Chapter 1

The Vast Utility of *Drosophila* Oogenesis

Michelle S. Giedt and Tina L. Tootle

Abstract

In this chapter, we highlight examples of the diverse array of developmental, cellular, and biochemical insights that can be gained by using *Drosophila melanogaster* oogenesis as a model tissue. We begin with an overview of ovary development and adult oogenesis. Then we summarize how the adult *Drosophila* ovary continues to advance our understanding of stem cells, cell cycle, cell migration, cytoplasmic streaming, nurse cell dumping, and cell death. We also review emerging areas of study, including the roles of lipid droplets, ribosomes, and nuclear actin in egg development. Finally, we conclude by discussing the growing conservation of processes and signaling pathways that regulate oogenesis and female reproduction from flies to humans.

Key words *Drosophila*, Oogenesis, Stem cells, Reproduction, Migration, Cell cycle, Cell death, Lipid droplets, Ovulation

1 Overview

Drosophila oogenesis has been, and continues to be, a useful tool to answer questions in cell biology, development, tissue homeostasis, and disease. Further, while morphologically distinct from vertebrate ovaries, many of the cellular and signaling mechanisms driving oogenesis in mammals also take place in the fly, making it an emerging model for studying female reproduction [1].

The utility of the *Drosophila* ovary lies in the presence of multiple cell types, processes, and an organized ovarian structure that permits simultaneous observation of spatiotemporal events across the whole process of oogenesis. Additionally, oogenesis depends upon basic cell biological processes including cell division, signaling, cell migration, and morphogenesis to create a mature egg capable of fertilization. This variety of cell types and biological processes makes the fly ovary a tremendous resource for investigating an array of questions. Further benefits of the system come from its low cost, ease of maintenance, the wide availability of mutants, and robust genetic tools, including genetic mosaic analysis and the

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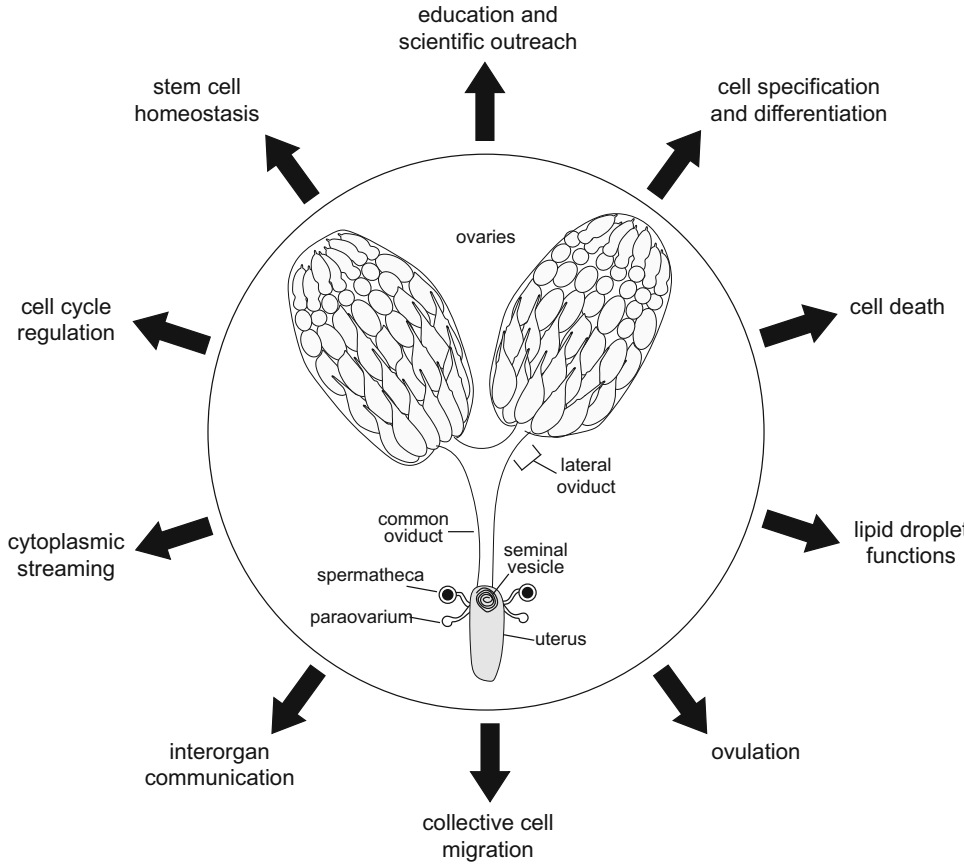

Fig. 1 The utility of the *Drosophila* ovary as a model tissue. Schematic illustrating the *Drosophila* female reproductive tissues and the numerous conserved cellular processes used during *Drosophila* oogenesis. Thus, this tissue is a valuable tool for advancing our understanding of and uncovering the underlying mechanisms driving these processes

ability to overexpress or knockdown genes in specific cells at particular times during development. Additionally, techniques including long-term live imaging, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) gene tagging and mutations, single cell RNA sequencing (scRNA-seq), chromatin immunoprecipitation sequencing (ChIP-seq), and proteomic studies have successfully been used to advance understanding of the processes during *Drosophila* oogenesis.

In this chapter, we highlight the utility of *Drosophila* oogenesis as a model (Fig. 1). First, we provide an overview of early ovary development from embryonic gonad coalescence through larval ovary morphogenesis to pupal ovary formation. Then we describe the key events during the 14 stages of adult follicle development. Next, we summarize how *Drosophila* oogenesis has been used to advance the field's understanding of: stem cell maintenance and differentiation using the germline stem cells (GSCs) as an example;

a diverse array of cell cycles from mitosis and meiosis to endocycling and gene amplification; collective cell migrations, including follicle elongation, border cell migration, and dorsal appendage formation; cytoplasmic streaming to establish embryonic polarity; nurse cell dumping; and cell death. Further, we discuss emerging areas of study, including the roles of lipid droplets, ribosome biogenesis and heterogeneity, and nuclear actin in follicle development. We end by highlighting how, in the last decade, *Drosophila* oogenesis has become a recognized model for understanding conserved principles of female reproduction, such as interorgan communication and ovulation.

2 Early Ovary Development

The cell types and events leading to the production of a mature ovary are conserved between flies and mammals [1–3]. Both require the specification and migration of special germline and somatic cell populations. These cells come together and, as development proceeds, undergo rearrangements to form the adult ovary.

The origins of the *Drosophila* ovary are determined during oogenesis in the mother, when a highly specialized, maternally supplied cytoplasm is sequestered at the posterior of the oocyte. This cytoplasm, termed germplasm, is required for germ cell specification and contains transcription factors, proteins, and mRNAs necessary for germline specification such as *oskar*, *vasa*, *tudor*, and *aubergine* [4, 5]. The germplasm is crucial during embryogenesis for specification of the primordial germ cells (PGCs).

Two classes of cells, the PGCs and somatic gonadal precursors (SGPs), are necessary for ovary development and oogenesis [4, 6–8]. These cells arise at different times and locations during embryogenesis. The PGCs appear in the germplasm during stages 4–5 of embryogenesis. The PGCs migrate along and through the posterior midgut until they reach the mesoderm. In contrast, the SGPs develop as clusters of cells in the mesodermal segments during stage 11 of embryogenesis. At stage 12, the PGCs migrate toward the SGPs where they form two nascent gonads on either side of the embryo. Migration and coalescence of primordial germline and somatic cell populations also occur during mammalian development [9, 10].

