Primordial germ cells migration: morphological and molecular aspects

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Abstract

Primordial germ cells (PGCs) are germline stem cells that give rise to gametes in vertebrates. They originate outside the embryo very early in development and migrate by a well-defined route into the genital ridges. During early embryogenesis in mammals, the PGCs are observed in an extra-embryonic region near the yolk sac, translocate to the endodermal epithelium of the hindgut as embryogenesis advances, and then separate from the gut epithelium to enter the dorsal mesentery, through which they finally migrate to form gonadal anlage. It is accepted that PGC migration occurs in three phases: separation, migration, and colonization. The PGCs move actively by amoeboid movements to cross the migratory pathway. These cells are oval or round in shape with irregular contours and large nuclei containing prominent nucleoli. An identifying characteristic of PGCs is their high alkaline phosphatase activity. Recently, interest has been focused on the mechanism of PGC migration. At least four mechanisms have been hypothesized to explain PGC migration: attraction by chemotactic factors, PGC-PGC interactions, substrate-guidance, and interaction with extracellular matrix molecules. We have demonstrated that a repertoire of extracellular matrix molecules, proteoglycans in particular, are temporo-spatially expressed in the migratory pathways of PGCs according to the phase of the migration process. It is of note that PGCs are pluripotent cells, from which two types of equally pluripotent stem cells are derived. *In vivo,* PGCs engender embryonic carcinoma cells, the stem cells of teratomas and benign tumors. *In vitro,* mouse PGCs give rise to embryonic germ cells, stem cells capable of producing a variety of different cell types, including hematopoietic cells and myogenic cells. Greater knowledge of the mechanisms that control embryonic carcinoma cell formation and the signaling pathways that control embryonic germ cell derivation could help us understand the molecular controls of developmental potency in mammals.

Keywords: germ cells; growth; cell movement; extracellular matrix; proteoglycans.

Origin of primordial germ cells

Primordial germ cells (PGCs) are germline stem cells that give rise to gametes in vertebrates. They originate outside the embryo itself very early in development and migrate by a well-defined route into the genital ridges (Witschi, 1948; Chiqouine, 1954). Since PGCs present high alkaline phosphatase activity, this activity has been used as a marker through which they have been identified in an extra-embryonic region near the yolk sac early in embryogenesis and traced along their migratory route toward the gonadal ridges. In the mouse, PGCs first appear in 7-day-old embryos in both early pregastrulation and the early-streak stage as a cluster of 50-100 alkaline-phosphatase positive cells at the base of allantoids (Ginsburg et al., 1990). In 8.5-day-old embryos, as consequence of morphogenetic events, they translocate into the hindgut epithelium. This is a rather efficient means of transportation for the PGCs and has the advantage of placing them in close proximity to the regions where the gonadal ridges will soon form. From this location, the PGCs move through the mesenchyme of the dorsal mesentery toward the genital ridges, where they later differentiate into either oogonia or spermatogonia (Fig. 1a; Table 1). During this migration, the PGCs proliferate. In the mouse embryo, the number of PGCs increases from less than 100 to approximately 4000 during the period of migration.

Classically, the PGC migration process is divided into three distinct phases. During the first phase, the separation phase, the PGCs leave the hindgut epithelium and enter the mesenchyme of the dorsal mesentery (Fig. 1b; Table 1). The second phase is the migration phase in which the PGCs use amoeboid movements to move between the mesenchymal cells of the dorsal mesentery and travel toward the genital ridges (Fig. 1c; Table 1). Finally, in the colonization phase, the PGCs reach and populate the genital ridges (Fig. 1d; Table 1; Fujimoto et al., 1977; 1989).

There is evidence in culture, that both the proliferation and the direction of migration of mouse PGCs are influenced by soluble factors released from their target tissue, the genital ridges. In the mouse, TGF-beta1 or a close related molecule was a chemotropic molecule released from genital ridges in culture

(Godin *et al*., 1990; Godin and Wylie, 1991). Studies on other stem cell populations have shown that complex combinations of growth factors control their proliferation, migration, and differentiation (See Farini *et al*., 2005).
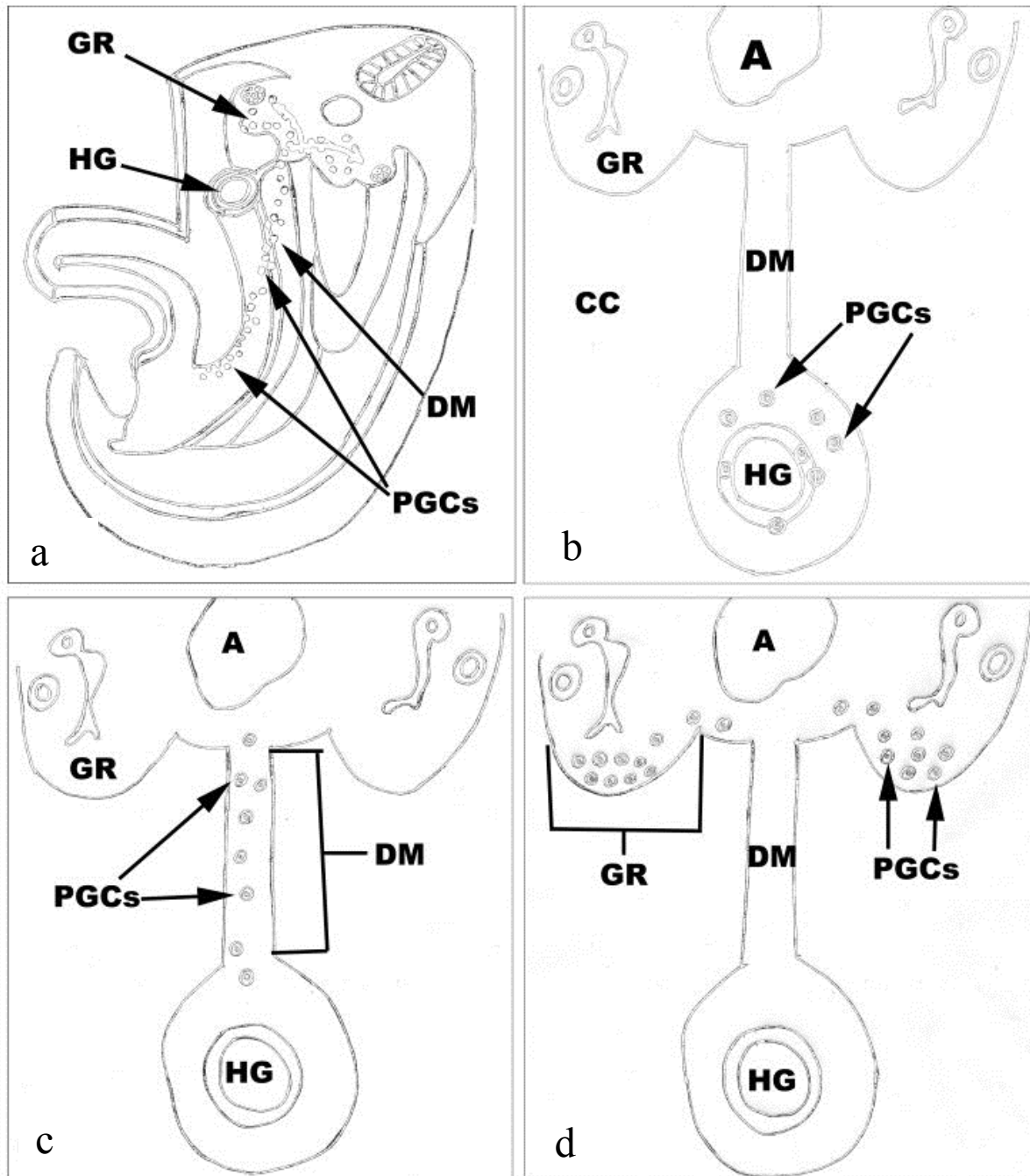

