Development 114, 805-813 (1992)
Printed in Great Britain © The Company of Biologists Limited 1992

Development of the spatial pattern of retinoic acid receptor-β transcripts in embryonic chick facial primordia

ANNIE ROWE[1,2,*], JOY M. RICHMAN[1,†] and PAUL M. BRICKELL[2]

[1]*Department of Anatomy and Developmental Biology*
[2]*The Medical Molecular Biology Unit, Department of Biochemistry and Molecular Biology, University College and Middlesex School of Medicine, The Windeyer Building, Cleveland Street, W1P 6DB, UK.*

*Author for correspondence
†Present address: Department of Preventive Dental Science, Faculty of Dentistry, University of Manitoba, 780 Bannatyne Avenue, Winnipeg, Manitoba, R3E 0W3, Canada

Summary

Retinoic acid causes a range of embryonic defects, including craniofacial abnormalities, in both birds and mammals and is believed to have a number of roles in normal development. We have previously shown that the distribution of retinoic acid receptor-β (RAR-β transcripts is spatially restricted within the neural-crest-derived upper beak primordia of the chick embryo. We have now used in situ hybridisation to trace the distribution of RAR-β transcripts during the migration of cranial neural crest cells and during formation of these primordia.

RAR-β transcripts were present in a subset of migrating neural-crest-derived cells in the head of the stage 10 embryo. These cells were situated in pathways followed by cells that migrate from the neural crest overlying the posterior prosencephalic/anterior mesencephalic region of the developing brain. Cells containing RAR-β transcripts accumulated around the developing eyes and in the regions of the ventral head from which the upper beak primordia later develop. We mapped the distribution of RAR-β transcripts as the facial primordia were forming, with particular reference to the development of the maxillary primordia. We found that these form in a region of the ventral head that includes the boundary between regions of high and low levels of RAR-β transcripts. The boundary between these two groups of cells persisted as the maxillary primordia developed.

The restriction of RAR-β transcripts to a subset of migrating neural crest cells which arise from a specific region of the neural crest, and which give rise to precisely distributed populations of cells, provides further evidence that there is some form of prepatterning in the neural crest from which the facial primordia originate.

Key words: chick embryogenesis, facial primordia, maxillary primordia, neural crest, RAR-β, retinoic acid receptor.

Introduction

The vitamin A metabolite retinoic acid (RA) can affect pattern formation in a number of developmental systems, including the developing chick limb bud (reviewed by Tickle and Brickell, 1991) and face (reviewed by Wedden et al., 1988), and the developing *Xenopus* central nervous system (Durston et al., 1989). Retinoids are also potent teratogens in mammals, where they cause a wide range of embryological defects, including abnormalities in limb and craniofacial development (Satre and Kocchar, 1989; Morriss and Thorogood, 1978; Lammer et al., 1985). Dencker et al. (1990) have shown that radiolabelled RA and RA analogues injected into pregnant mice accumulate in specific locations in the embryo, including the neural plate, neural crest, the roof of the midbrain, the hindbrain, neural tube, limb buds and upper jaw primordia. RA occurs endogenously in developing chick limb buds (Thaller and Eichele, 1987), in the floor plate of the chick neural tube (Wagner et al., 1990) and in the *Xenopus* embryo (Durston et al., 1989).

Application of a bead soaked in an appropriate concentration of RA to the chick wing bud between embryonic stages 18 and 21 results in failure of upper beak development in 100% of treated embryos (Tamarin et al., 1984; Wedden and Tickle, 1986). The chick face is formed from a set of primordia populated by mesenchymal cells originating from the cranial neural crest. The frontonasal mass, lateral nasal processes and maxillary primordia give rise to the upper beak, whilst the mandibular primordia give rise to the

lower beak. RA treatment results in failure of outgrowth of the frontonasal mass, which then fails to fuse with the lateral nasal processes and the maxillary primordia, resulting in severe bilateral clefting of the primary palate. Recombination experiments with epithelium and mesenchyme from normal and RA-treated embryos have shown that RA acts on the mesenchymal cells of the facial primordia, rather than on the epithelium (Wedden, 1987). The development of the mandibular primordia is unaffected by RA. These data, along with the evidence for the presence and active localisation of RA in embryos and its teratogenic effects, indicate a role for RA in normal facial development.

Chick facial primordia arise as buds of neural-crest-derived ectomesenchyme encased in epithelium. Noden (1975) has mapped the contributions of cells from different levels of the cranial neural crest to these primordia. Neural crest cells from the posterior prosencephalic and anterior mesencephalic levels of the early brain migrate rostrally around the optic lobes. They migrate into the ventral region of the head, ventral and posterior to the optic lobes, contributing mainly to the frontonasal mass, lateral nasal processes and maxillary primordia. Cells migrating laterally from the posterior mesencephalon contribute mainly to the maxillary primordia and, to a lesser extent, to the mandibular primordia. All of the facial primordia are distinct by embryonic stages 18-19.

The effects of RA on cells are thought to be mediated by nuclear RA receptors that belong to the steroid/thyroid hormone receptor family (Green and Chambon, 1988). Two classes of nuclear receptors that respond to RA have been identified in humans, mice and chickens. One class comprises RAR-α (Petkovich et al., 1987; Giguère et al., 1987; Noji et al., 1991), RAR-β (Brand et al., 1988; Benbrook et al., 1988; Smith and Eichele, 1991; Rowe et al., 1991a; Noji et al., 1991) and RAR-γ (Zelent et al., 1989; Krust et al., 1989). The other class comprises RXR-α (Manglesdorf et al., 1990), RXR-β (Manglesdorf et al., 1990; Hamada et al., 1989) and a third receptor, cRXR, identified in chickens (Rowe et al., 1991b).

