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Expression of the mouse *goosecoid* gene during mid-embryogenesis may mark mesenchymal cell lineages in the developing head, limbs and body wall

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SUMMARY

After an earlier, transient phase of expression in the developing primitive streak of 6.4- to 6.8-day mouse embryos, the homeobox gene *goosecoid* is now shown to be expressed in a later phase of mouse development, from 10.5 days onwards. The later, spatially restricted domains of *goosecoid* expression are detected in the head, limbs and ventrolateral body wall. At all sites, the domains of expression are first detected in undifferentiated tissue, and then expression persists as these tissues undergo subsequent morphogenesis. For example, *goosecoid* expression is noted in the first branchial arch at 10.5 days, and then expression persists as this tissue undergoes morphogenesis to form the lower jaw and the body of the tongue. Expression in tissues around the first branchial cleft persists as these undergo morphogenesis to form the base of the auditory meatus and eustachian tube. Expression in tissues around the newly formed nasal pits persists as these elongate to form the nasal chambers. Expression in the ventral epithelial lining of the otic vesicle persists as this eventually gives rise to the non-sensory epithelium of the cochlea. Expression in the proximal limb buds and ventrolateral body wall persists as these tissues undergo morphogenesis to form proximal limb structures and ventral ribs respectively. Our findings lead us to suggest that the *goosecoid* gene product plays a role in spatial programming within discrete embryonic fields, and possibly lineage compartments, during organogenesis stages of mouse development.

Key words: *goosecoid*, gene expression, branchial arches, tongue, ear, mandible, limb buds

INTRODUCTION

Work on *Drosophila* and vertebrate embryos has shown that morphogenesis and the development of pattern is often presaged by the expression, within undifferentiated tissues, of spatially restricted patterns of homeobox gene (homeogene) expression (reviewed, for example, by Gaunt, 1991). The body is thus divided into zones, within each of which a homeogene product, or unique combination of homeogene products, may act as 'selector' of a particular route of morphogenetic development (Garcia Bellido, 1975). Such a zone has been variously described as a 'field' (De Robertis et al., 1991; Ingham and Martinez Arias, 1992) or 'compartment' (Lawrence, 1990; Gaunt, 1991), the chief distinction being that the term 'compartment' also implies a strictly self-contained unit of cell lineage.

goosecoid is a homeobox-containing gene present in a wide variety of vertebrate species (Blum et al., 1992). It is expressed in the dorsal lip of the blastopore in *Xenopus* (Blumberg et al., 1991; Cho et al., 1991) and in the developing primitive streak region of the mouse (Blum et al., 1992). At these sites, *goosecoid* is expressed in anteriorly migrating cells that spearhead gastrulation movements. Microinjection of *goosecoid* mRNA into *Xenopus* embryos leads to formation of a new body axis, suggesting that this gene is part of the biochemical pathway leading to 'organizer' activity. The *goosecoid*-expressing cells of the early mouse embryo are fated to form the head process, which later gives rise to anterior notochord and endoderm, and head mesoderm (Beddington, 1983; Lawson et al., 1991). In both mouse and *Xenopus*, *goosecoid* expression ceases to be detectable in later stages of gastrulation (Blumberg et al., 1991; Blum et al., 1992). We have found, however, that *goosecoid* expression recommences during organogenesis stages of mouse development, from 10.5 days, and it is these patterns of expression, seen in restricted regions of the facial processes and arches, limbs and body wall that are the subject of this paper.

Fig. 1 shows the arrangement of the branchial arches and facial processes as seen in a mouse embryo of about 10.5 days. The mesenchymal component of these is derived almost entirely from cephalic neural crest cells (Noden, 1988; Hunt et al., 1991d). Expression of the *Antennapedia*-like homeobox (Hox) genes probably accounts for at least

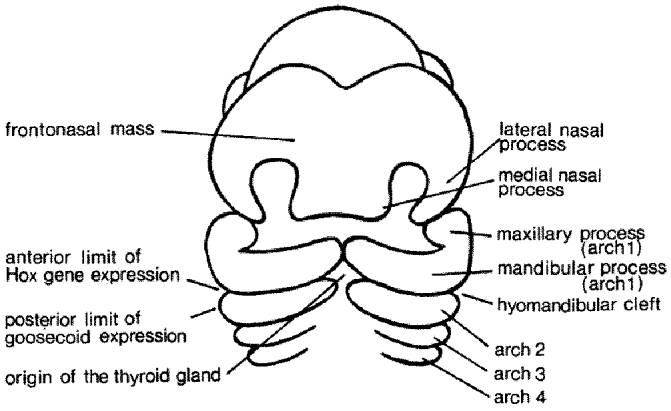

Fig. 1. The face of a 10.5 day mouse embryo, showing the arrangement of the branchial arches and facial processes. The list of derivatives is confined to those structures that are described in this paper. Comprehensive descriptions of derivatives are given by Sadler (1985) and Hamilton and Mossman (1972). The anterior limit of Hox gene expression is described by Hunt et al. (1991a-d).

some of the positional programming known to be intrinsic within neural crest-derived mesenchyme (Noden, 1983, 1988; Kirby, 1989; Richman and Tickle, 1989). Thus, anterior boundaries of Hox gene expression correspond with junctions between the branchial arches, and arches 2, 3 and 4 each display a unique combination of Hox gene products (Hunt et al., 1991a-d). Hox genes are not, however, expressed rostral to branchial arch 2 (Hunt et al., 1991a-d), and other genes must therefore be responsible for patterning in the mandibular, maxillary and frontonasal processes.

We now show how the *goosecoid* gene is expressed in discrete spatial domains within the facial processes and arches. These *goosecoid*-expressing domains, formed presumably within neural crest-derived mesenchyme, give rise to parts of the tongue, mandible, nasal chambers, palate and ears. Our findings also indicate that the *goosecoid* gene may play a role in spatial patterning within proximal regions of the limb and adjacent body wall.

MATERIALS AND METHODS

Methods used for ageing of embryos, tissue fixation, sectioning, preparation of ^{35}S-labelled riboprobes, and in situ hybridization were all as previously described (Gaunt, 1987).

The mouse *goosecoid* gene (Blum et al., 1992) contains three exons. Two different *goosecoid* probes ('probes 2 and 3'), prepared from genomic DNA, were used in the experiments of this study. Probe 2 (as described in Blum et al., 1992) was prepared from a 909 bp DNA fragment that comprises 180 bp of exon 2 together with exon 3 and the intervening 347 bp intron. Probe 3 was prepared from a 349 bp *Sau*3A-*Hin*cII DNA fragment (nucleotides 1808-2157; Blum et al., 1992) that contains most of exon 3 plus 3′ trailer sequences upstream of the polyadenylation signal. In all in situ hybridization experiments, probes 2 and 3 gave identical results for the expression pattern of *goosecoid*. Probe 2 gave slightly higher background over all tissues than probe 3, but it also gave a stronger signal that was better suited to low-power photography of autoradiograms. The autoradigrams shown in this paper were obtained using probe 2. Localized expression of *goosecoid* within the tongue, nasal chambers and limbs was also noted using a non-overlapping exon 1 probe ('probe 1', as described by Blum et al., 1992), Probe 1 was not, however, used extensively since it gave excessive background labelling over all tissues.

Dlx-1 and *Hox-1.5* probes were as used by Dollé et al. (1992) and Gaunt (1987) respectively.

