Int. J. Dev. Biol. 55: 335-340 (2011)
doi: 10.1387/ijdb.103258cg

THE INTERNATIONAL JOURNAL OF DEVELOPMENTAL BIOLOGY
www.intjdevbiol.com

CXCL14 expression during chick embryonic development

CHRISTOPHER T. GORDON*, CHRISTINE WADE, INIGO BRINAS and PETER G. FARLIE

Craniofacial Development Laboratory, Murdoch Children's Research Institute, Royal Children's Hospital, Parkville, Australia

ABSTRACT Chemokines are small secreted signalling molecules best known for their roles as chemoattractants for cells of the immune system. CXCL12 and its receptor CXCR4 comprise one chemokine signalling pathway with essential functions in non-immune cell types during embryonic development. *CXCL14*, a chemokine-encoding gene related to *CXCL12*, is developmentally regulated in zebrafish and *Xenopus* embryos, but its role during embryogenesis remains unknown. Here we describe the embryonic expression pattern of *CXCL14* in an amniote, the chick. Although expression in some regions is conserved with that of fish and frog, chick *CXCL14* displays a complex pattern of expression in several novel sites. We analyse the expression pattern in the branchial arches, trigeminal placode and ganglion, inner ear, dorsal midline of the brain, somites, trunk neural tube and limb bud. Expression in several domains raises the possibility that CXCL14 may be involved in some of the same developmental events during which CXCL12-CXCR4 signalling is known to play a role.

KEY WORDS: *chemokine, trigeminal, mesencephalon, somite, otic vesicle.*

Chemokines are a large family of secreted proteins that have historically been investigated in the context of immune cell trafficking. In this paradigm, chemokines act as chemoattractants, recruiting immune cells that express G-protein coupled chemokine receptors. However, some chemokines and their receptors have roles outside the haematopoietic system. The most extensively studied chemokine system during embryonic development consists of the CXCL12 ligand and CXCR4 receptor. Initial investigation of *Cxcl12* and *Cxcr4* knockout mice revealed that in addition to requirements in haematopoietic cells, these genes had essential roles in cardiac, cerebellar and vascular development (Ma *et al*., 1998; Nagasawa *et al*., 1996; Tachibana *et al*., 1998; Zou *et al*., 1998). Subsequently, it was demonstrated that the *cxcl12-cxcr4* pathway was required for migration of primordial germ cells in zebrafish and mice (Doitsidou *et al*., 2002; Knaut *et al*., 2003; Molyneaux *et al*., 2003), for migration of the lateral line primordium in zebrafish (Dambly-Chaudiere *et al*., 2007; David *et al*., 2002; Haas and Gilmour, 2006; Valentin *et al*., 2007), and for coordinating migration of germ layers during zebrafish gastrulation (Nair and Schilling, 2008). In the mouse cerebral cortex and cerebellum, positioning of Cajal-Retzius cells and granule cells, respectively, is regulated by *Cxcl12* and *Cxcr4* (Borrell and Marin, 2006; Ma *et al*., 1998; Paredes *et al*., 2006; Reiss *et al*., 2002; Zhu *et al*., 2002). Roles for *Cxcl12* and *Cxcr4* during migration of muscle progenitors (Vasyutina *et al*., 2005) and cranial neural crest migration (Olesnicky Killian *et al*., 2009; Theveneau *et al*., 2010) have also been described. A common theme in most of these examples is expression of *Cxcl12* in a tissue through which *Cxcr4*-expressing cells migrate, while in some cases *Cxcl12* is required to enable *Cxcr4*-positive cells to maintain a fixed position relative to the ligand (reviewed in Raz and Mahabaleshwar, 2009).

Initial characterisation of *CXCL14* indicated that although it was widely expressed in normal tissues (as assessed by Northern blotting), its expression was reduced in tumour cell lines and tumour-derived tissue (Cao *et al*., 2000; Frederick *et al*., 2000; Hromas *et al*., 1999; Sleeman *et al*., 2000). Also, forced expression in mice has demonstrated that Cxcl14 suppresses tumour growth (Izukuri *et al*., 2010; Ozawa *et al*., 2006; Tessema *et al*., 2010). On the other hand, CXCL14 can act as an autocrine stimulator of fibroblast growth and migration (Augsten *et al*., 2009). Although the identity of its receptor is unknown, CXCL14 has been shown to function as a chemoattractant for some immune cell types (Kurth *et al*., 2001; Schaerli *et al*., 2005; Shellenberger *et al*., 2004; Shurin

Abbreviations used in this paper: AER, apical ectodermal ridge; HH, Hamburger-Hamilton.

***Address correspondence to:** Christopher T. Gordon. INSERM U781, Tour Lavoisier – Deuxième Etage, Hôpital Necker-Enfants Malades, 149 Rue de Sèvres, 75743 Paris Cedex 15, France. E-mail: chris.gordon@inserm.fr

Supplementary Material (four figures) for this paper is available at: http://dx.doi.org/10.1387/ijdb.103258cg

Accepted: 11 January 2011. Final, author-corrected PDF published online: 15 June 2011.

