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Abstract—Half of the members of the nuclear receptors superfamily are so-called “orphan” receptors because the identity of their ligand, if any, is unknown. Because of their important biological roles, the study of orphan receptors has attracted much attention recently and has resulted in rapid advances that have helped in

the discovery of novel signaling pathways. In this review we present the main features of orphan receptors, discuss the structure of their ligand-binding domains and their biological functions. The paradoxical existence of a pharmacology of orphan receptors, a rapidly growing and innovative field, is highlighted.

Introduction

The cloning of genes encoding the specific receptors for known hormones such as steroids, thyroid hormones, and vitamin-derived compounds such as retinoids and vitamin D₃, as well as functional demonstration of their implication in fundamental biological processes of therapeutic interest, led to an intensive search for related proteins predicted to share similar features (Mangelsdorf et al., 1995; Chambon,

1996). The defining structural and functional features of nuclear receptors are a conserved zinc finger DNA-binding domain (DBD¹) and a ligand-binding domain (LBD). The evolutionary combination of these functional domains led to the generation of a diverse family of ligand-activated transcription factors that regulate gene expression in response to ligand binding. The high degree of similarity among the first receptors identified, both at the structural and functional levels, set the stage for the search for other family members, initially by low stringency screening of cDNA libraries and polymerase chain reaction screens with degenerate primers (Giguere et al., 1988; Wang et al., 1989; Becker-Andre et al., 1993) and more recently by genome sequence analysis (Robinson-Rechavi and Laudet, 2003). These efforts led to the successful identification of the vast majority of known nuclear receptors (NRs) without prior knowledge of their ligand and defined the gene family (Blumberg and Evans, 1998). In humans, these proteins, referred to as orphan nuclear receptors, still represent half of the total number of NRs (24 of a total of 48 different genes in human).

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¹ Abbreviations: DBD, DNA-binding domain; LBD, ligand-binding domain; NR, nuclear receptor; RXR, retinoid X receptor; PPAR, peroxisome proliferator-activated receptor; FXR, farnesoid X receptor; LXR, liver X receptor; CAR, constitutive androstane receptor; PXR, pregnane X receptor; RAR, retinoic acid receptor; TR, thyroid hormone receptor; HNF, hepatocyte nuclear factor; ROR, retinoid-related orphan receptor; SF-1, steroidogenic factor 1; DAX-1, dosage-sensitive sex reversal-adrenal hypoplasia congenital critical region on the X chromosome protein 1; SHP, small heterodimer partner; TLX, tailless; NGFI-B, nerve growth factor-induced clone B; COUP-TF, chicken ovalbumin upstream promoter transcription factor; TR2, testicular receptor 2; TR4, testicular receptor 4; NURR1, Nur-related factor 1; GCMF, germ cell nuclear factor; NOR1, neuron-derived orphan receptor 1; ERR, estrogen-related receptor; LRH, liver receptor homolog; DR, direct repeat; PNR, photo-receptor-specific nuclear receptor; EAR, ErbA-related protein; ER, estrogen receptor; AF, activation factor; LBP, ligand-binding pocket.

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The discovery of the orphan NRs has raised several questions concerning their physiological functions and the existence of specific ligand(s) and possibly new endocrine systems and has shifted “endocrinology into reverse” (Kliwer et al., 1999; Shiau et al., 2001). Thus, the search for biological function and ligands for orphan NRs has become the subject of intense investigation. In this introductory review we will briefly present these molecules and their diverse bio-

logical functions and discuss how the search for ligands has led to a refinement of our definition of a NR ligand.

What Are Orphan Receptors?

The definition of orphan receptors is a loose and paradoxical one because, by definition, orphan receptors are receptors for which no ligand is known. The term “receptor” itself implies that a physiological ligand should exist, even though there is still no consensus in the field as to whether this will be true for all orphan NRs. Because the absence of proof is not the proof of absence, it is extremely difficult to demonstrate that a given orphan NR truly has no endogenous ligand. Complicating the issue is the fact that once a natural ligand has been discovered for an orphan NR, the receptor is no longer considered to be an orphan, despite the fact that it may retain structural and functional features more similar to the other orphan NRs than to the classic steroid and thyroid hormone receptors. Two prime examples are the RXRs and PPARs, which were discovered as orphan NRs, but which are now clearly considered to be liganded receptors, although the precise identity of their physiological, endogenous ligands is somewhat controversial (Gottlicher et al., 1992; Heyman et al., 1992; Kitareewan et al., 1996; Lemotte et al., 1996; Mata de Urquiza et al., 2000; Willson et al., 2000; Lengqvist et al., 2004). Together with the RXRs and PPARs, the FXRs, LXRs, CAR, and PXR have been classified as a new type of NRs that are considered natural sensors (Janowski et al., 1996; Lehmann et al., 1998; Kawamoto et al., 1999; Makishima et al., 1999; Tzamelis et al., 2000; Tzamelis and Moore, 2001; Francis et al., 2003). The ligand-binding pocket of these receptors is larger than those of classic receptors (such as RARs, TRs, or steroid receptors), and they bind a large diversity of molecules with lower affinity (typically in the micromolar range) (Benoit et al., 2004). Even though some compounds were found inside the pocket of some orphan receptors such as HNF-4, RORs, or SF-1, they still are firmly part of the orphan receptor group because the regulatory role of the compound is unclear and/or the physiological relevance of the interaction with the receptor has not been clearly established (Dhe-Paganon et al., 2002; Kallen et al., 2004; Li et al., 2005; Stehlin et al., 2001; Wisely et al., 2002). Thus, the composition of the orphan receptor group is likely to continue to shrink in the future.

Following this definition, the orphan receptors form a highly diverse group. In fact, orphan receptors are not linked functionally or evolutionarily. In phylogenetic trees of NRs, they are scattered among the six defined subfamilies (Escriva et al., 2000). In addition, their structures are also highly diverse, not only at the structural level within the LBD as discussed below but also in the other domains (Fig. 1). Indeed, some orphan recep-

tors have only one of the two characteristic domains of the NR superfamily. In vertebrates, DAX-1 and SHP, which contain only an LBD and lack a classic DBD with conserved cysteines as do the other receptors, are examples of such divergent orphan receptors (Zanaria et al., 1994; Burris et al., 1996; Seol et al., 1996). In other species (e.g., *Drosophila* or nematodes), there are several other examples of receptors containing only the LBD or only the DBD sequence. The size of the other domains is also variable; the A/B region of some orphan receptors is extremely short: 8 amino acids for some isoforms of ROR β and 14 amino acids in TLX, whereas in other cases this domain is quite long (250–280 amino acids for NGFI-B/NR4A group members). Like some liganded receptors, such as the RARs, the HNF-4 group members contain an F domain that modulates their transcriptional activities (Ruse et al., 2002).

The diversity of orphan receptors is also illustrated by various modes of binding to DNA. Although most of them seem to bind to DNA as homodimers on direct repeat elements (HNF-4, COUP-TFs, and TR2/4), some interact with RXRs (NGFI-B and NURR1) (Perlmann and Jansson, 1995), and probably the most singular example of a DNA-binding mechanism is the oligomerization of the orphan GCNF upon binding to a direct repeat AGGTCAAGGTCA (Gu et al., 2005c). This divergent DNA-binding mechanism of GCNF, hexamer formation, is probably a reflection of its being the only member in the distant sub-branch 6 of the superfamily. Importantly, the study of several orphan receptors (Rev-erbs, RORs, SF-1, NGFI-B, NURR1, NOR1, and ERRs) allowed definition of a new type of interaction with DNA, namely, monomer binding to half-site sequences (Wilson et al., 1993). Even though such an ability has been found in a few cases for liganded receptors (e.g., TR α), the functional relevance of monomeric binding is clear only for orphan receptors. In all cases, binding occurs on a conserved A/GGGTCA binding motif that is preceded by an A/T-rich region in 5'. The sequence of this A/T-rich region is variable from one receptor type to another. SF-1, LRH-1, and ERRs bind to TCAA/GGGTCA elements (called SFRE or ERRE) (Honda et al., 1993; Sladek et al., 1997), whereas NGFI-B/NR4A group members bind to AAA/GGGTCA elements (called NBRE) (Wilson et al., 1991). Lastly, Rev-erbs and RORs bind to a less constrained sequence, the consensus of which is A/TAA/TNTA/GGGTCA and is termed a RevRE or a RORE (Harding and Lazar, 1993; Giguère et al., 1994). In addition, Rev-erbs have been described to bind as homodimers to special DR2 elements, called RevDR2, in which the 5' element, a RevRE, and the 3' element, a classic A/GGGTCA, are separated by two bases, most often CT (Harding and Lazar, 1995). In all of these cases of monomeric binding to extended half-site sequences, the interaction between the receptor and DNA is in the A/GGGTCA motif, with a recognition helix at the C-terminal part of the first zinc finger interacting with the

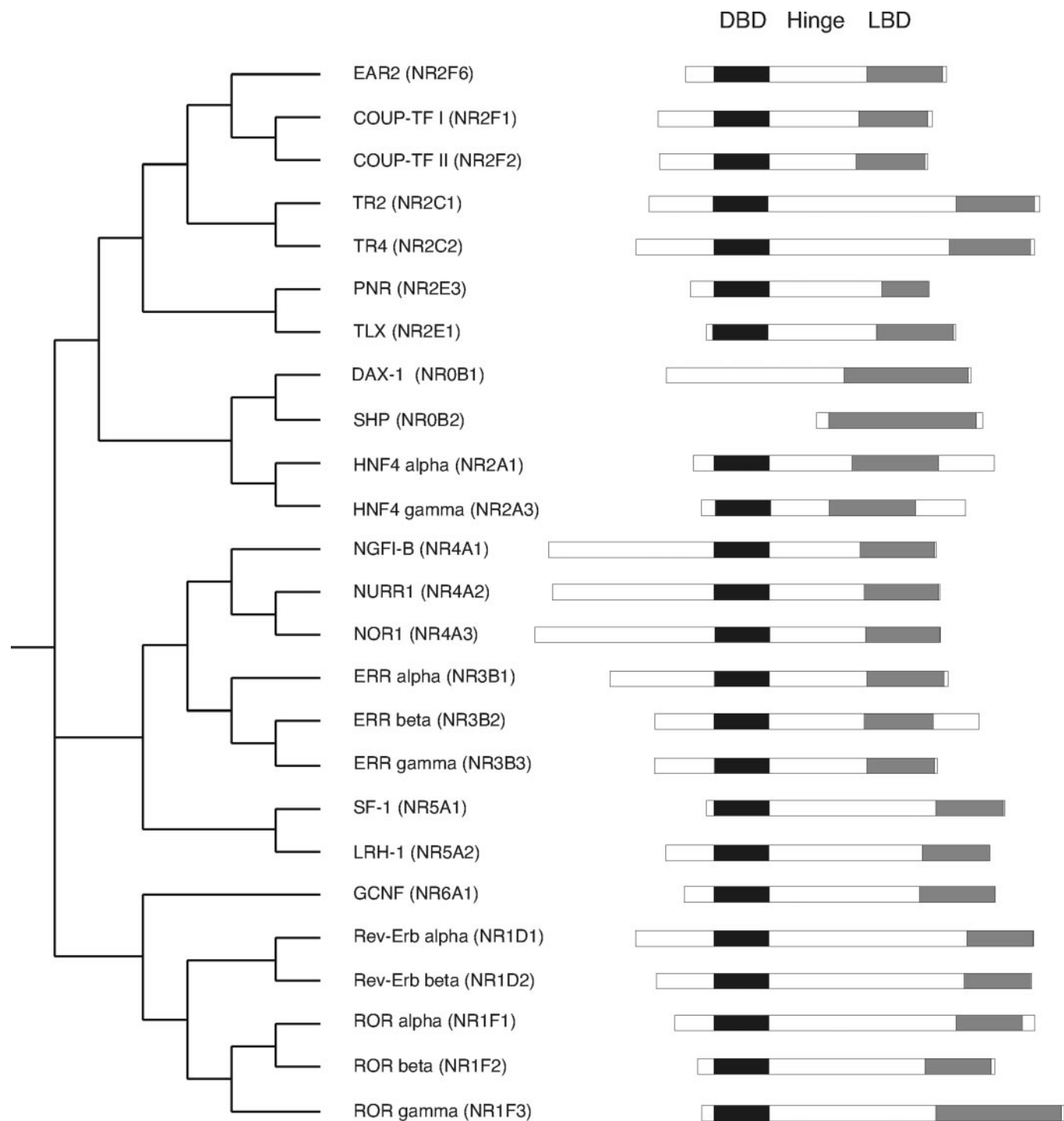


FIG. 1. Phylogenetic tree and schematic structure of orphan nuclear receptors present in human, mouse, and rat.

major groove of DNA and making specific contacts with the A/GGGTCA motif. A second helix in the second zinc finger stabilizes the interaction with DNA and allows dimerization with partners, when partners are present. In addition, the C-terminal part of the receptor is able to interact specifically with the extended 5' element. Several detailed functional studies plus structural analyses, including one of the Rev-erb DBD associated with DNA, led to the identification of

a region beyond the core DBD (C domain), called the A box, that forms a third α -helix of the DBD and is implicated in the recognition of the 5' extension of the DNA element (Wilson, 1993; Rastinejad et al., 1995). In fact, it has now been shown that variations of this structural element can be found in liganded receptors, such as TRs or RXRs. This is a nice illustration of the impact that orphan receptors can have on the study and understanding of liganded receptors.

