



**HAL**  
open science

## Function of retinoic acid receptors during embryonic development.

Manuel Mark, Norbert B. Ghyselinck, Pierre Chambon

► **To cite this version:**

Manuel Mark, Norbert B. Ghyselinck, Pierre Chambon. Function of retinoic acid receptors during embryonic development.. Nucl Recept Signal, 2009, 7, pp.e002. 10.1621/nrs.07002 . inserm-00384487

**HAL Id: inserm-00384487**

**<https://inserm.hal.science/inserm-00384487v1>**

Submitted on 15 May 2009

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# Function of retinoic acid receptors during embryonic development

Manuel Mark, Norbert B. Ghyselincx and Pierre Chambon

Corresponding Author: [norbert@igbmc.fr](mailto:norbert@igbmc.fr) [chambon@igbmc.fr](mailto:chambon@igbmc.fr)

Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), Département de Biologie Cellulaire and Développement (MM and NG) and Département de Génomique Fonctionnelle (PC), and Collège de France (PC), Strasbourg, France

**Retinoids, the active metabolites of vitamin A, regulate complex gene networks involved in vertebrate morphogenesis, growth, cellular differentiation and homeostasis. Studies performed *in vitro*, using either acellular systems or transfected cells, have shown that retinoid actions are mediated through heterodimers between the RAR and RXR nuclear receptors. However, *in vitro* studies indicate what is possible, but not necessarily what is actually occurring *in vivo*, because they are performed under non-physiological conditions. Therefore, genetic approaches in the animal have been used to determine the physiological functions of retinoid receptors. Homologous recombination in embryonic stem cells has been used to generate germline null mutations of the RAR- and RXR-coding genes in the mouse. As reviewed here, the generation of such germline mutations, combined with pharmacological approaches to block the RA signalling pathway, has provided genetic evidence that RAR/RXR heterodimers are indeed the functional units transducing the RA signal during prenatal development. However, due to (i) the complexity in “hormonal” signalling through transduction by the multiple RARs and RXRs, (ii) the functional redundancies (possibly artefactually generated by the mutations) within receptor isotypes belonging to a given family, and (iii) *in utero* or early postnatal lethality of certain germline null mutations, these genetic studies have failed to reveal all the physiological functions of RARs and RXRs, notably in adults. Spatio-temporally-controlled somatic mutations generated in given cell types/tissues and at chosen times during postnatal life, will be required to reveal all the functions of RAR and RXR throughout the lifetime of the mouse.**

Received February 6th, 2009; Accepted March 13th, 2009; Published April 3rd, 2009 | **Abbreviations:** AD: activating domain; AF: activation function; BA: branchial arch; CYP: cytochrome P450 hydroxylase; DORV: double outlet right ventricle; E: embryonic day; INZ: interdigital necrotic zone; NCC: neural crest cells; NR: nuclear receptor; PHPV: persistent and hyperplastic vitreous body; POM: periocular mesenchyme; R1 to R7: rhombomeres 1 to 7; RA: retinoic acid; RALDH: retinaldehyde dehydrogenase; RAR: retinoic acid receptor; RBP: retinol-binding protein; RDH: retinol dehydrogenase; RXR: retinoid X receptor; STRA: stimulated by retinoic acid; VAD: vitamin A-deficiency | Copyright © 2009, Mark et al. This is an open-access article distributed under the terms of the Creative Commons Non-Commercial Attribution License, which permits unrestricted non-commercial use, distribution and reproduction in any medium, provided the original work is properly cited.

Cite this article: Nuclear Receptor Signaling (2009) 7, e002

## Introduction

Both clinical findings and experimental approaches have revealed that vitamin A (retinol) and its active derivatives (i.e., the retinoids) exert a wide variety of effects on vertebrate embryonic body shaping and organogenesis, tissue homeostasis, cell proliferation, differentiation and apoptosis (reviewed in [Blomhoff, 1994; Kastner et al., 1995; Mark et al., 2006; Morriss-Kay and Ward, 1999; Sporn et al., 1994]). Following Hale's initial demonstration that vitamin A-deficiency (VAD) induces congenital ocular malformations [Hale, 1933], Warkany and his collaborators showed that a large array of congenital malformations affecting the ocular, cardiac, respiratory and urogenital systems (collectively referred as to the fetal VAD syndrome) occurred in fetuses from vitamin A-deficient (VAD) rats (reviewed in [Wilson et al., 1953]). It was shown, much later, that retinoic acid (RA) could replace vitamin A during embryogenesis, at least at certain stages and in certain organs [Dickman et al., 1997; White et al., 1998].

How RA can exert such pleiotropic effects was a long-standing question, which found its solution with the discovery of two classes of RA-binding transcriptional regulators, the retinoic acid receptors (the multiple isoforms of the RAR $\alpha$ ,  $\beta$  and  $\gamma$  isotypes), and the retinoid

X receptors (the multiple isoforms of the RXR $\alpha$ ,  $\beta$  and  $\gamma$  isotypes) (reviewed in [Chambon, 1996; Leid et al., 1992]).

The present review focuses on three main questions: (i) are RARs and RXRs involved in the transduction of RA signals *in vivo*? (ii) to what extent do the observations made *in vivo* support the molecular mechanisms deduced from *in vitro* studies? (iii) what are the developmental events controlled by RARs and RXRs? We summarize and discuss in this review the developmental phenotypes induced by mutations that have been introduced at loci encoding RARs and RXRs, and compare them to the phenotypes resulting either from VAD or from administration of pharmacological doses of antagonistic ligands for RARs in vertebrates, placing emphasis on results obtained in mammals.

## RARs and RXRs are instrumental to retinoic acid signalling during embryonic development

### Signalling through RARs is indispensable for embryonic patterning and organogenesis

*Rara*, *Arb*, and *Rarg* null mutant mice are viable. They display some aspects of the fetal (and postnatal) VAD syndromes, as well as a few additional congenital

malformations (Table 1). However, their abnormalities are restricted to a subset of tissues normally expressing these receptors, probably reflecting the existence of functional redundancies between RARs (discussed in [Kastner et al., 1995; Kastner et al., 1997a; Mark and Chambon, 2003; Mark et al., 1995; Mascrez et al., 1998]). To test this hypothesis, mutants lacking two RAR isotypes (*Rara/b-*, *Rara/g-* and *Rarb/g-* null mutants), or several isoforms belonging to distinct isotypes were generated. For the sake of clarity, only abnormalities displayed by double null mutants lacking a couple of RAR isotypes (all isoforms deleted) are listed in Table 2 and Table 3. Similar abnormalities, albeit often less penetrant, which are displayed by "isoform-specific" double null mutants are listed in [Kastner et al., 1995] (mutants lacking RAR $\alpha$ 1/RAR $\beta$ 2/4, RAR $\alpha$ 1/RAR $\gamma$ , RAR $\alpha$ 1 $\alpha$ 2<sup>+/</sup>/RAR $\gamma$  and RAR $\beta$ 2/4/RAR $\gamma$ ), in [Subbarayan et al., 1997] (mutants lacking RAR $\alpha$ /RAR $\gamma$ 1 and RAR $\alpha$ /RAR $\gamma$ 2), in [Luo et al., 1996] (mutants lacking RAR $\alpha$ 1/RAR $\beta$ ), in [Ghyselinck et al., 1998] (mutants lacking RAR $\alpha$ /RAR $\beta$ 1/3 and RAR $\beta$ 1/3/RAR $\gamma$ ), and in [Grondona et al., 1996] (mutants lacking RAR $\beta$ 2/RAR $\gamma$ 2).

*Rara/b-*, *Rara/g-* and *Rarb/g-* null mutants die *in utero* or at birth because of severe developmental defects that altogether include the complete spectrum of malformations belonging to the fetal VAD-induced syndrome reported by Warkany's group 55 years ago [Wilson et al., 1953] (Table 2). As with *Rar*-single-null mutants (see Table 1), *Rar*-double-null mutants (Table 3) also exhibit congenital abnormalities that were not described in Hale's and Warkany's pioneering fetal VAD studies [Hale, 1933; Wilson et al., 1953], encompassing agenesis of the Harderian glands, skeletal defects of the skull, face, vertebrae, limbs and forebrain [Ghyselinck et al., 1997; Lohnes et al., 1993; Lohnes et al., 1994; Luo et al., 1996; Mendelsohn et al., 1994; Subbarayan et al., 1997]. The occurrence of these "non-VAD" defects in *Rar*-single and double-null mutant mice is most probably accounted for by the difficulty to achieve, by dietary deprivation, a state of profound VAD compatible with pregnancy. In fact, almost all these "non-VAD" defects have been subsequently produced in rodent embryos (i) deficient in vitamin A, but supplemented with RA [Dickman et al., 1997; White et al., 2000; White et al., 1998]; (ii) deficient in both RBP (retinol-binding protein) and vitamin A [Quadro et al., 2005]; (iii) lacking the retinaldehyde synthesizing enzyme RDH10 (retinol dehydrogenase 10) [Sandell et al., 2007]; (iv) lacking the RA synthesizing enzymes RALDH2 (retinaldehyde dehydrogenase 2) [Halilagic et al., 2007; Molotkova et al., 2007; Niederreither et al., 2002a; Niederreither et al., 1999; Niederreither et al., 2001; Niederreither et al., 2000; Niederreither et al., 2002b; Ribes et al., 2006] or RALDH3 [Dupe et al., 2003; Halilagic et al., 2007] or (v) treated with synthetic retinoids possessing panRAR antagonistic activities [Kochhar et al., 1998; Wendling et al., 2000; Wendling et al., 2001]. In addition, it was recently found that the Matthew-Wood syndrome, which consists of a spectrum of congenital abnormalities also observed in *Rara/b*, *Rara/g* and *Rarb/g*-null mutants, is caused by mutations in the RBP receptor gene, STRA6 [Golzio et

al., 2007; Pasutto et al., 2007]. As STRA6 loss-of-function mutations most probably yield a state of RA insufficiency in embryonic tissues [Kawaguchi et al., 2007], this discovery provides evidence that RAR signalling performs similar functions during embryonic development in mice and humans.

The comparison of the *Rar*-null mutant phenotypes with those of rodents and humans carrying the aforementioned blocks in RA signal transduction, demonstrate that liganded RARs play crucial roles at many distinct stages of the development of numerous organs ([Kastner et al., 1995] and references therein). For example, the severe malformations found in *Rara/g*-null embryos [Wendling et al., 2001] are similar to those of *Aldh1a2* (formerly *Raldh2*)-null embryos [Niederreither et al., 1999], and reflect early roles of RAR signalling in axial rotation, segmentation and closure of the hindbrain, formation of otocysts, pharyngeal arches and forelimb buds, as well as in closure of the primitive gut. RARs are also indispensable for the ontogenesis of (almost) all the anatomical structures that are derived from mesectodermal cells, i.e. the cranial neural crest cells (NCC) that give rise to mesenchymal derivatives (reviewed in [Kastner et al., 1995; Mark et al., 1998; Mark et al., 1995]). RARs are involved in antero-posterior patterning of the somitic mesoderm and hindbrain neuroectoderm [Dupe et al., 1999b; Ghyselinck et al., 1997; Lohnes et al., 1993; Lohnes et al., 1994; Wendling et al., 2001], notably through controlling expression of homeobox genes [Allan et al., 2001; Dupe et al., 1997; Dupe et al., 1999b; Houle et al., 2000; Oosterveen et al., 2003; Serpente et al., 2005]. RARs are also involved in the establishment of the antero-posterior axis of the limbs [Dupe et al., 1999a; Lohnes et al., 1994; Mascrez et al., 1998]. RARs are required for the development of a large number of eye structures (Table 2 and Table 3) and for histogenesis of the retina [Ghyselinck et al., 1997; Grondona et al., 1996; Lohnes et al., 1994], cardiomyocyte differentiation [Kastner et al., 1994; Kastner et al., 1997b], as well as for the control of physiological apoptosis in the retina [Grondona et al., 1996], the frontonasal and interdigital mesenchymes [Crocoll et al., 2002; Dupe et al., 1999a; Ghyselinck et al., 1997; Lohnes et al., 1994], the conotruncal segment of the embryonic heart [Ghyselinck et al., 1998] and the embryonic nephric duct [Batourina et al., 2005]. In the embryonic urogenital tract, RARs control epithelial-mesenchymal interactions in the kidney through expression of the receptor tyrosine kinase Ret [Batourina et al., 2002; Batourina et al., 2001; Mendelsohn et al., 1999], as well as the formation of the genital ducts and ureters [Batourina et al., 2001; Batourina et al., 2005; Ghyselinck et al., 1997; Mendelsohn et al., 1994]. In the developing respiratory tract, RA-liganded RARs are necessary for the morphogenesis of the nasal cavities and for their communication with the more caudal airways [Dupe et al., 2003; Ghyselinck et al., 1997] (Table 2 and Table 3). RA-liganded RARs are also required for the partitioning of the primitive foregut into oesophagus and trachea, and regulate lung branching morphogenesis [Chen et al., 2007; Desai et al., 2006; Malpel et al., 2000;

