The orphan nuclear receptors at their 25-year reunion

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Abstract

The nuclear receptor superfamily includes many receptors, identified based on their similarity to steroid hormone receptors but without a known ligand. The study of how these receptors are diversely regulated to interact with genomic regions to control a plethora of biological processes has provided critical insight into development, physiology, and the molecular pathology of disease. Here we provide a compendium of these so-called orphan receptors and focus on what has been learned about their modes of action, physiological functions, and therapeutic promise.

Key Words

orphan nuclear receptor

Introduction

In the late 1980s the observation that the steroid hormone receptors shared highly conserved domains encouraged researchers to search for additional members of the nuclear receptor (NR) family (Mangelsdorf et al. 1995, Giguère 1999, Willson & Moore 2002, Benoit et al. 2006). Unlike that of the classic NRs that had previously been identified with prior knowledge of a naturally occurring ligand, these new members were initially without ligand, and therefore referred to as orphans. Over the span of a decade, using probes designed from conserved NR domains to screen cDNA libraries and degenerate primers for target amplification, as well as automated searches of EST databases, 36 vertebrate orphan NRs were identified (Fig. 1; Willson & Moore 2002).

Orphan NRs consist of the four major domains that characterize classic nuclear hormone receptors (Aranda & Pascual 2001, Huang et al. 2010, Helsen et al. 2012). The amino terminus contains the A/B domain consisting of activation function 1 (AF1) and among orphans, this region is quite variable in size. The DNA-binding domain (DBD) consists of two zinc finger motifs and confers response element specificity; it is typically highly conserved within orphan receptor subgroups. Linking the DBD to the carboxy-terminal ligand-binding domain (LBD) is the hinge region, whose length varies between subfamilies. The pocket formed by the LBD can also vary greatly in size and by the absence or presence of the AF2 region that mediates coactivator interaction.

Classic NRs are transcription factors regulated by the high affinity binding of naturally occurring small molecules, which dictate receptor subcellular localization and conformation. The latter determines coactivator–repressor interactions and thereby transactivation potential (Mangelsdorf et al. 1995, Aranda & Pascual 2001). In contrast, while the regulation of gene transcription by orphan NRs also depends on interactions with coactivator and corepressor complexes, the role of ligand varies (Benoit et al. 2004, Markov & Laudet 2011). Nevertheless, once an endogenous ligand has been identified, the corresponding orphan is then considered ‘adopted’ (Benoit et al. 2006).

Due to their potential ligand regulation, orphan NRs have the prospect of serving as therapeutic targets of small molecules (Mukherjee & Mani 2010). Thus, there has been an intense focus on the physiological roles and molecular
mechanisms of orphan NRs over the last 25 years (Benoit et al. 2006). All vertebrate orphan NRs have been globally deleted in mice and some have been overexpressed and/or selectively targeted spatially and/or temporally. To illustrate the enormous impact of this technology on our understanding of orphan NR biology, all relevant mouse models are summarized in Table 1.

There are many orphan NRs in mammals as well as in lower organisms. Orphan NRs in *Drosophila melanogaster* and *Caenorhabditis elegans* have been reviewed elsewhere (Taubert et al. 2011, Fahrbach et al. 2012). Here, we review current knowledge about each of the 36 orphan NRs that has a human ortholog. The entire NR superfamily has been categorized into six structurally distinct groups based on phylogenetic analysis, producing a unified nomenclature system that identifies each NR with less ambiguity (Laudet 1997, Nuclear Receptors Nomenclature Committee 1999, Germain et al. 2006). In this review, each orphan is introduced as part of its official NR group, then addressed by its most commonly used name. The discussion of each is necessarily brief, highlighting its discovery, regulation, and physiological functions, particularly those with therapeutic implications. For more detailed information on individual NRs, readers are directed to the NURSA website (www.nursa.org) and to more comprehensive reviews.

**The odd ones: orphans of the NR0B group**

**Nr0b1/Dax1 and Nr0b2/Shp**

DAX1 and SHP are atypical NRs harboring a classifiable NR LBD in their carboxyl-terminus, but lack a classic NR DBD and instead have a region resembling the NR interaction motifs characteristic of coactivators (Zanaria et al. 1994, Seol et al. 1996, Lalli & Sassone-Corsi 2003, Båvner et al. 2005, Ehrlund & Treuter 2012). The name small heterodimer partner (*Shp*) reflects the ability of SHP and DAX1 to bind to the AF2 of other NRs, preventing coactivator recruitment while recruiting corepressor complexes and acting as transcriptional repressors (Lalli & Sassone-Corsi 2003, Båvner et al. 2005, Ehrlund & Treuter 2012). While most reports indicate that DAX1 and SHP function as transcriptional repressors, they may also enhance transcription (Kim et al. 2001, Nishizawa et al. 2002, Xu et al. 2009, Kelly et al. 2010).
<table>
<thead>
<tr>
<th>Receptor</th>
<th>Embryonic lethal</th>
<th>Whole body mutants</th>
<th>Tissue-specific mutants</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAX1 (NR0B1)</td>
<td>No</td>
<td>KO: fertility (males)</td>
<td>NR</td>
</tr>
<tr>
<td>SHP (NR0B2)</td>
<td>No</td>
<td>KO: fertility; energy expenditure, cholesterol metabolism; hepatic inflammation (diet-induced)</td>
<td>Liver: cholesterol and TG metabolism</td>
</tr>
<tr>
<td>PPARα (NR1C1)</td>
<td>No</td>
<td>KO: fatty acid oxidation (fasting); cholesterol and TG metabolism; glycemic control (HFD); cardiac fibrosis; wound healing</td>
<td>Muscle (transgene): glycemic control (HFD)</td>
</tr>
<tr>
<td>PPARβ/δ (NR1C2)</td>
<td>Yes</td>
<td>KO: placentaition; growth; dyslipidemia; cancer; myelination; energy expenditure; cholesterol and TG metabolism</td>
<td>Heart (transgene): TG and cholesterol metabolism</td>
</tr>
<tr>
<td>PPARγ (NR1C3)</td>
<td>Yes</td>
<td>KO: death by E10.5 (placentaition); Ki (hypomorphic): premature death, AT deposition, glycemic control, TG metabolism; Ki (Pro12Ala): body weight; glycemic control; TG + cholesterol metabolism; Ki (P465L): embryonic lethal; BAT activity (het); Ki (L466A): embryonic lethal; AT deposition, TG metabolism, glycemic control (hets); Ki (S112A): glycemic control; KO (Mox2-Cre): AT deposition, glycemic control, blood pressure; circadian rhythm; KO (γ2 isofrom only): AT deposition (strain-dependent); glycemic control KO (γ2 isofrom only): AT deposition (strain-dependent); glycemic control</td>
<td>Muscle (transgene): glycemic control; normal Pan-hematopoietic: atherosclerosis; glycemic control (HFD); hematopoietic Myeloid: autoimmunity; glycemic control (HFD) Skeletal muscle: glycemic control; fiber type; regeneration Pancreas: insulin secretion Heart (transgene): glycemic control Pancreas: beta cell mass I-Pancreas: no beta cell defects Heart: hypertrophy, systolic function Myeloid: autoimmunity; glycemic control; polarization; glycemic control (HFD); immunity to infection; atherosclerosis Kidney: body weight, blood volume Liver: TG metabolism, glycemic control Muscle: AT deposition; glycemic control Lung epithelium: lung structure Mammary epithelium: no mammary defects Ovary: ovulation (pre-ovulatory follicles) Endothelium;BM: TG metabolism; blood pressure rhythms Treg: adipose inflammation (HFD) B cell: antibody production CNS: no abnormalities reported Adipose (aP2): AT deposition; body weight; glycemic control I-Adipose (aP2): AT maintenance</td>
</tr>
<tr>
<td>REV-ERBα (NR1D1)</td>
<td>No</td>
<td>KO: postnatal lethality; fertility; circadian (behavior, cholesterol and TG metabolism, immune response); bile acids; cerebellar</td>
<td>NR</td>
</tr>
<tr>
<td>REV-ERBβ (NR1D2)</td>
<td>No</td>
<td>KO: no reported abnormalities</td>
<td>NR</td>
</tr>
<tr>
<td>RORα (NR1F1)</td>
<td>No</td>
<td>ENU: postnatal lethality; cerebellar; paralysis</td>
<td>DKO liver: circadian; dyslipidemia</td>
</tr>
<tr>
<td>RORβ (NR1F2)</td>
<td>No</td>
<td>KO: postnatal lethality; growth; fertility; learning/memory; motor; ocular</td>
<td>NR</td>
</tr>
<tr>
<td>RORγ (NR1F3)</td>
<td>No</td>
<td>KO: hematopoietic</td>
<td>T cell: autoimmunity</td>
</tr>
<tr>
<td>LXRβ (NR1H2)</td>
<td>No</td>
<td>KO: fertility; skin; CNS (amyloid deposition)</td>
<td>NR</td>
</tr>
<tr>
<td>LXRα (NR1H3)</td>
<td>No</td>
<td>KO: cholesterol and TG homeostasis (high cholesterol diet); infection Ki: constitutively active LXRα confers resistance to LPS-induced lung injury</td>
<td>Liver: cholesterol and TG homeostasis (high cholesterol diet); atherosclerosis</td>
</tr>
</tbody>
</table>

