

# General molecular biology and architecture of nuclear receptors

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## Abstract

**Nuclear receptors (NRs) regulate and coordinate multiple processes by integrating internal and external signals, thereby maintaining homeostasis in front of nutritional, behavioral and environment challenges. NRs exhibit strong similarities in their structure and mode of action: by selective transcriptional activation or repression of cognate target genes, which can either be controlled through a direct, DNA binding-dependent mechanism or through crosstalk with other transcriptional regulators, NRs modulate the expression of gene clusters thus achieving coordinated tissue responses. Additionally, non genomic effects of NR ligands appear mediated by ill-defined mechanisms at the plasma membrane. These effects mediate potential therapeutic effects as small lipophilic molecule targets, and many efforts have been put in elucidating their precise mechanism of action and pathophysiological roles. Currently, numerous nuclear receptor ligand analogs are used in therapy or are tested in clinical trials against various diseases such as hypertriglyceridemia, atherosclerosis, diabetes, allergies and cancer and others.**

**MESH Keywords** Animals ; Humans ; Ligands ; Molecular Biology ; Receptors, Cytoplasmic and Nuclear ; chemistry ; genetics ; metabolism

**Author Keywords** transcriptional regulation ; nuclear receptors ; coactivators ; corepressors ; structure

## INTRODUCTION

The nuclear receptor superfamily comprises evolutionarily related transcription factors fulfilling multiple regulatory functions in growth, development and homeostasis. Nuclear receptors share a common architecture and functional behavior. The effector function of nuclear receptors is to modulate transcription through several distinct mechanisms, which include both transactivation and transrepression activities upon receptor-specific ligand binding. Nuclear receptors can also be the targets of other signaling pathways that modify the receptor, or their transcriptional comodulators, post-translationally and affect their activity and functions. According to phylogenetic studies, nuclear receptors emerged long before the divergence of vertebrates and invertebrates, during the earliest metazoan evolution [1 ]. The first cloned human receptors were the glucocorticoid receptor (GR/NR3C1, [2 ,3 ]) together with the estrogen receptor (ER) [4 ,5 ] and the thyroid hormone receptor (T3R/NR1A1, [6 ,7 ]). Forty eight nuclear receptors have since been identified in the human [8 ].

Nuclear receptors share a common structural organization which defines this gene superfamily (Figure 1 ). The N-terminal domain is highly variable depending on the receptor and contains a ligand-independent transactivation domain termed Activation Function 1 (AF-1). The most conserved central region is the DNA-binding domain (DBD), which contains the P-box, a short motif responsible for direct DNA interaction and DNA-binding specificity. Additional sequences in the DBD are involved in the homo- or heterodimerization of nuclear receptors. Nuclear receptors bind to sequence-specific elements located not only in the vicinity of target gene promoters, but also in intronic and enhancer regions, either as monomers (Nor1/NR4A3), as homodimers such as the steroid receptors [GR/NR3C1, estrogen receptors (ER $\alpha$ /NR3A1 and ER $\beta$ /NR3A2), progesterone receptor (PR/NR3C3), mineralocorticoid receptor (MR/NR3C2), androgen receptor (AR/NR3C4)] and retinoid X receptors (RXR $\alpha$ /NR2B1, RXR $\beta$ /NR2B2, RXR $\gamma$ /NR2B3), or as heterodimers with RXRs. The DBD and the C-terminal ligand-binding domain (LBD) are linked by the hinge region [9 ]. The C terminus of NRs harbors several functionally critical motifs, such as the activating function 2 (AF-2), conferring to many NRs a ligand-dependent transcriptional activity, a strong dimerization interface and a ligand binding pocket (LBP). The in-depth structural nuclear receptor architecture is delineated further in this review.

Nomenclatures of the nuclear receptor family have been proposed according to different criteria. Based on the sequence alignment of the two well-conserved domains (DBD and LBD) and phylogenetic tree construction, the nuclear receptor gene family has been divided into six subfamilies. Interestingly and importantly, a correlation exists between DNA-binding and dimerization abilities of each classified nuclear receptor and its phylogenetic position. Subfamily 1 comprises nuclear receptors forming heterodimers with RXR (T3Rs:NR1A; RARs: NR1B; VDR: NR1I1; PPARs: NR1C; RORs: NR1F; Rev-erbs: NR1D; CAR: NR1I3; PXR: NR1I2; LXRs: NR1H). Subfamily 2 is formed by HNF4s: NR2A1&2; COUP-TFs: NR2F; RXRs: NR1B. Subfamily 2 members can function in two configurations, either as homodimers and as heterodimers. Subfamily 3 includes the above mentioned steroid hormone receptors. Subfamily 4 contains the nerve growth factor-induced clone B group of orphan receptors NGFI-B/Nur77/NR4A1, Nurr1/NR4A2, and NOR1/NR4A3. The small subfamily 5 includes the steroidogenic factor 1 (SF1/NR5A1) and receptors related to Drosophila FTZ-F1 (LRH1/NR5A2). The sixth subfamily comprises only the GCNFI receptor. Finally, subfamily 0 encompasses 2 atypical nuclear receptors lacking the DBD (Dax1/NR0B1 and SHP/NR0B2), thereby displaying constitutive dominant-negative activities [10 ].

Another functional classification according to the ligand-binding properties splits the superfamily of nuclear receptors into three groups. The most characterized subfamily called thyroid/steroid hormone receptor subfamily comprises ER, AR, PR, MR and GR and also includes the thyroid receptors T3Rs, VDR, and RARs. The second 'orphan' subfamily is composed by nuclear receptors for which regulatory molecules have not been identified so far. They are represented by NR4 receptors and COUP-TFs. The function and molecular mechanism of action for many 'orphan' receptors is only poorly investigated. The third subfamily of nuclear receptors is known as 'adopted' orphan receptors. Members of this subfamily were initially characterized as 'orphans' and afterwards, natural ligands have been identified that convey physiological functions. These nuclear receptors are sensors of the metabolic status of cells, organs and the whole body and trigger responses to xenobiotics, dietary signals, diatomic gases and metabolites. In this class are found Rev-erba and  $\beta$ , PPARs, LXRs, FXRs, RORs, PXR and CAR.

The ability of nuclear receptors to be regulated by natural or synthetic molecules have led to intensive efforts to target nuclear receptors therapeutically. However, many currently available ligands have several deleterious side-effects, many of which seem to be related to their transactivating properties. It seems to be essential to determine the importance of positive and negative gene regulation in conferring the therapeutic benefits of nuclear receptor ligands in disease models. In this review we will discuss the relationship between the molecular structure and the molecular action of nuclear receptors.

