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Specific spatial and temporal distribution of retinoic acid receptor gamma transcripts during mouse embryogenesis

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Summary

Retinoic acid (RA), a putative morphogen in vertebrates, has profound effects on development during embryogenesis, chondrogenesis and differentiation of squamous epithelia. The distribution of the transcripts of the retinoic acid receptor gamma (RAR-γ) gene has been studied here by *in situ* hybridization during mouse development from days 6.5 to 15.5 post-coïtum (p.c.). RAR-γ transcripts are detected as early as day 8 p.c. in the presomitic posterior region. Between days 9.5 and 11.5 p.c., the transcripts are uniformly distributed in the mesenchyme of the frontonasal region, pharyngeal arches, limb buds and sclerotomes. At day 12.5 p.c., RAR-γ transcripts are found in all precartilaginous mesenchymal condensations. From day 13.5 p.c., the transcripts are specifically localized in all cartilages and differentiating squamous keratinizing epithelia, irrespective of their embryological origin. RAR-γ transcripts are also found in the developing teeth and whisker follicles. The developmental pattern of expression of the RAR-γ gene suggests that RAR-γ plays a crucial role for transducing RA signals at the level of gene expression during morphogenesis, chondrogenesis and differentiation of squamous epithelia.

Key words: RAR-γ, development, *in situ* hybridization, cartilage, squamous epithelia.

Introduction

Retinoic acid (RA) is a vitamin A (retinol) metabolite, which plays an important role in pattern formation during vertebrate limb development and regeneration (Tickle *et al.* 1982; Maden, 1985; Robertson, 1987; Slack, 1987; Thaller and Eichele, 1987; Brockes, 1989 and refs. therein). In mammals, high levels of retinoids during pregnancy are teratogenic, and result in a spectrum of craniofacial and limb malformations involving abnormal development of cartilage and skeletal elements (Morriss, 1972; Morriss and Thorogood, 1978; Sulik, 1986; Satre and Kochhar, 1989 and refs. therein). Furthermore, retinoids have a marked effect on chondrogenesis of craniofacial mesenchymal cells in culture (Wedden *et al.* 1987, 1988; Langille *et al.* 1989 and refs therein). Retinoids have also been observed to affect the development of the mammalian brain (Morriss, 1972; Lammer *et al.* 1985) and the regional differentiation within the developing brain of *Xenopus* (Durston *et al.* 1989). The spectacular effects of RA on amphibian limb regeneration and its ability to induce pattern duplications in chick and amphibian limbs [see Brockes (1989) for a review] has led to the idea that it could be a natural morphogen conferring positional values to the limb bud mesenchymal cells. This hypothesis has been strongly supported by the finding that there is an anteroposterior gradient of RA in the chick limb bud with the highest levels in the posterior margin (Thaller and Eichele, 1987; Slack, 1987). Furthermore, RA has marked effects on differentiation and maintenance of epithelial cells *in vivo* and *in vitro*. Skin is a major target organ for retinoids both in its normal (Roberts and Sporn, 1984; Brown *et al.* 1985; Shapiro, 1985; Asselineau *et al.* 1989; Kopan and Fuchs, 1989; and refs. therein) and pathological states (Peck, 1984).

The discovery that members of the steroid/thyroid hormone receptor superfamily are receptors for RA (Petkovich *et al.* 1987; Giguere *et al.* 1987) represents an important step towards the molecular understanding of how RA signals could be transduced to control genetic events. Indeed, nuclear receptors are inducible transcriptional enhancer transactivators (for reviews, see Green and Chambon, 1988; Evans, 1988). Three RA receptors (RARs) have been characterized: RAR-α (Petkovich *et al.* 1987; Giguere *et al.* 1987), RAR-β (Brand *et al.* 1988; Benbrook *et al.* 1988) and RAR-γ (Zelent *et al.* 1989; Krust *et al.* 1989). A comparison of the amino acid sequences of all six human and mouse RARs has indicated that the evolutionary interspecies

conservation of a given member of the RAR subfamily (either α, β or γ) is much higher than the conservation of all three receptors in a given species (Zelent *et al.* 1989; Krust *et al.* 1989). This almost complete interspecies conservation, as well as the differential distribution of the three RAR mRNAs in various mouse tissues, has suggested that each member of the RAR subfamily may play specific roles during development and in the adult animal (Zelent *et al.* 1989; Krust *et al.* 1989).

To identify the possible function of these RAR genes, we have undertaken a study of their RNA transcript distribution during embryogenesis and differentiation. We report here that the RAR-γ gene exhibits a restricted pattern of expression, which suggests that RAR-γ plays a unique role in morphogenesis, chondrogenesis and differentiation of squamous epithelia.

Materials and methods

Sample preparation

Embryos and foetuses from natural mating between C57BL/6×S3L/3 mice were collected between 6.5 and 15.5 days of gestation. Midday of the day of the vaginal plug was considered as day 0.5 p.c. Samples were fixed in a freshly prepared solution of 4% paraformaldehyde in PBS, dehydrated in ethanol, cleared with xylene and embedded in paraffin. Sections (6–8 μm) were collected on gelatinized slides, air dried and stored at 4°C. Prior to hybridization with oligonucleotides, the sections were deparaffined in xylene for 5 min, rehydrated in decreasing series of ethanol concentrations and air dried. For hybridization with RNA probes, after rehydration the sections were treated for 30 min at 37°C with 1 μg ml^{-1} of proteinase K, immersed for 10 min in a 0.1 M-triethanolamine pH 8.0/0.25% (v/v) acetic anhydride solution, dehydrated and air dried.

Preparation of probes

Oligonucleotides were complementary to bases 336–376 and 1576–1617 of the RAR-γ cDNA (Zelent *et al.* 1989) and labelled with [α-^{35}S]dATP by the terminal deoxynucleotidyl-transferase to specific activities of $\sim 1\times10^9$ cts min^{-1} μg^{-1} according to the suppliers directions (Boehringer Mannheim). The specificity of the oligomers was tested by Northern blot hybridization (data not shown).