RESULTS

Expression in branchial arches, facial processes, and their derivatives

At 10.5 days, strong expression of *goosecoid* was seen in the mandibular process of the first branchial arch, especially more posterior parts, and the anterior one-third of the second arch (Fig. 2A,B). Within the second arch, the posterior boundary of expression was sharp (arrowed in Fig. 2B). Expression of *goosecoid* was therefore abundant within the region of the first branchial cleft (hyomandibular cleft; Fig. 2B), the precursor of the eustachian tube and auditory meatus (Fig. 1). Expression was detected only within medial parts of the first arch (Fig. 3B,C) and, apart from the rostral-most limits of this arch (Fig. 3B), was continuous from the left to right sides (Figs 2B, 3C). Expression was not evident at this stage within the maxillary process of the first arch (Figs 3B, 4B). Fig. 4 allows comparison of the *goosecoid* expression domain with that of *Dlx-1*, another transcription factor known to be spatially restricted within the first arch (Dollé et al., 1992). *goosecoid* transcripts (Fig. 4B) were generally seen to lie medial to those of *Dlx-1* (Fig. 4C). In rostral parts of the first arch (seen on the right hand side of Fig. 4), *goosecoid* and *Dlx-1* transcripts occupied apparently exclusive and complementary domains. However, in more posterior parts (left hand side of Fig. 4) there was apparent overlap between the *goosecoid* and *Dlx-1* domains. Expression of *goosecoid* within the second arch was detected both medially and laterally (Fig. 3D), with medially located expression extending posteriorly beyond the limits of more lateral labelling (Fig. 3E). It was not clear whether or not this medial labelling (located within the floor of the pharynx) extended posteriorly beyond the limits of the second arch. The third and fourth arches were only clearly distinguishable in their more lateral parts (Fig. 3E,F) and these regions showed no expression of *goosecoid*. At 10.5 days, strong expression was also seen in the lateral and medial nasal processes surrounding the base of the nasal pits (Fig. 3A).

By 12.5 days, strong labelling persisted within the hyomandibular cleft (cf. Figs 5C, 2B). Strong labelling also persisted in the derivatives of the mandibular process (see Fig. 1), namely the lower jaw (Figs 5C, 6B) and parts of the tongue (Figs 5A,B, 6B). The ventral-most parts of the

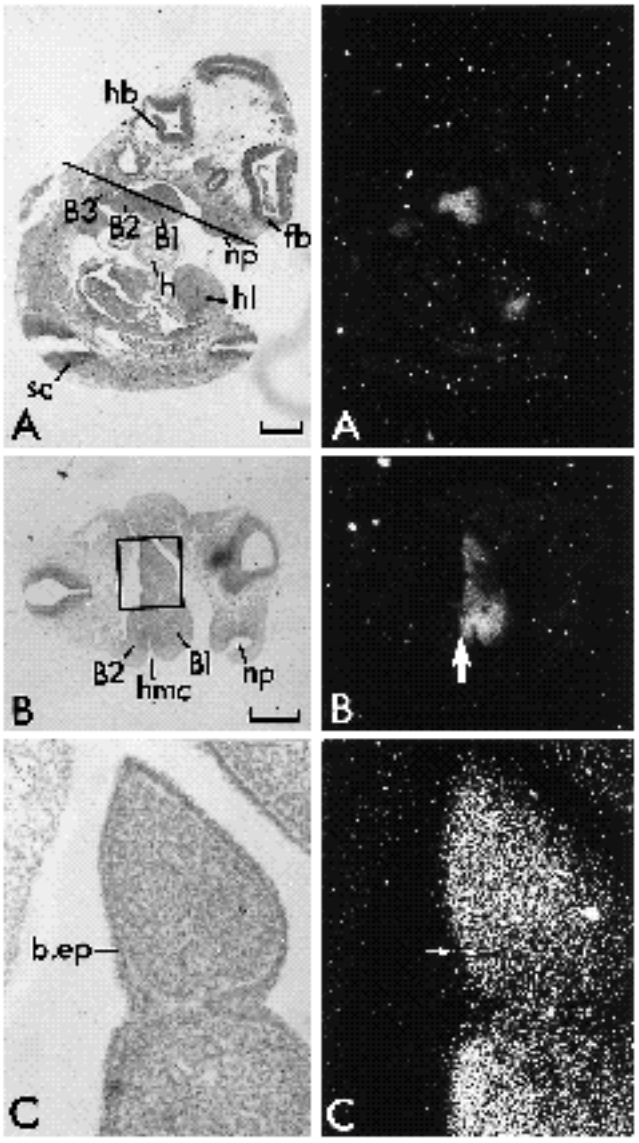

Fig. 2. *goosecoid* expression in the 10.5 day mouse embryo detected on parasagittal (A) and transverse (B,C) sections. The plane of section B is indicated by the line drawn in A. C is an enlargement of the boxed area in B. Left, bright-field; right, dark-field illumination. hb, hindbrain; B1, B2, B3, branchial arches 1, 2, 3; sc, spinal cord; hl, hindlimb bud; h, heart; np, nasal pit; fb, forebrain; hmc, hyomandibular cleft; b.ep, branchial epithelium; small arrows in C, upper and lower limits of b.ep. Bars, 0.5 mm.

tongue were not labelled (Fig. 6B). Within the tongue, there was a sharp boundary between labelled anterior and non-labelled posterior parts (Fig. 6B). This boundary, being at the level of the thyroid duct, lay at the anatomical boundary between the 'body' and 'root' of the tongue (Fig. 10). A continuous tract of labelled tissues was seen to extend posteriorly from the base of the thyroid duct to below, and around, the aortic arch (Fig. 6B). These tissues have been commonly observed to be labelled with Hox gene probes (e.g. *Hox-1.4*, *-2.6*, *-4.2*, *-1.5*; Gaunt et al., 1988, 1989) and were tentatively identified as the thyroid (anterior) and thymus (posterior) glands. Fig. 6 allows comparison of the *goosecoid* (Fig. 6B) and *Hox-1.5* (Fig. 6C) expression patterns within the tongue. As shown earlier (Gaunt et al., 1988), *Hox-1.5* is expressed in the root of the tongue, and in the sulcus terminalis (tissue lying just rostral to the thyroid duct; Fig. 10), but is not expressed in more rostral parts of the body of the tongue. By 12.5 days, the earlier expression around the olfactory pit (Fig. 3A) had developed into an extensive zone of labelled mesenchyme surrounding each nasal passage (Fig. 5A). This zone of expression extended continuously into the ingrowing palatal shelves (Fig. 5A).

By 14.5 days, strong labelling persisted around the nasal passages, in the palate and around the lower jaw (Fig. 7A). As seen in a cross-section through the mandible, labelling became markedly reduced in central parts already transformed into cartilage (Fig. 7A). Strong expression of *goosecoid* was seen at 14.5 days in mesenchymal tissues around the developing eustachian tube and base of the auditory meatus (Fig. 7C). Both of these regions are derived from tissues around the hyomandibular cleft (see Fig. 1), already noted to be expressing goosecoid at 10.5 and 12.5 days (Figs 2B, 5C). Strong labelling was seen in the developing malleus (Fig. 7C) but not in other middle ear ossicles (incus and stapes; Fig. 7C).

At all stages, the central nervous system was unlabelled. However, one exception to this was found at 14.5 days in a small region of tissue within the floor of the diencephalon (Fig. 7A).