ISSN: Online 1696-3547, Print 0214-6282

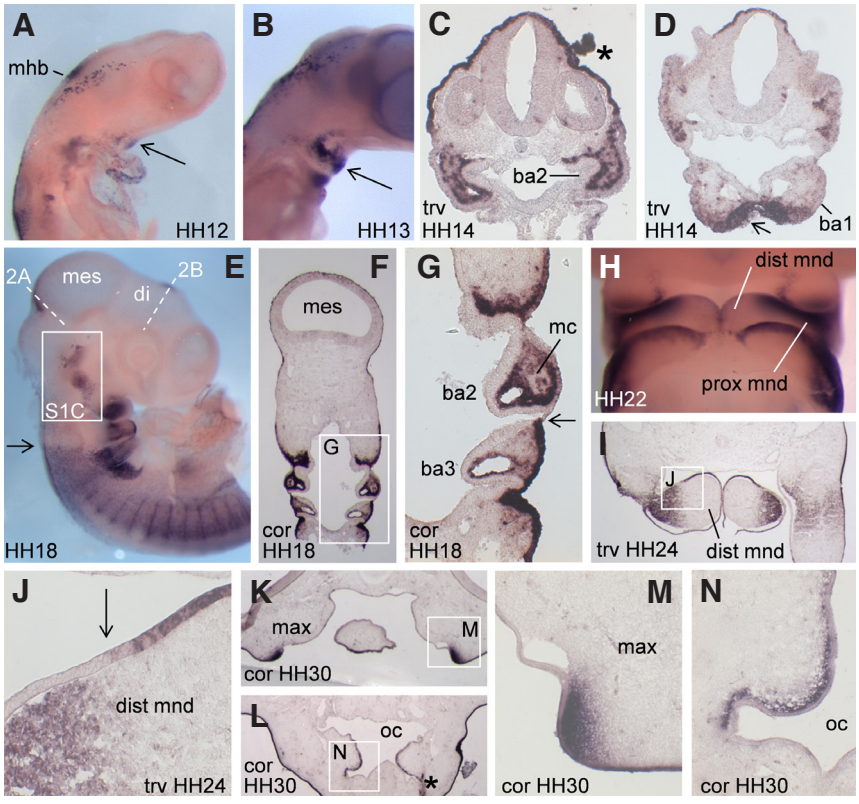

Fig. 1. Expression of *CXCL14* during development of the branchial arches. (A) *HH12.* **(B)** *HH13.* **(C)** *HH14, transverse section through the second branchial arch.* **(D)** *HH14, transverse section through the first branchial arch. Arrows in (A,B,D) indicate expression in first branchial arch epithelium.* **(E-G)** *HH18. White dashed lines in (E) indicate the approximate plane of sectioning through the mesencephalon and diencephalon in Fig. 2 A,B, respectively. The boxed region in (E) is shown at higher magnification in Supplementary Fig. 1C.* **(F)** *Coronal section.* **(G)** *Higher magnification image of the region boxed in (F). Black arrows in (E,G) indicate the boundary of surface ectoderm expression between the 2nd and 3rd branchial arches.* **(H)** *HH22.* **(I,J)** *HH24, transverse section.* **(J)** *Higher magnification image of the box in (I). The arrow in (J) indicates a mesenchymal-epithelial boundary of expression in the distal mandible.* **(K,L,M,N)** *HH30, coronal sections. (M,N) Higher magnification images of the boxed regions in (K,L), respectively. Asterisks indicate staining artifacts. mhb, midbrain-hindbrain boundary; ba1, 1st branchial arch; ba2, 2nd branchial arch; ba3, 3rd branchial arch; mes, mesencephalon; di, diencephalon; mc, mesodermal core; dist mnd, distal mandible; prox mnd, proximal mandible; max, maxilla; oc, oral cavity; trv, transverse; cor, coronal.*

et al., 2005; Starnes *et al.*, 2006). *Cxcl14* knockout mice have defects in feeding behaviour (Tanegashima *et al.*, 2010) and in glucose metabolism (Nara *et al.*, 2007), but have a normal immune system (Meuter *et al.*, 2007). Both groups that have knocked out *Cxcl14* reported that a lower than expected number of null mice were recovered in the perinatal period (Meuter *et al.*, 2007; Nara *et al.*, 2007; Tanegashima *et al.*, 2010). Although no major phenotypic abnormalities were noted, this finding may indicate a subtle developmental defect that initiates during embryonic stages. Embryonic functions for *Cxcl14* remain unexplored. In zebrafish embryos, *cxcl14* is expressed transiently in discrete domains of the brain and otic vesicle, and in cells of the anterior and posterior lateral line (Long *et al.*, 2000). In *Xenopus*, *CXCL14* is expressed in specific regions of cranial ectoderm, and in the dorsal retina and head mesenchyme (Park *et al.*, 2009). Park *et al.*, (2009) noted that the only region of potential overlap between *Xenopus* and zebrafish *CXCL14* expression was within the otic vesicle. In this report, we examine the embryonic *CXCL14* expression pattern in an amniote, the chick, and compare it to that reported for zebrafish and *Xenopus*. Our results indicate some similarities in expression amongst these species, but several sites of expression not reported in the anamniotes. Some of the developmental events during which chick *CXCL14* expression is observed raise the intriguing possibility that this chemokine may function in parallel to the CXCL12-CXCR4 ligand-receptor system.

Results and Discussion

Branchial arches

We examined *CXCL14* expression in chick by whole-mount and section *in situ* hybridisation at several stages during embryogenesis. During craniofacial development, *CXCL14* is regionally expressed in both branchial arch epithelium and cranial neural crest-derived mesenchyme (Fig. 1). At early stages of first branchial arch expansion, *CXCL14* is expressed within mandibular epithelium (Fig.1 A,B,D); this domain increases between Hamburger-Hamilton (HH) stages 12 and 13 (Fig. 1 A,B). In *Xenopus*, *CXCL14* is expressed specifically in the cement gland, a thickened layer of ectoderm immediately ventral to the prospective oral opening (Park *et al.*, 2009). This epithelial expression on the ventral side of the mouth in both species may indicate homologous regulation of expression. In the second branchial arch, *CXCL14* is widely expressed in mesenchymal but not epithelial cells, while dorsal ectoderm at the same axial level strongly expresses *CXCL14* (Fig. 1C). At HH18, expression is greatly reduced in cranial surface ectoderm relative to that posterior to the otic vesicle (Fig. 1E). This difference is also evident at the junction of surface ectoderm covering the second and third branchial arches (Fig. 1 F,G). Between HH18-24, *CXCL14* expression in mandibular mesenchyme is more extensive proximally than distally (Fig. 1 H,I and data not shown). Interestingly, the domain of mesenchymal expression along the proximodistal axis of the mandible ceases at a point where expression in the distal mandibular epithelium begins (Fig. 1 I,J). Note also there are scattered positive cells within the mesodermal core (Fig. 1 F,G); a similar pattern has been observed for some genes expressed within the neural crest cells that invade this tissue (Grenier *et al.*, 2009). In first branchial arch derivatives at HH30, a stage subsequent to the initiation of skeletal differentiation, *CXCL14* continues to be expressed in limited domains of the upper and lower jaws, including a maxillary mesenchyme domain at the junction of expressing and non-expressing epithelia (Fig. 1 K,M), and a thin layer of mesenchyme immediately subjacent to lateral oral epithelium (Fig. 1 L,N).

