Response to fibronectin of mouse primordial germ cells before, during and after migration

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Summary

The adhesive extracellular matrix glycoprotein fibronectin is thought to play a central role in cell migration during embryogenesis. In order to define this role, we have examined the response to fibronectin in cell culture of mouse primordial germ cells (PGCs) before, during and after their migration from the hindgut into their target tissue, the genital ridges. Using an explant culture system, we show that PGCs will emigrate from tissue fragments containing hindgut, and that fibronectin stimulates this migration. Adhesion assays show that the start of PGC migration is associated with a fall in adhesion to fibronectin. Double-labelling studies using in situ hybridization and histochemistry demonstrate that migrating PGCs do not contain detectable fibronectin mRNA, suggesting that they do not synthesize and secrete the fibronectin within their migratory substratum. Taken together, these findings are consistent with an important role for fibronectin in stimulating PGC migration. In addition, however, they suggest that the interaction between PGCs and fibronectin may be important in timing the start of migration, with the fall in adhesion allowing the PGCs to commence their migration towards the genital ridges.

Key words: fibronectin, primordial germ cells, cell migration, cell adhesion, extracellular matrix, alkaline phosphatase, in situ hybridization.

Introduction

Cell migration is a prominent feature of the development of multicellular organisms. A number of studies addressing the mechanisms that control this migration have focussed on the adhesive glycoproteins within the extracellular matrix (ECM) and have suggested important roles for fibronectin (for references see Hynes, 1990), laminin (Perris et al. 1989; Goodman et al. 1989), type-IV collagen (Chelberg et al. 1990), tenascin (Mackie et al. 1988; Tan et al. 1987; Stern et al. 1989; Wehrle and Chiquet, 1990) and thrombospondin (O'Shea et al. 1990). A family of cell surface receptors, termed integrins, that can mediate cell attachment to these different molecules have been identified (Hynes, 1987; Buck and Albelda, 1990; Humphries, 1990); As a result we have a fairly detailed knowledge of the molecular mechanisms by which the cell can interact with adhesive ECM glycoproteins. Less well understood, however, is the role of these interactions in the control of the four essential components of cell migration; the timing of start, the generation of movement, the guidance of the cells and the cessation of migration once the target is reached. In order to examine all these components, it is essential to study the same cell populations before, during and after migration. The difficulty of isolating and identifying early migratory cells at all these different stages has hampered such studies.

To overcome this difficulty, we have chosen to study the migration of mouse primordial germ cells (PGCs). PGCs, which form the gametes of the adult animal, can first be recognised in the extraembryonic mesoderm of 7-7.25 days post coitum (dpc) mouse embryos (Ginsburg et al. 1990). From here they move into their correct location within the genital ridges over the next 4 days. This movement occurs in two distinct phases. The first phase, in which they move from the endoderm into the hindgut between 7.5 and 8.5 dpc, may result from the passive carriage of cells along with the invaginating hindgut endoderm as the cells show no ultrastructural features of a migratory phenotype (Clark and Eddy, 1975). In contrast, the second phase, in which they move through the dorsal mesentery and into the genital ridge between 9.5 and 11.5 dpc, results from active migration as the PGCs now show ultrastructural features of a migratory phenotype (Clark and Eddy, 1975). In contrast, the second phase, in which they move through the dorsal mesentery and into the genital ridge between 9.5 and 11.5 dpc, results from active migration as the PGCs now show ultrastructural features of a migratory phenotype (Clark and Eddy, 1975).
and Wylie, 1986; Blandau et al. 1963). PGCs can be dissociated from the embryo before, during and after the phase of active migration and can be identified both in these cultures and in intact embryos by their cell-surface alkaline phosphatase activity (Donovan et al. 1986).

Our work examining the role of the ECM in the control of PGC migration has focussed on fibronectin, as several lines of evidence suggest an important role for fibronectin in embryonic cell migration. First, fibronectin stimulates cell migration in culture (Ali and Hynes, 1978; Rovasio et al. 1983) and is widespread in pathways of embryonic cell migration (Hynes, 1990). Second, antibodies against fibronectin can block cell migration in vivo (Boucaut et al. 1984; Poole and Thiery, 1986; Gratocos et al. 1988). Third, migrating cells express integrin receptors (Duband et al. 1986; Krotoski et al. 1986) that can bind a variety of ECM ligands including fibronectin (Humphries, 1990), and blocking these interactions can also block cell migration in vivo (Bronner-Fraser, 1985; Bronner-Fraser, 1986). Our previous work on amphibian PGCs suggests a role for fibronectin in PGC migration, as we found fibronectin to be essential for the maintenance of cell–cell contact between the PGCs and the coelomic epithelial cells that form the migratory substratum (Heasman et al. 1981). Here we have performed a more direct analysis of the role of fibronectin by developing assays to measure mouse PGC adhesion and migration in response to fibronectin and also by using in situ hybridization to determine the patterns of fibronectin synthesis during PGC migration in vivo.

