


# The varied roles of nuclear receptors during vertebrate embryonic development

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**Nuclear receptors comprise a superfamily of sequence-specific transcription factors whose members have diverse roles during development. This review will summarize the developmental roles of selected members of the nuclear receptor superfamily.**

Received May 13th, 2003; Accepted June 16th, 2003; Published June 27th, 2003 | **Abbreviations:** **DES:** diethylstilbestrol; **ES cells:** embryonic stem cells | Copyright © 2003, Chung and Cooney. This is an open-access article distributed under the terms of the Creative Commons Non-Commercial Attribution License, which permits unrestricted non-commercial use distribution and reproduction in any medium, provided the original work is properly cited.

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## Introduction

During larval development in *Drosophila*, eight nuclear receptors are specifically expressed and perform specific functions [Thummel, 1995]. Seven of them are orphan receptors, for which ligands have not yet been identified. This phenomenon suggests nuclear receptors play an important role in insect embryonic development. At the end of the third instar larval and prepupal stages, pulses of ecdysone, the molting hormone of insects, directly induce a small set of early genes, such as the orphan nuclear receptor E75. These early genes later repress their own expression and also induce a large set of the late genes, including the orphan nuclear receptors E78B and DHR3. Then the mid-prepupal genes, such as  $\beta$ FTZ-F1, are induced. This cascade of expression of nuclear receptors during *Drosophila* metamorphosis not only suggests that nuclear receptors play crucial roles in regulating normal embryonic development, but also raises a possibility that nuclear receptors participate in regulating each others expression. Although this aspect of vertebrate orphan receptor regulation has not yet been examined in great depth, it may potentially provide another point of convergence in the regulation and function of nuclear receptor superfamily members [Thummel, 1995]. In vertebrate embryonic development, homologs of some *Drosophila* orphan nuclear receptors are found in embryos and loss of function of these receptors causes severe defects. Nuclear receptors clearly play an important role in regulating vertebrate embryonic development. The goal of this review is to summarize the major findings about the expression and function of nuclear receptors during vertebrate embryonic development (Table 1).

## Hepatocyte Nuclear Factor 4 $\alpha$ (HNF4 $\alpha$ ) (NR2A1)

In the adult, HNF4 $\alpha$  is highly expressed at in liver, kidney, intestine, and pancreas and at low levels in the testis [Drewes et al., 1996; Miquerol et al., 1994; Sladek et al., 1990]. During embryonic development, HNF4 $\alpha$  expression is found in the primary endoderm at embryonic day (E) 4.5 and in the visceral endoderm between E5.5 and 8.5 [Duncan et al., 1994]. Hepatic HNF4 $\alpha$  expression is

detected in the liver primordia by E8.5. At E10.5, HNF4 $\alpha$  is expressed in the developing pancreas and the mesonephric tubules. HNF4 $\alpha$  binds as homodimers to hormone responsive elements (HREs) configured as direct repeats with one base pair spacing (DR1) and regulates the expression of genes involved in the metabolism of carbohydrates, lipids, and cholesterol, as well as xenobiotics and amino acids. Targeted deletion of the HNF4 $\alpha$  gene results in apoptosis of the embryonic ectoderm at E6.5, followed by abnormal mesoderm differentiation and embryonic death [Chen et al., 1994]. However, ablation of the HNF4 $\alpha$  gene in either ES cells or E8.5 embryos is associated with significantly reduced expression of glycolytic enzymes, as well as glucose and fatty acid transport proteins [Stoffel and Duncan, 1997]. Embryoid bodies derived from HNF4 $\alpha$ -/- ES cells differentiate into endoderm and express makers of the endoderm lineage, suggesting that it may control the differentiation of visceral endoderm [Duncan et al., 1997]. Its expression in visceral endoderm is regulated by the zinc finger transcription factor, GATA6 [Morrissey et al., 1998]. Recent studies show that HNF4 $\alpha$  can directly regulate pregnane X receptor (PXR) expression during fetal liver development and thus regulate PXR responses to xenobiotics [Kamiya et al., 2003]. Liver-specific knockout of HNF4 $\alpha$  in mice confirms its important role during differentiation of hepatocytes, hepatic storage of glycogen, and hepatic epithelium generation [Parviz et al., 2003]. These results suggest that HNF4 $\alpha$  is crucial for early embryonic development. HNF4 $\alpha$  is the earliest nuclear receptor to be expressed and functional in vertebrate embryos and HNF4 $\alpha$  regulation of PXR is also a prime example of a cascade of nuclear receptor expression.

