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 have 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 ckl12-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-Reetzus 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; Schaarli et al., 2005; Shellenberger et al., 2004; Shurin et al., 2006). The common theme in most of these examples is expression of CXCL14 in a tissue through which CXCR4-expressing cells migrate, while in some cases CXCL14 is required to enable CXCR4-positive cells to maintain a fixed position relative to the ligand (reviewed in Raz and Mahabaleshwar, 2009).

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.
et al., 2006). Cxcl14 knockout mice have defects in feeding behaviour (Tanegashima et al., 2007) 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 amniotes. 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. 1 C). At HH18, expression is greatly reduced in cranial surface ectoderm relative to that posterior to the otic vesicle (Fig. 1 E). 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 G,F); 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. 1C-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, ccr4b is required for correct positioning of sensory neurons within the developing trigeminal ganglion (Knauf 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.
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 epithysis (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 dorsoverentral 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
CXCL14 in chick embryonic development

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'-GA-GAAATCATCTTCTGCAGAGC (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 pCR II-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**


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