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Review

Localization of mitochondrial large rRNA in germinal granules and the consequent segregation of germ line

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ABSTRACT This article reviews recent studies on cytoplasmic factors for germ-line establishment in *Drosophila* and *Xenopus*. In a variety of animal groups, the cytoplasmic factors for germ-line differentiation have been postulated to be localized in the germ plasm. We have found that mitochondrial large rRNA (mtlrRNA) is present in germinal granules, the distinctive morphological markers of germ plasm in the fruit-fly and the frog. MtlrRNA has been identified as a cytoplasmic factor that induce pole cells, the progenitor of the germ line, in uv-sterilized *Drosophila* embryos. The developmental stages of *Xenopus* at which mtlrRNA is present in germinal granules correspond to the stages of germ line segregation. Based on our findings and available data on posterior class gene products as well as on classical developmental biology of germ cells, we discuss the probable role of mtlrRNA in germ-line segregation from the somatic line in these animals.

KEY WORDS: *pole cells, germ cells, germ plasm, Drosophila, Xenopus*

Introduction

The early developmental processes produce topologically localized, diversely committed cell lineage. In many animals, localized cytoplasmic factors, called determinants, have been postulated to play an essential role in the commitment of early embryonic cell lineage (for review see Davidson, 1986). One of the well studied examples is the determination of the germ line. In many animal groups, the factor required for germ-line establishment has been postulated to be localized in a histologically remarkable region in egg cytoplasm, or germ plasm (for reviews see Beams and Kessel, 1974; Eddy, 1975). Experimental studies with frogs and the fruit-fly *Drosophila*, have demonstrated that factors with sufficient ability to establish germ line are localized in germ plasm. Germ plasm is able to induce the germ line, when it is transplanted into an ectopic region of embryos (Illmensee and Mahowald, 1974, 1976; Ikenishi *et al.*, 1986). Furthermore, transplantation of germ plasm, but no other cytoplasm, restores fertility to the uv-sterilized embryos (Smith, 1966; Okada *et al.*, 1974).

Recently, we have found in *Drosophila melanogaster* that mitochondrial large ribosomal RNA (mtlrRNA) can restore the progenitor of the germ line in uv-sterilized embryos, and that mtlrRNA is a component of germ plasm. This leads to the idea that mtlrRNA is transported out of mitochondria into cytosol in germ plasm (Kobayashi and Okada, 1989; Kobayashi *et al.*, 1993a). Furthermore, we found that extra-mitochondrial mtlrRNA is restricted to germ plasm of early *Xenopus* embryos. These findings indicate that mtlrRNA is a common component of germ plasm in *Drosophila* and *Xenopus*, and probably plays a key role in germ-line establishment in these animals. This article focuses on cytological events commonly observed in germ-line establishment in the fruit-fly, frogs and other animals, and discusses possible roles of mtlrRNA in these events.

Drosophila melanogaster

Pole cell formation

In *Drosophila*, the germ line is derived from pole cells, which are formed at the posterior pole of the embryo (Illmensee, 1973; Illmensee and Mahowald, 1974, 1976; Okada *et al.*, 1974; Underwood *et al.*, 1980; Technau and Campos-Ortega, 1986). 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

Abbreviations used in this paper: mtlrRNA, mitochondrial large ribosomal RNA.

