

GENE ACTION IN INHERITANCE

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Summary

The sperm and egg, or the germ cells, are the specialized cells that can transmit genetic materials from one generation to the next in sexual reproduction. All the other cells of the body are somatic cells. This separation of germ and somatic cells is one of the oldest problems in developmental biology. In many animal groups, a specialized portion of egg cytoplasm, or germ plasm, is inherited by the cell lineage that gives rise to germ cells. This cell lineage is called the “germline.”

The germline progenitors eventually migrate into the gonads, where they differentiate as germ cells when the organisms are physically matured. Earlier investigators have demonstrated that germ plasm contains maternal factors required and sufficient for germline formation. In the fruit fly, *Drosophila*, this cytoplasm is histologically marked by the presence of polar granules, which act as a repository for the maternal factor required for germline formation.

Molecular screens have identified several factors stored in the polar granules. One of these factors is mitochondrial large rRNA, which functions to form the germline progenitors, or pole cells. The others are nanos mRNA and Pgc RNA, which are both required for pole cell differentiation. This article focuses on the molecular functions of these factors in germline formation.

1. Germline Development

In many organisms, the germline progenitors are formed in an embryonic region distinct from the gonads where they will eventually differentiate into germ cells. They move through and along different tissues to associate with somatic component of the gonad. In *Drosophila*, the germline is derived from pole cells, which are formed at the posterior pole of the embryo (Figure 1).

After fertilization, nine nuclear divisions take place without cytokinesis in the central yolk region of embryos (the cleavage stage). The nuclei then migrate to the periphery (the syncytial blastoderm stage). As the nuclei enter the posterior polar plasm (germ plasm), each of them is included in a cytoplasmic protrusion that contains germ plasm. These protrusions are segregated to become pole cells.

The nuclei penetrating the periplasm other than germ plasm divide four more times and are surrounded by the cell membrane to form somatic cells (the cellular blastoderm stage).

During the morphogenesis, the pole cells migrate through the midgut epithelium into the hemocoel to reach the embryonic gonads, and become primordial germ cells. During post-embryonic development, the primordial germ cells divide several more times to give rise to the stem cells, which later produce oocytes or sperm (Figure 2).

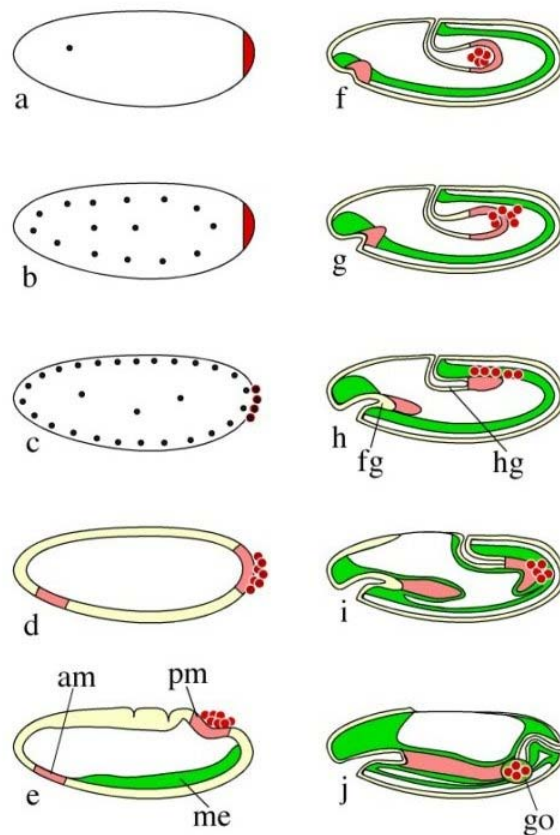


Figure 1. Schematic diagram of embryogenesis

Drosophila embryogenesis is divided into 17 stages (stages shown here follow the scheme proposed by Campos-Ortega and Hartenstein).

In a–c, black dots and red cytoplasm at the posterior poles represent nuclei and polar plasm, respectively. a, stage 1. b, stage 2 (the cleavage stage), the nuclei multiply in the central region of embryo without cytokinesis. c, stage 4 (the yncytial blastoderm stage), the nuclei migrate to the periphery of embryo.

