Stem cells orchestrate oogenesis: A lesson from the fruit fly, *Drosophila melanogaster*

Oogenesis, the production of oocytes, is a fundamental biological process in all animals and is necessary for species perpetuation. In most insects, females actively lay eggs throughout their life cycles [1]. Such active egg production is achieved by the supply of germline cells, and it is thought that germline stem cells (GSCs) are essential for the replenishment of germline cells. In coordination with GSCs, a type of somatic stem cells, called follicle stem cells (FSCs), also function to supply follicle cells, which support oocyte maturation in a coordinated manner.

In this issue, we focus on recent advances in the understanding of the regulatory mechanisms involved in the proliferation and maintenance of such stem cells in the fruit fly, *Drosophila melanogaster*. In fact, we initially intended to collect manuscripts covering a wide variety of insect species, as the mechanisms of oogenesis vary among species even within insects [1]. However, we immediately realized that the fine details of GSCs and FSCs have scarcely ever been documented in insects other than *D. melanogaster*, predominantly due to the lack of well-defined labelling methods and molecular genetic tools. In contrast, *D. melanogaster* has been studied extensively with regards to the molecular and cellular mechanisms of not only oogenesis but also other tissue stem cell systems. Even before the development of tools dissecting cellular architecture in the 1960s, Dr. Robert King and his colleagues had proposed, based on detailed observations made using electron microscopy, that GSCs reside at the anterior apex of the germarium, a small anteriorly located structure in *D. melanogaster* ovaries [2]. Subsequently, this proposal was validated by old-fashioned mosaic analysis with X-ray irradiation [3]. Later, the development of genetic tools in *D. melanogaster*, a landmark technological advance, enabled convenient linage tracing of stem cells as well as identification of unique cell types. Using these genetic techniques, Dr. Allan Spradling and his colleagues have made tremendous contributions to the field of stem cell biology in the last decades; these include defining and locating GSCs as well as FSCs in the germarium, and importantly, identifying the microenvironment of GSCs, i.e., the niche. In 1993, using site-specific recombination driven by FLP-recombinase to produce an active *lacZ* reporter, Drs. John Margolis and Spradling dissected the cellular architecture of the ovariole. They identified approximately two FSCs per germarium and located them at the at the region 2a/2b border, the site of encapsulation of germline

cysts by follicle cells [4]. Concurrently, Drs. Haifan Lin and Spradling reported that germaria transplanted into oogenesis-deficient host females produced egg chambers, thus proving the presence of GSCs in germaria [5]. In 1998, a milestone article by Drs. Ting Xie and Spradling reported that the BMP2/4 homolog Decapentaplegic (Dpp) functions to maintain female GSCs in the niche [6]. Since then, many studies have contributed to elucidating the intrinsic molecular mechanisms in GSCs and FSCs, as well as the signaling pathways controlling them [7,8].

Based on the historical background described above, *D. melanogaster* is an indispensable model system for understanding the behavior of GSCs and FSCs in insects. Therefore, considering the fact that stem cell studies in other insects are scarce, we decided to focus only on *D. melanogaster* studies in this issue. Thus, we commissioned 8 reviews by leading *D. melanogaster* scientists, covering recent progresses in the understanding of GSCs and FSCs at molecular, cellular, genomic, systemic, and organismal levels.

Two articles particularly focus on the niche signals and their signaling pathways to control GSC and FSC behaviors, respectively. The article by Zhang & Cai illustrates how GSC self-renewal and differentiation are tightly regulated by multiple niche-derived signals. In addition to providing the central signaling molecule, Dpp, for GSC self-renewal, the niche also provides additional molecules, Hedgehog (Hh) and Wnts, for promoting GSC differentiation. The article summarizes how the niche functions as a signaling hub to integrate these multiple niche-derived local signals as well as other organ-produced systemic signals to control GSC self-renewal and differentiation.

The article by Rust & Nystrul reviews the signaling pathways that control FSC behaviors. Unlike in the case of GSC self-renewal and differentiation, not Dpp, but multiple other signals, including Hh, Wnts, epidermal growth factor, Hippo, Notch, and JAK-STAT, are central to the regulation of FSCs. Rust & Nystrul illustrate the complex interactions of these signals with each other to coordinately regulate FSC self-renewal and differentiation. They also describe and discuss FSC behaviors at the cellular level. Notably, this article presents a view that is contradictory to several studies [9-11], particularly, with respect to how FSCs are maintained and contribute to generating the

follicle epithelium including escort cells as well as follicle cells. We hope that this article stimulates active discussions in the field.

Recent studies also reveal that extracellular and intracellular architectures, such as extracellular matrix and nuclear lamina, also contribute greatly to GSC behaviors. Two articles in this issue focus on this topic. Hayashi *et al.* review how GSCs and FSCs are maintained within a distinct extracellular microenvironment. While the content of this review overlaps with that of Zhang & Cai and Rust & Nystrul to some degree, the review highlights that the niche signals of GSCs and FSCs are tightly regulated by the regulatory components of the extracellular matrix, including Dally, Dally-like protein, and matrix-metalloprotease. Hayashi *et al.* also discuss the role of cell competition in regulating GSC and FSC behaviors.

Regarding the intracellular architecture, this issue includes an article focusing on a novel mechanism regulating GSCs in female insects, namely, nuclear architecture. Duan *et al.* highlight emerging data that support nuclear architecture, particularly the nuclear lamina, as an intrinsic regulator of female GSC maintenance and germ cell differentiation. The article suggests that the nuclear lamina and nucleolus integrate signals needed for the switch between GSC maintenance and germ cell differentiation.