Materials and methods

Collection of primordial germ cells for adhesion assays

Embryos were obtained from MF1 mice on the 8–12th day of timed pregnancies (8.5–12.5 dpc). The day after copulation (as determined by a vaginal plug) was considered day 0. Embryos were removed, dissected and dissociated in Ca2+/Mg2+-free phosphate-buffered saline (PBS). The regions dissected away from the embryos were chosen so as to obtain PGC-enriched cell populations and correspond to the localisation of PGCs at the different stages examined. At 8.5 dpc the entire allantois and posterior primitive streak was removed. At 9.5 dpc a cylinder of tissue containing the developing hind gut and associated mesentery could be peeled away from the more dorsal tissues of the embryo. At 12.5 dpc the gonads could be removed directly from the ventral wall without any other adherent tissue. In all cases, the tissue fragments were dissociated by leaving them in Ca2+/Mg2+-free PBS containing 1 mM EDTA for 15 min, then passing them up and down a Pasteur pipette fifty times before resuspending the cell suspension in an appropriate volume of serum-free medium (SF-1; Northumbria Biologicals).

Adhesion assays

Assays were performed in 35 mm bacteriological plastic dishes. Central spots of approximately 7 mm in diameter were preincubated in the desired concentration of fibronectin or laminin in PBS for one hour, then washed in PBS and coated with 0.3 % heat-inactivated BSA (Fraction V-Sigma) in PBS for a further hour to block any remaining non-specific protein-binding sites. The coated areas were washed three times in SF-1 immediately prior to the plating of the cells. Cell suspensions containing PGCs, which had been incubated in 5 μg ml−1 cyclohexamide (Sigma) for one hour, were then plated onto the coated areas in the same solution for exactly one hour. In control experiments to determine the number of PGCs in each experiment, dishes were also coated with 50 μg ml−1 poly-D-lysine (to which we would expect all PGCs to adhere) for two hours and an identical volume of suspension was plated onto these dishes at the time of the assay. Approximately 4, 2 and 0.5 embryo equivalents were used at 8.5, 9.5 and 12.5 dpc respectively in each of the assays. After one hour, the plates were gently washed twice with SF-1, fixed with 4 % paraformaldehyde and then stained for alkaline phosphatase to visualise the germ cells. Staining was carried out by washing the plates in a solution of 0.4 mg ml−1 alpha-naphthyl MX phosphate (Sigma), 1 mg ml−1 fast red TR (Sigma) and 4 mM MgCl2 for fifteen minutes (Donovan et al. 1986). PGCs were counted only if they did not contact any other cell type that could be binding them indirectly to the substratum. All assays were performed in duplicate. The number of adherent PGCs was then expressed as a percentage of the total plated out in that experiment (as measured by counting PGC numbers on the poly-D-lysine plate), so as to allow comparison between experiments.

Migration assays

PGC-containing regions of 8.5 and 9.5 dpc embryos were removed as described above, and cut into smaller pieces. 9.5 dpc hindgut fragments were always cut into three equal pieces, comprising the anterior, central and posterior regions of the tissue. Fragments from 5 embryos were then plated out in serum-free culture media (SF1; Northumbria Biologicals) onto 13 mm glass coverslips coated either with fibronectin or with an irradiated cell monolayer comprising STO cell embryonic fibroblasts. Fibronectin-coated coverslips were prepared by incubating the coverslips in fibronectin (40 μg ml−1 in PBS) for one hour and then washing with PBS before use. STO cells monolayers were grown on laminin/poly-D-lysine-coated coverslips, prepared by incubating the coverslips sequentially in PBS containing poly-D-lysine (50 μg ml−1) and then laminin (40 μg ml−1). STO cells were grown and irradiated as previously described (Donovan et al. 1986), and then plated onto the coverslips at a density of 2×105 ml−1. The cells were allowed to spread for 1–2 days and then washed thoroughly before use.