Trigeminal placode and ganglion

In surface ectoderm between HH7-9, *CXCL14* is expressed

in caudal regions of the embryo and as far anterior as the region overlying the caudal hindbrain, but is absent from cranial surface ectoderm during the same period (Supplementary Fig. 3A and data not shown). By HH12 however, expression is observed in scattered cells in a bilateral swathe adjacent to the midbrain (Fig. 1A and Supplementary Fig. 1A); this distribution is reminiscent of expression of *PAX3*, a marker of the ophthalmic trigeminal placode (Stark *et al.*, 1997). Transverse sections through this region at HH13 indicate that the positive cells are indeed within the surface ectoderm, rather than within underlying mesenchyme (Supplementary Fig. 1B). Between HH17-22 *CXCL14* is expressed in cells of both the ophthalmic and maxillomandibular lobes of the trigeminal placode and ganglion (Supplementary Fig. 1 C-I). At HH17, expression is seen in streams of cells ingressing from the placode into the ganglion (Supplementary Fig. 1D). By HH21, portions of the trigeminal ganglion retain a connection to the surface ectoderm, via *CXCL14*-expressing cells, while small clumps of cells in the surface ectoderm continue to express *CXCL14* without an obvious connection to the ganglion (Supplementary Fig. 1E). By comparing the distribution of ectodermal cells overlying the trigeminal ganglion at HH18 (Supplementary Fig. 1C) and HH22 (Supplementary Fig. 1F), it can be seen that loose clusters of *CXCL14*-expressing cells resolve into discrete clumps. The latter typically overlie the ophthalmic branch of the trigeminal ganglion, in some cases protruding prominently from the surface (Supplementary Fig. 1G), and may correspond to ectopic ganglia described previously within the chick surface ectoderm in a similar region (Kuratani and Hirano, 1990). Cells expressing *CXCL14* in the trigeminal ganglion are mainly localised in the distal portion of the ganglion (Supplementary Fig. 1I), which is typical of trigeminal placode-derived sensory neurons, in contrast to neural crest-derived neurons that reside more proximally (D'Amico-Martel and Noden, 1983). In zebrafish, *cxcr4b* is required for correct positioning of sensory neurons within the developing trigeminal ganglion (Knaut *et al.*, 2005), and based on our expression analysis, chick *CXCL14* may also be involved in assembly of this ganglion. Trigeminal expression was not reported for zebrafish or *Xenopus CXCL14* (Long *et al.*, 2000; Park *et al.*, 2009), however in *Xenopus*, the transcription factor PAX3 is necessary and sufficient for *CXCL14* expression in the hatching gland, a specialisation of the cranial ectoderm (Park *et al.*, 2009). In the future it will be of interest to test whether chick *CXCL14* is also downstream of PAX3 in the ophthalmic trigeminal placodal ectoderm. The lack of *PAX3* expression in the maxillomandibular lobe suggests that *CXCL14* would be downstream of other transcription factors in that portion of the trigeminal. In addition to the trigeminal, we observed expression of *CXCL14* in scattered cells of other cranial ganglia (data not shown). *CXCL14* is also expressed in the anterior half of Rathke's pouch, a placodal epithelium that develops as an outpocketing of the oral cavity (Supplementary Fig. 1J).

Otic vesicle and hindbrain

At HH13, *CXCL14* is expressed in the dorsal surface ectoderm surrounding the invaginating otic vesicle, but is excluded from the thickened epithelium of the vesicle itself (Supplementary Fig. 2A and 3B). However by HH22 two patches of expression can be discerned at the anterior and posterior limits of the otic vesicle

