Iranian Int. J. Sci. ***3****(2), 2002, p.155-164*

Movement of Primordial Germ Cells in the Endodermal Cell Mass of *Xenopus* Embryos

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(received: 8/1/2002 ; accepted: 12/8/2002)

Abstract

In *Xenopus* embryos, primordial germ cells (PGC), located ventrally in the endoderm, migrate into dorsal endodermal crest where they exit from the endoderm and migrate into genital ridges. The aim of this study was to investigate the nature of PGC movement from ventral to dorsal endoderm. A grafting experiment was planned in which the ventral part of one of three endodermal regions - anterior, middle or posterior endoderm labelled with fluorescein dextran amine (FDA) from stage 28 donor embryos was grafted to a similar position in unlabelled host embryos of the same stage. The grafted embryos were allowed to develop until stage 40 and 46 when they were examined histologically for presence of FDA positive cells. Regardless of some slight local distortion (especially in the anterior graft), labelled endodermal cells appeared as a cohesive group in the ventral region adherent to the ventral lateral plate mesoderm. In all cases there were sharp, straight, anterior, posterior and dorsal boundaries between the donor labelled cells and host endoderm. These results suggest that, there are no early endodermal cell movements, either as single cells or as a group of cells, involved in morphogenesis of the endoderm, indicating that the PGC could move actively through the endoderm.

Key words: *PGC, endoderm, cell movement, Xenopus embryos*

Introduction

A founder clone of four primordial germ cells (PGC) is segregated during cleavage of the *Xenopus* egg by the allocation of germ plasm to one daughter blastomere by successive asymmetrical mitoses (Whitington and Dixon, 1975). Germ plasm is located peripherally in

the cell during cell divisions, but late in cleavage it lies around the nucleus. Subsequent divisions of the founder clone, of which they are usually three (Dixon, 1981), produce about 32 germ cells in the endoderm. They remain there until stage 39/40 when they exit from the dorsal endodermal crest into incipient genital ridges. These are situated in either sites of dorsal root of the dorsal mesentery that suspends the posterior gut in the coelom, and are sometimes referred to as “the median genital ridge”. The first stage of the movement of the primordial germ cells is within the endoderm, and the second stage from the endoderm to the median genital ridge.

It is generally believed that the second stage requires active movement of the PGC first along the dorsal mesentery and then along the abdominal wall into the genital ridge (Reviewed by Wyile 1999). However, there is also some evidence showing that this stage of movement does not require active movement (Whitington and Dixon, 1975; Subtelny, 1980). Based on these evidence, the PGC are separated from the dorsal endodermal crest by ingrowing sheets of splanchnic mesoderm, come into contact with the body wall mesoderm, and adhere to it. Dorsal mesentery does not form until later, after stage 41, and therefore not normally used as a pathway of the germ cell migration.

There is also controversy in the nature of germ cell movement in the first stage that is from ventral endoderm to the dorsal endodermal crest. There are two sets of observations, which may lead to the conclusion that this movement is as results of active migration. The first group depends on histological examination of PGC *in situ* within the endoderm. Kamimura et al. (1980) and Delbos et al. (1980) showed that the germ cells are in spaces in the endoderm, with an irregular shape, and with obvious lobopodia and fillopodia. However, these conclusions are drawn from a series of static images and may not accurately reflect conditions *in vivo*. The second set of evidence come from *in vitro* studies in which active movement of germ cells has been directly observed (Heasman et al, 1981). In this experiment the germ cells are taken from the genital ridges which may not be an appropriate model.

On the other hand, there is some evidence showing that the first

stage of germ cells movement could be passive movement. Injecting dye into the yolky endodermal mass, Lewis et al. (unpublished data) observed movement of the dye from ventral to dorsal region of the endoderm, concluding that there are morphogenetic movements in the endoderm. Then they showed that latex beads injected into the endoderm are carried out of the endoderm, some even into genital ridges, again indicating the existence of massive endodermal movement enough to translocate germ cells from the ventral to dorsal region of the endoderm . On the basis of these findings, they suggest that movement of germ cells within the endoderm is a passive migration.

Because of this controversy, we have decided to re-examine the endodermal movement of PGC to see whether the morphogenetic movement involved in formation of the gut is responsible for movement of the PGC.

Materials and Methods

Eggs and embryos

Xenopus laevis eggs were obtained from frogs injected with human gonadotropins and fertilized with a fragment of testis (after Cleine and Dixon 1985). Embryos were cultured in 25% MMR and staged according to Nieuwkoop and Faber (1967).