The *Drosophila* ovary proper begins to form during larval development [7, 11]. Initially, the PGCs and SGPs proliferate, growing the nascent gonads over ~2 days, with some somatic cells taking on the fate of intermingled cells, which begin to wrap around the germ cells [11]. Additional somatic cell types arise throughout larval development. Indeed, scRNA-seq analysis reveals that the third instar larval ovary is comprised of seven types of ovary progenitor cells [12]. In addition to germline cells located at the

center of the larval ovary, a group of sheath cells are present at the anterior tip and eventually give rise to the ovarioles in the adult ovary [7]. Two other groups of cells, the terminal filament and cap cells, form the future GSC niche [13]. The fifth cell type is the intermingled cells which wrap the germline cells and regulate larval germline proliferation [14]. The swarm cells (also known as the basal cells) are a group of cells of unknown function that initially form at the anterior of the gonad and then migrate to the posterior of the developing ovary [11, 15]. The seventh group of ovary progenitor cells identified by scRNA-seq is a pool of follicle stem cell (FSC) precursors, which occupy the region between the intermingled cells and the swarm cells [12].

During larval ovary development, the nascent gonad goes from round to oval and ovariole specification begins (Fig. 2a) [7, 11]. The first step in ovariole formation is the differentiation of the terminal filament cells. Stacks of 8–10 of these cells form the terminal filament (TF) at the anterior end of each ovariole, producing 16–20 TFs per ovary. The terminal filament cells induce

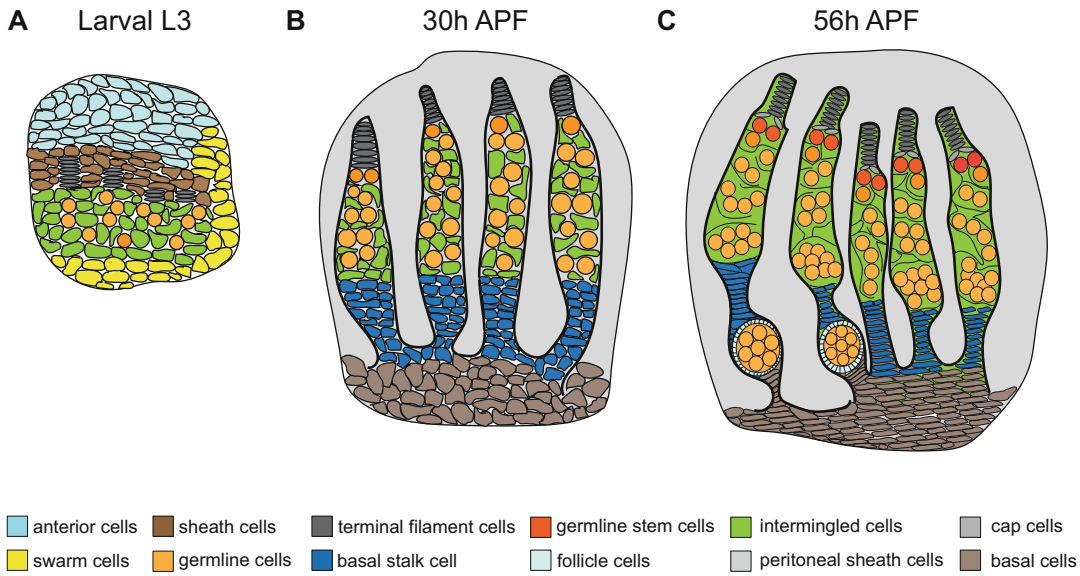

Fig. 2 Larval and pupal ovary development. (**a**) Schematic of a third instar larval ovary. Anterior cells = light blue. Sheath cells = brown. Terminal filament cells = dark gray. Germline cells = orange. Intermingled cells = green. Swarm cells = yellow. (**b**, **c**) Schematics of pupal ovaries at 30 and 56 h (h) after pupal formation (APF). Terminal filament cells = dark gray. Cap cells = light gray. GSCs = dark orange. Germline cells = orange. Intermingled cells = green. Basal stalk cells = dark blue. Basal cells = brown. Follicle cells = light blue. Peritoneal sheath cells are represented by light gray fill. In the third instar larvae, each ovary is a ball of cells that begin to undergo morphogenesis into the adult structure; specifically, the terminal filaments begin to form, delineating the future ovarioles (**a**). These ovarioles continue to form throughout the pupal stage. By 30 h APF, ovarioles are distinct and separated by the tunica propria (**b**). At 56 h APF, the germaria are more defined and the first follicles arise at the posterior of individual germarium, and the space between ovarioles begins to decrease (**c**)

intermingled cells to differentiate into cap cells, The cap cells then recruit the adjacent PGCs to differentiate into GSCs. PGCs distant from the TF and cap cells differentiate to produce the first germline cystoblasts. Apical cells then migrate posteriorly between TFs and their attached GSCs, cystoblasts, and associated somatic cells and lay down a basement membrane called the tunica propria; this separates the gonad into tube structures that will become the adult ovarioles.

The larval ovary undergoes morphogenesis during the pupal stage (Fig. 2b, c). Between 12 and 24 h after pupal formation germaria develop, including the specification of somatic lineages including the escort cells (ECs), FSCs, and differentiating follicle cells [7, 16]. For details on the structure of the germarium, see below and Fig. 3. As pupation progresses, oogenesis proceeds, and by 56 h after the onset of pupation, the first follicle (also referred to as an egg chamber) has budded off the germarium (Fig. 2c). Follicles continue to bud off individual germarium and mature throughout pupal development. For detail on the events occurring during pupal ovariole morphogenesis leading to the formation of the first follicles, please *see* Refs. [7, 16].

3 The Adult Ovary

Drosophila adult females possess a pair of ovaries located in the abdomen. Each ovary is connected to the uterus by oviducts, through which mature eggs pass. Attached to the uterus are sperm storage organs—spermatheca and the seminal vesicle—and secretory organs termed paraovaria (*see* Fig. 1). For more information, please refer to the following paper and reviews [17–19]. Each ovary is composed of 16–20 ovarioles, which are chains of sequentially maturing egg chambers or follicles. In each ovariole, a germarium, which houses the stem cell populations, is at the anterior, and chains of follicles are arranged in order of increasing maturity (Fig. 4) [20]. Oogenesis consists of 14 stages of follicle development. Each follicle is composed of a layer of somatic epithelial cells, termed follicle cells, surrounding sixteen germline-derived cells; fifteen of these are support cells, termed nurse cells, and the other is the oocyte [20].

The germarium, a specialized region at the anterior of each ovariole, contains two stem cell populations, the GSCs and the FSCs that are in separate niches and are required for follicle formation (Fig. 3a). The germarium has four regions—region 1, region 2a, region 2b, and region 3—going from its anterior to its posterior. Region 1 contains the GSC niche which is composed of somatic terminal filament and cap cells. The GSCs contact the cap cells and divide asymmetrically to form a self-renewing GSC and a daughter cell termed a cystoblast [20, 21]. Contributing to this