^{35}S-labelled RNA probes (specific activity of $\sim 5\times10^8$ cts min^{-1} μg^{-1}) were transcribed using T7 polymerase (according to the suppliers directions, Promega Biotec) from the *Eco*RI–*Eco*RI fragment of RARγ subcloned in pSG5 (Green *et al.* 1988) and linearized with *Bgl*II. The template for synthesis of the RAR-β antisense riboprobe was the *Eag*I–*Bam*HI fragment of RAR-β cDNA (Zelent *et al.* 1989) inserted into the *Bam*HI site of Bluescribe (Vector Cloning Systems), using a *Bam*HI–*Eag*I adaptor, and linearized with *Hin*dIII. Probe length was reduced to 100–150 nucleotides by limited alkaline hydrolysis (Cox *et al.* 1984).

Hybridization

Labelled probes diluted to 25.000 cts min^{-1} μl^{-1} were applied to each section in 20 μl of the hybridization buffer (50% formamide, 1 mM-EDTA, 1×Denhardt's, 500 μg ml^{-1} tRNA, 10% Dextran sulfate, 10 mM-DTT, 0.6 M-NaCl for oligomers or 0.3 M-NaCl for RNA probes). Sections were covered with parafilm strips and incubated in humid chambers at 50°C overnight.

Wash

After hybridization with oligonucleotide probes, the slides were washed at 50°C in 1×SSC and 0.1×SSC for 2 h and dehydrated in 70% and 95% ethanol solutions containing 0.3 M-ammonium acetate. Sections hybridized with riboprobes were immersed at 50°C for 1 h in washing buffer (50% formamide, 0.3 M-NaCl, 20 mM-Tris–HCl pH 7.5, 5 mM-EDTA, 10 mM-$NaPO_4$ pH 6.8), rinsed for 15 min at 37°C in NTE solution (0.5 M-NaCl, 10 mM-Tris–HCl pH 7.5, 5 mM-EDTA), treated for 30 min at 37°C with 20 μg ml^{-1} of RNaseA and immersed for a further 15 min in NTE. The slides were then placed for 1 h at 50°C in washing buffer, 30 min at 50°C in 0.1×SSC and dehydrated. The slides were then coated with Kodak NTB 2 emulsion and stored at 4°C. The exposure time was 15 days for RNA probes and two months for oligonucleotide probes. They were developed in Kodak D19 and stained with toluidine blue.

Results

The spatial distribution of RAR-γ RNA transcripts during mouse development has been studied by *in situ* hybridization on serial sections of embryos and foetuses from day 6.5 to day 15.5 p.c. Two kinds of antisense and sense ^{35}S-labelled probes were used: synthetic oligonucleotides complementary to the RAR non-conserved A and F regions, which do not cross-hybridize on Northern blots with RAR-α and RAR-β mRNAs, and RNA probes corresponding to the full coding sequence of RAR-γ cDNA (see Zelent *et al.* 1989, and Materials and methods). The same pattern of hybridization was obtained using either antisense RNA or oligonucleotide probes (Fig. 1B and C, respectively). No specific signals were detected with the corresponding sense probes on consecutive sections (data not shown). RNA probes were used in all subsequent experiments.

RAR-γ transcripts at early stages of development

Only diffuse RAR-γ signals, similar to those in the decidual tissue, could be detected in embryonic and extraembryonic tissues at day 6.5 (Fig. 2A) and 7.5 (Fig. 2B) p.c., which correspond to the gastrulation period when mesoderm develops from the primitive streak, thus establishing the anteroposterior axis of the embryo (see also legend to Fig. 2A).

At day 8 p.c., a strong hybridization signal is detected in the posterior region of the embryo, in all germ layers (Fig. 2C, pm). This restricted expression of the RAR-γ gene to the caudal region is confirmed on transverse sections (Fig. 2D), where the labelling is found in the neurectoderm of the open neural folds, in the presomitic (pm) and lateral mesoderm and in the endoderm. This posterior labelling pattern persists at later stages of development (Fig.3B, ng and pm) as long as somites have not condensed and the neural tube is not closed.

RAR-γ transcripts in mesodermal structures

From day 8.5 p.c., there are marked morphogenetic changes in the embryonic head, with the closure of the

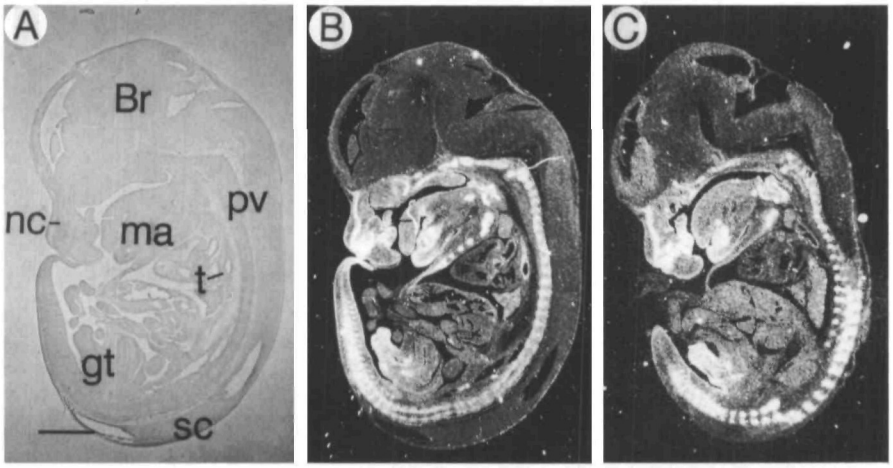


Fig. 1. *In situ* hybridization using RNA or oligonucleotide probes gave the same pattern of distribution of RAR-γ transcripts. (A and B) Bright- and dark-field photographs of a sagittal section of a day 13.5 p.c. embryo hybridized with RNA probes. (C) Dark field of a sagittal section of an embryo at the same stage of development hybridized with oligonucleotide probes. At this stage, transcripts are found in precartilaginous and cartilaginous skeletal elements and in the undifferentiated mesenchyme of the trachea and genital tubercle. nc, cartilage of nasal capsule; ma, mandible; pv, prevertebrae; t, trachea; gt, genital tubercle; sc, spinal cord; Br, brain. Bar 1 mm.