In experiments examining the effects of fibronectin on migration, plasma fibronectin (Sigma) was added to the culture medium to the desired concentration one hour after plating of tissue fragments. In experiments using carboxyfluorescein diacetate succinimidyl ester (CFSE-Molecular Probes Inc.) to determine the extent of spread of the tissue fragments in culture, the fragments were incubated in 33 μM CFSE in PBS for thirty minutes prior to plating. After 18 h in culture, all experiments were fixed and stained for alkaline phosphatase to visualize PGCs by light microscopy. In experiments using CFSE, fluorescein optics were also used to visualize the labelled cells. Cultures were analysed using a drawing tube to document the extent of migration of each PGC from the edge of the explant, which could be seen by phase contrast. The number of cells leaving each explant and the distance of their migration was measured. These distances were added to give the total migration from each explant, and the mean migration/explant was calculated for all the explants on one coverslip.
In situ hybridization

The method used is a modification of that described by ffrench-Constant and Hynes (1988), using low temperature polyethylene glycol disterate (PED) wax (Koch Chemicals Ltd) to allow double-labelling studies in which the alkaline phosphatase activity of the PGCs could be localised in the fixed and embedded tissue prior to hybridization. 10.5 dpc embryos were fixed in ice-cold 4% formaldehyde/85% ethanol for fifteen minutes then dehydrated through ethanol and embedded in PED wax (containing 1% cetyl alcohol) at 37°C overnight. After cooling of the blocks, 5 μm sections were cut onto silane-treated slides, dewaxed and incubated in 75 mM Tris pH 8.5 containing 0.8% NaCl, 0.1% fast red TR (Sigma) 0.05% alpha-napthyl phosphate (Sigma) 2% N,N-dimethylformamide and 0.08% MgCl₂ to visualize the PGCs (Hahnel and Eddy, 1986). Once a suitable level of reaction product could be observed over the PGCs, the sections were washed in 75 mM Tris pH 8.75 and processed for in situ hybridization using 35S-labelled single-stranded RNA probes. The probes used were synthesized from a 270 nt cDNA fragment of rat fibronectin as described in the results. The hybridization protocol followed was the same as ffrench-Constant and Hynes (1988), with the one difference that the sections were not dehydrated after hybridization in order to prevent loss of the reaction product in alcohol.

Results

PGC adhesion

PGC adhesion to fibronectin was examined at three different ages - 8.5, 9.5 and 12.5 dpc. Only embryos with less than 10 somites were included in the 8.5 dpc group. These three stages were chosen to provide PGC populations in which most, if not all, cells are at one of the three stages of their migration from the hindgut into the genital ridges; premigratory, migratory and postmigratory. At 8.5 dpc (less than 10 somites) PGCs are found within the invaginating hind gut and have not yet started to migrate actively out of the gut. By 9.5 dpc PGCs show active migration and are leaving the gut and moving into the dorsal mesentery. At 12.5 dpc virtually all PGCs have arrived at the genital ridges and are postmigratory (Clark and Eddy, 1975; Tam and Snow, 1981).

Regions of the embryo containing germ cells were dissected apart and then dissociated in a non-enzymatic buffer so as to preserve cell-surface receptors. PGCs in the suspension could be recognised by their cell-surface alkaline phosphatase (ALP) activity (Fig. 1). These ALP⁺ cells usually showed surface blebs, as described in previous studies (De Felici and McLaren, 1982). At 8.5 and 9.5 dpc they constituted less than 5% of the cells within the suspension. At 12.5 dpc, however, the genital ridge could be dissected away from the embryo and the subsequent dissociation yielded a cell suspension of which the majority were PGCs, as found by previous workers using this dissociation technique.

PGC adhesion to fibronectin was measured by counting the number of ALP⁺ cells adhering after 1 h to bacteriological plastic Petri dishes coated with different concentrations of fibronectin. Cells were washed before use, and the assays were performed in the presence of cyclohexamide (5 μg ml⁻¹) to reduce the level of protein synthesis by contaminating somatic cells (Cascio and Gurdon, 1987). The absence of binding to control plates coated only with BSA confirms the effectiveness of these measures in preventing binding to ECM components contaminating the cell suspension. Dose-dependent adhesion to fibronectin was observed at all three stages. However, as shown in Fig. 2, different degrees of adhesion were seen at the different stages. At 8.5 dpc virtually all PGCs adhered to fibronectin. At 9.5 dpc significantly less adhesion was seen with only 37% of the PGCs binding to the substratum. By 12.5 dpc adhesion to the substratum was reduced still further, although adhesion to other cells within the...
suspension was now more noticeable than at the other ages. This may reflect the adhesion of 12.5 dpc PGCs to Sertoli cells (De Felici and Siracusa, 1985). PGCs adhering to any other cell type were not counted in the assay at any of the ages examined in order to prevent those PGCs only bound indirectly to the substratum being classified as adherent.

