Cell, Vol. 111, 647–659, November 27, 2002, Copyright ©2002 by Cell Press

Guidance of Primordial Germ Cell Migration by the Chemokine SDF-1

Maria Doitsidou,[1] Michal Reichman-Fried,[1] Jürg Stebler,[1] Marion Köprunner,[1] Julia Dörries,[1] Dirk Meyer,[2] Camila V. Esguerra,[3] TinChung Leung,[3] and Erez Raz[1,4]
[1]Germ Cell Development
Max Planck Institute for Biophysical Chemistry
Am Fassberg 11
37077 Göttingen
Germany
[2]Department for Developmental Biology
Institute for Biology I
University of Freiburg
Hauptstrasse 1
D-79104 Freiburg
Germany
[3]Mermaid Pharmaceuticals GmbH
Falkenried 88
20251 Hamburg
Germany

Summary

The signals directing primordial germ cell (PGC) migration in vertebrates are largely unknown. We demonstrate that *sdf*-1 mRNA is expressed in locations where PGCs are found and toward which they migrate in wild-type as well as in mutant embryos in which PGC migration is abnormal. Knocking down SDF-1 or its receptor CXCR4 results in severe defects in PGC migration. Specifically, PGCs that do not receive the SDF-1 signal exhibit lack of directional movement toward their target and arrive at ectopic positions within the embryo. Finally, we show that the PGCs can be attracted toward an ectopic source of the chemokine, strongly suggesting that this molecule provides a key directional cue for the PGCs.

Introduction

Directional cell migration during early development and adult life is crucial for the establishment of the embryonic body plan, organogenesis, and organ function. Identification of the molecular cues governing cell migration in vivo is of major importance for understanding development and for therapy in cases of diseases resulting from aberrant cell movement. A useful model system for studying the process of directional migration is that of primordial germ cells (PGCs). In most organisms, the formation of a functional gonad depends on the migration of PGCs from their site of specification to the position where the somatic part of the gonad develops (Starz-Gaiano and Lehmann, 2001; Wylie, 1999, 2000). Therefore, the PGCs have to travel long distances within the embryo, which itself undergoes complex processes of morphogenesis and differentiation.

PGC migration has been studied in chick, mouse, *Xenopus*, *Drosophila*, and zebrafish (reviewed in Starz-Gaiano and Lehmann, 2001; Wylie, 1999). These studies showed that while migrating, PGCs interact with different somatic structures in the developing embryo. These structures can carry the PGCs along, as part of general morphogenetic movements, repel them from certain regions of the embryo, and attract them toward intermediate and final targets (Deshpande et al., 2001; Godin et al., 1990; Jaglarz and Howard, 1995; Kuwana and Rogulska, 1999; Starz-Gaiano et al., 2001; van Doren et al., 1998; Weidinger et al., 1999, 2002; Zhang et al., 1997). Yet, despite the fact that this migration process has been studied for several decades in different model organisms, the molecular nature of the actual signals that direct PGCs toward their intermediate and final targets has remained unknown.

We have chosen to study PGC migration in zebrafish, benefiting from the fast, extrauterine embryonic development, the optical clarity, and the availability of mutant strains and genomic tools. Previously, we have described the migration path of zebrafish PGCs and analyzed the requirement for specific somatic structures in allowing proper migration to take place (Weidinger et al., 1999, 2002). As in other organisms, PGC migration in zebrafish can be divided into several steps that appear to rely on the integrity of specific somatic structures. Nevertheless, even though PGC migration in zebrafish has been described in great detail and the available data support the notion that secreted factors are involved in the process, the identity of such molecules was not known (Braat et al., 1999; Weidinger et al., 1999, 2002; Yoon et al., 1997).

In this work, we have identified the chemokine stromal-cell-derived-factor (SDF)-1a as a pivotal component guiding PGC migration. We show that *sdf*-1a is expressed in domains toward which the PGCs migrate and that alterations in its expression pattern lead to corresponding alterations in the migration route of the cells. Importantly, inhibition of the translation of SDF-1a, or its seven transmembrane G protein-coupled receptor CXCR4b, lead to misguided PGC migration. Taken together, these findings indicate that SDF-1a and its receptor CXCR4b are key molecules directing the PGCs toward their intermediate and final targets.

Results

SDF-1a Is Expressed in Regions toward Which the PGCs Migrate, and Its Receptor Is Expressed in the Migrating PGCs

In the course of a large-scale antisense oligonucleotide-mediated knockdown screen for genes important for PGC migration, we discovered that the seven-transmembrane G protein-coupled receptor CXCR4b (Chong et al., 2001) plays a critical role in the directional migration of PGCs (see below). With significant relevance to our work, it has been previously shown that CXCR4 and its ligand, the chemokine SDF-1, are essential for stem cell homing and mobilization (Peled et al., 1999b; Petit et

[4]Correspondence: eraz@gwdg.de

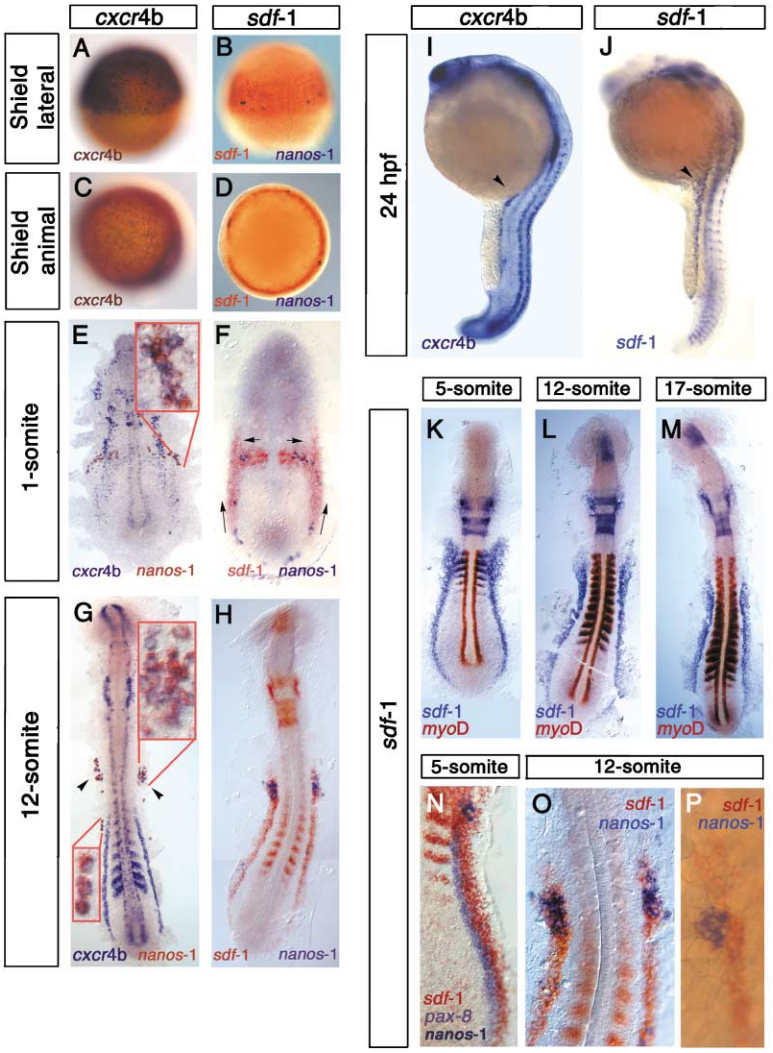

Figure 1. Expression Patterns of *cxcr*4b and *sdf*-1a

(A, C, E, and G) The distribution of the CXCR4b transcripts in wild-type embryos from gastrulation to mid-somitogenesis stages. (A and C) During early gastrulation the mRNA of the receptor is uniformly distributed. (E and G) Tissue-specific pattern is evident during somitogenesis stages when the PGCs coexpress *cxcr*4b and a PGC-specific RNA, *nanos*-1.

(I) The expression of the receptor persists in the PGCs after they arrive at the region of the gonad (arrowhead).

(B, D, F, and H) The expression pattern of *sdf*-1a mRNA relative to the position of the PGCs. (B and D) Expression of *sdf*-1 mRNA during early gastrulation. (F) *sdf*-1a expression during early somitogenesis. Depending on their position at this stage, the PGCs migrate laterally or anteriorly (arrows). (H) During somitogenesis, *sdf*-1a is expressed along the border of the trunk mesoderm, in the somites, and in specific domains in the head.

(J) By the end of the first day of development, *sdf*-1a is expressed in the somites, in the brain, and along the pronephric duct. In the region where the gonad develops, a larger number of cells express *sdf*-1a (arrowhead).

(K–M) Alterations in *sdf*-1a expression during somitogenesis.

(N) *sdf*-1a is expressed in the lateral plate mesoderm of the trunk lateral to the domain expressing *pax*8.

(O and P) Two examples showing that the position of the PGC clusters overlaps with the anterior border of high *sdf*-1a expression along the trunk mesoderm.

al., 2002), leuckocyte traffic (Aiuti et al., 1997; Baggiolini, 1998; Bleul et al., 1996a; Zou et al., 1998), neuronal cell migration (Zou et al., 1998), nerve growth cone guidance (Xiang et al., 2002), and determination of metastatic destination of tumor cells (Muller et al., 2001; Murphy, 2001). To establish direct relationships between this receptor and PGC migration, we first analyzed the RNA expression patterns of this molecule and its ligand in relation to the position of the PGCs at different steps of their migration.

During early gastrulation stages, at the onset of PGC migration, the *cxcr*4b RNA is uniformly distributed within the embryo and is thus expressed also in the migrating PGCs (Figures 1A and 1C). By the end of gastrulation, the RNA is expressed in the PGCs that align along the lateral plate mesoderm of the trunk and the border between the head and the trunk mesoderm (insert in Figure 1E). During somitogenesis stages, the PGC-specific expression of the receptor is evident in the clustered PGCs (arrowheads and insert in Figure 1G) as well as in cells migrating toward the clusters (small insert in Figure 1G). At 24 hr postfertilization (hpf), the PGCs, found at the anterior part of the yolk extension, strongly express *cxcr*4b (arrowhead in Figure 1I). Thus, PGCs express *cxcr*4b at the time when they migrate toward their clustering position and continue expressing it after arriving at the site where the gonad will develop.

