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Primordial germ cell migration

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ABSTRACT Mutational and antisense screens in *Drosophila* and zebrafish, and transcriptional profiling and time-lapse analysis in the mouse, have contributed greatly to our understanding of PGC development. In all three systems, the behavior of PGCs is controlled by growth factors which signal through G-protein coupled receptors and/or tyrosine kinase receptors. Additionally, regulated cell-cell and cell-substrate adhesion is important for PGC motility. Finally, localized growth factors may control PGC survival and consequently PGC position. Chemotaxis, regulated adhesion and cell survival are important for multiple migration processes which occur during development and disease. PGC migration shares these features.

KEY WORDS: *primordial germ cell, migration, Drosophila, zebrafish, mouse*

Introduction

Directed migration of cells in culture is often initiated by growth factor mediated activation of tyrosine kinase receptors or G-protein coupled receptors. These initiate changes in the cytoskeleton and alteration of the cell-substrate adhesion (reviewed in Ridley *et al.*, 2003). Actin dynamics at the leading edge of a cell results in the extension of filopodia/lamellopodia and translocation of the cell body. After extension, the trailing edge of the cell is retracted via a myosin dependent process. Cell culture models have allowed for the elegant analysis of the signaling machinery required for cell-shape changes and motility. It is likely that the general paradigm for cell migration established from *in vitro* studies will also apply to migratory populations that arise during embryogenesis or disease *in vivo*. However, the fact that these cell populations often migrate long distances and through tissue environments that change during migration adds an extra layer of complexity to the process of cell homing/metastasis in multicelled organisms. In this review, we will discuss the process of PGC migration as an example of how cells move within a complex and changing tissue environment.

PGCs are the embryonic precursors of the gametes. The identification of the PGC marker gene alkaline phosphatase in the mouse (Chiquoine, 1954) and more recently the RNA-binding factor, vasa in flies and zebrafish (Schupbach and Wieschaus, 1986; Hay *et al.*, 1988; Olsen *et al.*, 1997; Yoon *et al.*, 1997) have allowed PGCs to be visualized in the embryo. In all systems, PGCs form far from the site of the developing gonads and migrate to the sites of developing ovaries or testes. In the mouse (Fig. 1), PGCs can first be detected at embryonic day 7.5 (E7.5) as a cluster of alkaline phosphatase-positive cells at the base of the allantois (Ginsburg *et al.*, 1990). By E9.0, PGCs become incorporated into the hindgut. Between E9.0-E9.5, PGCs emerge from the dorsal side of the gut and migrate laterally to colonize the genital ridges. In *Drosophila* (Fig. 2), PGCs arise from the posterior pole of the developing embryo where localized maternal components (including vasa) become segregated into pole cells (reviewed in Starz-Gaiano and Lehmann, 2001). Pole cells become incorporated into the hindgut during the process of germ band extension. PGCs migrate out of the ventral side of the gut, migrate along the basal surface of the gut and into the lateral mesoderm where they coalesce with the somatic cells of the gonad. Similarly, in zebrafish (Fig. 3), PGCs are specified by maternal components (including vasa) that become segregated into four clusters within the cleaving embryo (reviewed in Raz, 2003). During gastrulation, these PGC clusters move dorsally and align at the border between the head and trunk mesoderm or align within the lateral mesoderm. Both lines of cells then migrate towards an intermediate target within the lateral mesoderm. At the 8-somite stage, PGCs leave this intermediate target and migrate posteriorly to colonize the developing gonad.

Abbreviations used in this paper: BMP, bone morphogenetic protein; Cx43, connexin 43; ECM, extracellular matrix; FGF, fibroblast growth factor; Foi, fear-of-intimacy; HmG-CoA reductase, 3-hydroxy-3-methylglutaryl coenzyme A reductase; LIF, leukemia inhibitory factor; LPP, lipid phosphate phosphohydrolase; PAP, phosphatidic acid phosphatase; PGCs, primordial germ cells; SDF-1, stromal derived factor 1; SGPs, somatic gonadal precursors; TGFβ, transforming growth factor β.