Null mutation	Abnormalities	References
<i>Rara1</i>	none	Li et al., 1993
<i>Rara2</i>	none	our unpublished results
<i>Rara</i>	Growth retardation (PnVAD); male sterility (testis degeneration #; PnVAD); impaired alveolar formation. CD: webbed digits; homeotic transformations and malformations of cervical vertebrae; pterygoquadrate cartilage; malformation of the squamosal bone; malformed laryngeal (i.e., cricoid) cartilage	Ghyselinck et al., 1997 ; Subbarayan et al., 1997 ; Massaro et al., 2003, Lufkin et al., 1993
<i>Rarb1/3</i>	none	Ghyselinck et al., 1998
<i>Rarb2/4</i>	CD: persistence and hyperplasia of the primary vitreous body (fetal VAD)	Grondona et al., 1996
<i>Rarb</i>	Growth retardation (PnVAD); behavioural defects; altered alveolar formation. CD: homeotic transformations and malformations of cervical vertebrae; persistence and hyperplasia of the primary vitreous body (fetal VAD)	Ghyselinck et al., 1997, Massaro et al., 2000; Massaro and Massaro, 2003 ; Krezel et al., 1998; Chiang et al., 1998
<i>Rarg1</i>	Growth deficiency (PnVAD). CD : malformations of cervical vertebrae # ; malformed laryngeal (i.e., cricoid) cartilage; abnormal differentiation of granular keratinocytes #	Subbarayan et al., 1997; our unpublished results
<i>Rarg2</i>	None	Lohnes et al., 1993
<i>Rarg</i>	Growth deficiency (PnVAD); male sterility (squamous metaplasia of the seminal vesicle and prostate gland epithelia # (PnVAD); squamous metaplasia of other epithelia (PnVAD); impaired alveolar formation. CD : webbed digits; homeotic transformations and malformations of cervical vertebrae # ; malformed laryngeal cartilages and tracheal rings #; agenesis of the Harderian glands; agenesis of the metoptic pillar of the skull; abnormal differentiation of granular keratinocytes #	Ghyselinck et al., 1997; Lohnes et al., 1993; McGowan et al., 2000; Chapellier et al., 2002; our unpublished results
<i>Rarb/Rarg1</i>	Hydronephrosis	Subbarayan et al., 1997
<i>Rarb2/4/Rarg2</i>	Dysplasia and degeneration # of the retina. CD : persistence and hyperplasia of the primary vitreous body (fetal VAD); blepharophimosis #; partial agenesis of the sclera and choroids #	Grondona et al., 1996
<i>Rarb1/3/Rarg</i>	Degeneration of the retina. CD : persistence and hyperplasia of the primary vitreous body (fetal VAD); partial agenesis of the sclera and choroids #	Ghyselinck et al., 1998
<i>Rxra+/-</i>	Growth retardation (PnVAD). CD: webbed digits	Kastner et al., 1994, 1997,
<i>Rxb</i>	Male sterility # (defective spermatogenesis); abnormal lipid metabolism in Sertoli cells; behavioral defects	Kastner et al., 1996; Krezel et al., 1998
<i>Rxrg</i>	Metabolic and behavioural defects	Krezel et al., 1996, 1998; Chiang et al., 1998 ; Brown et al., 2000

**Table 1. Postnatal manifestations of germline ablation of *Rar* and *Rxr* genes.** CD: congenital defects, PnVAD: abnormalities present in postnatal vitamin A-deficiency (Wolbach and Howe, 1925); fetal VAD: abnormalities present in vitamin A-deficiency during pregnancy (Wilson et al., 1953). #: these abnormalities are completely penetrant. *Rara1*, *Rara2*, *Rarb1/3*, *Rarb2/4*, *Rarg1* and *Rarg2* refer to isoform-specific ablations.

Mollard et al., 2000; Wang et al., 2006], as well as lung alveoli septation [Massaro and Massaro, 2003; Massaro et al., 2003; Massaro et al., 2000; McGowan et al., 2000] (Table 1, Table 2, and Table 3).

Altogether, the developmental abnormalities of *Rar*-null mutant mice faithfully recapitulate those of rodents lacking the RAR ligand either through dietary vitamin A deprivation or genetically-impaired RA synthesis. There are, however, two notable exceptions to this rule which are related to the control, through RA signalling, of (i) the

symmetry of somite patterning and (ii) germ cell differentiation in the embryonic ovary. Concerning somitogenesis, it has been firmly established, in several vertebrate species, that RA is instrumental to the generation of bilaterally symmetrical pairs of somites [Echeverri and Oates, 2007; Kawakami et al., 2005; Sirbu and Duester, 2006; Vermot and Pourquie, 2005]. It was also recently demonstrated that signalling by RA is required for expression of *Stra8* (stimulated by retinoic acid 8) which, in turn, triggers meiosis in the embryonic ovary [Baltus et al., 2006; Bowles et al., 2006; Koubova

Abnormalities of the fetal VAD syndrome	Genotypes of <i>Rar</i> -null mutants showing similar abnormalities	Abnormalities observed in <i>Rxra</i> -null mutants
<b>Respiratory system defects</b>		
• Agenesis or hypoplasia of the left lung	$A\alpha/A\beta$ #	no
• Hypoplasia of the right lung	$A\alpha/A\beta$ #	no
• Agenesis of the oesophago- tracheal septum	$A\alpha/A\beta$ #	
• Diaphragmatic hernia	$A\alpha/A\beta$	no
<b>Hypoplasia of the ventricular myocardium</b>	$A\alpha/A\gamma$	Yes #
<b>Heart outflow tract defects</b>		
• Persistent truncus arteriosus	$A\alpha/A\beta$ #, $A\alpha/A\gamma$ #	no
• High interventricular septal defect or double outlet right ventricle (E18.5)/ conotruncal septum defect (E14.5)	$A\alpha/A\beta$ #, $A\alpha/A\gamma$ #, $A\beta/A\gamma$	Yes
• Abnormal great arteries derived from aortic arches	$A\alpha/A\beta$ #, $A\alpha/A\gamma$ #, $A\beta/A\gamma$	no
<b>Kidney hypoplasia</b>	$A\alpha/A\beta$ #, $A\alpha/A\gamma$ #	no
<b>Ureteral defects</b>		
• Agenesis	$A\alpha/A\beta$ , $A\alpha/A\gamma$ #	no
• Ectopia	$A\alpha/A\beta$ , $A\beta/A\gamma$	no
<b>Genital tract defects</b>		
<b>- Female</b>		
• Agenesis of the oviduct and uterus (E18.5)/ agenesia of the Müllerian duct (E14.5)		
- Complete	$A\alpha/A\beta$ #	no
- Partial	$A\alpha/A\gamma$ #	no
• Agenesis of the cranial vagina	$A\alpha/A\beta$ #, $A\alpha/A\gamma$ #	NA
<b>- Male</b>		
• Agenesis or dysplasia of the vas deferens	$A\alpha/A\gamma$ #	NA
• Agenesis of the seminal vesicles	$A\alpha/A\gamma$ #	NA
<b>Ocular defects</b>		
• Coloboma of the retina	$A\alpha/A\gamma$ #	no
• Coloboma of the optic disc	$A\beta/A\gamma$ , $A\alpha/A\gamma$ #	Yes
• Persistence et hyperplasia of the primary vitreous body (PHPV)	$A\beta$ , $A\alpha/A\beta$ #, $A\beta/A\gamma$ #	Yes #
• Hypoplasia of the conjunctival sac	$A\alpha/A\gamma$ #	
• Thickening of the corneal stroma	$A\beta/A\gamma$ #, $A\alpha/A\gamma$ #	Yes #
• Ventral rotation of the lens	$A\beta/A\gamma$ #, $A\alpha/A\gamma$	Yes #
• Shortening of the ventral retina	$A\beta/A\gamma$ #	Yes #

**Table 2. Abnormalities of the fetal vitamin A deficiency (VAD) syndrome (Wilson et al., 1953) present in *Rarb*-null mutants ( $A\beta$ ), *Rxra*-null mutants and in compound *Rara/b*-, *Rara/g*- and *Rarb/g*-null mutants.** ( $A\alpha/A\beta$ ,  $A\alpha/A\gamma$  and  $A\beta/A\gamma$ , respectively). #, this abnormality is completely penetrant. NA, not applicable, as the corresponding structure is normally not found at E14.5, the time around which *Rxra*-null mutants die. From references (Ghyselinck et al., 1997; Lohnes et al., 1994; Kastner et al., 1994). Note that most of the abnormalities seen in *Rara/b*-null mutants occur at similar frequencies in *Rara/b2*-mutants (Mendelsohn et al., 1994).

et al., 2006]. However, defects in the symmetry of somite derivatives, such as vertebrae and muscles, or in the histology of the ovaries, were never observed in *Rara/b*-, *Rara/g*- and *Rarb/g*-null mutant mice. As mentioned above, the apparent lack of phenotypic convergence between the animal models lacking RA and those lacking RARs is probably a consequence of the artificial compensation of the functions of the RARs that are missing in the knockout mice by the remaining one.

### RARs have been instrumental to the phylogenesis of mesectodermal derivatives

In addition to the dramatic craniofacial skeletal deficiencies affecting *Rara/g*-null mutants [Lohnes et al., 1994], subtle defects which often alter the shape of a single skeletal piece are observed in several *Rar*-null mice, including: a cartilaginous or osseous connection between the incus middle ear bone and the alisphenoid bone (the pterygoquadrate element), a cartilage separating the trigeminal ganglion from the brain (the pila antotica) and an agenesis of the rostral ethmoturbinata



Congenital abnormalities not associated with the fetal VAD syndrome	Genotypes of <i>Rar</i> -null fetuses showing these defects
<p><b>Nervous system defects</b></p> <ul style="list-style-type: none"> <li>• Exencephaly</li> <li>• Agenesis of the corpus callosum</li> </ul>	<p><i>Aα/Aγ</i> <i>Aα/Aγ</i> #</p>
<p><b>Skeletal defects</b></p> <ul style="list-style-type: none"> <li>• Agenesis or multiple malformations of cranial skeletal elements</li> <li>• Homeotic transformation and malformations of cervical vertebrae</li> <li>• Agenesis and malformations of limb bones</li> <li>• Reappearance of atavistic skeletal elements               <ul style="list-style-type: none"> <li>- Pila antotica</li> <li>- Pterygoquadrate cartilage</li> </ul> </li> </ul>	<p><i>Aα/Aγ</i> # <i>Aα</i>, <i>Aβ</i>, <i>Aγ</i>, <i>Aα/Aγ</i> #, <i>Aα/Aβ</i> #, <i>Aβ/Aγ</i> # <i>Aα/Aγ</i> #</p> <p><i>Aα/Aγ</i> # <i>Aα</i>, <i>Aα/Aγ</i> #, <i>Aα/Aβ</i></p>
<p><b>Eye defects</b></p> <ul style="list-style-type: none"> <li>• Corneal-lenticular stalk</li> <li>• Agenesis of the lens</li> </ul>	<p><i>Aα/Aγ</i> <i>Aα/Aγ</i></p>
<p><b>Glandular defects</b></p> <ul style="list-style-type: none"> <li>• Agenesis or dysplasia, of the sub-maxillary and sub-lingual glands and/or of their excretory ducts</li> <li>• Agenesis of the Harderian gland</li> <li>• Agenesis or ectopia of the thyroid, thymus and parathyroid glands</li> </ul>	<p><i>Aα/Aγ</i> #, <i>Aβ/Aγ</i> #</p> <p><i>Aγ</i>, <i>Aα/Aγ</i> #, <i>Aβ/Aγ</i> # <i>Aα/Aβ</i>, <i>Aα/Aγ</i></p>
<p><b>Other defects</b></p> <ul style="list-style-type: none"> <li>• Webbed digits</li> <li>• Abnormal laryngeal cartilages and tracheal rings</li> <li>• Kidney agenesis</li> <li>• Agenesis of the anal canal</li> </ul>	<p><i>Aα</i>, <i>Aγ</i>, <i>Aβ<sup>+/-</sup>/Aγ</i> #, <i>Aβ/Aγ</i> # <i>Aγ</i> #, <i>Aα/Aγ</i> #, <i>Aα/Aβ</i> #, <i>Aβ/Aγ</i> # <i>Aα/Aγ</i> <i>Aα/Aβ</i> #</p>