We next sought to determine whether the CXCR4b ligand expression pattern is consistent with its putative role in PGC migration. CXCR4 has been shown to bind a single molecule, SDF-1, a CXC subfamily chemokine (Baggiolini et al., 1997; Bleul et al., 1996a, 1996b), and this ligand appears to interact exclusively with the CXCR4 receptor. We therefore cloned the zebrafish *sdf*-1a gene, which encodes a protein that exhibits a high level of conservation with the mouse SDF-1 protein (43% identity at the amino acid level; see Supplemental Figure S1 at http://www.cell.com/cgi/content/full/111/5/647/DC1).

Analysis of the *sdf*-1a spatio-temporal expression pattern revealed a remarkable correlation with the PGC localization at different developmental stages. Specifically, at the onset of gastrulation, *sdf*-1a is expressed around the blastoderm margin with the exception of the dorsal-most aspect of the embryo, coinciding with the position where the PGCs are found at this stage (Figures 1B and 1D; Weidinger et al., 1999; Yoon et al., 1997). During gastrulation and early somitogenesis, the PGCs align along the lateral plate mesoderm of the trunk and at the border between the head and trunk mesoderm

(Weidinger et al., 1999), and at this stage too, the PGCs are found mainly within domains of *sdf*-1a expression (Figure 1F). At later stages, when the PGCs cluster at the bilateral positions, strong expression of the ligand is observed in the lateral plate mesoderm where the PGCs are located (Figures 1H and 1N). During the last step of migration, the PGCs migrate posteriorly to the position where the gonad develops. Consistently, a high level of *sdf*-1a RNA is detected at this position as well (Figure 1J). In conclusion, we find a strong correlation between the position of the PGCs at different stages of their migration and the tissues expressing high levels of *sdf*-1a transcripts.

If *sdf*-1a indeed acts as an attractant for the PGCs, then one would expect that the dynamics of its expression pattern would mirror the migration pattern of these cells. To test this notion, we looked more closely at the expression pattern of the ligand at several stages relative to concurring steps of PGC migration.

During early somitogenesis stages, the PGCs which align along the tissues that express *sdf*-1a in the trunk (steps IIIa and IIIb in Weidinger et al., 1999; Figure 1F) migrate toward the clustering positions (steps IV and V in Weidinger et al., 1999; Figure 1F, arrows). Consistent with a role for SDF-1a in directing this migration, we find that its RNA is strongly expressed in a large number of cells at the clustering position (Figure 1K). In addition, in parallel to PGC migration toward the lateral clustering sites (Figure 1F, horizontal arrows pointing left and right) a reduction in the expression of the ligand at the first somite is observed while the expression in lateral positions at the same anteroposterior level is enhanced (compare Figures 1F and 1K). This presumably leads to cell migration toward the adjacent lateral positions expressing high levels of *sdf*-1a.

We then investigated another step of PGC migration where the PGCs migrate posteriorly away from the position where they initially cluster; that is, from the level of the first three somites toward the anteroposterior level of the eighth to tenth somites (step VI in Weidinger et al., 1999, 2002). At this stage too, the expression of the ligand is in perfect agreement with its proposed role in directing the PGCs' route of migration. Here, *sdf*-1a expression in the lateral plate mesoderm progressively recedes from the position of the first somites toward more posterior somites in dynamics identical to that observed for the posteriorly migrating PGCs (Figures 1K–1M; Weidinger et al., 1999). This striking correlation between the expression pattern of the ligand and the position of the PGCs can be visualized in double stainings where the anterior border of *sdf*-1a coincides with the position of the migrating cell cluster (Figure 1O). This point is clearly demonstrated in Figure 1P where residual *sdf*-1a expression can still be detected anterior to the PGC cluster that has migrated toward the region expressing a higher level of the ligand.

Alterations in the Expression Pattern of *sdf*-1a in Mutant Embryos Are Paralleled by Abnormal PGC Migration

Based on the analysis of PGC migration in mutant embryos that exhibit specific differentiation defects, we have previously demonstrated that normal development of certain structures within the embryo is essential for proper PGC migration (Weidinger et al., 1999, 2002). Therefore, we set out to reexamine some of those mutants in an attempt to correlate the PGC migration defects with possible altered expression pattern of the *sdf*-1a gene in these embryos.

The *spadetail* (*spt*) gene, encoding a T box protein, is important for normal development of trunk paraxial mesoderm (Griffin et al., 1998). In *spt* mutant embryos, the PGCs do not align along the border between the head and the trunk mesoderm (step IIIa in Weidinger et al., 1999), and as a result, some PGCs arrive at anterior positions where they eventually cluster (Figure 2D; Weidinger et al., 1999). Indeed, the abnormal PGC migration pattern observed in *spt* mutant embryos coincides with the altered expression pattern of *sdf*-1a. Specifically, at the stage when the PGCs normally align between the head and the trunk mesoderm, *sdf*-1a is not expressed along this border as it is in wild-type embryos, thus allowing some cells to reach anterior locations in the embryo (arrowheads in Figure 2B). Consistent with the proposed role for SDF-1a as an attractant for the PGCs, while clustering occurs at the normal positions, the ectopic cells that arrive at the very anterior positions are concentrated in a region where high levels of *sdf*-1a mRNA can be detected (arrowhead in Figure 2D).

As we previously described, in *spadetail* and *notail* (*ntl*) (*spt;ntl*) double mutant embryos, differentiation of the somatic tissue at the clustering position is defective as determined by marker gene expression, and consequently, no PGC clustering occurs at the normal position (Weidinger et al., 2002). In agreement with the proposed role for SDF-1a in attracting PGCs toward the clustering point, very low levels of *sdf*-1a RNA are detected at this position in *spt;ntl* mutant embryos (compare Figure 2E and Figure 2C).

Loss of function of the zebrafish chordin homolog *chordino,* a BMP antagonist, leads to an expansion of ventral-posterior tissues (Hammerschmidt et al., 1996; Schulte-Merker et al., 1997). In *chordino* (*din*) mutant embryos, starting at early somitogenesis stages, more PGCs are found in the posterior positions (Weidinger et al., 1999). Unlike the situation in wild-type embryos, these cells never leave this region and are found in the tail of 24-hour-old mutant embryos. The distribution of *sdf*-1a provides the basis for the *din* PGC phenotype. Here, instead of the posterior gap in *sdf*-1a expression observed in wild-type embryos (Figures 2A and 2C), *sdf*-1a is strongly expressed in the posterior-most part of the mutant embryos (Figure 2F). This result is therefore consistent with the idea that in wild-type embryos PGCs migrate toward more anterior positions where the ligand is expressed and eventually arrive at the clustering position. The strong expression of the ligand in posterior tissues of *din* mutant embryos therefore prevents the anterior migration of PGCs and consequently they remain in the tail (Figures 2F and 2G). While we do not know the basis for the ectopic posterior *sdf*-1a expression, a contributing factor to this phenomena could be the abnormal cell movements in this mutant (Myers et al., 2002). Specifically, the increase in nonconverging ventral cells in this mutant could interfere with the elimination of the ventral *sdf*-1a expression.

The last step in PGC migration that we have analyzed in mutant embryos is the migration of the cluster from the original clustering site at the level of the first somites

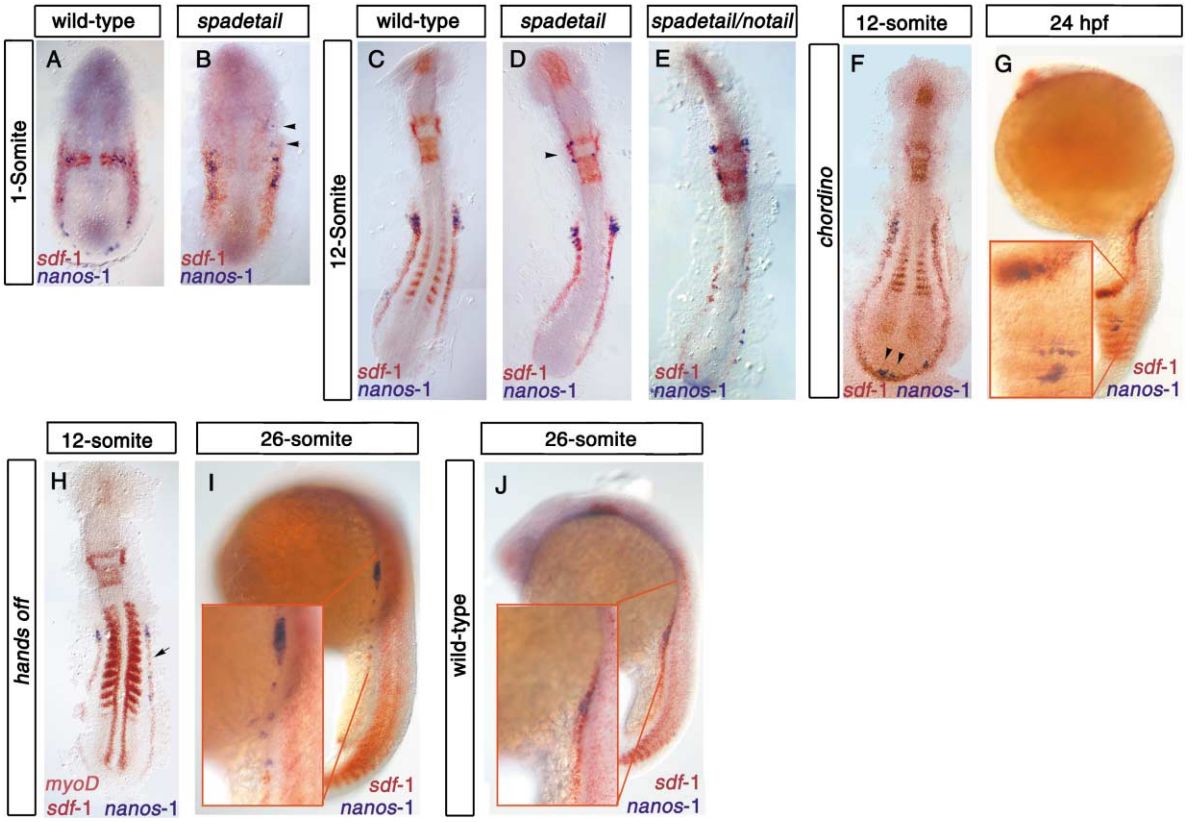

Figure 2. A Correlation between Specific Alterations in the Expression Pattern of *sdf*-1a in Mutant Embryos and the Direction of PGC Migration

(A) In wild-type embryos at 1 somite stage, the PGCs are found along the borders of the trunk mesoderm where *sdf*-1a is expressed.