Studies in the mouse have shown that transcripts encoding these receptors have distinct patterns of distribution in a broad range of embryonic tissues (Dollé et al., 1989, 1990; Ruberte et al., 1990, 1991). In the early facial primordia, murine RAR-α and RAR-γ transcripts were found to be uniformly distributed, with RAR-γ transcripts gradually becoming restricted to regions of chondrogenesis (Osumi-Yamashita et al., 1990). In contrast, in both the mouse (Osumi-Yamashita et al., 1990) and the chick (Rowe et al., 1991a; Smith and Eichele, 1991), RAR-β transcripts were found to be regionally localised within the facial primordia. cRXR transcripts are not detectable in the neural-crest-derived mesenchyme of the facial primordia, even though cRXR transcripts are abundant in other neural crest derivatives (Rowe et al., 1991b).

In the chick, at embryonic stages 20, 24 and 28, RAR-β transcripts were found to be abundant in parts of the upper beak primordia, which are affected by RA treatment, and present at lower levels in the mandibular primordia (Rowe et al., 1991a). Transcripts were abundant in the anterior part of the maxillary primordia, but were undetectable in the posterior parts of these primordia. High levels of transcripts were also localised to the edges and corners of the frontonasal mass, to a v-shaped region in the centre of the frontonasal mass and to the lateral nasal processes. The complex distribution of RAR-β transcripts to specific facial primordia and to certain regions within single primordia is intriguing. We have therefore used in situ hybridisation to investigate the development of this pattern of RAR-β transcript distribution by examining the embryonic chick head, between stages 10 and 18, when the facial primordia are forming. In particular, we have followed closely the development of the maxillary primordia where the localisation of RAR-β transcript levels within the primordia is most striking. At stages 10 and 12, as neural crest cells are migrating into the ventral head and visceral arches, we have used in situ hybridisation to cRXR transcripts as a marker for the location of migrating neural crest cells.

Materials and methods

Chick embryos

Fertilised chicken eggs were obtained from Poyndon Farm, Waltham Cross, Herts, UK and were incubated at 38±1°C. The embryos were staged according to Hamburger and Hamilton (1951) and dissected into sterile PBS.

RAR-β hybridisation probe

The ^{35}S-labelled antisense RNA probe for chicken RAR-β transcripts was synthesised as previously described, using the chicken RAR-β cDNA clone pRAR1 as a template after linearising with *Bam*HI (Rowe et al., 1991a). The probe contained sequences complementary to those encoding part of the A domain, the B, C and D domains and part of the E domain of RAR-β. The negative control sense strand probe was synthesised from the same template, linearised with *Sal*I.

cRXR hybridisation probe

The ^{35}S-labelled antisense RNA probe for cRXR transcripts was synthesised using the cDNA clone pR2BE as a template after linearising with *Bam*HI (Rowe et al., 1991b). The probe contained sequences complementary to those encoding the E and F domains of cRXR. The negative control sense strand probe was synthesised from the same template, linearised with *Eco*RI.

In situ hybridisation

Whole early embryos (stages 10 and 12) or dissected heads (stage 14 and later) were fixed and embedded in wax as described by Davidson et al. (1988). 7 μm sections were cut, collected on slides coated with 3-aminopropyltriethoxysilane (TESPA, Sigma) and baked for 6-16 hours at 60°C. Some sections were taken at this stage for histological examination and stained with Mallory's stain. Probes were prepared and in situ hybridisation was performed as described previously (Rowe et al., 1991a) After autoradiography, slides were stained in 0.5% (w/v) malachite green.

Results

Stage 10

At stage 10, cranial neural crest cells have begun to migrate away from the neural crest. Some cells migrate ventrally, posterior to the optic lobes, whilst others migrate anteriorly over the optic lobes before migrating ventrally (Noden, 1975). The distribution of RAR-β transcripts in the head of the stage 10 chick embryo is shown in Fig. 1. Low levels of RAR-β transcripts were detected in cells anterior and posterior to the optic lobes and in cells ventral to the developing midbrain. RAR-β transcripts were not detectable in the mesoderm-derived mesenchyme ventral to the developing mesencephalon and anterior rhombencephalon. RAR-β transcripts were also detectable at very low levels in the developing central nervous system anterior to rhombomere 5. There was a striking increase in the levels of RAR-β transcripts in the neural tube in rhombomere 5. This increase was graded along the anterior-posterior axis and did not appear to coincide with the boundary between rhombomeres 5 and 6. RAR-β transcripts were also present at high levels within the developing otic vesicle, in the rest of the hindbrain and in the neural tube.

In transverse sections through the head in the region of the optic lobes of stage 10 embryos, RAR-β transcripts were present at very low levels in cells dorsal to the optic lobes (Fig. 2C). Hybridisation of adjacent sections with a cRXR probe, which hybridises to migrating neural crest cells and most neural crest derivatives (Rowe et al., 1991b, and our unpublished observations), enabled us to identify the positions of neural crest cells in these sections (Fig. 2B), indicating that the cells dorsal to the optic lobes that contained RAR-β transcripts had the same location as migrating neural crest cells. In sections from more posterior regions of the developing head, RAR-β transcripts were undetectable other than in the developing central nervous system (data not shown). RAR-β transcripts were present at extremely low levels in the optic lobes themselves and in the developing brain (Fig. 2C).

Stage 12

At stage 12, the positions of migrating neural crest cells in transverse sections of the developing head were again visualised by in situ hybridisation with the cRXR probe (Fig. 3C, G, K). In sections anterior to the optic lobes, neural crest cells containing cRXR transcripts were located lateral to the developing prosencephalon (Fig. 3C). In sections through the head in the region of the optic lobes, migrating neural crest cells containing cRXR transcripts were present dorsal to the optic lobes, lateral to the prosencephalon (Fig. 3G). In the posterior prosencephalic/anterior mesencephalic region of the head, neural crest cells containing cRXR transcripts accumulated ventrolaterally relative to the developing brain (Fig. 3K). Sections in series with those hybridised with the cRXR probe were hybridised with the RAR-β probe (Fig. 3B, F, J). RAR-β transcripts were present at high levels in migrating neural crest