Grafting procedures

Embryos at stage 28 were used for grafting experiments. They were first demembranated with watchmaker's forceps and then anaesthetised with MS 222 (tricaine methane sulfonate, Calbiochem). During the grafting procedure the embryos were kept in sterile full strength Holtfreter's solution (NaCl, 60 mM; KCl, 0.6 mM; $CaCl_2$, 0.9 mM; $NaHCO_3$, 0.2 mM, from Peng et al. 1991) in plastic petri dishes with a 3 mm layer of agarose (Sigma, 2% in Holtfreter's solution. Tissue was excised with micro-scalpels made from two parallel chips (4 mm wide) of razor blade and grafted immediately to host embryos which were at the same stage as donor embryos (Fig. 1). Care was taken not to reverse the polarity of the graft with respect to that of the host embryo. The grafts were held in position by glass rods and healed within 10 to 15 minutes after which the rods were removed. Well-healed grafted embryos were allowed to develop until stage 40 or 46 when they were

examined morphologically and histologically.

Microinjection and visualization of cell lineage

Fluorescein dextran amine (FDA) was used as a cell lineage tracer to distinguish donor tissues from host tissues in grafting experiments (Gimlich and Braun 1985). Approximately 20 nl of FDA (Sigma) at a concentration of 50 mg/ml in distilled water (Gimlich 1986) was injected into eggs, in sterile 100% MMR containing 5% Ficoll, immediately after fertilization. Injected eggs were left undisturbed for a few hours after which they were gently transferred to 25% MMR and cultured at 16-18^{o}C until the stage needed for grafting experiments. Grafted embryos and recombinants containing FDA-labeled tissues were fixed with 4% paraformaldehyde, and then embedded, sectioned and stained as described above.

Results

Cell movement in the endoderm before coiling

To determine whether there are cell movement in the endoderm to move the PGC from ventral to dorsal region, a homotopic grafting experiment was planned. Endodermal tissue labelled with fluorescein dextran amine (FDA) (together with its mesodermal and ectodermal sheath) from stage 28 donor embryos was grafted to a similar position in unlabelled host embryos. Figure 1 shows a summary of the procedure. The ventral part of one of three endodermal regions- anterior, middle or posterior - was excised from donor embryos labelled with FDA and grafted into host embryos. Embryos in which the grafts healed well (Fig. 2A) and subsequently exhibited a normal morphology were allowed to develop until stage 40 and 46 when they were examined histologically for FDA-positive cells.

In all embryos receiving anterior grafts at stage 40, the graft showed a slight local distortion. However, there were still sharp boundaries between labelled and unlabelled endodermal tissues. Moreover, the labelled tissues always appeared to adhere ventrally to the lateral plate mesoderm. This result indicates that anterior grafts did not move, and the local dislocation of labelled tissue is the result of morphogenetic movements of endodermal tissue starting anteriorly from stage 35/36 and leading to primary segregation of anterior endodermal regions.

In all embryos with grafts of middle endoderm, where the PGCs are, labelled endodermal cells appeared as a cohesive group in the ventral region adherent to the ventral lateral plate mesoderm. In all cases there were sharp, straight, anterior, posterior and dorsal boundaries between the donor labelled cells and host endoderm (Fig. 2B). The same results were obtained in all embryos with grafts of posterior endoderm. This result indicates that the middle and posterior graft cells also did not move as a group.

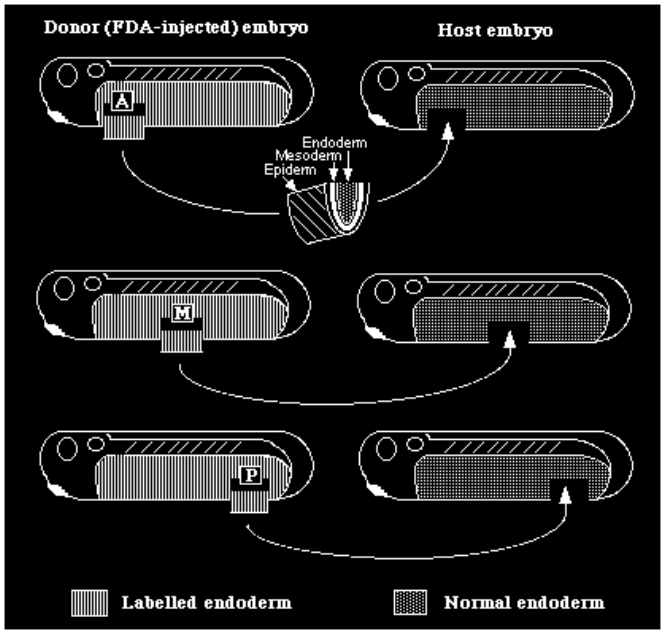

Figure 1 - Homotopic grafting procedure. Ventral endoderm together with its mesodermal and ectodermal sheath from anterior (A), mid (M) or posterior (P) regions of stage 28 donor labeled embryos were grafted to the similar position in uninjected host embryos at the same stage. To label donor tissues one cell stage embryos were injected with fluorescein dextran amine (FDA) and allowed to develop until stage 28.

