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A light- and electron-microscopic study on the migration of primordial germ cells in the teleost, *Oryzias latipes*

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Summary. The primordial germ cells (PGCs) of *Oryzias latipes* in migration to the gonadal anlage have been investigated by light and electron microscopy. The ultrastructure of the PGCs, which occur in the subendodermal space on the syncytial periblast, differ conspicuously from that of the surrounding endodermal cells. After the PGCs move to the cavity between lateral plate and ectoderm, they are taken into the somatomesodermal layer and transferred to the dorsal mesentery where they form gonadal anlage with mesodermal cells. During their translocation to the dorsal mesentery through the somatic mesoderm, apparently without formation of pseudopods, the PGCs are completely surrounded by mesodermal cells. Since these conditions seem unfavorable to the active translocation of the PGCs to the dorsal mesentery, it is more likely that the PGCs are transferred passively by the morphogenic activity of the lateral-plate mesoderm.

Counts of the number of the PGCs revealed that they are mitotically dormant during the migratory period. After the completion of the migration, they regain their proliferative activity. The PGCs in the female proliferate more actively than those in the male, which provides the first morphological indication of sex differentiation in this species of fish.

Key words: Migration – Primordial germ cell – Teleost – *Oryzias latipes* – Ultrastructure

There have been many investigations of the early developmental stages of germ cells (Nieuwkoop and Sutasurya 1979). Early segregation from the somatic cell line, migration to the gonadal anlage, and sex differentiation are the most prominent events in the germ-cell lineage. Changes in their proliferative activity in the various

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classes of the vertebrates have been described. In mammals and birds, primordial germ cells continue to divide throughout these events, whereas a mitotically quiescent period occurs in the reptiles, amphibians, and fish (Hardistry 1967). However, this conclusion is based mainly on histological observations or partial cell counts. Only for *Xenopus laevis* is there an accurate description of the pattern proliferation of PGCs, thanks to the investigations by Dixon and his coworkers. According to Zunst and Dixon (1977), four presumptive PGCs are segregated during cleavage. After "determination", PGCs divide two or three times before the mitotically dormant period, during which migration to the genital ridge occurs. After arrival at the genital ridge, PGCs begin to divide again, gonial cells formed, and sex differentiation of germ cells becomes evident.

The origin and migration of PGCs in *Oryzias latipes* were first studied by Gamo (1961). However, since the results of cell counts in his report do not agree with those by Tsuzuki et al. (1966), and Satoh and Egami (1972), information on the population of PGCs in early developmental stages remains incomplete.

Although, numerous reports of electron-microscopic observations of germ cells have been published recently, only a few consider the PGCs during migration (Zamboni and Merchant 1973, Spiegelman and Bennet 1973, mouse; Fujimoto, Ukeshima and Kiyofuji 1976, Lee et al. 1978, chick; Wylie and Heaseman 1976, Wylie et al. 1976, *Xenopus*). Among the fishes, germ cells in the gonadal anlage of *Oryzias latipes* have been investigated by electron microscopy (Satoh 1974), but the ultrastructure of PGCs in earlier stages of development remains to be clarified.

In this communication we describe the results of light- and electron-microscopical investigations of the morphology and change in numbers of PGCs at various stages of embryonic development of *Oryzias latipes*.

Materials and methods

Materials used were the embryos of the *d-rR* strain of the teleost fish, *Oryzias latipes*. The fertilized eggs were incubated in glass vessels placed in an air-conditioned room at $25 \pm 2°$ C. The stages of development of the embryos were identified according to Matsui's table of normal development (1949).

Histological preparations and germ cell counting

Embryos at stages earlier than 27 were fixed in Bouin's solution with the minor modification of Johnston (1951), dechorionized with sharpened forceps in the fixative and embedded in Paraplast (Sherwood Medical, USA). Embryos older than stage 28 were dechorionized enzymatically (Smithberg 1966) prior to the fixation.

Serial transverse sections were cut at the thickness of 5 μm and stained with Delafield's haematoxylin and eosin. From examination of all sections, the number of germ cells per embryo was determined.

Electron microscopy

Embryos at various stages of development were fixed in formaldehyde-glutaraldehyde-picric acid combination fixative (Ito and Karnovsky 1968), adjusted to pH 7.3 with phosphate buffer, for 90 min at room temperature. After rinsing in the buffer, they were post-fixed in cold 1 % OsO_4 for 2 h, dehydrated in ethanol, and embedded in epoxy resin. To facilitate the penetration of fixative in embryos younger than stage 26, the chorion was punctured with a needle just after the immersion of the eggs in fixative. The eggs were denuded of chorions with forceps after about 15 min. Embryos older than stage 27 were dechorionized enzymatically (Smithberg 1966). Thick sections were cut transversally, stained with toluidin blue, and examined with a light microscope for the identification of PGCs. Thin sections were cut, stained with uranyl acetate and lead citrate, and examined with HS-9 and H-300 electron microscopes (Hitachi, Japan).