(B) In *spadetail* embryos, the expression of *sdf*-1a along the border between the head and the trunk mesoderm is eliminated, thereby allowing some cells to reach the head (arrowheads).

(C) In wild-type embryos at 12 somite stage, most of the PGCs are found in two cell clusters around the level of the fourth somite.

(D) At the same stage in *spadetail* embryos, PGCs that arrived at the head cluster ectopically in a region that exhibits a high level of *sdf*-1a expression.

(E) In *spadetail/notail* double mutants, *sdf*-1a expression in the lateral plate mesoderm of the trunk is severely reduced, and consequently no clustering occurs at the normal position.

(F) Posterior expression of *sdf*-1a in *chordino* mutant embryos (arrowheads point at posterior PGCs).

(G) In 24-hour-old *chordino* embryos, the ectopic cells are concentrated in regions expressing high levels of the ligand.

(H) In *hands off* mutant embryos, the expression of *sdf*-1a at the trunk lateral plate mesoderm is reduced (arrow).

(I) During subsequent somitogenesis stages in *hands off* mutants, *sdf*-1a expression continues to decline and a dispersed anterior PGC cluster is observed.

(J) *sdf*-1a in a 24-hour-old wild-type embryo relative to the PGCs.

toward a more posterior position as well as anterior migration of trailing posterior cells toward the region where the gonad develops. We have previously found that in *hands off* mutant embryos, which carry a mutation in the bHLH transcription factor *hand*2, the migration of the PGC cluster posteriorly is disrupted (Weidinger et al., 2002; Yelon et al., 2000). We therefore examined the expression of *sdf*-1a in this mutant. From the 12 somite stage and onward, the expression domain of *sdf*-1a becomes increasingly restricted in *hands off* mutant embryos (compare Figure 2H, arrow, with Figure 2C), but this has yet no effect on the cells that cluster normally and align along the line defined by the expression pattern of the gene. It is only at later stages that the level of *sdf*-1a is reduced to a degree that leads to abnormal migration (compare Figure 2I with Figure 2J). The severe decline in the level of *sdf*-1a mRNA in the region where the gonad should develop presumably results in inability of the cluster to migrate posteriorly and of trailing posterior cells to migrate anteriorly.

In conclusion, the expression analysis described above in wild-type and mutant embryos shows a remarkable correlation between the positions at which *sdf*-1a is expressed at higher levels and positions toward which the PGCs migrate during the first 24 hr of zebrafish embryonic development. To examine more directly whether CXCR4b and SDF-1a are functionally essential for normal PGC migration, we studied the effect of knocking down the activity of each one of them on the migration process.

Knockdown of the Activity of CXCR4b and of the SDF-1a Ligand Result in Severe PGC Migration Defects

To determine how a reduction in the amount of CXCR4b and the chemokine SDF-1a would affect PGC migration,

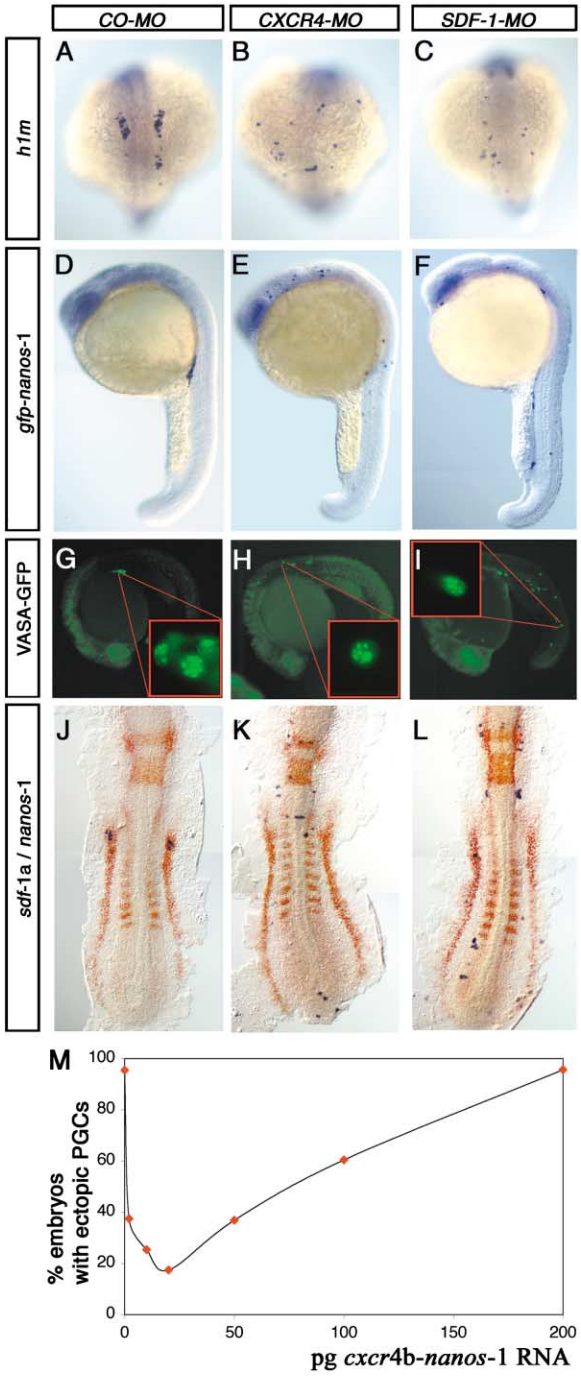

Figure 3. Knockdown of CXCR4b and SDF-1a by Morpholinos Disrupts PGC Migration without Affecting Their Identity

(A–C) *h1m* RNA is normally expressed in the PGC clusters of 14 somite stage control embryos as well as in ectopic PGCs in embryos injected with a morpholino directed against *cxcr*4b or *sdf*-1a.

(D–F) PGCs in embryos injected with the control morpholino and *gfp-nanos*-1 mRNA specifically protect the injected mRNA, similar to ectopic PGCs in embryos injected with morpholino directed against *cxcr*4b or *sdf*-1a.

(G–I) A GFP-Vasa fusion protein is subcellularly localized to perinuclear granules in the PGCs of control embryos similar to the localization in ectopic cells in embryos injected with morpholino directed against *cxcr*4b, or *sdf*-1a.

(J–L) Normal somatic development in embryos injected with the control morpholino or morpholinos against *cxcr*4b or *sdf*-1a as revealed by the expression of *sdf*-1a mRNA.

(M) The PGC migration phenotype in embryos injected with morpholinos against the CXCR4b is reversed by specific expression of the receptor in the PGCs.

we injected embryos with morpholino antisense oligonucleotides (Nasevicius and Ekker, 2000) directed against either one of these genes and examined the resulting PGC distribution by in situ hybridization using different PGC markers. Injection of these morpholino oligonucleotides (two different oligonucleotides were used for each gene with similar results) resulted in a dramatic migration phenotype where at mid somitogensis stages most of the PGCs were randomly scattered in the embryo (Figures 3A–3C).

Analysis of the morpholino-injected embryos using different markers demonstrates that the abnormal phenotype reflects primarily migration rather than a PGC or somatic differentiation defect. First, the ectopic PGCs show normal expression of a PGC RNA marker, the zebrafish *h*1*m* RNA (Figures 3A–3C; Müller et al., 2002). The regulation of *h*1*m* RNA tissue-specific expression is unique considering that it is not incorporated into the zebrafish germ plasm and is not subjected to "degradation-protection" regulation as seen in other PGC-specific genes (Köprunner et al., 2001; Müller et al., 2002; Wolke et al., 2002). At the same time, the PGCs exhibit normal expression of RNA molecules like *nanos*-1 (Figures 3J–3L) and *vasa* (data not shown) that characteristically reside in the germ plasm. Further, when a fusion RNA between GFP and the 3′ untranslated region (3′-UTR) of the *nanos*-1 gene was coinjected with the antisense oligonucleotides, the abnormally migrating cells were capable of specifically protecting this RNA from degradation, thereby obeying to the degradation-protection rule of regulation as do the PGCs in control embryos (Figures 3D–3F). Last, the knocked down PGCs contained perinuclear granules, a characteristic feature of germ cells (Eddy, 1974; Eddy and Ito, 1971; Hay et al., 1988; Knaut et al., 2000; Schisa et al., 2001; Strome and Wood, 1982). This point was demonstrated by expressing a Vasa-GFP fusion protein in the PGCs and observing proper subcellular localization to the perinuclear granules whether the cells arrived at the gonad region (Figure 3G; Wolke et al., 2002) or were found at ectopic positions (Figures 3H and 3I).

While the reduction in the activity of SDF-1a or CXCR4b results in severe migration defects, it is formally possible that this effect is indirect, resulting from defects in the differentiation of somatic tissues that normally support PGC migration. Several lines of evidence argue against this possibility. First, despite the adverse effect on PGC migration, the morphology and development of the embryos (e.g., Figures 3A–3I) appeared similar to embryos injected with the control morpholino oligonucleotides. To substantiate this point more rigorously, we analyzed the injected embryos using different molecular markers expressed in somatic tissues. Under the experimental conditions that affected PGC migration, we could not detect any obvious defects reflected by alteration in gene expression. For example, the lateral border of

the mesoderm, the clustering point at the anterior trunk, the developing somites, and specific structures in the head were expressing *sdf*-1a in a pattern similar to that in control embryos (Figures 3J–3L). Using other markers for somatic structures such as *Myo*D (somites and adaxial cells), *Pax*8, and *Pax*2.1 (midhindbrain boundary and pronephros), we did not detect any obvious abnormalities in the injected embryos (data not shown).

To prove that the activity of CXCR4b is indeed required specifically in the PGCs, we coinjected embryos with a morpholino against the receptor and different amounts of *cxcr*4b-*nanos*-1 RNA lacking the morpholino binding site. This way we targeted specific expression of CXCR4b to the PGCs in embryos globally depleted of the protein (see Experimental Procedures and Köprunner et al., 2001). Indeed, small amounts of this RNA were sufficient to reverse the morpholino-induced phenotype, allowing all of the PGCs to arrive at the region of the gonad in most of the embryos (Figure 3M). Interestingly, when high amounts of the RNA were injected, PGC migration appeared abnormal, presumably due to basal level of signaling of the overexpressed receptor.

Taken together, these experiments show that CXCR4b activity in PGCs is important for their directional migration. This finding allows us now to follow the chemokine-dependent migration in live embryos and to evaluate the effects of loss of this signaling pathway on cell behavior.