## STRUCTURAL FEATURES OF NUCLEAR RECEPTORS

Nuclear receptors reveal characteristic protein architecture that consists of five to six domains of homology designated A to F, starting from N-terminus to C-terminus of protein. The weakest conservancy is observed in the N-terminal A/B domain, D or hinge domain, and F region at the C-terminus which is not present in all nuclear receptors. The DBD and LBD are the most highly conserved domains (Figure 1). The most recent structural studies [11, 12] of RXR heterodimers bound to DNA showed asymmetric complexes of 150–200Å, with LBDs being located on one side of the DNA, 5' of the DNA response element (Figure 2). The hinge region plays an important structural role by specifying the relative orientation of the DBD with respect to the LBD.

### A/B domain

The poorly structurally defined N-terminal A/B region reveals a strong diversity among nuclear receptors and because of its high mobility, its tertiary structure has not been elucidated so far. Isoform-specific differences in amino termini are observed for several NRs and these sequence variations may induce differential binding affinities to response elements and/or with members of the transcription initiation complex, distinct transcriptional activities and different *in vivo* roles (see for examples [13–18]).

The A/B domain contains the activation function 1 (AF-1) which is ligand-independent. Hydrogen/deuterium exchange mass spectrometry of PPAR $\gamma$  revealed that the ordering of A/B portion is not substantially changed upon ligand binding [11]. By contrast, the N-terminus of T3R $\beta$ 1 may transmit thyroid hormone-dependent signaling to the general transcriptional machinery by a direct interaction of the receptor with transcription factor IIB (TFIIB, [13, 19]). Moreover, the N-terminal region is an interaction surface for multiple transcriptional coregulatory proteins: steroid receptor coactivator-1 (SRC-1/NCoA1), steroid receptor coactivator-2 (SRC-2/TIF2/NCoA2), p300 and CBP enable a functional synergism between AF-1 and AF-2 regions of steroid receptors, PPAR $\gamma$  or RARs and thus cooperatively enhances transactivation [20–23]. In addition, co-regulator-linked interactions with the N-terminal and C-terminal domains were found for AR, ER and PR [24]. Inter-domain communication also regulates ligand-independent transcriptional silencing: deletion of the PPAR $\gamma$  N-terminal domain prevents corepressor binding [25].

The A/B domains can be modified by phosphorylation and other post-translational, covalent modifications and confer distinct functional properties of nuclear receptors. In the case of ligand-activated receptors, AF-1 modifications have generally a tissue-specific modulatory effect on their transcriptional properties. For instance, the MR N-terminus harbors a serine/threonine-rich nuclear localization signal (NLS) that can be regulated by phosphorylation and influence receptor subcellular localization [26]. An elegant mechanism of regulation of the activity of RXR is provided by the piggyback nuclear exclusion of RXR upon association with Nur77, in a Nur77 AF-1 phosphorylation-dependent manner [27]. Similarly, MEK1-mediated phosphorylation of serine at position 84 inhibits PPAR $\gamma$ 1 nuclear localization [28], although an alternative mechanism involving Pin1-mediated proteasomal degradation of PPAR $\gamma$  has been recently proposed [29]. Preventing phosphorylation at this residue *in vivo* generates mice with increased insulin sensitivity when fed a high fat diet [30]. Taken together, these and other data suggest that translocation of NRs to the nucleus is a property which can be very rapidly regulated by various signaling cascades.

Post-translational modifications also affect the intrinsic transactivating potential of NRs, i.e. by modulating their ability to recruit transcriptional comodulators, or by modifying the polypeptide half-life, both properties being in some instances intimately linked [31, 32]. Very interestingly, phosphorylation of the A/B domain of GR by p38 MAPK was shown to induce stable tertiary structure formation in this domain, hence favoring its interaction with coregulatory proteins [33]. In turn, this tertiary structure may be stabilized by protein-protein interactions, as reported for the AR AF-1 [34]. More physiologically, the estrogenic effects of EGF are partially mediated

by the phosphorylation of ER AF-1 by EGF-activated MAPKs [35]. In the case of orphan receptors, whose transcriptional activity is strongly dependent on AF-1 integrity, covalent modifications of this region have a very strong impact on their transcriptional output. Amino acid motifs in the A/B domain of Nurr1 mediating ERK5- or ERK2-mediated transcriptional activation have been identified [36,37]. Evidence for other post-translational modifications occurring in the N-terminus of NRs are scarce. Phosphorylation-dependent SUMOylation the AF-1 of ERR $\gamma$  represses its transcriptional activity [38]. AR is SUMO-1ylated in its AF-1 domain at a SUMO consensus sequence found in all steroid receptors, thus inhibiting androgen-regulated signaling [39]. Conversely, N-terminal SUMO-1ylation of PPAR $\gamma$  strongly increases its transactivating potential [40]. However, as discussed below, SUMOylation in the C-terminal AF-2 region is now viewed as a critical mechanism regulating the balance between transactivating and transrepressive functions of NRs.

### The DNA-binding domain

The DNA-binding domain (DBD) or C domain is the most conserved domain within the nuclear receptor family. Its main function is to recognize and bind specific DNA regulatory sites called response elements (REs) [41]. The core DBD region contains about 66 amino acids, but many nuclear receptors additionally contain a less conserved C-terminus, a poorly structured motif of about 25 amino acids called the C-terminal extension region (CTE). As the CTE is located in the so-called hinge region, its features will be detailed in the corresponding paragraph.

The DBD is a highly structured, very compact globular domain composed by a pair of perpendicular  $\alpha$ -helices stabilized by two C4 zinc-binding domains each coordinating tetrahedrally a zinc atom, a short  $\beta$ -sheet, and a few stretches of amino acids [42,43]. Each receptor monomer establishes specific DNA contact through the first N-terminal helix (helix 1) which directly interacts with the major groove of the DNA half-site. A motif called the P box is critical for the DNA-binding specificity of the receptor [43–47]. Three amino acids of the  $\alpha$ -helical P box distinguish nuclear receptors that will bind to the core AGACA half-element (the “GSV-P box” initially found in GR) or to the AGGTCA half-element (the “EGG-P box” initially found in ER). Structural studies revealed that V and E amino acids make direct and unique contacts with the DNA half-site [48,49].

Nuclear receptor homo- or heterodimers establish contacts with two DNA half-sites that can be arranged in different geometry and separated by a spacer of varying length (see below and [50]). The C-terminal helix (helix 2) contributes to stabilization of the overall DBD structure, establishes weak, non-specific contact with DNA. A 5-amino acid loop defines a strong dimerization interface (D box) for homodimer formation and contributes, to a much lesser extent, to heterodimer stabilization [8,51–53].

DNA also provides a template for dimer assembly, which in turn induces conformational changes of the DNA double helix, most notably by inducing distortion of the minor groove to facilitate sequence recognition by the CTE [54]. This phenomenon is correlated with increased DNA bending *in vitro*, which has been documented for a number of nuclear receptors [55–58]. The relevance of this phenomenon when response elements are in a chromatinized environment is not clear however, although intrinsic DNA bendability affects GR binding to nucleosomal response elements *in vitro* [59]. The important role of nucleosome assembly and of histone post-translational modifications on the DNA binding affinity and transcriptional activity of nuclear receptors was demonstrated *in vitro* [60–63] and *in vivo* [64].