The loss of adhesion to fibronectin did not reflect an overall loss in adhesion to all extracellular matrix components. In control experiments using laminin as a substratum more than 75% of the PGCs adhered to the dish at both 8.5 and 12.5 dpc. Adhesion to fibronectin appears therefore to be specific and independently regulated from adhesion to laminin.

**PGC migration**

To measure PGC migration, we established an explant culture system to analyse the degree to which PGCs can migrate out of tissue fragments onto defined substrata or onto feeder cell layers. Regions of 8.5 and 9.5 dpc embryos containing PGCs were dissected from the embryo and then divided as described in Materials and Methods. When these fragments were plated onto fibronectin or laminin-coated glass coverslips for 18 h a sheet of migrating cells could be seen spreading from the explant. ALP⁺ PGCs could be seen within this sheet at both ages, but never left the explant to migrate directly over the substratum (Fig. 3A). In contrast, when 9.5 dpc explants were grown on a monolayer of STO embryonic fibroblasts, which we have found previously to support the proliferation and survival of mouse PGCs, individual PGCs could be seen to have emigrated from the explants over the feeder cell layer (Fig. 3C). No such emigration was ever observed from

![Image of PGC migration](image_url)
fragments of 8.5 dpc embryos; at this stage, all the PGCs remained within the explant both on fibronectin substrata and on STO cell monolayers.

To confirm that the 9.5 dpc PGCs were migrating independently, rather than being carried passively by migrating somatic cells, explants were labelled with carboxyfluorescein diacetate succinimidyl ester (CFSE) prior to plating on the fibronectin or STO cell substrata. Cells within these explants become labelled with intracellular fluorescent dye that can subsequently be visualised by examining the cultures with appropriate optics (Bronner-Fraser, 1985). After 18 h in culture a sheet of fluorescent-labelled cells can be seen spreading from the explant (Fig. 3B). In cultures grown on fibronectin-coated coverslips, PGCs could be seen within this labelled sheet but were never seen to separate from it and move independently on the fibronectin substratum (Fig. 3A,B). PGCs on the STO cell monolayer, in contrast, did separate from the labelled somatic cells. These PGCs could usually be seen to have moved further from the explant than the sheet of somatic cells and were not accompanied by any somatic cells (Fig. 3C,D). Although the CFSE will not have labelled every cell in the original explant most, if not all, the somatic cells that spread out of the explant did appear to be labelled in the experiments on fibronectin substrata (Fig. 3A,B). The consistency of the observation that PGCs cultured on STO cell substrata could be seen beyond the labelled somatic cells in 3 separate experiments, each with 12–15 explants, suggests therefore that PGCs are able to emigrate from the hindgut fragments and migrate independently over the STO cell monolayer.

Having established an in vitro assay for PGC migration, we next determined the effect of exogenous fibronectin on the extent of this migration. Human plasma fibronectin was added to the explant system 1 h after plating and the extent of migration was assessed 18 h later. In order to minimise the contribution by the STO cells to the fibronectin available for PGC migration STO cell monolayers were used within 1 or 2 days of being plated out. The results of 12 consecutive experiments are shown in Fig. 4. Different concentrations of exogenous fibronectin produced significant differences in the extent of PGC migration from each explant (P<0.05 using a one-way ANOVA on all four groups) with a sum of squares analysis showing the increase observed with 10 μg ml⁻¹ fibronectin to be significant at the level of P<0.025. The enhanced migration seen with 10 μg ml⁻¹ fibronectin resulted in an increase in the number of PGCs leaving each hindgut fragment in the presence of fibronectin (2.85±0.251 vs 2.06±0.251 in the control explants - P<0.0005 in a paired t-test). No significant increase in the distance migrated by each individual PGC was seen (86.2±5 μm vs 84.6±5 μm) suggesting that the speed of PGC migration was unaffected by the addition of fibronectin.