**Table 3.** Abnormalities absent from the fetal vitamin A deficiency (VAD) syndrome are found in *Rara*-, *Rarb*- and *Rarg*-null mutants (*Aα*, *Aβ*, *Aγ*), and in compound *Rara/b*-, *Rara/g*- and *Rarb/g*-null mutants (*Aα/Aβ*, *Aα/Aγ* and *Aβ/Aγ*). #: this abnormality is completely penetrant. From references (Ghyselinck et al., 1997; Lohnes et al., 1994; Kastner et al., 1994). Note that most of the abnormalities seen in *Rara/b*-null mutants occur at similar frequencies in *Rara/b2*-mutants (Mendelsohn et al., 1994).

and maxillary sinus (Table 3) [Ghyselinck et al., 1997; Lohnes et al., 1994; Mark et al., 1998]. The pterygoquadrate element and the pila antotica, which were lost during evolution from reptiles to mammals, represent atavistic features (discussed in [Mark et al., 1998; Mark et al., 1995]). Along the same lines, ethmoturbinate bones and paranasal sinuses (such as the maxillary sinus) are typical mammalian features not present in reptiles [Novacek, 1993]. It is thus conceivable that agenesis of these nasal structures in *Rar*-null mutants also mimics an atavistic condition. The presence of atavistic characteristics in *Rar*-null mutants supports the possibility that changes in the temporal or spatial patterns of expression of *Rar* genes has provided a general mechanism for modifying the number and shape of individual cranial skeletal elements during vertebrate evolution. Interestingly, recent teratogenic experiments using RA in excess have led to similar conclusions [Vieux-Rochas et al., 2007].

The persistent and hyperplastic vitreous body (PHPV) seen in *Rarb*-null mutants is comparable to the pecten oculi, a normal vascular and pigmented projection from

the optic disk found in some reptiles, which is thought to function in the nutrition of the retina [Mann, 1937]. The shortening of the ventral retina observed in *Rarb/g*-null mutants might also be interpreted as a modification towards an ancestral condition, since comparative embryology of the retina shows that the ventral retinal field increases in size as one ascends the vertebrate scale from fishes to amphibia, reptiles and mammals [Mann, 1937]. Thus, *RARβ* and *RARγ* might have been instrumental in the expansion of the ventral retinal field during vertebrate evolution.

### RXR $\alpha$ is the main RXR isotype involved in embryogenesis

*Rxrα*-null mutants all display a hypoplasia of the compact layer of the ventricular myocardium (Table 2), which appears to be the main cause of mutant death occurring by cardiac failure around E14.5 [Kastner et al., 1994; Kastner et al., 1997b; Ruiz-Lozano et al., 1998; Sucov et al., 1994]. That a similar myocardium defect is observed in VAD and *Rar*-null fetuses [Mendelsohn et al., 1994; Wilson et al., 1953], suggests that RXR $\alpha$  is involved in the transduction of the RA signal required for myocardial

growth. This requirement is unlikely to be cell-autonomous, as over-expressing RXR $\alpha$  in cardiomyocytes by means of transgenesis does not prevent the *Rxra*-null mutation-induced hypoplasia of the ventricular myocardium [Subbarayan et al., 2000]. Other data further indicate that *Rxra* expression in the epicardium is required for triggering a paracrine signal necessary for myocardial growth [Chen et al., 2002; Kang and Sucov, 2005; Merki et al., 2005].

About one third of the *Rxra*-null mutants lack the conotruncal septum, which normally divides the embryonic heart outflow tract (or conotruncus) into the intracardiac portions of the aorta and the pulmonary trunk [Kastner et al., 1994]. Interestingly, deficiencies of this septum represent both a classical VAD defect in rodents and a leading cause of human congenital heart defects, ranging from high interventricular septal defects to double outlet right ventricle (DORV). In *Rxra*-null mutants, the agenesis of the conotruncal septum appears secondary to an enhanced rate of cell death in both the mesenchymal cells of the conotruncal ridges and the parietal conotruncal cardiomyocytes, therefore indicating that RXR $\alpha$  is required for the transduction of the RA signal that controls apoptosis in the conotruncal segment of the embryonic heart [Ghyselinck et al., 1998].

In addition to heart defects, all *Rxra*-null fetuses display a characteristic ocular syndrome characterized by a PHPV, closer eyelid folds, a thickened ventral portion of the corneal stroma, a ventral rotation of the lens, an agenesis of the sclera and a shortening of the ventral retina (Table 2) [Kastner et al., 1994]. As similar defects are present in VAD fetuses and in *Rarb/g*-null mutants (Table 2) [Ghyselinck et al., 1997; Wilson et al., 1953], RXR $\alpha$  appears to play an essential role in the transduction of the RA signals required for several ocular morphogenetic processes, notably the formation of the ventral retinal field.

Importantly, the fact that mice lacking both RXR $\beta$  and RXR $\gamma$  (*Rarb/g*-null mutants) do not display any obvious morphogenetic defects, even when additionally lacking one allele of *Rxra*, clearly indicates that RXR $\alpha$  is functionally the most important RXR during morphogenesis of the embryo proper [Krezel et al., 1996].

### The AF-1-containing A/B domain and the ligand-dependent AF-2 of RXR $\alpha$ are differentially involved in development

The role played by RXRs as either “active” or “silent” heterodimerization partners in the transcription of target genes, inferred from *in vitro* studies, has been a controversial issue. To determine the transcriptional role of RXR $\alpha$  *in vivo*, mouse mutants were engineered that express truncated RXR $\alpha$  proteins lacking (i) the N-terminal activation function (AF)-1-containing A/B region (*Rxra*<sup>af1o</sup> mutants) [Mascres et al., 2001], (ii) the AF-2 activating domain (AD) core-containing helix 12 located at the C-terminus of the E region (*Rxra*<sup>af2o</sup> mutants) [Mascres et al., 1998] and (iii) both AF-1 and AF-2 (*Rxra*<sup>af0</sup> mutants) [Mascres et al., 2009].

The *Rxra*<sup>af2o</sup> mutants display the myocardium hypoplasia and the ocular syndrome that are hallmarks of the *Rxra*-null phenotype [Kastner et al., 1994], but at low frequency [Mascres et al., 1998]. This may reflect a functional compensation by RXR $\beta$ , as (i) the frequency of the myocardium hypoplasia increases from 5% in *Rxra*<sup>af2o</sup> mutants to 50% upon the additional inactivation of *Rxrb* (which, on its own, has no effect) [Kastner et al., 1996], and (ii) the frequency of the ocular syndrome increases from about 15% in *Rxra*<sup>af2o</sup> mutants to 100% upon further inactivation of *Rxrb* [Mascres et al., 1998]. The full penetrance of the *Rxra*-null ocular syndrome observed in *Rxra*<sup>af2o</sup>/*Rxrb*-null mutants [Mascres et al., 1998], as well as in *Rxra*<sup>af0</sup> mutants [Mascres et al., 2009], supports the view that the AF-2 of RXR $\alpha$  (and thus possibly a RXR agonistic ligand) is indispensable for ocular morphogenesis. On the other hand, the rare or modest penetrance of the myocardium hypoplasia in *Rxra*<sup>af2o</sup> (5%), *Rxra*<sup>af2o</sup>/*Rxrb*-null (50%) and *Rxra*<sup>af2o</sup>/*Rxrb/g*-null mutants (50%) suggests that the transcriptional activity of RXR $\alpha$  becomes necessary for myocardial growth only in “unfavourable” genetic backgrounds. Accordingly, we recently found that the heart histology is normal in 80% of *Rxra*<sup>af0</sup> fetuses [Mascres et al., 2009], indicating that a transcriptionally “silent” RXR $\alpha$  can promote myocardial growth.

Involvement of the primary vitreous body represents the developmental process which likely requires the highest concentration of RA-liganded retinoid receptors, since both VAD [Wilson et al., 1953], *Rarb* ablation [Ghyselinck et al., 1997], *Rxra* ablation [Kastner et al., 1994], deletion of RXR $\alpha$  AF-2 [Mascres et al., 1998] and deletion of RXR $\alpha$  AF-1 and AF-2 [Mascres et al., 2009] frequently yield a PHPV. Apart from an occasional PHPV, the *Rxra*<sup>af1o</sup> mutants never display any of the *Rxra*-null developmental defects [Mascres et al., 2001]. This near-absence of defects does not reflect a functional compensation by RXR $\beta$  or RXR $\gamma$ , as *Rxra*<sup>af1o</sup>/*Rxrb*-null and *Rxra*<sup>af1o</sup>/*Rxrb/g*-null mutants display no other developmental defect than a PHPV. However, the frequency of this PHPV increases from 10% in *Rxra*<sup>af1o</sup> mutants to 100% in *Rxra*<sup>af1o</sup>/*Rxrb/g*-null mutants [Mascres et al., 2001]. Altogether, these observations indicate that involution of the primary vitreous body requires both RXR $\alpha$  AF-1 and AF-2, while the other RA-dependent ocular morphogenetic events require RXR $\alpha$  AF-2 only, as they normally take place in the absence of the RXR $\alpha$  AF-1-containing A/B domain.

Thus, these data support the view that the activation functions of RXR $\alpha$  are differentially required for eye morphogenesis and that they can be dispensable for heart development. They also indicate that, due to functional redundancy, the role played by each activation function can be revealed only in genetic backgrounds impaired for RA-signalling (or under RA-insufficiency conditions). Assuming that the frequent requirement of RXR $\alpha$  AF-2 for developmental events reflects the binding of an agonistic ligand, this raises the question of the possible existence and nature of a physiological RXR ligand(s) *in vivo*. The fact that 9-cis RA is undetectable in rodent

embryos [Horton and Maden, 1995; Matt et al., 2005b] makes it doubtful that this RXR physiological ligand could be 9-cis RA (reviewed in [Wolf, 2006]; see also [Calleja et al., 2006]).

### The RXR $\alpha$ AF-1-containing A/B domain has a specific function in the involution of the interdigital mesenchyme

The AF-2 of RXR $\alpha$  appears functionally more important during development than its AF-1, as (i) *Rxra*<sup>af2o</sup>/*Rxb/g*-null fetuses all display a large array of congenital defects and die *in utero*, while *Rxra*<sup>af1o</sup>/*Rxb/g*-null fetuses display only a few congenital defects and are often viable; and (ii) transcription of a RA-responsive *lacZ* reporter transgene in the mouse requires RXR $\alpha$  AF-2, but not AF-1 [Mascrez et al., 1998; Mascrez et al., 2001]. However, the RXR $\alpha$  AF-1-containing A/B region has a unique role in the RA-dependent disappearance of the interdigital mesenchyme.

