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Review

Review

Structural overview and perspectives of the nuclear receptors, a major family as the direct targets for small-molecule drugs

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Abstract

The nuclear receptors (NRs) are an evolutionarily related family of transcription factors, which share certain common structural characteristics and regulate the expressions of various genes by recognizing different response elements. NRs play important roles in cell differentiation, proliferation, survival and apoptosis, rendering them indispensable in many physiological activities including growth and metabolism. As a result, dysfunctions of NRs are closely related to a variety of diseases, such as diabetes, obesity, infertility, inflammation, the Alzheimer's disease, cardiovascular diseases, prostate and breast cancers. Meanwhile, small-molecule drugs directly targeting NRs have been widely used in the treatment of above diseases. Here we summarize recent progress in the structural biology studies of NR family proteins. Compared with the dozens of structures of isolated DNA-binding domains (DBDs) and the striking more than a thousand of structures of isolated ligand-binding domains (LBDs) accumulated in the Protein Data Bank (PDB) over thirty years, by now there are only a small number of multi-domain NR complex structures, which reveal the integration of different NR domains capable of the allosteric signal transduction, or the detailed interactions between NR and various coregulator proteins. On the other hand, the structural information about several orphan NRs is still totally unavailable, hindering the further understanding of their functions. The fast development of new technologies in structural biology will certainly help us gain more comprehensive information of NR structures, inspiring the discovery of novel NR-targeting drugs with a new binding site beyond the classic LBD pockets and/or a new mechanism of action.

Key words nuclear receptor, structure, drug target, small-molecule drug

Introduction

The study on nuclear receptors (NRs) became a unique research filed in the middle of 1980s, when the molecular cloning of several hormone receptors revealed that they share a common architecture especially in the domain composition [1]. Due to their critical functions in many physiological processes and direct connections to a variety of human diseases, NRs have long been exploited as therapeutic drug targets. By some estimates, NR ligands constitute about 15%–20% of the small-molecule drugs on the pharmaceutical market worldwide [2–4]. At present, drugs directly targeting different NRs have been widely used in the treatment of various diseases, such as tamoxifen and evista targeting the estrogen receptor (ER) used in breast cancer and osteoporosis respectively [5], casodex targeting the androgen receptor (AR) used in prostate cancer [6,7], targretin targeting the retinoid X receptor (RXR) used in skin cancer [8,9], and glitazones targeting the peroxisome proliferator-activated receptor-gamma (PPARγ) used in type II diabetes [5]. In addition, a large number of compounds with stronger binding affinities and better specificities are currently in the research or development stages for new NR-targeting drugs [10,11].

There are totally 48 human NRs, half of which have known endogenous ligands (Table 1). Based on the protein sequence homology and functional analysis, a typical NR polypeptide can be roughly divided into 5 to 6 regions, which are represented by A-F from the N- to C-terminus respectively (Figure 1) [12,13]. N-terminal regulatory domain (NTD, i.e. the A/B region) is also called the ligand-independent transcription activation function-1 (AF-1) domain, which shows a low sequence conservation and a

