Spatial and developmental changes in the respiratory activity of mitochondria in early *Drosophila* embryos

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**Summary**

Mitochondria of early *Drosophila* embryos were observed with a transmission electron microscope and a fluorescent microscope after vital staining with rhodamine 123, which accumulates only in active mitochondria. Rhodamine 123 accumulated particularly in the posterior pole region in early cleavage embryos, whereas the spatial distribution of mitochondria in an embryo was uniform throughout cleavage stages. In late cleavage stages, the dye showed very weak and uniform accumulation in all regions of periplasm. Polar plasm, sequestered in pole cells, restored the ability to accumulate the dye. Therefore, it is concluded that the respiratory activity of mitochondria is higher in the polar plasm than in the other regions of periplasm in early embryos, and this changes during development. The temporal changes in rhodamine 123-staining of polar plasm were not affected by u.v. irradiation at the posterior of early cleavage embryos at a sufficient dosage to prevent pole cell formation. This suggests that the inhibition of pole cell formation by u.v. irradiation is not due to the inactivation of the respiratory activities of mitochondria. In addition, we found that the anterior of *Bicaudal-D* mutant embryos at cleavage stage was stained with rhodamine 123 with the same intensity as the posterior of wild-type embryos. No pole cells form in the anterior of *Bic-D* embryos, where no restoration of mitochondrial activity occurs in the blastoderm stage. The posterior group mutations that we tested (*staufen, oskar, tudor, nanos*) and the terminal mutation (*torso*) did not alter staining pattern of the posterior with rhodamine 123.

Key words: *Drosophila* embryo, polar plasm, mitochondria, pole cell, bicaudal, posterior mutant.

**Introduction**

In many animal groups, the establishment of certain cell lineages depends on the function of cytoplasmic determinants. The determinants are often described as being allotted to locally specialized cytoplasm in oocytes and early embryos and inherited by cell lineages during cleavage (Davidson, 1986).

One of the most common cytological features of cytoplasm allotted to a determinant is an accumulation of mitochondria. For example, teloplasm, which determines the teloblasts in annelid embryos (Shimizu, 1986), myoplasm, which determines the muscle cells in ascidian embryos (Zalokar and Sardet, 1984; Fujiwara and Satoh, 1990) and the polar lobes, which determine the mesodermal cells in mollusca (Reverberi, 1970), are all reported to contain considerable numbers of mitochondria. Thus it is postulated that high mitochondrial activity is required for the function of determinants.

The establishment of the germ cell lineage depends on a specialized cytoplasm that is called germ plasm in insects and amphibians (Smith, 1966; Beams and Kessel, 1974; Illmensee and Mahowald, 1974; Okada et al., 1974; Eddy, 1975; Warn, 1975). The distribution of mitochondria in insect germ plasm has not yet been made clear, although an accumulation of mitochondria has been reported in amphibian germ plasm (Mahowald and Hennen, 1971).

To investigate mitochondria in germ plasm of *Drosophila* embryos, we used transmission electron microscopy and vital staining with the fluorescent dye rhodamine 123. Rhodamine 123 accumulates in mitochondria only when they possess high transmembrane potential, which is an indication of a high respiratory activity (Johnson et al., 1980, 1981; Chen et al., 1982). Here we will show that rhodamine 123 accumulates particularly in germ plasm at early cleavage stage, in spite of the uniform distribution of mitochondria in periplasm throughout the cleavage stages. We will also present evidence for developmental changes of mitochondrial activity in germ plasm of cleavage and blastoderm embryos. In addition, we have compared the patterns of rhodamine 123-staining between normal and u.v. irradiated embryos, and found that u.v. irradiation at a dose high enough to inhibit pole cell formation (Geigy, 1931; Togashi and Okada, 1983) did not hinder the local mito-
chodrial activity in germ plasm. Furthermore, we asked if mutations affecting pole cell formation and/or antero-posterior body pattern influence the staining pattern of rhodamine 123. The Bicaudal-D was the only mutation showing a difference from wild type in the staining pattern in early cleavage embryos.

Materials and methods

Vital staining of embryos with rhodamine 123

Drosophila melanogaster mutant strains, mwh e^{11}, cn staufen^{D3}/CyO, ru st oskar^{166} e^{TM3}, tudor^{BM3}/SM1, st nanos^{L7} e^{TM3}, Bicaudal-D^{71.34}/CyO, torso^{M51} cn bw/CyO and a wild-type Oregon R were used in this study.