The issue also covers the recent progress on the impact of transposable elements (TEs) on GSC behaviors. It is well known that TEs spread through the host genomes by replicating in germline cells. Kelleher *et al.* describe that TEs are involved in the replication of GSCs in female *D. melanogaster* and discuss the relationship between transposition and GSC loss, which is arbitrated to reduced signaling for self-renewal, increased signaling for differentiation, and impairment of DNA damage response pathways.

Besides the above-mentioned articles that describe the regulatory mechanisms at the molecular, cellular, and genomic levels, this issue also includes three articles that focus on GSC behavioral regulation at the systemic and organismal levels. It is common in animals that the age-related decline in activities of adult stem cells leads to loss of tissue homeostasis. Ishibashi *et al.* highlight recent progress in stem cell aging in the *D. melanogaster* ovary and connect the discoveries in the fly to long-standing questions in stem cell aging.

Nutrients also greatly affect insect GSC proliferation and maintenance. Nutrient availability, which impacts proper allocation of limited nutritional resources to

oogenesis, influences GSC proliferation and maintenance through several systemic factors. The review by Lin & Hsu provides an overview of the *D. melanogaster* ovarian GSC-niche-adipocyte system and major nutrient-sensing pathways that intrinsically or extrinsically regulate GSC responses to nutrient signals.

Lastly, Ote & Yamamoto describe the impact of the infection with the insect-symbiotic bacteria, *Wolbachia pipientis*, on female *D. melanogaster* GSCs. Interestingly, in some *D. melanogaster* mutant contexts, *Wolbachia* colonizing female GSCs supports GSC maintenance and rescues defective ovarian development. The article also describes that he manipulation of host RNA in GSCs by *Wolbachia* is the key to successful vertical transmission of this bacterium.

Fueled by the advances in the understanding of ovarian stem cells in *D. melanogaster*, studies on GSCs and FSCs in other insects, including some with oogenesis mechanisms differing vastly from that of *D. melanogaster* are now emerging. For example, in the red flour beetle, *Tribolium castaneum*, germline cells do not divide in adult ovaries, but actively do so only in embryonic and larval stages [12]. Therefore, unlike that of *D. melanogaster*, *T. castaneum* ovary does not harbor typical GSCs in the adult stage. By contrast, a recent study using molecular markers suggested that the *T. castaneum* germarium likely houses FSCs supporting active egg production [13], although such stem cells are yet to be identified cytologically. We hope that this issue will stimulate research on GSCs and FSCs in not only *D. melanogaster* but also other insect species.

Last, but not least, we deeply thank all the authors for contributing to this issue and for their time and dedication.

References

1. Büning J: *The Insect Ovary: Ultrastructure, previtellogenc growth and evolution.* Netherlands: Springer; 1994.
2. Koch EA, King RC: **The origin and early differentiation of the egg chamber of *Drosophila melanogaster***. *J. Morphol.* 1966, 119:283-303.
3. Wieschaus E, Szabad J: **The development and function of the female germ line in *Drosophila melanogaster*: A cell lineage study**. *Dev. Biol.* 1979, 68:29-46.
4. Margolis J, Spradling A: **Identification and behavior of epithelial stem cells in the *Drosophila* ovary**. *Development* 1995, 121:3797-3807.

5. Lin H, Spradling A: **Germline stem cell division and egg chamber development in transplanted *Drosophila* Germaria**. *Dev. Biol.* 1993, 159:140-152.
6. Xie T, Spradling AC: ***decapentaplegic* Is Essential for the Maintenance and Division of Germline Stem Cells in the *Drosophila* Ovary**. *Cell* 1998, 94:251-260.
7. Spradling A, Drummond-Barbosa D, Kai T: **Stem cells find their niche**. *Nature* 2001, 414:98-104.
8. Yoshinari Y, Kurogi Y, Ameku T, Niwa R: **Endocrine regulation of female germline stem cells in the fruit fly *Drosophila melanogaster***. *Curr. Opin. Insect Sci.* 2019, 31:14-19.
9. Vied C, Reilein A, Field NS, Kalderon D: **Regulation of stem cells by intersecting gradients of long-range niche signals**. *Dev Cell.* 2012, 23:836-848.
10. Reilein A, Melamed D, Park KS, Berg A, Cimetta E, Tandon N, Vunjak-Novakovic G, Finkelstein S, Kalderon D: **Alternative direct stem cell derivatives defined by stem cell location and graded Wnt signalling**. *Nat Cell Biol.* 2017, 19:433-444.
11. Reilein A, Melamed D, Tavaré S, Kalderon D: **Division-independent differentiation mandates proliferative competition among stem cells**. *Proc. Natl. Acad. Sci. USA*. 2018, 115:E3182-E3191.
12. Bäumer D, Trauner J, Hollfelder D, Cerny A, Schoppmeier M: **JAK-STAT signalling is required throughout telotrophic oogenesis and short-germ embryogenesis of the beetle *Tribolium***. *Dev. Biol.* 2011, 350:169-182.
13. Teuscher M, Ströhlein N, Birkenbach M, Schultheis D, Schoppmeier M: ***TC003132* is essential for the follicle stem cell lineage in telotrophic *Tribolium* oogenesis**. *Front. Zoolog.* 2017, 14:26.