Table 1. The number of NR-related structures in PDB database till now

Names	Nomenclature	Ligand	DBD	LBD	Others	LBD-LBD	Multi	Reference
TRα	NR1A1	Thyroid hormones	0	8		1		[14–16]
TRβ	NR1A2	Thyroid hormones	1	22		1		[17–20]
RAR-α	NR1B1	Retinoic acid	4	6		2		[21–24]
RAR-β	NR1B2	Retinoic acid		8		1	1	[24–26]
RAR-γ	NR1B3	Retinoic acid		11				[27,28]
PPARα	NR1C1	Fatty acids		55				[29–31]
PPARβ	NR1C2	Fatty acids		44				[32,33]
PPARγ	NR1C3	Fatty acids		238				[34–37]
Rev-erbα	NR1D1	Orphan	4	4				[38–40]
Rev-erbβ	NR1D2	Orphan		6				[41]
RORα	NR1F1	Cholesterol		3				[42–44]
RORβ	NR1F2	Retinoic acid		3				[45,46]
RORγ	NR1F3	Orphan		141				[47,48]
LXRα	NR1H3	Oxysterols		8		3		[49,50]
LXRβ	NR1H2	Oxysterols		24			1	[51–53]
FXR	NR1H4	Bile acids		82		3		[54,55]
VDR	NR1I1	Vitamin D	4	100				[56–59]
PXR	NR1I2	Xenobiotics		42		2		[60–62]
CAR	NR1I3	Xenobiotics		1		3		[63,64]
HNF4α	NR2A1	Orphan	1	5			1	[65–67]
HNF4γ	NR2A2	Orphan		1				[68]
RXRα	NR2B1	Retinoic acid	18	58		20		[52,64,69–71]
RXRβ	NR2B2	Retinoic acid		6		1		[72,73]
RXRγ	NR2B3	Retinoic acid		2				[73]
TR2	NR2C1	Orphan						
TR4	NR2C2	Orphan		1				[74]
TLL	NR2E2	Orphan						
PNR	NR2E3	Orphan		1				[75]
COUP-TFI	NR2F1	Orphan		1				
COUP-TFII	NR2F2	Orphan		1				[76]
EAR2	NR2F6	Orphan						
ERα	NR3A1	Estradiol-17-β	2	298				[77–80]
ERβ	NR3A2	Estradiol-17-β		36				[81–83]
ERRα	NR3B1	Orphan		4				[84]
ERRβ	NR3B2	DES	1	2				[85,86]
ERRγ	NR3B3	DES		29				[87–89]
GR	NR3C1	Cortisol	40	28				[90–93]
MR	NR3C2	Aldosterones	2	28				[94,95]
PR	NR3C3	Progesterone	1	18				[96–98]
AR	NR3C4	Testosterone	1	95	1			[96,99–101]
Nur77	NR4A1	Orphan	4	17				[102–106]
NURR1	NR4A2	Orphan	3	5				[107,108]
NOR1	NR4A3	Orphan						
SF1	NR5A1	Orphan	1	8				[109,110]
LRH-1	NR5A2	Orphan	2	21	1	1		[109,111–113]
GCNF	NR6A1	Orphan	1					[114]
DAX-1	NR0B1	Orphan				2		[113]
SHP	NR0B2	Orphan		7				[115–118]

DBD, DNA-binding domain; LBD, ligand-binding domain; Others, other family proteins; LBD-LBD, homo- or heterodimer of NR LBD; Multi, Multi-domain complex of NRs.

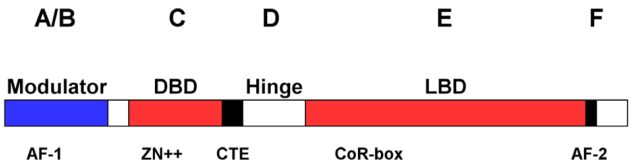

Figure 1. Structural organization of nuclear receptors Typical NRs can be roughly divided into five to six functional regions from N-terminus to C-terminus, designated as A–F respectively. A/B domain containing activation function-1 (AF-1) is a regulatory domain; C region is the DNA-binding domain; D is the hinge area; and E/F is the ligand-binding domain containing AF-2.

high conformational flexibility, with no high-resolution structure available yet [119]. DNA-binding domain (DBD), locating at the C region, has been well understood as one of the hallmarks of this NR family of transcription factors. The D region of NRs is a highly variable and flexible hinge region, which connects DBD and the ligand-binding domain (LBD). Nuclear localization signals (NLSs) of some NRs are located in the C and D regions, which affect the intracellular transport and subcellular distribution of NRs. The E/F region, i.e. the LBD, is the largest and most targetable domain of NRs. Transcription activation function-2 (AF-2) domain is also located in this region [119].

