Int. J. Dev. Biol. 39: 111-121 (1995)

Roles of retinoic acid receptors and of *Hox* genes in the patterning of the teeth and of the jaw skeleton

MANUEL MARK[1,2]*, DAVID LOHNES[1+], CATHY MENDELSOHN[1#], VALÉRIE DUPÉ[1], JEAN-LUC VONESCH[1], PHILIPPE KASTNER,[1] FILIPPO RIJLI[1], AGNÈS BLOCH-ZUPAN[2] and PIERRE CHAMBON[1]

[1]*Institut de Génétique et de Biologie Moléculaire et Cellulaire, CNRS/INSERM/ULP, Strasbourg and* [2]*Institut de Biologie Médicale, Faculté de Médecine, Strasbourg, France*

ABSTRACT Retinoic acid receptors and transcriptional factors encoded by *Hox* genes play key roles in vertebrate development and belong to an integrated functional network. To investigate the actual functions of these molecules during ontogenesis and in particular in the patterning of the cranial neural crest cells giving rise to the teeth and to the jaw bones, we have generated null mutant mice lacking functional retinoic acid receptors or *Hox* genes by gene targeting in embryonic stem cells.

KEY WORDS: *odontogenesis, jaws, pattern formation, neural crest, retinoic acid, retinoic acid receptors, Hox genes, atavisms, evolution*

Introduction

Odontogenic mesenchymal cells, which include the odontoblasts, the dental pulp cells, the cementoblasts, the osteoblasts of the alveolar bone and the fibroblasts of the periodontal ligament, are derived from the mesectoderm (Lumsden, 1988 and references therein). In the mouse embryo, putative odontogenic precursors exist within two streams of migrating cranial neural crest cells (NCC), as assessed *in vivo* by labeling premigratory NCC precursors with non-toxic fluorescent dyes (Lumsden *et al.*, 1991; Serbedzija *et al.*, 1992; Sechrist *et al.*, 1993; Scherson *et al.*, 1993). The stream originating from the prosencephalon and rostral mesencephalon, which populates the periocular and frontonasal regions, contributes to the upper incisors (Lumsden and Buchanan, 1986). The stream originating from the rostral rhombencephalon (i.e. rhombomeres 1 and 2) and caudal mesencephalon populates the 1st (maxillo-mandibular) pharyngeal arch, thus contributing to the lower incisors, to the lower and upper molars and perhaps to a small part of the upper incisors (Lumsden and Buchanan, 1986; Peterkova *et al.*, 1993). All cranial NCC have ceased to emigrate from the neural folds at 9.0 days post coitum (dpc) (Serbedzija *et al.*, 1992). Besides the odontogenic mesenchyme, the mesectoderm notably forms the majority of the craniofacial membranous and endochondral bones, as well as the mesenchyme of almost all the craniofacial striated muscles (Noden, 1988; Couly *et al.*, 1993; Le Douarin *et al.*, 1993). Whether premigratory precursors of cranial NCC present in the cephalic neural plate are multipotent or form a mosaic of developmentally distinct subpopulations with already restricted potentials is still an open question (reviewed in Selleck, 1993). Migration is not required for the acquisition by NCC of odontogenic, osteogenic or chondrogenic potentials in the mouse, but these are only expressed following induction by an ectodermal epithelium (reviewed in Lumsden, 1988; Hall, 1991 and Thorogood, 1993).

The dentition of a mouse consists of one pair of monocuspal incisors and of 3 pairs of molars, in each jaw. The crown of each molar comprises 3 (for the 3rd lower molar) to 8 (for the 1st upper molar) cusps (Gaunt, 1955). Teeth can be considered as serially-repeated anatomical units and since heterodonty derives from homodonty, the fundamental morphological unit of the dentition would be the cusp. The question remains as to how the patterning of the cusp founder cells is controlled (Ruch, 1995). The nature of the patterning mechanisms of mesectodermal structures, in general, is a matter of controversy. Noden (1983), employing the quail-chick marking system, showed that when premigratory NCC destined for the 1st pharyngeal arch are transplanted in place of 2nd or 3rd arch precursors, the host develops with a supernumerary jaw skeleton located ectopically in the 2nd or the 3rd pharyngeal arch. His main conclusion was that the morphogenetic fate of 1st pharyngeal arch osteogenic and chondrogenic NCC has already been determined within the neural plate, thus prior to the onset of migration. Noden's host embryos also developed a beak-like, cornified projection from the 2nd and the 3rd pharyngeal arches as well as a supernumerary external auditory meatus, indicating that these specializations of the surface ectoderm were normally induced by 1st arch mesectodermal cells. On the other hand,

Abbreviations used in this paper: GPP, Ground Patterning Program; NCC, neural crest cells; RA, retinoic acid; RAR and RXR are retinoic acid receptors; VAD, vitamin A deficiency.

***Address for reprints:** Institut de Génétique et de Biologie Moléculaire et Cellulaire, CNRS/INSERM/ULP, BP 163, 67404 Illkirch Cedex, C.U. de Strasbourg, France. FAX: 33.88653203.

Present addresses: +Institut de Recherche Clinique de Montreal, 110 Pina Ave. West, Montreal, Quebec H2W1R2, Canada and #College of Physicians and Surgeons, Columbia University, Dept. of Physiology and Cellular Biophysics, New York, NY 10032, USA.

