The formation of the gonadal ridge in *Xenopus laevis*

III. The behaviour of isolated primordial germ cells *in vitro*

By C. C. WYLIE[1] AND T. B. ROOS[2]

From the Department of Anatomy, University College London

SUMMARY

Previous studies have described the morphology, including the ultrastructure, of primordial germ cells (PGCs), and the cells with which they associate to form the gonadal ridge, in *Xenopus laevis*. In order to test their capacity for active movement we have studied single, isolated PGCs *in vitro*. Time-lapse studies of these cells reveal that they are motile, using broad cytoplasmic processes. The fact that these cells are very large and easy to manipulate *in vitro* makes them an attractive subject of study, particularly with respect to the mechanism of their movement and the surface phenomena which guide them to the site of the gonadal ridge.

INTRODUCTION

In vitro experiments in vertebrates have proved useful in the analysis of germ-cell migration and gonad formation. In the chick embryo the primordial germ cells (PGCs) have amoeboid properties (Dubois, 1967) and are capable of migrating through embryonic tissue in organ culture (Dubois, 1964a, b). Their migration appears to be non-random, the direction being imposed by a chemo-attractive stimulus from the germinal epithelium of the gonadal ridge (Dubois, 1968). In the mouse embryo (Mintz, 1960; Peters, 1970) PGCs also migrate actively, up the dorsal mesentery of the gut to the gonadal ridge. When tissue squashes of the early gonad are studied, the PGCs are found to exhibit amoeboid activity (Blandau, White & Rumery, 1963).

Previous work to elucidate the position in the anuran *Xenopus laevis* (Kalt & Gall, 1974; Whittington & Dixon, 1975; Wylie & Heasman, 1975; Wylie, Bancroft & Heasman, 1975) has shown that the PGCs appear to migrate from the embryonic gut to the root of its dorsal mesentery and then laterally to a region of the coelomic lining. This part of the coelomic lining differentiates concomitantly with PGC arrival to form the germinal epithelium of the gonadal ridge.

[1] *Author's address:* The Department of Anatomy, University College London, Gower Street, London WC1E 6BT, U.K.

[2] *Author's address:* The Department of Biological Sciences, Dartmouth College, Hanover, New Hampshire 03755, U.S.A.

The mechanism of PGC movement in *X. laevis* is uncertain: some of the PGCs appear to be passively pushed into position by the morphogenetic movements of mesentery formation, whilst others must move relative to the structures around them during the formation of the paired gonadal ridges. It is difficult to conceive of the PGCs, with their relatively enormous size, moving freely amongst the tissues of the embryo. It was therefore decided to study their behaviour *in vitro*. This paper describes the isolation and culture of single PGCs from *X. laevis* embryos and the fact that that they are capable of independent locomotion *in vitro*.

MATERIALS AND METHODS

X. laevis embryos were fertilized and grown to stages 44–45 (Nieuwkoop & Faber, 1956) as previously described. This takes about 6 days under our conditions. At this time most of the PGCs are found in the median germ ridge at the root of the dorsal mesentery. The tadpoles were anaesthetized with MS 222,

Fig. 1. Photomicrograph (× 35) taken through a binocular dissecting microscope, of a dissected tadpole in Steinberg's saline.

and dissected as far as the posterior body wall in sterile Steinberg's saline, containing 100 units/ml penicillin and 100 μg/ml streptomycin. This involves removal of the front and sides of the abdominal cavity, followed by careful removal of the viscera, head and tail. The result of such a dissection is shown in Fig. 1. The specimens were then pipetted into sterile Ca^{2+}- and Mg^{2+}-free Steinberg's saline containing 0·1 % EDTA, 100 units/ml penicillin, 100 μg/ml streptomycin, 0·25 % trypsin and 1 mg/ml Collagenase (type 1, Sigma). After 2 or 3 min of this treatment the cells of the dorsal mesentery and posterior body wall round up. The PGCs are clearly visible at this stage as shining white spheres,

due to their high content of refractile yolk granules. The PGCs, together with other cells of the region, are then gently scraped off and allowed to settle to the bottom of the dish. Using a finely drawn micro-pipette connected to a mouth-piece, individual PGCs were then picked up from the dish and transferred to a 50 mm plastic tissue culture dish (Sterilin) containing sterile Steinberg's saline plus penicillin and streptomycin, 10 % calf serum (Biocult) and 0·3 % glutamine.

