The homeobox gene Hox 7.1 has specific regional and temporal expression patterns during early murine craniofacial embryogenesis, especially tooth development *in vivo* and *in vitro*

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Summary

Hox 7.1 is a murine homeobox-containing gene expressed in a range of neural-crest-derived tissues and areas of putative epithelial–mesenchymal interactions during embryogenesis. We have examined the expression of *Hox 7.1* during craniofacial development in the mouse embryo between days 8 and 16 of development. Whereas facial expression at day 10 of gestation is broadly localised in the neural-crest-derived mesenchyme of the medial nasal, lateral nasal, maxillary and mandibular processes, by day 12 expression is restricted to the mesenchyme immediately surrounding the developing tooth germs in the maxillary and mandibular processes. *Hox 7.1* expression in the mesenchyme of the dental papilla and follicle is maximal at the cap stage of development and progressively declines in the bell stage prior to differentiation of odontoblasts and ameloblasts.

Hox 7.1 expression in tooth germs is independent of overall embryonic stage of development but is dependent on stage of development of the individual tooth. Similar patterns of transient *Hox 7.1* expression can also be detected in tooth germs *in vitro* in organ cultures of day 11 first branchial arch explants cultured for up to 7 days.

Hox 7.1 is also expressed early in development (days 10/11) in the epithelium of the developing anterior pituitary (Rathke's pouch), the connective tissue capsule and meninges of the developing brain, and specific regions of neuroepithelium in the developing brain.

Key words: homeobox, craniofacial development, tooth development, embryogenesis, epithelial–mesenchymal interactions, neural crest.

Introduction

Development of the mammalian dentition involves regional (incisors, canines, premolars and molars) and temporal patterning of the individual tooth anlage. Moreover development of an individual tooth requires an extensive series of reciprocal epithelial–mesenchymal interactions commencing with a thickening of the jaw epithelium, proceeding through morphogenesis and cytodifferentiation to result in a fully formed rudiment whose shape varies with position in the jaws (and is bilaterally symmetrical) and whose cell layers differentiate into specialised secreting cells (ameloblasts from the epithelium, odontoblasts from the mesenchyme) which synthesise and assemble the unique dental extracellular matrices of enamel and dentine (Thesleff and Hurmerinta, 1981; Ruch, 1984, 1987; Partanen and Thesleff, 1989; Thesleff *et al.* 1989). This combination of extensive, reciprocal, epithelial–mesenchymal interactions characterised by synthesis of unique cell layer specific molecules, complex development of organ shape at late stages of embryogenesis, feasibility of *in vivo* experimentation and *in vitro* culture under chemically defined, serum-free conditions, makes the developing tooth a favourite and well-studied model for analysis of embryonic epithelial–mesenchymal interactions (Kollar and Baird, 1969, 1970a,b; Slavkin, 1974; Kollar, 1976; Yamada *et al.* 1980; Thesleff and Hurmerinta, 1981; Ruch *et al.* 1982, 1983; Partanen *et al.* 1985; Ruch, 1987; Lumsden, 1988; Partanen and Thesleff, 1989; Thesleff *et al.* 1989).

The first sign of tooth development is a thickening of the jaw epithelium, followed by intrusion of the latter into the jaw mesenchyme to form a dental lamina, development of distal swellings on the lamina and condensation of neural-crest-derived jaw mesenchyme around the latter (Cohn, 1957; Lumsden and Buchanan, 1986; Westergaard and Ferguson, 1986, 1987, 1990; Thesleff *et al.* 1989). The developing tooth germ then

goes through named and well-characterised morphogenetic and differentiation stages: bud, cap, bell, to form the adult tooth (Mjor and Fejerskov, 1986; Ruch, 1987; Thesleff *et al.* 1989; Ferguson, 1990). Initiation and patterning of the dentition appear to be first regulated by the epithelium (Miller, 1969; Mina and Kollar, 1987; Lumsden, 1988), but shape of individual rudiments and ameloblast differentiation may be specified later by the dental papilla mesenchyme (Kollar and Baird, 1969, 1970*a,b*), whilst gradation of shape along the anteroposterior axis of the jaws has been postulated as a morphogenetic field phenomenon (Kollar, 1981, Osborn, 1984). These morphogenetic and differentiative events are characterised by changes in extracellular matrix molecules e.g. fibronectin, collagen type III (Thesleff *et al.* 1979, 1981, 1989; Lesot *et al.* 1981; Kubler *et al.* 1988), tenascin (Thesleff *et al.* 1987); chondroitin sulphates (Mark *et al.* 1990); matrix receptors e.g. syndecan (Thesleff *et al.* 1988; Vainio *et al.* 1989); growth factors e.g. EGF (Snead *et al.* 1989), FGF (Wilkinson *et al.* 1989), TGFB$_1$ (Heine *et al.* 1987; Lehnert and Akhurst, 1988), growth factor receptors e.g. EGF (Partanen and Thesleff, 1987, 1989), and NGF (Yan and Johnson, 1988) receptors and transcription factors e.g. Egr-1 (McMahon *et al.* 1990), particularly at the later stages of cytodifferentiation into ameloblasts and odontoblasts. Almost nothing is known about the molecular basis of patterning in the dentition.

mRNA from the *Hox 7.1* gene localises in regions of cephalic neural crest cell migration and differentiation as well as in the heart and in the developing limb buds of mouse embryos (Robert *et al.* 1989; Hill *et al.* 1989). These early reports surveyed sagittal sections of mouse embryos and indicated that *Hox 7.1* may be expressed at, and important in, sites of epithelial–mesenchymal interactions. We have independently cloned *Hox 7.1* and analysed its expression in developing embryonic mouse heads throughout the period of dental and palatal development.

Materials and methods

Embryonic mouse heads

Mice, strain Manchester, were mated overnight and the day of finding a vaginal plug designated day zero. At embryonic days 8–16, mothers were killed by ether overdose, the uteri and embryos aseptically removed, and the embryos decapitated and fixed in 10 % paraformaldehyde solution for 24–48 h. Heads were dehydrated through ascending grades of alcohol, cleared in Xylene and embedded in Fibrowax (Raymond A. Lamb, London). Serial histological sections were cut in the coronal plane and mounted on aminoalkylsilane-coated slides under RNAase-free conditions. Sections of embryos from day 12 onwards were screened for the presence of either palatal shelves or first or second molar tooth germs prior to *in situ* hybridisation.

Gene isolation, preparation of probes, in situ hybridisation

Hox 7.1 cDNAs were isolated from a mouse day 8½ embryonic cDNA library following a screen at reduced stringency with
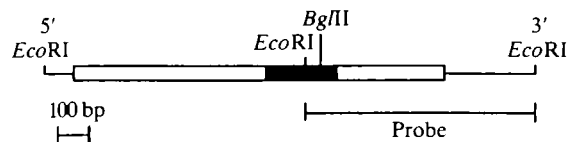

Fig. 1. Restriction map of *Hox 7.1* cDNA insert showing fragment subcloned to provide probe for *in situ* hybridisation. The homeobox region is shown in black.

the *Drosophila* bicoid homeobox. Sequencing confirmed that two clones isolated were derived from *Hox 7.1* transcripts by comparing the sequences to those published by Robert *et al.* (1989) and Hill *et al.* (1989). For *in situ* hybridisation a 600 bp *Eco*RI fragment from one cDNA clone, containing 375 bp of coding sequence (Fig. 1) was subcloned into pSP72. Sense and anti-sense ^{35}S-labelled riboprobes were generated by standard SP6 and T7 polymerase reactions. The *in situ* hybridisation protocol was as previously described using paraformaldehyde fixation (Sharpe *et al.* 1988).

In vitro culture

Embryonic heads were obtained aseptically, as described earlier, at 11 days gestation. The lower jaw was removed by cutting posterocaudally from the jaw angles so as to include the presumptive molar regions. Explants were placed on Millipore filters and cultured at the air–gas interface above Eagles minimum essential medium supplemented with glutamine, glycine, ascorbate and transferrin, as previously described (Yamada *et al.* 1980; Ferguson *et al.* 1984). Explants were removed from culture after 3 and 7 days, fixed and processed for *in situ* hybridisation as described earlier.