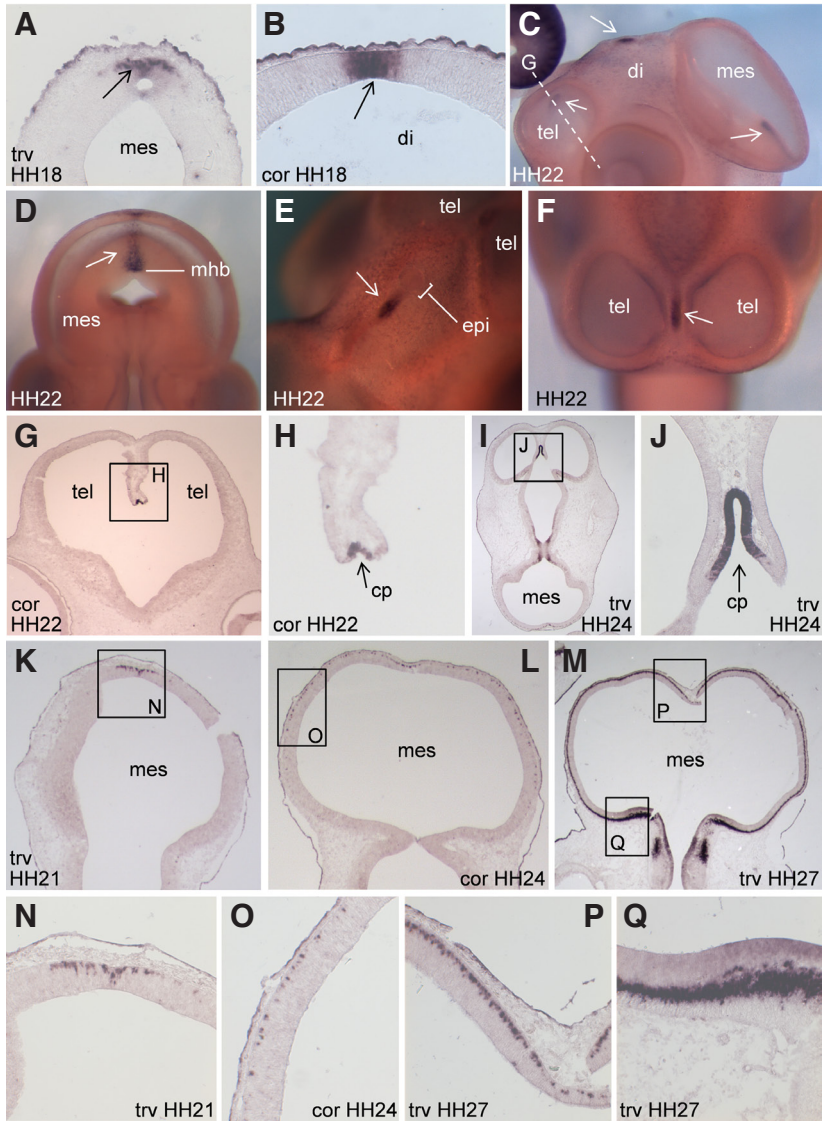

Fig. 2. Expression of *CXCL14* in the dorsal mesencephalon, diencephalon and telencephalon. (A,B) *HH18. (A) Transverse section through the caudal mesencephalon; (B) coronal section through the diencephalon, at the positions indicated in Fig. 1E.* **(C-H)** *HH22. White dashed line in (C) indicates the approximate plane of sectioning through the telencephalon in (G). In (D), the rostral mesencephalon has been removed to allow visualisation of* CXCL14 *expression in the dorsal midline of the caudal mesencephalon. Arrows in (A-F) indicate expression in the dorsal midline of the cephalic vesicles. (G,H) Coronal section; (H) is a magnified view of the box in (G).* **(I,J)***HH24, transverse section; (J) is a magnified view of the box in (I).* **(K-Q)** *expression of* CXCL14 *in migratory cells of the dorsal mesencephalon. (K,N) HH21, transverse section. (N) is a magnified view of the box in (K). (L,O) HH24, coronal section. (O) Magnified view of the box in (L). (M, P, Q) HH27, transverse section. (P,Q) Magnified images of the boxed regions in (M). tel, telencephalon; di, diencephalon; mes, mesencephalon; mhb, midbrain-hindbrain boundary; epi, epiphysis; cp, choroid plexus; trv, transverse; cor, coronal.*

(Supplementary Fig. 2B, and similarly at HH24 in Supplementary Fig. 2C). By HH26 a third major domain is distinct (Supplementary Fig. 2E). These three areas most likely correspond to the anterior, posterior and lateral cristae, based on comparison with published markers of these prosensory domains, such as *BMP4* and *SER1* (Adam *et al.*, 1998; Cole *et al.*, 2000; Wu and Oh, 1996). Prosensory patches give rise to the mechanotransducing hair cells and their support cells. *CXCL14* may additionally be expressed within the saccular macula, another sensory patch of the vestibule (Supplementary Fig. 2G). Notably, zebrafish *cxcl14* is also expressed specifically in sensory patches of the inner ear (Long *et al.*, 2000), suggesting conservation of transcriptional regulation between fish and birds, in this organ.

Between HH22-26, we observed prominent expression of *CXCL14* in the hindbrain (Supplementary Fig. 2 B,D-F), with the strongest expression occurring in a region adjacent to the otic vesicle. In zebrafish, *cxcl14* is also expressed in specific populations of cells within the hindbrain (Long *et al.*, 2000).

Telencephalon, diencephalon and mesencephalon

CXCL14 expression is successively activated in a caudal to rostral fashion in the brain. At HH12-13, the sole site of *CXCL14* expression in the central nervous system is a patch in the caudal-most region of the dorsal mesencephalon (Fig. 1A and Supplementary Fig. 1 A,B). Although expression in the dorsal midline of the forebrain is not evident at these stages, by HH18 a small domain of expression is observed in the dorsal diencephalon (Fig. 1E and 2B), with expression in the mesencephalon continuing at this stage (Fig. 1E and 2A). By HH22 *CXCL14* is expressed in the dorsal telencephalon (Fig. 2 C,F-H), with maintenance of expression in the mesencephalon and diencephalon at the same stage (Fig. 2 C-E). Central nervous system expression for *Xenopus CXCL14* was not reported (Park *et al.*, 2009), however Long *et al.,* (2000) described zebrafish *cxcl14* expression in the presumptive epiphyseal region of the diencephalon, the midbrain-hindbrain boundary, and the cerebellum. The expression domain for chick *CXCL14* in the dorsal diencephalon lies immediately posterior to the epiphysis (Fig. 2E); therefore this site plausibly represents regulatory conservation between chick and zebrafish. Expression at the midbrain-hindbrain boundary also appears similar between the two species, however we did not observe cerebellar expression for chick *CXCL14*. Indeed, the expression at the midbrain-hindbrain boundary is confined to the midbrain side of this boundary in chick (Fig. 2D). The *CXCL14* expression domain in the dorsal telencephalon may correspond to a portion of the prospective choroid plexus (Fig. 2F-J). This *CXCL14*-expressing region expands between HH22 (Fig. 2H) and HH24 (Fig. 2J).