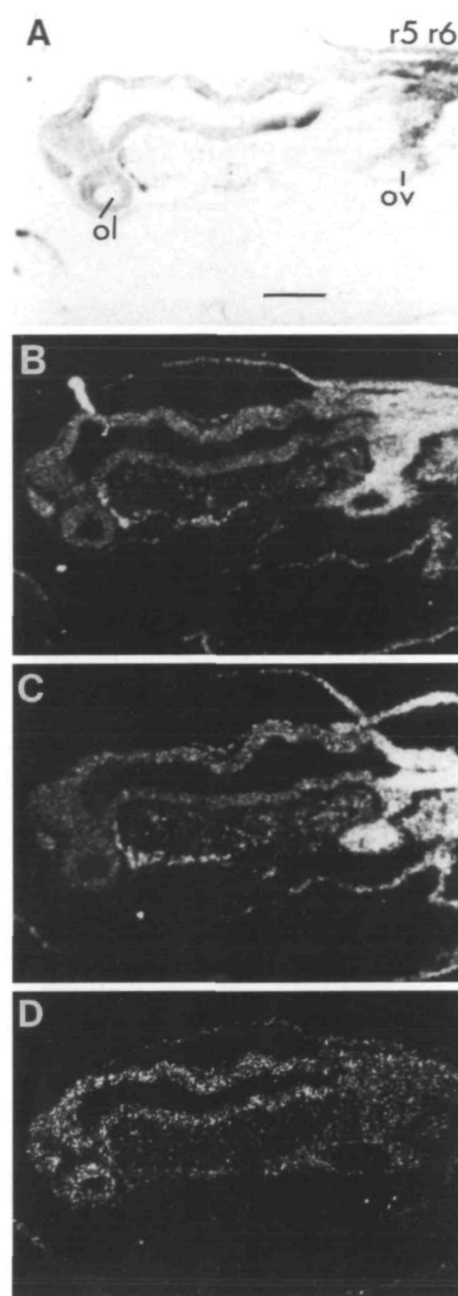

Fig. 1. Distribution of RAR-β transcripts at stage 10. Adjacent sections through the head region of a stage 10 embryo. (A) Stained with malachite green and photographed under bright-field illumination; (B and C) hybridised with the probe for RAR-β transcripts and photographed under dark-field illumination; (D) hybridised with a negative control probe and photographed under dark-field illumination. ol, optic lobe; ov, otic vesicle; r5, rhombomere 5; r6, rhombomere 6. Scale bar: 200 μm.

cells in the same regions of the developing head as those where cRXR transcripts were present. RAR-β transcripts were present in the neural-crest-derived cells lateral to the prosencephalon anterior to the optic lobes (Fig. 3B). In the region of the optic lobes, RAR-β transcripts were also present in the neural-crest-derived

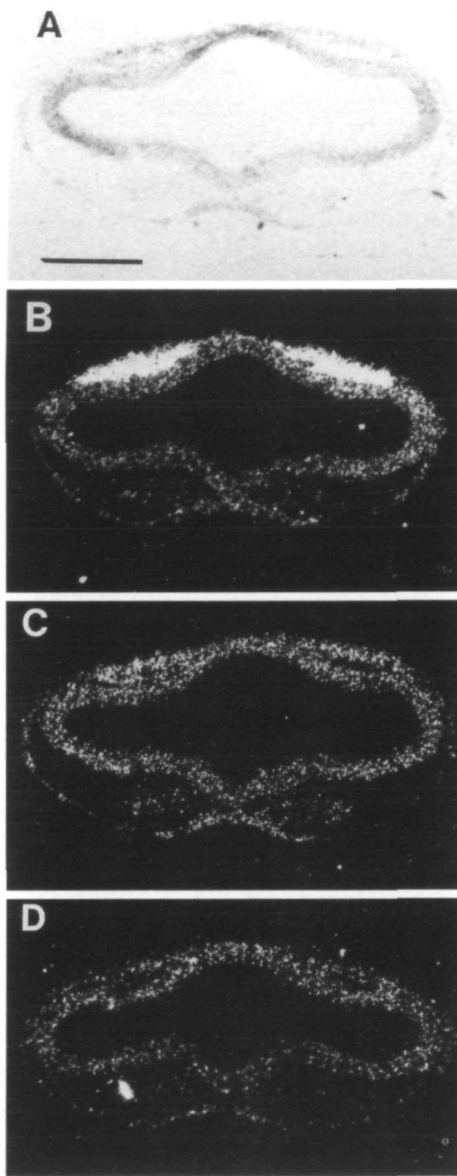

Fig. 2. Distribution of RAR-β transcripts at stage 10. Adjacent transverse sections through the head of a stage 10 embryo at the level of the optic lobes. (A and C) Hybridised with the probe for RAR-β transcripts; (B) hybridised with the probe for cRXR transcripts; (D) hybridised with a negative control probe. (A) Photographed under bright-field illumination; (B, C, and D) photographed under dark-field illumination. Scale bar: 200 μm.

cells dorsal to the optic lobes as well as in the small population of cells which have by this stage migrated ventral to the developing brain (Fig. 3F). Posterior to the optic lobes, RAR-β transcripts were present in neural-crest-derived cells ventrolateral to the developing posterior prosencephalon/anterior mesencephalon. RAR-β transcripts were present at very low levels in the developing cranial and viseral arch neural-crest-derived mesenchyme. Low levels of RAR-β transcripts were also detected in the developing optic lobes and brain.

Stage 14

By stage 14, neural crest cells have migrated ventrally in the head, both anterior and posterior to the developing eye, and into the first branchial arch (Noden, 1975). Mallory's stained sections show that although cell density is higher in the first branchial arch mesenchyme than in the ventral head mesenchyme (Fig. 4A), it was in the ventral head mesenchyme that RAR-β transcripts were most abundant (Fig. 4B). Transcripts were particularly concentrated in the ventral head mesenchyme around the optic cups.