Results

In vivo

Days 8 and 9

Intensive labelling was present in the neural folds and in the neural crest cells adjacent to the neural folds. Labelling was also present in the heart region, as previously described by Robert *et al.* (1989) and Hill *et al.* (1989).
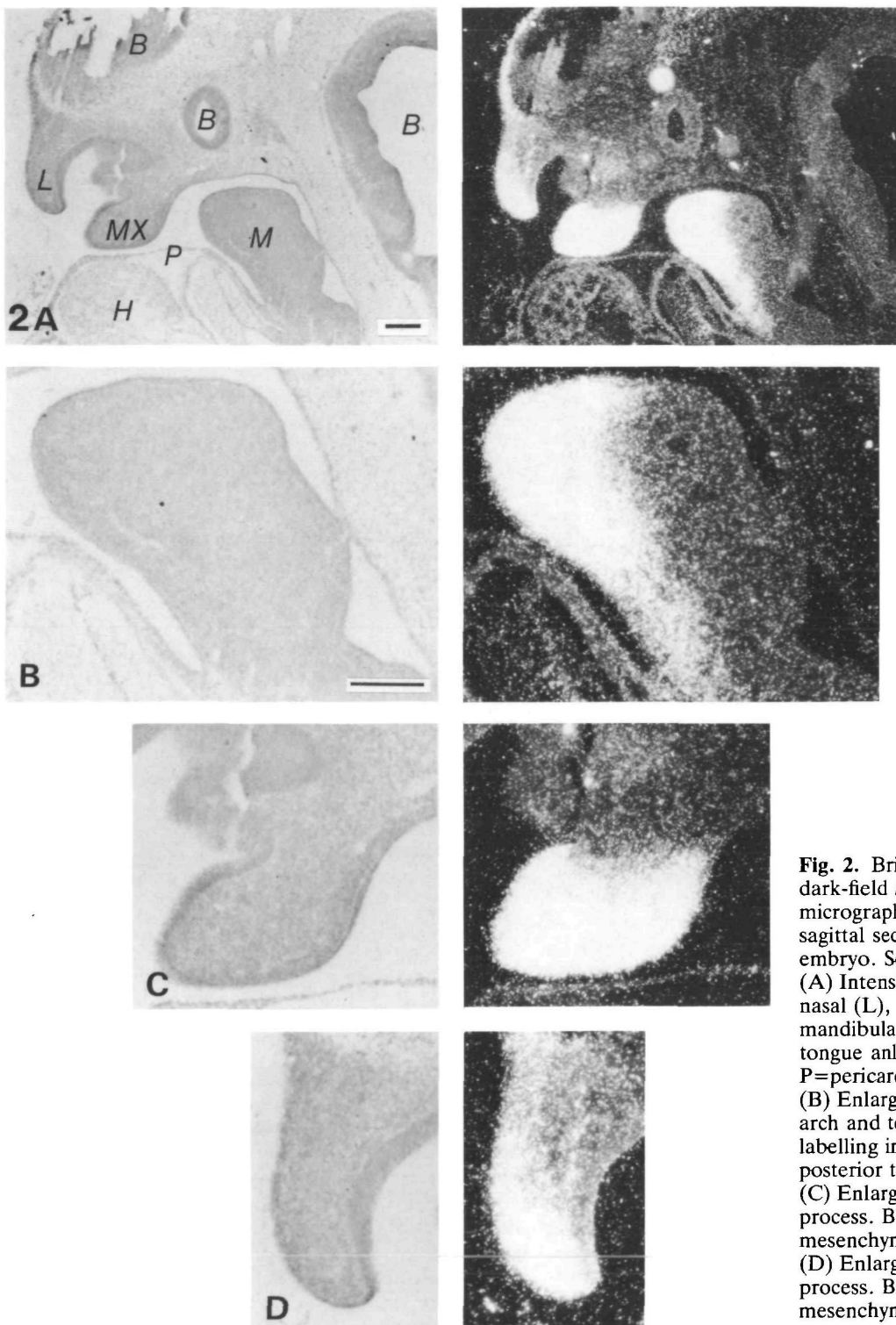
Day 10

Labelling was present in the brain and neural tube, progress zone of the limb bud and in the heart and pericardium (Figs 2A, 3B). In the head, the mesenchyme of the medial nasal, lateral nasal and maxillary processes and first branchial (mandibular) arch were all intensely labelled (Fig. 2A), again as previously described by Robert *et al.* (1989) and Hill *et al.* (1989).

In coronal section, the caudal tips of the medial nasal, lateral nasal and maxillary process (Fig. 4A) mesenchyme labelled intensely (Fig. 3A,B). There was little change in this pattern in posterior regions where the processes merge to form the primary nares. The epithelium of the invaginating nasal pit was unlabelled (Fig. 3A).

The mesenchyme surrounding the top and sides of the brain (future skull bones) and the developing brain meninges labelled intensely for Hox 7.1 (Fig. 3A,B).

The first branchial (mandibular) arch labelled intensely anteriorly throughout the mesenchyme, but

Fig. 2. Bright-field and corresponding dark-field *in situ* hybridisation micrographs of *Hox 7.1* expression in sagittal sections of a day 10 mouse embryo. Scale bar=50 µm.
(A) Intensive labelling in the lateral nasal (L), maxillary (mx) and mandibular (m) processes (including the tongue anlage). H=heart; P=pericardium; B=brain.
(B) Enlargement of the mandibular arch and tongue. Note the absence of labelling in the epithelium and in the posterior tongue anlagen.
(C) Enlargement of the maxillary process. Both epithelia and mesenchyme label intensely.
(D) Enlargement of the frontonasal process. Both epithelia and mesenchyme label intensely.

labelling fell off dramatically in the middle third of the mandible and the posterior one third was barely labelled above background (Figs 2A, 2B, 3B). Labelling was more intense in the top (oral) half of the mandibular mesenchyme than in the bottom (aboral) half (Figs 2A, 2B, 3B). The anterior component of the tongue anlage (of presumptive first arch origin) was heavily labelled whereas the posterior component (of presumptive second and third arch origin) was unlabelled (Fig. 2A,B). Labelling of the first branchial arch was however, confined to the mesenchyme components: epithelial labelling was not above background (Figs 2B, 3B). By contrast, both the mesenchymal and epithelial components of the maxillary (Figs 2C, 3B) medial nasal and lateral nasal (Figs 2D, 3A) processes labelled intensely, particularly at their
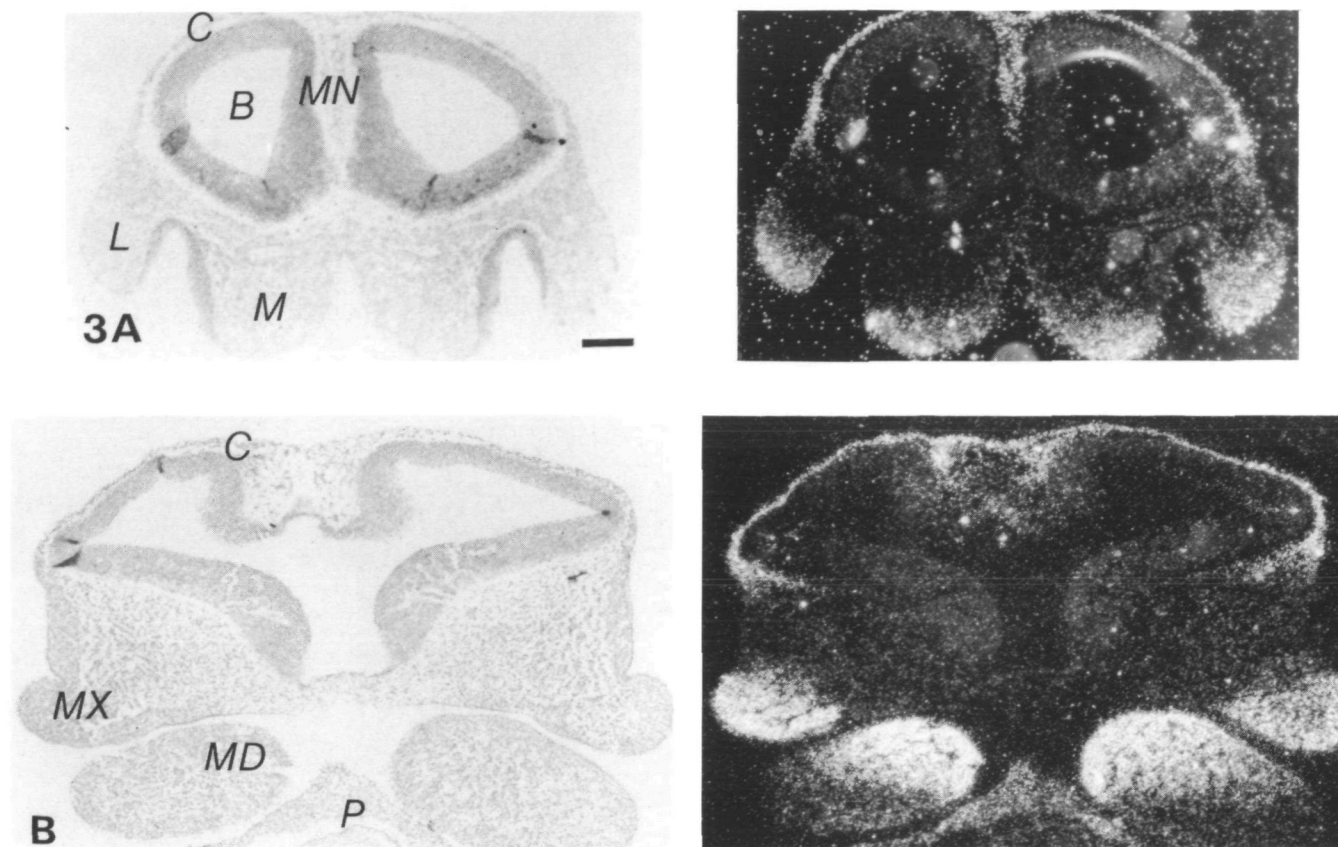
Fig. 3. Bright-field and corresponding dark-field *in situ* hybridisation micrographs of *Hox 7.1* expression in coronal sections of embryonic day 10 mouse heads. Scale bar=50 μm. (A) Intense labelling in the caudal tips of the medial nasal (M), and lateral nasal (L) processes (see Fig. 4 for orientation), in the mesenchymal capsule (C) surrounding the top and sides of the brain (B) and in the meninges (MN). (B) More posterior section to that in A illustrating intense labelling on the oral sides of the maxillary (MX) and mandibular (MD) processes, the mesenchymal capsule (C) surrounding the top and sides of the brain (B) and the pericardium (P).

tips. The maxillary mesenchyme also labelled more intensely nearer the oral side than the aboral side (Fig. 3B).