Results

1. Path of the migration of PGCs

In the embryos at stages 20–23, PGCs were found in the peripheral region of the endodermal layer (Fig. 1 A). They were identified by their large cell-size, slightly eosinophilic cytoplasm, the distinct outline of the nucleus, and the existence of a prominant nucleolus. Some of them seemed to be situated beneath the endodermal

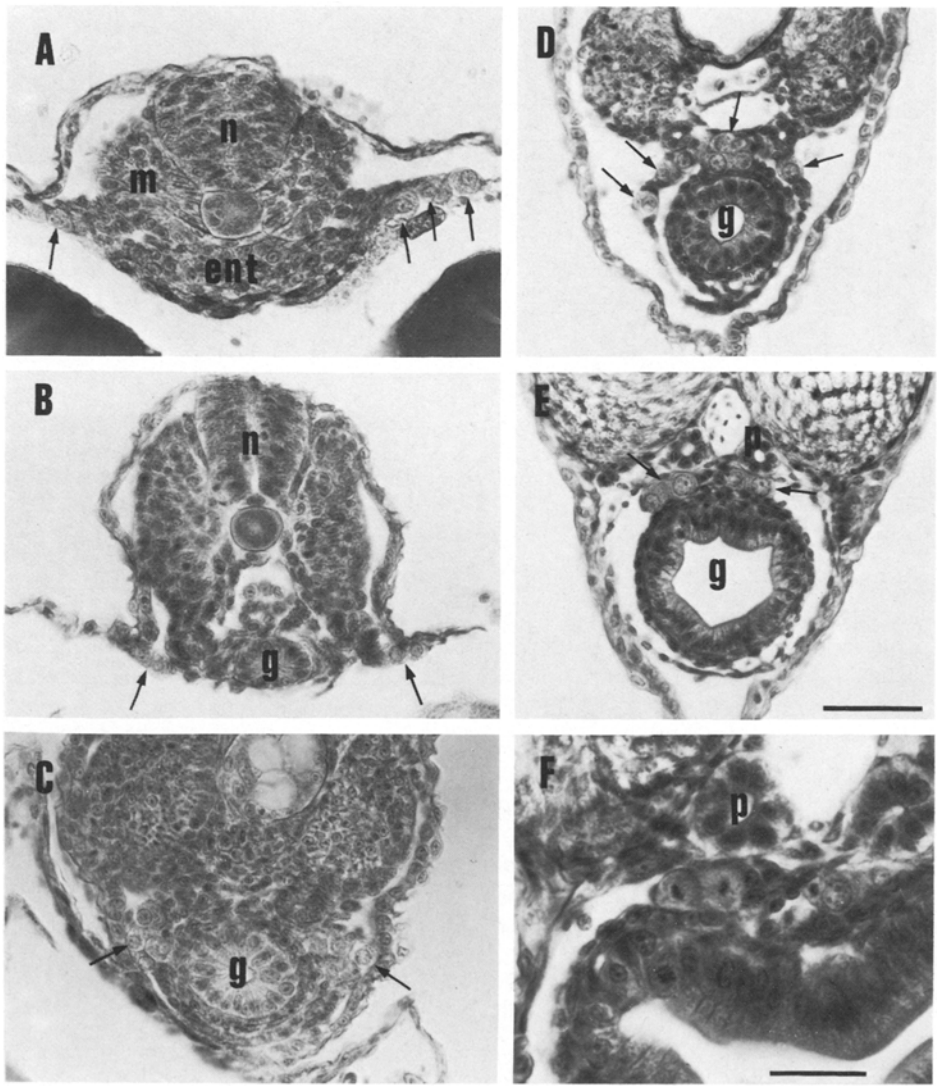

Fig. 1. The PGCs (*arrows*) in migration to the gonadal anlage; *n* Neural tube; *m* mesoderm; *ent* endoderm; *g* gut; *p* pronephric duct. **A** stage 23. **B** stage 25. **C** stage 26. **D** stage 29. Some germ cells have reached the gonadal region, others are in the somatic mesoderm. **E** stage 30. All germ cells are in the gonadal anlage between pronephric duct and the gut. Bottom bar, 40 μm. **F** stage 32. A germ cell in mitosis. Bottom bar, 20 μm