Table 1: Phenotypes of orphan receptor mutant mouse strains. Orphan NR mutant strains that have been published and are listed in the Mouse Genome Informatics (MGI) database. We have listed the general physiological systems and functions affected by each mutation. For more details, see the MGI resource (www.informatics.jax.org) from which this information was collated.
<table>
<thead>
<tr>
<th>Receptor</th>
<th>Embryonic lethal</th>
<th>Whole body mutants</th>
<th>Tissue-specific mutants</th>
</tr>
</thead>
<tbody>
<tr>
<td>LXRαβ</td>
<td>No</td>
<td>DKO: CNS (lipid deposits); cholesterol and fertility</td>
<td>TG metabolism; immunity to infection;</td>
</tr>
<tr>
<td>FXR (NR1H4)</td>
<td>No</td>
<td>KO: bile acids; dyslipidemia</td>
<td>Liver: bile acids; dyslipidemia</td>
</tr>
<tr>
<td>PXR (NR1I2)</td>
<td>No</td>
<td>KO: xenobiotic response</td>
<td>Intestine: bile acids; dyslipidemia</td>
</tr>
<tr>
<td>CAR (NR1I3)</td>
<td>No</td>
<td>KO: xenobiotic response; lipid homeostasis; body weight (caloric restriction)</td>
<td>Liver: bile acids; dyslipidemia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>KO: species-specific xenobiotic responses (human)</td>
<td>Intestine: bile acids; dyslipidemia</td>
</tr>
<tr>
<td>HNF4α (NR2A1)</td>
<td>Yes</td>
<td>KO: embryogenesis, die before E10.5</td>
<td>Liver: hepatogenesis; glycemic control; lipid homeostasis body weight; viability</td>
</tr>
<tr>
<td></td>
<td></td>
<td>KO: glycemic control (a1 isoform only)</td>
<td>Beta cell: insulin secretion</td>
</tr>
<tr>
<td>HNF4γ (NR2A2)</td>
<td>No</td>
<td>KO: body weight; locomotor activity and energy expenditure (circadian)</td>
<td>Liver: lipid and cholesterol metabolism, bile acids (high cholesterol diet); body</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>weight, glycemic control (HFD)</td>
</tr>
<tr>
<td>RXRα (NR2B1)</td>
<td>Yes</td>
<td>KO: cardiac, ocular and placental defects, die before E16.5</td>
<td>I-Liver: regeneration</td>
</tr>
<tr>
<td></td>
<td></td>
<td>KO: AF-1 only; partial postnatal lethality; cardiac; ocular; growth retardation</td>
<td>I-Adipose (α2): body weight</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Macrophage: autoimmunity; endotoxin response</td>
</tr>
<tr>
<td>RXRβ (NR2B2)</td>
<td>Partial</td>
<td>KO: viability, fertility</td>
<td>Epicardium: cardiovascular developmental defects</td>
</tr>
<tr>
<td>RXRγ (NR2B3)</td>
<td>No</td>
<td>KO: AF2 only: cholesterol metabolism</td>
<td>Retina: photoreceptors</td>
</tr>
<tr>
<td></td>
<td></td>
<td>KO: thyroid; cholinergic neurons; CNS re-myelination</td>
<td>Skin: hair and coat color</td>
</tr>
<tr>
<td>RXRαβγ (NR2B1)</td>
<td>Multiple</td>
<td>KO: viability, fertility</td>
<td>Prostate: pre-neoplastic lesions</td>
</tr>
<tr>
<td></td>
<td>combinatorial</td>
<td>KO: AF2 only: cholesterol metabolism</td>
<td></td>
</tr>
<tr>
<td></td>
<td>KO, see MGI</td>
<td>KO: thyroid; cholinergic neurons; CNS re-myelination</td>
<td></td>
</tr>
<tr>
<td>TR2 (NR2C1)</td>
<td>No</td>
<td>KO: fetal hemoglobin</td>
<td></td>
</tr>
<tr>
<td>TR4 (NR2C2)</td>
<td>Partial</td>
<td>KO: viability; aging; growth; fertility; cerebellar; body weight, glycemic control,</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>lipid homeostasis, energy expenditure, inflammation (HFD)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TR2/4</td>
<td>Yes</td>
<td>DKO: embryogenesis, die before E12</td>
<td>CNS: aggression</td>
</tr>
<tr>
<td>TLX (NR2E1)</td>
<td>No</td>
<td>KO: CNS (limbic development); neurogenesis (adult); growth; fertility; oculor;</td>
<td>I-CNS: spatial learning</td>
</tr>
<tr>
<td></td>
<td></td>
<td>aggression</td>
<td></td>
</tr>
<tr>
<td>PNR (NR2E3)</td>
<td>No</td>
<td>KO: ocular (retina, rods and cones)</td>
<td></td>
</tr>
<tr>
<td>COUP-TFI</td>
<td>No</td>
<td>KO: viability (brain development)</td>
<td></td>
</tr>
<tr>
<td>COUP-TFIi</td>
<td>No</td>
<td>KO: angiogenesis and cardiac development</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>KO: lymphatic (induction of systemic Cre at E8.5); fertility (induction of Cre at</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>P14)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>COUP-TFIi/</td>
<td>No</td>
<td>KO: circadian rhythm; thermal nociception; autoimmunity</td>
<td>Uterus: placentation</td>
</tr>
<tr>
<td>II</td>
<td></td>
<td>KO: adipose deposition; stress susceptibility (cardiac, temperature, infection);</td>
<td>Endothelium: venous and lymphatic development</td>
</tr>
<tr>
<td>EAR-2 (NR2F6)</td>
<td>No</td>
<td>bone structure</td>
<td>Beta cells: insulin secretion</td>
</tr>
<tr>
<td>ERRα (NR3B1)</td>
<td>No</td>
<td>KO: viability; cardiac; spinal cord; kidney</td>
<td>Stomach: morphogenesis</td>
</tr>
<tr>
<td>ERRβ (NR3B2)</td>
<td>Yes</td>
<td>KO: death by E10.5 (placentation)</td>
<td>DKO eye: morphogenesis</td>
</tr>
<tr>
<td>ERRγ (NR3B3)</td>
<td>No</td>
<td>KO: viability; cardiac; spinal cord; kidney</td>
<td></td>
</tr>
</tbody>
</table>

Table 1 Continued
To date, no ligands for the NR0B receptors have been well-established, although retinoid-related molecules have been reported to bind and enhance the repressive function of SHP (Miao et al. 2011, Ehrlund & Treuter 2012). Indeed, a crystal structure study of DAX1 demonstrated a ligand-binding pocket (LBP) filled with amino acid side chains, suggesting typical ligand regulation of this orphan to be unlikely (Sablin et al. 2008). Sequence conservation between Nr0b group members suggests this to be a shared characteristic, and thus, it appears that the Nr0b group is regulated at the level of expression, alternative splicing, posttranslational modifications, and miRNA targeting (Hossain et al. 2004, Miao et al. 2009, Xiao et al. 2012, Seok et al. 2013).