Neural crest-derived cells, which account for most of the mesenchymal component of the face (Noden, 1988), have completed their migration into the branchial arches by 9 days (Hunt et al., 1991d). At 9.5 days, however, no significant expression of *goosecoid* above background was detected (Fig. 8). We therefore conclude that expression of *goosecoid* in the developing tissues of the head commences after migration of neural crest from the brain, at some time between 9.5 and 10.5 days.

Expression in epidermal derivatives of the head

goosecoid expression within the branchial arch tissue did not, apparently, extend into the overlying branchial epithelium (Fig. 2C). Similarly, at 12.5 days, the epithelium overlying the tongue and lining the anterior edge of the thyroid duct showed little or no labelling above background (Fig. 6B). At 10.5 days (Fig. 3A), 12.5 days (Fig. 5A) and 14.5 days (Fig. 7A) labelling around the developing nasal cavities was confined to mesenchymal tissues, and did not include the lining epithelium.

goosecoid expression was detected in a discrete ventral region of the otic vesicle (precursor of the inner ear) at 10.5 days (arrowed in Fig. 3D,E) and 12.5 days (arrowed in Fig. 5B). By 14.5 days, the labelling persisted within discrete regions of the cochlear duct (Fig. 7A-C). This labelling was restricted to part of the wall adjacent to, but not including, the sensory organ of Corti (Fig. 7B). At all stages, expression appeared to be confined solely to the epithelium. Thus, although some spread of silver grains was apparent in the immediately underlying mesenchyme, the range of this spread (about half a cell width) was no greater than that seen in the overlying lumen (not shown).

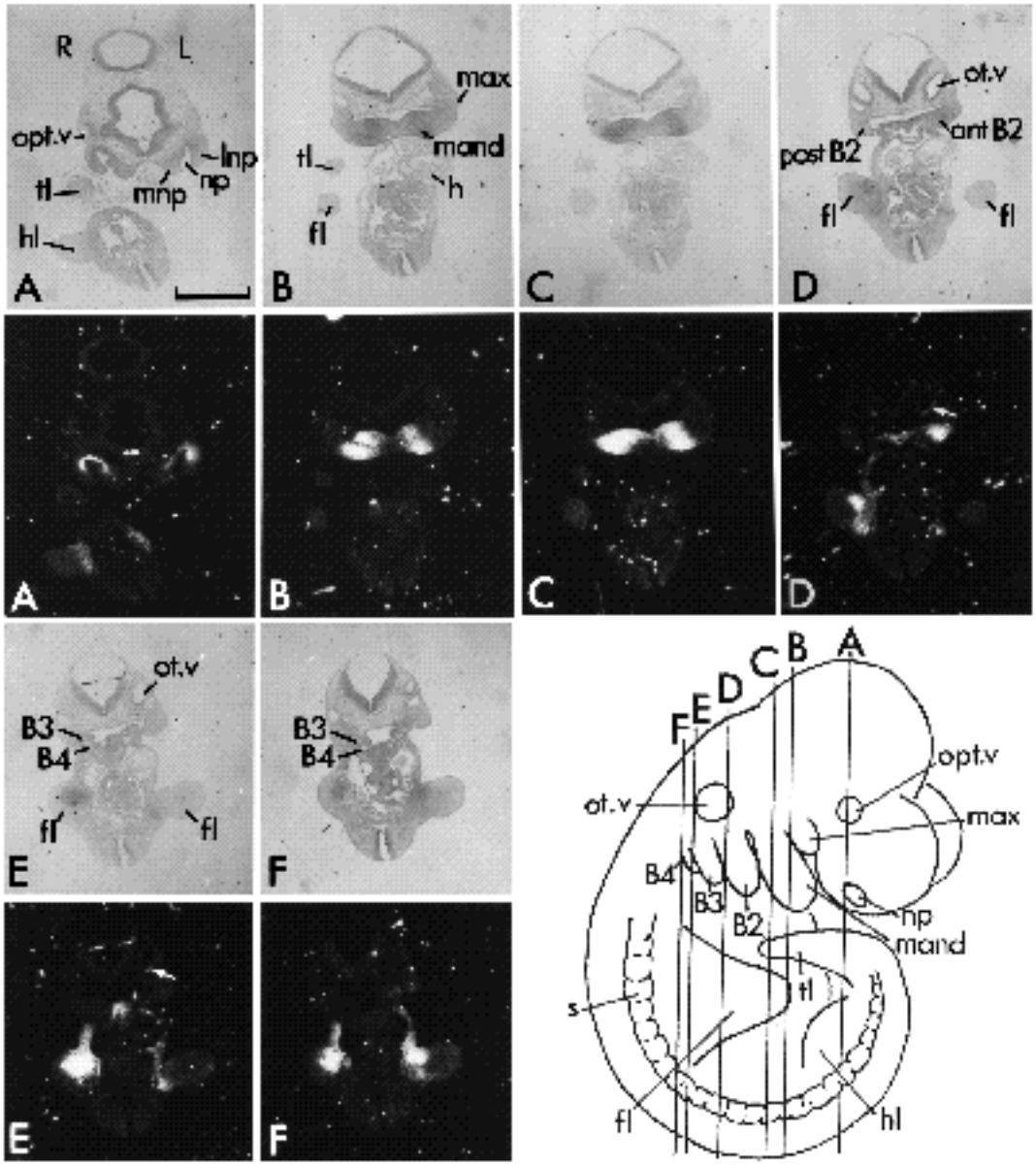

Fig. 3. *goosecoid* expression in the 10.5-day mouse embryo detected on parallel frontal sections. Upper panels, bright-field; lower panels, dark-field. R, L, right and left; opt.v, optic vesicle; ot.v, otic vesicle; tl, tail; hl, fl, hindlimb and forelimb buds; mnp, lnp, medial and lateral nasal processes; np, nasal pit; mand, mandibular process; max, maxillary process; h, heart; ant, post B2, anterior and posterior branchial arch 2; B3, B4, branchial arches 3 and 4; arrows in D and E, labelling within the otic epithelium; s, somite. Bar, 1.0 mm.

Expression in the limbs and trunk

No expression of *goosecoid* was detected in 9.5-day limb buds (Fig. 8B). However, strong expression was seen by 10.5 days (Figs 2A, 3A, 3D-F). In both fore- and hindlimb buds the expression was restricted to proximal parts, and serial sections through the forelimb bud (Fig. 3D-F) showed that the bulk of labelling was proximal, ventral and anterior. This domain of labelling therefore excludes that part of the limb (distal, dorsal and posterior) that includes the zone of polarizing activity, around which Hox-4 gene transcripts radiate in a series of partially overlapping domains (Dollé et al., 1989). Expression within the proximal limb buds was seen to extend into the adjacent body wall (Fig. 3A,D-F). This expression within the body wall did not extend continuously between the hind- and forelimbs (Fig. 3B,C). Anterior to the forelimb, expression was seen within lateral parts of the thoracic body wall overlying the heart (Fig. 3F, left hand side of Fig. 3D,E) but not within more dorsal (not shown) or ventral (right hand side of Fig. 3D) parts of the thoracic wall.