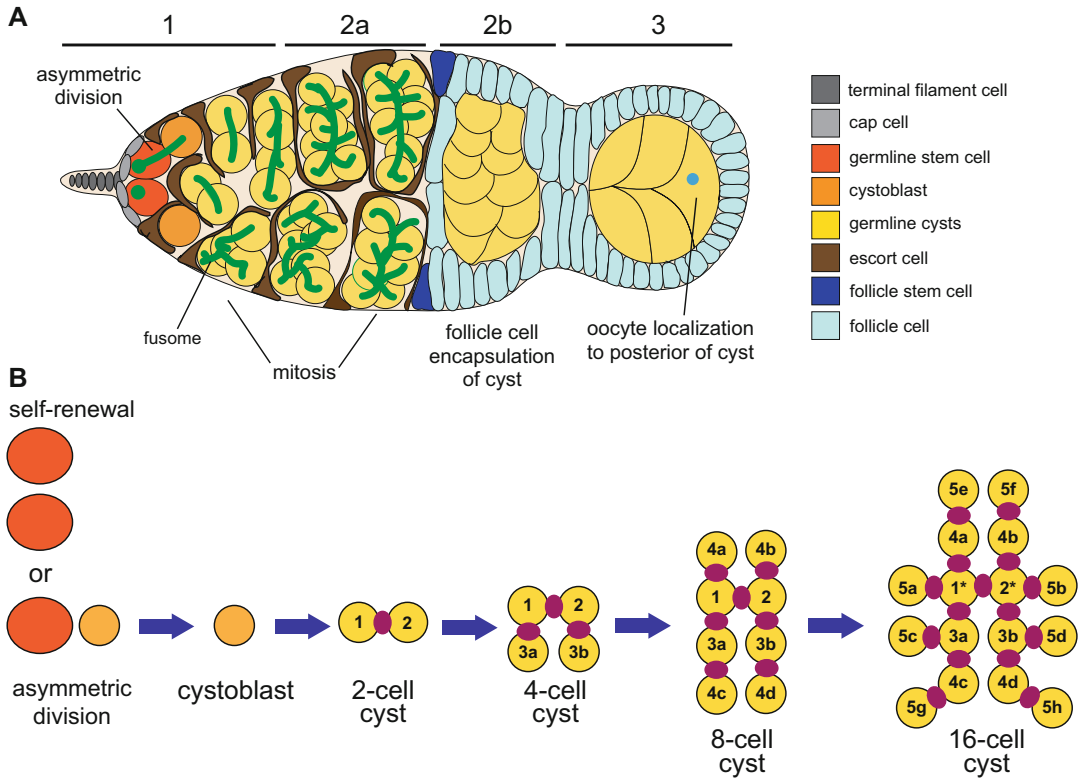

Fig. 3 The germarium and cyst division. (**a**) Diagram of a germarium, with key structures and events indicated. (**b**) Diagram of GSC and cyst division. Terminal filament cells = dark gray. Cap cells = light gray. Escort cells = brown. Germline stem cells (GSCs) = dark orange. Cystoblast = lighter orange. Cysts = yellow. Spectrosome and fusome = green. Cystoblast = lighter orange and cysts = lighter shades of orange. Follicle stem cells (FSCs) = dark blue. Follicle cells = light blue. Ring canals = maroon. The germarium possesses four regions (**a**). Region 1 extends from the GSC niche through the 4-cell cysts. Region 2a includes the 8- and 16-cell cysts. Throughout regions 1 and 2a, each cyst is surrounded by projections from the escort cells. At the 2a/2b boundary, the escort cells handoff the cysts to the follicle cells, which are produced by the two FSCs. In region 2b, the follicle cells encapsulate the 16-cell cyst and the oocyte is specified. In region 3, the oocyte localizes to the posterior of the cyst. Stem cells can either divide symmetrically to maintain the stem cell pool or undergo asymmetric division to produce a daughter cell, termed a cystoblast (**b**). The cystoblast undergoes four rounds of mitosis to produce a 16-cell cyst of interconnected cells. The cyst cells are connected via structures termed ring canals. The number of ring canals per germline cell varies from 1 to 4, with only two cells within the cyst possessing four ring canals (asterisks in 16-cell cyst). Of these two cells, one will be specified as the oocyte

asymmetric division is the GSC spectrosome, a membrane and cytoskeletal-based structure. During mitosis, the spectrosome localizes to the niche side of the mitotic spindle [22], such that after division one cell remains in contact with the cap cells, retains a larger portion of the spectrosome, and maintains stem cell identity, while the other cell orients away from the niche and differentiates (Fig. 3a). The differentiating germ cell then undergoes a series of four synchronous but incomplete mitotic divisions to ultimately

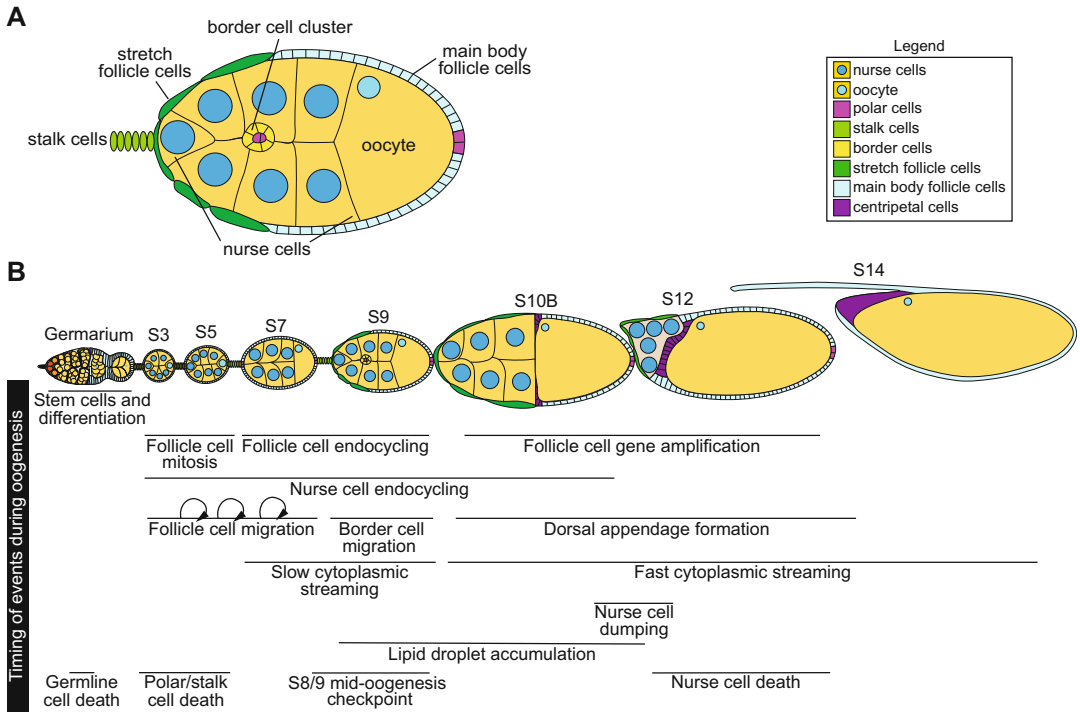

Fig. 4 The *Drosophila* follicle and an ovariole illustrating the developmental timing of events occurring during oogenesis. (**a**) A schematic of a stage 9 follicle, illustrating the various cell types that comprise a *Drosophila* follicle. (**b**) Schematic of a *Drosophila* ovariole and the developmental timing of the cellular events discussed in this chapter. Germline stem cells (GSCs) = dark orange. Nurse cells = yellow with blue nuclei. Oocytes = yellow with cyan nuclei. Escort cells = brown. Follicle stem cells (FSCs) = dark blue. Follicle cells = light blue. Stalk cells = neon green. Polar cells = pink. Border cells = bright yellow. Stretch follicle cells = green. Centripetal cells = purple. For details on events and cell-types within the germarium, please refer to Fig. 3. Each *Drosophila* follicle is comprised of 16 germline cells—15 nurse cells and one oocyte—and a layer of somatic follicle cells (**a**). The follicle cells differentiate into specific subtypes, including stalk cells, polar cells, border cells, squamous stretch follicle cells, and main body follicle cells. *Drosophila* oogenesis requires multiple cell biological processes at specific developmental stages to produce a mature egg (**b**). This temporal precision makes this system a robust model to study both the regulation of and the underlying mechanisms driving these processes

produce a cyst of sixteen interconnected germline cells by region 2a (Fig. 3) [20, 21]. Connecting the cysts cells are ring canals, remnants of the cytokinetic furrows, which permit communication between the cyst cells [23, 24]. Extending through the ring canals is the fusome, the differentiated and branched extension of the spectrosome [25–28]. By region 2b, the 16-cell cyst differentiates into fifteen nurse cells and one oocyte, which is meiotic [20]. This oocyte arises from one of the two cyst cells with four ring canals. The oocyte contains more fusome material than the other germline cells [25, 29]. Associating with both the ring canals and the fusome are proteins and mRNAs that specify the oocyte, including oo18 RNA-binding protein (Orb) and *oskar*. In region 3, the oocyte

becomes localized to the posterior end of the cyst [20]. For more information on cyst differentiation and oocyte specification, please refer to [20, 21, 29, 30].

