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Corepressor interaction differentiates the permissive and non-permissive retinoid X receptor heterodimers

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Abstract

Nurr1 is an orphan nuclear receptor regulating transcription both as a monomer and as a heterodimer with retinoid X receptor (RXR). RXR–Nurr1 heterodimers are permissive RXR heterodimers as they activate transcription in response to RXR ligands. In contrast, heterodimers formed by RXR and retinoic acid receptor (RAR) are non-permissive as they activate transcription only upon RAR ligand binding. We studied the mechanism mediating permissiveness and non-permissiveness by creating receptor chimeras between Nurr1 and RAR. We show that the amino-terminal part of the Nurr1 ligand binding domain conveys permissiveness to RXR–Nurr1 heterodimers. This region is involved in interactions with the corepressors SMRT and NcoR. The corepressors were released from RXR–Nurr1 heterodimers by RXR ligand binding. In contrast, RXR ligand increased the interaction between RXR–RAR heterodimers and the corepressors. The corepressors were released only upon binding of RAR ligand. In conclusion, corepressor interaction differentiates the permissive RXR–Nurr1 heterodimers from the non-permissive RXR–RAR heterodimers.
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Keywords: Nuclear receptor; Nurr1; NGFI-B; Retinoid X receptor; Retinoic acid receptor; Heterodimer; Permissiveness

Nuclear receptors (NRs)[1] are ligand-inducible transcription factors mediating the effects of small lipophilic ligands such as steroid hormones, retinoids, thyroid hormones, and vitamin D. In addition, there are a large number of NRs lacking identified physiological ligands. These receptors are referred to as orphan NRs [1,2]. NRs act as monomers, homodimers, or heterodimers. The most common heterodimerization partner among NRs is the retinoid X receptor (RXR). RXR-heterodimers can be classified as either permissive or non-permissive dimers depending on if they are activated by RXR ligands or if they require the binding of the ligand of the heterodimeric partner receptor in order to be activated. The subordination of RXR in the non-permissive RXR heterodimers has been studied over the years and different mechanisms have been proposed. RXR was originally regarded to be unable to bind its ligands when heterodimerized with a non-permissive partner but it has later been shown that RXR is capable of ligand binding and coactivator interaction in non-permissive heterodimers [3]. However, RXR is unable to release corepressors in response to RXR ligands when engaged in a non-permissive heterodimer and fails therefore to activate transcription. Corepressors are only released upon ligand binding by the heterodimerizing partner receptor [3]. Furthermore, heterodimerization with a non-permissive partner has also been suggested to enhance the interaction between RXR and corepressors [4]. The heterodimerization interface in

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[1] *Abbreviations used:* NR, nuclear receptor; RXR, retinoid X receptor; RAR, retinoic acid receptor; TR, thyroid hormone receptor; VDR, vitamin D receptor; LBD, ligand-binding domain; AF-2, activation function 2; βRE, retinoic acid receptor response element from the human RARβ2 gene; NBRE, NGFI-B response element; PPARγ, peroxisome proliferator-activated receptor γ; SMRT, silencing mediator for retinoic acid and thyroid hormone receptors; NCoR, nuclear receptor corepressor.

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permissive PPARγ–RXRα dimers has been shown to differ from that of the non-permissive RXR/RAR heterodimer as PPARγ forms an asymmetric heterodimer with RXRα in which the activation function-2 helix (AF2) of PPARγ interacts with helices 7 and 10 of RXRα [5]. This structural arrangement stabilizes the AF2 of PPARγ in an active position even in the absence of PPARγ ligands. In contrast, the non-permissive RXR–RAR heterodimer is symmetric and no interaction between the AF2 of RARα and RXRα was reported [6]. The ability of the AF2 helix to interact with RXR was suggested to associate with the length of AF2 helix [5].

Nurr1 (NR4A2) is an orphan nuclear receptor that acts both as a monomer activating transcription constitutively and as a heterodimer with RXR. The RXR–Nurr1 heterodimer is efficiently activated by RXR ligands making RXR–Nurr1 heterodimers permissive heterodimers [7,8]. Nurr1 is closely related to two other orphan NRs, NGFI-B (NR4A1) and Nor1 (NR4A3) that activate transcription as monomers. NGFI-B also forms permissive heterodimers with RXR whereas Nor1 is unable to heterodimerize with RXR [7,9,10]. According to the recently solved crystal structures of NR4A ligand binding domains (LBDs), these receptors are unique among NRs as they do not posses cavities for ligand binding in their LBDs [11,12]. It thus seems likely that the activities of these NRs are not regulated by classical lipophilic ligands but rather their activities are regulated by other mechanisms such as post-translational modifications and protein–protein interactions. For example, the constitutive monomeric activity of Nurr1 is repressed by heterodimerization with *apo*-RXR whereas RXR ligand binding stimulates the transcriptional activity of the RXR–Nurr1 heterodimer [7,13]. In addition to revealing the lack of a ligand binding pocket in Nurr1 LBD, the crystal structure showed that Nurr1 LBD is devoid of the classical coactivator binding surface [11]. The hydrophobic coactivator binding cleft present in most other NR LBDs is completely transformed into a charged surface in Nurr1. In line with this, Nurr1 failed to interact with several tested coactivator proteins in transfection assays [8]. As coactivators and corepressors bind an overlapping site in the NR LBDs these results indicate that Nurr1 interacts with neither coactivators nor corepressors through the classical binding site.