Although being a domain poorly accessible when receptor dimers are bound to nucleosomal DNA, the DBD can be the target of post-translational modifications. Much attention has been paid to kinase-mediated regulation of nuclear receptor affinity, and consequently a wealth of data document the generally inhibitor role of DBD phosphorylation. Indeed, as expected from the introduction of a repulsive charge, phosphorylation of the DBD of HNF4 [65,66], T3R [67] and ER [68] decreases their DNA binding activity. In a possibly related fashion, phosphorylation of a number of nuclear receptors in this region alters their nuclear retention and decreases their transcriptional activity [65,69,70]. In contrast, phosphorylation of TR2 [71] and of FXR [72] increased their DNA binding activity and interaction with PGC-1 $\alpha$  respectively. Other covalent modifications such as RAR $\alpha$  methylation or 15d-PGJ(2) adduct formation on ER $\alpha$  favor or inhibit receptor activity, respectively [73,74].

### The hinge region

The flexible hinge, or D region, also called the C-terminal extension of the DBD (CTE) links the C-domain to the multifunctional C-terminal E/F ligand-binding domain and displays very low amino acid identity and similarity between nuclear receptors. Being located between two functionally and structurally important domains, it seems likely that its functions, deduced mostly from deletion and/or site-directed mutagenesis, may also reflect structural and functional alterations of these neighboring domains. Nevertheless, hinge regions of numerous nuclear receptors have been extensively dissected from a molecular point of view and shown to contain motifs responsible for regulating the subcellular distribution of nuclear receptors. Such a function has been demonstrated for ER [75], AR [76], VDR [77] and Dax1 [78] and reflect the presence of conserved nuclear localization sequences (NLS). The hinge region is also involved in tethering activities. The GR hinge region interacts with GR corepressors HEXIM1 and Bag-1 [79,80]. A natural variant (V227A) in the PPAR $\alpha$  hinge region is associated with dyslipidemia and this mutation increases PPAR $\alpha$  interaction with the nuclear corepressor NCoR [81]. Quite

similarly, natural hinge variants of T3R display impaired dissociation of NCoR and recruitment of the coactivator SRC-1 upon agonist binding [82]. Supporting its role as a flexible link between the DBD and the C terminal LBD, hinge domain mutations affect the synergy between the AF-1 and AF-2 domains of ER [83]. Furthermore, the conserved 3D structure of receptor heterodimers, irrespective of the geometry of the bound DNA response element, highlights this physical property [12]. The hinge domain integrity is also conditioning the DNA binding affinity: *in vitro* assays showed that alternative splice variants affecting the hinge region sequence of FXR display distinct DNA binding affinities [84].

The CTE of monomeric receptor and of some dimeric receptors (also called T box and/or A box) adopts specific conformations which are context-dependent [85,86]. T and A boxes of dimeric receptors such as T3R, RARs, RXRs and VDR form an alpha-helical structure in solution and establish non-specific contacts with DNA [87–91] which can convert to an extended conformation favoring DNA binding in RXR homodimers and RXR-RAR heterodimers [92,93]. In contrast, the CTE of monomeric receptors such as rev-erbs, nur77 and ERRs establish specific contacts with DNA sequences located immediately 5' of the NR response element through the A box, which adopts an extended loop conformation [94–96]. The CTE is the major determinant of heterodimer polarity on half-site DNA [11].

The other function of CTE via the T-box is to provide an additional dimerization interface with the second zinc finger helix of RXR. Recent crystallographic analysis of PPAR-RXR-DNA complexes revealed a previously unknown dimerization interface between the RXR CTE and the PPAR $\gamma$  LBD [11], although the relevance of this structure has been challenged [12]. In contrast, the PPAR $\gamma$  CTE makes extensive DNA interaction by binding to the AAACCT DNA sequence upstream of the core response element. The interaction with this 5' flanking sequence is similar to that observed with the Rev-Erb CTE [97,98].

As other domains, the hinge domain can be regulated by post-translational modifications such as methylation, acetylation, phosphorylation and sumoylation. p300-catalyzed acetylation of ER $\alpha$  hinge region regulates its transactivation properties and ligand sensitivity [99]. SUMOylation of ROR $\alpha$  by both SUMO-1 and SUMO-2, as well as that of ER $\alpha$  has been reported, and mutations preventing SUMOylation generate transcription-defective receptors [100,101]. In contrast, hPPAR $\alpha$  SUMOylation on lysine 185 increases the selective recruitment of NCoR and decreased transcriptional activity [102]. Phosphorylation of serine residues in RAR $\alpha$ , ROR $\alpha$ 4 and Nur77 are detrimental for receptor-mediated transactivation, either by decreasing DNA recognition or by preventing receptor dimerization [103], while phosphorylation of the PPAR $\alpha$  hinge domain favors transactivation over tethered transrepression [104]. No investigations were carried out to identify structural changes induced by these covalent modifications, nor are reports describing when such modifications occur on such a sterically hindered environment.

### The E or ligand-binding domain

As the domain accommodating lipophilic ligands capable of activating or repressing the transcriptional activities of nuclear receptors, it has attracted considerable interest as a paradigm for a transcriptional molecular switch, and as a target for synthetic analogs since these receptors control signaling pathways involved in a wide range of pathophysiological processes. Since the first crystallization of the RXR LBD [105], more than 600 3D structures related to nuclear receptor LBD structures have been reported, and about 3000 publications relate to some aspects of LBD structure and function. For more details, readers may refer to recent reviews of this fascinating field, linking 3D structure determination and modeling to pharmacology and therapeutics [51,106].

The E domain (or LBD) of nuclear receptors is a multi-functional unit comprising, in addition to the ligand binding pocket, homo- and heterodimerization interfaces and a comodulator binding region. The LBD acts as a molecular switch by interpreting the ligand structure into conformational changes which will convert the receptor in a transcriptional activator or repressor. Although the ligand has been long considered as the sole conformational modifier, it is now recognized that DNA response elements also induce structural transitions (see below). Nevertheless, the LBD remains the main architectural feature triggering biological responses to very diverse lipophilic molecules.

X-ray crystallography established the E domain as organized as a three-layered antiparallel  $\alpha$ -helical sandwich composed by 12  $\alpha$ -helices, including a  $\beta$ -sheet (s1–s2) which is part of the ligand binding pocket (LBP). The LBP is located inside of this structure and is composed of a group of surrounding helices [51]. The LBP of nuclear receptors is a highly variable region, both in volume, ranging from 300 to 1500Å<sup>3</sup>, and in structure. Such a diversity allows the binding of a variety of molecules ranging from phospholipids to heme, including steroid and fatty acid derivatives, xenobiotics..., and highlights the broad spectrum of physiological actions of nuclear receptors.

Ligand-LBP interactions involve amino acids located in most receptors in helices 3, 5 and 10/11. Additional interactions are brought into play as a function of the receptor and the chemical structure of the ligand. Hydrophobic interactions, hydrogen bonding networks and the steric size and shape of LBPs determine the strength and specificity of LBD-ligand complex [107]. This atomic network is variable according to receptor isoforms, allowing the design of isoform-selective agonists or antagonists [108].