The first evidence implicating RA in the involution of the interdigital mesenchyme was provided by organ culture experiments using whole limb in a RA-deprived medium [Lussier et al., 1993]. Subsequently, it was shown that mice lacking both alleles of either *Rara* or *Rarg*, as well as mice heterozygous for the *Rxra*-null mutation occasionally exhibit mild forms of interdigital webbing (i.e., soft tissue syndactyly) (Table 1) [Ghyselinck et al., 1997; Kastner et al., 1994; Lohnes et al., 1993; Lufkin et al., 1993]. Surprisingly, this defect was absent in *Rarb*-null mutants, even though *Rarb* is strongly and specifically expressed in the interdigital necrotic zones (INZs) [Ghyselinck et al., 1997]. However, disruption of one (or both) allele(s) of *Rarb* in a *Rarg*-null genetic background consistently yields interdigital webbing [Ghyselinck et al., 1997]. The persistence of the fetal interdigital mesenchyme responsible for digit webbing is caused by a marked decrease in programmed cell death, as well as by an increase of cell proliferation in the mutant INZs [Dupe et al., 1999a]. As *Rarb* and *Rarg* are not co-expressed in the INZs, interdigital mesenchyme involution must involve paracrine interactions between this mesenchyme, which expresses *Rarb*, and either the cartilaginous blastema of the digits or the surface epidermis, which both express *Rarg* (discussed in [Dupe et al., 1999a]).

The RXR $\alpha$  AF-1-containing A/B region is indispensable for the function of RXR $\alpha$ /RAR $\beta$  and/or RXR $\alpha$ /RAR $\gamma$  heterodimers involved in interdigital mesenchyme involution, since the majority of *Rxra*<sup>af1o</sup> mutants and all *Rxra*<sup>af1o</sup>/*Rxb/g*-null mutants display a soft tissue syndactyly [Mascrez et al., 2001]. In contrast, *Rxra*<sup>af2o</sup> and *Rxra*<sup>af2o</sup>/*Rxb/g*-null mutants never display this defect [Mascrez et al., 1998], indicating a specific requirement of the RXR $\alpha$  AF-1-containing A/B region in the involution of the interdigital mesenchyme. Interestingly, phosphorylation of RXR $\alpha$  at a specific serine residue located in the A domain is necessary for the anti-proliferative response of F9 teratocarcinoma cells to RA [Bastien et al., 2002; Rochette-Egly and Chambon,

2001]. Phosphorylation of the RXR $\alpha$  A domain may therefore play an important function in the cascade of molecular events that, *in vivo*, leads to the normal disappearance of the interdigital mesenchyme.

### Retinoic acid signals are transduced by specific RXR $\alpha$ /RAR heterodimers during development

Compound mutants, in which a null mutation of a given RAR isotype is associated either with a *Rxra*-null, a *Rxra*<sup>af1o</sup> or a *Rxra*<sup>af2o</sup> mutation, altogether recapitulate the abnormalities exhibited by *Rar*-null mutants (Table 4) [Kastner et al., 1994; Kastner et al., 1997a; Mascrez et al., 1998; Mascrez et al., 2001]. This synergism between *Rar* and *Rxra* loss-of-function mutations supports the conclusion that RXR $\alpha$ /RAR $\alpha$ , RXR $\alpha$ /RAR $\beta$  and RXR $\alpha$ /RAR $\gamma$  heterodimers are the functional units transducing RA signals during embryogenesis. Moreover, that *Rxb/g*-null mutants develop normally indicates that RXR $\alpha$  is functionally the most important RXR isotype during development [Krezel et al., 1996]. This conclusion is further supported by a lack of synergism, during embryogenesis, between *Rara*-, *Rarb*- or *Rarg*-null mutations and *Rxb*- or *Rxrg*-null mutations [Kastner et al., 1997a].

Comparing the severity and penetrance of a given abnormality between various mutants led to the identification of heterodimers preferentially involved in transducing RA signals in a given developmental process. For instance, myocardium hypoplasia is found in none of the *Rxra*<sup>af1o</sup> mutants and in 5% of the *Rxra*<sup>af2o</sup> mutants. It is observed in 45% of the *Rxra*<sup>af1o</sup>/*Rara*-null and in 80% of the *Rxra*<sup>af2o</sup>/*Rara*-null mutants, but in none of the *Rxra*<sup>af1o</sup>/*Rarb*-null or *Rxra*<sup>af1o</sup>/*Rarg*-null mutants and in only 20% of the *Rxra*<sup>af2o</sup>/*Rarb*-null or *Rxra*<sup>af2o</sup>/*Rarg*-null mutants [Mascrez et al., 1998; Mascrez et al., 2001]. Together, these genetic data indicate that RXR $\alpha$ /RAR $\alpha$  heterodimers are preferentially transducing the RA signal acting on myocardial growth.

Similarly, although all three RARs are expressed in developing ocular structures [Mori et al., 2001], several lines of evidence indicate the involvement of RXR $\alpha$ /RAR $\beta$  and RXR $\alpha$ /RAR $\gamma$  but not of RXR $\alpha$ /RAR $\alpha$  heterodimers during eye morphogenesis. Firstly, there is a strong synergism between *Rxra*- and *Rarb*- or *Rarg*-null mutations, which is manifested by a marked increase in the severity of the *Rxra*-null ocular defects, whereas no synergism is observed with the *Rara*-null mutation (Table 4) [Kastner et al., 1997a]. Secondly, there is also a strong synergism between the *Rarb*- or *Rarg*-null mutations and ablation of either the AF-1-containing A/B domain or the AF-2 of RXR $\alpha$  for the generation of ocular defects (Table 4) [Mascrez et al., 1998; Mascrez et al., 2001]. In fact, apart from the PHPV discussed above, the characteristic *Rxra*-null ocular syndrome is never observed in *Rxra*<sup>af1o</sup> mutants and is found in less than 15% of the *Rxra*<sup>af2o</sup> mutants. On the other hand, this ocular syndrome is observed in 100% of the *Rxra*<sup>af1o</sup>/*Rarb*-null, *Rxra*<sup>af1o</sup>/*Rarg*-null, *Rxra*<sup>af2o</sup>/*Rarb*-null and *Rxra*<sup>af2o</sup>/*Rarg*-null mutants, while it is absent in all *Rxra*<sup>af1o</sup>/*Rara*-null and *Rxra*<sup>af2o</sup>/*Rara*-null mutants [Mascrez et al., 1998; Mascrez



Abnormalities	Genotypes of <i>Rxr/Rar</i> and <i>Rar/Rar</i> compound mutants showing similar defects		
	Compound <i>Rxra/Rar</i> -null mutants	Compound <i>Rar</i> -null mutants	Compound <i>Rxra<sup>af10</sup></i> or <i>Rxra<sup>af20</sup>/Rar</i> -null mutants
<b>Ocular defects (VAD)</b> • Severe shortening or agenesis of the ventral retina	$X\alpha/A\beta$ , $X\alpha/A\gamma^{+/-}$ #, $X\alpha/A\gamma$ #	$A\beta/A\gamma$ #	$X\alpha.af20/A\beta$ , $X\alpha.af20/A\gamma$ , $X\alpha.af10/A\beta$
<b>Respiratory system defects (VAD)</b> • Lung hypoplasia	$X\alpha/A\alpha$ *	$A\alpha/A\beta$ #	$X\alpha.af20/A\alpha$ #, $X\alpha.af20/A\beta$ , $X\alpha.af10/A\alpha$ *
• Agenesis of the oesophagotracheal septum	$X\alpha/A\alpha$ *	$A\alpha/A\beta$ #	$X\alpha.af20/A\alpha$ , $X\alpha.af20/A\beta$ , $X\alpha.af10/A\alpha$
<b>Heart outflow tract defects (VAD)</b> • Persistent truncus arteriosus	$X\alpha/A\alpha^{+/-}$ , $X\alpha/A\alpha$ #, $X\alpha/A\beta$ , $X\alpha/A\gamma$	$A\alpha/A\beta$ #, $A\alpha/A\gamma$ #	$X\alpha.af20/A\alpha$ , $X\alpha.af10/A\alpha$
• Abnormal arteries derived from aortic arches	$X\alpha/A\alpha^{+/-}$ , $X\alpha/A\alpha$ *, $X\alpha/A\beta$ , $X\alpha/A\gamma$	$A\alpha/A\beta$ #, $A\alpha/A\gamma$ #	$X\alpha.af20/A\alpha$ , $X\alpha.af20/A\beta$ , $X\alpha.af10/A\alpha$
<b>Urogenital system defects (VAD)</b> • Kidney hypoplasia	$X\alpha/A\alpha$ #	$A\alpha/A\beta$ #	$X\alpha.af20/A\alpha$ , $X\alpha.af10/A\alpha$
• Complete agenesis of Müllerian ducts	$X\alpha/A\alpha$ #	$A\alpha/A\beta$ #	$X\alpha.af10/A\alpha$
<b>Hypoplasia of the sub-maxillary gland</b>	$X\alpha/A\gamma$ #	$A\alpha/A\gamma$ #	$X\alpha.af20/A\gamma$ #
<b>Skeletal defects</b> • Multiple Cranio-facial defects	$X\alpha/A\gamma$	$A\alpha/A\gamma$ #	$X\alpha.af20/A\gamma$ #
• Limb defects	$X\alpha/A\gamma$	$A\alpha/A\gamma$ #	$X\alpha.af20/A\gamma$

**Table 4. Evidence that RXR $\alpha$  and RAR act synergistically in embryonic development.** Similar congenital defects absent (or very rare) in *Rxra*-null, *Rxra<sup>af10</sup>*, *Rxra<sup>af20</sup>*, and *Rara*-, *Rarb*-, *Rarg*-null mutants are observed in compound *Rxra/Rara*-, *Rxra/Rarb*-, and *Rxra/Rarg*-null mutants ( $X\alpha/A\alpha$ ,  $X\alpha/A\beta$  and  $X\alpha/A\gamma$ ), in compound *Rxra<sup>af20</sup>/Rara*-, *Rxra<sup>af20</sup>/Rarb*-, and *Rxra<sup>af20</sup>/Rarg*-null mutants ( $X\alpha.af20/A\alpha$ ,  $X\alpha.af20/A\beta$  and  $X\alpha.af20/A\gamma$ ), in compound *Rxra<sup>af10</sup>/Rara*-, *Rxra<sup>af10</sup>/Rarb*-, and *Rxra<sup>af10</sup>/Rarg*-null mutants ( $X\alpha.af10/A\alpha$ ,  $X\alpha.af10/A\beta$  and  $X\alpha.af10/A\gamma$ ), as well as in compound *Rara/b*-, *Rara/g*- and *Rarb/g*-null mutants ( $A\alpha/A\beta$ ,  $A\alpha/A\gamma$  and  $A\beta/A\gamma$ ). \*: this abnormality is present in a majority of the mutants. #: this abnormality is fully penetrant. VAD, these abnormalities belong to the fetal vitamin A deficiency syndrome (Wilson et al., 1953). From references (Kastner et al., 1997a and 1997b; Mascres et al., 1998; Kastner et al., 1994; Mascres et al., 2001).

et al., 2001]. Altogether, these genetic data indicate that RXR $\alpha$ /RAR $\beta$  and RXR $\alpha$ /RAR $\gamma$  are the heterodimers which are instrumental to ocular morphogenesis.

## Pharmacological and somatic mutagenesis approaches provide clues on RAR-controlled mechanisms operating in the head region during development

### The endoderm of branchial arches is the target of RA action mediated by RAR $\alpha$ and/or RAR $\beta$

*Rara/b*-null mutants, analysed at fetal stages of gestation, display the complete set of defects that can be generated in the chick by surgical ablation of post-otic NCC, namely thymus and parathyroid gland agenesis or ectopias, aberrant patterning of cephalic arteries, absence of pulmonary arteries and aorticopulmonary septum ([Ghyselinck et al., 1997; Mark et al., 1998; Mendelsohn et al., 1994]and references therein). These defects are

also present in the CATCH22 syndrome, which is an archetype of human neurocristopathy (i.e., congenital malformations of NCC-derived structures). These and other observations led to the proposal that cranial NCC giving rise to mesenchymal derivatives (i.e., the mesectodermal cells) are major targets of RA action (reviewed in [Mark et al., 2004]).

