Developmental expression of retinoic acid receptors (RARs)

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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 (*Rxrg*) 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.

Received February 6th, 2009; Accepted April 21st, 2009; Published May 12th, 2009 | **Abbreviations:** **CYP26:** cytochrome P450, subfamily 26; **E:** embryonic day; **r:** rhombomere; **RA:** retinoic acid; **RALDH:** retinaldehyde dehydrogenase; **RAR:** retinoic acid receptor; **RT-PCR:** reverse transcriptase-polymerase chain reaction; **RXR:** retinoid X (rexinoid) receptor; **ZPA:** zone of polarizing activity

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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 (RARβ, RARγ and RXRγ) 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*, *rarab*, *rarga*, *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*, *Rxrg* and *Rxrb*, 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;

	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, cristae	+ (high in cristae, 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

Table 1. Summary of *Rar* 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.

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,

1997]. The functional significance of *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 – presomitic stages

Differential distributions of *Rar* mRNAs become evident in gastrulating embryos of various species. In the mouse, *Rara* and *Rarg* distributions remain diffuse at presomitic stages (E7.5) [Ang and Duester, 1997; Ruberte et al., 1991; Ulven et al., 2000], whereas *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 *raraa* and *rarab* genes have been reported, whereas the two *rarg* genes exhibit diffuse – or poorly detectable – expression [Hale et al., 2006; Waxman and Yelon, 2007]. Studies in Xenopus indicate an accumulation of *Rara2*, and a downregulation of *Rara1*, at early gastrulation stages [Koide et al., 2001; Shiotsugu et al., 2004]. Expression of all *Rxrs* 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 *Rars* and *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 *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., 1992; 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]). *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). *Rarb* (Figure 1D-F) and *Rarg* (Figure 1G-I) show strikingly distinct distributions along the rostro-caudal axis of the embryo. *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, *Rarg* expression is downregulated, both in the neural plate and in mesodermal derivatives, while these tissues initiate differentiation. *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. *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 *Rarb* and *Rarg* (as well as *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 occuring in *Cyp26a1*-null mutant mice are prevented by a compound inactivation of *Rarg* [Abu-Abed et al., 2003].

Nervous system, craniofacial and sensory organ development

Early head, hindbrain and branchial region

Two murine *Rar* genes (*Rara* and *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. *Rara* is expressed until the rhombomere (r)6/7 boundary, and is additionally expressed in r4 [Ruberte et al., 1991]. Although both *Rara1* and *Rara2*