For time-lapse cinematography, PGCs were transferred to slide tissue culture chambers (Sterilin) and photographed using direct light.
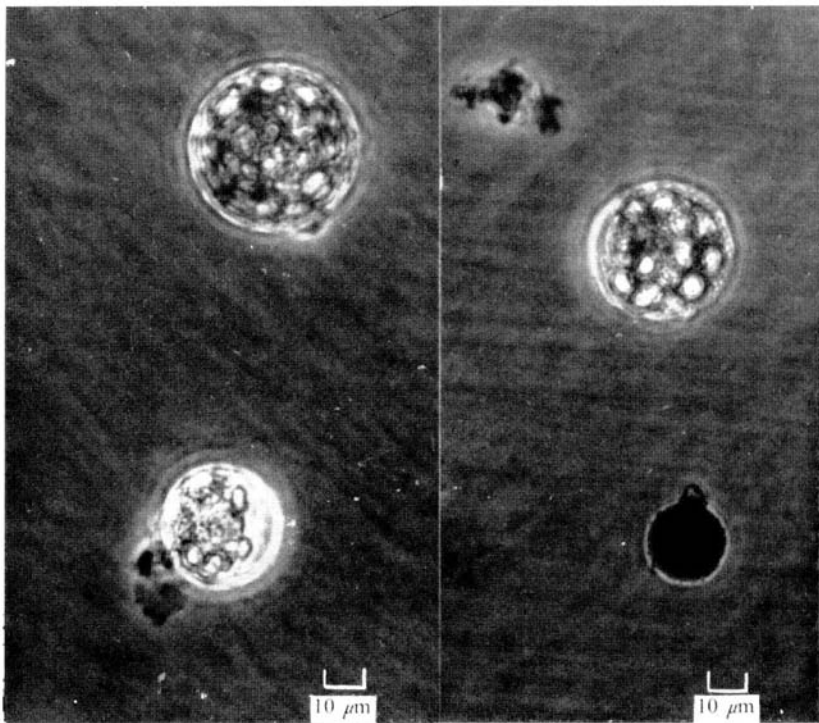

Fig. 2. Phase-contrast photomicrograph showing the initial appearance of PGCs while settling down on the bottom of the culture dish. A rounded-up pigment cell, from the posterior wall is shown for size comparison.

RESULTS

As the PGCs settle down to the bottom of the culture dish they can be seen clearly by phase-contrast microscopy as large spheres packed with refractile granules (Fig. 2). These yolk granules distinguish them immediately from any contaminating somatic cells, as does their size; the three PGCs shown in Fig. 2 measure between 35 and 45 μm in diameter.

After about 2 h in culture they adopt a variety of shapes and some of them start to show transient processes. Fig. 3 shows two phase-contrast photomicro-

graphs, taken 4 min apart, of the same PGC. The yolk platelets in the cytoplasm can be clearly seen. The cytoplasmic processes seen, which are free of yolk platelets, are extended and withdrawn very rapidly. In the 4 min interval between the two photographs the two processes inferiorly have been withdrawn and a large new process extended upwards and to the right.