Ligand binding causes conformational changes of nuclear receptors, which involve repositioning of H3, H4, L3-L4 and H12. Helix 12 (initially termed the AF-2 activating domain or AF-2 AD) is stabilized against the LBD core, generating a hydrophobic groove made of helices 12, 3, 4 and 5. This structure allows the LBD to interact with the LXXLL signature motif found in most if not all reported primary

nuclear receptor coactivators [109]. This interaction is further stabilized by a charge clamp made in most cases of a lysine in H3 and a glutamic acid in H12, which is required for optimal binding of coactivator molecules [110–113]. Subtle changes in ligand structure seems to affect the coactivator binding interface, providing a molecular basis for the varying efficacy and potency of nuclear receptor agonists [112]. In a more extreme fashion, antagonist binding positions helix 12 to cause a steric obstruction of the LXXLL binding groove. Importantly, the helix 12 region contains a degenerated LXXLL motif allowing for this interaction. Alternatively, antagonism can be exerted by generating a structure favoring the recruitment of corepressor molecules such as SMRT and NCoR or by preventing H12 proper folding. [113–117]. Intriguingly, some nuclear receptors act, in the absence of ligand, as transcriptional repressors. While it is acknowledged that this repressive action is physiologically important, the structural basis for this ligand-independent repression was unknown until recently. Two reports described a specific structure in RAR $\alpha$  and rev-erb- $\alpha$  in the LBD that forms an anti-parallel  $\beta$ -sheet with corepressor amino acids, identifying a novel interaction interface [118, 119] and documenting a structural basis for the mechanism of derepression, which necessitates the active removal of corepressor molecules.

Finally, the LBD harbors a dimerization interface, the core of which mapping to H7, H9, H10, H11, loops L8-L9 and L9-L10. Although ligand binding has been long suspected to promote nuclear receptor dimerization [119–124], structural studies did not provide evidence for ligand-induced reshaping of this dimerization interface [106, 125].

As other domains, the LBD is the target of posttranslational modifications. While it is beyond the scope of this review to provide an exhaustive list of identified covalent modifications (see also [126]), it is worth noting here SUMOylation plays an important role in channeling the transcriptional activity towards transactivation or tethered transrepression. SUMOylation of PPAR $\gamma$  at K365 is required for transrepression of the iNOS promoter in macrophages and targets PPAR $\gamma$  to the NCoR complex bound to NF-kappa-B regulated promoters [127]. This mechanism is detailed below. In an analogous manner, agonist-induced SUMOylation of LXR $\beta$  in the LBD promotes its interaction with GPS2 and binding to the NCoR complex associated to acute phase response genes [128]. PPAR $\alpha$  also controls negatively hepatic gene expression in a sex-specific manner. Such a repression is exerted for example on Cyp7b1 expression, known to divert DHEA from the testosterone biosynthesis pathway. This occurs through the SUMOylation-dependent PPAR $\alpha$  docking to the Cyp7b1 transactivating GA-binding protein, corepressor and HDAC recruitment to this promoter and DNMT3-catalyzed DNA methylation of a neighboring cis-activating SP-1 site [129]. Phosphorylation can exert opposite effects on NR activity through very diverse mechanisms. ATPase class 1 type 8B member [familial intrahepatic cholestasis 1 (FIC1) protein] activates FXR via PKC $\zeta$ -dependent phosphorylation of FXR at Thr-442. This covalent modification promotes the nuclear translocation of FXR and subsequent FXR target gene activation [130]. Through the combination of non genomic and genomic effects, retinoid acid activates the p38MAPK/MSK1 pathway, leading to phosphorylation of two serines in N-terminal domain and in RAR $\alpha$  LBD and of histone H3. Phosphorylation of RAR $\alpha$  increases the binding efficiency of cyclin H to the loop L8-L9 and promotes the right positioning of cdk7 and phosphorylation of RAR $\alpha$  AF-1, to finally trigger RAR $\alpha$  target genes activation [131]. This non-limitative set of examples thus point to the very complex integration of signaling events into nuclear receptor-mediated events.

### The F domain

The F domain is located at the extreme C-terminus of NR. Because of its high variability in sequence, little is known about its structure and functional role. The length of the domain F can vary from no to 80 amino acids [132]. Crystal structure of progesterone receptor revealed that the F domain adopts an extended  $\beta$ -strand conformation [133] which may, in the case of RAR dimers, contact the dimerization partner [134]. Differences in ER isotype transcriptional activity is partly due to a variable F domain structure. Based on amino acid sequence, it is predicted that ER $\alpha$  F domain is an  $\alpha$ -helical region followed by an extended  $\beta$ -strand-like region, separated by a random coil stretch. In contrast, ER $\beta$  domain F is more likely not to adopt an  $\alpha$ -helical structure [135]. Mutagenesis and functional studies showed that domain F does not exert its activity independently and that it is dispensable for ligand binding or transcriptional activity. Nevertheless, deletion of the domain F or part of it may perturb NR activity and interactions with co-regulators. Deletion of the domain F eliminates the ability of human ER $\alpha$  to activate transcription via interaction with SP-1 [136]. HNF4, which harbors the longest domain F in its alternatively spliced isoform HNF4 $\alpha$ 2, is transcriptionally more active and is more responsive to overexpression of the co-activators NCoA2 and CBP [137]. The F domain of HNF4 $\alpha$ 1 interacts also with NCoR2/SMRT [138]. Interestingly, deletion of the F domain of RAR $\alpha$  increased co-activator binding but decreased co-repressor binding [134]. Thus the F domain can be engaged in interactions with transcriptional co-regulators [139]. Moreover, different point mutations among domain F of ER suggested its involvement in ligand-receptor interaction, and impacts on the ligand responsiveness of ER tethered to an AP-1 response element [140]. Finally, the F domain can be covalently modified by phosphorylation and affect ER basal transcriptional activity. O-GlcNAcylation of this domain leads to decreased ability of ER to bind to an estrogen response element in vitro [141].

## DNA RESPONSE ELEMENTS GEOMETRY, ARCHITECTURE AND RECOGNITION BY NUCLEAR RECEPTORS

DNA sequence recognition and binding is the initial step of the transactivation process mediated by nuclear receptors. Consequently, NR monomers or dimers are positioned on RE which are made of one or two hexameric half-site motifs. Adopting a different geometry,

they form palindromes, direct (DR), everted (ER) or inverted repeats (IR) separated by a spacer of varying length and sequence. Four conditions can be distinguished that determine the uniqueness of the response element. They are (i) the nucleotide sequence of the DNA-half sites, (ii) their relative orientation (iii) the sequence of the spacer and (iv) the length of the spacer.