Biological Functions of Orphan Receptors

Given the wide diversity of orphan receptors, it is, of course, very difficult to summarize their biological functions (Giguere, 1999). Two points are nevertheless important to mention and to discuss. 1) All orphan receptors have a very important function that is specific to each one of them. Thus, they are not inert molecules, less important than classic receptors. Indeed, gene targeting in the mouse has revealed important, often essential, roles for orphan NRs in development and adult physiology. 2) Orphan receptors often play an important role in modulating the action of classic liganded receptors.

It is possible to generate a very short summary of the functions played by these molecules and because the function of most of them has been inactivated in the mouse or in other biological models, we have a fairly clear understanding of their role, even if, of course, many questions remain. Many orphan receptors are important players in development and cell differentiation. For example, HNF-4 α is critical for early mouse development as well as for the development of the liver in vertebrates and arthropods (Watt et al., 2003). COUP-TFs have a conserved fundamental role in nervous system development as illustrated in mouse, zebrafish, and even hydra (Cooney et al., 2001) as well as in organogenesis of various organs (Park et al., 2003). The three NGFI-B members are also important players in brain development (Perlmann and Wallen-Mackenzie, 2004) and in T-cell biology (He, 2002). ERR α in zebrafish (Bardet et al., 2005) and ERR β in mouse (Luo et al., 1997) have crucial roles in very early development, for example, during gastrulation. In addition ERR α has been proposed to regulate osteoblastic differentiation at later stages of mouse embryogenesis (Bonnelye et al., 1997). GCNF has been shown to play an important role in early mouse development (Chung et al., 2001). GCNF plays a pivotal role in the silencing of pluripotency gene expression at gastrulation and ES cell differentiation (Fuhrmann et al., 2001; Gu et al., 2005b). DAX-1 is specifically implicated in germ cell differentiation in mouse (Achermann, 2005; Zechel, 2005), whereas the TLX receptor and PNR also play an important role during development, for example, in retina formation (Kobayashi et al., 1999). SF-1 plays a central role in the development of steroidogenic tissues, the adrenals and gonads, whereas its close homolog LRH-1 plays a role in endoderm differentiation and maintenance of pluripotency in early embryos (Pare et al., 2004; Gu et al., 2005a).

In addition, orphan receptors have also an important role in adult physiology in regulating metabolism. This is the case for ERR α , which is important for adipogenesis and energy metabolism (Sladek and Giguere, 2000; Luo et al., 2003), but also for the previously mentioned LRH-1 and SF-1, which are critical players in the regulation of cholesterol metabolism in the liver as well as in

steroidogenic tissues (Fayard et al., 2004). HNF-4s, COUP-TFs, Rev-erbs, and RORs also play a role in regulating metabolism (especially in cholesterol and fatty acid metabolism) (Jetten et al., 2001; Jetten, 2004; Laitinen et al., 2005), although their specific functions are not yet well understood.

Finally, an emerging, yet poorly characterized, role for orphan receptors is in the regulation of circadian rhythm, a function probably tightly linked to their role in metabolism (Inoue et al., 2005). Rev-erbs and RORs are prominent members of the circadian pacemaker in peripheral tissues as well as in the master clock organ, the suprachiasmatic nucleus (Alvarez and Sehgal, 2002; Preitner et al., 2002, 2003; Emery and Reppert, 2004; Jetten, 2004; Triqueneaux et al., 2004; Guillaumond et al., 2005). Other orphan receptors, such as ERR α , SHP, or EAR2, are also expressed in a circadian manner, and it is interesting to note that the knockout of EAR2 in the mouse exhibits a circadian phenotype (Horard et al., 2004; Warnecke et al., 2005).

Many experiments have demonstrated that orphan receptors regulate the activity of liganded receptors. This is the case for COUP-TFs, TR2, and TR4 (and also to a lesser extent for HNF-4s), which have been shown to repress the activation mediated by liganded receptors such as RAR, TR, or PPAR (Lee et al., 2002; Park et al., 2003). DAX-1 and in a broader sense SHP are regulators of the activity of other receptors (either orphan or liganded) by direct interaction with these receptors (Zhang and Dufau, 2004; Bavner et al., 2005). These highly unusual members of the NR superfamily can even be described as corepressors because they do not bind DNA and do not dimerize with other NRs through the canonical homo-/heterodimerization interface but rather through the cofactor interface. Another case is the connection that exists between ERRs and estrogen signaling. It has been shown that ERRs and ERs share both structural and functional attributes, such as synthetic ligands, interactions with coactivators, and binding to similar DNA sequences *in vitro* (Giguere, 2002). Many of these connections were found during the early days of orphan receptor research, when researchers were avidly searching for a functional role of these molecules. Thus, it has to be emphasized that many of these experiments were done in transient transfection assays and that in some cases their biological relevance *in vivo* still awaits confirmation.

Unorthodox LBD for Unorthodox NRs

Two main strategies were developed to search for orphan receptor ligands. These were based either on the search for the ligand *per se* by focused or random screening of naturally occurring or synthetic compounds (Chawla et al., 2001) or alternatively through the resolution of the structure of the NR LBD by X-ray crystallography. The successful identification of fatty acids,

oxysterols, and bile acids as naturally occurring agonists of the PPARs (Gottlicher et al., 1992), the LXRs (Janowski et al., 1996), and the FXR (Makishima et al., 1999; Parks et al., 1999), respectively, led to the suggestion that all NRs may be ligand-regulated. However, it seems that some orphan NRs were resistant to the traditional screening approaches, especially those displaying some level of constitutive activation (i.e., NGFI-B and NURR1) or repression (i.e., Rev-erbs). More recently, evolutionary studies have suggested and structural studies have shown that there are orphan NRs, in which the LBD can carry out its regulatory functions without the need for a ligand.

In contrast to classic liganded receptors, many orphan receptors show a “constitutive” AF-2-dependent transcriptional activity in different biological systems. How this group of receptors (RORs, ERRs, HNF4s, NURRs, and LRH-1) achieves this constitutive activity is of considerable interest to the understanding the function of these orphan NRs. This question was recently elucidated by analysis of the crystal structure of their LBDs. Interestingly, the answers seem to be just as diverse as the LBD structure is conserved, further indicating that the NR family evolved in multiple directions and took advantage of a single structure, namely the LBD, to achieve different physiological functions. In contrast to the screening approaches, which focused on the ligand only, the characterization of the crystal structure of NR LBDs yielded insights into the capacity of a given NR to be regulated by a ligand and in some cases even led to the direct identification of cocrystallized chemical compounds. In addition, and most importantly, the resolution of these structures has led to a refinement of the definition of what is a ligand of a NR. There are four different possibilities for orphan receptors and their potential ligand: 1) receptors with no ligand-binding pocket at all; 2) receptors with empty ligand-binding pockets; 3) receptors with structural ligands; and 4) receptors regulated by ligands, but the physiological relevance of those remains an open question. There are still many receptors, for which we simply have no clear information. This fifth category is the largest one and contains the COUP-TFs, GCNF, TLX, PNR, TR2/4, and DAX/SHP. We will now briefly examine the four possibilities.

The case of NURR1 is probably the most convincing for a receptor containing no ligand-binding pocket (LBP) (Wang et al., 2003). Because the *Drosophila* homolog of NURR1 (Baker et al., 2003), called DHR38, has the same features as NURR1, it has been suggested that the two other members of the group are characterized by a comparable structure. This hypothesis was since verified for NGFI-B (Flaig et al., 2005). The NGFI-B/NR4A group members form a branch of nuclear receptor homologs expressed in various cell types. When transfected into mammalian cells, all NR4A family members act as constitutively active transcription factors, and all

early attempts to define ligands for them have failed. Interestingly, the crystallographic analysis of the NURR1 and DHR38 LBDs reveals that these proteins lack a ligand-binding pocket. The overall structures are very similar to the canonical LBD fold (Wurtz et al., 1996), but bulky amino acid side chains occupy the space that would normally form the LBP. In the crystal structure of the NURR1 LBD, helix 12 is in the active conformation explaining the known constitutive activity of NR4A receptors. It was also revealed that residues conserved between canonical receptors forming the so-called “charge clamp” region, which is required for coactivator binding, are substituted in the NURR1 LBD. These observations and the fact that the NURR1 LBD does not interact with classic p160 coactivators raise questions about the mechanism by which NURR1 activates transcription. More recently, several studies revealed the existence of an alternative coactivator binding cleft (Codina et al., 2004, 2005; Flaig et al., 2005; Volakakis et al., 2006), but cofactors for NR4A family members that use that cleft have not yet been identified. It is now evident that despite obvious similarity to canonical LBDs, NR4A family members are not receptors and are regulated at the level of their expression or via post-translational modifications triggered by intracellular signaling pathways. The Rev-erbs, which are potent transcriptional repressors, are also good candidates to be orphan receptors with no LBP, because modeling studies have suggested that, as for NURR1, the LBP is filled with amino acid side chains (Renaud et al., 2000). A note of caution is nevertheless required here, because this result is only based on modeling studies, not on the experimental determination of the structure. Interestingly, the *Drosophila* homolog of Rev-erb, E75, is regulated by a unique mechanism. E75, in fact, contains a heme prosthetic group in the LBP, which by controlling the oxidation state of the heme iron, gases, such as nitric oxide or carbon monoxide, controls the activity of the receptor (Reinking et al., 2005). This exemplifies the unexpected diversity of mechanisms that regulate orphan NR activity.

The second interesting case is represented by orphan receptors that have a constitutive activity but in which an empty LBP has been observed. This is the case for ERR γ , for which the structure of the LBD in complex with the SRC-1 peptide was determined (Greschik et al., 2002). This structure reveals a helix 12 in active conformation and a small, but empty, LBP. Similar results were obtained more recently with the structure of the ERR α LBD in complex with a PGC1 peptide (Kallen et al., 2004). There are known examples of compounds able to block the constitutive activity of ERR γ . Notably, these are widely used synthetic antiestrogens, such as 4-hydroxytamoxifen (Coward et al., 2001). Interestingly, and in contrast to other cases such as ROR β (see below), the activity of ERR γ does decline after LBP is blocked with bulky amino acid side chains. Antiestrogens, however,

no longer inactivate such mutants. In summary, $ERR\gamma$ and possibly $ERR\alpha$ and $ERR\beta$ are nuclear receptors that are activated by default, but which are also capable of responding to deactivating ligands (Coward et al., 2001; Tremblay et al., 2001a,b), although the physiological relevance of these ligands has not yet been demonstrated. Mouse LRH-1 is another example of an orphan receptor with a large and empty hydrophobic pocket (Sablin et al., 2003), but because this feature seems to be different for the human LRH-1, it will be further discussed below.

Several receptors contain ligands that are unable to leave the receptor and are in fact part of the structure itself. These “structural ligands” that behave as prosthetic groups are usually fatty acids or fatty acid derivatives. This fact is illustrated by the HNF-4s, which form another distinct group of constitutively active nuclear receptors (Dhe-Paganon et al., 2002; Wisely et al., 2002). Analysis of the structure of the HNF-4 γ LBD revealed the presence of fatty acids, which could not be displaced from the LBP without protein denaturation (Wisely et al., 2002a). The helix 12 was in an active conformation, and mutations of the LBP designed to prohibit the binding of fatty acids reduced the constitutive activity of the receptor. Thermal denaturation studies of mutated HNF-4 α derivatives, however, indicated reduced stability of variants unable to bind fatty acids. Taken together, these studies suggest that HNF4s evolved to position their AF-2 into active conformation without the involvement of the ligand but instead need the help of lipophilic fatty acids to globally fold the LBD. Therefore, HNF-4s may not be nuclear receptors in the classic sense. However, the activity of HNF-4s is regulated at the transcriptional level and by coexpression of regulatory factors, such as SHP. Two other examples in insects, *Drosophila* USP (Billas et al., 2001; Clayton et al., 2001) and E75 (as mentioned above), are also cases of receptors bound to prosthetic groups, namely, a phospholipid and a heme, respectively.