At the posterior, pole cells (red) are formed. d, stage 5 (the cellular blastoderm stage), the nuclei at the periphery are surrounded by cell membrane and then cellularized. e, stage 7, pole cells are moved into embryo with the posterior midgut primordium (pm). am: posterior midgut primordium, me: mesoderm. f, stage 9, pole cells are in the pouch of the posterior midgut epitherium. g, stage 10, pole cells migrate through the midgut epitherium into the haemocoel. h, stage 11. fg: foregut, hg: hindgut. i, stage 12, pole cells are attached with the overlying mesoderm. j, stage 14, pole cells form gonads with the gonadal mesodermal cells.

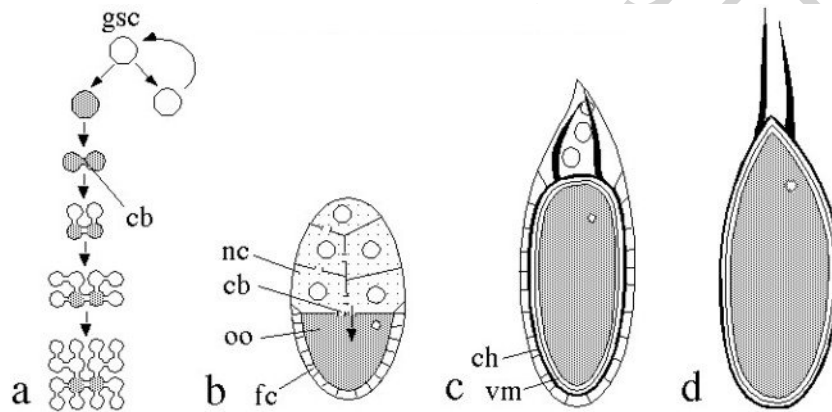


Figure 2. Schematic diagram of oogenesis

a, the germline stem cells (gsc) repeatedly divide at the tip of ovary. One of the two daughter cells becomes the germline stem cell again, and the other undergoes four rounds of mitosis with incomplete cytokinesis to produce 16 cells interconnecting with the cytoplasmic bridge (cb). b, stage 10 (stages are according to King), among the 16 cells, one is determined as oocyte (oo) and the remaining 15 become nurse cells (nc). Oocyte is surrounded by the follicle cells (fc). c, stage 13, the follicle cells produce the vitelline membrane (vm) and chorion (ch) around oocyte. d, stage 14 (mature oocyte).

2. Polar Granules, the Distinctive Organelles of Germ Plasm

In many animal groups, the factor required for germline establishment has been postulated to be localized in germ plasm. Experimental studies with frogs and *Drosophila* have demonstrated that factors with sufficient ability to establish germline are localized in germ plasm. Germ plasm is able to induce the germline, when it is transplanted into an ectopic region of embryos. Furthermore, transplantation of germ plasm, but no other cytoplasm, restores fertility to uv-sterilized embryos.

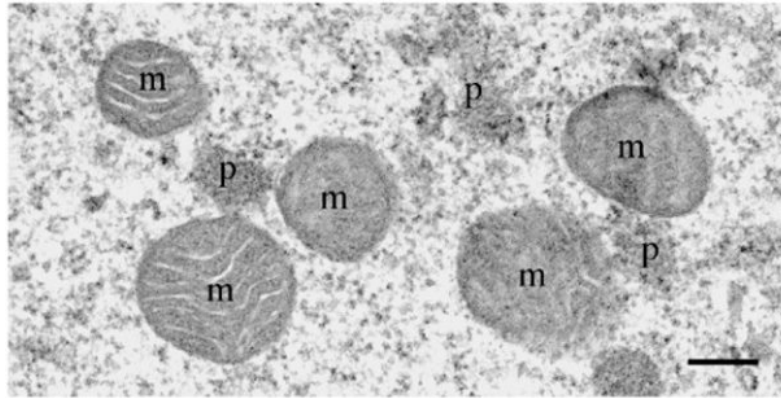


Figure 3. Electron micrograph of a section through polar plasm

p and m show polar granule and mitochondria, respectively. Polar granules are noted as electron-dense structures without being surrounded by membrane. Bar: 0.2 μm .

Within the polar plasm, specialized organelles called polar granules have been observed (Figure 3). Polar granules and their derivatives are present in the germline throughout most of the life cycle of *Drosophila*. In electronmicrographs, polar granules appear as electron-dense, fibro-granular structures. The granules of mature oocytes and early cleavage embryos are composed of RNA and proteins. The RNA disappears by the initiation of pole cells. Based on these observations, it is proposed that the RNA synthesized during oogenesis and stored in the polar granules is used for germline establishment. Thus the polar granules are regarded as a repository for the factors required for germline establishment.