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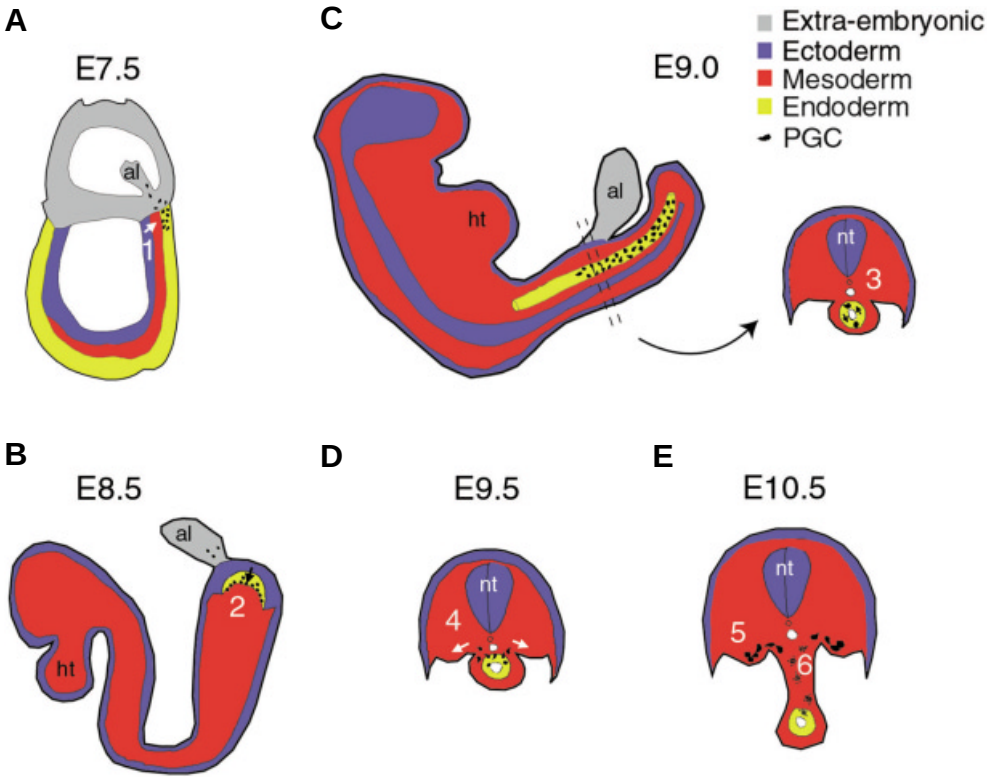

Fig. 1. Germ cell migration in the mouse. *The behavior of PGCs can be divided into six stages based on time lapse analysis of PGCs in tissue dissected from E7.5 (Anderson* et al., *2000) and from E9.0-E10.5 embryos (Molyneaux* et al., *2001). The behavior of PGCs at E8.5 is inferred from PGC position and morphology at this stage. Arrows indicate the net direction of PGC movements.* **(A)** *PGCs are induced to form in the proximal epiblast. During gastrulation, they move through the primitive streak and invade the definitive endoderm, parietal endoderm and allantois (step 1).* **(B)** *PGCs in the definitive endoderm are incorporated into the hindgut pocket (step 2). This process may be passive or active. Some PGCs remain in the allantois at this stage and the fate of these cells is uncertain.* **(C)** *By E9.0, the hindgut has extended and closed to form a tube. PGCs are confined to the hindgut, but move freely around the cells of the hindgut epithelium (step 3). PGC motility appears random with respect to the body axes at this stage. PGCs circle the gut and/or move towards the anterior or posterior.* **(D)** *Betweeen E9.0 and E9.5, PGCs emerge from the dorsal side of the hindgut and migrate towards the developing genital ridges (step 4).* **(E)** *At E10.5, PGCs begin to cluster forming a network of migrating cells (step 5). PGCs are slowing. PGCs left in midline structures fragment and die (step 6). By E11.5 most PGCs have colonized the genital ridge. The entire migration process takes approximately four days (E7.5-E11.5). Abbreviations: al, allantois; ht, heart; nt, neural tube.*

Within the past six years, a molecular model for PGC development has started to emerge. Forward genetic screens in *Drosophila* (Moore *et al.*, 1998) and *in situ*, antisense and genetic screens in Zebrafish have identified genes required at various stages of PGC migration (Doitsidou *et al.*, 2002; Knaut *et al.*, 2003; Weidinger *et al.*, 2003). In the mouse, single cell transcriptional profiling has been used to identify genes expressed in nascent PGCs (Saitou *et al.*, 2002; Tanaka and Matsui, 2002) and chip analysis has been used to identify genes that are differentially expressed in migratory and non-migratory germ cells (Molyneaux *et al.,* 2004). In addition, lines of mice have been established that express a live marker (GFP) under control of germ cell specific promoters allowing for the first time direct observation of PGC behavior in the mammalian embryo (Anderson *et al.*, 1999). From this wealth of new genetic and behavioral data, we can begin to piece together how PGCs initiate migration and how they home in on the positions of the gonads.

How is pgc migration initiated?

