Histol Histopathol (2009) 24: 1097-1103

http://www.hh.um.es

Histology and Histopathology

Cellular and Molecular Biology

The expression of a novel *cxcr4* gene in *Xenopus* embryo

Edurne Alonso[1], Laura Gómez-Santos[1], Juan Francisco Madrid[2] and Francisco José Sáez[1]

[1]Department of Cell Biology and Histology, University of the Basque Country, Leioa (Vizcaya), Spain and [2]Department of Cell Biology and Histology, University of Murcia, Espinardo, Murcia, Spain

Summary. The aim of the present work was to identify a homologue of zebrafish cxcr4b in *Xenopus*, which could be involved in primordial germ cell (PGC) guidance migration. Following a BLAST analysis, the clone gi 27519681, homologous to the zebrafish gene z-cxcr4b, was identified, inserted into pCMV-SPORT6 plasmid and cloned in *Escherichia coli*. Embryonic expression of x-cxcr4b was analyzed by RT-PCR. X-cxcr4b was weakly expressed maternally but sharply increased after the mid-blastula transition (MBT), declining significantly at stage 45 when PGCs migration is complete. In contrast, RT-PCR of isolated presumptive PGCs showed strong maternal expression at stage 8, which decreased by stage 10 post-MBT and was not detected at stage 14. Whole mount in situ hybridization of x-cxcr4b mRNA showed that this gene is expressed in neural and haematopoietic tissues, and should be linked to important processes during embryonic development of these organs. Although weak staining could be seen in some samples within the anterior endoderm, expression of x-cxcr4b was never coincident with that of Xpat mRNA, which labels PGCs restricted to the posterior endoderm. Therefore, maternal x-cxcr4b is specifically downregulated within PGCs at pre-migratory stages while it is expressed in other tissues.

Key words: Primordial germ cells, Migration, Whole mount in situ hybridization, Haematopoiesis, Gene expression

Offprint requests to: Francisco José Sáez, Universidad del Pais Vasco/Euskal Herriko Unibertsitatea, Departamento de Biología Celular e Histología, Facultad de Medicina y Odontología, Bº Sarriena s/n, E-8949 Leioa, Vizcaya, Spain. e-mail: francisco.saez@ehu.es

Introduction

cxcr4 is the receptor for stromal cell-derived factor-1 (sdf1) (Ma et al., 1998; Horuk, 2001). A major function of the cxcr4/sdf1 signal system is to positively regulate guidance of cell migration with sdf1 attracting cxcr4 positive cells (Bleul et al., 1996; Zou et al., 1998; David et al., 2002).

The receptor cxcr4, a member of the G-protein-coupled-receptor family, was originally identified in leukocytes but is now known to function in chemotaxis in other cell types, including lymphocytes, neurons, endothelial cells and lateral line cells (Bleul et al., 1996; Nagasawa et al., 1998; Tachibana et al., 1998; Nagasawa, 2001; David et al., 2002; Lu et al., 2002) as well as in pathological situations, including metastasis and HIV-1 entry (Feng et al., 1996; Müller et al., 2001). Recently, several papers have shown that the cxcr4/sdf1 system is involved in primordial germ cell (PGC) migration in both mouse (Ara et al., 2003; Molyneaux et al., 2003) and zebrafish (Doitsidou et al., 2002; Knaut et al., 2003).

Two related *cxcr4* genes have been described in zebrafish, *z-cxcr4a* and *z-cxcr4b*, which are related to mammalian *cxcr4* (Chong et al., 2001). The function of mammalian cxcr4 could be split between the two zebrafish genes, *z-cxcr4a* and *b*; the former may function mainly in interneurons and the endoderm and the latter in sensory neurons, motoneurons and cerebellum (Chong et al., 2001). Some works have shown that *z-cxcr4b* is involved in PGC migration in zebrafish (Doitsidou et al., 2002; Knaut et al., 2003; Dumstrei et al., 2004), a role that has not been reported for *z-cxcr4a*.

