The Role of Mitochondrial rRNAs and Nanos Protein in Germline Formation in Drosophila Embryos

Authors: Kobayashi, Satoru, Sato, Kimihiro, and Hayashi, Yoshiki

Source: Zoological Science, 22(9) : 943-954

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.22.943

ZOOLOGICAL SCIENCE **22**: 943–954 (2005)

[REVIEW]

The Role of Mitochondrial rRNAs and Nanos Protein in Germline Formation in *Drosophila* Embryos

Satoru Kobayashi[1, 2*], Kimihiro Sato[1, 2] and Yoshiki Hayashi[1, 2]

[1]*Okazaki Institute for Integrative Bioscience, National Institute for Basic Biology, National Institutes of Natural Sciences, Higashiyama, Myodaiji, Okazaki 444-8787, Japan*
[2]*Core Research for Evolutional Science and Technology (CREST), Japan Science and Technology Agency*

ABSTRACT—Germ cells, represented by male sperm and female eggs, are specialized cells that transmit genetic material from one generation to the next during sexual reproduction. The mechanism by which multicellular organisms achieve the proper separation of germ cells and somatic cells is one of the longest standing issues in developmental biology. In many animal groups, a specialized portion of the egg cytoplasm, or germ plasm, is inherited by the cell lineage that gives rise to the germ cells (germline). Germ plasm contains maternal factors that are sufficient for germline formation. In the fruit fly, *Drosophila*, germ plasm is referred to as polar plasm and is distinguished histologically by the presence of polar granules, which act as a repository for the maternal factors required for germline formation. Molecular screens have so far identified several of these factors that are enriched in the polar plasm. This article focuses on the molecular functions of two such factors in *Drosophila*, mitochondrial ribosomal RNAs and Nanos protein, which are required for the formation and differentiation of the germline progenitors, respectively.

Keywords: germ plasm, germ cell, *Drosophila*, mitochondria, *nanos*

GERMLINE DEVELOPMENT IN *DROSOPHILA*

In many organisms, the germline progenitors are formed in an embryonic region distinct from the gonads, where they will eventually differentiate into germ cells. These cells move along different tissues to associate with the somatic component of the gonad. In *Drosophila*, the germline is derived from pole cells, which are formed at the posterior pole of the embryo (Zalokar and Erk, 1976; Technau and Campos-Ortega, 1986; Campos-Ortega and Hartenstain, 1997; Williamson and Lehmann, 1996; Santos and Lehmann, 2004) (Fig. 1). After fertilization, nine nuclear divisions take place in the absence of cytokinesis in the central yolk region of the embryo (the cleavage stage). The nuclei then migrate to the periphery (the syncytial blastoderm stage). The subsequent penetration of these nuclei into the posterior polar plasm (polar plasm, or germ plasm) leads to the formation of cytoplasmic protrusions known as pole buds, which then segregate to form pole cells (Fig. 1). The nuclei that penetrate the periplasm, which is distinct from the germ plasm, divide four more times and are then surrounded by the cell membrane to form somatic cells (the cellular blastoderm stage). During morphogenesis, the pole cells migrate through the midgut epithelium into the hemocoel, where they separate into two bilateral groups, condense in the embryonic gonads (Fig. 1), and differentiate into germ cells (Mahowald and Kambysellis, 1980; Lindsley and Tokuyasu, 1980; Williamson and Lehmann, 1996).

In many animal groups, the factors required for germline establishment have been postulated to be localized in germ plasm (Beams and Kessel, 1974; Eddy, 1975; Extavour and Akam, 2003). Experimental studies in frogs and in *Drosophila* have demonstrated that factors which are both necessary and sufficient to establish the germline are localized in the germ plasm. It has been shown that the germ plasm can induce the formation of the germline when transplanted into an ectopic region of an embryo (Illmensee and Mahowald, 1974, 1976; Ikenishi *et al.*, 1986). Furthermore, transplantation of germ plasm, but no other part of the egg cytoplasm, restores fertility to UV-sterilized embryo (Smith, 1966; Okada *et al.*, 1974). Within the germ plasm,

* Corresponding author. Phone: +81-564-59-5875
FAX : +81-564-59-5879
E-mail: skob@nibb.ac.jp

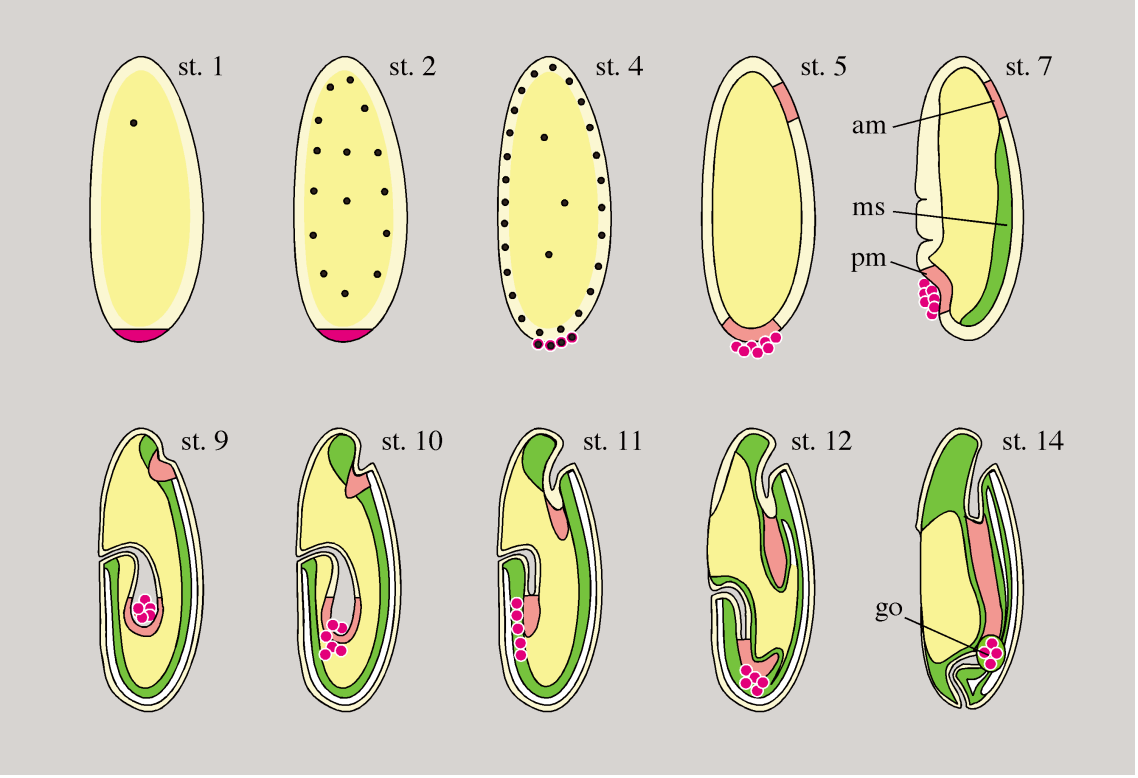

Fig. 1. Schematic representation of *Drosophila* embryogenesis. *Drosophila* embryogenesis is divided into 17 stages according to Campos-Ortega and Hartenstein (1997). **Stages1-4**: black dots and magenta cytoplasm at the posterior represent the nuclei and polar plasm, respectively. **Stage 2** (cleavage stage): the nuclei multiply in the central region of the embryo in the absence of cytokinesis. **Stage 4** (syncytial blastoderm stage): the nuclei migrate to the periphery of the embryo. In the posterior region, pole cells (magenta) are formed. **Stage 5** (cellular blastoderm stage): the nuclei at the periphery are surrounded by the cell membrane and then cellularized. **Stage 7**: pole cells migrate into the embryo with the posterior midgut primordium (pm); am, anterior midgut primordium; ms, mesoderm. **Stage 9**: pole cells are in the pouch of the posterior midgut epithelium. **Stage 10**: pole cells migrate through the midgut epithelium into the haemocoel. **Stage 11/12**: pole cells are attached to the overlying mesoderm. **Stage 14**: pole cells form gonads (go), together with the gonadal mesodermal cells.

specialized organelles known as polar granules have been identified, and these structures and their derivatives are present in the germline throughout most of the life cycle in *Drosophila*. In electron micrographs, polar granules appear as electron dense, fibro-granular structures (Mahowald, 1962, 1968, 1971a, 1992) (Fig. 2). The granular component of the germ plasm in mature oocytes and early cleavage embryos is composed of RNA and proteins. The RNA factors disappear by the time pole cells are formed, and it has therefore been proposed that maternal RNAs in the polar granules function during pole cell formation (Mahowald, 1968, 1971b). Hence, the polar granules are regarded as a repository of the factors required for germline establishment.