Stages 15 and 16

At stage 15, the nasal placodes are forming as thickenings of head ectoderm (for a description see Yee and Abbott, 1978). RAR-β transcripts were most abundant in the mesenchyme lateral and medial to the placodes (Fig. 5A, B, C). Deeper frontal sections and parasagittal sections (Fig. 5D to I) show that RAR-β transcripts were still most abundant around the optic cup and in the ventral head mesenchyme. The maxillary primordia are not yet formed at this stage and each appears only as a slight bulge in the mesenchyme just posterior to the developing eye and anterior to the first branchial arch (Fig. 5G, H). At stages 15 and 16, there was already a demarcation in the ventral head between regions containing high and low levels of RAR-β transcripts (Fig. 5H) and this demarcation lay within the bulges that develop into the maxillary primordium (indicated by arrow in Fig. 5H). Therefore, like the more mature maxillary primordia, the anterior part of these bulges contain cells with high levels of RAR-β transcripts, whilst the posterior part of these of the bulges contain cells with low levels of RAR-β transcripts. The first branchial arch mesenchyme and the mesenchyme just anterior to it contain relatively low levels of RAR-β transcripts. Shallower parasagittal sections of stage 16 heads (Fig. 6A, B, C) also revealed that RAR-β transcripts were not particularly abundant in the first branchial arch as compared to the rest of the developing head. RAR-β transcripts were still most abundant in the periocular and ventral regions of the head and were undetectable in the dorsal head mesenchyme.

Stage 18

By stage 18, the maxillary processes are prominent buds, lying anterior to the mandibular primordia. RAR-β transcripts were detected in cells in a continuous region extending from a postion anterior and ventral to the developing eyes into the anterior halves of the maxillary processes (Fig. 6D to I). The posterior halves of the maxillary processes and the mandibular primordia did not contain RAR-β transcripts. RAR-β transcripts were absent from the dorsal head mesenchyme, but were still present in the periocular region of the head mesenchyme. This distribution of RAR-β transcripts persists in the head mesenchyme at stages 20, 24 and 28, as we have previously described (Rowe, 1991a).

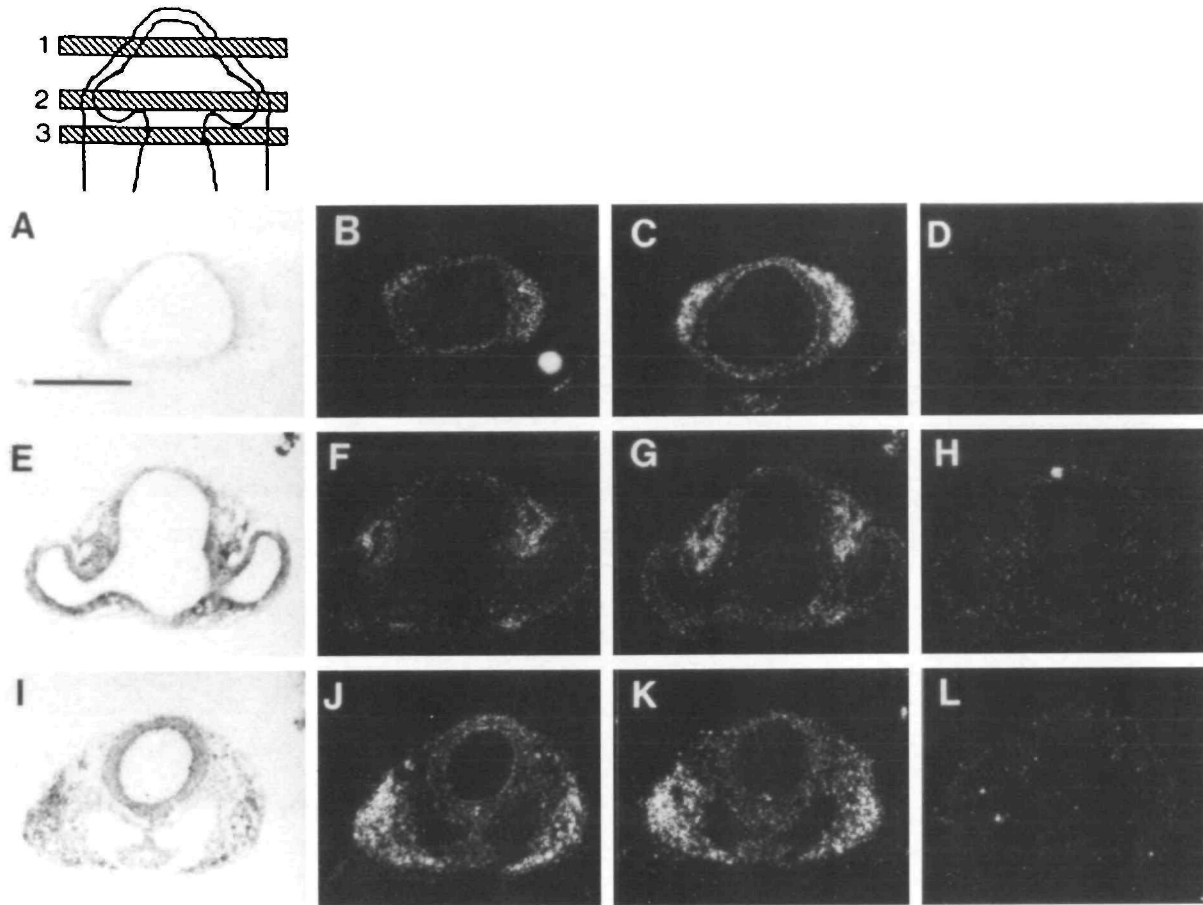

Fig. 3. Distribution of RAR-β transcripts at stage 12. Transverse sections through the head of a stage 12 embryo. The line drawing shows the three areas from which sections were taken. A to D from area 1, anterior to the optic lobes; E to H from area 2, through the optic lobes; I to L from area 3, posterior to the optic lobes, through the anterior mesencephalon. B, E, F, I and J, hybridised with the probe for RAR-β transcripts. C, G and K, hybridised with the probe for cRXR transcripts. A, D, H and L, hybridised with a negative control probe. A, E, I were photographed under bright-field illumination and all other sections were photographed under dark-field illumination. Scale bar: 200 μm.

