

OOGENESIS. One reason for the evolutionary success of insects is their remarkable capacity to generate large numbers of progeny. Since most insects reproduce sexually, this requires the efficient formation of specialized gametes, a process in females

called oogenesis. Females produce eggs that have to accomplish a number of functions. The egg must contain sufficient nutrients to sustain embryonic development, allow gas exchange for respiration, and protect the embryo from a variety of environmental stresses, including large shifts in temperature and water loss by desiccation. This is particularly true for insects that lay their eggs in the external environment (as opposed to ovoviviparous insects that store eggs in the body until hatching). In addition, early stages of insect embryogenesis are very much dependent on regulatory factors stored in the egg during oogenesis, often requiring that these factors be specifically localized. Hence, the relatively simple appearance of the egg masks a surprisingly complex structure that serves a number of different functions all essential for the development of the next generation.

Ovary structure

The germline consists of those cells that can directly produce gametes. All other cells are defined as somatic. Oogenesis requires extensive interactions between the soma and germline, with all developmental events taking place in the ovaries. In the adult there are typically two ovaries, each made up of multiple hollow tubes called ovarioles. At the distal tip of the ovariole is a region called the germarium, which contains undifferentiated germ cells called oogonia. Oogenesis begins when an oogonial cell becomes committed to forming an oocyte and initiates meiosis, which is usually arrested until fertilization. Somatic derived follicle cells then envelop the oocyte to form the egg chamber. Soon the oocyte will begin to take up and store yolk protein, entering the region of the ovariole known as the vitellarium. As new eggs form, older chambers are pushed down the length of the ovariole, differentiating along the way such that the most mature eggs are eventually located at the proximal end next to the oviduct. This developmental sequence results in a linear arrangement of sequentially more mature egg chambers, a morphology that is characteristic of insects. As one might expect, there is large species-specific variation in the number of ovarioles per ovary, ranging from a single ovariole in some aphids to over 2000 in termite queens.

Ovarioles are subdivided into two major types based on the presence and organization of specialized cells that provide trophic support to the oocyte. Panoistic ovarioles are the simplest in morphology and represent the most primitive design. In this case,

there are no specialized nutritive (trophic) cells to support oocyte development. Instead the oocyte directly absorbs food released by the surrounding follicular epithelium. This means that all germ cells can become oocytes. Panoistic ovarioles are found in Apterygota, Ephemera, Odonata, Orthoptera, and Siphonaptera.

In contrast, most insects have trophic cells whose primary function is to assist in oocyte development. These form meroistic ovaries that can be subdivided into two types. Telotrophic ovarioles (found in Hemiptera and Coleoptera) contain a cluster of trophic cells located near the germarium (in a region sometimes called the tropharium). These are linked by a nutritive cord to oocytes located some distance away in a separate cluster. The cord grows as the oocyte moves proximally, eventually breaking during vitellogenesis.

In polytrophic ovarioles, the trophic cells (also called nurse cells because they 'nurse' the oocyte) are grouped near the oocyte to provide necessary macromolecules. They are physically joined to each other and to the oocyte by cytoplasmic bridges. In some cases, the follicle layer surrounds both the nurse cells and the future oocyte, forming a composite egg chamber. The nurse cells eventually degenerate upon maturation of the oocyte. In the other case, the nurse cells form a separate chamber that lies adjacent to the oocyte they are nursing, creating an alternate arrangement of egg chambers and nutritive chambers in the ovariole.

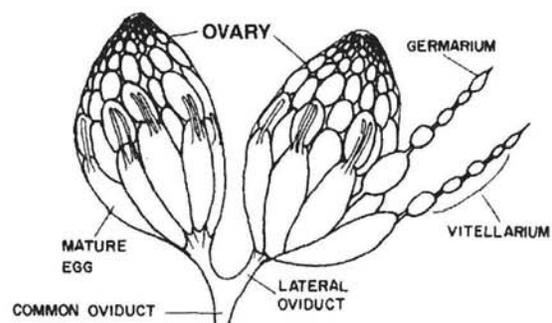


Fig. 741 Structure of the female gonads in *Drosophila*. The ovaries are shown from the dorsal side; two ovarioles are shown on the right. (From Mahowald, A. P., and M. P. Kambyzellis. 1980. Oogenesis. pp. 141–224 in M. Ashburner and T. R. F. Wright (eds.), *The genetics and biology of Drosophila*. Vol. 2c. Academic Press, London, United Kingdom.)