Over the past 30 years, the structures of a large number of NRs have been solved, since the cloning of first NR glucocorticoid receptor (GR/NR3C1) by Ronald Evans and colleagues (Table 1) [120]. As a result, the basic structural information of NRs is relatively clear, especially for the well-studied DBD and LBD regions. A systematic summary of the structural information on NRs has been presented by Fraydoon Rastinejad and colleagues [2,121,122], with the emphasis that the crystal structures of multi-domain homo- and hetero-dimeric complexes provide important insights, to fully reveal the allosteric regulatory mechanisms of NRs.

To date, the majority of NRs have had their structures (at least for certain domains) published, except for the testicular receptor 2 (TR2/NR2C1), tailless (TLL/NR2E2), V-erbA-related protein 2 (EAR2/NR2F6) and neuron-derived orphan receptor 1 (NOR1/NR4A3) (Table 1). However, it is worth noting that most of the structural information available has been heavily concentrated on several NRs [i.e. PPARγ, retinoid-related orphan receptor-gamma (RORγ), farnesoid X-activated receptor (FXR), 1,25-dihydroxyvitamin D3 receptor (VDR), RXRα, ERα, GR, and AR], whose functions are very important and well-studied. At present, PPARγ and ERα have contributed the largest number of NR structures (both over 200) in PDB, and most of them are LBDs in complex with synthetic derivatives of endogenous ligands (Table 1). Meanwhile, there is little information about structures of NRs in complex with other family of proteins, when excluding the classic LXXLL motif-containing peptides derived from the steroid receptor coactivator (SRC) proteins. In recent years, structures of several large complexes containing NRs, SRC and p300 have been visualized using the cryo-electron microscopy (cryo-EM), although at only very low resolutions. With the rapid development of cryo-EM technology, the structural basis of how NRs function in concert with other partner proteins and in response to various ligands, would be fully illustrated soon. In addition, new drug discovery strategies such as proteolysis targeting chimera (PROTAC) have emerged and enriched the approaches to develop novel drugs targeting NRs directly.

In this review, we present a comprehensive overview of structures related to NRs in the Protein Data Bank (PDB) database, as well as some perspectives in the future development of small-molecule drugs directly targeting NRs.

DBD Structures of NRs

The DBDs of NRs possess highly conserved protein sequences, and their structures and functions have been extensively studied. The typical NR DBD structure conforms to a Zn-finger subtype of transcription factors, in which eight highly conserved cysteine residues coordinate two zinc ions, setting the NRs apart from other DNA-binding proteins (Figure 2A). In the PDB database, RXR and GR have contributed the most DBD-DNA complex structures of NRs (Table 1). RXR can act as the dimer partner for several NRs including itself. Then these NR dimers bind to different response elements on the genomic DNA, and regulate transcription in a cell and gene-specific manner. The number of spacer nucleotides between two NR-binding half-sites, was initially defined for various RXR heterodimers in a simplified manner by the 'DR (direct repeat) 1–5 rule': i.e. RXR-RXR, and retinoic acid receptor (RAR)-RXR (DR1), RXR-RAR (DR2), RXR-VDR (DR3), RXR-TR (DR4) and RXR–RAR (DR5) (Figure 2B–G) [21,23,59,123–126]. Interestingly, a recent study reported the structure of RXR DBD homodimer in complex with *Hoxb13* DR0 DNA (Figure 2H) [23]. On the contrary, receptors for androgens (AR), corticosteroids (GR/MR), progesterone (PR) or estrogens (ER) typically bind DNA sequences configured as inverted repeats of the half-site sequences AGAACA or AG/AGTCA, respectively (Figure 2I–J); while the remaining members of the family bind half-sites of the sequence AGGTCA/AGGCTA, arranged singly (Figure 2K,L) or as direct repeats [2,127]. The advent of next-generation sequencing, in combination with chromatin immunoprecipitation (ChIP-seq), has allowed for the identification of NR-binding sites in a genome-wide manner and in various cell types under different pathophysiological conditions [128]. Furthermore, these studies have revealed that NR response elements can vary from monomeric half-sites to nearly perfect inverted or direct repeats, and have demonstrated the cooperation between NRs and other transcription factors, such as the forkhead box A1 (FOXA1) (for AR and ER), activating protein-1 (AP-1) [for GR and liver X receptor (LXR)], and CCAAT/enhancer-binding protein (C/EBP) (for PPAR-γ and LXR) [128].