Finally, researchers are currently in the process of identifying ligands for some orphan NRs such as RORs, LRH-1 and SF-1, the members of subfamily 5. $ROR\alpha$ is constitutively active, and its LBD can efficiently recruit p300 and glucocorticoid receptor interacting protein coactivators, which shifts the AF-2 into the active conformation. A surprising feature of the $ROR\alpha$ LBP is that this domain copurifies with a cholesterol molecule inside (Kallen et al., 2002, 2004). Interestingly, cholesterol bound in the LBP can be exchanged with cholesterol sulfate, which, using structural predictions, is expected to be a more potent ligand. In addition, changes in the intracellular level of cholesterol modulate $ROR\alpha$ transcriptional activity. These results suggest that $ROR\alpha$ could potentially serve as a cellular cholesterol “sensor” (Willson, 2002). Cholesterol fills the $ROR\alpha$ LBP either to stabilize helix 12 in an active conformation or to globally assist the folding of the LBD. The latter possibility is

compatible with the cholesterol receptor hypothesis for $ROR\alpha$. In summary, $ROR\alpha$ differs from canonical nuclear receptors in that it is bound to its ligand constitutively, but reversibly. The LBP of the $ROR\beta$ nuclear receptor, like its homolog $ROR\alpha$, was originally crystallized together with a fortuitously captured molecule of stearic acid (Stehlin et al., 2001). Additional experiments established that stearate did not fulfill the criteria for a true $ROR\beta$ ligand. Because mutagenesis studies designed to block the $ROR\beta$ ligand-binding pocket yielded inactive receptors, the search for a $ROR\beta$ ligand continued. This resulted in the discovery that the well-known RAR natural agonist all-*trans*-retinoic acid binds the $ROR\beta$ LBD with low, but biologically relevant, affinity (Stehlin-Gaon et al., 2003). In addition, all-*trans*-retinoic acid acts as a partial cell-type specific antagonist for $ROR\beta$. Many questions remain concerning the in vivo relevance of these interesting observations, and more work is needed before $ROR\alpha$ can be considered a real cholesterol sensor and $ROR\beta$ a third type of retinoic acid receptor.

An even more striking scenario is represented by LRH-1 and SF-1. The mouse LRH-1 LBD assembles into the active conformation with a large, but empty, LBP (Sablin et al., 2003). The ability of helix 12 of LRH-1 to associate with the LBD core was attributed to an unusual helix 2 structure, which forms a unique fourth outer layer of the LBD and actively contributes to the maintenance of the basal activity of the receptor as demonstrated by site-directed mutagenesis (Sablin et al., 2003). Additional experiments aimed at artificially “filling” the LRH-1 LBP with bulky amino acid side chains resulted in an increase of basal activity of the receptor, suggesting that mouse LRH-1 is still ligand-responsive. Strikingly, the determination of the structure of the human LRH-1 as well as of mouse and human SF-1 shows that these receptors, in contrast to mouse LRH-1, bind phosphatidyl inositol second messengers and that ligand binding is required for maximal activity (Krylova et al., 2005; Li et al., 2005; Wang et al., 2005). In line with these findings, mutations of specific amino acids that are part of the LBP of mouse SF-1 induce a loss of activity. The question, of course, remains whether these “fortuitous” ligands that were discovered because they were captured in the LBP during overexpression in bacteria are natural ligands or are at least indicative of the existence of natural ligands. An important and still unanswered question is whether these ligands can really enter and leave the LBD freely (i.e., act as bona fide signaling molecules) and by doing so regulate its transcriptional activity.

All of these observations illustrate the tremendous diversity that exists for orphan receptors with respect to their relationships with small molecules and allows us to redefine the term “ligand” for nuclear receptors. Historically, since the discovery of the superfamily started with the characterization of steroid receptors (i.e., recep-

tors with nanomolar affinity for very selective ligands that are typically hormones synthesized in specific tissues in the organism), it was thought that most, if not all, NRs should have ligands with similar characteristics. The characterization of “sensors” such as PPARs, LXRs, FXRs, PXR/CAR, and even RXRs has prompted a reevaluation of this definition because the LBDs of these receptors bind a large number of molecules, often derived from food or intermediate metabolism products and present at very high physiological concentrations relative to steroid hormones, with a much lower affinity (typically in the micromolar range). Thus, it became clear that NRs do bind not only hormones or morphogens, such as retinoic acid, but also a much broader set of small molecules. If we consider all orphan receptors, we can see that there is a continuum between small molecules forming prosthetic groups tightly linked to the receptor and exchangeable molecules with signaling activities. The main challenge for future work will be to decipher which of the newly discovered “ligands” of orphan receptors are physiologically relevant molecules with a signaling role (i.e., carrying biological information). For this, the emphasis will have to shift from structural studies that were extremely powerful in revealing the nature of these molecules to in vivo analyses of their biological role.

However, in trying to physiologically link a new ligand to an orphan receptor, an instructive paradigm to look at is the estrogen receptors and the enzyme, aromatase, that produces their ligand. The reproductive roles of the estrogen receptors α and β were succinctly determined by gene targeting (Lubahn et al., 1993; Krege et al., 1998; Dupont et al., 2000). The reproductive phenotype of the aromatase knockout only reinforced its role in producing the signal that regulates the estrogen receptors (Fisher et al., 1998; Honda et al., 1998; Nemoto et al., 2000). Thus, inactivation of an enzyme that produces a putative ligand should phenocopy part or all of a receptor phenotype.

The Paradox: Toward Pharmacology of Orphan Receptors?

To conclude, one cannot help but comment that this wide diversity of mechanisms is very good news for pharmacologists. Examples such as $ERR\gamma$ clearly show that even if an orphan NR has apparently no ligand and an empty pocket, it can still be a valid pharmaceutical target, potentially bound and regulated by drug molecules. The same is likely to be true for other receptors, such as LRH-1, SF-1, and the RORs, making them promising pharmaceutical targets as well.

Finally, it is also important to note that numerous orphan receptors form heterodimers with RXR (Mangelsdorf and Evans, 1995). This functional property seems to be critical for the true orphans of the NR4A subfamily (NGFI-B and NURR1 but not NOR1

(Perlmann and Jansson, 1995; Zetterstrom et al., 1996), because these heterodimers were shown to be responsive to RXR specific ligands, adding yet another mechanism by which the transcriptional activity of these physiologically essential receptors can be regulated.

Tables 1 through 25 summarize the functions, biologic activities, structural properties, and ligands of these receptors.

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TABLE 1
DAX-1

| | |
|---------------------------------|--|
| Receptor nomenclature | NR0B1 |
| Receptor code | 4.10.1.OR:0:B1 |
| Other names | AHCH |
| Molecular information | Hs: 470aa, P51843, chr. Xp21 ¹ Rn: 472aa, P70503, chr. Xq22 Mm: 472aa, Q61066, chr. X C1 ² |
| DNA binding | |
| Structure | Homodimer, heterodimer |
| HRE core sequence | DAX-1 lacks the conventional DNA-binding domain |
| Partners | SF-1 (physical, functional): inhibition of SF-1-dependent transactivation by recruiting the nuclear receptor corepressor NCOR to SF-1 ^{3,4} ; LRH-1 (physical, functional): inhibition of LRH-1-dependent transactivation ⁴ ; ER (physical, functional): inhibition of ER-dependent transactivation ⁵ ; AR (physical, functional): cellular localization, inhibition of ligand-dependent transcriptional activation, relocalization of AR in the cytoplasm and nucleus ^{6,7} ; PR (physical, functional): inhibition of PR ligand-dependent transactivation via destabilization of the receptor dimers ⁷ |
| Agonists | |
| Antagonists | |
| Coactivators | |
| Corepressors | NCOR1, NORR2, COPS2 ^{3,7,8} |
| Biologically important isoforms | DAX-1 α (Hs): lacks the last 70aa of the DAX-1 protein; abundantly expressed in the adrenal gland, brain, kidney, ovary, and testis; can bind SF-1 and DNA but is unable to repress SF-1-mediated transactivation; may act as an antagonist to DAX-1 ^{9,10} |
| Tissue distribution | Developmental: gonadal urogenital ridge, adrenal primordium, pituitary, diencephalon; adult: adrenal cortex, ovarian granulosa and theca cells, testicular Leydig and Sertoli cells, anterior pituitary gonadotrope cells, neurons of the ventromedial nucleus of the hypothalamus (Hs, Mm) [Northern blot, in situ hybridization, immunohistology] ^{1,2,11–13} |
| Functional assays | |
| Main target genes | Repressed: DAX-1 (Hs, Mm, Rn) ¹⁴ StAR (Hs, Mm, Rn) ¹⁴ |
| Mutant phenotype | XY mice carrying extra copies of mouse DAX-1 as a transgene show delayed testis development when the gene is expressed at high levels but do not normally show sex reversal except when the transgene is introduced into mice strains carrying weak Sry alleles, confirming the notion that DAX-1 is responsible for DSS syndrome (Mm) [disruption caused by insertion of a vector] ¹⁵ ; female mice lacking the DAX-1 receptor do not exhibit abnormal ovarian development or fertility; male mice lacking the DAX-1 receptor exhibit progressive degeneration of the testicular germinal epithelium, suggesting DAX-1 is essential for spermatogenesis; they also exhibit abnormalities in gonadotropin and testosterone production, further stressing the role of DAX-1 in steroidogenesis and HPA axis regulation (Mm) [disruption caused by insertion of a vector] ¹⁵ |
| Human disease | HHG: all types of missense mutations in DAX-1 resulting in HHG localize in the ligand-binding domain; many mutations are frameshift or nonsense mutations that lead to a truncated DAX-1 protein ¹⁶ ; DSS syndrome: due to a duplication of the DAX-1 gene and not to an alteration of the receptor ^{2,17,18} ; X-linked AHC: all types of missense mutations in DAX-1 resulting in AHC localize in the ligand-binding domain; many mutations are frameshift or nonsense mutations that lead to a truncated DAX-1 protein ^{16,19,20} |

aa, amino acids; chr., chromosome; HRE, hormone response element; AR, androgen receptor; PR, progesterone receptor; HHG, hypogonadotropic hypogonadism; HPA, hypothalamo-pituitary-adrenal; AHC, adrenal hypoplasia congenita; DSS, dosage-sensitive sex reversal; StAR, steroidogenic acute regulatory protein.

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TABLE 2
SHP

| | |
|---------------------------------|---|
| Receptor nomenclature | NR0B2 |
| Receptor code | 4.10.1:OR:0:B2 |
| Other names | |
| Molecular information | Hs: 257aa, Q15466, chr. 1p36 ¹ Rn: 260aa, P97947, chr. 5q36 ² Mm: 260aa, Q62227, chr. 4 D3 ¹ |
| DNA binding | |
| Structure | |
| HRE core sequence | SHP seems to be unable to bind DNA |
| Partners | LRH-1 (physical, functional): inhibition of LRH-1 mediated gene expression modulation ³ ; FXR (physical, functional): inhibition of FXR mediated gene expression modulation ⁴ ; CAR (physical, functional): inhibition of CAR mediated gene expression modulation ^{1,5,6} ; HNF-4 (physical, functional): inhibition of HNF-4 mediated gene expression modulation ⁷ ; LXR α and LXR β (physical, functional): inhibition of LXR mediated gene expression modulation ⁸ |
| Agonists | |
| Antagonists | |
| Coactivators | |
| Corepressors | HDAC1, HDAC3, Sin3A, CREBBP, NCOR1, NCOR2 ^{5,6,9–12} |
| Biologically important isoforms | |
| Tissue distribution | Liver, heart, adrenal gland, spleen, pancreas {Mm} [Northern blot, in situ hybridization] ^{1,13,14} |
| Functional assays | |
| Main target genes | Repressed: CYP7A1 (Hs, Mm, Rn), ^{4,15} NTCP (Hs, Mm, Rn), ¹⁶ ABCA1 (Hs, Mm, Rn), ⁸ ACOX1 (Hs, Mm, Rn), ¹⁷ PEPCK (Hs, Mm, Rn) ¹⁸ |
| Mutant phenotype | SHP-null mice show gross accumulation and increased bile acid synthesis caused by derepression of the rate-limiting enzymes CYP7A1 and CYP8B1 (Mm) [disruption caused by insertion of a vector] ^{19,20} |
| Human disease | Obesity (in relation with MODY): a recent study identified mutations in the NR0B2 gene that segregated with mild or moderate early onset obesity in Japanese subjects ²¹ |

aa, amino acids; chr., chromosome; HRE, hormone response element; CREBBP, cAMP response element-binding protein binding protein; MODY, maturity onset of diabetes; NTCP, Na⁺/taurocholate-cotransporting protein; PEPCK, phosphoenolpyruvate carboxykinase.