Embryos were dechorionated in 3% sodium hypochlorite solution, rinsed in distilled water and lined up on a glass slide. The following procedures were all performed on the glass slide. A drop of n-octane was applied to the embryos to permeabilize the vitelline membrane. After the n-octane was removed, embryos were stained for 10 minutes with rhodamine 123 (Sigma) dissolved in a basic medium (Limbourg and Zalokar, 1973) at a concentration of 10 µg/ml. Following a brief rinse with two changes of a dye-free basic medium, embryos were covered with a drop of fluorocarbon oil (Shinetsu Kagaku, FL100-450CS) for observation under a laser confocal scanning fluorescence microscope (MRC500, BIO RAD) using 488 nm excitation wavelength.

The intensity of fluorescence was measured with the system built in the MRC500 microscope, at the anterior pole, posterior pole and middle regions in periplasm, and the posterior subcortical region. In blastoderm embryos, somatic cells in the anterior, middle and posterior regions, and pole cells were measured. Measurements were carried out in an area of 82.4 µm² in an optical section through a region to be measured. The intensity of fluorescence was expressed with brightness being evaluated relatively by an index between 0 (minimum) and 255 (maximum) at each of 121 pixels in an 82.4 µm² area. The brightness of an area was expressed as the average of indices at all 121 pixels.

Transmission electron microscopy

Embryos were fixed with the trialddehyde fixative, containing glutaraldehyde, paraformaldehyde and acrolein (Mahowald et al., 1979), and embedded in epoxy resin according to the method of Spurr (Spurr, 1969). Thin sections were cut with an LKB Nova Ultramicrotome and were observed under a JEOL 100CX2 electron microscope. The area occupied by mitochondria in a section expressed as the average of indices at all 121 pixels.

U.v. irradiation

Embryos 30±15 minutes after egg laying (AEL) were irradiated posteriorly with 280 nm u.v. at 200 J/m², which is reported to be the most efficient condition for incapacitating the function of polar plasm without affecting the somatic line (Togashi and Okada, 1983).

Results

Vital staining of normal and u.v.-irradiated early embryos with rhodamine 123

In embryos shortly after fertilization, the fluorescence from rhodamine 123 was strongest in the posterior pole region, and weakest in the middle (lateral, ventral and dorsal) regions of the periplasm (Fig. 1A, F). The relative intensity of fluorescence measured with a laser confocal scanning fluorescence microscope was 2.7 times as strong in the posterior as in the middle regions of the periplasm, and twice as strong in the posterior as in the anterior (Table 1). Since the measurement provides the relative intensity of fluorescence in an optical section of 0.7 µm thickness, no adjustment for the thickness and shape of the embryo was necessary.

At the stage when nuclei migrate to the periplasm, the fluorescence in the posterior pole plasm was reduced so that the whole periplasm became approximately uniform in the intensity of fluorescence (Fig. 1B). At the stage of pole bud formation (stage 3 according to Campos-Ortega and Hartenstein, 1985), the periplasm was still uniform in fluorescence (Fig. 1C). However, at the syncytial blastoderm stage (stage 4), pole cells had accumulated much rhodamine 123, whereas the whole nucleated periplasm remained weak in fluorescence from rhodamine 123 (Fig. 1D, G). Pole cells were 2.4 times stronger in fluorescence than the nucleated periplasm (Table 1). At the cellular blastoderm stage (stage 5), this prominent fluorescence in the pole cells was still retained; pole cells were 2.4 times as bright as somatic cells (Fig. 1E, Table 1).

In embryos that were u.v.-irradiated posteriorly at an early cleavage stage, pole cell formation did not occur. The patterns of rhodamine 123-staining in u.v. irradiated embryos were found to be exactly the same as in normal embryos at early cleavage (Fig. 2A, Table 2) and nuclear migration (Fig. 2B, Table 2) stages. At syncytial and cellular blastoderm stages, no difference in the fluorescence was noticed between somatic cells in the posterior and the other regions of the irradiated embryos. Although these observations were performed with the mwh e^{11} strain, the same results were obtained with the Oregon R strain (data not shown).