Alterations in the Level of the SDF-1 Signaling Interferes with Directional PGC Migration

To account for the abnormal distribution of the cells in which the chemokine signaling level was altered, we first analyzed PGC migration in knocked down embryos at low magnification using time-lapse microscopy. A detailed description of the effects of this treatment on the dynamics of cell behavior and cytoskeletal architecture will be presented elsewhere (M.R-F., M.D., and E.R., unpublished), but a clear phenotype is evident already at this level of resolution. The PGCs in which the chemokine signaling was knocked down were capable of migrating relative to their neighboring somatic cells (Figures 4A–4C). However, in sharp contrast to the directed migration of wild-type PGCs toward the borders of the trunk mesoderm, the knocked down cells exhibit irregular nondirectional movement (Figures 4A–4C and see Supplemental Movies S1–S3 at http://www.cell.com/cgi/content/full/111/5/647/DC1). As a result, the cells remained in regions that they normally vacate, such as the segmental plate and the notochord, and exhibit no specific anteroposterior positioning.

To observe the cellular morphology and movement at a higher resolution, individual cells whose membrane was labeled with GFP were examined at higher magnification. Here again, a clear difference was observed between the knocked down PGCs and the control. Although PGCs depleted of CXCR4b generated lamellipodia, in striking contrast to control cells, these cellular protrusions extended in multiple directions, leading to a nonpolarized appearance of the cells (Figures 5A and 5B and see Supplemental Movies S5 and S6 at http://www.cell.com/cgi/content/full/111/5/647/DC1). Similar apolar cellular behavior was observed when the SDF-1a level was reduced (data not shown).

The Chemokine SDF-1 Directs PGC Migration

The migration phenotype described above is consistent with the notion that SDF-1a functions by introducing a bias in the direction of cell migration, leading the PGCs toward the source of the ligand. A prediction of this proposal is that expression of the ligand at high levels within the PGCs themselves would interfere with their ability to detect the endogenous signal. Indeed, overexpression of the ligand in the PGCs results in pronounced migration defects in otherwise wild-type embryos (Figure 4D and see Supplemental Movie S4 at http://www.cell.com/cgi/content/full/111/5/647/DC1). Specifically, PGCs overexpressing the ligand exhibit limited migration relative to their somatic neighbors and form cellular protrusions all around the circumference of the cell. As a result, the PGCs overexpressing SDF-1a are randomly distributed within the embryo (Figure 4D). Interestingly, the morphology of the cellular extensions in this case was different from that of the knocked down cells. PGCs overexpressing the SDF-1a develop longer and thinner cellular extensions (Figure 5C and Supplemental Movie S7), suggesting that high level of CXCR4b activation promotes the stabilization of these protrusions.

In view of these results, coupled with the strong correlation between the expression pattern of SDF-1a and the direction of PGC migration, it is reasonable to consider SDF-1a as the molecule that attracts the PGCs toward their intermediate and final targets. To examine this assumption, we designed experiments in which the endogenous SDF-1a activity was inhibited and tested the ability of exogenously introduced SDF-1a to attract the PGCs to ectopic positions (Figure 6).

To overcome the activity of the endogenous SDF-1a, we inhibited its translation by injecting specific anti-*sdf*-1a morpholinos into 1 cell stage embryos as well as mRNA encoding EYFP fused to the *nanos*-1 3′-UTR for the purpose of labeling the PGCs. We then raised the embryos to the 32 cell stage and injected cells with morpholino-resistant *sdf*-1a mRNA and with mRNA encoding the ECFP protein, thereby generating an ECFP- and SDF-1a-expressing clone of cells. The ability of the EYFP-labeled PGCs to arrive at areas in the embryo expressing high level of SDF-1a (marked by ECFP expression) was monitored and compared to that in control experiments. Indeed, while the cells exhibited random distribution with respect to the ECFP expression in the control experiment in which SDF-1a was knocked down (Figures 6B and 6E), they specifically arrived at regions in which SDF-1a was expressed (Figures 6C and 6E). Furthermore, the ability of the PGCs to respond to the exogenously supplied SDF-1a depended on the expression of CXCR4b. Injection of anti-*cxcr*4b antisense oligonucleotides at the 1 cell stage interfered with the directed PGC migration toward the SDF-1a source (Figures 6D and 6E).

Discussion

Starting their migration from random positions within the embryo, zebrafish PGCs execute a number of migration steps, passing through several intermediate targets on their way toward two clustering positions on either side

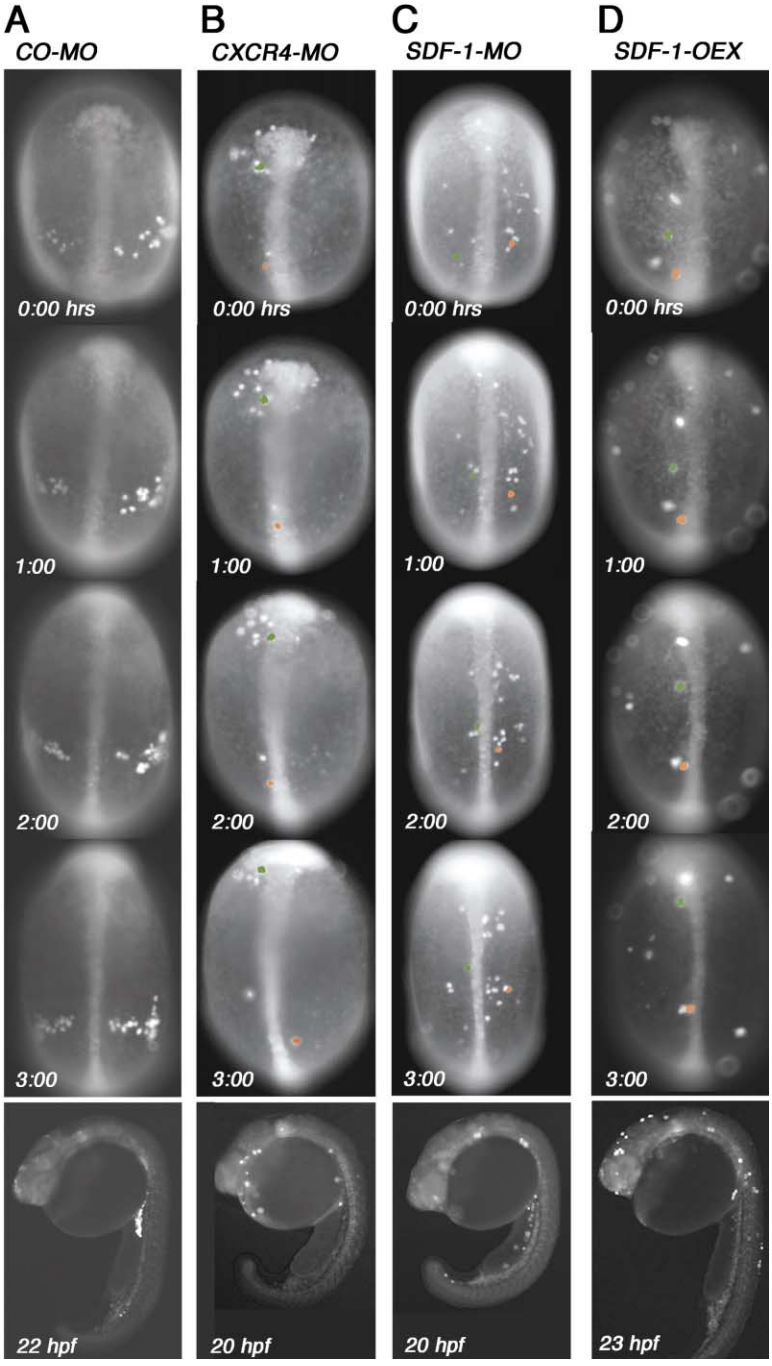

Figure 4. Alterations in the Level of the SDF-1a Signaling in the PGCs Result in Nondirected Cell Migration as Revealed by Time Lapse Analysis

(A) In embryos injected with control morpholino, the PGCs migrate toward the border between the head and the trunk mesoderm.

(B and C) Inhibiting the translation of the *cxcr*4b or *sdf*-1a mRNA results in random migration with cells arriving at ectopic positions.

(D) Overexpression of SDF-1a in the PGCs leads to a similar phenotype. In each image, four time points are shown, representing 3 hr of development starting at late gastrulation (80% epiboly). In each image, the filmed embryo is shown at the end of the first day of development. In the experimental embryos, two individual cells were artificially colored in red and green (B–D). The filmed embryos were injected with *gfp-nanos*-1 mRNA, leading to GFP expression in their PGCs, and express GFP under the control of the *goosecoid* promoter, which labels their axial mesoderm (see corresponding Supplemental Movies S1–S4 at http://www.cell.com/cgi/content/full/111/5/647/DC1).

of the body axis in the region where the gonad will be formed. In this study, we provide strong evidence for the involvement of the chemokine SDF-1a in providing directional information to the migrating cells.