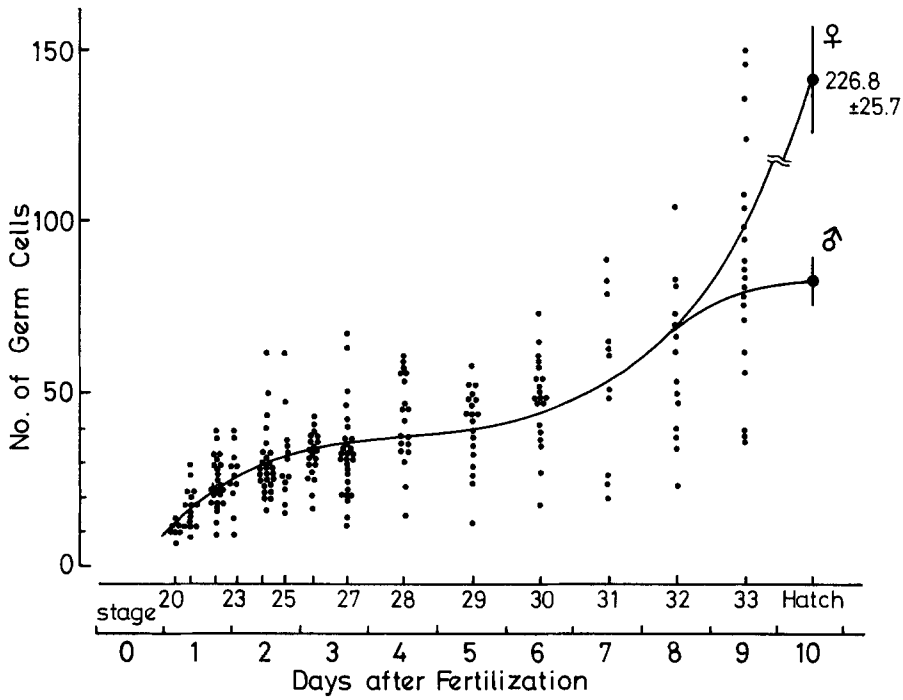

Fig. 2. Change in the number of germ cells in an embryo during normal development. Each dot designates the number of germ cells in an embryo. At the hatching time, the sex of the fry can be distinguished by the presence of the oocyte in meiotic prophase in the female. Means and standard errors are shown

layer and on the periblast. The PGCs in the embryos at stage 25 were located alongside of the newly formed digestive tract (Fig. 1 B). At stages 26–27, PGCs came together between the lateral plate and the ectoderm (Fig. 1 C). Between stages 27 and 29, the lateral-plate mesoderm differentiated into two layers, the somatic mesoderm and the splanchnic mesoderm, between which the body cavity was formed. During the process, the PGCs were transferred into the somatic mesoderm, through which they move towards the dorsal mesentery (Fig. 1 D). By stage 30, the gonadal anlage was completed, and all the germ cell were accomodated in it (Fig. 1 E). No mitotic figures of PGCs were found during their migratory period from stage 20 to 29, though some mitotic PGCs were occasionally observed in the gonadal anlage in the embryos between stages 30–33 (Fig. 1 F). Ectopic or degenerating PGCs during the migration were seldom found.

2. Change in the germ cell population

The change in the number of PGCs per embryo is shown in Fig. 2. The number of PGCs increased between stages 20–25. During this period, no mitotic figures of PGCs were observed in the present investigation, which does not conclusively eliminate the possibility of PGC-division because a small number of PGCs were present in the embryo. Between stages 26–30, there was hardly any significant increase in number of germ cells.