LBD Structures of NRs

The NR LBDs usually show a similar overall conformation, consisting of 11–13 α helices and 1 β-turn that together form a three-layered helical sandwich (Figure 3A). The typical ligand binding pocket is embedded in the interior of LBD, with the core position composed of amino acids derived from the helices H2, H5, H7, H11, and H12. The volume of pockets can vary from zero to more than 1500 $Å^3$ (Figure 3B) [129]. LBD plays an important role in the transcriptional regulation of classical NRs, and its mechanism of action has been clearly revealed. Structural analysis shows that LBD works like a "mousetrap" and ligand binding can induce the conformational change of helix H12 [27]. When ligands bind into pockets, H12 as the "gate" of the "mousetrap", will move towards LBD and fold back to close the entrance of the ligand binding pocket (Figure 3C,D). Moreover, the H12 adopts different conformations when the pocket binds agonists or antagonists. For agonists, H12, H3 and H4 form a hydrophobic coactivator binding surface, where the LXXLL motif can be well integrated (Figure 3D,E). While for

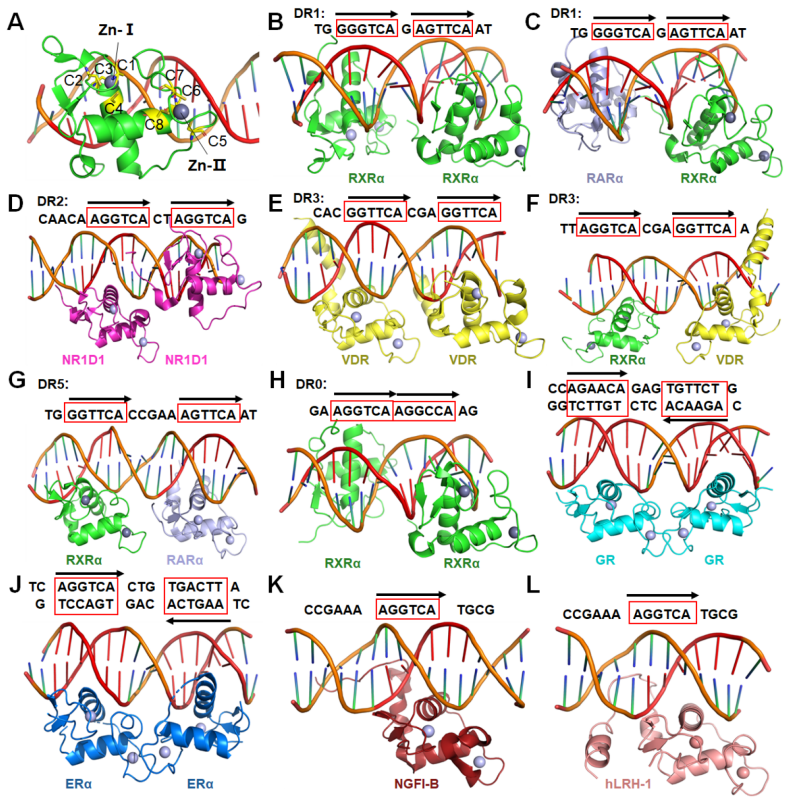

Figure 2. Interactions of DBDs with different response elements (A) The typical structure of NR DBD (PDB code: 4CN5). (B–H) Homo- or hetero-dimers of NR DBD complexes with DR0 to DR5 response elements (PDB codes: 4CN5, 1DSZ, 1GA5, 1KB2, 1YNW, 6XWG, and 6XWH, respectively). (I,J) Homodimers of GR and ERα bound to inverted repeats of the half-site sequences (PDB codes: 5CBX, 4AA6). (K,L) NGFI-B and hLRH-1 bound to single half-sites of DNA sequence (PDB codes: 1CIT, 5L0M).

