Developmental expression of retinoic acid receptors (RARs)

Pascal Dollé

IGBMC (Institut de Génétique et de Biologie Moléculaire et Cellulaire), France; Inserm, U 964, France; CNRS, UMR 7104, France; Faculté de Médecine, Université de Strasbourg, France

Here, I review the developmental expression features of genes encoding the retinoic acid receptors (RARs) and the ‘retinoid X’ or rexinoid receptors (RXRs). The first detailed expression studies were performed in the mouse over two decades ago, following the cloning of the murine Rar genes. These studies revealed complex expression features at all stages of post-implantation development, one receptor gene (Rara) showing widespread expression, the two others (Rarb and Rarg) with highly regionalized and/or cell type-specific expression in both neural and non-neural tissues. Rxr genes also have either widespread (Rxra, Rxrb), or highly-restricted (Rarg) expression patterns. Studies performed in zebrafish and Xenopus demonstrated expression of Rar and Rxr genes (both maternal and zygotic), at early pre-gastrulation stages. The eventual characterization of specific enzymes involved in the synthesis of retinoic acid (retinol/retinaldehyde dehydrogenases), or the triggering of its catabolism (CYP26 cytochrome P450s), all of them showing differential expression patterns, led to a clearer understanding of the phenomenons regulated by retinoic acid signaling during development. Functional studies involving targeted gene disruptions in the mouse, and additional approaches such as dominant negative receptor expression in other models, have pinpointed the specific, versus partly redundant, roles of the RARs and RXRs in many developing organ systems. These pleiotropic roles are summarized hereafter in relationship to the receptors’ expression patterns.

Introduction

The expression patterns of RARs and RXRs have been studied in various species. Initial studies, published shortly after the cloning of RARs and RXRs, have been performed on mouse embryos [Dolle et al., 1989; Dolle et al., 1990; Ruberte et al., 1991; Ruberte et al., 1990]. Reports on other species have often focused on early developmental stages (e.g., [Hale et al., 2006; Tallafuss et al., 2006] for recent reports on zebrafish rar and rxr gene expression), or on specific organ systems. In a few instances, expression of specific Rar isoforms has been studied [Koide et al., 2001; Mollard et al., 2000; Smith, 1994]. Most studies have analyzed the Rar and Rxr expression patterns at the mRNA level, using in situ hybridization (ISH) and occasionally RT-PCR (e.g., [Ulven et al., 2000]), but there are some reports of RAR/RXR protein distributions analyzed by immunohistochemistry (e.g., [Mori et al., 2001]). Unless otherwise mentioned, the data reviewed hereafter are mRNA expression data. As a general trend, three of the retinoid receptors (RARα, RXRα and RXRβ) have ubiquitous, or quite widespread, expression patterns, with the others (RARγ, RXRγ and RXRδ) showing complex, tissue-specific expression. Here, I review the expression features of the Rar and Rxr genes during successive steps of early embryonic development, and for later stages in the various differentiating organ systems. These expression features (see Table 1 for a summary) are discussed in relationship with functional data and current models about the roles of retinoic acid signaling during development.

Early development and morphogenesis

Pregastrulation stages

Various lines of evidence indicate that Rar and Rxr mRNAs are already expressed at early developmental stages. In zebrafish, all rar and rxr genes are maternally-expressed; expression appears to be diffuse at the cleavage and blastula stages, with the first signs of localized expression being seen at the gastrula stage [Hale et al., 2006; Tallafuss et al., 2006; Waxman and Yelon, 2007]. Currently, four Rar genes (raraa, rARB, rargb, and four Rxr genes (rxra, rxrba, rxrbb, rXrg), have been characterized in zebrafish. Thus, the zebrafish genome contains duplicated co-orthologs of mammalian Rara, RARg and RXr, but no identified orthologue to RARB ([Hale et al., 2006; Tallafuss et al., 2006] and references therein). In Xenopus, Rara and Rarg were reported as the main Rar genes expressed, both maternally and zygotically, at pregastrulation stages [Blumberg et al., 1992; Ellinger-Ziegelbauer and Dreyer, 1991; Ellinger-Ziegelbauer and Dreyer, 1993; Koide et al., 2001; Pfeffer and De Robertis, 1994; Shiotsugu et al., 2004]. There are no specific reports of Rar/RXr gene expression at pre-implantation stages in mouse or rat embryos. Rara, Rarg, RXra and RXrb transcripts were detected by RT-PCR in pre-implantation bovine embryos at all stages, from oocyte to hatched blastocyst [Mohan et al., 2001;
### Table 1. Summary of Rara gene expression patterns in the main developing organ systems

Data were essentially obtained in the mouse. See main text for references. + = ubiquitous (diffuse) expression. - = no expression detected.

| Organ System         | Rara                                                                 | Rarb                                                                 | Rarg                                                                 |
|----------------------|----------------------------------------------------------------------|----------------------------------------------------------------------|----------------------------------------------------------------------|
| Brain                | segmented hindbrain (rhombomeres 4 and 7), olfactory bulb, cortex, hippocampus, hypothalamus, cerebellum | segmented hindbrain (rhombomere 7), olfactory tubercle, caudate/putamen, accumbens, hypothalamus, meninges, choroid plexuses | -                                                                    |
| Spinal Cord          | + (high in ventricular neuroepithelium)                              | ventricular neuroepithelium, ventral horns (brachial-lumbar levels) | early neural plate (transient)                                        |
| Eye                  | + (including neural retina)                                          | ocular/periocular mesenchyme, pigmented epithelium                   | Ocular/periocular mesenchyme                                         |
| Inner Ear            | + (high in sensory epithelia)                                        | mesenchyme, basilar membrane, crista                              | + (high in crista, spiral limbus)                                    |
| Nasal Structures     | + (including olfactory epithelium)                                   | mesenchyme, olfactory epithelium (regionalized/apical)             | nasal capsule (precartilage)                                         |
| Pituitary Gland      | +                                                                    | + (regionalized)                                                   | -                                                                    |
| Palate               | +                                                                    | + (regionalized)                                                   | precartilage                                                        |
| Salivary Glands      | +                                                                    | mesenchyme                                                          | epithelium                                                          |
| Thyroid Gland        | +                                                                    |                                                                      | -                                                                    |
| Trachea              | +                                                                    |                                                                      | mesenchyme                                                          |
| Lung                 | +                                                                    | proximal bronchi                                                   | +                                                                    |
| Heart                | +                                                                    | myocardium, conotruncal mesenchyme                                 | endocardial cushions                                                |
| Stomach              | +                                                                    | + (regionalized)                                                   | squamous epithelium                                                 |
| Intestine            | +                                                                    | epithelium, outer mesenchyme (regionalized)                        |                                                                      |
| Liver                | +                                                                    | liver capsule                                                       | -                                                                    |
| Pancreas             | +                                                                    | mesenchyme                                                          | -                                                                    |
| Kidney               | +                                                                    | stroma                                                              | -                                                                    |
| Gonad                | +                                                                    |                                                                      | -                                                                    |
| Skin                 | +                                                                    |                                                                      | epidermis                                                           |
| Skeletal System      | +                                                                    |                                                                      | precartilaginous condensations (both neural crest and non-neural crest derived) |
| Limbs                | +                                                                    | proximal mesenchyme, interdigital regions                           | early mesenchyme, precartilaginous condensations                     |

Mohan et al., 2002]. An in situ hybridization study reported ubiquitous expression of Rara in extraembryonic and embryonic tissues, and of Rarg in embryonic tissues, in E6.5 (pregastrula) mouse embryos [Ang and Duester,
The functional significance of \textit{Rar/Rxr} genes being expressed at early embryonic stages – or being maternally inherited – is unclear, as current evidence indicates that RA is first produced within the embryo at gastrulation stages (see below).