Mouse

PGCs in the mouse may be motile from their inception (E7.25) to the point where they colonize the genital ridge (E11.5). Expression of *BMP4/BMP8b* in extraembryonic tissue induces the formation of PGCs in the proximal epiblast (Lawson *et al.*, 1999; Ying *et al.*, 2001). Shortly after formation, PGCs move through the posterior primitive streak and invade the definitive endoderm and posterior extraembryonic structures. PGCs at this stage have a polarized morphology and have been observed extending processes in time lapse movies (Anderson *et al.*, 2000). The initiation of PGC motility may have its roots in the formation of PGCs or in the subsequent gastrulation process. Two genes, *stella* and *fragillis/mil-1* were recently identified in cDNA libraries created from nascent PGCs (Saito *et al.,* 2002, Tanaka and Matsui, 2002). *Stella* is a novel gene with no known function. *Fragilis* is a member of an interferon inducible family of genes implicated in homotypic cell-cell adhesion and cell-cycle control. At E7.25, *fragilis* is expressed in the posterior epiblast with highest expression overlapping the region where PGCs are formed. At this stage, PGCs are clustered and appear non-motile. 24 hrs. later *fragilis* expression has been downregulated and PGCs have scattered and moved into the endoderm (Saitou *et al.,* 2002). This suggests that regulated adhesion may play a role in initiating PGC motility. However, nascent PGCs were found to express two other members of the *fragilis* gene family (*fragilis 2* and *3*) and *fragilis2* expression is maintained in migrating PGCs (Lange *et al.,* 2003). Hence, the relationship between fragilis-mediated adhesion and PGC motility is unclear. Cell-cell adhesion may also play a role in PGC formation. It was recently been shown that blocking antibodies against E-cadherin can prevent the formation of PGCs in a cell culture system (Okamura *et al.*, 2003). Newly formed PGCs were also found to express *Brachyury* (Saito *et al.,* 2002) and based on Affymetrix chip experiments, expression of this T-box transcription factor is retained in PGCs until E10.5, but is subsequently downregulated in non-motile PGCs (E12.5) (Molyneaux *et al.,* 2004). *Brachyury* is required for mesoderm formation and may either positively (Viebahn *et al.*, 2002) or negatively (Kwan and Kirschner, 2003) regulate cell re-arrangements during gastrulation. It is possible that the general motile characteristics of PGCs may be controlled by expression of *Brachyury* and PGCs may retain the motility characteristics of

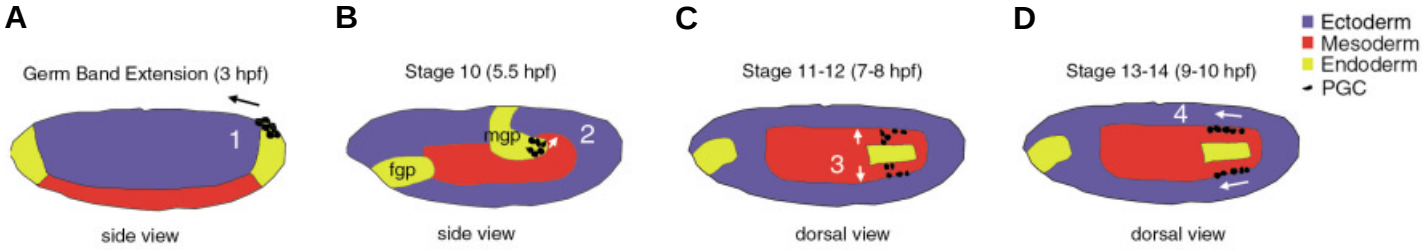

Fig. 2. Germ cell migration in *Drosophila*. *The behavior of PGCs can be divided into four stages based on the position and morphology of PGCs (Warrior, 1994) and the analysis of mutations affecting PGC position (Moore* et al.*, 1998). Pole cells form at the posterior end of the embryo at the blastoderm stage (stage 5, 2 hpf (hours post fertilization)).* **(A)** *During germ band extension, the pole cells move anteriorly and become internalized along with the posterior midgut (step 1). This process may be passive or active.* **(B)** *PGCs emerge from the ventral side of the midgut and move into the overlying mesoderm (step 2).* **(C)** *Germ cells move towards the somatic gonadal precursors, forming two bilateral lines within parasegments 10-13 (step 3).* **(D)** *PGCs and SGPs adhere and re-arrange forming tight clusters of cells within parasegment 10 (step 4). The entire migration process takes approximately 7 hours (Stage 8-Stage14). Staging is based on a modification of the Hartenstein stages described in Weigmann* et al. *(2003). Abbreviations: fgp, fore gut pocket; mgp, midgut pocket.*

gastrulating cells for days after the process of gastrulation is complete.

