akt* regardless of nutrient condition. Overexpression of *Ilp1*, *Ilp2*, *Ilp3*, *Ilp5* and *Ilp6* also reduced centrosome misorientation even in poor food, but this effect was not seen in *Ilp4* and *Ilp7* overexpression. These suggest that GSC centrosome orientation or GSC proliferation is controlled by insulin signalling pathway and nutrient availability (**Figure 3**) [57].

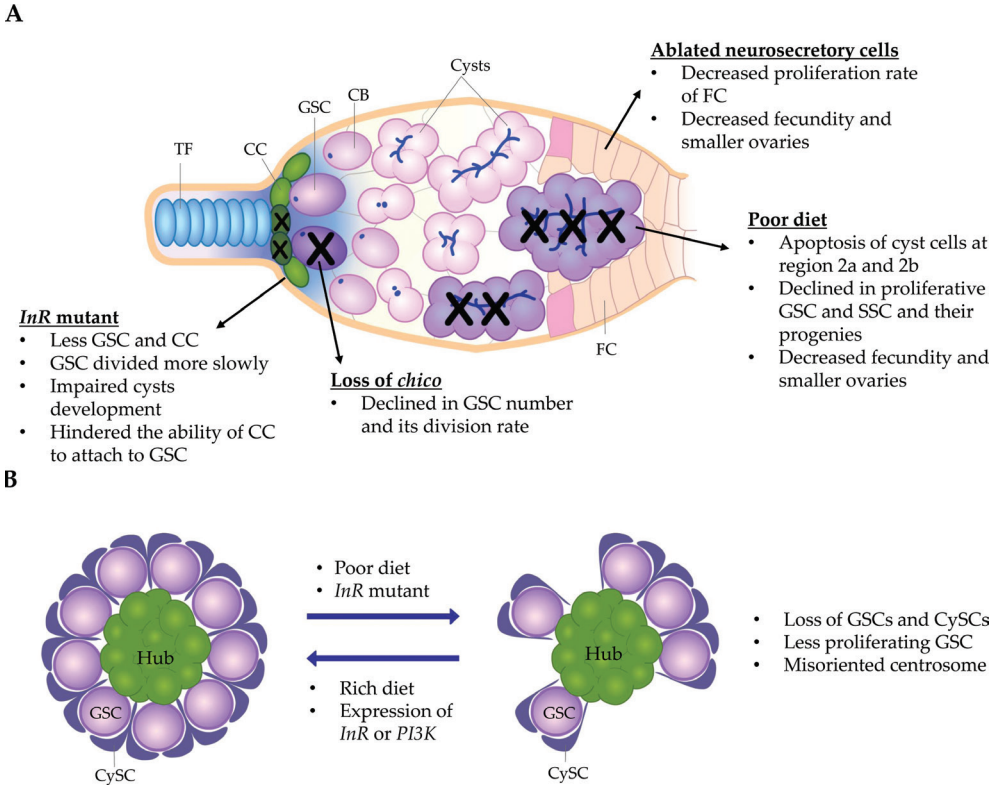


Figure 3. The effect of nutrient availability and insulin signalling pathway on the *Drosophila* ovary and testis. (**A**) A summary of the effect of poor diet and disrupted insulin signals on the *Drosophila* ovary. (**B**) The effect of poor diet and compromised insulin signalling pathway on the stem cell niche of the *Drosophila* testis and such effects are reversible when conditions become favourable again.

4. Steroid signalling regulates the development of *Drosophila* ovary and testis

The endocrine system plays a role in development, metamorphosis, oogenesis and stem cell maintenance in *Drosophila* [58–60]. The major steroid hormone in *Drosophila* is ecdysteroids or its active form, twenty-hydroxyecdysone (20E) which is analogous to the human sex steroids [61]. The 20E acts by binding to a heterodimeric nuclear receptor complex which comprises of an ecdysone receptor (EcR) and ultraspiracle (Usp). EcR and usp have mammalian orthologues, franesoid X receptor/liver X receptor and retinoid X receptor, respectively [62, 63]. The 20E/EcR/Usp complex will then bind to the ecdysone response elements (EcREs) to activate transcription or repression of various genes [64–66]. The early response genes of ecdysone signalling consist of E74, E75 and Broad-Complex (BR-C) which all play a role in egg chamber development [67, 68]. The ecdysteroids were first discovered in the ovaries of adult mosquitoes and subsequently found to be expressed in the ovaries of adult *Drosophila* [69–72]. *EcR* null mutation caused very few female flies (approximately 2% of females) to lay eggs, and they stop laying eggs at day 4–5, suggesting the requirement of ecdysone signalling for oogenesis. Besides, the enzyme important for steroid hormone synthesis was also found to regulate egg chamber development. When *dare*, the *Drosophila* homologue for the enzyme *adrenodoxin reductase*, was mutated, fewer female flies laid eggs, and they progressively lost their ability to lay eggs [67].