**Gastrula – presomatic stages**

Differential distributions of \textit{Rar} mRNAs become evident in gastrulating embryos of various species. In the mouse, \textit{Rara} and \textit{Rarb} distributions remain diffuse at presomitic stages (E7.5) [Ang and Duester, 1997; Ruberte et al., 1991; Ulven et al., 2000], whereas \textit{Rarb} expression is low in headfold and posterior midline tissues, and higher in lateral regions of the egg-cylinder [Ruberte et al., 1991]. In zebrafish, differential dorso-ventral distributions of the \textit{raraa} and \textit{rarab} genes have been reported, whereas the two \textit{rarg} genes exhibit diffuse – or poorly detectable – expression [Hale et al., 2006; Waxman and Yelon, 2007]. Studies in Xenopus indicate an accumulation of \textit{Rara2}, and a downregulation of \textit{Rara1}, at early gastrulation stages [Koide et al., 2001; Shiotsugu et al., 2004]. Expression of all \textit{Rrxa} has also been detected (by RT-PCR in the mouse [Ulven et al., 2000] and ISH in the zebrafish [Tallafuss et al., 2006]) at these stages. Collectively, these data show that there is relatively widespread and overlapping expression of \textit{Rars} and \textit{Rxrs} in early gastrulating vertebrate embryos. At this stage, retinoic acid (RA) is first synthesized in posterior mesodermal cells by the retinaldehyde dehydrogenase 2 (RALDH2) enzyme [Niederreither et al., 1997], and it has been shown to act in a region-specific manner as demonstrated, for instance, by using a RA-activated lacZ transgene [Ribes et al., 2009; Rossant et al., 1991]. RA starts to have signaling functions during gastrulation in the posterior region of the embryo, which includes the primitive streak, the node and the newly-formed mesoderm [Niederreither et al., 1999; Ribes et al., 2009; Shiotsugu et al., 2004]. On the other hand, anterior regions of the embryo are devoid of RA signaling, due to the action of the metabolizing enzymes CYP26A1 and CYP26C1 [Hernandez et al., 2007; Ribes et al., 2007; Uehara et al., 2007]. Koide et al. [Koide et al., 2001] showed that in the Xenopus gastrula, RAR(s) need to function as transcriptional repressors in the prospective head region, most likely to prevent any inappropriate activation of genes acting as posterior determinants.

**Neurula – early somitic stages**

Many morphogenetic events occur while gastrulation proceeds in the posterior region of the embryo. These include induction and regional patterning of the neurectoderm, migration of cranial neural crest cells and formation of the branchial arches, segmentation of the paraxial mesoderm into somites, fusion and looping of the heart tube, etc. Distinct patterns of expression are observed for all \textit{Rar} genes during this period, which are summarized hereafter for murine genes (see Figure 1; additional data on zebrafish and Xenopus can be found in [Blumberg et al., 1982; Dreyer and Ellinger-Ziegelbauer, 1996; Ellinger-Ziegelbauer and Dreyer, 1991; Ellinger-Ziegelbauer and Dreyer, 1993; Hale et al., 2006; Koide et al., 2001; Pfeffer and De Robertis, 1994; Waxman and Yelon, 2007]). \textit{Rara} (Figure 1A-C) expression progressively switches from a diffuse pattern, to a prominent expression within the neural ectoderm, with a discrete rostral expression boundary at the level of the prospective hindbrain (for the sake of clarity, expression features at later stages of head and brain development are detailed in the next section). \textit{Rarb} (Figure 1D-F) and \textit{Rarg} (Figure 1G-I) show strikingly distinct distributions along the rostro-caudal axis of the embryo. \textit{Rarg} is expressed in the regressing primitive streak [Ruberte et al., 1991], and during somitogenesis and caudal axis extension its expression is specifically maintained in the caudalmost tissues [Abu-Abed et al., 2003]. Thus, \textit{Rarg} expression is downregulated, both in the neural plate and in mesodermal derivatives, while these tissues initiate differentiation. \textit{Rarb} expression is relatively complementary, being undetectable in the caudalmost tissues, and present in the neural tube and some of the mesodermal derivatives at more rostral levels [Ruberte et al., 1991].

At these stages, RA is produced by RALDH2 in the newly-formed somites and the rostral presomitic mesoderm. It has been shown, both in avian models and mouse mutants deficient for RA synthesis, that retinoid signaling is indispensable for controlling neurogenesis and patterning in the developing spinal cord, and regulating somite size and left-right symmetry [Diez del Corral et al., 2003; Diez del Corral and Storey, 2004; Duester, 2007; Maden, 2006; Reijntjes et al., 2005; Ribes et al., 2009; Vermot et al., 2005a; Vermot and Pourquie, 2005; Wilson and Maden, 2005]. In these processes, RA appears to antagonize posterior signals, including Wnt3a and FGF8, required for the maintenance of an undifferentiated ‘stem’ zone within the embryonic tail bud. \textit{Rar} single or compound knockout mice have not been found to display such defects in spinal cord neurogenesis or mesodermal segmentation (for details and references, see accompanying review by [Mark et al., 2009]). This could be because RA has critical functions in the transition region between the posterior ‘stem’ zone and the differentiating tissues, where \textit{Rarb} and \textit{Rarg} (as well as \textit{Rara}) would be coexpressed. The posterior (tail bud) region needs to be actively protected from RA signaling by the action of the CYP26A1 enzyme [Abu-Abed et al., 2001; Sakai et al., 2001], and interestingly, the caudal defects occurring in \textit{Cyp26a1-null} mutant mice are prevented by a compound inactivation of \textit{Rarg} [Abu-Abed et al., 2003].