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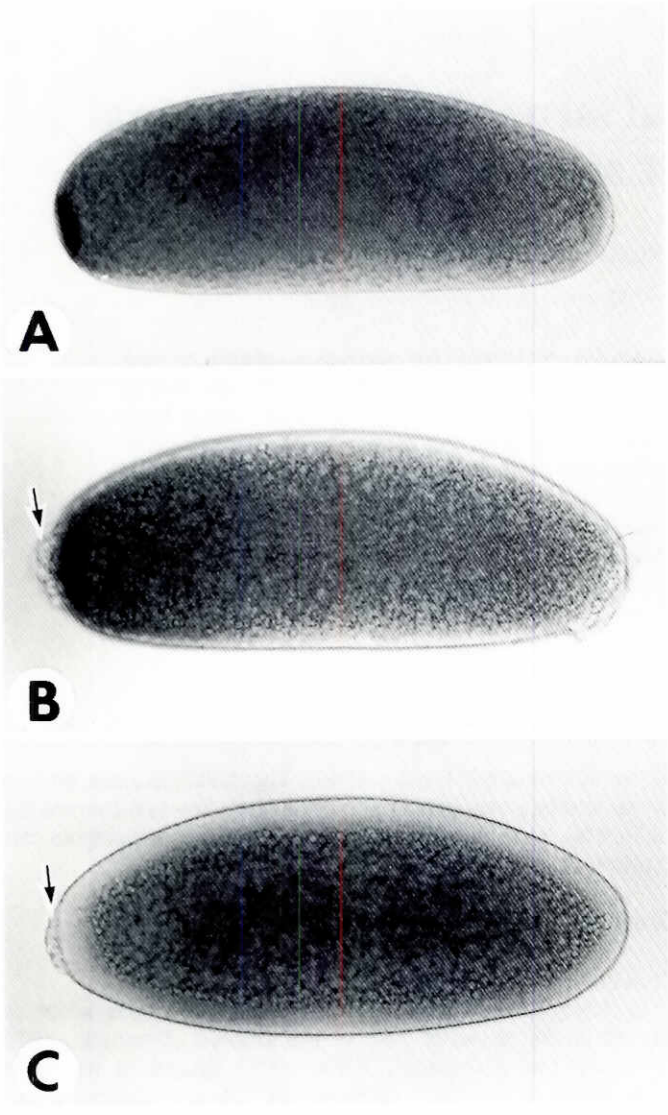

Fig. 1. Distribution of mtlrRNA in early *Drosophila* embryos. *In situ hybridization of embryos at the cleavage stage* **(A)**, *the pole cell formation stage* **(B)**, *the syncytial blastodermal stage* ***(C)***. *In all panels, anterior to the right. Arrows point to pole cells.*

development, the primordial germ cells divide several more times to give rise to the stem cells, which later produce oocytes or sperm (Lindsley and Tokuyasu, 1980; Mahowald and Kambysellis, 1980).

Several lines of evidence indicate that germ plasm includes the factors required for pole cell formation and subsequent differentiation of pole cells. Cytoplasmic transplantation experiments have demonstrated that germ plasm is sufficient to induce the germ line at ectopic locations in embryos (Illmensee and Mahowald, 1974, 1976). Further evidence in support of the importance of germ plasm is produced from ultraviolet (uv) irradiation experiments. When a cleavage embryo is irradiated with uv at the posterior pole region, where germ plasm is located, the embryo fails to form pole cells at the blastodermal stage (Okada *et al.*, 1974; Togashi and Okada, 1983). The adults developing from the embryos without pole cells are sterile. However, transplantation of intact germ plasm into the posterior pole restores fertility to the uv-sterilized embryos (Okada *et al.*, 1974).

Polar granules, histological and functional markers of germ plasm

Polar granules were noted as the distinctive organelles of germ plasm and as being accompanied with the germ line (Counce, 1973). Mahowald verified with electron microscopy that polar granules and their derivatives are present in the germ line throughout most of the life cycle of *Drosophila*. In electron micrographs, polar granules appear as electron dense, fibro-granular structures (Mahowald, 1962, 1968, 1971a, 1992). The granular components of the germ plasm of mature oocytes and early cleavage embryos are composed of RNA and proteins. The RNA disappears by the initiation of pole cells (Mahowald, 1971b). Based on these observations, Mahowald proposed that the RNA synthesized during oogenesis and stored in the polar granules is used for germ-line establishment or the development of posterior region of embryos (Mahowald, 1968, 1971b). Further evidence to support the view that polar granules are associated with determinants came from genetic analyses of seven maternally acting genes, or the posterior class genes *(cappuccino (capu), spire (spir), staufen (stau), mago nash (mago), oskar (osk), vasa (vas), valois (vls),* and *tudor (tud))* (Boswell and Mahowald, 1985; Lehmann and Nüsslein-Volhard, 1986; Schüpbach and Wieschaus, 1986; Manseau and Schüpbach, 1989; Boswell *et al.*, 1991; for reviews see Lehmann, 1992; Mahowald, 1992; Lehmann and Rongo, 1993). The mutation of any one of these genes is known to affect polar granule assembly as well as pole cell and abdominal pattern formation. Thus polar granules are regarded as associating with both pole cell factors and the abdominal factor. The factor for abdomen formation is encoded by a posterior class gene *nanos (nos)*, mutation of which results in the failure to form abdomens but affects neither polar granule nor pole cell formation (Lehmann and Nüsslein-Volhard, 1991; Wang and Lehmann, 1991). However, none of the posterior class genes so far cloned has been shown to encode a specific factor that is sufficient on its own to cause pole cell formation.