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TABLE 3
Rev-erb α

| | |
|---------------------------------|--|
| Receptor nomenclature | NR1D1 |
| Receptor code | 4.10.1:OR:1:D1 |
| Other names | EAR1, EAR1 A, Rev-erbA α |
| Molecular information | Hs: 614aa, P20393, chr. 17q21 ¹⁻³ Rn: 614aa, Q63503, chr. 10q31 ⁴ Mm: 615aa, Q3UJJ1, chr. 11 D ⁵ |
| DNA binding | |
| Structure | Monomer, homodimer |
| HRE core sequence | A/T A A/T N T PuGGTCA (DR-2, half-site) |
| Partners | Rev-erb α (physical, functional): DNA binding ⁶ |
| Agonists | Homology modeling of the LBD of the NR1D subgroup suggests that the pocket is occupied by bulky side chains and cannot accommodate a classic ligand ⁷ |
| Antagonists | |
| Coactivators | NCOA5 ⁸ |
| Corepressors | NCOR1, C1d, HDAC3, NCOA5 ⁸⁻¹¹ |
| Biologically important isoforms | Rev-erb α 2 {Hs, Mm, Rn}: encoded by an mRNA transcribed from an alternative promoter; lacks the first 114aa in the N-terminal domain of Rev-erb α ¹² |
| Tissue distribution | Developmental: heart, eyes, brain (Purkinje cells of the cerebellum, olfactory granule cells, cerebral cortex, hippocampus); adult: skeletal muscle, brown fat, liver, heart, brain, pituitary, kidney, testis, lung, hypothalamus {Hs, Mm} [Northern blot, Q-PCR, in situ hybridization, Western blot, immunohistology] ^{2,3,13-18} |
| Functional assays | |
| Main target genes | Repressed: Rev-erb α {Hs, Mm, Rn}, ⁶ ApoA1 {Rn}, ¹⁹ ApoCIII {Hs, Mm, Rn}, ^{20,21} Bmal1 {Hs, Mm, Rn} ^{12,18} |
| Mutant phenotype | Knockout mice exhibit abnormalities in the cerebellum after 2 weeks of life, such as alterations in the development of Purkinje cells, a delay in the proliferation and migration of granule cells, and an increase in apoptosis of neurons in the internal granule cell layer {Mm} [knockout] ¹³ ; knockout mice have also been shown to exhibit defects in their circadian rhythm {Mm} [knockout] ¹⁷ |
| Human disease | |

aa, amino acids; chr., chromosome; HRE, hormone response element; Q-PCR, quantitative polymerase chain reaction.

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TABLE 4
Rev-erb β

| | |
|---------------------------------|---|
| Receptor nomenclature | NR1D2 |
| Receptor code | 4.10.1:OR:1:D2 |
| Other names | EAR1 β BD73, RVR, HZF-2 |
| Molecular information | Hs: 579aa, Q14995, chr. 3p24 ¹ Rn: 578aa, Q63504 ^{2,3} Mm: 576aa, Q60674, chr. 14 B ^{4,5} |
| DNA binding | |
| Structure | Monomer, homodimer |
| HRE core sequence | A/T A A/T N T PuGGTCA (DR-2, half-site) |
| Partners | Rev-erb β (physical, functional): DNA binding ⁶ |
| Agonists | Homology modeling of the LBD of the NR1D subgroup suggests that the pocket is occupied by bulky side chains and cannot accommodate a classic ligand ⁷ |
| Antagonists | |
| Coactivators | NCOA5 ⁸ |
| Corepressors | NCOR1, NCOA5 ⁸⁻¹⁰ |
| Biologically important isoforms | |
| Tissue distribution | Heart, brain, lung, liver, skeletal muscle, kidney, spleen, testis, CNS (cerebellar cortex, dentate gyrus, hippocampus) {Hs, Mm} [Northern blot, Q-PCR, in situ hybridization, Western blot, immunohistology] ^{1-3,11} |
| Functional assays | |
| Main target genes | Repressed: Rev-erb α {Hs, Mm, Rn}, ¹² N-Myc {Hs, Mm, Rn} ¹³ |
| Mutant phenotype | |
| Human disease | |

aa, amino acids; chr., chromosome; HRE, hormone response element; CNS, central nervous system; Q-PCR, quantitative polymerase chain reaction.

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8. Saue F, McBroom LD, Gallant J, Moraitis AN, Labrie F, and Giguere V (2001) CIA, a novel estrogen receptor coactivator with a bifunctional nuclear receptor interacting determinant. *Mol Cell Biol* **21**:343-353.

9. Downes M, Burke LJ, Bailey PJ, and Muscat GE (1996) Two receptor interaction domains in the corepressor, N-CoR/RIP13, are required for an efficient interaction with Rev-erbA α and RVR: physical association is dependent on the E region of the orphan receptors. *Nucleic Acids Res* **24**:379-386.

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TABLE 5
ROR α

| | |
|---------------------------------|--|
| Receptor nomenclature | NR1F1 |
| Receptor code | 4.10.1:OR:1:F1 |
| Other names | RZR α , RORA |
| Molecular information | Hs: 556aa, P35398 chr. 15q21-q22 ¹ Rn: 523aa, chr. 8q24 Mm: 523aa, P51448, chr. 9 D ^{2,3} |
| DNA binding | |
| Structure | Monomer, homodimer |
| HRE core sequence | T/A A/T T/A C A/T A/GGGTCA (DR-2, half-site) |
| Partners | MyoD1 (physical, functional): interaction mediated by the N-terminal activation domain of the bHLH protein, MyoD, and ROR α 1 DNA-binding domain/C region ⁴ ; Nm23-1 (physical) ⁵ ; Nm23-2 (physical) ⁵ |
| Agonists | Cholesterol, cholesterol sulfate ⁶⁻⁸ |
| Antagonists | |
| Coactivators | NCOA2, PPARBP, EP300 ^{4,9} |
| Corepressors | NCOR1, NCOR2, ¹⁰ HR ¹¹ |
| Biologically important isoforms | ROR α 1 {Hs, Rn} ^{1,12} ; ROR α 2 {Rn} ^{1,12} ; ROR α 3 {Rn} ^{1,12} ; ROR α 4 {Rn} ^{1,12} ; the four ROR α isoforms differ in their N-terminal domain and exhibit differential DNA binding preferences ^{1,12} |
| Tissue distribution | Lung, muscle, brain (retinal ganglion cells, cerebellum, thalamus, suprachiasmatic nucleus), heart, leukocytes, spleen, liver, ovary, testis, cartilage, skin, lens, intestinal epithelium {Hs, Mm, Rn} [Northern, in situ hybridization] ^{1,4,13-19} |
| Functional assays | |
| Main target genes | Activated: N-Myc {Hs}, ²⁰ ApoA5 {Hs}, ²¹ laminin B1 {Hs}, ²² Bmal1 {Mm}, ^{23,24} fibrinogen β {Hs}, ²⁵ Rev-erbA α {Hs}, ^{26,27} |
| Mutant phenotype | The inactivation of ROR α causes the stagger phenotype in the cerebellum; no apparent morphological effects on the thalamus, hypothalamus, retina, or regions in which ROR α is expressed were detected; however, the pelage is significantly less dense and has growth difficulties when shaved {Mm} [knockout] ^{3,16,28,29} |
| Human disease | |

aa, amino acids; chr., chromosome; HRE, hormone response element; bHLH, basic helix-loop-helix; PPARBP, PPAR-binding protein; HR, hairless.

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TABLE 6
ROR β

| | |
|---------------------------------|---|
| Receptor nomenclature | NR1F2 |
| Receptor code | 4.10.1:OR:1:F2 |
| Other names | RZR β , ROR β |
| Molecular information | Hs: 459aa, Q92753, chr. 9q21 Rn: 459aa, P45446, chr. 1q43 ¹ Mm: 459aa, Q8R1B8, chr. 19 B ² |
| DNA binding | |
| Structure | Monomer, homodimer |
| HRE core sequence | T/A A/T T/A C A/T A/GGGTCA (half-site) |
| Partners | Nm23–2 (physical) ³ |
| Agonists | |
| Antagonists | ALRT 1550 (39 pM),* all- <i>trans</i> -retinoic acid (150 pM), all- <i>trans</i> -4-oxoretinoic acid (520 pM) [IC ₅₀] ⁴ |
| Coactivators | NCOA1 ⁵ |
| Corepressors | Nrip2, HR ^{6,7} |
| Biologically important isoforms | ROR β 2 {Rn}: differing in the N-terminal region, expression found only in the pineal gland and retina, more restricted DNA-binding properties, probably to regulate different sets of genes ⁸ |
| Tissue distribution | Developmental: retina; adult: pineal gland, hypothalamus, thalamus, spinal cord, pituitary, eye (retinal progenitor cells), spleen {Hs, Mm, Rn} [Northern blot, in situ hybridization, immunohistology] ^{9–11} |
| Functional assays | |
| Main target genes | Activated: Bmal1 {Hs, Mm, Rn} ¹² |
| Mutant phenotype | Knockout mice exhibit duck-like gait, disrupted reproduction in males, disorganization of the retina resulting in blindness, and abnormal circadian rhythm {Mm} [knockout] ⁹ |
| Human disease | |

aa, amino acids; chr., chromosome; HRE, hormone response element; HR, hairless.

* Radioligand.

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TABLE 7
ROR γ

| | |
|---------------------------------|--|
| Receptor nomenclature | NR1F3 |
| Receptor code | 4.10.1:OR:1:F3 |
| Other names | TOR, RORC |
| Molecular information | Hs: 518aa, P51449, chr. 1q21 ¹ Rn: 508aa, chr. 2q34 Mm: 516aa, P51450, chr. 3 F2 ² |
| DNA binding | |
| Structure | Homodimer |
| HRE core sequence | AGGTCA nnnnn AGGTCA (DR-4, DR-5, half-site) |
| Partners | Mi-2 β (physical, functional): inhibition of ROR γ transcriptional activity ³ |
| Agonists | |
| Antagonists | ALRT 1550, ⁴ all- <i>trans</i> -retinoic acid ⁴ |
| Coactivators | NCOA1 ⁵ |
| Corepressors | HR ⁶ |
| Biologically important isoforms | ROR γ b (Hs, Mm): differs in the 5'-UTR and coding region; resulting isoform is shorter and has a distinct N terminus ⁷ |
| Tissue distribution | Skeletal muscle, thymus, testis, pancreas, prostate, heart, liver, tongue, diaphragm; no expression found in the spleen or bone marrow (Hs, Mm, Rn) [Northern blot, in situ hybridization, immunohistology] ^{1,2,8,9} |
| Functional assays | |
| Main target genes | |
| Mutant phenotype | Homozygous mutants lack peripheral and mesenteric lymph nodes and Peyer's patches, reduced numbers of thymocytes and increased apoptosis with loss of thymic expression of antiapoptotic factor Bcl-xL (Mm) [knockout] ¹⁰ |
| Human disease | |

aa, amino acids; chr., chromosome; HRE, hormone response element; HR, hairless.

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TABLE 8
HNF α

| | |
|---------------------------------|--|
| Receptor nomenclature | NR2A1 |
| Receptor code | 4.10.1:OR:2:A1 |
| Other names | HNF-4, MODY1, TCF14 |
| Molecular information | Hs: 465aa, P41235, chr. 20q13 ^{1,2} Rn: 465aa, P22449, chr. 3q42 ^{3,4} Mm: 465aa, P49698, chr. 2 H3 ^{5,6} |
| DNA binding | |
| Structure | Homodimer |
| HRE core sequence | AGGTCA n AGGTCA (DR-1, DR-2) ^{4,7} |
| Partners | HIF (physical, functional): transactivation ^{8–10} ; HNF-1A (physical, functional): transactivation ^{11–13} ; COUP-TFI, COUP-TFII (functional): transactivation and competition for DNA binding ^{14–16} ; SHP (physical, functional): transactivation ^{17,18} ; SMADs (physical, functional): transactivation ^{19,20} |
| Agonists | |
| Antagonists | |
| Coactivators | NCOA1, NCOA2, CREBBP, PPARGC1A, PPARGC1B, PPARBP ^{21–27} |
| Corepressors | NCOR2 ²⁸ |
| Biologically important isoforms | HNF-4 α 1 {Hs, Mm, Rn}: main isoform ^{1,4,5} ; HNF-4 α 2 (variant B){Hs, Mm, Rn}: contains an additional 10 amino acids in the F domain and is the most prominent form in the liver and kidney ^{1,5,21} ; HNF-4 α 3 (variant C) {Hs, Mm}: displays reduced transcriptional activity and liver expression compared with isoforms 1 and 2 ²⁹ ; HNF-4 α 4 {Hs, Mm}: this variant has an insertion in the AF-1 of HNF-4 α 1 ³⁰ ; HNF-4 α 7 and HNF-4 α 8 {Hs, Mm, Rn}: transcribed from a different promoter and have a different N terminus from the isoforms above but the same F domain as HNF-4 α 1 and HNF-4 α 2 ^{31–34} |
| Tissue distribution | Developmental: primary endoderm, liver, kidney, pancreas, stomach, intestine; adult: HNFα-1 and -2 —liver (hepatocytes), kidney, small intestine and colon but not in the pancreas; HNFα-3 and -4 —liver; HNFα-7 —pancreas, adult liver, small intestine, colon, stomach but not in the liver {Hs, Mm, Rn} [Northern blot, in situ hybridization, Western blot, immunohistology] ^{4,35–37} |
| Functional assays | Measurement of receptor activity using CAT and luciferase reporter genes in HeLa, HepG2, Hep3B, Saos2, Caco-2, and HEK 293 cells {Hs} ^{4,28,38} ; ectopic overexpression of HNF-4 α in fibroblasts induces a mesenchymal-to-epithelial transition, indicating that HNF-4 α is a dominant regulator of the epithelial phenotype {Mm} ³⁹ |
| Main target genes | Activated: ApoC3 {Hs, Mm, Rn}, ^{4,40,41} ApoB {Hs}, ^{41,42} HNF1A {Hs, Mm, Rn}, ^{41,43,44} PEPCK {Hs, Mm, Rn}, ^{41,45} CYP3A4 {Hs} ^{34,46,47} |
| Mutant phenotype | Targeted disruption of the HNF-4 α gene results in embryonic lethality; the embryos initiate but do not complete gastrulation in the absence of HNF-4 α {Mm} [knockout] ^{48,49} ; adult mice lacking hepatic HNF-4 α expression accumulated lipid in the liver and exhibited greatly reduced serum cholesterol and triglyceride levels and increased serum bile acid concentrations {Mm} [knockout] ^{39,50,51} ; mice lacking HNF-4 α in pancreatic β cells have hyperinsulinemia and, paradoxically, impaired glucose tolerance, as well as impaired glucose-stimulated insulin secretion and dysfunction of the K _{ATP} channel activity {Mm} [conditional knockout] ^{52,53} |
| Human disease | Early-onset type 2 diabetes: due to the three SNPs (Asp ¹²⁶ →Tyr, Asp ¹²⁶ →His, Arg ¹⁵⁴ →Gln) ⁵⁴ ; late-onset type 2 diabetes: due to missense mutations in the LBD and F domain and 13 SNPs in the P2 promoter ^{55–58} ; MODY1: caused by mutations in several different human populations affecting either the DBD or LBD ^{32,59–63} ; factor VII deficiency: caused by mutations in the HNF-4 α -binding site in the blood coagulation factor VII gene ⁶⁴ ; hemophilia B Leyden: caused by mutations in the HNF-4 α -binding site in the blood coagulation factor IX gene ^{65–67} |