The expression pattern of SDF-1a perfectly overlaps with the positions toward which the PGCs migrate, and alterations in the expression pattern of SDF-1a in mutant embryos lead to a predictable migration phenotype. Furthermore, reduction in the level of SDF-1a or of its putative receptor CXCR4b results in migration defects. Last, we show that migration of the PGCs can be redirected toward sites of ectopically expressed SDF-1a. Our interpretation of these findings is that SDF-1a acts as a natural chemoattractant for zebrafish PGCs. A formal possibility is that SDF-1a is not the actual attractant, but rather that the chemokine signal leads to the production of another molecule that acts as the actual cue for the PGCs. We consider the idea of such a relay mechanism unlikely. First, consistent with our model, in a wide range of experimental systems, SDF-1 has been shown to act as the chemotactic factor per se, directing cells toward their target. Second, we observe exceedingly tight temporal relationships between the RNA expression of SDF-1a and the cellular response, a finding

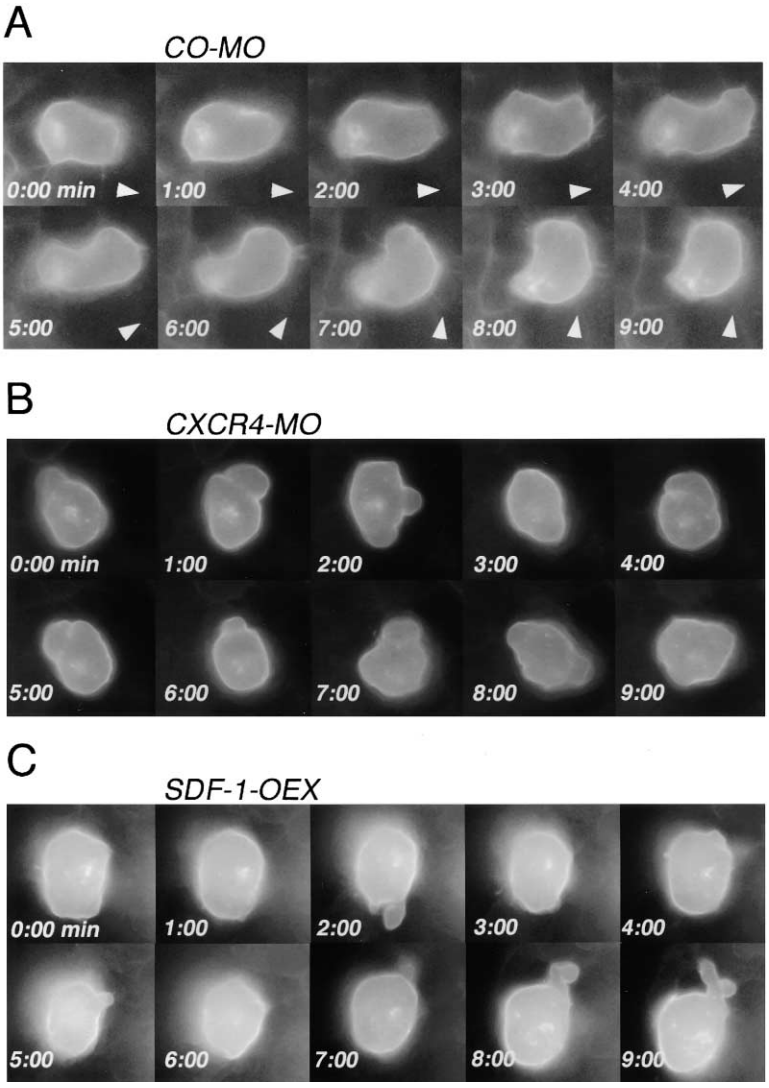

Figure 5. Alterations in the Level of the SDF-1a Signaling Affects PGC Polarity and Cellular Morphology as Revealed by High-Magnification Time Lapse Analysis

(A) Migrating PGCs are characteristically polarized, exhibiting protrusions that are extended primarily in the direction of their movement (indicated by arrowheads) and are normally elongated in shape.

(B) Inhibition of *cxcr4*b translation leads to apolar cell morphology.

(C) Overexpression of SDF-1a in the PGCs similarly leads to loss of polarity with cellular extensions that appear longer and more elaborate. In each image, snapshots from a time lapse video recorded over 9 min (see Supplemental Movies S5–S7 at http://www.cell.com/cgi/content/full/111/5/647.DC1).

that renders a relay mechanism less likely. Third, our ability to redirect cells toward ectopic positions by applying SDF-1a to these positions and to misguide the PGCs by expressing the ligand within them is less compatible with a relay mechanism in which a vast array of cell types would be required to be capable of mediating this process. Last, restoring the activity of CXCR4b specifically in the PGCs was sufficient to reverse the abnormal migration phenotype in embryos globally depleted for the receptor, providing further support to the notion that the activity of the receptor is required in the migrating cells themselves.

The PGCs appear to be very sensitive to changes in the level of SDF-1a protein, which is reflected by the tight correlation between high *sdf*-1a expression and the position of the cells or their direction of migration. This high sensitivity of the cells to small changes in the attractant level is consistent with studies revealing particularly high potency of SDF-1-mediated lymphocyte chemoattraction relative to other chemokines (Bleul et al., 1996a). Such high sensitivity of the responding cells to SDF-1a allows precise migration of PGCs toward their target despite complex and sometimes opposing morphogenetic movements that occur at the same time during development (Weidinger et al., 1999). The robust manner in which SDF-1a guides PGCs was demonstrated here by its ability to attract PGCs to ectopic locations. Conversely, PGCs transplanted into the animal pole of the zebrafish blastula, a region normally devoid of PGCs, are able to arrive at the correct position by the end of the first day of development (Ciruna et al., 2002), notwithstanding the ectopic origin of migration and the coinciding complicated gastrulation movements in the embryo. Analogously, the potency of SDF-1 enables efficient stem cell homing and mobilization (Peled et al., 1999b; Petit et al., 2002) and leuckocyte traffic (Aiuti et al., 1997; Baggiolini, 1998; Bleul et al., 1996a; Zou et al., 1998). It will be interesting to determine if the analogy between zebrafish PGC migration and leukocyte trafficking can be extended to other levels such as regulated cell adhesion, which plays an important role in targeting of leukocytes (Campbell et al., 1998; Mazo et al., 1998; Peled et al., 1999a; Springer, 1994).

Unlike other chemokines whose function and expres-

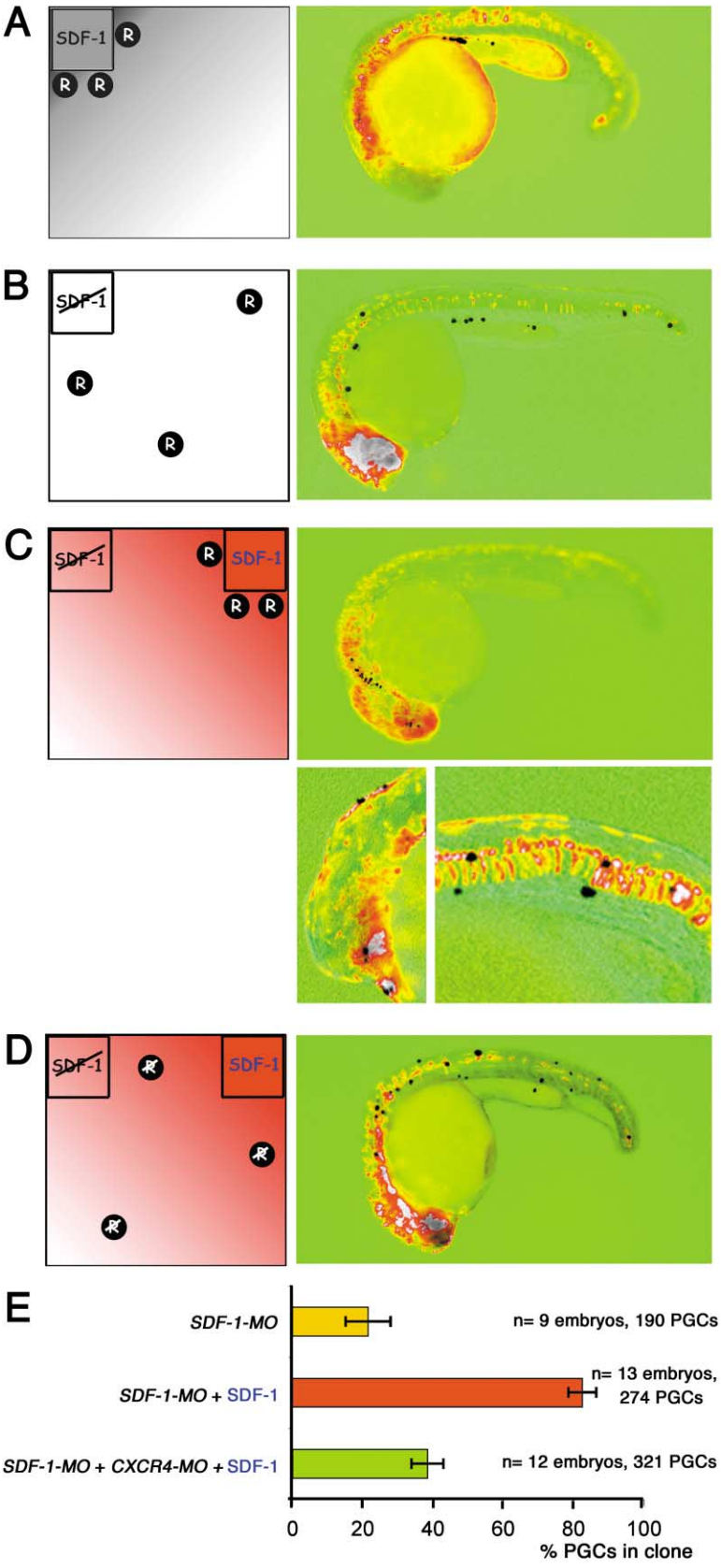

Figure 6. PGCs Are Attracted toward an Ectopic Source of SDF-1a

(A) An embryo injected with control morpholino and *eyfp-nanos*-1 RNA at 1 cell stage and *ecfp-globin* at 32 cell stage. The EYFP-labeled PGCs (black) are found at the normal position.

(B) An embryo injected at the 1 cell stage with *SDF-1a-MO* and *eyfp-nanos*-1 mRNA, followed by a later injection of ECFP mRNA into cells at the 32 cell stage. The PGCs are randomly distributed with respect to the ECFP-expressing clone of cells (pseudocolors of red and white represent high and higher levels of ECFP expression, respectively).

(C) Top: an embryo injected at the 1 cell stage with *SDF*-1a-*MO* and *eyfp-nanos*-1 mRNA followed by injection of cells at the 32 cell stage with ECFP and morpholino-resistant *sdf*-1a mRNA. The PGCs are preferentially found in close proximity to the ECFP- and SDF-1a-expressing cells (pseudocolors represent the levels of ECFP- and therefore SDF-1a expression). Bottom: high-magnification pictures of the head region (left) and the notochord (right) of a different embryo treated as above.

(D) An embryo injected at the 1 cell stage with *SDF*-1a-*MO*, *CXCR4b-MO*, and *eyfp-nanos*-1 mRNA followed by injection of cells at the 32 cell stage with ECFP and morpholino-resistant *sdf*-1a mRNA. In these embryos, many PGCs are randomly distributed relative to the ECFP-expressing clone of cells.