Mitochondrial lrRNA is a component of polar granules

Mitochondrial large ribosomal RNA (mtlrRNA) has been identified as a cytoplasmic factor that induces pole cell formation in embryos whose pole cell formation ability has been abolished with uv irradiation (Kobayashi and Okada, 1989). The temporal and spatial distribution of mtlrRNA is determined by *in situ* hybridization for light and electron microscopy (Kobayashi *et al.*, 1993a). The *in situ* hybridization technique used in this study enables us to detect only mtlrRNA outside mitochondria, since the mtlrRNA probe hardly permeates mitochondria under the condition used (Amikura *et al.*, 1994). In cleavage embryos, the mtlrRNA signal is strongest in germ plasm, and very weak in the other regions (Fig. 1A). During and after pole cell formation, the mtlrRNA signal is hardly detectable in pole buds and pole cells, but it is prominent in periplasm beneath pole cells instead (Fig. 1B). After a late syncytial-blastoderm or cellular-blastoderm stage, the localized mtlrRNA signal is no longer discernible (Fig. 1C). *In situ* hybridization at ultrastructural level reveals that mtlrRNA is present on the surface of polar granules in germ plasm of cleavage embryos (Fig. 2D). In contrast, no mtlrRNA signal is found in polar granules in pole cells of syncytial blastoderm embryos. These results clearly show the existence of mtlrRNA outside mitochondria particularly on the polar granules during the cleavage stage. Since mtlrRNA is encoded