## Estrogen related receptor $\beta$ (ERR $\beta$ ) (NR3B2)

Slightly later during embryogenesis, at E5.5, ERR $\beta$  transcripts are first detected in extraembryonic ectoderm [Luo et al., 1997; Pettersson et al., 1996]. At E8.5, ERR $\beta$  is specifically expressed in the chorion, suggesting that ERR $\beta$  may play a role in early placental development. The ERR $\beta$  knockout embryos have severely impaired

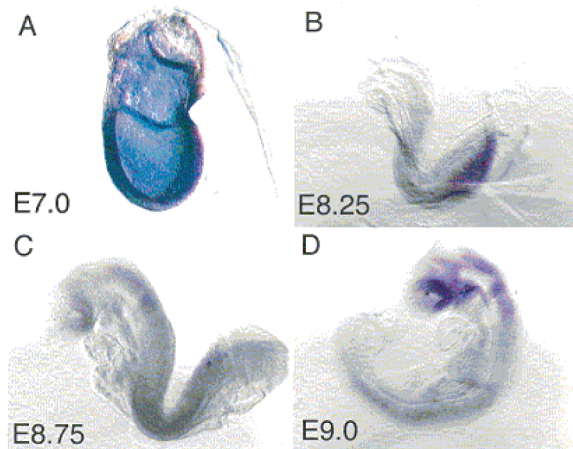
Name	Drosophila Homolog	Expression	Ligand	Lethality	Null mutant	Defects	Reference
HNF4-alpha	HNF4	E4.5 primary endoderm, E5.5 in visceral endoderm, E8.5 in liver primordia, E10.5 in pancreas and mesonephric tubules	NA	Die at E6.5	Apoptosis of embryonic ectoderm at E6.5, abnormal mesoderm differentiation	(Chen et al., 1994; Duncan et al., 1994)	
ERR-beta		E5.5 in extraembryonic ectoderm, E8.5 in chorion	NA	Die at E10.5	Abnormal chorion development and over abundance of trophoblast giant cells	(Luo et al., 1997)	
GCNF		E6.5 in 3 germ layers, E8.5 mainly in the posterior of the trunk	NA	Die at E10.5	Anteroposterior defects Posterior truncation, open neural tube, protruded tail bud,	(Chung et al., 2001)	
PPAR-gamma		E9.5 Anterior part of the embryo		Die at E10	Severe myocardial thinning, improper trophoblast differentiation	(Barak et al., 1999)	
RAR-alpha		In syncytiotrophoblasts and cytotrophoblasts	<i>All trans</i> RA	-	Decreased viability, growth deficiency,	(Ang and Duester, 1997; Lohnes et al., 1994)	
RAR-beta		E6.5 in embryo proper, E8.9 in neural epithelium, E7.5 in presumptive hindbrain, E8.25 in hindbrain and hindgut	<i>All trans</i> RA	-	-	(Ang and Duester, 1997; Lohnes et al., 1994)	
RAR-gamma		E8.5 neural epithelium near posterior neuropore, E13.5 limbs and skin	<i>All trans</i> RA	-	Decreased viability, growth deficiency	(Ang and Duester, 1997; Lohnes et al., 1994)	
RXR-alpha	USP	liver, kidney, spleen	<i>9 cis</i> RA	E12.5-16.5	Vitamin A deficiency syndromes	(Kastner et al., 1994; Mangelsdorf et al., 1992)	
RXR-beta	USP	Central nervous system	<i>9 cis</i> RA	-	-	(Kastner et al., 1994; Mangelsdorf et al., 1992)	
RXR-gamma	USP	Peripheral nervous system	<i>9 cis</i> RA	E12.5-16.5	-	(Kastner et al., 1994; Mangelsdorf et al., 1992)	
COUP-TFI	SVP	E7.5 in neural ectoderm, E8.5 in neural crest cells	NA	perinatal	Fusion of the IX and X cranial nerves, malfunction of sensory and motor function of pharynx and tongue	(Qu et al., 1997)	
COUP-TFII	SVP	In sinus venous, common atrium	NA	E10	Heart and vascular defects	(Pereira et al., 1999)	
SF1	FTZ	E9.0 in urogenital ridge, E10.0 in adrenal steroidogenic tissue and gonadal steroid-producing cells	NA	Perinatal	Complete adrenal and gonadal agenesis	(Ikeda et al., 1994; Luo et al., 1994)	