cranial neural tube and development of the pharyngeal arches. At day 8.5 p.c., RAR-γ transcripts are found for the first time in the anterior portion of the embryo; the strongest signals are in the frontonasal mesenchyme (fm, in Fig. 3A and B) and in the mandibular arch (I in Fig. 3A), whereas a weaker signal is found in the rest of the head mesenchyme (Fig. 3A). There is no clear signal above background in the neural epithelium (Fig. 3A and B). At day 9¼ p.c., the second pharyngeal arch has appeared. At this stage, the transcripts are found in the mesenchyme of the first and second pharyngeal arches (I, II), where they are homogeneously distributed, and at a weaker level in the non-arch mesenchyme; they are absent from the neural epithelium and the heart (ht) (Fig. 3C). At day 10.5 p.c., all the pharyngeal arches have developed, as well as the ectoderm-derived olfactory pits and optic and otic vesicles (Fig. 3E and F). RAR-γ transcripts are found in all of the pharyngeal arches and in the frontonasal mesenchyme (I, II, fm, Fig. 3E and F). On the other hand, no significant hybridization signal could be detected in the overlying ectoderm (Fig. 3 and data not shown). No labelling is seen in the forebrain (fb), or in the olfactory pits (of) and optic vesicle (opv) (Fig. 3F and data not shown).

On day 9 p.c., both forelimb and hindlimb buds appear. Embryo sections at days 9¼ (Fig. 3C and D) and 9¾ (data not shown) show RAR-γ transcripts homogeneously distributed in both limb buds (fl in Fig. 3D) and lateral somatopleuric mesoderm (lm in Fig. 3C and D). By day 10.5 p.c., the RAR-γ transcripts are still homogeneously distributed in the mesenchyme of both limb buds (Fig. 3E, fl and hl), whereas the apical ectodermal ridge, and more generally the whole ectodermal layer, are not labelled to a significant level by the RAR-γ probe (Fig. 3E and data not shown).

At the same stage (10.5 days p.c.), RAR-γ transcripts are also found along the body axis in the sclerotomal portion of the differentiating somites (sk in Fig. 3E). This is the first time that RAR-γ transcripts can be seen in a somitic structure. One day later, the sclerotomes which are more distinct, are more intensely labelled (data not shown). Note that there is no significant labelling in any of the structures of the central nervous system at this or any subsequent stage of development.

RAR-γ transcripts in precartilage and cartilage

Later stages of development are characterized by a more restricted pattern of expression of the RAR-γ gene. At day 12.5 p.c., the transcripts are still found in most of the frontonasal and pharyngeal arch mesenchyme derivates (Fig. 4A), with higher levels of RAR-γ transcripts now present in the first precartilaginous mesenchymal condensations [e.g. in the mandibles (ma) and the precartilage of the otic capsule (oc); no signal is seen in the otocyst-derived tissue of the developing inner ear]. A very distinct and strong hybridization signal is also seen in the sclerotome-derived precartilage where the prevertebrae (pv in Fig. 4A and B, and Fig. 5A) are easily recognized. A similar restriction of RAR-γ gene expression, from a homogeneous distribution in all mesenchymal components to the precartilaginous mesenchymal condensations, is clearly seen in the limbs. This phenomenon is illustrated in Figs 4B and 5C, where labelling is highly restricted to precartilage condensations in the shoulder girdle (hu and sp) and proximal segments of the forelimb (fl). At this stage, RAR-γ transcripts are still found throughout the forefoot (Fig. 4B) and hindfoot (Fig. 5E) plates, although the signal is more intense in the precartilaginous condensed areas. At day 13.5 p.c., the RAR-γ transcripts are seen in all precartilage and chondrified structures (see Fig. 1, e.g. in the head, the sternum and the prevertebrae; see also Fig. 6 central

