Available online at www.sciencedirect.com

SCIENCE DIRECT®

ELSEVIER

Developmental Biology 272 (2004) 351–361

DEVELOPMENTAL BIOLOGY

www.elsevier.com/locate/ydbio

Primordial germ cell migration in the chick and mouse embryo: the role of the chemokine SDF-1/CXCL12☆

Jürg Stebler,[a] Derek Spieler,[b] Krasimir Slanchev,[a] Kathleen A. Molyneaux,[c] Ulrike Richter,[b] Vlad Cojocaru,[d] Victor Tarabykin,[e] Chris Wylie,[c] Michael Kessel,[b] and Erez Raz[a,]*

[a] *Germ Cell Development, Max Planck Institute for Biophysical Chemistry, 37077 Göttingen, Germany*
[b] *Department of Molecular Cell Biology, Max Planck Institute for Biophysical Chemistry, 37077 Göttingen, Germany*
[c] *Division of Developmental Biology, Children's Hospital Research Foundation, Cincinnati, OH 452229, USA*
[d] *Department of Molecular Biology, Max Planck Institute for Biophysical Chemistry, 37077 Göttingen, Germany*
[e] *Max Planck Institute for Experimental Medicine, 37075 Göttingen, Germany*

Received for publication 18 December 2003, revised 2 April 2004
Available online 2 July 2004

Abstract

As in many other animals, the primordial germ cells (PGCs) in avian and reptile embryos are specified in positions distinct from the positions where they differentiate into sperm and egg. Unlike in other organism however, in these embryos, the PGCs use the vascular system as a vehicle to transport them to the region of the gonad where they exit the blood vessels and reach their target. To determine the molecular mechanisms governing PGC migration in these species, we have investigated the role of the chemokine stromal cell-derived factor-1 (SDF-1/CXCL12) in guiding the cells towards their target in the chick embryo. We show that *sdf*-1 mRNA is expressed in locations where PGCs are found and towards which they migrate at the time they leave the blood vessels. Ectopically expressed chicken SDF-1α led to accumulation of PGCs at those positions. This analysis, as well as analysis of gene expression and PGC behavior in the mouse embryo, suggest that in both organisms, SDF-1 functions during the second phase of PGC migration, and not at earlier phases. These findings suggest that SDF-1 is required for the PGCs to execute the final migration steps as they transmigrate through the blood vessel endothelium of the chick or the gut epithelium of the mouse.
© 2004 Elsevier Inc. All rights reserved.

Keywords: PGC; Chicken; Cell migration; Chemotaxis; Guidance; Chemokine; SDF-1; CXCL12; CXCR4; Dead end

Introduction

Cell migration plays a central role in a wide variety of biological processes such as development, tissue repair and disease. These processes rely on directed cell migration along and through cell layers and epithelia (e.g. (Butcher et al., 1999; Springer, 1994; Starz-Gaiano and Lehmann, 2001)). The chemokine stromal cell-derived factor 1 (SDF-1/CXCL12), the ligand of CXCR4, is involved in providing cells with directional cues as well as in controlling proliferation and differentiation (Horuk, 2001; Lazarini et al., 2003). SDF-1 is an efficacious chemoattractant for lymphocytes, monocytes and CD34-expressing hematopoietic progenitor cells (Aiuti et al., 1997; Bleul et al., 1996; Kim and Broxmeyer, 1998). Analysis of mice in which SDF-1 or CXCR4 were knocked out showed that the signaling pathway activated by these molecules controls B-cell lymphopoiesis and bone marrow myelopoiesis (Ma et al., 1998; Nagasawa et al., 1996), vascularization of the gastrointestinal tract (Tachibana et al., 1998) and cerebellar neuronal cell migration (Zhu et al., 2002; Zou et al., 1998). In addition to regulating organogenesis and homeostatic processes, SDF-1 and CXCR4 have been shown to be involved in the pathogenesis of tumors and metastasis (Muller et al., 2001; Staller et al., 2003; Zeelenberg et al., 2003), as well as in infectious and inflammatory processes (Abi-Younes et al., 2000; Buckley et al., 2000; Feng et al., 1996; Gonzalo et al., 2000; Hernandez et al., 2003).

Recent work has shown that the G-protein-coupled receptor CXCR4b (Doitsidou et al., 2002; Knaut et al.,

☆ Supplementary data associated with this article can be found, in the online version, at doi: 10.1016/j.ydbio.2004.05.009.

* Corresponding author. Germ Cell Development, Max Planck Institute for Biophysical Chemistry, Am Fassberg 11, 37077 Göttingen, Germany. Fax: +49-551-201-1504.

E-mail address: eraz@gwdg.de (E. Raz).

0012-1606/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.ydbio.2004.05.009

2003) and its ligand SDF-1a (Doitsidou et al., 2002) provide zebrafish primordial germ cells (PGCs) with directional cues in the course of their migration towards the gonad. In mice too, colonization of the gonad by germ cells is impaired in animals lacking functional SDF-1 or CXCR4 (Ara et al., 2003; Molyneaux et al., 2003). The role of SDF-1 signaling in germ cell migration in avians is of particular interest since the migratory route of PGCs is different from those of fish and mice (Niewkoop and Sutasurya, 1979). In the chick embryo, germ cells are specified in the epiblast in the central zone of the *area pellucida*. From this position, the cells are translocated to the anterior extraembryonic region called the germinal crescent where they incorporate into the forming extraembryonic vascular network [stages 8–10 according to Hamburger and Hamilton (1951) (HH 8–10)] and start to circulate within the blood stream (stage HH 11) (Ginsburg and Eyal-Giladi, 1987; Tsunekawa et al., 2000). Later in development, germ cells leave the vascular system and migrate into the region of the future gonad (HH 15–29) (reviewed by Niewkoop and Sutasurya, 1979). The migration path of PGCs in the chick is strikingly similar to the route taken by leukocytes during normal development and in response to an immune challenge as well as to the route followed by metastatic cells on their way to form secondary tumors. In all of those cases, the vascular system serves as a vehicle to transport the cells to distant locations and migration through the vessel wall takes place in the vicinity of the target tissue. We therefore sought to determine whether SDF-1 might play a role in guiding PGC migration in the chick. Here, we provide evidence suggesting that SDF-1α regulates germ cell migration in the chick embryo at the time of extravasation from the vascular system and in the following steps of migration towards the prospective gonad.

Materials and methods

Cloning of chicken SDF-1α and SDF-1β and amino-acid sequence alignment

The chicken SDF-1α (GenBank accession number AY429472) and SDF-1β (GenBank accession number AY429473) were identified based on their homology to the mouse SDF-1 protein. The full-length sequences were determined by 5′ rapid amplification of cDNA ends (RACE). Amino acid sequence alignment of SDF-1 from chicken, mouse, rat, human, frog and zebrafish was performed using ClustalW (Thompson, 1994). The signal peptide of chicken SDF-1α and SDF-1β was predicted using a signal sequence detecting algorithm (von Heijne, 1986).