Drosophila

In flies, localized maternal components control the formation of PGCs (reviewed in Starz-Gaiano and Lehmann, 2001) and initially these cells have a spherical non-motile morphology. There is confusion in the literature as to when PGCs in this system become motile. Based on the morphology of PGCs stained with antibodies to Vasa, it was proposed that PGCs are passively incorporated into the posterior midgut and initiate motility between stages 10 and 11 when they crawl out of the gut (Warrior, 1994). This process does not require inductive signals from the mesoderm or gonad (Jaglarz and Howard, 1994; Warrior, 1994), but may be initiated by changes in the epithelium of the midgut (Callaini *et al.*, 1995; Jaglarz and Howard, 1995). Mutations that perturb midgut formation prevented PGCs from assuming a migratory shape. It was also found that PGCs arising from fluorescently labeled transplanted pole cells that remained outside the embryo assumed a migratory morphology much earlier than PGCs within the midgut pocket suggesting that the early embryonic environment may have a repressive influence on PGC motility (Jaglarz and Howard, 1995). In support of this, explanted pole cells were found to be motile in culture (Jaglarz and Howard, 1995). More recent data demonstrated that PGCs in the embryo assume a migratory morphology earlier than initially proposed and this initiation of pole cell motility may be activated via Torso (Li *et al.*, 2003). *Torso* is a maternally inherited transcript encoding for a tryosine kinase receptor involved in patterning the dorsal and ventral poles of the *Drosophila* embryo (reviewed in Duffy and Perrimon, 1994). Signaling via Torso was shown to be required for activation of Jak/Stat and Ras pathways within PGCs (Li *et al.*, 2003). Hyperactivation of these pathways resulted in an increase in pole cell numbers and premature activation of migration. Likewise a loss of Torso signaling resulted in a reduction of pole cell numbers and sluggish PGC migration and poor internalization. Hence PGCs may actively migrate into the

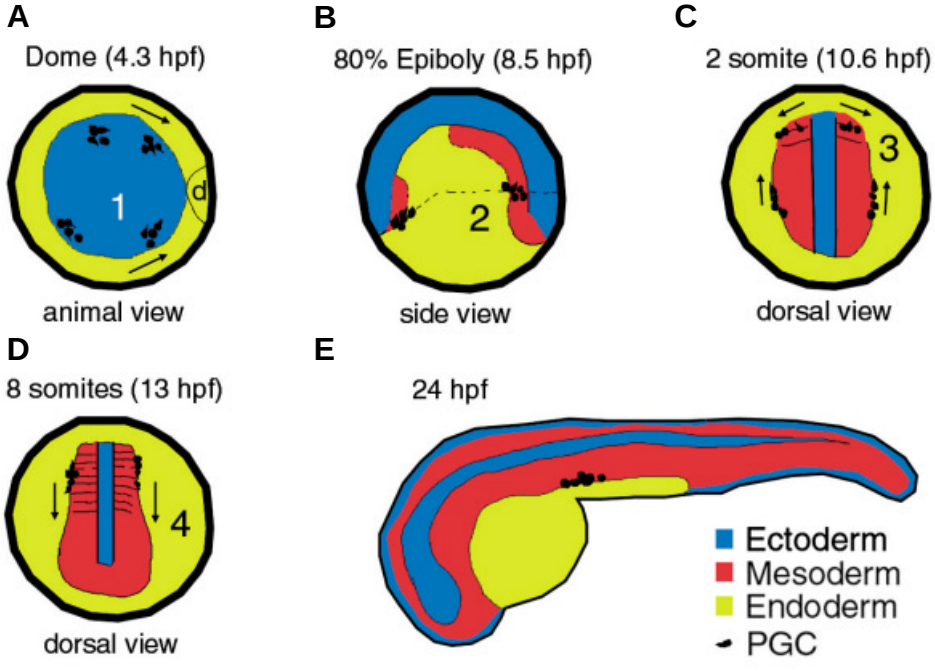

Fig. 3. Germ cell migration in Zebrafish. *This figure is a simplification adapted from Weidinger* et al. *(1999). Four clusters of PGCs are formed during the cleavage stages.* **(A)** *At the dome stage (4.3 hpf), PGC clusters within the deep blastoderm begin to move dorsally (step 1). They are excluded from dorsal midline structures (d).* **(B)** *At 80% epiboly (8.5 hpf), PGCs form a line at the border between the head and trunk mesoderm (cluster 1), or align within the lateral mesoderm (cluster 2) (step 2).* **(C)** *PGC clusters move towards an intermediate target within the lateral mesoderm (step 3). They form bilateral clusters between the 1st and 3rd somite and may temporarily cease moving.* **(D)** *PGC clusters move posteriorly to colonize the gonad (step 4).* **(E)** *By 24 hpf, PGCs have formed clusters between the 8th and 10th somite. The entire migration process takes approximately 20 hours. (dome stage - 24 hpf). Stages are a modification of the Kimmel stages described in Sprague* et al. *(2001).*

midgut invagination (Li *et al.,* 2003). Also, Torso signaling in flies may be analogous to the role of the receptor tyrosine kinase c-kit in PGC development in the mouse (see below) (Li *et al.,* 2003).