**Nervous system, craniofacial and sensory organ development**

### Early head, hindbrain and branchial region

Two murine \textit{Rar} genes (\textit{Rara} and \textit{Rarb}) are coexpressed within the developing neural tube by E9.5-10.5, but show differential rostral boundaries in the hindbrain neuroepithelium during its transient segmentation into rhombomeres. \textit{Rara} is expressed until the rhombomere (r)6/7 boundary, and is additionally expressed in r4 [Ruberte et al., 1991]. Although both \textit{Rara1} and \textit{Rara2}
mRNA isoforms are expressed in the neural tube, it is the Rara$^2$ isoform that shows highest expression and displays a clear rhombomeric boundary [Mollard et al., 2000]. Rarb (principally the Rarb$^2$ isoform) is strongly expressed in
the neural tube epithelium, up to the r6/r7 boundary (see Figure 2) and ([Mollard et al., 2000; Ruberte et al., 1991; Serpente et al., 2005]; see [Hale et al., 2006; Waxman and Yelon, 2007] for data on zebrafish rar genes). RA is synthesized by RALDH2 in the occipital somites and the posterior hindbrain menenchyme, and is thought to act by diffusion towards the hindbrain neuroepithelium (see [Glover et al., 2006; Maiden, 2002] for reviews). Hence, the discrete, segmental expression patterns of Rara and Rarb may be critical for proper rhombomeric expression of RA-regulated target genes. These include several Hox genes, including Hoxa1 and Hoxb1 ([Dupe et al., 1997; Studer et al., 1998] and references therein). Combinatorial roles of RARα and RARβ for patterning of posterior rhombomeres have been demonstrated by the analysis of compound knockout mutants ([Dupe et al., 1999b]; see also the accompanying review by [Mark et al., 2009]).

In contrast to Rara and Rarb, the murine Rarg gene shows no detectable expression within the developing brain and spinal cord neuroepithelium, apart from its early expression in the caudalmost neural plate (see above). From E9 onwards, it is expressed throughout the cranial menenchyme, both at the level of the frontonasal mass and the branchial arches (Figure 3A,B) [Ruberte et al., 1990]. The cranial menenchyme is composed of two cell components, the primary menenchyme and the neural crest cells originating from forebrain, midbrain and hindbrain levels. The homogeneous pattern of Rarg expression (Figure 3A) suggests that both components may express this receptor. Eventually, Rarg expression becomes localized to precartilaginous cell condensations (Figure 3B), which in the head, derive from neural crest cells. Rarg expression is found in precartilaginous condensations throughout the body (including non-skeletal presumptive cartilages, e.g., laryngeal and tracheal cartilages) (Figure 3C). Both Rara and Rarb are also expressed in cranial mesenchyme. Rara (mainly the Rara1 isofrom) is expressed in a relatively diffuse manner, whereas Rarb (mainly RARB2) is strongly expressed in the frontonasal and pericocular mesenchyme (see Figure 2B); it is not expressed in most of the maxillo-mandibular region derived from the first branchial arch, and weaker levels are found in more posterior branchial arches [Mollard et al., 2000; Ruberte et al., 1991]. Gene knockout experiments revealed that RARα and RARγ are the prevalent receptors in neural crest-derived cranial mesoderm [Lohnes et al., 1994]. Rarb (especially RARB2) is also strongly expressed in the embryonic foregut endoderm (Figure 1E,F). This expression appears before formation of the branchial arches [Ruberte et al., 1991; Smith, 1994] and is eventually found in the branchial pouch endoderm (Figure 2E), another important target tissue of retinoid signaling ([Niederreither et al., 2003; Wendling et al., 2000]; also see accompanying review by [Mark et al., 2009]).

Among murine Rxr genes, Rxra and Rrxb are expressed in a diffuse manner throughout these stages, whereas Rrxb is specifically expressed in myogenic cells [Dolle et al., 1994]. Tallafuss et al. [Tallafuss et al., 2006] reported distinct distributions of zebrafish rxr mRNAs at corresponding stages.

**Brain development**

As described above, none of the Rar murine gene transcripts were detected in the early embryonic neuroectoderm rostrally to the hindbrain, i.e., at midbrain and forebrain levels (e.g., Figure 2D,E and Figure 3B). This is surprising, since the RA-producing enzyme RALDH2 is transiently expressed in the rostralmost forebrain epithelium before outgrowth of the telencephalic vesicles, and RALDH2−/− knock-out embryos have severe forebrain deficiencies [Ribes et al., 2006]. RALDH2 function is partly redundant with that of RALDH3, which produces RA in the frontonasal, non-neural surface ectoderm [Halagic et al., 2007; Schneider et al., 2001]. Possibly, RAR(s) are expressed at low levels in the early forebrain neuroepithelium, where they might transduce these RA effects. Xenopus Rara is expressed during early brain development [Shiotsugu et al., 2004], and RAR knock-down in this species reduces forebrain development [Koide et al., 2001]. Also, one of the zebrafish Rara orthologues (rarab) is expressed in the prospective diencephalon [Hale et al., 2006].

Expression of Rar genes during later brain development has been studied in most detail in the mouse [Ruberte et al., 1993] and the rat [Zetterstrom et al., 1999]. In both species, Rarg transcripts were not detected in brain structures. Rara and Rarb are expressed in the developing myelencephalon (medulla oblongata), in regions that derive from their early hindbrain expression domains, and expression of Rarb in particular, becomes localized to somatic and visceral motor nuclei [Ruberte
et al., 1993]. Rara expression is very low or absent in other brain structures, except in the corpus striatum and pallidum, where the murine Rara2 isoform is expressed from E12.5 onwards in a domain adjacent to that of Rarb (Figure 4A-D) [Mollard et al., 2000; Ruberte et al., 1993]. Both Rarb1 and Rarb2 are coexpressed in the corpus striatum (caudate-putamen and nucleus accumbens) from E12.5 onwards (Figure 4C,D), as well as in the olfactory tubercle [Mollard et al., 2000; Ruberte et al., 1993]. Rarb transcripts are also detected in the choroid plexuses and the developing meninges, which also express the RA-synthesizing enzyme Raldh2 [Niederreither et al., 1997]. Among Rxs, Rxra and Rxrb are relatively ubiquitously expressed in the developing mouse [Dolle et al., 1994] and rat [Zetterstrom et al., 1999] brain, whereas Rxrg appears in specific brain regions at newborn stages [Zetterstrom et al., 1999]. Specific distributions of RARs and RXRs have also been reported, both at mRNA and protein levels, in the adult mouse brain [Krezel et al., 1999]. In particular, RARβ expression persists in the caudate-putamen and nucleus accumbens, where it is coexpressed with RXRγ, and both RARα and RARγ are coexpressed in the hippocampal fields, where RARβ is also detected. Through the analysis of viable Rar/Rxr compound mutant mice, it has been possible to characterize some of the corresponding functions in locomotor control [Krezel et al., 1998] or learning and memory [Chiang et al., 1998; Wietrzych et al., 2005].

**Spinal cord**

The Rara and Rarb genes remain expressed in the differentiating mouse spinal cord, at least until E14.5 [Colbert et al., 1995; Ruberte et al., 1993]. Rara is expressed throughout the spinal cord, and at higher levels in the ventricular zone neuroepithelium. At early stages (E10.5-11.5), Rarb transcripts are also found in the ventricular neuroepithelium, and by E12.5 they appear in cells of the ventral horns (presumptive motor neurons) within the mantle zone. Eventually, they become restricted to dorsal and intermediate regions of the ventricular neuroepithelium. Along the anterior-posterior (rostro-caudal) axis, they become localized to cervical/brachial and lumbar levels, where limb-innervating neurons are being generated [Colbert et al., 1995]. Although both Rarb isoforms are expressed in the spinal cord, it is the Rarb1 isoform which is most abundant [Mollard et al., 2000]; (see also [Mendelsohn et al., 1994a; Mendelsohn et al., 1991] for lacZ reporter transgene analysis). Rxrg transcripts are restricted to the ventral horns of the spinal cord, where they are coexpressed with Rarb [Dolle et al., 1994].