Although DAX1 and SHP are mechanistically very similar, their tissue expression patterns are distinct, suggesting different physiological roles (Ehrlund & Treuter 2012). Nr0b1 was originally identified as a gene frequently deleted or mutated in X-linked adrenal hypoplasia congenita (AHC) and also duplicated in dosage-sensitive sex reversal (DSS), leading to its more common name, DSS-AHC critical region on the X, gene 1, or Dax1 (Muscatelli et al. 1994, Zanaria et al. 1994, Lalli & Sassone-Corsi 2003). Dax1 is highly expressed in the hypothalamic–pituitary–adrenal–gonadal axis and has essential roles in gametogenesis and sex specification mediated in part by repression of NR5A1 (SF1; Lalli & Sassone-Corsi 2003, Iyer & McCabe 2004). In contrast, Shp is highly expressed in the liver and small intestine, where it represses NR5A2 (LRH1) to regulate cholesterol, bile acid (BA), and glucose metabolism (Båvner et al. 2005, Chanda et al. 2008).

Both members of the Nr0b group have also been reported to repress the transcriptional activity of other factors besides NRs (Kim et al. 2004, 2012, Suh et al. 2006, Kinsey et al. 2009, Sun et al. 2009, Yuk et al. 2011). For example, SHP functions as a corepressor of NF-κB during inflammatory Toll-like receptor signaling (Yuk et al. 2011). Moreover, the ability to bind and repress OCT4 activity has identified DAX1 as a critical component in maintaining embryonic stem cell pluripotency (Wang et al. 2006, Kim et al. 2008, Sun et al. 2009). Finally, the potential role of Nr0b receptors in carcinogenesis is highlighted by the functional interaction of DAX1 with the EWS/FLI fusion protein in Ewing’s sarcoma (Kinsey et al. 2009).

There for dinner: orphans of the NR1C group

Nr1c1/Pparγ, Nr1c2/Pparβ or δ, and Nr1c3/Pparγ

Peroxisome proliferator-activated receptors (PPARs) regulate carbohydrate and lipid metabolism in major metabolic organs (liver, adipose, and muscle) as well as

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Embryonic lethal</th>
<th>Whole body mutants</th>
<th>Tissue-specific mutants</th>
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</thead>
<tbody>
<tr>
<td>NURR77 (NR4A1)</td>
<td>No</td>
<td>KO: body weight (HFD); atherosclerosis</td>
<td>NR</td>
</tr>
<tr>
<td>NURR1 (NR4A2)</td>
<td>No</td>
<td>KO: complete postnatal lethality by 12 h; selective loss of dopaminergic neurons</td>
<td>NR</td>
</tr>
<tr>
<td>NOR1 (NR4A3)</td>
<td>Yes</td>
<td>KO: death by E8.5 (gastrulation); seizures; inner ear; atherosclerosis</td>
<td>NR</td>
</tr>
<tr>
<td>NURR77/NOR1 DKO</td>
<td>No</td>
<td>KO: viability; leukemia (growth, hepatomegaly, anemia)</td>
<td>Anterior pituitary: fertility (gonad maturation)</td>
</tr>
<tr>
<td>SF1 (NR5A1)</td>
<td>No</td>
<td>KO: viability (adrenals); gonads; hypothalamus</td>
<td>Gonads: fertility (gonad maturation)</td>
</tr>
<tr>
<td>LRH-1 (NR5A2)</td>
<td>Yes</td>
<td>KO: death before E7.5</td>
<td>CNS: behavior (anxiety); body weight (HFD)</td>
</tr>
<tr>
<td>GCF (NR6A1)</td>
<td>Yes</td>
<td>KO: death by E10.5 (cardiac development)</td>
<td>Liver: slight dyslipidemia; bile acid composition</td>
</tr>
</tbody>
</table>

KO, knock-out; DKO, double knock-out; KI, knock-in; I, inducible Cre transgene; NR, not yet reported; AT, adipose tissue; HFD, high-fat diet; TG, triglyceride.

Table 1 Continued
in peripheral tissues with metabolic roles (intestine, hematopoietic, and hypothalamic). The ‘PPAR’ name followed the discovery that PPARα was activated by clofibrate, a lipid-lowering drug that causes peroxisomal proliferation in the rodent liver (Issemann & Green 1990); however, the β/δ and γ subtypes have not been shown to cause peroxisomal proliferation. Despite ongoing searches for dominant endogenous ligands, the PPARs are now commonly thought of as ‘sensors’ for a wide range of fatty acid molecules, a promiscuity that may be explained by their large LBP (Nolte et al 1998, Uppenberg et al 1998, Xu et al 1999, 2001, Michalik 2006). However, high affinity and relatively specific synthetic ligands exist for each PPAR (Bensinger & Tontonoz 2008). As noted earlier, lipid-lowering fibrates activate PPARα, and agonists of PPARγ function as anti-diabetic drugs (Lehrke & Lazar 2005).

Activation of PPARα and PPARβ/δ supports fatty acid oxidation (FAO) in the heart, liver, and muscle, while PPARγ promotes lipid storage in adipose tissue (Michalik 2006). Thus, loss of PPARα in the liver blocks elevated FAO in response to fasting, leading to hepatic and myocardial lipid accumulation, hypoglycemia, and elevated serum–free fatty acids (Kersten et al 2006). Similarly, PPARβ/δ activity in the muscle promotes formation of highly oxidative slow-twitch fibers, in part through its positive regulation of PGC1α (Evans et al 2004, Schuler et al 2006). Interestingly, these two receptors have opposing roles in cardiac myocytes of the diabetic heart, where PPARβ/δ promotes glucose uptake, utilization, and overall cardiac health, while the PPARα pathway decreases glucose utilization and promotes the myocardial lipid accumulation associated with cardiac disease (Burkart et al 2007). PPARγ is strongly induced during adipocyte differentiation and its activity is necessary and, in some cases, sufficient for adipogenesis (Chawla & Lazar 1994, Tontonoz et al 1994, Tontonoz & Spiegelman 2008). Two isoforms, γ1 and γ2, identical except for an additional 30 amino acids at the N-terminus of γ2, are highly expressed in adipocytes, while other cell types express comparatively lower levels of the γ1 isoform (Tontonoz et al 1994, Nagy et al 2013). Loss of PPARγ expression causes lipodystrophy in mice as do dominant negative mutants of PPARγ in both mice and humans (Barak et al 2002, Freedman et al 2005, Semple 2006, Duan et al 2007). PPARγ also regulates lipid metabolism in macrophages and appears to have an overlapping but non-redundant role with PPARδ in the regulation of macrophage polarization and homeostatic scavenging of apoptotic cells (Ahmadian et al 2013).

PPARs bind DNA as obligate heterodimers with Nr2b1 (Rxr) family members to a direct repeat (DR) of the sequence AGGTCA separated by one nucleotide, usually an adenine (Kliewer et al 1992b, IJpenberg et al 1997, Varga et al 2011). This DR1 element was initially defined using in vitro binding and transactivation assays and has been confirmed as the predominant recognition motif at PPAR binding sites identified on a genome-wide scale in vivo (Dreyer et al 1992, Kliewer et al 1992b, Tugwood et al 1992, Letterova et al 2008, 2010, Nielsen et al 2008, Mikkelsen et al 2010, Boergesen et al 2012).