3. Maternal Genes Required for Germ Plasm Assembly

Recent genetic screens have identified several maternally acting genes, or posterior class genes, such as *cappuccino* (*capu*), *spire* (*spir*), *staufer* (*stau*), *mago nash* (*mago*), *oskar* (*osk*), *vasa* (*vas*), *valois* (*vls*), and *tudor* (*tud*), whose functions are all required for germ plasm assembly. The mutation of any one of these genes is known to affect polar granule assembly as well as pole cell and abdomen formation. Among these genes, *osk* has a central role in the pathway leading to polar plasm assembly. Mislocalization of *osk* mRNA to the anterior pole leads to induction of polar granules as well as pole cells and abdomen at the ectopic site. Furthermore, the posterior class genes, *vas* and *tud*, are required downstream of *osk* for the induction of polar plasm at the ectopic site.

The recent molecular analysis of several posterior class genes has revealed that *osk*, *vas*, and *tud* all encode products that are components of polar granules. These products are synthesized in the nurse cells and later translocated to the posterior pole region of the oocytes during oogenesis. The first molecules to localize at the posterior pole of the oocytes are Staufen (Stau) protein and *osk* mRNA. Stau is essential for the transportation of *osk* mRNA to the posterior. Once *osk* mRNA is localized in the posterior region, it is translated *in situ* to produce Osk protein, which in turn directs the localization of Vas and Tud protein until stage 10 of oogenesis. These findings are compatible with the observation at the ultra-structural level that polar granules become

discernible at the posterior pole region of stage 10 oocytes.

The posterior cytoplasm taken from stage 13–14 oocytes can induce ectopic pole cell formation when injected into the anterior pole of recipient embryos, whereas the cytoplasm from stage 10–12 oocytes cannot. This clearly shows that some additional molecules other than Osk, Vas and Tud are needed for polar plasm function, and are accumulated in the posterior pole region of oocytes late in oogenesis.

Recent molecular analysis has identified four additional RNA species—*nanos* (*nos*) mRNA, *germ cell-less* (*gcl*) mRNA, mitochondrial large ribosomal RNA (mtlrRNA) mitochondrial small rRNA (mtrRNA) and *Polar granule component* (*Pgc*) RNA—that accumulate to the posterior pole region during late oogenesis and early embryogenesis. In contrast to Osk, Vas, and Tud, individual later localized RNAs are only required for a part of the polar plasm function.

4. Maternal Factors Required for Pole Cell Formation

4.1. mtlrRNA

The polar plasm contains mitochondria in addition to polar granules. Earlier ultrastructural studies have shown that both organelles become associated with each other at stages prior to pole cell formation, suggesting that mitochondria contribute to pole cell formation. *In situ* hybridization at the ultrastructural level has revealed that mtlrRNA and mtrRNA (mtrRNAs) are present on the surface of polar granules during the cleavage stage and are no longer localized on the granules in pole cells.

Prior to pole cell formation, mtrRNAs are released from polar granules, and degenerate without entering pole cells. Since mtrRNAs are encoded exclusively in mitochondrial genome and are transcribed *in situ*, it is reasonable to postulate that mtrRNAs are transported out of mitochondria to reach polar granules only in polar plasm. This transportation occurs after the completion of oogenesis.

No mtrRNAs are discernible on the polar granules in the mature oocytes (stage 14), unless the oocytes are activated within the oviducts. In freshly laid eggs at embryonic stage 1, polar granules and mitochondria are closely associated with each other and mtrRNAs are localized at the boundaries between them. At stage 2, when polar granules are detached from mitochondria, mtrRNAs remain associated with polar granules until pole cell formation.

mtlrRNA has been identified as a molecule that induces pole cells in embryos whose ability to form pole cells has been abolished by treatment with ultraviolet light. This observation suggests that mtlrRNA is required for pole cell formation.

This is further evidenced by the fact that reducing the amount of extra-mitochondrial mtlrRNA by injecting targeted ribozymes into polar plasm causes a failure to form pole cells. These observations show that the extra-mitochondrial mtlrRNA on polar granules has an essential role in pole cell formation, presumably cooperating with mtrRNA.

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Detailed information on the molecular traits and function of the gene products presented here are available through <<http://hedgehog.lbl.gov:7081/>>.

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Biographical Sketch

Satoru Kobayashi graduated from the University of Tsukuba in 1983, and from the Doctoral Program (Biological Sciences) of University of Tsukuba at 1988. He became Research Associate at the University

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