RA is required for proper patterning and neurogenesis within the spinal cord ([Diez del Corral and Storey, 2004; Maden, 2006; Wilson and Maden, 2005] and references therein), and conditional gene knockouts have shown that it acts in a complex manner, being produced both by the adjacent mesoderm, and eventually, by specific motor neuron progenitor populations at brachial and lumbar levels [Ji et al., 2006; Vermot et al., 2005b]. RARα and RARβ may act redundantly to transduce the RA signal within the spinal cord, as the corresponding null mutant mice do not display phenotypic abnormalities that may be related to abnormal spinal cord development. Molecular patterning of the spinal cord has yet to be studied in compound Rara/Rarb mutants.

**Eye development**

The developing retina is particularly rich in RA, and is one of the first structures in which region-specific patterns of RA synthesis were reported ([McCaffery et al., 1992; Wagner et al., 2000] and references therein). At early stages of murine eye development, however, expression of Rar genes is most prominent in extra-retinal tissues: Rarb is strongly expressed in the periocular mesenchyme (including the presumptive choroid and corneal mesenchyme) and Rxrg is expressed more homogeneously throughout the head mesenchyme [Dolle
et al., 1990]. A detailed immunohistochemistry study has been performed for retinoid receptor expression throughout eye development, which confirmed the presence of the corresponding proteins in these mesenchymal tissues (Figure 4E-H) [Mori et al., 2001]. RARα was the only receptor to be detected within the neural retina (Figure 4F), especially in the inner nuclear and ganglion cell layers. RARα and RARβ were also detected in the retinal pigmented epithelium (Figure 4F,G). RXRα and RXRγ were detected in specific retinal cell layers, whereas RXRβ was ubiquitously expressed [Mori et al., 2001]. \textit{Rar} and \textit{Rxr} transcript distributions have also been described in the developing chick retina [Hoover et al., 2001].

Although RA is actively synthesized within the developing neural retina of various vertebrate species ([Li et al., 2000; McCaffery et al., 1992; Wagner et al., 2000] and references therein), current functional evidence in mouse points to a paracrine mode of action, with RA diffusing and acting within the pigmented epithelium and pericellular mesenchyme [Matt et al., 2005; Molotkov et al., 2006]. Possible intrinsic functions of RA within the neural retina, for instance to regulate proper differentiation of retinal cell types (see [Kelley et al., 1994; Prabhudesai et al., 2005] for a description of the effects of exogenous RA on photoreceptor differentiation), remain to be characterized.

**Auditory system**

The expression of retinoid receptors has been studied in detail in the developing mouse inner ear [Raz and Kelley, 1999; Romand et al., 2002; Romand et al., 1998]. Expression of all three \textit{Rar} genes was observed in the developing otocyst as early as E10.5 [Romand et al., 2002], and persisted until prenatal stages [Romand et al., 1998]. The expression patterns were largely non-overlapping, \textit{Rara} being predominantly expressed in the developing sensory epithelium, \textit{Rarb} in inner ear mesenchymal tissues, and \textit{Rarg} in the differentiating otic capsule. Interestingly, expression of the three \textit{Rar} genes was further detected in the adult inner ear. \textit{Rara} and \textit{Rarg}, in particular, are expressed in the organ of Corti (the cochlear auditory epithelium) [Romand et al., 2002]. \textit{Rarα} and \textit{Rarγ} were shown to be necessary for embryonic inner ear development [Romand et al., 2002]. Whether the retinoid receptors may play a role postnatally in the auditory system remains unclear. However, \textit{Rara}-null mutants exhibit a hearing deficiency related in part to middle ear functional deficits (R. Romand, personal communication).

**Nasal, palatal and tooth development**

RA plays important roles for the development of nasal structures [Dupe et al., 2003; Hallilagic et al., 2007]. \textit{Rarb} exhibits region-specific expression in differentiating nasal structures, being present both in the mesenchyme and in specific areas of the olfactory epithelium [Doile et al., 1990] (see Figure 4D). Knockout mouse models have shown that \textit{Rarα} and \textit{Rarg} are important for the development of nasal structures [Lohnes et al., 1994]. Whether there is a function of \textit{RARβ} in later development of the nasal cavities and/or differentiation of olfactory neurons is unclear [Ghyselinck et al., 1998; Luo et al., 1995]. It would be of interest to investigate whether \textit{Rarb}-null mutant mice have an impaired olfaction.

Cleft palate is one of the major malformations induced by excess RA in rodent models ([Cuervo et al., 2002] and references therein). Lack of fusion of the palatal shelves
is also seen in compound Rara/Rarg mutants [Lohnes et al., 1994], and mice deficient for endogenous RA synthesis (P. Dollé, unpublished observations). All three Rars are expressed during palatal shelf development, with Rarb levels increasing by E13.5 [Naitoh et al., 1998]. Distinct Rar expression patterns have also been described during tooth bud development [Bloch-Zupan et al., 1994], although the functional involvement of RARs in odontogenesis remains unclear.

**Glandular structures**

Two Rar genes show prominent expression in the developing pituitary gland: Rara (mainly the Rara1 isoform), expressed throughout the anterior pituitary anlage, and Rarb2, preferentially expressed in the periphery of the gland [Mollard et al., 2000]. Rarg is also specifically expressed in the developing pituitary gland [Dolle et al., 1994], which furthermore expresses RA-synthesizing enzymes [Fujinava et al., 2007]; the involvement of retinoid signaling in pituitary development has not been elucidated, however. Apart from Rara, no Rar transcripts were detected in the developing thyroid gland [Dolle et al., 1990]. Rarb and Rarg show differential distributions within the developing salivary glands, in mesenchymal and epithelial cells, respectively [Dolle et al., 1990]. Other glandular systems with Rarg expression include the ocular Harderian glands, which are absent in the corresponding null mutants [Lohnes et al., 1993].

**Tissue differentiation and organogenesis**

**Tissue-specific versus ubiquitous expression**

Expression of the retinoid receptors during organogenesis has mainly been studied in the mouse [Dolle et al., 1994; Dolle et al., 1990; Ruberte et al., 1990] (see Table 1 for a summary). Although three of the receptors (RARβ, RARγ and RXRγ) exhibit complex, differential expression features, only in some instances do their distributions correlate with specific differentiating cell- or tissue-types throughout the organism. This is the case for Rarg, which is expressed in all precardilaginous cell condensations (see Figure 3B,C and Figure 4D,E), irrespective of their embryological origin [Ruberte et al., 1990]. The same receptor is also expressed in the developing skin epithelium, as well as in all prospective squamous keratinizing epithelia, including the esophagus and left wall of the stomach (Figure 3D) [Ruberte et al., 1990]. Loss of function of mouse RARγ does not lead to any overall defect in chondrogenesis, or histogenesis of the skin and squamous epithelia (although Rarg−/− mutants have squamous metaplasia of the seminal vesicles and prostate [Lohnes et al., 1993]). As observed at early embryonic stages, murine Rara is expressed nearly ubiquitously in differentiating organs [Dolle et al., 1990]. Rarb has more discrete expression features, which are reviewed below.