Migration of the PGCs has been studied in *Xenopus*, but little is known about the mechanisms involved, including what guides them from the endoderm to the genital ridges (Gomperts et al., 1994; Houston and King, 2000; Horvay et al., 2005). Recent reports have identified *Xenopus* sdf1 and cxcr4 homologues during

early stages of embryo development, but no evidence exists about their involvement in PGC migration in this species (Moepps et al., 2000; Braun et al., 2002). The aim of the present work was to determine if a second *x-cxcr4* gene exists, analogous to z-cxcr4b in zebrafish, which could be involved in PGC migration. Here we report finding such a candidate gene.

Materials and methods

Sample preparation

Defolliculated oocytes were obtained from adult *Xenopus* females (Blades Biological, UK) by incubating ovarian fragments in 0.2% collagenase (type II, Sigma) in 0.1M sodium phosphate pH 7.4. Eggs were obtained after inducing females to ovulate by hCG injection (1000 U; Profasi HP; Serono). Eggs were fertilized in vitro by gently rubbing them with testis fragments. Embryos were reared in darkness in 1/10 Normal Amphibian Medium (NAM; Slack, 1984) and gentamicyn antibiotic (2.5 mg/100ml; Sigma) at 20°C in a Selecta Prebatem refrigerated incubator until the appropriate embryonic stage. Embryos were staged following the normal table from Nieuwkoop and Faber (1956). Eggs were dejellied with 1.25% cysteine (Sigma) pH 8.0 in NAM.

Cloning the x-cxcr4b gene

A BLAST analysis allowed us to identify the Xenopus clone gi 27519681 (accession number CA9870008), and the clone gi: 27924173 (accession number BC044963), as being homologous to the zebrafish gene *cxcr4b*. The second clone (BC044963) was acquired from a cDNA *Xenopus* stage 10 library (6863554 Cat# EXL1051, Open Biosystems). The clone was inserted into pCMV-SPORT6 plasmid, transformed in *Escherichia coli* XL 10 competent cells grown in ampicillin containing media. Plasmid mini preparations were made using the "JET quick Spin Column Technique" kit (Genomed).

RT-PCR

Total RNA was extracted from oocytes, eggs, and whole *Xenopus* embryos at different stages of embryonic development (4, 7, 10, 14, 25, 28, 32, 40 and 45) using Trizol Reagent (Invitrogen). The extracted RNA was purified with chloroform and acid phenol: chloroform:isoamilalcohol (Ambion) and finally precipitated with ethanol. The pellet was air dried and DNA impurities were eliminated by DNAseI digestion (Ambion) 15 minutes at 37°C. Samples were stored at -80°C.

Transcription reaction was carried out with the "MMLV" kit (Maloney murine leukaemia virus reverse transcriptase, GIBCO). The cDNA was amplified by PCR (30 cycles: 94°C 0:30 min; 60°C 0:30 min; 72°C 1:00 min, followed by a single incubation at 72°C for 10 min) using ^{32}P-dCTP labelled RT-primers. The primers have the following sequences: ACTGCATTTGGGA GAACACC (forward) and CTCAGAACGCATTCA CCTCA (reverse). The same procedure was carried out for the positive control odc amplification and the amplified cDNA fragments were fractionated on a 4% acrylamide gel at 100V.

RT-PCR of cDNA of isolated PGCs

Presumptive PGCs were isolated from embryos at stages 8, 10 and 14 as previously described (Venkataraman et al., 2004). Embryos were dissociated with Ca^{2+}- Mg^{2+} free Media (CMCF) and the PGCs selected manually. RNA was extracted from 50 isolated PGCs from each stage (8, 10 and 14) using the "Absolutely RNA Micropep Kit" (Stratagene). Transcription reaction was carried out with the "Super Smart PCR cDNA syntesis kit" (Klontech), the cDNA purified by column chromatography ("NucleoTrap PCR purification BD Biosciences" kit; Klontech), and amplified by PCR (30 cycles: 94°C 0:30 min; 60°C 0:30 min; 72°C 1:00 min, followed by a single incubation at 72°C for 10 min) using the same ^{32}dCTP RT-primers cited before.