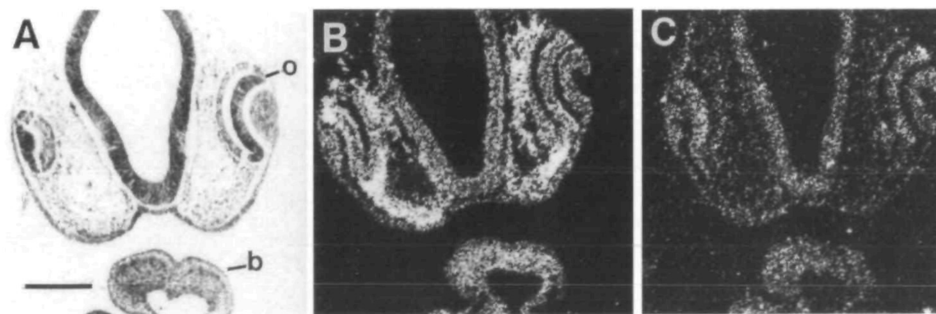

Fig. 4. Distribution of RAR-β transcripts at stage 14. Adjacent frontal sections through the optic vesicles (o) and first branchial arch (b) of a stage 14 embryo. (A) Section stained with Mallory's stain and photographed under bright-field illumination; (B) hybridised with the probe for RAR-β transcripts; (C) hybridised with a negative control probe. (B and C) Photographed under dark-field illumination. Scale bar: 200 μm.

Discussion

RAR-β transcripts in migrating neural crest cells

At embryonic stage 10, streams of cells containing RAR-β transcripts were found anterior and posterior to the optic lobes, and ventral to the developing mesencephalon. At later stages, cells containing RAR-β transcripts were found surrounding the optic cups and accumulating in the ventral head mesenchyme. Transcripts were present at much lower levels in the first branchial arch mesenchyme. By stage 18, cells containing high levels of RAR-β transripts were found in the

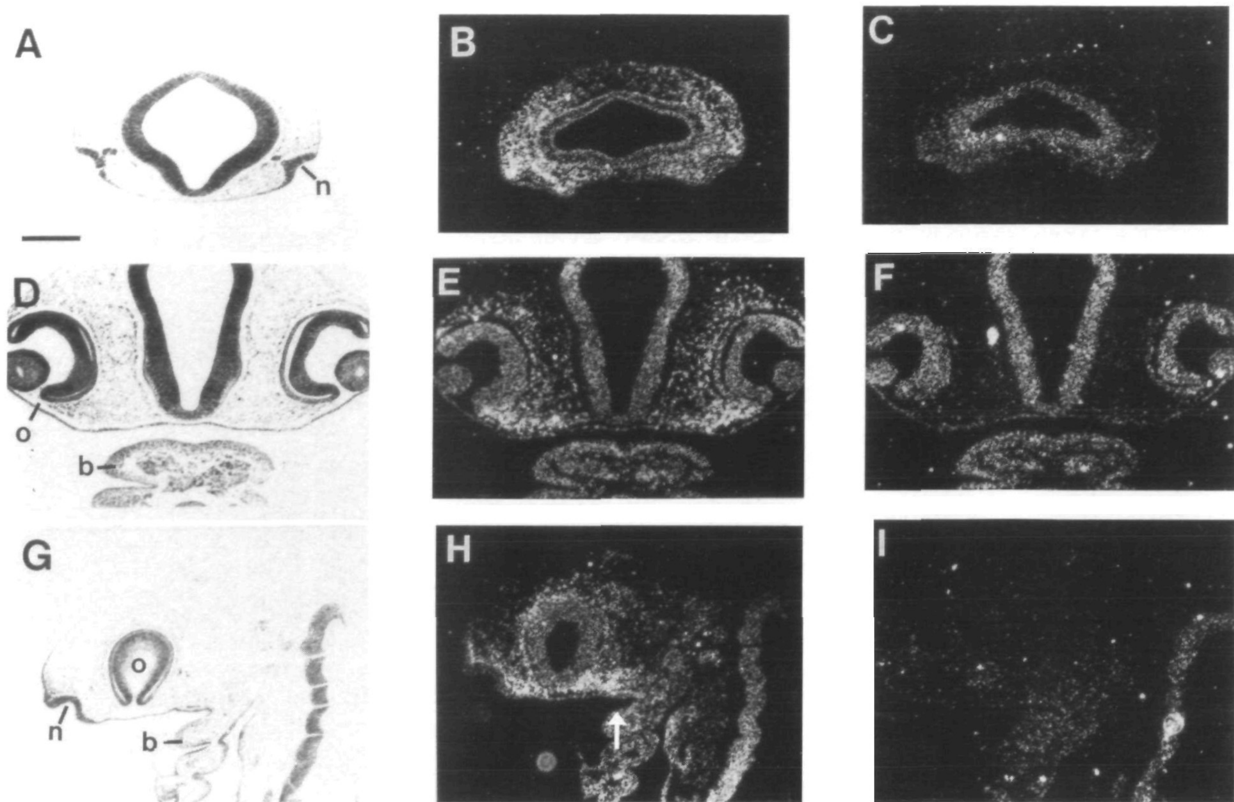

Fig. 5. Distribution of RAR-β transcripts at stage 15. A, B and C are adjacent frontal sections through the head of a stage 15 embryo at the level of the nasal placodes (n). D, E and F are deeper sections in the same plane as A, B and C, through the optic cups (o) and the first branchial arch (b). G, H and I are parasagittal sections through the head at the same stage, the arrow on H indicates the demarcation between areas of high and low RAR-β transcript levels. (A, D and G) Stained with Mallory's stain and photographed under bright-field illumination; (B, E and H) hybridised with the probe for RAR-β transcripts; (C, F and I) hybridised with a negative control probe. (B, C, E, F, H and I) Photographed under dark-field illumination. Scale bar: 200 μm.

frontonasal mass, the lateral nasal processes and the anterior part of the maxillary primordia, but not in the posterior part of the maxillary primordia or in the mandibular primordia; a pattern closely resembling that which we have previously described for stage 20, 24 and 28 chick embryos (Rowe et al., 1991a).