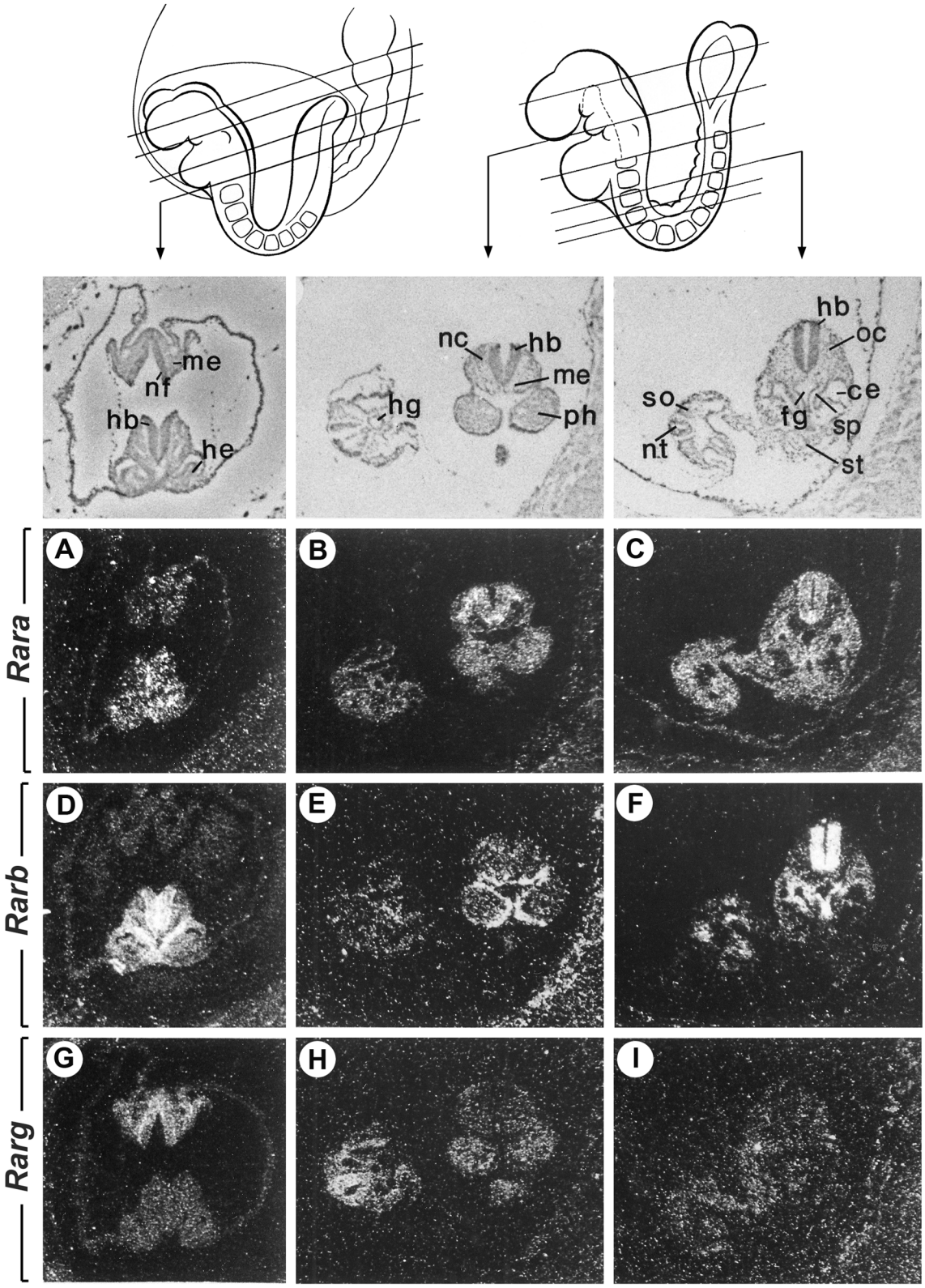

Figure 1. Expression of murine *Rar* genes in somite-stage embryos. Adjacent histological sections (planes of section as indicated by arrows on the schemes) from two embryos at the 8 somite (A,D,G) and 14 somite (B,C,E,F,H,I) stages were hybridized with ^{35}S-labelled riboprobes for *Rara* (A-C), *Rarb* (D-F) and *Rarg* (G-I). Following emulsion autoradiography, sections are viewed under dark-field illumination showing the signal grains in white. Bright-field views are shown above for histology. Abbreviations: ce, celomic cavity; fg, foregut; hb, hindbrain; he, heart; hg, hindgut; me, mesoderm (primary mesenchyme); nc, neural crest; nf, neural folds; nt, neural tube; oc, occipital somite; ph, first pharyngeal (mandibular) arch; so, somite; sp, splanchnopleure; st, stomach primordium. From (Ruberte et al., 1991).

mRNA isoforms are expressed in the neural tube, it is the *Rara2* isoform that shows highest expression and displays a clear rhombomeric boundary [Mollard et al., 2000]. *Rarb* (principally the *Rarb2* 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 mesenchyme, and is thought to act by diffusion towards the hindbrain neuroepithelium (see [Glover et al., 2006; Maden, 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]).

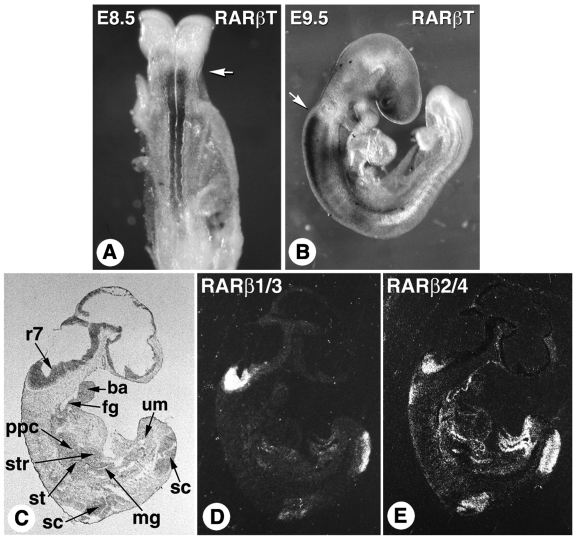

Figure 2. Early expression features of murine *Rarb*. A,B: Whole-mount ISH of E8.5 (dorsal view) and E9.5 (profile view) embryos hybridized with a digoxigenin-labelled *Rarb* probe recognizing all isoforms ('*Rarb* total', RARβT). Arrows point to the sharp expression boundary in the post-otic hindbrain neuroepithelium (r6/7 boundary). C-E: Comparative ISH with ^{35}S-labelled probes specific for RARβ1/3 and RARβ2/4 isoforms, on serial parasagittal sections of an E9.5 embryo. Abbreviations: ba, first branchial arch; fg, foregut; mg, midgut; ppc, peritoneal-pericardial region; r7, rhombomere 7; sc, spinal cord; st, stomach primordium; str, septum transversum; um, umbilical region. (A,B) from (Serpente et al., 2005). (C-E) from (Mollard et al., 2000).