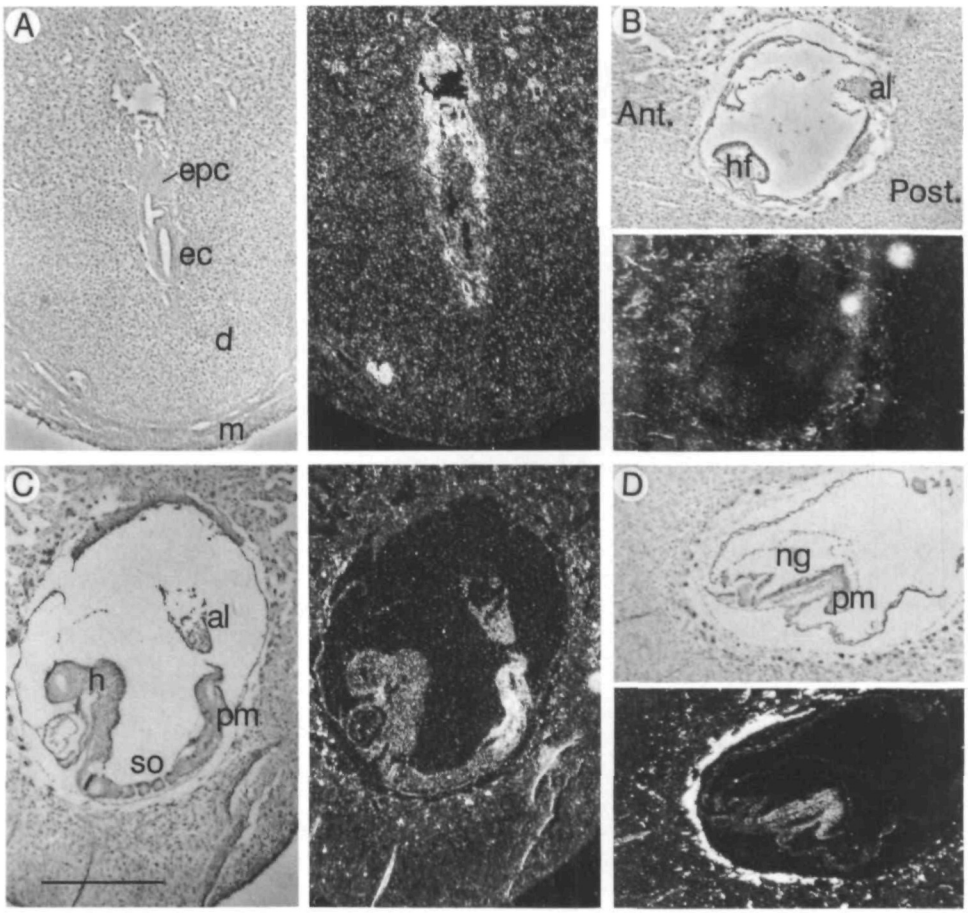


Fig. 2. Distribution of RAR-γ transcripts at early stages of development. (A and B) Sections of uterus with 6.5 and 7.5 day p.c. embryos, respectively. No specific labelling over background levels could be detected in embryos at these stages. The bright signal seen in the uterus in A and D corresponds to optical artefacts and not to high silver grain density. (C) Longitudinal and (D) transverse sections through uterus with 8.0 day p.c. embryos. (C) RAR-γ transcripts are restricted to the caudal region of the embryo. Note that the already condensed somites show no labelling with the RAR-γ probe. (D) A transverse section through the posterior half of the embryo shows transcripts in the presomitic mesoderm, the overlying ectoderm and endoderm. epc, ectoplacental cone; ec, egg cylinder; d, decidual swelling; m, myometrium; al, allantois; hf, head fold; Ant, anterior; Post, posterior; pm, presomitic mesoderm; so, somites; h, head; ng, neural groove. Bar 0.5 mm.

panels, in hind foot and otic capsule cartilage). At days 12.5 and 13.5 p.c., labelling is also detected in the mesenchyme surrounding the tracheal (Fig. 1B, t) and bronchial (Fig. 4B, br) epithelium, as well as in the genital tubercle mesenchyme (Fig. 1, gt).

One day later, at day 14.5 p.c., the first ossification centers appear. They are visible in some cervical vertebrae, where they correspond to regions from which RAR-γ transcripts have disappeared, whereas the not-yet-ossified cartilage elements are still labelled (Fig. 5B, compare with Fig. 5A). A similar pattern can be seen in the scapula, where ossification is taking place and only the perichondrium and the cartilage remain labelled (Fig. 5D, compare with Fig. 5C). At the same

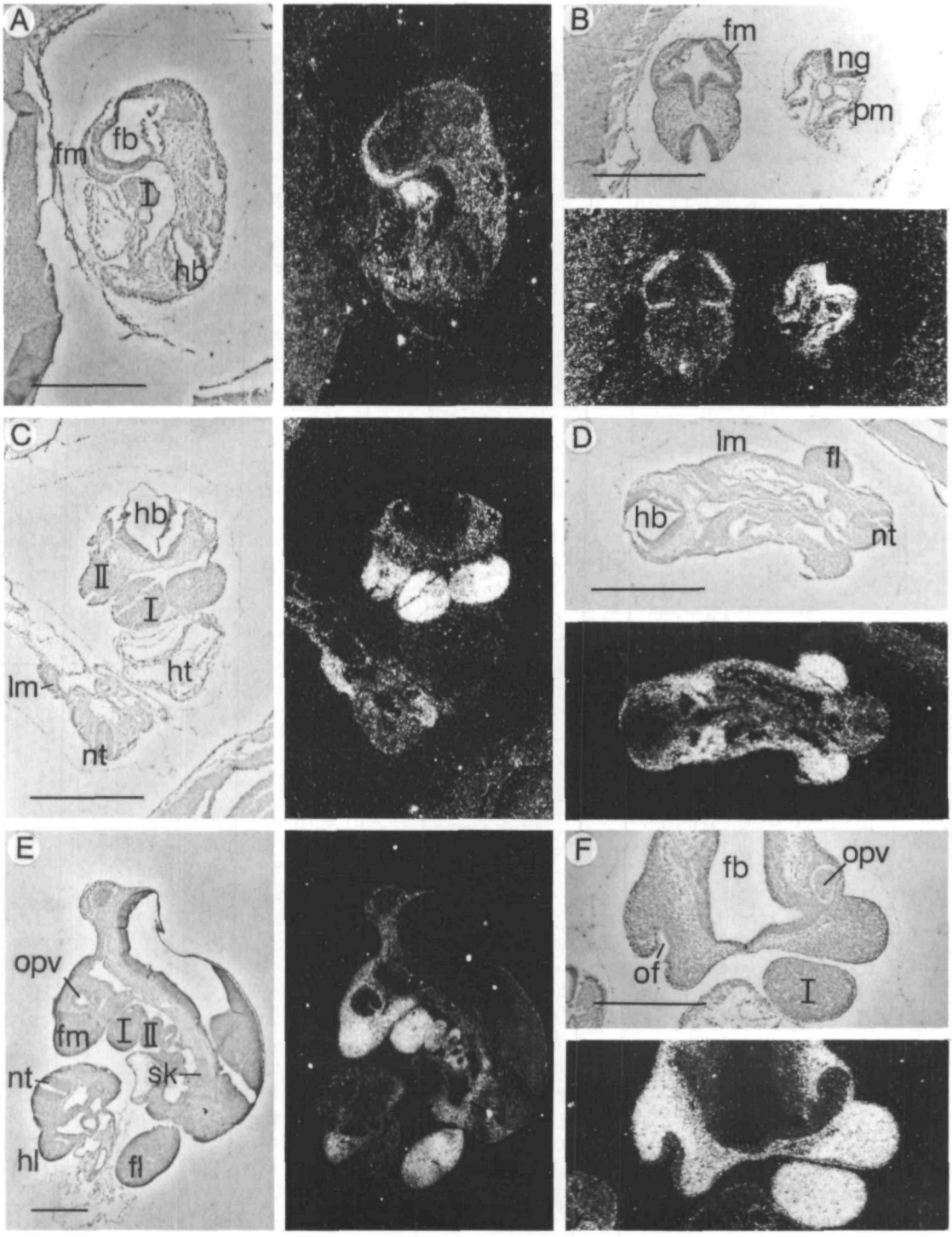
Fig. 3. Distribution of RAR-γ transcripts in 8.5 to 10.5 day embryos. Near sagittal (A) and transverse (B) sections of 8.5 day p.c. embryos in the uterus. (A) A hybridization signal is detected in the head mesenchyme with higher levels in the frontonasal mesenchyme and in the first pharyngeal arch. (B) In the posterior regions of the embryo, transcripts are found in the neural groove and the underlying undifferentiated mesenchyme. (C) Transverse and (D) frontal sections of embryos at 9 days p.c. (C) As in the previous stages high levels of transcripts are found in frontonasal and pharyngeal arch mesenchyme, lateral mesoderm, and (D) in undifferentiated mesoderm of the limb buds. (E and F) sections of 10.5 day embryos. (E) Parasagittal section through all the pharyngeal arches. Transcripts are found in frontonasal and pharyngeal arch mesenchyme as well as in the sclerotome, lateral mesoderm and hind and forelimb buds. (F) Frontal section through the head where transcripts are detected in the mesoderm whereas the optical vesicle, olfactory pit and forebrain show no hybridization signal. fm, frontonasal mesenchyme; I, II, first and second pharyngeal arches; nt, neural tube; fb, forebrain; hb, hindbrain; ng, neural groove; pm, presomitic mesoderm; lm, lateral mesoderm; fl, forelimb bud; hl, hindlimb bud; sk, sclerotome; opv, optic vesicle; of, olfactory pit; ht, heart; Bar 0.5 mm.