aa, amino acids; chr., chromosome; HRE, hormone response element; HIF, hypoxia-inducing factor; CREBBP, cAMP response element-binding protein binding protein; PPARGC, PPAR coactivator gene; PPARBP, PPAR binding protein; SNP, single-nucleotide polymorphism; MODY1, maturity-onset diabetes of the young type 1; CAT, chloroamphenicol acetyl transferase; PEPCK, phosphoenolpyruvate carboxykinase.

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TABLE 9
HNF-4 γ

| | |
|---------------------------------|--|
| Receptor nomenclature | NR2A3 |
| Receptor code | 4.10.1:OR:2:A3 |
| Other names | HNF4B |
| Molecular information | Hs: 408aa, Q14541, chr. 8q21 ¹ Rn: chr. 2q24 Mm: 418aa, Q9WUU6, chr. 3 A1 ² |
| DNA binding | |
| Structure | Homodimer |
| HRE core sequence | AGGTCA n AGGTCA (DR-1) ^{3,4} |
| Partners | |
| Agonists | |
| Antagonists | |
| Coactivators | |
| Corepressors | |
| Biologically important isoforms | |
| Tissue distribution | Endocrine, pancreas, kidney, small intestine, and testis; not found in the liver and only very weakly in the colon (Hs, Mm, Rn) [Northern blot, in situ hybridization, immunohistology] ^{2,5} |
| Functional assays | Measurement of receptor activity using CAT and luciferase reporter genes in HeLa, HepG2, Hep3B, Saos2, Caco-2, and Hek 293 cells (Hs) ^{1,2,4,5} |
| Main target genes | Activated: ApoA4 (Mm), ^{4,5} ApoC3 (Mm), ² Tat (Mm), ² HNF-1 α (Hs), ¹ AKR1C4 (Hs) ⁶ |
| Mutant phenotype | |
| Human disease | |

aa, amino acids; chr., chromosome; HRE, hormone response element; CAT, chloroamphenicol acetyl transferase.

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TABLE 10
TR2

| | |
|---------------------------------|---|
| Receptor nomenclature | NR2C1 |
| Receptor code | 4.10.1:OR:2:C1 |
| Other names | TR2-11 |
| Molecular information | Hs: 603aa, Q15625, chr. 12q22 ¹ Rn: 590aa, Q8VIJ4, chr. 7q12 ² Mm: 590aa, Q505F1, chr. 10 C3 ³ |
| DNA binding | |
| Structure | Homodimer, heterodimer |
| HRE core sequence | AGGTCA n AGGTCA (DR-1, DR-2, DR-3, DR-4, DR-5, DR-6) |
| Partners | TR4 (physical, functional): DNA binding, exerts a stronger repressive activity than expressing either receptor alone ² ; AR (physical, functional): DNA binding, repression of TR2 target genes ³ ; ER (physical, functional): DNA binding ⁴ |
| Agonists | |
| Antagonists | |
| Coactivators | |
| Corepressors | NR1P1, HDAC3, HDAC4 ^{5,6} |
| Biologically important isoforms | TR2-5 (Hs): shorter LBD ^{1,7} ; TR2-7 (Hs): lacking LBD ^{1,7} ; TR2-9 (Hs): shorter LBD ^{1,7} |
| Tissue distribution | Developmental: testis (seminiferous tubules), kidney, and intestine; adult: prostate, liver, testis, seminal vesicle, and kidney {Mm, Rn} [Northern blot, in situ hybridization] ^{1,7} |
| Functional assays | |
| Main target genes | Activated: CNTFR α (Hs), ² aldolase A (Hs) ⁸ ; repressed: HRH1 (Hs), ⁹ EPO (Hs) ¹⁰ |
| Mutant phenotype | Both male and female TR2 knockout mice are fertile; male mutants have functional testes, including normal sperm number and motility {Mm} [knockout] ¹¹ |
| Human disease | |

aa, amino acids; chr., chromosome; HRE, hormone response element; CNTFR, ciliary neurotrophic factor receptor; EPO, erythropoietin.

1. Chang C, Kokontis J, Acakpo-Satchivi L, Liao S, Takeda H, and Chang Y (1989) Molecular cloning of new human TR2 receptors: a class of steroid receptor with multiple ligand-binding domains. *Biochem Biophys Res Commun* **165**:735–741.

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8. Chang C, Lee HJ, and Lee YF (1997) Identification of the human aldolase A gene as the first induced target for the TR2 orphan receptor, a member of the steroid hormone receptor superfamily. *Biochem Biophys Res Commun* **235**:205–211.

9. Lee HJ, Lee YF, and Chang C (1999) Identification of the histamine H1 receptor gene as a differentially repressed target of the human TR2 orphan receptor. *Mol Cell Biochem* **194**:199–207.

10. Lee HJ, Young WJ, Shih CY, and Chang C (1996) Suppression of the human erythropoietin gene expression by the TR2 orphan receptor, a member of the steroid receptor superfamily. *J Biol Chem* **271**:10405–10412.

11. Shyr CR, Collins LL, Mu XM, Platt KA, and Chang C (2002) Spermatogenesis and testis development are normal in mice lacking testicular orphan nuclear receptor 2. *Mol Cell Biol* **22**:4661–4666.

TABLE 11
TR4

| | |
|---------------------------------|---|
| Receptor nomenclature | NR2C2 |
| Receptor code | 4.10.1.OR:2:C2 |
| Other names | TAK1 |
| Molecular information | Hs: 596aa, P49116, chr. 3p25 ^{1,2} Rn: 596aa, P55094, chr. 4q34 ¹ Mm: 596aa, P49117, chr. 6 D2 ³ |
| DNA binding | |
| Structure | Monomer, homodimer, heterodimer |
| HRE core sequence | AGGTCA n AGGTCA (DR-1, DR-2, DR-3, DR-4, DR-5, half-site) |
| Partners | TR2 (physical, functional): DNA binding, exerts a stronger repressive activity than expressing either receptor alone ⁴ ; ER (physical, functional): DNA binding ⁵ ; AR (physical, functional): DNA binding, repression of TR4 target genes ⁶ |
| Agonists | |
| Antagonists | |
| Coactivators | |
| Corepressors | TRA16, TIP27 ^{7,8} |
| Biologically important isoforms | TAK1 (Hs); TR4a1 (Hs, Rn): differs in the A/B domain—present in brain, ovary, and placenta; TR4a2 (Hs, Rn): differs in the A/B domain—present in brain, ovary, and placenta |
| Tissue distribution | Developmental: neuronal precursors Adult: brain (hippocampus, cerebellum, hypothalamic area), CNS, adrenal gland, spleen, testis (spermatocytes), prostate, lungs (Mm, Rn) [Northern blot, in situ hybridization] ^{3,9} |
| Functional assays | |
| Main target genes | Activated: HIV1-LTR (Hs), ¹⁰ LHcGR (Hs), ¹¹ steroid 21-hydroxylase (Hs), ¹² CNTFR α (Hs), ⁴ ApoE (Hs) ¹³ |
| Mutant phenotype | Knockout mice exhibit delayed spermatogenesis and reduced sperm production (Mm) [knockout] ¹⁴ ; knockout mice have a significantly reduced number of offspring; they demonstrate high rates of early postnatal mortality, as well as significant growth retardation; in addition, female mutants show defects in reproduction and maternal behavior, with pups dying soon after birth with no indication of milk intake (Mm) [knockout] ¹⁵ ; knockout mice exhibit behavior deficits in motor coordination, suggesting impaired cerebellar function (Mm) [knockout] ¹⁶ |
| Human disease | |

aa, amino acids; chr., chromosome; HRE, hormone response element; CNS, central nervous system; CNTFR, ciliary neurotrophic factor receptor; LTR, long terminal repeat; LHR, luteinizing hormone receptor.

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- Collins LL, Lee YF, Heinlein CA, Liu NC, Chen YT, Shyr CR, Meshul CK, Uno H, Platt KA, and Chang C (2004) Growth retardation and abnormal maternal behavior in mice lacking testicular orphan nuclear receptor 4. *Proc Natl Acad Sci USA* **101**:15058–15063.
- Chen YT, Collins LL, Uno H, and Chang C (2005) Deficits in motor coordination with aberrant cerebellar development in mice lacking testicular orphan nuclear receptor 4. *Mol Cell Biol* **25**:2722–2732.

TABLE 12
TLX

| | |
|---------------------------------|---|
| Receptor nomenclature | NR2E1 |
| Receptor code | 4.10.1:OR:2:E1 |
| Other names | MTLL |
| Molecular information | Hs: 385aa, Q9Y466, chr. 6q21 ¹ Rn: Mm: 385aa, Q64104, chr. 10 B2 ² |
| DNA binding | |
| Structure | Monomer, homodimer |
| HRE core sequence | AAGTCA n AAGTCA (DR-1, half-site) |
| Partners | |
| Agonists | |
| Antagonists | |
| Coactivators | |
| Corepressors | |
| Biologically important isoforms | |
| Tissue distribution | Developmental: head ectoderm (developing telencephalon and dorsal midbrain), eye, nose, and proangiogenic astrocytes [Mm] [Northern blot, in situ hybridization, immunohistology] ^{2–4} |
| Functional assays | |
| Main target genes | Activated: RAR β (Hs, Mm, Rn) ⁵ ; repressed: PAX2 (Hs, Mm, Rn), ³ Gfap (Mm) ⁴ |
| Mutant phenotype | TLX knockout mice exhibit a marked forebrain phenotype with a reduction in the size of rhinencephalic and limbic structures; in addition, both males and females are more aggressive than usual, and the females lack normal maternal instincts; the knockout mice also exhibit a progressive retinal and optic nerve degeneration with associated blindness [Mm] [knockout] ^{6–12} ; a spontaneous mouse mutation exists for the NR2E1 gene called fierce (frc)—this mutation is genetically and phenotypically similar to NR2E1-targeted mutations [Mm] [spontaneous mutation] ¹³ |
| Human disease | |

aa, amino acids; chr., chromosome; HRE, hormone response element; RAR, retinoic acid receptor.