Unexpectedly, *Rara/b*-null mutants analysed at embryonic stages of gestation display very small caudal branchial arches (BA), but do not show NCC alterations [Dupe et al., 1999b]. BA are transient bulges of the embryonic head and neck, partially filled with NCC, and separated from one another by evaginations of the endoderm, the pharyngeal pouches. Caudal BA and pouches give rise to the adult organs affected in the aforementioned NCC ablation experiments. As the BA defects observed in *Rara/b*-null embryos are less severe than those displayed by embryos lacking RALDH2 (i.e., embryos devoid of RA) [Niederreither et al., 1999], they do not reflect a complete block in RA signal transduction. To analyse NCC

migration, as well as formation of BA and pharyngeal pouches, in a situation where the degree of the block in RA signal transduction could be precisely controlled, a culture system was designed in which wild-type embryos are exposed, at selected times, to the panRAR antagonist BMS493 [Wendling et al., 2000].

Treatment with BMS493, induces a lack of caudal BA and pharyngeal pouches, and slightly disturbs the paths of post-otic NCC migration, without affecting the amount of NCC. Moreover, and most interestingly, this treatment inhibits caudal BA development only during a narrow window of time, which does not correspond to the period of post-otic NCC migration. Thus, contrary to what was expected from the set of abnormalities displayed by *Rara/b*-null fetuses [Dupe et al., 1999b], migrating NCC destined for the caudal BA do not represent exclusive primary targets of RA action. On the other hand, BMS493-induced alterations in endodermal expression of “patterning” genes and of genes encoding peptides involved in paracrine signalling pathways indicate that RA signalling (i) is required to specify the pharyngeal endoderm, and (ii) may provide a permissive environment for NCC migration through secretion of specific paracrine factors by the pharyngeal endoderm [Mark et al., 2004; Wendling et al., 2001]. These data also raise the possibility that genes responsible for the human CATCH22 syndrome are actually expressed in the endoderm, under the control of RA during the fourth and fifth weeks of gestation.

### RARs act on top of a genetic cascade controlling hindbrain segmentation

The embryonic hindbrain is transiently divided into segments (rhombomeres), of which seven (R1 to R7) are visible in mammals. Although early and transient, hindbrain segmentation is instrumental to organize adult structures such as cranial nerves. The hindbrain of *Rara/g*-null embryos shows a posterior expansion of R3 and R4 markers, but fails to express *kreisler*, a specific marker of R5 and R6 [Wendling et al., 2001]. In contrast, the neuroectodermal territory corresponding to R5 and R6 is markedly enlarged in *Rara/b*-null embryos [Dupe et al., 1999b]. Treating embryonic day (E)7.0 wild-type embryos with the panRAR antagonist BMS493 duplicates the abnormal hindbrain phenotype of *Rara/g*-null embryos, while BMS493 administration started at E8.0 results in a *Rara/b*-null-like phenotype [Wendling et al., 2000]. Therefore, the distinct phenotypes in *Rara/b*- and *Rara/g*-null embryos are related to RA actions during different windows of time. At E7.5 (when RA synthesis begins in the embryo) [Niederreither et al., 1999], RAR $\gamma$  and RAR $\alpha$  transduce a signal required to specify the R5/R6 territory. At E8.0, RAR $\beta$  and RAR $\alpha$  mediate a local increase in RA signalling in the posterior portion of the hindbrain to control the position of the R6 caudal boundary, thus allowing the next caudal rhombomere, R7, to be specified [Mark et al., 2004; Serpente et al., 2005; Wendling et al., 2000].

That the expression domains of several important hindbrain patterning genes are altered in *Rara/b*- and

*Rara/g*-null embryos provides evidence that RA acts on top of the genetic hierarchy controlling hindbrain patterning [Wendling et al., 2001]. Moreover, generation of a graded embryonic block in RA signal transduction through varying the concentrations of the panRAR antagonist in the cultures, demonstrates that individual rhombomeres are specified by distinct thresholds of RA signalling, and support the view that RA acts as a posteriorizing signal for the patterning of the embryonic hindbrain ([Dupe and Lumsden, 2001; Serpente et al., 2005; Wendling et al., 2001]and references therein). Threshold levels of RA signalling can be set up through modulations of the expression levels of RAR or of RA-synthesising and catabolising enzymes (RALDHs and CYP26s, respectively) [Abu-Abed et al., 2001; MacLean et al., 2001; Niederreither et al., 2000; Tahayato et al., 2003; Uehara et al., 2007].

### Retinoic acid-dependent eye morphogenesis is orchestrated by the neural crest

As mentioned above, *Rarb/g*-, *Rxra/Rarb*- and *Rxra/Rarg*-null mutants, but not *Rxra/Rara*-null mutants, display similar, albeit much more severe ocular defects than *Rxra*-null mutants, indicating that RXR $\alpha$ /RAR $\beta$  and RXR $\alpha$ /RAR $\gamma$  heterodimers are instrumental to the morphogenesis of eye structures, including the ventral retina (Table 2, Table 3, and Table 4). Both *Rarb* and *Rarg* are expressed in the periocular mesenchyme (POM), but not in the retina [Ghyselinck et al., 1997; Mori et al., 2001]. Therefore, RA signalling in the POM (a NCC-derived tissue) may be instrumental to the morphogenesis of the retina (a neuroectoderm-derived tissue). Using a somatic mutagenesis approach (reviewed in [Metzger and Chambon, 2001]), we have recently demonstrated that selective excision of both *Rarb* and *Rarg* genes in POM precursor cells (*Rarb/g*<sup>NCC $\alpha\alpha$</sup>  mutants) recapitulates the eye defects generated upon germline ablation of the same receptor genes. In POM cells, RXR $\alpha$ /RAR $\beta$  and RXR $\alpha$ /RAR $\gamma$  heterodimers appear to control the extent of cell-death involved in POM remodelling and the expression of *Foxc1* and *Pitx2* genes [Matt et al., 2005a], which play crucial roles the development of the anterior eye segment in mice and humans [Cvekl and Tamm, 2004]. Interestingly, the POM does not express any RA-synthesizing enzymes (RALDHs), and is therefore unable to synthesise RA. Instead, the neural retina, the retinal pigment epithelium and the corneal ectoderm express both RALDH1 and RALDH3 ([Matt et al., 2005a] and references therein). The fact that in mutants lacking both RALDH1 and RALDH3 (i) the activity of a RA-sensitive reporter transgene is abolished in the POM and (ii) the ocular defects that are generated recapitulate those observed in *Rarb/g*-null mutants establishes that these two RA-synthesizing enzymes provide the RA required to activate RXR $\alpha$ /RAR $\beta$  and RXR $\alpha$ /RAR $\gamma$  heterodimers in the POM. Therefore, in the developing eye, RA acts as a paracrine signal: it is synthesised by epithelial compartments (i.e., the retina, retinal pigment epithelium and corneal ectoderm), but exerts its effect on the mesenchymal compartment (i.e., the POM). Conversely,

the mesenchymal compartment appears to respond to the RA signal by synthesising a yet unknown paracrine factor required for the growth of the ventral retina [Matt et al., 2005a; Matt et al., 2008].

### Conclusions and perspectives

The phenotypic analyses of mutants lacking retinoid receptors have provided compelling evidence that RA is actually the metabolite of vitamin A, which is active during early embryogenesis, organogenesis, as well as postnatally. This conclusion was subsequently strengthened by the demonstration that RA, synthesized by the retinaldehyde dehydrogenases (RALDH1, RALDH2 and RALDH3), acts as an indispensable developmental hormone [Dupe et al., 2003; Matt et al., 2005a; Mic and Duester, 2003; Mic et al., 2002; Mic et al., 2003; Mic et al., 2004; Niederreither et al., 2002a; Niederreither et al., 1999; Niederreither et al., 2001; Niederreither et al., 2000; Niederreither et al., 2002b; Vermot et al., 2003]. Furthermore, the results from genetic and pharmacological studies conducted in the mouse have proven that the molecular mechanisms underlying transduction of the RA signal by retinoid receptors, which were suggested from *in vitro* studies, are actually instrumental to retinoid signalling under physiological conditions. They have also conclusively established that the teratogenic effects resulting from administration of exogenous RA to embryos do not reflect the physiological role of endogenous RA in the corresponding developmental processes (discussed in [Mark et al., 2006]).

RXR $\alpha$ /RAR heterodimers are clearly the main functional units transducing RA signals during development, and specific heterodimers are involved in given developmental processes (reviewed in [Mark and Chambon, 2003]). This strongly supports the initial proposal that the pleiotropic effects of RA reflect sophisticated combinatorial mechanisms, through which multiple RXR/RAR heterodimers differentially transduce retinoid signals to selectively control the expression of numerous sets of RA target genes [Leid et al., 1992]. Secondly, *in vivo*, the RXR partner can be either transcriptionally active (thus acting in synergy with its RAR partner) or inactive within RXR/RAR heterodimers, depending on the developmental event under consideration. Thirdly, the transcriptional activity of RXR is subordinated to ligand binding to the RAR partner *in vivo* [Elmazar et al., 2001; Matt et al., 2003; Mic et al., 2003], as is the case in cultured cells *in vitro* [Durand et al., 1994; Rochette-Egly and Chambon, 2001; Roy et al., 1995], and when RXR $\alpha$  is transcriptionally active, either one or both activation functions (AF-1 and AF-2) can be involved, their activity depending on the nature of the RA-controlled developmental event [Mascrez et al., 2009; Mascrez et al., 1998; Mascrez et al., 2001].

The genetic studies summarized in this review have revealed an extensive functional redundancy within the members of each family (RARs or RXRs), although each of these members appears to individually exert at least one specific physiological function. Since the members

of each family share a common ancestor [Escriva et al., 2004], such a redundancy is not surprising. However, it raises the question as to whether it is physiologically relevant or artefactually generated when a given receptor is missing, as is the case in cultured F9 cells [Rochette-Egly and Chambon, 2001; Taneja et al., 1995; Taneja et al., 1996]. In this respect, note that the existence of two fully redundant genes is, in an evolutionary sense, unlikely [Brookfield, 1992; Thomas, 1993]. It is also noteworthy that the occurrence of a given morphological defect in *Rar* double-null mutants contrasting with its absence in *Rar* single-null mutants should not be taken as a definite proof of a cell-autonomous functional redundancy. Another possible explanation could imply the action of distinct RARs in different tissues, which may independently direct the making of a given structure. Such a possibility may apply to the case of the interdigital soft tissue, which involutes normally in all *Rarb*- and in almost all *Rarg*-null mutants, but persists in all *Rarb/g*-null mutants yielding webbed digits (Table 1 and Table 3). In this instance, a functional cell-autonomous redundancy between RAR $\beta$  and RAR $\gamma$  is hardly conceivable, as *Rarb* and *Rarg* exhibit non-overlapping expression patterns in the limbs [Ghyselinck et al., 1997].

Importantly, there is much less functional redundancy in RXR $\alpha$ /RAR compound mutants (Table 2, Table 3, and Table 4). As RXR $\alpha$ /RAR heterodimers are the functional transducing units, the easiest way to interpret these observations is to postulate that redundancy occurs only when a single partner of the “physiological heterodimer” is missing [Kastner et al., 1997a; Mascrez et al., 1998; Mascrez et al., 2001]. In other words, the activity of the “alternative heterodimer” may still be above the functional threshold level when either the RXR or the RAR partner of the “physiological heterodimer” is missing, but not when both are deleted. According to this interpretation, the selective involvement of a given RAR or RXR could be revealed only under conditions where the functional threshold level is not reached. This would notably account for the observations that the role of the RXR $\alpha$  AF-1 or AF-2 cannot be fully revealed, unless the activity of the “physiological heterodimer” is altered by the additional ablation of either the RAR partner or the redundant RXR isotypes [Mascrez et al., 1998; Mascrez et al., 2001]. Thus, any conditions which would lower the activity of RXR/RAR heterodimers (for instance a decreased availability of RA), may reduce or abrogate functional redundancy. As the supplies in vitamin A, the precursor of RA, could be more limiting in wild-life than in the context of an animal facility, the functional redundancies between RAR and RXR may therefore be much less prominent in natural environments.