By 12.5 days, the limbs contain mesenchymal condensations destined to give rise to skeletal elements (Figs 5D, 9). *goosecoid* expression at this time was restricted within patches that lay mainly between, rather than within, these condensations (Figs 5D, 9). The proximal restriction of *goosecoid* expression seen at 10.5 days (Fig. 3D-F) was

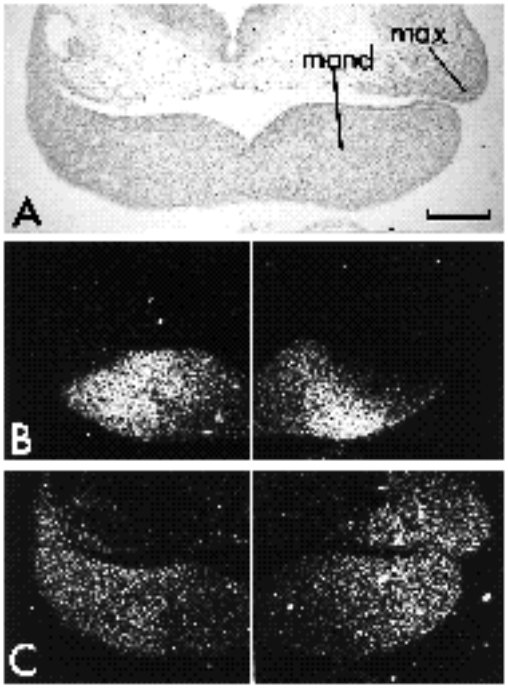

Fig. 4. *goosecoid* (B) and *Dlx-1*(C) expression compared on adjacent sections through the first branchial arch of a 10.5 day mouse embryo. The plane of sectioning is obliquely frontal (right hand side is rostral relative to left). (A) Bright-field view of section C; (B, C) dark-field illumination; mand, mandibular process; max, maxillary process. Bar, 0.25 mm.

maintained, so that 12.5-day expression was seen within the region of the developing hip, tibia and fibula (Fig. 5D), shoulder, radius and ulna (not shown), ankle and wrist (Fig. 9), but not around the digits (Fig. 9). At 12.5 days, expression of *goosecoid* was also maintained within the lateral body wall. This was confined to expression around the lateral and ventral, but not dorsal, parts of the developing ribs. As already described above for the limbs at 12.5 days (Fig. 5D), and the mandible at 14.5 days (Fig. 7A), expression around the ribs was excluded from the condensing skeletal elements themselves (Fig. 5D).

By 14.5 days, *goosecoid* expression within the forelimb was seen to be reduced to a few flecks, seen mainly within the shoulder and wrist regions (Fig. 7A).

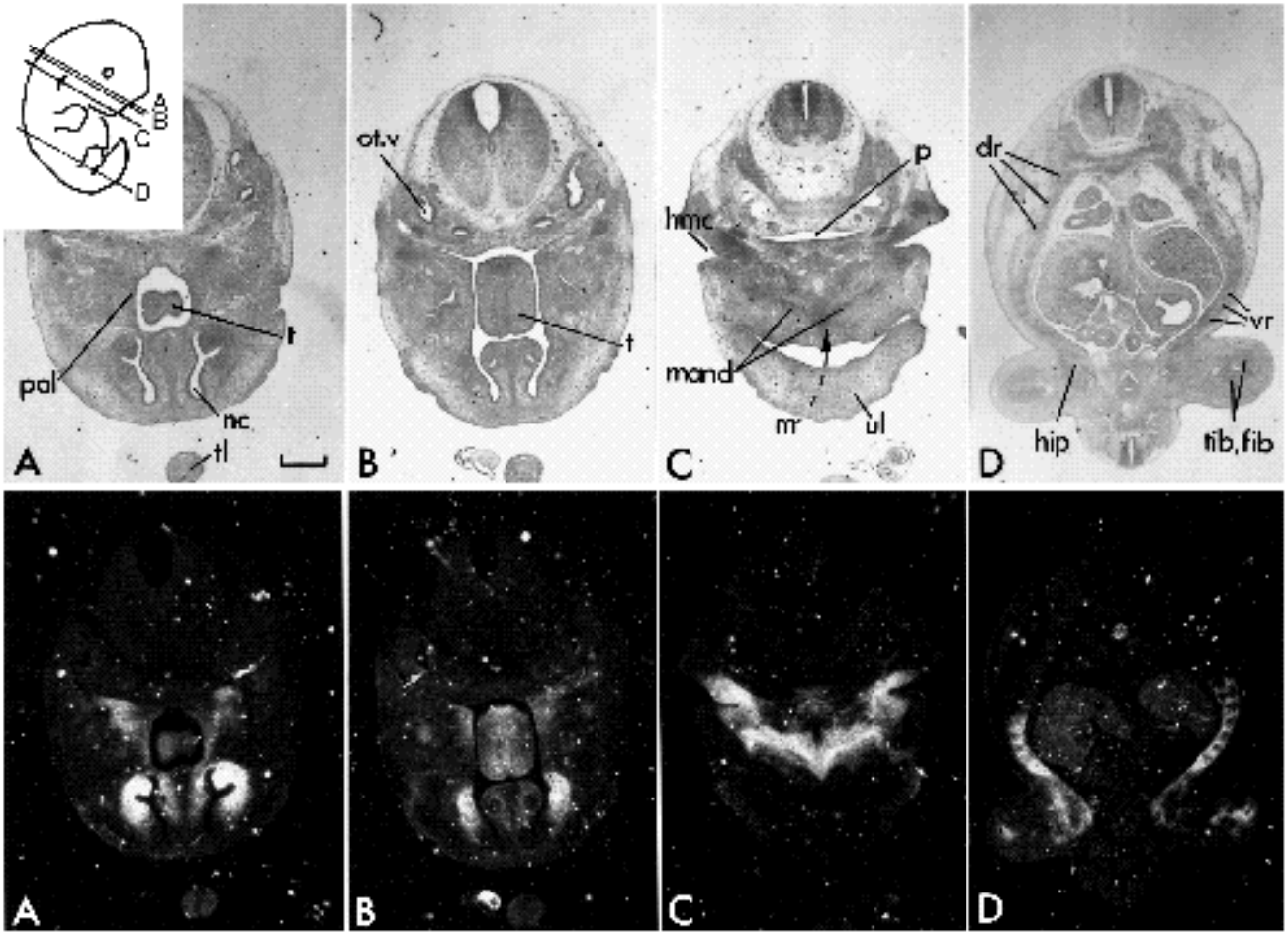

Fig. 5. *goosecoid* expression in the 12.5-day mouse embryo detected on parallel sections. Upper panels, bright-field; lower panels, dark-field. ot.v, otic vesicle; pal, palatal shelf; nc, nasal cavity; tl, tail; t, dorsum of tongue; hmc, hyomandibular cleft; m, mouth opening; ul, upper lip; mand, mandible; p, pharynx; dr, vr, dorsal and ventral ribs; tib, tibia; fib, fibula; arrow in B, labelling within the otic epithelium. Bar, 0.5 mm.

