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Methods for studying oogenesis

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ABSTRACT

Drosophila oogenesis is an excellent system for the study of developmental cell biology. Active areas of research include stem cell maintenance, gamete development, pattern formation, cytoskeletal regulation, intercellular communication, intercellular transport, cell polarity, cell migration, cell death, morphogenesis, cell cycle control, and many more. The large size and relatively simple organization of egg chambers make them ideally suited for microscopy of both living and fixed whole mount tissue. A wide range of tools is available for oogenesis research. Newly available shRNA transgenic lines provide an alternative to classic loss-of-function F2 screens and clonal screens. Gene expression can be specifically controlled in either germline or somatic cells using the Gal4/UAS system. Protein trap lines provide fluorescent tags of proteins expressed at endogenous levels for live imaging and screening backgrounds. This review provides information on many available reagents and key methods for research in oogenesis.

1. Introduction

Oogenesis in Drosophila [1,2] supports an impressively high level of fecundity. Ovaries in adult females consist of ~16 parallel tubes called ovarioles that contain developing egg chambers arranged a linear array of progressive developmental stages (Fig. 1). Movement of egg chambers is facilitated by peristaltic contractions of circular muscles surrounding each ovariole and a muscle mesh surrounding the whole ovary [3–5]. The ovarioles of each ovary converge at a lateral oviduct, which connects to a common oviduct and then the uterus. Mature stage 14 eggs move into the uterus where they are fertilized by a single sperm and then laid. The development of each egg takes about eight days: roughly half of this time is spent in the germarium for egg chamber formation, and the remaining four days are required for egg chamber development. Each of the ovarioles produces approximately two eggs per day, or over 60 eggs from a young, well-fed female [6]. This high output of eggs depends on an abundant source of food, major contributions from support cells in egg chambers and a wide range of cellular interactions. An overview of oogenesis and examples of how its study has contributed to our understanding of developmental biology are summarized in this section.

1.1. The germarium and early oogenesis

The formation of egg chambers (also called follicles) takes place during the first four days of oogenesis in the germarium. Germline stem cells at the apical end of germaria are maintained by signaling from adjacent niche cells called cap cells. Stem cell daughters called cystoblasts leave the niche and undergo four mitotic divisions to produce a cyst of 16 cells. Incomplete cytokinesis in these divisions leaves intercellular bridges that are later stabilized by the accumulation of filamentous actin to form ring canals that persist until the end of oogenesis. Somatic escort cells encase the dividing cysts. After completing mitosis, escort cells are exchanged for follicle cells to complete stage 1 egg chamber assembly. The follicle cells are generated by two follicle-cell stem cells located between germarium regions 2a and 2b. Thus, the germarium contains two types of stem cells maintained in separate niches whose division rates must be coordinated to produce cells necessary for egg chamber production. Only one of the 16 germline-derived cells develops into an oocyte while the remaining 15 differentiate into nurse cells with polyploid nuclei. By the time an egg chamber emerges from the germarium, the oocyte is already positioned at the posterior as a result of a signaling cascade emanating from the next most mature egg chamber. Thus, the first four days of oogenesis produce an oocyte already endowed with anterior/posterior axis information and accompanied by a suite of nurse cells and follicle cells poised to support oocyte development. Examination of egg chamber assembly carried out in many laboratories has yielded a huge amount of information on stem cell and stem cell niche biology [7,8] and the

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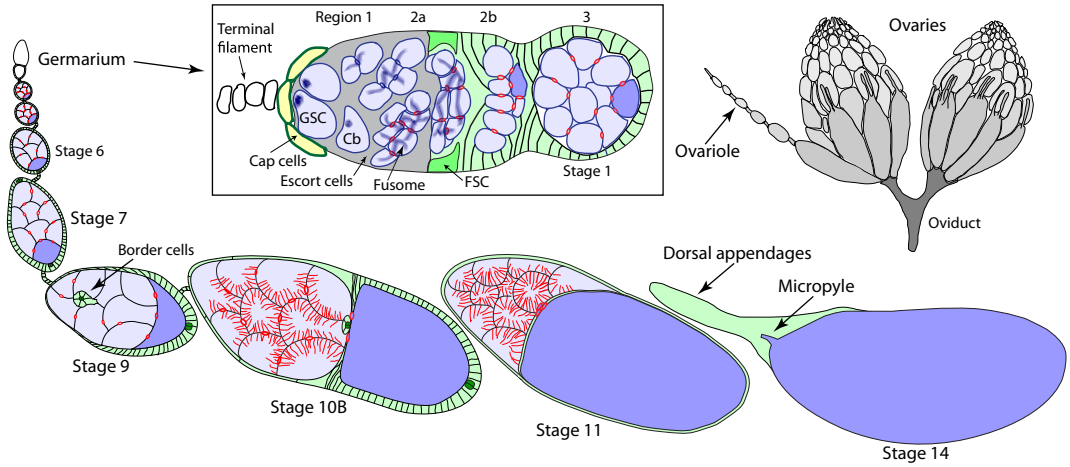

Fig. 1. Overview of oogenesis. Drosophila females have a pair of ovaries (top right), each of which consists of ~15 ovaioles. Oogenesis begins in the germarium (center box), where germline and somatic stem cells (GSC and FSC, respectively) divide continuously to support the formation of new egg chambers. Somatic follicle cells: green; nurse cells: light purple; oocytes: dark purple; ring canals and nurse cell F-actin bundles: red. See text for further details.

origins of polarity [9]. This research takes advantage of powerful genetic and cytological tools developed over many years.