**Table 1. A summary of nuclear receptors during early embryonic development** See text for more details

placental formation and die owing to a lack of nutrients by E10.5 [Luo et al., 1997]. The ERR $\beta$  knockout embryos exhibit abnormal chorion development associated with an over abundance of trophoblast giant cells and an absence of diploid trophoblasts. The ERR $\beta$  null phenotype can be rescued by aggregation of ERR $\beta$ <sup>-/-</sup> embryos with tetraploid wild-type cells that contribute exclusively to extraembryonic tissues [Luo et al., 1997]. Recently a synthetic estrogen diethylstilbestrol (DES) have been shown to act as an inverse agonist of ERR $\beta$ . DES regulates ERR $\beta$  transcriptional activity and affects normal placenta development [Tremblay et al., 2001]. These results show that ERR $\beta$  is required for normal trophoblast proliferation and differentiation.

## Germ Cell Nuclear Factor (GCNF) (NR6A1)

In the adult, GCNF is highly expressed in germ cells. GCNF binds to DR0 HREs as a homodimer and acts as a transcriptional repressor in the absence of ligand [Hummelke and Cooney, 2001]. GCNF expression is also found in both frog and mouse embryos [Chung et al., 2001; David et al., 1998; Joos et al., 1996]. In *Xenopus*, levels of GCNF expression increase from the gastrula stage to the mid-neural stage [David et al., 1998]. An anteroposterior gradient of GCNF expression then develops at the late neurula stage. In the mouse, GCNF expression is turned on as early as the egg cylinder stage (Figure 1)A. After gastrulation, GCNF expression is detected in the head-fold and throughout the primitive streak [Chung et al., 2001]. Unlike *Xenopus*, a posterior-anterior gradient of GCNF expression develops by the neural stage with the highest levels at presomitic mesoderm in the posterior (Figure 1B). Slightly later, it is expressed at its highest levels throughout the neuroectoderm (Figure 1C). By the late neural stage (Figure 1D), GCNF expression is markedly reduced and GCNF expression turns off subsequently. Changes in GCNF expression in embryos affect normal embryonic development in both frog and mouse. Injection of the full-length or dominant negative (dn) GCNF transcripts into frog embryos disrupts normal embryonic development [David et al., 1998; Joos et al., 1996]. In the mouse, loss of GCNF function by gene targeting results in embryonic lethality, due to cardiovascular complications [Chung et al., 2001]. As GCNF has been shown to repress the *in vitro* expression of Oct4, which is essential for the maintenance of the mammalian germ line, ablation of GCNF in the mouse also shows a failure to repress Oct4 expression in somatic cells [Fuhrmann et al., 2001]. Thus, GCNF is critical for repressing Oct4 gene activity as pluripotent stem cells differentiate and thus play role in



**Figure 1. GCNF expression pattern during post-gastrulation and neuralation stages.** (A-D) Whole mount in situ hybridization of GCNF at E7.0 (A), E8.25 (B), E8.75 (C), and E9.0 (D).

confining Oct4 expression to the germ line. In addition, GCNF<sup>-/-</sup> embryos fail to undergo axial rotation and to close the neural tube. Recent studies in *Xenopus* suggest that GCNF participates in the process of neurulation [Barreto et al., 2003a]. In addition, loss of GCNF affects expression of genes involved in anteroposterior axis formations. GCNF<sup>-/-</sup> embryos also show a halt in somitogenesis, leading to a posterior truncation. These results of the GCNF knockout mice suggest that GCNF is essential for embryonic survival and it is required for normal posterior development, somitogenesis and neural tube closure. Recent studies in *Xenopus* suggests that GCNF can interfere with retinoic acid (RA) signaling during early embryonic development by affecting the expression of, an important catabolic enzyme of RA, cyp26A1 [Barreto et al., 2003b]. This finding suggests that cross-talk exists between GCNF and RA signaling, which is an area ripe for further investigation.