<table>
<thead>
<tr>
<th>Stage of Normal Embryos</th>
<th>Brightness±s.e.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early cleavage anterior</td>
<td>76.0±13.6</td>
</tr>
<tr>
<td>Early cleavage middle</td>
<td>55.2±23.9</td>
</tr>
<tr>
<td>Early cleavage posterior</td>
<td>147.4±27.3</td>
</tr>
<tr>
<td>Posterior subcortex</td>
<td>53.8±8.0</td>
</tr>
<tr>
<td>Late cleavage anterior</td>
<td>66.0±8.4</td>
</tr>
<tr>
<td>Late cleavage middle</td>
<td>45.6±2.9</td>
</tr>
<tr>
<td>Late cleavage posterior</td>
<td>60.0±12.7</td>
</tr>
<tr>
<td>Pole bud anterior</td>
<td>64.7±2.9</td>
</tr>
<tr>
<td>Pole bud middle</td>
<td>52.1±7.8</td>
</tr>
<tr>
<td>Pole bud posterior</td>
<td>68.0±4.2</td>
</tr>
<tr>
<td>Pole subcortex anterior</td>
<td>55.3±10.8</td>
</tr>
<tr>
<td>Pole subcortex middle</td>
<td>60.1±1.6</td>
</tr>
<tr>
<td>Pole subcortex posterior</td>
<td>141.8±16.5</td>
</tr>
<tr>
<td>Syncytial blastoderm</td>
<td>64.0±1.7</td>
</tr>
<tr>
<td>Cellular blastoderm</td>
<td>79.8±13.8</td>
</tr>
<tr>
<td>Cells under pole cells</td>
<td>85.3±12.3</td>
</tr>
</tbody>
</table>

*Each value is the average of measurements of three embryos. For details see Materials and methods.
Vital staining of early embryos with mutations for the anteroposterior body pattern and pole cell formation

In fertilized eggs laid by mothers homozygous for any one of the mutations in the posterior group genes (stau, osk, tud or nos) or homozygous for the mutation tor, fluorescence from rhodamine 123 was strongest in the posterior polar plasm, weak in the anterior pole region, and faint in the periplasm in the middle (Fig. 3A–D, Table 3). This was exactly the same as in the wild type. Late cleavage embryos with tud or nos mutation showed reduced intensity of fluorescence in the posterior polar plasm as in wild-type embryos (Fig. 3E, H, Table 3).

Since tud embryos form no pole cells, no restoration of fluorescence occurred in the posterior region of tud embryos at the syncytial blastoderm stage (Fig. 3F, Table 3). The somatic cells formed in the posterior pole region of tud embryos were exactly the same as cells in the other regions in the intensity of fluorescence. Pole cells formed in nos embryos accumulated rhodamine 123 as much as the pole cells of wild-type embryos, while the nucleated periplasm in the somatic regions remained low in the intensity of fluorescence (Fig. 3I, Table 3).

In contrast to the wild type and the other mutants that we observed, Bic-D embryos shortly after fertilization exhibited bright rhodamine 123 fluorescence in the anterior as well as in the posterior polar plasm (Fig. 3J, Table 3). In late cleavage stages, the fluorescence in both the anterior and the posterior polar plasm of Bic-D embryos had largely disappeared (Fig. 3K, Table 3). Up to this stage, the anterior region of Bic-D embryos is identical with the posterior of wild-type embryos. However, at the syncytial blastoderm stage, the anterior and the posterior of Bic-D embryos become quite different. Pole cells formed in the posterior of Bic-D embryos accumulate as much rhodamine 123 as pole cells of wild-type embryos, while the intensity of fluorescence in the anterior remains low (Fig. 3L, Table 3).

Transmission electron microscopy

Electron micrographs of early cleavage embryos did not show any particular region in periplasm where mitochondria were more concentrated (Fig. 4A, B, C). Conditions were similar in late cleavage embryos (Fig. 4D, E, F), and also in the pole bud stage embryos (stage 3) (Fig. 4G, H, I). To quantitate these observations, the ratio of the area occupied by mitochondria to the area of cytoplasm was calculated from the measurement of the areas on electron micrographs using a digitizer (Fig. 6). There was no significant difference in the ratio between regions in periplasm. Therefore, the increase in rhodamine 123-staining in the