It's about time: orphans of the NR1D group

Nr1d1/Rev-erbα and Nr1d2/Rev-erbβ

Rev-erbα was discovered as a coding sequence on the reverse strand of the c-erbα gene, which encodes a thyroid hormone receptor, and the identification of Rev-erbβ followed several years later (Lazar et al 1989, Dumas et al 1994, Forman et al 1994, Retnakaran et al 1994). Structurally, the Rev-erbs are unique among NRs because they lack a critical alpha-helix (H12) in the AF2 domain that mediates coactivator interaction, resulting in a conformation more suitable for corepressor recruitment (Renaud et al 2000, Ramakrishnan & Muscat 2006, Yin et al 2010). Both Rev-erbα and Rev-erbβ act as receptors for heme, which binds within the classic NR LBP (Reinking et al 2005, Raghuram et al 2007, Yin et al 2007, Burris 2008). Several synthetic agonists have been developed and show potential for modulating Rev-erb in vivo (Yin et al 2010, Solt et al 2012). Besides ligand binding, regulation of Rev-erb activity also occurs at the level of gene expression, posttranslational modifications, and protein–protein interactions (Yin et al 2010, Chini et al 2013). For example, Rev-erb expression in most tissues is circadian and is tightly controlled by the core circadian clock TFs, BMAL1 and CLOCK, as well as repressing its own transcription (Feng & Lazar 2012). Furthermore, Rev-erbα is stabilized by GSK3β-mediated phosphorylation that can be blocked by lithium exposure; in hippocampal neurons, oligophrenin 1 alters cellular localization of Rev-erbα, blocking its repressive function (Valnegri et al 2011, Feng & Lazar 2012).

Acting as dedicated transcriptional repressors, both Rev-erbs bind to extended half-sites (AGGTCA) with an A/T-rich 5′ end (RORE), a sequence identical to that recognized by NR1F (ROR) orphan receptors but with opposing effects. Importantly, Rev-erb monomer’s association with isolated ROREs is not sufficient for active transcriptional repression (although it can still compete...
with ROR binding to the RORE); however, two neighboring monomeric Rev-erbs can cooperate to recruit corepressors to repress gene expression (Yin et al. 2010). Heme stabilizes the interaction with NCoR, augmenting repression of transcription and defining the Rev-erb group as ligand-stimulated transcriptional repressors (Pardee et al. 2009). Rev-erbs α and β also dimerize and function at a DR of their unique half-site spaced by two additional nucleotides (Rev-DR2; Yin et al. 2010).

The Rev-erbs have been implicated in a variety of physiological processes, including cerebellar development, osteoarthritis, adipogenesis, and mitochondria biogenesis, but the most well-known cellular function is to act as integrators of circadian rhythm and metabolic pathways (Chomze et al. 2000, Chaturvedi et al. 2006, Ramakrishnan & Muscat 2006, Wang & Lazar 2008, Yin et al. 2010, Woldt et al. 2013). Genome-wide studies have indicated that the cyclic expression and genomic presence of both Rev-erbs result in the proper rhythmic expression of circadian clock and lipid metabolism genes and underscore the importance of this convergence, Rev-erb deficiency in the liver results in hepatic steatosis and dysregulation of the cell autonomous hepatic clock (Bugg et al. 2012). Recent work has also described that Rev-erbs represent a key link between the cellular clock and macrophage function, where they function cyclically to limit the expression of cytokines impacting responses to pathogens (Fontaine et al. 2008, Gibbs et al. 2012, Chandra et al. 2013, Lam et al. 2013).

More time for discussion: orphans of the NR1F group

Nr1f1/Rorα, Nr1f2/Rorβ, and Nr1f3/Rorγ


Rors bind as monomers to the same RORE sequence bound by Rev-erbs and are described as constitutive activators, recruiting coactivators in absence of ligand (Jetten 2009). However, independent investigations have suggested that oxysterols and cholesterol derivatives act as agonist and inverse agonist ligands of Rorα and γ (Kallen et al. 2002, Jin et al. 2010, Wang et al. 2010). In addition, retinoids and retinoic acid have been shown to antagonize the activity of Rorβ (Stehlin-Gaon et al. 2003). Despite the uncertainty surrounding endogenous ligand regulation of Rors, the generation of synthetic agonists and inverse agonists has great therapeutic promise, particularly in modulating the function of the Rorγ-T isoform in T117 lymphocytes during autoimmunity (Yang et al. 2008, Huh et al. 2011, Solt et al. 2011).

Like other NRs, activity of RORS can be regulated by posttranslational modifications, including phosphorylation and sumoylation (Hwang et al. 2009, Onishi et al. 2009, Lee et al. 2010). Intriguingly, a ‘methyl degron’ sequence has been recently described in Rorα, making it the first non-histone substrate targeted by methyltransferases to regulate protein stability (Lee et al. 2012). Antagonism with other TFs also mediates ROR activity, demonstrated by the recent observation that Rorγ function is inhibited by direct interaction with Foxp3 (Ichiyama et al. 2008, Zhou et al. 2008). Furthermore, the ability of Rorα and Rev-erbs to bind to overlapping sites and counteract each other’s activity at target genes has been demonstrated in the circadian control of Bmal1 and Rev-erb expression (Guillaumond et al. 2005). In addition to direct transcriptional regulation at specific response elements, the transrepression of Wnt/β-catenin-mediated gene activation has been reported (Lee et al. 2010). Furthermore supporting the DNA-binding independent functions of RORS, Rorα has been reported to bind and stabilize p53 protein and thereby indirectly regulate p53 target gene expression (Kim et al. 2011, Wang et al. 2012).

Watching what they eat: orphans of the NR1H group

Nr1h2/Lxrβ, Nr1h3/Lxrα, and Nr1h4/Fxrα

The Nr1h family includes liver X receptors, Lxrα and Lxrβ, and farnesol X receptors, Fxrα and Fxrβ, although Fxrβ is found in mice but not in humans (Moore et al. 2006). Lxrs and Fxrs have metabolically intertwined roles that converge on minimizing the buildup of cholesterol by responding to elevated levels of steroids and BAs respectively (Calkin & Tontonoz 2012). Endogenous LXR ligands that have been reported include: 24(S), 25-epoxycholesterol in the liver, 24(S)-hydroxycholesterol in the brain, and 27-hydroxycholesterol in macrophages (Moore et al. 2006). Synthetic, high affinity pan-LXR
agonists are in wide experimental use. These compounds promote the cholesterol-lowering activity of LXRs, but this benefit is offset by activation of the SREBP1C pathway of de novo lipogenesis in the liver, which has hindered the advancement of LXR agonists as therapies for human metabolic disorders (Repa et al. 2000, Joseph et al. 2002, Tangirala et al. 2002, Levin et al. 2005). FXR was initially named based on its activation by very high concentrations of farnesol and its metabolites in vitro, but was later found to respond to physiological concentrations of BAs and is now widely viewed as a nuclear BA receptor and central regulator of BA homeostasis (Forman et al. 1995, Makishima et al. 1999, Parks et al. 1999, Wang et al. 1999). BAs vary in their affinity for FXR with the strongest agonist being the primary BA chenodeoxycholic acid (CDCA; Modica et al. 2010). Importantly, synthetic FXR agonists are efficacious in treating mouse models of cholestatic diseases (Moschetta et al. 2004).

Subtype and cell type-specific deletions of LXR have uncovered their roles in the regulation of whole-body cholesterol homeostasis. In atherosclerosis-prone mouse strains, LXRs are required for preventing cholesterol overload in several tissues including the liver, where LXR activation cannot be compensated for by LXRβ; macrophages, where it contributes equally with LXRβ; and likely other tissues including adipose and intestine (Lehrke et al. 2005, Bradley et al. 2007, Hong et al. 2012, Zhang et al. 2012). Similarly, LXRβ has a non-redundant role in preventing cholesterol accumulation in BA-metabolizing cholangiocytes, the CNS, spinal cord, and male gonadal cells (Gabbi et al. 2009, Xia et al. 2012).