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 mesenchyme, both at the level of the frontonasal mass and the branchial arches (Figure 3A,B) [Ruberte et al., 1990]. The cranial mesenchyme is composed of two cell components, the primary mesenchyme 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 precartilages) (Figure 3C). Both *Rara* and *Rarb* are also expressed in cranial mesenchyme. *Rara* (mainly the *Rara1* isoform) is expressed in a relatively diffuse manner, whereas *Rarb* (mainly *Rarb2*) is strongly expressed in the frontonasal and periocular 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 mesectoderm [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 *Rxrb* are expressed in a diffuse manner throughout these stages, whereas *Rxrg* 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*$^{-/-}$ knockout 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 [Halilagic 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

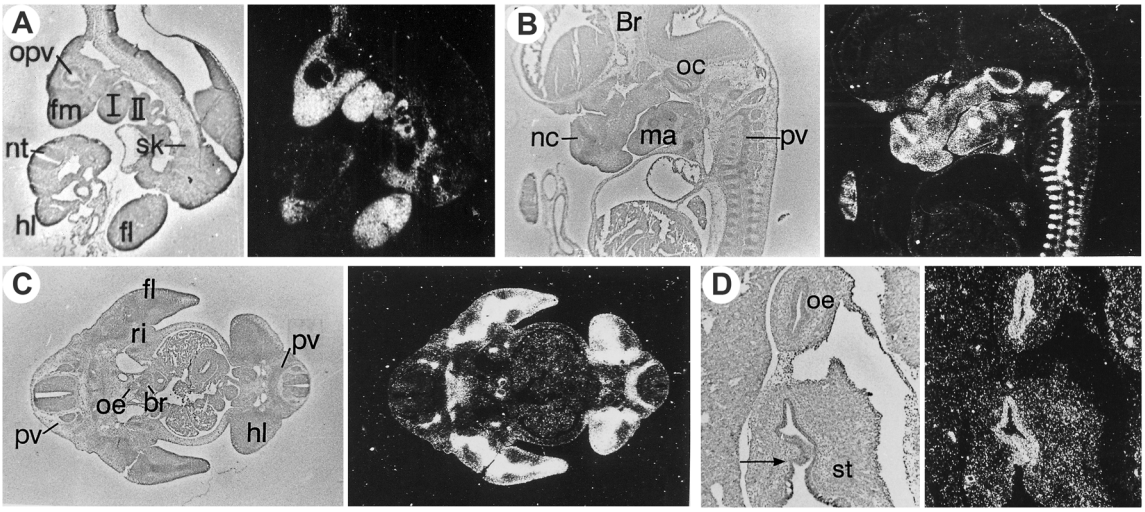

Figure 3. Expression features of murine *Rarg*. Parasagittal sections of E9.5 (A) and E12.5 (B) embryos; transverse section of an E12.5 embryo (C); detail of the oesophagus (oe) and stomach (st) of an E14.5 fetus (D), arrow pointing to the expression boundary at the limit of the squamous stomachal epithelium. ^{35}S-labelled probes. Other abbreviations: I,II, branchial arches; Br, brain; br, stem bronchus; fl, forelimb bud; fm, frontonasal mesenchyme; hl, hindlimb bud; ma, mandible primordium; nc, nasal precartilage; nt, neural tube; oc, otic capsule; opv, optic vesicle; pv, prevertebra; ri, rib anlage; sk, sclerotome. From (Ruberte et al., 1990).