![Fig. 3. Vital staining of mitochondria with rhodamine 123 in mutant embryos. Computer processed images produced with a laser scanning confocal fluorescence microscope. Anterior poles are to the left. (A) An early cleavage embryo from a stau110/stau116 female. (B) An early cleavage embryo from a osk110/osk116 female. (C) An early cleavage embryo from a tof1/1+ tof1/1+ female. (D) An early cleavage embryo from a osk110/osk116 female. (E) An early cleavage embryo from a nos1/2/nos1/2 female. (F) A syncytial blastoderm embryo from a tudBM-3/tudBM-3 female. (G) An early cleavage embryo from a tudBM-3/tudBM-3 female. (H) A late cleavage embryo from a tudBM-3/tudBM-3 female. (I) A late cleavage embryo from a tudBM-3/tudBM-3 female. (J) A syncytial blastoderm embryo from a tudBM-3/tudBM-3 female. (K) A late cleavage embryo from a tudBM-3/tudBM-3 female. (L) A syncytial blastoderm embryo from a tudBM-3/tudBM-3 female. (M) A late cleavage embryo from a tudBM-3/tudBM-3 female. Arrowheads point to autofluorescence in vitelline membrane. (N) A syncytial blastoderm embryo from a tudBM-3/tudBM-3 female. Bar, 100 μm. Colours represent relative fluorescence intensity. Colour indicator is in Fig. 1.](image-url)
posterior region of early cleavage embryos is not due to an increase in the number of mitochondria there.

On electron micrographs of embryos at syncytial blastoderm (stage 4) and cellular blastoderm (stage 5), there were fewer mitochondria in pole cells than in somatic cells (Fig. 5A-F, 6). Thus the high intensity of fluorescence in pole

Fig. 4. Transmission electron micrographs of normal embryos. The surfaces of the embryos are to the left. A-C are from the same early cleavage stage embryo. (A) The anterior pole region, (B) the middle region, (C) the posterior pole region. Arrows and arrowheads indicate polar granules and mitochondria respectively. D-F are from the same late cleavage stage embryo. (D) The anterior pole region, (E) the middle region, (F) the posterior pole region. G-I are from the same embryo at the pole bud stage. (G) The anterior pole region, (H) the middle region, (I) pole bud. Bar, 5 µm.
cells is also ascribed to an increase in respiratory activity of mitochondria. For some unknown reason, the number of mitochondria in somatic cells at the posterior end of blastoderm embryo was greater than that in somatic cells in the other regions of the embryo (Fig. 5G, 6).

**Discussion**

*Mitochondrial activity varies between the regions and developmental stages of embryos*

It is known that rhodamine 123 only stains respiratory active mitochondria; i.e. the rate of incorporation of the dye into mitochondria is in proportion to their inner membrane potential generated by electron transport (Johnson et al., 1980, 1981; Chen et al., 1982). Using this property of the dye, we demonstrated that the cytoplasm was stained with rhodamine 123 more strongly in the posterior pole region than in the other regions of early cleavage embryos. However, electron microscopy did not reveal any significant accumulation of mitochondria in the posterior pole cytoplasm. These observations suggest that the respiratory activity of mitochondria is higher in the posterior pole region than in the other regions. At the late cleavage stage

**Fig. 5.** Transmission electron micrographs of normal embryos. The surfaces of the embryos are to the top. A-C are from the same syncytial blastoderm stage embryo. (A) The anterior pole region, (B) the middle region, (C) pole cell. Arrows and arrowheads point to polar granules and mitochondria. D-G are from the same cellular blastoderm stage embryo. (D) A somatic cell in the anterior pole region, (E) a somatic cell in the middle region, (F) a pole cell, (G) a somatic cell under pole cells. Bar, 5 μm.
when nuclei had reached the subcortex, the posterior pole cytoplasm lost most of its fluorescence, resulting in homogeneous weak rhodamine 123 fluorescence throughout periplasm. In blastoderm embryos, the pole cells, in which the posterior pole cytoplasm had been sequestered, showed conspicuous rhodamine 123 incorporation, although the density of mitochondrial population was lower in pole cells than in the other cells. Since there was no difference between early and late cleavage embryos in the number and the distribution pattern of mitochondria, we presume that mitochondria in the posterior pole cytoplasm are highly activated in early cleavage embryos and come to a state of low activity at late cleavage stages. Mitochondria in polar plasm returned to their highly activated state after the polar plasm was sequestered in pole cells. We have not determined, however, if the same mitochondria are activated in early cleavage embryos and again in pole cells.

In some animal phyla, morphological and functional variations have been noted among mitochondria in an individual. For example, in Xenopus embryos, the activity of enzymes in the mitochondrial matrix varies with the developmental stage and the tissue (Kistler and Weber, 1974). In embryos of the mouse and a slug (Arion ater rufus), a remarkable spatiotemporal heterogeneity in the fine structure of mitochondria has been reported (Sathananthan, 1970; Stern et al., 1971). Moreover, it has been reported in mice that the average diameter of mitochondria differs depending on the enzymes that they contain, namely ornithine aminotransferase or malate dehydrogenase (Thomson et al., 1974). These reports suggest the possibility that Drosophila embryos possess two types of mitochondria, one of which is localized in polar plasm. However, on rhodamine 123-stained preparations no individual mitochondria can be recognized. Furthermore, no heterogeneity has been noticed in the mitochondrial population in electron micrographs.