Assembly of the polar granules requires the function of maternal effect genes (Boswell and Mahowald, 1985; Lehmann and Nüsslein-Volhard, 1986; Schüpbach and Wieschaus, 1986; Manseau and Schüpbach, 1989; Boswell *et al*., 1991; Williamson and Lehmann, 1996; Mahowald, 2001; Starz-Gaiano and Lehmann, 2001; Santos and Lehmann, 2004). Among these, *oskar* (*osk*), *vasa* (*vas*) and *tudor* (*tud*) are all essential for the formation of pole cells. These genes produce proteins that localize at the polar granules in a stepwise and hierarchical manner (Hay *et al*., 1988; Ephrussi and Lehmann, 1992; Bardsley *et al*., 1993; Breitwieser *et al*., 1996; Williamson and Lehmann, 1996; Mahowald, 2001; Santos and Lehmann, 2004). These gene products are synthesized in the nurse cells and then later translocated to the posterior pole region of the oocytes during oogenesis. The first molecule to localize at the posterior pole of the oocyte is *osk* mRNA (Ephrussi *et al*., 1991; St Johnston *et al*., 1991). After *osk* transcript localizes at the posterior region, it is translated *in situ*, and its protein product directs the localization of Vas and Tud proteins until stage 10 of oogenesis (Ephrussi *et al*., 1991; Bardsley *et al*., 1993; Liang *et al*., 1994).

Mahowald *et al*. (1976) have reported that polar plasm from stage 13–14 oocyte can induce ectopic pole cell formation when injected into the anterior pole of recipient embryo, whereas cytoplasm from stage 10–12 oocyte does not exert this effect. This strongly indicates that additional molecules other than Osk, Vas and Tud are required for polar plasm function, and that these factors accumulate in the posterior pole region of oocyte, late in oogenesis. The completion of polar granule assembly is accompanied by the localization

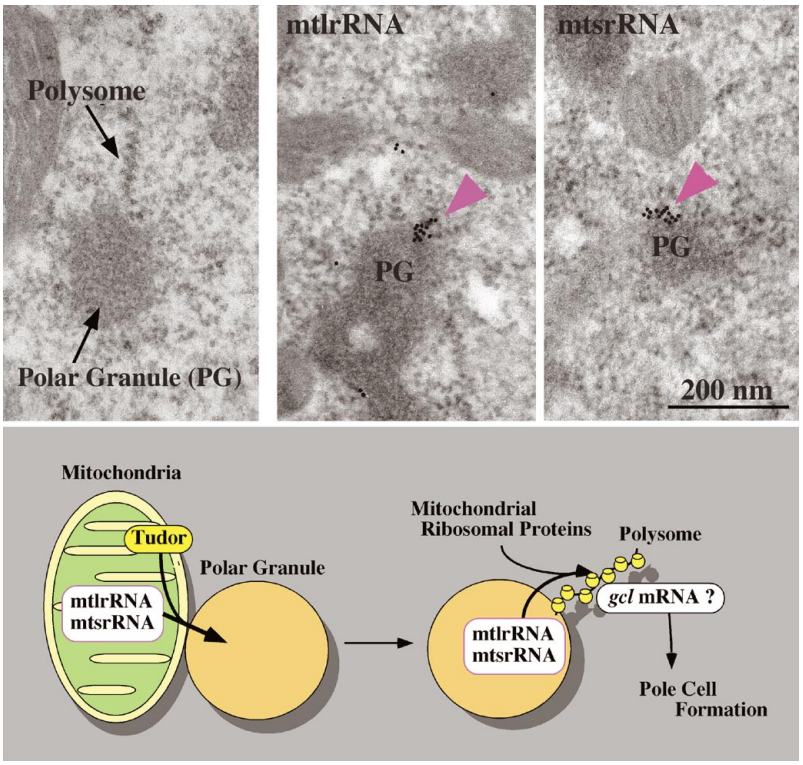

Fig. 2. Distribution of mitochondrial rRNAs in polar plasm. Electron micrographs showing a well-developed polysome on the surface of a polar granule at stage 2 (upper left), and sections hybridized with probes for mtlrRNA (upper middle) and mtsrRNA (upper right). Signals are arranged linearly from the surfaces of polar granules (arrowheads). The lower panel summarizes our results on the distribution and function of mtrRNAs (see text).

of various RNA species at the granules. These include mitochondrial ribosomal RNAs (mtrRNAs) and *germ cell-less* (*gcl*) mRNA, which are localized via the activities of *osk*, *vas,* and *tud* (Jongens *et al*., 1992; Kobayashi *et al*., 1993; Amikura *et al*., 1996; Kashikawa *et al*., 1999; Amikura *et al*., 2001a). In contrast to Osk, Vas and Tud, however, individual RNA molecules that are localized at the granules at a later stage are only required for a part of polar plasm function.

DISTRIBUTION OF MITOCHONDRIAL RIBOSOMAL RNAs IN THE POLAR PLASM

Mitochondria originated from an eubacterial symbiont and became functionally integrated into eukaryotic cells during evolution (Blackstone, 1995; Margulis, 1996). Whereas the primary roles of the mitochondria include oxidative phosphorylation and the biosynthesis of a number of metabolites, it has now become evident that they are also involved in cellular events that play critical roles in development. One remarkable example of this is their involvement in germline formation. Ultrastructural studies have previously shown that the germ plasm is primarily composed of germinal granules and mitochondria (Beams and Kessel, 1974; Eddy, 1975). Furthermore, earlier ultrastructural studies have shown that these two organelles form an association with each other prior to pole cell formation (Mahowald, 1962, 1968, 1971a, 1971b), suggesting that mitochondria contribute to this process. *In situ* hybridization studies at the ultrastructural level have further revealed that mtrRNAs, namely mitochondrial large ribosomal RNA (mtlrRNA) and mitochondrial small ribosomal RNA (mtsrRNA), are present on the surface of polar granules during the cleavage stage and are thus no longer localized on the granules in pole cells (Kobayashi *et al*.,1993; Amikura *et al*., 1996; Kashikawa *et al*., 1999) (Fig. 2). Since mtrRNAs are encoded exclusively by the mitochondrial genome and are transcribed *in situ*, it is reasonable to postulate that they are transported out of the mitochondria to the polar granules only in the polar plasm (Kobayashi and Okada, 1989; Kobayashi *et al*., 1993). This transportation occurs after the completion of oogenesis (Amikura *et al*., 1996; Kashikawa *et al*., 1999; Amikura *et al*., 2001a). No mtrRNAs are discernible on the polar granules in mature oocytes (stage 14), unless they are activated within the oviducts. In freshly laid eggs at embryonic stage 1, both the polar granules and the mitochondria are closely associated with each other, and the mtrRNAs are localized at the boundaries between them. At stage 2, when polar granules are detached from the mitochondria, mtrRNAs remain associated with polar granules until pole cell formation.

THE ROLE OF MITOCHONDRIAL RIBOSOMAL RNAs IN POLE CELL FORMATION

mtlrRNA has been identified as a molecule which restores the pole-cell-forming ability of embryo in which this has been abolished by treatment with UV (Kobayashi and Okada, 1989). This observation suggests that mtlrRNA is required for pole cell formation. This is supported by the fact that a reduction in the levels of extra-mitochondrial mtlrRNA, by injection of targeted ribozymes into the polar plasm, results in the failure to form pole cells (Iida and Kobayashi, 1998). These findings show that the extra-mitochondrial mtlrRNA on the polar granules has an essential role in pole cell formation, presumably in cooperation with mtsrRNA.

Since both mtrRNAs are major components of mitochondrial ribosomes, it has been speculated that they function to form ribosomes on the polar granules. This idea is consistent with a previously proposed model in which the transcripts encoding proteins that function in pole cell formation are stored in the polar granules and are translated on the developing polysomes at their surface (Mahowald, 1968, 1971b, 1992). Ultrastructural analysis has revealed that both mtrRNAs are localized at the polysomes that form on the surface of the polar granules during the short period prior to pole cell formation at stage 3 (Amikura *et al*., 2001b) (Fig. 2). Furthermore, the mitochondrial ribosomal proteins S12 and L7/L12 are enriched both in the polysomes at the polar granules and in mitochondria (Amikura *et al*., 2001b). Smaller ribosomes exist in the polysomes around the polar granules; they are almost identical in size to the mitochondrial ribosomes, but are smaller than those of the cytosol (Amikura *et al*., 2001b). These observations strongly suggest that mtrRNAs form mitochondrial-type ribosomes on polar granules, cooperating with mitochondrial ribosomal proteins.