Figure 1. A schematic, three-dimensional representation of a transverse section of an 11-day-old mouse embryo (a) showing the migratory route of the primordial germ cells from the hindgut toward the gonadal ridge and the three phases of primordial germ cell migration; b) separation phase (9-day-old embryo), primordial germ cells are dislodging from the hindgut epithelium; c) migration phase (10-day-old embryo), primordial germ cells are migrating through dorsal mesentery; and d) colonization phase (12-day-old embryo), primordial germ cells are found in the developing gonad after the migration process has finished. A: Aorta; HG: hindgut; DM: dorsal mesentery; PGCs: primordial germ cells; GR: gonadal ridge; CC: coelomic cavity; AL: allantoids; NT: neural tube.

Table 1. PGC location, morphology, and markers of the cell surface.

DAY	PGCs Location	PGCs Morphology	Molecules expressed on the cell surface of PGC
6.0-6.5 DD	Proximal epiblast close to the extraembryonic ectoderm	nd	APase
7.0-7.5 DD	Extraembryonic mesoderm at the base of the amniotic fold	nd	APase, E-Cadherin
8.0 - 8.5DD	Most are still embedded in the epithelium of the developing hindgut	Round or oval shape with smooth contour	APase, PECAM-1
9.0-9.5 DD	Migrate out of the hindgut into its mesentery	Irregular shape with pseudopodium-like projections	APase, SSEA-1, C-Kit, P- and E-Cadherins, PECAM-1
10.0 DD	Reached the peak of migration and they were concentrated in the dorsal mesentery mesenchyme	Irregular shape with pseudopodium-like projections	APase, SSEA-1, C-Kit, integrins α6, α5, α3, β1, P-and E-Cadherins, PECAM-1
11.0 DD	In the genital ridge, but some are still migrating among the mesenchymal cells of the dorsal mesentery	Irregular shape with pseudopodium-like projections	APase, SSEA-1, C-Kit, integrins α6, α5, α3, β1, P-and E-Cadherins, PECAM-1
12.0 DD	Genital ridges	Round shape	APase, SSEA-1, C-Kit, integrins α6, α5, α3, β1, P-and E-Cadherins, PECAM-1

Legend: APase: alkaline phostatase; SSEA: stage-specific embryonic antigen; PCAM-1: platelet endothelial cell adhesion molecule-1; C-Kit: Stem cell factor receptor C-Kit; nd: not described.

Recently, studies have been conducted to identify mutations affecting the development of PGCs (Sasado *et al*., 2004; Molyneaux *et al*., 2004). Studies that used embryos derived from a mutagenized founder, Medaka (Oryzias latpes) fish, showed that several mutations caused altered PGC distribution, most of which were associated with morphological abnormalities and were grouped in four phenotypic classes. Other mutations caused a decrease in the number of PGCs. This decrease was observed in the offspring of heterozygous mothers, indicating the contribution of a maternal factor determining the number of PGC.

Several efforts have been made to elucidate the mechanism for specification of PGCs. Recently, Blim1, a transcriptional repressor of the histone methyltranferase subfamily, was identified as a key regulator of mouse PGC specification (Ohinata *et al*., 2005; Saitou, 2005). It is believed that the specification of approximately 40 founder PGCs and their aggregation from somatic neighbors are important events in early development. In addition, it is proposed that this specification requires repression of a genetic program that is adopted by neighboring cells. Surani´s group has shown that Blimp1 (also know as Prdm1) has a critical role in the foundation of the mouse germ cell lineage, as its disruption causes a block early in the process of primordial germ cell formation (Ohinata *et al*., 2005). The genetic, lineage-tracing experiment indicates that the Blimp-1 positive cells, originating from the proximal-posterior epiblast cells, are in fact the lineage-restricted PGC precursors (Ohinata *et al*., 2005; Saitou, 2005). Moreover, disruption of Blimp1 function resulted in aberrant PGC-like cells with a deregulated intrinsic gene expression program at a very early stage, thus demonstrating that Blimp1 is a critical determinant of the germ line in mice (Saitou *et al*., 2005).

Primordial germ cell morphology

The characteristic PGC morphology is oval or round in shape with an irregular contour and large nuclei containing prominent nucleoli (Fig. 2). However, these cells experience slight morphological modifications depending on the migration phase in which they are observed. In the separation and migration phases, the PGCs are amoeboid in shape, and consequently, the nucleus has a somewhat irregular contour. Electron microscopy studies have shown that, in these phases, PGCs have a conspicuous nucleolus, abundant glycogen particles, and lipid droplets as well as ribosomes and mitochondria. In contrast, the endoplasmic reticulum and Golgi complex are not yet well developed (Fig. 3. Jeon and Kennedy, 1973; Spiegelman and Bennett, 1973; Clark and Eddy, 1975; Fukuda, 1976; Fujimoto et al., 1977, Pereda et al., 1988; 1991; Pereda and Motta, 1991; Fujimoto et al., 1989; Makabe et al., 1989; Makabe et al., 1991). These features are common to rodents and humans; however, separation- and migration-phase rodent PGCs in particular contain little glycogen and lipids but exhibit an abundance of ribosomes (Fujimoto et al., 1977; 1989).

In the colonization stage, the PGCs are round or elliptical, and some change in their fine morphology can be observed including the following: diminished glycogen particles and lipid droplets, a well-defined golgi complex and endoplasmic reticulum, and an increase in the number of mitochondria (Jeon and Kennedy, 1973; Clark and Eddy, 1975; Fukuda, 1976; Fujimoto et al., 1977; 1989; Makabe et al, 1989; 1991; Pereda et al., 1991).