Zebrafish

Dead end was the first factor found to play a specific role in the initiation of PGC motility. *Dead end* encodes a novel RNA-binding protein and was identified in zebrafish in a large-scale expression screen for transcripts expressed in PGCs (Weidinger *et al.*, 2003). In zebrafish, PGCs are non-motile during the cleavage period and through most of the blastula period. They become motile at the dome stage (4.3 hrs. post-fertilization) and during gastrulation, the four PGC clusters found within the deep blastoderm move dorsally. Blocking *dead end* translation in zebrafish embryos blocked this initial migration step. At the dome stage, in *dead-end* depleted embryos, PGCs were no longer found in the deep blastoderm but were instead located in ectopic locations in the surface layer. These ectopic cells never assumed a motile phenotype, remained tightly adherent to each other and eventually died. *Dead-end* homologues have been identified in PGCs in *Xenopus*, chicken and mouse. However, in the mouse, *dead end* is expressed in PGCs after the migratory stages hence its function in PGC development may not be entirely conserved.

How do pgcs find their targets?

Mouse

Time lapse analysis of PGCs in tissue dissected from mouse embryos identified six distinct stages of PGC behavior (Fig. 1) including invasion of the endoderm (1), passive or active migration into the hindgut (2), random migration within the hindgut (3), migration from the gut to the genital ridges (4), clustering at the ridges (5) and cell death within midline structures (6). At E7.5, PGCs move through the primitive streak and into the definitive endoderm. Some PGCs also end up in the allantois and/or parietal endoderm. The fate of PGCs in extraembryonic structures remains uncertain, but PGCs in the definitive endoderm become incorporated into the hindgut and by E9.0, can be found moving within and around the cells of the hindgut epithelium (Molyneaux *et al.*, 2001). Morphogenetic movements of the embryo between E7.5 and E8.5 have made it difficult to observe PGC behavior during formation of the hindgut; hence it is unclear whether the colonization of the gut is an active or a passive process for PGCs. However, at E8.5, PGCs on the lip of the hindgut pocket have a rounded non-motile morphology suggesting that PGCs are passively incorporated into the gut and then re-initiate motility. At least two proteins regulate PGC behavior within the gut, E-cadherin and C-kit/Steel. Cells of the hindgut epithelium express the Ca++ dependent adhesion molecule E-cadherin; whereas PGCs within the gut do not (Bendel-Stenzel *et al.*, 2000). The lack of strong adhesive interactions may allow PGCs to move freely within the gut. PGCs upregulate *E-cadherin* expression upon leaving the gut and this transition may be involved in the emigration process. The Steel-c-kit interaction is required for PGCs to colonize, survive and/or migrate within the gut. Mutations in the tyrosine-kinase receptor, *Kit* and its membrane-bound ligand, *Steel Factor* cause a dramatic reduction in PGC numbers by E9.5 (Besmer *et al.*, 1993). Additionally, PGCs remaining in *Kit* mutant embryos are mislocalized. At E9.0, PGCs can normally be found scattered around the entire circumference of the gut. However, PGCs in W^e/W^e (the White extreme allele of *c-Kit*) embryos are confined to the ventral side of the gut and many can be found in the allantois and vitelline artery (Buehr *et al.*, 1993). PGCs express *c-Kit* and the cells of the hindgut express *Steel* and this interaction may be required for PGC motility as well as survival in this environment. Between E9.0-E9.5, PGCs exit from the dorsal aspect of the gut, split into two streams and migrate towards the positions of the developing genital ridges. It was proposed that PGC migration was not directional at E9.5 (Molyneaux *et al.*, 2001). This was based on time lapse movies of PGCs moving in tissue dissected from E9.5 embryos and cultured in medium supplemented with fetal calf serum. It was later found that directed migration of PGCs can occur at this stage in serum-free medium (Molyneaux *et al.*, 2003 and unpublished observations) suggesting that factors in fetal calf serum inhibit this process. This directed migration of PGCs appears to be controlled by secreted factors released by the genital ridge. Godin *et al.,* have shown that genital ridge conditioned medium can attract PGCs in culture (Godin *et al.*, 1990) and TGFβ can mimic this effect (Godin and Wylie, 1991), although it is still uncertain what role TGFβ plays in germ cell development *in vivo*. Both *in vivo* and *in vitro* data suggest that the peptide growth factor stromal derived factor 1 (SDF-1) acts as an attractant for PGCs (Molyneaux *et al.*, 2003). Addition of SDF-1 to cultured embryo slices blocked the directional movement of PGCs and implantation of SDF-1 coated beads into embryo slices caused local accumulation of PGCs. Also, mutations in *SDF-1* and it's receptor *CXCR4* caused reduced numbers of PGCs to reach the genital ridges (Ara *et al.*, 2003; Molyneaux *et al.*, 2003). However, based on *in situ* and immunostaining data, *SDF-1* is broadly expressed in the E9.5 mouse embryo hence it is remains unclear precisely how this factor controls PGC position *in vivo*. One possibility is that containment mechanisms prevent PGCs from moving towards other sites of *SDF-1* expression. Regions of the ECM may be non-permissive for PGC motility and this could confine PGCs to a narrowly defined track. PGCs lacking β1 integrin were unable to colonize the genital ridges demonstrating that interactions between the PGCs and the ECM are vital for this process (Anderson *et al.*, 1999). However, transplantation experiments have recently shown that ectopically placed PGCs still move towards the genital ridges suggesting that a large region of the dorsal body wall is permissive for directed migration (Molyneaux *et al.*, 2004). Interactions between PGCs may also be important for their homing behavior. PGCs emerge from the gut individually, but during migration, they interact with each other via long, thin processes forming a migrating network of cells (Gomperts *et al.*, 1994). This network becomes progressively aggregated into clusters of cells towards the end of migration. Antibodies against E-cadherin blocked the process of PGC aggregation in cultured embryo slices and prevented PGCs from forming tight clusters at the genital ridges (Bendel-Stenzel *et al.*, 2000). Likewise, neural crest cells have been shown to migrate as a network of cells. Junctions between neural-crest cells are thought to allow cell-cell communication during the migration process (Lo *et al.*, 1997). Loss of the gap-junction protein Cx43 interferes with neural crest migration (Xu *et al.*, 2001). PGCs were found to express *Cx43* during the migratory stages, but downregulate its expression after colonizing the genital ridge (Molyneaux *et al.*, 2004). E11.5 embryos lacking *Cx43* have reduced numbers of PGCs within the genital ridges (Juneja *et al.*, 1999). This suggests that *Cx43* controls some aspect of PGC behavior in the early embryo.