Based on these previous findings, we now speculate that the mitochondrial-type ribosomes that are localized on polar granules are specifically required for the production of the proteins necessary for pole cell formation (Fig. 2). This idea is supported by our observations that inhibitors of mitochondrial (prokaryotic)-type translation, kasugamycin (KA) and chloramphenicol (CH), suppress pole cell formation when injected into early embryos (Amikura *et al*., 2005). Since KA is known to inhibit the initiation step of prokaryotic translation (Poldermans *et al*., 1979), it is expected that it would act to eliminate mitochondrial-type ribosomes from the polysomes. Indeed, KA treatment significantly decreases the number of mitochondrial, but not cytosolic, ribosomes around the polar granules.

mRNAs TRANSLATED BY MITOCHONDRIAL RIBOSOMES

The above observations strongly suggest that the impairment of pole cell formation by specific inhibitors is a result of the suppression of translation by mitochondrial-type ribosomes in the polar granule polysomes. We therefore speculate that the mRNAs encoding the proteins required for pole cell formation are translated on these ribosomes. The most probable candidate transcript is *germ cell-less* (*gcl*), which encodes a protein known to be necessary for pole cell formation (Jongens *et al*., 1992, 1994; Robertson *et al*., 1999). *gcl* mRNA is stored in the polar granules, and their translation is initiated at about stage 2 (Jongens *et al*., 1992; Amikura *et al*., 2005). Furthermore, this coincides with the appearance of mitochondrial ribosomes in the polar granule polysomes (Amikura *et al*., 2001b). As the nuclei penetrate the polar plasm, the Gcl protein becomes associated with them, and its localization persists around these pole cell nuclei until they begin migration. In mutant embryos lacking maternal *gcl* transcripts, pole cell formation and pole cell survival are disrupted (Jongens *et al*., 1992; Robertson *et al*., 1999). In addition, females overexpressing *gcl* mRNA produce progeny with an increased number of pole cells (Jongens *et al*., 1994).

In KA- and CH-treated embryos, the nuclear accumulation of Gcl is significantly impaired (Amikura *et al*., 2005), even in pole cells that are successfully formed, showing that these compounds inhibit its production. Since *gcl* mRNA accumulates at normal levels in the pole cells of KA- and CH-treated embryos, this inhibition must occur at the level of translation. In contrast, KA and CH do not affect the production of Nanos (Nos) protein. Maternal *nos* mRNA is enriched in the polar plasm (Wang *et al*., 1994) and is translated by cytosolic ribosomes immediately following fertilization (Amikura *et al*., 2005). Taken together, these results suggest that mitochondrial-type translation on polar granules is necessary for the production of proteins involved in pole cell formation, such as Gcl. Further studies will be required, however, to determine whether the translation of *gcl* transcripts uses the mitochondrial genetic code and whether the factors involved in translational initiation or polypeptide elongation in mitochondria also participate in these translational pathways.

TRANSPORT OF mtrRNAs FROM THE MITOCHONDRIA TO THE POLAR GRANULES

The transport of mtrRNAs from the mitochondria to the polar granules is a critical step in pole cell formation. It has been reported previously that the localization of mtrRNAs in polar plasm is impaired by mutations of any one of the maternal genes *osk, vas*, or *tud* (Ding *et al*., 1994; Kobayashi *et al*., 1995; Kashikawa *et al*., 1999). The most downstream of these genes, *tud*, encodes a protein that is localized in both the mitochondria and the polar granules (Bardsley *et al*., 1993). This observation leads to the hypothesis that the Tud protein may mediate the transport of mtrRNAs from the mitochondria to the polar granules. This idea is supported by a number of findings (Amikura *et al*., 2001a). In early embryo derived from *tud* mutant female, Vas protein is normally localized in the polar plasm, whereas

extra-mitochondrial mtrRNAs are undetectable throughout the cytoplasm. Consistent with this observation, *tud* mutant embryo contains polar granules, although their number and size are both reduced. These polar granules in the mutant are associated with mitochondria during the early cleavage stage, but no mtrRNA signals are detectable. In normal embryos, Tud protein and mtrRNAs colocalize at the boundaries between the mitochondria and polar granules, when the transport of mtrRNAs occurs. These ultrastructural data strongly suggest that Tud mediates the transport of mtrRNAs from the mitochondria to the polar granules. At present, however, it is not known how mtrRNAs move across the mitochondrial membranes, which are impermeable to macromolecules. In addition, it remains to be elucidated whether these rRNAs are transferred to the polar granules as ribosomes, and how this is mediated by Tud. Further studies will be required to identify the factors that interact with Tud, as this is likely to address some of these questions.

THE ROLE OF MITOCHONDRIA IN GERMLINE FORMATION IN *DROSOPHILA*

The above observations strongly suggest that there is an important functional role for mitochondria during germline formation. However, it remains unclear why the RNA molecules involved in germline development are encoded by the mitochondrial genome. As mitochondria are symbionts derived from ancestral microbes (Margulis, 1996), they have likely developed a strong association with the germline in order to propagate themselves to subsequent generations. An example of this is a *Rickettsia* observed to be incorporated into pole cells in *Drosophila* embryos (O'Neill and Karr, 1990). It is probable, therefore, that mitochondria have adopted an effective strategy for their survival, in which they produce factors to form the germline as a vehicle to carry them to the next generation. Alternatively, this mechanism might be a selective step to ensure that the germline progenitors are supplied with "intact" mitochondria. The mitochondrial genome has a high rate of mutation. It has been proposed that a bottleneck in the number of mitochondria that pass through the germline and a selection of hosts with fewer deleterious mutations are required for the maintenance of viable mitochondria (Bergstrom and Pritchard, 1998). During *Drosophila* oogenesis, a fraction of the mitochondria form aggregates known as Balbiani bodies that associate with the polar plasm, and these bodies may act as a mitochondrial bottleneck (Cox and Spradling, 2003). Subsequently, if the polar plasm contains mostly intact mitochondria, the pole cells that form will transmit these competent organelles to the next generation.

THE ROLE OF MATERNAL NANOS PROTEIN IN POLE CELL MIGRATION

The pole cells induced by mtlrRNA in UV-irradiated *Drosophila* embryo never develop into functional germ cells, suggesting that additional factors in the germ plasm are required and that these are essential for the differentiation of pole cells (Kobayashi and Okada, 1989). Nos, a CCHC zinc-finger protein, has been identified as the critical factor both for pole cell differentiation and abdomen formation (Lehmann and Nüsslein-Volhard, 1991; Wang and Lehmann, 1991; Wang *et al*., 1994; Kobayashi *et al*., 1996; Forbes and Lehmann, 1998; Arrizabalage and Lehmann, 1999). Maternally transcribed *nos* mRNA is concentrated in the polar plasm at a late stage of oogenesis via the actions of *osk* and *vas*. After egg laying, it is translated *in situ* to form a Nos protein gradient with the highest concentration in the polar plasm (Baker *et al*., 1992; Ephrussi and Lehmann, 1992; Smith *et al*., 1992; Wang *et al*., 1994; Thomson and Lasko, 2004). The Nos gradient then specifies the abdomen by repressing the translation of maternal *hunchback* (*hb*) mRNA, which otherwise inhibits abdomen formation (Tautz, 1988; Hülskamp *et al*., 1989; Irish *et al*., 1989; Struhl, 1989; Tautz and Pfeifle, 1989; Baker *et al*., 1992). Nos protein is only transiently present in the abdominal anlage, however, and becomes undetectable by the cellular blastoderm stage. In contrast, Nos protein in the polar plasm is incorporated into the pole cells and remains detectable throughout pole cell migration (Wang *et al*., 1994).