The mechanism mediating permissive activation of RXR–Nurr1 heterodimers in response to RXR ligands has remained elusive. As corepressor interaction and release have been suggested to associate with the permissiveness of RXR heterodimers, we studied whether the permissive RXR–Nurr1 heterodimers interacted with the corepressors SMRT and NCoR in a different manner compared to the non-permissive RXR–RAR and RXR–TR heterodimers. We show here that Nurr1 LBD interacts very weakly with SMRT and NCoR and that in RXR–Nurr1 heterodimers RXR is the partner that mainly interacts with the corepressors and releases them in response to ligand binding. In contrast, in RXR–RAR and RXR–TR heterodimers, RAR and TR are the receptors that monitor the interaction and release of corepressors in response to their ligands. Taken together, we show that the ability to release corepressors in response to RXR ligand differentiates permissive and non-permissive RXR heterodimers.

Materials and methods

Plasmid constructions

The receptor expression plamids (pCMX-Nurr1, pCMX-Gal4-Nurr1 LBD, pCMX-Nurr1 KLL(554-556)AAA, pCMX-NGFI-B, pCMX-Gal4-NGFI-B, pCMX-Nor1, pCMX-Gal4-Nor1, pCMX-RXRα, pCMX-Gal4-RXRα, pCMX-VP16-RXRα, pCMX-RXRα A416K, pCMX-RARγ, and pCMX-Gal4-RARγ), the β-galactosidase internal control plasmid (pCMX-βgal), and the reporters ($NBRE_3$tk-LUC, βRE_3tk-LUC, and MH100tk-LUC) have been described previously [7,8,10,13,14]. pCMX-RXRα L294R and pCMX-RARγ V242R were generated using the GeneEditor™ *in vitro* Site-Directed Mutagenesis System (Promega) according to the manufacturer's instructions. pCMX-Nurr1/RAR LBD receptor chimera was created by replacing the Nurr1 residues 318–598 in pCMX-Nurr1 with a ScaI–NheI fragment from pCMX-RARγ (RARγ residues 144–454). pCMX-RAR/Nurr1 LBD was created by replacing the RARγ residues 145–454 in pCMX-RARγ with a ScaI–NheI fragment from pCMX-Nurr1 (Nurr1 residues 318–598). Nurr1/RAR DIM was created by amplifying RARγ residues 314–404 by PCR and cloning to pCMX-Nurr1 to replace Nurr1 amino acids 490–517. To create Nurr1/RAR AF2, RARγ residues 408–454 were amplified by PCR and cloned to pCMX-Nurr1 to replace Nurr1 residues 584–598. All PCR products were verified by sequencing. To obtain Gal4-fusions of the Nurr1/RAR chimeras, the fragments of the chimeras encoding the hinge region and LBD were cloned as ScaI–NheI fragments to pCMX-Gal4 vector obtained from Dr. R. Evans (Salk Institute, San Diego, CA). The GST-Nurr1 LBD plasmid was created by cloning the Nurr1 amino acids 354–598 to the pGEX 2TK vector. The VP16-fusions of SMRT and NcoR IDs were kindly provided by Dr. M. Lazar (University of Pennsylvania School of Medicine, Philadelphia, PA). pCMX-SMRT was a generous gift form Dr. R. Evans (Table 2).

Table 1
Summary of the ligand binding properties of the constructs

Construct	9-*cis*-RA	SR11237	TTNPB	T3
RXR	+	+	−	−
TR	−	−	−	+
Nurr1	−	−	−	−
RAR	−	−	+	−
RAR/Nurr1 LBD	−	−	−	−
Nurr1/RAR LBD	−	−	+	−

Table 2
Summary of the responsiveness of the different constructs to RXR ligands as heterodimers with RXR

Construct	Permissiveness for activation by RXR ligands
Nurr1	Permissive
RAR	Non-permissive
RAR/Nurr1 LBD	Permissive
Nurr1/RAR LBD	Non-permissive
Nurr1/RAR DIM	Permissive
Nurr1/RAR AF2	Permissive
$RAR^{-corepr}$	Non-permissive
TR	Non-permissive

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