Among the RXRs, Rxra is expressed rather ubiquitously, with higher levels in developing skin epidermis by late gestation [Dolle et al., 1994]. Rxb is also expressed ubiquitously, and at low levels. Rxrg is expressed in all developing skeletal muscles. The functional significance of this expression is unclear, as the Rxrg−/− mouse mutants are viable and have no muscular defect, even in compound mutant combinations.

**Limb development**

Much attention has been devoted to the developing limbs, as early studies performed even before the cloning of RARs have shown (i) that RA applied locally can lead to mirror-image digit duplications in the chick wing bud, thus mimicking ectopic grafts of the posterior ‘zone of polarizing activity’ (ZPA) ([Tickle, 2006] and references therein); (ii) that endogenous RA is present at high concentrations in the posterior wing bud mesenchyme, which suggested a putative role as a diffusible morphogen [Thaller and Eichele, 1987]. The three Rar genes are expressed during early limb bud development, Rara being ubiquitous, Rarg being found throughout the limb bud mesenchyme before being localized to precardilaginous blastemas, and Rarb being present only in the proximal mesenchyme [Dolle et al., 1989; Smith and Eichele, 1991]. Eventually, it was shown that RA is produced in the flank and proximal limb bud cells by the RALDH2 enzyme, from which it likely acts in conjunction with posteriorly-restricted factors (such as Hand2) to induce a functional ZPA [Mic et al., 2004; Niederreither et al., 2002]. Another, later function of RA concerns the involution of the interdigital mesenchyme (Figure 5). Rarb (mainly the Rarb2 isoform) is specifically expressed in the prospective interdigital zones of the fore- and hindlimbs (Figure 5B,C), similar to the RA-synthesizing enzyme RALDH2 [Dolle et al., 1989; Mollard et al., 2000; Niederreither et al., 1997]. Abnormal interdigital webbing is indeed seen in several Rar and/or Rxra compound mutant genotypes [Dupe et al., 1999a] and references therein).

**Heart and vascular system**

As observed in other tissues, Rara is expressed rather ubiquitously during heart development, both of its isoforms being detected in the myocardium [Mollard et al., 2000]. The other Rars have more restricted distributions, Rarb1 being present in the conotruncal mesenchyme [Ghyselinck et al., 1998], and Rarb2 throughout the developing myocardium [Mollard et al., 2000]. Rarg transcripts are specifically detected in the endocardial cushion tissue and the developing large vessels at E12.5 [Dolle et al., 1990]. None of the Rxr genes display restricted expression patterns in the cardiovascular system.

The heart is a major target organ of retinoid signaling during development, with RA being involved in morphogenetic events [Dickman and Smith, 1996; Niederreither et al., 2001; Zile et al., 2000], outflow tract septation and large vessel patterning [Ghyselinck et al., 1998; Gruber et al., 1996; Mendelsohn et al., 1994b] and regulation of cardiomyocyte differentiation [Kastner et al., 1997; Niederreither et al., 2001]. Gene knockout studies have highlighted the receptors involved in these
RAR expression in the differentiating limb. Comparative ISH of RARβ1/3, RARβ2/4, RARγ1 and RARγ2 on serial sections through the extremity (footplate) of an E13.5 hindlimb. 35S-labelled probes. Abbreviations: dc, digit precartilaginous condensation; fp, footplate; id, interdigital domain; mg, midgut; mu, muscle; zp, zeugopod (tibia/fibula region). From (Mollard et al., 2000).

processes (for review and references, see [Mark et al., 2009]), in particular they have revealed an important function of RARβ isoforms for the development of conotruncal ridges [Ghyselinck et al., 1998]. RXRa, on the other hand, is indispensable for proper cardiomyocyte differentiation and development of the trabecular myocardium. The exact tissue- and cell-types where retinoid-induced effects take place are not fully characterized, although a recent conditional gene knockout study has demonstrated that RXRa function is critical within the epicardium [Merki et al., 2005]. RA has also been shown to be required for proper morphogenesis and remodeling of the extra-embryonic vascular network, and RARα isoforms are thought to be involved in this process [Bohnsack et al., 2004].

Lung

The Rar genes are differentially expressed during lung development. Rarb is expressed at high levels in the foregut endoderm prior to lung budding (see Figure 2B,E) [Ruberte et al., 1991; Smith, 1994], and is most likely present in the early lung bud endoderm. During branching morphogenesis, it remains expressed along the endoderm of the trachea and the proximal (large) bronchi, but is absent in the distal bronchi and the developing alveolar epithelia (Figure 6B) [Dolle et al., 1990]. Rarg is also expressed, albeit in a more homogeneous manner, in developing lung tissues (Figure 6C). There is ample evidence that RA regulates lung development [Desai et al., 2006; Mendelsohn et al., 1994b], and the RA-dependent molecular interactions are beginning to be unravelled [Chen et al., 2007; Wang et al., 2006]. It appears that RA signaling is required for initial budding and early lung branching, but needs to be downregulated for more distal branching and distal airway formation to proceed to completion [Malpel et al., 2000; Wongtrakool et al., 2003]. In rat, there is persistent expression of RARs in the fetal and neonatal lung [Grummer et al., 1994; Grummer and Zachman, 1995], suggesting that RA might also function during lung maturation. Indeed, exogenous RA stimulates alveoli formation in immature rat and mouse lung [Massaro and Massaro, 2000] and murine RARs and RARβ do regulate the septation of alveoli at distinct time points of postnatal lung maturation ([Massaro and Massaro, 2000; Massaro
et al., 2003]; also see [Hind et al., 2002; Maden, 2004; Maden and Hind, 2004; Stinchcombe and Maden, 2008]).

Digestive tract

Whereas Rara is expressed throughout the developing digestive tract, Rarb expression is spatially restricted: it is found in both the epithelial and mesenchymal layers of the mesentery and the anterior (cardiac) portion of the stomach, is absent at pyloric and duodenal levels (Figure 6D,E), and reappears more posteriorly at midgut levels [Dolle et al., 1990]. As previously mentioned, Rarg transcripts are specific to the differentiating squamous epithelium in the oesophagus and left wall of the stomach (Figure 3D) [Ruberte et al., 1990]. The Rarb2 isoform is expressed in the developing diaphragm and liver capsule, whereas both Rarb isoforms are expressed in the pancreas primordium (Figure 6D,E). Whether their expression is restricted to a given cell lineage has not been determined. RA produced by the RALDH2 enzyme was recently shown to be required for early development of the dorsal pancreas in the mouse [Martin et al., 2005; Molotkov et al., 2005].