1. Finley KD, Taylor BJ, Milstein M, and McKeown M (1997) *dissatisfaction*, a gene involved in sex-specific behavior and neural development of *Drosophila melanogaster*. *Proc Natl Acad Sci USA* **94**:913–918.
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9. Miyawaki T, Uemura A, Dezawa M, Yu RT, Ide C, Nishikawa S, Honda Y, Tanabe Y, and Tanabe T (2004) *Tlx*, an orphan nuclear receptor, regulates cell numbers and astrocyte development in the developing retina. *J Neurosci* **24**:8124–8134.
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TABLE 13
PNR

| | |
|---------------------------------|--|
| Receptor nomenclature | NR2E3 |
| Receptor code | 4.10.1:OR:2:E3 |
| Other names | RNR |
| Molecular information | Hs: 410aa, Q9Y5X4, chr. 15q23 ¹ Rn: Mm: 395aa, Q9QXZ7, chr. 9 B ² |
| DNA binding | |
| Structure | Homodimer |
| HRE core sequence | AAGTCA n AAGTCA (DR-1) ¹ |
| Partners | Crx (physical): PNR and Crx interact via the DBD of each protein; the promoter/enhancer occupancy of PNR is Crx-dependent, suggesting that PNR is associated with photoreceptor gene targets by interacting with Crx ³ |
| Agonists | |
| Antagonists | |
| Coactivators | |
| Corepressors | |
| Biologically important isoforms | PNR α (Hs): this is the longest transcript, but it encodes the shorter isoform; PNRn (Hs): differs from PNA α in the 3'-UTR and coding region—the resulting isoform contains a longer C terminus compared with PNR α |
| Tissue distribution | Exclusively expressed in the retina in the outer nuclear layer, which contains the nuclei of cone and rod photoreceptor cells (Hs, Mm) [Northern blot, in situ hybridization] ^{1,2,4-6} |
| Functional assays | |
| Main target genes | Activated: Rhodopsin (Hs, Mm, Rn) ³ ; repressed: S-cone opsin (Hs, Mm, Rn), ³ M-cone opsin (Hs, Mm, Rn) ³ |
| Mutant phenotype | Spontaneous mutation associated with retinal degeneration: this mutation is a deletion of exons 4 and 5, resulting in the absence of 380 base pairs from the transcript; the predicted protein expressed from this allele would lack 127 amino acids, including sequences corresponding to the DNA binding domain; the deletion also introduces a frameshift and creates a premature stop codon {Mm} [spontaneous mutation] ⁵ |
| Human disease | Enhanced S-cone syndrome: due to several mutations affecting NR2E3 ^{4,7} ; retinitis pigmentosa: Crypto-Jews in Portugal with retinitis pigmentosa have a Arg ³¹¹ →Gln mutation in exon 6 of the NR2E3 gene ⁸ ; Goldmann-Favre syndrome: an Arg ³¹¹ →Gln mutation in the NR2E3 gene was found in a family with classic Goldmann-Favre syndrome ⁹ |

aa, amino acids; chr., chromosome; HRE, hormone response element; CRX, cone-rod homeobox.

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TABLE 14
COUP-TFI

| | |
|---------------------------------|--|
| Receptor nomenclature | NR2F1 |
| Receptor code | 4.10.1:OR:2:F1 |
| Other names | COUP α , COUP-TFA, EAR3, SVP44 |
| Molecular information | Hs: 423aa, P10589, chr. 5q15 ¹ Rn: 419aa, Q62681, chr. 2q11 ² Mm: 422aa, Q60632, chr. 13 C2 ³ |
| DNA binding | |
| Structure | Homodimer, heterodimer |
| HRE core sequence | AGGTCA n AGGTCA (DR-0, DR-1, DR-3, DR-4, DR-5, DR-6, DR-8, DR-11, palindrome) |
| Partners | RXR (physical, functional): sequesters RXR partners, thereby reducing its availability for use by TR, VDR, RAR, and PPAR ⁴⁻⁷ ; HNF-4 (physical, functional): transactivation, competition for DNA binding ⁸⁻¹⁰ ; TR (physical, functional): heterodimerization interferes with TR-dependent transcriptional regulation ^{7,11} ; RAR (physical, functional): heterodimerization interferes with RAR-dependent transcriptional regulation ^{7,11} ; ER α (physical, functional): formation of a ER α complex results in an increased recruitment of ERK2/p42 MAPK, phosphorylation of the human ER α on Ser ¹¹⁸ , and enhanced transcriptional activity; COUP-TF has also been shown to antagonize ER activation of the lactoferrin and oxytocin promoters ^{12,13} |
| Agonists | |
| Antagonists | |
| Coactivators | BCL11B1, NCOA1, CREBBP ^{10,14,15} |
| Corepressors | NCOR1, NCOR2, BCL11A ^{14,16} |
| Biologically important isoforms | |
| Tissue distribution | Developmental: rostral brain, presumptive hindbrain, anterior somites, CNS (neural tubes, motor neurons), tongue, follicles of the vibrissae, the cochlea, and nasal septum stroma; in organs that require mesenchymal and epithelial interactions, COUP-TFI is expressed in the mesenchymal cells but not in the terminally differentiated epithelium; adult: rostral and caudal part of brain, supraoptic nucleus (Mm) [Northern blot, in situ hybridization, immunohistology] ^{3,7,17} |
| Functional assays | |
| Main target genes | Activated: NGFI-A (Rn), ¹⁵ PEPCK (Hs, Mm, Rn), ^{18,19} TF (Hs) ⁸ ; repressed: CYP3A1 (Hs, Mm, Rn), ²⁰ MHC class I (Mm) ²¹ |
| Mutant phenotype | Animals die at birth from starvation and dehydration; these animals exhibit defects in morphogenesis of the ninth cranial ganglion and nerve resulting from an excess cell death in the ganglionic precursor cells (Mm) [knockout] ²²⁻²⁵ |
| Human disease | |

aa, amino acids; chr., chromosome; HRE, hormone response element; VDR, vitamin D receptor; CNS, central nervous system; ERK, extracellular signal-regulated kinase; MAPK, mitogen-activated protein kinase; CREBBP, cAMP response element-binding protein binding protein; MHC, major histocompatibility class.

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TABLE 15
COUP-TFII

| | |
|---------------------------------|--|
| Receptor nomenclature | NR2F2 |
| Receptor code | 4.10.1:OR:2:F2 |
| Other names | COUP β , COUP-TFB, ARP1, SVP40 |
| Molecular information | Hs: 414aa, P24468, chr. 15q26 ¹ Rn: 414aa, O09018, chr. 1q0.31 Mm: 414aa, P43135, chr. 7 ² |
| DNA binding | |
| Structure | Homodimer, heterodimer |
| HRE core sequence | A/GGGTCA n AGGGTCA (DR-0, DR-1, DR-3, DR-4, DR-5, DR-6, DR-8, DR-10, DR-11, palindrome, inverted repeats) |
| Partners | RXR (physical): sequesters RXR partners, thereby reducing its availability for use by TR, VDR, RAR, and PPAR receptors ³⁻⁵ ; HNF-4 (physical, functional): transactivation, competition for DNA binding ⁶⁻⁸ ; EAR2 (physical) ⁹ ; RAR ¹⁰ ; TR ¹⁰ |
| Agonists | |
| Antagonists | |
| Coactivators | BCL11B, SQSTM1 ^{11,12} |
| Corepressors | NCOR1, NCOR2, BCL11A ^{11,13} |
| Biologically important isoforms | |
| Tissue distribution | Developmental: at 7.5 days postcoitum expression identical to that of COUP-TFI except for a set of neuromeres in the diencephalic neuromeres, rhombomeres in the hindbrain, and expression restricted to motoneurons in the neural tube; COUP-TFII expression is greater than that of COUP-TFI in salivary gland, lung, esophagus, stomach, pancreas, kidney, and prostate but less than that of COUP-TFI in the testis, ovary, retina, skin, inner ear, or limb bud (Hs, Mm, Rn) [Northern blot, in situ hybridization, Western blot] ^{1,14} |
| Functional assays | |
| Main target genes | Activated: CYP7A (Hs, Mm, Rn), ¹⁵⁻²⁰ arrestin (Hs, Mm, Rn) ²¹ ; repressed: Apo AI (Hs, Mm, Rn), ^{1,22,23} MHC class I (Mm) ²⁴⁻²⁶ |
| Mutant phenotype | Homozygous mutants die around embryonic day 10 with growth retardation, hemorrhage, and edema; histological analysis revealed enlarged blood vessels, lack of normal heart development, and malformed cardinal veins; two-thirds of heterozygous mutants die during the first weeks of life with growth and reproductive defects due to reduced expression of enzymes important for progesterone synthesis in the ovary and defective decidual response in the uterus (Mm) [knockout] ²⁷⁻³¹ |
| Human disease | |

aa, amino acids; chr., chromosome; HRE, hormone response element; VDR, vitamin D receptor; MHC, major histocompatibility class.

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TABLE 16
EAR2

| | |
|---------------------------------|---|
| Receptor nomenclature | NR2F6 |
| Receptor code | 4.10.1:OR:2:F6 |
| Other names | |
| Molecular information | Hs: 403aa, P10588, chr0.19p13 ¹ Rn: 390aa, O09017, chr. 16p14 Mm: 389aa, P43136, chr. 8 B3.3 ² |
| DNA binding | |
| Structure | Homodimer, heterodimer |
| HRE core sequence | AGGTCA n AGGTCA (DR-1) |
| Partners | TR β (physical, functional): heterodimerization with TR β 1 inhibits TR β 1 binding to its response element ³ ; COUP-TFII (physical, functional): DNA binding ⁴ ; CBFA2 (physical, functional): interaction with CBFA2 inhibits activity of CBFA2 ⁵ ; ER α (physical) ³ ; GR (physical) ³ |
| Agonists | |
| Antagonists | |
| Coactivators | NCOA1 ³ |
| Corepressors | |
| Biologically important isoforms | |
| Tissue distribution | Developmental: liver; adult: placenta, heart, muscle, pancreas, kidney, but not in the lung or brain—also expressed in myeloid progenitor cells and epithelial cells {Hs, Mm} [Northern blot, in situ hybridization, immunohistology] ^{1–6} |
| Functional assays | |
| Main target genes | Repressed: renin {Mm}, ⁷ LH receptor {Hs, Mm, Rn}, ⁸ GRIK5 {Hs, Mm, Rn}, ⁹ oxytocin {Hs, Mm, Rn} ⁶ |
| Mutant phenotype | EAR2-null mice exhibit defects in the development of the locus coeruleus and in circadian behaviors and circadian gene expression {Mm} [knockout] ⁹ |
| Human disease | |

aa, amino acids; chr., chromosome; HRE, hormone response element; GR, glucocorticoid receptor; LH, luteinizing hormone.

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TABLE 17
ERR α

| | |
|---------------------------------|---|
| Receptor nomenclature | NR3B1 |
| Receptor code | 4.10.1:OR:3:B1 |
| Other names | ERR1, ESRL1 |
| Molecular information | Hs: 519aa, P11474, chr. 11q13 ¹ Rn: 421aa, Q5QJV7, chr. 1q43 Mm: 462aa, O08580, chr. 19 A ² |
| DNA binding | |
| Structure | Monomer, homodimer |
| HRE core sequence | TNA AGGTCA (ERE, SFRE) |
| Partners | ER α (physical, functional): may play a role in the response of some genes to estrogen via heterodimerization with ERs ^{3,4} |
| Agonists | 5,7,4'-Trihydroxyisoflavone, 7,4'-dihydroxyisoflavone, 5,7-dihydroxy-4'-methoxyisoflavone ⁵ |
| Antagonists | XCT790 (300–500 nM), diethylstilbestrol (5–15 μ M) [IC ₅₀] ^{6,7} ; toxaphene, chlordane ⁸ |
| Coactivators | PNRC2, PPARGC1A, NCOA3, NCOA2, NCOA1, PNCR ^{9–14} |
| Corepressors | |
| Biologically important isoforms | |
| Tissue distribution | Developmental: nervous system, muscles, epidermis, and several endodermal derivatives, such as the epithelium of the intestine and urogenital system; adult: cerebellum, hippocampus, kidney, gut, heart, hypothalamus, liver, lung, uterus, vagina, cervix (Hs, Mm) [Northern blot, in situ hybridization, Western blot, immunohistology] ^{1,2,4,15–18} |
| Functional assays | |
| Main target genes | Activated: MCAD (Hs, Mm, Rn), ¹⁷ osteopontin (Hs, Mm, Rn), ¹⁹ lactoferrin (Hs, Mm, Rn), ³ TR α (Hs, Mm, Rn), ²⁰ ApoA4 (Hs, Mm, Rn) ²¹ |
| Mutant phenotype | Knockout mice have reduced body weight and peripheral fat deposits and are resistant to high-fat diet-induced obesity (Mm) [knockout] ²² |
| Human disease | Osteoporosis: there is a statistically significant association between ERR α promoter polymorphism and lumbar spine BMD, suggesting a link between ERR α regulation and osteoporosis ²³ ; obesity: a recent study found a significant association between ERR α promoter polymorphism and elevated BMI ²⁴ |

aa, amino acids; chr., chromosome; HRE, hormone response element; PPARGC, PPAR coactivator gene; BMD, bone mineral density; BMI, body mass index; ERE, estrogen response element; SFRE, SF-1 response element; MCAD, medium-chain acyl-coenzyme A dehydrogenase.