Whole mount in situ hybridization

Whole mount in situ hybridization was done as previously described with slight modifications (Harland, 1991). In brief, oocytes, eggs and embryos (stages 10, 14, 25, 28, 36, 40 and 45) were fixed for 90 min at RT in MEMFA [0.1 M MOPS, pH 7.4, 2mM EGTA, 1mM $MgSO_4$ and 4%(w/v) paraformaldehyde] and then stored at -20°C in ethanol. Whole mount in situ hybridization was performed with digoxigenin-labelled cRNA probes. The plasmid was digested with SalI and NotI restriction enzymes. In situ hybridization was performed with digoxigenin-labeled antisense RNA transcribed in vitro using T7 RNA polymerase from an x-cxcr4b cDNA fragment using the DIG RNA labelling kit (SP6/T7) (Roche). Dig-labeled *Xpat* probe was used as a positive control, and the sense RNA sequence for *x-cxcr4b* as the negative one.

Results

Following a BLAST analysis, two *Xenopus* clones being homologous to the zebrafish gene *cxcr4b* were identified. Both clones (accession number CA9870008 and BC044963) were compared by Clustal analysis and a 99% consensus was shown. Thus, they represent the same gene now referred to as x-cxcr4b. X-cxcr4b amino acid sequence has a 97% similarity to an *x-cxcr4* gene previously cloned and a 60% similarity to z-cxcr4b. Furthermore, when cxcr4 from mouse, human, rat, zebrafish, and *Xenopus* were compared, sequence similarity was also very high (Fig. 1).