Some NRs, mainly orphans, bind to DNA as monomers. The monomeric Nurr1 binds to a hormone response element 5'-AGGTCA-3' flanked by a 5' 1 to 6-bp long A/T-rich sequence [142]. This sequence referred to as an Nur77/NGFI-B response element (NBRE) [143] is also the target of Nur77 monomers [144]. Nurr1 can however dimerize with RXR, and in this configuration can display significant affinity for DR with spacing ranging from 10 to 27 bases [145]. A similar promiscuity in binding to naked DNA is observed for SF-1, FTZ-F1, rev-Erb- $\alpha$  and ROR $\alpha$  which target a single copy of this extended core recognition sequence, although rev-Erb- $\alpha$  can also bind to a specific DR2 RE [146,147].

Receptors binding to DNA as homodimers, exemplified by the steroid hormone receptors GR, MR, AR and PR recognize two consensus half-sites 5'-AGAACA-3' or in case of ER 5'-AGGTCA-3' arranged as inverted repeats spaced by 3 bp (IR3) [148]. Formation of stable head-to-head homodimers is dependent on discrete dimerization interfaces located in both the DBD and the LBD (Figure 3).

Nuclear receptors that form heterodimers with RXRs recognize REs composed of two half-site motifs arranged as direct (DR), inverted (IR) or everted repeats (ER), the core consensus sequence being 5'-AGGTCA-3'. For instance PPARs, RARs, VDR and T3R recognize direct repeats following a specificity rule called the 1-2-3-4-5 rule [149–151]. Some RXR partners display a more relaxed specificity: PXR can bind to a variety of DNA response elements with various spacing, which includes direct repeats DR-3, DR-4, and DR-5, and everted repeats ER-6 and ER-8 [152,153]. FXR prefers binding to an inverted repeat of the ideal sequence 5'-AGGTCA-3' separated by 1 bp (IR-1) [154], but several different response elements have been reported, including ER8 [155] and DR1 [156].

RXR partners can be divided into two groups depending on their functionality as heterodimers. Permissive RXR-containing heterodimers can be activated by RXR agonists in the absence of the agonist for the RXR partner. This group includes PPAR, LXR and FXR. Nonpermissive heterodimers formed by RXR and RAR, TR, VDR cannot be activated by RXR agonists and require agonists of the RXR partner to be activated.[157–159].

Heterodimers can adopt various polarities when bound to different REs, and RXR can be positioned either upstream or downstream of the heterodimer partner. This relative orientation and its impact on the transcriptional activity of receptors has been dissected for RAR-RXR heterodimers. On DR2 and DR5 elements, RXR occupies the 5' hexameric motif, whereas the RAR partner occupies the 3' motif. The polarity is reversed on DR1 response elements. This structural arrangement has dramatic consequences on the transactivation properties of RXR-RAR heterodimers, as RAR agonists are unable to activate transcription from a DR1 RE. This relates to the allosteric control of NCoR assembly on these various DR REs [157,160,161] whose geometry imposes an important structural adaptation of receptor domains. In support of this, DNA binding of RXR-VDR dimers was shown to alter VDR H12 structure [125]. Crystallographic structures of isolated GR DBD bound to DNA identified the so-called "lever arm", located between the two GR zinc fingers, which adopts different conformations according to the RE geometry and influences coactivator recruitment [162]. Other heterodimers such as PPAR $\alpha$ -RXR $\alpha$  bind to DNA similarly to RAR-RXR $\alpha$  and form a polar head-to-tail interaction with DR1, where RXR $\alpha$  binds exclusively to the 3' site [11,92]. For VDR assembled on a DR3, TR and LXR on a DR4 and NGFI-B on a NBRE, the RXR DBD was found to bind to the 5' upstream half-site [50,89,163].

Thus several structural features are brought into play to limit nuclear receptor DNA binding promiscuity, in addition to tissue- and cell-specific expression and limited ligand availability. It is worth noting that these rules have been defined using naked DNA templates. However, a genome-wide bioinformatic search for any of these consensus sequences will yield at least a hit every 500–1000 bp. This number is at odds with the number of actual NR binding sites determined by Chip-seq experiments (several thousands for ER and PPAR $\gamma$ , [164–167]) and the number of regulated genes determined in similar conditions (a few hundreds). Moreover, many of these sequences are located very distal to the transcriptional start site (TSS) when considering a linear sequence, either 5' or 3' to the TSS. Chromosomal conformational studies revealed that enhancer sequences act in cis with respect to promoter sequences, implying chromatin looping between TSS and enhancer sequences [165,168,169]. Quite intriguingly, the functionality of such an association is characterized by the induction of the so-called enhancer-templated non-coding RNA (eRNA) emanating from the distal binding site [170], a phenomenon whose functional significance has not yet been elucidated but which is not restricted to NR-mediated transcriptional control [171]. Genome-wide mapping of nuclear receptor binding sites also revealed the statistically- and biologically-significant association of a fraction of REs with other transcription factor binding sites. This led to the identification of cell-specific "pioneering factors" such as FoxA1, which act by priming NR DNA binding sites to bind their cognate NRs [172,173].

There are thus multiple mechanisms controlling the association of NRs with DNA, all of them having a significant impact on the assembly of NRs on chromatin templates and productive recruitment of the transcription machinery.

## GENERAL MECHANISMS OF TRANSCRIPTIONAL REGULATION BY NUCLEAR RECEPTORS

As already mentioned above, nuclear receptors can control transcriptional events by exerting either a positive, direct effect or by imposing a repressed state to regulated promoters. They can also mediate, through protein-protein interaction, a repressive effect on a variety of other signaling pathways under the control of transcription factors such as AP-1, NF-kappa-B or C/EBP. Each of these aspects will be described below to provide a global view of the most recent concepts which have emerged in the field in the past years (Figure 4).

### Transcriptional activation

An important feature of steroid hormone receptors and of most of the heterodimeric nuclear receptors is the ability to activate transcription of target genes upon ligand binding. In general, this mechanism comprises ligand-dependent conformational changes of the nuclear receptor associated to chromatinized REs, that trigger co-repressor complex release and the sequential recruitment of co-activator complexes that modify chromatin structure and promote the assembly of the transcription initiation complex at regulated promoters. Various co-activators were identified for NRs and the repertoire is specific for certain cell types, genes and signals. Thus binding of agonists stimulate the exchange of co-repressors for co-activators necessary for transcriptional activation. Of note, the ligand-dependent association of NR corepressors such as LCoR and RIP140, through LXXLL motifs may play a significant role in transcription attenuation [174,175], however this mechanism has not been studied in great detail and will not be discussed here.