Posteriorly the mesenchymal core of the second branchial arch labelled intensely. The epithelial invagination (Rathke's pouch) from the roof of the oronasal cavity to form the anterior pituitary labelled intensely (see Fig. 6). Selected regions of the brain neuroepithelium e.g. in the areas of the future thalamus, third and fourth ventricles and in the connective tissue supporting the choroid plexus also labelled strongly (Fig. 3B – see also Fig. 6).

Day 11

Labelling in the facial processes (Fig. 4B), future skull bones, meninges, anterior pituitary and brain followed the same general pattern as day 10 (Figs 5A–F, 6). In the region of closure between the medial nasal, lateral nasal and maxillary processes (Fig. 4B,D) *Hox 7.1* was expressed throughout the mesenchyme and in the epithelia of the oral stem of the Y-shaped nares (Fig. 5B).

In the mandibular arch and maxillary processes, a

continuous line of thickened epithelium marked the site of the future tooth germs (Fig. 5C–E). In the maxillary processes, the expression of *Hox 7.1* was broadly localised to the mesenchyme surrounding such thickened epithelium and fell off progressively in an anteroposterior gradient (Fig. 5C–E). The mesenchyme at the tip of the mandibular arch labelled intensely for *Hox 7.1* throughout its entirity (Fig. 5C). In the anterior and middle third of the mandibular arch the expression of *Hox 7.1* decreased progressively in the caudal, aboral mesencyhme and in more posterior arch mesenchyme (Fig. 5D,E). Labelling was intense in the anterior two thirds of the tongue anlage (Fig. 5D) but absent in the posterior third (Fig. 6). In the posterior third of the mandibular arch, *Hox 7.1* was expressed for the first time in broad areas of mesenchyme surrounding the thickened dental epithelium (Fig. 6) and in the base of the mandibular arch mesenchyme in the region of the future hyoid bone (Fig. 6). At the very back of the mandibular arch, where the thickened dental epithelium was absent, there was no labelling in the mandibular mesenchyme; however, the mesenchyme of the second branchial arch was intensely labelled (Fig. 5F). No expression was
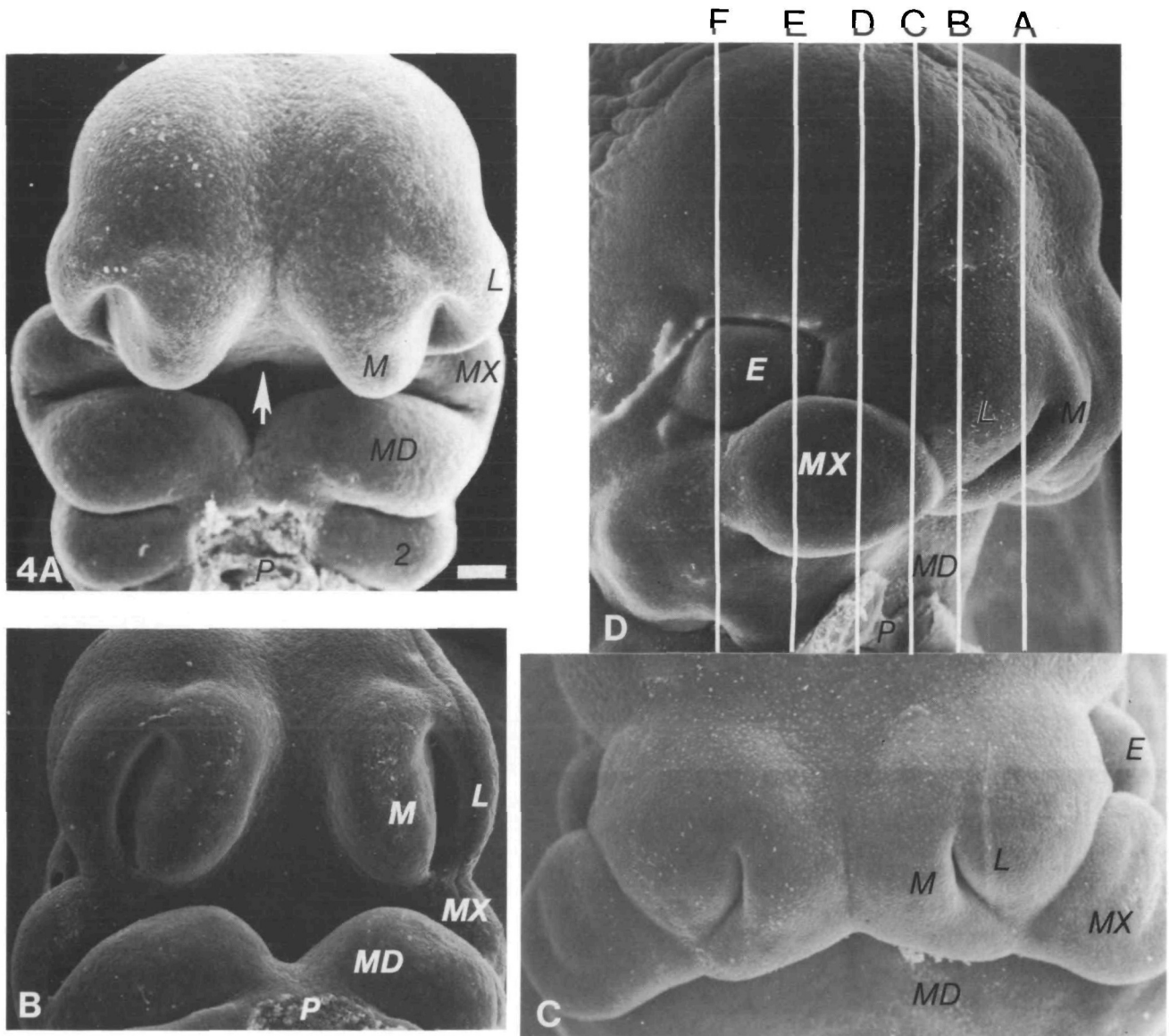
Fig. 4. Scanning electron micrographs of day 10 (A), 11 (B) and 12 (C) mouse embryos viewed *en face* to illustrate the medial nasal (M), lateral nasal (L), maxillary (MX) and mandibular (MD) processes. E=eye, P=pericardium (removed) and arrow=invagination of Rathke's pouch in the roof of the oronasal cavity. D=lateral oblique view of a day 11 mouse embryonic head with lines indicating the levels of section in Fig. 5A–F. Scale bar=100 µm.

observed in any region of the mandibular epithelium (Fig. 5C–F).

The epithelium of the anterior pituitary was heavily labelled (Fig. 6) as were various areas of neuroepithelium and the mesenchyme adjacent to the developing eye (Fig. 6).

Between days 11 and 12, expression of *Hox 7.1* became progressively restricted to the mandibular mesenchyme surrounding the thickened dental epithelium. In the anterior two thirds of the mandible this meant that the expression of *Hox 7.1* decreased in the rest of the mesenchyme, whereas in the posterior one

third, it meant that *Hox 7.1* was expressed for the first time in the mesenchyme surrounding the thickened dental epithelium.