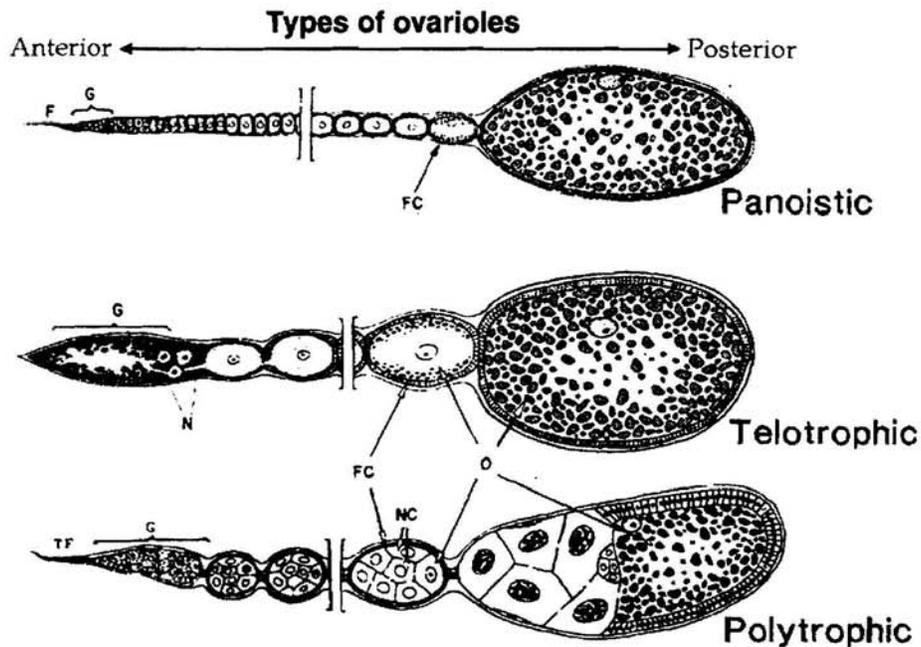


Fig. 742 Variations in the organization of insect ovarioles. A panoistic ovariole contain no specialized nutritive (trophic) cells to support oocyte development. Meroistic ovarioles are of two types. Telotrophic ovarioles contain a cluster of trophic cells (N) located near the germarium. In polytrophic ovarioles, the trophic cells or nurse cells (NC) are grouped near the oocyte to provide necessary macromolecules. FC: follicle cells. G: germarium. N: nutritive cord. NC: nurse cell. O: oocyte. TF: terminal filament. (Modified from Mahowald, A. P. 1972. Oogenesis. pp. 1-47 in Counce, S. J., and C. H. Waddington (eds.), *Developmental systems: Insects*. Vol. 1. Academic Press, London, United Kingdom.)

Major stages of oogenesis

What follows is a description of the major oogenic events using primarily examples from the fruit fly, *Drosophila melanogaster*. This provides an example of a polytrophic ovary in which the nurse cells are derived from the same germ cell lineage as the oocyte. The first step in oogenesis occurs when an oogonial stem cell undergoes an asymmetric division to produce a daughter stem cell (thereby maintaining the oogonial population) and the precursor to the egg called a cystoblast. In *Drosophila melanogaster*, the cystoblast undergoes a set of four synchronous mitotic divisions to product 16 daughter cells. The divisions are associated with incomplete cytokinesis such that intercellular bridges called ring canals connect the cells. This arrangement allows the rapid exchange of cytoplasmic material, hence the cell cluster can be seen as forming a syncytium or cyst with each interconnected cell called a cystocyte. During these divisions, one cystocyte is committed to become the oocyte and initiates meiosis, which arrests prior to the first meiotic division. The remaining 15 cystocytes differentiate into nurse cells.

The number of cystocyte divisions can vary between species, with as many as 127 nurse cells found in some eggs of *Carabus violaceus*, while the honey bee has 47. In some species, the number of divisions even within the same mother is variable, resulting in eggs with different nurse cell numbers. Variation can also occur in the origin of the nurse cells, as there are examples of some being derived from somatic cells, such as the follicular epithelium.

Once the cystocyte divisions are completed in *Drosophila*, a single layer of approximately 80 somatically derived follicle cells surround the oocyte and nurse cell syncytium to form the egg chamber. Within the next 30 hours, the follicle layer will undergo four mitotic divisions to give about 1,200 cells. The oocyte positions itself at the posterior end of the egg, while the nurse cells expand in size and undergo endomitosis (DNA replication without cell division). The result is a polyploid nucleus containing about 1,000 times the haploid DNA content. At their largest point the nurse cell nuclei expand to a volume about 2,000 times that of the original cystocyte nucleus. Polyploidy is believed to facilitate

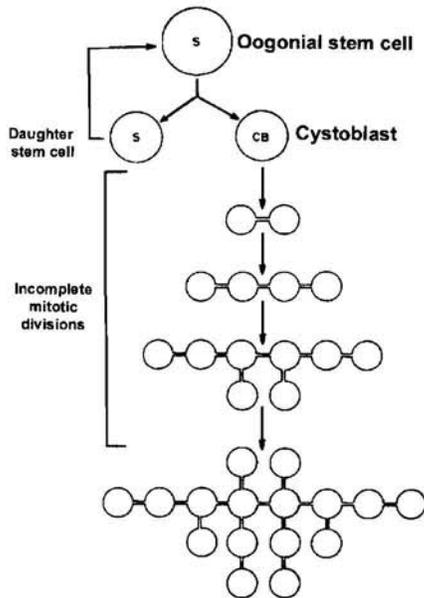


Fig. 743 Pattern of cell division in the formation of the *Drosophila* egg chamber. Cystocytes are connected by intercellular bridges called ring canals. (From King, R. C., J. D. Cassida, and A. Rousset. 1982. The formation of clones of interconnected during gametogenesis in insects. R. C. King and H. Akai (eds.), *Insect ultrastructure*. Plenum Press, New York, New York.)

the production of the massive amounts of macromolecules the nurse cells must produce to support oocyte development. In contrast, the oocyte nucleus remains arrested in meiosis and is mostly inactive.