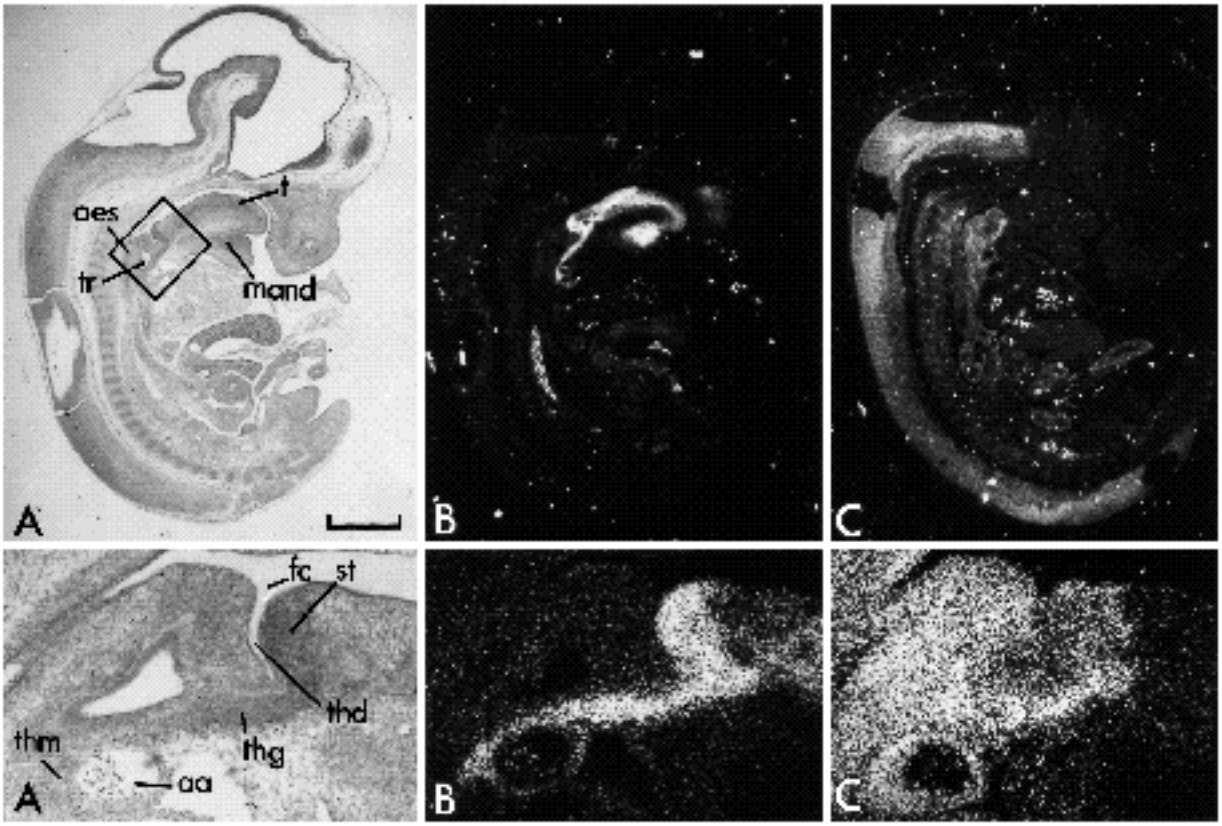

Fig. 6. *goosecoid* (B) and *Hox-1.5* (C) expression compared on parasagittal sections of a 12.5 day mouse embryo. (A) Bright-field view of section B; (B,C) dark-field illumination. The box in A shows the position of the high power views illustrated in the three lower panels. oes, oesophagus; tr, trachea; mand, mandible; t, tongue; thm, thymus; aa, aortic arch; thg, thyroid gland; thd, thyroid duct; st, sulcus terminalis; fc, foramen cecum. Bar, 1.0 mm.

DISCUSSION

goosecoid expression: a possible role in spatial patterning

From our findings, we note four striking features of *goosecoid* expression during the period of organogenesis in the mouse. These features are also shown by the Hox genes, a group of homeobox-containing genes known to be required for spatial programming in the developing embryo (e.g. Gaunt, 1991). First, *goosecoid* expression is spatially restricted within tissues that otherwise appear morphologically homogeneous in cellular composition (for example, the branchial arches and limbs). Second, expression of *goosecoid* commences in undifferentiated tissues and then persists as these undergo morphogenesis. Third, earlier (10.5-day) and later (12.5- to 14.5-day) patterns of expression are, at least to some extent, clearly linked by cell lineage (for example, *goosecoid* is expressed in first branchial arch tissue, and also its derivatives such as the mandible and tongue). Finally, *goosecoid* is expressed according to the position of a cell rather than its tissue-specific pathway of differentiation (for example, cells in the mandible and tongue pursue different pathways of differentiation). These findings, taken together with the fact that *goosecoid* encodes a homeodomain protein (Blumberg et al., 1991), presumably a transcription factor (Treisman et al., 1992), lead us to suggest that *goosecoid* product may be required for spatial programming within discrete embryonic fields (De Robertis et al., 1991; Ingham and Martinez Arias, 1992), and possibly lineage compartments (Lawrence, 1990; Gaunt, 1991), during organogenesis stages of mouse development.

Already by 10.5 days, the branchial arches and limbs contain both mesenchymal (Hunt et al., 1991d) and myogenic (Ott et al., 1991; Sassoon et al., 1989) cell populations. In facial processes, mesenchymal and myogenic cell types are derived from cephalic neural crest (except, in branchial arches, for a minor component from lateral mesoderm; Noden, 1988) and head mesoderm respectively (e.g. Noden, 1988). In limbs, these two cell populations are derived from lateral plate mesoderm and trunk myotomes respectively (e.g. Tabin, 1991). We believe that the patterns of *goosecoid* expression now described are largely, if not entirely, attributable to expression within mesenchyme. Thus, many of the areas of *goosecoid* expression form mesenchymal rather than muscular derivatives: for example, the mandible, walls of the nasal chambers and eustachian tubes, palate and ventral ribs. However, owing to a lack of available cell markers, and to the limited resolution of the in situ hybridization technique, we cannot rule out the additional possibility of myogenic cell expression.

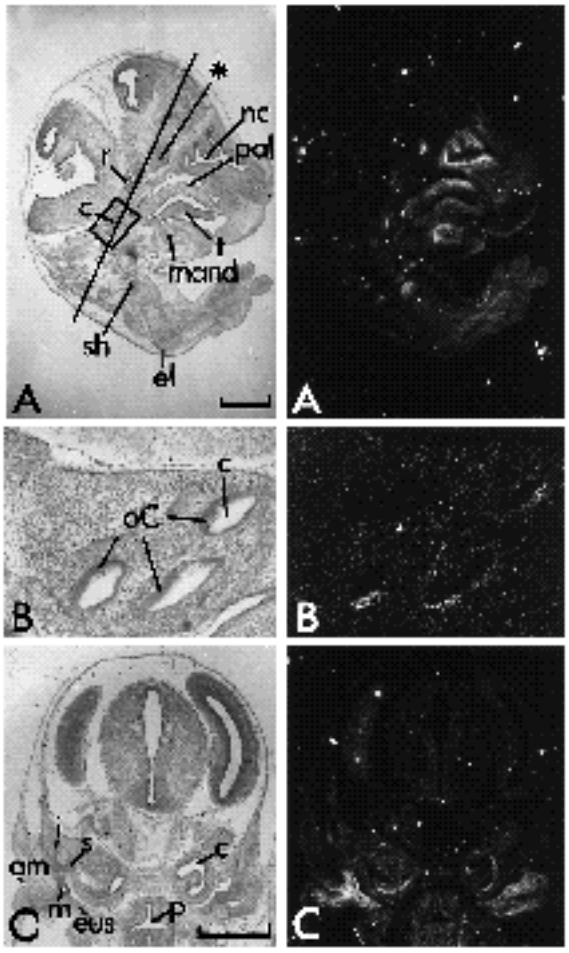

Fig. 7. *goosecoid* expression in the 14.5-day mouse embryo. The box in A shows the position, in a parallel section, of the high power view shown in B. The plane of section C is indicated by the line drawn on section A. Left, bright-field; right, dark-field. r, Rathke's pocket; c, cochlear duct; sh, shoulder; el, elbow; mand, mandibular cartilage; t, tongue; pal, palate; nc, nasal cavity; *, patch of labelled tissue within diencephalon; oC, organ of Corti; m, i, s, malleus, incus and stapes; am, auditory meatus; eus, eustachian tube; p, pharynx. Bars, 1.0 mm.