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 *Rxrs*, *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 *Rarg* is expressed more homogeneously throughout the head mesenchyme [Dolle

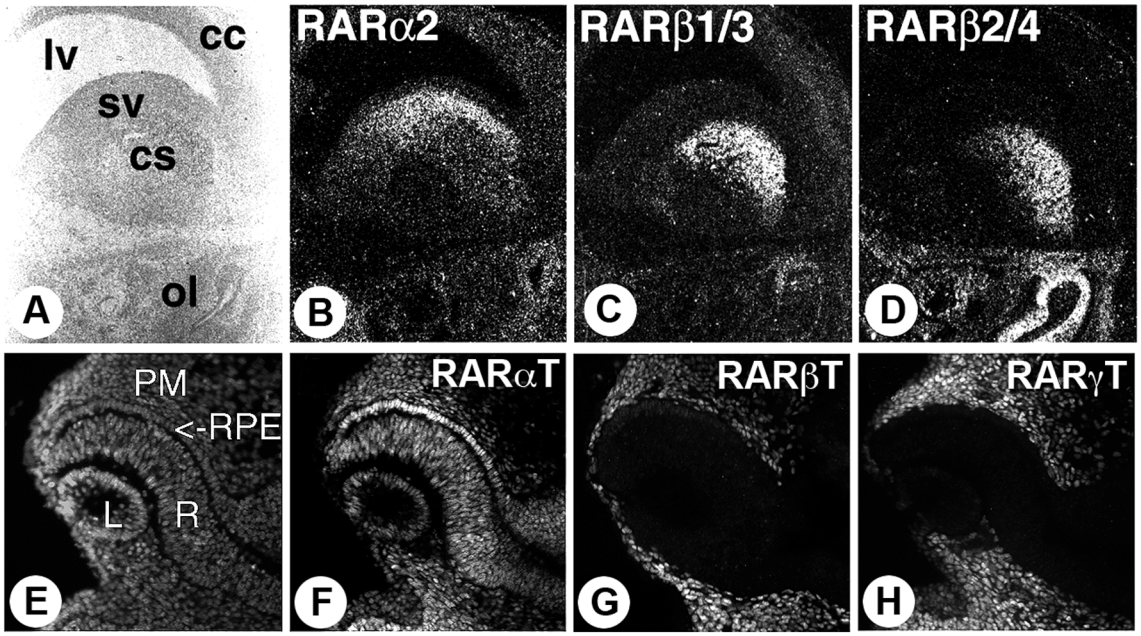

Figure 4. RAR gene and protein expression in the developing brain and eye. (A-D) Comparative ISH of RARα2, RARβ1/3 and RARβ2/4 on neighboring sections of the corpus striatum (lateral ganglionic eminence) of an E13.5 fetal mouse brain. ^{35}S-labelled probes. (E-H) Immunofluorescence detection of RARαT, RARβT and RARγT on serial sections of an E10.5 eye. Abbreviations: cc, cerebral cortex; cs, corpus striatum; L, lens; lv, lateral ventricle; ol, olfactory epithelium; PM, periocular mesenchyme; R, neural retina; RPE, retinal pigmented epithelium; sv, subventricular zone. (A-D) from (Mollard et al., 2000). (E-H) from (Mori et al., 2001).

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]. *Rar* and *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 periocular 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 *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, *Rara* being predominantly expressed in the developing sensory epithelium, *Rarb* in inner ear mesenchymal tissues, and *Rarg* in the differentiating otic capsule. Interestingly, expression of the three *Rar* genes was further detected in the adult inner ear. *Rara* and *Rarg*, in particular, are expressed in the organ of Corti (the cochlear auditory epithelium) [Romand et al., 2002]. RARα and 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, *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; Halilagic et al., 2007]. *Rarb* exhibits region-specific expression in differentiating nasal structures, being present both in the mesenchyme and in specific areas of the olfactory epithelium [Dolle et al., 1990] (see Figure 4D). Knockout mouse models have shown that RARα and RARγ are important for the development of nasal structures [Lohnes et al., 1994]. Whether there is a function of 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 *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]. *Rxrg* is also specifically expressed in the developing pituitary gland [Dolle et al., 1994], which furthermore expresses RA-synthesizing enzymes [Fujiwara 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 precartilaginous 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]. *Rxrb* 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 precartilaginous 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