Construction of expression vectors

The chicken SDF-1α and SDF-1β were subcloned into pcDNA 3.1(−) after being amplified using the primers 5′-GCTCTAGAATGGACCTCCGCGCC-3′ and 5′-CCCAAGCTTCCTTACTTGTTTAATGCTTTCTCCAG-3′ for SDF-1α and 5′-GCTCTAGAATGGACCTCCGCGCC-3′ and 5′-GGAATTCGCTTACAATGCTTGCCTCTTA-3′ for SDF-1β.

Electroporation and whole embryo culture

Stage HH 4 and HH 10 chick (Hamburger and Hamilton, 1951) embryos (White Leghorn) were electroporated as previously described (Endo et al., 2002) and cultivated in Early-Chick (EC)-whole embryo culture (Chapman et al., 2001) at 38°C until stage HH 17 when they were imaged (on average, approximately 15% of tissue expressed GFP) and analyzed following in situ hybridization. CMV-SDF-1α 2.5 μg/μl) was co-injected with CMV-GFP 2.5 μg/μl) or as control only CMV-GFP 5 μg/μl) alone between the blastoderm and the vitelline membrane. The DNA was electroporated using three pulses of 7 V for 25 ms within 200 ms (using Electro Square Porator ECM 830, Btx Inc.).

Cell culture, transfection and implantation

Cos 7 cells were co-transfected with either CMV-SDF-1α and CMV-GFP or CMV-SDF-1β and CMV-GFP using Lipofectamine2000 (Gibco-BRL) according to manufacturer's instructions. As a control, the same total DNA amount of CMV-GFP was used. Twenty-four hours after transfection, an aggregate of transfected Cos 7 cells was grafted between ectoderm and endoderm into embryonic and extra embryonic tissues at different anterior–posterior positions of stage HH 4 or HH 10 chicken embryos. Chicken embryos were cultured in Early-Chick (EC)-whole embryo culture (Chapman et al., 2001) either up to stage HH 10 or HH 17, imaged and analyzed after in situ hybridization.

Whole mount in situ hybridization and histology

Mouse and chicken in situ hybridization were performed as previously described (Knoetgen et al., 1999; Wilkinson, 1992). In situ hybridization probes were synthesized using a PCR amplified template containing only the sequence of the probe and polymerase promoter. The following probes were used for whole mount in situ hybridization analysis: mouse Stella (Saitou et al., 2002), mouse SDF-1α and SDF-1β (Nagasawa et al., 1996), chicken CVH (Tsunekawa et al., 2000), chicken *dead end* (Weidinger et al., 2003), chicken SDF-1 (this work; discrimination between SDF-1α and SDF-1β RNA expression pattern was technically not possible presumably due to the small size of the splice specific probes) and chicken CXCR4 (Liang et al., 2001). Stained embryos were dehydrated, embedded in Paraplast Plus (Sherwood medicals) and sectioned (8 μm).

Mouse organ culture and time lapse

Transverse slices from the hindgut regions of heterozygous Oct4ΔPE:GFP transgenic mouse embryos expressing GFP in the germ cell lineage were cultured and filmed as previously described (Molyneaux et al., 2003). Images for time lapse were captured every 7 min starting at E 9.5.

Results

Identification of chicken SDF-1

Avian and reptile primordial germ cells (PGCs) are transported by the vascular system to the region of the gonad where they exit the blood vessels and migrate into the genital ridge. The mechanisms that direct the PGCs along this route are largely unknown. Considering recent evidence implicating SDF-1 signaling in guiding germ cell migration in zebrafish (Doitsidou et al., 2002), SDF-1 is a good candidate molecule for providing PGCs with directional cues in these organisms. To investigate this point, we cloned the chick SDF-1 cDNA and identified two transcripts, which were termed SDF-1α and SDF-1β . These two transcripts are identical except for the C-terminal domain in which the β form is 42 amino acids longer. SDF-1 amino acid sequence alignment of chicken, mouse, rat, human, *Xenopus laevis* and zebrafish shows extensive conservation of the chick SDF-1 with its mammalian counterparts (Fig. 1A and supplementary data S1A). For example, the chicken SDF-1α protein is 90% identical to the mature mouse SDF-1α (identical residues in respect to chicken SDF-1α are indicated in black). Six residues that are essential for binding to glycosaminoglycans (i.e. Lys[1] Lys[24], His[25], Lys[27], Arg[41], Lys[43]) (Fig. 1A, arrowheads) (Amara et al., 1999; Sadir et al., 2001) as well as the R[12]FFESH (Arg[12]PhePheGluSer-His) motif (Fig. 1A, stars), proposed to be an important initial docking site of mouse SDF-1 with its receptor (Crump et al., 1997), are identical except His[17] which is replaced by a similar amino acid Asn. The chicken SDF-1β protein is more similar in length to the recently cloned rat SDF-1γ for which a function has not been assigned yet (Gleichmann et al., 2000). The similarity between the mouse and the chick proteins at the primary sequence level can be extended by way of calculation to imply conservation at the level of the tertiary structures (supplementary data S1B).

Chick SDF-1 is expressed in positions where PGCs are found during the last stages of their migration

To determine whether SDF-1 and CXCR4 play a role in chicken PGC migration, we examined the expression of the chicken SDF-1 and CXCR4 RNAs relative to the position of the PGCs at different stages. The PGCs were detected by employing a cocktail of the chick *vasa* (*cvh*) and *dead end* (*dnd*) probes, while SDF-1 was detected using a probe recognizing both the α and the β forms (see Materials and methods). At stage HH 6, SDF-1 is expressed in the posterior

Fig. 1. (A) SDF-1 amino acid sequence alignment of chicken (chSDF1α and chSDF1β), mouse (mSDF1α and mSDF1β), rat (rSDF1α, rSDF1β and rSDF1γ), human (hSDF1α and hSDF1β), *Xenopus laevis* (xSDF1) and zebrafish (zSDF1a and zSDF1b) shows extensive conservation of the chick SDF-1α with its mammalian counterparts including essential residues for binding to CXCR4 and glycosaminoglycans (identical residues in respect to chicken SDF-1α are indicated in black).

region of the ectoderm and mesoderm (Figs. 2K and P) but not in the hypoblast of the germinal crescent, the site where most PGCs are located (Figs. 2A and F). Later in development, during the first phases of somitogenesis, the PGCs migrate out of the hypoblast and enter the developing vascular network (Figs. 2F and G, arrowheads). At this stage, a few PGCs are frequently found in the developing head (data not shown). As the heart starts to beat (HH 11), PGCs are transported passively by the blood stream and are distributed throughout the entire vascular network (Niewkoop and Sutasurya, 1979). Finally, between embryonic stage HH 15 and HH 23, PGCs start to transmigrate the vascular endothelium and migrate within the lateral plate mesoderm (lpm) towards the genital ridge (Figs. 2C–E and H–J).