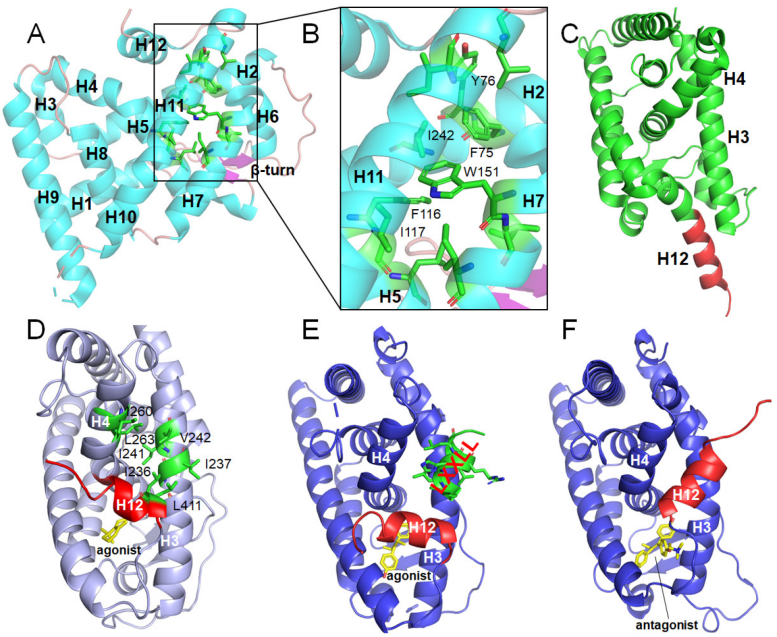

Figure 3. Structural characteristics and classical transcriptional regulation mechanisms of NR LBDs (A) Overall conformation of Nur77 LBD shown as cartoon in cyan (PDB code: 3V3E). (B) Ligand binding pocket of Nur77 is occupied by several hydrophobic amino acids. (C) Inactive state of the apo-LBD, H12 color in red (PDB code: 6HN6). (D) Active state of RARγ holo-LBD, agonist shown as sticks in yellow, hydrophobic amino acids (shown as sticks in green) in H3, H4, H5 forming a hydrophobic interaction surface (PDB code: 2LBD). (E) Active state of ERα holo-LBD, agonist shown as sticks in yellow, coactivator GRIP1 with the LXXLL motif color in green (PDB code: 3ERD). (F) ERα LBD bound with antagonist and H12 color in red occupying the co-activator binding site (PDB code: 3ERT).

antagonists, H12 moves away from the excited position, masks or destroys the binding sites of coactivators, and forms the binding interface for corepressors (Figure 3F) [130]. Under different signal stimulations, NRs interact with different coregulators and conduct different functions, activating or repressing the expression of downstream genes. However, there are also several NRs not conforming to the above mechanism. For example, RORγ, immediate-early response protein NOT (Nurr1) and nuclear hormone receptor Nur77 are in the transcriptionally activated conformation independent of ligands (Figure 3A). Especially, the pockets of three members of the NR4A subfamily (Table 1) are completely occupied by hydrophobic amino acids, and the hydrophobic binding regions of co-regulators are also occupied by some polar amino acids (Figure 3B). As a result, the transcriptional regulation mechanism of this type of NRs is still unclear.