TABLE 1

COMPARISON OF SOME MOTILE CELL TYPES

Cell Type	Environment	Average Velocity (μm/h)	Reference
E10.5 PGCs	Tissue	10[a]	Molyneaux *et al.* (2001)
Tumor cells	Collagen gel	6-18	Entschladen and Zanker (2000)
Fibroblasts	Collagen gel	6-18	Entschladen and Zanker (2000)
Muscle cells	Tissue	37[b]	Knight *et al.* (2000)
Dendritic cells	Collagen gel	120-180	Entschladen and Zanker (2000)
Neural crest cells	Tissue	170	Kulesa *et al.* (2000)
Neutrophil granulocytes	Collagen gel	240	Entschladen and Zanker (2000)

[a] With spurts up to 30 μm/h. [b] With spurts up to 60 μm/h.

Regulated PGC survival also appears to be an important mechanism for controlling PGC position in the mouse embryo. At E10.5, lagging PGCs found within the hindgut mesentery and the midline of the body wall fragment and die during time lapse analysis (Molyneaux *et al.*, 2001). Loss of *Bax*, a pro-apoptotic member of the *Bcl-2* family blocked this process and resulted in the accumulation of ectopic PGCs in midline structures (Stallock *et al.*, 2003). Localized survival factors are likely to control PGC survival and hence, PGC position during the migratory stages. Many growth factors have been found to affect PGC survival in culture (reviewed in Donovan and de Miguel, 2003) and a cocktail of LIF, FGF2 and steel factor can immortalize PGCs (Matsui *et al.*, 1992, Resnick *et al.*, 1992). As discussed above, interactions between Steel and c-Kit are necessary to support PGC survival during the early migratory stages, but it is uncertain whether localized expression of Steel controls PGC behavior during directed migration. Also, the role of FGF2 and LIF in germ cell development *in vivo* remains unclear.