stage, ossification has not yet started in the forefoot and hindfoot plates where the cartilage is labelled (Fig. 5F). The presence of RAR-γ transcripts is clearly seen at day 14.5 p.c. in cartilaginous tracheal rings (tr, in Fig. 7A) and in all laryngeal cartilages (lc, in Fig. 7A). We note that some labelling is also found in the lung, notably surrounding the bronchioles (Fig. 7A, lu).

RAR-γ transcripts in epithelia

From day 13.5 p.c., as epithelia begin to differentiate, RAR-γ transcripts accumulate for the first time in non-neural ectodermal and endodermal derivatives. Hybridization is clearly seen at day 14.5 p.c. in the epithelia of the oral cavity (Fig. 7A, arrow), the oesophagus (Fig. 7B, oe) and the left wall of the stomach (st), where it stops abruptly at the beginning of the glandular epithelium (Fig. 7B, arrow).

From day 12.5 p.c., the whisker follicles develop as an epithelial thickening over the undifferentiated mesenchyme and then as invaginations of this epithelium into the underlying mesenchyme. RAR-γ transcripts can be detected in these thickened epithelium and



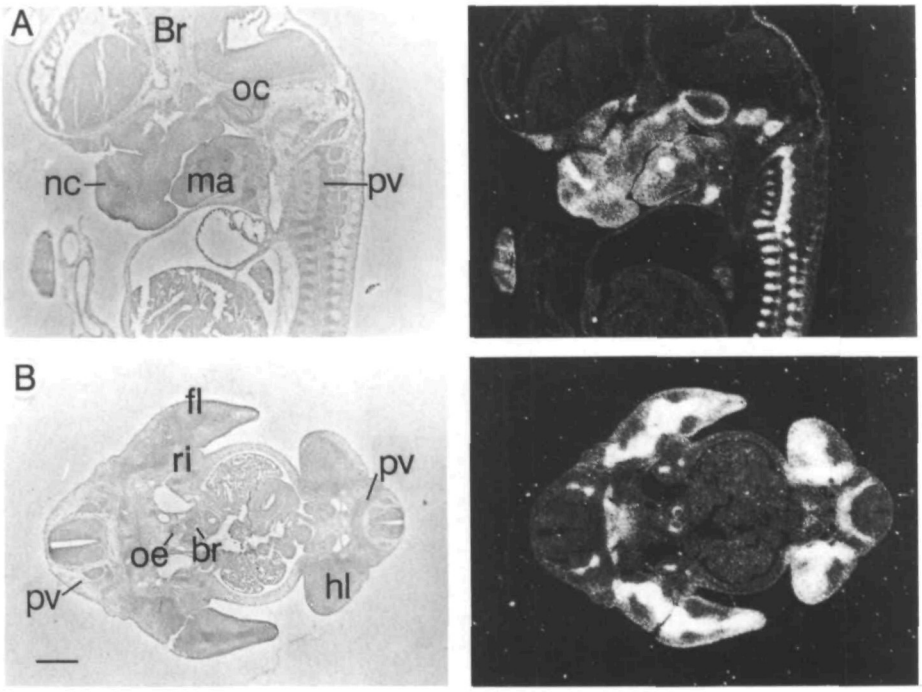


Fig. 4. (A) Parasagittal section of the head of a 12.5 day p.c. embryo. Transcripts are seen in the mesenchyme of the head and in the precartilaginous condensations, e.g. otic capsule and prevertebrae. (B) Transverse section of an embryo at the same stage. A hybridization signal is seen in the prevertebrae, precartilage of the limbs, the ribs and in the undifferentiated mesenchyme of the bronchi. Note that no hybridization signal is found in the brain or neural tube. Br, brain; oc, otic capsule; ma, mandibular region; pv, prevertebrae; nc, nasal cartilages; fl, forelimb; hl, hindlimb, ri, ribs; oe, oesophagus; br, bronchi. Bar 0.5 mm.