Analysis of the spatiotemporal expression of *sdf*-1 revealed a strong correlation between *sdf*-1 expression domains and the position of the PGCs during these developmental stages. During HH 8–10, SDF-1 expression becomes restricted to the posterior part of the embryo and to the head region (Figs. 2Q and L), thus raising the

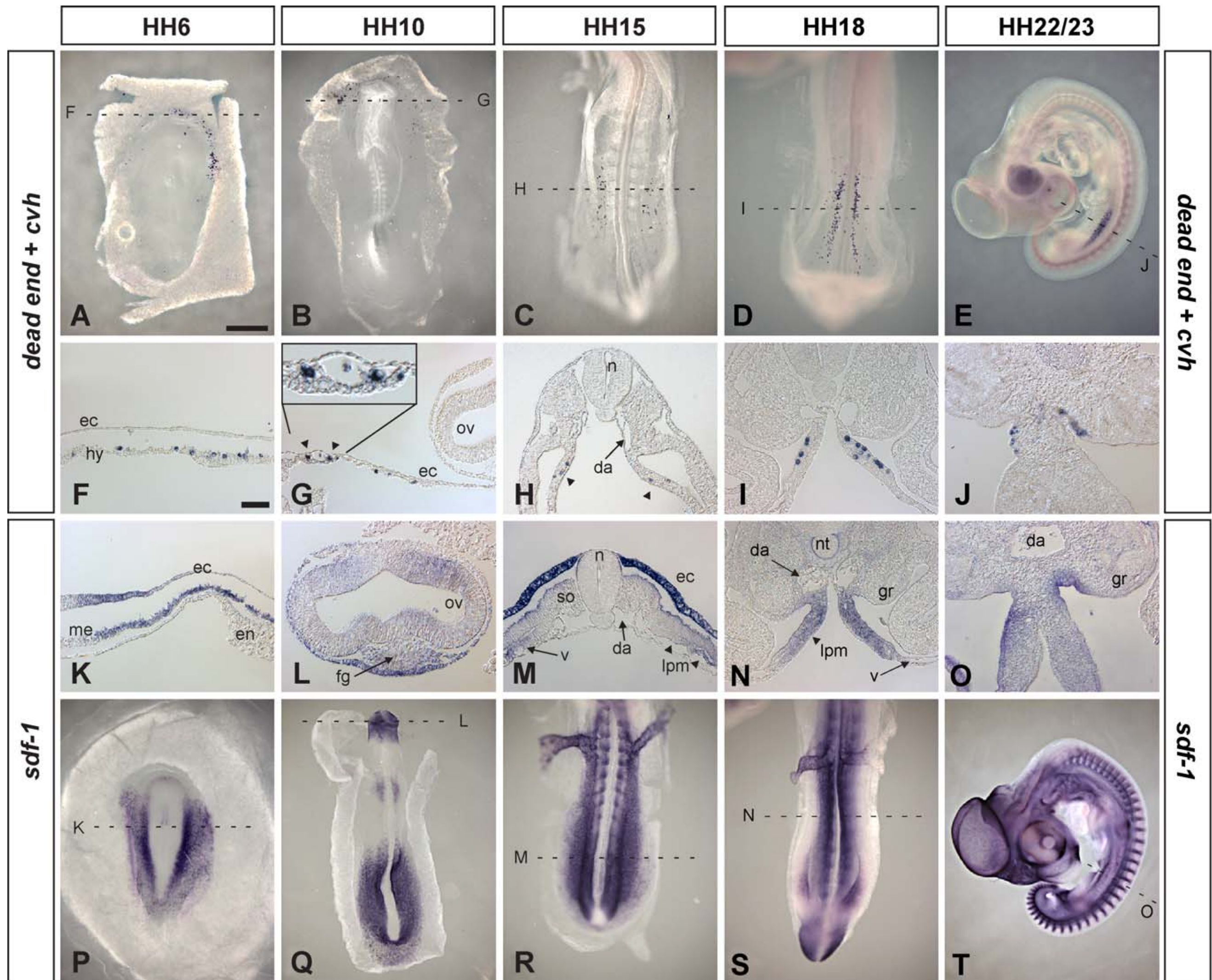

Fig. 2. Correlation between *sdf*-1 expression and the position of the germ cells. (A–E) Whole mount in situ hybridization pictures of chicken embryos stained with PGC marker *dead end* and *cvh* or (P–T) *sdf*-1. (F–O) Sections from the corresponding embryo performed at the level indicated by the black dashed line. (A, B, F, G) PGCs marked by *dead end* and *cvh* are in the germinal crescent within the hypoblast at stage HH 6 and within forming blood vessels (arrowhead and insert) at stage HH 10. (K, L, P, Q) At these stages, *sdf*-1 is expressed more posterior in the ectoderm and mesoderm and later additionally in the head region. (C and H) At HH 15, circulating PGCs (arrowhead) leave the vesicular endothelium and migrate within the lateral plate mesoderm. (M and R) *sdf*-1 is expressed in the lateral plate mesoderm and in the ectoderm while expression in the head region is almost completely diminished (data not shown). (D, E, I, J) Germ cells within the lateral plate mesoderm migrate towards the genital ridge between HH 18 and HH 23. (N, O, S, T) Expression of *sdf*-1 within the lateral plate mesoderm (arrowhead) becomes more restricted and overlaps with the position of the germ cells. Indicated are dorsal aorta (da), ectoderm (ec), endoderm (en), foregut (fg), genital ridge (gr), hypoblast (hy), lateral plate mesoderm (lpm), neural tube (n), notochord (nt), mesoderm (me), optic vesicle (ov), somite (so) and vein (v). The scale bar in A represents 1 mm in A and P, 0.8 mm in B and Q, 0.5 mm in C–D and R–S and 1.5 mm in E and T. The scale bar in F–O represents 200 μm and in insert 600 μm.

possibility that SDF-1 is responsible for the localization of some PGCs to the developing head. As expression in the head region is strongly reduced at stage HH 15 (data not shown), *sdf*-1 is expressed in a broad area of the posterior part of the embryo in the lateral plate mesoderm where the germ cells are found after leaving the blood vessels (Figs. 2C, H, M and R). In the following stages, *sdf*-1 expression becomes stronger and more spatially restricted in the lateral plate mesoderm. Thus, during later development, the expression pattern of *sdf*-1 becomes gradually overlapping with the sites at which PGCs are found (Figs. 2D, E, I, J, N, O, S and T).

The correlation between the position of the PGCs during later stages of migration and the expression pattern of SDF-1 raises the possibility that SDF-1 provides the cells with directional cues by activating its receptor, CXCR4, expressed by the migrating cells. To examine this supposition, the distribution of *cxcr*4 mRNA was examined by in situ hybridization. At stage HH 4 and HH 8, *cxcr*4 is weakly expressed in the germinal crescent, where PGCs reside (Figs. 3A and B, arrowheads). Before stage HH 14, *cxcr*4 is widely expressed in the lateral plate mesoderm (data not shown). Significantly, at the time of PGC migration, *cxcr*4-positive cells can be clearly detected showing the typical morphology (large cell size, 14–17 μm) and location characteristic of PGCs at this stage (Figs. 3C and D). At the time PGCs are colonizing the gonad (Fig. 3E), *cxcr*4 is expressed in numerous tissues whereas mRNA expression in the region where the germ cells reside is significantly reduced (Fig. 3F).