0214-6282/95/$03.00

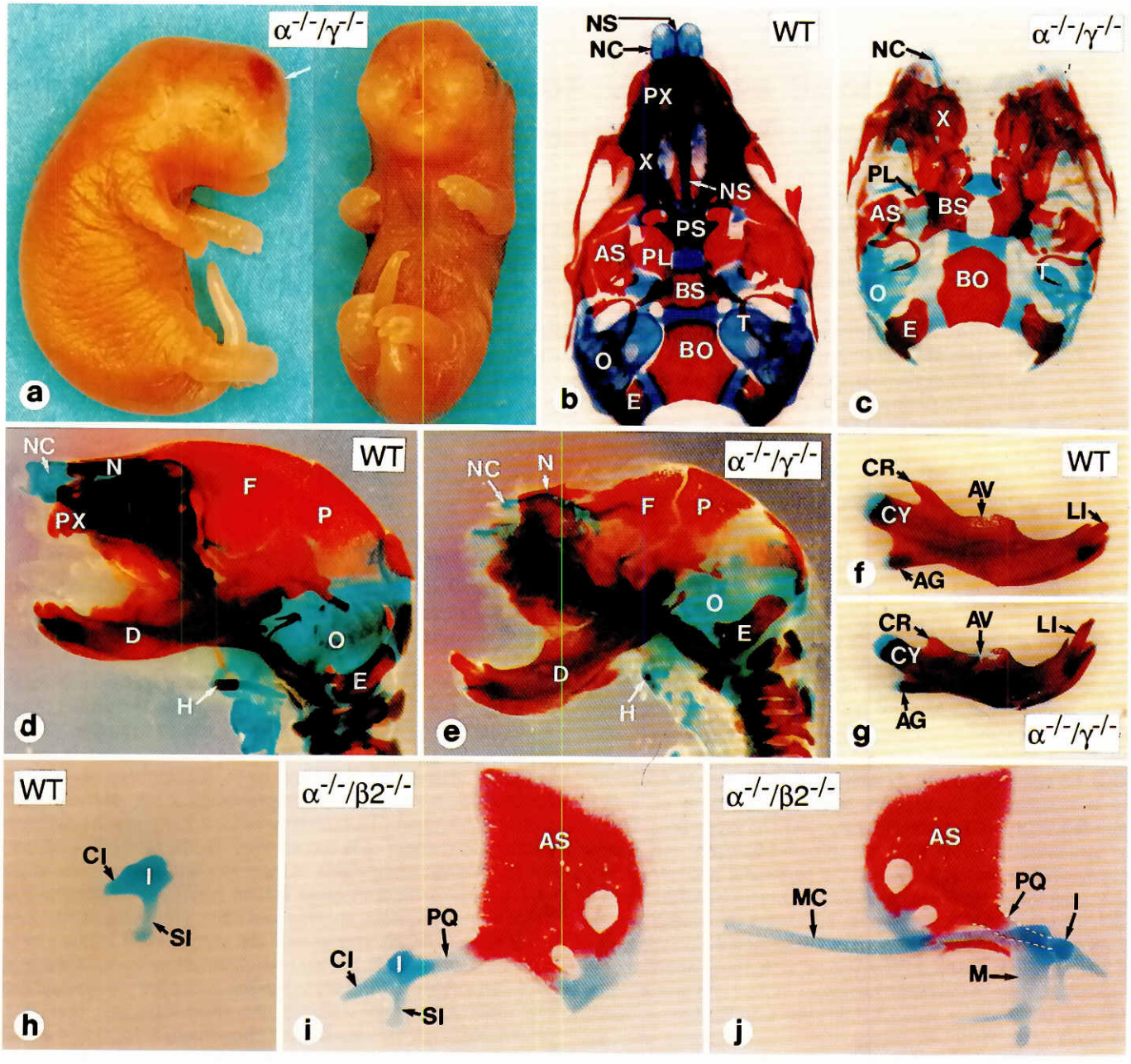

Fig. 1. External aspect and craniofacial skeletal features of 18.5 dpc wildtype (WT) and RAR double mutant fetuses (the genotype is indicated on each photograph). (a) *Typical external aspect of a non-exencephalic RARα$^{-/-}$/γ$^{-/-}$ mutant: note the shortening of the snout, the median facial cleft and the bulging of the forebrain beneath the skin (arrow), which reflects the agenesis of the median portion of the frontal bone.* **(b-g)** *Comparison of ventral* **(b,c)** *and lateral* **(d-g)** *views of the skull and of the dentary bone between wildtype* **(b,d, and f)** *and RARα$^{-/-}$/γ$^{-/-}$mutant* **(c,e and g)** *fetuses. In the mutant, note the near absence of the nasal capsule (NC), the complete agenesis of the nasal septum (NS) and of the premaxillary (PX) and presphenoid (PS) bones, the wide median cleft in the basisphenoid bone (BS) and the aplasia of the hyoid bone (H). On the other hand, note that the different parts of the mutant dentary bone (D) are readily identifiable [e.g. angular process (AG), condylar process (CY), coronoid process (CR), alveolar bone of the molars (AV)].* **(h-i)** *Medial aspect of the wildtype incus and of the complex formed by the incus (I) pterygoquadrate cartilage (PQ) and alisphenoid bone (AS) in a RARα$^{-/-}$/β2$^{-/-}$ mutant fetus. Note the lengthening of the caudal process of the mutant incus (CI).* **(j)** *Lateral aspect of all skeletal elements derived from the lower and upper jaw cartilages in a RARα$^{-/-}$/β2$^{-/-}$ fetus. The pterygoquadrate cartilage which passes behind the malleus (M) is indicated by broken lines. AG, angular process of the dentary bone; AS, alisphenoid bone; AV, alveolar bone of the molar; BO, basiocciptal bone; BS, basisphenoid bone; CI, caudal process of the incus; CR and CY, coronoid and condylar processes of the dentary bone respectively; D, mandibular (dentary) bone; E, exoccipital bone; F, frontal bone; H, hyoid bone; I, incus; LI, lower incisor; M, malleus; MC, Meckel's cartilage; N, nasal bone; NC, nasal capsule; NS, nasal septum; O, otic capsule; P, parietal bone; PL, palatine bone; PQ, pterygoquadrate cartilage; PS, presphenoid bone; PX, incisive (premaxillary) bone; SI, stapedial process of the incus; T, Tympanic bone; X, maxillary bone. The same magnifications were used for* **(b-g)** *and* **(h-i)**. *For further details see Lohnes et al. (1994).*

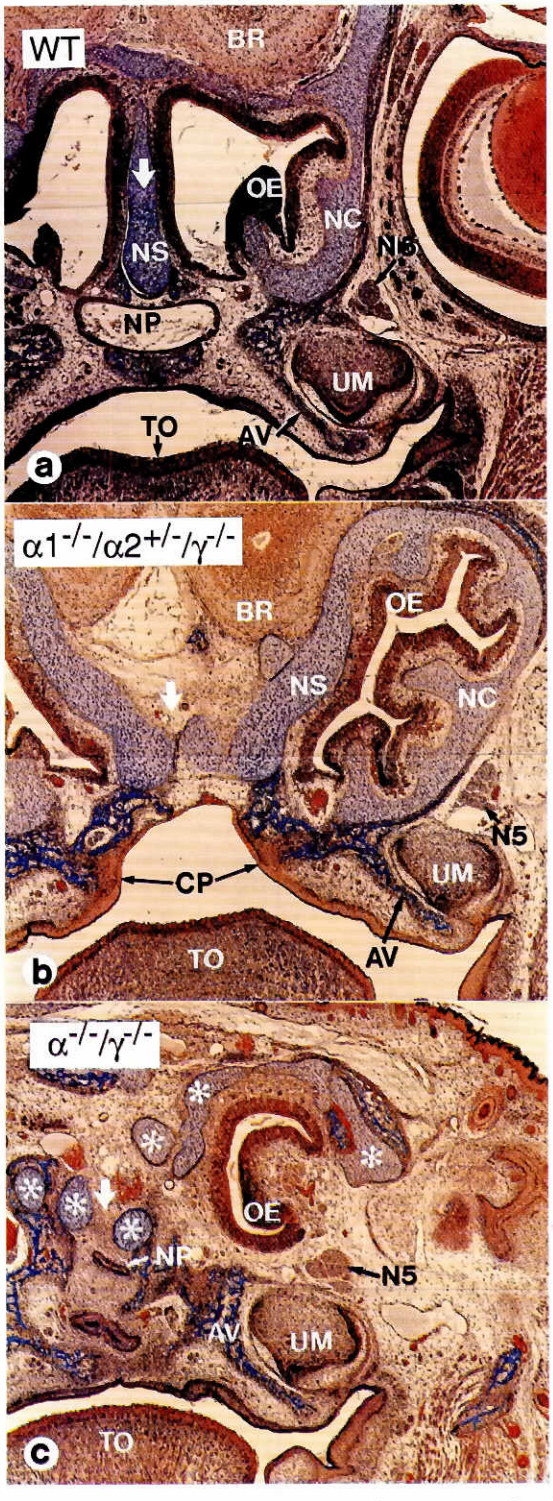

Fig. 2. Frontal sections at similar levels of the first upper molars in 18.5 dpc wildtype (a), RARα1$^{-/-}$ / α2$^{+/-}$ / γ$^{/-}$ (b), and RARα$^{-/-}$ / γ$^{/-}$ (c) fetuses. *Note that the nasal septum (NS) is duplicated in* **(b)**. *The nasal septum and the nasal capsule are both lacking in* **(c)** *and are replaced by irregular rods and nodules of cartilage (asterisks). AV, alveolar bone; BR, brain; CP, cleft of the secondary palate; N5, maxillary branch of the trigeminal nerve (5th cranial nerve); NC, nasal capsule; NP, nasopharyngeal duct; NS, nasal septum; OE, olfactory epithelium; TO, tongue; UM, first upper molar. The large arrow indicates the midline*

Lumsden recombined mouse premigratory NCC with enzyme-dissociated limb bud or 1st pharyngeal arch ectoderm. His results indicated that both cranial and trunk NCC progenitors had odontogenic potentials but that these were only expressed in association with 1st arch ectoderm. From these data and from the results of other epithelial-mesenchymal recombinations, Lumsden suggested that the oral portion of the 1st arch epithelium was the site of tooth specification and patterning (reviewed in Lumsden, 1988).

Hox and retinoic acid receptor genes encode transcriptional factors which are thought to play key roles in the patterning of the limb and axial skeleton. That these genes might belong to an integrated functional network is supported by several lines of evidence (reviewed in Hofmann and Eichele, 1994; see also references in Kastner *et al.*, 1994; Lohnes *et al.*, 1994; Mendelsohn *et al.*, 1994). First, *Hox* gene expression in cultured embryonal carcinoma cells is controlled by retinoic acid (RA). Second, a number of these responsive *Hox* genes have functional retinoic acid response elements (RAREs) in their promoter regions. Third, there are striking similarities between the vertebral transformations observed in some *Hox* null mutant mice and retinoic acid receptor null mutant mice. Members of both gene families have restricted expression domains in the frontonasal process and/or in the branchial region of the head (i.e. the first 3 pharyngeal arches), thus representing potential candidates for controlling the spatial organization of mesectoderm-derived structures, including teeth. Analysis of developmental defects in mice carrying null mutations, generated by targeted gene disruption in embryonic stem cells (Chisaka and Capecchi, 1991), is a means for gaining insight into the roles of these genes. We review here recent analyses of the phenotypic consequences of the disruption of *Hox* and retinoic acid receptor genes on cranial mesectodermal cells.

Mesectodermal cells of the frontonasal process and of the 1st pharyngeal arch exhibit different requirements for retinoic acid

Vitamin A (retinol) is an essential component of the vertebrate diet. Vitamin A deficiency (VAD) studies during pregnancy and following birth have shown that vitamin A is required both during development and in the adult animal. After birth, retinol is required for survival, normal growth, reproduction, vision and maintenance of numerous tissues (including teeth and alveolar bones: Wolbach

TABLE 1

GRADED INCREASE IN THE SEVERITY OF THE DEFECTS IN SOME FRONTONASAL MESECTODERMAL STRUCTURES OF MUTANT FETUSES ANALYZED AT 18.5 DPC

	RAR Double Null Mutant Genotype		
	α1-/- / γ-/-	α1-/- / α2+/- / γ-/-	α-/- / γ-/-
External midfacial features	All normal	Median cleft of upper lip (a)	Agenesis of the prolabium
Bones derived from fronto-nasal mesectoderm			
Frontal	All normal	Shortened	Median portion agenic
Ethmoid		Nasal septum duplicated	Nasal septum agenic
Premaxillary		Misshapened	Absent
Upper incisors	Normal	Malformed (b)	Absent

(a) This abnormality was only incompletely penetrant. (b) Unilateral agenesis was observed in one (out of 5) fetuses.