Although at HH18 *CXCL14* expression in the dorsal mesencephalon is confined to a small patch (Fig. 2A), we noticed that between HH21-27, individual *CXCL14*-expressing cells are progressively distributed more ventrally (Fig. 2 K-M; magnified images of the ventral-most cells in the respective sections are shown in Fig. 2 N,O,Q). This pattern is suggestive of expression in a tangentially migrating population. Note that the positive cells are confined to a very superficial layer throughout most of this putative migratory route (Fig. 2 O,P), but are located in a deeper layer in the most ventral region of the mesencephalon (Fig. 2Q). By HH30, *CXCL14*-positive cells along this entire putative migratory route also appeared to have shifted to a deeper layer within the mesencephalon (data not shown). The expression pattern of *CXCL14* in the mesencephalon is intriguing in light of known roles of Cxcl12 and Cxcr4 in neuronal migration. For example, in the mouse telencephalon *Cxcr4* is expressed in Cajal-Retzius cells, a population that migrates tangentially within the marginal zone, while *Cxcl12* is expressed in the meningeal layer underlying the marginal zone. Studies in targeted mice suggest that Cxcl12, secreted by the meninges, is required for correct positioning of Cxcr4-positive Cajal-Retzius cells. In the case of chick *CXCL14*, although it may seem counterintuitive for a secreted chemokine ligand to be expressed in migrating cells, it has been reported that a chemotactic response of myeloid dendritic cells to activin A depends on the production of CXCL14 and CXCL12 from the migrating cells themselves (Salogni *et al.*, 2009). The identity of the *CXCL14*-expressing cells in the mesencephalon awaits further investigation. The possibility that *Cxcl14* has developmental or regulatory functions in the mammalian brain is supported by the recent finding that *Cxcl14* knockout mice have defects in feeding behaviour when placed in a novel environment (Tanegashima *et al.*, 2010).

Somitogenesis

At HH9-10, we observed prominent *CXCL14* expression in somites and surface ectoderm, but not in endoderm, lateral plate mesoderm or neuroepithelium (Supplementary Fig. 3 A,D). During early somitogenesis, the intensity of expression within individual somites varies according to the axial level, such that expression in the oldest somites (ie, the most anterior) is lower than that in younger somites (Supplementary Fig. 3 A-C). At HH13, there is a striking rostrocaudal polarity of expression within individual somites, with expression restricted to the posterior half (Supplementary Fig. 3C). By HH18, a dorsoventral restriction within the somite is apparent, with exclusion from the dorsal and ventral lips of the dermomyotome (Supplementary Fig. 3E). Several other genes with polarised rostrocaudal somitic expression have been reported, including some members of the NOTCH signalling pathway (Rodrigues *et al.*, 2006). Chick *CXCL14* may therefore be regulated by this pathway. Notably, in zebrafish *cxcl12a* displays restriction to the posterior portion of early somites, similar to chick *CXCL14*, while the duplicated receptor genes *cxcr4a* and *cxcr4b* are expressed in the anterior portion (Chong *et al.*, 2001; Chong *et al.*, 2007). In contrast, chick *CXCR4* expression appears restricted to the posterior half of early somites (Yusuf *et al.*, 2005). Although we have observed complex somitic expression for chick *CXCL14*, expression of the zebrafish and *Xenopus* orthologues of *CXCL14* was not reported in somites (Long *et al.*, 2000; Park *et al.*, 2009). An intriguing possibility is that different vertebrates utilise different chemokine signalling components during anteroposterior somitic patterning.

Spinal cord

At HH18, *CXCL14* is expressed in a salt and pepper fashion within the ventral half of the trunk neural tube (Supplementary Fig. 3F). However by HH23, this pattern resolves into a ventral to dorsal gradient within the ventral neural tube, with very prominent expression within the floorplate (Supplementary Fig. 3I). In the dorsal neural tube, *CXCL14* is expressed in a narrow bilateral strip of cells that extends throughout most of the trunk rostrocaudal axis (Supplementary Fig. 3G-I). These cells may correspond to dorsal interneurons (Liem *et al.*, 1997). Expression of *CXCL14* in the trunk neural tube of *Xenopus* or zebrafish was not reported. Further

studies will be required to identify the cell types expressing chick *CXCL14* in the neural tube.

Limbs

Although *CXCL14* expression is widespread in the dorsal and ventral surface ectoderm of the limb bud, it is excluded from the apical ectodermal ridge (AER) throughout limb bud development (Supplementary Fig. 4A,B). The AER is a specialised epithelium that provides growth and patterning signals to the underlying mesoderm (reviewed in Fernandez-Teran and Ros, 2008); one possibility is that CXCL14 acts to restrict these AER functions. Within the mesenchyme of the autopod, two major bands of expression were observed; one within the anterior third of the autopod, and a thinner band immediately subjacent to the anterior-most epithelium (Supplementary Fig. 4C,D). Anterior mesenchymal patches persist through to stages at which cartilage elements have appeared, but expression was not observed in chondrocytes (Supplementary Fig. 4E,F). Note that expression is widespread in limb and head surface ectoderm at least as late as HH30 (Fig. 1L and Supplementary Fig. 4E), consistent with reports that *CXCL14* is strongly expressed in the epidermis of mouse and human skin (Meuter and Moser, 2008).