Synthesis of fibronectin during PGC migration
To determine the source of the fibronectin present in the migratory pathway, we developed a double-labelling procedure to localise fibronectin mRNA and ALP⁺ PGCs in the 10.5 dpc dorsal mesentery. This age was chosen in preference to 9.5 dpc as we found that the PGC cell-surface alkaline phosphatase activity withstood fixation and wax embedding better at the older age. Although some PGCs have reached their target in the genital ridge by 10.5 dpc, many have not yet completed their migration (Tam and Snow, 1981) and these migrating cells can be identified within the dorsal mesentery. Appropriate sections of 10.5 dpc embryos were incubated as described in the materials and methods to detect ALP and so localise PGCs, then processed for in situ hybridization using single-stranded RNA probes labelled with 35S as described in ffrench-Constant and Hynes (1988). The probe used was synthesized from a 279nt cDNA fragment encoding a region of rat fibronectin that is included in all the different forms of fibronectin (C-Fn in ffrench-Constant et al. (1989)). Control experiments using sense probes showed no specific labelling. Antisense probes showed intense labelling over the wall of the developing aorta and a lower level of labelling within the mesenchymal tissue of the dorsal mesentery (Fig. 5). No labelling was seen over the migrating PGCs themselves, which could be seen clearly between the coelomic epithelium (which was also unlabelled) and the mesenchymal cells of the mesentery (Fig. 5).

Discussion

The effects of fibronectin on mouse PGCs
The results of our experiments can be summarised as follows. First, adhesion of PGCs to fibronectin falls coincident with the start of their emigration from the
Fig. 5. Migrating primordial germ cells do not contain fibronectin mRNA. The schematic diagram (A) shows the pathway of PGC migration (arrows) from the gut (G) to the genital ridge (GR). PGCs leave the gut mesentery at the coelomic angle, where they pass between the coelomic epithelium (CE) and the aorta (AO). A transverse section of the coelomic angle and adjacent aorta (shown in the box marked X) has been reacted for alkaline phosphatase to visualize PGCs and then processed by *in situ* hybridization to detect fibronectin mRNA. Three different views of the section as shown; phase contrast (B), bright field to show the alkaline phosphatase reaction product over the PGCs (C) and dark field to show the distribution of fibronectin mRNA as visualized by autoradiography (D). PGCs (arrows in C) can be seen migrating within the mesentery or adjacent to the coelomic epithelium (CE), and contain little if any fibronectin mRNA as shown by the absence of significant labelling in D. Note that cells within the mesentery (M) are weakly labelled in the *in situ* hybridization experiment while the cells of the wall of the developing aorta (AO) are intensely labelled. Scale bar=50 μm.

hindgut, and decreases still further when this migration is complete. Second, migratory PGCs will move out of embryonic hindgut explant cultures and migrate over a feeder layer of embryonic fibroblasts, and this migration is enhanced by exogenous fibronectin. Third, migrating PGCs within the dorsal mesentery do not contain fibronectin mRNA suggesting that they not contribute significant levels of fibronectin to their migratory substratum.

While the adhesion of PGCs prior to their emigration from the hindgut has not previously been examined, our demonstration that about one third of PGCs migrating between the hindgut and genital ridge adhere to fibronectin is in agreement with a previous study of de Felici and Dolci (1989). These authors found that 30 % of 10.5 dpc PGCs adhered to fibronectin, close to the 37 % we observed with 9.5 dpc PGCs in this present study. We did not examine 10.5 dpc PGCs as a small proportion of these cells have reached their target and may therefore no longer be migratory (Tam and Snow, 1981). The majority of PGCs are still migrating, however, so that the similar degree of adhesion at 9.5
dpc and 10.5 dpc would be expected. A significant difference between the two studies emerges, however, when the adhesion of postmigratory germ cells is considered. We observed a significant decrease in adhesion to fibronectin in postmigratory (12.5 dpc) PGCs when compared with migratory PGCs. In contrast de Felici and Dolci (1989) observed an increase between 10.5 and 12.5 dpc, with a subsequent decrease occurring by 15.5 dpc. This discrepancy between the two studies may reflect differences in the assays used to measure adhesion. We used bacteriological plastic rather than tissue culture plates for the assays, as we found unpredictable levels of background adhesion in experiments using 12.5 dpc PGCs with tissue culture plates. In addition, we performed all assays in the presence of cyclohexamide to block protein synthesis by the contaminating somatic cells within the assay. We reasoned that such protein might coat the plastic dishes and so lead to falsely high levels of adhesion. The absence of any adhesion in our BSA-coated control plates confirms the effectiveness of these measures in eliminating background adhesion. Whatever the cause of the discrepancy at 12.5 dpc, however, an important point emerges from both studies is that PGC adhesion to ECM components is developmentally regulated with changes occurring in association with the different phases of PGC behaviour.