Kidney and urogenital tract

Expression of Rarb is seen in the mesonephros (the embryonic kidney derived from the intermediate mesoderm), and eventually appears in the mesonephros or definitive kidney (Figure 6F,G). Expression is found in the kidney stromal mesenchyme, rather than in cells of the developing nephrons [Mendelsohn et al., 1999], and it is the Rarb2 isoform which is predominantly (or exclusively) expressed [Mollard et al., 2000]. Rara is also expressed throughout the developing kidney, and Rara/Rarb2 compound mutants display abnormal kidney development [Batourina et al., 2001; Mendelsohn et al., 1999]. Both Rarb isoforms are also expressed according to distinct spatial domains in the mesenchyme surrounding the urogenital sinus, ureters and developing genital tract. Additional abnormalities in Rara/Rarb2 mutant mice include an incorrect insertion of the ureters into the developing bladder, as well as agenesis of the Mullerian ducts (precursors of the oviduct and uterus) [Batourina et al., 2001; Mendelsohn et al., 1994b].

Expression of RARs and RXRs in prenatal gonads and germ cells has not been studied in the same detail, for instance, as in adult mouse testis ([Vernet et al., 2006] and references therein). Thus, it is currently unclear which receptor(s) mediate the recently-discovered role of RA as a meiosis-inducing factor in female germ cells during development ([Bowles and Koopman, 2007] for review, and references therein).

Acknowledgements

I would like to deeply thank Prof. P. Chambon for his confidence, and many past and present colleagues including C. Mendelsohn, K. Niederreither, C. Rochette-Egly, R. Taneja, M. Petkovich, D. Lohnes, T. Lufkin, P. Bouillet, N. Ghyselinck, P. Kastner, W. Krezel, M. Mark, D. Metzger, M. Oulad-Abdelghani, F. Rijli, and R. Romand, for sharing their expertise and for great interactions. Current work is funded by the CNRS, INSERM, Agence Nationale de la Recherche, Fondation pour la Recherche Médicale, and European Union (EURExpress: LSHG-CT-2004-512003; EVI-GENORET: LSHG-CT-2005-512036).

References

Abu-Abed, S., Dolle, P., Metzger, D., Wood, C., MacLean, G., Chambon, P. and Petkovich, M. (2003) Developing with lethal RA levels: genetic ablation of Rarg can restore the viability of mice lacking Cyp26a1 Development 130, 1449-59.

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

Ang, H. L. and Duester, G. (1997) Initiation of retinoid signaling in primitive streak mouse embryos: spatiotemporal expression patterns of receptors and metabolic enzymes for ligand synthesis Dev Dyn 206, 536-43.

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.

Bloch-Zupan, A., Decimo, D., Loriot, M., Mark, M. P. and Ruch, J. V. (1994) Expression of nuclear retinoid acid receptors during mouse odontogenesis Differentiation 57, 195-203.

Blumberg, B., Mangelsdorf, D. J., Dyck, J. A., Bittner, D. A., Evans, R. M. and De Robertis, E. M. (1992) Multiple retinoid-responsive receptors in a single cell: families of retinoid “X” receptors and retinoid acid receptors in the Xenopus egg Proc Natl Acad Sci U S A 89, 2321-5.

Bohnsack, B. L., Lai, L., Dolle, P. and Hirschi, K. K. (2004) Signaling hierarchy downstream of retinoid acid that independently regulates vascular remodeling and endothelial cell proliferation Genes Dev 18, 1345-58.

Bowles, J. and Koopman, P. (2007) Retinoid acid, meiosis and germ cell fate in mammals Development 134, 3401-11.

Chen, F., Desai, T. J., Qian, J., Niederreither, K., Lu, J. and Cardoso, W. V. (2007) Inhibition of Tgf β signaling by endogenous retinoid acid is essential for primary lung bud induction Development 134, 2969-73.

Chiang, M. Y., Misner, D., Kempermann, G., Schikorski, T., Giguere, V., Sucov, H. M., Gage, F. H., Stevens, C. F. and Evans, R. M. (1998) An essential role for retinoid receptors RARbeta and RXRgamma in long-term potentiation and depression Neuron 21, 1353-61.

Colbert, M. C., Rubin, W. W., Linney, E. and LaMantia, A. S. (1995) Retinoid signaling and the generation of regional and cellular diversity in the embryonic mouse spinal cord Dev Dyn 204, 1-12.

Cuervo, R., Valencia, C., Chandraratna, R. A. and Covarrubias, L. (2002) Programmed cell death is required for palate shelf fusion and is regulated by retinoic acid Dev Biol 245, 145-56.

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 α and β in early lung morphogenesis Dev Biol 291, 12-24.
Dickman, E. D. and Smith, S. M. (1996) Selective regulation of cardiomyocyte gene expression and cardiac morphogenesis by retinoic acid Dev Dyn 206, 39-48.

Diez del Corral, R., Olivera-Martinez, I., Gorily, A., Gale, E., Maden, M. and Storey, K. (2003) Opposing FGF and retinoid pathways control ventral neural pattern, neuronal differentiation, and segmentation during body axis extension Neuron 40, 65-79.

Diez del Corral, R. and Storey, K. G. (2004) Opposing FGF and retinoid pathways: a signalling switch that controls differentiation and patterning onset in the extending vertebrate body axis Bioessays 26, 857-69.

Dolle, P., Fraulob, V., Kastner, P. and Chambon, P. (1994) Developmental expression of murine retinoid X receptor (RXR) genes Mech Dev 45, 91-104.

Dolle, P., Ruberte, E., Kastner, P., Petkovich, M., Stoner, C. M., Gudas, L. J. and Chambon, P. (1989) Differential expression of genes encoding α, β and γ retinoic acid receptors and CRABP in the developing limbs of the mouse Nature 342, 702-5.

Dolle, P., Ruberte, E., Leroy, P., Morris-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, 1133-51.

Dreyer, C. and Ellinger-Ziegelbauer, H. (1996) Retinoic acid receptors and nuclear orphan receptors in the development of Xenopus laevis Int J Dev Biol 40, 255-62.

Duester, G. (2007) Retinoic acid regulation of the somitogenesis clock Birth Defects Res C Embryo Today 81, 84-92.

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., 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 α and β in the patterning of the caudal hindbrain, pharyngeal arches and otocyst in the mouse Development 126, 5051-9.

Ellinger-Ziegelbauer, H. and Dreyer, C. (1991) A retinoic acid receptor expressed in the early development of Xenopus laevis Genes Dev 5, 94-104.

Ellinger-Ziegelbauer, H. and Dreyer, C. (1993) The pattern of retinoic acid receptor γ (RAR γ) expression in normal development of Xenopus laevis and after manipulation of the main body axis Mech Dev 41, 33-46.

Fujiiwara, K., Maekawa, F., Kikuchi, M., Takigami, S., Yoda, T. and Yashiro, T. (2007) Expression of retinaldehyde dehydrogenase (RALDH)2 and RALDH3 but not RALDH1 in the developing anterior pila of the rats Cell Tissue Res 328, 129-35.

Ghyselinck, N. B., Wendling, O., Messadegh, N., Dierich, A., Lampron, C., Decimo, D., Viville, S., Chambon, P. and Mark, M. (1998) Contribution of retinoic acid receptor β isoforms to the formation of the conotruncal septum of the embryonic heart Dev Biol 198, 303-5.