Mutation in the biosynthesis of ecdysone or *EcR* encourages GSCs to progress through G2 phase of cell cycle which showed increase in G2/M fusomes. However, these mutations also caused a rapid loss in GSC number. The ecdysone early response gene *E74* but not others was found to regulate GSCs as well. When mutated, GSCs showed significant decline in their division rate. There was also a surge in apoptopic cysts and decline in late-stage cysts which were not seen in *usp* inactivation and may suggest that *E74* is required for the survival of cysts. This seems to be similar to what was found in insulin signalling which also promotes GSC progression through G2 phase. However, E74 acts independently of insulin signalling because removal of a downstream target of insulin signalling, forkhead box, subgroup O (FOXO) has no effect in division of *E74* mutant GSCs [73]. The chromatin remodelling factor called imitation SWI (ISWI) was known to be involved in the self-renewal of *Drosophila* ovary GSCs [74]. This study found ecdysone signalling to work together with ISWI-containing nucleosome remodelling factor (NURF) complex to regulate GSCs in the *Drosophila* ovary. There was an amplified loss in GSCs in combined mutation of *nurf301* and *EcR*, *ISWI* and *EcR* and *ISWI* and *E74*. The BMP signalling was also regulated by ecdysone, whereby phosphorylated Mad (pMad), which is the downstream effector of BMP signalling, was decreased in *usp* and *E74* mutation GSCs. A decline or loss in GSCs was also seen in combined mutation of *EcR* and *dpp*, a BMP ligand [73]. These results show that steroid hormones can alter the epigenetic status of stem cells to influence their fate as well as affecting their capability to receive signals from the niche.

Another finding showed that downregulation of *taiman* (a steroid receptor co-activator) in ECs increased the number of GSCs and CCs and disruption of ecdysone signalling or the

biosynthesis of ecdysone caused excessive germ cells with single spectrosome [75]. In another study, mutation in the biosynthesis of ecdysone and knockdown of *usp*, *EcR* or the response gene, *E75*, in the somatic cells can all affect early oogenesis, whereby regions 1 and 2a of the germarium became significantly reduced in size. Mutation in ecdysone and its signalling in the germarium also caused a rapid loss in GSC number as well as reduced 16-cell cysts. Besides that, depleted ecdysone signalling caused severe impairment in the development of new 16-cell cyst and entry into meiosis [76].

The male *Drosophila* is known to have lower titers of 20E as compared to the females. Nevertheless, the hormone is present in the *Drosophila* testis, and the ecdysone signalling is also required for stem cell maintenance. Male flies deprived of ecdysone caused by mutation led to far less GSCs. Mutation in *EcR* caused significant loss of CySCs and GSCs with 8-cell spermatogonia detected right next to the hub. Besides that, knocking down *EcR* or *usp* specifically in the CySCs and its lineages resulted in a significant decline in CySCs as well as GSCs. This may suggest that *EcR* and *usp* are required nonautonomously in CySCs for GSC maintenance. Interestingly, expression of *EcR-B2* (an isoform of *EcR*) in the CySC lineage can rescue the loss of both stem cell populations seen earlier in *EcR* mutation [77].