The locations of these cells, from stage 10 onwards, correspond to those of migrating neural crest cells which arise from the neural crest at the posterior prosencephalic/anterior mesencephalic level of the developing brain and which subsequently populate the frontonasal mass, the lateral nasal processes and the maxillary primordia of the developing face. (Noden, 1975; Le Lievre, 1978). Neural crest cells arising from the posterior mesencephalic/anterior rhombencephalic level of the developing brain, which migrate to form the first branchial arch and subsequently give rise to the mandibular primordia (Noden, 1975; Le Lievre, 1978), contained extremely low levels of RAR-β transcripts, as did neural crest cells migrating from more posterior regions of the hindbrain. It therefore appears that migrating neural crest cells destined to participate in forming regions of the head that contain high levels of RAR-β transcripts, already express the RAR-β gene by stage 10. It is not clear whether these cells contain RAR-β transcripts prior to migration, or whether transcripts only begin to accumulate as the cells migrate. In either case, the difference in RAR-β gene expression between cells arising from different positions within the neural crest is intriguing. It is possible that positional cues acting within the neural crest could activate RAR-β gene expression directly, or could prime cells to activate RAR-β gene expression shortly after they begin to migrate. Since RAR-β gene expression can be induced by RA both in vitro (de Thé et al., 1990) and in vivo (Noji et al., 1991; Rowe et al., 1991a), it is possible that the local concentration of free RA is one such cue, although it is likely that other factors also have a role in regulating RAR-β gene expression (Rossant et al., 1991).

The function of RAR-β in cells derived from the cranial neural crest is unclear. In particular, it is not known whether these cells require RAR-β prior to migration, during migration or once they have reached their destinations in the developing face. Grafting experiments indicate that cells arising at different levels of the cranial neural crest are prepatterned in terms of the craniofacial structures to which they will give rise (Noden, 1983). In view of the apparent restriction of RAR-β transcripts to neural crest cells arising from the

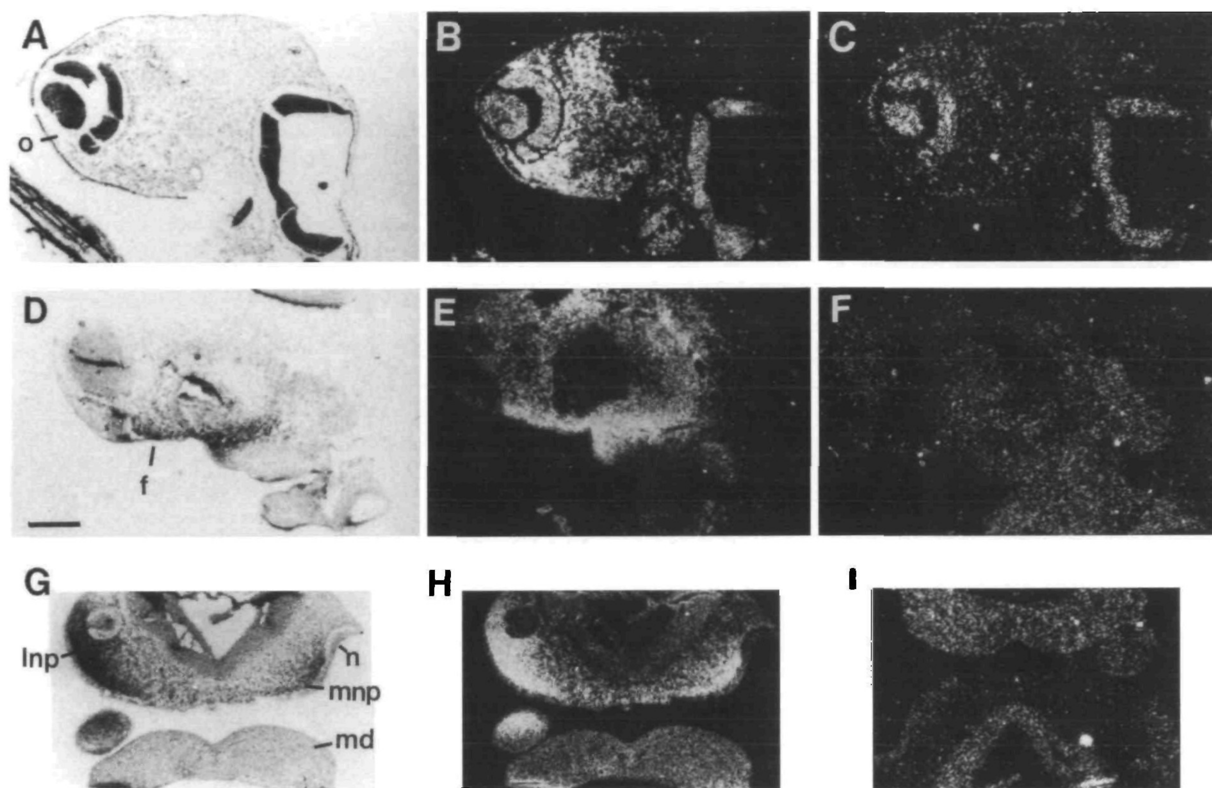

Fig. 6. Distribution of RAR-β transcripts at stages 16 and 18. A, B and C are adjacent parasagittal sections through the head of a stage 16 embryo. D, E and F are parasagittal sections through the head of a stage 18 embryo. G, H and I are frontal sections through a stage 18 embryo. (A) Stained with Mallory's stain; (B, D, E, G and H) hybridised with the probe for RAR-β transcripts; (C, F and I) hybridised with a negative control probe. (A, D and G) photographed under bright-field illumination. All other sections photographed under dark-field illumination. f, frontonasal mass; lnp, lateral nasal process; mnp, medial nasal process; md mandibular primordium; n, nasal slit; o, optic cup. Scale bar: 200 μm.

posterior prosencephalic/anterior mesencephalic level of the developing brain, it is possible that RAR-β is involved in the specification of these cells. RAR-β could regulate, or act in concert with, other genes that are required to specify these cells, either within the neural crest or during migration. The identity of these genes is unknown. There are no known homeobox-containing genes that are expressed in this region of the developing brain, although homeobox-containing genes that are expressed in regions lying either anterior or posterior to this region have been described (Price et al., 1991; Hunt et al., 1991).