As mentioned earlier, most of NR structures reported in PDB are the complexes of LBDs with endogenous ligands and their derivatives. By now, the numbers of LBD structures for PPARγ and ERα have reached 238 and 298, respectively (Table 1). Rosiglitazone is one of the thiazolidinedione (TZD) drugs for diabetes treatment, and it is an insulin sensitizer with a high affinity and specificity to activate PPARγ. However, TZDs also display severe adverse effects, giving rise to fluid retention, weight gain, liver toxicity and cardiovascular diseases, which are prevalent among diabetic patients [131–133]. Based on the structure of PPARγ LBD in complex with rosiglitazone, researchers designed and discovered more effective agonists. Ionomycin interacts with the PPARγ LBD in a unique binding mode (Figure 4A) and effectively improves hyperglycaemia and insulin resistance, with reduced side effects compared with TZDs in the mouse model of diabetes [133]. Another example among NR drugs is raloxifene, which is a selective ERα modulator used in osteoporosis. The crystal structures of ERα LBD in complex with the endogenous estrogen, 17β-oestradiol, and the selective antagonist raloxifene, indicate that agonist and antagonist bind at the same site within the core pocket of LBD, but demonstrate different binding modes (Figure 4B) [134]. These small-molecule bound complex structures not only help reveal their mechanisms of action, but also provide valuable information for the structure-based drug design targeting NRs.

For the majority of NRs, their activations and functions depend on the dimerization. Generally, NRs possess two dimerization sites, one in DBD and the other in LBD. LBD is considered as the major dimerization site for NRs [22]. Ligand binding not only changes the conformation of LBDs to coordinate with the coregulator proteins, but also promotes the dimerization of NRs [22]. According to the analysis of LBD structures in the PDB database, most of the NRs utilize the classical dimerization sites (H10 and H9 or even Loops 8-9 and H7) to form dimers (Figure 5A–C). The size of dimerization interface of ERα homodimer is about 1700 Å^2, while those for the RXRα homodimer and its heterodimer with RARα LBD are only 950 Å^2 and 970 Å^2, respectively (Figure 5A,B) [135]. The smaller dimerization interface of RXRα is related to its biological function, making RXRα relatively easy to depolymerize and form new homo- or heterodimers with other NRs [136]. The heterodimerization of RXR with RAR, TR and VDR via LBDs mainly depends on the I-box

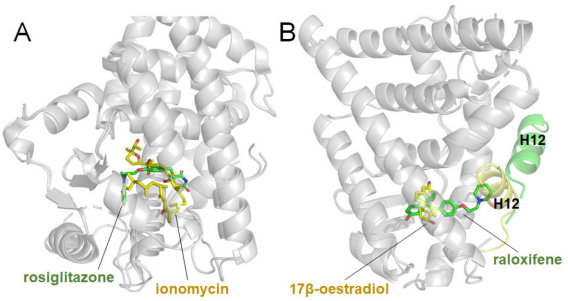

Figure 4. NR LBD structures in complex with different small molecules (A) Superimposition of PPARγ LBD in complex with ionomycin (stick in yellow) and rosiglitazone (stick in green) (PDB codes: 4FGY, 2PRG). (B) Superimposition of ERα LBD in complex with endogenous agonist, 17β-oestradiol (stick in yellow) and antagonist raloxifene (stick in green), related H12 shown as cartoon color in yellow and green respectively (PDB codes: 1ERE, 1ERR).