5. Mating acts as an external stimulus that regulates GSC number in the *Drosophila* ovary

Another external stimulus that can affect GSC number is mating. During mating, the male-derived sex peptide (SP) is received by the sex peptide receptor (SPR), which is expressed in the female genital tract and its nervous system [78, 79]. Female flies that mated had more GSCs compared to the virgin females, and such increase in GSCs lasted for only 6 days, consistent with the period that sperm can sustain upon mating. There was no increase in GSC number when female flies mated with male flies depleted of *SP* or in female flies with loss of *SPR* function, suggesting the involvement of SP signalling in regulating mating-induced GSC number. Expression of *SP* gene in the neurons of virgin females resulted in increase of GSC number, but such effect was not detected when the somatic cells of the ovaries were overexpressed with *SP* [80]. Another study showed that mating caused a surge in the level of ecdysteroid in *Drosophila* ovaries and, hence, the steroid hormone regulated the mating-induced increase in GSCs [80]. The sensory neurons in the female uterus and oviduct expressed neuronal markers like *fruitless* (*fru*) and *pickpocket* (*ppk*). Disrupting the synaptic transmission of these neurons impersonated the presence of *SP* and caused virgin female flies to become less receptive to mating and stimulated egg-laying. These might suggest that activation of *SPR* by *SP* reduced the synaptic transmission of the sensory neurons. It was also established that the neuronal signal initiated by the *SP* is delivered to the central nervous system [79]. These suggest that mating acts as an external stimulus which sends signal to the sensory neurons in the female genital tract to the central circuits which then alter the female reproductive behaviour leading to more egg production to ensure the survival of the species (**Figure 4**).

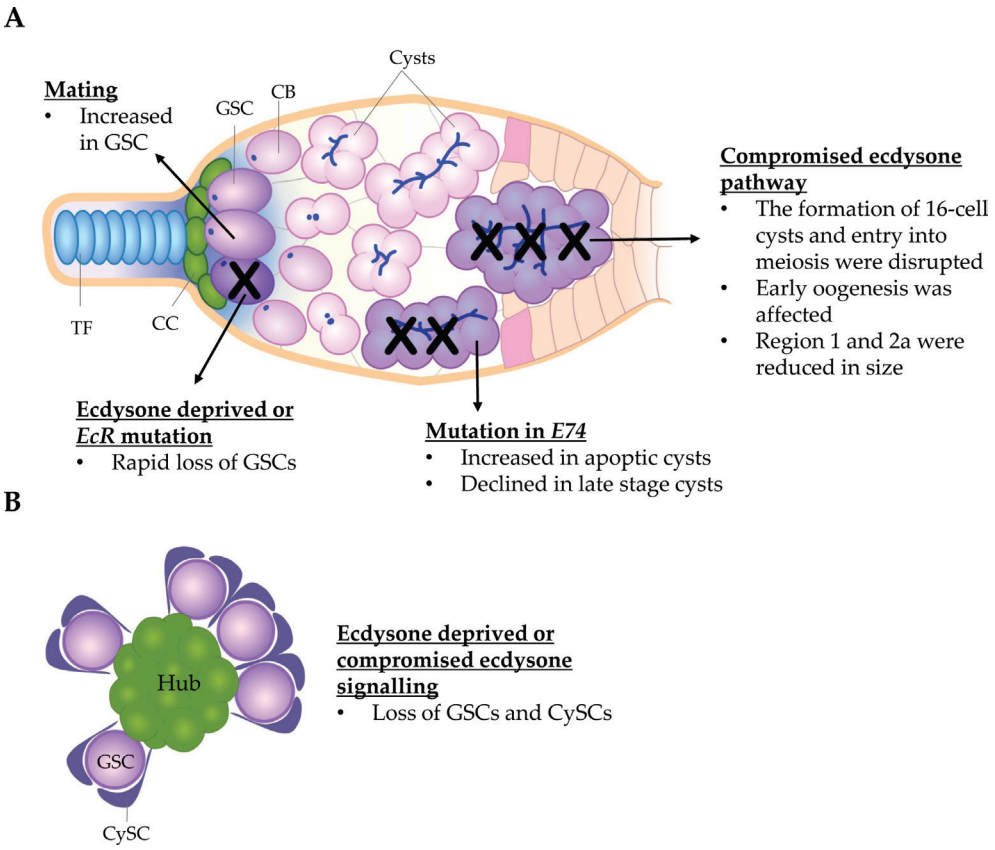


Figure 4. The effect of the ecdysone hormone and its signalling pathway on the *Drosophila* ovary and testis. (**A**) A summary of the effect of mating, deprived ecdysone and its disrupted signalling pathway on the *Drosophila* ovary. (**B**) Hormone deprivation and its compromised signalling pathway can affect the GSC and CySC number of the *Drosophila* testis.