The expression of x-cxcr4b was analyzed by RT-

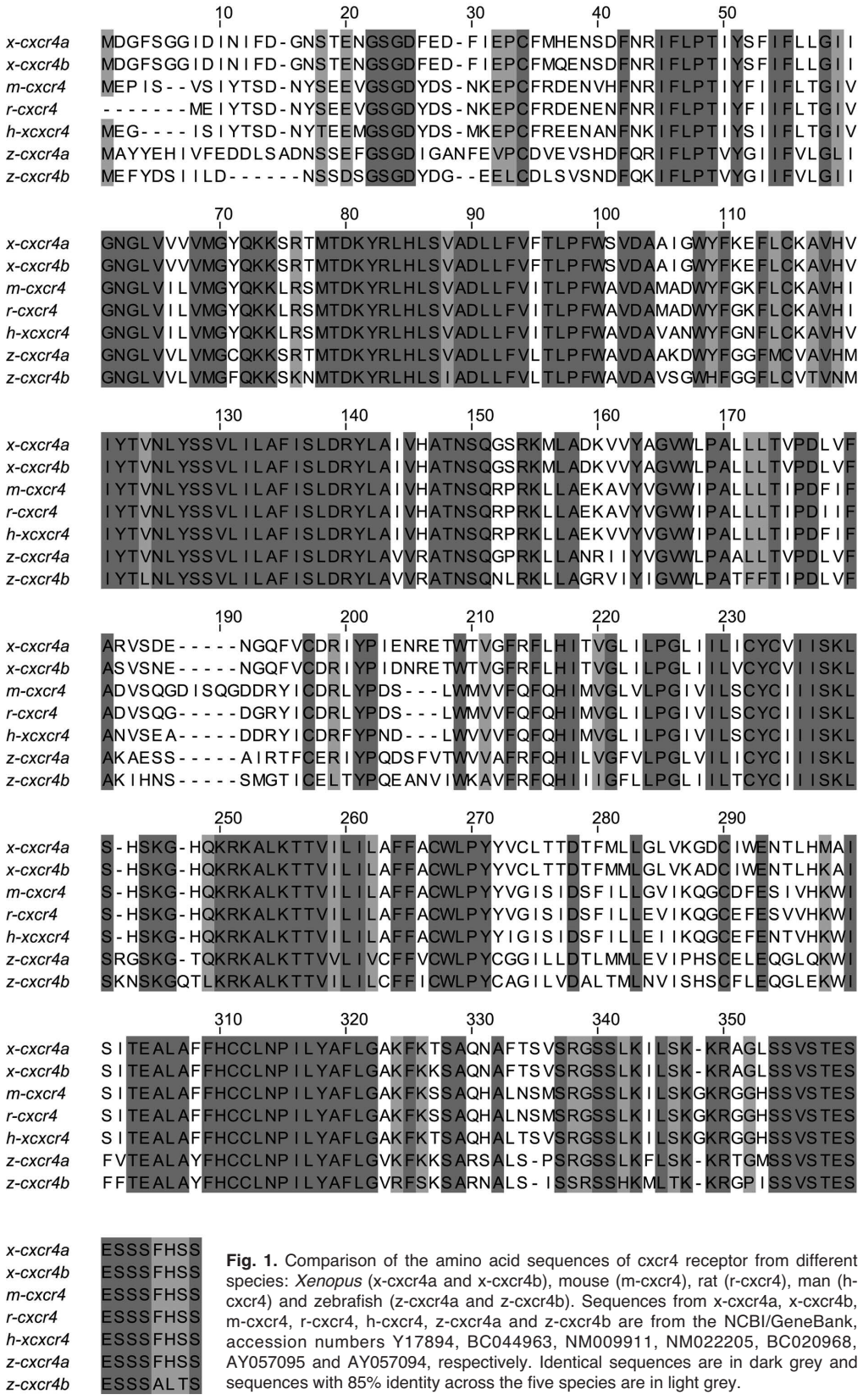

Fig. 1. Comparison of the amino acid sequences of cxcr4 receptor from different species: *Xenopus* (x-cxcr4a and x-cxcr4b), mouse (m-cxcr4), rat (r-cxcr4), man (h-cxcr4) and zebrafish (z-cxcr4a and z-cxcr4b). Sequences from x-cxcr4a, x-cxcr4b, m-cxcr4, r-cxcr4, h-cxcr4, z-cxcr4a and z-cxcr4b are from the NCBI/GeneBank, accession numbers Y17894, BC044963, NM009911, NM022205, BC020968, AY057095 and AY057094, respectively. Identical sequences are in dark grey and sequences with 85% identity across the five species are in light grey.

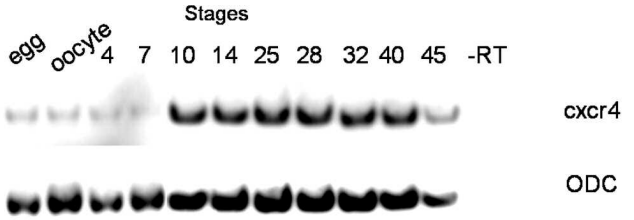

Fig. 2. RT-PCR analysis of x-cxcr4b m-RNA expression in *Xenopus* laevis embryos. X-cxcr4b shows a weak expression in oocytes, eggs and embryos before gastrulation (stages 4 and 7), but expression increases after stage 10 until stage 40. At stage 45, expression decreases. The gene odc was employed as a positive control. –RT: negative control.

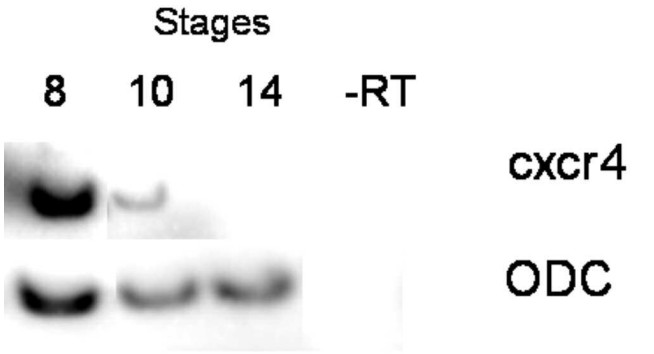

Fig. 3. RT-PCR analysis of x-cxcr4b m-RNA expression in *Xenopus* laevis presumptive PGCs isolated from embryos at stages 8, 10 and 14. Expression level was high at stage 8, decreased at stage 10 and was not detected at stage 14. The gene odc was used as positive control. –RT: negative control.

PCR in oocytes, eggs, and whole embryos at several stages of development, from early blastula to tailbud stage. In oocytes and early stages of development x-cxcr4b expression was weak, while zygotic expression at gastrulation remained high during early organogenesis, until stage 45, when expression diminished (Fig. 2).

Expression of x-cxcr4b was also detected by RT-PCR analysis in isolated presumptive PGCs (Fig. 3) isolated from embryos at blastula (stage 8) and early gastrulation (stage 10), but not at neurula (stage 14). No other stage was tested because identification and isolation of presumptive PGCs is uncertain in later stages.