Conclusions

Our analysis of embryonic chick *CXCL14* expression leads to a number of testable hypotheses. Based on comparison with published expression patterns, *CXCL14* is potentially downstream of several key developmental regulators. For example, *CXCL14* may be regulated by PAX3 in the ophthalmic trigeminal placode, and by NOTCH signalling within early somites. These possibilities could be tested by electroporation of overexpression, dominant negative, or knockdown constructs for putative upstream regulators of *CXCL14*, followed by analysis of changes in expression of *CXCL14*, by *in situ* hybridisation. Our data also highlight the possibility that CXCL14 participates in some of the embryonic events during which CXCL12-CXCR4 signalling is known to play a role. An important task will be to examine in detail the extent of overlap, temporally and spatially, between expression of *CXCL14* and *CXCL12* or *CXCR4*, in the same species. Such analysis may then raise the possibility of functional redundancy between developmental chemokine pathways, thereby requiring parallel loss of function approaches. The major puzzle confronting all studies of CXCL14 is the identity of its receptor; in the future, comparison with our expression data may serve to highlight the likelihood that a given candidate receptor is physiologically relevant. Previous work has indicated that CXCL14 can act as a tumour suppressor, or a growth factor, depending on the context. It is possible that CXCL14 may also regulate tissue growth during embryonic development. Given that several of the *CXCL14* expression sites are within tissues that have traditionally been amenable to electroporation in the chick embryo, including the neural tube, placodal ectoderm, epithelial somites and inner ear, *CXCL14* misexpression experiments utilising *in ovo* electroporation may therefore be fruitful in the study of the embryonic functions of this chemokine in the future.

Materials and Methods

The primers 5'-GAGGACGGGAACACAAGACAG (forward) and 5'-GAGAAATCATCTTCTGCAGAGC (reverse) were used to amplify a 430 bp fragment of *CXCL14* (spanning the coding sequence of all four exons of the gene), from an embryonic chick cDNA library. The fragment was cloned into pCRII-TOPO (Invitrogen), and the presence of *CXCL14* was confirmed by sequencing. Following digestion with XbaI or BamHI, digoxigenin (Roche)-labelled antisense or sense probes were generated using SP6 or T7 primers, respectively. Chicken eggs were obtained from Research Poultry Farm, Research, Victoria. Embryos were processed for whole-mount and section *in situ* hybridisation essentially as described (Acloque *et al*., 2008). Probe hybridisation was visualised by incubation with an α-digoxigenin antibody conjugated to alkaline phosphatase (Roche) followed by addition of the colorimetric substrates NBT and BCIP (Roche). No specific signal was detected in whole embryos or sections hybridised with the sense probe, at all embryonic stages tested (data not shown).

References

ACLOQUE, H., WILKINSON, D.G. and NIETO, M.A. (2008). *In situ* hybridization analysis of chick embryos in whole-mount and tissue sections. *Methods Cell Biol* 87: 169-185.

ADAM, J., MYAT, A., LE ROUX, I., EDDISON, M., HENRIQUE, D., ISH-HOROWICZ, D. and LEWIS, J. (1998). Cell fate choices and the expression of Notch, Delta and Serrate homologues in the chick inner ear: parallels with *Drosophila* sense-organ development. *Development* 125: 4645-4654.

AUGSTEN, M., HAGGLOF, C., OLSSON, E., STOLZ, C., TSAGOZIS, P., LEVCHENKO, T., FREDERICK, M.J., BORG, A., MICKE, P., EGEVAD, L. *et al.,* (2009). CXCL14 is an autocrine growth factor for fibroblasts and acts as a multi-modal stimulator of prostate tumor growth. *Proc Natl Acad Sci USA* 106: 3414-3419.

BORRELL, V. and MARIN, O. (2006). Meninges control tangential migration of hem-derived Cajal-Retzius cells via CXCL12/CXCR4 signaling. *Nat Neurosci* 9: 1284-1293.

CAO, X., ZHANG, W., WAN, T., HE, L., CHEN, T., YUAN, Z., MA, S., YU, Y. and CHEN, G. (2000). Molecular cloning and characterization of a novel CXC chemokine macrophage inflammatory protein-2 gamma chemoattractant for human neutrophils and dendritic cells. *J Immunol* 165: 2588-2595.

CHONG, S.W., EMELYANOV, A., GONG, Z. and KORZH, V. (2001). Expression pattern of two zebrafish genes, cxcr4a and cxcr4b. *Mech Dev* 109: 347-354.

CHONG, S.W., NGUYET, L.M., JIANG, Y.J. and KORZH, V. (2007). The chemokine Sdf-1 and its receptor Cxcr4 are required for formation of muscle in zebrafish. *BMC Dev Biol* 7: 54.

COLE, L.K., LE ROUX, I., NUNES, F., LAUFER, E., LEWIS, J. and WU, D.K. (2000). Sensory organ generation in the chicken inner ear: contributions of bone morphogenetic protein 4, serrate1, and lunatic fringe. *J Comp Neurol* 424: 509-520.

D'AMICO-MARTEL, A. and NODEN, D.M. (1983). Contributions of placodal and neural crest cells to avian cranial peripheral ganglia. *Am J Anat* 166: 445-468.

DAMBLY-CHAUDIERE, C., CUBEDO, N. and GHYSEN, A. (2007). Control of cell migration in the development of the posterior lateral line: antagonistic interactions between the chemokine receptors CXCR4 and CXCR7/RDC1. *BMC Dev Biol* 7: 23.

DAVID, N.B., SAPEDE, D., SAINT-ETIENNE, L., THISSE, C., 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., KOPRUNNER, M., DORRIES, 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.

FERNANDEZ-TERAN, M. and ROS, M.A. (2008). The Apical Ectodermal Ridge: morphological aspects and signaling pathways. *Int J Dev Biol* 52: 857-871.

FREDERICK, M.J., HENDERSON, Y., XU, X., DEAVERS, M.T., SAHIN, A.A., WU, H., LEWIS, D.E., EL-NAGGAR, A.K. and CLAYMAN, G.L. (2000). *In vivo* expression of the novel CXC chemokine BRAK in normal and cancerous human tissue. *Am J Pathol* 156: 1937-1950.