PGCs can be attracted towards a source of SDF-1α

To directly test the notion that SDF-1 can attract chick PGCs, SDF-1α and SDF-1β were ectopically expressed and the ensuing PGC response was examined. Aggregates of Cos 7 cells, co-transfected with either the α or β form of SDF-1 and GFP, were implanted into the chicken embryo at stages HH 4 and HH 10 at different regions (Fig. 4A). Embryos manipulated at stage HH 4 were cultured until stage HH 10, at which time the PGC localization relative to the site of the implant was examined. Embryos harboring implants expressing either SDF-1α and GFP ($n = 16$), SDF-1β and GFP ($n = 13$) or GFP alone as control ($n = 10$) in the *area opaca* or head region did not show any effect of SDF-1 on germ cell localization as no PGCs were found adjacent to the implants (data not shown). Considering that SDF-1-expressing cells were transplanted adjacent to the region where PGCs reside and close to positions where blood vessel develop, we conclude that at least under the conditions of this experiment, PGCs do not respond to SDF-1 between stages HH 4 and HH 10.

To explore the possible function of SDF-1 in germ cell migration during later stages, embryos were similarly manipulated at stage HH 10 and analyzed at stage HH 17 (Figs. 4B–G, at these stages, all embryos showed high numbers of PGCs in the gonad which responded to the endogenous signals, but these cells can be visualized only from the ventral side of the embryo). Four of the thirteen embryos containing implanted Cos 7 cells that express SDF-1α and GFP (Figs. 4B and D) showed ectopic *dead end* and *cvh* labeled PGCs exactly and exclusively at the position of the GFP-labeled graft (Figs. 4C and E). This result was further validated by sectioning the embryos and confirming the overlapping localization of the SDF-1α source and the attracted PGCs (data not shown). In contrast, none of the embryos in which SDF-1β/GFP- or GFP-expressing cells were implanted showed PGCs near the graft ($n = 17$, data not shown and $n = 17$, Figs. 4F and G, respectively). Taking into consideration that the exogenous SDF-1α source has to be precisely positioned near a blood vessel such that it can subsequently be presented on the luminal side to circulating PGCs and that it has to compete with the endogenous SDF-1α, the number of embryos showing these results supports the idea that SDF-1α acts as a chemoattractant for chicken PGCs.

To confirm this finding, we employed an independent method, in which we electroporated chicken embryos at stages HH 4 and HH 10 with SDF-1α and GFP or GFP alone to generate an ectopic SDF-1α source at different positions (Fig. 4H). These embryos were cultured in EC-whole embryo culture until stage HH 17. Indeed, 19.1 ± 5.5% (SEM) of PGCs in embryos electroporated with SDF-1α and GFP ($n = 10$ embryos, $n = 519$ cells) reached the region of high SDF-1α expression (marked by high GFP expression) away from the region of the gonad (Figs. 4I–L). In contrast, only 1.7 ± 0.6% of the PGCs in GFP electroporated control embryos ($n = 20$ embryos, $n = 1472$ cells $P < 0.0001$) were found within high GFP expressing regions (Figs. 4M and N).

These data, along with *sdf*-1 expression pattern, clearly implicate SDF-1α, but does not support a role for SDF-1β, in guidance of migrating PGC in the chick. Interestingly, in zebrafish too, only one of the two SDF-1 proteins (SDF-1a which differs from SDF-1b in its C-terminus) is considered to be relevant for activating CXCR4b in the PGCs (Doitsidou et al., 2002). According to our findings, the function of SDF-1 as a PGC attractant in the chick appears to be temporally restricted to the second phase of PGC migration, the stage at which germ cells leave the vascular network and migrate towards the genital ridge (HH 15–29).

Mouse PGCs transmigrate through the hindgut epithelia exclusively in the region of SDF-1 expression

Analogous to our findings in chick, mouse SDF-1 has been recently shown to be essential for PGC migration from the hindgut via the mesenchyme to the gonad (Ara et al., 2003; Molyneaux et al., 2003), but is not required for the earlier migration phases. Based on these results, it was proposed that during early steps of PGC migration, SDF-1 might be complemented by other chemokines or cytokines