Like the germline cells, there are multiple populations of somatic cells in the germarium that have region-specific functions (Fig. 3a) [21]. In regions 1 and 2a are escort cells (ECs), which encapsulate the developing cysts. At the boundary of region 2a and 2b, the ECs hand-off the cysts to the follicle cells. Follicle cells arise from the two FSCs at the 2a/2b boundary [31, 32]. One FSC resides on each side of the germarium, asymmetrically dividing to maintain one FSC and producing proliferative follicle cells that either migrate across the anterior of the 16-cell cyst where they can either differentiate into follicle cells or replace the other FSC, or the daughter follicle cell can move posteriorly to generate the follicle cells that will surround the cyst [32, 33]. In regions 2b and 3, the follicle cells differentiate into multiple subclasses required for the formation of the follicle [34]. In region 2b, the first group of follicle cells specified are the polar cells; additional polar cells arise during stages 1–2 [31, 33]. The polar cells, as their name implies, signal to establish anterior and posterior identities within the developing follicle and ultimately, the oocyte [35]. In region 3, the anterior polar cell induces a second group of follicle cells to differentiate into the stalk cells which separate each developing follicle from the next [36]. The remaining follicle cell precursors will give rise to the main body follicle cells, which will differentiate into multiple types later in follicle development.

Fully formed follicles bud off the germarium, resulting in a circular follicle composed of a layer of follicle cells surrounding 15 nurse cells and one oocyte (Fig. 4). The follicle then dramatically increases in size by a combination of follicle cell proliferation and increasing nurse cell size by endocyling [20]. At the same time, the follicle goes from round to oval, increasing its length to width ratio; this elongation is driven by collective cell migration and is discussed later in this chapter [37]. Multiple follicle cell-types also arise and contribute to follicle development. For example, at stage 9 the anterior polar cells induce 4–6 surrounding follicle cells to differentiate into border cells (Fig. 4a) [38–40]. The border cells delaminate from the surrounding epithelium and undergo a collective cell migration between the nurse cells to the oocyte, where the cluster then migrates dorsally. Border cell migration is required for the formation of the micropyle, a cone-shaped eggshell structure through which sperm enter to fertilize the egg [41]; border cell migration is discussed in more detail below. Coinciding with border cell migration, the ~50 anterior follicle cells go from cuboidal to squamous and differentiate into stretch follicle cells; by stage 10A, the stretch cells cover the nurse cells, while cuboidal main body follicle cells cover the oocyte [36]. At stage 10B, 30–40 of these follicle cells differentiate into centripetal cells and begin to migrate

between the nurse cells and the oocyte to ultimately seal the anterior of the oocyte [36]. As this is occurring (stage 11), the nurse cells transfer their content into the oocyte in a process termed nurse cell dumping [42]. During stage 12, the nurse cells undergo cell death, leaving the follicle to be composed of only the oocyte and the surrounding somatic cells [42, 43]. During late oogenesis (stages 10B–13), the follicle cells secrete the vitelline envelope and eggshell [36], with the centripetal cells producing the operculum and a specialized group of dorsal follicle cells producing the dorsal appendages, paddle-shaped structures that oxygenate the developing embryo [44]. Together, these developmental processes result in the production of a mature, ready to ovulate stage 14 egg.

4 *Drosophila* GSCs: A Model for Stem Cell Maintenance and Differentiation

Stem cells are pluripotent cells that divide asymmetrically to both maintain the stem cell pool and produce cells that will differentiate and maintain tissue homeostasis [45]. *Drosophila* oogenesis has been, and continues to be, a powerful model for uncovering the mechanisms regulating stem cell maintenance and differentiation [46–49]. The power of this system comes from the genetic tools, including generating mosaic clones using the FLP/FRT system and cell-specific gene knockdown and overexpression [50, 51]. Further, the germarium houses two stem cell populations: the GSCs and FSCs. Here we focus on the GSCs, their cellular niche, and the roles of adhesion, nuclear architecture, and signaling in stem cell maintenance and differentiation; for detailed reviews on FSCs, please *see* Refs. [48, 49].

The GSC niche is located at the anterior end of the germarium and is composed of several cell types, the terminal filament cells, cap cells, and ECs (Fig. 3a) [47, 52]. Specifically, at the germarium tip is a stack of 8–10 terminal filament cells, the posterior of which contacts 5–7 cap cells. Attached to the cap cells and lining the outside of the germarium are 4–6 anterior ECs. These ECs extend protrusions which surround the GSCs [13, 53, 54]. Together, these three cell-types comprise the niche and maintain a population of 2–4 GSCs.

Bidirectional communication between the niche and the GSCs is required to both maintain the GSC population and control differentiation. Indeed, direct DE-cadherin cell-cell adhesions attach the GSCs to the cap cells [55, 56]; this keeps the GSCs in contact with the source of the signals required for maintaining stem cell fate. For example, Bone Morphogenetic Protein (BMP) family members Decapentaplegic (Dpp) and Glass bottom boat (Gbb) are secreted from the niche and signal to the GSCs [57–59]. This signal causes the transcriptional repression of the differentiation factor Bag of marbles (Bam) in the GSCs. When the GSC divides, one

cell maintains its contact with the niche and its stem-cell promoting signals, and the other cell, the cystoblast, moves away from the niche, which drives the expression of Bam (Fig. 3a) [59–61]. Loss of either Dpp or Gbb leads to depletion of the GSC pool [57–59], whereas loss of Bam leads to germline tumors of undifferentiated cells [62–64].

Multiple mechanisms restrict signaling to the GSCs, including the extracellular matrix (ECM) secreted by the niche cells. This ECM not only provides physical support but restricts the diffusion of signals by sequestering signaling molecules [47, 65, 66]. For example, Division Abnormally Delayed (Dally), a heparin-sulfate proteoglycan (HSPG) within the ECM, restricts the diffusion of Dpp [66, 67]. Intriguingly, the expression of *dally* is repressed in the ECs by Epidermal Growth Factor (EGF) signaling coming from the GSCs. Decreased EGFR expression within the niche expands the expression domain of *dally*, altering the distribution of Dpp and increasing the number of GSCs [68]. The ECM also regulates adhesion of the GSCs to the niche, as the perlecan homolog Terribly reduced optic lobes (Trol) is required for the DE-Cadherin adhesions between the GSCs and the cap cells, in addition to its earlier role in establishing the niche [65]. Another means of restricting the niche signaling to the GSCs is by the Janus kinase (Jak)-signal transducer and activator of transcription (Stat) pathway [69–71]. Indeed, increased Jak/Stat activity in the niche cells expands the number of GSCs [71]. Thus, the niche is a critical regulator of the *Drosophila* GSCs. Similarly, interactions between niche cells, ECM, and stem cells play important roles in stem cell maintenance and differentiation in other organisms and tissues, including hematopoietic stem cells, hair follicle stem cells, intestinal stem cells, and muscle satellite cells [45].

Dynamic crosstalk between the germline and the ECs is also required for GSC maintenance and differentiation. The germline activates Jak/STAT and EGFR signaling in the ECs to drive their protrusions [72, 73]. These protrusions encapsulate the germline cysts, promoting the differentiation of the germline [74, 75]. In particular, the ECs downregulate the stem cell factor Dpp, which drives the pro-differentiation factor Bam [76]. For more in-depth reviews of GSC regulation, we refer readers to [47, 52, 77].