(E) Quantitative analysis of the experimental results. In embryos treated as described in (B), approximately 20% of the somatic cells exhibit ECFP expression, and a similar proportion (yellow bar, 21.5% $\pm$ 6.4%) of PGCs were found within the clone. In embryos treated as described in (C), the PGCs are preferentially found within the ECFP/SDF-1a-expressing clone (red bar, 82.5% $\pm$ 3.9%, $p < 0.001$ in t test). The attraction of PGCs toward the SDF-1a clone is inhibited when CXCR4b is knocked down (green bar, 38.4% $\pm$ 4.5%, $p < 0.001$ in t test).

sion are centered around their role in leukocyte trafficking, both SDF-1 and its receptor CXCR4 were found to be expressed in a wide variety of cell types and tissues. Consistently, mice lacking either the SDF-1 or its receptor exhibit defects that extend beyond the immune system, revealing roles for this chemokine in the genesis of the circulatory and central nervous systems (Nagasawa et al., 1996; Tachibana et al., 1998; Zou et al., 1998). Our finding that PGC migration depends on directional signaling by SDF-1a allows us to model these processes in a more accessible system at a higher resolution. Furthermore, although embryos in which SDF-1a or CXCR4b activity was knocked down did not exhibit increased lethality or obvious somatic defects, given the widespread expression of these genes, a thorough analysis of behavior of other cell types may reveal additional requirements for SDF-1a and its receptor in zebrafish.

Our initial observations indicate that the activity of the chemokine signaling pathway is not required for the formation of the cellular extensions characteristic of migrating cells, nor is it essential for the ability of the cell to move. Rather, the most prominent feature of cells in which SDF-1a function is inhibited is the loss of stable cell polarity and directed cell migration. Consistent with our findings, G protein-linked signaling has been shown to polarize cells such as neutrophils that respond to chemoattractants by polarizing the distribution of PIP_3 and directed migration (Servant et al., 2000). The most detailed understanding of establishment of cell polarity in chemotaxis was obtained in *Dictyostelium discoideum* (reviewed in Iijima et al., 2002; Parent and Devreotes, 1999). In this model system, directional migration toward the chemoattractant is also controlled by asymmetric activation of a G protein-coupled receptor. This is translated into asymmetrical distribution of PIP_3 by spatially and temporally regulated production and subsequent degradation of this phosphoinositide (Funamoto et al., 2002; Iijima and Devreotes, 2002). In accordance with our findings, an important conclusion derived from studying chemotaxis in *Dictyostelium* is that while directional sensing depends on receptor activation, the actual movement of the cell is independent of it (Parent and Devreotes, 1999). An interesting avenue of research would be to determine whether these striking similarities between *Dictyostelium* chemoattraction and PGC migration includes the molecular cascade downstream of the receptor. These questions can be addressed now in vivo in the context of a live vertebrate at a resolution approaching that obtained in *Dictyostelium*.

As mentioned above, the process of PGC migration takes place in many different organisms. The question of whether PGC migration in these organisms is regulated by molecular mechanisms similar to those described here for the zebrafish remains open. While strong tools are available for following PGC migration in mouse (Anderson et al., 2000; Molyneaux et al., 2001), they have not yet been applied to study mice deficient for SDF-1 or for CXCR4. In addition, it is not clear whether molecules that are functionally homologous to SDF and its receptor are present in *Drosophila*. The question of conservation is especially intriguing in the chick (reviewed in Niewkoop and Sutasurya, 1979). In this organism, following their segregation, the PGCs penetrate the vascular system and are found in the extraembryonic blood vessels and then in the blood circulation of the embryo proper. The PGCs then leave the blood vessels and begin to penetrate the gonadal epithelium. This route of PGC migration is noticeably reminiscent of leukocyte trafficking, where the cells migrate into and out of blood vessels to arrive at their target. The involvement of attracting signals directing PGC migration in the chick has been suggested, for example, by showing that an ectopically transplanted target tissue is capable of attracting and then being colonized by PGCs (Kuwana and Rogulska, 1999). In addition to seeking a generalized concept underlying the findings presented here and PGC migration in other organisms, it would be necessary to determine the relationships between SDF-1 signaling and the corresponding pathways that are involved in PGC migration in other organisms (Starz-Gaiano and Lehmann, 2001; Wylie, 1999, 2000). For example, the possible relevance of lipid-metabolizing enzymes whose function is important for PGC migration in *Drosophila* (Starz-Gaiano et al., 2001; van Doren et al., 1998; Zhang et al., 1996) should be investigated in the fish. Similarly, the involvement of specific cell-cell and cell-matrix adhesion molecules shown to participate in mouse PGC migration (Anderson et al., 1999; Bendel-Stenzel et al., 2000) should also be examined in the zebrafish system.

Besides their importance in normal processes such as leukocyte migration and organ development, SDF-1 and CXCR4 have been shown to be involved in several pathological conditions. CXCR4 plays a central role in T cell accumulation in rheumatoid arthritis synovium (e.g., Buckley et al., 2000; Gu et al., 2002; Nanki et al., 2000), it determines the metastatic destination of tumor cells (Muller et al., 2001), and it acts as a coreceptor for the HIV-1 entry and membrane fusion with the target cell (Muller et al., 2001). Using the available genetic and genomic tools in zebrafish as a model for these diseases would enhance our understanding of these disorders. Moreover, compounds that may provide a possible prospective treatment for conditions of this kind (e.g., Matthys et al., 2001; Schols et al., 1997a, 1997b) could be screened for, using PGC migration as an in vivo assay for the SDF-1-CXCR4 signaling pathway (Peterson et al., 2000).

Experimental Procedures

Cloning of CXCR4b and SDF-1a

The *cxcr*4b (Chong et al., 2001) cDNA was PCR amplified from early somitogenesis AB cDNA using the primers 5′-CCGGACTTAT TGCGCCTTT-3′ and 5′-TGATTTTTAGGTTTTATTATGAAATGG-3′. Another *cxcr*4 gene, the *cxcr*4a (Chong et al., 2001), has been previously described. Based on the expression pattern of this gene and the fact that inhibition of its translation does not affect PGC migration, we consider it unlikely that it plays a role in this process. The cDNA of *sdf*-1a (GenBank accession number AY147915) was amplified from early somitogenesis AB cDNA using the primers 5′-CAGTGCGGATCTCTTCTTCA-3′ (forward) and 5′-AAACACGGAG CAAACAGGAC-3′ (reverse) based on expressed sequence tags (ESTs) sequences identified using BLAST search for molecules homologous to the mouse *sdf*-1. An EST for another *sdf*-1-related gene was identified (termed *sdf*-1b, accession number BM070896). Based on the expression pattern of this gene and the fact that its overexpression in the PGCs did not affect their migration, we consider it unlikely that this gene is involved in PGC migration.

Knockdown Experiments

Two nonoverlapping morpholino oligonucleotides were used to inhibit the translation of *cxcr*4b with similar results: *R4b*-1-*MO* TGC TCAAAAAGGTGCAATAAGTCCG and *R4b*-2-*MO* AAATGATGCTAT CGTAAAATTCCAT. As a control, a morpholino oligonucleotide that includes four mismatching bases was injected: *R4b*-2-*CO*-*MO* AAT TGAAGCTATCGTAATATTGCAT. To inhibit the translation of *sdf*-1a mRNA, two different morpholino oligonucleotides were used with similar results: *SDF-1a*-1-*MO* 5′-CTACTACGATCACTTTGAGATC CAT-3′ and *SDF-1a-2-MO* 5′-TTGAGATCCATGTTTGCAGTGTGAA-3′.

For the knockdown experiments, 0.4 pmol of these morpholino oligonucleotides dissolved in 10 mM HEPES (pH 7.6) were injected into 1 cell stage embryos.

Construction of RNA Expression Vectors and RNA Synthesis

gfp-nanos-1 is a construct that includes the mmGFP open reading frame (ORF) (Siemering et al., 1996) fused to the 3′-UTR of the *nanos*-1 gene as previously described (Korpuner, 2001). Injection of RNA generated from this construct results in specific GFP expression in the PGCs.

yefp-nanos-1-3′-UTR is a construct similar to *gfp-nanos*-1, with YEFP replacing the mmGFP ORF.

vasa-gfp-nanos-1 is a construct that encodes a fusion between Vasa and mmGFP ORFs as previously described (Wolke et al., 2002), with the 3′-UTR of *vasa* exchanged for that of the *nanos*-1 gene. The resulting fusion protein is subcellularly localized to perinuclear granules in the PGCs (Wolke et al., 2002).

Farnesylated *egfp-nanos*-1 is a construct that was cloned for the purpose of targeting GFP expression to the membrane of PGCs (Weidinger et al., 2002).

cefp-globin was made by CEFP cloned into pSP64T. Injection of RNA produced from this construct leads to CEFP expression in cells that inherit it.

For *cxcr*4b-*nanos*-1, the CXCR4b ORF was fused to the 3′-UTR of *nanos*-1 for the purpose of expressing the protein specifically in PGCs. The coding region of *cxcr*4b was amplified by PCR using the primers 5′-AAAGGATCCGAACAAAATGGAATTTTACGATAGC-3′ and 5′-AAACTCGAGACATGCACACACTAACTCGTCA-3′ and was cloned upstream of the *nanos*-1 UTR.

For *sdf*-1a- *nanos*-1, the SDF-1a ORF was fused to the 3′-UTR of *nanos*-1 for the purpose of overexpressing the protein in PGCs. The coding region of *sdf*-1a was amplified by PCR using the primers 5′-AAAGGATCCAACATGGATCTCAAAGTG-3′ (forward) and 5′-AAA CTCGAGTTAGACCTGCTGCTGTTG-3′ (reverse). The *sdf*-1a coding region was cloned into the *gfp-nanos*-1 construct replacing the GFP ORF.

Morpholino-resistant *sdf*-1a-*globin* was used for ectopic expression of SDF-1a in *SDF-1a-MO*-injected embryos. The SDF-1a ORF was amplified using the primers 5′-AAAGGATCCAACATGGACTT GAAGGTCATCGTAGT-3′ (underline, nucleotide exchanges relative to the wild-type *sdf*-1a which should eliminate the binding of the *SDF-1a-MO*-1) and 5′-AAAACTAGTTTAGACCTGCTGCTGTTG-3′. The amplification product that encodes a wild-type SDF-1a was cloned into CEFP-*globin* construct replacing the CEFP ORF.

Phenotypic Rescue and Overexpression Experiments

To express CXCR4b specifically in the PGCs in embryos globally depleted of CXCR4b, embryos were coinjected at 1 cell stage with 0.4 pmol of *R4b-1-MO* and different amounts of *cxcr*4b-*nanos*-1 and *gfp-nanos*-1 RNA. The injected amounts of *cxcr*4b-*nanos*-1 were 0, 2, 10, 20, 50, 100, and 200 pg per embryo, and the total amount of injected RNA was supplemented with *gfp-nanos*-1 to 300 pg. For each combination, 40 embryos or more were analyzed. The *cxcr*4b-*nanos*-1 RNA lacks the sequence recognized by the *R4b-1-MO*.