Day 12

Labelling was not intense in the fused medial nasal, lateral nasal, maxillary (Fig. 4C) and mandibular mesenchyme, except in the region of epithelial invagination for the incisor and first molar tooth germs (Fig. 7A). The mesenchyme surrounding the dental epithelial thickenings on the jaw margin was more intensely labelled (Fig. 7A,B). Where the epithelium
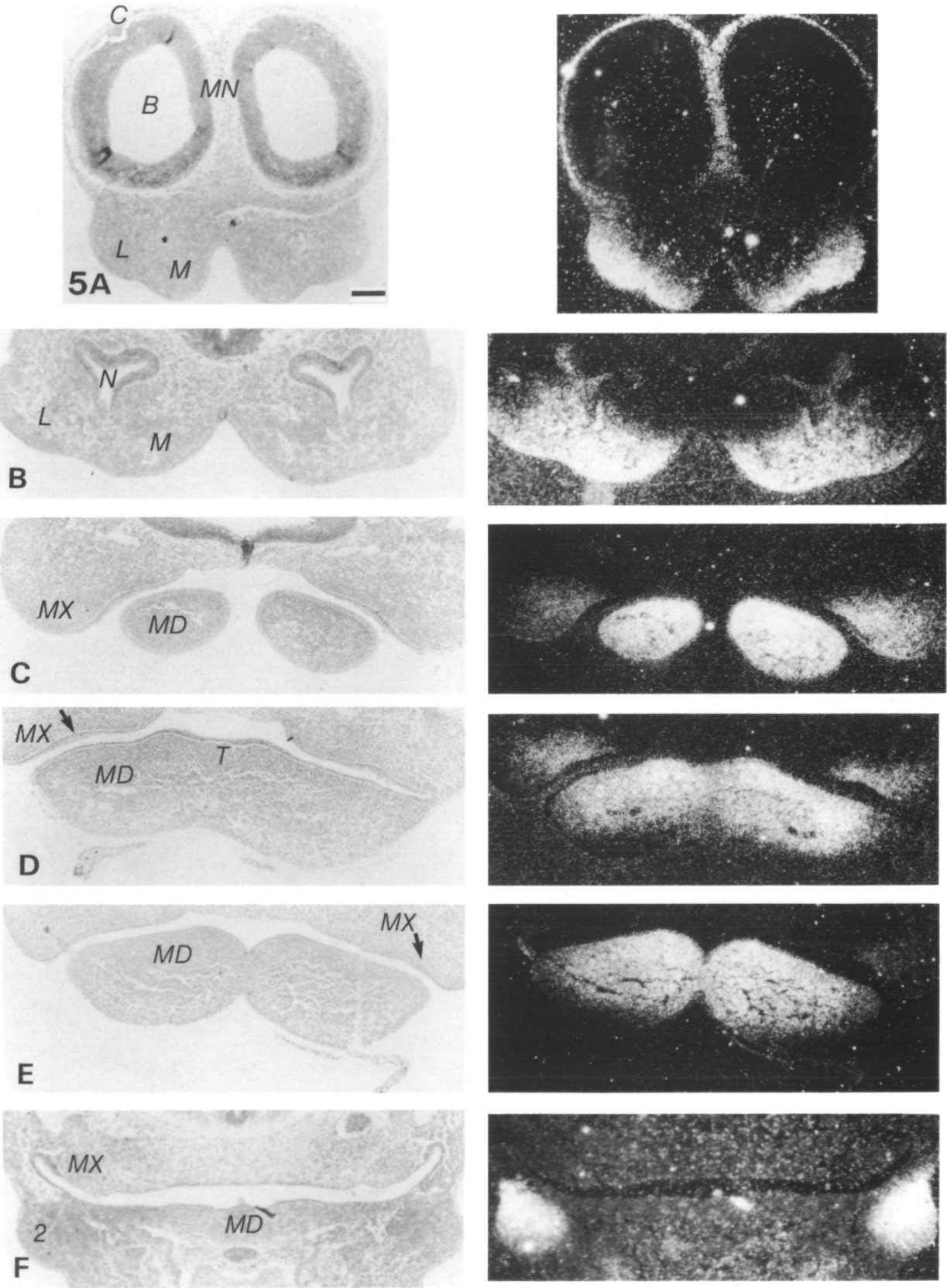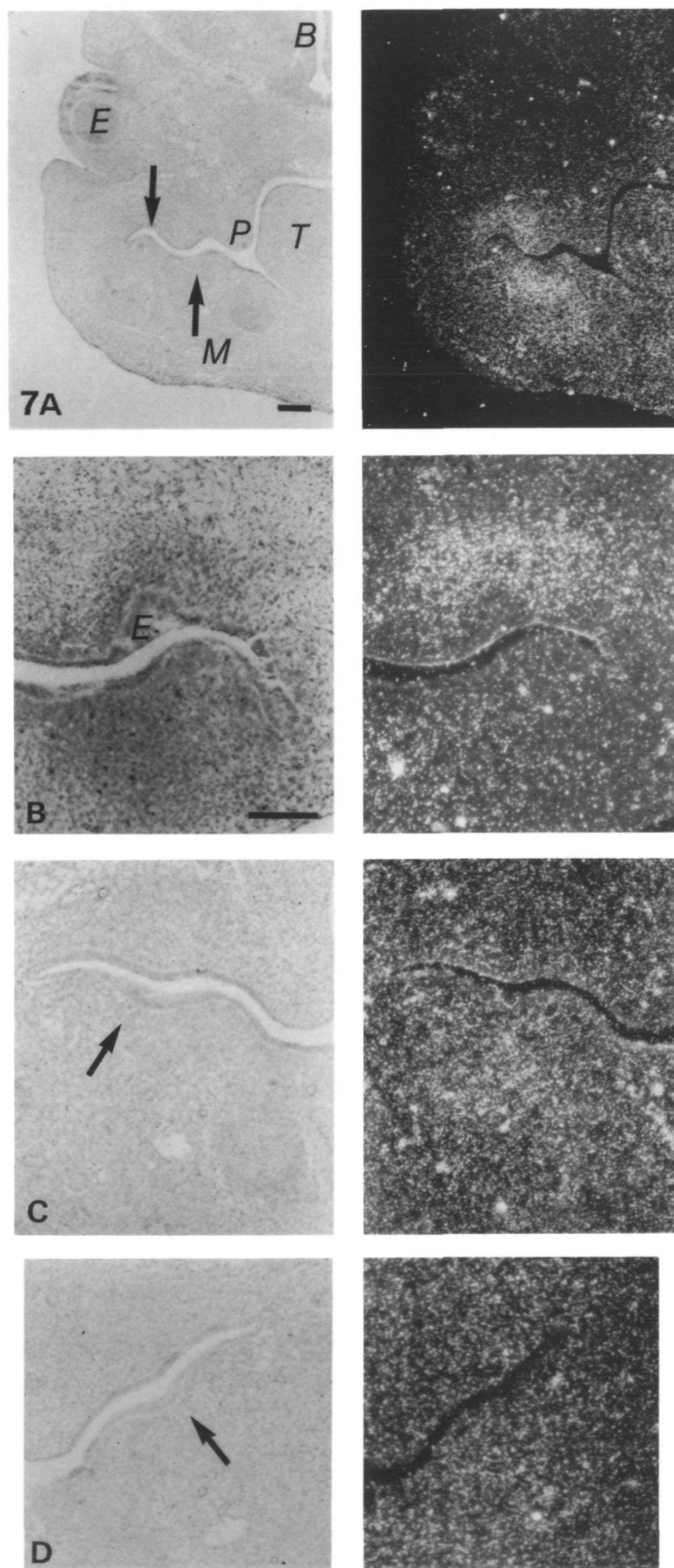
Fig. 5. Bright-field and corresponding dark-field *in situ* hybridisation micrographs of *Hox 7.1* expression in coronal sections of embryonic day 11 mouse heads. Scale bar=50 μm. The level of sectioning of A–F is indicated in Fig. 4D and the *en face* orientation view in Fig. 4B. A=intense labelling in the mesenchyme of the medial (M) and lateral (L) nasal processes, the mesenchymal capsule (C) surrounding the top and sides of the brain (B) and in the meninges (MN). B= intense labelling in the mesenchyme of the medial (M) and lateral (L) nasal processes in the region of their fusion (see Fig. 4B and D). The epithelium of the primary nares (N) labels at the oral base of its Y shape in the region of fusion of the medial and lateral nasal processes: elsewhere it is unlabelled. The silver grains in the nares are non-specific trapping. C=intense labelling throughout the mesenchyme of the tip of the mandibular process (MD) and in the mesenchyme of the maxillary process (MX) particularly near its oral margins. D=intense labelling in the mesenchyme of the mandibular process (MD) and tongue anlage (T), but this is no longer uniformly distributed: the caudal aboral mesenchyme does not label. Labelling in the maxillary process (MX) mesenchyme is becoming localised to areas surrounding the thickened dental epithelia (arrowed). E= intense labelling in the mesenchyme of the mandibular process (MD) of a different embryo from that in A–D, again illustrating progressive loss of labelling as one moves caudally from the oral cavity. Maxillary process (MX) mesenchyme labelling is becoming restricted to surround areas of thickened dental epithelium (arrowed). F=absence of labelling in the posterior mesenchyme and epithelia of the mandibular (MD) and maxillary (MX) processes, but intense labelling in the second branchial arch (2).
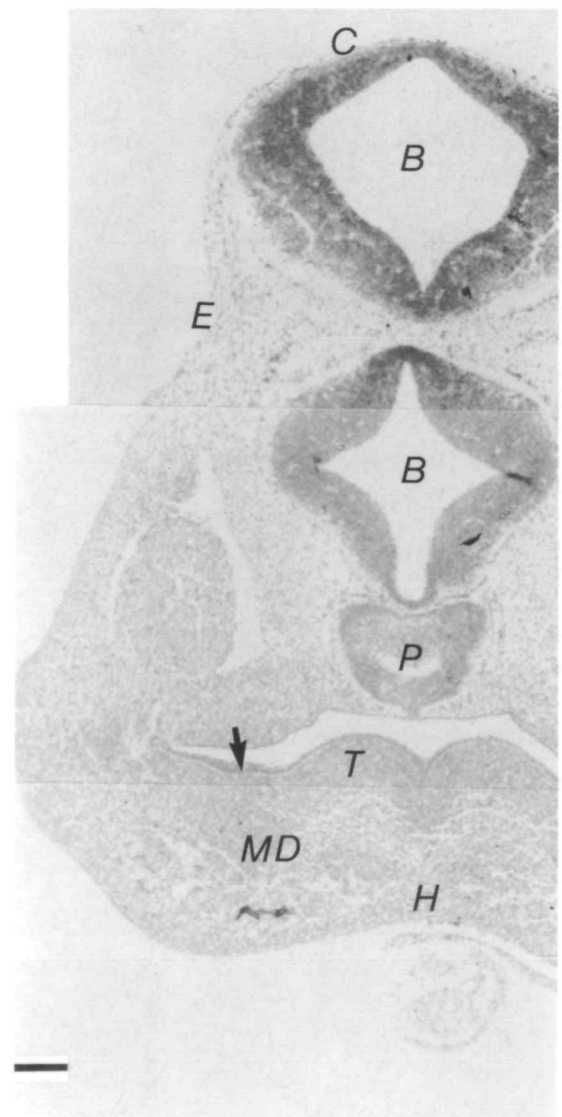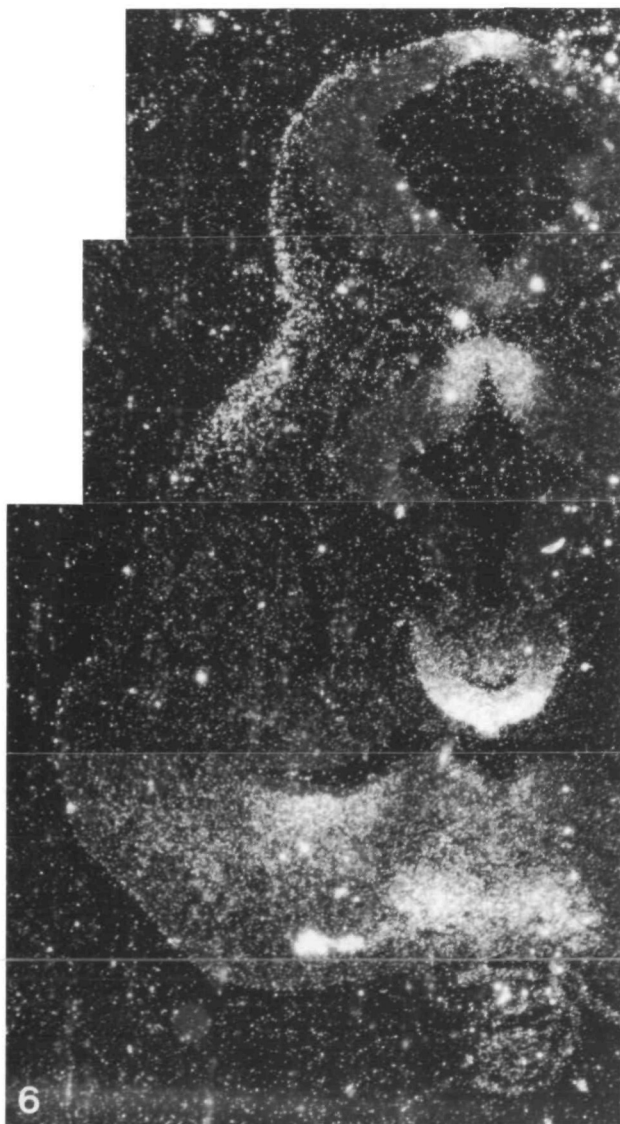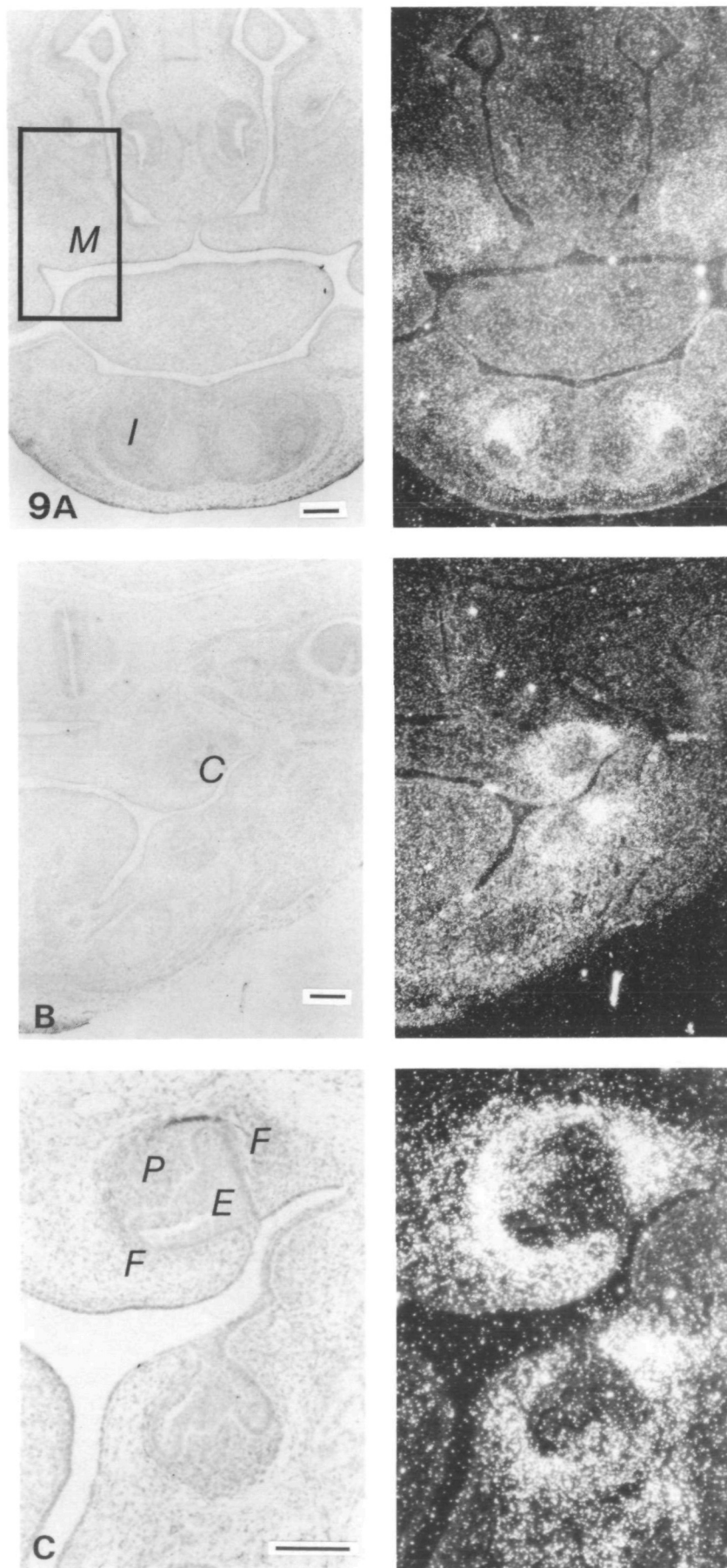