For FXR, activation by BAs initiates a negative feedback loop that limits BA synthesis and promotes their transit from hepatocytes to the enterohepatic circulation. Consistent with this, FXR-deficient mice challenged with high levels of dietary BAs suffer severe hepatotoxicity (Sinai et al. 2000).

All Nr1h members heterodimerize with RXR and recent genome-wide binding studies have demonstrated widespread use of sequence motifs identified using older low-throughput methods. In liver, the vast majority of FXR-binding sites identified by ChIP-seq, contained an IR1 motif (inverted repeats of the canonical NR half site AGGTCA separated by one nucleotide) which was initially reported as the preferred motif for FXR-RXR heterodimers (Laffitte et al. 2000, Chong et al. 2010, Thomas et al. 2010). Similarly, genome-wide profiling confirmed co-occupancy of LXR–RXR at DR4 motifs, initially characterized almost 20 years ago (Willy et al. 1995, Boergesen et al. 2012).

The defenders: orphans of the NR11 group
Nr1i2/Pxr and Nr1i3/Car

The pregnane X receptor (PXR) and constitutive androstane receptor (CAR) play a key role in the body’s defense against xenobiotics (foreign materials encountered in the environment). Mouse and human PXR were discovered almost simultaneously, revealing PXR as a conserved, direct regulator of Cyp3a family enzymes important in xenobiotic and endobiotic metabolism (Bertilsson et al. 1998, Blumberg et al. 1998, Kliwer et al. 1998, Lehmann et al. 1998). PXR can bind structurally distinct ligands that range in size from 268 to 823 Da and are highly species-specific, in accordance with the relatively low conservation of the PXR LBP among orthologs (Reschly & Krasowski 2006). This diverse array of xenobiotics, dietary compounds, and endobiotics includes rifampicin, clotrimazole, phenobarbital, the herbal antidepressant St John’s wort, HIV protease inhibitors, and BAs (Zhou et al. 2009, Tolson & Wang 2010). Initial cloning of CAR reported its transcriptional activity in the absence of an exogenous ligand, leading to the name constitutive active receptor before androstanes were identified as inverse agonists (Baes et al. 1994, Forman et al. 1998). To date, many xenobiotic agonists have been identified with phenobarbital-like compounds being the most widely used to study CAR’s function (Gao & Xie 2010).

Both receptors induce phase I and II drug metabolizing enzymes in the liver and intestine (Tolson & Wang 2010, Ihunnah et al. 2011). Though this activity is clearly beneficial, it can also enhance drug toxicity, such as during the PXR-mediated accumulation of toxic aspirin metabolites (Guo et al. 2004). Similarly, CAR deficiency is protective against hepatotoxicity caused by the breakdown of phenobarbital and related compounds (Wei et al. 2000). Roles of PXR and CAR in endobiotic metabolism have also come to light. PXR can promote BA excretion from the liver into the urine, which may explain the profound susceptibility of PXR-deficient mice to challenge with a high cholesterol-BA diet (Staudinger et al. 2001, Xie et al. 2001, Sonoda et al. 2005). Ligand-activated PXR can also suppress hepatic gluconeogenesis and fasting-induced FAO and has been implicated in the regulation of reverse cholesterol transport, steroid hormone synthesis, and the catabolism of androgen, bilirubins, and retinoic acid (Ihunnah et al. 2011). Ligand-activated CAR protects against diet-induced obesity and its related metabolic complications (Gao et al. 2009).

Ligand activation of both PXR and CAR induces cytoplasmic to nuclear shuttling that can be regulated by
phosphorylation (Squires et al. 2004, Pondugula et al. 2009). Once in the nucleus, PXR binds DNA as a heterodimer with RXR, predominantly at DR4 motifs, as revealed by recent genome-wide binding analysis in the liver (Kliwer et al. 1998, Cui et al. 2010). Notably, this study found no evidence for PXR binding to everted repeats (ER6 or ER8), as was previously reported (Kliwer et al. 2002). CAR also binds as an obligate heterodimer with RXR to DR4 motifs, but this preference has yet to be shown on a genome-wide scale (Kawamoto et al. 1999, Sueyoshi & Negishi 2001).

Arrested development: orphans of the NR2A group

Nr2a1/Hnfα and Nr2a2/Hnfγ

The hepatocyte nuclear factors HNF4α and HNF4γ are encoded by two distinct but highly homologous genes (Drewes et al. 1996). Cloning of HNF4α from human liver revealed several variants regulated by alternative promoter usage and splicing (Chartier et al. 1994, Kritis et al. 1996). There are two predominant isoforms: the longer HNF4α containing an additional N-terminal AF1 domain expressed highly in liver, and the shorter HNF4α expressing highly in the pancreas (Eckhoute et al. 2003). HNF4γ was cloned from a human kidney cDNA library and an early report found it in the pancreas, liver, brain, and lung (Plengvidhya et al. 1999). HNF4α appears to function as a ligand-independent, constitutively active receptor (Ruse et al. 2002). However, several crystal structures of purified LBDs identified fatty acids in its binding pocket and there is evidence that linoleic acid binds to HNF4α in the livers of fed, but not fasted, mice (Dhe-Paganon et al. 2002, Wisely et al. 2002, Yuan et al. 2009).

Mutations in HNF4α are associated with a rare form of early-onset, autosomal dominant diabetes called maturity onset diabetes of the young, and therefore HNF4α is considered a MODY1 gene (Yamagata et al. 1996, Navas et al. 1999). Mouse models have shown that it is required in the pancreas for glucose-stimulated insulin secretion and beta cell expansion (Gupta et al. 2002, Wisely et al. 2010). The production of 9-cis-RA in vivo is controversial, but several specific and high affinity pharmacological ligands have been developed (Wolf 2006, Pérez et al. 2012).

Partners for life: orphans of the NR2B group

Nr2b1/Rxrα, Nr2b2/Rxrβ, and Nr2b3/Rxrγ

The Nr2b family includes retinoid X receptors, RXRα, RXRβ, and RXRγ. Cloning of RXRα as a retinoic acid-responsive factor that shared modest homology with retinoic acid receptors (RARs) was followed shortly by identification of RXRβ and RXRγ (Mangelsdorf et al. 1990, Rowe et al. 1991, Yu et al. 1991). RXRs dimerize with and strengthen the DNA-binding and transcriptional activity of other NRs, a list that now includes TR, RARs, VDR, PPARs, LXR, FXR, PXR, CAR, NGFIB, and NURR1 (Yu et al. 1991, Kliwer et al. 1992a, Leid et al. 1992, Marks et al. 1992, Mark & Chambon 2003). RXRs bind 9-cis-retinoic acid with high affinity, and their heterodimer partners can be defined as ‘permissive’ or ‘non-permissive’ based on whether an RXR ligand activates the complex (Levin et al. 1992, Mangelsdorf et al. 1992, Lefèbvre et al. 2010). The production of 9-cis-RA in vivo is controversial, but several specific and high affinity pharmacological ligands have been developed (Wolf 2006, Pérez et al. 2012).

Because of their wide array of binding partners, RXRs may have the most disparate biological roles of all NRs. For instance, RXRα ablation is embryonic lethal due to cardiac defects likely to result from its participation in complexes with RAR (Kastner et al. 1994, Sucov et al. 1994, Gruber et al. 1996). In the liver, where RXRα is the most abundant subtype, hepatocyte-specific deletion results in elevated serum triglycerides, serum cholesterol, and dramatic intolerance to a high cholesterol diet, likely via a loss of LXR, FXR, and possibly PXR and CAR activities (Mangelsdorf et al. 1992, Wan et al. 2000). Ablation of
RXRβ is lethal in some genetic backgrounds and it has a non-redundant role in spermatogenesis (Kastner et al. 1996). RXRγ is not essential for development but appears to be important for proper sensitivity to thyroid hormone, as knock-out mice have increased metabolic activity, serum T₄ and TSH, and are resistant to diet-induced obesity (Brown et al. 2000, Haugen et al. 2004). It is also required in the CNS for proper functioning of cholinergic and dopaminergic pathways and for oligodendrocyte differentiation (Saga et al. 1999, Krzyzosiak et al. 2010, Huang et al. 2011). Compound mutants in RXR family members have been generated and have been comprehensively reviewed elsewhere (Mark et al. 2006).