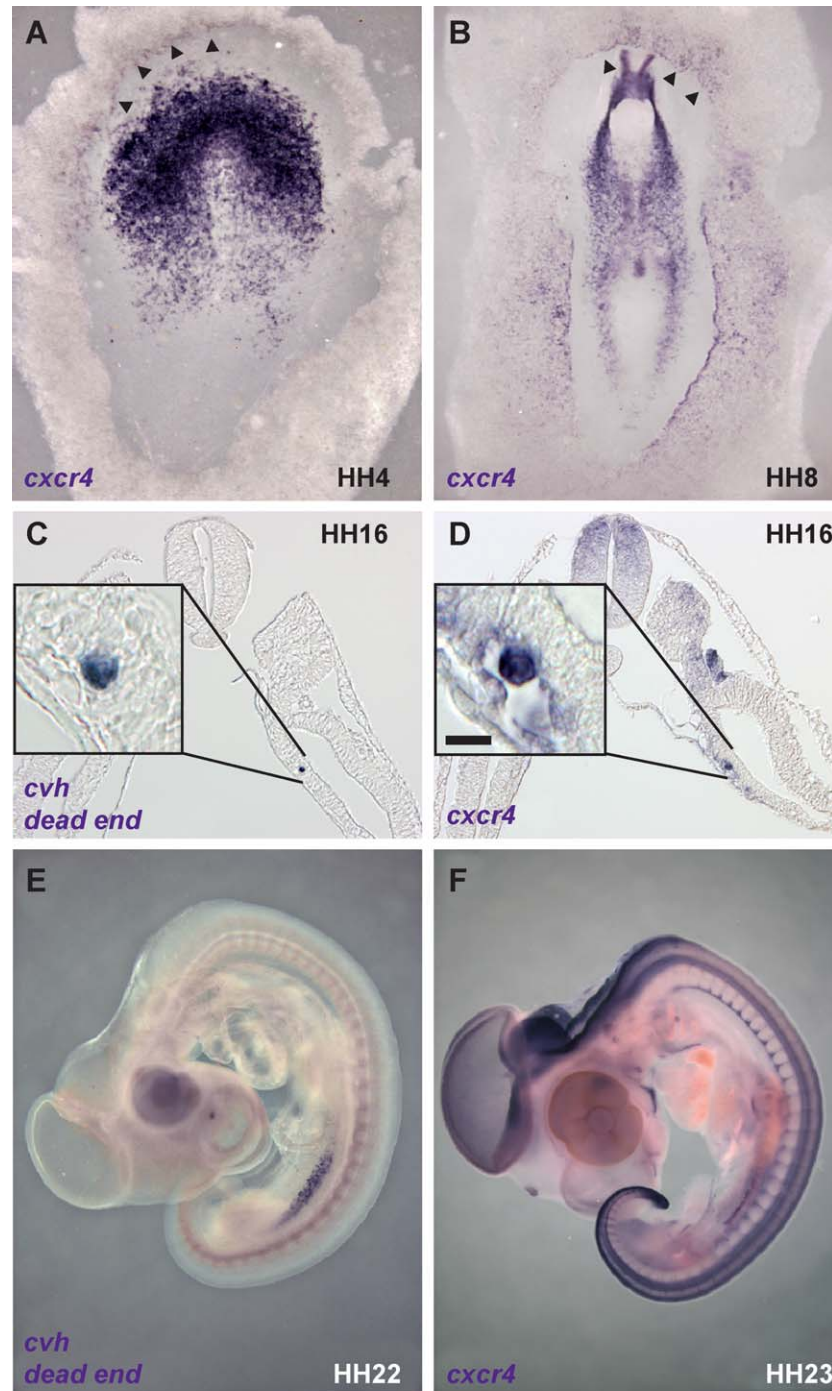

Fig. 3. *cxcr*4 is expressed in the germinal crescent and in cells with typical morphological characteristics of PGCs in the lateral plate mesoderm. (A and B) At stage HH 4–8, *cxcr*4 is expressed in low amount in the germinal crescent, the site where germ cells are (arrowheads). (C) At stage HH 16, PGCs expressing *dead end* and *cvh* are in the lateral plate mesoderm. (D) At this stage, cells in the lateral plate mesoderm showing the typical morphology and size of germ cells express *cxcr*4. (E and F) At stage HH 23, PGCs expressing *dead end* and *cvh* are in the region of the gonad (E) and *cxcr*4 is expressed in numerous tissues including the region of the gonad (F). The scale bar in B represents 280 μm in A and B, 200 μm in C and D, 17 μm in inserts and 0.9 mm in E and F.

that play a redundant role (Ara et al., 2003). To address this notion, we examined the expression pattern of mouse SDF-1α and SDF-1β with respect to the position of the germ cells by in situ hybridization from developmental stages E8.5–11.5. During this time window, the PGCs exit the hindgut and migrate towards the gonad. *sdf*-1β (and *sdf*-1α, data not shown) is expressed along the migratory route in the dorsal mesentery and in the genital ridge (Figs. 5B, D–I and supplementary data S2A–D). However, at E9, before the PGCs migrate dorsally towards the point where they exit the gut, *sdf*-1β (and *sdf*-1α, data not shown) is expressed in the dorsal half of the hindgut, but is clearly absent from the

ventral half (Figs. 5A and A′), where PGCs are predominantly found at this stage (Figs. 5C and C′). These data show that most PGCs are distant from SDF-1 expression domain before the time point at which they migrate dorsally (E9–9.5). We conclude that SDF-1 does not play a role in guiding the cells anteriorly along the ventral side of the