Drosophila

PGC migration in flies can be divided into four stages, internalization of the pole cells (1), emigration of PGCs from the gut (2), lateral migration of PGCs (3) and gonad coalescence (4) (Fig. 2). Torso activity is required for efficient incorporation of pole cells into the hindgut pocket (see above). Loosening of cell-cell contacts between the cells of the midgut epithelium allows PGCs to emigrate from the gut (Callaini *et al.*, 1995; Jaglarz and Howard, 1995) and a repulsive signal mediated via Wunen/Wunen2 forces PGCs away from the gut towards the overlying mesoderm (Starz-Gaiano *et al.*, 2001; Zhang *et al.*, 1997). *Wunen* and *wunen2* are *Drosophila* homologues of the mammalian *lipid phosphate phosphohydrolases (LPPs), which are* also called *phosphatidic acid phosphatases (PAPs).* There are three homologues in the mouse (*LPP1/Pap2a*, *LPP2/Pap2c* and *LPP3/Pap2b*). Only one of the mammalian *LPPs (LPP2)* has been targeted (Zhang *et al.*, 2000). These animals have no obvious defect in PGC development hence it remains uncertain whether *LPPs* have a conserved role in PGC development. Wunens/LPPs are responsible for the turnover of small lipids implicated in chemotaxis and cell survival. Hence, their repulsive activity could be mediated directly (by generation of a lipid breakdown product that acts as a PGC repellent) or indirectly (by degradation of a lipid attractant). PGCs may be attracted to lipid intermediates generated by activity of *HmG-CoA reductase* as loss of this enzyme was found to prevent PGCs from colonizing the gonads and ectopic expression of Hmg-CoA reductase was found to attract PGCs away from their normal migration route (Van Doren *et al.,* 1998). Germ cell migration in the mouse may also be controlled by lipid attractants. In a recent chip screen, migratory PGCs were found to express *Edg2*, a transcript encoding a G-protein coupled receptor for lysophosphatidic acid (Molyneaux *et al.*, 2004). Additional attractants have been proposed for PGCs in *Drosophila*. Misexpression of the secreted factors hedgehog (Deshpande *et al.*, 2001) and unpaired (Li *et al.*, 2003) have been shown to perturb PGC development. Gonad coalescence is the final step in PGC migration in flies. During this process, PGCs associate with the somatic cells of the gonad and form a tight cluster of cells in parasegment 10. Coalescence is thought to be mediated via the somatic gonadal precursors (SGPs) and is probably driven by cell-cell adhesive interactions. Mutations in *E-cadherin* and the recently identified FICL family member, *Foi* block gonad coalescence (Van Doren *et al.*, 2003). FICL (named after the founding members Foi, Iar1, Catsup and Livi) comprises a family of membrane proteins that share weak homology to the ZIP family of metal transporters. FICL proteins may form membrane channels. Expression of *FOI* in the SGPs may control expression of *E-cadherin* (Van Doren *et al.,* 2003).

Zebrafish

There are four stages of PGC migration in zebrafish. In this system, PGCs form as four clusters, which are randomly oriented with respect to the body axes of the embryo. Hence, germ cells in this system home in on their target zones from very divergent starting positions. This complicated migration process requires several intermediate targets (Weidinger *et al.*, 2002). PGCs move dorsally during gastrulation (1) and then form a line at the border between the head and trunk mesoderm (2). Next, PGCs at this border, move towards an intermediate target within the lateral plate mesoderm (3). Finally, PGCs leave this intermediate target and migrate posteriorly to colonize the gonad (4). Despite this complicated series of steps, PGC position appears to be controlled via a very simple molecular mechanism in Zebrafish. PGCs are attracted to their intermediate and final targets via SDF-1 (Doitsidou *et al.*, 2002; Knaut *et al.*, 2003). The expression of *SDF-1* in Zebrafish correlates much more strongly with PGC position than it does in the mouse. *SDF-1* is expressed in a stripe between the head and trunk mesoderm at the time PGCs accumulate at this position. Later, *SDF-1* expression becomes elevated in the lateral mesoderm surrounding the intermediate target. Finally, its expression recedes posteriorly as PGCs migrate towards the gonad. Morpholino antisense oligos were used to block translation of *SDF-1* and its receptor *CXCR4* and in both cases PGC migration was perturbed. Finally, both studies demonstrated that ectopic expression of *SDF-1* could lure germ cells away from their normal migration routes.

Conclusions

From analysis of PGC development in mouse, flies and zebrafish some general mechanisms can be proposed that control PGC behavior. Initiation of PGC motility is currently poorly understood and it may be controlled by species-specific mechanisms. In mouse, PGCs are formed via an inductive signal; whereas in flies and zebrafish, maternal components specify germ cell identity and possibly early germ cell behavior. Despite their different origins, the