### Peroxisome Proliferator-Activated Receptor $\gamma$ (PPAR $\gamma$ ) (NR1C3)

PPARs mediate the effects of fatty acids and their derivatives at the transcriptional level by forming a heterodimer with RXR [Hihi et al., 2002]. PPAR  $\gamma$  is expressed in syncytiotrophoblasts and cytotrophoblasts in human placental villi [Schaiff et al., 2000]. PPAR  $\gamma$  deficiency causes embryonic lethality at E10 because of severe myocardial thinning [Barak et al., 1999]. In addition, PPAR  $\gamma$  is required for epithelial differentiation of trophoblast tissue, which is important for proper placental vascularization. Unlike PPAR $\beta/\delta$  mutants [Barak et al., 2002], defects in PPAR  $\gamma$ <sup>-/-</sup> placentas affects differentiation of the labyrinthine trophoblast but do not disrupt the placental-decidual interface clearly distinguishing between the placental functions of either PPAR. PPAR  $\gamma$ /RXR $\alpha$  heterodimers have been recently shown to play this crucial role in human trophoblast differentiation [Fournier et al., 2002; Tarrade et al., 2001a; Tarrade et al., 2001b]. Although the loss of PPAR $\beta/\delta$  function causes embryonic lethality at E10.5, this result is contributed by a maternal effect of PPAR $\beta/\delta$  in the uterus and placenta [Barak et al., 2002].

### Retinoid receptors (RARs and RXRs) (NR1B1, 2 & 3 and NR2B1, 2 & 3)

The function and regulation of RA signaling during embryonic development is extremely complex and detailed discussion is beyond the scope of this review. It has been reviewed in detailed elsewhere [Begemann and Meyer, 2001; Kastner et al., 1995; Morriss-Kay and Ward, 1999; Ross et al., 2000; Zile, 2001]. In this minireview we will briefly highlight some important aspects of retinoid receptor function during embryonic development. Expression of different subtypes of RARs and RXRs has been demonstrated in embryos of various animals. In mouse embryos, RAR  $\alpha$  is ubiquitously expressed at E6.5-7.5 [Ang and Duester, 1997; Ruberte et al., 1991]. At E8, RAR  $\alpha$  transcript appears in the neural epithelium of the brain and then at E8.5 it is widely and abundantly expressed in the lateral neural epithelium of both forebrain

and hindbrain. At gastrulation stages, RAR  $\beta$  is expressed primarily in the presumptive hindbrain ectoderm and the adjacent mesenchyme [Ang and Duester, 1997; Ruberte et al., 1991]. At E8.25, RAR  $\beta$  expression is found in the caudal hindbrain region and hind gut [Ruberte et al., 1991; Ruberte et al., 1990]. RAR $\gamma$  is expressed after E8.5 in the open portion of the neural epithelium of the posterior neuropore [Ang and Duester, 1997; Ruberte et al., 1991]. After the neural tube closes, RAR $\gamma$  neural expression disappears. At E13.5, RAR $\gamma$  expression reappears and is strongly expressed in the developing limbs and skin [Dolle et al., 1990].

There is no close relationship between the expression of RARs and RXRs [Mangelsdorf et al., 1992; Mangelsdorf et al., 1990]. In rodent embryos, RXR  $\alpha$  is expressed abundantly in liver, kidney, spleen, visceral tissues and skin [Mangelsdorf et al., 1992]. The expression of RXR  $\beta$  is mainly in the central nervous system, while RXR $\gamma$  is found in the peripheral nervous system and in muscle.

Most of the single RAR gene knockouts are viable and have less significant phenotypes [Lohnes et al., 1994; Mendelsohn et al., 1994]. Double knockouts, however, show more severe phenotypes [Kastner et al., 1997a; Lohnes et al., 1994; Mendelsohn et al., 1994]. The RAR  $\alpha/\gamma$  double mutants display rhombencephalic defects [Lohnes et al., 1994]. The developmental defects generated include axial transformations. Some of the RAR knockout mice have phenotypes resembling those generated by inactivation of the Hox genes, which are targets of RA signaling [Durstun et al., 1989]. Other defects belong to the fetal vitamin A deficiency syndrome [Lohnes et al., 1994].