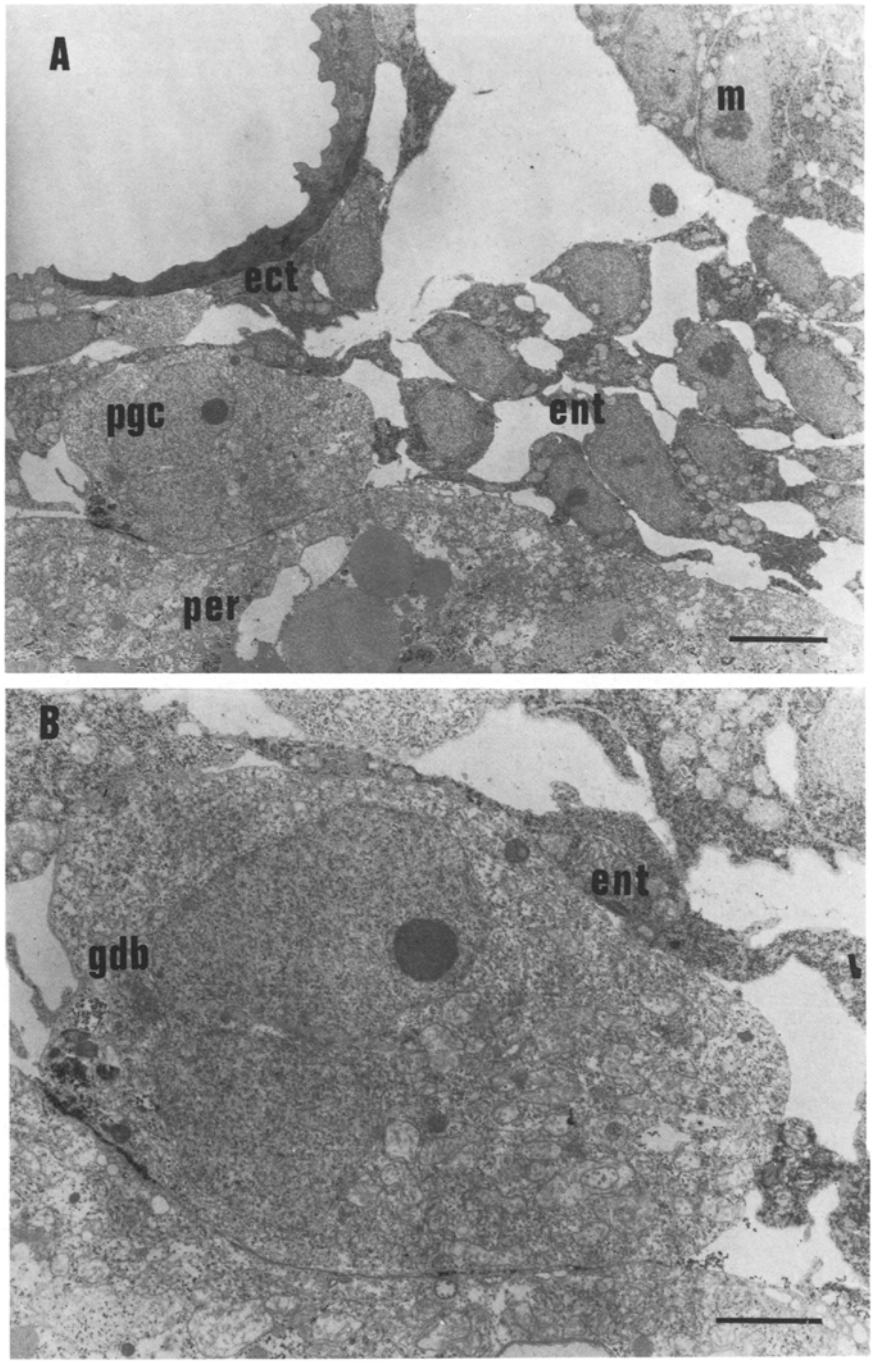

Fig. 3. Stage 23. **A** The PGC is in the sub-endodermal space on the syncytial periblast. Bottom bar, 5 μm. **B** The PGC is in contact with the endodermal cells and the syncytial periblast. Bottom bar, 2 μm; *ect* ectoderm; *ent* endoderm; *m* mesoderm; *per* syncytial periblast; *pgc* primordial germ cell; *gdb* germinal dense body

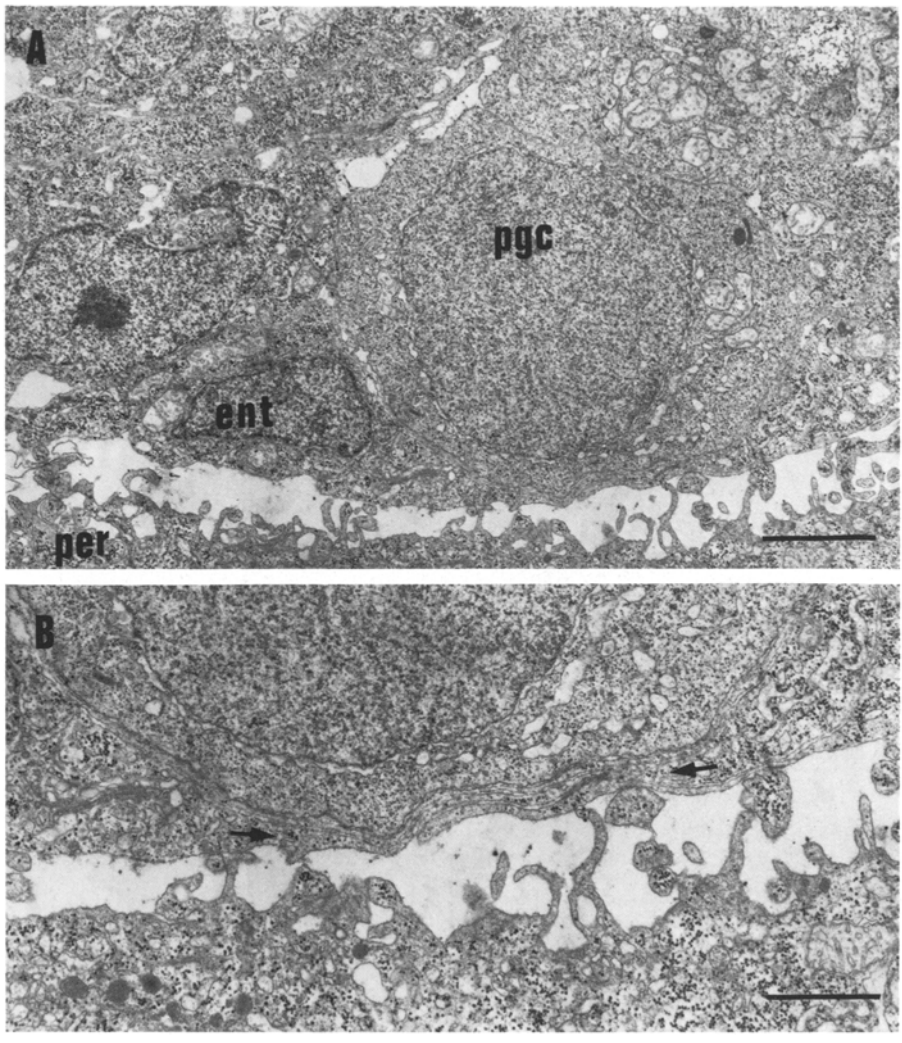

Fig. 4. Stage 25. **A** Between PGC and syncytial periblast, endodermal cells extend thin cytoplasmic processes and separate them. Bottom bar, 2 μm. **B** High magnification of Fig. 4 A. The thin cytoplasm of endodermal cells (*arrows*) are piled up. Bottom bar, 1 μm

After the completion of the migration to the gonadal anlage, the germ cells resumed their mitotic activity. Between the onset of this proliferative period and hatching, the germ cells in the male embryos doubled in number, whereas those in the females increased about fourfold. This difference in the proliferation rate is the first morphological indication of sex differentiation in this species.