In addition to extrinsic mechanisms regulating stem cell maintenance and differentiation, there are also intrinsic factors. Here we discuss one intrinsic regulator of the *Drosophila* GSCs, the nuclear lamina; for a more in-depth review, please *see* Ref. [78]. The nuclear lamina consists of a protein network of lamins and hundreds of lamin-binding proteins on the inside of the nuclear envelope and regulates both nuclear structure and chromatin organization [79, 80]. In relation to structure, the type of lamin controls nuclear stiffness, with higher levels of Lamin A increasing stiffness [81]. Across organisms and systems, stem cell nuclei are softer

and stiffness increases with differentiation. In *Drosophila*, the GSCs express only the B-type Lamin (Lamin B, *Drosophila* Lamin Dm0), and its level decreases as differentiation proceeds; Lamin A (*Drosophila* Lamin C) is present starting from the 16-cell cysts in region 2b [78].

This differential lamin expression also results in a patterned expression of interacting proteins, including the Lap2-emerin-MAN1 domain (LEM-D) family member Emerin (D-emerin/Otefin). D-emerin is highly expressed in the GSCs and decreases with differentiation [78]. D-emerin, like other LEM-D proteins, connects the nuclear lamina to Barrier-to-Autointegration Factor (BAF), which in turn binds chromatin [82, 83]. Loss of either D-emerin or BAF activates kinases of the DNA damage checkpoint, blocking differentiation and causing GSC loss [84–86]. Further, the GSC nuclear lamina plays a critical role in chromatin organization necessary for maintaining stemness, as heterochromatin coalesces in *d-emerin* mutant GSCs [84]. D-emerin and the nuclear lamina also regulate GSC mitosis, see below for more details [87]. Importantly, alterations in the nuclear lamina are linked to patient stem cell dysfunction in the progression of laminopathies [88, 89], making the *Drosophila* GSCs a robust model for uncovering the mechanisms by which specific nuclear lamina components act in stem cell maintenance and tissue homeostasis.

5 Cell Cycle Regulation

Drosophila oogenesis provides a robust system to define the mechanisms controlling cell cycle transitions. In addition to being a model for studying meiosis, during follicle development multiple cell types undergo mitosis, including a noncanonical mitosis in the GSCs. Other mitotic cells transition to endocycling, and follicle cells also undergo gene amplification. This diversity of cell cycle events has made *Drosophila* oogenesis a key system for advancing the field's understanding of cell cycle regulation (Fig. 4b).

Recent work by the Geyer Lab discovered that GSCs undergo a noncanonical mitosis. Indeed, while most eukaryotic cells examined breakdown their nuclear envelope during mitosis, the *Drosophila* ovarian GSCs retain a permeable but intact nuclear envelope [87]. This noncanonical mitosis is highly sensitive to perturbations in the nuclear lamina, with defects resulting in chromosome segregation errors and activation of checkpoints that drive GSC loss. It is tempting to speculate that this noncanonical mitosis is a more broadly used mechanism of assessing stem cell quality across tissues and organisms [87].

The early adult germ cells also provide a system to study how cells transition from mitosis to meiosis. The differentiating germ cells undergo 4 mitotic divisions to produce a 16-cell cyst (Fig. 3b).

In region 2a, the two pro-oocytes initiate prophase I of meiosis, with only one remaining in meiosis by region 3 [90, 91]. At stage 5, the oocyte arrests in diplotene of prophase I and remains arrested until stage 13 when meiosis resumes. In stage 14, metaphase I arrest occurs and is maintained until egg activation during ovulation and transport through the oviduct induces the resumption and completion of meiosis [92, 93].

The germline-derived nurse cells exit mitosis in the germarium and endocycle through stage 10. Endocycling cells go from gap (G) to synthesis (S) phases without intervening mitosis or cytokinesis, generating polyploid cells; this allows for cell and tissue growth without disruptions in tissue integrity due to mitosis. In the nurse cells, oscillating levels of Cyclin E drive 10–12 endocycles over just a few days [94]. This results in massive nurse cell growth, and thereby, contributes to the growth of the follicle. Further, the increased DNA content allows the nurse cells to rapidly synthesize contents for the future egg. Thus, nurse cell endocycling facilitates rapid oogenesis.

The *Drosophila* follicle cells also transition from mitosis to endocycling [95]. From region 3 of the germarium to stage 6 of follicle development, the follicle cells are mitotic, producing ~650 cells. Signaling from the nurse cells then activates Notch within the follicle cells, inducing the switch from mitosis to endocycling [96–101]. The follicle cells undergo 3 rounds of endocycling during stage 7-10A, supporting the rapid growth of the follicle.

Endocycling is found across tissues and organisms. Indeed, many mammalian cells undergo endocycling as part of developmental programs [102, 103]. For example, placental trophoblast giant cells require their polyploid size to form and maintain a barrier between the maternal and embryonic tissues [104–106]. In the liver, hepatocyte polyploidization occurs during both development and in response to stress. This stress response allows hepatocytes to survive genetic instability and to rapidly regenerate the liver in response to injury, aging, or hepatectomy [103, 107, 108]. Thus, the nurse and follicle cells of the *Drosophila* ovary provide a robust system to uncover the underlying mechanisms and functions of endocycling, providing insights that can be used to improve human health.

During stage 10B, the *Drosophila* follicle cells undergo another cell cycle transition, switching from endocycling to gene amplification; this transition is regulated by ecdysone signaling [98]. Gene amplification is when only specific regions of chromosomes are replicated. In the follicle cells, there are 6 sites of gene amplification that exhibit distinct temporal patterns of initiation and elongation of the amplified regions [109–111]. The amplified sites contain genes encoding eggshell components and are required for the temporally controlled, rapid expression, and production of the eggshell at the end of oogenesis. Thus, the multiple cell cycle

transitions that follicle cells undertake during oogenesis are required to produce a viable egg and thereby influence fertility. Further, gene amplification in the follicle cells provides a system to study the mechanisms controlling the different steps in DNA replication, from origin recognition, to initiation, to elongation [109, 110, 112]. Additionally, gene amplification also occurs in mammalian cells in response to stress and is routinely observed in cancer [113, 114].

6 Collective Cell Migration

Cell migration is critical for life. In multicellular organisms, cell migration drives not only the morphogenic movements during development, including organ formation, but it is also critical for organ homeostasis and repair, wound healing, and diseases, such as cancer [115–117]. While some cellular migrations occur as single cells moving independently, most in vivo cell migrations occur as groups of cells adhering to each other and migrating as a cohesive cluster, termed collective cell migration [118]. Cell migration is controlled by both signaling molecules and properties of the microenvironment, including stiffness [119–121]. The fly ovary is an excellent model for studying the mechanisms controlling collective cell migration as specific populations of follicle cells undergo developmentally timed collective cell migrations to drive follicle elongation, micropyle formation, and dorsal appendage formation (Fig. 4b). These migratory events, in combination with temporal and cell-specific gene manipulation and advances in live imaging, make *Drosophila* oogenesis a great, in vivo model to understand the mechanisms controlling collective cell migration, and how such migrations mediate morphogenic events across organisms.

From the moment follicles detach from the germarium through stage 8, the outer follicle cells collectively migrate on the surrounding ECM (also referred to as the basement membrane) to lengthen the follicle [37, 122, 123]. All the cells within the follicle cell layer coordinate their behavior and form a polarized actin network. On the basal surface, which faces the outside of the follicle, lamellopodial and filopodial leading edge protrusions and parallel arrays of stress fibers attach to integrin-based adhesions on the ECM to drive the collective migration [122, 124–129]. Genetic perturbations of the actin cytoskeleton, the ECM or its cellular receptors, and the signaling pathways involved result in a round egg phenotype and ultimately sterility. Importantly, similar epithelial collective cell migrations drive tissue remodeling during other morphogenic events, including wound healing and cancer metastasis [115, 116, 118].

During stage 9, another collective cell migration, termed border cell migration, occurs. The border cell cluster ultimately contributes to the formation of the micropyle, the eggshell structure

through which sperm enter to fertilize the egg [41], and thus, border cell migration is required for fertility. For detailed reviews on border cell migration, *see* Refs. [38–40]. At the beginning of stage 9, the anterior polar cells specify a group of 4–6 surrounding follicle cells to become border cells. These border cells surround the polar cells and delaminate from the follicular epithelium and migrate as a cohesive cluster between the nurse cells to the oocyte (Fig. 4a). Once at the oocyte (late stage 9, early stage 10A), the border cells migrate dorsally.