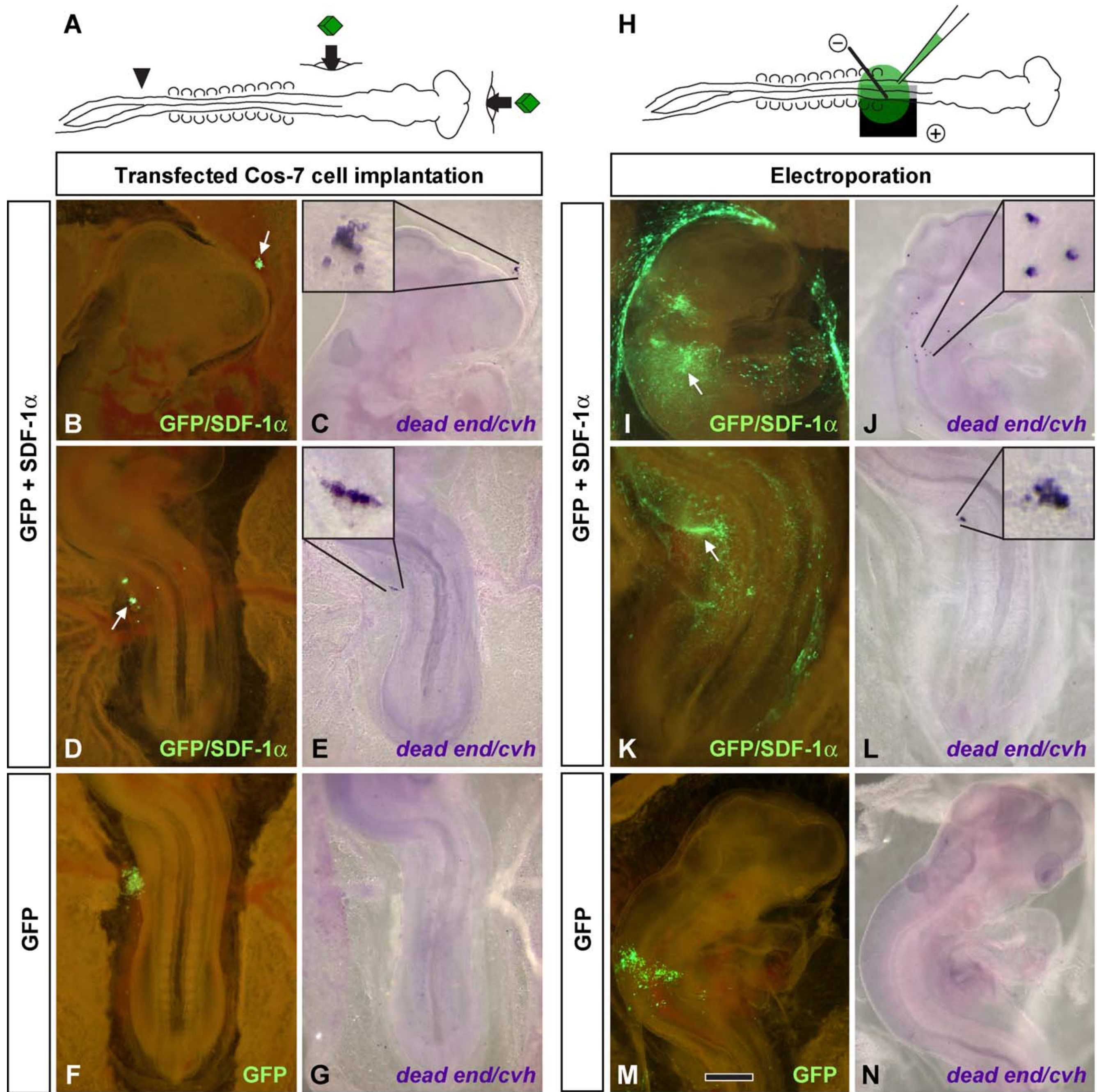

Fig. 4. Germ cells are attracted towards ectopically expressed SDF-1α. (A) Aggregates of Cos 7 cells expressing either SDF-1α and GFP, SDF-1β and GFP or GFP alone were implanted at different anterior–posterior position into the chicken embryo at stage HH 10. Embryos were cultured until stage HH 17 and after in situ hybridization using the PGC markers *dead end* and *cvh* analyzed for the position of germ cells relative to the graft. Arrowhead indicates region of the future gonad. (B, C, D, E) GFP and SDF-1α expressing Cos 7 cells (white arrow) attracted numerous germ cells (insert), which are marked with *dead end* and *cvh* (dorsal view). (F and G) GFP expressing Cos 7 cells do not attract germ cells. (H) CMV-SDF-1α and CMV-GFP or CMV-GFP alone were co-electroporated at different positions into the chicken embryo, cultured until stage HH 17 and analyzed for the position of the PGCs relative to cells expressing high levels of SDF-1α. (I, J, K, L) Germ cells (insert) are attracted to areas of CMV-GFP and CMV-SDF-1α electroporated cells (white arrow). (M and N) Germ cells are not attracted to areas of CMV-GFP electroporated cells. Insert shows PGCs from the area indicated by the arrow. All embryos showed high numbers of PGCs in the gonad, which were attracted by the endogenous signals (visible from ventral side view only, data not shown). The scale bar in M represents 515 μm in B and C, 500 μm in D–G and I–N.

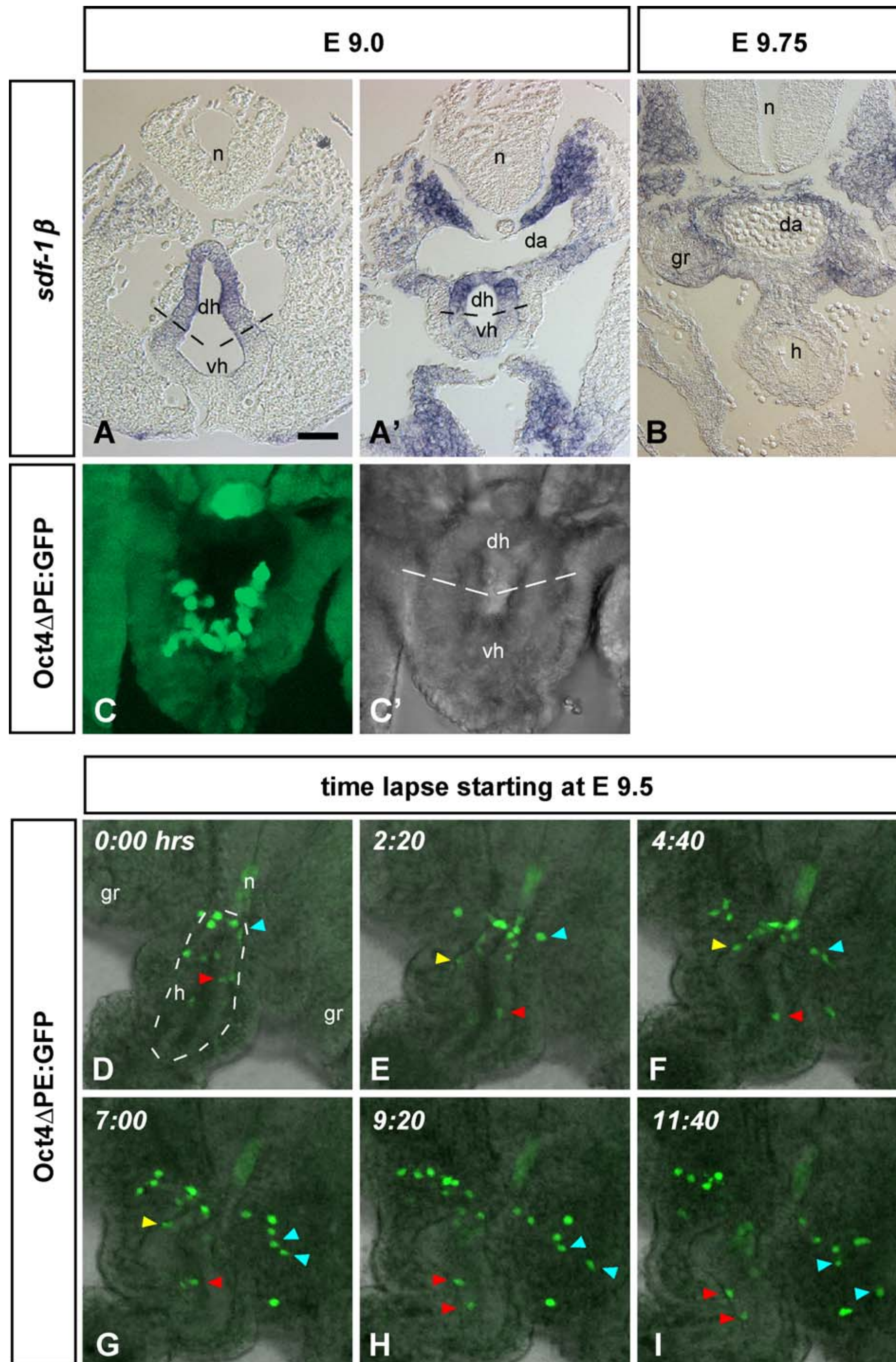

Fig. 5. Mouse SDF-1 expression correlates with germ cell position from the time PGCs start to emerge from the hindgut. (A and A′) Two sections of a stage E9 embryo show *sdf*-1β staining in the dorsal half of the hindgut but not in the ventral half. Both sections are from the same embryo whereas A is more posterior than A′ . (B) Section through the gonad of the E9.75 embryo displayed in supplementary data Fig. S2C shows that *sdf*-1β is expressed along the PGC migration route in the dorsal mesentery and in the gonad but not anymore in the hindgut. (C) GFP expressing germ cells in cultured hindgut transverse slices are in the ventral half of the hindgut. C′ shows C in bright field. (D–I) Six snapshots from a time-lapse movie showing GFP expressing PGCs, which emerge from the dorsal-most part of the hindgut and migrate towards the genital ridge (blue arrowhead). Germ cells in the ventral half of the hindgut (red and yellow arrowheads) do not transmigrate the gut epithelia and eventually die (supplementary Movie 1). Indicated are dorsal aorta (da), dorsal hindgut (dh), genital ridge (gr), hindgut (h), neural tube (n) and ventral hindgut (vh). The scale bar in C represents 1 mm in A and C and 2.5 mm in B and D. The scale bar in A represents 200 μm in A–B, 300 μm in D–I and 360 μm in C and C′.

hindgut. The migration of the cells to the position adjacent to the genital ridge is therefore guided by other chemokines or cytokines.