3. Ultrastructural observation of the migrating PGCs

Ultrastructural observations were performed on 5–9 embryos per stage from stage 23 to stage 30. Since no serial sections were used, the following descriptions are

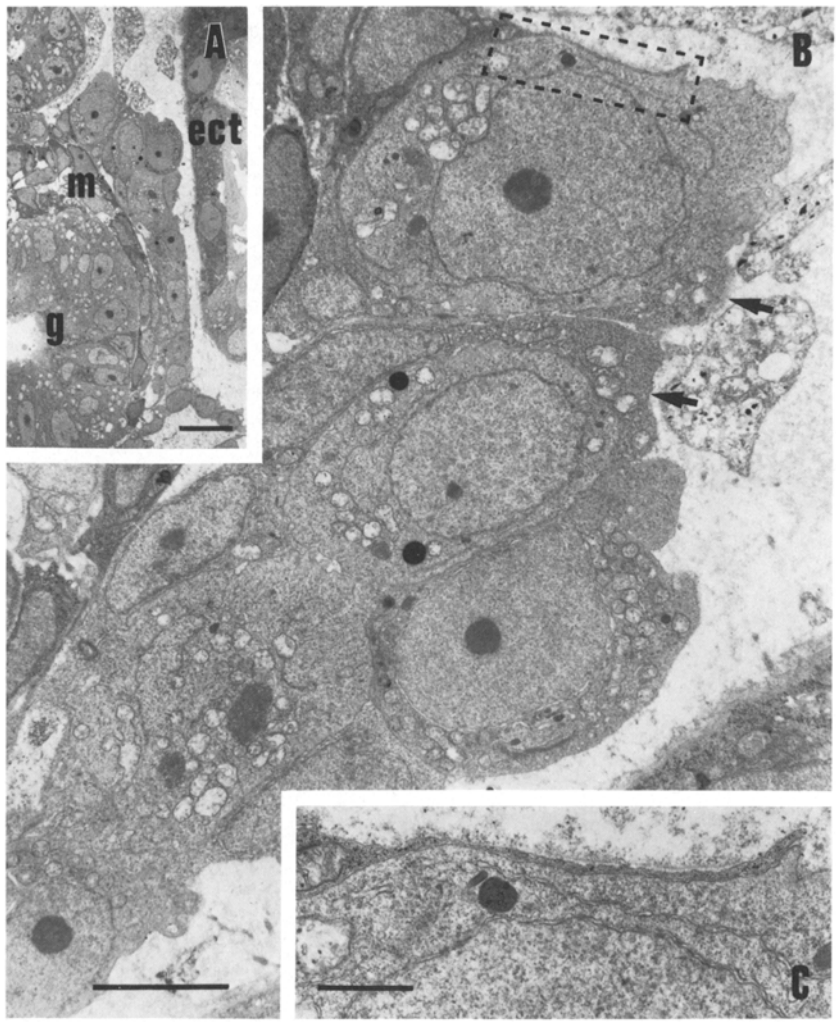

Fig. 5. Stage 27. **A** PGCs are located in the cavity between lateral plate and ectoderm. Bottom bar, 10 μm. **B** The PGCs are in contact with each other. However, the mesodermal cells begin to intervene between them (*arrows*). Bottom bar, 4 μm. **C** High magnification of the region enclosed with perforated lines in Fig. 5 B. Mesodermal cells extend thin cytoplasm to cover the PGC. Bottom bar, 1 μm; *p* pronephric duct; *g* gut

based on the observations of discrete thin sections. In some cases, several planes of one cell were observed.

Figure 3 shows a PGC in the embryo at stage 23. The PGC was in contact with the syncytial periblast and endodermal cells. The homogeneous distribution of chromatin in the nucleus, light profile of the cytoplasmic matrix, the existence of the germinal dense bodies, and the large cell-size identify it as a germ cell. The cytoplasm of the surrounding endodermal cells was rich in ribosomes, and the difference in the ultrastructural feature between PGC and somatic cells was