1.2. Previtellogenic development, stages 2–8

The second half of oogenesis (~3.5 days) takes place in stage 2–14 egg chambers as they move within ovarioles toward the oviducts. Most of this time is needed for previtellogenic egg chamber development during stages 2–8, when oocyte growth is mediated entirely by intercellular movement of cytoplasm from nurse cells to the oocyte through ring canals. The diameter of ring canals slowly expands as egg chambers grow, mediated by active actin filament polymerization in the ring canal rim [10]. The polarization of oocytes is established during these stages as a consequence of the posterior location of the oocyte nucleus. The *gurken* mRNA accumulates at the posterior with the oocyte nucleus, and produces a ligand for EGFR activation in posterior follicle cells [11]. EGFR activation transmits a signal back to the germline that causes microtubule reorganization in the anterior/posterior axis. In addition, many maternal mRNAs and RNA-binding proteins accumulate specifically in oocytes through a combination of microtubule-based directed transport and trapping within the oocyte [12].

Follicle cells also make crucial contributions to previtellogenic oogenesis. Egg chambers change shape from spherical to elongated starting during stage 5. Interestingly, this shape change is driven by egg chamber rotations that occur between stages 5 and 9. Follicle cells drive the rotations as they migrate perpendicular to the axis of the ovariole, laying down a girdle of polarized extracellular matrix as they go. As a result, expansion of egg chambers takes place anisotropically toward the poles [13]. A major change in cell cycle also takes place in follicle cells. Mitotic divisions cease at the end of stage six, followed by three endoreplication cycles during stages 7–10A [14]. The Notch-Delta signaling pathway controls this transition [15,16], providing an excellent opportunity for studying cell cycle changes under developmental control. Like the germline cysts, the follicle cells are also syncytial, as they remain interconnected with a number of sibling cells by small (~200 nm diameter) ring canals that result from incomplete cytokinesis [17–19]. These ring canals do not grow in size during oogenesis, but they are able to support intercellular movement of protein between cells, raising the interesting possibility that they serve an important function in oogenesis [20,21].

Stage 8 egg chambers do not progress into vitellogenesis (yolk uptake) if egg chambers have severe patterning defects or if environmental conditions are unlikely to support the survival of progeny. Limiting the availability of protein in the diet of females causes egg chamber apoptosis at the end of stage 8, thus avoiding the metabolic cost of completing egg development and depleting the female's energy stores. During egg chamber apoptosis, follicle cells lose their epithelial organization and become phagocytic, engulfing the cytoplasm of germline cells [22,23]. If egg chambers are sound and protein is restored to the food, oogenesis resumes and stage 14 eggs can develop from surviving stage 8 egg chambers within one day. Thus, stage 8 serves as a metabolic checkpoint that triggers egg chamber destruction while preserving less mature egg chambers poised to resume development when conditions improve.

1.3. Completing oogenesis, stages 9–14

The final day of oogenesis produces a huge increase in oocyte volume due to yolk uptake from hemolymph and the complete transfer of nurse cell cytoplasm to the oocyte. Yolk uptake beginning at the end of stage 8 causes the rate of oocyte growth to overtake nurse cell growth so that the oocyte takes up half the volume of egg chambers by stage 10A. During these stages, several key patterning molecules are localized within the oocyte: *oskar* mRNA at the posterior, *bicoid* mRNA at the anterior, and *gurken* mRNA at the dorsal anterior domain [24]. The movement and anchoring of these maternal mRNAs are active areas of research that benefit from the ability to do live-cell imaging to reveal conserved mechanisms of mRNA localization [25,26]. The final phase of oocyte growth happens during stage 11 when nurse cells contract and squeeze (or 'dump') their remaining cytoplasm into the oocyte in about 30 min, accompanied by robust microtubule-mediated mixing of the oocyte cytoplasm. Nurse cell death after dumping has some of the hallmarks of apoptosis, although it is a caspase-independent process [23,27]. In preparation for this nurse cell dumping, stage 10B egg chambers produce cables of unipolar actin filaments that grow from the nurse cell membranes inward until they reach the nuclear envelope [28,29]. The actin cables prevent nurse cell nuclei from being squeezed into ring canals where they would block the flow of cytoplasm to the oocyte. Prostaglandin signaling is involved with triggering the formation of nurse cell actin cables [30].

Follicle cells form a secretory epithelium in egg chambers with apical/basal polarity and extensive microvilli on their apical surfaces facing the germline cells. They secrete yolk protein during vitellogenesis, vitelline membrane proteins during stage 9–11,