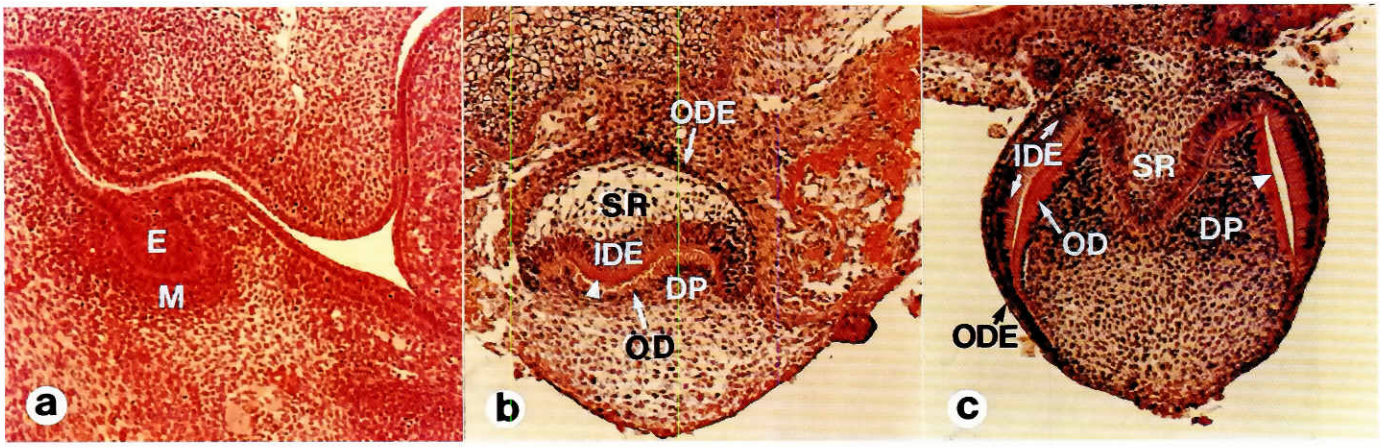

Fig. 3. Effect of a synthetic retinoid (Ch55) at a final concentration of 10-^{9}M on tooth morphogenesis in a chemically-defined culture medium. *Frontal histological sections through 13.0 dpc first lower molars in situ* **(a)**, *and after 10 days in culture* **(b and c)** *either in the absence* **(b)** *or in the presence* **(c)** *of Ch55. Retinoid-deficiency* **(b)** *selectively impairs tooth morphogenesis since it has little effect on the histogenesis of the dental epithelium (IDE, ODE and SR) and terminal differentiation of the odontoblasts (OD). DP, dental papilla; E, dental epithelial bud, representing the anlage of the inner and outer dental epithelia and of the stellate reticulum; IDE, inner dental epithelium; M, dental mesenchyme, containing the anlage of the dental papilla; ODE, outer dental epithelium; SR, stellate reticulum; arrowheads, predentin. Same magnification in* **(a-c)**. *For further details see Mark et al. (1992).*

and Howe, 1925; Mellanby, 1941; McDowell *et al.*, 1987 and references therein). With the exception of defects in vision, retinoic acid administration can prevent most of the defects generated by a post-natal VAD diet. Conceptuses of VAD dams exhibit a large number of congenital malformations (known as the fetal VAD syndrome) that affect the patterning and development of many structures including, among others, the eye, genito-urinary tract, aortic arch derivatives, heart, lungs and kidneys. The multiple teratogenic effects of excess maternal RA administration, as well as the morphogenetic effects of topical administration of RA on limb development and regeneration, led to the suggestion that RA could also be the active retinoid, indispensable during vertebrate development (for reviews and references see Lohnes *et al.*, 1993, 1994; Chambon, 1994; Hofmann and Eichele, 1994; Kastner *et al.*, 1994; Mendelsohn *et al.*, 1994).

The discovery of a nuclear receptor for RA (Giguère *et al.*, 1987; Petkovich *et al.*, 1987) has greatly advanced our understanding as to how the retinoid signal is transduced and could exert so many diverse effects. The three types of retinoic acid receptors, RARα, β, and γ and the three types of retinoid X receptors, RXRα, β, and γ are ligand-inducible trans-regulators which modulate the transcription of target genes by interacting with cis-acting DNA response elements in the promoter region of target genes. RARs are efficiently activated by either all-trans or 9-cis RA, whereas RXRs are efficiently activated only by 9-cis-RA. RAR isoforms (e.g. RARα1, RARβ2) are generated through the differential usage of two promoters and/or alternative splicing (reviewed in Chambon, 1994).

Mesectoderm-derived craniofacial structures were not deficient in double null mutant mice lacking both the RARα and RARβ2 genes (RARα$^{-/-}$/β2$^{-/-}$ mutants) or both the RAR β2 and RARγ genes (RARβ2$^{-/-}$/γ$^{-/-}$ mutants) (Lohnes *et al.*, 1994; Mendelsohn *et al.*, 1994). In particular, the patterning of the dentition and that of the cusps appeared normal in all of these RAR double mutants at 18.5 dpc, which corresponds to the last developmental stage that can be analyzed since mutant newborns all die within a few hours.

In contrast almost all the derivatives of cranial mesectoderm were profoundly affected in RARα$^{-/-}$/γ$^{-/-}$ embryos and fetuses (Lohnes *et al.*, 1994; Mendelsohn *et al.*, 1994). In these double null mutants at 18.5 dpc, all the structures derived from the frontonasal process were partially or completely agenic. These structures included: (i) external midfacial structures (resulting in a median facial cleft; Fig. 1a), (ii) bones : the frontal (F), nasal (N), premaxillary (PX), ethmoid (comprising the nasal capsule, NC, and the nasal septum, NS) and presphenoid (PS) bones were missing for the most part (compare Fig. 1b and d with 1c and e, and Fig. 2a with 2c), and (iii) teeth: the upper incisors were bilaterally absent in all 5 RARα$^{-/-}$/γ$^{-/-}$ fetuses analyzed at 18.5 dpc. Likewise, the skeletal derivatives of the 2nd and 3rd pharyngeal arches either were not identifiable (e.g. stapes) or appeared severely malformed (e.g. hyoid bone; compare H, Fig. 1d with 1e). However, the 1st pharyngeal skeletal elements were all identifiable in 18.5 dpc RARα$^{-/-}$/γ$^{-/-}$ fetuses, and some appeared nearly normal [i.e. dentary bone (compare D, Fig. 1d and f with 1e and g), temporo-mandibular joint, Meckel's cartilage, malleus, and tympanic bone (compare T, Fig. 1b with 1c)]. Likewise, the spatial arrangement and the shape of the lower incisors and of the lower and upper molars (compare UM, Fig. 2a with 2c) were apparently normal in all RARα$^{-/-}$/γ$^{-/-}$ fetuses.

These malformations in the structures derived from cranial mesectodermal cells in RARα$^{-/-}$/γ$^{-/-}$ mutants are likely to reflect RA requirements for events occurring during or after NCC migration. Indeed, RARα and RARγ are both highly expressed in the frontonasal process and in all pharyngeal arches after (and possibly during) NCC migration, but expression of RARγ has not been detected in presumptive cranial NCC progenitors by *in situ* hybridization (Ruberte *et al.*, 1990). The agenesis of midfacial structures is preceded by increased cell death in the frontonasal mesectoderm at 10.5 dpc, indicating that RA is normally required for the survival of post-migratory NCC in this location (Lohnes *et al.*, 1994).

RARα and RARγ are functionally redundant for the development of frontonasal mesectodermal structures as indicated both by the absence of craniofacial defects in RARα$^{-/-}$ single mutants and in RARα1$^{-/-}$/γ$^{-/-}$ double mutants and by the graded increase in the

severity of the defects with the subsequent loss of one and then of both copies of the RARα2 gene from the RARα1$^{-/-}$/γ$^{-/-}$ genetic background (Lufkin *et al.*, 1993; Lohnes *et al.*, 1993, 1994; Table 1). Contrary to RARα1$^{-/-}$/γ$^{-/-}$ mutants, RARα1$^{-/-}$/α2$^{+/-}$/γ$^{-/-}$ mutants exhibited malformations of the structures derived from the frontonasal mesectoderm, but these were less severe than in RARα$^{-/-}$/γ$^{-/-}$ mutants (Fig. 2a-c; Table 1). For instance, unilateral agenesis of an upper incisor was observed only in 1 (out of 5) fetuses. However, the RARα1$^{-/-}$/α2$^{+/-}$/γ$^{-/-}$ upper incisors were always hypoplastic and misshapened, again in contrast to the lower incisors and to the molars, which were apparently unaffected (e.g. UM, compare, Fig. 2a with 2b).

Although RARα and γ transcripts are apparently as abundant in the 1st pharyngeal arch as in the frontonasal region and in other, more caudal, arches (Dollé *et al.*, 1990; Ruberte *et al.*, 1990, 1991), it is striking that only structures derived from the 1st arch are unaffected or only mildly affected in RARα$^{-/-}$/γ$^{-/-}$ mutants. It is noteworthy (i) that the level and patterns of expression of RARβ were apparently unaffected in RARα$^{-/-}$/γ$^{-/-}$ mutants (Lohnes *et al.*, 1994) and (ii) that RARβ transcripts are much less abundant in the 1st arch than in the frontonasal process. The absence of effects of RARs inactivations on some 1st arch-derived structures, including teeth, might indicate that RA is not critically required for their development. It is clear however that, *in vitro*, RA is indispensable for tooth morphogenesis: when molar explants are cultured on a chemically-defined semi-solid medium in the absence of retinoids, morphogenesis is arrested at the dental cap stage. The addition of RA at a final concentration of 10^{-7} M or of Ch55 (a synthetic retinoid) at a concentration of 10^{-9} M to the culture medium restores a normal pattern of cell proliferation in the dental epithelium and dental mesenchyme and permits the formation of the dental bell and subsequently of dental cusps in molar explants (Mark *et al.*, 1992; Fig. 3a-c.). It also permits the normal asymmetrical development of the lingual and labial sides in incisor explants (Bloch-Zupan *et al.*, 1994b). Taken together, our *in vivo* and *in vitro* data do not rule out the possibility of a functional redundancy between RARα, RARβ and RARγ for the morphogenesis of the tooth crown. Alternatively, RA may be able to substitute for some other *in vivo* odontogenic factor when added *in vitro*.

RAR/RXR heterodimers bind *in vitro* much more efficiently to cis-acting DNA response elements than homodimers of either RAR or RXR (reviewed in Chambon, 1994). Unlike RAR double null mutants, which may survive until birth, RARγ$^{-/-}$/RXRα$^{-/-}$ double mutants die *in utero* because the RXRα null mutation is embryonic lethal (Kastner *et al.*, 1994). Therefore, in RARγ$^{-/-}$/RXRα$^{-/-}$ mutants the completion of crown morphogenesis (which takes place around birth in the 1st molars) could not be analyzed. However, in the two 14.5-15.5 dpc RARγ$^{-/-}$/RXRα$^{-/-}$ fetuses examined, the shape and spatial arrangement of the teeth anlagen appeared normal. These observations suggest that heterodimers between RARγ and RXRα, both of which are expressed in the dental mesenchyme at these developmental stages and before (Bloch-Zupan *et al.*, 1994a and references therein), are not critically involved in the patterning of the dentition.