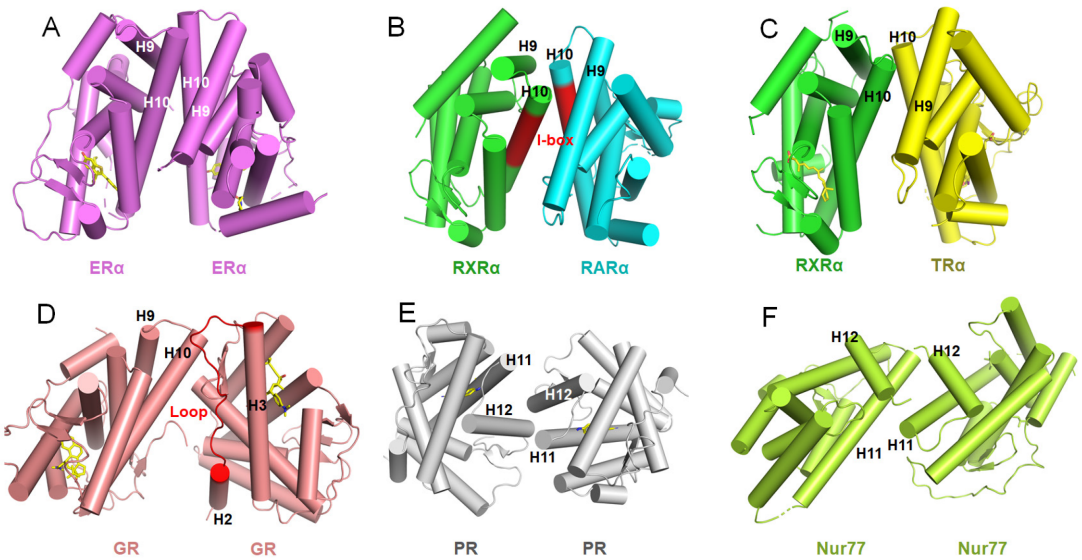

Figure 5. Different dimerization interfaces of NR LBDs (A–C) Homo- or hetero-dimers through H9 and H10. The I-box of RXRα and RARα colored in red (PDB codes: 1ERR, 1DKF, 3UVV, respectively). (D) Homodimer interface of GR (PDB code: 6DXK). (E,F) Homodimers of PR and Nur77 through H11 and H12 (PDB codes: 1ZUC, 3V3E).

located in H9-H10 region, as shown by their dimeric crystal structures (Figure 5B,C) [22,137–139]. However, not all NRs dimerize in this way. GR homodimerizes through the hydrophobic interactions of some hydrophobic amino acids in the H2-H3 loop region, with a dimerization area of 623 Å^2 (Figure 5D) [140]. PR homodimerizes through hydrogen bonds and salt bridges formed in the H11-H12 region, and the dimerization surface area is about 600 Å^2, which is much smaller than those of classical NRs (Figure 5E) [97]. Another NR with a small dimer interface is Nur77, whose asymmetry interface is mediated by hydrogen bonds and salt bonds in the H11-H12 region with a surface area of only about 590 Å^2 (Figure 5F) [105,106].

Structures of Full-length and Multi-domain Receptor Complexes

NRs are multi-domain transcription factors that bind to specific DNA elements and regulate the expressions of downstream genes. How DNA binding influences the NR conformation, and how the conformations of DBD and LBD change collaboratively in the process of transcriptional regulation, are still poorly understood. Previous structural studies have mainly focused on the isolated DNA or ligand-binding segments, i.e. DBDs and LBDs. To date, only 4 crystal structures of full-length or multi-domain NR complexes bound to DNA have been reported, with 3 of them by Fraydoon Rastinejad and colleagues.

The structure of PPARγ and RXRα, published in 2008 as a heterodimer bound to DNA, ligands and coactivator peptides, is the first structure of multi-domain NR complex, in which PPARγ-RXRα polypeptides reside in a polar arrangement set by the 5′ extension of the DR1 and the lone spacer base pair [70]. PPARγ occupies most of the DR1 including its specific 5′ element. Three interfaces link PPARγ and RXRα, including those that are DNA-dependent. The PPARγ LBD cooperates with both DBDs to enhance the response-element binding (Figure 6A).