Pole cells that lack Nos protein are unable to develop into functional germ cells (Kobayashi *et al*., 1996; Forbes and Lehmann, 1998). Embryo derived from female homozygous for the *nos* mutation do form pole cells (*nos* pole cells), and when transplanted into normal embryo, these cells migrate through the midgut epithelium into the hemocoel; however, they are never incorporated into the gonads of the host embryo (Kobayashi *et al*., 1996) (Fig. 3). Furthermore, these mutant pole cells are unable to contribute to egg production in adult female (Kobayashi *et al*., 1996; Forbes and Lehmann, 1998). These results indicate that the autonomous deficiency of maternal *nos* activity in pole cells leads to their inability to penetrate into the gonads and, consequently, to their failure to become functional germ cells.

In the pathways leading to abdomen formation, Nos protein acts in concert with the RNA binding protein Pumilio (Pum), which is distributed ubiquitously in the embryo, to repress translation of maternal *hb* mRNA (Tautz, 1988; Hülskamp *et al*., 1989; Irish *et al*, 1989; Struhl, 1989; Tautz and Pfeifle, 1989; Baker *et al*., 1992). Translational repression of *hb* is mediated by discrete target sites known as *nos* response elements (NREs) in its 3' UTR (Wharton and Struhl, 1991; Wharton *et al*., 1998). Pum binds directly to the *hb* NREs in a sequence-specific manner, and the interaction of Nos with Pum is essential for the translational repression of *hb* (Murata and Wharton, 1995; Wharton *et al*., 1998; Sonoda and Wharton, 1999). In pole cells, Pum, in a similar manner to Nos, is autonomously required for pole cell migration (Asaoka-Taguchi *et al*., 1999) (Fig. 3). Thus, we speculate that Nos acts together with Pum to regulate germline-specific events in pole cells by repressing the translation of specific transcripts in these cells.

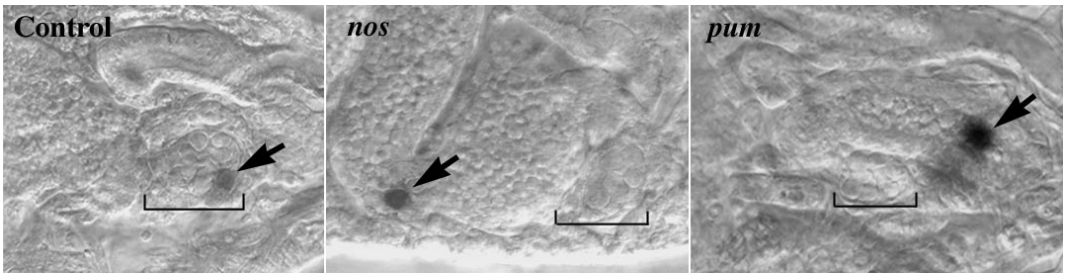

Fig. 3. Nos is essential in pole cells for their migration into the gonads. Photomicrographs showing pole cells (arrows) transplanted from control (normal) (left), *nos* (middle) and *pum* (right) embryos into host embryos. Control pole cells are observed within the gonad of the host at stage 15. In contrast, *nos* and *pum* pole cells are outside the gonads. Square brackets indicate the gonads.

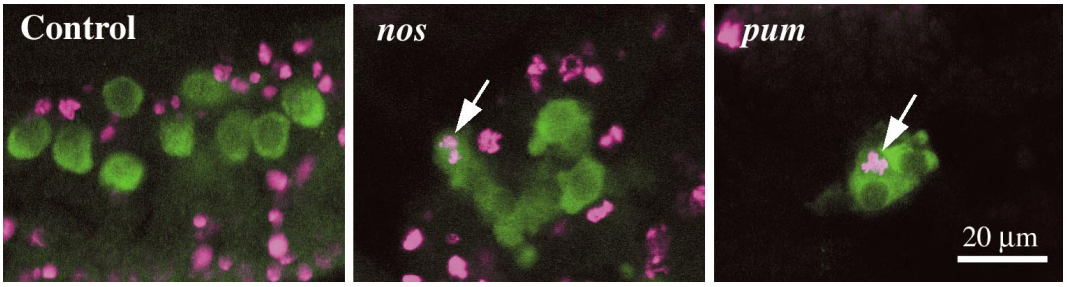

Fig. 4. Nos is required to repress mitosis of pole cells during their migration. Confocal images of migrating pole cells in control (left), *nos* (middle) and *pum* (right) embryos at stage 12, double-stained with antibodies against a phosphorylated form of histone H3 (PH3) (magenta) as a mitotic marker, and Vas (green) as a germline marker. Arrows show PH3-positive pole cells.

MITOTIC ARREST OF MIGRATING POLE CELLS BY MATERNAL NOS

One of the regulatory targets of both Nos and Pum in pole cells is maternal *cyclin B* (*cycB*) mRNA (Asaoka-Taguchi *et al*., 1999), which contains NRE-like sequences within its 3' UTR (Dalby and Glover, 1993). This transcript is localized in the polar plasm and is partitioned into the pole cells, but its translation is repressed until the pole cells reach the gonads (Dalby and Glover, 1993). Consistent with this observation, pole cells cease mitosis at gastrulation and remain quiescent in the G2 phase of the cell cycle, whereas somatic cells continue to proliferate. Moreover, in embryo lacking either Nos or Pum, the migrating pole cells produce CycB, and are then released from G2 arrest and enter into mitosis (Asaoka-Taguchi *et al*., 1999) (Fig. 4). Furthermore, the induction of CycB in wild-type pole cells is sufficient to drive them from the G2 phase through mitosis and into G1 (Asaoka-Taguchi *et al*., 1999). In addition, Nos and Pum bind *cycB* mRNA in NRE-dependent manner (Sonoda and Wharton, 2001). These findings clearly demonstrate that Nos and Pum inhibit the transition from G2 to mitosis in migrating pole cells by repressing CycB production, and this leads us to speculate that the inhibition of sequential cell cycling has an important role in early germline development. One possible role of this mechanism is to prevent dilution of the maternal factors that have been incorporated in the pole cells. Nos and Pum may thus repress the G2/M transition to maintain a sufficiently high concentration of these factors to facilitate proper pole cell migration and zygotic gene regulation. Since pole cells that are deficient in either Nos or Pum undergo a G1 arrest after mitosis, the G1/S transition may also be suppressed by another maternal factor(s) to ensure that these cells remain quiescent.

REPRESSION OF APOPTOSIS BY MATERNAL NOS

Pole cells lacking either Nos or Pum fail to properly migrate into the embryonic gonads. However, the repression of CycB by Nos and Pum is not required for pole cell migration, and its induction does not affect pole cell migration, although it does initiate a single round of mitosis (Asaoka-Taguchi *et al*., 1999). These findings suggest that CycB is not the only regulatory target of Nos and Pum in pole cells. Our observations (Hayashi *et al*, unpublished) further suggest that an additional target of Nos and Pum is *head involution defective* (*hid*) mRNA, which also contains an NRE in its 3' UTR and encodes a protein required for the induction of apoptosis (Grether *et al*., 1995). In the absence of Nos or Pum, migrating pole cells are eliminated by an apoptotic mechanism which is initiated at stage 9/10 in the developing embryo (Hayashi *et al*., 2004, unpublished) (Fig. 5). We have also found that *Df(3L)H99* (H99), a small deletion within the genomic region that includes the *hid* gene, suppresses apoptosis in *nos* pole cells (Hayashi *et al*., 2004). In embryo lacking both maternal Nos and zygotic H99 activity (*nos*-H99 embryo), there is no apoptotic death of any pole cells (Hayashi *et al*., 2004). In addition, and to our surprise, *nos*-H99 pole cells have the ability to migrate into the gonads when transplanted into normal host embryo

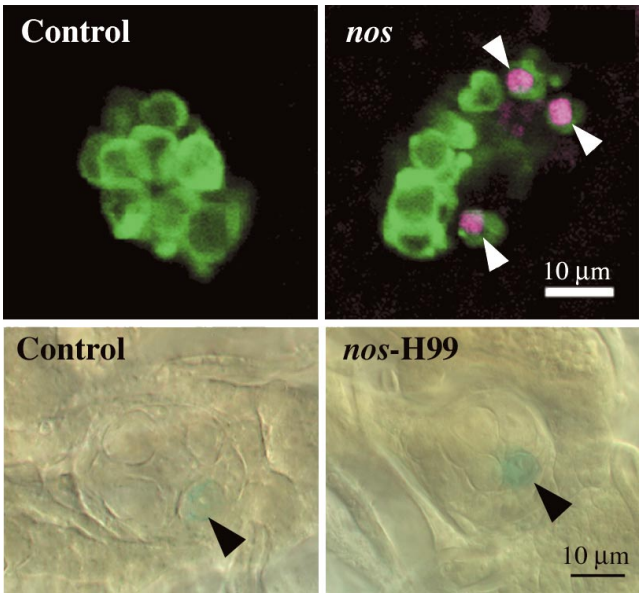

Fig. 5. Nos prevents apoptosis of pole cells. Confocal images of pole cells in control (upper left) and *nos* (upper right) embryos at stage 13, stained with TUNEL labeling (magenta) and an antibody against Vas (green). Arrowheads show TUNEL-positive pole cells. Lower panels: Photomicrographs showing pole cells (arrowheads) transplanted from control (lower left) and *nos*-H99 (lower right) embryos into host embryos. The transplanted control and *nos*-H99 pole cells are observed within the gonads of the host at stage 15-17.