RAR double null mutant mice exhibit atavistic changes of the primary jaw joint

Independently from the presence of craniofacial deficiencies, some RAR double mutant fetuses (e.g. RARα$^{-/-}$/γ$^{-/-}$, RARα$^{-/-}$/β2$^{-/-}$) showed a specific enlargement of the incus (I, Fig. 1h, i and j) and/or a supernumerary skeletal element (PQ, Fig. 1i and j) linking the latter to the alisphenoid bone (AS, Fig. 1i and j). These skeletal alterations resemble normal features of the reptilian jaw skeleton.

Reptiles (Fig. 4) have lower jaw with a tooth-bearing dermal bone, the dentary bone (D), several post-dentary dermal bones [e.g. splenial (SP) and coronoid (C) bones, angular bone (A) and gonial (or prearticular) bone (G)] and the articular bone (AR), which is an endochondral bone derived from the caudal portion of Meckel's (lower jaw) cartilage (MC). Dorsally the articular bone meets the quadrate bone (Q), which belongs to the upper jaw (see below) at the primary (articular-quadrate) jaw joint. There is a large body of paleontological evidence indicating that during the evolution of the reptilian progenitors of mammals (the mammal-like reptiles or therapsids), the dentary bone enlarged caudally until it met the squamosal bone to establish a secondary jaw joint, the dentary-squamosal or temporo-mandibular joint (reviewed in Allin, 1975; DeBeer, 1985 and Walker, 1987).

Some therapsids indeed possessed a double articulation between the skull and the lower jaw involving both reptilian and mammalian elements (i.e. both the articular-quadrate and the dentary-squamosal jaw joints) (Olson, 1959 and references therein).

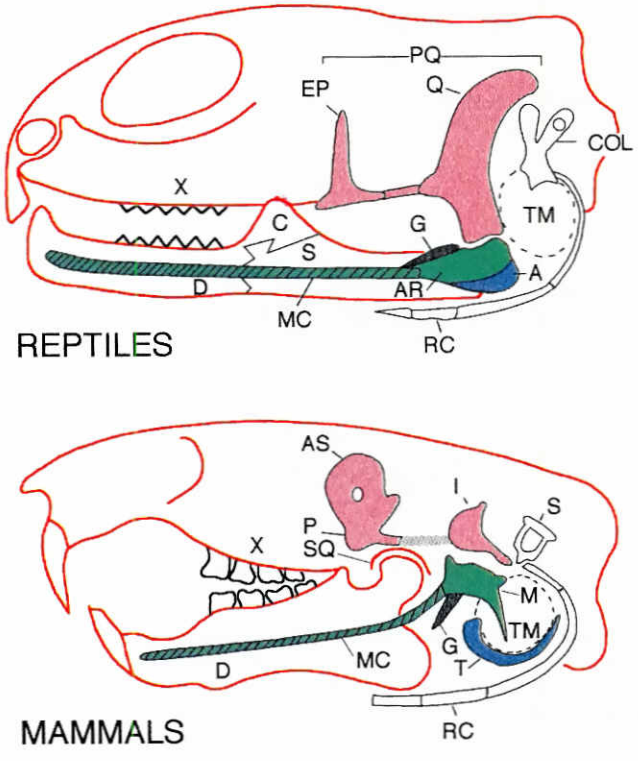

Fig. 4. Schematic representation of the jaws and middle ears of reptilian and mammalian fetuses. *A, angular bone; AR, articular bone; AS, alisphenoid bone; C, coronoid bone, COL, columella; D, dentary bone; EP, epipterygoid bone; G, gonial (prearticular) bone; I, incus; M, malleus; MC, Meckel's cartilage; P, pterygoid bone (i.e. the pterygoid process of the alisphenoid bone); PQ, pterygoquadrate cartilage; Q, quadrate bone; RC, Reichert's (second arch) cartilage; S, stapes; SP, splenial bone; SQ, squamosal bone (note that although not represented here, the squamosal bone also exists in reptiles); T, tympanic bone; TM, tympanic membrane; X, maxillary bone. See the text for further details. Scheme adapted from Goodrich (1930).*

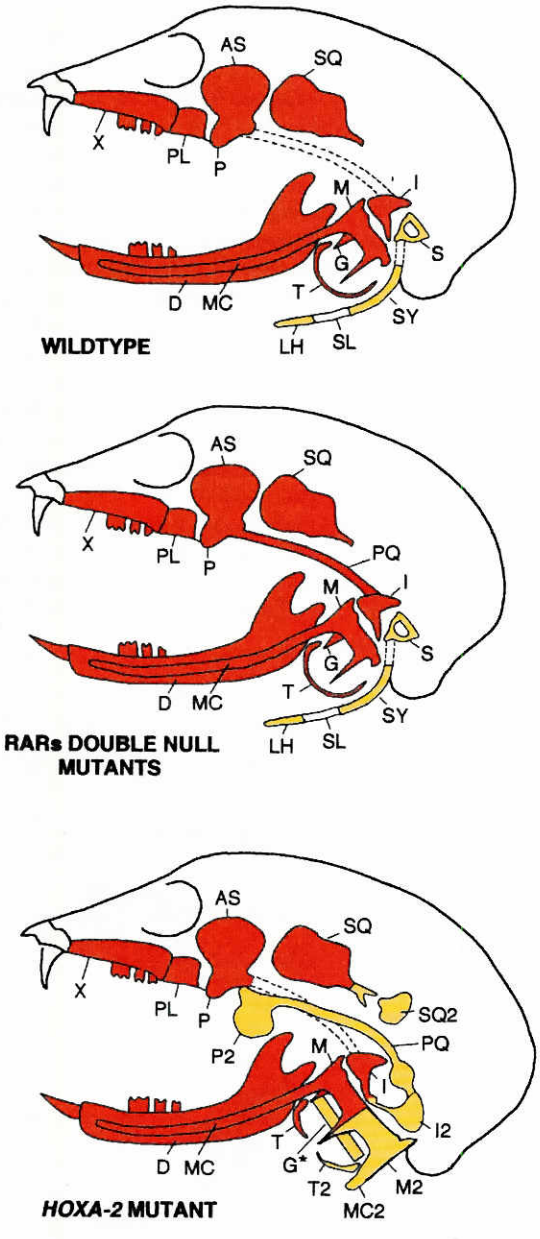

Fig. 5. Schematic representation of the relationships of the skeletal elements derived from the 1st pharyngeal arch (in orange) and from the 2nd pharyngeal arch (in yellow) in wildtype, RAR double null mutant and *Hoxa-2* null mutant fetuses seen from the lateral aspect. *Note that (i) in wildtype fetuses, the stapes (S), styloid bone (SY), stylohyoid ligament (SL) and lesser horn of the hyoid bone (LH) all derive from the cartilage of the second arch (Reichert's cartilage) and (ii) in the Hoxa-2 null mutant, the pterygoquadrate cartilage (PQ) is ectopically located as compared to its homolog in RAR double null mutants. AS, alisphenoid bone; D, dentary bone; G and G*, wildtype and Hoxa-2$^{-/-}$ gonial bone respectively. I and I2, orthotopic and ectopic incus respectively; LH, lesser horn of the hyoid bone; M and M2, orthotopic and ectopic malleus respectively; MC, Meckel's cartilage; P and P2, orthotopic and ectopic pterygoid bone respectively, PL, palatine bone; PQ, pterygoquadrate cartilage; S, stapes; SL, stylohyoid ligament; SQ, orthotopic and ectopic squamosal bone respectively; SY, cartilaginous anlage of the styloid bone; T and T2, orthotopic and ectopic tympanic bone; X, maxillary bone. For further details see Rijli et al. (1993).*

However, at some stage during the therapsid phase of mammalian evolution the primary jaw joint likely became superfluous for feeding activities and its element became reorganized to fulfil a new function, the transmission of air-born vibrations to the inner ear: the articulo and quadrate bones and the articulo-quadrate joint were annexed to the middle ear as the malleus (M; Fig. 4), incus (I) and the malleo-incudal joint, and were intercalated between the tympanic membrane (TM) and the stapes (S), thus giving rise to the mammalian-specific three-ossicular chain (Allin, 1975; DeBeer, 1985; Walker, 1987). Likewise, the many post-dentary reptilian dermal bones were either incorporated into the mammalian hearing apparatus, the gonial bone becoming part of the adult malleus and the angular bone becoming the tympanic bone (T; Fig. 4), or they disappeared (e.g. splenial, coronoid). In the therapsid phase of mammalian evolution the quadrate bone also underwent size reduction (Allin, 1975). In this respect, it is noteworthy that the caudal (or short) process of the incus, which appears specifically enlarged in RAR double null fetuses (compare CI, Fig. 1h with Fig. 1i), is the only part of this ossicle which can be homologized with a part of the reptilian quadrate bone, the stapedial (or long) process of the incus (SI, Fig. 1h and i) probably representing a mammalian neomorph (Presley, 1989).

Comparative anatomical and embryological data indicate that (i) the reptilian epipterygoid bone (EP, Fig. 4) is homologous to the mammalian alisphenoid bone (AS) and that (ii) in reptilian fetuses, the quadrate and epipterygoid bones develop from a single cartilaginous anlage, the pterygoquadrate (or upper jaw) cartilage (PQ), whereas the mammalian homologs of these two bones always chondrify separately (Goodrich, 1930; De Beer, 1985; Fig. 4). The connection between the rostral and caudal skeletal elements derived from the pterygoquadrate cartilage (i.e. epipterygoid/alisphenoid and quadrate/incus respectively), which was lost at some stage of the reptilian-mammalian transition, is recovered in RAR double null fetuses in the form of a supernumerary cartilaginous or osseous rod linking the incus to the alisphenoid bone (PQ, Fig. 1i and j; Fig. 5).