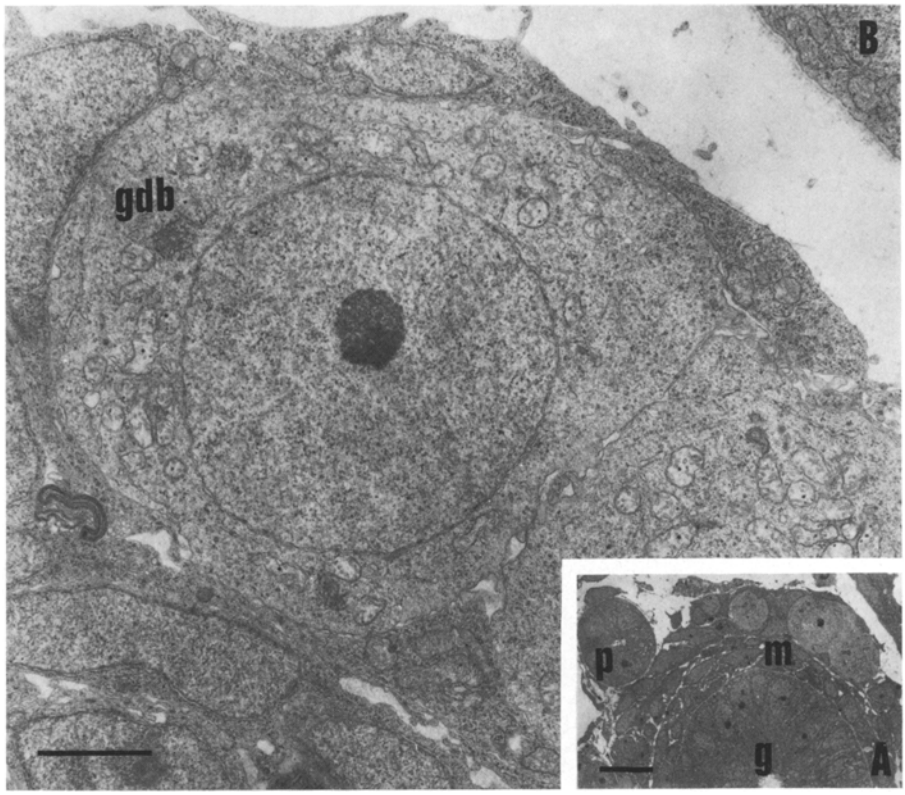

Fig. 6. Stage 27^{+}. **A** PGCs have been taken into the lateral plate. Bottom bar, 10 μm. **B** The mesodermal cells completely cover the PGCs. Bottom bar, 2 μm

conspicuous even at this early stage of embryonic development. The indentation of the nuclear membrane of PGCs was noticeable.

At stage 25, endodermal cells extended a thin sheet of cytoplasm that seperated the PGCs from the periblast (Fig. 4). The periblast streched many filopodia-like protrusions, which were not observed in the embryos at other stages of development.

As was stated above, the PGCs in the embryos at stage 27 were found on the lateral plate (Fig. 5 A). They adhered to each other with their free surface exposed to the cavity between the lateral plate and ectoderm (Fig. 5 B). The cytoplasm of the mesodermal cells began to intervene between the PGCs and to cover their surface (Fig. 5 B, C). The nuclei of the PGCs had smooth contours at and after stage 27. Fig. 6 shows PGCs of stage-27-embryo which is at a more advanced stage than that shown in Fig. 5. The PGCs were incorporated in the lateral plate (Fig. 6 A), and completely covered with mesodermal cells (Fig. 6 B).

At stage 28, the PGCs were found in the somatic mesoderm (Fig. 7 A). Slightly electron-dense substance appeared in the body cavity. The PGCs were surrounded by somato-mesodermal cells, the cytoplasm of which became very thin (Fig. 7 B). Occasionally, the desmosomes joined the mesodermal cells. The PGCs in the

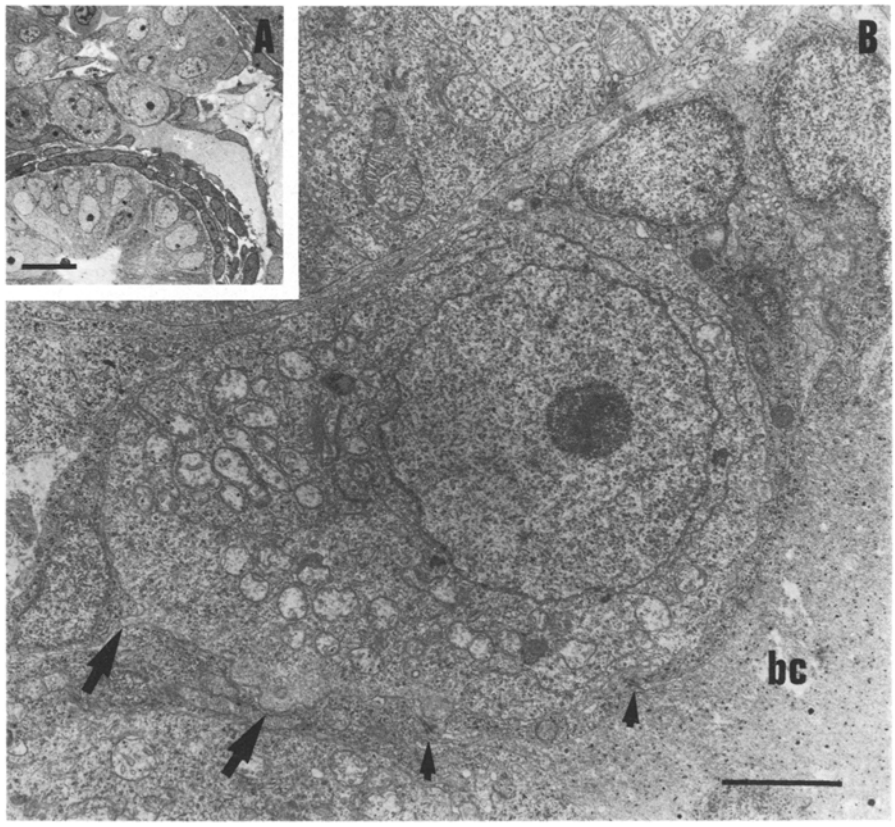

Fig. 7. Stage 28. **A** Two PGCs have reached the gonadal region and one is in the somatic mesoderm. Bottom bar, 10 μm. **B** High magnification of the PGC in the somatic mesoderm shown in Fig. 7A. The cytoplasmic sheet of mesodermal cells completely wraps arround the PGC. Desmosomes are seen between mesodermal cells (*small arrows*). Small cytoplasmic protrusions of the PGC are noticed (*large arrows*). Bottom bar, 2 μm; *bc* body cavity

embryos before stage 27 had rather irregular shapes, whereas most of the germ cells found in the somatic mesoderm were round or oval.