Single knockouts of RXR  $\beta$  and RXR $\gamma$  are viable and do not show defects related to the vitamin A deficiency syndrome [Durstun et al., 1989]. RXR  $\beta$  was shown to be necessary for spermatogenesis [Kastner et al., 1994; Sucov et al., 1994]. In RXR $\gamma$ <sup>-/-</sup> mice, the expression of choline acetyltransferase in the cholinergic interneurons has recently been shown to be down-regulated [Saga et al., 1999]. These results suggest that RXR $\gamma$  may play an important role in the proper functioning of neurons.

RXR $\alpha$  has been shown to be involved in cardiac and liver organogenesis and homozygous mutant fetuses die from embryonic days 12.5 to 16.5 [Kastner et al., 1994; Sucov et al., 1994]. The defects of these fetuses resemble those with the fetal vitamin A deficiency syndromes, including a myocardial hypoplasia, conotruncal and ocular defects. In addition, the lethality of RXR  $\alpha$  fetuses may be due to cardiac, hepatic, and placental defects [Kastner et al., 1997b; Tran and Sucov, 1998]. In addition, a synergistic effect was observed in RXR  $\alpha$ /RAR mutants, however, no synergy was observed between the effects of mutations of either RXR  $\beta$  or RXR $\gamma$  and those of the RAR mutations [Kastner et al., 1997a]. These results suggest that RXR  $\alpha$ /RAR heterodimers are the most common functional unit in the RA signaling pathway during embryogenesis.



With availability of various agonists, antagonists and double knockouts, more specific roles of retinoid receptors in embryonic development have been demonstrated, such as the development of branchial arch, hindbrain, inner ear, and pulmonary alveolus [Massaro et al., 2003; Matt et al., 2003; Romand et al., 2002; Wendling et al., 2001].

In early studies, ectopic addition of RA has been shown to have a teratogenic effect on normal embryonic development [Durstun et al., 1989]. A RA gradient has been shown to exist along the anterior-posterior axis of the embryo [Maden et al., 1998]. The findings from knockouts of the key enzymes, retinaldehyde dehydrogenase 2 (Raldh2) and *cyp26A1*, involved in RA metabolism have demonstrated that these enzymes are essential for mouse embryonic development and early morphogenesis [Niederreither et al., 1999]. Raldh2 is responsible for embryonic RA synthesis while *cyp26A1* metabolizes the active RA to hydroxylated retinoids [Marill et al., 2003]. Raldh2 and *cyp26A1* are mutually exclusively expressed in vertebrate embryos to generate RA sensitive and RA free regions in the embryos [Abu-Abed et al., 2001; Swindell et al., 1999]. Raldh2 deficiency cause embryonic lethality at E10 and the null mutant embryos suffer severe defects, such as failure in axial rotation and dilated heart [Niederreither et al., 1999]. Ablation of *cyp26A1* also causes embryonic lethality at E11.5 and the *cyp26A1*<sup>-/-</sup> embryos suffer severe posterior truncation [Abu-Abed et al., 2001]. Taken together, RARs and RXRs transduce the RA signaling, which is important for normal embryonic development.

### Chicken Ovalbumin Upstream Promoter Transcription Factors (COUP-TFs) (NR2F1 and NR2F2)

COUP-TF binds to HREs as a homodimer and acts as transcriptional repressor [Pereira et al., 2000]. Similar to its *Drosophila* homologs, SVP, two members of mouse COUP-TFs are expressed in embryos. COUP-TFI is first expressed in the neural ectoderm at E7.5 [Qiu et al., 1994; Qiu et al., 1997]. At E8.5 it is expressed in premigratory and migratory neural crest cells. In *Xenopus laevis*, misexpression of COUP-TFI leads to anterior truncations and malformations within the embryonic brain [Schuh and Kimelman, 1995]. Targeted disruption of mouse COUP-TFI results in perinatal death because of multiple defects in central and peripheral nervous system development [Qiu et al., 1997]. Most of COUP-TFI<sup>-/-</sup> embryos have fusions of the glossopharyngeal (IX) and vagus (X) cranial nerves. These defects impair both sensory and motor functions of the pharynx and the tongue, and lead to malnutrition, dehydration and usually death. Moreover, axonal projection and arborization are significantly reduced in the cervical plexus region and in the ophthalmic branch of the trigeminal nerve. Taken together, these results strongly suggest that COUP-TFI is a crucial component in the regulation of axon guidance, neurogenesis and cellular differentiation during embryonic development [Zhou et al., 2001]. A different pattern of expression for COUP-TFII offers clues about its distinct functions during mouse embryogenesis. At early stages