Fig. 6. Bright-field and corresponding dark-field *in situ* hybridisation micrographs of *Hox 7.1* expression in a coronal section of a day 11 mouse head. Scale bar=50 μm. Note the intense labelling in the epithelium of the developing anterior pituitary/Rathke's pouch (P), selected regions of the neuroepithelium of the brain (B), the mesenchymal capsule (C) covering the top of the brain and a layer of mesenchyme extending laterally down the sides of the head beneath the epithelium, particularly adjacent to the developing eye (E), the mesenchyme surrounding the thickened dental epithelium (arrowed) of the mandibular processes (MD) and the relative absence of labelling elsewhere in the mandible and tongue (T) except for the future anlage of the hyoid bone (H).

Fig. 7. Bright-field and corresponding dark-field *in situ* hybridisation micrographs of *Hox 7.1* expression in coronal sections of a day 12 mouse embryo head. Scale bar=50 μm. (A) Labelling in the jaw mesenchyme surrounding the invaginating dental epithelia for the upper and lower first molar teeth (arrowed). B=brain, E=eye, P=palate, T=tongue, M=mandible. (B) Localised labelling in the mesenchyme surrounding the invaginating dental epithelia (E) for the upper first molar tooth. The epithelia is unlabelled. (C) No localised labelling in either the mesenchyme near, or epithelia of, the thickened dental epithelia for the second molar tooth (arrowed). (D) Control section through the second molar tooth germ hybridised with the sense probe, illustrating no localisation of any silver grains.
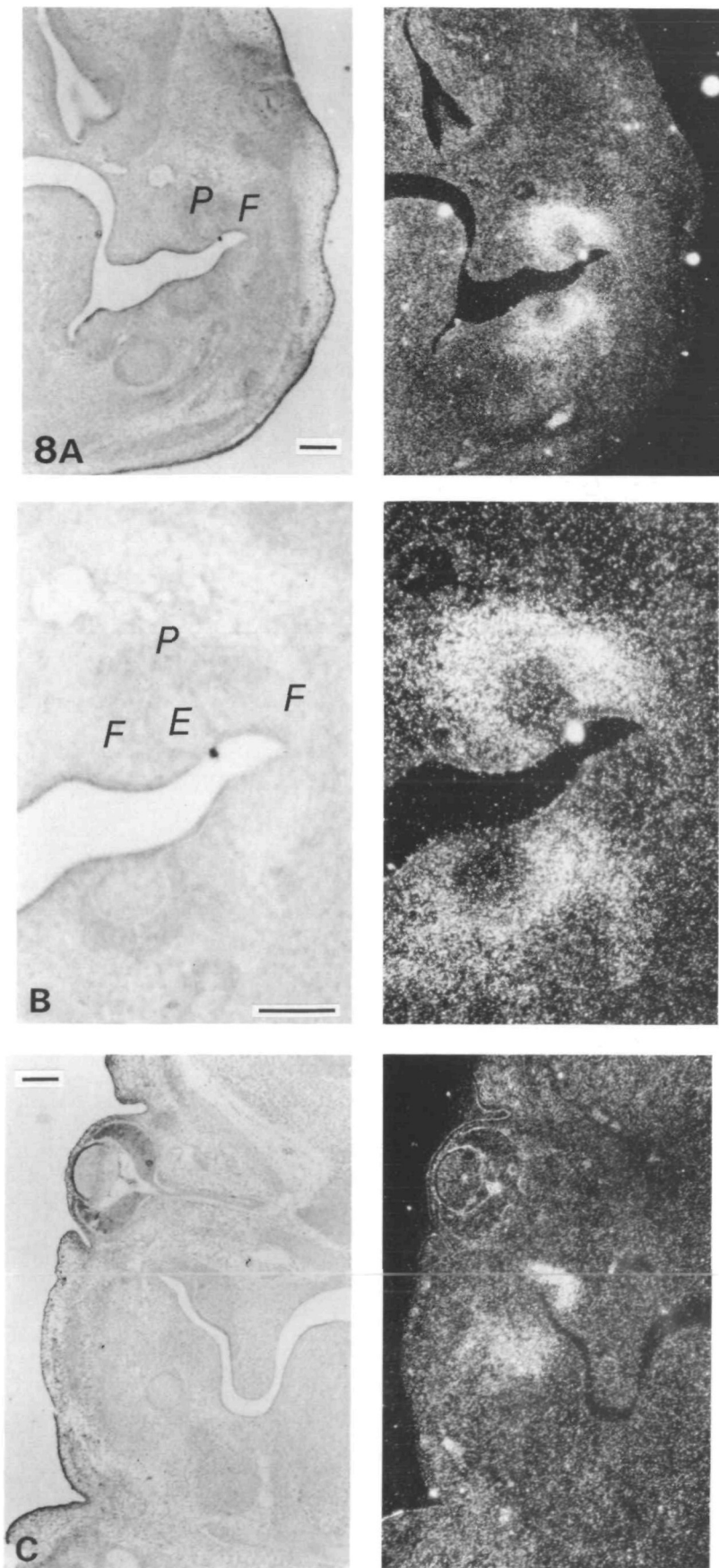
Fig. 8. Bright-field and corresponding dark-field *in situ* hybridisation micrographs of *Hox 7.1* expression in coronal sections of a day 13 mouse embryo head. Scale bar=50 μm. (A) Intensive labelling in the dental papillae (P) and follicles (F) of the first molar tooth germs. (B) Enlargement of the bud stage first molar tooth germs illustrating intensive labelling in the dental papilla (P) and follicle (F) mesenchyme but absence in the enamel organ (E) and jaw epithelia. (C) Labelling of the mesenchyme surrounding the invaginating epithelia for the second molar tooth (compare with Fig. 7).