In contrast to the endoderm, in the majority of the grafted embryos, FDA-positive lateral plate mesoderm cells were observed posterior to the location of the graft surrounding FDA-negative endodermal mass (Fig.3). This result may indicate that there is an anterior to posterior movement of

lateral plate mesoderm during tailbud- and tadpole-stage embryo.

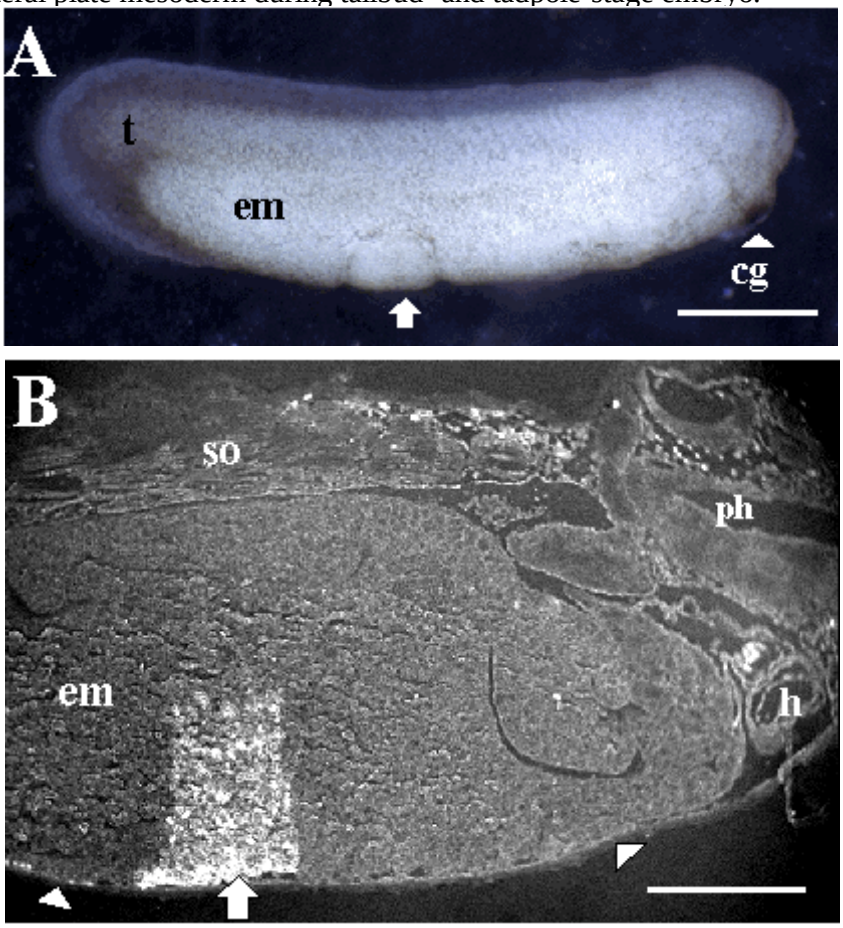

Figure 2 - Homotopic grafting experiment. Ventral endoderm together with its mesodermal and ectodermal sheath from anterior, mid or posterior endodermal region of stage 28 labelled donor embryos were grafted to a similar position in unlabelled host embryos at the same stage. One cell-stage embryos were injected with 20 nl of 50mg/ml fluorescein dextran amine (FDA) and allowed to develop until stage 28 when they were used as donor embryos.

A. A grafted embryo a few hours after operation. The graft (arrow) healed well.

B. Sagittal section of stage 40 grafted embryo. FDA-labelled endodermal tissue remain as a cohesive group of cells in the ventral region adherent to the ventral lateral plate mesoderm (arrowhead). Also note that there are sharp boundaries between the donor labelled cells and host endoderm.

cg, cement gland; em, endodermal mass; h, heart; ph, pharynx; so, somite;

t, tail.
Scale bars: 700 ?5m in A; 280 ?5m in B.

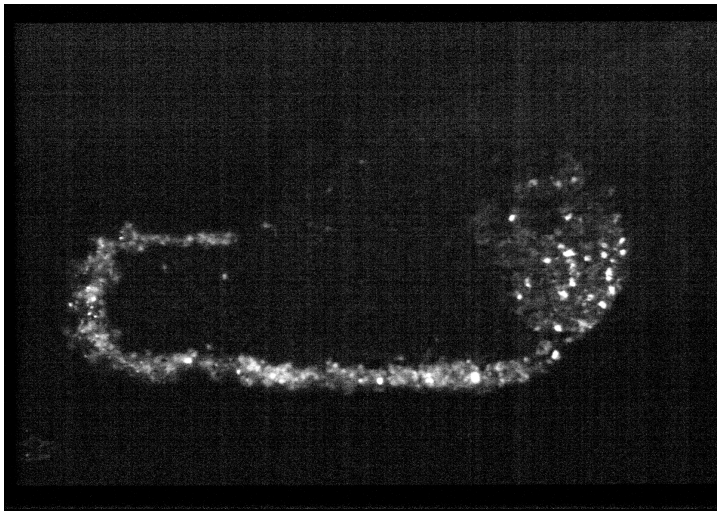

Figure 3 - sagittal section through the endoderm in stage-40 grafted embryo. While the FDA positive endodermal cells remain as cohesive group of cells (arrow), FDA positive lateral plate mesodermal cells (arrowhead) show extensive migration toward posterior of the embryo. Scale bar: 400?m