Therefore, this supernumerary skeletal element as well as the enlarged short process of the incus likely represent atavistic changes — according to Hall (1984) "the reappearance of a lost character typical of remote ancestors and not seen in the parents or recent ancestors of the organism displaying the atavistic character" — corresponding to an evolutionary state intermediate between the therapsids and the mammals. The re-emergence of ancestral skeletal features in RAR double mutants not only indicates that the underlying mesectodermal ontogenetic program is still present in mammals, but also that RA-dependent mechanisms have been recruited during the reptilian-mammalian transition to modify the reptilian jaw (Lohnes *et al.*, 1994).

Disruption of *Hoxa-2* results in a homeotic transformation of 2nd to 1st pharyngeal arch NCC identity and reveals the existence of a ground patterning program which underlies the evolution of the jaws

Hox genes are the vertebrate counterparts of the homeotic (HOM) genes of *Drosophila*, the latter being defined by mutations causing the transformation of the phenotype of specific segments into structures normally found at a different location along the rostro-caudal axis. The HOM/*Hox* genes encode a family of transcription factors with a conserved DNA binding domain, the

Antennapedia-like homeodomain. *Hox* genes are present in 4 paralogous clusters, HoxA, -B, -C and-D, each located on a different chromosome and showing clear structural homology to the prototypic homeotic complex HOM-C of *Drosophila*. Ectopic expression and disruption of *Hox* genes in transgenic mice often result in the homeotic transformation of specific vertebrae into the likeness of caudal or rostral neighbors, thus indicating that the function of *Hox* genes, like that of HOM genes, is to specify the identity of segments according to their position along the rostro-caudal axis (reviewed in McGinnis and Krumlauf, 1992 and Botas, 1993).

Hox gene expression domains, determined by *in situ* hybridization, in the neurectoderm and/or NCC of the branchial region of the head exhibit anterior boundaries that coincide with specific rhombomeric junctions and interfaces between pharyngeal arches (reviewed in Wilkinson, 1993 and Krumlauf, 1993). The NCC populating the 1st pharyngeal arch unlike those of other, more caudal, arches do not express *Hox* genes. However, *Hoxa-2,* which is the rostral-most *Hox* gene, is expressed in the 2nd rhombomere at the time of NCC production (Prince and Lumsden, 1994). The NCC populating the 2nd (hyoid) arch express two *Hox* genes, which are *Hoxa-2* and its only paralog *Hoxb-2* (Krumlauf, 1993 and references therein).

Hoxa-2 null mutant fetuses (Gendron-Maguire *et al.*, 1993; Rijli *et al.*, 1993) analyzed at 18.5 dpc selectively lacked the skeletal elements normally derived from the mesectoderm of the 2nd pharyngeal arch, namely the stapes (S), the styloid bone (SY), and the lesser horn of the hyoid bone (LH) (Fig. 5). Instead, they possessed a caudal set of 1st arch membranous and endochondral bones which formed a mirror image of its orthotopic counterpart (Figs. 5, 6a-c) and comprised: (i) supernumerary malleus (M2) (fused to an ectopic truncated Meckel's cartilage, MC2), incus (I2), tympanic (T2) and squamosal (SQ2) bones; (ii) a rod-like cartilaginous element which was fused rostrally to the alisphenoid bone and whose caudal end was always in close contact to, and eventually continuous with, the supernumerary incus (see I2 and PQ, Fig. 6c). This latter element had no counterpart in wildtype mice but, based on its anatomical relationships, it was homologized to the reptilian upper jaw cartilage (PQ, pterygoquadrate cartilage; see above and Figs. 5 and 6c). In addition, the external auditory meatus, which derives from the ectoderm of the 1st pharyngeal cleft, was duplicated in *Hoxa-2* null fetuses (Fig. 7a and b). *Hoxa-2* null embryos analyzed at 9.5 dpc, i.e. just after the completion of cranial NCC migration, were morphologically normal. Furthermore, in these null embryos, the molecular identity of the pharyngeal arches and rhombomeres was normal as assessed by *in situ* hybridization using specific positional markers such as antisense probes for *Hox* genes and for the *Krox-20* gene (Rijli *et al.*, 1993). Hence, inactivation of *Hoxa-2* results in a homeotic transformation of the 2nd arch skeletal elements into a subset of 1st arch skeletal elements. On the other hand, *Hoxa-2* expression at the level of rhombomere 2 likely corresponds to a vestigial situation, since it appears to be dispensable for the determination of all the structures in this location.

Our data indirectly support Noden's conclusion that some 1st pharyngeal arch mesectodermal osteogenic and chondrogenic cells are embodied with intrinsic (i.e. cell-autonomous) patterning information and that the 1st arch mesectoderm controls the patterning of some ectodermal derivatives (e.g. external auditory meatus) (Noden, 1983). On the other hand, many first arch structures, including the upper and lower jaw dermal bones (i.e.

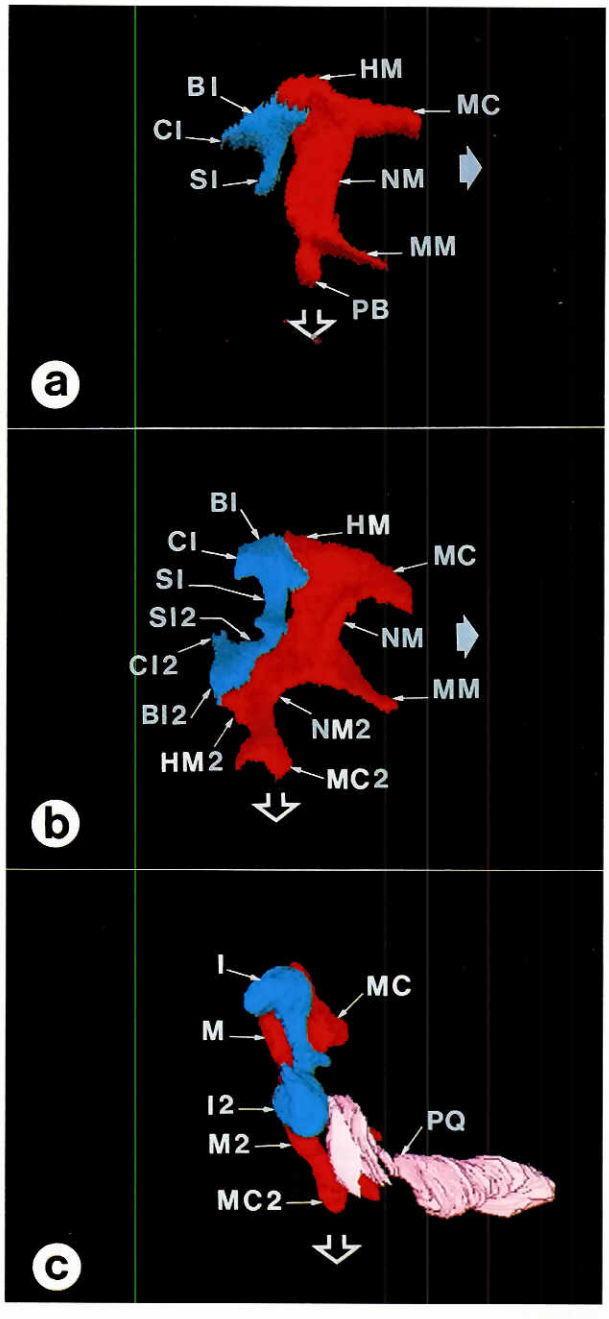

Fig. 6. Medial (a,b) and caudal (c) aspects of three-dimensional computer reconstructions from serial histological sections of the left incudo-mallear complex in 18.5 dpc wildtype (a) and *Hoxa-2* null mutant mice (b and c). *The mutant incudes are fused only at the tip of their stapedial processes (SI and SI2) and thus each moiety of the double incus possesses its own body (BI and BI2) and caudal process (CI and CI2). In* **(c)**, *we have represented the caudal-most portion of the pterygoquadrate (upper jaw) element (PQ) to show its proximity to the caudal incus. The two mutant mallei are fused at the level of their manubrium (MM) and thus each moiety of the double malleus possesses its own head (HM and HM2) and neck (NM and NM2) but lacks the processus brevis (PB). The mutant double malleus is continuous rostrally with the orthotopic Meckel's cartilage (MC, note that only the caudal-most portion of the Meckel's cartilage has been reconstructed) and caudally with an ectopic truncated Meckel's cartilage (MC2), reconstructed completely. The arrow points rostrally (i.e. towards the snout); the open arrow points ventrally (i.e. towards the neck).*