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TABLE 18
ERRβ

| | |
|---------------------------------|--|
| Receptor nomenclature | NR3B2 |
| Receptor code | 4.10.1:OR:3:B2 |
| Other names | ERR2, Estrrb |
| Molecular information | Hs: 500aa, O95718, chr. 14q24 ¹ Rn: 433aa, P11475, chr. 6q31 ² Mm: 433aa, Q61539, chr. 12 E ^{1,3} |
| DNA binding | |
| Structure | Monomer, homodimer |
| HRE core sequence | TNA AGGTCA (DR-3, ERE, SFRE, half-site) |
| Partners | HSP90 (physical, functional): efficient homodimerization and DNA binding ² |
| Agonists | 5,7,4'-Trihydroxyisoflavone, 7,4'-dihydroxyisoflavone, 5,7-dihydroxy-4'-methoxyisoflavone, N'-((1E)-[4-(diethylamino)phenyl]methylene)-4-hydroxybenzohydrazide ^{4,5} |
| Antagonists | Diethylstilbestrol (5–15 μM) [IC ₅₀] ⁶ |
| Coactivators | PNRC, NCOA3, NCOA1, NCOA2 ^{4,7} |
| Corepressors | |
| Biologically important isoforms | Short-form hERRβ {Hs}: lacks the F domain found in hERRβ and is the matched homolog of mouse and rat ERR proteins in humans; it is widely expressed, whereas the other two isoforms are restricted to testis and kidney ⁸ ; hERRβ2-δ 10 {Hs}: lacks the exon 10 present in the canonical transcript and encodes a protein isoform only differing in the F domain of the protein; the canonical transcript and this variant are primate-specific and present a restricted expression in testis and kidney ⁸ |
| Tissue distribution | Developmental: trophoblast progenitor cells (these extraembryonic cells are implicated in placental formation); adult: liver, stomach, skeletal muscles, kidney, heart, supraoptic nucleus {Hs, Mm, Rn} [Northern blot, RT-PCR, in situ hybridization, Western blot, immunohistology] ^{1,2,3,8,9} |
| Functional assays | |
| Main target genes | |
| Mutant phenotype | Homozygous knockout mice have severely impaired placental formation and die at 10.5 days postcoitum; the mutants display abnormal chorion development associated with an overabundance of trophoblast giant cells and a severe deficiency of diploid trophoblast {Mm} [knockout] ⁹ |
| Human disease | |

aa, amino acids; chr., chromosome; HRE, hormone response element; h, human; RT-PCR, reverse transcription-polymerase chain reaction; ERE, estrogen response element; SFRE, SF-1 response element.

1. Chen F, Zhang Q, McDonald T, Davidoff MJ, Bailey W, Bai C, Liu Q, and Caskey CT (1999) Identification of two hERR2-related novel nuclear receptors utilizing bioinformatics and inverse PCR. *Gene* **228**:101–109.

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5. Yu DD and Forman BM (2005) Identification of an agonist ligand for estrogen-related receptors ERRβ/γ. *Bioorg Med Chem Lett* **15**:1311–1313.

6. Tremblay GB, Kunath T, Bergeron D, Lapointe L, Champigny C, Bader JA, Rossant J, and Giguere V (2001) Diethylstilbestrol regulates trophoblast stem cell differentiation as a ligand of orphan nuclear receptor ERRβ. *Genes Dev* **15**:833–838.

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TABLE 19
ERR γ

| | |
|---------------------------------|---|
| Receptor nomenclature | NR3B3 |
| Receptor code | 4.10.1:OR:3:B3 |
| Other names | ERR3, ESRRG |
| Molecular information | Hs: 458aa, P62508, chr. 1q41 ^{1,2} Rn: 458aa, P62510, chr. 13q26 Mm: 458aa, P62509, chr. 1 H5 ³ |
| DNA binding | |
| Structure | Monomer, homodimer |
| HRE core sequence | TNA AGGTCA (DR-3, ERE, SFRE) ⁴ |
| Partners | Calmodulin (physical, functional): interaction with calmodulin in vitro in a Ca ²⁺ -dependent manner ⁵ ; DAX1 (physical, functional): inhibition of PGC1 α -mediated ERR γ transactivation by competing for the AF-2 binding domain ⁶ ; SHP (physical, functional): inhibition of transcriptional activity ⁷ |
| Agonists | 5,7,4'-Trihydroxyisoflavone, 7,4'-dihydroxyisoflavone, 5,7-dihydroxy-4'-methoxyisoflavone, N'-((1E)-[4-(diethylamino)phenyl]methylene)-4-hydroxybenzohydrazide ^{8,9} ; GSK9089 (substituted phenolic acyl hydrazones) (0.66 μ M), GSK4716 (substituted phenolic acyl hydrazones) (2 μ M) [IC ₅₀] ¹⁰ |
| Antagonists | 4-Hydroxytamoxifen (35 nM) [K _d] ¹¹ ; diethylstilbestrol (5–15 μ M) [IC ₅₀] ^{11,12} |
| Coactivators | PNRC2, PPARGC1A, PPARGC1B, NCOA1, TLE1 ^{13–15} |
| Corepressors | |
| Biologically important isoforms | ERR γ 2 (Hs, Mm): differs from ERR γ 1 by an additional 23 N-terminal residues ¹⁶ ; ERR γ 3 (Hs): ERR γ 3 variant consists of eight exons including three unique 5'-terminal exons and lacks the exon encoding the second zinc finger motif; the expression of ERR γ 3 was confined to adipocytes and prostate, whereas that of ERR γ 2 was fairly widespread; the ERR γ 3 variant was shown by transactivation assay to have no ability to activate ERE-controlled transcription; however, ERR γ 3 has an ability to modulate the transcriptional activity of other nuclear hormone receptors ¹⁷ |
| Tissue distribution | Brain, kidney, testis, lung, adrenal gland, pancreas, and bone marrow (Hs, Mm) [Northern blot, RT-PCR, in situ hybridization, Western blot, immunohistochemistry] ^{1,2,3,18} |
| Functional assays | |
| Main target genes | Activated: ERR α (Hs), ¹⁹ DAX-1 (Mm), ⁶ MAOA, and MAOB (Hs, Mm, Rn) ²⁰ |
| Mutant phenotype | Homozygous mutant mice do not survive to weaning age; heterozygous mice exhibit a significant increase in overall startle amplitude, indicating a possible hypersensitivity to sound-induced motor reflex in these mice (Mm) [disruption caused by insertion of a vector] |
| Human disease | |

aa, amino acids; chr., chromosome; HRE, hormone response element; PPARGC, coactivator gene; RT-PCR, reverse transcription-polymerase chain reaction; MAO, monoamine oxidase; ERE, estrogen response element; SFRE, SF-1 response element.

1. Chen F, Zhang Q, McDonald T, Davidoff MJ, Bailey W, Bai C, Liu Q, and Caskey CT (1999) Identification of two hERR2-related novel nuclear receptors utilizing bioinformatics and inverse PCR. *Gene* **228**:101–109.

2. Eudy JD, Yao S, Weston MD, Ma-Edmonds M, Talmadge CD, Cheng JJ, Kimberling WJ, and Sumeji J (1998) Isolation of a gene encoding a novel member of the nuclear receptor superfamily from the critical region of Usher syndrome type IIa at 1q41. *Genomics* **50**:382–384.

3. Hong H, Yang L, and Stallcup MR (1999) Hormone-independent transcriptional activation and coactivator binding by novel orphan nuclear receptor ERR3. *J Biol Chem* **274**:22618–22626.

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9. Yu DD and Forman YM (2005) Identification of an agonist ligand for estrogen-related receptors ERR β / γ . *Bioorg Med Chem Lett* **15**:1311–1313.

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13. Hentschke M and Borgmeyer U (2003) Identification of PNRC2 and TLE1 as activation function-1 cofactors of the orphan nuclear receptor ERR γ . *Biochem Biophys Res Commun* **312**:975–982.

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TABLE 20
NGFI-B

| | |
|---------------------------------|---|
| Receptor nomenclature | NR4A1 |
| Receptor code | 4.10.1.OR:4:A1 |
| Other names | NAK1, ST-59, TR3, nur77, N10, TIS1, NGFI-B α |
| Molecular information | Hs: 598aa, P22736, chr. 12q13 ¹⁻³ Rn: 597aa, P22829, chr. 7q36 ^{4,5} Mm: 601aa, P12813, chr. 15 F3 ^{6,7} |
| DNA binding | |
| Structure | Monomer, homodimer, heterodimer, RXR partner |
| HRE core sequence | AAAGGTCA (DR-5, half-site, NBRE, NuRE) |
| Partners | NURR1 (physical, functional): DNA binding ⁸ ; NOR1 (physical, functional): DNA binding ⁸ ; GR (physical, functional): DNA binding and antagonism of NuRE-dependent transcription induced by all members of the NR4A subfamily ⁹ ; BCL-2 (physical, functional): cellular localization—NGFI-B binding induces a BCL-2 conformational change that exposes its BH3 domain, resulting in conversion of BCL-2 from a protector to a killer ¹⁰ ; AKT (physical, functional): DNA binding and phosphorylation of Ser ³⁵⁰ on the NGFI-B protein within its DNA-binding domain ^{11,12} ; Notch-1 (physical, functional): interaction with NGFI-B to repress NGFI-B-dependent transcription and rescue T-cell receptor-mediated apoptosis ¹³ |
| Agonists | Receptor lacks ligand-binding pocket |
| Antagonists | Receptor lacks ligand-binding pocket |
| Coactivators | NCOA1, NCOA2, NCOA3, EP300, PPARBP ¹⁴ |
| Corepressors | |
| Biologically important isoforms | TRC β (Hs): contains a shorter and distinct C terminus compared with NGFI-B |
| Tissue distribution | Nervous system, pituitary, adrenal, thyroid, liver, testis, ovary, thymus, muscle, lung, prostate (Hs, Mm, Rn) [Northern blot, in situ hybridization, Western blot, immunohistochemistry] ^{14,6,7,15-18} |
| Functional assays | |
| Main target genes | Activated: POMC (Hs, Mm, Rn), ¹⁹ steroid 21-hydroxylase (Hs, Mm, Rn), ²⁰ steroid 17-hydroxylase (Hs, Mm, Rn), ²¹ INSL3 (Hs, Mm, Rn) |
| Mutant phenotype | Knockout mice exhibit no clear phenotype, suggesting functional redundancy between NR4A subfamily members in vivo; however, an altered neuropeptide expression pattern is observed (Mm) [knockout] ^{22,23} |
| Human disease | |

aa, amino acids; chr., chromosome; HRE, hormone response element; GR, glucocorticoid receptor; PPARBP, PPAR binding protein; NBRE, NGFI-B response element; NuRE, Nur response element; POMC, pro-opiomelanocortin.

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4. Milbrandt J (1988) Nerve growth factor induces a gene homologous to the glucocorticoid receptor gene. *Neuron* 1:183–188.

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TABLE 21
NURR1

| | |
|---------------------------------|---|
| Receptor nomenclature | NR4A2 |
| Receptor code | 4.10.1.OR:4:A2 |
| Other names | NOT, TINUR, HZF-3, RNR-1, NGFI-B β |
| Molecular information | Hs: 598aa, P43354, chr. 2q24 ¹ Rn: 598aa, Q07917, chr. 3q12 ² Mm: 598aa, Q06219, chr. 2 C2 ³ |
| DNA binding | |
| Structure | Monomer, homodimer, heterodimer, RXR partner |
| HRE core sequence | AAAGGTCA (DR-5, half-site, NBRE, NuRE) |
| Partners | NGFI-B (physical, functional): DNA binding ⁴ ; NOR1 (physical, functional): DNA binding ⁴ ; RXR (physical, functional): DNA binding ^{5,6} ; P57KIP2 (physical, functional): inhibition of NURR1 transcriptional activity ⁷ ; PIAS γ (physical, functional): repression of NURR1 transcriptional activity ⁸ |
| Agonists | Receptor lacks ligand-binding pocket ⁹ |
| Antagonists | Receptor lacks ligand-binding pocket ⁹ |
| Coactivators | |
| Corepressors | |
| Biologically important isoforms | NURR2 (Hs, Mm, Rn): has a novel cryptic exon located upstream in the NURR1 promoter region and is generated by alternative splicing at exons 1, 2, and 6; lacks the C-terminal sequences corresponding to the ligand-binding domain or dimerization domain; inactive by itself, but may be able to inhibit transactivation by interaction with members of the NGFI-B family ¹⁰ |
| Tissue distribution | Nervous system (mesencephalic dopaminergic neurons of the ventral tegmental area and of the substantia nigra), liver, pituitary, thymus, osteoblasts {Hs, Mm, Rn} [Northern blot, Q-PCR, in situ hybridization, Western blot, immunohistology] ^{2,3,6,11-13} |
| Functional assays | |
| Main target genes | Activated: osteopontin {Mm}, ¹⁴ osteocalcin {Rn}, ¹⁵ tyrosine hydroxylase {Mm}, ¹⁶ neuropilin {Mm} ¹⁷ |
| Mutant phenotype | Homozygous knockout mice exhibit a complete loss of ventral mesencephalic dopaminergic neurons and altered gene expression in the dorsal motor nucleus of the brainstem; they have respiratory dysfunction and die at birth {Mm} [knockout] ¹⁸⁻²² |
| Human disease | PD: in 8 of 107 individuals with familial PD, a T deletion was found at transcribed nucleotide position 291 upstream of the initiator AUG codon of NR4A2 and a T→G substitution at transcribed nucleotide position 245; these mutations did not affect the ORF but seem nevertheless dominant; later studies have not confirmed the importance of these mutations in PD ²³⁻²⁵ |

