Scanning Electron Microscopy

Volume 1986
Number 3 *Part III*

Article 37

7-2-1986

The Migration of Amphibian Primordial Germ Cells in the Chick Embryo

Marjorie A. England
University of Leicester

Alma P. Swan
University of Leicester

P. Dane
University of St. Andrews

SCANNING ELECTRON MICROSCOPY/1986/III (Pages 1175-1182)
SEM Inc., AMF O'Hare (Chicago), IL 60666-0507 USA

0586-5581/86$1.00+05

THE MIGRATION OF AMPHIBIAN PRIMORDIAL GERM CELLS IN THE CHICK EMBRYO

Marjorie A. England*, Alma P. Swan** and P. Dane ***

Departments of Anatomy* and Zoology**,
University of Leicester,
Leicester, LE1 7RH
Department of Zoology***,
University of St. Andrews,
Fife, Scotland

(Received for publication March 01, 1986, and in revised form July 02, 1986)

Abstract

A fibrous band of extracellular materials on the chick embryo area pellucida/area opaca border is a preferential migratory pathway for chick embryo primordial germ cells (PGC). This band contains fibronectin, collagen Type I and sulphated glycosaminoglycans. It is known that PGCs from Xenopus laevis interact with fibronectin as they undergo migration in the embryo from their site of origin to the gonads. To establish whether this pathway is species specific in chick embryos it was decided to transplant PGC from Xenopus laevis embryos stage 48 on to chick embryos stage 4 fibrous band. Their rapid migration on this extracellular matrix and their subsequent re-orientation of the basement membrane has been studied by scanning electron microscopy.

KEY WORDS: Amphibian embryo, Cell migration, extracellular matrix, Chick embryo, Primordial germ cells, Collagen, Embryonic development, Cell interactions, Basement membrane, Cell movement.

* Address for correspondence:
Marjorie A. England,
Department of Anatomy,
University of Leicester,
Leicester LE1 7RH Phone No:(0533) 551234

Introduction

Vertebrate primordial germ cells (PGCs) originate extragonadally (Swift, 1914; Bounoure, 1934) and then migrate to the site of gonad formation. Several studies have noted and documented the pathways of PGC migration but the mechanisms of this process remain unclear. In the anuran amphibian Xenopus laevis PGCs arising at the vegetal pole of the blastula become incorporated into the endoderm layer and subsequently in the embryonic gut tube. During early larval development PGCs leave this region and migrate through the mesentery to the dorsal body wall in the region of the future gonadal ridges. These PGCs are very invasive and will penetrate and invade adult tissues (Swan et al., 1981) as well as the intact epithelial lining of the embryonic mesentery. The Xenopus laevis PGCs have been shown to be invasive both in vivo and in vitro (Heasman and Wylie, 1978). On a cellular substrate they insert processes downwards (Heasman and Wylie, 1978) and induce the cells of the substrate to respond by migrating over the PGC surface and enveloping them (Swan et al., 1981). The PGCs end up beneath the cell layer by a combined process of active invasion on their part and envelopment of the substrate cells.

Chick embryo primordial germ cells have been shown to adhere to, and migrate along, a fibrous band lying on the anterior area pellucida/area opaca border (Wakely and England, 1979; Critchley et al., 1979) on the ventral ectoderm basement membrane. This band of fibres forms an arc along the anterior margins of the area pellucida and with further incubation this band increases both in width and in length Wakely and England, 1979). The fibres have been shown to contain a high concentration of collagen Type I (England et al., 1982); fibronectin and sulphated glycosaminoglycans (Wakely and England, 1979). Chick PGCs leave the endoderm layer and contact this band. Those not in the immediate proximity of the band actively migrate towards it (England, 1980). After contacting the band the PGCs move along it and actively deform the fibres. A transmission electron microscope study of the band has shown it consists of folds of basement membrane studded with materials resembling proteoglycans (Kordylewski and England, 1980).

In the present study we investigated further the conditions necessary for the migration of Xenopus PGCs, including whether this band, which is known to influence chick PGC migration (England 1983) is capable of being utilised by Xenopus laevis PGCs. Both chick and amphibian PGCs are influenced by the presence of fibronectin on their normal migratory routes and both cell types are invasive. We have studied the gross migratory movements of the amphibian germ cells in vitro; in a timed interval study and by scanning electron microscopy (SEM).

Materials and Methods

Chick embryos

Fertile White Leghorn hens' eggs were incubated at 37.5°C until the embryos reached stages 4 and 5 (Hamburger and Hamilton, 1951). The embryos were then removed from the eggs and mounted as for New culture (New, 1955). The embryo in this preparation is facing endoderm side uppermost. The glass rings of the New culture were flooded with saline and the anterior area pellucida/area opaca endoderm margin dissected off with cactus needles (England, 1981) mounted on cocktail sticks. The endoderm margin is discarded. This procedure exposes the ventral ectoderm layer with its associated basement membrane and fibrous band. Most of the saline was then removed from the New culture ring and the preparation left on the bench at room temperature.