### Nuclear receptor corepressor binding

In the unliganded state, NRs are associated to corepressor complexes. These complexes are composed of a subunit (SMRT/NCoR2 or NCoR1) directly interacting with the receptor through a degenerated LXXLL motif, which harbor a consensus sequence L/I-X-X-I/V-I or LXXXI/LXXXI/L also called the CoRNR box [176,177]. This CoRNR box motif interacts, as the coactivator LXXLL motif, with amino acids from the LBD hydrophobic groove. This interaction interface is remodeled upon agonist binding and helix 12 positioning occludes part of the CoR binding interface. As mentioned above, additional CoR binding interfaces, as well as novel CoRNR boxes have been described [118,119,178], suggesting the use of alternative mechanisms for NR-corepressor interaction. Corepressor complexes are built around the SMRT or NCoR subunits, which harbor a conserved repression domain on which the core repressive machinery (including HDAC3, GPS2 and TBL1 or TBLR1) is assembled. Recent structural and functional studies highlighted a central role for TBL1 in assembling this very large complex (ca. 1–2 MDa) [179]. In some cases, ligand-binding is sufficient to inhibit co-repressor recruitment (e.g. for RXR and TR), but more generally the active removal of the co-repressor complex is required. This points again to the critical role of TBL1/TBLR1 which encompass a F-box domain interacting with the ubiquitin-conjugating enzyme H5 (UBCH5) and a 19S-proteasome complex, which mediates ubiquitination and proteosomal degradation of SMRT- or NCoR-GPS2-HDAC3 complexes [180].

### Nuclear receptor coactivator binding

Since the seminal discovery of SRC-1/NCoA1 as a progesterone receptor coactivator [181], more than 350 coactivators have been identified so far. This prodigious amount of polypeptides exhibit various enzymatic activities involved in the regulation of histone modification and chromatin remodeling, initiation of transcription, elongation of RNA transcripts, mRNA splicing and elongation, and proteasomal termination of nuclear receptor complexes. Their involvement and relative activity in nuclear receptor-controlled processes is modulated by their cell-specific expression levels and post-translational modifications, conditions which have been reviewed recently [182,183]. It is also nowadays accepted that many of these coregulators participate in molecular events driven by other transcription factors.

The coactivator family has been divided in two subfamilies. The first one defines coactivators which interact directly with NR AF-1&2 regions such as the SRC coactivators, CBP and p300. The second one includes other proteins which interact with primary coactivators such as CARM1, CoCoA, Fli-I... Primary and secondary coactivators are recruited to regulated promoters in an orchestrated fashion [184]. Since this issue is devoted to nuclear receptors involved in metabolism control, only coactivators associated to such an activity will be briefly described here.

### The p160 and p300 families

Co-activators belonging to the p160 family [NCoA1/SRC-1, NCoA2/TIF2 (known as SRC-2 or GRIP1) and NCoA3/RAC3 (also known as SRC-3, ACTR, pCIP or TRAM-1)], p300 and the cAMP response element-binding protein (CBP) bind to the NR LBD via an alpha-helical LXXLL motif [185,186]. Co-activators such as CBP and p300 possess histone acetylase transferase (HAT) activity, which has a critical role in regulating NR-mediated transcription [187]. N-terminal tail acetylation of histone H4, which is likely to establish contacts with the histone H2A/H2B dimer, prevents this interaction and destabilizes chromatin compaction. Additionally, acetylation weakens the interaction of the histone tails with DNA [188]. Consequently, the chromatin is decondensated allowing the promoter initiation complex to bind at the promoter site.

Data emerging from studies of knockout animals suggest that the SRCs play critical and distinct roles in controlling energy homeostasis. SRC-1<sup>-/-</sup> mice have decreased energy expenditure and are prone to obesity. In opposition, SRC-2<sup>-/-</sup> mice are protected against high-fat diet-induced obesity, but can lead to a condition reminiscent of a glycogen storage disease type 1a. The ablation of SRC-3 generates mice highly resistant to high-fat diet-induced obesity. Collectively, these data and others point to a complex, but critical role of SRCs in metabolic regulation which has been in most instances related to the control of PPAR $\gamma$  transcriptional activity [189]. However, given the pleiotropic role of SRCs, it is very likely that other mechanisms contribute to these metabolic effects.

#### ***The ATP-dependent remodelling complex SWI/SNF***

the SWI/SNF complex has a role in metabolic control, as it was identified in yeast to be essential for mating-type switching and growth on sucrose. The SWI/SNF family is evolutionary conserved and plays an important role in ATP-dependent chromatin remodeling [190] by catalyzing the disruption of DNA-histone interactions and sliding of the nucleosome along DNA [191]. The human homolog BAF complex is a multimeric entity of 1.2 MDa including BRG1/hBRM, BAF polypeptides (BAF155/170, BAF60, BAF57, BAF53a/b, BAF47, BAF250a/b, BAF200, BAF45a/b/c/d, Brd9, and Brd7) and actin. Several of these subunits harbor LXXLL motifs and have been identified not only as nuclear receptors coactivators for ER [192, 193], AR [194], RAR [193, 195], FXR [196] and GR [197], but also as corepressors of SHP [198], as SWI/SNF components can be integrated in corepressor complexes [199]. Interestingly, the BAF60a subunit displays a circadian expression in mouse liver and, acting as a coregulator of ROR $\alpha$ , regulates the expression of clock and metabolic genes [200].

#### ***The mediator complex***

Like the SWI/SNF complex, the Mediator complex has been originally identified in yeast and subsequently characterized in other eukaryotic cells. A number of studies described its role as a catalyzer of the transcription preinitiation complex (PIC) assembly at activated promoters. Through direct interaction with RNA polymerase II, general transcription factors (TFIID, TFIIF) and elongation factors, Mediator plays a key role in RNA polymerase II-controlled transcription [201]. Investigations about the role of Mediator in NR research gained momentum when it was realized that Mediator-like complexes bind directly to NRs [202–206]. Mediator is organized in four structural modules and includes more than 20 subunits, of which the Med1 subunit contains LXXLL motifs [207]. The liver-specific Med1 KO induces hepatic steatosis in a PPAR $\gamma$ -dependent manner [208], in agreement with its adipogenic [209] and PPAR $\gamma$  coactivator roles [210]. Skeletal muscle-specific KO of Med1 enhances insulin sensitivity and improves glucose tolerance and confers resistance to high-fat diet-induced obesity [211]. Thus given its broad and key roles in transcriptional regulation through a direct interaction with RNA polymerase II, Mediator is viewed as being the last complex recruited cyclically to NR-regulated promoters [184].

### **Transcriptional repression**

#### ***Transcriptional repression by unliganded receptors***

Some nuclear receptors can actively repress transcription in the absence of ligand. This process is related to the recruitment of co-repressor complexes. There are several co-repressor complexes characterized, but the most commonly studied complex comprises nuclear receptor co-repressor (NCoR), silencing mediator for retinoid and thyroid hormone receptors (SMRT), histone deacetylase 3 (HDAC3), transducin- $\alpha$ -like 1 (TBL1), TBL-1-like related protein (TBLR1) and G-protein-pathway suppressor 2 (GPS2) [212, 213]. HDACs possess a well-characterized role in transcriptional repression by deacetylating N-terminal lysines of histone proteins thus generating a condensed, transcriptionally inactive chromatin structure. It was reported that SMRT and NCoR contain a deacetylase-activating domain which can trigger the enzymatic activity of HDAC3 [214].