Fig. 9. Bright-field and corresponding dark-field *in situ* hybridisation micrographs of *Hox 7.1* expression in coronal sections of a day 14 mouse embryo head. Scale bar=50 μm. (A) Anterior section illustrating labelling in the developing bony blastemata for the maxillary bones (M) and the dental papillae and follicles of the incisor tooth germs (I). Boxed area is enlarged in E. (B) Intensive labelling in the dental papillae and follicles of the cap stage first molar tooth germs (c). (C) Enlargement of the cap stage first molar tooth germs illustrating intensive labelling in the dental papillae (P) and follicle (F) mesenchyme and absence in the enamel organ epithelia (E). (D) Intensive labelling in the dental papilla and follicle mesenchyme around the bud stage second molar tooth germs (T). Less intense labelling is also present in the muscle blastematae for the masseter (M) and lateral pterygoids (L). (E) Enlargement of the boxed area in A illustrating labelling in the maxillary bone blastemata.

had invaginated into the mesenchyme, labelling was more highly localised to the mesenchymal condensation for the dental papilla, dental follicle and future alveolar bone (Fig. 7B). This labelling did not continue along the anteroposterior length of the jaws, indeed posteriorly where the epithelium had thickened to be only 2–3 layers thick, no intense labelling was localised in the underlying mesenchyme (Fig. 7C,D). No localised labelling, above background, was evident in the palatal shelves (except very anteriorly), maxillae, mandible, tongue or any epithelia, including dental, (Fig. 7A).

Day 13
The first molar tooth germs are at the bud stage of development (Fig. 8A,B). Intense labelling is highly localised to the dental papillae, dental follicles and future alveolar bone of the first molars (Fig. 8A,B). Intense labelling is also present anteriorly in the dental papillae and follicles of the incisor tooth germs and

posteriorly in the mesenchymal condensations around the invaginating epithelium for the second molars (Fig. 8C). The epithelia of all tooth germs (and of the jaws) remain unlabelled.

Day 14
Anteriorly, the dental papillae and follicles of the incisor tooth germs are intensely labelled (Fig. 9A). This labelling now extends posteriorly in the jaw mesenchyme, the full length of the incisor tooth germs. The osteogenic blastemata for the maxillary bones are also lightly labelled (Fig. 9A,E). The first molar tooth germs are at the cap stage of development: the mesenchymal condensations for the dental papillae and dental follicles are intensely labelled (Fig. 9B,C). Labelling is slightly less intense than at the previous bud stage. All the enamel organs and jaw epithelia do not label above background (Fig. 9A–C). Posteriorly, the
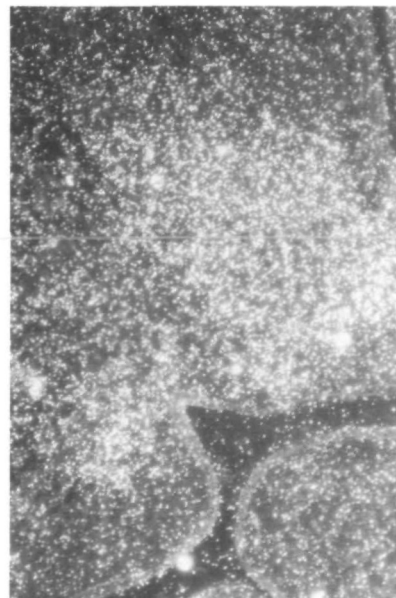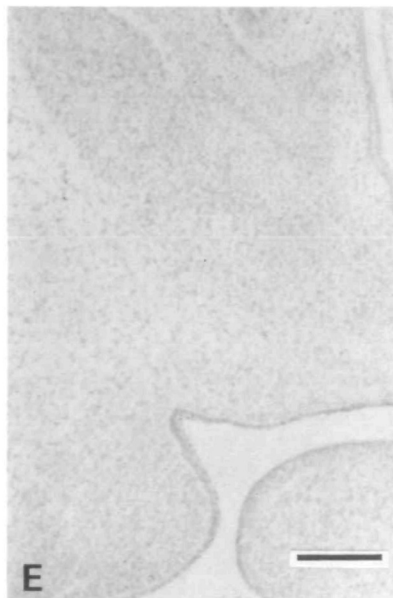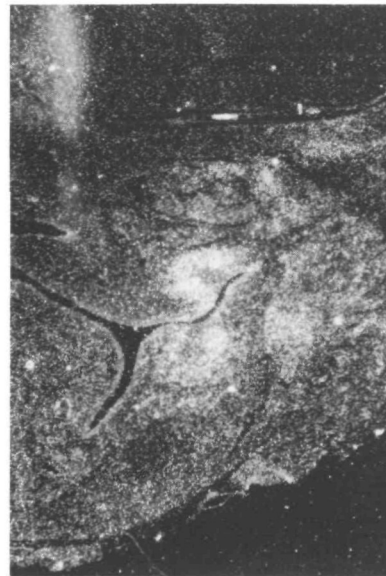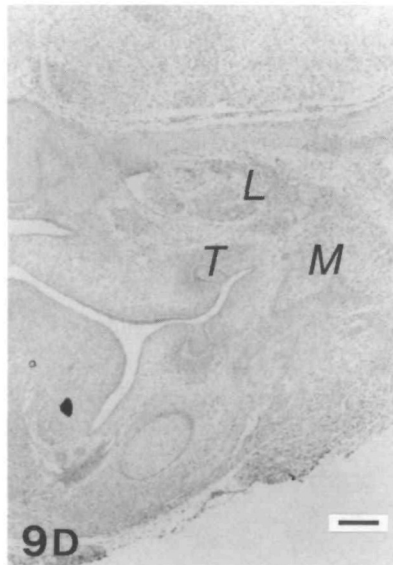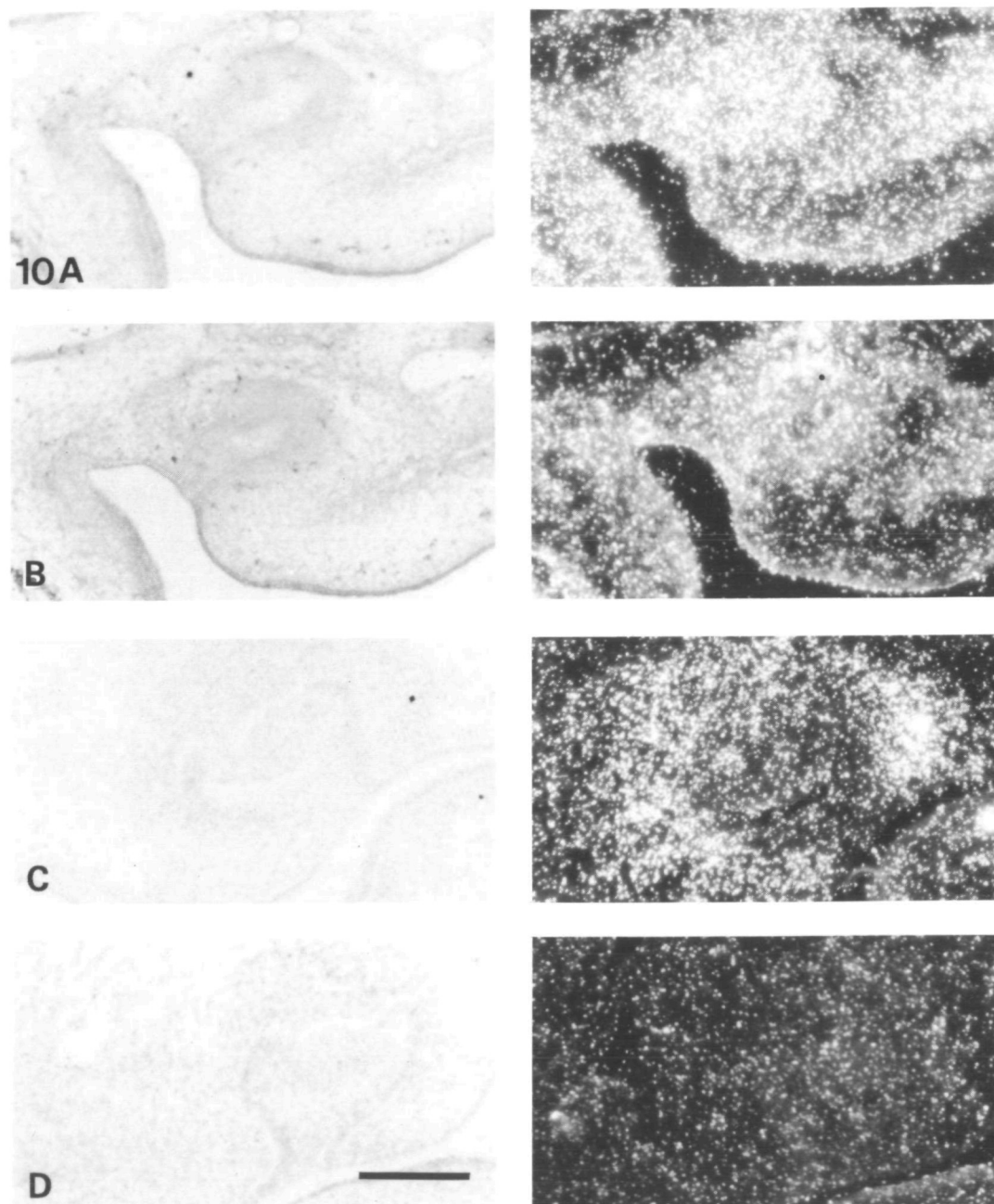
Fig. 10. Bright-field and corresponding dark-field *in situ* hybridisation micrographs of *Hox 7.1* expression in coronal sections of first and second molar tooth germs in day 15 mouse embryo heads. Scale bar=50 μm. (A) First molar tooth germ (at the late cap/early bell stage) treated with the antisense probe. There is no localised labelling above background, compared to control sections (B) treated with the control sense probe. (C) Second molar tooth germ (at the cap stage) with localised labelling of the antisense probe in the mesenchyme of the dental papilla and follicle (compare Fig. 9C). (D) Second molar tooth germ (at the cap stage) treated with the control sense probe. Note no localised labelling: compare with Fig. 10C.