of development, COUP-TFII is expressed in the sinus venosus, the elongating umbilical veins and the neuroepithelium [Pereira et al., 1999]. Later it is expressed in the common atrium. In addition, COUP-TFII is expressed most highly in the mesenchymal compartments of developing organs such as the salivary gland, prostate, lung, kidney, pancreas primordium and stomach. COUP-TFII knockout mice die at around E10, showing a variety of heart and vascular defects [Pereira et al., 1999]. Atrial development is halted at E9.0 in COUP-TFII<sup>-/-</sup> embryos. These results suggest that COUP-TFII may play a role in the regulation of mesenchymal-epithelial interactions during organogenesis, and in development of the cardiovascular system.

### Steroidogenic factor (SF1) (NR5A1)

During mouse embryonic development, SF1 expression is first detected at E9.0 in the urogenital ridge, and subsequently in adrenal steroidogenic primordium (E11) and gonadal steroid-producing cells (E13) [Ikeda et al., 1994]. SF1 expression is also detected in the ventromedial hypothalamic nucleus (VMH) after E11.5 and in the pituitary gland after E13.5. Pituitary SF1 expression precedes the onset of FSH expression in gonadotropes, suggesting that SF1 might either directly regulate FSH gene transcription or regulate gonadotrope differentiation [Ingraham et al., 1994]. Using both *in vitro* and *in vivo* assays, SF1 can directly regulate expression another orphan receptor, DAX1, which is also important in mammalian gonad development and sex determination. [Hoyle et al., 2002]. The Wilms tumor suppressor (*Wt1*) and the Lim homeobox protein, *Lhx9m*, have been recently shown to directly activate SF1 expression and thus mediate early gonadogenesis. [Wilhelm and Englert, 2002]. SF1<sup>-/-</sup> embryos have complete adrenal and gonadal agenesis, male-to-female sex-reversal, and persistence of Mullerian structures in males [Luo et al., 1994; Luo et al., 1995; Sadovsky et al., 1995]. SF1 null mutants are viable at birth, but die during the first 8 days of life due to adrenocortical insufficiency. These gene knockout experiments have provided strong evidence for a direct role for SF1 in regulating mammalian sexual development as well as the differentiation of steroidogenic tissues.

### Conclusion

In summary, knockout mouse models currently offer great opportunities to understand the functions of individual nuclear receptors during embryonic development. Recent results from *in vitro* and *in vivo* studies show possible functional relationships between nuclear receptors. For instance, RA is capable of inducing expression of COUP-TFs and GCNF [Fuhrmann et al., 2001]. HNF4 $\alpha$  regulates PXR expression [Kamiya et al., 2003], SF1 controls DAX1 expression [Hoyle et al., 2002], Oct4 expression is sequentially regulated by SF1, GCNF and COUP-TF [Fuhrmann et al., 2001]. *Xenopus* GCNF is able to regulate RA signaling by controlling the expression of its catabolic enzyme, *cyp26A1* [Barreto et al., 2003b]. These results come closer to the Thummel's speculation

about the convergent regulation of nuclear receptors in invertebrates and vertebrates [Thummel, 1995]. Do nuclear receptors expressed in the early embryonic stages induce the expression of the nuclear receptors in later stages? Although there is, as yet, no clear answer to this question, new technologies, such as, transgenic mouse techniques, RNAi, and gene array technology, as well as genomic information and bioinformatic resources should be capable of enhancing our opportunity to explore this area. An area that is ripe to be explored is the degree of cross-talk between different NR pathways in vertebrate development.

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