In addition, other corepressor complexes have been described, such as SWI/SNF-containing complexes as mentioned above, PRC1&2 and CoRest complexes. Like the NCoR/SMRT complex, tethering these multiprotein entities to promoters leads to histone and DNA covalent modifications, followed by chromatin compaction and/or DNA masking. A critical step in NR-mediated transcriptional activation is the dismissal of corepressor complex from the DNA-bound receptor. In vitro assays have demonstrated that agonist-induced conformational changes are sufficient for SMRT or NCoR dissociation from the receptor, in agreement with crystal structure data. However, dynamic models of de-repression involving post-translational modifications of corepressor complex subunits leading either to their nuclear exclusion and/or degradation have been described [215]. The mechanism(s) by which such an active derepression takes place is as of yet unknown.

#### ***Direct transrepression by liganded receptors***

Ligand-bound NRs repress the transcription of some genes by a mechanism called negative regulation. This process occurs with multiple NRs and genes and was detailed for GR and TR. It has been suggested that these NRs recognize and bind negative response elements and downregulate specific target genes. The analysis of specific DNA sites revealed that negative glucocorticoid response elements (nGRE) and negative thyroid response elements (nTRE) are different from positive response elements that mediate transcriptional activation [216, 217]. Overlapping binding sites for transcription factors such as Oct-1/Pbx, AP-1 and SP1 were found for negative response elements of GR and TR, and found to dictate the transcriptional cis effect of the response element [218–221]. These data thus



posit that negative cis-acting glucocorticoid response elements exert such an activity by interacting with other transcription factors. However, a recent report described a novel class of negative glucocorticoid REs, organized as inverted repeats with a 1bp spacer, on which glucocorticoids promote the recruitment of GR-corepressor complexes [222]. Such a mechanistic principle does not seem to hold true for T3-mediated transcriptional repression. As detailed for the  $\alpha$ TSH gene, the corepressor SMRT is recruited to the nTRE and promotes histone deacetylation. Upon agonist treatment, SMRT dismissal is correlated with histone acetylation and gene repression [223, 224]. Furthermore, functional studies have shown a role for SRC-1 in transcriptional repression mediated by liganded TR [225, 226]. The mechanistic basis for such a reversal of transcriptional activity is not known, but could be mediated by post-translational modifications such as phosphorylation, acetylation or SUMOylation of promoter-associated histones and/or of coregulatory proteins [227–229]. Thus direct repression occurs via distinct mechanisms which are receptor- and context-dependent. These studies also pinpoint to the versatility of coregulator complexes, which may exert either positive or negative effects on the transcriptional outcome following NR agonist stimulation.

### ***Tethered transrepression by liganded receptors***

The mechanism referred to as tethered transrepression engages negative crosstalk of ligand-activated nuclear receptors with other signal-dependent transcription factors, including NF-kappa-B and activator protein-1 (AP-1). This process modulates inflammation in various cells of the central nervous system, the immune system as well as the liver, *etc* and interferes with cellular proliferation in various tissues.

Several mechanisms can be proposed to account for such a repression: (i) repression of PIC assembly on NF-kappa- or AP-1 regulated promoters [127, 128, 230, 231]; (ii) inhibition of RNA polymerase II conversion towards an elongation-competent form [232, 233]; (iii) upregulation of the expression of the inhibitor of NF-kappa-B [234]; (iv) interaction with upstream components of the NF-kappa-B or AP-1 activating cascade [235–237]; (v) coactivator exclusion by competition [238, 239] and (vi) direct physical interaction with AP-1 or NF-kappa-B (mostly p65) subunits [240–243], although this process is much more complex and requires multiple factors in living cells [244].

Interestingly, inflammatory programs triggered by TLR-3, 4 or 9 activation in macrophages are only partially inhibited by GR, LXR and PPAR $\gamma$  agonists, each receptor inhibiting about one-third to one-half of the induced genes. Intriguingly, inhibited clusters of genes by each receptor were only partially overlapping [238].

NR co-repressors such as NCoR and SMRT play an important role in ligand-dependent tethered transrepression. NCoR-deficient macrophages display a derepressed expression of various AP-1 and NF-kappa-B-related genes, an effect linked to NCoR (or SMRT, [245]) association to these DNA-bound transcription factors [246]. Much like NR-mediated transcription, activation of signaling pathways leads to the transcription of NF-kappa-B-driven genes by removal of the corepressor complex through a proteasome-dependent pathway. NR activation upon ligand binding promotes tethering of sumoylated NR to NF-kappa-B complexes, which interrupts corepressor complexes clearance, hence maintaining the promoter in a repressed state [127]. More recently, sumoylated LXRs were found to be targeted at transrepressed promoters through interaction with a NCoR complex component, coronin A. This interaction prevents corepressor turnover by preventing oligomeric actin recruitment [247]. This very elaborate process has been described for LXR. In mouse macrophages, whereas transrepression of the acute phase reaction (APR) in mouse liver by LXR involves GPS2 rather than coronin A [128, 247]. Thus, as suspected from many previous studies, tethered transrepression follows different mechanistic schemes which are receptor-, gene- and cell type-specific.

The structural features of NR specifically involved in transrepression are not clearly defined. Extensive mutagenesis studies of T3R, RAR, PPAR $\gamma$ , GR and ER (see for examples [239, 248–261]) did not yield a clear-cut and unifying model for tethered transrepression. Taken as a whole, it clearly appeared that coactivator recruitment through the AF-2 domain is not required for this activity, as well as direct DNA binding. There are also strong evidences suggesting that homo- or hetero dimerization is not mandatory [239, 262]. The lack of well-defined molecular structures involved in transrepression is an important pitfall in designing screening methods aiming at identifying dissociated ligands which would preferentially elicit tethered transrepression in inflammatory diseases.

## **NUCLEAR RECEPTORS AND NON-GENOMIC SIGNALING PATHWAYS**

NR ligands regulate gene expression by genomic actions which are described above. Nevertheless, NR ligands also exhibit non-genomic effects manifested by the rapid and transient activation of several kinase cascades, which can be attributable to a subpopulation of NRs located at the cell membrane, although this point is still debated. Accordingly, conserved palmytoylation sites have been identified in GR and ER [263–265], and together with MR, these receptors have been detected in lipid rafts [265–268].