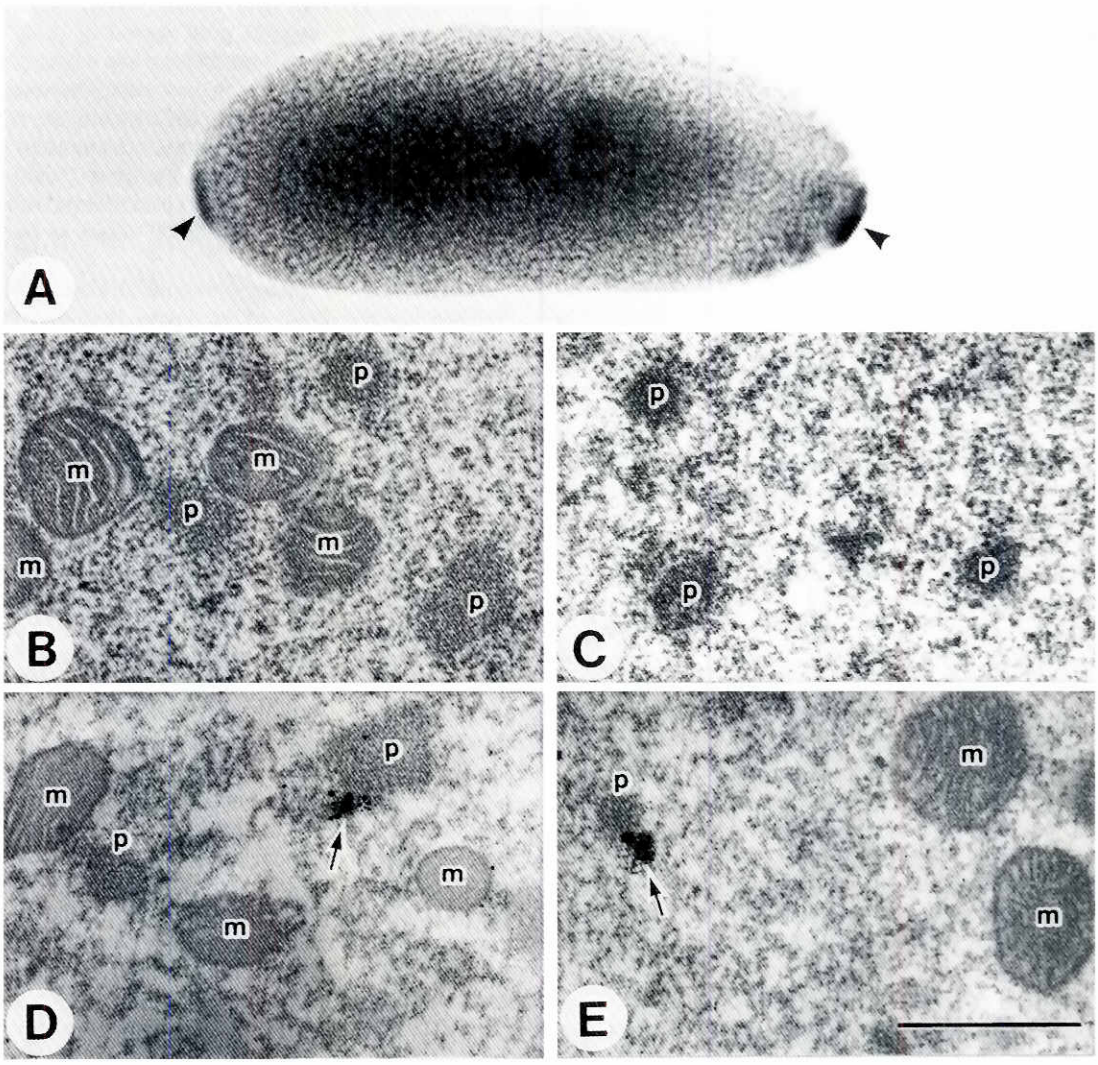

Fig. 2. Distribution of mtlrRNA in posterior polar plasm of cleavage embryos from wild type females, and in the anterior region of late cleavage embryos from osk-bcd3'UTR females. *The osk-bcd3'UTR females produce embryos with osk mRNA and protein mislocalized to the anterior pole region. In these embryos, pole cells are formed in the ectopic region (Ephrussi and Lehmann, 1992).* **(A)** *Distribution of mtlrRNA in osk-bcd3'UTR embryos. MtlrRNA signal is observed at both poles (arrowheads). Posterior to the left.* **(B,D)** *Electron micrographs of sections through posterior polar region of wild-type embryos hybridized with mtlrRNA probe* **(D)** *and embryos not processed for in situ hybridization* **(B)**. **(C,E)** *Electron micrographs of anterior polar regions of osk-bcd3'UTR embryos hybridized with mtlrRNA probe* **(E)** *and osk-bcd3'UTR embryos not processed for in situ hybridization* **(C)**. *In situ hybridization was carried out according to the protocol by Amikura et al. (1994). Arrows show mtlrRNA signal; p, polar granules; m, mitochondria. Bar, 0.5 mm.*

exclusively in mitochondrial genome and is transcribed *in situ* (Kobayashi and Okada, 1989), it is reasonable to postulate that mtlrRNA is transported out of mitochondria to reach polar granules only in germ plasm. This probably occurs prior to the cleavage stage, and mtlrRNA is released from the polar granules during pole cell formation.

Transportation of mtlrRNA from mitochondria to polar granules depends on the function of posterior class genes, since the mutation of any one of the seven posterior class genes *(capu, spir, stau, osk, vas, vls, tud)* disrupts localization of extra-mitochondrial mtlrRNA in germ plasm (Ding, Whittaker and Lipshitz, personal communication). Recently it has been shown that mislocalization of osk RNA to the anterior pole leads to induction of functional pole cells at the ectopic site. Furthermore, among the seven posterior class genes, only two, *vas* and *tud*, are required for the induction of pole cells at the ectopic site (Ephrussi and Lehmann, 1992). In the anterior pole cytoplasm where osk is mislocalized, polar granules are observed, as in the posterior pole (Bardsley *et al.*, 1993; observations by Amikura). Extra-mitochondrial mtlrRNA is concentrated at both poles of the embryos (Fig. 2A). *In situ* hybridization for electron microscopy shows that mtlrRNA is present on the anterior polar granules (Fig. 2E) (Amikura, unpublished data). These results show that the transportation of mtlrRNA is regulated by the function of *osk*, *vas* and *tud* genes. In immuno-electron microscopic studies the protein encoded by *tud* gene is reported to be present in mitochondria as well as polar granules

during the cleavage stage (Bardsley *et al.*, 1993). In contrast, vas and osk proteins are found only in polar granules (Hay *et al.*, 1988; Lehmann and Rongo, 1993). So far tud gene is the only posterior class gene of which product is found in mitochondria. These findings lead us to the hypothesis that transport of mtlrRNA from mitochondria to polar granules is mediated by tud protein, although interaction between mtlrRNA and tud protein in mitochondria or in polar granules remains to be tested.