early development of PGCs in flies and mouse is quite similar and the survival and early migration of PGCs in these systems require signaling via tyrosine kinase receptors (torso and c-kit respectively) (Besmer *et al.*, 1993; Li *et al.*, 2003). A tyrosine kinase receptor with a role similar to Torso/c-kit has yet to be identified in zebrafish. Initiation of PGC motility in zebrafish is controlled by the mRNA binding protein Dead end (Weidinger *et al.*, 2003). PGC guidance mechanisms have been well studied in all three species and require chemoattractants that signal via G-protein coupled receptors (SDF-1 and/or lipid-based attractants) (Van Doren *et al.*, 1998, Doitsidou *et al.*, 2002; Knaut *et al.*, 2003; Molyneaux *et al.*, 2003), cell-cell adhesion (E-cadherin and foi) (Bendel-Stenzel *et al.*, 2000; Van Doren *et al.*, 2003) and probably require specific interactions between PGCs and the ECM (β1 integrin) (Anderson *et al.*, 1999). There is currently no data from any system on how PGCs downregulate motility upon reaching their final target. To complicate this issue even further, zebrafish PGCs (Knaut *et al.*, 2002) and mouse PGCs may undergo periods of migration interspersed with stops at intermediate targets (lateral mesoderm in zebrafish and the lip of the hindgut pocket in mouse). The final loss of motility characteristics appears to correlate with the formation of strong PGC-PGC adhesive contacts in all three species. In the mouse, germ cell adhesion may be controlled via a PGC intrinsic clock as PGCs in culture cluster and become non-motile at approximately the same time as PGCs that have colonized the gonads (Gomperts *et al.*, 1994). Similarly, a PGC intrinsic clock has been proposed to control entry of PGCs into meiosis in the mouse (reviewed in McLaren, 2003). However, PGCs in ectopic locations in the mouse were found to retain motility and expression of early germ cell markers (Stallock *et al.,* 2003). This data makes a clock mechanism unlikely and instead suggests that interactions between the PGCs and the cells of the genital ridge stop PGCs from moving. Broad comparisons can be drawn between PGC behavior and the behavior of other migratory cell populations. Regulated cell-cell and cell-substrate adhesion is important for multiple migratory cell types. Also chemokines that signal via tyrosine kinase and/or G-protein coupled receptors provide guidance cues for many cell types. That said, PGC motility has some unique features. Germ cells are extremely slow moving cells (Table 1). In tissue they move with an average velocity of 10 μm/h and exhibit spurts up to 30 μm/h (Molyneaux *et al.*, 2001). PGCs in culture move more quickly (50 μm/h for mouse and from 12-144 μm/h for *Drosophila*) (Donovan *et al.*, 1987; Jaglarz and Howard, 1995). Slow migration interspersed with spurts may be characteristic of cells moving through tissue (Knight *et al.*, 2000) and may reflect physical restraints imposed by the environment through which the cells migrate. Other slow moving cell types (fibroblasts and tumor cells) form strong integrin-dependent attachments to the ECM and the duration of these attachments is believed to control their speed (reviewed in Entschladen and Zanker, 2000). PGC migration resembles that of other embryonic cell populations (migrating muscle cells and neural crest) and it shares some features proposed for tumor cells undergoing metastasis (reviewed in Bogenrieder and Herlyn, 2003). Downregulation of cell-cell adhesion and upregulation of cell-substratum adhesion is thought to initiate the process of metastasis. This is followed by the production of proteolytic enzymes (matrix metalloprotienases, ADAMs and uPA) in the tumor cells (or more commonly) in the stroma surrounding the tumor. Tumor cells then migrate into the vasculature and colonize other tissue via three proposed mechanisms, selective adhesion, selective survival and directed migration. In some species (avians) PGCs are carried to their target tissue via the vasculature (Meyer, 1964) and chick PGCs were found to accumulate in gonadal tissue grafted into ectopic locations (Kuwana and Rogulska, 1999) suggesting that this guidance process is active. PGCs in the mouse, flies and zebrafish take other routes to reach the gonads. Regardless of the pathway, selective adhesion, selective survival and directed migration are all important aspects of PGC development. Regulation of cell-cell adhesion is vital for PGC motility. *E-cadherin* is expressed shortly after PGCs form and is required for their formation. It is downregulated when PGCs are in the gut and is then upregulated again as PGCs colonize the genital ridges. *E-cadherin* expression has a similar bi-phasic pattern in metastatic cancer. Loss of *E-cadherin* is associated with tumor invasiveness whereas expression is regained within secondary tumors. Also, regulation of PGC survival is important for PGC homing behavior. PGCs in ectopic locations die via apoptosis which is probably initiated by a lack of localized survival factors (Stallock *et al.*, 2003). Finally, chemotaxis is vital for PGC development. SDF-1/CXCR4 directs PGC migration in mouse and zebrafish and this chemokine-receptor interaction has been shown to be involved in metastasis of many tumor cell-types (reviewed in Moore, 2001).

In conclusion, despite being slow and surrounded by a bewildering array of tissue environments, PGC migration is quite similar to other migratory processes. Further confirmation of this will emerge as new tools allow for germ cell specific targeting of components implicated in cell-migration in culture. Additionally, it will be quite interesting to see whether localized proteolysis of the ECM is involved in PGC migration.

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