The gonadal anlage of the embryo at stage 30 was composed of flat somatic cells and round germ cells (Fig. 8 A). These somatic cells enveloped the germ cells completely so that none of them had any free surface exposed to the coelom (Fig. 8 B). They inserted their cytoplasm between the germ cells (Fig. 8 C). In its consequence, most of the germ cells appeared to be wrapped in the somatic cells, though in rare cases some germ cells were in contact with each other (Fig. 8 D).

Throughout the present observation period, the germinal dense bodies were present in the PGCs. The morphology of the germinal dense bodies changed according to the stages of embryonic development, the details of which will be reported elsewhere (S. Hamaguchi, in preparation). The long agranular endoplasmic reticulum, which has been reported by Hogan (1978) as "... the membranous structure parallel to the nuclear envelop", was also observed. This

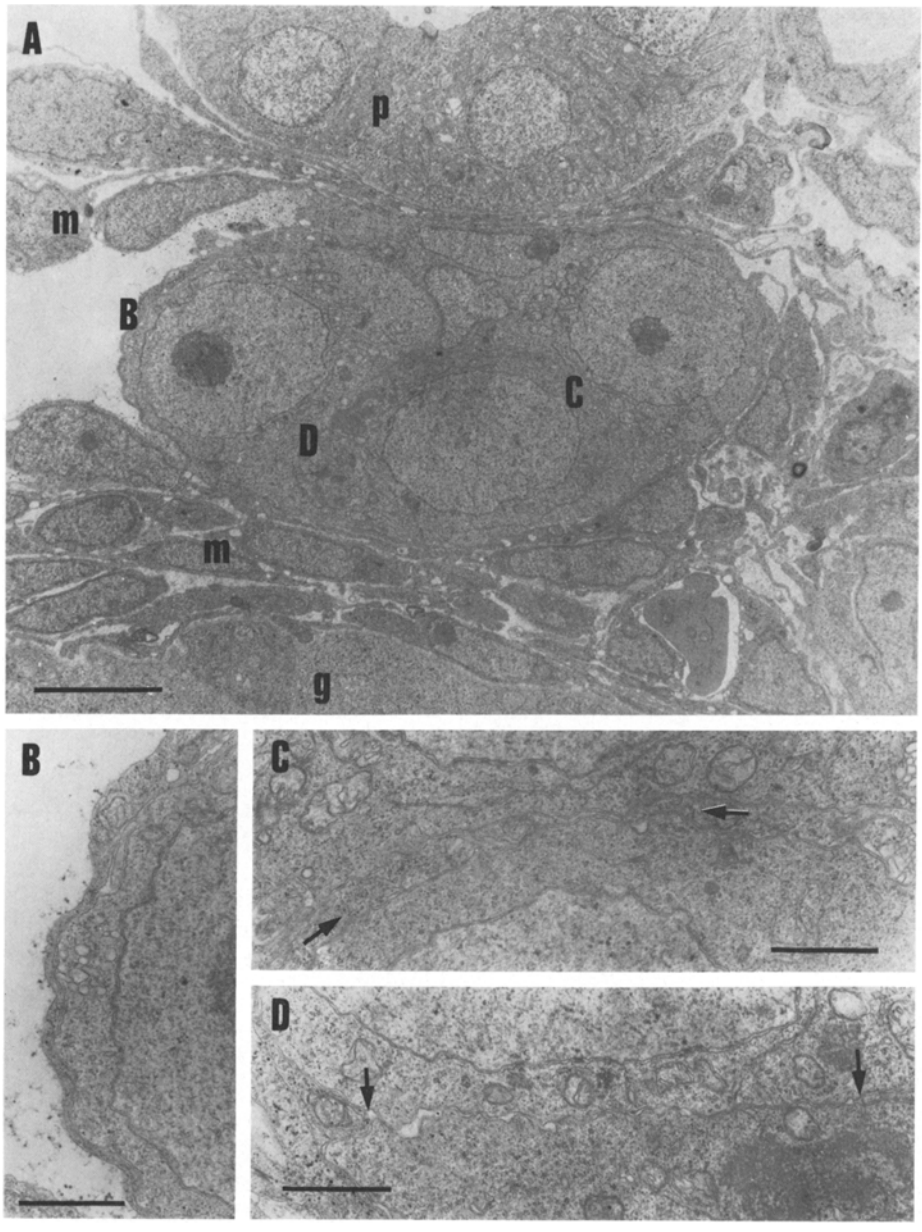

Fig. 8. Stage 30. **A** Three PGCs can be seen in the gonadal anlage. Bottom bar, 4 μm. **B** High magnification of the region B in Fig. 8A. Thin cytoplasm of the mesodermal cell covers the PGC. Bottom bar, 1 μm. **C** High magnification of the region C in Fig. 8A. The PGCs are separated by thin cytoplasm of the mesodermal cells (*arrows*). Bottom bar, 1 μm. **D** High magnification of the region D in Fig. 8A. The PGCs are in contact with each other in this region (*between arrows*). Bottom bar, 1 μm

structure was more conspicuous in germ cells at later stages of the migration. As reported by Satoh (1974), annulate lamellae were occasionally found.