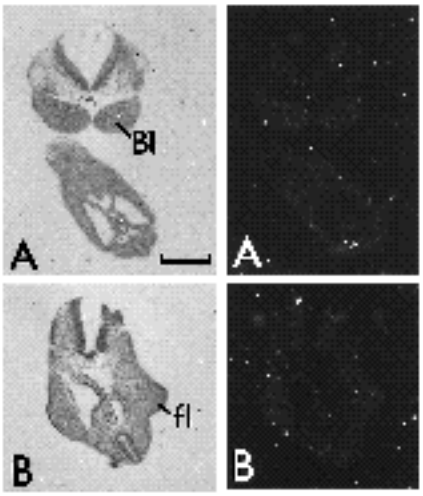

Fig. 8. Absence of *goosecoid* transcripts within frontal sections of the 9.5-day embryo. The planes of section are comparable with sections 3B and 3F. Left, bright-field; right, dark-field. B1, branchial arch 1; fl, forelimb bud. Bar, 0.25 mm.

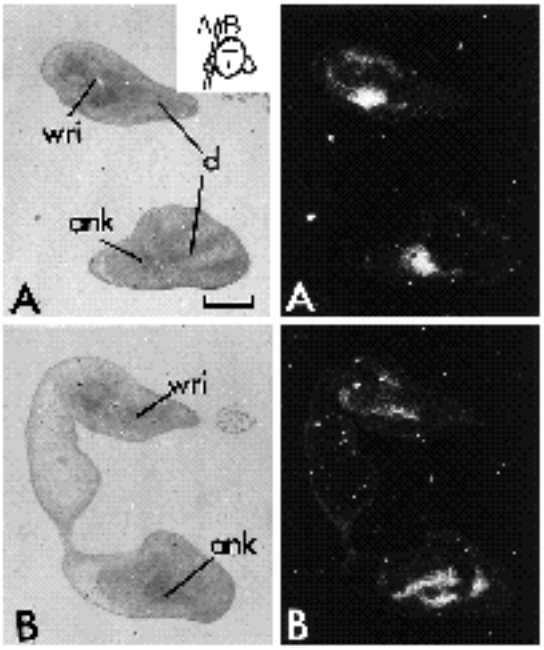

Fig. 9. *goosecoid* expression within the developing limbs of the 12.5-day embryo. The levels of the obliquely parasagittal sections are indicated on the diagram. Left, bright-field; right, dark-field. T, top of head, wri, wrist; ank, ankle; d, digits. Bar, 0.5 mm.

Expression in cephalic neural crest derivatives

The principal sites of *goosecoid* expression seen in the head are within anterior arch 2, arch 1, and the frontonasal mass. The neural crest-derived mesenchymal cells within all of these structures first arise by an orderly ventral streaming of cells from the dorsal surface of the brain (data from cell lineage studies in the chick; e.g. Lumsden et al., 1991). By analogy with the chick (Le Douarin, 1982; Lumsden et al., 1991; Lumsden, personal communication), we conclude that *goosecoid* expression in the head spans a region derived from the anterior part of the fourth rhombomere of the hindbrain (which contributes cells to the anterior part of the second branchial arch) to the anterior midbrain (which contributes cells to the frontonasal mass).

We found that the *goosecoid* gene is expressed in discrete spatial domains within the facial arches and processes. This suggests that *goosecoid* has a role in patterning *within* facial arches and processes. For Hox genes in contrast, expression patterns within mesenchyme have so far suggested a role in differential patterning *between* arches. Thus, anterior boundaries of Hox gene expression coincide with junctions between adjacent branchial arches (Hunt et al., 1991a-d; Frohman et al., 1990).

For Hox genes, the expression patterns within branchial arches are first established in neural crest cells prior to their migration from the central nervous system (Hunt et al.

1991a-d). This is not, apparently, the case for *goosecoid*, since *goosecoid* expression is not detected until after neural crest migration is completed (Hunt et al., 1991d). Most of the *goosecoid* expression domain is rostral to the limits of Hox gene expression (the anterior boundary of arch 2; Hunt et al., 1991a-d), yet it is known that such rostral tissue (e.g. arch 1 mesenchyme) is, prior to migration, already determined, at least to some extent (Noden, 1983, 1988), with respect to anteroposterior position. Some yet-unknown patterning mechanism must therefore precede *goosecoid* in this event. As one possibility, this primary mechanism, distinguishing neural crest rostral to rhombomere 2, might simply be absence of any Hox gene product. Following this primary anteroposterior patterning of cephalic neural crest, we assume that the discrete domains of *goosecoid* expression within head mesenchyme must be established by secondary events that take place in the environment of the facial processes and arches. These might, for example, include interaction between mesenchyme and overlying epithelia (reviewed by Hall, 1987), or diffusion of signalling polypeptides within the facial mesenchyme (e.g. *Wnt-5*; Gavin et al., 1990).

The possibility that the *goosecoid*-expressing cells seen from 10.5 days are, alternatively, simply descendants of the *goosecoid*-expressing cells seen earlier in the developing primitive streak (Blum et al., 1992) and fated to form the head process does not seem likely. Thus, no signs of *goosecoid* expression were detected in the embryo between 7.5 and 10.5 days, and *goosecoid*-expressing cells within the head process are not known to contribute to the domains of *goosecoid* expression seen later within the limbs and body wall.

In addition to *goosecoid* and Hox genes, other genes encoding transcription factors are also expressed in neural crest-derived head mesenchyme (*Dlx-1*, Dollé et al., 1992; *Hox-7*, Hill et al., 1989; Robert et al., 1989; Mackenzie et al., 1991; *AP-2*, Mitchell et al., 1991; *M-twist*, Wolf et al., 1991; *Pax-3*, Goulding et al., 1991). These also display spatial restriction in expression, suggestive of a role in spatial programming, although all of the genes are apparently expressed in all of the branchial arches. Most such genes (*Hox-7*, *AP-2*, *M-twist*, *Pax-3*), like the Hox genes, commence expression prior to neural crest migration. However, the homeobox-containing gene *Dlx-1*, like *goosecoid*, commences expression in facial mesenchyme at about 10.5 days. Moreover, we have now shown that the spatial domains of *Dlx-1* and *goosecoid* are apparently complementary at sites within the first branchial arch.

Patterning in the tongue and thyroid

Expression of *goosecoid* in the developing tongue was noted anterior, but not posterior, to the level of the thyroid duct (which opens to the pharynx through the foramen cecum). It is of interest to consider the position of this expression boundary in relation to the patterns of cell lineage that contribute to the tongue.