In order to test whether the PGCs are moving during this process of formation and retraction, two time-lapse films have been made of their behaviour in culture. It is clear from these films that the PGCs can move over the surface of the tissue culture plate. Ten frames from one of the films are shown in Fig. 4, with an
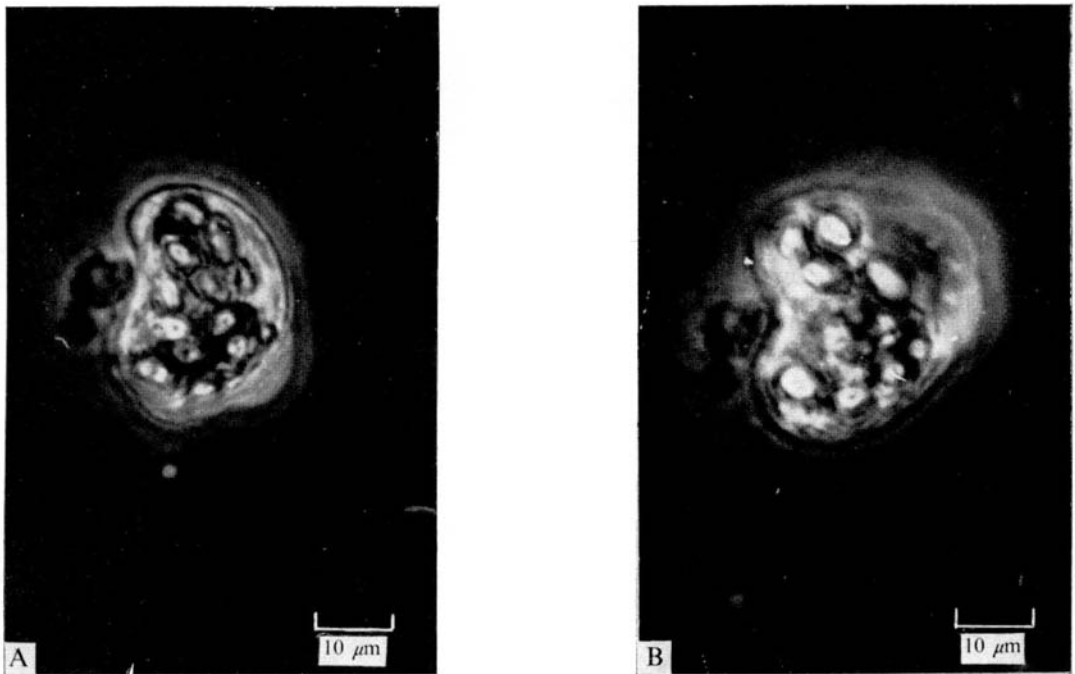

Fig. 3. Phase-contrast photomicrographs, taken 4 min apart, of the same PGC in culture to show process formation.

interval between frames of 10 sec. The field of view was not adjusted during this part of the film so that the edges of the frame serve as fixed points of reference, allowing movement of the cell to be followed. Using this method we can describe the events seen in the ten frames:

Frame 1. The PGC starts off with a broad process superiorly.
Frame 2. The superior process is still present. A new process is being extended to the left.
Frame 3. No change.
Frame 4. The process to the left is expanding and moving inferiorly.
Frame 5. The left/inferior process is extending further.
Frame 6. The left/inferior process is still large and has started to extend further inferiorly. The superior process has started to withdraw.
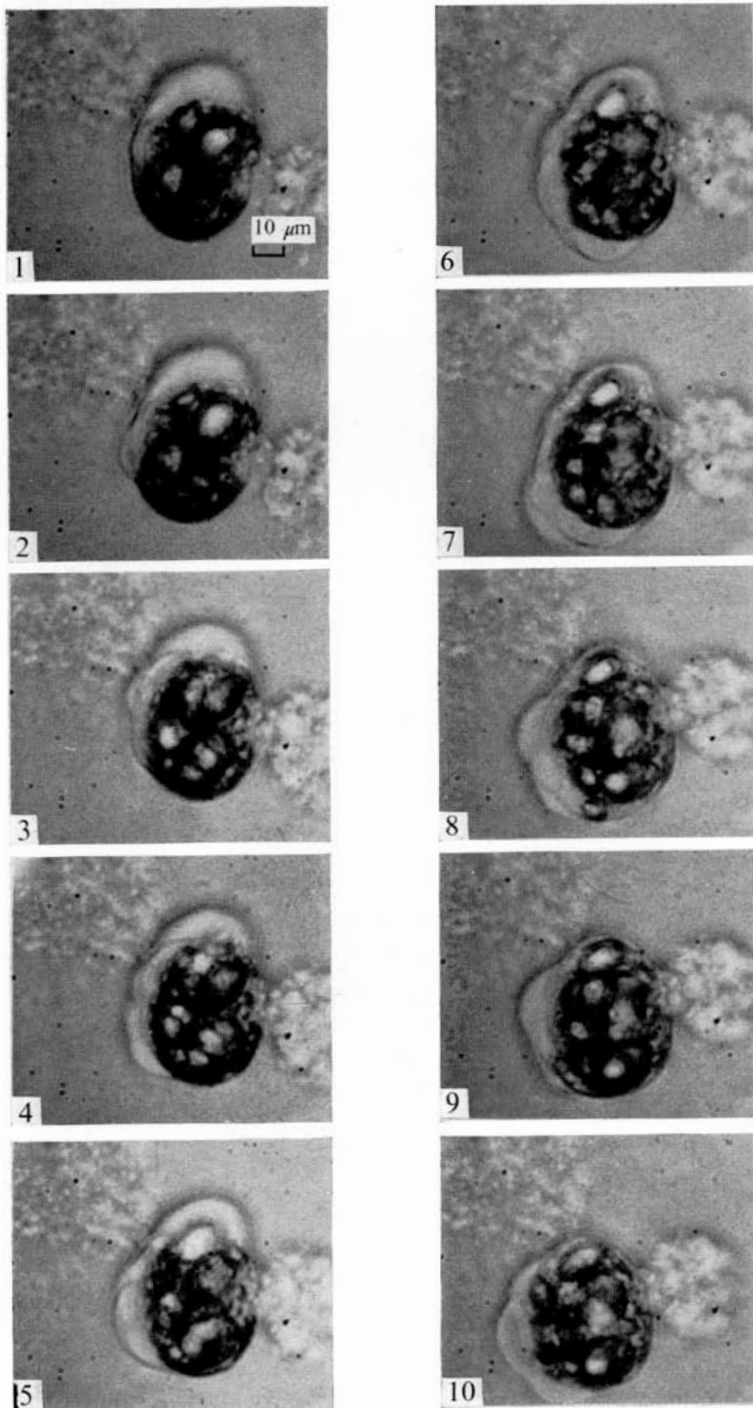
Fig. 4. Ten frames from a time-lapse film (interval between frames = 10 sec) to show sequence of movements of cell processes and contents.

Frame 7. The superior process is now almost completely withdrawn. The processes to the left and inferiorly have extended further. The yolky contents of the cell have started to move downwards.

Frame 8. The cell contents have moved downwards to fill the inferior process. A new process is extending to the left.

Frame 9. The process to the left has moved slightly further and the cell contents have started to move to the left into this process.

Frame 10. The cell contents have continued to move to the left but still do not fill the cytoplasmic process, which has extended even further. A new process has been put out inferiorly.
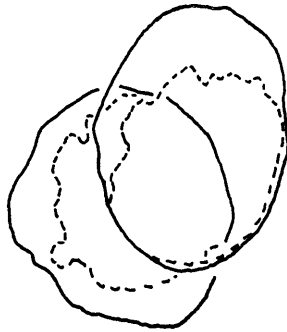
10 μm

Fig. 5. Tracing to show overall movement of PGC during the sequence shown in Fig. 4.

The overall movement of the PGC during this 100 sec period is shown in Fig. 5. Here, the first and last frames have been taken and their shape and position, relative to fixed reference points, traced on to melanex and super-imposed. It can be seen in Fig. 5 that the change in position of the PGC is not solely due to the repositioning of its processes, but also by movement of its cytoplasmic contents downwards and to the left.