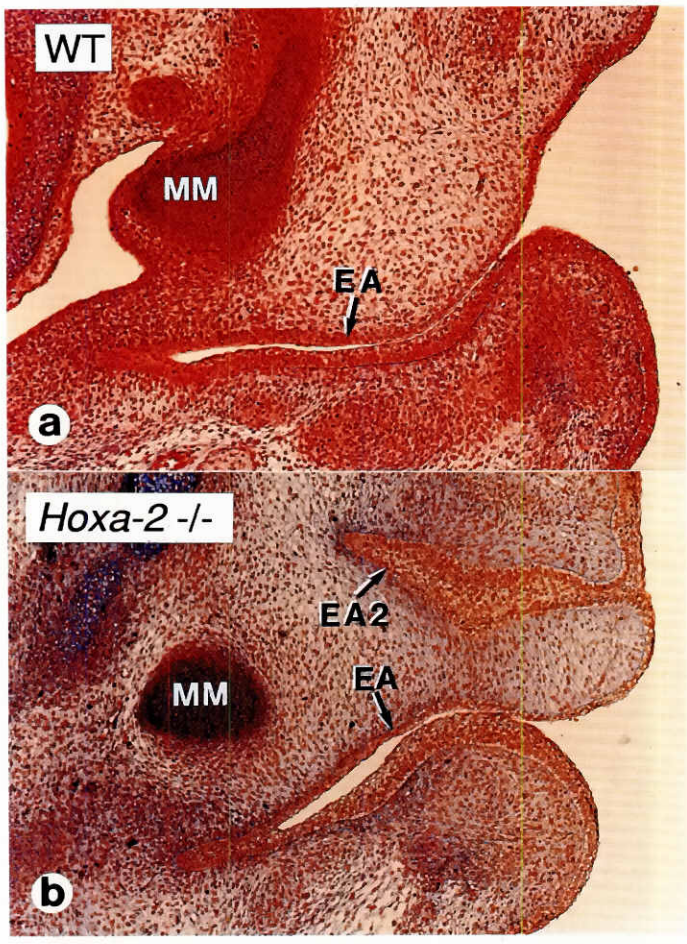

Fig. 7. Frontal sections through the head of 14.5 dpc fetuses at the level of the external auditory meatus. (a) *Wildtype fetus.* **(b)** *Hoxa-2 null fetus. EA and EA2, orthotopic and supernumerary external auditory meatus respectively. MM, manubrium of the malleus.*

dentary, maxillary and palatine bones), the distal Meckel's cartilage and the teeth, were not duplicated in *Hoxa-2* null fetuses. There are at least three, non mutually exclusive, explanations for this incomplete transformation of the 2nd to a 1st arch NCC identity (Rijli *et al.*, 1993; Mark *et al.*, 1995): (i) the existence of 1st arch-specific osteogenic, chondrogenic and odontogenic, ectodermal signals; (ii) the selective duplication of the 1st arch mesectodermal elements which are normally derived from the rostral rhombencephalon; (iii) the selective inhibition of teeth and jaw bone duplications by the expression of *Hoxb-2* in the 2nd arch, which would exert a posteriorizing effect. The first scenario takes into account the results from from Lumsden's tissue recombination experiments (see above and Lumsden, 1988) as well as the finding that mouse 1st arch mesectoderm at 9.0 to 11.0 dpc is apparently unable to form teeth when recombined with isochronic 2nd arch ectoderm (Mina and Kollar, 1987). The second hypothesis is supported by the results of NCC grafting experiments in avians suggesting that the most proximal 1st arch-derived skeletal elements (e.g. quadrate cartilage, proximal Meckel's cartilage, proximal angular bone, and squamosal bone), which are phylogenetically homologous to structures duplicated in the *Hoxa-2* null mutants, are almost exclusively formed from rostral rhombencephalic (i.e. rhombomere 1 and 2-derived) NCC, whereas the distal elements of the lower jaw (e.g. dentary, bone, distal Meckel's cartilage) are contributed by both rostral rhombencephalic and caudal mesencephalic NCC, and the distal elements of the upper jaw (e.g. maxillary and palatine bones) are formed exclusively from mesencephalic NCC (Noden, 1978, 1983). The last hypothesis will be tested by inactivating *Hoxb-2* in the mouse: the *Hoxb-2* and the compound *Hoxa-2/Hoxb-2* null mutations will reveal whether *Hox* gene combinatorial effects are required to pattern different subpopulations of mesectodermal cells.

"Homology is resemblance caused by a continuity of information" (Van Valen, 1982). The homeotic transformation of second (hyoid) to 1st (maxillo-mandibular) pharyngeal arch identity in *Hoxa-2* null mutants indicates that these arches are serially homologous. It also indicates that, indeed, the morphogenetic program of the R1- and R2-derived NCC corresponds to a Ground (or default) skeletogenic Patterning Program (GPP) which is common to mesenchymal NCC of at least the 1st and 2nd pharyngeal arches and does not require *Hox* gene expression (Rijli *et al.*, 1993; Mark *et al.*, 1995).

In wildtype mice the GPP is respecified by *Hoxa-2*, which, like *Drosophila* homeotic genes, acts as a selector gene to yield the NCC 2nd arch-specific morphogenetic program. In the absence of a functional *Hoxa-2* gene, the 2nd arch mesenchymal NCC apparently execute a GPP which corresponds to an ancestral one, since it results in the appearance of a reptilian pterygoquadrate element. Moreover, the presence of both a pterygoquadrate and an incus among the duplicated elements suggests that the mouse GPP corresponds to that of the therapsid phase of mammalian evolution (see above). Subsequently, the present-day mammalian 1st arch skeletal pattern has been generated from the therapsid pattern by a process involving a *Hox* gene-independent genetic system, resulting in the disappearance of the quadrate. We have provided evidence that this process involves the expression of retinoic acid responsive gene(s) (see above and Lohnes *et al.*, 1994).

That the first two mammalian arches share the same GPP is not completely unexpected, since they are likely to be derived from the branchial basket of agnathan ancestors [lamprey-like, jawless vertebrates lying in the phylogenetic hierarchy between the "headless" protochordates and the gnathostome (jawed) vertebrates] that was formed by a series of identical cartilages supporting the gills. Agnathan arches may in fact correspond to the primordial NCC skeletal ground pattern, which was subsequently modified during gnathostome radiation to yield the present-day mouse skeletal ground pattern (Langille and Hall, 1989, and references therein). We have previously reported that the ectopic expression of a single *Hox* gene *(Hoxd-4)* (Lufkin *et al.*, 1992) in the occipital somites is sufficient to transform the occipital bones into vertebrae. Thus, a vertebral ground pattern may also exist, whose realization can be modified by the expression of a single *Hox* selector gene. This ground pattern may have evolved from the original ground pattern of the agnathans, which had occipital vertebrae instead of occipital bones. It is tempting to speculate that the retreat of *Hoxa-2* expression from the rostral-most agnathan gill-bearing arch (leaving behind a vestigial expression domain in rhombomere 2) and that of *Hoxd-4* from the agnathan occipital somites might have been required for the acquisition of the 2 craniofacial features characteristic of gnathostomes: the jaws and the post-otic skull (neocranium). In this context, it is noteworthy that vertebrate teeth are homologous to the odontodes (or dendicles) which formed the