The PGCs are usually in close proximity to or accompanied by somatic cells (Fig. 3), which are believed to modify or maintain the environment of these cells (Clark and Eddy, 1975). In the separation phase, adherent junctions, desmosomes, and tight junctions are detected between these two cell types. During the migration phase however, cytoplasmic bridges supplant the intercellular junctions connecting PGCs to the neighboring somatic cells (Pereda et al., 1988; Fujimoto et al., 1977; 1989). According to Fujimoto et al. (1977; 1989), these cytoplasmic bridges may be important for the exchange of gases, nutrients, and molecular information by which the PGCs recognize their migratory pathway. The PGCs have pseudopodial cytoplasmic projections. Confocal microscopy studies have shown that PGCs extend long processes by which they link up with each other in order to form an extensive network of connecting cells (Gomperts et al., 1994b). As expected, migratory PGCs have a well-developed cytoskeleton rich in microtubules and actin microfilaments (Fujimoto et al., 1977; 1989; Spiegelman and Bennett, 1973; Pereda et al., 1991).

Identification of primordial germ cells

Due to the three typical morphological characteristics described above, PGCs are easily distinguished from the other cells found in the migratory pathway. This distinction can be made through the examination of semithin sections of samples embedded in resin (Fig. 2; Fujimoto et al., 1977). Histochemical detection of alkaline phosphatase activity can also be used to identify PGCs (Fig. 4; McKay et al., 1953, Jeon and Kennedy, 1973; Clark and Eddy, 1975). However, currently PGCs are most precisely identified by the use of monoclonal antibodies such as the stage-specific embryonic antigens α-SSEA-1 (Fox et al., 1981), α-SSEA-3, and SSEA-4 (Shevinski et al., 1982) as well as EMA-1 (Hahnel and Eddy, 1986) and TG-1 (Donovan et al., 1987), all of which bind glycoprotein molecules present on the surface of these cells. Of these, α-SSEA-1 is the most widely used marker for PGCs. Alpha-SSEA-1 is a trisaccharide (galactose [β1-4] N-acetylglucosamine [α1-3] fucose) found in mouse embryonic carcinoma (EC) cells and embryonic cells from the 8-cell stage embryo. Pluripotent marker genes such as Oct-1 (Park et al., 2004) and the c-kit receptor for tyrosine kinase (De Miguel et al., 2002) have also been used for CGP identification in cell cultures.
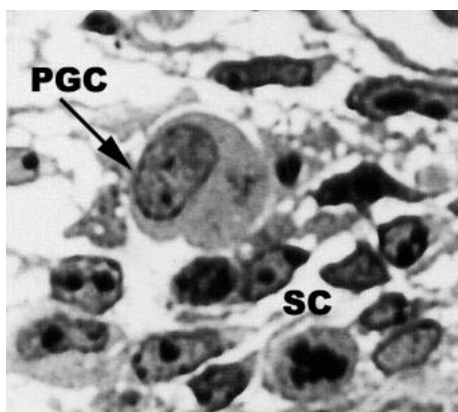

Figure 2. In the semi-thin section of a resin-embedded 10-day-old embryo, primordial germ cells (PGC) are distinguished from the adjacent somatic cells (SC) by their larger size, round form, and conspicuous nucleolus (680x).
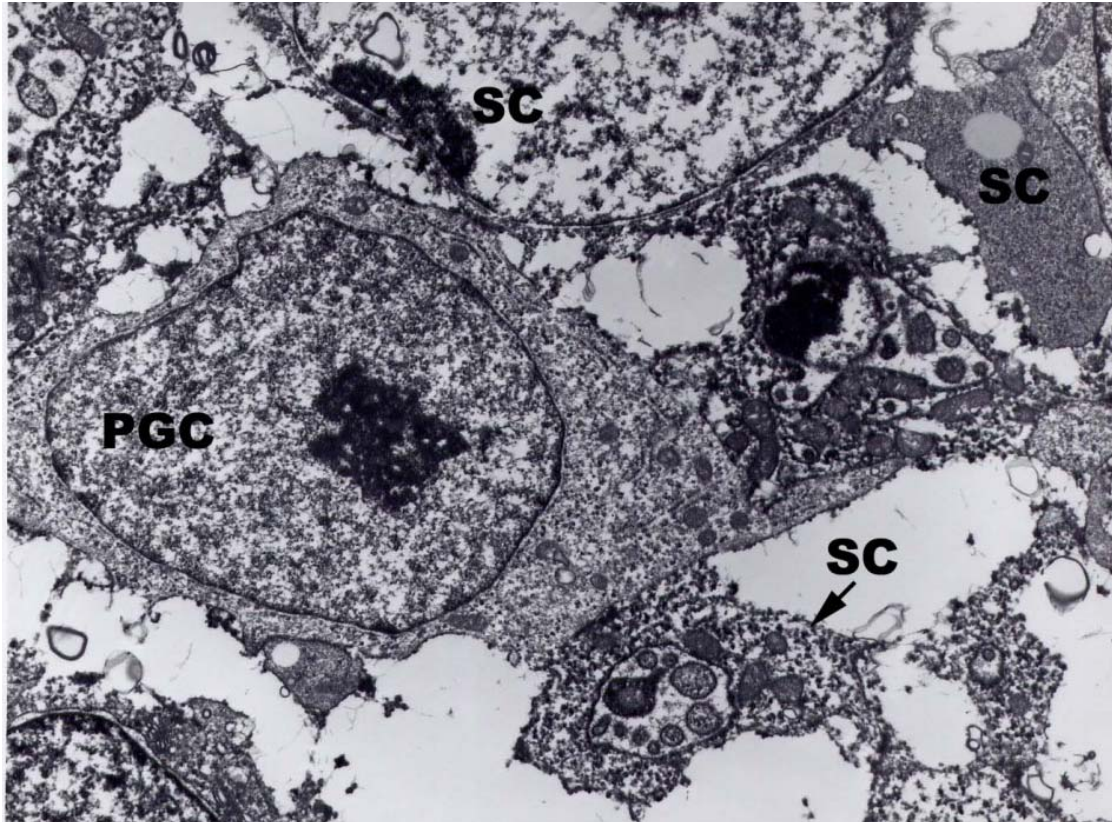
Figure 3. Electron micrograph of an 11-day-old mouse embryo showing a typical primordial germ cell (PGC) surrounded by somatic cells (SC) in the dorsal mesentery (6500x).
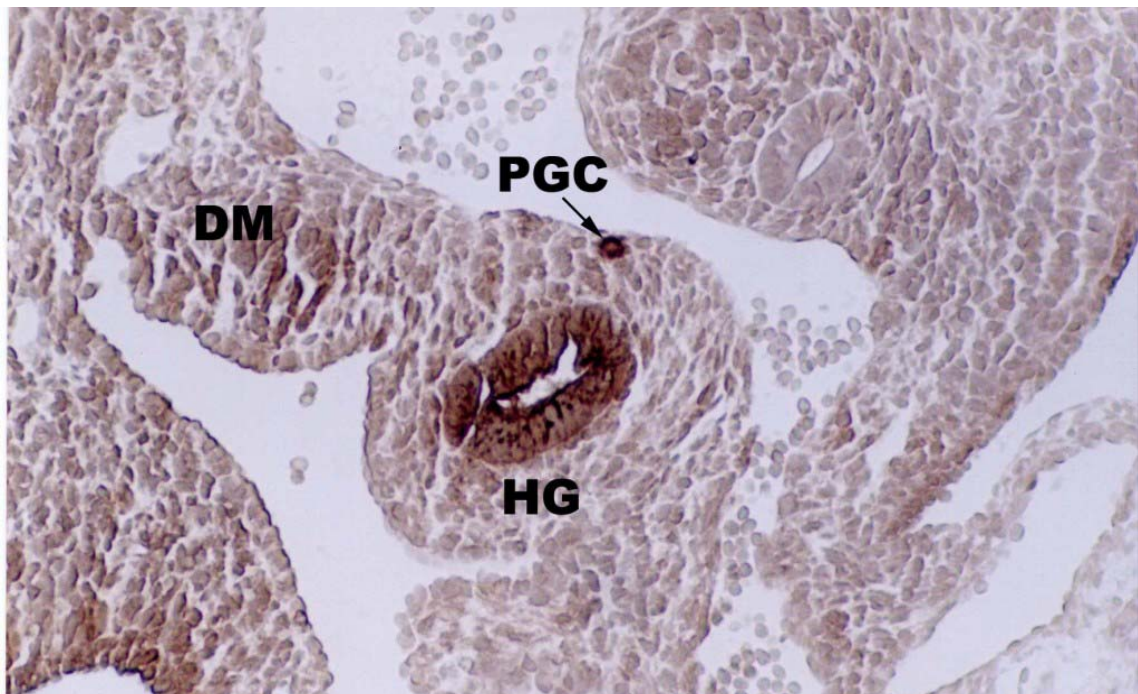