Discussion

The present observations on the path of migration of PGCs agree with those of Gamo (1961). The germinal path leads from the peripheral region of the subendodermal space on the syncytial periblast into the cavity between the lateral plate and the ectoderm. Thereafter, the PGCs are taken into the somatic mesoderm and transferred to the dorsal mesentery, where they form a gonadal anlage with the mesodermal cells.

The history of germ cells in the fish has been studied since the beginning of this century. With *Fundulus,* Richards and Thompson (1921) have reported the path of migration to be similar to that observed in the present study; similar investigations were performed by Hann (1927) on *Cottus bairdii* and extensively by Johnston (1951) on *Micropterus salmoides.* The PGCs of all of these fish move from the ventral to the dorsal region of the gut in the splanchnic mesoderm. Those in *Oryzias* pass through the somatic mesoderm.

In anurans, the early displacement of PGCs in the endodermal cell mass is thought to be mainly passive and due to the morphogenic movement of the surrounding tissue, while the later process from the endoderm to the gonadal ridge through the mesentery is attributed to the active migration of the PGCs (Nieuwkoop and Sutasurya 1979). Among other classes of vertebrates, pseudopodia of PGCs have been observed in mouse (Zamboni and Merchant 1973), in human (Fujimoto et al. 1977), and in chick (Fujimoto, Ukeshima and Kiyofuji 1976). Isolated PGCs move by the amoeboid extrusions of lobopodia in vitro (Fujimoto, Ninomiya and Ukeshima 1976, chick; Wylie and Roos 1976, Heasman et al. 1977, *Xenopus*). As to the mode of the migratory activity of PGCs in teleosts, Richards and Thompson (1921) inferred the passive displacement by the surrounding tissues, whereas Johnston (1951) by light microscopy observed pseudopod formation on the surface of PGCs and suggested the occurence of active migratory movement. In the present study on *Oryzias latipes*, the examination of more than 700 PGCs in 19 embryos at stage 28 by light microscopy, and 41 in 9 embryos by electron microscopy, revealed no obvious pseudopodia of PGCs. Also, mesodermal cells were observed to stretch their thin cytoplasm over the PGCs (Fig. 5), and to incorporate them into the somatic mesodermal layer (Fig. 6, 7). During the displacement from the lateral-plate region to the gonadal anlage, the PGCs were completely surrounded with mesodermal cells, which seems unfavorable to an active translocation of PGCs by the ameboidal movement. The gonad in this species forms simultaneously with the differentiation of the lateral plate into a mesotherium. In other words, the gonad formation can be regarded as part of the morphogenesis of the lateral plate mesoderm. It is likely that the PGCs are forced to take part in this morphogenic movement, and are transferred passively.

In *Xenopus* embryos, PGCs in the dorsal mesentery are also covered with a thin layer of cytoplasm of the mesentery cells by Wylie and Heasman (1976), who did not report the existence of pseudopodia, but did note the cytoplasmic process of PGCs, whose size was approximately comparable to microvilli. In the present study

on *Oryzias*, small cytoplasmic protrusions of PGCs were occasionally noted (Fig. 7). However, there is no clue as to their possible role in the translocation of PGCs.

From our cell counts, the embryonic stages of germ cells can be classified into three phases according to their proliferative activity: 1) First proliferative phase, when the germ cells are segregated from somatic cells. 2) First nonproliferative phase, when they migrate to the gonadal anlage. 3) Second proliferative phase, when sex differentiation occurs. Based on the incorporation of ^{3}H-TdR (Dziadek and Dixon 1975, 1977) and cell counts, Zunst and Dixon (1977) after analysis of the stages of germ cell development in *Xenopus*, proposed a proliferation kinetics of PGCs similar to the results reported here.

The germ cells of *Oryzias* resume mitosis just after the completion of migration. There is no information available concerning the induction of these changes. However, the coincidence of the onset of mitosis with the entrance of PGCs into the gonadal anlage suggests the significance of the micro-milieu surrounding the germ cells. The ultrastructural observations presented here show that PGCs are enclosed within the thin cytoplasmic sheet of the mesodermal cells (Fig. 7, 8), making it likely that this enclosure serves as the structural basis for the local environment of PGCs in the gonad.

The embryos could not be classified into two groups on the basis of cell count at stage 30. This agrees with the report of Quirk and Hamilton (1973) that the number of PGCs in the known male genotype is not different from that in females. The first indication of sex differentiation was provided by the difference in the rate of proliferation of the germ cells during the second proliferative phase, between stages 30 and 33. In females, these mitoses are immediately followed by the divisions of the oogonia and the initiation of meiosis, whereas in males, the germ cells divide only once and then mitosis is interrupted at hatching (Satoh and Egami 1972; Hamaguchi 1979). This suggests that in females the germ cells mature as stem cells of gametogenesis earlier than in males. On the basis of the ultrastructural architecture of the gonad and germ cells of the pre-hatching embryo, no distinction could be made between males and females.

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