Stimulation of PGC migration by fibronectin has been described in a previous study by Alvarez-Buylla and Merchant-Larios (1986). These authors took advantage of the high proportion of PGCs in suspensions prepared from 11.5–12.5 dpc genital ridges to perform time-lapse microcinematography on individual PGCs. They showed increased translocation of these cells when fibronectin was included in the sustratum. A potential disadvantage of this approach, however, is that the PGCs being examined are those that have reached their target and that may have subsequently altered their response to ECM components. We chose therefore to examine migrating PGCs directly using a novel explant system to facilitate quantitation of the migration of the small number of PGCs in each culture. Our results confirm that fibronectin stimulates PGC migration, in this case when added into the culture medium rather than directly into the substratum. However, further analysis shows that, in our experimental system, stimulation results from an increase in the number of cells leaving the explant rather than an increase in the distance travelled by each cell. This suggests that the added fibronectin is incorporated into the substratum around the explant making it more attractive for PGC migration but does not increase the speed of their migration once they leave the explant. This may reflect the presence of other ECM molecules being produced by the STO cell feeder layer that can influence the speed of PGC movement and further work is required to test this possibility. An alternative explanation, that the soluble fibronectin stimulates migration by inhibiting the interaction between the PGCs and the substratum-bound fibronectin and so reducing adhesion, is unlikely. The concentration of soluble fibronectin at which we observed a stimulation of migration (10 μg ml⁻¹) is at least two orders of magnitude lower than that shown by Yamada and Kennedy (1984) to act as an inhibitor of fibronectin-mediated cell spreading when in solution.

The requirement for a feeder cell layer in the migration assays makes it impossible to exclude the possibility that some of the observed increase in migration might result from an indirect effect of fibronectin on the STO cells, resulting in the production of other migration-enhancing products. In addition, two other potential problems must be considered in the interpretation of the results. First, it is necessary to establish that germ cells are actually leaving the explant and migrating over the feeder layer, rather than being carried by the migration of somatic cells. Second, the feeder layer that provides the migratory substratum will itself synthesize and incorporate fibronectin into the pericellular matrix and this endogenous fibronectin would be expected to mask the effect of added fibronectin. We used explants preincubated in CFSE to overcome the first problem. These control experiments established that migrating PGCs do leave the explant on feeder cell substrata, while this was never seen in explants in the absence of a feeder layer. The second problem, that of fibronectin synthesis by the feeder cells, was minimised by using feeder layers that had been plated out within the previous 2 days. However, even within this time period fibronectin synthesized and incorporated into the pericellular matrix by the feeder cells can be demonstrated by immunofluorescence (ffrench-Constant, unpublished observations). The use of cells expressing antisense RNA to inhibit selectively the synthesis of fibronectin or other ECM components represents one approach to this problem, and such experiments are underway.

The source of the exogenous fibronectin used in the adhesion and migration assays was soluble plasma fibronectin. A number of previous studies have shown that plasma fibronectin supports cell migration in culture (Ali and Hynes, 1978; Rovasio et al. 1983; Hynes, 1990), suggesting that this form of fibronectin can be used to analyse cell migration in vitro. However, we have shown previously that, as a result of alternative splicing of the fibronectin primary gene transcript, plasma fibronectin differs from the type of fibronectin present in the embryo at the time of cell migration and proliferation. These early embryonic fibronectins include three regions termed EIIIB, EIIIA and V (ffrench-Constant and Hynes, 1989; ffrench-Constant and Hynes, 1988) while plasma fibronectin completely lacks EIIIB and EIIIA and includes V in only 50% of the molecules (Norton and Hynes, 1987). At present, plasma fibronectin is the only form of fibronectin available in sufficient quantities to perform the adhesion and migration assays described in this study. It is possible, however, that interesting quantitative differences will emerge between the different forms of fibronectin in experiments using the individual spliced forms manufactured in recombinant expression systems (Guan et al. 1990).
The presence of fibronectin in the dorsal mesentery forming the migratory pathway of mouse PGCs is well documented (Fujimoto et al. 1985). The use of in situ hybridization to detect fibronectin mRNA allows the source of this fibronectin to be established, and our results show the PGCs themselves contribute little, if any, fibronectin to their migratory substratum. The higher levels of fibronectin mRNA in the mesenchymal cells adjacent to the PGCs within the mesentery suggests that these cells do synthesize and secrete fibronectin, in agreement with the previous demonstration by immunoelectron microscopy that fibronectin is present in the endoplasmic reticulum of these cells (Fujimoto et al. 1985). The conclusions from our in situ hybridization study on mouse PGCs in vivo are similar to those of our previous work on amphibian PGCs grown in cell culture. In amphibians such as Xenopus, PGCs are large enough to be isolated from the mesentery by dissection. Fibronectin is present on the surface of freshly dissected PGCs but is not resynthesized after enzymatic removal, suggesting that these PGCs do not synthesize fibronectin and that the fibronectin present within the mesentery is derived from other cell types (Heasman et al. 1981).