Glover, J. C., Renaud, J. S. and Rijli, F. M. (2006) Retinoic acid and hindbrain patterning J Neurobiol 66, 705-25.

Gruber, P. J., Kubalak, S. W., Pexieder, T., Sucov, H. M., Evans, R. M. and Chien, K. R. (1996) RXR α deficiency confers genetic susceptibility for aortic sac, constriction, atrioventricular cushion, and ventricular muscle defects in mice J Clin Invest 98, 1332-43.

Grummer, M. A., Thet, L. A. and Zachman, R. D. (1994) Expression of retinoic acid receptor genes in fetal and newborn rat lung Pediatr Pulmonol 17, 234-8.

Grummer, M. A. and Zachman, R. D. (1995) Postnatal rat lung retinoic acid receptor (RAR) mRNA expression and effects of dexamethasone on RAR β mRNA Pediatr Pulmonol 20, 234-40.

Hale, L. A., Tallafuss, A., Yan, Y. L., Dudley, L., Eisen, J. S. and Postlethwait, J. H. (2006) Characterization of the retinoic acid receptor genes raraa, rarab and rarg and during zebrafish development Gene Expr Patterns 6, 546-56.

Halliagac, R., Ribes, V., Ghyselinck, N. B., Zile, M. H., Dolle, P. and Studer, M. (2007) Retinoic acids control anterior and dorsal properties in the developing forebrain Dev Biol 303, 362-75.

Hernandez, R. E., Putzke, A. P., Myers, J. P., Pargrearta, L. M. and Moens, C. B. (2007) Cyp26 enzymes generate the retinoic acid response pattern necessary for hindbrain development Development 134, 177-87.

Hind, M., Corcoran, J. and Maden, M. (2002) Temporal/spatial expression of retinoid binding proteins and RAR isoforms in the postnatal lung Am J Physiol Lung Cell Mol Physiol 282, L468-76.

Hoover, F., Gundersen, T. E., Ulven, S. M., Michaille, J. J., Blanchet, S., Blohmoff, R. and Glover, J. C. (2001) Quantitative assessment of retinoid signaling pathways in the developing eye and retina of the chicken embryo J Comp Neurol 436, 324-35.

Ji, S. J., Zhuang, B., Falco, C., Schneider, A., Schuster-Gossler, K., Gossler, A. and Sockanathan, S. (2006) Mesenterial and neuronal retinoids regulate the induction and maintenance of limb innervating spinal motor neurons Dev Biol 297, 249-61.

Kastner, P., Messadegh, N., Mark, M., Wendling, O., Grondona, J. M., Ward, S., Ghyselinck, N. and Chambon, P. (1997) Vitamin A deficiency and mutations of RXRalpha, RXRbeta and RARalpha lead to early differentiation of embryonic ventricular cardiomyocytes Development 124, 4749-58.

Kelley, M. W., Turner, J. K. and Reh, T. A. (1994) Retinoic acid promotes differentiation of photoreceptors in vitro Development 120, 2091-102.

Koide, T., Downes, M., Chandraratna, R. A., Blumberg, B. and Umesono, K. (2001) Active repression of RAR signaling is required for head formation Genes Dev 15, 2111-21.

Krezel, W., Kastner, P. and Chambon, P. (1999) Differential expression of retinoic receptors in the adult mouse central nervous system Neuroscience 89, 1291-300.

Krezel, W., Ghyselinck, N., Samad, T. A., Dupe, V., Kastner, P., Borrelli, E. and Chambon, P. (1998) Impaired locomotion and dopamine signaling in retinoic acid receptor mutant mice Science 279, 863-7.

Li, H., Wagner, E., McAfferty, P., Smith, D., Andreas, A. and Drager, U. C. (2000) A retinoic acid synthesizing enzyme in ventral retina and telencephalon of the embryonic mouse Mech Dev 95, 263-9.

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, 643-58.

Lohones, D., Mark, M., Mendelsohn, C., Dolle, P., Dierich, A., Gorry, P., Ganssmuller, 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.

Luo, J., Pasceri, P., Conlon, R. A., Rossant, J. and Giguere, V. (1995) Mice lacking all isoforms of retinoic acid receptor β develop normally and are susceptible to the teratogenic effects of retinoic acid Mech Dev 53, 61-71.


**Review**

Maden, M. and Hind, M. (2004b) Retinoic acid in alveolar development, maintenance and regeneration *Philos Trans R Soc Lond B Biol Sci* **359**, 799-808.

Maden, M. (2002) Retinoid signalling in the development of the central nervous system *Nat Rev Neurosci* **3**, 843-53.

Maden, M. (2006) Retinoids and spinal cord development *J Neurobiol* **66**, 726-38.

Maden, M. (2004a) Retinoids in lung development and regeneration *Curr Top Dev Biol* **61**, 153-89.

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

Mark, M., Ghyselinck, N. B. and Chambon, P. (2009) Function of retinoic acid receptors during embryonic development *Nucl Recept Signal* **7**, e002.

Martin, M., Gallego-Llanas, J., Ribes, V., Kedinger, M., Niederreither, K., Chambon, P., Dolle, P. and Gradwohl, G. (2005) Dorsal pancreas agenesis in retinoic acid-deficient Raldh2 mutant mice *Dev Biol* **284**, 399-411.

Massaro, G. D., Massaro, D. and Chambon, P. (2003) Retinoic acid receptor-α 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. and Massaro, D. (2000) Retinoic acid treatment partially rescues failed septation in rats and in mice *Am J Physiol Lung Cell Mol Physiol* **278**, 1955-60.

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

McCaffery, P., Lee, M. O., Wagner, M. A., Sladek, N. E. and Drager, U. C. (1992) Asymmetrical retinoic acid synthesis in the dorsolateral axis of the retina *Development* **115**, 371-82.

Mendelsohn, C., Ruberto, E., LeMeur, M., Morriss-Kay, G. and Chambon, P. (1991) Developmental analysis of the retinoic acid-inducible RARβ 2 promoter in transgenic animals *Development* **113**, 723-34.

Mendelsohn, C., Lohnes, D., Decimo, D., Luftin, T., LeMeur, M., Chambon, P. and Mark, M. (1994b) 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., Larkin, S., Mark, M., LeMeur, M., Clifford, J., Zelent, A. and Chambon, P. (1994a) RARβ isoforms: distinct transcriptional control by retinoic acid and specific spatial patterns of promoter activity during mouse embryonic development *Mech Dev* **45**, 227-41.

Mendelsohn, C., Batourina, 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., Kubakal, S. W., Kaliman, P., Belmonte, J. C., Chien, K. R. and Ruiz-Lozano, P. (2005) Epicardial retinoid X receptor α is required for myocardial growth and coronary artery formation *Proc Natl Acad Sci U S A* **102**, 18455-60.

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

Mohan, M., Malayer, J. R., Geisert, R. D. and Morgan, G. L. (2001) Expression of retinol-binding protein messenger RNA and retinoic acid receptors in preattachment bovine embryos *Mol Reprod Dev* **60**, 289-96.