MtlrRNA is one of the cytoplasmic factors required for germ-line establishment

The pole cell forming factor(s) is anticipated to satisfy the following conditions: to be crucial for pole cell formation; to be regulated by the seven posterior class genes for its localization in germ plasm; to have no effect on abdomen formation. We propose that extra-mitochondrial mtlrRNA is a candidate for a pole cell forming factor, based on the following evidence. First, the extra-mitochondrial mtlrRNA is localized on the surface of polar granules in germ plasm of cleavage embryos (Kobayashi *et al.*, 1993a). The mutation in any one of the seven posterior class genes disrupts localization of the extra-mitochondrial mtlrRNA, but a mutation in *nos*, which specifies the abdomen, never affects the localization of mtlrRNA. Second, in embryos from *Bicaudal-D (Bic-D)* females, the mtlrRNA is enriched only in the posterior pole region (Kobayashi *et al.*, 1993a). Bic-D embryos develop an ectopic abdomen with reverse polarity at the expense of head and thorax, but form pole cells only at the posterior pole (Mohler and Wieschaus, 1986). This fits the idea that mtlrRNA is indispensable for pole cell formation but dispensable for abdomen formation. Third, uv irradiation of germ plasm at a dose sufficient to prevent pole cell formation but not enough for affecting abdomen formation reduces or abolishes extra-mitochondrial mtlrRNA in germ plasm (Kobayashi *et al.*, 1993a). Finally, mtlrRNA is able to rescue embryos from uv-caused pole cell formation inability, when injected (Kobayashi and Okada, 1989).

We have previously reported that the pole cells induced by mtlrRNA in the uv-irradiated posterior region never develop into germ cells (Kobayashi and Okada, 1989). This may suggest a requirement for an additional factor that is localized in germ plasm and is essential for differentiation of pole cells. Furthermore, failure of pole cell induction at the anterior pole by mtlrRNA leads to the idea that pole cell formation requires not only mtlrRNA but also an additional factor localized in germ plasm. The recently reported gene *germ cell-less (gcl)* is a likely candidate for a gene encoding the additional factor required for pole cell formation (Jongens *et al.*, 1992). Maternal *gcl* RNA is localized in germ plasm, and is incorporated in pole cells. The localization depends on the posterior class genes required for pole cell formation. Although no mutation in *gcl* gene has been identified, embryos with reduced *gcl* RNA caused by the administration of antisense RNA fail to form pole cells. These characteristics reported for *gcl* products suggest that *gcl* plays an important role in pole cell formation.

Xenopus laevis

Description of germ-line development

In anuran development, germ plasm has been convincingly shown to be required for germ-line development. Blastomeres that inherit the germ plasm during the cleavage stage migrate to the genital ridge, and become primordial germ cells (Whitington and Dixon, 1975; Ressen and Dixon, 1988). Anuran germ plasm assembles in the vegetal pole region of early embryos. After fertilization germ plasm is partitioned into 4 blastomeres by the first 2 cleavage divisions. Although the 4 blastomeres continue to divide 10-11 more times during the rest of the cleavage stage, the number of cells including germ plasm does not increase (Whitington and Dixon, 1975). After gastrulation, the germ plasm-containing cells start to increase in number in the endodermal region. These cells then migrate dorsally to the genital ridges to become primordial germ cells.