Recent ChIP-seq studies have profiled the binding specificities of RXRs on a genome-wide scale, revealing extensive co-occupancy of RXRs with PPARγ in adipocytes and LXR in liver. These studies also reported substantial numbers of RXR-only binding sites, suggesting the likely cooperation of RXR with additional NR partners in these tissues (Nielsen et al. 2008, Boergesen et al. 2012). A similar study found RXRα bound near ~80% of genes expressed in the liver, though expression of only a small fraction were affected by hepatocyte-specific RXR knockout, illustrating the challenge of elucidating the role of RXRs in tissue-specific regulatory networks (Zhan et al. 2012).

**What’s in a name: orphans of the NR2C group**

**Nr2c1/Tr2 and Nr2c2/Tr4**

Testicular orphan NR 2 (Tr2) was originally identified by screening a human testis cDNA library for novel genes containing sequences similar to previously identified NR DBDs and the identification of related receptor Tr4 (Tak1) was reported a few years later (Chang et al. 1989, 1994, Hirose et al. 1994, Law et al. 1994, Lee et al. 2002). Tr2 and Tr4 display a widespread expression pattern in embryonic and adult tissues, suggesting pleiotropic physiological functions (Lee et al. 2002). Indeed, loss of function studies implicated Tr4 in many diverse biological systems, including the CNS, reproduction, and metabolism (Kim et al. 2003, Chen et al. 2005b, Kang et al. 2011, Lin et al. 2012). Interestingly, mice lacking Tr2 appeared normal suggesting that the Nr2c group may function redundantly and this is supported by the dramatic defect in stem cell self-renewal, commitment, and differentiation observed in mice lacking both orphan NRs (Shyr et al. 2002a, 2009).

Tr2 and 4 can bind to response elements consisting of a DR of the canonical NR half site (AGGTCA) with various types of spacing (DR1–5) and, consistent with this relatively promiscuous binding, part of the group’s functions seems to be mediated by sharing or competing with other NRs for response elements (Lin et al. 1995, Yan et al. 1998, Xie et al. 2009a). Direct regulation of transcription appears to be dependent on homo- or heterodimerization between the Nr2c group members (Lee et al. 1998, Zhou et al. 2011). In contrast, the analysis of Tr4-enriched genomic regions obtained with ChIP-seq revealed no obvious DR element, suggesting that the genomic localization of NR2C members may be indirect (O’Geen et al. 2010). Consistent with this, several studies have reported interaction with and regulation of other NRs independent of NR2C binding to DNA (Hu et al. 2002, Shyr et al. 2002b, Mu & Chang 2003).

To date, ligands for Nr2c group members have not been described, although there is some evidence that they can be bound and activated by polyunsaturated fatty acids (Xie et al. 2009a). A recent crystal structure showed ligand-free Tr4 to be in an auto-repressed conformation that can be modulated by retinoid treatment introducing another potential ligand of the Nr2c group (Zhou et al. 2011). Receptor phosphorylation, acetylation, and sumoylation status can also cause the exchange of co-repressors and co-activators and thereby convert NR2C receptors from transcriptional repressors to activators and vice versa (Khan et al. 2005, Gupta et al. 2009, Xie et al. 2011). This is exemplified by the regulation of the Oct4 promoter in stem cells by Tr2, where phosphorylation of the receptor causes repression of Oct4, a critical switch in the balance of pluripotent cell self-renewal and differentiation (Gupta et al. 2008). In addition to posttranslational modification, the expression level of TR2/4 appears to be an important means of activity modulation and showing therapeutic promise, genetic overexpression of Tr2/Tr4 in the erythroid lineage of a sickle cell disease model conferred enhanced expression of target gene fetal hemoglobin and alleviated disease symptoms (Campbell et al. 2011).

**Seeing is believing: orphans of the NR2E group**

**Nr2e1/Tlx and Nr2e3/Pnr**

Tlx is the vertebrate homologue of the *Drosophila* gene tailless (tl), which was characterized in 1990 (Gui et al. 2011). A screening for similar genes led to the photoreceptor NR (Pnr), making it the latest vertebrate NR to be described (Chen et al. 1999, Kobayashi et al. 1999). TLX and PNR negatively regulate target gene transcription as
monomers or homodimers, recruiting corepressors to the half sites or DRI elements although there have been reports of gene activation as well (Chen et al. 2005a, Zhang et al. 2006, Sun et al. 2007, Yokoyama et al. 2008, Qu et al. 2010). Furthermore, a recent study has reported the ability of PNR to interact with and positively regulate p53 in a manner independent of PNR DNA-binding (Wen et al. 2012). While very little is known about the ligand dependency of the Nr2e group, activity of PNR and TLX is regulated by interaction with other NRs, posttranslational modification, alternative splicing, and miRNA targeting (Cheng et al. 2004, Wolkenberg et al. 2006, Onishi et al. 2009, Zhao et al. 2009, 2010, Shibata et al. 2011, Qin et al. 2013b).

In contrast to most orphan NRs, the Nr2e members have very specialized physiological roles limited mainly to the development of the rods and cones of the retina and CNS function (Gui et al. 2011, Forrest & Swaroop 2012). The essential role for PNR in retinal development is reflected in its mutation being closely associated with enhanced S-cone syndrome, retinitis pigmentosa, and other retinopathy in humans (Forrest & Swaroop 2012). Retinal degeneration also occurs in mice with either a spontaneous or targeted deletion (Forrest & Swaroop 2012). TLX also functions in preventing retinal degeneration and in addition, mouse models have demonstrated a critical role in the development of the limbic system, where deletion leads to extreme aggression (Monaghan et al. 1997, Yu et al. 2000, Young et al. 2002). More recently, the essential role of TLX in maintaining adult neural stem cells in an undifferentiated, proliferative state has come to light and this cellular function has been reported to impact both spatial learning and brain tumor expansion, suggesting its potential as a therapeutic target (Shi et al. 2004, Liu et al. 2010, Zou et al. 2012).

**Leaders of the chicken dance: orphans of the NR2F group**

**Nr2f1/Coup-tfi, Nr2f2/Coup-tfil, and Nr2f6/Ear2**

Chicken ovalbumin upstream promoting TF 1 (COUP-TFI) was identified as a long-sought after regulator of the chicken ovalubin gene (Wang et al. 1989). It, as well as ERBA-related protein 2 (EAR2), was originally described as the genes encoding proteins with homology to thyroid receptor, while COUP-TFI, a Nr2f member highly related to COUP-TFI, was identified in a later screen (Miyajima et al. 1988, Ritchie et al. 1990, Ladis & Karathanasis 1991).

COUP-TFI and II bind as dimers to repress transcription via DRI elements (Tsai & Tsai 1997, Alfano et al. 2013). The COUP-TFs can also promiscuously recognize many other direct, inverted and everted NR half-site (AGGTCA) repeats, enabling them to compete with and antagonize the action of other NRs (Tsai & Tsai 1997, Alfano et al. 2013). These receptors also have transpressive effects mediated by heterodimerization with other TFs or their partner RXR. EAR2 also appears to be a transcriptional repressor able to heterodimerize with other NRs including the COUP-TFs (Jonk et al. 1994, Zhu et al. 2000, Warnecke et al. 2005). For example, EAR2 was recently demonstrated to bind and inhibit the function of RORγ in Th17 lymphocytes (Hermann-Kleiter et al. 2012). Despite the large body of evidence describing this family as transcriptional repressors, there have been some reports of NR2F-mediated gene activation but in vivo relevance of these findings has not been substantiated (Tsai & Tsai 1997). To date, the Nr2f members remain as true orphan NRs, but in addition to their level of gene expression, activity of these TFs is regulated by posttranslational modifications including phosphorylation (Tsai & Tsai 1997, Hermann-Kleiter et al. 2008).