GRENIER, J., TEILLET, M.A., GRIFONE, R., KELLY, R.G. and DUPREZ, D. (2009). Relationship between neural crest cells and cranial mesoderm during head muscle development. *PLoS One* 4: e4381.

HAAS, P. and GILMOUR, D. (2006). Chemokine signaling mediates self-organizing tissue migration in the zebrafish lateral line. *Dev Cell* 10: 673-680.

HROMAS, R., BROXMEYER, H.E., KIM, C., NAKSHATRI, H., CHRISTOPHERSON, K., 2ND, AZAM, M. and HOU, Y.H. (1999). Cloning of BRAK, a novel divergent CXC chemokine preferentially expressed in normal versus malignant cells. *Biochem Biophys Res Commun* 255: 703-706.

IZUKURI, K., SUZUKI, K., YAJIMA, N., OZAWA, S., ITO, S., KUBOTA, E. and HATA, R. (2010). Chemokine CXCL14/BRAK transgenic mice suppress growth of carcinoma cell transplants. [corrected]. *Transgenic Res* 19: 1109-1117.

KNAUT, H., BLADER, P., STRAHLE, U. and SCHIER, A.F. (2005). Assembly of trigeminal sensory ganglia by chemokine signaling. *Neuron* 47: 653-666.

KNAUT, H., WERZ, C., GEISLER, R. and NUSSLEIN-VOLHARD, C. (2003). A zebrafish homologue of the chemokine receptor Cxcr4 is a germ-cell guidance receptor. *Nature* 421: 279-282.

KURATANI, S.C. and HIRANO, S. (1990). The appearance of trigeminal ectopic ganglia within the surface ectoderm in the chick embryo. *Arch Histol Cytol* 53: 575-583.

KURTH, I., WILLIMANN, K., SCHAERLI, P., HUNZIKER, T., CLARK-LEWIS, I. and MOSER, B. (2001). Monocyte selectivity and tissue localization suggests a role for breast and kidney-expressed chemokine (BRAK) in macrophage development. *J Exp Med* 194: 855-861.

LIEM, K.F., JR., TREMML, G. and JESSELL, T.M. (1997). A role for the roof plate and its resident TGFbeta-related proteins in neuronal patterning in the dorsal spinal cord. *Cell* 91: 127-138.

LONG, Q., QUINT, E., LIN, S. and EKKER, M. (2000). The zebrafish scyba gene encodes a novel CXC-type chemokine with distinctive expression patterns in the vestibulo-acoustic system during embryogenesis. *Mech Dev* 97: 183-186.

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.

MEUTER, S. and MOSER, B. (2008). Constitutive expression of CXCL14 in healthy human and murine epithelial tissues. *Cytokine* 44: 248-255.

MEUTER, S., SCHAERLI, P., ROOS, R.S., BRANDAU, O., BOSL, M.R., VON ANDRIAN, U.H. and MOSER, B. (2007). Murine CXCL14 is dispensable for dendritic cell function and localization within peripheral tissues. *Mol Cell Biol* 27: 983-992.

MOLYNEAUX, K.A., ZINSZNER, H., KUNWAR, P.S., SCHAIBLE, K., STEBLER, J., SUNSHINE, M.J., O'BRIEN, W., RAZ, E., LITTMAN, D., WYLIE, C. *et al.,* (2003). The chemokine SDF1/CXCL12 and its receptor CXCR4 regulate mouse germ cell migration and survival. *Development* 130: 4279-4286.

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.

NAIR, S. and SCHILLING, T.F. (2008). Chemokine signaling controls endodermal migration during zebrafish gastrulation. *Science* 322: 89-92.

NARA, N., NAKAYAMA, Y., OKAMOTO, S., TAMURA, H., KIYONO, M., MURAOKA, M., TANAKA, K., TAYA, C., SHITARA, H., ISHII, R. *et al.,* (2007). Disruption of CXC motif chemokine ligand-14 in mice ameliorates obesity-induced insulin resistance. *J Biol Chem* 282: 30794-30803.

OLESNICKY KILLIAN, E.C., BIRKHOLZ, D.A. and ARTINGER, K.B. (2009). A role for chemokine signaling in neural crest cell migration and craniofacial development. *Dev Biol* 333: 161-172.

OZAWA, S., KATO, Y., KOMORI, R., MAEHATA, Y., KUBOTA, E. and HATA, R. (2006). BRAK/CXCL14 expression suppresses tumor growth *in vivo* in human oral carcinoma cells. *Biochem Biophys Res Commun* 348: 406-412.

PAREDES, M.F., LI, G., BERGER, O., BARABAN, S.C. and PLEASURE, S.J. (2006). Stromal-derived factor-1 (CXCL12) regulates laminar position of Cajal-Retzius cells in normal and dysplastic brains. *J Neurosci* 26: 9404-9412.

PARK, B.Y., HONG, C.S., SOHAIL, F.A. and SAINT-JEANNET, J.P. (2009). Developmental expression and regulation of the chemokine CXCL14 in *Xenopus*. *Int J Dev Biol* 53: 535-540.

RAZ, E. and MAHABALESHWAR, H. (2009). Chemokine signaling in embryonic cell migration: a fisheye view. *Development* 136: 1223-1229.

REISS, K., MENTLEIN, R., SIEVERS, J. and HARTMANN, D. (2002). Stromal cell-derived factor 1 is secreted by meningeal cells and acts as chemotactic factor on neuronal stem cells of the cerebellar external granular layer. *Neuroscience* 115: 295-305.

RODRIGUES, S., SANTOS, J. and PALMEIRIM, I. (2006). Molecular characterization of the rostral-most somites in early somitic stages of the chick embryo. *Gene Expr Patterns* 6: 673-677.