To examine the events occurring at this stage more directly, we monitored the behavior of GFP-expressing mouse germ cells in cultured transverse slices (Anderson

et al., 1999; Molyneaux et al., 2001) from E9.5 hindgut by time-lapse microscopy (Figs. 5D–I; supplementary Movie 1). We found that the germ cells emerge exclusively from the dorsal-most part of the hindgut (blue arrowhead), where *sdf*-1 is expressed, whereas ventrally positioned PGCs move while colliding with the epithelium but do not transmigrate through it (red and yellow arrowhead) and eventually die (Molyneaux et al., 2001). Together, these results are consistent with the idea that SDF-1 is required specifically for directing the dorsal migration of PGCs to the position in the hindgut from which they migrate into the mesentery. Our data suggest a common function of SDF-1 in promoting PGC transmigration through epithelial-like structures such as the hindgut epithelium in mouse and the endothelium in chick.

Discussion

The migration path of PGCs in chick is remarkably similar to that taken by leukocytes during normal development and disease as well as by metastatic cells. In this study, we provide evidence that implicates SDF-1α in guiding chicken germ cells as they leave the blood vessels on their way to the region of the gonad.

Ectopic expression of SDF-1α, but not SDF-1β, could attract PGCs at the time at which they migrate through the endothelium but not in earlier stages. Our interpretation of these results is that SDF-1 does not play a role in PGC migration before stage HH 11, nor is it responsible for the commonly observed early ectopic localization of PGCs in the head region (Nakamura et al., 1988), as could have been inferred from SDF-1 expression domains at early developmental stages. We therefore favor the idea that early migration of chick PGCs, as well as the early ectopic positioning of PGCs, reflects the function of another signaling molecule or results from non-guided cell movements. As we were not able to label the PGCs simultaneously with germ cell markers and CXCR4, we could not exclude the formal possibility that the cells do not respond to SDF-1 at these stages due to lack of receptor on their cell membrane.

The proposed role for SDF-1 in migration of chick PGCs is highly reminiscent of the mechanism underlying the migration of hematopoietic precursor cells and lymphocytes as they exit the blood vessels. Here, SDF-1 stimulates integrin-mediated arrest of CD34-expressing cells (Peled et al., 1999) and mediates transendothelial migration of T lymphocytes (Phillips and Ager, 2002) possibly through rapid increase of integrin avidity (Campbell et al., 1998; Grabovsky et al., 2000).

The demonstration that SDF-1/CXCR4 signaling is important for germ cell migration in birds (aves) together with similar findings in ray-finned fish (actinopterygii) and mammals (mammalia) suggests that this mechanism had evolved before the phylum Chordata diverged. Moreover, the requirement for seven transmembrane domain receptors for PGC migration could represent a more ancient mechanism since *Drosophila* PGCs, which transmigrate through the gut epithelium on their way to the gonad also appear to depend on the function of such proteins (Starz-Gaiano, 2002). Despite the apparent evolutionary conservation with respect to the use of the CXCR4-SDF-1 signaling in PGC migration, an important difference should be pointed out. In contrast to the role of SDF-1a in fish, which is essential for PGC guidance throughout their migration (Doitsidou et al., 2002), chicken and mouse germ cells appear to perform the first phases of migration independently of SDF-1 function (Molyneaux et al., 2003, and this work).

The observation that transepithelial migration of mouse PGCs occurs specifically in the dorsal-most part of the hindgut is consistent with the suggestion that the cells are attracted towards this region. Interestingly, cells located at ventral positions never exit the hindgut although they clearly bounce against its walls. It would be interesting to determine whether in analogy to the situation in *Drosophila* (Jaglarz and Howard, 1994, 1995; Starz-Gaiano and Lehmann, 2001), only parts of the mouse hindgut epithelium become competent for transmigration due to specific alterations in epithelial morphology.

Acknowledgments

We thank M. Reichman for comments on the manuscript, L. Wittler and H.R. Chung for help and advice, W. Behrens for technical support and P. Murphy for reagents. J.S. is supported by the Swiss National Science Foundation and E.R. is supported by the DFG and Volkswagen-Stiftung. D.S. is supported by the Studienstiftung des deutschen Volkes. K.M. is supported by an NRSA fellowship (1 F32 HD008678-01) from the NIH and C.W. is supported by NIH RO1-33440.

References

Abi-Younes, S., Sauty, A., Mach, F., Sukhova, G.K., Libby, P., Luster, A.D., 2000. The stromal cell-derived factor-1 chemokine is a potent platelet agonist highly expressed in atherosclerotic plaques. Circ. Res. 86, 131–138.

Aiuti, A., Webb, I., Bleul, C., Springer, T., 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.

Amara, A., Lorthioir, O., Valenzuela, A., Magerus, A., Thelen, M., Montes, M., Virelizier, J.L., Delepierre, M., Baleux, F., Lortat-Jacob, H., Arenzana-Seisdedos, F., 1999. Stromal cell-derived factor-1alpha associates with heparan sulfates through the first beta-strand of the chemokine. J. Biol. Chem. 274, 23916–23925.

Anderson, R., Fassler, R., Georges-Labouesse, E., Hynes, R., Bader, B., Kreidberg, J., Schaible, K., Heasman, J., 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.

Ara, T., Nakamura, Y., Egawa, T., Sugiyama, T., Abe, K., Kishimoto, T., Matsui, Y., Nagasawa, T., 2003. Impaired colonization of the gonads by primordial germ cells in mice lacking a chemokine, stromal cell-derived factor-1 (SDF-1). Proc. Natl. Acad. Sci. U. S. A. 100, 5319–5323.

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

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., 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.

Butcher, E.C., Williams, M., Youngman, K., Rott, L., Briskin, M., 1999. Lymphocyte trafficking and regional immunity. Adv. Immunol. 72, 209–253.

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

Chapman, S.C., Collignon, J., Schoenwolf, G.C., Lumsden, A., 2001. Improved method for chick whole-embryo culture using a filter paper carrier. Dev. Dyn. 220, 284–289.

Crump, M.P., Gong, J.H., Loetscher, P., Rajarathnam, K., Amara, A., Arenzana-Seisdedos, F., Virelizier, J.L., Baggiolini, M., Sykes, B.D., Clark-Lewis, I., 1997. Solution structure and basis for functional activity of stromal cell-derived factor-1; dissociation of CXCR4 activation from binding and inhibition of HIV-1. EMBO J. 16, 6996–7007.

Doitsidou, M., Reichman-Fried, M., Stebler, J., Koprunner, M., Dorries, J., Meyer, D., Esguerra, C.V., Leung, T., Raz, E., 2002. Guidance of primordial germ cell migration by the chemokine SDF-1. Cell 111, 647–659.

Endo, Y., Osumi, N., Wakamatsu, Y., 2002. Bimodal functions of Notch-mediated signaling are involved in neural crest formation during avian ectoderm development. Development 129, 863–873.

Feng, Y., Broder, C.C., Kennedy, P.E., Berger, E.A., 1996. HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor. Science 272, 872–877.

Ginsburg, M., Eyal-Giladi, H., 1987. Primordial germ cells of the young chick blastoderm originate from the central zone of the area pellucida irrespective of the embryo-forming process. Development 101, 209–219.