Quite surprisingly, RXR loss-of-function mutants do not display defects in morphogenesis other than those observed in the fetal VAD syndrome or upon ablation of the RA-synthesizing enzymes. This suggests that RXRs are involved in morphogenesis solely through their heterodimerization with RARs. Accordingly, mice harbouring null mutations for the other nuclear receptors



(NRs) dimerizing with RXRs and for which a ligand is known (i.e., PPAR $\alpha$ , PPAR $\beta$ , TR $\alpha$ , TR $\beta$ , VDR, LXR $\alpha$ , LXR $\beta$ , FXR, PXR, CAR) do not display morphological abnormalities [Flamant and Samarut, 2003; Kato, 2000; Lee and Gonzalez, 1996; Peet et al., 1998; Peters et al., 2000; Sinal et al., 2000; Staudinger et al., 2001; Wei et al., 2000; Xie et al., 2001], with the exception of PPAR $\gamma$ -null mice [Barak et al., 1999]. In this latter case, the embryonic heart defect is clearly secondary to a severe placental hypoplasia [Barak et al., 1999]. Thus, amongst the multiple “hormone-like” signals that RXR/NR heterodimers can integrate, RA appears to be the most crucial, if not the only one involved in morphogenesis of the embryo proper.

The genetic approach summarized in this review has provided valuable insights on the functions of RA receptors during development. However, this strategy has intrinsic limitations, which are mostly due to the introduction of mutations in the germline. First, the effect of a germline mutation may be functionally compensated for during development, thus precluding the appearance of a defect. On the other hand, the mutation can be lethal *in utero* (e.g., the *Rxra* knockout and the RAR compound null mutants), thus preventing analysis of the functions of the gene at postnatal stages. The mutation can also arrest the development of a given organ at an early stage, thus precluding further analysis of the gene functions at a later stage. Moreover, introducing mutations in germline makes it difficult to distinguish cell-autonomous from non-cell autonomous functions of genes belonging to families, such as RARs and RXRs that are involved in pleiotropic signalling pathways. In many instances, these limitations may actually prevent the determination of the function of a given gene product in a defined cell type/tissue and/or at a given time of the life of the animal. This is obviously the case for RARs and RXRs.

To overcome all these limitations, strategies for spatio-temporally-controlled somatic mutagenesis of RARs and RXRs in mice have been designed, which are based on the cell type-specific expression of a tamoxifen-inducible form of the Cre recombinase (called Cre-ER<sup>T</sup> and Cre-ER<sup>T2</sup>) (reviewed in [Metzger and Chambon, 2001; Metzger et al., 2003]). The combined use of transgenic mice expressing chimeric tamoxifen-inducible Cre recombinases in specific cell types and of mouse lines harbouring loxP-flanked conditional alleles for RAR and RXR genes will provide invaluable models to elucidate the postnatal functions of retinoid receptors.

## References

Abu-Abed, S., Dolle, P., Metzger, D., Beckett, B., Chambon, P. and Petkovich, M. (2001) The retinoic acid-metabolizing enzyme, CYP26A1, is essential for normal hindbrain patterning, vertebral identity, and development of posterior structures *Genes Dev* **15**, 226-40.

Allan, D., Houle, M., Bouchard, N., Meyer, B. I., Gruss, P. and Lohnes, D. (2001) RARgamma and Cdx1 interactions in vertebral patterning *Dev Biol* **240**, 46-60.

Baltus, A. E., Menke, D. B., Hu, Y. C., Goodheart, M. L., Carpenter, A. E., de Rooij, D. G. and Page, D. C. (2006) In germ cells of mouse

embryonic ovaries, the decision to enter meiosis precedes premeiotic DNA replication *Nat Genet* **38**, 1430-4.

Barak, Y., Nelson, M. C., Ong, E. S., Jones, Y. Z., Ruiz-Lozano, P., Chien, K. R., Koder, A. and Evans, R. M. (1999) PPAR $\gamma$  is required for placental, cardiac, and adipose tissue development *Mol Cell* **4**, 585-95.

Bastien, J., Adam-Stitah, S., Plassat, J. L., Chambon, P. and Rochette-Egly, C. (2002) The phosphorylation site located in the A region of retinoic X receptor  $\alpha$  is required for the antiproliferative effect of retinoic acid (RA) and the activation of RA target genes in F9 cells *J Biol Chem* **277**, 28683-9.

Batourina, E., Tsai, S., Lambert, S., Sprenkle, P., Viana, R., Dutta, S., Hensle, T., Wang, F., Niederreither, K., McMahon, A. P., Carroll, T. J. and Mendelsohn, C. L. (2005) Apoptosis induced by vitamin A signaling is crucial for connecting the ureters to the bladder *Nat Genet* **37**, 1082-9.

Batourina, E., Choi, C., Paragas, N., Bello, N., Hensle, T., Costantini, F. D., Schuchardt, A., Bacallao, R. L. and Mendelsohn, C. L. (2002) Distal ureter morphogenesis depends on epithelial cell remodeling mediated by vitamin A and Ret *Nat Genet* **32**, 109-15.

Batourina, E., Gim, S., Bello, N., Shy, M., Clagett-Dame, M., Srinivas, S., Costantini, F. and Mendelsohn, C. (2001) Vitamin A controls epithelial/mesenchymal interactions through Ret expression *Nat Genet* **27**, 74-8.

Blomhoff, R. (1994) Transport and metabolism of vitamin A *Nutr Rev* **52**, S13-23.

Bowles, J., Knight, D., Smith, C., Wilhelm, D., Richman, J., Mamiya, S., Yashiro, K., Chawengsaksophak, K., Wilson, M. J., Rossant, J., Hamada, H. and Koopman, P. (2006) Retinoid signaling determines germ cell fate in mice *Science* **312**, 596-600.

Brookfield, J. (1992) Can genes be truly redundant? *Curr Biol* **2**, 553-4.

Calleja, C., Messaddeq, N., Chapellier, B., Yang, H., Krezel, W., Li, M., Metzger, D., Mascrez, B., Ohta, K., Kagechika, H., Endo, Y., Mark, M., Ghyselinck, N. B. and Chambon, P. (2006) Genetic and pharmacological evidence that a retinoic acid cannot be the RXR-activating ligand in mouse epidermis keratinocytes *Genes Dev* **20**, 1525-38.

Chambon, P. (1996) A decade of molecular biology of retinoic acid receptors *Faseb J* **10**, 940-54.

Chen, T. H., Chang, T. C., Kang, J. O., Choudhary, B., Makita, T., Tran, C. M., Burch, J. B., Eid, H. and Sucof, H. M. (2002) Epicardial induction of fetal cardiomyocyte proliferation via a retinoic acid-inducible trophic factor *Dev Biol* **250**, 198-207.

Chen, F., Desai, T. J., Qian, J., Niederreither, K., Lu, J. and Cardoso, W. V. (2007) Inhibition of Tgf  $\beta$  signaling by endogenous retinoic acid is essential for primary lung bud induction *Development* **134**, 2969-79.

Crocoll, A., Herzer, U., Ghyselinck, N. B., Chambon, P. and Cato, A. C. (2002) Interdigital apoptosis and downregulation of BAG-1 expression in mouse autopods *Mech Dev* **111**, 149-52.

Cvekl, A. and Tamm, E. R. (2004) Anterior eye development and ocular mesenchyme: new insights from mouse models and human diseases *Bioessays* **26**, 374-86.

Desai, T. J., Chen, F., Lu, J., Qian, J., Niederreither, K., Dolle, P., Chambon, P. and Cardoso, W. V. (2006) Distinct roles for retinoic acid receptors  $\alpha$  and  $\beta$  in early lung morphogenesis *Dev Biol* **291**, 12-24.

Dickman, E. D., Thaller, C. and Smith, S. M. (1997) Temporally-regulated retinoic acid depletion produces specific neural crest, ocular and nervous system defects *Development* **124**, 3111-21.

Dupe, V., Matt, N., Garnier, J. M., Chambon, P., Mark, M. and Ghyselinck, N. B. (2003) A newborn lethal defect due to inactivation of retinaldehyde dehydrogenase type 3 is prevented by maternal retinoic acid treatment *Proc Natl Acad Sci U S A* **100**, 14036-41.