Amphibian PGCs

Adult pairs of Xenopus laevis frogs were injected with chorionic gonadotrophin and induced to mate. Embryos were collected and maintained in N/10 Gurdon's Modified Barth's saline (Gurdon, 1974) until stage 48 (Nieuwkoop and Faber, 1967). Stage 48 embryos were anaesthetised with MS.222 and the anterior and lateral body walls of the embryo dissected off and the abdominal contents removed. PGCs were collected from the rest of the mesentery using a disaggregating saline (0.06% Tris, 0.34% NaCl, 0.0005% KCl, 0.1 ethylene diamine tetraacetic acid buffered to pH 7.8 with HCl) and physical manipulation with fine tungsten needles. The isolated PGCs were transferred using a fine glass tube connected to a mouthpiece. They were placed on agarose in 70% L-15 medium containing 10% fetal calf serum, 2 mM glutamine and gentamycin (20 µg/ml) (complete medium) until used.

Chick embryo-amphibian PGC preparation

The amphibian PGCs were transferred to the prepared New cultures in the region of the fibrous band. The PGCs were carefully collected in a group at one end of the fibrous band (Figure 1). Any excess saline was carefully pipetted off and the culture left at room temperature for varying periods of time up to three hours.

Preparation of mesentery monolayers

Adult Xenopus laevis were anaesthetised with MS. 222 and the gut mesentery removed and rinsed briefly in Barth X (G.M.B. (20)). After rinsing, the gut mesentery was cut into 3-4 mm square pieces and pinned beneath sterile coverslip fragments on a sterile glass coverslip in tissue culture medium. This preparation was incubated at 25°C for 2 weeks until the mesentery cell monolayer covers the glass coverslip. The coverslip fragments were removed and the monolayer left in culture medium at 20°C until needed.

Preparation of the extracellular matrix (E.C.M.)

The mesentery monolayers were washed twice in 70% Calcium, Magnesium-free Phosphate Buffered Saline (C.M.F. P.B.S.) at 20°C. This was followed by 3 washes in buffer (0.1 M Na_2HPO_4, 2 mM $MgCl_2$, 2 mM E.G.T.A. at pH 9.6) at 20°C. After washing, monolayers were incubated for 15 minutes in 70% lysis buffer (8 mM Na_2HPO_4, 1% N.P. 40 - pH 9.6) at 25°C and then re-incubated for 60 minutes in fresh lysis buffer at 25°C. The cell-free E.C.M. was then washed in five changes of 70% P.B.S. and distilled water at 20°C and left in P.B.S. at 4°C until needed.

Scanning Electron Microscopy of PGCs

The New cultures at the end of each time period were carefully rinsed with saline several times to remove any remaining L-15 medium and calf serum. Specimens not rinsed were covered in a precipitate making it impossible to study the results by SEM.

The embryos were then fixed in half-strength Karnovsky's fixative (Karnovsky, 1965) in 0.1 M cacodylate buffer (Plumel, 1948) for 2 hours and then transferred to cacodylate buffer overnight. The specimens were postfixed in 1% OsO_4 in 0.1 M cacodylate buffer, dehydrated in an ascending graded series of ethanol/water until 100% ethanol. They were then transferred to 100% acetone and critical point dried in gelatin capsules (England, 1981) in a Polaron apparatus by replacing acetone with liquid carbon dioxide. Dried specimens were mounted ventral side uppermost on Cambridge stubs with silver paint. They were then coated with 20 nm of gold and viewed on the International Scientific Instruments DS 130 scanning electron microscope at an operating voltage of 9.15kV.

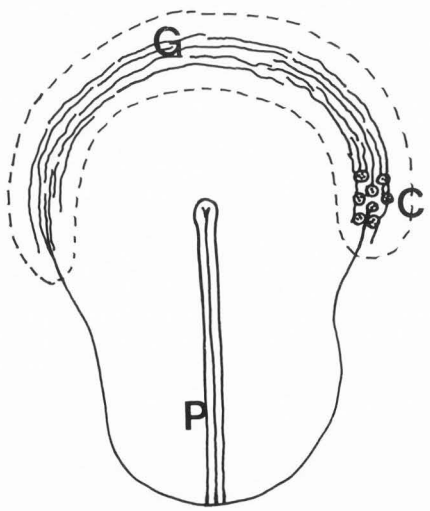

Fig. 1: Diagram to show position of seeded PGCs on chick fibrous band (stage 4). P = primitive streak; C = primordial germ cells from amphibians; G = fibrous band of germinal crescent, dotted line = endoderm removed over germinal crescent area. The embryo is ventral surface uppermost.