(Hayashi *et al*., 2004) (Fig. 5). Hence, the ability of *nos* pole cells to migrate into the gonads is fully restored by the suppression of apoptosis in our transplantation experiments. This clearly demonstrates that Nos inhibits the apoptotic response in pole cells to permit their proper migration into the gonads.

The above observations suggest that pole cells have the potential to enter into apoptosis, which somewhat contradicts the notion that the germline is fundamentally immortal, as it is required for the propagation of any given species. We speculate, however, that this apoptotic pathway may be part of a mechanism that eliminates “aberrant pole cells” that have inherited an insufficient quantity of germ plasm components, such as maternal Nos protein.

nos-H99 pole cells that are incorporated within the embryonic gonads appear to be intact, as they express the Vas germline marker (Hayashi *et al*., 2004). However, they do not complete the gametogenic process, which suggests that maternal Nos has an additional function in the later stages of germline development (Hayashi *et al*., 2004). It has been reported that maternal *nos* activity is required for the formation of a germline-specific organelle, the spectrosome, that plays an important role in the asymmetric division of germline stem cells (Deng and Lin, 1997; Wawersik and Van Doren, 2005). Furthermore, zygotic Nos has been shown to be required by germline cells to prevent their premature entry into oogenesis during larval development (Wang and Lin, 2004). In larvae lacking zygotic Nos, the germline cells form premature cyst aggregates but fail to execute oogenesis and eventually degenerate. It is possible therefore that maternal Nos may also be required by the pole cells to repress their premature differentiation. Alternatively, the defect that characterizes *nos*-H99 pole cells could simply result from their failure to establish proper germline fates (see below).

TRANSCRIPTIONAL QUIESCENCE IN POLE CELLS

In addition to their mitotic arrest and migration to the gonads, pole cells can be distinguished by their transcriptional regulation. Pole cells are transcriptionally quiescent until the onset of gastrulation, whereas transcription is initiated in the soma during the syncytial blastoderm stage (Lamb and Laird, 1976; Zalokar and Erk, 1976; Kobayashi *et al*., 1988; Pritchard and Schbiger, 1996; Van Doren *et al*., 1998). Consistent with this, RNA polymerase II (RNAP II) remains inactive in early pole cells (Seydoux and Dunn, 1997; Leatherman and Jongens, 2003; Martinho *et al*., 2004). Furthermore, pole cells lack a subset of nucleosomal histone modifications, such as methylated lysine 4 on histone H3 (H3meK4), that correlates well with transcriptional ability (Schaner *et al*., 2003; Martinho *et al*., 2004). Hence, the ability to express zygotic mRNA-encoding genes is suppressed only in pole cells in early embryo.

Within pole cells, Nos is involved in maintaining transcriptional quiescence (Deshpande *et al*., 1999) and is also required for the maintenance of a germline-specific chromatin status that correlates with transcriptional inactivity (Schaner *et al*., 2003). In the absence of maternal Nos activity, somatic genes such as *fushi tarazu* (*ftz*), *even-skipped* (*eve*) and *Sex-lethal* (*Sxl*) are expressed ectopically in pole cells (Deshpande *et al*., 1999). In this instance, the phosphorylation of serine resides 2 and 5 in the carboxy-terminal domain (CTD) of RNAPII, both of which are required for transcriptional activation, and also the methylation of histone H3 on lysine 4 (H3meK4) are derepressed (Schaner *et al*., 2003; Deshpande *et al*., 2005). These findings indicate that Nos is a component of the mechanism that maintains transcriptional quiescence in pole cells.

We have found that maternal Nos, along with Pum, maintains transcriptional quiescence in pole cells by repressing the production of Importin-α2 (Impα2) protein (Asaoka *et al*., unpublished). Impα2 is a *Drosophila* homologue of Importin α required for the nuclear import of karyophilic proteins, including transcription factors, and *impα2* mRNA has an NRE-like sequence in its 3’ UTR (Török *et al*., 1995). At the blastoderm stage, Impα2 protein is distributed throughout the soma but not the pole cells, although *impα2* transcripts are detectable in pole cells. Moreover, the ectopic expression of Impα2 in pole cells causes nuclear import of a transcriptional factor, Ftz-F1, which in turn activates *ftz*. These data suggest that Nos and Pum repress somatic gene expression in pole cells by inhibiting nuclear import of transcriptional activators.

It is noteworthy that somatic genes are not activated in

every pole cell lacking maternal Nos. Increased H3meK4 signal and elevated phosphorylation of RNAPII CTD serines 2 and 5 are observed in a subset of *nos* pole cells (Deshpande *et al*., 1999, 2005; Schaner *et al*., 2003). These observations suggest that additional factors contribute to the transcriptional quiescence of pole cells. Indeed, Gcl and *Polar granule component* (*Pgc*) RNA have also now been shown to be required for transcriptional quiescence (Martinho *et al*., 2004; Letherman *et al*., 2002; Deshpande *et al*., 2004). In the absence of maternal *gcl* activity, the expression of the somatic genes, *sisterless A* (*sisA*) and *sisterless B* (*sisB*), and the phosphorylation of RNAPII CTD serine 5 can be detected ectopically in the nuclei of the pole buds (Leatherman *et al*., 2002). The failure of transcriptional repression thus appears to cause a defect in pole cell formation (Leatherman *et al*., 2002).

Immediately after pole cell formation, *Pgc* is required for transcriptional repression (Deshpande *et al*., 2004; Martinho *et al*., 2004). *Pgc* has been identified as a RNA that is highly concentrated in the polar plasm of cleavage embryo and is incorporated only into pole cells (Nakamura *et al*., 1996). During early pole cell development, *Pgc* represses somatic genes such as *zerknullt* (*zen*), *tailless* (*tll)* and *slow as molasses* (*slam*), and is also required for the suppression of both phosphorylation of CTD on serine 2 and methylation of histone H3 on lysine 4 (Martinho *et al*., 2004). *Pgc* RNA appears to act independently of Nos to repress transcription in early pole cells, as *eve* expression is still repressed in the absence of *Pgc* activity, and *zen* and *tll* are not activated in pole cells that lack Nos (Deshpande *et al*., 1999; Martinho *et al*., 2004). In contrast, in later pole cells, *Pgc* appears to be required for *nos* function, as a reduction in its activity decreases the concentration of *nos* mRNA and causes defects in pole cell migration and survival, similar to *nos* mutation (Nakamura *et al*., 1996).

REPRESSION OF THE SOMATIC DIFFERENTIATION OF POLE CELLS BY MATERNAL NOS

Previous findings lead us to speculate that pole cells lacking Nos may adopt a somatic cell fate. To test this hypothesis, *nos*-H99 pole cells are utilized, as most *nos* pole cells are eliminated by apoptosis in developing embryo. When transplanted into normal host embryo, *nos*-H99 pole cells are integrated within somatic tissues, such as the midgut epithelium, tracheal epithelium and gastric caeca (Hayashi *et al*., 2004) (Fig. 6). Furthermore, *nos*-H99 pole cells within the somatic tissues are observed to be morphologically indistinguishable from their neighboring host somatic cells. Moreover, these transplanted pole cells express somatic markers ectopically (Fig. 6). Conversely, the germline marker Vas is not detectable or is found to be significantly reduced in these transplanted cells. These results clearly show that *nos* pole cells can differentiate into somatic cells when their normal apoptotic pathways are suppressed.