Figure 4. Transverse section through the hindgut (HG) of 10-day-old mouse embryo stained for alkaline phosphatase (ALP; 550x). One ALP positive primordial germ cell is depicted by the arrow in the mesenchyme close to the hindgut epithelium (HG). DM: dorsal mesentery.

Hypothesis regarding the mechanism of primordial germ cells guidance to the genital ridges

The greatest unsolved question in the field of embryology is exactly which mechanisms maintain the PGCs during their migration as well as which factors control PGC migration and homing within the genital ridges. This process requires integrated signals involving contact of PGCs with extracellular matrix molecules and cellular substrates or repulsion from them, adhesion among PGCs themselves, and attraction by developing gonads (De Felici *et al.*, 2005). Therefore, these mechanisms are of great interest to the embryologist. The control of PGC guidance has been extensively studied. Although the results have been fragmentary, they have led to at least five attractive and not necessarily mutually exclusive hypotheses.

PGCs actively migrate.

The first hypothesis is that PGCs actively migrate to the developing gonads. The *in vivo* observation of pseudopodial structures has led to the conclusion that PGCs migrate by amoeboid movements (Jeon and Kennedy, 1973; Spiegelman and Bennett, 1973; Clark and Eddy, 1975; Fukuda, 1976; Fujimoto *et al.*, 1977; 1989; Pereda *et al.*, 1988; Makabe and Motta, 1989; Makabe *et al.*, 1991). In addition, active *in vitro* locomotion of PGCs has been demonstrated in samples obtained from mice (Blandau *et al.*, 1963; Stott and Wylie, 1986), birds (Kuwana and Fujimoto, 1986), and humans (Kuwana and Fujimoto, 1983). Despite the evidence for active migration of PGCs obtained from studies using mice embryos, it is possible that variation in this pattern may exist in other species. Recent studies, performed in presumptive primordial germ cells (pPGC) in Xenopus embryos at Stages 7-40 (Nishiumi *et al.*, 2005), showed that the F-actin, an essential molecule for active cell migration, was only recognized on pPGC in embryos stages later than Stage 24. In addition, a molecule like the CXC chemokine receptor 4 (CXCR4) was detected on pPGC only in later stages. These results suggest the existence of a passive (before Stage 24) and active (after Stage 24) migration of pPGCs in the endoderm cell mass of Xenopus mouse embryos. Accordingly, F-actin, CXCR4, and probably beta-1-integrin and collagen type IV, which are indispensable for the formation of F-actin, are thought to be involved in the active migration of PGCs in the endoderm cell mass.

Chemotactic signal

The second hypothesis suggests the existence of a chemotactic signal, meaning that PGCs are attracted by some chemical factor emitted from the genital ridges and/or by somatic cells positioned along their migratory pathway. Chemotropic agents are well known to play a role in the migration of several cell populations both in adult and in particular, in embryonic phase (reviewed by Davies, 1987), and there is some evidence of the involvement of such tropic agents in PGC migration. Rogulska *et al.* (1971) implanted migratory mouse PGCs into chick embryos and observed that implanted PGCs were attracted to the genital ridges. Apparently, the chemotactic effect is not species specific. An *in vitro* study, using PGCs obtained at 8.5 days *post-coitum* (the beginning of their migratory phase), showed that PGC numbers increased in culture medium conditioned by homogenate of 10.5 days post coitus (dpc) genital ridges (Godin *et al.*, 1990). Moreover, PGCs migrate toward 10.5 dpc genital ridges in preference to other explanted organs. This and other experiments indicate that the genital ridges release tropic factors that have long-range effects on the migrating population of PGCs, exerting a strong attractive effect on PGC guidance (Kuwana and Fujimoto, 1986; Godin *et al.*, 1990).