For SDF-1a overexpression in the PGCs, embryos were injected at 1 cell stage with 100 pg *sdf*-1a-*nanos*-1 RNA.

Whole-Mount In Situ Hybridization

One- and two-color in situ hybridization was performed as previously described (Jowett and Lettice, 1994) with modification according to Hauptmann and Gerster (1994) and Weidinger et al. (2002). The following probes were used: *cmlc*2 (Yelon and Stainier, 1999), *cxcr*4b (Chong et al., 2001), *myo*D (*myod*; Zebrafish Information Network) (Weinberg et al., 1996), *nos*1 (Köprunner et al., 2001), *ntl* (Schulte-Merker et al., 1994), *pax*2.1 (*pax2a*; Zebrafish Information Network) (Krauss et al., 1991), *pax*8 (Pfeffer et al., 1998), and *sdf*-1a (this work).

Time Lapse Analysis of PGC Migration

For the time lapse analysis at low magnification (Figure 4, 10× objective), the PGCs were labeled by microinjection of 150 pg *gfp-nanos*-1 RNA (Köprunner et al., 2001) into embryos obtained by mating wild-type AB females with *goosecoid-GFP* transgenic males. The embryos were oriented in 1.5% agarose ramps and overlaid with 0.3× Danieau's solution (Westerfield, 1995). The time lapse movies were generated using the Metamorph software (Universal Imaging) controlling a Zeiss Axioplan2 microscope. Pictures were taken at 1 min intervals. For the time lapse analysis at high magnification (Figure 5, 63× objective), wild-type embryos were microinjected with 140 pg of farnesylated -*egfp-nanos-1* RNA leading to GFP expression in the membrane of the PGCs. To obtain faint labeling of somatic cells, 2 pg farnesylated *egfp-globin* was also injected. Pictures were taken at 10 s intervals.

Ectopic Expression of SDF-1a

To express SDF-1a in ectopic positions in the embryo, the embryos were injected at the 1 cell stage with 0.4 pmol *SDF-1a-1 MO*, 0.2 pmol *R4b-2-CO-MO*, and 120 pg YEFP-*nanos*-1-3′-UTR RNA. The embryos were raised to the 32 cell stage and cells were injected with 100 pg morpholino-resistant *sdf*-1a-globin RNA and 100 pg *cefp*-globin RNA. As a control, the embryos were treated at 1 cell stage similarly to the experimental embryos and at 32 cell stage were injected only with 200 pg *cefp*-globin RNA. As a second control, the embryos were injected at 1 cell stage with 0.4 pmol *SDF*-1*a-MO*, 0.2 pmol *R4b-2-MO*, and 120 pg *yefp-nanos*-1 and at the 32 cell stage were treated as the experimental embryos. The injected embryos were photographed at around 24 hpf using ECFP and EYFP specific fluorescent filters. Pseudocolors were used to improve the visualization of the fluorescence intensities. The proportion of PGCs in contact with red and white spots that correspond to high and higher intensities, respectively, of ECFP (and therefore of SDF-1a) was counted and the results were analyzed using student's t test.

Additional information regarding experimental procedures is provided in the Supplemental Data at http://www.cell.com/cgi/content/full/111/5/647/DC1.

Acknowledgments

This work was supported by grants from the DFG and the Volkswagen-Stiftung to E.R. We thank members of the Raz lab for discussions, Karin Dumstrei and Krasimir Slanchev for comments on the manuscript, and Peter Gruss and Herbert Jäckle for help and encouragement.

Received: September 9, 2002
Revised: October 25, 2002

References

Aiuti, A., Webb, I., Bleul, C., Springer, T., and Gutierrez-Ramos, J. (1997). The chemokine SDF-1 is a chemoattractant for human CD34+ hematopoietic progenitor cells and provides a new mechanism to explain the mobilization of CD34+ progenitors to peripheral blood. J. Exp. Med. *185*, 111–120.

Anderson, R., Fassler, R., Georges-Labouesse, E., Hynes, R., Bader, B., Kreidberg, J., Schaible, K., Heasman, J., and Wylie, C. (1999). Mouse primordial germ cells lacking beta1 integrins enter the germline but fail to migrate normally to the gonads. Development *126*, 1655–1664.

Anderson, R., Copeland, T.K., Scholer, H., Heasman, J., and Wylie, C. (2000). The onset of germ cell migration in the mouse embryo. Mech. Dev. *91*, 61–68.

Baggiolini, M. (1998). Chemokines and leukocyte traffic. Nature *392*, 565–568.

Baggiolini, M., Dewald, B., and Moser, B. (1997). Human chemokines: an update. Annu. Rev. Immunol. *15*, 675–705.

Bendel-Stenzel, M.R., Gomperts, M., Anderson, R., Heasman, J., and Wylie, C. (2000). The role of cadherins during primordial germ cell migration and early gonad formation in the mouse. Mech. Dev. *91*, 143–152.

Bleul, C., Fuhlbrigge, R., Casasnovas, J., Aiuti, A., and Springer, T. (1996a). A highly efficacious lymphocyte chemoattractant, stromal cell-derived factor 1 (SDF-1). J. Exp. Med. *184*, 1101–1109.

Bleul, C.C., Farzan, M., Choe, H., Parolin, C., Clark-Lewis, I., Sodroski, J., and Springer, T.A. (1996b). The lymphocyte chemoattractant SDF-1 is a ligand for LESTR/fusin and blocks HIV-1 entry. Nature *382*, 829–833.

Braat, A., Zandbergen, T., van de Water, S., Goos, H., and Zivkovic, D. (1999). Characterization of zebrafish primordial germ cells: morphology and early distribution of vasa RNA. Dev. Dyn. *216*, 153–167.

Buckley, C.D., Amft, N., Bradfield, P.F., Pilling, D., Ross, E., Arenzana-Seisdedos, F., Amara, A., Curnow, S.J., Lord, J.M., Scheel-Toellner, D., and Salmon, M. (2000). Persistent induction of the chemokine receptor CXCR4 by TGF-beta 1 on synovial T cells contributes to their accumulation within the rheumatoid synovium. J. Immunol. *165*, 3423–3429.

Campbell, J.J., Hedrick, J., Zlotnik, A., Siani, M.A., Thompson, D.A., and Butcher, E.C. (1998). Chemokines and the arrest of lymphocytes rolling under flow conditions. Science *279*, 381–384.

Chong, S., Emelyanov, A., Gong, Z., and Korzh, V. (2001). Expression pattern of two zebrafish genes, cxcr4a and cxcr4b. Mech. Dev. *109*, 347–354.

Ciruna, B., Weidinger, G., Knaut, H., Thisse, B., Thisse, C., Raz, E., and Schier, A.F. (2002). Production of maternal-zygotic mutant zebrafish by germ-line replacement. Proc. Natl. Acad. Sci. USA, in press.

Deshpande, G., Swanhart, L., Chiang, P., and Schedl, P. (2001). Hedgehog signaling in germ cell migration. Cell *106*, 759–769.

Eddy, E. (1974). Fine structural observations on the form and distribution of nuage in germ cells of the rat. Anat. Rec. *178*, 731–758.

Eddy, E.M., and Ito, S. (1971). Fine structural and radioautographic observations on dense perinuclear cytoplasmic material in tadpole oocytes. J. Cell Biol. *49*, 90–108.

Funamoto, S., Meili, R., Lee, S., Parry, L., and Firtel, R.A. (2002). Spatial and temporal regulation of 3-phosphoinositides by PI 3-kinase and PTEN mediates chemotaxis. Cell *109*, 611–623.

Godin, I., Wylie, C., and Heasman, J. (1990). Genital ridges exert long-range effects on mouse primordial germ cell numbers and direction of migration in culture. Development *108*, 357–363.

Griffin, K.J., Amacher, S.L., Kimmel, C.B., and Kimelman, D. (1998). Molecular identification of spadetail: regulation of zebrafish trunk and tail mesoderm formation by T-box genes. Development *125*, 3379–3388.

Gu, J., Marker-Hermann, E., Baeten, D., Tsai, W.C., Gladman, D., Xiong, M., Deister, H., Kuipers, J.G., Huang, F., Song, Y.W., et al. (2002). A 588-gene microarray analysis of the peripheral blood mononuclear cells of spondyloarthropathy patients. Rheumatology (Oxford) *41*, 759–766.

Hammerschmidt, M., Pelegri, F., Mullins, M.C., Kane, D.A., van Eeden, F.J., Granato, M., Brand, M., Furutani-Seiki, M., Haffter, P., Heisenberg, C.P., et al. (1996). dino and mercedes, two genes regulating dorsal development in the zebrafish embryo. Development *123*, 95–102.

Hauptmann, G., and Gerster, T. (1994). Two-color whole-mount in situ hybridization to vertebrate and *Drosophila* embryos. Trends Genet. *10*, 266.

Hay, B., Ackerman, L., Barbel, S., Jan, L., and Jan, Y. (1988). Identification of a component of *Drosophila* polar granules. Development *103*, 625–640.

Iijima, M., and Devreotes, P. (2002). Tumor suppressor PTEN mediates sensing of chemoattractant gradients. Cell *109*, 599–610.

Iijima, M., Huang, Y.E., and Devreotes, P. (2002). Temporal and spatial regulation of chemotaxis. Dev. Cell *3*, 469–478.

Jaglarz, M.K., and Howard, K.R. (1995). The active migration of *Drosophila* primordial germ cells. Development *121*, 3495–3503.

Jowett, T., and Lettice, L. (1994). Whole-mount in situ hybridizations on zebrafish embryos using a mixture of digoxigenin- and fluorescein-labelled probes. Trends Genet. *10*, 73–74.

Knaut, H., Pelegri, F., Bohmann, K., Schwarz, H., and Nusslein-Volhard, C. (2000). Zebrafish vasa RNA but not its protein is a component of the germ plasm and segregates asymmetrically before germline specification. J. Cell Biol. *149*, 875–888.

Köprunner, M., Thisse, C., Thisse, B., and Raz, E. (2001). A zebrafish nanos-related gene is essential for the development of primordial germ cells. Genes Dev. *15*, 2877–2885.