- Dupe, V., Ghyselinck, N. B., Thomazy, V., Nagy, L., Davies, P. J., Chambon, P. and Mark, M. (1999b) Essential roles of retinoic acid signaling in interdigital apoptosis and control of BMP-7 expression in mouse autopods *Dev Biol* **208**, 30-43.
- Dupe, V. and Lumsden, A. (2001) Hindbrain patterning involves graded responses to retinoic acid signalling *Development* **128**, 2199-208.
- Dupe, V., Davenne, M., Brocard, J., Dolle, P., Mark, M., Dierich, A., Chambon, P. and Rijli, F. M. (1997) In vivo functional analysis of the Hoxa-1 3' retinoic acid response element (3'RARE) *Development* **124**, 399-410.
- Dupe, V., Ghyselinck, N. B., Wendling, O., Chambon, P. and Mark, M. (1999a) Key roles of retinoic acid receptors  $\alpha$  and  $\beta$  in the patterning of the caudal hindbrain, pharyngeal arches and otocyst in the mouse *Development* **126**, 5051-9.
- Durand, B., Saunders, M., Gaudon, C., Roy, B., Losson, R. and Chambon, P. (1994) Activation function 2 (AF-2) of retinoic acid receptor and 9-cis retinoic acid receptor: presence of a conserved autonomous constitutive activating domain and influence of the nature of the response element on AF-2 activity *Embo J* **13**, 5370-82.
- Echeverri, K. and Oates, A. C. (2007) Coordination of symmetric cyclic gene expression during somitogenesis by Suppressor of Hairless involves regulation of retinoic acid catabolism *Dev Biol* **301**, 388-403.
- Elmazar, M. M., Ruhl, R. and Nau, H. (2001) Synergistic teratogenic effects induced by retinoids in mice by coadministration of a RARalpha- or RARGamma-selective agonist with a RXR-selective agonist *Toxicol Appl Pharmacol* **170**, 2-9.
- Escriva, H., Bertrand, S. and Laudet, V. (2004) The evolution of the nuclear receptor superfamily *Essays Biochem* **40**, 11-26.
- Flamant, F. and Samarut, J. (2003) Thyroid hormone receptors: lessons from knockout and knock-in mutant mice *Trends Endocrinol Metab* **14**, 85-90.
- Ghyselinck, N. B., Wendling, O., Messaddeq, N., Dierich, A., Lampron, C., Decimo, D., Viville, S., Chambon, P. and Mark, M. (1998) Contribution of retinoic acid receptor  $\beta$  isoforms to the formation of the conotruncal septum of the embryonic heart *Dev Biol* **198**, 303-18.
- Ghyselinck, N. B., Dupe, V., Dierich, A., Messaddeq, N., Garnier, J. M., Rochette-Egly, C., Chambon, P. and Mark, M. (1997) Role of the retinoic acid receptor  $\beta$  (RARbeta) during mouse development *Int J Dev Biol* **41**, 425-47.
- Golzio, C., Martinovic-Bouriel, J., Thomas, S., Mougou-Zrelli, S., Grattagliano-Bessieres, B., Bonniere, M., Delahaye, S., Munnich, A., Encha-Razavi, F., Lyonnet, S., Vekemans, M., Attie-Bitach, T. and Etchevers, H. C. (2007) Matthew-Wood syndrome is caused by truncating mutations in the retinol-binding protein receptor gene STRA6 *Am J Hum Genet* **80**, 1179-87.
- Grondona, J. M., Kastner, P., Gansmuller, A., Decimo, D., Chambon, P. and Mark, M. (1996) Retinal dysplasia and degeneration in RARbeta2/RARGamma2 compound mutant mice *Development* **122**, 2173-88.
- Hale, F. (1933) Pigs born without eyeballs *J Hered* **24**, 105-6.
- Halliglic, A., Ribes, V., Ghyselinck, N. B., Zile, M. H., Dolle, P. and Studer, M. (2007) Retinoids control anterior and dorsal properties in the developing forebrain *Dev Biol* **303**, 362-75.
- Horton, C. and Maden, M. (1995) Endogenous distribution of retinoids during normal development and teratogenesis in the mouse embryo *Dev Dyn* **202**, 312-23.
- Houle, M., Prinos, P., Iulianella, A., Bouchard, N. and Lohnes, D. (2000) Retinoic acid regulation of Cdx1: an indirect mechanism for retinoids and vertebral specification *Mol Cell Biol* **20**, 6579-86.
- Kang, J. O. and Sucov, H. M. (2005) Convergent proliferative response and divergent morphogenic pathways induced by epicardial and endocardial signaling in fetal heart development *Mech Dev* **122**, 57-65.
- Kastner, P., Mark, M., Leid, M., Gansmuller, A., Chin, W., Grondona, J. M., Decimo, D., Krezel, W., Dierich, A. and Chambon, P. (1996) Abnormal spermatogenesis in RXR  $\beta$  mutant mice *Genes Dev* **10**, 80-92.
- Kastner, P., Grondona, J. M., Mark, M., Gansmuller, A., LeMeur, M., Decimo, D., Vonesch, J. L., Dolle, P. and Chambon, P. (1994) Genetic analysis of RXR  $\alpha$  developmental function: convergence of RXR and RAR signaling pathways in heart and eye morphogenesis *Cell* **78**, 987-1003.
- Kastner, P., Mark, M., Ghyselinck, N., Krezel, W., Dupe, V., Grondona, J. M. and Chambon, P. (1997a) Genetic evidence that the retinoid signal is transduced by heterodimeric RXR/RAR functional units during mouse development *Development* **124**, 313-26.
- Kastner, P., Mark, M. and Chambon, P. (1995) Nonsteroid nuclear receptors: what are genetic studies telling us about their role in real life? *Cell* **83**, 859-69.
- Kastner, P., Messaddeq, N., Mark, M., Wendling, O., Grondona, J. M., Ward, S., Ghyselinck, N. and Chambon, P. (1997b) Vitamin A deficiency and mutations of RXRalpha, RXRbeta and RARalpha lead to early differentiation of embryonic ventricular cardiomyocytes *Development* **124**, 4749-58.
- Kato, S. (2000) The function of vitamin D receptor in vitamin D action *J Biochem* **127**, 717-22.
- Kawaguchi, R., Yu, J., Honda, J., Hu, J., Whitelegge, J., Ping, P., Wiita, P., Bok, D. and Sun, H. (2007) A membrane receptor for retinol binding protein mediates cellular uptake of vitamin A *Science* **315**, 820-5.
- Kawakami, Y., Raya, A., Raya, R. M., Rodriguez-Esteban, C. and Belmonte, J. C. (2005) Retinoic acid signalling links left-right asymmetric patterning and bilaterally symmetric somitogenesis in the zebrafish embryo *Nature* **435**, 165-71.
- Kochhar, D. M., Jiang, H., Penner, J. D., Johnson, A. T. and Chandraratna, R. A. (1998) The use of a retinoid receptor antagonist in a new model to study vitamin A-dependent developmental events *Int J Dev Biol* **42**, 601-8.
- Koubova, J., Menke, D. B., Zhou, Q., Capel, B., Griswold, M. D. and Page, D. C. (2006) Retinoic acid regulates sex-specific timing of meiotic initiation in mice *Proc Natl Acad Sci U S A* **103**, 2474-9.
- Krezel, W., Dupe, V., Mark, M., Dierich, A., Kastner, P. and Chambon, P. (1996) RXR  $\gamma$  null mice are apparently normal and compound RXR  $\alpha$  +/-RXR  $\beta$  +/-RXR  $\gamma$  +/- mutant mice are viable *Proc Natl Acad Sci U S A* **93**, 9010-4.
- Lee, S. S. and Gonzalez, F. J. (1996) Targeted disruption of the peroxisome proliferator-activated receptor  $\alpha$  gene, PPAR  $\alpha$  *Ann NY Acad Sci* **804**, 524-9.
- Leid, M., Kastner, P. and Chambon, P. (1992) Multiplicity generates diversity in the retinoic acid signalling pathways *Trends Biochem Sci* **17**, 427-33.
- Lohnes, D., Kastner, P., Dierich, A., Mark, M., LeMeur, M. and Chambon, P. (1993) Function of retinoic acid receptor  $\gamma$  in the mouse *Cell* **73**, 643-58.
- Lohnes, D., Mark, M., Mendelsohn, C., Dolle, 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**, 2723-48.
- 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  $\alpha$  mutant mice *Proc Natl Acad Sci U S A* **90**, 7225-9.
- Luo, J., Sucov, H. M., Bader, J. A., Evans, R. M. and Giguere, V. (1996) Compound mutants for retinoic acid receptor (RAR)  $\beta$  and RAR  $\alpha$  1 reveal developmental functions for multiple RAR  $\beta$  isoforms *Mech Dev* **55**, 33-44.

Lussier, M., Canoun, C., Ma, C., Sank, A. and Shuler, C. (1993) Interdigital soft tissue separation induced by retinoic acid in mouse limbs cultured in vitro *Int J Dev Biol* **37**, 555-64.

MacLean, G., Abu-Abed, S., Dolle, P., Tahayato, A., Chambon, P. and Petkovich, M. (2001) Cloning of a novel retinoic-acid metabolizing cytochrome P450, Cyp26B1, and comparative expression analysis with Cyp26A1 during early murine development *Mech Dev* **107**, 195-201.

Malpel, S., Mendelsohn, C. and Cardoso, W. V. (2000) Regulation of retinoic acid signaling during lung morphogenesis *Development* **127**, 3057-67.

Cambridge University PressMann, I. (1937) *Developmental abnormalities of the eye*. (Cambridge University Press Cambridge)

Mark, M., Ghyselinck, N. B. and Chambon, P. (2006) Function of retinoid nuclear receptors: lessons from genetic and pharmacological dissections of the retinoic acid signaling pathway during mouse embryogenesis *Annu Rev Pharmacol Toxicol* **46**, 451-80.

Mark, M. and Chambon, P. (2003) Functions of RARs and RXRs in vivo: Genetic dissection of the retinoid signaling pathway *Pure Appl Chem* **75**, 1709-1732.

Mark, M., Ghyselinck, N. B., Kastner, P., Dupe, V., Wendling, O., Krezel, W., Mascrez, B. and Chambon, P. (1998) Mesectoderm is a major target of retinoic acid action *Eur J Oral Sci* **106 Suppl 1**, 24-31.

Mark, M., Ghyselinck, N. B. and Chambon, P. (2004) Retinoic acid signalling in the development of branchial arches *Curr Opin Genet Dev* **14**, 591-8.

Mark, M., Lohnes, D., Mendelsohn, C., Dupe, V., Vonesch, J. L., Kastner, P., Rijli, F., Bloch-Zupan, A. and Chambon, P. (1995) Roles of retinoic acid receptors and of Hox genes in the patterning of the teeth and of the jaw skeleton *Int J Dev Biol* **39**, 111-21.

Mascrez, B., Ghyselinck, N. B., Chambon, P. and Mark, M. (2009) A transcriptionally silent RXR $\alpha$  supports early embryonic morphogenesis and heart development *Proc Natl Acad Sci U S A* **106**, 4272-4277.

Mascrez, B., Mark, M., Krezel, W., Dupe, V., LeMeur, M., Ghyselinck, N. B. and Chambon, P. (2001) Differential contributions of AF-1 and AF-2 activities to the developmental functions of RXR  $\alpha$  *Development* **128**, 2049-62.

Mascrez, B., Mark, M., Dierich, A., Ghyselinck, N. B., Kastner, P. and Chambon, P. (1998) The RXR $\alpha$  ligand-dependent activation function 2 (AF-2) is important for mouse development *Development* **125**, 4691-707.

Massaro, G. D., Massaro, D. and Chambon, P. (2003b) Retinoic acid receptor- $\alpha$  regulates pulmonary alveolus formation in mice after, but not during, perinatal period *Am J Physiol Lung Cell Mol Physiol* **284**, L431-3.

Massaro, G. D., Massaro, D., Chan, W. Y., Clerch, L. B., Ghyselinck, N., Chambon, P. and Chandraratna, R. A. (2000) Retinoic acid receptor- $\beta$ : an endogenous inhibitor of the perinatal formation of pulmonary alveoli *Physiol Genomics* **4**, 51-7.

Massaro, D. and Massaro, G. D. (2003a) Retinoids, alveolus formation, and alveolar deficiency: clinical implications *Am J Respir Cell Mol Biol* **28**, 271-4.

Matt, N., Schmidt, C. K., Dupe, V., Dennefeld, C., Nau, H., Chambon, P., Mark, M. and Ghyselinck, N. B. (2005a) Contribution of cellular retinol-binding protein type 1 to retinol metabolism during mouse development *Dev Dyn* **233**, 167-76.

Matt, N., Ghyselinck, N. B., Pellerin, I. and Dupe, V. (2008) Impairing retinoic acid signalling in the neural crest cells is sufficient to alter entire eye morphogenesis *Dev Biol* **320**, 140-8.

Matt, N., Dupe, V., Garnier, J. M., Dennefeld, C., Chambon, P., Mark, M. and Ghyselinck, N. B. (2005b) Retinoic acid-dependent eye morphogenesis is orchestrated by neural crest cells *Development* **132**, 4789-800.

Matt, N., Ghyselinck, N. B., Wendling, O., Chambon, P. and Mark, M. (2003) Retinoic acid-induced developmental defects are mediated by RAR $\beta$ /RXR heterodimers in the pharyngeal endoderm *Development* **130**, 2083-93.

McGowan, S., Jackson, S. K., Jenkins-Moore, M., Dai, H. H., Chambon, P. and Snyder, J. M. (2000) Mice bearing deletions of retinoic acid receptors demonstrate reduced lung elastin and alveolar numbers *Am J Respir Cell Mol Biol* **23**, 162-7.

Mendelsohn, C., Lohnes, D., Decimo, 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**, 2749-71.

Mendelsohn, C., Baturina, E., Fung, S., Gilbert, T. and Dodd, J. (1999) Stromal cells mediate retinoid-dependent functions essential for renal development *Development* **126**, 1139-48.

Merki, E., Zamora, M., Raya, A., Kawakami, Y., Wang, J., Zhang, X., Burch, J., Kubalak, S. W., Kaliman, P., Belmonte, J. C., Chien, K. R. and Ruiz-Lozano, P. (2005) Epicardial retinoid X receptor  $\alpha$  is required for myocardial growth and coronary artery formation *Proc Natl Acad Sci U S A* **102**, 18455-60.

Metzger, D. and Chambon, P. (2001) Site- and time-specific gene targeting in the mouse *Methods* **24**, 71-80.

Metzger, D., Indra, A. K., Li, M., Chapellier, B., Calleja, C., Ghyselinck, N. B. and Chambon, P. (2003) Targeted conditional somatic mutagenesis in the mouse: temporally-controlled knock out of retinoid receptors in epidermal keratinocytes *Methods Enzymol* **364**, 379-408.

Mic, F. A., Haselbeck, R. J., Cuenca, A. E. and Duester, G. (2002) Novel retinoic acid generating activities in the neural tube and heart identified by conditional rescue of Raldh2 null mutant mice *Development* **129**, 2271-82.

Mic, F. A. and Duester, G. (2003a) Patterning of forelimb bud myogenic precursor cells requires retinoic acid signaling initiated by Raldh2 *Dev Biol* **264**, 191-201.

Mic, F. A., Sirbu, I. O. and Duester, G. (2004) Retinoic acid synthesis controlled by Raldh2 is required early for limb bud initiation and then later as a proximodistal signal during apical ectodermal ridge formation *J Biol Chem* **279**, 26698-706.

Mic, F. A., Molotkov, A., Benbrook, D. M. and Duester, G. (2003b) Retinoid activation of retinoic acid receptor but not retinoid X receptor is sufficient to rescue lethal defect in retinoic acid synthesis *Proc Natl Acad Sci U S A* **100**, 7135-40.

Mollard, R., Ghyselinck, N. B., Wendling, O., Chambon, P. and Mark, M. (2000) Stage-dependent responses of the developing lung to retinoic acid signaling *Int J Dev Biol* **44**, 457-62.

Molotkova, N., Molotkov, A. and Duester, G. (2007) Role of retinoic acid during forebrain development begins late when Raldh3 generates retinoic acid in the ventral subventricular zone *Dev Biol* **303**, 601-10.

Mori, M., Ghyselinck, N. B., Chambon, P. and Mark, M. (2001) Systematic immunolocalization of retinoid receptors in developing and adult mouse eyes *Invest Ophthalmol Vis Sci* **42**, 1312-8.