Recently, evidence has been accumulated showing that chemokines, a family of structurally related glycoproteins with potent leukocyte activation and/or chemotactic activity, play a role in PGC migration (reviewed by Raz, 2004; Dumstrei *et al.*, 2004; Stebler *et al.*, 2004; Blaser *et al.*, 2005; Nishiumi *et al.*, 2005). Using the promoter of the novel gene askopos and RNA elements of nanos-1 to drive GFP expression in zebrafish PGCs, Blaser *et al.* (2005) showed that, as PGCs begin the migratory phase, they acquire competence to respond to the chemokine stromal cell-derived factor-1a (SDF-1a) secreted by surrounding somatic cells. In addition, Reichman-Fried *et al.* (2004) showed that in zebrafish embryos, individually migrating PGCs alternate between migratory and pausing modes. The coordinated migration appears to work in response to local variation in SDF-1a distribution**.**

Apparently, the actions of SDF-1a do not restrict zebrafish PGC migration and act also in avian and mouse PGC migration. SDF-1a mRNA was demonstrated to be expressed in locations where PGCs are found and towards which they migrate at the time they leave the blood vessels in avian embryos (Stebler *et al.*, 2004). According to these authors, these results, as well as the analysis of gene expression and PGC behavior in the mouse embryo, suggest that SDF-1a is required for the PGCs to execute the final migration steps as they transmigrate through the endothelium of blood vessels in the chick or the gut epithelium of the mouse. A new important view coming from these studies is that the migration of PGCs in the chicken is similar to that of leukocyte migration during normal development and disease as well as metastatic cell

migration (Stebler *et al.*, 2004).

Putting these data together, we should recognize that studies designed to identify the intracellular signals controlling directional migration and cell motility would be important for understanding PGC migration. In this regard, recent studies have identified CXCR4b as the receptor for SDF-1a, and the intracellular CXCR4b activation is mediated by two important biochemical pathways, G-protein-dependent and phosphoinositide 3-kinase (PI3K)-dependent signaling (Dumstrei *et al.*, 2005). In addition, studies performed in zebrafish PGCs (Dumstrei *et al.*, 2005) showed that G-protein-dependent signaling is essential for directional migration whereas the PI3K pathway is important for the motility of PGCs.

Substrate-guidance

The third hypothesis is that PGCs are guided by a substrate-guided mechanism. Using scanning microscopy, Wylie *et al.* (1979) showed that, in *Xenopus embryos*, PGCs appear to be guided *in vitro* by the shape of, or contact with underlying cells. In this study, PGCs were always found to move in the direction of stress fibers in the underlying cells. As will be further discussed in this review, there is accumulated evidence that shows that the motility of migrating PGCs relies on integrated signals from extracellular matrix (ECM) molecules and the surrounding somatic cells (reviewed by De Felici, 2000).

Basement membrane

The fourth hypothesis proposes that the basement membrane of the epithelium of the migratory pathway guides the PGCs towards the genital crest. Heasman *et al.* (1985) showed that, in *Xenopus laevis,* the appearance of the basement membrane in the PGC migratory pathway was associated with PGC acquisition of migratory ability. In fact, histochemical studies have demonstrated that PGCs migrate along a basal lamina underlying the coelomic epithelium (Clark and Eddy, 1975). There is evidence that PGCs interact with the ECM around them as they migrate (Donovan *et al.*, 1987; De Felici and Dolci, 1998). Immunohistochemical studies have indicated the existence of a temporo-spatial distribution of basement membrane molecules along the migratory pathway such as laminin, type IV collagen (Garcia-Castro *et al.*, 1997; Soto *et al.*, 1998), and perlecan and heparan sulfate (Soto-Suazo *et al.*, 2002a). Recently, syndecan-4, a cell surface proteoglycan containing heparan sulfate, was shown to abound in the migratory pathway of PGCs (Soto-Suazo *et al.*, 2002a). In addition, other ECM molecules have been observed as associated with the basement membrane during PGC migration including fibronectin (Fujimoto *et al.*, 1985), hyaluronan (Soto-Suazo *et al.*, 2002b), versican (Soto-Suazo *et al.*, 2002a), tenascin-C, and type I and III collagens (Soto-Suazo *et al.*, 2004).

PGC-PGC interactions

The fifth hypothesis is that PGC-PGC interactions may play a role in their accumulation in the genital ridges. Gomperts *et al.* (1994a), using laser confocal microscopy, showed that PGCs exit the hindgut independently but then extend long processes, by which they link to each other, in order to form extensive networks. These PGC networks aggregate into groups of closely apposed cells in the genital ridges. According to Gomperts *et al.* (1994b), these observations change the previous view that PGCs migrate as individuals as was believed from observing semithin sections only. The principal movements seem to involve PGC interactions with each other, first via long processes and then by aggregation. Cell adhesive molecules, belonging to three adhesion molecules families such as cadherins (E-P and N-cadherins), integrins, and the IgG superfamily (PECAM-1), have been identified in mammalian PGCs, mainly in the mouse. Recent studies, which have investigated how genes encoding adhesive molecules are regulated in PGCs (see De Felici *et al.*, 2005), have reinforced the importance of these molecules not only in the control of PGC migration but also in the differential fate of PGCs.

Primordial germ cell surface

Human PGCs frequently present a delicate, fibrillar coat on their free surface during the migration phase (Pereda and Motta, 1991). This PGC surface coat may be associated with the binding sites of specific membrane macromolecular components and with ECM macromolecules. Various molecules have been identified on the cell surface of migratory PGCs in different animal species. These include the enzyme alkaline phosphatase (Chiqouine, 1954), ovomucin-like protein (Halfter *et al.*, 1996), and hyaluronan, chondroitin, and dermatan sulfate (Pereda *et al.*, 1998). Besides these, the following molecules are also associated with cell adhesion: carbohydrate epitope SSEA-1 (stage specific embryonic antigen 1; D´Costa and Petitte, 1999); oligossacarides such as Lewis X; growth factor receptors c-kit (Wakayama *et al.*, 2003); IGg superfamily (PECAM-1; Wakayama *et al.*, 2003); and α_6, α_5, α_3, and β_1 subunits of integrin receptors (De Felici and Dolci, 1998). In addition, E-cadherin and P-cadherin (Bendel-Stenzel *et al.*, 2000) are likely involved in PGC adhesion, recognition, and movement phenomena. However, mutant mice lacking the enzyme alkaline phosphatase present no deficits in the numbers of PGCs arriving at the genital ridges (MacGregor *et al.*, 1995). In fact, knockout experiments have demonstrated that embryos with targeted deletion of α_6, α_5, and α_3

integrin subunits present no major defects in PGC migration. However, PGCs lacking integrin β1 subunits fail to migrate normally to the gonads (Anderson and Beams, 1999).

Extracellular matrix of the primordial germ cells migratory pathway

It is currently recognized that ECM molecules are important for PGC migration. Pioneering studies performed by Fujimoto *et al.* (1985) showed that adhesive proteins, such as fibronectin, are present in the ECM of the PGC migratory pathway. Subsequent *in vitro* experiments have shown that fibronectin plays a role in the PGC migration process, promoting both PGC adhesion to substrates and PGC migration (Alvarez-Buylla and Merchant-Larios, 1986; Ffrench-Constant *et al.*, 1991).