Results

Interactions of Xenopus PGCs with ECM components in vitro

Xenopus PGCs when seeded onto a mesentery cell monolayer in vitro adhere rapidly, attachments being made preferentially at the margins of the underlying cells (P. Dane, unpublished). Many PGCs then elongate and move and burrow between the substrate cells until they come to lie beneath them. This behaviour probably has two components: a tendency on the part of the PGCs to invade and also to gain contact with a preferable substratum for locomotion. In this case, the latter is a matrix of collagen, fibronectin and glycosaminoglycans secreted by the overlying mesentery cells onto the plastic dish. If, after two weeks of culture, the mesentery cells are lysed and washed off the substrate, this matrix is left behind, firmly attached to the tissue culture plastic (Figs. 2a and 2b). When PGCs are seeded onto this substrate they adhere firmly, elongate and translocate (Fig. 3). Immunofluorescence studies with anti-fibronectin on this substrate show that it is rich in fibronectin. The migratory route of these PGCs in vivo is also rich in fibronectin (Heasman et al., 1981). To investigate further the role of fibronectin in the interaction of Xenopus PGCs with their substrate, we examined their adhesion and locomotion on an area of the chick embryo which has been shown to contain much fibronectin (Critchley et al., 1979) and is the normal migratory route for PGCs of the chick.

Time study of Xenopus PGCs on chick wholemount embryos

As the PGCs were seeded onto one end of the chick fibrous band at zero hours, their subsequent movements were easily noted. The Xenopus PGCs are large and easily identified by reflected light as they migrate over the fibrous band. After one half-hour many of the PGCs had migrated along the arc of the fibrous band and were scattered from their zero position to approximately one-half the length of the arc of the band (Fig. 4). By 45 minutes - one hour a few of the PGCs had migrated approximately 2/3rds of the distance of the band. Between 2 and 3 hours the rate of migration dropped dramatically and, although many PGCs were visible, others were no longer easily discernible.

Scanning electron microscopy

As the amphibian PGCs are large (50 μm) and are approximately 2 to 3 times larger than the chick PGCs on the band, it is easy to distinguish the two types of PGCs. Additionally, the amphibian cells are characterised by large yolk granules and lipid droplets in their cytoplasm. The freshly seeded Xenopus PGCs are rounded and are often present on the substrate with no apparent flattening or ruffling (Fig. 5). The chick fibrous band is composed of numerous approximately parallel fibres (Fig. 6). It arcs along the anterior area pellucida/area opaca border. Some punctate material is also present on the fibres. Within a few minutes, however, some of the PGCs produce blunt cellular protrusions (Fig. 7). These protrusions contact the fibrous band and fibres are visible separated from the band (Fig. 8)

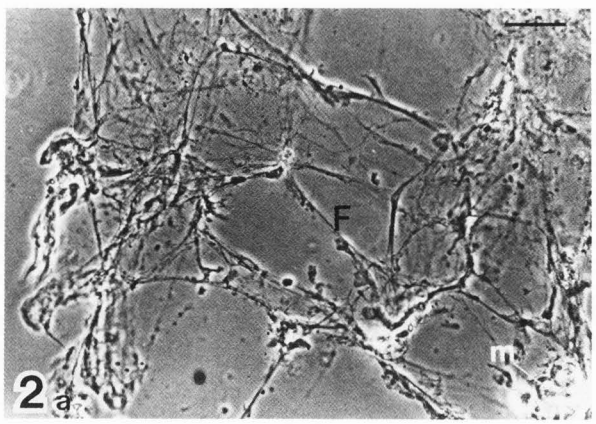

Fig. 2a: Phase contrast photomicrograph of ECM laid down by mesentery in culture. Note fibrous appearance of the collagen network. m = remains of a mesentery cell membrane; F = collagen fibres. Scale bar = 10 μm.

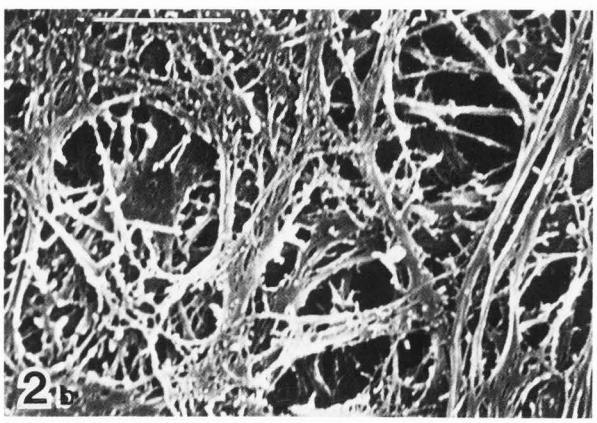

Fig. 2b: SEM of matrix shown in 2a. Scale bar = 1 μm.

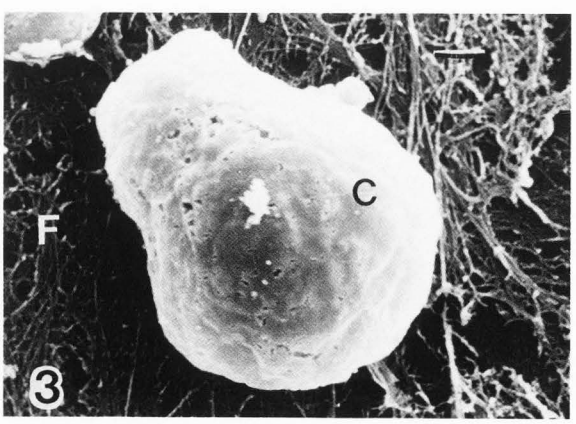

Fig. 3: Xenopus PGC on mesentery matrix in vitro. The cell has adhered and is elongating. C = germ cell; F = fibrous matrix. Scale bar = 1 μm.