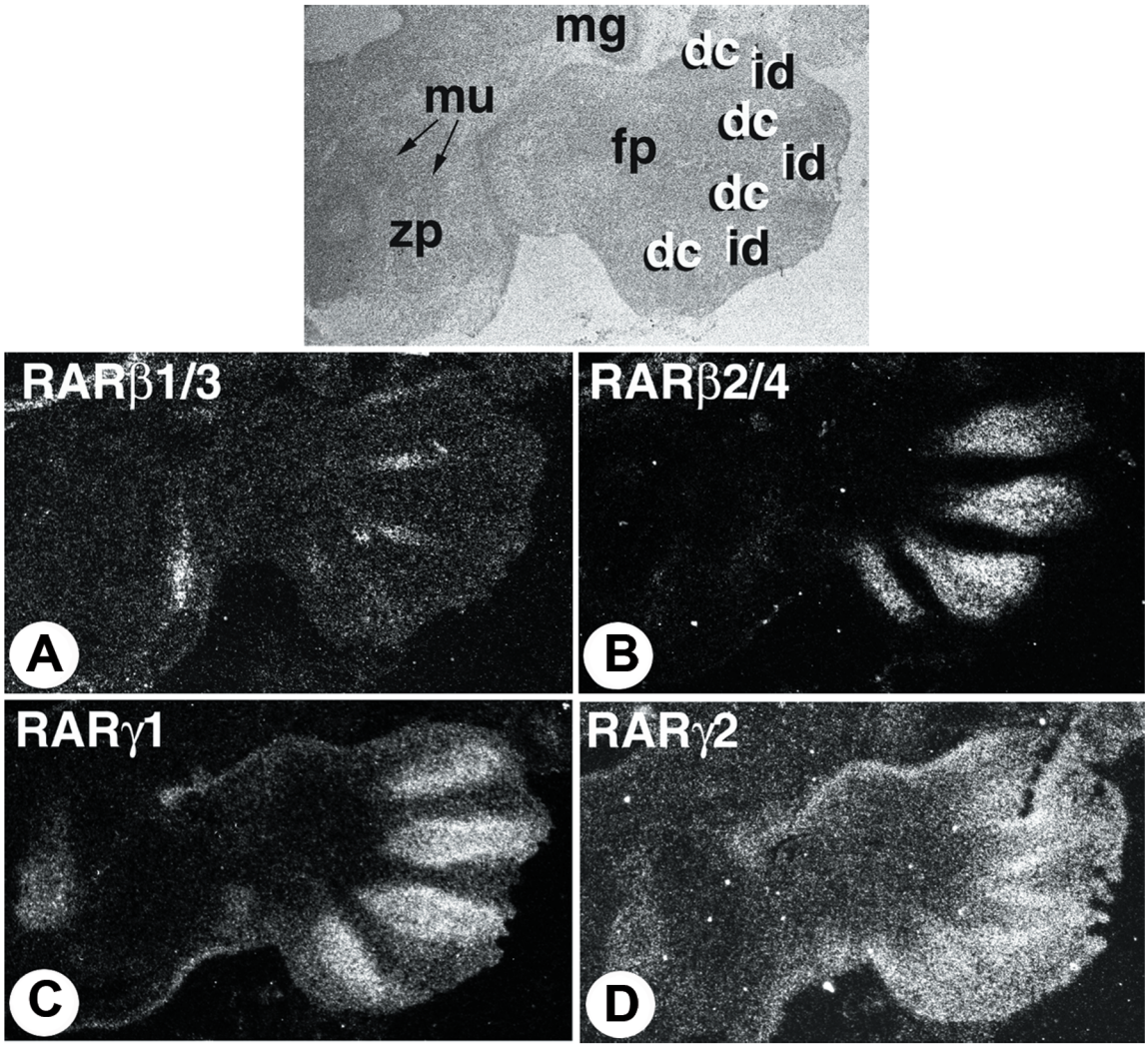

Figure 5. RAR expression in the differentiating limb. Comparative ISH of RARβ1/3, RARβ2/4, RARγ1 and RARγ2 on serial sections through the extremity (footplate) of an E13.5 hindlimb. ^{35}S-labelled probes. Abbreviations: dc, digit precartilaginous condensation; fp, footplate; id, interdigital domain; 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]. RXRα, 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 RXRα 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 lung 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 RARα 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]).

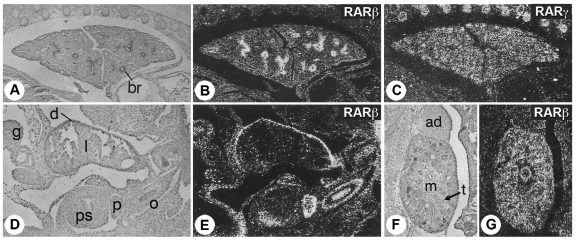

Figure 6. RAR expression in differentiating organs. (A-C) Comparative ISH of *Rarb* and *Rarg* on neighboring sections of the lung of an E14.5 mouse fetus. (D-G) Details of *Rarb* expression in abdominal organs at E12.5, and in the developing kidney at E14.5. ^{35}S-labelled probes. Abbreviations: ad, adrenal gland; br, bronchus; d, diaphragm; g, gut; l, liver; m, metanephros; o, oesophagus; p, pancreas; ps, pyloric region of the stomach; t, metanephric tubules. From (Dolle et al., 1990).

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 oesophagus 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 epithelia 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 metanephros 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 uterers 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).

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