The Nr2f group is widely expressed and loss of function models has been a key in the dissection of their many physiological roles (Lin et al. 2011). By influencing cellular processes including, survival, migration, fate determination, and differentiation, COUP-TFI and II have unique and essential functions in neural development and organogenesis respectively (Lin et al. 2011). However, they do share nearly identical DBDs and LBDs and recent analyses of mice deficient in both receptors have uncovered a redundant role in retinal development (Satoh et al. 2009, Tang et al. 2010). In addition to loss of function studies, genome-wide profiling of histone modifications and analyses of enhancer sequences predicted a role for both COUP-TFI and II in the phenotype of embryonic neural crest cells (NCC) which was further supported by knockdown experiments in human NCCs (Rada-Iglesias et al. 2012). Besides direct developmental roles, the NR2F receptors have recently been implicated as potential targets in cancer therapeutic intervention (Litchfield & Klinge 2012). For example, overexpression of COUP-TFI results in the inhibition of SMAD4-mediated transactivation, which normally prevents cancer progression in prostate epithelium (Qin et al. 2013a). In contrast to COUP-TFI and II, much less is known about EAR2; however, knock-out mouse studies have suggested the roles in the CNS and immune system (Warnecke et al. 2005, Hermann-Kleiter et al. 2008).
Lots of energy: orphans of the NR3B group

**Nr3b1/Errα, Nr3b2/Errβ, and Nr3b3/Errγ**

Estrogen-related receptors, α (ERRα) and β (ERRβ) were discovered by screening libraries with a probe consisting of the sequence of the DBD of estrogen receptor (ER) and were the first orphan NRs to be discovered (Giguère et al. 1988, DeBlois & Giguère 2011). Nearly a decade later, ERRγ was discovered as a gene deleted in a critical region of Usher syndrome, a genetic disorder resulting in hearing and vision loss, and subsequently described by two additional groups (Eudy et al. 1998, Hong et al. 1999, Heard et al. 2000). The ERRs are constitutive activators and crystal structure studies suggest that classic ligand binding modulation is unlikely due to an inaccessible LBP. However, the presence of certain inverse agonists are able to induce a conformational change favoring corepressor interaction (Kallen et al. 2007, Xie et al. 2009b). Although no endogenous ligand for the ERRs has been reported, many inverse agonists and antagonists have been described, including pesticides, synthetic and phyto-estrogens, and 4-hydroxytamoxifen (4-OHT), underscoring the notion of functional crosstalk between ERR and ER signaling (Coward et al. 2001, Tremblay et al. 2001a,b, Bonnelye & Aubin 2013). Adding to this complexity, bisphenol A, the synthetic estrogen and endocrine disruptor found in many plastics, binds ERRγ and prevents 4-OHT-induced coactivator dissociation (Takayanagi et al. 2006). Posttranslational modifications including phosphorylation, acetylation, and sumoylation also influence ERR activity (Ariazi et al. 2007, Tremblay et al. 2008, Wilson et al. 2010). The constitutive activity of the ERR group is also negatively regulated by DAX1 and SHP (Sanyal et al. 2002, Uranishi et al. 2013). The ERRs all recognize an extended half-site termed the ERRE (TNAAGGTCA) as monomers, homodimers, or heterodimers (Dufour et al. 2007, Tremblay & Giguère 2007). Although ERRs may also have affinity for classic estrogen and thyroid receptor elements in vitro, recent genome wide localization studies have suggested that the ERR is the main site of ERR occupancy (Johnston et al. 1997, Vanacker et al. 1998, Dufour et al. 2007, Tremblay & Giguère 2007).

ERRα and ERRγ are widely expressed and regulate genes that modulate cellular energy metabolism by directing mitochondrial biogenesis and function impacting a variety of tissues under various physiological stresses including bone, adipose, heart, immune cells, kidney, and liver, implicating them as important therapeutic targets in a variety of metabolic disorders (Tremblay & Giguère 2007, Giguère 2008, DeBlois & Giguère 2011). In addition, ERRs have the potential to modulate cancer cell energy production and, recently, ERRγ has been identified as a miRNA target in breast cancer cells, leading to a shift from oxidative to glycolytic metabolism (Eichner et al. 2010, DeBlois & Giguère 2013). ERRβ has emerged as a component of cell pluripotency, being a key target of both NANOG and GSK/TCF3 pathways that modulates the expression of genes important in self-renewal (Ivanova et al. 2006, Chen et al. 2008, Festuccia et al. 2012, Martello et al. 2012). ERRβ also functions during development of the placenta, retina, and endolymph of the inner ear and, and mutations have been linked to hearing loss in humans (Chen & Nathans 2007, Collin et al. 2008, Onishi et al. 2010, Ben Saïd et al. 2011, Lee et al. 2011b).

First to the party: orphans of the NR4A group

**Nr4a1/Nur77, Nr4a2/Nurr1, and Nr4a3/Nor1**


NR4a orphans harbor a unique LBD filled with bulky hydrophobic residues that is unlikely to accommodate a typical ligand (Baker et al. 2003, Wang et al. 2003). Furthermore, nonclassical coactivator interfaces have been described for these receptors (Wansa et al. 2002, Codina et al. 2004). Therefore, regulation of the NR4A receptor activity depends on gene expression, alternative splicing, posttranslational modification, interaction with other NRs, microRNA targeting, and cellular localization (Maruyama et al. 1998, Maxwell & Muscat 2006, Malewicz...
et al. 2011, McMorrow & Murphy 2011, Yang et al. 2012, Li et al. 2013). It should be noted that despite the unique structure of the LBD, pharmacological agonists have been described including 1,1-bis(3-indolyl)-1-(p-anisyl)-methane, anti-neoplastic and anti-inflammatory agent 6-mercaptopurine, and cytosporone B (Wansa et al. 2003, Chintharlapalli et al. 2005, Zhan et al. 2008).

NURR1, NUR77, and NOR1 can bind as monomers to half-site recognition motifs called the NBRE (AAAGGTCA), but they can also homodimerize or heterodimerize with each other and with RXR and bind NurRE, a DR element (Maruyama et al. 1998, Maxwell & Muscat 2006). The importance of the transactivation function of the NR4A receptors has been demonstrated in vivo. For example, Nor1 is a gene component within chromosomal translocations that occur frequently in human extraskeletal myxoid sarcoma (Labelle et al. 1995, Clark et al. 1996). The oncogenic fusion protein product of this translocation is frequently a strong activation domain of another TF such as EWS linked to a full length NOR1 leading to misregulation of NOR1 gene targets (Filion & Labelle 2012). Critical gene regulation by NR4A receptors is also exemplified by the direct regulation of tyrosine hydroxylase in neurons by NURR1 to maintain the dopaminergic phenotype which is hampered in Parkinson’s disease and by obligatory direct regulation of Foxp3 by all three NR4A members acting redundantly during the development of regulatory T cells thereby preventing autoimmunity (Sakurada et al. 1999, Luo 2012, Sekiya et al. 2013). Like other NRs, transrepression by the NR4A group has been reported. Specifically, NURR1 in concert with the CoREST complex is able to dock on the p65 subunit of NFκB and drive its clearance from inflammatory target genes in astrocytes and microglia, providing an additional mechanism for the protective function of NURR1 in Parkinson’s disease (Saijo et al. 2009). Finally, NUR77 and NOR1 play essential roles in apoptosis in many cells and there is mounting evidence that this is accomplished in a non-genomic manner by converting BCL-2 proteins into pro-apoptotic molecules at the mitochondria (Li et al. 2006, Mohan et al. 2012). Under-scoring the potential impact of this finding, small peptide mimics of NUR77 have been designed successfully to induce cancer cell death (Kolluri et al. 2008).