underlying mesenchyme at day 12.5 p.c. (data not shown) and are clearly seen at day 14.5 in the roots of the developing whisker follicles (Fig. 7C, wf). Similarly, from day 13.5 p.c., RAR-γ transcripts are detected in the regions where the teeth develop as epithelial invaginations into the underlying mesenchyme (data not shown). At day 14.5 p.c., the three skin layers have differentiated. All three peridermal, epidermal and dermal layers contain RAR-γ transcripts (Fig. 5D, arrow; Fig. 5F and Fig. 7C; compare with day 12.5 p.c., in Fig. 5C and E, where no significant labelling is seen in the non-differentiated surface ectoderm).

Discussion

Using *in situ* hybridization, we report here the distribution of RAR-γ transcripts during mouse development. The restricted developmental pattern of expression of the RAR-γ gene suggests that it could be involved in early morphogenetic events, in the specification of the chondrogenic pattern and in the differentiation of cartilage and squamous epithelia. This spatiotemporal distribution of the RAR-γ transcripts does not necessarily imply the concomitant appearance or disappearance of the RAR-γ protein.

The RAR-γ gene does not appear to be transcribed at a significant level before or during early gastrulation. Note, however, that no hybridization signal above the background does not exclude low levels of RAR-γ gene transcription. RAR-γ transcription has been detected late in gastrulating embryos in the mesoderm where somites have not yet differentiated and in the overlying neurectoderm (Fig. 2C). It is unknown whether the formation of the first somites is also preceded by the expression of RAR-γ in the mesoderm. Within the trunk, the transcripts seem to disappear in a craniocaudal direction, concomitantly with both the appearance of new somites and the closure of the neural tube (Fig. 3B). No RAR-γ transcripts could be detected in any part of the central or peripheral nervous system subsequently in development. This early pattern of expression suggest that RAR-γ may play a role in somite formation and/or in early differentiation of the neurectoderm.

The earliest strong signals of RAR-γ transcripts in the cranial region of the embryo were observed in the mesenchymal cell populations that will participate in the formation of the craniofacial structures, i.e. frontonasal and pharyngeal arch mesenchyme. The subsequent modifications in the spatial distribution of RAR-γ transcripts are closely related to the sequence of morphogenetic events in this region. At early stages, RAR-γ transcripts are homogeneously distributed throughout the mesenchymal cells. Higher levels of transcripts are then found in the first mesenchymal precartilaginous condensations that appear, whereas the hybridization signal decreases concomitantly in the surrounding non-chondrogenic mesenchyme. At later stages, the transcripts become restricted to the cartilagi-