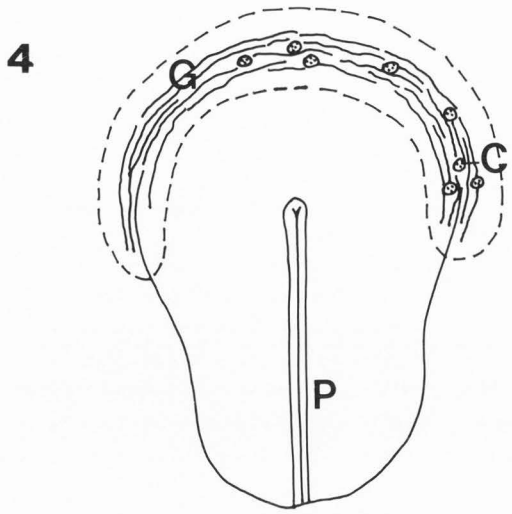

Fig. 4: Diagram to show amphibian PGC migration 30 minutes after seeding onto chick fibrous band. P = primitive streak; C = primordial germ cells from amphibian; G = fibrous band; dotted line = endoderm removed from germinal crescent area.

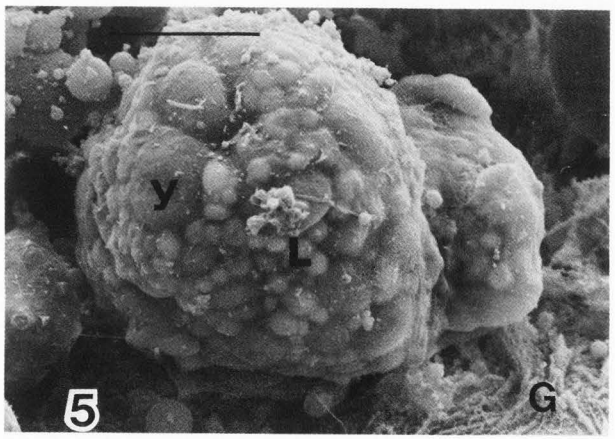

Fig. 5: Freshly seeded PGC on chick fibrous band. Y = yolk platelets; L = lipid droplets; G = fibrous band. Scale bar = 10 μm

and contacting the PGC. Several normal chick PGCs are present migrating along the fibrous band. One hour after seeding, several PGCs are draped with the fibres from the chick band. Sheets of basement membrane are lifted from the surface of the chick ectoderm and orientated vertically to the substrate (Fig. 9). Each PGC is surrounded by whorls of fibrous band whose parallel fibres are disturbed from their normal configurations. By two hours, the fibrous band is disturbed in several areas (Fig. 10) and its parallel orientation has been converted to a meshwork. In other regions on the same embryo PGCs are surrounded by large areas depleted of fibrous band materials (Fig. 11).

By three hours several PGCs are covered with numerous ruffles and microvilli (Fig. 12). Some of these cells are embedded in the substrate (Fig. 13) and others have long cellular processes pene-

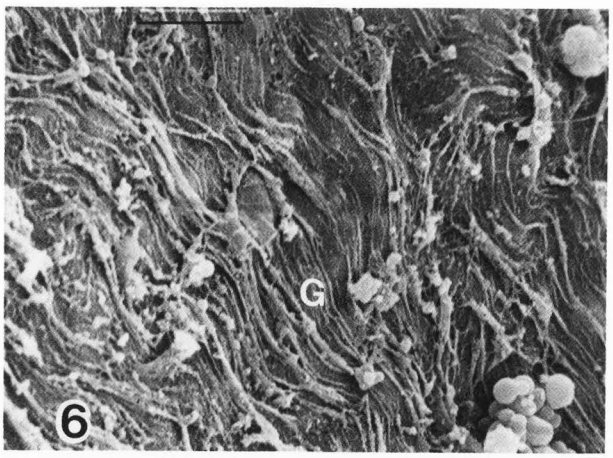

Fig. 6: Stage 4 chick fibrous band. Note the approximately parallel array of fibres. G = fibres. Scale bar = 10 μm

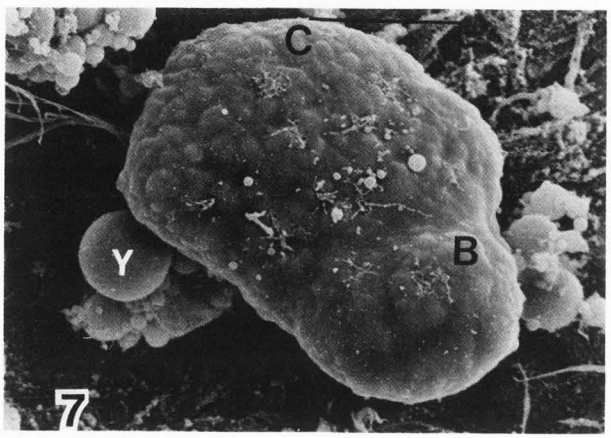

Fig. 7: PGC elongating on fibrous band. C = cell body; B = blunt process; Y = contaminating yolk droplets. Scale bar = 10 μm.