SALOGNI, L., MUSSO, T., BOSISIO, D., MIROLO, M., JALA, V.R., HARIBABU, B., LOCATI, M. and SOZZANI, S. (2009). Activin A induces dendritic cell migration through the polarized release of CXC chemokine ligands 12 and 14. *Blood* 113: 5848-5856.

SCHAERLI, P., WILLIMANN, K., EBERT, L.M., WALZ, A. and MOSER, B. (2005). Cutaneous CXCL14 targets blood precursors to epidermal niches for Langerhans cell differentiation. *Immunity* 23: 331-342.

SHELLENBERGER, T.D., WANG, M., GUJRATI, M., JAYAKUMAR, A., STRIETER, R.M., BURDICK, M.D., IOANNIDES, C.G., EFFERSON, C.L., EL-NAGGAR, A.K., ROBERTS, D. *et al.,* (2004). BRAK/CXCL14 is a potent inhibitor of angiogenesis and a chemotactic factor for immature dendritic cells. *Cancer Res* 64: 8262-8270.

SHURIN, G.V., FERRIS, R.L., TOURKOVA, I.L., PEREZ, L., LOKSHIN, A., BALKIR, L., COLLINS, B., CHATTA, G.S. and SHURIN, M.R. (2005). Loss of new chemokine CXCL14 in tumor tissue is associated with low infiltration by dendritic cells (DC), while restoration of human CXCL14 expression in tumor cells causes attraction of DC both *in vitro* and in vivo. *J Immunol* 174: 5490-5498.

SLEEMAN, M.A., FRASER, J.K., MURISON, J.G., KELLY, S.L., PRESTIDGE, R.L., PALMER, D.J., WATSON, J.D. and KUMBLE, K.D. (2000). B cell- and monocyte-activating chemokine (BMAC), a novel non-ELR alpha-chemokine. *Int Immunol* 12: 677-689.

STARK, M.R., SECHRIST, J., BRONNER-FRASER, M. and MARCELLE, C. (1997). Neural tube-ectoderm interactions are required for trigeminal placode formation. *Development* 124: 4287-4295.

STARNES, T., RASILA, K.K., ROBERTSON, M.J., BRAHMI, Z., DAHL, R., CHRISTOPHERSON, K. and HROMAS, R. (2006). The chemokine CXCL14 (BRAK) stimulates activated NK cell migration: implications for the downregulation of CXCL14 in malignancy. *Exp Hematol* 34: 1101-1105.

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.

TANEGASHIMA, K., OKAMOTO, S., NAKAYAMA, Y., TAYA, C., SHITARA, H., ISHII, R., YONEKAWA, H., MINOKOSHI, Y. and HARA, T. (2010). CXCL14 deficiency in mice attenuates obesity and inhibits feeding behavior in a novel environment. *PLoS One* 5: e10321.

TESSEMA, M., KLINGE, D.M., YINGLING, C.M., DO, K., VAN NESTE, L. and BELINSKY, S.A. (2010). Re-expression of CXCL14, a common target for epigenetic silencing in lung cancer, induces tumor necrosis. *Oncogene* 29: 5159-5170.

THEVENEAU, E., MARCHANT, L., KURIYAMA, S., GULL, M., MOEPPS, B., PARSONS, M. and MAYOR, R. (2010). Collective chemotaxis requires contact-dependent cell polarity. *Dev Cell* 19: 39-53.

VALENTIN, G., HAAS, P. and GILMOUR, D. (2007). The chemokine SDF1a coordinates tissue migration through the spatially restricted activation of Cxcr7 and Cxcr4b. *Curr Biol* 17: 1026-1031.

VASYUTINA, E., STEBLER, J., BRAND-SABERI, B., SCHULZ, S., RAZ, E. and BIRCHMEIER, C. (2005). CXCR4 and Gab1 cooperate to control the development of migrating muscle progenitor cells. *Genes Dev* 19: 2187-2198.

WU, D.K. and OH, S.H. (1996). Sensory organ generation in the chick inner ear. *J Neurosci* 16: 6454-6462.

YUSUF, F., REHIMI, R., DAI, F. and BRAND-SABERI, B. (2005). Expression of chemokine receptor CXCR4 during chick embryo development. *Anat Embryol (Berl)* 210: 35-41.

ZHU, Y., YU, T., ZHANG, X.C., NAGASAWA, T., WU, J.Y. and 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. and LITTMAN, D.R. (1998). Function of the chemokine receptor CXCR4 in haematopoiesis and in cerebellar development. *Nature* 393: 595-599.

Further Related Reading, published previously in the *Int. J. Dev. Biol.*

Early expression of axon guidance molecules in the embryonic chick mesencephalon and pretectum
Kerry-Lyn Riley, Sarah Gledhill and Frank R. Schubert
Int. J. Dev. Biol. (2010) 54: 743-753

Developmental expression and regulation of the chemokine CXCL14 in Xenopus
Byung-Yong Park, Chang-Soo Hong, Faraz A. Sohail and Jean-Pierre Saint-Jeannet
Int. J. Dev. Biol. (2009) 53: 535-540

Mouse G-protein gamma3 expression in the developing CNS and neural crest cell derivatives
Gregory M. Kelly, Yukio Saijoh, Ariel Finkielsztein and Steve Mangos
Int. J. Dev. Biol. (2008) 52: 1143-1150

Stromal-derived factor-1 (SDF-1) expression during early chick development
Rizwan Rehimi, Nargis Khalida, Faisal Yusuf, Fangping Dai, Gabriela Morosan-Puopolo and Beate Brand-Saberi
Int. J. Dev. Biol. (2008) 52: 87-92

Isthmus organizer and regionalization of the mesencephalon and metencephalon
Harukazu Nakamura and Yuji Watanabe
Int. J. Dev. Biol. (2005) 49: 231-235

5 yr ISI Impact Factor (2009) = 3.253