Whole mount *in situ* hybridization analysis (Fig. 4) showed that expression of x-cxcr4b starts at gastrulation at the region of the blastopore in somatic cells but is not observed in PGCs (Fig. 4a-c). From stage 25 the expression was strongest in the nervous system, including forebrain, hindbrain, sensory organs (eyes and otic vesicles) and neural crest cells (Fig. 4d-f). At stage 36, x-cxcr4b mRNA was also detected abundantly in the structures which will develop into the embryonic and definitive haematopoietic cells: the dorsolateral plate and the ventral blood islands. In addition, some sparse endodermal cells were weakly labelled, but this labelling was clearly different to that obtained after in situ hibridization for Xpat, a marker for PGCs (Fig. 4g). At stage 40, ventral blood island labelling was weaker and a weak labelling at the dorsal side of the endoderm could be seen (Fig. 4h).

Discussion

CXCR4b is a conserved protein among vertebrates

Comparison of sequences by Clustal analysis of both acquired clones (accession number CA9870008 and BC044963) indicated a 99% consensus. Comparison of this gene with other *cxcr4* genes from other species, including another *x-cxcr4* previously cloned (Moepps et al., 2000) shows a very high sequence similarity. This high degree of conservation at the amino acid level for cxcr4 suggests it may have similar functions in all the organisms listed. Sdf1, the cxcr4 ligand, is also a conserved protein. Sdf1 from cat and human are identical, and differ only by 2% with respect to mouse sdf1 (Braun et al., 2002). Moreover, sdf1 from humans can activate x-cxcr4 (Moepps et al., 2000). We infer that the cxcr4/sdf1 system is a mechanism highly conserved during evolution because it is involved in basic and conserved developmental processes, such as migration of sensory neuron progenitors or neural stem cells, migration of the lateral line primordium, lymphocyte and endothelial maturation and, in some vertebrates, PGC migration (Bleul et al., 1996; Nagasawa et al., 1998; Tachibana et al., 1998; Nagasawa, 2001; David et al., 2002; Lu et al., 2002; Molyneaux et al., 2003; Dumstrei et al., 2004).

Maternal expression of X-cxcr4b increased in embryos but decreased in presumptive PGCs after MBT

RT-PCR of x-cxcr4b shows that expression increased in early embryos. These data suggest that after the midblastula transition, some embryonic cells increase their level of x-cxcr4b expression, preparing some tissues for morphogenetic movements. Two isoforms of sdf1, the cxcr4 ligand, have been identified in Xenopus. RT-PCR analysis of x-sdf1 has shown that expression starts at stage 18, with a weak signal that increases at stage 26, i.e., in later stages than x-cxcr4 (Braun et al., 2002; Fukui et al., 2007). However, the expression of another isoform, x-sdf1α, starts at stage 10-11 (Fukui et al., 2007). Thus, it is possible that x-sdf1α could behave as the ligand of x-cxcr4b at the first stages of embryonic development. This early expression of x-cxcr4b and x-sdf1α could be related with the morphogenetic movements carried out during gastrulation (Fukui et al., 2007).