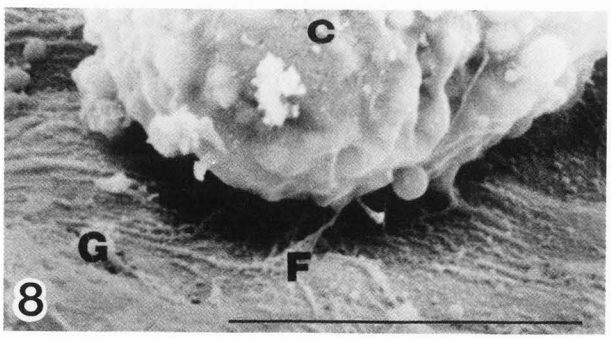

Fig. 8: Area of contact between PGC and fibrous band. C = PGC; G = fibrous band: F = fibres attached to cell surface. Scale bar = 10 μm.

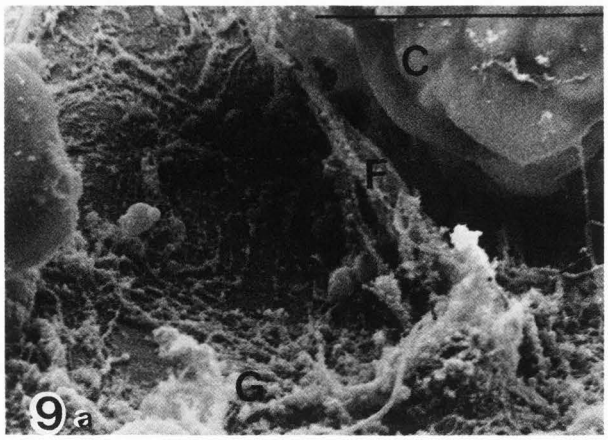

Fig. 9a: Disruption of fibrous band by PGC. SEM shows fibres displaced by cell. C = PGC; G = fibrous band; F = fibres. Scale bar = 10 μm.

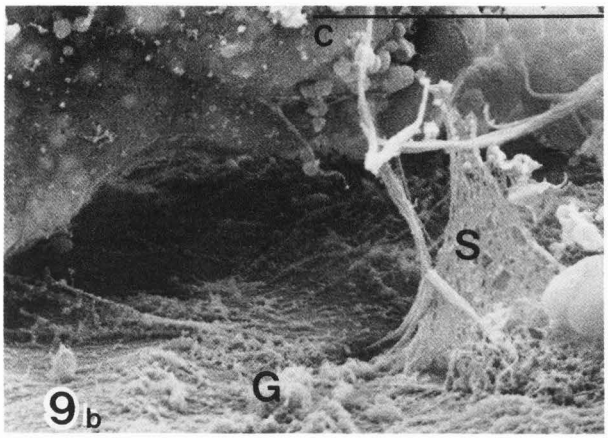

Fig. 9b: Disruption of fibrous band by PGC. A sheet of matrix material is attached to cell surface. C = PGC; G = fibrous band; S = sheet of matrix. Scale bar = 10 μm.

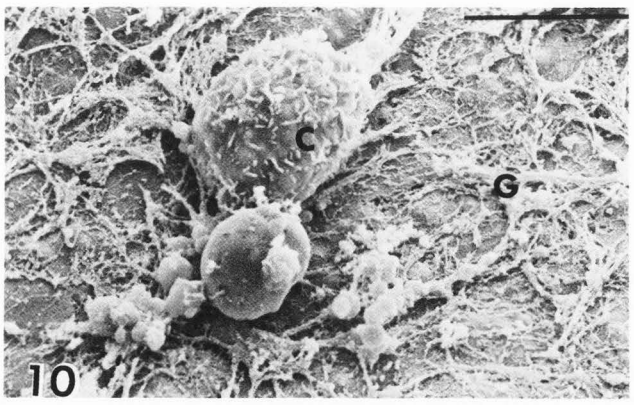

Fig. 10: Disruption of parallel array of fibres by adherent PGC. C = PGC; G = fibrous band. Scale bar = 10 μm.

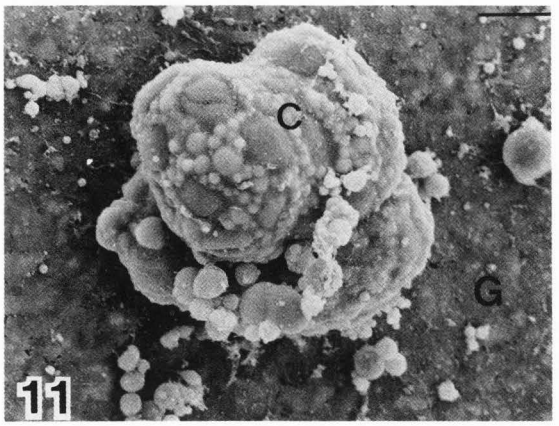

Fig. 11: Adherent PGC on area of band depleted of matrix fibres. C = PGC; G = ectodermal basement membrane cleared of fibres. Scale bar = 10 μm.