Previous studies using vital fluorescent dye for probing mitochondrial activities in certain embryonic regions have shown that the total mitochondrial activity of a region of cytoplasm is in proportion to the number of mitochondria existing in the region. In the embryos of the ascidian Phallusia, the myoplasm, which has been presumed to include the determinant for the muscle cell lineage, contains a considerable number of mitochondria (Zalokar and Sardet, 1984). The myoplasm of Halocynthia (Asciidae) embryos can be distinctly visualized with a monoclonal antibody specific to mitochondria (Fujiwara and Satoh, 1990). The pole plasm of Tubifex embryos has a mass of mitochondria, which are handed down to the teloblast cell lineage (Shimizu, 1986). Furthermore, in the polar lobe of Dentalium (mollusca), which determines the mesodermal cells, mitochondria were concentrated (Reverberi, 1970). The dense mitochondrial population observed in myoplasm, teloplasam and polar lobes may support the idea that a high mitochondrial activity is required for cytoplasm to function as a determinant. If the idea is generally acceptable, our finding will support it in the sense that Drosophila polar plasm fulfills the requirement by increasing the activities instead of the number of mitochondria.

**U.v. irradiation does not reduce mitochondrial activities**

Since u.v.-irradiated embryos and normal embryos were practically identical in their pattern of rhodamine 123-staining at the cleavage stage, it is reasonable to conclude that the mitochondria in the posterior pole cytoplasm have normal respiratory function even when irradiated with u.v. at a dose high enough to prevent pole cell formation. This suggests that the inhibition of pole cell formation in u.v. irradiated embryos is not the result of damage to mitochondrial respiratory activity. This is supported by transmission electron microscopic observations that the fine structure of mitochondria in the posterior pole region of Drosophila embryos remains normal after u.v. irradiation (Yamazaki and Okada, unpublished observation).

**Higher respiratory activity of mitochondria in germ plasm at early cleavage stage**

It has been postulated that there are at least two cytoplasmic factors that are necessary for pole cell formation: one is u.v.-sensitive and the other is u.v.-resistant (Ueda and Okada, 1982; Togashi et al., 1986; Kobayashi and Okada, 1989). Mitochondrial large ribosomal RNA (MitrRNA) has been shown to be a probable candidate for the u.v.-sensitive factor on the basis that MitrRNA is capable of restoring pole cell-forming activity to u.v. irradiated embryos, when injected immediately after u.v. irradiation of the posterior pole at early cleavage stage (Kobayashi and Okada, 1989). MitrRNA alone is incapable, but it is capable of inducing pole cells in ectopic sites when co-injected with u.v. irradiated posterior pole plasm, suggesting that a u.v.-
resistant factor which is necessary for pole cell formation is localized in the posterior pole cytoplasm (Kobayashi and Okada, 1989). The fact that u.v. irradiation does not alter the high mitochondrial respiratory activity in the posterior of early cleavage embryos rules out the possibility that the high mitochondrial activity is a crucial factor for pole cell formation. However, there still remains the possibility that this activity represents a u.v.-resistant cytoplasmic factor postulated to work in cooperation with MtlrRNA.

Among the anteroposterior polarity mutants that we have studied, only Bic-D affected the staining pattern of rhodamine 123. Both the anterior and posterior polar regions were stained strongly with rhodamine 123 in Bic-D embryos at early cleavage stages. However, all the posterior group mutants that we examined were exactly as wild type in the rhodamine 123-staining pattern of early cleavage embryos. For example, in oskar and staufer embryos in which neither abdominal segments nor pole cells are formed, mitochondrial respiratory activities in the posterior increased at early cleavage stages then decreased at later cleavage stages. The same developmental and spatial changes in the staining pattern were observed in nanos embryos, in which abdominal segments are deficient but pole cells are produced.