The role of fibronectin in mouse PGC migration

Our results suggest three distinct roles for fibronectin in the control of mouse PGC migration. First, fibronectin stimulates PGC migration. This property of fibronectin has been demonstrated with a number of different embryonic cell types, suggesting that it is an important general role for fibronectin during embryogenesis. Interestingly fibronectin is present at very low levels in the genital ridge (Fujimoto et al. 1985; Wylie et al. 1986), and the absence of any fibronectin to stimulate migration may be one important factor responsible for the cessation of PGC migration once they reach their target.

A second role for fibronectin in PGC migration may be determining the timing of active migration from the hindgut to the genital ridge. Prior to leaving the hindgut, PGCs adhere tightly to fibronectin, and this level of adhesion would facilitate the passive carriage of the PGCs into the hindgut by the invaginating endoderm (Clark and Eddy, 1975). Once this phase of PGC movement is complete, however, the fall in adhesion seen in migratory PGCs may allow the cells to leave the hindgut by active migration. This hypothesis emphasises that, as with all migrating cell types, the behaviour of PGCs on fibronectin will reflect a balance between adhesion and migration. Fibronectin may stimulate cell migration but high levels of adhesion to fibronectin, resulting either from high concentrations of fibronectin or from expression of appropriate receptors, may prevent cell movement. In keeping with this, we found that high concentrations of fibronectin (100 µg ml⁻¹) did not stimulate cell migration in 9.5 dpc PGCs. Very low levels of adhesion, in contrast, may be insufficient to support migration because the cells are unable to generate sufficient traction. The fall in adhesion seen in postmigratory PGCs may therefore result in a loss of the migratory ability of these cells as they complete their proliferation within the genital ridge.

These observations emphasize that the relationship between adhesion and migration may be complex, with moderate levels of adhesion being optimal for cell migration. In keeping with this, Duband et al. (1991) have recently shown that binding of cell surface integrin receptors by substratum-bound antibodies of moderate avidity promoted migration more effectively than antibodies with high avidity. A prediction of the hypothesis that the timing of migration is regulated by changes in the level of adhesion to fibronectin is that the start of active migration will be associated with a decrease in the affinity of PGC fibronectin receptors. The integrin family of receptors, which contains at least two well-characterised fibronectin receptors (Humphries, 1990), are excellent candidates for a role in the timing of PGC migration and experiments to examine the different integrins on premigratory and migratory PGCs will be required to test this prediction.

Finally, fibronectin may be involved in the guidance of PGC migration. Migrating cells such as neural crest cells that do not synthesize fibronectin will be more sensitive to exogenous fibronectin in the migratory pathway (Newgreen and Thiery, 1980; ffrench-Constant and Hynes, 1988). As a result, the distribution of this fibronectin could act as a guidance cue. Both this and our previous study (Heasman et al. 1981) show that PGCs, like neural crest cells, do not synthesize fibronectin. Electron microscopic studies of the ECM fibrils in the pathway of migrating trunk neural crest cells shows them to be orientated in the direction of cell migration (Newgreen, 1989) and we have observed a similar orientation of ECM fibrils in the dorsal mesentery that forms the migratory pathway of amphibian PGCs (Heasman, unpublished observations). The presence of fibronectin in such an orientated substratum may therefore provide an important cue in the guidance to their targets of migrating cells that do not synthesize fibronectin such as PGCs and neural crest cells.

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References


Fibronectin and PGC migration


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