Distribution of RAR-β transcripts in the maxillary primordia

Perhaps the most striking feature of the distribution of RAR-β transcripts in the facial primordia is the restriction of high levels of transcripts to the anterior part of the maxillary primordia, which is apparent from stage 18 onwards. One possibility is that this difference in RAR-β transcript levels reflects the presence of two distinct cell populations within the maxillary primordia. The precise origins of the maxillary primordia are unclear. It has been suggested on the basis of morphological studies that they arise from the first branchial arch, although data from analyses of neural crest migration do not support this view (for review see Le Douarin, 1982). Our data suggest that the cells that populate the posterior part of the maxillary primordia share a common origin with the cells that populate the first branchial arch and the ventral head mesenchyme just anterior to it.

Distribution of RAR-β transcripts in the neural epithelia

As well as being present in a subset of cells derived from the cranial neural crest, RAR-β transcripts were present in the neural epithelia at all stages examined. At stage 10, transcripts were abundant in the neural tube and posterior hindbrain, showed a graded decrease in levels within rhombomere 5 and were present at extremely low levels in more anterior regions. Transcripts were abundant in the otic vesicle. This distribution is maintained until at least stage 20 of chick development (Smith and Eichele, 1991) and differs from that observed in the mouse, where the anterior limit of RAR-β gene expression has been reported to correspond to the boundary between rhombomeres 6 and 7, and where no transcripts were detected in the otic vesicle (Ruberte et al., 1991).

This difference is suprising and may have implications for models of how RAR-β gene expression is

regulated in the vertebrate hindbrain. Since RAR-β gene expression can be induced by RA, it has been suggested that the distribution of RAR-β transcripts in the hindbrain may reflect the distribution of free endogenous RA and that this could in turn reflect the distribution of the cellular retinoic acid binding protein (CRABP), which is thought to regulate the amount of free RA within the cell (Morriss-Kay, 1991). This seems unlikely, however, since the distribution of CRABP in the chick (Maden et al., 1991) and mouse (Dencker et al., 1990) hindbrain at equivalent stages is similar. This indicates that there may not be a simple relationship between the levels of CRABP and RAR-β gene expression in the hindbrain, and suggests that factors other than RA influence RAR-β gene expression.

It will be of interest to compare the expression domains in mouse and chick of other key regulatory molecules that are expressed in the developing hindbrain, such as *int*- 2, *Krox*-20 and genes within the *Hox*-1 and *Hox*-2 clusters. In the one comparison reported so far, the *Ghox-lab* and *Hox*-2.9 genes have similar domains of expression in the chick and the mouse embryonic hindbrain (Sundin and Eichele, 1990).

In the developing head, high levels of RAR-β transcripts are confined to two separate regions, part of the neural-crest-derived facial mesenchyme and the posterior half of the developing hindbrain. This defines two very different tissues where the receptor may function and suggests that RAR-β is involved in a variety of morphogenetic processes during head development. In addition, the restriction of RAR-β transcripts to a subset of migrating neural crest cells provides further evidence that there is some form of prepatterning in the neural crest from which the facial primordia arise.

We thank Cheryll Tickle for her support and helpful discussions. This research was supported by a grant from the Wellcome Trust. J. M. R. was supported by a fellowship from the Medical Research Council of Canada.

References

Benbrook, D., Lernhardt, E. and Pfahl, M. (1988). A new retinoic acid receptor identified from a hepatocellular carcinoma. *Nature* 333, 669-672.

Brand, N. J., Petkovich, M., Krust, A., Chambon, P., De Thé, H., Marchio, A., Tiollais, P. and Dejean, A. (1988). Identification of a second human retinoic acid receptor. *Nature* **332**, 850-853.

Davidson, D., Graham, E., Sime, C. and Hill, R. (1988). A gene with sequence similarity to *Drosophila engrailed* is expressed during the development of the neural tube and vertebrae in the mouse. *Development* **104**, 305-316.

Dencker, L., Annerwall, E., Busch, C. and Eriksson, U. (1990). Localisation of specific retinoid binding sites and expression of cellular retinoic acid-binding protein (CRABP) in the early mouse embryo. *Development* **110**, 343-352.

De Thé, H., Del Mar Vivanco-Ruiz, M., Tiollais, P., Stunnenberg, H. and Dejean, A. (1990). Identification of a retinoic acid responsive element in the retinoic acid receptor β gene. *Nature* **343**, 177-180.

Dollé, P., Ruberte, E., Kastner, P., Petkovich, M., Stoner, C., Gudas, L. and Chambon, P. (1989). Differential expression of genes encoding α, β and γ retinoic acid receptors and CRABP in the developing limbs of the mouse. *Nature* **342**, 702-705.

Dollé, P., Ruberte, E., Leroy, P., Morriss-Kay, G. and Chambon, P. (1990). Retinoic acid receptors and cellular retinoid binding proteins. I. A systematic study of their differential patterns of transcription during mouse organogenesis. *Development* **110**, 1133-1151.

Durston, A. J., Timmermans, J. P. M., Hage, W. J., Hendricks, H. F. J., De Vries, N. J., Heidenveld, M. and Nieukoop, P. (1989). Retinoic acid causes an anteroposterior transformation in the developing central nervous system. *Nature* **340**, 140-144.

Giguère, V., Ong, E. S., Segui, P. and Evans, R. M. (1987). Identification of a receptor for the morphogen retinoic acid. *Nature* **330**, 624-629.

Green, S. and Chambon, P. (1988). Nuclear receptors enhance our understanding of transcriptional regulation. *Trends Genet.* **4**, 309-314.

Hamada, K., Gleason, S. L., Levi, B-Z., Hirschfield, S., Appella, E. and Ozato, K. (1989). H-2RIIBP, a member of the nuclear hormone receptor superfamily that binds to both the regulatory element of major histocompatibility class I genes and the oestrogen response element. *Proc. natn. Acad. Sci. U.S.A.* **86**, 8289-8293.

Hamburger, V. and Hamilton, H. L. (1951). A series of normal stages in the development of the chick embryo. *J. Morph.* **88**, 49-52.

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

Krust, A., Kastner, P., Petkovich, M., Zelent, A. and Chambon, P. (1989). A third human retinoic acid receptor. *Proc. natn. Acad. Sci. U.S.A.* **86**, 5310-5314.