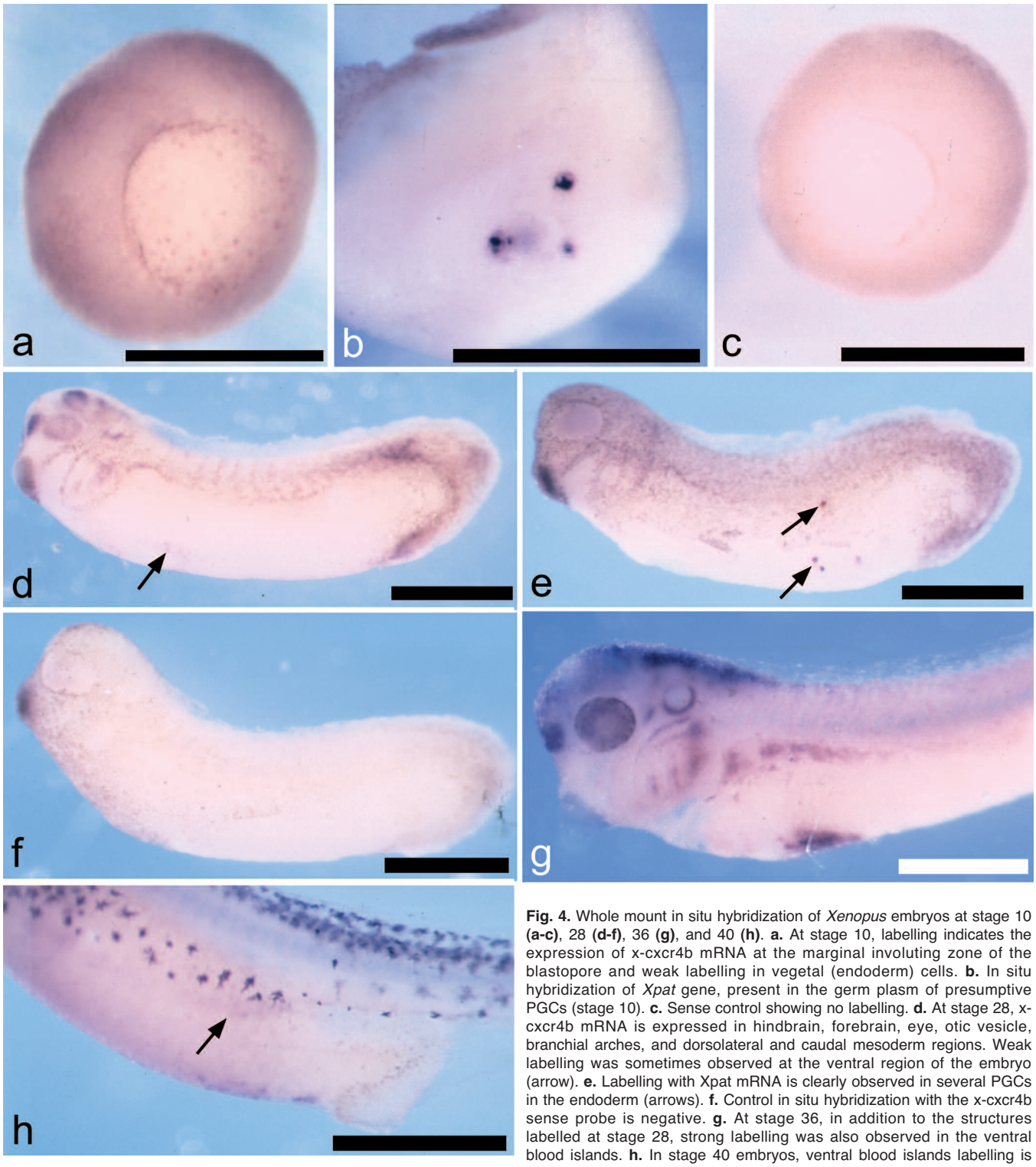

Fig. 4. Whole mount in situ hybridization of *Xenopus* embryos at stage 10 **(a-c)**, 28 **(d-f)**, 36 **(g)**, and 40 **(h)**. **a.** At stage 10, labelling indicates the expression of x-cxcr4b mRNA at the marginal involuting zone of the blastopore and weak labelling in vegetal (endoderm) cells. **b.** In situ hybridization of *Xpat* gene, present in the germ plasm of presumptive PGCs (stage 10). **c.** Sense control showing no labelling. **d.** At stage 28, x-cxcr4b mRNA is expressed in hindbrain, forebrain, eye, otic vesicle, branchial arches, and dorsolateral and caudal mesoderm regions. Weak labelling was sometimes observed at the ventral region of the embryo (arrow). **e.** Labelling with Xpat mRNA is clearly observed in several PGCs in the endoderm (arrows). **f.** Control in situ hybridization with the x-cxcr4b sense probe is negative. **g.** At stage 36, in addition to the structures labelled at stage 28, strong labelling was also observed in the ventral blood islands. **h.** In stage 40 embryos, ventral blood islands labelling is weaker, and endodermic staining is more dorsal (arrow), perhaps due to migratory PGCs at dorsal positions of the endoderm, reaching the dorsal mesentery. Bars: 1 mm.