Mohan, M., Malayer, J. R., Geisert, R. D. and Morgan, G. L. (2002) Expression patterns of retinoid X receptors, retinol dehydrogenase, and peroxisome proliferator activated receptor γ in bovine preattachment embryos *Biol Reprod* **66**, 692-700.

Mollard, R., Vville, S., Ward, S. J., Decimo, D., Chambon, P. and Dolle, P. (2000) Tissue-specific expression of retinoic acid receptor isoform transcripts in the mouse embryos *Mech Dev* **94**, 223-32.

Molotkov, A., Molotkova, N. and Duester, G. (2005) Retinoid acid generated by Raldh2 in mesoderm is required for mouse dorsal endodermal pancreas development *Dev Dyn* **232**, 950-7.

Molotkov, A., Molotkova, N. and Duester, G. (2006) Retinoic acid guides eye morphogenetic movements via paracrine signaling but is unnecessary for retinal dorsosventral patterning *Development* **133**, 1901-10.

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.

Naitoh, H., Mori, C., Nishimura, Y. and Shiota, K. (1998) Altered expression of retinoic acid (RA) receptor mRNAs in the fetal mouse secondary palate by all-trans and 13-cis RAs: implications for RA-induced teratogenesis *J Craniofac Genet Dev Biol* **18**, 202-10.

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., Schuhbaur, 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., Schuhbaur, B., Chambon, P. and Dolle, P. (2002) Embryonic retinoic acid synthesis is required for forelimb growth and anteroposterior patterning in the mouse *Development* **129**, 3583-74.

Niederreither, K., McCaffery, P., Drager, U. C., Chambon, P. and Dolle, P. (1997) Restricted expression and retinoic acid-induced downregulation of the retinaldehyde dehydrogenase type 2 (RALDH-2) gene during mouse development *Mech Dev* **62**, 67-78.

Niederreither, K., Vermot, J., Le Roux, I., Schuhbaur, B., Chambon, P. and Dolle, P. (2003) The regional pattern of retinoic acid synthesis by RALDH2 is essential for the development of posterior pharyngeal arches and the enteric nervous system *Development* **130**, 2525-34.

Pfeffer, P. L. and De Robertis, E. M. (1994) Regional specificity of RAR γ isoforms in Xenopus development *Molec Dev* **45**, 147-53.

Prabhudesai, S. N., Cameron, D. A. and Stenkamp, D. L. (2005) Targeted effects of retinoic acid signalling upon photoreceptor development in zebrafish *Dev Biol* **287**, 157-67.

Raz, Y. and Kelley, M. W. (1999) Retinoic acid signaling is necessary for the development of the organ of Corti *Dev Biol* **213**, 180-93.

Rejintjes, S., Blentic, A., Gale, E. and Maden, M. (2005) The control of morphogen signalling: regulation of the synthesis and catabolism of retinoic acid in the developing embryo *Dev Biol* **285**, 224-37.

Ribes, V., Le Roux, I., Rhinn, M., Schuhbaur, B. and Dolle, P. (2009) Early mouse caudal development relies on crosstalk between retinoid, Shh and Fgf signalling pathways *Development* **136**, 665-76.

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 signalling *Development* **133**, 351-61.

Ribes, V., Fratol, V., Petkovich, M. and Dolle, P. (2007) The oxidizing enzyme CYP26A1 tightly regulates the availability of retinoic acid in the gastrulating mouse embryo to ensure proper head development and vasculogenesis *Dev Dyn* **236**, 644-53.

Romand, R., Sapin, V. and Dolle, P. (1998) Spatial distributions of retinoic acid receptor gene transcripts in the prenatal mouse inner ear *J Comp Neurol* **393**, 299-308.

Romand, R., Hashino, E., Dolle, P., Vonesch, J. L., Chambon, P. and Ghyselinck, N. B. (2002) The retinoic acid receptors RARα and
Ulven, S. M., Gundersen, T. E., Weedon, M. S., Landaas, V. O., Sakhi, of migratory cranial neural crest cells in the mouse anterior-posterior patterning of the developing brain and the production and Sakai, Y. (2007) CYP26A1 and CYP26C1 cooperatively regulate Uehara, M., Yashiro, K., Mamiya, S., Nishino, J., Chambon, P., Dolle, P. and Le Roux, I. (2005c) Retinoldehydrogenase 2 and Hoxc8 are required in the murine brachial spinal cord for the specification of Lmx1+ motoneurons and the correct distribution of Islet1+ motoneurons Development 132, 1611-21.

Vermot, J., Gallego Llanas, J., Fraulob, V., Niederreither, K., Chambon, P. and Dolle, P. (2005a) Retinoic acid controls the bilateral symmetry of somite formation in the mouse embryo. Science 308, 563-6.

Vermot, J. and Pourquie, O. (2005b) Retinoic acid coordinates somitogenesis and left-right patterning in vertebrate embryos Nature 435, 215-20.

Vernet, N., Dennefeld, C., Rochette-Egly, C., Oualid-Abdelghani, M., Chambon, P., Ghyselinck, N. B. and Mark, M. (2006) Retinoic acid metabolism and signaling pathways in the adult and developing mouse testis Endocrinology 147, 96-110.

Wagner, E., McCaffery, P. and Drager, U. C. (2000) Retinoic acid in the formation of the dorsalventral retina and its central projections Dev Biol 222, 460-70.

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.

Waxman, J. S. and Yelon, D. (2007) Comparison of the expression patterns of newly identified zebrafish retinoic acid and retinoid X receptors Dev Dyn 236, 587-95.

Wendling, O., Dennefeld, C., Chambon, P. and Mark, M. (2000) Retinoic signaling is essential for patterning the endoderm of the third and fourth pharyngeal arches Development 127, 1553-62.

Wietrzych, M., Meziane, H., Sutter, A., Ghyselinck, N., Chapman, P. F., Chambon, P. and Kreuzel, W. (2005) Working memory deficits in retinoid X receptor γ-deficient mice Learn Mem 12, 318-26.

Wilson, L. and Maden, M. (2005) The mechanisms of dorsalventral patterning in the vertebral neural tube Dev Biol 282, 1-13.

Wongtrakool, C., Malpel, S., Gorenstein, J., Sedita, J., Ramirez, M. I., Underhill, T. M. and Cardoso, W. V. (2003) Down-regulation of retinoic acid receptor α signaling is required for sacculaon and type I cell formation in the developing lung J Biol Chem 278, 46911-8.

Zetterstrom, R. H., Lindqvist, E., Mata de Urquiza, A., Tomac, A., Eriksson, U., Perlmann, T. and Olson, L. (1999) Role of retinoic acid in the CNS: differential expression of retinoid binding proteins and receptors and evidence for presence of retinoic acid Eur J Neurosci 11, 407-16.

Zile, M. H., Kostetskii, I., Yuan, S., Kostetskaia, E., St Amand, T. R., Chen, Y. and Jiang, W. (2000) Retinoid signaling is required to complete the vertebrate cardiac left/right asymmetry pathway Dev Biol 233, 323-38.