Lammer, E. J., Chen, D. T., Hoar, R. M., Agnish, N. D., Benke, P. J., Braun, J. T., Curry, C. J., Fernhoff, P. M., Grix, A. W., Lott, I. T., Richard, J. M. and Sun, S. C. (1985). Retinoic acid embryopathy. *New Engl. J. Med.* **313**, 837-841.

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

Le Lievre, C. S. (1978). Participation of neural-crest-derived cells in the genesis of the skull in birds. *J. Embryol. exp. Morph.* **47**, 17-37.

Maden, M., Hunt, P., Eriksson, U., Kuriowa, A., Krumlauf, R. and Summerbell, D. (1991). Retinoic acid-binding protein, rhombomeres and the neural crest. *Development* **111**, 35-44.

Manglesdorf, D. J., Ong, E. S., Dyck, J. A. and Evans, R. M. (1990). Nuclear receptor that identifies a novel retinoic acid response pathway. *Nature* **345**, 224-229.

Morriss, G. M. and Thorogood, P. V. (1978). An approach to cranial neural crest migration and differentiation in mammalian embryos. In *Development in Mammals* vol. 3 (ed. M.H. Johnson), pp. 363-411. Amsterdam: Elsevier North Holland.

Morriss-Kay, G. (1991). Retinoic acid, neural crest and craniofacial development. *Sem. Dev. Biol.* **2**, 211-218.

Noden, D. M. (1975). An analysis of the migratory behaviour of avian cephalic neural crest cells. *Dev. Biol.* **42**, 106-130.

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

Noji, S., Nohno, T., Koyama, E., Muto, K., Ohyama, K., Aoki, Y., Tamura, K., Ohshugi, K., Ide, H., Taniguchi, S. and Saito, T. (1991). Retinoic acid induces polarising activity but is unlikely to be a morphogen in the chick limb bud. *Nature* **350**, 83-86.

Osumi-Yamashita, N., Noji, S., Nohno, T., Koyama, E., Doi, H., Eto, K. and Taniguchi, S. (1990). Expression of retinoic acid receptor genes in neural-crest-derived cells during mouse facial development. *FEBS Letts.* **264**, 71-74.

Petkovich, M., Brand, N. J., Krust, A. and Chambon, P. (1987). A human retinoic acid receptor which belongs to the family of nuclear receptors. *Nature* **330**, 444-450.

Price, M., Lemaistre, M., Pischetola, M., Di Lauro, R. and Duboule, D. (1991). A mouse gene related to *Distal-less* shows a restricted expression in the developing forebrain. *Nature* **351**, 748-751.

Rossant, J., Zirngibl, R., Cado, D., Shago, M. and Giguère, V. (1991). Expression of a retinoic acid response element-*hsplacZ* transgene defines specific domains of transcriptional activity during mouse embryogenesis. *Genes Dev.* **5**, 1333-1344.

Rowe, A., Eager, N. S. C. and Brickell, P. M. (1991b). A member of the RXR nuclear receptor family is expressed in the neural-crest-

derived cells of the developing chick peripheral nervous system. *Development* **111**, 771-778.

Rowe, A., Richman, J. M. and Brickell, P. M. (1991a). Retinoic acid treatment alters the distribution of retinoic acid receptor-β transcripts in the embryonic chick face. *Development* **111**, 1007-1016.

Ruberte, E., Dollé, P., Krust, A., Zelent, A., Morriss-Kay, G. and Chambon, P. (1990). Specific spatial and temporal distribution of retinoic acid receptor gamma transcripts during mouse embryogenesis. *Development* **108**, 213-222.

Ruberte, E., Dollé, P., Chambon, P. and Morriss-Kay, G. (1991). Retinoic acid receptors and cellular retinoid binding proteins. II. Their differential pattern of transcription during early morphogenesis in mouse embryos. *Development* **111**, 45-60.

Satre, M. A. and Kochhar, D. M. (1989). Elevations in the levels of the putative morphogen retinoic acid in embryonic mouse limb-buds associated with limb dysmorphogenesis. *Dev. Biol.* **133**, 529-536.

Smith, S. M. and Eichele, G. (1991). Temporal and regional differences in the expression pattern of distinct retinoic acid receptor-β transcripts in the chick embryo. *Development* **111**, 245-252.

Sundin, O. and Eichele, G. (1990). A homeo domain protein reveals the metameric nature of the developing chick hindbrain. *Genes Dev.* **4**, 1267-1276.

Tamarin, A., Crawley, A., Lee, J. and Tickle, C. (1984). Analysis of upper beak defects in chicken embryos following treatment with retinoic acid. *J. Embryol. Exp. Morph.* **84**, 105-123.

Thaller, C. and Eichele, G. (1987). Identification and spatial distribution of retinoids in the developing chick limb bud. *Nature* **327**, 625-628.

Tickle, C. and Brickell, P. M. (1991). Retinoic acid and limb development. *Sem. Dev. Biol.* **2**, 189-197.

Wagner, M., Thaller, C., Jessell, T. and Eichele, G. (1990). Polarising activity and retinoid synthesis in the floor plate of the neural tube. *Nature* **345**, 819-822.

Wedden, S. E. (1987). Epithelial-mesenchymal interactions in the development of chick facial primordia and the target of retinoid action. *Development* **99**, 341-351.

Wedden, S. E., Ralphs, J. R. and Tickle, C. (1988). Pattern formation in the facial primordia. *Development* **103 Supplement**, 31-40.

Wedden, S. E. and Tickle, C. (1986). Quantitative analysis of the effect of retinoids on facial morphogenesis. *J. Craniofac. Genet. Dev. Biol.* **2**, 169-178.

Yee, G. W. and Abbott, U. K. (1978). Facial development in normal and mutant chick embryos, 1. Scanning electron microscopy of primary palate formation. *J. Exp. Zool.* **206**, 307-322.

Zelent, A., Krust, A., Petkovich, M., Kastner, P. and Chambon, P. (1989). Cloning of murine α and β retinoic acid receptors and a novel receptor predominantly expressed in skin. *Nature* **339**, 714-717.

(*Accepted 20 December 1991*)