This extranuclear localization provides a mean for steroid receptors to interact with various kinases. Estrogens trigger protein-protein interaction between ER and Src/p21ras/Erk and PI3K/Akt, through the SH2 domain of c-Src and the regulatory subunit of PI3K respectively. Estrogen-mediated induction of these kinase cascades play an important role in cell proliferation in breast cancer and vascular

function [269–273]. Progesterins can induce the Src/Erk1/2 pathway mediated by the interaction of two domains of the progesterone receptor (PR) with the LBD of ER. This crosstalk is essential for progesterin induction of DNA synthesis and cell proliferation in breast cancer [274]. A complex of activated PR, ERK and its target kinase Msk1 is recruited to the promoter after hormone treatment and phosphorylates serine 10 of histone H3, where it induces the recruitment of SRC-1, RNA polymerase II and chromatin remodeling complex (hSnf2h and Brg1). This example constitutes a link between kinase cascade activation in the cytoplasm, chromatin remodeling, and transcriptional activation in the nucleus [275] which is possibly conserved for retinoid receptors [276]. It has been suggested that aldosterone can counteract vasoconstriction via stimulation of endothelial NO production. This occurs through a mechanism which engages PI3 kinase and its interaction with MR [277]. A similar mechanism seems to underlie the decreased vascular inflammation and reduced myocardial infarct size following ischemia and reperfusion injury induced by glucocorticoids [278].

Recent evidences show that dexamethasone, a synthetic GR agonist, reduces cPLA2 activation which releases arachidonic acid. This mechanism seems to be glucocorticoid receptor-dependent but transcription-independent [279,280]. Plasma membrane-bound GR [281] has indeed been described in a variety of cell types [282,283] and GR has been shown to associate to Src in lipid rafts [268].

Non-genomic effect events similar to those described for steroid hormones occur for retinoids. It has been reported that RAR is present in the cytoplasm and in membranes where it associates with PI3K or Src [284]. Retinoic acid (RA) rapidly activates mitogen-activated protein kinases (MAPKs) such as ERK and p38MAPK in fibroblasts, mouse embryocarcinoma cells, mammary breast tumor cells and leukemia cells [131,285,286]. A novel unexpected non-genomic activity has been demonstrated for RAR $\alpha$ : RAR $\alpha$  is transported to neuronal dendrites where associates with glutamate receptor 1 (GluR1) mRNA, via its C-terminal F region and, as a result, inhibits the translation of this mRNA. RA binding abrogates this translational repression. These effects have been correlated to the regulation of synaptic functions and neuronal plasticity controlled by RA [287–289].

Non-genomic effects were also observed for nuclear receptor ligands involved in metabolic control. Although there is no evidence for membrane-bound PPAR $\gamma$ , the synthetic agonist rosiglitazone (RGZ) as well as the natural agonist 15 $\Delta$ PGJ2 regulate glucose and lipid metabolism and sperm activation in human spermatozoa by a rapid mechanism involving protein phosphorylation [290]. In human microvascular endothelial cells, RGZ interferes with pro-inflammatory actions of TNF and IFN $\gamma$  by direct inhibition of ERK1/2 phosphorylation in a PPAR $\gamma$ -dependent manner [291]. RGZ-mediated ERK1/2 regulation and PI3K inhibition was observed in human adrenocortical cells and PC3 prostate cells [292,293]. Conversely, in vascular smooth muscle cells 15 $\Delta$ PGJ2 and TZD activated the MEK/ERK pathway via PI3K [294]. Importantly, the energy-sensitive AMP kinase is activated by TZD-stimulated PPAR $\gamma$ , inducing acetyl CoA carboxylase phosphorylation, stimulation of glucose uptake and fatty acid oxidation in skeletal muscle, liver and adipose tissue [295,296].

Thus non genomic effects of NR ligands, mediated or not by an extranuclear subpopulation of NRs introduce a new layer of complexity in NR biology which must be determined when studying biological and pharmacological effects of NR ligand administration. Although impaired by technical limitations, the study of the subcellular localization of NRs in pathophysiological conditions may help deciphering mechanisms controlling the broad spectrum of biological responses controlled by NRs. Worth noting, the mitochondrial effects of some NRs such as SHP [297], GR [298] and Nur77 [299–301] which play an important role in apoptosis regulation through protein-protein interaction, deserve further investigations for other members of the NR family.

## CONCLUSION

NRs are modular transcription involved in multiple pathophysiological processes. They can be viewed as an assembly platform on chromatin for multimeric coregulators which will dictate the cell-specific and even gene-selective transcriptional output of target cells. In addition to direct ligand binding, these multi-proteic complexes are integration modules of other signaling pathways which can additionally adjust NR-driven promoters response to their extracellular cues. With the advent of high throughput genomic, epigenetic and proteomic techniques, a NR system biology can now be elaborated to bring a global and detailed view of NR contribution to human biology and diseases.

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## Footnotes:

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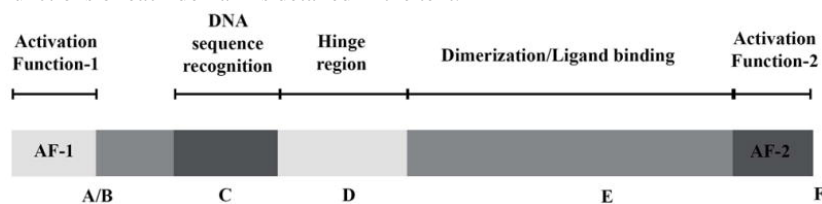
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**Figure 1**

General structural organization of nuclear receptors

Letters from A to F represent nuclear receptor domains from N-terminus to C-terminus of the nuclear receptor respectively. The structure and functions of each domain is detailed in the text.



**Figure 2**

Crystal structure of PPAR $\gamma$ -RXR $\alpha$  complex bound to a DR-1 response element

Crystallographic coordinates were obtained from the RCSB protein databank (PDB 3E00) and visualized using the Jmol software. PPAR $\gamma$  is purple and RXR $\alpha$  is blue.



**Figure 3**

Different architecture of selected response elements of nuclear receptors

IR -inverted repeat, ER - everted repeat, DR - direct repeat, 'N' indicates any nucleotide, "n" indicates negative response elements.

IR-0		RXR $\alpha,\beta,\gamma$ RAR $\alpha,\beta,\gamma$ TR $\alpha,\beta$
nIR-0		TR
IR-1		FXR
nIR-1		GR
IR-3		GR, ER $\alpha,\beta$
ER-6		TR $\alpha,\beta$
ER-8		TR $\alpha,\beta$ RAR $\alpha,\beta,\gamma$
DR-1		RXR -RXR $\alpha,\beta,\gamma$ RAR $\alpha,\beta,\gamma$ PPAR $\alpha,\beta,\gamma$ COUP-TF HNF4
DR-2		RXR - PPAR RAR $\alpha,\beta,\gamma$
DR-3		RXR - VDR
DR-4		RXR - TR $\alpha,\beta$ LXR $\alpha,\beta$ CAR
DR-5		RXR - TR NGFI-B NURR1

**Figure 4**

## General mechanism of NR action

Nuclear receptors may act in two different ways. Upon ligand binding nuclear receptors forming heterodimers with RXR interact with a specific positive gene response element (pRE) and activate mRNA transcription of target genes. Alternatively, they may interact directly with repressive, negative response elements (nRE). The major suppressive effect of nuclear receptors is however thought to be mediated by monomers interaction with subunits of AP-1 and NF- $\kappa$ B transcription factors, and hamper the expression of inflammatory-related genes (see text for details).