Krauss, S., Johansen, T., Korzh, V., and Fjose, A. (1991). Expression pattern of zebrafish pax genes suggests a role in early brain regionalization. Nature *353*, 267–270.

Kuwana, T., and Rogulska, T. (1999). Migratory mechanisms of chick primordial germ cells toward gonadal anlage. Cell Mol. Biol. (Noisy-le-grand) *45*, 725–736.

Matthys, P., Hatse, S., Vermeire, K., Wuyts, A., Bridger, G., Henson, G.W., De Clercq, E., Billiau, A., and Schols, D. (2001). AMD3100, a potent and specific antagonist of the stromal cell-derived factor-1 chemokine receptor CXCR4, inhibits autoimmune joint inflammation in IFN-gamma receptor-deficient mice. J. Immunol. *167*, 4686–4692.

Mazo, I.B., Gutierrez-Ramos, J.C., Frenette, P.S., Hynes, R.O., Wagner, D.D., and von Andrian, U.H. (1998). Hematopoietic progenitor cell rolling in bone marrow microvessels: parallel contributions by endothelial selectins and vascular cell adhesion molecule *1*. J. Exp. Med. *188*, 465–474.

Molyneaux, K.A., Stallock, J., Schaible, K., and Wylie, C. (2001). Time-lapse analysis of living mouse germ cell migration. Dev. Biol. *240*, 488–498.

Muller, A., Homey, B., Soto, H., Ge, N., Catron, D., Buchanan, M.E., McClanahan, T., Murphy, E., Yuan, W., Wagner, S.N., et al. (2001). Involvement of chemokine receptors in breast cancer metastasis. Nature *410*, 50–56.

Müller, K., Thisse, C., Thisse, B., and Raz, E. (2002). Expression of a linker histone-like gene in the primordial germ cells in zebrafish. Mech. Dev. *117*, 253–257.

Murphy, P.M. (2001). Chemokines and the molecular basis of cancer metastasis. N. Engl. J. Med. *345*, 833–835.

Myers, D.C., Sepich, D.S., and Solnica-Krezel, L. (2002). Bmp activity gradient regulates convergent extension during zebrafish gastrulation. Dev. Biol. *243*, 81–98.

Nagasawa, T., Hirota, S., Tachibana, K., Takakura, N., Nishikawa, S., Kitamura, Y., Yoshida, N., Kikutani, H., and Kishimoto, T. (1996). Defects of B-cell lymphopoiesis and bone-marrow myelopoiesis in mice lacking the CXC chemokine PBSF/SDF-1. Nature *382*, 635–638.

Nanki, T., Hayashida, K., El-Gabalawy, H.S., Suson, S., Shi, K., Girschick, H.J., Yavuz, S., and Lipsky, P.E. (2000). Stromal cell-derived factor-1-CXC chemokine receptor 4 interactions play a central role in CD4+ T cell accumulation in rheumatoid arthritis synovium. J. Immunol. *165*, 6590–6598.

Nasevicius, A., and Ekker, S.C. (2000). Effective targeted gene 'knockdown' in zebrafish. Nat. Genet. *26*, 216–220.

Niewkoop, P.D., and Sutasurya, L.A. (1979). Primordial Germ Cells in the Chordates. (Cambridge, UK: Cambridge University Press).

Parent, C.A., and Devreotes, P.N. (1999). A cell's sense of direction. Science *284*, 765–770.

Peled, A., Grabovsky, V., Habler, L., Sandbank, J., Arenzana-Seisdedos, F., Petit, I., Ben-Hur, H., Lapidot, T., and Alon, R. (1999a). The chemokine SDF-1 stimulates integrin-mediated arrest of CD34(+) cells on vascular endothelium under shear flow. J. Clin. Invest. *104*, 1199–1211.

Peled, A., Petit, I., Kollet, O., Magid, M., Ponomaryov, T., Byk, T., Nagler, A., Ben-Hur, H., Many, A., Shultz, L., et al. (1999b). Dependence of human stem cell engraftment and repopulation of NOD/SCID mice on CXCR4. Science *283*, 845–848.

Peterson, R.T., Link, B.A., Dowling, J.E., and Schreiber, S.L. (2000). Small molecule developmental screens reveal the logic and timing

of vertebrate development. Proc. Natl. Acad. Sci. USA *97*, 12965–12969.

Petit, I., Szyper-Kravitz, M., Nagler, A., Lahav, M., Peled, A., Habler, L., Ponomaryov, T., Taichman, R.S., Arenzana-Seisdedos, F., Fujii, N., et al. (2002). G-CSF induces stem cell mobilization by decreasing bone marrow SDF-1 and up-regulating CXCR4. Nat. Immunol. *17*, 687–694.

Pfeffer, P.L., Gerster, T., Lun, K., Brand, M., and Busslinger, M. (1998). Characterization of three novel members of the zebrafish Pax2/5/8 family: dependency of Pax5 and Pax8 expression on the Pax2.1 (noi) function. Development *125*, 3063–3074.

Schisa, J.A., Pitt, J.N., and Priess, J.R. (2001). Analysis of RNA associated with P granules in germ cells of C. elegans adults. Development *128*, 1287–1298.

Schols, D., Este, J.A., Henson, G., and De Clercq, E. (1997a). Bicyclams, a class of potent anti-HIV agents, are targeted at the HIV coreceptor fusin/CXCR-4. Antiviral Res. *35*, 147–156.

Schols, D., Struyf, S., Van Damme, J., Este, J.A., Henson, G., and De Clercq, E. (1997b). Inhibition of T-tropic HIV strains by selective antagonization of the chemokine receptor CXCR4. J. Exp. Med. *186*, 1383–1388.

Schulte-Merker, S., van Eeden, F.J.M., Halpern, M.E., Kimmel, C.B., and Nüsslein-Volhard, C. (1994). *no tail (ntl)* is the zebrafish homologue of the mouse *T (Brachyury)* gene. Development *120*, 1009–1015.

Schulte-Merker, S., Lee, K.J., McMahon, A.P., and Hammerschmidt, M. (1997). The zebrafish organizer requires *chordino*. Nature *387*, 862–863.

Servant, G., Weiner, O.D., Herzmark, P., Balla, T., Sedat, J.W., and Bourne, H.R. (2000). Polarization of chemoattractant receptor signaling during neutrophil chemotaxis. Science *287*, 1037–1040.

Siemering, K., Golbik, R., Sever, R., and Haseloff, J. (1996). Mutations that suppress the thermosensitivity of green fluorescent protein. Curr. Biol. *6*, 1653–1663.

Springer, T.A. (1994). Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. Cell *76*, 301–314.

Starz-Gaiano, M., and Lehmann, R. (2001). Moving towards the next generation. Mech. Dev. *105*, 5–18.

Starz-Gaiano, M., Cho, N.K., Forbes, A., and Lehmann, R. (2001). Spatially restricted activity of a *Drosophila* lipid phosphatase guides migrating germ cells. Development *128*, 983–991.

Strome, S., and Wood, W.B. (1982). Immunofluorescence visualization of germ-line-specific cytoplasmic granules in embryos, larvae, and adults of *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. USA *79*, 1558–1562.

Tachibana, K., Hirota, S., Iizasa, H., Yoshida, H., Kawabata, K., Kataoka, Y., Kitamura, Y., Matsushima, K., Yoshida, N., Nishikawa, S., et al. (1998). The chemokine receptor CXCR4 is essential for vascularization of the gastrointestinal tract. Nature *393*, 591–594.

van Doren, M., Brohier, H.T., Moore, L.A., and Lehmann, R. (1998). HMG-CoA reductase guides migrating primordial germ cells. Nature *396*, 466–469.

Weidinger, G., Wolke, U., Koprunner, M., Klinger, M., and Raz, E. (1999). Identification of tissues and patterning events required for distinct steps in early migration of zebrafish primordial germ cells. Development *126*, 5295–5307.

Weidinger, G., Wolke, U., Koprunner, M., Thisse, C., Thisse, B., and Raz, E. (2002). Regulation of zebrafish primordial germ cell migration by attraction towards an intermediate target. Development *129*, 25–36.

Weinberg, E.S., Allende, M.L., Kelly, C.S., Abdelhamid, A., Murakami, T., Andermann, P., Doerre, O.G., Grunwald, D.J., and Riggleman, B. (1996). Developmental regulation of zebrafish MyoD in wild-type, no tail and spadetail embryos. Development *122*, 271–280.

Westerfield, M. (1995). The Zebrafish Book (Oregon: University of Oregon Press).

Wolke, U., Weidinger, G., Köprunner, M., and Raz, E. (2002). Multiple levels of post-transcriptional control lead to germ line specific gene expression in the zebrafish. Curr. Biol. *12*, 289–294.

Wylie, C. (1999). Germ cells. Cell *96*, 165–174.

Wylie, C. (2000). Germ cells. Curr. Opin. Genet. Dev. *10*, 410–413.

Xiang, Y., Li, Y., Zhang, Z., Cui, K., Wang, S., Yuan, X.B., Wu, C.P., Poo, M.M., and Duan, S. (2002). Nerve growth cone guidance mediated by G protein coupled receptors. Nat. Neurosci. *5*, 843–848.

Yelon, D., and Stainier, D.Y. (1999). Patterning during organogenesis: genetic analysis of cardiac chamber formation. Semin. Cell Dev. Biol. *10*, 93–98.

Yelon, D., Ticho, B., Halpern, M.E., Ruvinsky, I., Ho, R.K., Silver, L.M., and Stainier, D.Y. (2000). The bHLH transcription factor hand2 plays parallel roles in zebrafish heart and pectoral fin development. Development *127*, 2573–2582.

Yoon, C., Kawakami, K., and Hopkins, N. (1997). Zebrafish vasa homologue RNA is localized to the cleavage planes of 2- and 4-cell-stage embryos and is expressed in the primordial germ cells. Development *124*, 3157–3165.

Zhang, N., Zhang, J., Cheng, Y., and Howard, K. (1996). Identification and genetic analysis of wunen, a gene guiding *Drosophila melanogaster* germ cell migration. Genetics *143*, 1231–1241.

Zhang, N., Zhang, J., Purcell, K.J., Cheng, Y., and Howard, K. (1997). The *Drosophila* protein Wunen repels migrating germ cells. Nature *385*, 64–67.

Zou, Y.R., Kottmann, A.H., Kuroda, M., Taniuchi, I., and Littman, D.R. (1998). Function of the chemokine receptor CXCR4 in haematopoiesis and in cerebellar development. Nature *393*, 595–599.