As in *Drosophila*, the experimental studies on anuran eggs have provided several lines of evidence that germ plasm has the autonomous capacity of canalizing a cell into germ line. Smith (1966) showed that uv irradiation of *Rana* eggs at the vegetal pole during early cleavage stages caused absence of primordial germ cells from the genital ridges of the irradiated embryos. Transplantation of vegetal cytoplasm, including germ plasm, restores the capability of forming primordial germ cells to the irradiated embryos. If single somatic blastomeres isolated from 32-cell *Xenopus* embryos are injected with germ plasm, then implanted into host embryos, the injected cells migrate into the genital ridges and differentiate as primordial germ cells (Ikenishi *et al.*, 1986). This strongly suggests that germ plasm is able to change the fate of a given blastomere into a germ-line cell. On the contrary, Wylie *et al.* (1985) reported that germ plasm-bearing cells were reversible in their developmental fate. They demonstrated that the germ plasm-bearing cells isolated from their migratory route in tadpoles can differentiate into a variety of cell types when implanted into an ectopic region of a late blastula. This result may indicate that the role of germ plasm is restricted to migration of the germ plasm-bearing cells to the genital ridges, rather than extended to determination of their developmental fate. Indeed, uv-irradiation to the vegetal pole region affects the migratory behavior of germ plasm-bearing cells in anuran eggs (Züst and Dixon, 1975, 1977; Williams and Smith, 1984). Of course, we can not rule out the possibility that some components of germ plasm act in migration and subsequently some others play to determine germ cells at a later stage. In *Drosophila*, pole cells differentiate into nothing other than the germ line (Underwood *et al.*, 1980; Technau and Campos-Ortega, 1986).

The similarities between *Drosophila* and frogs are noted in the structure of germ plasm (Mahowald and Hennen, 1971; Czolowska, 1972; Beams and Kessel, 1974; Ikenishi *et al.*, 1974; Eddy, 1975; Ikenishi and Kotani, 1975). The germ plasm of *Rana* and *Xenopus* embryos is principally composed of mitochondria and electron dense bodies, called germinal granules, which are very similar to the polar granules of *Drosophila*. In cleavage embryos and blastulae, germinal granules are associated with mitochondria and appear to contain some RNA. To understand the function of the granules, further information is needed concerning the molecular nature of the RNA in these granules.

Localization of mtlrRNA in germ plasm

The similarities between *Drosophila* and frogs in the structures of germ plasm raise a question whether mtlrRNA is localized in germinal granules during early development of frogs. We have examined the localization of mtlrRNA in *Xenopus* embryos with *in situ* hybridization for light and electron microscopy, and found that mtlrRNA is present on germinal granules exclusively at the stages from 4-cell to blastula (Kobayashi *et al.*, in preparation). Thus

mtlrRNA is a common component of germ plasm in *Xenopus* and *Drosophila*. This RNA probably plays a key role in germ-line development in both these animals. However, some difference should be noted. In *Drosophila*, mtlrRNA disappears from the polar granules after pole cell formation, while in *Xenopus* the RNA remains associating with the germinal granules until blastula. To understand these observations, we need further cytological information about germ-line cells at the stages when mtlrRNA is present on these cytoplasmic granules. In *Xenopus*, segregation of germ line from somatic lines takes place during the developmental stages from 4-cell to blastula. As mentioned above, germ plasm is partitioned into 4 blastomeres by the first 2 cleavage divisions. At subsequent cleavages, germ plasm always associates with only one of two poles of a mitotic spindle. This means that germ plasm is included in only one of the two daughter cells. Consequently, the number of germ plasm-bearing cells in an embryo is maintained at four through 10-11 rounds of cleavage mitoses. In contrast, during and after gastrulation, both daughter cells receive germ plasm after a mitosis, resulting in an increase in the number of germ plasm-bearing cells in an embryo (Whitington and Dixon, 1975). It is worthwhile to note that mtlrRNA localizes in germ plasm only at the stages when germ plasm is sequestered in either of daughter cells at every mitosis. A similar situation is observed in *Drosophila*. Segregation of germ plasm in pole cells is initiated shortly before the disappearance of mtlrRNA from germ plasm. Besides, we know that mtlrRNA in germ plasm is required for pole cell formation (Kobayashi and Okada, 1989).

Orientation of a mitotic spindle in a cell is determined by the position of the centrosomes, which function as the microtubule-organizing centers (for review see Strome, 1993). In the germ plasm-bearing cells of *Xenopus* embryos, one of the two centrosomes or the aster seems to associate with germ plasm, ensuring that one pole of the mitotic spindle associates with germ plasm (Whitington and Dixon, 1975). In contrast to *Xenopus*, at pole cell formation in *Drosophila*, no mitotic spindle appears. However, it has been reported that centrosomes but not nuclei are responsible for pole cell formation (Raff and Glover, 1989). In aphidicolin-injected embryos, the centrosomes migrate apart from nuclei to the cortex, although migration of nuclei is completely inhibited. The centrosomes migrating into germ plasm can reorganize the cortical cytoskeleton to initiate pole cell formation in the absence of nuclei. Neither somatic cell formation nor pole-cell-type cell formation is initiated by the centrosomes in periplasm in the somatic region. Thus, it is very probable that interaction between centrosomes and germ plasm is required for proper segregation of germ plasm into a limited number of cells, both in *Drosophila* and in *Xenopus*, although the process of the segregation is quite different between these animals. The fact that extra-mitochondrial mtlrRNA is present in germ plasm only at the stages when germ plasm segregation occurs leads us to the idea that mtlrRNA mediates the interaction between centrosomes and germ plasm.