The expression and distribution of the glycoprotein laminin and type IV collagen have also been correlated with the PGC migration process (Garcia-Castro *et al.*, 1997; Soto *et al.*, 1998). Closer analysis of the adhesion of PGCs to laminin revealed that PGCs adhere particularly strongly to the E3 domain of laminin, and *in vitro* blocking experiments suggest that they adhere to this domain using a cell-surface,

heparin-sulfate proteoglycan (Garcia-Castro *et al.*, 1997). According to Nishiumi *et al* (2005), collagen type IV is indispensable for the formation of F-actin that is thought to be involved in the active migration of Xenopus presumptive PGCs in the endodermal cell mass.

An ultrastructural, cytochemistry study showed that proteoglycans in the PGC migratory pathway are organized as a meshwork of granules interconnected by thin filaments (Fig. 5; Soto-Suazo *et al.*, 1999). The authors demonstrated that these granules prevailed in the ECM throughout the entire migration process, whereas the number of filamentous structures increased during the PGC migration phase. In another study, treatment with hyaluronidase disrupted the filamentous structures indicating that they are composed of hyaluronan (Pereda *et al.*, 1998). Soto-Suazo *et al.* (2002b) followed the distribution of hyaluronan during the separation, migration, and colonization phases of the PGC migration process and showed that high expression of hyaluronan correlates with the presence of PGCs in each compartment of the PGC migratory pathway (Table 2). The presence of hyaluronan along the migratory pathway may provide a hydrated environment that facilitates PGC migration.
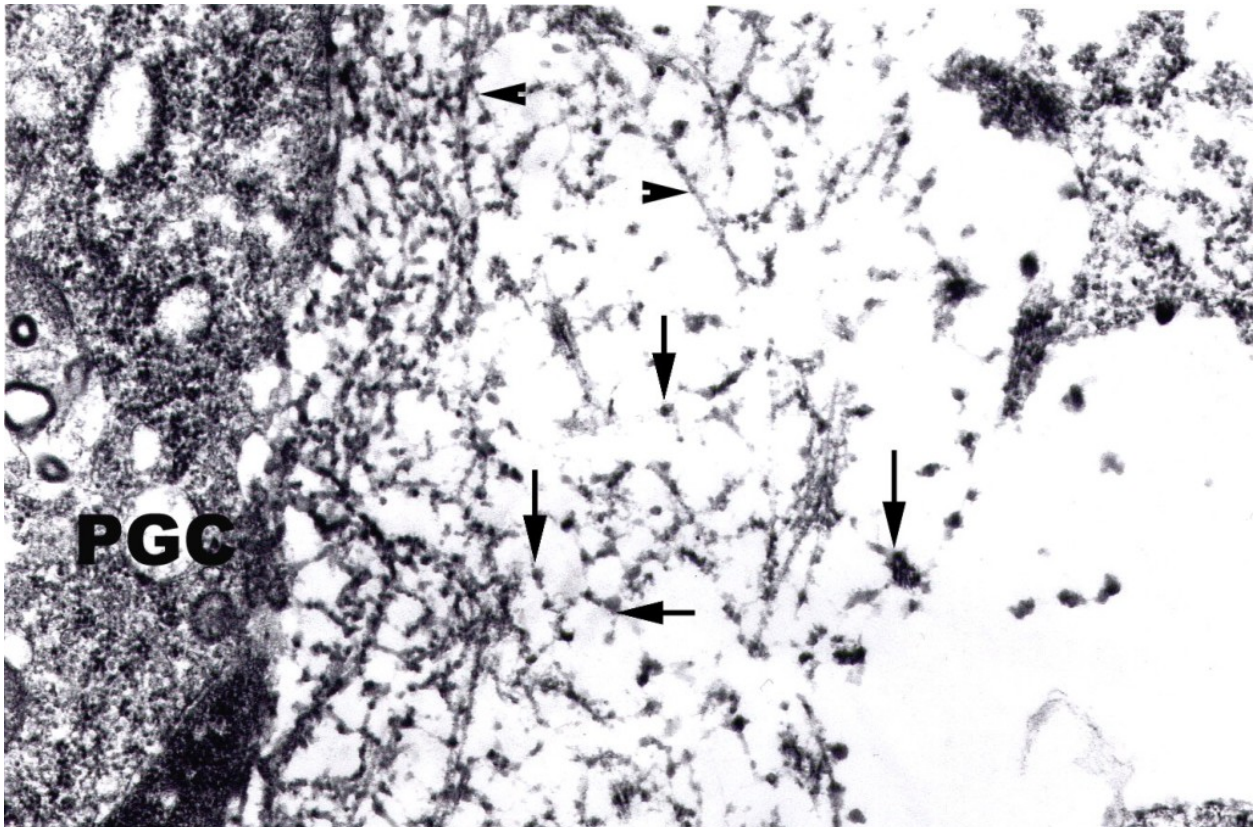

Figure 5. Extracellular spaces of the migratory pathway of the PGCs after treatment of tissues with ruthenium hexammine trichloride (RHT; 40,000x). The proteoglycans are observed as electron-dense granules (arrows) interconnected by thin filaments (arrowheads).

Table 2. Glycosaminoglycans, proteoglycans, and glycoproteins expressed in the extracellular spaces and the basement membranes of different regions of the migratory route of primordial germ cells (PGCs) on different days of embryonic development.

Molecule	9 DD SEPARATION PHASE			10 DD MIGRATION PHASE			11 DD COLONIZATION PHASE			12DD COLONIZATION PHASE		
	HG	DM	GR	HG	DM	GR	HG	DM	GR	HG	DM	GR
	PGCs			PGCs			PGCs		PGCs			PGCs
CH-0-S	***	*	*	**	**	**	-	-	-	-	-	-
CH-6-S	***	**	**	***	***	*	*	*	***	*	*	***
HS	**	**	**	**	**	**	*	*	**	*	*	**
HY	***	***	***	***	***	***	*	*	*	*	*	*
VRN	***	***	***	***	***	***	*	*	***	*	*	***
SYN-4	**	**	**	**	**	**	*	*	**	*	*	**
DCN	-	-	-	-	-	-	-	-	*	-	-	*
BGN	-	-	-	-	-	-	-	-	*	-	-	*
PLN	**	-	*	***	***	***	***	***	***	***	***	***
COLL I	*	*	*	**	**	**	***	***	***	***	***	***
COLL III	***	*	*	***	***	***	***	***	***	***	***	***
COLL IV	*	*	*	**	*	*	***	***	*	***	***	*
COLL V	*	*	**	**	**	**	**	**	*	**	**	*
TEN-C	-	-	-	-	**	-	-	-	*	-	-	*
FN	**	**	**	**	***	**	-	-	-	-	-	-
LAM	**	**	**	***	**	*	***	***	**	***	***	**

Semiquantitative evaluation of the staining of the immunocytochemical reaction: *** strong reaction; ** moderate reaction; * weak reaction; - no reaction. PGCs: indicates the location of primordial germ cells in a specific region along the migratory route. HG: hindgut; DM: dorsal mesentery; GR: gonadal ridge; G: gonad; CH-0-S: chondroitin-0-sulfate; CH-6-S: chondroitin-6-sulfate; HS: heparin sulphate; HY-hyaluronan; VRN: versican; SYN-4: syndecan-4; DCN: decorin; BGN: biglycan; PLN: perlecan; COLL I: collagen type I; COLL III: collagen type III; COLL V: collagen type V; TEN-C: tenascin-C; FN: fibronectin; LAM: laminin.