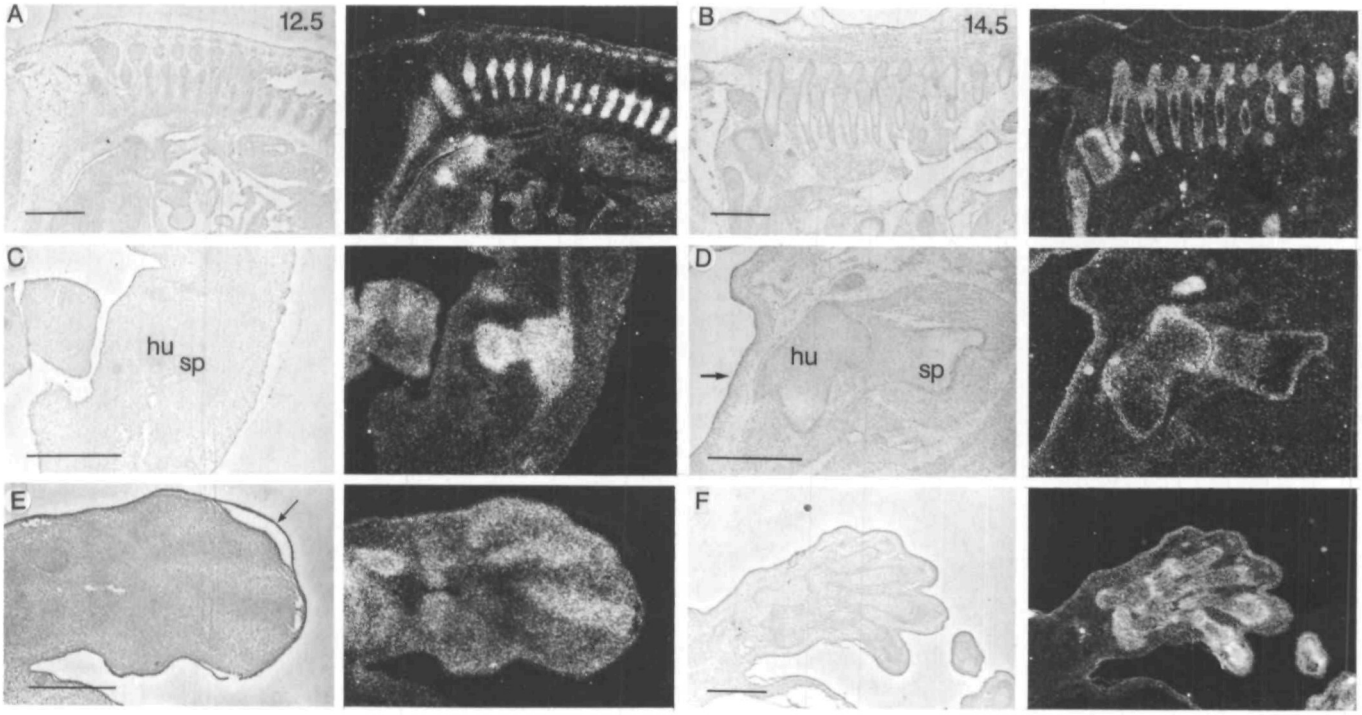


Fig. 5. Sections of cervical prevertebrae, scapula and hindlimb of 12.5 (A,C,E) and 14.5 (B,D,F) day p.c. embryos. Embryos of 12.5 days p.c. show transcripts uniformly distributed in the precartilaginous blastema while, at 14.5 days of development, as ossification starts, labelling becomes restricted to the cartilaginous cells and no signal is detected in the appearing ossification centers (compare A,B and C,D). Note the appearance of labelling in the skin by day 14.5 (arrow in D, compare with C and arrow in E). (E) At 12.5 days p.c., the hindfoot plate is still mainly mesenchymal, but the first precartilaginous condensations are appearing. Note that labelling is found in most of the mesenchyme, while higher levels are seen in the appearing blastema. (F) At 14.5 days of development labelling is found in the cartilaginous cells of the foot. sp, scapula; hu, humerus. Bar 0.5 mm.

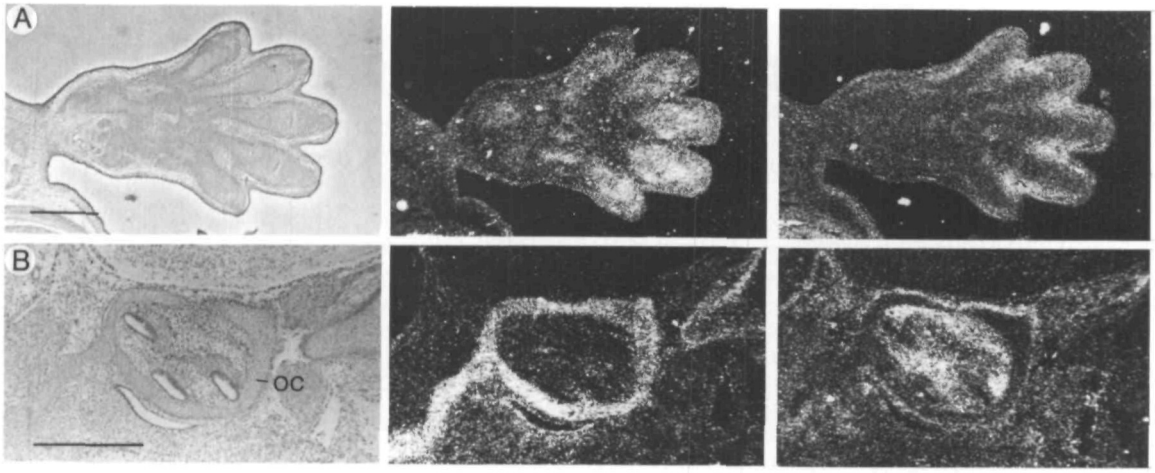


Fig. 6. Sections of hindfoot (A) and inner ear (cochlea) (B) of a 13.5 day p.c. embryo hybridized with a RAR-γ probe (central panels) and RAR-β probe (right panels). RAR-γ transcripts are found in cartilage of the foot and otic capsule, whereas RAR-β transcripts are found in the interdigital mesenchyme and the mesenchyme of the inner ear. oc, otic capsule. Bar 0.5 mm.

nous elements. A similar transition in the expression of the RAR-γ gene from undifferentiated mesenchyme to precartilaginous blastema and cartilage is observed in the developing limbs. Note also that, although at early stages there is no RAR-γ gene expression in the non-differentiated somites, RAR-γ transcripts appear specifically in the sclerotomes, which correspond to the region of the somitic mesoderm from which the axial skeleton will derive. In fact, at later stages of skeletal development, there is a striking restriction of RAR-γ transcripts to structures that will remain cartilaginous in the adult mouse. RAR-γ gene expression can be found