Border cell migration is directed by both Platelet-Derived Growth Factor (PDGF) and EGF signaling originating from the oocyte [130, 131] and the physical environment [132]. In particular, the stiffness of the nurse cells, the substrate on which the border cells migrate, is a critical regulator of migration. Increasing the stiffness of the nurse cells impedes migration [133, 134]. Recent work suggests that the border cells modulate the nurse cell stiffness, perhaps creating a durotactic gradient to promote their own migration or altering the nurse cells to mediate later developmental events [135]. Additionally, many signaling pathways control the polarity of the cluster, driving its directed migration by modulating the actin cytoskeleton and its regulators within the border cell cluster [38–40]. For example, prostaglandin signaling regulates both border cell migration and cluster cohesion [136]. In summary, the conserved processes observed in border cell migration—delamination, invasion, and collective migration—along with the ability to visualize migration dynamics live [130, 137, 138] have made border cell migration an ideal model for understanding the conserved signals, microenvironmental properties, and cellular changes driving collective cell migration, including cancer invasion and metastasis.

The formation of the dorsal appendages, respiratory eggshell structures, also depends on collective cell migration (*see* Ref. [44] for a detailed review). During stage 10, the oocyte initiates both EGFR activation and inhibition to specify two separate populations of dorsal follicle cells [139–142]. Further specification of the dorsal-appendage primordia along the anterior-posterior axis comes from Dpp signaling [143–147]. Ultimately, 65–70 cells undergo migration and synthesize the tubular dorsal appendages. Each tube is made from two subpopulations, the cells that produce the roof and sides (termed roof cells) and those that produce the floor [44]. During stage 11, dorsal appendage morphogenesis begins, with the apical constriction of the roof cells, and the floor cells migrating underneath them [148, 149]. In stages 12–13, these cells migrate anteriorly over the stretch follicle cells. Throughout their migration, they secrete the eggshell. By stage 13, the migration stops, and the distal cells shorten along the apical-basal axis to produce the flattened paddle of the appendages [148–150]. Defects in patterning and collective migration result in

aberrant dorsal appendages, including a single dorsal appendage, altered separation of the two appendages, and abnormal appendage morphologies, including an "antler" appearance [44]. Thus, dorsal appendage formation is a great model for studying the mechanisms driving tubulogenesis.

7 Cytoplasmic Streaming

A reoccurring tool used to mediate development is fluid flow. For example, fluid flow is required for the development of the vasculature, lungs, and kidneys in vertebrates [151]. Intracellular fluid flow, termed streaming, plays critical roles in oogenesis across organisms [152]. Notably, streaming is generally observed in large cells and is implicated in long-distance transport and compartmentalization. *Drosophila* oogenesis provides a robust system to uncover the mechanisms driving and the functions of cytoplasmic streaming; *see* Ref. [152] for a detailed review.

Two types of streaming occur during *Drosophila* oocyte development (Fig. 4b): slow streaming during mid-oogenesis (stages 7–10A) and fast streaming during late oogenesis (stages 10B–14) [153–155]. Both streaming events depend on microtubules and the plus-end-directed motor protein, Kinesin-1 [155–157]. During slow streaming, the oocyte is filled with an actin mesh, and disassembly of this is required for the transition to fast streaming [158]. Thus, cytoplasmic streaming within the *Drosophila* oocyte is a model for studying the mechanisms regulating both microtubule and actin dynamics. One function of streaming in the *Drosophila* oocyte is to help establish embryonic polarity [152, 159, 160]. The nurse cells transcribe mRNAs that are transported via the ring canals into the oocyte; these mRNAs are delivered to specific locations within the oocyte, and this localization is then maintained. Briefly, head and thorax identity are specified by mRNAs, including *bicoid*, localizing at the anterior end of the oocyte, whereas abdominal and germline identity are controlled by mRNAs such as *nanos* at the posterior end.

8 Nurse Cell Dumping

A conserved cytoplasmic streaming event during oogenesis from flies to mice is when sister germ cells, often called nurse cells, within a germline cyst transfer their cytoplasmic contents into the oocyte, driving its growth as the sister cells shrink and ultimately die [42, 161, 162]. In mice, this process is mediated by both microtubule-dependent transport and actin fibers [161, 162]. Conversely, in late *Drosophila* oogenesis, the actin cytoskeleton is primarily responsible. Specifically, during stage 10B, actin filament

bundles initiate at the nurse cell plasma membranes and extend to the nuclei, forming a cage-like structure surrounding each nucleus [163, 164]. The cortical actin also thickens [165]. At stage 11, the nurse cells rapidly transfer mRNAs, proteins, and organelles through the ring canals into the oocyte; this is termed nurse cell dumping.

Recent live-imaging and mathematical modeling work reveals nurse cell dumping occurs in two phases [166]. The first phase is driven by fluid flow from smaller cells into larger cells, with the nurse cells immediately adjacent to the oocyte dumping their contents first, and the more anterior nurse cells transferring their contents through the posterior nurse cells. The second phase of dumping occurs when the nurse cell cytoplasmic volume is comparable to its nuclear volume and is driven by actomyosin contractility of the cortical actin. If nurse cell dumping fails, follicle morphogenesis is disrupted, including failure of the nurse cells to die during stage 12, generation of short eggs, and abnormal dorsal appendage formation. Nurse cell dumping defects can be classified based on their distinct outcomes. In the first case, nurse cell nuclei are observed plugging the ring canals into the oocyte, and thereby, blocking dumping; this phenotype indicates defects in actin bundle formation and/or organization [167–169]. In the second case, dumping fails but no nuclei plug the ring canals; such a phenotype is observed when myosin activity is impaired [165]. Thus, nurse cell dumping during *Drosophila* oogenesis is widely used to study actin cytoskeletal dynamics.

While the functions of numerous actin-binding proteins have been uncovered using nurse cell dumping as a model, the mechanisms initiating this process have largely remained elusive. As discussed above, one means of driving nurse cell dumping is simply cellular size and fluid force [166]. Whether this serves as a mechanical signal to drive actin remodeling events is unknown. Additionally, prostaglandin signaling is required for nurse cell dumping [170]. Loss of prostaglandin signaling results in a severe reduction and often complete loss of actin bundle formation, and cortical actin breakdown [171]. Strikingly, even though the bundles are defective, nurse cell nuclei do not plug the ring canals, suggesting prostaglandins also regulate nurse cell contraction. While the detailed prostaglandin signaling pathway remains to be determined, multiple actin-binding proteins—Fascin (*Drosophila* Singed), Enabled, and Non-muscle myosin II—have been identified as downstream effectors of prostaglandins [172–174]. Whether fluid flow and prostaglandin signaling are conserved mechanisms used to control germline cyst cytoplasmic transfer across organisms is unknown.

The primary purpose of *Drosophila* nurse cell dumping is to provide the oocyte with everything it needs for successfully completing embryogenesis, which occurs outside the mother. During

the first ~2 h of embryogenesis, there is no zygotic transcription, and therefore, the embryo is completely dependent on maternally supplied factors [175]. Indeed, roughly 60% of the genome is supplied as maternal RNA, and regulation of its translation, stability, and localization within the embryo are essential for embryogenesis. In the first 2 h, the embryo undergoes 13 rapid nuclear divisions in a shared cytoplasm; this rapid cell cycle depends on maternally supplied proteins. During nuclear cycle 14, the embryo cellularizes to package maternally supplied organelles into individual cells and begins zygotic transcription. One maternally supplied organelle that plays critical roles both early in embryogenesis and after cellularization is the lipid droplet [176]. Lipid droplets consist of a neutral lipid core surrounded by a phospholipid monolayer decorated with an array of proteins. Across organisms, lipid droplet accumulation, composition, and localization are dynamic during oocyte maturation [177–179], and changes in lipids droplets are associated with infertility [180–182]. These lipid droplets are known to support the metabolic needs of the embryo, both when embryonic development occurs outside the mother, such as in *Drosophila*, and prior to implantation in placental mammals [183, 184]. Additionally, in *Drosophila*, maternally supplied lipid droplets sequester and buffer the histone supply prior to cellularization [185–188]; whether this is conserved in higher eukaryotes remains to be determined.