An interesting case of pole cell formation has been reported in Miaster, a kin of *Drosophila* (for review see Beams and Kessel, 1974). In this insect, first 2 nuclear divisions take place without cytokinesis in the central yolk mass as in *Drosophila*. At the third nuclear division, one nucleus reaches the posterior pole where the nucleus proceeds to mitosis. One of the poles of the mitotic spindle gets associated with germ plasm. As a result, one of the resulting daughter nuclei becomes embedded in germ plasm, then is sequestered with germ plasm in a pole cell. This type of cytokinesis in *Miaster* pole cell formation seems very similar to the cleavage mitosis of germ plasm-bearing cells in *Xenopus* embryos. Localization of mtlrRNA in germ plasm of *Miaster* embryos remains to be tested.

Germ plasm segregation in other animal groups

The intimate association between germ plasm and centrosomes during the segregation of germ plasm is widely observed in the animal kingdom. Germ plasm that is identified as a histologically remarkable region of ooplasm is described as exclusively transferred to germ line in many animal groups, such as Arthropoda, Nematoda, Annelida, Molluska, Chaetognatha and Chordata (for review see Eddy, 1975). In some animals, e.g. *Miaster, Drosophila, C. elegans, Cyclops* and *Xenopus*, interaction between germ plasm and centrosomes has been reported during the segregation of germ plasm (Beams and Kessel, 1974). A correlation between the orientation of spindles and the distribution of granules that define the germ line has been well documented in a nematode *C. elegans*. In this animal, cytoplasmic granules termed P granules, detectable with immunofluorescence microscopy, are segregated into the germ line or P lineage during early cleavages (Strome and Wood, 1982). P granules are electron dense structures comparable to the polar granules of *Drosophila* and germinal granules of *Xenopus* (Wolf *et al.*, 1983). The function of the P granules in germ-line development remains elusive, partly because the genes encoding the components of the granules have not been identified. P granules are dispersed throughout the embryo at the time of fertilization, and are localized in the posterior pole region by the time pronuclei meet (Strome and Wood, 1983). At the first mitosis, the nucleus accompanying a pair of centrosomes rotates 90° so that the resulting mitotic spindle is oriented along the antero-posterior axis (Albertson, 1984). As the mitosis proceeds, the spindle is shifted posteriorly resulting in the posterior pole of the spindle being much closer to the posterior pole of the embryo (Strome and Wood, 1983). The rotation of centrosome-nuclear complex and subsequent posterior shift of the spindle bring about a larger anterior cell (AB cell) and a smaller posterior cell (P1 cell) in which P granules, gathering at the posterior pole, are incorporated. This process is repeated at every subsequent mitosis of P lineage, consequently P granules are finally sequestered in P4 (Strome and Wood, 1982).

These observations seemingly suggest that direct or indirect interaction between P granules and one of the two centrosomes causes asymmetric cell divisions. However, there is no causal relationship between P granule localization and the rotation of centrosomes in P lineage (Strome and Wood, 1983; Hyman and White, 1987). This rotation is mediated by shortening of microtubules extending from centrosomes to the anterior cortex of the cells, since laser ablation of the cytoplasmic region between the centrosome and the anterior cortex inhibits the rotation (Hyman, 1989). Hill and Strome (1990) claim that the unequal division of the P1 cell is coupled with P granule partitioning, based on their observations that P granules are always sequestered in smaller daughter cells in cytochalasin-D treated embryos, in which the pattern of cell division and P granule distribution are altered. Perhaps, there is a mechanism controlling both the spindle positioning and P granule segregation.