RT-PCR of isolated presumptive PGCs from early embryos shows that x-cxcr4b was decreasing after gastrulation (Fig. 3). This finding is in contrast to the x-cxcr4b expression pattern found in somatic cells, where maternal expression was low (Fig. 2). This suggests that early presumptive PGCs may down regulate x-cxcr4b RNA prior to neurulation in contrast to somatic cells. PGCs may translate X-CRCR4 early to prepare for their migratory movements later. However, it must be noted again that other endodermal cells express cxcr4 during gastrulation (Fukui et al., 2007). Thus, the diminished maternal expression of x-cxcr4b in PGCs at the onset of gastrulation may reveal another early function for it in PGCs and not be related to PGC migration and differentiation.

X-cxcr4b is expressed in neural and haematopoietic tissues

The expression pattern of x-cxcr4b shows by whole mount in situ hybridization during gastrulation at the region of the blastopore in an expression pattern also reported by Fukui et al. (2007). Whole mount in situ hybridization at tailbud stages (from stages 25 and next) shows expression in the nervous system and in the dorsolateral plate and the ventral blood islands, which develop in haematopoietic tissues (Lane and Sheets, 2002). We cannot state that PGCs are expressing x-cxcr4b, because the labelling observed in some endodermal cells differs to that of the PGC marker Xpat (Hudson and Woodland, 1998). However, the weak labelling of the dorsal endoderm at stage 40 is coincident with the position of PGCs at this stage, and it could be suggested that this labelling is due to migratory PGCs. In other previous work, immunocytochemistry with anti-mouse CXCR4 antibody labels a small number of PGC in embryos at stage later than 24, and not all of them (see fig 7A'' from Nishiumi et al., 2005), but this labelling is rarely observed in any PGC of stage 40 embryos (Nishiumi et al., 2005). If we assume that not all the PGCs are expressing cxcr4, then the question that must be answered is if the cxcr4/sdf1 system is involved in *Xenopus* PGC migration. Preliminary experiments performed in our laboratory showed that presumptive PGCs isolated from gastrulating *Xenopus* embryos migrate in vitro towards heparin beads embedded with the product of x-sdf1 (unpublished data). RT-PCR analysis of x-cxcr4b expression in isolated PGCs at these later stages could resolve this issue.

In summary, we have identified and cloned the x-cxcr4b in *Xenopus* and analysed its expression by RT-PCR and in situ hybridization in early embryos. The results suggest that this gene is expressed in the central nervous system, neural crest cells, pronephric ducts and haematopoietic tissues. Furthermore, this gene is maternally expressed in PGCs, declines during a quiescent period while PGCs are in the endoderm and may be re-expressed later when PGCs are actively migrating, as suggested by weak dorsal endoderm labelling.

Acknowledgements. This work was supported by grants from the University of the Basque Country (EA 137-97 and E-15927/2004) to FJS. EA was supported by a fellowship from the Spanish Ministerio de Educación y Ciencia. EA made a stage in the laboratory of Mary Lou King (University of Miami, supported by a grant from the NIH R01 (GM33932) to MLK). Thanks are given to Mary Lou King for advice and for a critical review of the manuscript; E. Dancause and Thiagarajan Venkataraman for expert assistance during Edurne Alonso's visit (University of Miami, Miller School of Medicine, Miami); and M. Portuondo and C. Otamendi (Universidad del País Vasco/Euskal Herriko Unibertsitatea) for his expert technical assistance. M. J. Aldasoro (Universidad del País Vasco/Euskal Herriko Unibertsitatea) assists the authors in office work.

References

Ara T., Nakamura Y., Egawa T., Sugiyama T., Abe K., Kishimoto T., Matsui Y. and 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. USA 100, 5319-5323.

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

Braun M., Wunderlin M., Spieth K., Knöchel W., Gierschik P. and Moepps B. (2002). *Xenopus laevis* stromal cell-derived factor 1: conservation of structure and function during vertebrate development. J. Immunol. 168, 2340-2347.

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

David N.B., Sapede D., Saint-Etienne L., Thisse B., Dambly-Chaudiere C., Rosa F.M. and Ghysen A. (2002). Molecular basis of cell migration in the fish lateral line: role of the chemokine receptor CXCR4 and of its ligand, SDF1. Proc. Natl. Acad. Sci. USA 99, 16297-16302.