Gleichmann, M., Gillen, C., Czardybon, M., Bosse, F., Greiner-Petter, R., Auer, J., Muller, H.W., 2000. Cloning and characterization of SDF-1gamma, a novel SDF-1 chemokine transcript with developmentally regulated expression in the nervous system. Eur. J. Neurosci. 12, 1857–1866.

Gonzalo, J.A., Lloyd, C.M., Peled, A., Delaney, T., Coyle, A.J., Gutierrez-Ramos, J.C., 2000. Critical involvement of the chemotactic axis CXCR4/stromal cell-derived factor-1 alpha in the inflammatory component of allergic airway disease. J. Immunol. 165, 499–508.

Grabovsky, V., Feigelson, S., Chen, C., Bleijs, D.A., Peled, A., Cinamon, G., Baleux, F., Arenzana-Seisdedos, F., Lapidot, T., van Kooyk, Y., Lobb, R.R., Alon, R., 2000. Subsecond induction of alpha4 integrin clustering by immobilized chemokines stimulates leukocyte tethering and rolling on endothelial vascular cell adhesion molecule 1 under flow conditions. J. Exp. Med. 192, 495–506.

Hamburger, V., Hamilton, H., 1951. A series of normal stages in the development of the chick embryo. J. Morphol. 88, 49–82.

Hernandez, P.A., Gorlin, R.J., Lukens, J.N., Taniuchi, S., Bohinjec, J., Francois, F., Klotman, M.E., Diaz, G.A., 2003. Mutations in the chemokine receptor gene CXCR4 are associated with WHIM syndrome, a combined immunodeficiency disease. Nat. Genet. 34, 70–74.

Horuk, R., 2001. Chemokine receptors. Cytokine Growth Factor Rev. 12, 313–335.

Jaglarz, M., Howard, K., 1994. Primordial germ cell migration in *Drosophila melanogaster* is controlled by somatic tissue. Development 120, 83–89.

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

Kim, C.H., Broxmeyer, H.E., 1998. In vitro behavior of hematopoietic progenitor cells under the influence of chemoattractants: stromal cell-derived factor-1, steel factor, and the bone marrow environment. Blood 91, 100–110.

Knaut, H., Werz, C., Geisler, R., Nusslein-Volhard, C., 2003. A zebrafish homologue of the chemokine receptor Cxcr4 is a germ-cell guidance receptor. Nature 421, 279–282.

Knoetgen, H., Viebahn, C., Kessel, M., 1999. Head induction in the chick by primitive endoderm of mammalian, but not avian origin. Development 126, 815–825.

Lazarini, F., Tham, T.N., Casanova, P., Arenzana-Seisdedos, F., Dubois-Dalcq, M., 2003. Role of the alpha-chemokine stromal cell-derived factor (SDF-1) in the developing and mature central nervous system. Glia 42, 139–148.

Liang, T., Hartt, J., Lu, S., Martins-Green, M., Gao, J., Murphy, P., 2001. Cloning, mRNA distribution, and functional expression of an avian counterpart of the chemokine receptor/HIV coreceptor CXCR4. J. Leukocyte Biol. Feb;69, 297–305.

Ma, Q., Jones, D., Borghesani, P.R., Segal, R.A., Nagasawa, T., Kishimoto, T., Bronson, R.T., Springer, T.A., 1998. Impaired B-lymphopoiesis, myelopoiesis, and derailed cerebellar neuron migration in CXCR4- and SDF-1-deficient mice. Proc. Natl. Acad. Sci. U. S. A. 95, 9448–9453.

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

Molyneaux, K., Zinszner, H., Kunwar, P., Schaible, K., Stebler, J., Sunshine, M., O'Brien, W., Raz, E., Littman, D., Wylie, C., Lehmann, R., 2003. The chemokine SDF1/CXCL12 and its receptor CXCR4 regulate mouse germ cell migration and survival. Development 130, 4279–4286.

Muller, A., Homey, B., Soto, H., Ge, N., Catron, D., Buchanan, M.E., McClanahan, T., Murphy, E., Yuan, W., Wagner, S.N., Barrera, J.L., Mohar, A., Verastegui, E., Zlotnik, A., 2001. Involvement of chemokine receptors in breast cancer metastasis. Nature 410, 50–56.

Nagasawa, T., Hirota, S., Tachibana, K., Takakura, N., Nishikawa, S., Kitamura, Y., Yoshida, N., Kikutani, H., 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.

Nakamura, M., Kuwana, T., Miyayama, Y., Fujimoto, T., 1988. Extragonadal distribution of primordial germ cells in the early chick embryo. Anat. Rec. 222, 90–94.

Niewkoop, P.D., Sutasurya, L.A., 1979. Primordial Germ Cells in the Chordates. Cambridge Univ. Press, Cambridge, UK, pp. 118–123.

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

Phillips, R., Ager, A., 2002. Activation of pertussis toxin-sensitive CXCL12 (SDF-1) receptors mediates transendothelial migration of T lymphocytes across lymph node high endothelial cells. Eur. J. Immunol. 32, 837–847.

Sadir, R., Baleux, F., Grosdidier, A., Imberty, A., Lortat-Jacob, H., 2001. Characterization of the stromal cell-derived factor-1alpha-heparin complex. J. Biol. Chem. 276, 8288–8296.

Saitou, M., Barton, S.C., Surani, M.A., 2002. A molecular programme for the specification of germ cell fate in mice. Nature 418, 293–300.

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

Staller, P., Sulitkova, J., Lisztwan, J., Moch, H., Oakeley, E.J., Krek, W., 2003. Chemokine receptor CXCR4 downregulated by von Hippel–Lindau tumour suppressor pVHL. Nature 425, 307–311.

Starz-Gaiano, M., 2000. Molecular guidance cues and intracellular signaling in *Drosophila* germ cell migration. Thesis, New York Univ.

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

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

Thompson, J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22, 4673–4680.

Tsunekawa, N., Naito, M., Sakai, Y., Nishida, T., Noce, T., 2000. Isolation of chicken vasa homolog gene and tracing the origin of primordial germ cells. Development 127, 2741–2750.

von Heijne, G., 1986. A new method for predicting signal sequence cleavage sites. Nucleic Acids Res. 14, 4683–4690.

Weidinger, G., Stebler, J., Slanchev, K., Dumstrei, K., Wise, C., Lovell-Badge, R., Thisse, C., Thisse, B., Raz, E., 2003. dead end, a novel vertebrate germ plasm component, is required for zebrafish primordial germ cell migration and survival. Curr. Biol. 13, 1429–1434.

Wilkinson, D., 1992. In Situ Hybridisation; a Practical Approach Oxford Univ. Press, London.

Zeelenberg, I.S., Ruuls-Van Stalle, L., Roos, E., 2003. The chemokine receptor CXCR4 is required for outgrowth of colon carcinoma micrometastases. Cancer Res. 63, 3833–3839.

Zhu, Y., Yu, T., Zhang, X.C., Nagasawa, T., Wu, J.Y., Rao, Y., 2002. Role of the chemokine SDF-1 as the meningeal attractant for embryonic cerebellar neurons. Nat. Neurosci. 5, 719–720.

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