9 Lipid Droplets: Roles in Oogenesis

The conservation of lipid droplet accumulation during oogenesis raises the possibility that in addition to mediating early embryogenesis lipid droplets play roles in follicle development. During *Drosophila* mid-oogenesis, large numbers of lipid droplets are generated in the nurse cells [176]. Lipid droplet accumulation begins at stage 8, such that by stage 10B the nurse cell cytoplasm is packed with lipid droplets (Fig. 4b). This temporal accumulation of lipid droplets is driven by steroid hormone signaling activating the Sterol regulatory-element-binding protein (SREBP) transcription factor, which promotes the expression of lipophorin receptors (LpR1/2) [189]. LpR1/2 take up lipoproteins from the circulating hemolymph, providing the lipids needed for lipid droplet assembly [190, 191]. Diacylglycerol O-acyl transferase 1 (DGAT1, *Drosophila* Midway) incorporates these lipids into triglycerides, driving lipid droplet formation; in its absence, nurse cells and oocytes lack lipid droplets.

Genetic studies on DGAT1 and LpR1/2 suggest lipid droplets play critical roles in oogenesis. Loss of DGAT1 results in a loss of lipid droplet formation and follicles arrest at stage 8, display premature actin bundles, and trigger the mid-oogenesis checkpoint,

resulting in follicle death [192]. Thus, DGAT1 is required for follicle development. However, LpR1/2 germline clones also lack lipid droplets, but these have been reported to develop past the mid-oogenesis checkpoint [190]. One difference in these mutants is that *LpR1/2* mutants will fail to take up fatty acids from the hemolymph, while *DGAT1* mutants will have excess free fatty acids in the nurse cells that cannot be sequestered into lipid droplets. A number of free fatty acids are cytotoxic, including arachidonic acid [193, 194]. Indeed, arachidonic acid is found in triglycerides in ovary lysates and loss of the lipid droplet-associated adipose triglyceride lipase (ATGL, also called Brummer) increases such triglycerides. Further, exogenous arachidonic acid inhibits the development of *Drosophila* follicles ex vivo, and reducing ATGL suppresses this toxicity [195]. These data lead to the idea that lipid droplets sequester free fatty acids, including arachidonic acid, to prevent lipotoxicity.

A recent study uncovered another role of lipid droplets in *Drosophila* oogenesis—mediating prostaglandin signaling [195]. Genetic and pharmacological studies support the model that during stage 10B, ATGL releases arachidonic acid from lipid droplet triglycerides. This arachidonic acid is then used as the substrate for prostaglandin production. These prostaglandins activate signaling cascades that drive the actin remodeling events necessary for nurse cell dumping and late-stage follicle morphogenesis. Thus, *Drosophila* oogenesis is emerging as a system to decipher the roles of lipid droplets in oocyte development.

10 Cell Death

Cell death is an essential process for eliminating defective cells and mediating development across organisms. *Drosophila* oogenesis has been widely used to understand the mechanisms and different types of cell death, as cell death is restricted to specific cells and periods of follicle development (Fig. 4b).

In early oogenesis there are three cell death events [43]. Germline cysts can undergo cell death in region 2 of the germarium. Such death, which is a combination of autophagy and apoptosis, is triggered by a checkpoint that can be activated by defects in the cyst or in the environment, such as a shortage of nutrients [196–198]. There are also two types of somatic cell death in early oogenesis. Initially, there are 3–6 polar cells at each end of the follicle, and prior to stage 5, the number is reduced to 2 by apoptosis [199, 200]. Similarly, if there are too many stalk cells, they undergo apoptosis during stages 2–8 [201].

After the follicle has the correct number of polar and stalk cells, it then undergoes a final assessment of both follicle health and the fly's environment prior to the energy-intensive process of

vitellogenesis; this is termed the mid-oogenesis checkpoint [43, 196, 202, 203]. Failure to pass this checkpoint results in the elimination of the whole stage 8/9 follicle; this is a mechanism to avoid wasting energy on building an oocyte that is unlikely to produce a viable offspring. This follicle death occurs by all the nurse cell nuclei simultaneously condensing and losing their nuclear integrity. The surrounding follicle cells then act as phagocytes and engulf the dying germline, and subsequently undergo cell death.

In late oogenesis, the 15 nurse cells within the follicle undergo a phagocyte-dependent cell death, leaving only the oocyte and the surrounding follicle cells [42, 43]. After the completion of nurse cell dumping (stage 12), the stretch follicle cells invade, but do not engulf, the nurse cells; this initiates nurse cell death. During stage 13, the nurse cell nuclei become acidified by the stretch follicle cells and then degrade asynchronously, ultimately resulting in the mature stage 14 egg [204–206]. During ovulation, the follicle cell layer is removed, allowing the oocyte, encased by its eggshell, to move through the oviduct. These follicle cells will later die and be phagocytized by the epithelial cells of the lateral oviduct [207, 208].

11 *Drosophila* Oogenesis: A Model for Other Cell Biology Processes

Drosophila oogenesis is a robust model for studying a diverse array of cell biology, from understanding the functions of organelles to uncovering the development roles of understudied factors using both genetics and more recently proteomics. Here we illustrate the utility of *Drosophila* oogenesis to study the roles of ribosomes, nuclear actin, and metabolic filaments.

Control of protein synthesis, at the level of ribosomal activity, is a critical conserved regulator of stem cell function, differentiation, and tissue homeostasis, including during *Drosophila* oogenesis [209]. Indeed, GSCs have higher levels of ribosomal RNA (rRNA) transcription by RNA Polymerase I (RNAPI) than their differentiating daughters but decreased protein synthesis [78, 210, 211]. Inhibiting RNAPI activity results in GSC loss, whereas increasing it drives differentiation [210]. Further, an unbiased RNAi screen discovered that ribosome assembly impacts GSC cytokinesis, and differentiation requires increased ribosome biogenesis and protein synthesis [211]. Recent work indicates three DExD/H-box proteins are required to sense ribosome biogenesis levels to control the GSC cell cycle, and thus, differentiation [212]. Ribosomal activity also plays critical roles in later stages of oogenesis. Two studies analyzing paralogs of ribosome protein S5 (RpS5) found that loss of RpS5b but not RpS5a results in numerous defects in oogenesis, including disruption of vitellogenesis, posterior

follicle cell hyperplasia, and mid-oogenesis checkpoint arrest [213, 214]. Contributing to these defects is both increased translation and altered mRNA translation efficiency, resulting in striking transcriptional changes including in metabolic processes, yolk granule proteins, and cytoskeletal regulators. These findings, along with growing evidence of germline-specific differential ribosomal paralog expression [215], suggest that ribosomal specialization is particularly critical for germline function. Together, these studies establish *Drosophila* oogenesis as a model for not only understanding how ribosomal activity controls stemness and differentiation, but how ribosomal protein paralogs drive ribosomal heterogeneity and tissue specialization.

Drosophila oogenesis is also becoming a model to study the functions of actin in the nucleus. While actin has been extensively studied for its cytoskeletal roles, actin localizes to the nucleus in a highly regulated manner where it has numerous functions, including binding to and regulating all three RNA polymerases, functioning in chromatin remodeling complexes, mediating DNA repair, and contributing to nuclear architecture [216–219]. How these different functions of nuclear actin are used to drive development remains poorly understood, and *Drosophila* oogenesis is poised to contribute to this area. The first evidence suggesting nuclear actin has a role in *Drosophila* oogenesis came from the observation that germline expression of tools for imaging actin cytoskeletal dynamics results in a stage-dependent accumulation of thick nuclear actin filaments, often called nuclear actin rods [220]. As these tools stabilize endogenous actin filaments, this finding led to the idea that there was endogenous nuclear actin during *Drosophila* oogenesis.