Once the egg chamber has formed, it moves into the vitellarium where uptake of yolk proteins begins. Yolk is the principle component of the mature egg, making up about 90% of the total weight. It is a combination of lipids and protein that serves as a nutritive source for the developing embryo. About 60% or more of yolk proteins come from large molecules called vitellogenins made up of a complex of glycolipophosphoproteins. Vitellogenins are synthesized primarily by the fat bodies and are secreted into the hemolymph where they are selectively taken up by the oocyte. This process requires vitellogenin-specific receptors and is regulated by insect steroid hormones. Once in the oocyte, the vitellogenins are frequently processed to a more refined structure called vitellin. The absorption and deposition of vitellogenin results in a continuing increase in the size of oocyte. By the end of vitellogenesis, the oocyte will have increased in volume approximately 90,000-fold.

In addition to vitellogenesis, the oocyte receives substantial amounts of macromolecules and organelles from the nurse cells via the ring canals. These include ribosomes, mitochondria, and a large collection of regulatory and structural proteins and RNAs. Much of this movement is probably nonspecific and passive, but there is also evidence for specific transport of macromolecules. This includes a kinesin and microtubule-dependent process that selective moves regulatory RNAs and proteins to the oocyte. Near the end of oogenesis in *Drosophila*, there is a short period of extremely rapid transport in which the bulk of the nurse cell cytoplasmic contents flows into the oocyte. The oocyte expands from being 50% of egg volume to almost completely filling the available space in less than 30 minutes. This period of nurse cell 'dumping' is associated with nurse cell degeneration and requires the action of actin microfilaments.

Structure of the mature egg

The completion of vitellogenesis is associated with the secretion of the vitelline membrane by the follicle cells. The vitelline membrane is a rigid, protein-dense layer that efficiently protects the egg from desiccation and also has a role in embryonic pattern formation. Just above the vitelline membrane is a thin waxy layer followed by the chorion, which makes up the bulk of the eggshell. The chorion is composed of at least 20 proteins, many of which are evolutionarily conserved among insects. They form an intricate cross-linked array providing structural strength and rigidity. Choriogenesis also involves the formation of specialized eggshell structures. The micropyle is the opening through which the sperm enters and is located in the anterior end of the *Drosophila* egg. Near the micropyle are long dorsal filaments that extend outward. These are respiratory structures important for gas exchange. Also in this area is a weakened 'collar' called the operculum from which the larva will eventually hatch.

Studies in *Drosophila* and other insects have shown that the internal architecture of the egg is surprisingly complex. The egg displays both anterior-posterior and dorsal-ventral asymmetry with spatially localized collections of regulatory macromolecules. For example, determinants for the specification of germ cells are anchored at the posterior end of the egg while RNAs and proteins required for the patterning of the embryo are distributed in gradients along the anterior-posterior axis. Hence, the spatial

polarity of the egg influences the establishment of analogous polarity in the embryo. How this asymmetric distribution of regulatory factors occurs is a complex process involving extensive interactions between the nurse cells, oocyte, and surrounding follicle layer during oogenesis. Additional and more broadly distributed maternally contributed products include those that control early embryonic cell division, gene expression, the cytoskeletal network, and contribute to the determination of embryonic sex. For example, tubulin protein subunits make up about 3% of total egg protein while histone mRNA amounts to 2% of total maternal mRNAs.

Regulation of oogenesis

The regulation of oogenesis requires a complex interplay of interactions between the soma and germline. Direct contact with the somatic cells at the apical tip of the ovariole (the terminal filament) controls the asymmetric division of the oogonial stem cells in the germarium. Direct physical interactions between the follicle cells and oocyte are necessary to define the shape and polarity of the egg as well as control the differentiation and migration of the follicle cells. Long-range hormonal regulation of oogenesis occurs with juvenile hormone and ecdysteroids, which can alter yolk protein synthesis in the fat bodies and yolk protein uptake in the ovary. Both the maturation and release of eggs are also influenced by environmental factors, such as changes in light cycle, low temperature, and nutrition. These may also involve modulation of steroid hormone levels.

See also, HORMONAL REGULATION OF INSECT REPRODUCTION, EMBRYOGENESIS, JUVENILE HORMONE, ECDYSTEROIDS, DIA-PAUSE, STERILE INSECT TECHNIQUE.

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