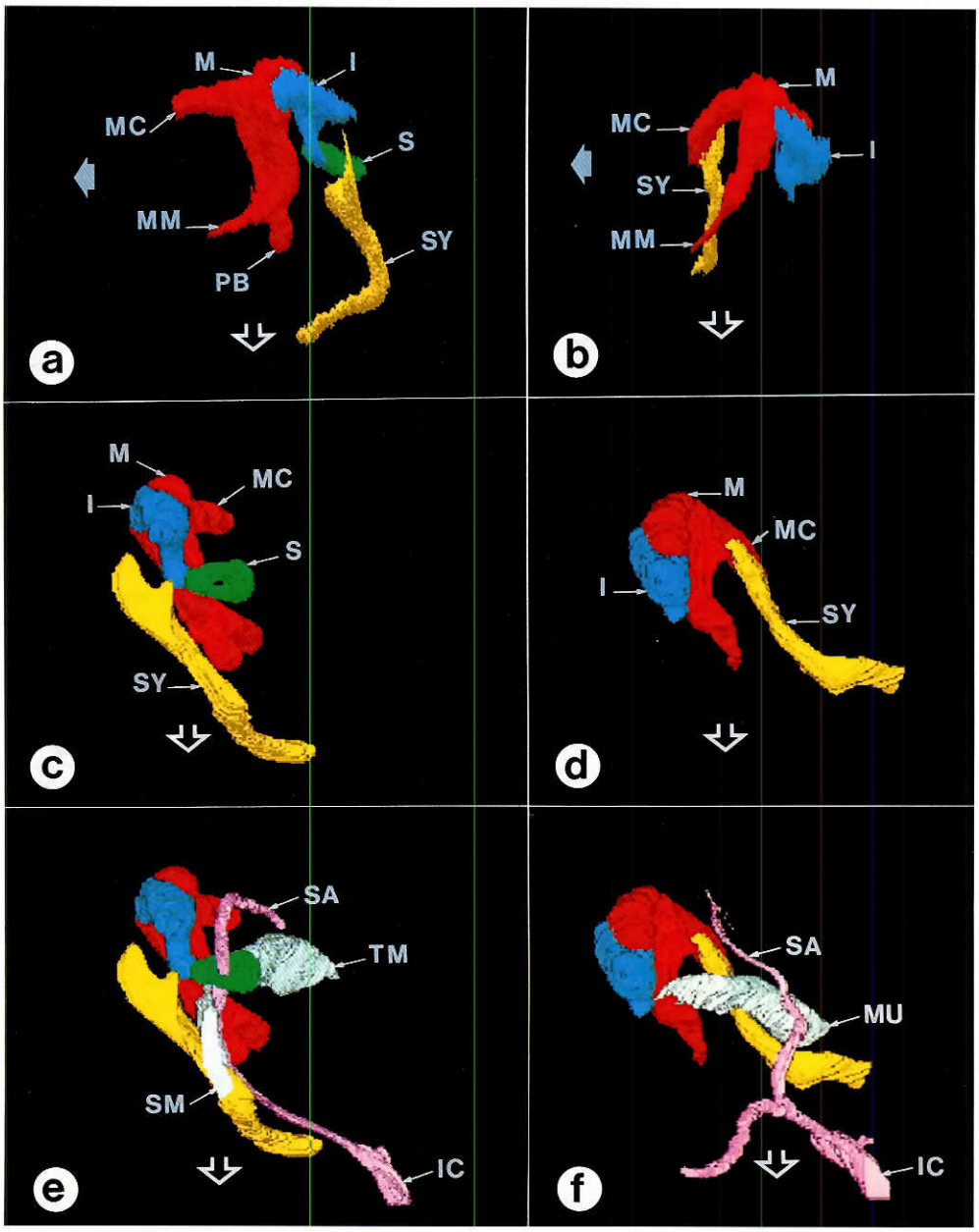

Fig. 8. Lateral (a and b) and caudal (c-f) aspects of three-dimensional computer reconstructions from serial histological sections of the left middle ear of 18.5 dpc wildtype (a,c and e) and *Hoxa-1* null (b,d and f) mutant mice. *The mutant styloid bone was identified on the basis of its muscular insertions (not shown). However, the stapes was not recognizable in this mutant. Note the marked secondary distortions of the mutant malleus (which lacks the processus brevis: PB in the wildtype) and of the mutant incus (which lacks most of the caudal and stapedial processes: CI and SI, in the wildtype) and the presence of the single large muscle (MU) replacing the tensor tympani and the stapedius muscles. I, incus; IC, internal carotid artery; M, malleus; MM, manubrium of the malleus; MC, Meckel's cartilage, MU, single large muscle of the Hoxa-1 null mutant middle ear; PB, processus brevis of the malleus; S, stapes; SA, stapedial artery; SM, stapedius muscle; SY, styloid bone; TM, tensor tympani muscle. The arrow points rostrally (i.e. towards the snout); the open arrow points ventrally (i.e. towards the neck).*

body armor of extinct agnathans (the "bony-skinned" ostracoderms) (Jollie, 1968; Smith and Hall, 1990). Whether *Hox* selector genes might have been involved in the dramatic spatial restriction of the odontogenic potential in the course of gnathostome evolution is an open question.

Disruption of *Hox* genes normally expressed in the hindbrain interferes with the development of 1st arch-derived structures

It has been shown by *in situ* hybridization that, in the mouse embryo, the neurectoderm rostral to the 2nd rhombomere and the NCC populating regions rostral to the 2nd pharyngeal arch do not express *Hox* genes (reviewed in Wilkinson, 1993; Krumlauf 1993). These data and the results of the *Hoxa-2* knockout (see, above and below) indicate that *Hox* genes are not directly involved in the patterning or morphogenesis of 1st arch and of more rostral craniofacial structures. Rather other homeobox genes (e.g. *Msx* genes, *Dlx* genes and the *Goosecoid* gene) might play roles in the ontogeny of these regions (Satokata and Maas, 1994; Sharpe, 1995). However, it is noteworthy that 1st arch derivatives may be secondarily altered in some *Hox* null mutant mice.

About eighty per cent of the *Hoxa-2* null newborn showed a wide cleft of the secondary palate (Rijli *et al.*, 1993). However, before the time of normal shelf elevation the *Hoxa-2* null mutant palatal shelves were morphologically normal. This observation indicates that the cleft is not caused by a NCC deficiency, but rather is due to a failure of palatal shelf elevation or fusion. This could occur as a result of the mechanical stress induced by a more caudal skeletal

defect (Ferguson, 1994), e.g. the fusion of the pterygoquadrate element to the base of the skull (see Fig. 5).

The *Hoxa-3* null mutant mice exhibited agenesis of the thymus, parathyroid glands and carotid arteries (Chisaka and Capecchi, 1991; Condie and Capecchi, 1993). These structures are contributed by mesectodermal cells originating from the caudal rhombencephalon (rhombomeres 6 and 7), which represent the rostral-most NCC-expressing this gene. The cause of the shortening of the mandible and maxilla in the *Hoxa-3* mutant mice is unclear. A possible explanation is that these 1st arch defects might be secondary to the absence of carotid arteries since these vessels normally make a major contribution to the vascularization of facial structures.

Hoxa-1 is expressed in the hindbrain neurectoderm caudal to the boundary between rhombomeres 3 and 4. That a majority (5 out of 7) of *Hoxa-1* null fetuses did not display gross anatomical defects in structures derived from the 1st or 2nd arches is in agreement with the absence of *Hoxa-1* expression in NCC (Lufkin *et al.*, 1991; Mark *et al.*, 1993 and references therein). However, in two *Hoxa-1* null mutant fetuses, the styloid bone (SY, Fig. 8a-d), which is a part of the Reichert's cartilage (2nd arch), was bilaterally fused with the Meckel's cartilage (1st arch), rostral to the malleus (compare Fig. 8a and c with 8b and d). Moreover, in these two mutants the stapedius muscle (SM, Fig. 8e), which is contributed by 2nd arch NCC, was bilaterally fused with the tensor tympani muscle (TM, Fig. 8e), which is contributed by 1st arch NCC to form a single large muscle (MU, Fig. 8f and data not shown). These observations strongly suggest that NCC normally destined for the 2nd arch have migrated into the 1st arch. That in these 2 mice the geniculate (i.e. facial nerve) ganglion was fused to the Gasser's (i.e. trigeminal nerve) ganglion further supports this interpretation since these neuronal structures are contributed by gangliogenic NCC which have migrated from the rhombencephalon at identical axial levels as those forming the Reichert's and Meckel's cartilages respectively. We think that the ectopic, rostral migration of 2nd arch NCC is a mechanical consequence of a rostral shift in the position of the otocyst. Indeed, this change in the position of the otocyst which is secondary to a localized shortening of the rhombencephalon is observed in every *Hoxa-1* embryo (Lufkin *et al.*, 1991; Chisaka *et al.*, 1992; Carpenter *et al.*, 1993; Dollé *et al.*, 1993; Mark *et al.*, 1993). Interestingly, the presence of 2nd arch mesectodermal cells in the vicinity of developing 1st arch skeletal elements seem to alter the shape of the latter (compare M and I, Fig. 8a and c with 8b and d). That mesectodermal cells normally destined for the 2nd arch form a 2nd arch cartilaginous structure (see SY, Fig. 8b and d) when they are forced to migrate and to differentiate into the 1st pharyngeal arch supports the view that NCC are already specified before leaving the neural plate (reviewed in Noden, 1988). In the same context, it is noteworthy that supernumerary teeth were not observed in *Hoxa-1* null fetuses.

Clinicians and geneticists searching for candidate genes for mutations in human craniofacial disorders should be aware that defects may occur in structures developing outside the normal expression domain of a disrupted gene.

Acknowledgments

We thank Dr. Jean-Victor Ruch and the members of the Strasbourg homeogene and retinoid group for useful discussions; Dr. S. Ward for proof reading the manuscript; B. Weber, C. Fisher and V. Giroult for technical help; C. Werlé for help with illustrations and the secretariat staff for typing the manuscript. This work was supported by the Institut National de la Santé et de la Recherche Médicale, the Centre National de la Recherche Scientifique, the Centre Hospitalier Universitaire Régional, the Association pour la Recherche sur le Cancer and the Fondation pour la Recherche Médicale.

References

ALLIN, E.F. (1975). Evolution of the mammalian middle ear. *J. Morphol. 147:* 403-437.

BLOCH-ZUPAN, A., DÉCIMO, D., LORIOT, M., MARK, M.P. and RUCH, J.V. (1994a). Expression of nuclear retinoid acid receptors during mouse odontogenesis. *Differentiation 57:* 195-203.

BLOCH-ZUPAN, A., MARK, M.P., WEBER, B. and RUCH, J.V. (1994b). *In vitro* effects of retinoic acid on mouse incisor development. *Arch. Oral Biol. 39:* 891-900.

BOTAS, J. (1993). Control of morphogenesis and differentiation by HOM/Hox genes. *Curr. Opin. Cell Biol. 5:* 1015-1022.

CARPENTER, E.M., GODARD, J.M., CHISAKA, O., MANLEY, N.R. and CAPECCHI, M.R. (1993). Loss of *Hox-A1 (Hox-1.6)* function results in the reorganization of the murine hindbrain. *Development 118:* 1063-1075.

CHAMBON, P. (1994). The retinoid signaling pathway: molecular and genetic analyses. *Semin. Cell Biol. 5:* 115-125.

CHISAKA, O. and CAPECCHI, M.R. (1991). Regionally restricted developmental defects resulting from targeted disruption of the mouse homeobox gene *Hox-1.5. Nature 350:* 473-479.

CHISAKA, O., MUSCI, T.S. and CAPECCHI, M.R. (1992). Developmental defects of the ear, cranial nerves and hindbrain resulting from targeted disruption of the mouse homeobox gene *Hox-1.6. Nature 355:* 516-520.

CONDIE, B.G. and CAPECCHI, M.R. (1993). Mice homozygous for a targeted disruption of *Hoxd-3 (Hox-4.1)* exhibit anterior transformations of the first and second cervical vertebrae, the atlas and the axis. *Development 119:* 579-595.

COULY, G.F., COLTEY, P.M. and LE DOUARIN, N.M. (1993). The triple origin of skull in higher vertebrates: a study in quail-chick chimeras. *Development 117:* 409-429.

DE BEER, G. (1985). *The Development of the Vertebrate Skull.* The University of Chicago Press, Chicago and London.

DOLLÉ, P., LUFKIN, T., KRUMLAUF, R., MARK, M., DUBOULE, D. and CHAMBON, P. (1993). Local alterations of *Krox-20* and *Hox* gene expression in the hindbrain suggest lack of rhombomeres 4 and 5 in homozygote null *Hoxa-1 Hox-1.6* mutant embryos. *Proc. Natl. Acad. Sci. USA 90:* 7666-7670.

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 pattern of transcription during mouse organogenesis. *Development 110:* 1133-1151.

FERGUSON, M.W.J. (1994). Craniofacial malformations: towards a molecular understanding. *Nature Genet. 6:* 329-330.