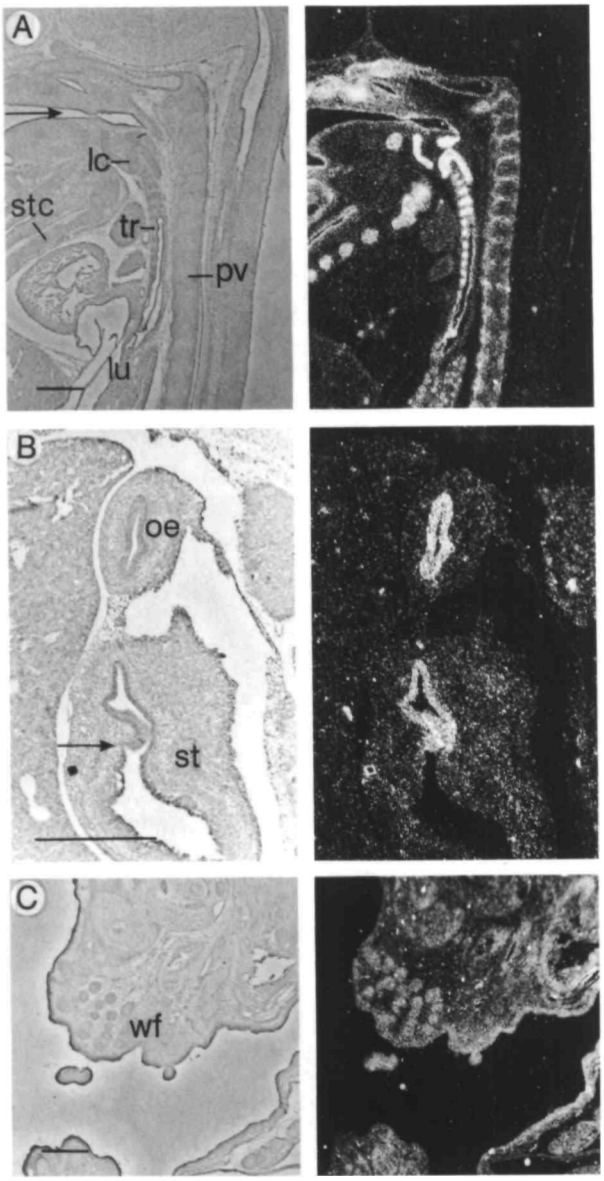


Fig. 7. (A) Sagittal section of a 14.5 day p.c. embryo. Labelling is seen in the cartilages of the trachea, larynx, sternum and prevertebrae. RAR-γ transcripts are seen in the lungs and in the epithelium of the oral cavity (arrow) and, as shown in B, in the oesophagus and the left wall of the stomach; note the disappearence of signal at the boundary with the glandular epithelium (arrow). (C) At the same stage, a signal is found in the root of the whisker follicles. tr, tracheal cartilages; pv, prevertebrae; stc, sternal cartilages; lu, lung; oe, oesophagus; st, stomach; wf, whisker follicles. Bar 0.5 mm.

in the differentiating mesenchyme and later in the cartilages of the larynx, trachea and bronchi. Thus, the expression of the RAR-γ gene is specific to cells that have a common developmental fate in terms of differentiated tissue-type, although they do not belong to a common embryological lineage, since the craniofacial cartilage cells are derived from the ectodermal neural crest, and the limb and axial skeletal cartilage cells are derived from mesoderm (Noden, 1984; Morriss-Kay and Tan, 1987; Hall, 1978).

RAR-γ transcripts are also specifically found late during embryonic development in differentiating squamous epithelia, i.e. those of the skin (in both dermis and epidermis) and digestive tract (oral cavity, oesophagus, and left wall of the stomach), which are all cornified in the adult mouse (Hebel and Stromberg, 1986). As in the case of cartilage cells, the expression of the RAR-γ gene in these epithelia is linked to a common developmental fate and not a common embryological origin, since the epidermis derives from the ectoderm and the digestive tract squamous epithelia from the endoderm (Rugh, 1968).

The distribution pattern of RAR-γ transcripts observed in this study is similar to that of cells and tissues known to be specifically sensitive to high levels of retinoids *in vitro*, e.g. primary mesenchyme (Morriss, 1975), cranial neural crest (Thorogood *et al.* 1982), cartilage (Gallandre and Kistler, 1980; Kochhar *et al.* 1984; Horton *et al.* 1987; and refs. therein) and skin (Roberts and Sporn, 1984; Kopan and Fuchs, 1989; Asselineau *et al.* 1989). In whole embryos, *in vivo* and *in vitro*, retinoids appear to affect the migration of cranial neural crest cells from which the craniofacial structures are derived (Morriss and Thorogood, 1978; Webster *et al.* 1986). Retinoids are known to modulate chondrogenesis of craniofacial cells in culture (Wedden *et al.* 1987, 1988; Langille *et al.* 1989 and refs therein) and to affect macromolecular synthesis in chondrocytes and osteoblasts in culture (Gallandre and Kistler, 1980; Kochhar *et al.* 1984; Horton *et al.* 1987; Heath *et al.* 1989, and refs. therein). The effect of retinoids on epithelia and notably on the maintenance and differentiation of skin are also well established (Roberts and Sporn, 1984; Peck, 1984; Brown *et al.* 1985; Shapiro, 1985; Asselineau *et al.* 1989; Koppen and Fuchs, 1989; and refs. therein).

In addition to RAR-γ, two retinoic acid receptors, α and β, have been characterized (Petkovich *et al.* 1987; Giguere *et al.* 1987; Brand *et al.* 1988; Benbrook *et al.* 1988). Our *in situ* hybridization studies with RAR-α and RAR-β probes indicate that the RAR-α gene is rather ubiquitously expressed in mouse embryos, whereas the RAR-β gene is very restricted in its expression. RAR-β transcripts are not found in the developing limbs with the exception of very specific areas, e.g. the interdigital mesenchyme (Dollé *et al.* 1989; see also Fig. 6A). This non-overlapping distribution of RAR-β and RAR-γ transcripts is not limited to the developing limb; for instance, in the inner ear region, the RAR-γ transcripts are restricted to the cartilage of the otic capsule, whereas the RAR-β transcripts are found in the mesenchyme surrounding the inner ear epithelium (Fig. 6B). Similarly, in agreement with our previous Northern blot results (Zelent *et al.* 1989), our *in situ* hybridization studies indicate that there is very little expression of the RAR-β gene in the skin (Dollé *et al.* 1989 and our unpublished results). Thus, the effect of retinoic acid on morphogenesis, chondrogenesis and squamous epithelial differentiation

may be specifically mediated by RAR-γ whose spatio-temporal pattern of expression is closely related to these events. Furthermore, the distribution of RAR-γ in the early embryo may be functionally correlated with the specific vulnerability of certain cell populations to excess retinoids and to the onset of retinoid-induced abnormal development. That some of the effects caused by RA excess may be due to abnormal expression of some of the homeogenes whose expression is known to be regulated by RA *in vitro* (Colberg-Poley *et al.* 1985*a*,*b*; Deschamps *et al.* 1987; Kessel *et al.* 1987; Murphy *et al.* 1988; LaRosa and Gudas, 1988), is suggested by the recent report of Balling *et al.* (1989) showing that ectopic expression of Hox-1.1 in transgenic mice induces craniofacial abnormalities. However, it is not clear from their results that these craniofacial abnormalities bear a real resemblance to those of retinoic acid embryopathy. Finally, several differentially spliced transcripts of the RAR-γ gene have been recently found (Krust *et al.* 1989; our unpublished results). Whether they could be specifically distributed during embryogenesis in the cells expressing the RAR-γ gene remains to be seen.

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