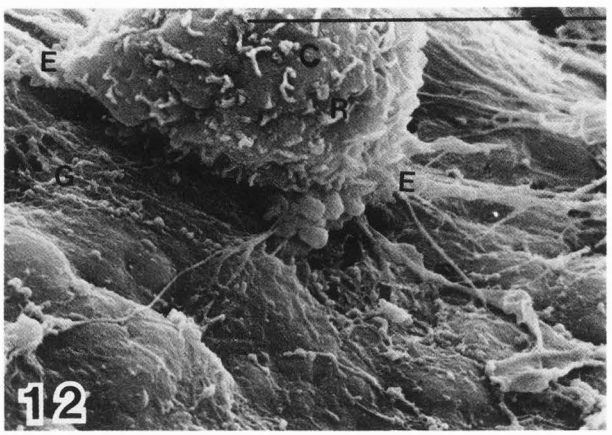

Fig. 12: PGC with extended processes and covered with ruffles and microvilli. C = PGC; G = fibrous band; R = ruffles; E = processes extended from cell. Scale bar = 10 μm.

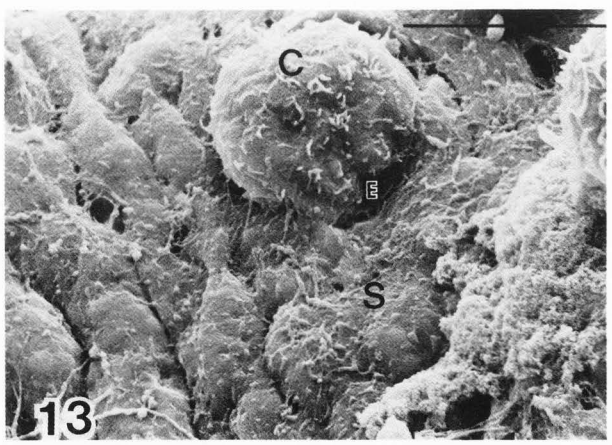

Fig. 13: PGC beginning to invade between ectodermal cells. C = PGC; S = chick ectoderm substrate; E = extended process. Scale bar = 10 μm.

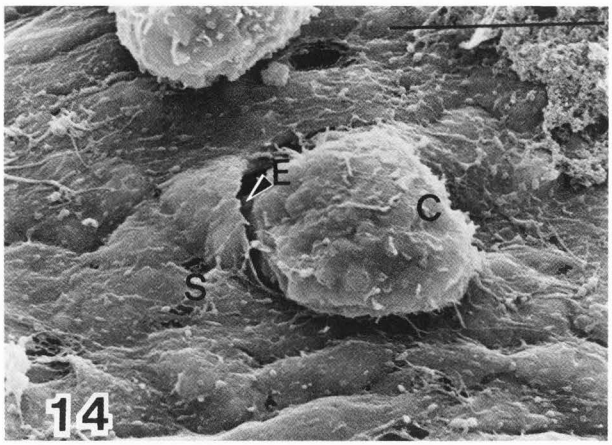

Fig. 14: PGC inserting process between ectodermal cells. C = PGC; S = chick ectoderm substrate; E = extended process. Scale bar = 10 μm.

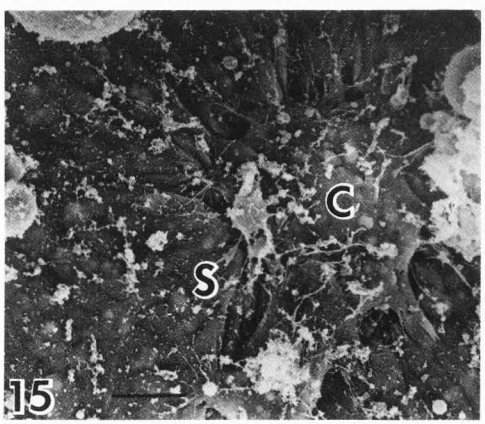

Fig. 15: PGC insinuated in chick ectoderm cell layer. C = PGC; S = chick ectoderm substrate. Scale bar = 10 μm.

trating the substrate (Fig. 14). Still others have insinuated themselves in the chick ectoderm layer (Fig. 15).

A control specimen of fibrous band left for three hours and unseeded with amphibian PGCs remained similar to the zero hour chick fibrous band.

Discussion

Previous studies in both the Xenopus leavis embryo and chick embryo have demonstrated the importance of fibronectin in PGC morphogenetic movements. In the amphibian embryo the PGCs migrate through the gut mesentery where extracellular materials, including fibronectin, are plentiful. Additionally, the PGCs' surfaces also have a fibronectin-rich surface demonstrable by immunofluorescent labelling, though this was shown to be adherent to - but not synthesized by - the PGCs (Heasman et al., 1981). This was demonstrated by trypsinizing the cells and studying their recovery over a 48-hour period (Heasman et al., 1981). They concluded that PGCs lost their invasive and migratory properties if deprived of their surface fibronectin. Furthermore, they did not recover unless fibronectin was replaced in their environment. In the chick embryo the PGCs migrate along a fibronectin-rich band of extracellular fibres. These fibres also have a high concentration of sulphated glycosaminoglycans (Wakely and England, 1979) and collagen Type I (England et al., 1982). The PGCs preferentially choose this substrate over the surrounding substrate of the ectoderm basement membrane (England, 1980).