On steroids: orphans of the NR5A group

Nr5a1/Sf1 and Nr5a2/Lrh1

Steroidogenic factor 1 (SF1) was discovered by Lala et al. (1992) as a major regulator of steroidogenic enzyme gene expression. Liver receptor homolog 1 (LRH-1) was cloned by several groups as a regulator of heptatitis virus and albumin gene expression (Tsukiyama et al. 1992, Becker-Andreé et al. 1993, Galanreau et al. 1996, Li et al. 1998, Nitta et al. 1999). SF1 and LRH1 have large, hydrophobic LBDs (Schimmer & White 2010). Initial studies reported stable active state conformations of both receptor LBDs without addition of an exogenous ligand (Desclozeaux et al. 2002, Sabin et al. 2003). However, in 2005, three groups discovered phospholipid species in the LBDs of SF1 and LRH1 purified from bacteria (Krylova et al. 2005, Li et al. 2005, Ortlund et al. 2005). Intriguingly, an unusual phosphatidylcholine species was identified as an LRH1 ligand that, when administered to WT, but not Lrh1−/− mice, improved their glucose homeostasis and ameliorated hepatic steatosis during high fat diet feeding (Lee et al. 2011a).

SF1 and LRH1 are transcriptional activators that bind DNA as monomers recognizing consensus of AGGTCA sequences. An apparent preference for YCA (where Y is a pyrimidine) 5’ to the hexamer may be due to a C-terminal extension (CTE) of the DBD shared with other monomeric DNA-binding NRs (Wilson et al. 1993, Solomon et al. 2005). SF1 and LRH1 also contain an additional 20 amino acid extension after the CTE called the Ftz-f1 motif (after Drosophila Ftz-f1) that is unique to NR5A family members (Ingraham & Redinbo 2005). Crystal structures revealed that this motif does not contact DNA but rather affects interactions with co-activating proteins (Solomon et al. 2005). Recent genome-wide profiling of SF1 binding in adrenocortical cells found the NR half site as its preferential motif, but did not find enrichment of the predicted 5’ pyrimidine, suggesting that it may not be required for SF1 binding to DNA in chromatin (Doghman et al. 2013).

SF1 is required for the differentiation of steroidogenic tissues, with homozygous null mice dying shortly after birth due to corticosteroid insufficiency (Luo et al. 1994, Sadovsky et al. 1995). SF1 function is also required for cell autonomously in pituitary gonadotropes, developing gonads and postnatal ovarian granulosa cells (Schimmer & White 2010). These mouse models of sexual maturation defects are important to human disease, as shown by the reproductive dysfunction in patients with naturally occurring SF1 mutations (El-Khairi & Achermann 2012, Lalli et al. 2013). LRH1 is involved in the regulation of steroid, BA and cholesterol homeostasis, processes that are consistent with its restricted expression in the pancreas, liver, intestines, and ovaries (Fernandez-Marcos et al. 2011). Hepatocyte-specific deletion of LRH1 changes the composition of the BA pool, resulting in decreased...
intestinal lipid absorption and BA recycling (Mataki et al. 2007, Lee et al. 2008). LRH1 has also been implicated in regulating glucose metabolism in the liver and proliferation in the intestine and pancreas (Schoonjans et al. 2005, Oosterveer et al. 2012). Intriguingly, GWAS studies have found a link between LRH1 SNPs and pancreatic cancer susceptibility (Petersen et al. 2010).

The family jewel: orphans of the NR6A group

Nr6a1/Gcnf

Germ cell nuclear factor (Gcnf, Rtr, Ncnf) is unique enough within the NR superfamily to be the only member of the NR6 subfamily (Hummelke & Cooney 2001, Germain et al. 2006). Originally named because of its robust and restricted expression in the germ cells, GCNF lacks a classic AF2 domain and acts as a transcriptional repressor through ligand-independent interactions with corepressor complexes (Chen et al. 1994a, Hirose et al. 1995, Bauer et al. 1997, Hummelke & Cooney 2001, Mullen et al. 2007). To date, a ligand for GCNF has not been identified, and its activity appears to be controlled by regulation of its gene expression (Heinzer et al. 1998, Gurtan et al. 2013, Krill et al. 2013, Wang et al. 2013). While recombiant GCNF forms homodimers upon binding to a DR of the classic NR half-site (AGGTCA) with no additional central nucleotides (DR0), endogenous DR0-bound GCNF exists as an oligomer in a DNA-dependent manner unlike that of typical NR dimerization (Gu et al. 2005b).

One of the direct targets of GCNF is Oct4, a core TF in the maintenance of embryonic stem cell pluripotency (Fuhrmann et al. 2001). Indeed, loss of function studies in mice has demonstrated that the GCNF-mediated repression of Oct4 is essential for stem cell differentiation during embryogenesis (Chung et al. 2001, Gu et al. 2005a). The regulation of Oct4 and other pluripotency factors makes GCNF an attractive target for therapeutic manipulation and biomarking in stem cells (Mullen et al. 2007, Akamatsu et al. 2009, Wang et al. 2013).

Summary and future perspectives

Advances in chemistry and molecular biology have led to an enormous accumulation of knowledge about the number, regulation, and function of orphan NRs over the past 25 years since their initial discovery. This review has attempted to provide a high-level overview of this progress, focusing on how each orphan was discovered, its regulation by ligand, and its regulation of gene expression in different tissues in health and disease. Much more is known about each orphan NR, and readers are encouraged to seek out additional information from the printed and virtual scientific literature.

Although the family is much grown up, current rapid progress in high throughput nucleotide sequencing, proteomics, metabolomics, and computational biology promises to lead to a more complete and integrated understanding of the orphan NRs and how they are regulated in different tissues, and what they regulate at many developmental stages, and in disease states. This approach is presently somewhat limited by the lack of adequate antibodies and data analysis pipelines, and by the added complexity of many orphan NRs playing critical roles in cell types that are minority components of heterogenous tissues. Technical advances in these areas will be critical for the discovery of novel therapeutic targets as well as strategies that avoid target effects while maintaining beneficial actions.

Possibly the largest question still unanswered concerns the identification of the most relevant endogenous ligands for most, if not all, of the orphan NRs. Beginning shortly after the initial discovery of orphan NRs, the issue of whether they would all have endogenous ligands was hotly debated (‘Do Orphan Receptors Have Ligands?’, see www.sarah-greene.net/previous/hmsbeagle/html/content/03/cutedge/overview.htm) and this question still remains. While endogenous and synthetic ligands have been discovered for many of the orphan NRs, others seem likely to be bona fide ligand-independent TFs, yet this is almost impossible to prove (Schupp & Lazar 2010). Further, in most cases, there is not a consensus about the physiological role of putative endogenous ligands. This is in clear contrast with NR superfamily members that are receptors for hormones, where the hormones were discovered before the ligands and, in most cases, are produced by discrete endocrine organs such as the thyroid and adrenals, whose function is largely based on secretion of their cognate hormones.

Nevertheless, the identification of biological functions and ligands that activate the orphan NRs has yielded remarkable insight into dozens of diverse physiological processes, from embryonic stem cell self renewal and differentiation to inflammation, circadian rhythm, and metabolism. This has led to a more complete understanding of the mechanism of action for certain drug classes, the identification of targets mediating adverse effects of environmental pollutants, better strategies for the reprogramming of pluripotent cells, and development of novel small molecules for the treatment of human disease. Further dissection of orphan NR networks, including their
mechanisms of action and the genes and cellular processes that they regulate, continues to have great potential to elucidate the molecular pathology of diseases as well as the underlying physiology, leading to safer, more specific, and more effective therapeutic strategies that are likely to be evident at the next orphan NR reunion.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

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