Some of the grafted embryos allowed to develop further until stage 46 when they were examined histologically. In all embryos, the histological slides clearly showed normally developed gut in which the grafted labelled tissue integrated into host tissue developing gut structures (Fig. 4). This result indicates that the grafting procedure does not affect the normal development of the host embryos and the morphogenesis of the grafted tissues. In the embryos with middle labelled graft, cross sections showed labelled cells, most probably PGC, in the dorsal mesentery (data not shown). This observation indicates that in the absence of endodermal movement, the PGC could move out of the endoderm.

Taken together, these results suggest that, there are no early endodermal cell movements, either as single cells or as a tissue, involved in morphogenesis of the endoderm. This conclusion does not affect the reality of the local morphogenetic movements responsible for primary segregation of anterior endodermal regions and intestinal

coiling which take place later at stage 39. On the basis of these results, we conclude that the PGC could move actively through the endoderm.

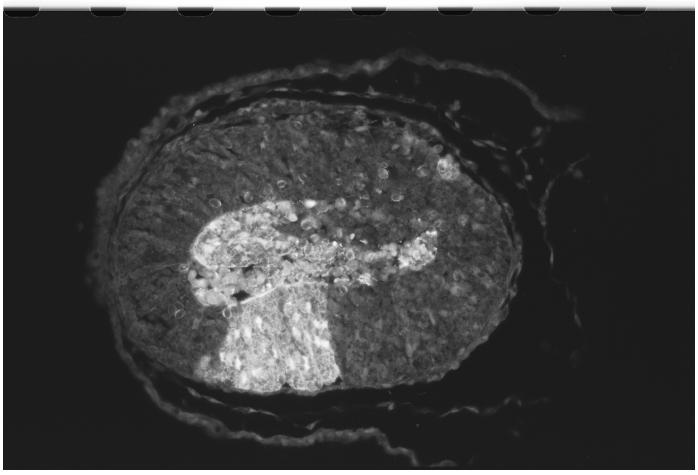

Figure 4 - Transverse section of the intestine in stage-46 grafted embryo. FDA positive cells are integrated into host endodermal cells developing normal intestine. Scale bar: 42?m

DISCUSSION

The aim of this study was to determine the nature of the PGC migration within the endoderm in *Xenopus* embryos. At the end of cleavage, the founder germ line cells are located over the vegetal pole; after gastrulation, cells at the vegetal pole end up in the middle of the endoderm (Cline and Dixon, 1985) and hence the PGC are also located in this region, ventral to the archenteron. They remain there until stage 35/36 when they move round archenteron into the dorsal endoderm and finally into endodermal crest, arriving there at stage 38/39 (Kamimura et al., 1976).

To address the nature of PGC movement within the endoderm, we asked whether there is extensive cell movement within the endoderm that could translocate the PGC from ventral to dorsal region of the endoderm. We have applied a standard technique, that is, grafting labeled endoderm from stage-28 donor embryos into host embryos at the same stage and examining the behavior of labeled cells in further development.

Our results revealed that, in stage 40 grafted embryos, labeled tissue always appeared as a cohesive group with sharp boundary with unlabelled tissue and adhere ventrally to the lateral plate mesoderm. This result indicates that the grafts did not move, suggesting that there are no early endodermal cell movement, either as a single cells or group of cells, involved in the morphogenesis of the endoderm. However, in stage 46 grafted embryos, the labeled tissue integrated into host tissue developing normal gut structure. This evidence together with the observation that some labeled cells, most probably PGC, could be seen out of the endoderm on the dorsal mesentery rule out the possibility of the encapsulation of the grafted tissue so that labeled cells could not move out of the graft.

From the evidence presented here, we suggest that there are no early endodermal cell movements before stage 40 when PGC leave the endoderm. This indicates that the migration of the PGC within the endoderm cannot be relied on the endodermal movement (passive movement) and therefore is an active migration. This conclusion is consistent with the histological examination of Kamimura et al. (1980) and Delbos et al. (1980) who showed that PGC has ultrastructural characteristic of active motile cells, and the studies of isolated germ cells in culture, where active movement of PGC has been directly observed (heasman *et al.,* 1981).

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