second molar tooth germs are at the bud stage of development and label intensely in the dental papillae and dental follicle mesenchyme (Fig. 9D). The muscle blastematae for the medial pterygoids, lateral pterygoids, masseter and temporalis also label, though less intensely than the tooth germs (Fig. 9D). Other muscle blastemata e.g. tongue, eye do not label (Fig. 9B,D).

Day 15

The first molar tooth germs are at the late cap/early bell stage of development (Fig. 10A,B). There is no localised labelling in the dental papillae and follicles (Fig. 10A,B). Labelling is much less intense in the incisor tooth germs. By contrast the second molars, at the cap stage of development, label with similar

localised intensity (Fig. 10C,D) to the first molar at day 14 (Fig. 9C).

Day 16

Labelling of the first molar and incisor tooth germs is neither localised nor above background, whilst the second molar tooth germs resemble the first molar pattern at day 15.

In vitro

First branchial arches explanted at day 11 and cultured for 3 days had first molars at the bud stage of tooth development (Fig. 11A). The mesenchymal condensations for the dental papillae and follicles labelled intensely (Fig. 11A). First molar tooth germs explanted with their branchial arches at day 11 and cultured for various periods showed similar tooth developmental stage labelling patterns to those described *in vivo*, including the cessation of labelling at the late cap/early bell stage after 7 days in culture (Fig. 11B,C). Likewise, explants containing second molar tooth germs cultured for 7 days showed specific labelling patterns similar to those observed *in vivo* for comparable stages of tooth development (Fig. 11D). These culture experiments confirmed that the transient stage-specific expression of Hox 7.1 in selected tissues of developing tooth germs also occurs when they are cultured *in vitro* under defined conditions.

Discussion

Hox 7.1 appears to be expressed initially in most or all cephalic neural crest cells at days 8 and 9 of development. It is then expressed throughout the neural-crest-derived mesenchyme of the medial nasal, lateral nasal, maxillary and mandibular processes, the fibrous tissue surroundings of the brain, the meninges (day 10 of development) and in some epithelia e.g. Rathke's pouch and the tips of the medial nasal processes. Labelling of the medial nasal and lateral nasal processes and the epithelia of their tips is reminiscent of a limb progress zone pattern. Alternatively, the heavy labelling of all facial process mesenchyme surrounding the oral cavity may indicate that a stimulatory molecule (e.g. growth factor) is absorbed from the amniotic fluid by the oral epithelia. In the mandibular arch mesenchyme *Hox 7.1* is initially expressed anteriorly but not posteriorly. Expression also falls off in a posterior to anterior gradient with time. Anteriorly in the mandibular and maxillary processes *Hox 7.1* expression persists only in a localised fashion around the developing dental epithelium, whereas posteriorly *Hox 7.1* expression appears for the first time highly localised to the mesenchyme beneath the dental epithelium. Between embryonic days 12–15 *Hox 7.1* is expressed predominantly in areas of dental development, but disappears completely from the developing first molar tooth germs by embryonic day 16. Throughout this time it is not expressed in other neural-crest-derived jaw mesenchyme, but at embry-

onic days 14–16 begins to be expressed, at lower levels, in muscle and bone anlage. Whilst bony anlage consist of neural-crest-derived cells, the myotubes of the facial muscles are of lateral plate mesoderm origin (Noden, 1983, 1986, 1988). *Hox 7.1* is therefore not expressed exclusively in neural-crest-derived mesenchyme, as is also evident from its expression in the limb bud, brain and Rathke's pouch epithelium. In the head, its expression in particular epithelia of the brain and anterior pituitary as well as in certain neural crest cell populations may relate to a common region of expression (potential expression) in the rhombomeres of the developing brain. *Hox 7.1* expression in developing muscle is not surprising considering that it is the mammalian homologue of the *Drosophilia* muscle segment homeobox gene (msh) (Robert *et al.* 1989; Hill *et al.* 1989). Why it is expressed in some (e.g. jaw) but not all (e.g. tongue) cranial muscles at embryonic day 14 is unclear, but may relate to the timing of differentiation and warrants further detailed investigation.

Expression of *Hox 7.1* in the developing dentition is tooth stage specific: the pattern of expression at the bud, cap and bell stages being similar regardless of: the tooth in question (1st/2nd molar), the chronological age of the embryo, or whether it was developing *in vivo* or *in vitro*. Expression was low at the dental lamina stage, maximal at the bud/cap stages and fell of rapidly in the bell stage of development. Expression of *Hox 7.1* correlated with the appearance of the mesenchymal condensation for the dental papilla and follicle. At no time was expression detected in any epithelial components: dental or jaw. This pattern suggests that the thickening/invaginating dental epithelium induces *Hox 7.1* expression in the underlying mesenchyme. As the expression patterns are similar *in vitro* this hypothesis is currently being tested by heterotypic, heterochronic epithelial–mesenchymal recombination experiments. Expression of *Hox 7.1* declines when the tooth germ begins to undergo cytodifferentiation at the bell stage, into odontoblasts, ameloblasts and dental follicle cells. This period corresponds to a time of epithelial–mesenchymal interactions between the dental papilla mesenchyme and the enamel organ epithelia (Thesleff and Hurmerinta, 1981).