- aa, amino acids; chr., chromosome; HRE, hormone response element; Q-PCR, quantitative polymerase chain reaction; PD, Parkinson's disease; ORF, open reading frame.
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TABLE 22
NOR1

| | |
|---------------------------------|--|
| Receptor nomenclature | NR4A3 |
| Receptor code | 4.10.1:OR:4:A3 |
| Other names | TEC, MINOR, CHN, NGFI-B γ |
| Molecular information | Hs: 626aa, Q92570, chr. 9q31 ^{1,2} Rn: 628aa, P51179, chr. 5q22 ³ Mm: 627aa, Q9QZB6, chr. 4 B2 |
| DNA binding | |
| Structure | Monomer, homodimer, heterodimer |
| HRE core sequence | AAAGGTCA (half-site, NBRE, NuRE) |
| Partners | NGFI-B (physical): DNA binding ⁴ ; NURR1 (physical): DNA binding ⁴ |
| Agonists | Receptor lacks ligand-binding pocket ⁵⁻⁷ |
| Antagonists | Receptor lacks ligand-binding pocket ⁵⁻⁷ |
| Coactivators | SIX3, PPARBP, EP300, NCOA2, PCAF ⁸⁻¹⁰ |
| Corepressors | |
| Biologically important isoforms | NOR1 α (Hs, Mm): contains an additional segment in the coding region introducing a stop codon into the sequence, thereby creating a shorter and distinct C terminus compared with NOR1 ^{11,12} ; NOR1 β (Hs, Mm, Rn): differs in the 5'-UTR and coding region and contains a longer N terminus than NOR1 ¹¹ |
| Tissue distribution | Nervous system, pituitary, adrenal, heart, muscle, thymus, kidney (Hs, Mm, Rn) [Northern blot, in situ hybridization, Western blot, immunohistology] ^{1-3,13,14} |
| Functional assays | |
| Main target genes | Activated: POMC (Hs, Mm, Rn) ^{15,16} |
| Mutant phenotype | Knockout mice have been shown to exhibit inner ear defects and partial bidirectional circling behavior (Mm) [knockout] ¹⁷ ; knockout mice embryos have also been shown to fail to complete gastrulation and display distinct morphological abnormalities (Mm) [knockout] ¹⁸ |
| Human disease | EMC: three versions of EMCs are the result of reciprocal translocations between this gene and other genes; the translocation breakpoints are associated with NR4A3 (chr. 0.9) and either Ewing sarcoma breakpoint region 1 (chr. 0.22), RNA polymerase II, TATA box-binding protein-associated factor (chr. 0.17), or transcription factor 12 (chr. 0.15) ^{1,19-21} |

aa, amino acids; chr., chromosome; HRE, hormone response element; PPARBP, PPAR binding protein; EMC, extraskeletal myxoid chondrosarcoma; NBRE, NGFI-B response element.

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TABLE 23
SF-1

| | |
|---------------------------------|--|
| Receptor nomenclature | NR5A1 |
| Receptor code | 4.10.1:OR:5:A1 |
| Other names | FTZ-F1, ELP, AD4BP |
| Molecular information | Hs: 461aa, Q13285, chr. 9q33 ¹ Rn: 462aa, P50569, chr. 3q11 ² Mm: 462aa, P33242, chr. 2 B ³ |
| DNA binding | |
| Structure | Monomer |
| HRE core sequence | YCA AGG YCR (half-site) |
| Partners | DAX-1 (physical, functional): inhibits SF-1 transcriptional activation and blocks interaction of WT-1/SF-1 ^{4,5} ; WT-1 (physical, functional): enhancement of SF-1 transcriptional activity ⁵ ; GATA4 (physical, functional) ⁶ ; Ptx1 (physical, functional): enhancement of SF-1 transcriptional activity ⁷ ; SOX9 (physical, functional): enhancement of SF-1 transcriptional activity ⁸ |
| Agonists | 1,2-Dimyristoyl- <i>sn</i> -glycero-3-phosphoethanolamine (64 nM), 1,2-didodecanoyl- <i>sn</i> -glycero-3-phosphoethanolamine (66 nM), 1,2-dihexadecanoyl- <i>sn</i> -glycero-3-phosphocholine (80–120 nM) [EC ₅₀] ⁹ ; phosphatidyl inositols PIP ₂ and PIP ₃ ¹⁰ |
| Antagonists | 1,2-Dilinoleonyl- <i>sn</i> -glycerol-3-phosphocholine (100–300 nM) [IC ₅₀] ⁹ |
| Coactivators | CREBBP, NCOA1, NCOA2, EDF1, PNRC2 ^{10,11–15} |
| Corepressors | NCOR2 ¹² |
| Biologically important isoforms | ELP1 {Mm}: differs in its N- and C-terminal domains due to alternative splicing and promoter usage ¹⁶ ; ELP2 {Mm}: differs in its N-terminal domain due to alternative splicing and promoter usage ¹⁶ ; ELP3 {Mm}: encoded by a slightly longer mRNA due to alternative splicing and promoter usage ¹⁶ |
| Tissue distribution | Developmental: carcinoma cells, urogenital ridge, somatic cells (steroidogenic and nonsteroidogenic), adrenal cortex (but not in the adrenal medulla), ovary and testis (Sertoli and Leydig cells), pituitary (gonadotrope cells), ventromedial hypothalamic nucleus; adult: spleen, eutopic endometriotic tissue, adrenal glands, and gonads (Sertoli and Leydig cells) {Hs, Mm} [Northern blot, in situ hybridization, Western blot, immunohistology] ^{17–19} |
| Functional assays | Overexpression of SF-1 in embryonic carcinoma cells results in steroidogenesis (progesterone production) {Mm} ²⁰ |
| Main target genes | Activated: CYP11A1 {Hs, Mm, Rn}, ^{21,22} CYP17 {Hs, Mm, Rn}, ^{23,24} MC2R {Hs}, ^{25–27} VNN1 {Mm} ²⁸ |
| Mutant phenotype | Knockout mice lack adrenal glands and gonads, male-to-female sex reversal of the internal and external urogenital tracts, impaired expression of markers in gonadotrophs that regulate steroidogenesis, lack of ventromedial hypothalamic nucleus {Mm} [knockout] ^{29–32} ; heterozygous mutants exhibit adrenal insufficiency resulting from defects in adrenal development and organization; compensatory mechanisms help to maintain (nearly) normal adrenal function under basal conditions—however; stressful conditions reveal adrenal defects {Mm} [knockout] ^{33–35} |
| Human disease | Adrenocortical insufficiency: associated with an Arg ²⁵⁵ →Leu mutation in the hinge region of the SF-1 receptor ³⁶ ; sex reversal, XY, with adrenal failure: associated with an Arg ⁹² →Gln mutation in the DNA-binding domain of the SF-1 receptor ³³ ; sex reversal, XY, without adrenal failure: associated with premature termination upstream of sequences encoding the AF-2 domain; this mutated receptor has no transcriptional activity and inhibits the function of the wild type in most cases ³⁷ |

aa, amino acids; chr., chromosome; HRE, hormone response element; PIP₂, phosphatidylinositol bisphosphate; PIP₃, phosphatidylinositol triphosphate; CREBBP, cAMP response element-binding protein binding protein.

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TABLE 24
LRH-1

| | |
|---------------------------------|--|
| Receptor nomenclature | NR5A2 |
| Receptor code | 4.10.1:OR:5:A2 |
| Other names | FTF, CPF, Hb1F, FTZ-F1 β |
| Molecular information | Hs: 541aa, O00482, chr. 1q32 ¹ Rn: 560aa, chr. 13q13 ² Mm: 560aa, P45448, chr. 1 E4 |
| DNA binding | |
| Structure | Monomer |
| HRE core sequence | YCA AGG YCR (half-site) |
| Partners | DAX1 (physical, functional): inhibition of LRH-1-dependent transactivation ³ ; SHP (physical, functional): inhibition of LRH-1-dependent transactivation ^{3,4} ; β -catenin (physical, functional): DNA binding and increased transcriptional activity of cyclin E1 gene and cyclin D1 gene ⁵ |
| Agonists | Phosphatidyl-(3,4,5)-inositol triphosphate, phosphatidyl-(3,4)-inositol biphosphate, phosphatidyl-(3,5)-inositol biphosphate, phosphatidyl-(4,5)-inositol biphosphate, phosphatidylethanolamine C16:1, C18:1, and C18:3, phosphatidylglycerol C16:1 and C18:1 ⁶⁻⁹ |
| Antagonists | |
| Coactivators | NCOA1, NCOA3, EP300, NCOA62, EDF1 ^{8,10-12} |
| Corepressors | Prox1 ^{13,14} |
| Biologically important isoforms | LRH-1v1 (Hs): contains a larger A/B domain ^{1,15} ; LRH-1v2 (Hs): smallest isoform, contains deletions within the D and E domains caused by another alternative splicing event in exon 5, cannot activate transcription although the transcription factors have not yet been identified ^{15,16} |
| Tissue distribution | Liver, pancreas, intestine, ovary, and preadipocyte and at lower levels in the placenta; in the adrenal gland and testis, expression is species-specific (Hs, Mm, Rn) [Northern blot, in situ hybridization, Western blot, immunohistology] ^{1,17-25} |
| Functional assays | |
| Main target genes | Activated: CYP11A1 (Hs), ²⁶ ApoA1 (Hs, Mm, Rn), ²⁷ cyclin E1 (Mm), ⁵ StAR (Hs, Mm, Rn), ²⁸ ABCG5/ABCG8 (Hs) ²⁹ |
| Mutant phenotype | LRH-1 ^{-/-} embryos die at embryonic days 6.5–7.5 with features typical of visceral endoderm dysfunction (Mm) [knockout] ^{5,30,31} ; LRH-1 ^{+/-} adult mice are hypocholesterolemic and express liver FTF at about 40% of the normal level (Mm) [knockout] ^{5,30,31} |
| Human disease | |

aa, amino acids; chr., chromosome; HRE, hormone response element; FTF, fetoprotein transcription factor; StAR, steroidogenic acute regulatory.

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TABLE 25
GCNF

| | |
|---------------------------------|---|
| Receptor nomenclature | NR6A1 |
| Receptor code | 4.1:OR:6:A1 |
| Other names | RTR, NCNF, TRIF |
| Molecular information | Hs: 480aa, Q15406, chr. 9q33 ¹⁻⁵ Rn: 453aa, chr. 3q11 ⁶ Mm: 495aa, Q64249, chr. 2 B ⁷⁻¹¹ |
| DNA binding | |
| Structure | Homodimer |
| HRE core sequence | TCA AGGTCA (DR-0, half-site) ^{7,11-19} |
| Partners | SF-1 (functional): DNA binding ^{7,17,19} ; CREM τ (functional): DNA binding ²⁰ ; ERR α , ERR β , ERR γ (functional): DNA binding ²¹ ; COUP-TFI, COUP-TFII (functional): DNA binding ¹⁹ ; LRH-1 (functional): DNA binding ²² |
| Agonists | |
| Antagonists | |
| Coactivators | RAP80 ²³ |
| Corepressors | NCOR1, NCOR2 ^{19,24} |
| Biologically important isoforms | GCNF2 {Hs}: uses two alternate in-frame splice sites resulting in an isoform that has the same N and C termini but is shorter than GCNF ⁵ ; GCNF3 {Hs}: this variant lacks an alternate in-frame segment and uses an alternate in-frame splice site, resulting in an isoform that has the same N and C termini but is shorter than GCNF ⁵ |
| Tissue distribution | Developmental: brain, ectodermal cells, primitive streak, nervous system; adult: testis, ovary, liver, kidney, germ cells {Hs, Mm, Rn} [Northern blot, in situ hybridization, Western blot, immunohistology] ^{1,2,6-9,17,19,25-29} |
| Functional assays | |
| Main target genes | Repressed: Oct4 {Hs, Mm, Rn}, ^{17,19} PRM1 {Mm}, ²⁰ PRM2 {Mm}, ²⁰ BMP15 {Mm}, ³⁰ GDF-9 {Mm} ³⁰ |
| Mutant phenotype | Homozygote GCNF-null mice have cardiovascular abnormalities, defective trunk development, impaired somite formation, failure to turn, open neural tube, hindgut, protrusion of the tailbud outside the yolk sac, and die by embryonic day 10.5 {Mm} [knockout] ^{17,19,28,31} ; hypofertility because of prolonged diestrus phase of the estrous cycle and aberrant steroidogenesis {Mm} [tissue-specific Cre/Lox knockout in the oocyte] ³⁰ |
| Human disease | |

aa, amino acids; chr., chromosome; HRE, hormone response element; CREM, cAMP-response element modulator.

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