Fig. 10 shows how, according to the commonly held view, the tongue develops from branchial arches 1, 3 and 4. From this, we suggest that patterns of *goosecoid* expression in the developing tongue may simply follow the known patterns of cell lineage. Thus, *goosecoid* expression

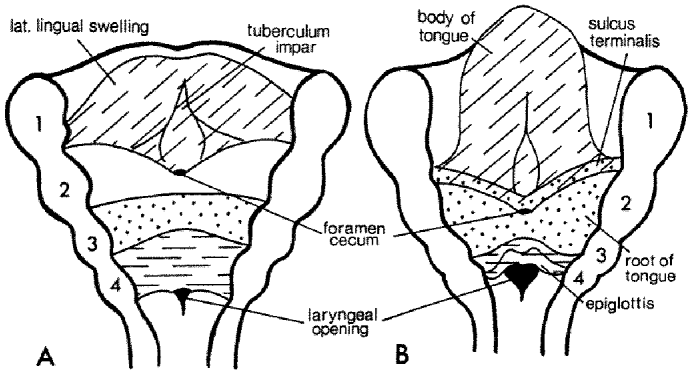

Fig. 10. Development of the tongue from branchial arches 1, 3 and 4 (from Sadler, 1985; Hamilton and Mossman, 1972). The figures show the floor of the pharynx (viewed from above after cutting horizontally through the branchial arches) as seen before (A) and after (B) development of the tongue. The mesenchymal component of the anterior part of the tongue (the 'body') arises from neural crest-derived cells located within the first arch. Sensory innervation of this region is therefore derived from nerves of the first arch: the lingual branch of the trigeminal nerve (for touch sensitivity) and the chorda tympani branch of the facial nerve (for taste). The posterior margin of the body of the tongue is marked at all stages in development by the foramen cecum. The mesenchymal component of the posterior part of the tongue (the 'root') arises mainly from third branchial arch tissue. This tissue overgrows the second arch, separating it from the surface of the tongue, and advances rostrally in the form of a V to give the sulcus terminalis. Most of the root of the tongue therefore receives sensory innervation from the glossopharyngeal nerve, the nerve of the third arch. The sulcus terminalis, located just rostral to the foramen cecum and innervated by both trigeminal and glossopharyngeal nerves, apparently represents a region where first and third arch tissues are mixed (Hamilton and Mossman, 1972). The extreme posterior part of the tongue is derived from fourth arch tissue, and receives sensory innervation from the vagus nerve.

is seen in the body of the tongue (that is, anterior to the foramen cecum) at 12.5 days because this is derived from *goosecoid*-expressing parts of the first arch as seen at 10.5 days. *goosecoid* is not expressed in the root of the tongue (posterior to the foramen cecum) at 12.5 days because this is derived from *goosecoid* non-expressing regions in the third and fourth arches.

Furthermore, we suggest that the pattern of *Hox-1.5* expression in the developing tongue similarly follows the pattern of cell lineage. Thus, *Hox-1.5* is expressed in the root of the tongue, and the sulcus terminalis (just anterior to the foramen cecum), because these parts contain cells derived from *Hox-1.5*-expressing tissues located in the third arch (Hunt et al., 1991a). *Hox-1.5* is not expressed anterior to the sulcus terminalis because this region is derived from *Hox-1.5* non-expressing first arch tissue (Hunt et al., 1991a). One finding not easily accommodated within this interpretation is the expression pattern noted for *Hox-2.6*. Although *Hox-2.6* is expressed in the fourth, and not third, branchial arches (Hunt et al., 1991a-d) its expression pattern in the tongue has been seen to be similar to, although much weaker than, the pattern for *Hox-1.5* (Gaunt et al., 1989). *Dlx-1* (Dollé et al. ,1992), a gene expressed in lat-

eral parts of the first arch at 10.5 days, is not expressed in the tongue or in medial parts of the mandible at 13.5 days. This observation, taken together with our results for *goosecoid*, suggest that it is medial first arch tissue that gives rise to the tongue and to medial parts of the mandible.

Commencing at the thyroid duct, we observed a tract of *goosecoid*-expressing tissue extending posteriorly towards the base of the heart. We tentatively identified the labelled structures within this tract as the mesenchymal components of the thyroid, thymus and aortic arch. Expression within thyroid mesenchyme is readily understood, since this structure originates at the ventral junction of the first and second branchial arches (at the level of the foramen cecum; Fig. 10), a region seen to be expressing *goosecoid* in the 10.5-day embryo. Expression within the thymus and aortic arch (derivatives of the third and fourth branchial clefts, and the fourth branchial arch respectively; Hamilton and Mossman, 1972; Sadler, 1985) is not so readily explained since we have not, so far, located *goosecoid* expression within any part of the third and fourth arches of 10.5-day embryos.

Patterning in the ear

As for the tongue and mandible, there was evidence of a lineage relationship between cells that express *goosecoid* at earlier and later stages of development of the ear. Thus, both the mesenchyme surrounding the hyomandibular cleft at 10.5-12.5 days and also its derivatives, seen at 14.5 days as tissue around the eustachian tube and part of the external auditory meatus (Hamilton and Mossman, 1972; Sadler, 1985), were found to express *goosecoid*. The ossicles of the middle ear showed expression of *goosecoid* within the malleus, but not incus or stapes. This may be explained by our finding at earlier times (10.5 days) of more widespread labelling within the mandibular process (origin of the malleus; Sadler, 1985) than in the maxillary process or hyoid arch (origins of the incus and stapes respectively; Sadler, 1985). At 14.5 days, *goosecoid* expression within the cochlear duct was restricted to a discrete part of the epithelial lining adjacent to, but not including, the developing sensory organ of Corti. These labelled cells may be derived from the discrete patch of labelled cells seen at earlier times (10.5-12.5 days) in the ventral part of the otic vesicle. In keeping with this proposal, it is known to be the ventral part of the otic vesicle that gives rise to the cochlear duct (Sadler, 1985). It is of interest to note that *int-2*, another gene likely to be involved in spatial patterning within the inner ear, is expressed in the cochlea only within the sensory regions (Wilkinson et al., 1989).

Patterning in the limbs

From 10.5 days, *goosecoid* was found to be expressed strongly in the proximal limb buds and adjacent body wall. So far, all transcription factor genes found to be expressed in facial mesenchyme (including *Hox-7*, *AP-2*, *M-twist Pax-3* and *Dlx-1*) have also been seen to be expressed in the limbs. Of these genes, however, only *goosecoid* has been found to be expressed proximally, rather than distally. At present, the significance of this remains unclear. However, if the limb is patterned by similar mechanisms to those of the main body axis (Dollé et al., 1989; De Robertis et al., 1991) then it is of interest to note that *goosecoid* expression within these structures occurs in parts that have been deemed to be analogous: that is, the anterior part of the body and the proximal part of the limb.

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REFERENCES

Beddington, R. S. P. (1983). The origin of the foetal tissues during gastrulation in the rodent. In *Development in Mammals*, vol. 5. (ed. M. H. Johnson), pp. 1-32. Amsterdam: Elsevier.

Blum, M., Gaunt, S. J., Cho, K. W. Y, Steinbeisser, H., Blumberg, B., Bittner, D. and De Robertis, E. M. (1992). Gastrulation in the mouse: the role of the homeobox gene *goosecoid*. *Cell* **69,** 1097-1106.

Blumberg, B., Wright, C. V. E., De Robertis, E. M. and Cho, K. W. Y. (1991). Organizer-specific homeobox genes in *Xenopus Laevis* embryos. *Science* **253,** 194-196.

Cho, K. W. Y., Blumberg, B., Steinbeisser, H. and De Robertis, E. M. (1991). Molecular nature of Spemann's organizer: the role of the *Xenopus* homeobox gene *goosecoid*. *Cell* **67,** 1111-1120.

De Robertis, E. M., Morita, E. A. and Cho, K. W. Y. (1991). Gradient fields and homeobox genes. *Development* **112,** 669-678.