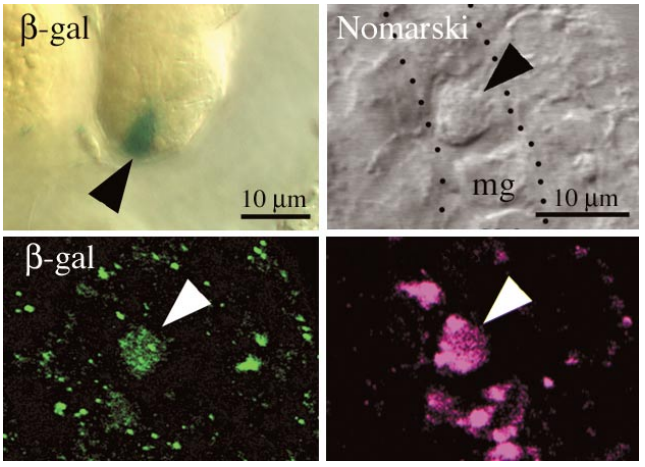

Fig. 6. *nos*-H99 pole cells are able to adopt somatic fate. Photomicrograph showing pole cells (upper left) transplanted from a *nos*-H99 embryo into a host embryo. The transplanted pole cells, identified by expression of β–galactosidase (β-gal), are integrated within the midgut epithelium of the host at stage 17. *nos*-H99 pole cells within the midgut epithelium (mg) of the host embryo at stage 14 (upper right) are able to express midgut marker genes (*CG11267*/*dGATAe*) (magenta) (lower right), as well as β-gal (green) (lower left). Arrowheads show *nos*-H99 pole cells integrated within the midgut epithelium.

These results also indicate that pole cells are multipotent, as they are capable of adopting both germline and somatic cell fates, and of undergoing apoptosis. Nos is required to repress the pathways that promote somatic differentiation and apoptosis, and thus to direct germline development. Consequently, the removal of Nos and H99 activities causes some pole cells to differentiate into soma. However, not all *nos*-H99 pole cells become somatic cells in these experiments. This suggests that they must be separable into two distinct types, those with and those without the ability to adopt a somatic cell fate. Apoptosis is suppressed in both types of pole cell by maternal Nos. When apoptosis is experimentally suppressed in Nos-negative pole cells by H99, the existence of these two populations of pole cells becomes evident. A possible alternative explanation may be that the different behaviors of *nos*-H99 pole cells are due to differences in the cellular environments encountered by them. The former explanation of pole cell behavior is supported by the observation that they possess Nos-independent transcriptional repression mechanisms (Deshpande *et al*., 1999, 2005; Schaner *et al*., 2003). Nos represses somatic gene expression in a subset of pole cells by suppressing Impα2 production (see above). We therefore propose that transcriptional derepression of pole cells is a prerequisite for their somatic differentiation. This is further supported by our preliminary data showing that the somatic differentiation of *nos*-H99 pole cells is suppressed by the reduction of Impα2 activity (Hayashi *et al*., unpublished).

THE WIDESPREAD ROLE OF NOS DURING GERMLINE FORMATION IN MULTICELLULAR ORGANISMS

The proper segregation of the germline and somatic line is a phylogenetically very old phenomenon and probably represents the primary step in the differentiation of multicellular organisms. This necessarily implies that molecules involved in germline establishment are highly and widely conserved in animal groups from invertebrates to vertebrates. Indeed, *nos*-like genes are widely conserved across the Metazoa and play an important role in germline development (Extavour and Akam, 2003; Extavour *et al*., 2005). In nematodes, zebrafish and mouse embryos, *nos* homologs are required for the maintenance of the germline progenitors (Subramaniam and Seydoux, 1999; Köprunner, *et al*., 2001; Tsuda *et al*., 2003). These results, and those we have presented here, indicate that *nos* is involved in evolutionarily conserved mechanisms that are required for germline maintenance. Moreover, in *C. elegans* and in *Drosophila*, *nos* is required for the establishment of germline-specific histone modifications that correlate with transcriptionally inactive chromatin (Schaner *et al*., 2003). We propose that *nos* also acts as part of a conserved mechanism that represses somatic gene expression and differentiation in order to establish the germ/soma dichotomy. It has also been reported that Pie1 and Blimp1 repress somatic programming in the germline progenitors to guide them towards germline development in nematode and mouse, respectively (Seydoux *et al*., 1996; Seydoux and Strome, 1999; Unhavaithaya *et al*., 2002; Ohinata *et al*., 2005). These data are consistent with the idea that germline cells are restricted to locations and/or stages that will exclude them from body patterning processes, and that the role of the germ plasm is to protect them from somatic development (Dixon, 1994).

There are thus at least two distinct modes of germline specification in animals (Dixon, 1994; Extabour and Akam, 2003). The germline is specified either by maternally inherited molecules (preformation), as in *Drosophila*, or by inductive signals from surrounding somatic tissues (epigenesis). The most striking example of epigenesis is seen in the mouse embryo, in which the primordial germ cells are specified in the proximal epiblast by signals from the neighboring extraembryonic tissues (Lawson *et al*., 1999). In mouse embryo, *nos* genes are zygotically expressed in the primordial germ cells (Tsuda *et al*., 2003). This is in contrast to *Drosophila*, where *nos* mRNA is maternally supplied to the embryos and is partitioned into pole cells (Wang *et al*, 1994). It has been proposed that epigenesis might be of early Metazoan origin, and that preformation might have then evolved from this ancestral mechanism (Extavour and Akam, 2003; Extavour *et al*., 2005). Further studies on the expression of *nos*-related genes and their functions during embryonic and post-embryonic development, in a variety of animal groups other than model organisms, will provide a better understanding of the evolution of epigenesis and preformation, as well as of the molecular mechanisms underlying germline specification.

ACKNOWLEDGMENTS

This work was supported in part by a grant from the Ministry of Education, Culture, Sports, Science, and Technology; by Research Project for Future Program funding from the Japan Society for the Promotion of Science; by a grant from the National Institute of Agrobiological Sciences; and by funding as a Core Research for Evolutional Science and Technology (CREST) project from the Japan Science and Technology Agency.

REFERENCES

Amikura R, Kobayashi S, Saito H, Okada M (1996) Changes in subcellular localization of mtlrRNA outside mitochondria embryogenesis of *Drosophila melanogaster*. Dev Growth Differ 38: 489–498

Amikura R, Hanyu K, Kashikawa M, Kobayashi S (2001a) Tudor protein is essential for the localization of mitochondrial RNAs in polar granules of *Drosophila* embryos. Mech Dev 107: 97–104

Amikura R, Kashikawa M, Nakamura A, Kobayashi S (2001b) Presence of mitochondria-type ribosomes outside mitochondria in germ plasm of *Drosophila* embryos. Proc Natl Acad Sci USA 98: 9133–9138

Amikura R, Sato K, Kobayashi S (2005) Role of mitochondrial ribosome-dependent translation in germline formation in *Drosophila* embryos. Mech Dev 122: 1087–1093

Arrizabalaga G, Lehmann R (1999) A selective screen reveals discrete functional domains in *Drosophila* Nanos. Genetics 153: 1825–1838

Asaoka-Taguchi M, Yamada M, Nakamura A, Hanyu K, Kobayashi S (1999) Maternal Pumilio acts together with Nanos in germline development in *Drosophila* embryos. Nat Cell Biol 1: 431–437

Bardsley A, McDonald K, Boswell RE (1993) Distribution of tudor protein in the *Drosophila* embryo suggests separation of functions based on site of localization. Development 119: 207–219

Barker DD, Wang C, Moore J, Dickinson LK, Lehmann R (1992) Pumilio is essential for function but not for distribution of the *Drosophila* abdominal determinant Nanos. Genes Dev 6: 2312–2326

Beams HW, Kessel RG (1974) The problem of germ cell determinant. Int Rev Cyt 39: 413–479

Bergstrom CT, Pritchard J (1998) Germline bottlenecks and the evolutionary maintenance of mitochondrial genomes. Genetics 149: 2135–2146

Blackstone N (1995) A units-of-evolution perspective on the endosymbiont theory of the origin of the mitochondrion. Evolution 49: 785–796

Boswell RE, Mahowald AP (1985) *tudor*, a gene required for assembly of the germ plasm in *Drosophila melanogaster*. Cell 43: 97–104

Boswell RE, Prout ME, Steichen JC (1991) Mutations in newly identified *Drosophila melanogaster* gene *mago nashi* disrupt germ cell formation and result in the formation of mirror-image symmetrical double abdomen embryos. Development 113: 373–384