Based on the presence of fibronectin as a preferential pathway for both chick PGCs and amphibian PGCs in vitro and in vivo we have decided to examine further the conditions necessary for PGC adhesion and migration. We have looked at the behaviour of these cells on substrates composed of ECM components in vitro and in wholemount chick embryos.

Extra-cellular materials (ECM) in both the early chick embryo (Wakely and England, 1979) and early amphibian embryo (Nakatsuji et al., 1982; Heasman et al., 1985) are composed of collagen with fibronectin and glycosaminoglycans present. It has been shown in the present study that the amphibian PGCs migrating on mesentery cells in vitro adhere to this substrate and actively invade the cell layer to reach the underlying ECM (Swan et al., 1981). Heasman et al. (1985) reported in a recent study on PGCs penetrating basement membranes that, although PGCs migrate between mesentery cells through an area rich in ECM, they do not penetrate basement membranes in vivo or in vitro. In the present study the PGCs did not penetrate the ECM, but rather adhered to this substrate and subsequently translocated on it between the gut mesentery cells and the ECM. This migration and translocation mimics the in vivo situation and we would suggest the ECM produced by the gut mesentery cells in vivo and in vitro are similar.

We have previously noted that PGCs seeded onto a 2-dimensional pure collagen substrate prepared from commercially supplied collagen will adhere but not translocate. The PGCs will, however, modify the matrix surrounding them (Boswell and Swan, 1984). If glycosaminoglycans, particularly chondroitin sulphate, are added to this system, the PGCs will adhere and elongate but they will not translocate (P. Dane, unpublished observation). Equally, if the PGCs and the collagen substrate are trypsinized independently before the experiment and the PGCs and collagen substrate are incubated in a fibronectin-free medium, the PGCs will adhere but cannot elongate and move (P. Dane, unpublished observation).

These findings clearly indicate that

fibronectin is important for normal amphibian PGC migratory behaviour. In addition, the topography of the substrate seems to be very important, since PGCs will adhere to and elongate and translocate within a 3-dimensional matrix of collagen (Swan et al., 1983).

We decided, therefore, to investigate the behaviour of the Xenopus PGCs on the chick embryo fibrous band which is both rich in fibronectin and is normally a substrate for chick PGC migration. In addition, the chick band has physical, as well as chemical, cues for guiding moving cells to a predestination. It was also possible by presenting amphibian PGCs with a naturally formed basement membrane as a substrate to monitor their individual interactions with this substrate. As the band is cell-free, with the exception of the chick PGCs, individual identification of the amphibian cells was possible. By fixing the preparations at intervals, we were able to record their migratory behaviour by light microscopy and subsequently examine the same cells by SEM.

Our studies confirm the migratory nature of the Xenopus PGCs as demonstrated by their ability to rapidly migrate over a fibronectin-rich substrate also containing collagen I and glycosaminoglycans in vivo. These findings would imply that the cues for amphibian and chick PGCs are similar for the different species. The combination of chemical and physical cues similarly promotes rapid cell migration in preference to cellular adhesion and non-motility. After the initial burst of PGC migration in the first half hour, however, the PGCs invade the underlying ectoderm layer. This is brought about by two characteristics of PGCs; their ability to re-organise their environment of extra-cellular materials and their penetration of cellular layers by invading between the substrate cells. It is clear from the present in vitro study that the PGCs penetrated between the mesentery cells to contact the extra-cellular materials beneath, whilst in the chick host the reverse is true. In the chick the band is re-organised by the PGCs and the underlying cells exposed. The PGCs then invade between them. This behaviour would suggest that PGCs prefer to migrate within a 3-dimensional substrate, rather than on a 2-dimensional one. We have noted this previously (Swan et al., 1983) and the present results corroborate this evidence.

Acknowledgements

The authors are grateful to Miss S. Uppal and Mr. S. Byrne for their excellent technical assistance. Mr. G.L.C.McTurk of the Leicester University Scanning Electron Microscope Unit produced the scanning electron micrographs.

We are also grateful to Professor C.C.Wylie, St. George's Hospital Medical School, London, who conducted a preliminary study with us.

The authors are particularly grateful to The Medical Research Council and M.A.E. also thanks The Gunnar Nilsson Cancer Research Trust Fund for their grants in aid of our research.

We also thank the departmental secretaries, Mrs. A. Dean, Miss D. Dolman and Miss M. Reeve, who typed the manuscript.

References

Boswell JM, Swan AP. (1984) Mononuclear phagocytes and collagen matrices - a Review. Scanning Electron Microsc. 1984; IV: 2045-2058.

Bounoure L. (1934) Recherches sur la lignee germinale chez la grenouille rousse aux premieres stades du developpement. Ann. Sci. Nat. Zool. Biol. Anim. 17: 67-248.