The pattern of expression of some glycosaminoglycans and proteoglycans in the PGC migratory pathway changes according to the different phases of the migration process as demonstrated in an immunohistochemical study conducted by Soto-Suazo et al. (2002a). Some molecules, such as Chondroitin-0-sulfate, decorin proteoglycan, and biglycan proteoglycan, are present only during certain phases of the PGC migration process (Table 2). Decorin and biglycan proteoglycans have not been detected in the PGC migratory pathway in either the separation or migration phase. However, small amounts of these proteoglycans have been observed in the developing gonads during the colonization phase. It is known that, as well as playing a structural role in the organization of the ECM, decorin proteoglycan binds to TGF-beta thereby participating in the regulation of cell proliferation. This interaction is competitively inhibited by biglycan proteoglycan. In vivo studies performed in embryos lacking TGF-beta signaling via type I receptor ALK5 (Chuva de Sousa Lopes et al., 2005) demonstrated that TGF was neither a chemo-attractant for PGCs nor did it affect their proliferation during migration towards the genital ridges up the 10th day of the embryo development (E10). On the contrary, the authors proposed that the absence of TGF-beta resulted in significant facilitation of PGC migration out of the hindgut. The authors concluded that TGF-beta signaling plays no role in regulating the proliferation of PGCs or acting as a chemo-attractant until E10. They suggested that by regulating collagen type I deposition around the hindgut, TGF-beta signaling indirectly restricts the migration of PGCs from the hindgut to the dorsal mesentery.

Heparan sulfate and chondroitin-6-sulfate, as well as the proteoglycans versican, perlecan, and syndecan-4, although exhibiting some degree of differential expression, have been detected during all phases of the migration process (Soto-Suazo et al., 2002a). Versican was highly expressed in the extracellular compartments of the migratory pathway (Fig. 6), whereas its expression was clearly diminished in the colonization phase. However, versican immunoreactivity was higher in the developing gonads during the colonization phase. The space-time distribution of versican in the PGC migratory pathway (Table 2) strongly suggests that this proteoglycan may favor displacement of the PGCs. On the other hand, the high expression of versican observed in the developing gonads suggests that this molecule could be related to the arrest of PGC migration, and consequently, avoiding PGCs

escape from the gonads (Soto-Suazo et al., 2002a). Soto-Suazo et al. (2002a) detected syndecan-4 in small amounts in both the separation and migration phases (Fig. 6) indicating that, by regulating the adhesion of PGCs, it might contribute to controlling the migration process. The authors found that, during the various phases of the migration process, perlecan expression was progressively increased in the basement membranes of the PGC migratory pathway (Fig. 6). The same authors observed sequential expression of perlecan (Table 2) during PGC migration, suggesting that this proteoglycan acts as a barrier to the return of the PGCs to the hindgut epithelium as well as preventing them from escaping the developing gonads (Soto-Suazo *et al*., 2002a).
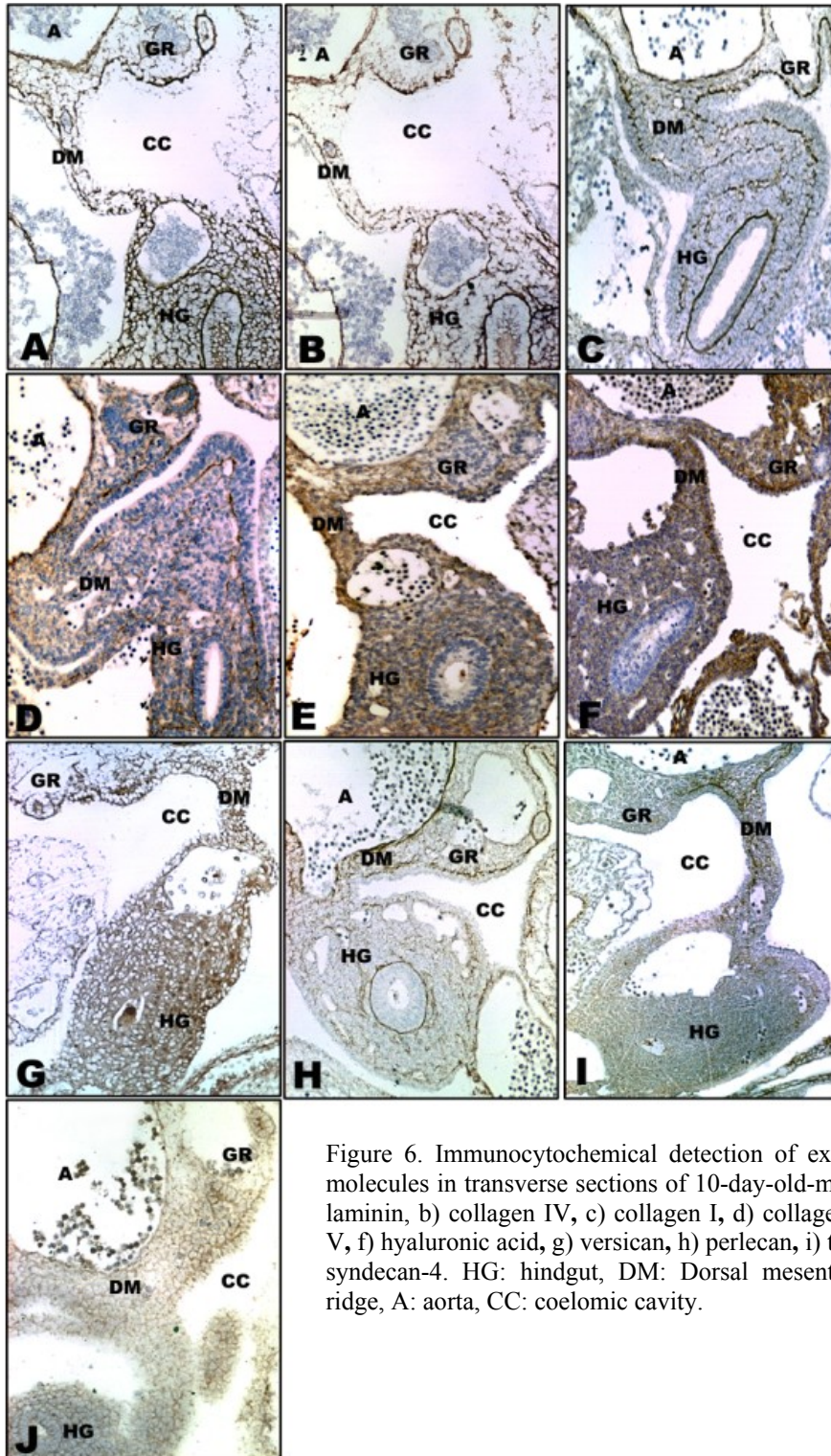