Tenascin and syndecan are also expressed in the dental papilla mesenchyme and dental follicle in a broadly similar fashion to *Hox 7.1* (except that syndecan is also present in the jaw and dental epithelium) at the bud/cap stage of development (Thesleff *et al.* 1987, 1988, Vainio *et al.* 1989). However, unlike *Hox 7.1*, tenascin and syndecan continue to be expressed in the dental tissues even into adulthood (Thesleff *et al.* 1987, 1988). Fibronectin and collagen type III also appear around the bud/cap stage in a generally similar pattern to *Hox 7.1* but their precise and subsequent localisations differ greatly (Thesleff *et al.* 1979, 1981, Lesot *et al.* 1981). Growth factors and oncogene products such as EGF, int 2, $TGFB_1$ and IGFII also localise, with their receptors, in the dental papillae and follicles at the bud/cap stage (Partanen
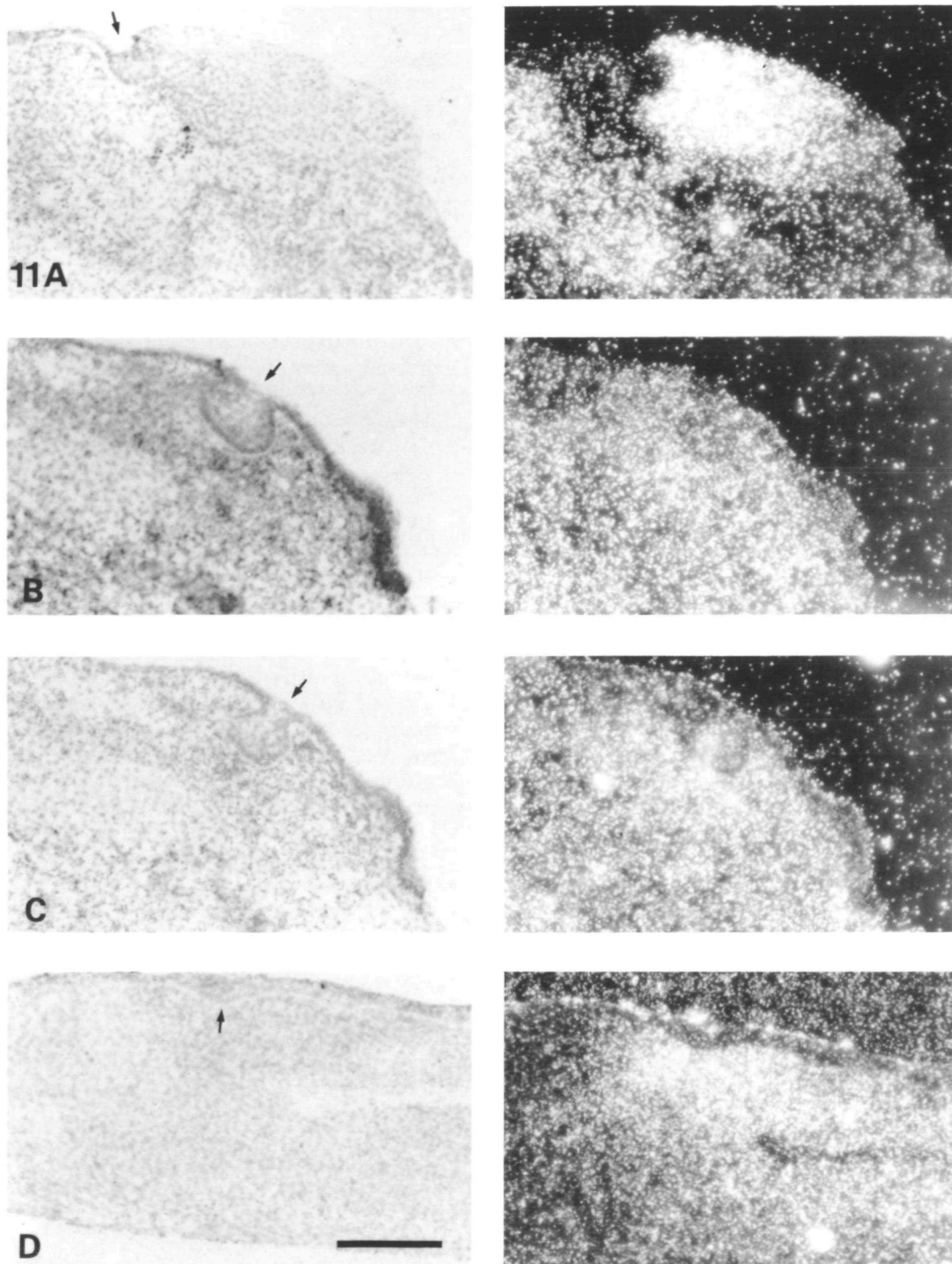
Fig. 11. Bright-field and corresponding dark-field *in situ* hybridisation micrographs of Hox 7.1 expression *in vitro* in cultured mouse first branchial arches explanted at embryonic day 11 and cultured for 3 (A) or 7 (B–D) days. (A) Culture day 3 hybridised with the antisense probe. The first molar tooth germ (arrowed) is at the bud stage of development and there is intense localised labelling in the dental papilla and follicle (compare equivalent *in vivo* pattern in Fig. 8A,B). (B) Culture day 7 hybridised with the antisense probe. There is no localised labelling around the first molar tooth germ (arrowed) which is at the late cap stage of development (this section is at the periphery of the enamel organ). (Compare with equivalent *in vivo* pattern in Fig. 10A). (C) Culture day 7 hybridised with the control sense probe. There is no localised labelling around the first molar tooth germ. Compare Fig. 10B and 11B. (D) Culture day 7 hybridised with the antisense probe. There is localised labelling in the mesenchyme beneath the epithelial invagination (arrowed) for the second molar tooth germ.

and Thesleff, 1987, 1989, Heine *et al.* 1987; Lehnert and Akhurst, 1988; Wilkinson *et al.* 1989; Snead *et al.* 1989; Sharpe and Ferguson unpublished) but none show the precise localisation pattern either temporally or spatially as *Hox 7.1.* Thus TGFB$_1$ transcripts are present in the mesenchyme beneath the bud stage enamel organ but in the epithelia of the enamel organ of the cap-stage tooth germ (mesenchyme negative): the TGFB$_1$ polypeptide is present in the dental papilla mesenchyme of the cap stage (Lehnert and Akhurst, 1988). Int-2 transcripts are not located in the epithelium or mesenchyme of the invaginating dental epithelium but present only in the dental papilla (not follicle) mesenchyme of the bud, cap and bell stages (Wilkinson *et al.* 1989). EGF transcripts are present in both the enamel organ and dental papilla mesenchyme of bell-stage (embryonic day 17) tooth germs (Snead *et al.* 1989). It remains to be demonstrated where the *Hox 7.1* protein localises (antibodies are not yet available). Nonetheless, the fact that growth factor and extracellular matrix gene expression is sometimes temporally different from that of *Hox 7.1* may indicate regulatory interactions. It therefore remains to investigate whether expression of any of these growth factor or extracellular matrix molecules is regulated by *Hox 7.1,* or whether they might regulate *Hox 7.1* transcription: such hypotheses are being tested in experimentally manipulated organ cultures.

Equally, expression of *Hox 7.1* in the dental papillae and dental follicles may be a cell-lineage-related phenomenon. The dental papillae and follicles have a similar lineage and mesenchymal cells in these tissues differ from those elsewhere in the jaws/embryo in a number of parameters (Thesleff, 1986; Ruch, 1987). Moreover odontoblasts (which differentiate from the dental papilla mesenchyme) and alveolar bone osteoblasts, (which differentiate from the dental follicle) appear to control the transcription of the $\alpha 1(1)$ collagen gene by using *cis* regulatory sequences different from those of other fibroblast cells (Kratochwil *et al.* 1989; Schwarz *et al.* 1990).

The peculiar expression pattern of *Hox 7.1* in the anterior but not posterior mesenchyme of the mandibular processes may relate to its restriction to specific neural crest cell populations or the migration of such populations. Migration of neural crest cells, begins at the 4+ somite stage (day 8 in the mouse) from mesencephalic and rostral metencephalic levels (Nichols, 1981). These cells emerge from the wide open neuroepithelium approximately lateral to the dorsal region of the pharynx, near the base of the mandibular arch (Nichols, 1986). These are the first cells to migrate into the mandibular arch and they form its anterior region. By the 7- to 8-somite stage, the first arch swells with the further migration of neural crest cells, but migration dwindles about the 11 somite stage (day 8.5 in the mouse) and is largely complete by embryonic days 9–10 in the mouse (Lumsden, 1988). Indeed after day 9 most of the increase in cell number in the mandibular processes is due to mitosis of cells *in situ* and little further migration of new cells. Therefore *Hox 7.1*

expression may initially be restricted to the first waves of neural crest cells that leave the neural folds, diminishing both in latter waves and also as the first formed cells divide into daughters. This would explain the expression patterns in the mesenchyme of embryonic day 10–12 heads, along with the postulate that the thickening dental epithelium is responsible for either the *de novo* expression or persistance of expression of *Hox 7.1* in the immediately surrounding mesenchyme. Interestingly the expression of other homeobox genes can be influenced experimentally by retinoic acid application (Deschamps *et al.* 1987; La Rosa & Gudas, 1988; Mavillio *et al.* 1988). High levels of expression of mRNA for the retinoic acid receptor (Ruberete *et al.* 1990) and the cellular retinoic acid binding protein (Perez-Castro *et al.* 1989; Maden *et al.* 1990) are found in the developing mouse head, particularly in areas of neural-crest-derived mesenchyme including the teeth. It therefore remains to investigate whether retinoids have any effect on *Hox 7.1* expression, and whether such effects might explain either the apparent inducing ability of dental epithelium or the pathogenesis of retinoic-acid-induced craniofacial malformations (Sulik *et al.* 1988). In man retinoic acid embryopathy is sometimes associated with dental anomalies e.g. fused or missing teeth (E. Lammer personal communication) whilst patients with Wolf–Hirschhorn syndrome, which is associated with a putative mutation in human *Hox 7.1* (Ivens *et al.* 1990), often have dental anomalies.

Hox 7.1 is the first homeobox gene to be localised in developing teeth. Its precise function remains to be determined but it may be important in establishing the patterning of individual dental rudiments (and hence the dentition) and in regulating epithelial–mesenchymal interactions (as in the limb – Robert *et al.* 1989; Hill *et al.* 1989) during early dental development. Importantly its expression in a mammalian system capable of extensive experimental manipulation *in vivo* and *in vitro*, makes the developing tooth a potentially important experimental model for investigating the function and regulation of mammalian homeobox gene expression.

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