6. Conclusion

The germline stem cell system in both female and male *Drosophila* has been advantageous in providing a platform to address fundamental questions in stem cell biology. As many features of the *Drosophila* stem cell biology are conserved, the studies done on *Drosophila* can have an extensive implication on our understanding of the mammalian stem cell system and, hence, aid in the development of regenerative medicine. The recent work on the *Drosophila* ovaries and testes has shed light on our general understanding of stem cell behaviour. It has revealed the complex regulatory network of the stem cell niche that constantly maintains GSCs, which then develop progressively to give rise to functional gametes when required. Moreover, it is remarkable to see how the brain is involved in safeguarding an

organ or cells that are so far apart from itself. Not only does the brain produce insulin-like peptides that regulate germ cells, but mating can also send signals to the brain to induce egg production in germline or amend the reproductive behaviour in the females. Besides that, the stem cell niche proved to be resilient and flexible at the same time, whereby they can sense and respond to internal and external changes as described above. It is also worth to note that compromised internal or external changes very often just reduced the number of GSCs and the surrounding somatic cells or caused them to divide more slowly instead of going through severe programmed cell death. It is as though nature has its way to preserve these GSCs in bad times and is definitely better to have less functional GSCs than to have none. Besides that, in times when conditions become favourable again, the very few GSCs left can repopulate the lost GSCs by symmetric division or turning on or off specific signalling pathway to quickly get back on track. Given the requirement of GSCs to pass on the genome to its future generation, this is definitely an intelligent way to ensure that the species is being preserved.

7. Future directions

The *Drosophila* ovary and testis stem cell niches are a complex system to a certain extent; however, more insights can be gained through various genome-wide assays such as large-scale RNAi screening or gene expression profiling to identify new players whose loss of function either enhance or inhibit stem cell self-renewal. Ongoing and future studies will persist to disclose the complex network of signalling pathways that control the maintenance of GSC and the adjacent somatic cells and how these signalling pathways function and respond to changes in their external and internal environment. The somatic cells that surround the germ cells have not received enough attention despite their importance in maintaining the germ cells. It would be interesting to reveal how the two different cell populations exchange signals from each other, especially under unfavourable conditions. Besides that, it has been known that the GSCs in both the female and male *Drosophila* can be replenished through mechanisms such as dedifferentiation of differentiated germ cells or via symmetric division of the GSCs. However, what drives such phenomena to occur remains largely unexplored. Since poor nutrition or unfavourable hormone signalling can cause loss of GSCs, will these GSCs be replenished after prolong exposure to unfavourable external or internal environment and would it be through dedifferentiation or symmetric division? In addition, although both the ovary and testis systems are very similar in many aspects, there are still obvious differences between the two. For example, there are multiple stem cell niches present in the ovary due to the presence of several ovarioles compared to only one stem cell niche in the *Drosophila* testis. Furthermore, there might be more distinct mechanisms underlying GSC maintenance which are present in one system but not the other. There are endless interesting questions to be explored and will take many more years of research for us to fully understand these complex systems. Most importantly, the studies done on the *Drosophila* ovary and testis will help us understand adult stem cells and design therapeutic interventions for stem cell-related disorders in a whole new level.

Abbreviations

20E	Twenty-hydroxyecdysone
bam	Bag of marbles
bgcn	Benign gonial cell neoplasm
BMP	Bone morphogenetic protein
BR-C	Broad-Complex
CC	Cap cell
CB	Cystoblast
CySC	Cyst stem cell
dpp	*Decapentaplegic*
E74	Ecdysone-induced protein 74EF
E75	Ecdysone-induced protein 75B
EB	Enteroblast
EC	Escort cell
EcR	Ecdysone receptor
EcRE	Ecdysone response element
ERT	Endoreplicating tissue
FC	Follicle cell
FOXO	Forkhead box, subgroup O
FSC	Follicle stem cell
fru	Fruitless
GB	Gonialblast
gbb	Glass-bottom boat
GSC	Germline stem cell
Hh	Hedgehog
Hts	Hu-li tai shao
InR	Insulin receptor
Ilp	Insulin-like peptide
ISC	Intestinal stem cell
ISWI	Imitation SWI
JAK-STAT	Janus kinase-signal transducers and activators of transcription
Mad	Mothers against *dpp*
NURF	Nucleosome remodelling factor
Pdk1	Phosphoinositide-dependent kinase 1
Piwi	P-Element-induced wimpy testis
pMad	Phosphorylated Mad
Ppk	Pickpocket
SP	Sex peptide
SPR	Sex peptide receptor
SSC	Somatic stem cell
TF	Terminal filament
Tor	Target of rapamycin

Upd	Unpaired
Usp	Ultraspiracle
Yb	Female sterile (1) Yb
Zfh1	Zn-finger homeodomain protein 1

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