Figure 6. Immunocytochemical detection of extracellular matrix molecules in transverse sections of 10-day-old-mouse embryos**:** a) laminin, b) collagen IV, c) collagen I**,** d) collagen III**,** e) collagen V**,** f) hyaluronic acid**,** g) versican**,** h) perlecan**,** i) tenascin C**,** and j) syndecan-4. HG: hindgut, DM: Dorsal mesentery, GR: genital ridge, A: aorta, CC: coelomic cavity.

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The expression of tenascin-C, as well as that of the collagen types I, III, and V (Fig. 6), has been shown to change in the various compartments along the PGC migratory pathway depending on the migration phase (Soto-Suazo et al., 2004). These results suggest that the collagen types I and III form suitable substrates for migratory PGCs, and that collagen V may act as a barrier, preventing both the return and the ectopic migration of PGCs. Primordial germ cell migration and homing within the genital ridge is a not, as of yet, well-clarified event that requires a net balance between contact or repulsion of PGCs with extracellular matrix molecules. There is a suggestion that migratory PGCs adhere strongly to collagen; therefore, reduced collagen type I along the gut might diminish adhesion thus facilitating PGC migration into dorsal mesentery and genital ridges (Chuva de Sousa Lopes et al., 2005). The expression and distribution of tenascin-C along the PGC migratory pathway were more restricted during PGC migration. This suggests that tenascin-C plays a role in the PGC migration process although it has not been clarified. Taken as a whole, these data lead us to assume that each successive step of PGC migration process requires coordinated expression of specific ECM molecules, which probably interact with each other in order to provide an appropriate environment for PGC migration. Table 2 summarizes these results showing a schematic representation of the distribution of a representative group of ECM molecules along the migratory route during different phases of the PGC migration process.

A series of observations performed by De Felici´s group showed that throughout the migratory period, PGCs receive signals from the surrounding somatic cells that: 1) secure their survival (at the same time perhaps promoting their apoptotic degeneration in ectopic sites), 2) control their proliferation, and 3) guide them to the developing gonad. At least some such signals are mediated by adhesive interactions through molecules of the distinct adhesion family. Primordial germ cells are probably able to modulate their adhesiveness according to different ECM molecules and somatic cells encountered during their migration.

Derivation of pluripotent embryonic stem cells from primordial germ cells

Embryonic stem cells are derived from the inner cell mass of pre-implantation embryos (reviewed by Evans and Kaufman, 1981; Martin, 1981), and embryonic germ (EG) cells are derived from PGCs (Matsui et al., 1998; Resnick et al., 1992). Germline cells, contrary to the somatic lineage of the embryo, carry the genome from generation to generation. Therefore, PGCs are the only stem cells that retain true developmental totipotency after gastrulation, express markers typical of a totipotent/pluripotent status, and give rise to pluripotent stem cells such as EC and EG cells both in vivo and in vitro (Klinger et al., 2003). Paradoxically, when mouse PGCs are introduced into a host blastocyst, they do not contribute to either the germline or to the soma, suggesting that their developmental potency is restricted. In contrast, two types of pluripotent stem cells arise from PGCs. In vivo, PGCs give rise to EC cells, the pluripotent stem cells of teratomas and benign tumors, containing derivatives of the three primary germ layers (Donovan et al., 1998; Donovan and Miguel, 2003). When mouse PGCs are cultured in vitro on feeder layers supplemented with a specific cocktail of growth factors, they give rise to EG cells, pluripotent stem cells capable of giving rise to somatic and germline chimeras (Labosky et al., 1994; Donovan et al., 1998; Durcova-Hills et al., 2001; McLaren and Durcova-Hills, 2001; Donovan and Miguel, 2003). The EG cells constitute a stem cell population capable of producing a variety of different cell types including hematopoietic (Rich, 1995) and myogenic cells (Klinger et al., 2003). According to Donovan and Miguel (2003), the conversion of PGCs into pluripotent stem cells is a process remarkably similar to nuclear reprogramming in the oocyte cytoplasm. Using 5- to 9-week-old (postfertilization) human embryos obtained as a result of the therapeutic termination of pregnancy, Shamblott et al. (1998) cultivated human genital ridges and mesenteries to establish pluripotent stem cells. The authors successfully developed human PGC-derived cultures that met the criteria for pluripotent stem cells and most closely resembled EG cells. According to these authors, human pluripotent stem cells, with their potential to differentiate into a wide variety of cell types in culture, would be invaluable for studies of some aspects of human embryogenesis and for transplantation therapies. Greater knowledge of the mechanisms that control EC cell formation and of the signaling pathways that control EG cell derivation could help us understand the molecular controls of developmental potency in mammals.

In conclusion, during the last years, the interest in PGC migration has advanced significantly, principally as a result of the identification of molecules that provide directional cues for PGCs. These findings make the study of PGC migration a classical topic in developmental biology. They also are directly relevant to work on the development of other organs, stem cell homing, leukocyte trafficking, and neuronal cell migration; all are phenomena in which the same molecules are used (reviewed in De Felici, 2000).

The evidence currently available indicates that the ECM is capable of inducing specific gene expression in developing tissues (Bissell et al., 1982; Martins-Green and Bissell, 1990). Further studies evaluating the relationship between the ECM molecules and PGC gene expression are warranted in order to better understand the genetic control affecting PGC survival, proliferation, differentiation, and directional

control during migration. Moreover, recent studies have shown that migration of PGCs from diverse species exhibit several behavioral modes that are likely to be relevant for other migratory cells, particularly those guided by chemokines (Reichman-Fried *et al.*, 2004)

Future studies with mutant mice deficient in specific extracellular molecules will allow us to better understand the role of these molecules during the migration and differentiation of the PGCs.

Acknowledgments

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