Subsequent work uncovered three tools that label both overlapping and distinct pools of endogenous nuclear actin [221, 222]. Specifically, monomeric nuclear actin labeled with DNase I is found in the nucleolus of every cell during oogenesis. The C4 actin antibody colocalizes with DNase I in a subset of cells during early oogenesis, but it also labels distinct nucleoplasmic regions in the early germ cells and the oocyte. The AC15 actin antibody labels the chromatin in all cells starting around stage 6 and increases in intensity through stage 10. Additionally, during stages 9–10B, puncta of nuclear actin within the nurse cell nucleoli are labeled with the AC15 actin antibody. Thus, all pools of endogenous nuclear actin exhibit some localization to the nucleolus, suggesting nuclear actin may play a critical role there.

Nuclear actin also regulates transcription during *Drosophila* oogenesis. Using ChIP-seq of whole ovary lysates, the Vartiainen Lab found actin colocalizes with RNAPII on most gene promoters and along the gene body of highly expressed genes, including eggshell genes [223]. Impairing the nuclear import of actin results in decreased eggshell gene expression, supporting that nuclear actin

plays a critical role in transcription during oogenesis. Future work using *Drosophila* oogenesis is expected to advance the mechanistic understanding of nuclear actin in development and tissue homeostasis.

The power of using *Drosophila* oogenesis to study cell biology is amplified by the biochemistry capabilities of the ovary. Indeed, due to the ease of obtaining large quantities of either individual follicles or whole ovaries, *Drosophila* oogenesis is becoming more widely used to identify protein-protein interactions. For example, both proximity-dependent biotinylation using the APEX enzyme and tandem affinity purification followed by mass spectrometry were used to uncover the protein interaction partners of the ring canal protein Kelch and its molecular functions [224, 225]. TurboID-mediated biotinylation has also been used to understand the interaction partners of cytidine triphosphate (CTP) synthase when it can or cannot form filamentous cytophidia [226]. Cytophidia are conserved intracellular compartments observed from yeast to humans. Such filamentous structures have been observed with multiple metabolic enzymes and evidence suggests the purpose is to modulate enzymatic activity [227]. As cytophidia are present in both the nurse and follicle cells during *Drosophila* oogenesis, this model has played a critical role in both the discovery and understanding of this cellular structure [227, 228].

12 *Drosophila* as a Female Reproductive Model

While the organization and morphology of the *Drosophila* ovary are distinct from those found in mammals, growing evidence supports that numerous processes during oogenesis and their regulation are conserved. As discussed above, interconnected germline cysts are common, and recent work supports that mammalian germline cells act like *Drosophila* nurse cells, providing contents to the surviving oocyte and then undergoing cell death [42, 161, 162]. Lipid droplet accumulation is also a conserved process during oogenesis [177–179], although it remains unknown whether the roles of lipid droplets in regulating both prostaglandin signaling and lipotoxicity observed in flies extend to higher eukaryotes [195]. Further, across organisms, prostaglandin signaling regulates female reproduction, including follicle maturation [229–232]. However, whether, like in *Drosophila* [136, 171–174], prostaglandins regulate nurse cell dumping, actin cytoskeletal remodeling, and cell migration during mammalian oogenesis remains to be determined. Here we discuss two additional examples—interorgan communication and ovulation—of how *Drosophila* oogenesis serves as a model to uncover conserved mechanisms regulating female reproduction.

Across organisms, female reproduction—oogenesis, ovulation, and fertilization—depends on the status of the whole organism, and therefore, it is critical for the reproductive system to respond to

signals from other organs; this is termed interorgan communication. *Drosophila* is a robust model for discovering how interorgan communication controls oogenesis due to the ability to use the UAS/GAL4 system to drive RNA interference (RNAi) knockdown in specific organs and cell-types [233, 234].

One conserved environmental factor driving interorgan control of oogenesis is nutrition. In *Drosophila*, a protein poor diet results in decreased GSC proliferation, GSC loss, increased mid-oogenesis checkpoint death, and a block in ovulation, resulting in increased numbers of stage 14 follicles [43, 196, 197, 235]. The nutritional status of the fly is conveyed to the ovary by a number of organs. For example, upon feeding, the brain secretes insulin-like peptides to signal to the germline, promoting GSC proliferation, follicle growth, and progression through vitellogenesis [236, 237]. Adipose tissue, known as the fat body in *Drosophila*, also plays a critical role in regulating oogenesis in response to nutrition. In adipocytes, insulin signaling promotes GSC maintenance, early germline cyst survival, and vitellogenesis [238]. Adipocytes also use the amino acid response pathway to promote germline cyst survival and progression through vitellogenesis [239]. Further, Target of rapamycin (TOR)-dependent amino acid sensing in adipocytes promotes ovulation [239]. Genetic studies also support that fatty acid oxidation in the fat body is required for GSC maintenance [240].

Another conserved means of regulating oogenesis is through nuclear receptors, ligand-dependent transcription factors. For example, loss of the nuclear receptor *seven up* (*svp*) in adipocytes results in increased GSC loss and early germline cyst death, while loss in oenocytes increases follicle death at the mid-oogenesis checkpoint [241]. The orphan nuclear receptor, Hormone Receptor 4 (Hr4), also acts in multiple tissues to regulate oogenesis [242]. Within the ovary, Hr4 acts in the niche and GSCs, whereas in the muscle Hr4 is required for GSC maintenance and follicle growth. Hr4 acts in other, currently undefined tissues to promote early germline cyst survival and vitellogenesis. Thus, multiple organs use diverse mechanisms to control key aspects of *Drosophila* oogenesis, allowing it to avoid wasting energy producing eggs when the organism is undergoing a stress.

Over the past decade, *Drosophila* has also emerged as a model for studying the conserved mechanisms controlling ovulation. Ovulation in mammals is induced by luteinizing hormone (LH) signaling to the granulosa cells of the preovulatory follicles [243]. This activates multiple pathways, including EGF, progesterone, and prostaglandins, to drive meiotic resumption, somatic cell expansion, and proteolytic breakdown of the follicle wall [244–246]. In *Drosophila*, octopamine signals to the follicle cells on mature stage 14 follicles, activating NADPH oxidase [247] and matrix metalloprotease 2 [248]. The latter, in combination with ecdysone signaling, leads to the breakdown of the follicle wall

[249]. Specifically, the posterior follicle cells are degraded, allowing the mature oocyte to rupture from the follicle cells and be released into the oviduct. The rest of the follicle cells remain at the end of the ovariole to form a corpus luteum-like endocrine structure similar to that in mammals [250]. Further illustrating the utility of *Drosophila* as a model for understanding ovulation, this system has been used to screen for nonsteroidal contraceptive compounds [251]. Thus, *Drosophila* is poised to uncover new and conserved mechanisms regulating the poorly understood process of ovulation.

13 Conclusion

While *Drosophila* oogenesis has already taught us much about fundamental principles of biology and development, with the expanse of technological and genetic tools it remains a model that continues to advance multiple fields, from stem cells and tissue homeostasis to conserved mechanisms controlling female reproduction. Further, the breadth of processes that can be studied, ease of obtaining large amounts of tissue, and simple readouts of defects in follicle development (i.e., fertility and follicle morphology) make *Drosophila* oogenesis poised to transform science education, from outreach activities and classroom exercises for K-12 to undergraduate course-based research experiences [252]. For excellent examples of how-to bring *Drosophila* oogenesis into the classroom, please *see* Refs. [253, 254].

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