Prospects for the role of mtlrRNA

MtlrRNA, which has been identified as a factor inducing pole cells in uv-irradiated embryos, is most probably transported from mitochondria to polar granules in germ plasm of *Drosophila* embryos. This finding may give a novel role to a mitochondrially encoded product in the establishment of germ line. However, important questions still remain to be answered. How does mtlrRNA function to form pole cells? How is mtlrRNA transported from mitochondria to polar granules? One approach to answering the questions is to generate mutants with inability to form pole cells. Unfortunately the technology to manipulate mitochondrial genes has not yet developed to the extent that one can routinely establish strains with mutations in the mtlrRNA gene.

Recent genetic and molecular analyses have revealed that some components of germ plasm are encoded by posterior class genes, and are assembled stepwise during oogenesis (for review see Mahowald, 1992; Lehmann, 1992; Lehmann and Rongo, 1993). In the genetic cascade of the seven posterior genes, *tud* is placed most downstream. In addition, tud is the only posterior gene product known to be localized in mitochondria (Bardsley *et al.*, 1993). On these grounds, we presume that tud protein mediates transport of mtlrRNA from mitochondria to polar granules, although there is a point to be considered in that tud protein seems to be present in mitochondria throughout embryos (Bardsley *et al.*, 1993).

No experiments have been successful to show that mtlrRNA is translated into protein in rabbit reticulocyte lysate (Uozumi, unpublished). This raises the possibility that mtlrRNA functions without being translated into protein. Perhaps, molecular complexes of mtlrRNA and some proteins with an RNA binding motif participate in pole cell formation. We are to test how pole cell formation is affected by reduction of the extra-mitochondrial mtlrRNA in germ plasm with the recently reported ribozyme technique (Zhao and Pick, 1993). On the other hand, identification of molecules binding to mtlrRNA must be informative for clarifying the mode of function of mtlrRNA.

Our previous studies suggested that germ-line development is regulated in at least two steps: pole cell formation and commitment of pole cells to the germ line (Ueda and Okada, 1982; Togashi *et al.*, 1986; Kobayashi and Okada, 1989). This is also supported by the phenotype of a maternal-effect mutation, *agametic*. This mutation does not affect pole cell formation, but causes degeneration of pole cells in the following embryogenesis (Engstrom *et al.*, 1982). Interestingly, similar phenotype is observed in *mes* mutants in *C. elegans*. The mutations in maternally active mes-2, -3, -4 genes affect post-embryonic germ-line development, but none of the mutations disrupts generation of germ-line progenitor cells (Capowski *et al.*, 1991). MtlrRNA is required for the first step, pole cell formation. Note that the pole cells induced by this RNA in uv-irradiated embryos never develop as germ cells. The genes required for the second step, differentiation of pole cells as germ cells, remain to be identified. We reported that the splicing activity for the third P-element intron is accommodated only to a small fraction of pole cells that are capable of differentiating as germ cells, and proposed that the splicing activity in pole cells is involved in the mechanism underlying germ-line differentiation (Kobayashi *et al.*, 1993b). It is probable that the gene(s) of which transcript is processed by this splicing activity in pole cells takes part in differentiation of pole cells as germ cells. We plan to identify these genes.

It is a very suggestive finding that extra-mitochondrial mtlrRNA is present in germ plasm in both *Drosophila* and *Xenopus*. Considering the embryonic stages of these animals when extra-mitochondrial mtlrRNA is localized in germ plasm, we hypothesize that mtlrRNA is involved in the mechanism that drives the segregation of germ plasm in a small fraction of cells in both animals. Tobler (1986) stated that the segregation of germ and somatic lines is phylogenetically a very old phenomenon and represents probably the first step in differentiation of multicellular organisms. This statement implies that molecules involved in germ-line establishment are conserved widely in animal groups covering invertebrates and vertebrates. Our observations mentioned above support this idea. It will thus be important to know whether mtlrRNA is present outside mitochondria during germ-line segregation in other organisms, such as *C. elegans* and mice.

The question that needs to be answered but is not readily tackled is why the RNA involved in the germ-line development is encoded in mitochondria. Mitochondria are believed to be symbionts originated from ancient microbes. When symbionts are deeply involved in host's life cycle, they keep strong association with germ line, ensuring their transmission to the next generation. For example, a rickettia observed in *Drosophila* embryos is incorporated into pole cells (O'Neill and Karr, 1990). It would be reasonable, if mitochondria had adopted the most effective strategy for existence, for them to produce a factor to create the germ line as a vehicle to carry them to the next generation.

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