Critchley DR, England MA, Wakely J, Hynes RO. (1979) Distribution of fibronectin in the ectoderm of gastrulating chick embryo. Nature 280: 498-500.

England MA. (1980) Fibronectin-related cell shape changes in vivo. Cell Biol. Int. Rep. 4: 801.

England MA. (1981) Applications of the SEM to the analysis of morphogenetic events. J. Micros. 123: 133-146.

England MA. (1983) The migration of primordial germ cells in avian embryos. In 'Current Problems in Germ Cell Differentiation', (eds.) McLaren A, Wylie CC. Cambridge Univ. Press. pp. 91-114.

England MA, Critchley DR, Shellswell G. (1982) Collagen Type I in the early chick embryos. J. Anat. 134: 626-628.

Gurdon JB. (1974) 'The Control of Gene Expression in Animal Development'. Oxford: Clarendon Press, p. 123.

Hamburger V, Hamilton HL. (1951) A series of normal stages in the development of the chick. J. Embryol. exp. Morph. 88: 49-92.

Heasman J, Wylie CC. (1978) Electron microscopic studies on the structure of motile primordial germ cells from Xenopus laevis in vitro. J. Embryol. exp. Morph. 46: 119-133.

Heasman J, Hynes RO, Swan AP, Thomas V, Wylie CC. (1981) Primordial germ cells of Xenopus embryos: the role of fibronectin in their adhesion during migration. Cell 27: 437-447.

Heasman J, Wylie CC, Holwill S. (1985) The importance of basement membranes in the migration of primordial germ cells in Xenopus laevis. Dev. Biol. 112: 18-29.

Karnovsky MJ. (1965) A formaldehyde-glutaraldehyde fixative of high osmolarity for use in electron microscopy. J. Cell Biol. 27: 137a.

Kordylewski L, England MA (1980) Transmission electron microscopy of extra-cellular materials in the developing chick embryo. J. Anat. 130: 660-662.

Nakatsuji N, Gould AC, Johnson KE. (1982) Movement and guidance of migrating mesodermal cells in Ambystoma maculatum gastrulae. J. Cell Sci. 56: 207-222.

New DAT. (1955) A new technique for the cultivation of the chick embryo in vitro. J. Embryol. exp. Morph. 3: 326-331.

Nieuwkoop PD, Faber J. (1967) 'Normal Table of Xenopus laevis'. Amsterdam: North Holland,p.1-252.

Plumel M. (1948) Tampon au cacodylate de sodium. Bull. Soc. Chim. Biol. (Paris) 30: 129-130.

Swan AP, Dane P, Baker JE. (1983) The role of extra-cellular matrix components on the adhesion and locomotion of primordial germ cells of Xenopus laevis. Eur. J. Cell Biol. Suppl. 1: 39.

Swan AP, Heasman J, Wylie CC. (1981) The invasion of cultured cell layers and intact epithelia. Scanning Electron Microsc. 1981; IV: 99-104.

Swift CH (1914) Origin and early history of the primordial germ cells in the chick. Amer. J. Anat. 15: 483-516.

Wakely J, England MA (1979) Scanning electron microscopical and histochemical study of the structure and function of basement membranes in the early chick embryo. Proc. Roy. Soc. Lond. (B). 206: 329-352.

Discussion with Reviewers

J.M. Boswell: The net movement of the PGCs is out from the position they were intitially placed at the extreme right side of the arc. Have you tried placing the cells mid-way along the arc, and if so do they tend to migrate out from this position in one or both directions? Do you have any ideas on how the cells "know" in which direction to move or is it purely random?
D.S. Packard: I would like to ask the authors if they have thought of placing the Xenopus PGCs at the cephalic end of the chick fibrous band. If the Xenopus PGCs migrated caudally, it would suggest that there is no directionality to the PGC-substratum interaction.
Authors: When we began this series of experiments we placed the PGCs centrally (i.e. cephalically) on the germinal crescent. The PGCs moved out randomly in both directions from the initial cluster, making it difficult to measure migration distances accurately. We, therefore, modified the experiments from then on, placing the PGCs at one end of the germinal crescent. We could then easily monitor migration distance from the origin.

H. Eyal-Giladi: I have a criticism concerning the timing of PGC migration. It is simply not true that the PGCs of stage 4 already start to move posteriorly. On the contrary, at stage 4 they are on their way anteriorly in the direction of the GC, a process which continues at least until stage 10.
Authors: The papers already published in the literature generally agree with our statement that the PGCs at stage 4 in the chick embryo are moving posteriorly already. Our observations throughout our investigations are consistent with this.

H. Eyal-Giladi: Still another point I am uneasy about is the "burial procedure" of the PGCs in the epiblast.
Authors: Our observations of the "burial procedure" means just that: we have investigated the tendency of amphibian PGCs during their migratory phase to invade any suitable substratum they encounter. The experimental conditions here reproduced their normal in vivo situation, where the PGCs migrate between layers of tissue cells in the mesentery of the gut.

Finally, chick PGCs at these stages during their migration use the basement membrane of the epiblast as their migratory substratum.