GAUNT, W.A. (1955). The development of the molar pattern of the mouse *(Mus musculus). Acta Anat. 24:* 249-268.

GENDRON-MAGUIRE, M., MALLO, M., ZHANG, M. and GRIDLEY, T. (1993). *Hoxa-2* mutant mice exhibit homeotic transformation of skeletal elements derived from cranial neural crest. *Cell 75:* 1317-1331.

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.

GOODRICH, E.S. (1930). *Studies on the Structure and Development of Vertebrates,* Vol. 1. Constable and Co. Ltd, London.

HALL, B.K. (1984). Developmental mechanisms underlying the formation of atavisms. *Biol. Rev. 59:* 89-124.

HALL, B.K. (1991). Cellular interactions during cartilage and bone development. *J. Craniofac. Genet. Dev. Biol. 11:* 238-250.

HOFMANN, C. and EICHELE, G. (1994). Retinoids in development. In *The Retinoids, Biology, Chemistry and Medicine* (Eds. M.B. Sporn, A.B. Roberts and D.S. Goodman). Raven Press, New York, pp. 387-435.

JOLLIE, M. (1968). Some implications of the acceptance of a delamination principle. In *Current Problems of Lower Vertebrate Phylogeny* (Ed. T. Orvig). Almqvist and Wiksell, Stockholm, pp. 89-107.

KASTNER, P., GRONDONA, J., MARK, M., GANSMULLER, A., LEMEUR, M., DÉCIMO, D., VONESCH, J-L., DOLLÉ, P. and CHAMBON, P. (1994). Genetic analysis of RXRα developmental function: convergence of RXR and RAR signalling pathways in heart and eye morphogenesis. *Cell 78:* 987-1003.

KRUMLAUF, R. (1993). *Hox* genes and pattern formation in the branchial region of the vertebrate head. *Trends Genet. 9:* 106-112.

LANGILLE, R.M. and HALL, B.K. (1989). Developmental processes, developmental sequences and early vertebrate phylogeny. *Biol. Rev. 64:* 73-91.

LE DOUARIN, N., ZILLER, C. and COULY, G. (1993). Patterns of neural crest derivatives in the avian embryos: *in vivo* and *in vitro* studies. *Dev. Biol. 159:* 24-49.

LOHNES, D., KASTNER, P., DIERICH, A., MARK, M., LEMEUR, M. and CHAMBON, P. (1993). Function of retinoic acid receptor γ in the mouse. *Cell 73:* 643-658.

LOHNES, D., MARK, M., MENDELSOHN, C., DOLLÉ, P., DIERICH, A., GORRY, P., GANSMULLER, A. and CHAMBON, P. (1994). Function of the retinoic acid receptors (RARs) during development. I. Craniofacial and skeletal abnormalities in RAR double mutants. *Development 120:* 2723-2748.

LUFKIN, T., DIERICH, A., LEMEUR, M., MARK, M. and CHAMBON, P. (1991). Disruption of the *Hox*-1.6 homeobox gene results in defects in a region corresponding to its rostral domain of expression. *Cell 66:* 1105-1119.

LUFKIN, T., LOHNES, D., MARK, M., DIERICH, A., GORRY, P., GAUB, M.P., LEMEUR, M. and CHAMBON P. (1993). High postnatal lethality and testis degeneration in retinoic acid receptor α mutant mice. *Proc. Natl. Acad. Sci. USA 90:* 7225-7229.

LUFKIN, T., MARK, M., HART, C.P., DOLLÉ, P., LEMEUR, M. and CHAMBON, P. (1992). Homeotic transformation of the occipital bones of the skull by ectopic expression of a homeobox gene. *Nature 359:* 835-841.

LUMSDEN, A.G.S. (1988). Spatial organization of the epithelium and the role of neural crest cells in the initiation of the mammalian tooth germ. *Development 103 (Suppl.):* 155-169.

LUMSDEN, A.G.S. and BUCHANAN, J.A.G. (1986). An experimental study of timing and topography of early tooth development in the mouse embryo with an analysis of the role of innervation. *Arch. Oral. Biol. 31:* 301-311.

LUMSDEN, A.G.S., SPRAWSON, N. and GRAHAM, A. (1991). Segmental origin and migration of neural crest cells in the midbrain region of the chick embryo. *Development 113:* 1281-1291.

MARK, M., BLOCH-ZUPAN, A. and RUCH, J.V. (1992). Effects of retinoids on tooth morphogenesis and cytodifferentiations, *in vitro. Int. J. Dev. Biol. 36:* 517-526.

MARK, M., LUFKIN, T., VONESCH, J.L., RUBERTE, E., OLIVO, J.C., DOLLÉ, P., GORRY, P., LUMSDEN, A. and CHAMBON, P. (1993). Two rhombomeres are altered in *Hoxa-1* null mutant mice. *Development 119:* 319-338.

MARK, M., RIJLI, F.M., LUFKIN, T., DOLLÉ, P., GORRY, P. and CHAMBON, P. (1995). Hox gene function and the development of the head. In *Neural CEll Specification: Molecular Mechanisms and Neurotherapeutic Implications* (Eds. B. Juurlink, W. Kulyk, P. Krone, V. Verge and R. Doucette). Plenum Press. (In press).

McDOWELL, E.M., SHORES, R.L., SPANGLER, E.F., WENK, M.L. and DE LUCA, L. (1987). Anomalous growth of rat incisor teeth during chronic intermittent vitamin A deficiency. *J. Nutr. 117:* 1265-1274.

McGINNIS, W. and KRUMLAUF, R. (1992). Homeobox genes and axial patterning. *Cell 68:* 283-302.

MELLANBY, H. (1941). The effect of maternal dietary deficiency of vitamin A on dental tissues in rats. *J. Dent. Res. 20:* 489-503.

MENDELSOHN, C., LOHNES, D., DÉCIMO, D., LUFKIN, T., LEMEUR, M., CHAMBON, P. and MARK, M. (1994). Function of the retinoic acid receptors (RARs) during development. II. Multiple abnormalities at various stages of organogenesis in RAR double mutants. *Development 120:* 2749-2771.

MINA, M. and KOLLAR, E.J. (1987). The induction of odontogenesis in non-dental mesenchyme combined with early murine mandibular arch epithelium. *Arch. Oral Biol. 32:* 123-127.

NODEN, D.M. (1978). The control of avian cephalic neural crest cytodifferentiation. *Dev. Biol. 67:* 296-312.

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

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

OLSON, E.C. (1959). The evolution of mammalian characters. *Evolution 13:* 344-353.

PETERKOVA, R., PETERKA, M., VONESCH, J.L. and RUCH, J.V. (1993). Multiple developmental origin of the upper incisor in mouse: histological and computer assisted 3-D-reconstruction studies. *Int. J. Dev. Biol. 37:* 581-588.

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.

PRESLEY, R. (1989). Ontogeny and the evolution of the mammalian jaw complex. In *Complex Organismal Functions: Integration and Evolution in Vertebrates* (Eds. D.B. Wake and G. Roth). John Wiley and Sons, pp. 53-61.

PRINCE, V. and LUMSDEN, A. (1994). Hoxa-2 expression in normal and transposed rhombomeres: independent regulation in the neural tube and neural crest. *Development 120:* 911-923.

RIJLI, F.M., MARK, M., LAKKARAJU, S., DIERICH, A., DOLLÉ, P. and CHAMBON, P. (1993). A homeotic transformation is generated in the rostral branchial region of the head by disruption of *Hoxa-2,* which acts as a selector gene. *Cell 75:* 1333-1349.

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.

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.

RUCH, J.V. (1995). Tooth crown morphogenesis and cytodifferentiations: candid questions and critical comments. *Connect. Tissue Res. 31:* 1-8.

SATOKATA, I. and MAAS, R. (1994). *Msx1* deficient mice exhibit cleft palate and abnormalities of craniofacial and tooth development. *Nature Genet. 6:* 348-356.

SCHERSON, T., SERBEDZIJA, G., FRASER, S. and BRONNER-FRASER, M. (1993). Regulative capacity of the cranial neural tube to form neural crest. *Development 118:* 1049-1061.

SECHRIST, J., SERBEDZIJA, G.N., SCHERSON, T., FRASER, S.E. and BRONNER-FRASER, M. (1993). Segmental migration of the hindbrain neural crest does not arise from its segmental generation. *Development 118:* 691-703.

SELLECK, M.A.J., SCHERSON ,T.Y. and BRONNER-FRASER, M. (1993). Origins of neural crest cell diversity. *Dev. Biol. 159:* 1-11.

SERBEDZIJA, G.N., BRONNER-FRASER, M. and FRASER, S.E. (1992). Vital dye analysis of cranial neural crest cell migration in the mouse embryo. *Development 116:* 297-307.

SHARPE, P.T. (1995). Homeobox genes and orofacial development. *Connect. Tissue Res.* (In press).

SMITH, M.M. and HALL, B.K. (1990). Development and evolutionary origins of vertebrate skeletogenic and odontogenic tissues. *Biol. Rev. 65:* 277-373.

THOROGOOD, P. (1993). The problems of building a head. *Curr. Biol. 3:* 705-707.

VAN VALEN, L. (1982). Homology and causes. *J. Morphol. 173:* 305-312.

WALKER, W.F., Jr. (1987). *Functional Anatomy of the Vertebrates. An Evolutionary Perspective.* Saunders College Publishing, Philadelphia.

WILKINSON, D.G. (1993). Molecular mechanisms of segmental patterning in the vertebrate hindbrain and neural crest. *BioEssays 15:* 499-505.

WOLBACH, S.B. and HOWE, P.R. (1925). Tissue changes following deprivation of fat-soluble A vitamin. *J. Exp. Med. 42:* 753-777.