Breitwieser W, Markussen FH, Horstmann H, Ephrussi A (1996) Oskar protein interaction with Vasa represents an essential step in polar granule assembly. Genes Dev 10: 2179–2188

Campos-Ortega JA, Hartenstain VE (1997) The embryonic development of *Drosophila melanogaster*. Springer-Verlag, Heidelberg

Cox RT, Spradling AC (2003) A Balbiani body and the fusome medi-

ate mitochondrial inheritance during *Drosophila* oogenesis. Development 130: 1579–1590

Dalby B, Glover DM (1993) Discrete sequence elements control posterior pole accumulation and translational repression of maternal *cyclin B* RNA in *Drosophila*. EMBO J 12: 1219–1227

Deng W, Lin H (1997) Spectrosomes and fusomes anchor mitotic spindles during asymmetric germ cell divisions and facilitate the formation of a polarized microtubule array for oocyte specification in *Drosophila*. Dev Biol 189: 79–94

Deshpande G, Calhoun G, Yanowitz JL, Schedl PD (1999) Novel functions of *nanos* in downregulating mitosis and transcription during the development of the *Drosophila* germline. Cell 99: 271–281

Deshpande G, Calhoun G, Schedl P (2004) Overlapping mechanisms function to establish transcriptional quiescence in the embryonic *Drosophila* germline. Development 131: 1247–1257

Deshpande G, Calhoun G, Jinks TM, Polydorides AD, Schedl P (2005) Nanos downregulates transcription and modulates CTD phosphorylation in the soma of early *Drosophila* embryos. Mech Dev 122: 645–657

Ding D, Whittaker KL, Lipshitz HD (1994) Mitochondrially encoded 16S large ribosomal RNA is concentrated in the posterior polar plasm of early *Drosophila* embryos but is not required for pole cell formation. Dev Biol 163: 503–515

Dixson K (1994) Evolutionary aspects of primodial germ cell formation. Ciba Foundation Symposium 182: 92–113

Eddy EM (1975) Germ plasm and differentiation of the germ line. Int Rev Cyt 43: 229–280

Ephrussi A, Lehmann R (1992) Induction of germ cell formation by *oskar*. Nature 358: 387–392

Ephrussi A, Dickinson LK, Lehmann R (1991) *oskar* organizes the germ plasm and directs localization of the posterior determinant *nanos*. Cell 66: 37–50

Extavour CG, Akam M (2003) Mechanisms of germ cell specification across the metazoans: epigenesis and preformation. Development 130: 5869–5884

Extavour CG, Pang K, Matus DQ, Martindale MQ (2005) *vasa* and *nanos* expression patterns in a sea anemone and the evolution of bilaterian germ cell specification mechanisms. Evol Dev 7: 201–215

Forbes A, Lehmann R (1998) Nanos and Pumilio have critical roles in the development and function of *Drosophila* germline stem cells. Development 125: 679–690

Grether ME, Abrams JM, Agapite J, White K, Steller H (1995) The *head involution defective* gene of *Drosophila melanogaster* functions in programmed cell death. Genes Dev 9: 1694–1708

Hay B, Ackerman L, Barbel S, Jan LY, Jan YH (1988) Identification of a component of *Drosophila* polar granules. Development 103: 625–640

Hayashi Y, Hayashi M, Kobayashi S (2004) Nanos suppresses somatic cell fate in *Drosophila* germ line. Proc Natl Acad Sci USA 101: 10338–10342

Hülskamp M, Schroder C, Pfeifle C, Jackle H, Tautz D (1989) Posterior segmentation of the *Drosophila* embryo in the absence of a maternal posterior organizer gene. Nature 338: 629–632

Iida T, Kobayashi S (1998) Essential role of mitochondrially encoded large rRNA for germ-line formation in *Drosophila* embryos. Proc Natl Acad Sci USA 95: 11274–11278

Ikenishi K, Nakazato S, Okuda T (1986) Direct evidence for the presence of germ-cell determinant in vegetal pole cytoplasm of *Xenopus laevis* and in a subcellular fraction of it. Dev Growth Differ 28: 563–568

Illmensee K, Mahowald AP (1974) Transplantation of posterior polar plasm in *Drosophila*. Induction of germ cells at the anterior pole cell of the egg. Proc Natl Acad Sci USA 71: 1016–1020

Illmensee K, Mahowald AP (1976) The autonomous function of germ plasm in a somatic region of the *Drosophila* egg. Exp Cell Res 97: 127–140

Irish V, Lehmann R, Akam M (1989) The *Drosophila* posterior-group gene *nanos* functions by repressing *hunchback* activity. Nature 338: 646–648

Jongens TA, Hay B, Jan LY, Jan YN (1992) The *germ cell-less* gene product: a posteriorly localized component necessary for germ cell development in *Drosophila*. Cell 70: 569–584

Jongens TA, Ackerman LD, Swedlow JR, Jan LY, Jan YN (1994) *germ cell-less* encodes a cell type-specific nuclear pore-associated protein and functions early in the germ-cell specification pathway of *Drosophila*. Genes Dev 8: 2123–2136.

Kashikawa M, Amikura R, Nakamura A, Kobayashi S (1999) Mitochondrial small ribosomal RNA is present on polar granules in early cleavage embryos of *Drosophila melanogaster*. Dev Growth Differ 41: 495–502

Kobayashi S, Okada M (1989) Restoration of pole-cell-forming ability to u.v.-irradiated *Drosophila* embryos by injection of mitochondrial lrRNA. Development 107: 733–742

Kobayashi S, Mizuno H, Okada M (1988) Accumulation and spatial distribution of poly(a)+RNA in oocytes and early embryos of *Drosophila melanogaster*. Dev Growth Differ 30: 251–260

Kobayashi S, Amikura R, Okada M (1993) Presence of mitochondrial large ribosomal RNA outside mitochondria in germ plasm of *Drosophila melanogaster*. Science 260: 1521–1524

Kobayashi S, Amikura R, Nakamura A, Saito H, Okada M (1995) Mislocalization of *oskar* product in the anterior pole results in ectopic localization of mitochondrial large ribosomal RNA in *Drosophila* embryos. Dev Biol 169: 384–386

Kobayashi S, Yamada M, Asaoka M, Kitamura T (1996) Essential role of the posterior morphogen *nanos* for germline development in *Drosophila*. Nature 380: 708–711

Köprunner M, Thisse C, Thisse B, Raz E (2001) A zebrafish *nanos*-related gene is essential for the development of primordial germ cells. Genes Dev 15: 2877–2885

Lamb MM, Laird CD (1976) Increase in nuclear poly(A)-containing RNA at syncytial blastoderm in *Drosophila melanogaster* embryos. Dev Biol 52: 31–42

Lawson KA, Dunn NR, Roelen BA, Zeinstra LM, Davis AM, Wright CV, Korving JP, Hogan BL (1999) Bmp4 is required for the generation of primordial germ cells in the mouse embryo. Genes Dev 13: 424–436

Leatherman JL, Jongens TA (2003) Transcriptional silencing and translational control: key features of early germline development. Bioessays 25: 326–335

Leatherman JL, Levin L, Boero J, Jongens TA (2002) *germ cell-less* acts to repress transcription during the establishment of the *Drosophila* germ cell lineage. Curr Biol 12: 1681–1685

Lehmann R, Nüsslein-Volhard C (1986) Abdominal segmentation, pole cell-formation, and embryonic polarity require the localized activity of *oskar*, a maternal gene in *Drosophila*. Cell 47: 141–152

Lehmann R, Nüsslein-Volhard C (1991) The maternal gene *nanos* has a central role in posterior pattern formation of the *Drosophila* embryo. Development 112: 679–691

Liang L, Diehljones W, Lasko P (1994) Localization of Vasa protein to the *Drosophila* pole plasm is independent of its RNA-binding and helicase activities. Development 120: 1201–1211

Lindsley DL, Tokuyasu KT (1980) Spermatogenesis. In the genetics and biology of *Drosophila*. Academic Press, London

Mahowald AP (1962) Fine structure of pole cells and polar granules in *Drosophila melanogaster*. J Exp Zool 151: 201–215

Mahowald AP (1968) Polar granules of *Drosophila* II. Ultrastructural changes during early embryogenesis. J Exp Zool 167: 237–262

Mahowald AP (1971a) Polar granules of *Drosophila* III. The continuity of polar granules during life cycle of *Drosophila*. J Exp Zool 176: 329–343