The yolk granules, which are such a predominant feature of the PGCs in culture, are in a continuous state of motion relative to each other. This is shown to greatest advantage in the films, but can also be seen in Figs. 4 and 5.

The speeds at which PGCs move *in vitro* were found to be very variable and sometimes prodigious. Using timed sequences of photographs and marked culture dishes, speeds varying from zero to 150 μm/h have been recorded. A detailed study of this kind is not possible, however, due to the fact that the cells do not remain unidirectional for long enough. They often stop, reverse, go round in circles or merely oscillate to and fro in the culture dish.

DISCUSSION

There is good evidence from *in vitro* experiments in mammalian embryos and from organ culture experiments in chick embryos that the PGCs from these species show amoeboid activity. Furthermore, from chick embryos there is evidence to suggest that they move in response to chemotactic stimuli, produced by the undifferentiated gonadal ridge (see Introduction for references).

The problem of germ-cell migration and gonadal ridge formation has been studied in several anuran amphibian species. Much of this work has been devoted to the study of the 'germ plasm' (Beams & Kessel, 1974), but several techniques have also been applied to the actual migration of PGCs. These include micro-scopical studies (Witschi, 1929; Kalt & Gall, 1974; Whitington & Dixon, 1975) and extirpation and grafting experiments (Giorgi, 1974; Gipouloux, 1964a, b, 1970). It is often tacitly assumed from these experiments that the PGCs migrate actively to the gonadal ridges, although there has been no demonstration of PGC movement. This is not altogether surprising, since the enormous amount of yolk present in the embryonic cells at the time of mesentery formation tends to obscure cell boundaries in histological sections. Many PGCs are already at the root of the dorsal mesentery when it first forms, and this has led several authors to suggest that the PGCs might not move at all, and that they may be passively pushed into position by the events of mesentery formation (Witschi, 1929; Whittington & Dixon, 1975). The results presented here suggest strongly that the PGCs, in *X. laevis* at least, move actively to form the early gonadal ridge.

Grafting (Giorgi, 1974) and extirpation experiments (Gipouloux, 1964a, b, 1970) have suggested that the dorsal tissues of the embryo may exert a chemo-attractive effect on the PGCs. In the chick embryo (Cuminge & Dubois, 1974) it is thought that chemo-attractive molecules are released from the germinal epithelium of the undifferentiated gonadal ridge. In *X. laevis* there is no pre-existing gonadal ridge to which the PGCs migrate. However, we have demon-strated (Wylie *et al.* 1975) the presence of a specialized band of cells, derived from the coelomic lining, which surrounds the PGCs as they move laterally and differentiates to form the germinal epithelium. It is possible that it is these cells which exert a chemo-attractive influence. The results presented here suggest that this possibility may now be tested *in vitro*, using pure populations of disaggregated PGCs.

The mechanism by which PGCs move *in vitro* is not certain. The cytoplasmic processes observed do not correspond to many of the traditional organs of cell locomotion, e.g. lamellae (Harris, 1973). They resemble most closely the lamel-lipodia described in *Fundulus blastulae* (Trinkaus, 1973), particularly as the processes seen in PGCs often continue to bulge around the surface in a propa-gated wave. PGCs in culture represent an attractive system for the study of cell movement in vertebrates. They are known to be a migratory cell type, possibly responding to definable chemotactic stimuli. They are very large, thus rendering

them easily amenable to surface marking experiments, as well as intracellular manipulation.

In this paper and in previous papers (Wylie & Heasman, 1975; Wylie *et al.* 1975) we have shown that PGCs are capable of active, independent movement in culture. We have also described microscopic and ultrastructural details of the cells over which they move and with which they associate to form the developing gonad. If we can reproduce these phenomena *in vitro* it will be an exciting opportunity to study the molecular bases of cell locomotion and interaction.

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