Dollé, P., Izpisùa-Belmonte, J. C., Falkenstein, H., Renucci, A. and Duboule, D. (1989). Coordinate expression of the murine Hox-5 complex homeobox-containing genes during limb pattern formation. *Nature* **342,** 767-772.

Dollé, P., Price, M. and Duboule, D. (1992). Expression of the murine *D1x-1* homeobox gene during facial, ocular and limb development. *Differentiation* **49,** 93-99.

Frohman, M. A., Boyle, M. and Martin, G. A. (1990). Isolation of the mouse *Hox-2. 9* gene; analysis of embryonic expression suggests that positional information along the anterior-posterior axis is specified by mesoderm. *Development* **110,** 589-607.

Garcia-Bellido, A. (1975). Genetic control of wing disc development in *Drosophila. In Cell Patterning Ciba Foundation Symposium,* pp. 161-182. Amsterdam: Elsevier.

Gaunt, S. J. (1987). Homeobox gene *Hox-1. 5* expression in mouse embryos: earliest detection by in situ hybridization is during gastrulation. *Development* **101,** 51-60.

Gaunt, S. J. (1991). Expression patterns of mouse Hox genes: clues to an understanding of developmental and evolutionary strategies. *BioEssays* **13,** 505-513.

Gaunt, S. J., Krumlauf, R. and Duboule, D. (1989). Mouse homeogenes within a subfamily, *Hox-1. 4, -2. 6* and *-5. 1*, display similar anteroposterior domains of expression in the embryo, but show stage- and tissue-dependent differences in their regulation. *Development* **107,** 131-141.

Gaunt, S. J., Sharpe, P. T. and Duboule, D. (1988). Spatially restricted domains of homeogene transcripts in mouse embryos: relation to a segmented body plan. *Development Suppl.* **104,** 169-179.

Gavin, B. J., McMahon, J. A. and McMahon, A. P. (1990). Expression of multiple novel *Wnt-1/int-1*-related genes during fetal and adult mouse development. *Genes Dev.* **4,** 2319-2332.

Goulding, M. D., Chalepakis, G., Deutsch, U., Erselius, J. R. and Gruss, P. (1991). *Pax-3*, a novel murine DNA binding protein expressed during early neurogenesis. *EMBO J.* **10,** 1135-1147.

Hall, B. (1987). *Developmental and Evolutionary Aspects of the Neural Crest*. pp. 215-259. New York: John Wiley.

Hamilton, W. J. and Mossman, H. W. (1972). *Hamilton, Boyd and Mossman's Human Embryology.* Fourth Edition. Baltimore: Williams & Wilkins.

Hill, R. E., Jones, P. F., Rees, A. R., Sime, C. M., Justice, M. J., Copeland, N. G., Jenkins, N. A., Graham, E. and Davidson, D. R. (1989). A new family of mouse homeobox-containing genes: molecular structure, chromosomal location, and developmental expression of *Hox-7. 1. Genes Dev.* **3,** 26-37.

Hunt, P., Gulisano, M., Cook, M., Sham, M., Faiella, A., Wilkinson, D.,

Boncinelli, E. and Krumlauf, R. (1991a). A distinct Hox code for the branchial region of vertebrate head. *Nature* **353,** 861-864.

Hunt, P. and Krumlauf, R. (1991b). Deciphering the Hox code: clues to patterning branchial regions of the head. *Cell* **66,** 1075-1078.

Hunt, P., Whiting, J., Muchamore, I., Marshall, H. and Krumlauf, R. (1991c). Homeobox genes and models for patterning the hindbrain and branchial arches. *Development* **Supplement 1**, 187-196.

Hunt, P., Wilkinson, D. and Krumlauf, R. (1991d). Patterning the vertebrate head: murine Hox 2 genes mark distinct subpopulations of premigratory and migrating cranial neural crest. *Development* **112,** 43-50.

Ingham, P. W. and Martinez Arias A. (1992). Boundaries and fields in early embryos. *Cell* **68,** 221-235.

Kirby, M. L. (1989). Plasticity and predetermination of mesencephalic and trunk neural crest transplanted into the region of the cardiac neural crest. *Dev. Biol.* **134,** 402-412.

Lawrence, P. A. (1990). Compartments in vertebrates? *Nature* **334,** 382-383.

Lawson, K. A., Meneses, J. J. and Pedersen, R. A. (1991). Clonal analysis of epiblast fate during germ layer formation in the mouse embryo. *Development* **113,** 891-911.

Le Douarin, N. (1982). *The Neural Crest*. Cambridge: Cambridge University Press.

Lumsden, A., Sprawson, N. and Graham, A. (1991). Segmental origin and migration of neural crest cells in the hindbrain region of the chick embryo. *Development* **113,** 1281-1291.

Mackenzie, A., Ferguson, M. W. J. and Sharpe, P. T. (1991). *Hox-7* expression during murine craniofacial development. *Development* **113,** 601-611.

Mitchell, P. J., Timmons, P. M., Hebert, J. M., Rigby, P. W. J. and Tjian, R. (1991). Transcription factor *AP-2* is expressed in neural crest cell lineages during mouse embryogenesis. *Genes Dev.* **5,** 105-119.

Noden, D. M. (1983). The role of the neural crest in patterning of avian cranial skeletal, connective, and muscle tissues. *Dev. Biol.* **96,** 144-165.

Noden, D. M. (1988). Interactions and fates of avian craniofacial mesenchyme. *Development* **103Supplement,** 121-140.

Ott, M., Bober, E., Lyons, G., Arnold, H. and Buckingham, M. (1991). Early expression of the myogenic regulatory gene, *myf*-5, in precursor cells of skeletal muscle in the mouse embryo. *Development* **111,** 1097-1107.

Richman, J. M. and Tickle, C. (1989). Epithelia are interchangeable between facial primordia of chick embryos and morphogenesis is controlled by the mesenchyme. *Dev. Biol.* **136,** 201-210.

Robert, B., Sassoon, D., Jacq, B., Gehring, W. and Buckingham, M. (1989). *Hox-7*, a mouse homeobox gene with a novel pattern of expression during embryogenesis. *EMBO J.* **8,** 91-100.

Sadler, T. W. (1985). *Langman's Medical Embryology*. Fourth Edition. Baltimore: Williams & Wilkins.

Sassoon, D., Lyons, G., Wright, W. E., Lin, V., Lassar, A., Weintraub, H. and Buckingham, M. (1989). Expression of two myogenic regulatory factors myogenin and Myo D1 during mouse embryogenesis. *Nature* **341,** 303-307.

Tabin, C. J. (1991). Retinoids, homeoboxes, and growth factors: toward molecular models for limb development. *Cell* **66,** 199-217.

Treisman, J., Harris, E., Wilson, D. and Desplan, C. (1992). The homeodomain: a new face for the helix-turn-helix. *BioEssays* **14,** 145-150.

Wilkinson, D. G., Bhatt, S. and McMahon, A. P. (1989). Expression pattern of the FGF-related proto-oncogene *int-2* suggests multiple roles in fetal development. *Development* **105,** 131-136.

Wolf, C., Thisse, C., Stoetzel, C., Thisse, B., Gerlinger, P. and Perrin-Schmitt, F. (1991). M-twist gene of *Mus* is expressed in subsets of mesodermal cells and is closely related to the *Xenopus x-twi* and the *Drosophilatwist* genes. *Dev. Biol.* **143,** 363-373.

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