The posterior group mutant embryos all display a deficiency in the localization of nanos products in the posterior pole cytoplasm. Consequently, the localization of nanos products may not be necessary for mitochondrial activities to increase in the posterior cytoplasm in early cleavage embryos. Furthermore, torso mutations did not affect normal transition of mitochondrial activities in the posterior cytoplasm. This suggests that activated torso products are not involved in the pathway to stimulate mitochondrial activities. We did not test a vasa mutant. However, considering that tudor and nanos embryos have vasa protein localized in the posterior pole cytoplasm, but there is no localization of vasa protein in the polar plasm in oskar embryos (Lasko and Ashburner, 1990), whereas none of these mutants are distinguishable from wild type in rhodamine 123 staining, vasa products in the polar plasm could not be a critical factor.

In the anterior of Bic-D embryos, abdominal structures with the inverted polarity are formed. However, no pole cells form. Consequently, no germ cells are produced in the ectopic abdomen of Bic-D embryos. Considering that bicoid products are replaced with nanos products in the anterior of Bic-D embryos (Ephrussi et al., 1991), and that at least one of the pole cell-forming factors must be deficient or impaired in the anterior of Bic-D embryos, the absence of bicoid products and/or the pole cell-forming factor(s) may be an underlying cause of mitochondrial stimulation. However, this does not explain the low mitochondrial activities in the middle regions of embryos. The local mitochondrial activities can be regulated by cooperation of germ line-determining factors and cytoplasmic factors depending on posterior group genes. More precise genetical and developmental analyses of the posterior pole cytoplasm, and of the anterior of Bic-D embryos will shed light on this problem, as well as on the roles of the cytoplasmic factors in polar plasm.

Higher respiratory activity of mitochondria in pole cells at blastoderm stage

In embryos that have been u.v. irradiated posteriorly at an early cleavage stage, germ plasm is sequestered in somatic cells at the posterior end of the blastoderm because of failure in pole cell formation in these embryos. In the same way, the posterior pole plasm of tud embryos and the anterior pole plasm of Bic-D embryos, both of which showed strong rhodamine 123-staining at early cleavage stages, were sequestered in somatic cells. However, restoration of high mitochondrial activities did not occur in these somatic cells. This suggests that mitochondria in the posterior are activated again only when they are sequestered in pole cells. In blastoderm embryos, RNA synthesis in the pole cells is hardly detectable whereas this occurs in the somatic cells. In contrast, protein synthesis in the pole cells is significantly higher than in the somatic cells and is thought to be the translation of maternal mRNA (Zalokar, 1976). The reactivation of mitochondria in pole cells may be related to this increase in protein synthesis. It is unknown, however, what causes pole cells to be different from blastodermal somatic cells in the rate of protein synthesis even though both cell types are determined at the blastoderm stage.

Based on transplantation of polar plasm (Ueda and Okada, 1982; Togashi et al., 1986; Kobayashi and Okada, 1989) and on the phenotype of agamic mutant (Engstrom et al., 1982), it is suggested that at least two maternal cytoplasmic factors are involved in the developmental pathway of pole cells to germ cell lineage: one responsible for pole cell formation and the other for differentiation of pole cells as germ cells. It remains to be tested whether a higher activity of mitochondria in pole cells is required for a germ cell determinant proper to function in the differentiation of pole cells to germ cells.

We thank Dr Osamu Numata for advice in electron microscopy, Dr. Takayoshi Tsuchiya for allowing us to use his digitizer, and Dr Shin Sugiyama for discussion. This work was supported in part by a Grant-in-Aid from the Ministry of Education, Science and Culture, a grant from The Naito Foundation and a grant from University of Tsukuba Special Project Research.

References


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Fig. 1. Vital staining of mitochondria with rhodamine 123 in normal embryos. Computer processed images produced with a laser scanning confocal fluorescence microscope. Anterior poles are to the left. (A) An early cleavage embryo. (B) A late cleavage embryo. (C) An embryo at the stage of pole bud formation. (D) A syncytial blastoderm embryo. (E) A cellular blastoderm embryo. (F) The posterior pole region at a higher magnification of the embryo shown in A. (G) The posterior pole region of the embryo shown in D at a higher magnification. Colours represent relative fluorescence intensity. Colour indicator is shown at the bottom left corner, where white refers to the maximum intensity and blue to the minimum. A-E are at the same magnification. Arrowheads point to autofluorescence in vitelline membrane. Bars, 100 µm (A) and 25 µm (F).

Fig. 2. Vital staining of mitochondria with rhodamine 123 in u.v. irradiated embryos. Computer-processed images produced with a laser scanning confocal fluorescence microscope. Anterior poles are to the left. (A) An early cleavage embryo. (B) A late cleavage embryo. Colours represent relative fluorescence intensity. Colour indicator is in Fig. 1. Bar, 100 µm.