Morriss-Kay, G. M. and Ward, S. J. (1999) Retinoids and mammalian development *Int Rev Cytol* **188**, 73-131.

Niederreither, K., Subbarayan, V., Dolle, P. and Chambon, P. (1999) Embryonic retinoic acid synthesis is essential for early mouse post-implantation development *Nat Genet* **21**, 444-8.

Niederreither, K., Vermot, J., Messaddeq, N., Schuhbauer, B., Chambon, P. and Dolle, P. (2001) Embryonic retinoic acid synthesis is essential for heart morphogenesis in the mouse *Development* **128**, 1019-31.

Niederreither, K., Vermot, J., Schuhbauer, B., Chambon, P. and Dolle, P. (2002b) Embryonic retinoic acid synthesis is required for forelimb growth and anteroposterior patterning in the mouse *Development* **129**, 3563-74.

- Niederreither, K., Abu-Abed, S., Schuhbauer, B., Petkovich, M., Chambon, P. and Dolle, P. (2002a) Genetic evidence that oxidative derivatives of retinoic acid are not involved in retinoid signaling during mouse development *Nat Genet* **31**, 84-8.
- Niederreither, K., Vermot, J., Schuhbauer, B., Chambon, P. and Dolle, P. (2000) Retinoic acid synthesis and hindbrain patterning in the mouse embryo *Development* **127**, 75-85.
- University of Chicago Press/Novacek, M. J. (1993) Patterns of diversity in mammalian skull :*The skull* (University of Chicago Press Chicago), 438-548.
- Oosterveen, T., Niederreither, K., Dolle, P., Chambon, P., Meijlink, F. and Deschamps, J. (2003) Retinoids regulate the anterior expression boundaries of 5' Hoxb genes in posterior hindbrain *Embo J* **22**, 262-9.
- Pasutto, F., Sticht, H., Hammersen, G., Gillissen-Kaesbach, G., Fitzpatrick, D. R., Nurnberg, G., Brasch, F., Schirmer-Zimmermann, H., Tolmie, J. L., Chitayat, D., Houge, G., Fernandez-Martinez, L., Keating, S., Mortier, G., Hennekam, R. C., von der Wense, A., Slavotinek, A., Meinecke, P., Bitoun, P., Becker, C., Nurnberg, P., Reis, A. and Rauch, A. (2007) Mutations in STRA6 cause a broad spectrum of malformations including anophthalmia, congenital heart defects, diaphragmatic hernia, alveolar capillary dysplasia, lung hypoplasia, and mental retardation *Am J Hum Genet* **80**, 550-60.
- Peet, D. J., Janowski, B. A. and Mangelsdorf, D. J. (1998) The LXRs: a new class of oxysterol receptors *Curr Opin Genet Dev* **8**, 571-5.
- Peters, J. M., Lee, S. S., Li, W., Ward, J. M., Gavrilova, O., Everett, C., Reitman, M. L., Hudson, L. D. and Gonzalez, F. J. (2000) Growth, adipose, brain, and skin alterations resulting from targeted disruption of the mouse peroxisome proliferator-activated receptor  $\beta$  ( $\delta$ ) *Mol Cell Biol* **20**, 5119-28.
- Quadro, L., Hamberger, L., Gottesman, M. E., Wang, F., Colantuoni, V., Blaner, W. S. and Mendelsohn, C. L. (2005) Pathways of vitamin A delivery to the embryo: insights from a new tunable model of embryonic vitamin A deficiency *Endocrinology* **146**, 4479-90.
- Ribes, V., Wang, Z., Dolle, P. and Niederreither, K. (2006) Retinaldehyde dehydrogenase 2 (RALDH2)-mediated retinoic acid synthesis regulates early mouse embryonic forebrain development by controlling FGF and sonic hedgehog signaling *Development* **133**, 351-61.
- Rochette-Egly, C. and Chambon, P. (2001) F9 embryocarcinoma cells: a cell autonomous model to study the functional selectivity of RARs and RXRs in retinoid signaling *Histol Histopathol* **16**, 909-22.
- Roy, B., Taneja, R. and Chambon, P. (1995) Synergistic activation of retinoic acid (RA)-responsive genes and induction of embryonal carcinoma cell differentiation by an RA receptor  $\alpha$  (RAR  $\alpha$ -), RAR  $\beta$ - or RAR  $\gamma$ -selective ligand in combination with a retinoid X receptor-specific ligand *Mol Cell Biol* **15**, 6481-7.
- Ruiz-Lozano, P., Smith, S. M., Perkins, G., Kubalak, S. W., Boss, G. R., Sucov, H. M., Evans, R. M. and Chien, K. R. (1998) Energy deprivation and a deficiency in downstream metabolic target genes during the onset of embryonic heart failure in RXR $\alpha$ -/- embryos *Development* **125**, 533-44.
- Sandell, L. L., Sanderson, B. W., Moiseyev, G., Johnson, T., Mushegian, A., Young, K., Rey, J. P., Ma, J. X., Staehling-Hampton, K. and Trainor, P. A. (2007) RDH10 is essential for synthesis of embryonic retinoic acid and is required for limb, craniofacial, and organ development *Genes Dev* **21**, 1113-24.
- Serpente, P., Tumpel, S., Ghyselinck, N. B., Niederreither, K., Wiedemann, L. M., Dolle, P., Chambon, P., Krumlauf, R. and Gould, A. P. (2005) Direct crossregulation between retinoic acid receptor  $\beta$  and Hox genes during hindbrain segmentation *Development* **132**, 503-13.
- Sinal, C. J., Tohkin, M., Miyata, M., Ward, J. M., Lambert, G. and Gonzalez, F. J. (2000) Targeted disruption of the nuclear receptor FXR/BAR impairs bile acid and lipid homeostasis *Cell* **102**, 731-44.
- Sirbu, I. O. and Duester, G. (2006) Retinoic-acid signalling in node ectoderm and posterior neural plate directs left-right patterning of somitic mesoderm *Nat Cell Biol* **8**, 271-7.
- Raven Press/Sporn, M. B., Roberts, A. B. and Goodman, D.S. (1994) *The Retinoids. Biology, Chemistry and Medicine* (Raven Press New York)
- Staudinger, J. L., Goodwin, B., Jones, S. A., Hawkins-Brown, D., MacKenzie, K. I., LaTour, A., Liu, Y., Klaassen, C. D., Brown, K. K., Reinhard, J., Willson, T. M., Koller, B. H. and Kliewer, S. A. (2001) The nuclear receptor PXR is a lithocholic acid sensor that protects against liver toxicity *Proc Natl Acad Sci U S A* **98**, 3369-74.
- Subbarayan, V., Kastner, P., Mark, M., Dierich, A., Gorry, P. and Chambon, P. (1997) Limited specificity and large overlap of the functions of the mouse RAR  $\gamma$  1 and RAR  $\gamma$  2 isoforms *Mech Dev* **66**, 131-42.
- Subbarayan, V., Mark, M., Messadeq, N., Rustin, P., Chambon, P. and Kastner, P. (2000) RXR $\alpha$  overexpression in cardiomyocytes causes dilated cardiomyopathy but fails to rescue myocardial hypoplasia in RXR $\alpha$ -null fetuses *J Clin Invest* **105**, 387-94.
- Sucov, H. M., Dyson, E., Gumeringer, C. L., Price, J., Chien, K. R. and Evans, R. M. (1994) RXR  $\alpha$  mutant mice establish a genetic basis for vitamin A signaling in heart morphogenesis *Genes Dev* **8**, 1007-18.
- Tahayato, A., Dolle, P. and Petkovich, M. (2003) Cyp26C1 encodes a novel retinoic acid-metabolizing enzyme expressed in the hindbrain, inner ear, first branchial arch and tooth buds during murine development *Gene Expr Patterns* **3**, 449-54.
- Taneja, R., Roy, B., Plassat, J. L., Zusi, C. F., Ostrowski, J., Reczek, P. R. and Chambon, P. (1996) Cell-type and promoter-context dependent retinoic acid receptor (RAR) redundancies for RAR  $\beta$  2 and Hoxa-1 activation in F9 and P19 cells can be artefactually generated by gene knockouts *Proc Natl Acad Sci U S A* **93**, 6197-202.
- Taneja, R., Bouillet, P., Boylan, J. F., Gaub, M. P., Roy, B., Gudas, L. J. and Chambon, P. (1995) Reexpression of retinoic acid receptor (RAR)  $\gamma$  or overexpression of RAR  $\alpha$  or RAR  $\beta$  in RAR  $\gamma$ -null F9 cells reveals a partial functional redundancy between the three RAR types *Proc Natl Acad Sci U S A* **92**, 7854-8.
- Thomas, J. H. (1993) Thinking about genetic redundancy *Trends Genet* **9**, 395-9.
- Uehara, M., Yashiro, K., Mamiya, S., Nishino, J., Chambon, P., Dolle, P. and Sakai, Y. (2007) CYP26A1 and CYP26C1 cooperatively regulate anterior-posterior patterning of the developing brain and the production of migratory cranial neural crest cells in the mouse *Dev Biol* **302**, 399-411.
- Vermot, J., Niederreither, K., Garnier, J. M., Chambon, P. and Dolle, P. (2003) Decreased embryonic retinoic acid synthesis results in a DiGeorge syndrome phenotype in newborn mice *Proc Natl Acad Sci U S A* **100**, 1763-8.
- Vermot, J. and Pourquie, O. (2005) Retinoic acid coordinates somitogenesis and left-right patterning in vertebrate embryos *Nature* **435**, 215-20.
- Vieux-Rochas, M., Coen, L., Sato, T., Kurihara, Y., Gitton, Y., Barbieri, O., Le Blay, K., Merlo, G., Ekker, M., Kurihara, H., Janvier, P. and Levi, G. (2007) Molecular dynamics of retinoic acid-induced craniofacial malformations: implications for the origin of gnathostome jaws *PLoS ONE* **2**, e510.
- Wang, Z., Dolle, P., Cardoso, W. V. and Niederreither, K. (2006) Retinoic acid regulates morphogenesis and patterning of posterior foregut derivatives *Dev Biol* **297**, 433-45.
- Wei, P., Zhang, J., Egan-Hafley, M., Liang, S. and Moore, D. D. (2000) The nuclear receptor CAR mediates specific xenobiotic induction of drug metabolism *Nature* **407**, 920-3.
- Wendling, O., Denfeld, C., Chambon, P. and Mark, M. (2000) Retinoid signaling is essential for patterning the endoderm of the third and fourth pharyngeal arches *Development* **127**, 1553-62.
- Wendling, O., Ghyselinck, N. B., Chambon, P. and Mark, M. (2001) Roles of retinoic acid receptors in early embryonic morphogenesis and hindbrain patterning *Development* **128**, 2031-8.

White, J. C., Shankar, V. N., Highland, M., Epstein, M. L., DeLuca, H. F. and Clagett-Dame, M. (1998) Defects in embryonic hindbrain development and fetal resorption resulting from vitamin A deficiency in the rat are prevented by feeding pharmacological levels of all-trans-retinoic acid *Proc Natl Acad Sci U S A* **95**, 13459-64.

White, J. C., Highland, M., Kaiser, M. and Clagett-Dame, M. (2000) Vitamin A deficiency results in the dose-dependent acquisition of anterior character and shortening of the caudal hindbrain of the rat embryo *Dev Biol* **220**, 263-84.

Wilson, J. G., Roth, C. B. and Warkany, J. (1953) An analysis of the syndrome of malformations induced by maternal vitamin A deficiency. Effects of restoration of vitamin A at various times during gestation *Am J Anat* **92**, 189-217.

Wolf, G. (2006) Is 9-cis-retinoic acid the endogenous ligand for the retinoic acid-X receptor? *Nutr Rev* **64**, 532-8.

Xie, W., Radomska-Pandya, A., Shi, Y., Simon, C. M., Nelson, M. C., Ong, E. S., Waxman, D. J. and Evans, R. M. (2001) An essential role for nuclear receptors SXR/PXR in detoxification of cholestatic bile acids *Proc Natl Acad Sci U S A* **98**, 3375-80.