Mahowald AP (1971b) Polar granules of *Drosophila* IV. Cytochemi-

cal studies showing loss of RNA from polar granules during early stages of embryogenesis. J Exp Zool 176: 345–352
Mahowald AP (1992) Germ plasm revisited and illuminated. Science 255: 1216–1217
Mahowald AP (2001) Assembly of the *Drosophila* germ plasm. Int Rev Cytol 203: 187–213
Mahowald AP, Kambysellis MP (1980) Oogenesis. In the genetics and biology of *Drosophila*. Academic Press, London
Mahowald AP, Illmensee K, Turner FR (1976) Interspecific transplantation of polar plasm between *Drosophila* embryos. J Cell Biol 70: 358–373
Manseau LJ, Schüpbach T (1989) *cappuccino* and *spire* — two unique maternal-effect loci required for both the anteroposterior and dorsoventral patterns of the *Drosophila* embryo. Genes Dev 3: 1437–1452
Margulis L (1996) Archaeal-eubacterial mergers in the origin of Eukarya: phylogenetic classification of life. Proc Natl Acad Sci USA 93: 1071–1076
Martinho RG, Kunwar PS, Casanova J, Lehmann R (2004) A noncoding RNA is required for the repression of RNApolIII-dependent transcription in primordial germ cells. Curr Biol 14: 159–165
Murata Y, Wharton RP (1995) Binding of Pumilio to maternal *hunchback* mRNA is required for posterior patterning in *Drosophila* embryos. Cell 80: 747–756
Nakamura A, Amikura R, Mukai M, Kobayashi S, Lasko PF (1996) Requirement for a noncoding RNA in *Drosophila* polar granules for germ cell establishment. Science 274: 2075–2079
O'Neill SL, Karr TL (1990) Bidirectional incompatibility between conspecific populations of *Drosophila* simulans. Nature 348: 178–180
Ohinata Y, Payer B, O'Carroll D, Ancelin K, Ono Y, Sano M, Barton SC, Obukhanych T, Nussenzweig M, Tarakhovsky A *et al*. (2005) Blimp1 is a critical determinant of the germ cell lineage in mice. Nature 436: 207–213
Okada M, Kleinmann IA, Schneiderman HA (1974) Restoration of fertility in sterilized *Drosophila* eggs by transplantation of polar cytoplasm. Dev Biol 37: 43–54
Poldermans B, Goosen N, Van Knippenberg PH (1979) Studies on the function of two adjacent N6,N6-dimethyladenosines near the 3' end of 16 S ribosomal RNA of Escherichia coli. I. The effect of kasugamycin on initiation of protein synthesis. J Biol Chem 254: 9085–9089
Pritchard DK, Schubiger G (1996) Activation of transcription in *Drosophila* embryos is a gradual process mediated by the nucleocytoplasmic ratio. Genes Dev 10: 1131–1142
Robertson SE, Dockendorff TC, Leatherman JL, Faulkner DL, Jongens TA (1999) *germ cell-less* is required only during the establishment of the germ cell lineage of *Drosophila* and has activities which are dependent and independent of its localization to the nuclear envelope. Dev Biol 215: 288–297
Santos AC, Lehmann R (2004) Germ cell specification and migration in *Drosophila* and beyond. Curr Biol 14: 578–589
Schaner CE, Deshpande G, Schedl PD, Kelly WG (2003) A conserved chromatin architecture marks and maintains the restricted germ cell lineage in worms and flies. Dev Cell 5: 747–757
Schüpbach T, Wieschaus E (1986) Germline autonomy of maternal-effect mutations altering the embryonic body pattern of *Drosophila*. Dev Biol 113: 443–448
Seydoux G, Dunn MA (1997) Transcriptionally repressed germ cells lack a subpopulation of phosphorylated RNA polymerase II in early embryos of *Caenorhabditis elegans* and *Drosophila melanogaster*. Development 124: 2191–2201
Seydoux G, Strome S (1999) Launching the germline in *Caenorhabditis elegans*: regulation of gene expression in early germ cells. Development 126: 3275–3283
Seydoux G, Mello CC, Pettitt J, Wood WB, Priess JR, Fire A (1996) Repression of gene expression in the embryonic germ lineage of *C. elegans*. Nature 382: 713–716
Smith LD (1966) The role of "germinal cytoplasm" in the formation of primordial germ cells in *Rana pipiens*. Dev Biol 14: 330–347
Smith JL, Wilson JE, Macdonald PM (1992) Overexpression of *oskar* directs ectopic activation of *nanos* and presumptive pole cell formation in *Drosophila* embryos. Cell 70: 849–859
Sonoda J, Wharton RP (1999) Recruitment of Nanos to *hunchback* mRNA by Pumilio. Genes Dev 13: 2704–2712
Sonoda J, Wharton RP (2001) *Drosophila* Brain tumor is a translational repressor. Genes Dev 15: 762–773
St Johnston D, Beuchle D, Nüsslein-Volhard C (1991) *staufen*, a gene required to localize maternal RNAs in the *Drosophila* egg. Cell 66: 51–63
Starz-Gaiano M, Lehmann R (2001) Moving towards the next generation. Mech Dev 105: 5–18
Struhl G (1989) Differing strategies for organizing anterior and posterior body pattern in *Drosophila* embryos. Nature 338: 741–744
Subramaniam K, and Seydoux, G. (1999) *nos-1* and *nos-2*, two genes related to *Drosophila* nanos, regulate primordial germ cell development and survival in *Caenorhabditis elegans*. Development 126: 4861–4871
Tautz D (1988) Regulation of the *Drosophila* segmentation gene *hunchback* by two maternal morphogenetic centres. Nature 332: 281–284
Tautz D, Pfeifle C (1989) A non-radioactive *in situ* hybridization method for the localization of specific RNAs in *Drosophila* embryos reveals translational control of the segmentation gene *hunchback*. Chromosoma 98: 81–85
Technau GM, Campos-Ortega JA (1986) Lineage analysis of transplanted individual cells in embryos of *Drosophila melanogaster* III. Commitment and proliferative capabilities of pole cells and midgut progenitors. Rouxs Arch Dev Biol 195: 489–498
Thomson T, Lasko P (2004) *Drosophila tudor* is essential for polar granule assembly and pole cell specification, but not for posterior patterning. Genesis 40: 164–170
Török I, Strand D, Schmitt R, Tick G, Török T, Kiss I, Mechler BM (1995) The overgrown hematopoietic organs-31 tumor suppressor gene of *Drosophila* encodes an Importin-like protein accumulating in the nucleus at the onset of mitosis. J Cell Biol 129: 1473–1489
Tsuda M, Sasaoka Y, Kiso M, Abe K, Haraguchi S, Kobayashi S, Saga Y (2003) Conserved role of *nanos* proteins in germ cell development. Science 301: 1239–1241
Unhavaithaya Y, Shin TH, Miliaras N, Lee J, Oyama T, Mello CC (2002) MEP-1 and a homolog of the NURD complex component Mi-2 act together to maintain germline-soma distinctions in *C. elegans*. Cell 111: 991–1002
Van Doren M, Williamson A, Lehmann R (1998) Regulation of zygotic gene expression in *Drosophila* primordial germ cells. Curr Biol 8: 243–246
Wang C, Lehmann R (1991) Nanos is the localized posterior determinant in *Drosophila*. Cell 66: 637–647
Wang C, Dickinson LK, Lehmann R (1994) Genetics of nanos localization in *Drosophila*. Dev Dyn 199: 103–115
Wang Z, Lin H (2004) Nanos maintains germline stem cell self-renewal by preventing differentiation. Science 303: 2016–2019
Wawersik M, Van Doren M (2005) *nanos* is required for formation of the spectrosome, a germ cell-specific organelle. Dev Dyn 234: 22–27
Wharton RP, Struhl G (1991) RNA regulatory elements mediate control of *Drosophila* body pattern by the posterior morphogen *nanos*. Cell 67: 955–967
Wharton RP, Sonoda J, Lee T, Patterson M, Murata Y (1998) The Pumilio RNA-binding domain is also a translational regulator.

Mol Cell 1: 863–872
Williamson A, Lehmann R (1996) Germ cell development in *Drosophila*. Annu Rev Cell Dev Biol 12: 365–391
Zalokar M, Erk I (1976) Division and migration of nuclei during early embryogenesis of *Drosophila melanogaster*. J Microsc Biol Cell 25: 97–106

(Received August 18, 2005 / Invited Review)