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

Dumstrei K., Mennecke R. and Raz E. (2004). Signaling pathways controlling primordial germ cell migration in zebrafish. J. Cell Sci. 117, 4787-4795.

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

Fukui A., Goto T., Kitamoto J., Homma M. and Asashima M. (2007). SDF-1α regulates mesendodermal cell migration during frog gastrulation. Biochem. Biophys. Res. Commun. 354, 472-477.

Gomperts M., Wylie C. and Heasman J. (1994). Primordial germ cell migration. Ciba Found. Symp. 182, 121-139.

Harland R.M. (1991). In situ hybridization: an improved whole-mount method for *Xenopus* embryos. Methods Cell. Biol. 36, 685-695.

Horuk R. (2001). Chemokine receptors. Cytokine Growth Factor Rev. 12, 313-335.

Horvay K., Claußen M., Katzer M., Landgrebe J. and Pieler T. (2005). *Xenopus* Dead end mRNA is a localized maternal determinant that serves a conserved function in germ cell development. Dev. Biol. 291, 1-11.

Houston D.W. and King M.L. (2000). A critical role for Xdazl, a germ plasm-localized RNA, in the differentiation of primordial germ cells in *Xenopus*. Development 127, 447-456.

Hudson C. and Woodland H.R. (1998). *Xpat*, a gene expressed specifically in germ plasm and primordial germ cells of *Xenopus* laevis. Mech. Dev. 73, 159-168.

Knaut H., Werz C., Geisler R., Tübingen 2000 Screen Consortium and Nüsslein-Volhard C. (2003). A zebrafish homologue of the chemokine receptor Cxcr4 is a germ-cell guidance receptor. Nature 421, 279-282.

Lane M.C. and Sheets M.D. (2002). Primitive and definitive blood share a common origin in Xenopus: a comparison of lineage techniques used to construct fate maps. Dev. Biol. 248, 52-67.

Lu M., Grove E.A. and Miller R.J. (2002). Abnormal development of the hippocampal dentate gyrus in mice lacking the CXCR4 chemokine receptor. Proc. Natl. Acad. Sci. USA 99, 7090-7095.

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

Moepps B., Braun M., Knopfle K., Dillinger K., Knochel W. and Gierschick P. (2000). Characterization of a *Xenopus laevis* CXC chemokine receptor 4: implications for hematopoietic cell development in the vertebrate embryo. Eur. J. Immunol. 30, 2924-2934.

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

Müller A., Homey B., Soto H., Ge N., Catron D., Buchanan M.E., McClanaham T., Murphy E., Yuan W., Wagner S.N., Barrera J.L., Mohar A., Verástegui E. and Zlotnik A. (2001). Involvement of chemokine receptors in breast cancer metastasis. Nature 410, 50-56.

Nagasawa T. (2001). Role of chemokine SDF-1/PBSF and its receptor CXCR4 in blood vessel development. Ann. N. Y. Acad. Sci. 947, 112-115. discussion 115-116.

Nagasawa T., Tachibana K. and Kishimoto T. (1998). A novel CXC chemokine PBSF/SDF-1 and its receptor CXCR4: their functions in development, hematopoiesis and HIV infection. Semin. Immunol. 10, 179-185.

Nieuwkoop P.D. and Faber J. (1956). Normal table of *Xenopus laevis* (Daudin). North-Holland Publishing. Amsterdam.

Nishiumi F., Komiya T. and Ikenishi K. (2005). The mode and molecular mechanisms of the migration of presumptive PGC in the endoderm cell mass of Xenopus embryos. Dev. Growth Differ. 47, 37-48.

Slack J.M.W. (1984). Regional biosynthetic markers in the early amphibian embryo. J. Embryol. Exp. Morphol. 80, 289-319.

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

Venkataraman T., Dancause E. and King M.L. (2004). PCR-Based cloning and differential screening of RNAs from *Xenopus* primordial germ cells: cloning uniquely expressed RNAs from rare cells. Methods Mol. Biol. 254, 67-78.

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

Accepted March 4, 2009