HNF-4α is the most abundant DNA-binding protein in liver, where about 40% of the actively transcribed genes have at least one HNF-4α response element [141,142]. To understand the extent of domain integration of HNF-4α, Rastinejad group solved and analyzed the crystal structure of HNF-4α, an obligate homodimer, bound to its DNA element and coactivator-derived peptides in 2013 (Figure 6B) [65]. The LBD and DBD portions match their previously determined isolated structures. Both DBDs are in register with their half-sites, interacting with the major grooves. The DBD of the upstream subunit and the hinge region of the downstream subunit, form an important domain-domain interface of the complex, which is similar to the one in the PPARγ-RXRα complex. The manner in which the two LBDs cooperate to interact with the upstream DBD suggests that the physical integration of all three domains may be required for a high-affinity DNA binding, as further proved by the physiological and biochemical experiments [143].

The LXRs are physiologically important oxysterol-dependent NRs. LXRs are master regulators of lipid and cholesterol metabolism [144,145], inflammation [146], neural development [147], cancer [148,149], and other physiological processes. Furthermore, LXRβ has protective effects upon dopamine neurons [150,151] and modulates the cytotoxic functions of microglia [152]. Thus, LXRs are key pharmaceutical targets in a variety of diseases. Gustafsson group reported the crystal structure of the RXRα-LXRβ heterodimer on its cognate element, an AGGTCA direct repeat spaced by 4 nt (DR4) in 2014 (Figure 6C) [52]. This complex shows an extended X-shaped arrangement, with DBD and LBD crossed, which is different from the other 3 multi-domain complexes (Figure 6C). Compared with previous NR structures, it reveals the flexibility in NR organization and suggests a role for RXRα in the adaptation of heterodimeric complexes to DNA.

The RAR and RXR proteins are among the most intensively studied NRs for their structural properties, but most structural characterizations to date have focused on the isolated LBD domains (Table 1). In 2017, Rastinejad group reported the crystal structure of the multi-domain RARβ-RXRα heterodimer bound to DR1 DNA, ligands and coactivator peptides [26]. The DBD and LBD of RARβ are physically connected. However, the corresponding two domains of RXRα are spatially displaced from each other without any physical contacts, and each of them locates on the opposite side of the double-strand DNA. Both RXRα and RARβ adopt the active conformation at their LBDs. This conformation is defined by both receptors having their H12 appropriately positioned by ligands to facilitate the recruitment of coactivator LXXLL motifs (Figure 6D).

By analyzing the structures of 4 multi-domain complexes and comparing with the structures of their individual LBDs and DBDs, we found that they are essentially identical at the single domain level. These observations suggest that none of these domains undergoes major internal distortions in adopting the quaternary state of the multi-domain heterodimers. Through structural comparison and analysis, we can also learn the following lessons. (1) The flexible hinge region not only physically connects DBD and LBD, but also plays important regulatory roles in the interactions between

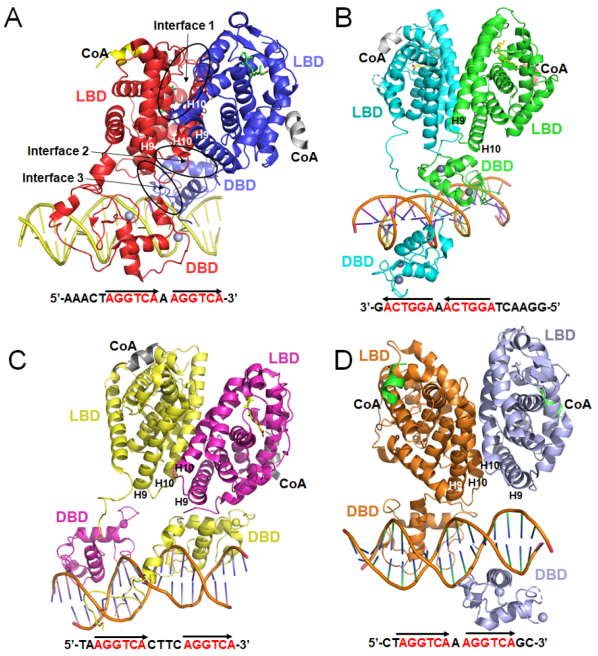

Figure 6. Four multi-domain complex structures of NRs (A) The PPARγ-RXRα (colored in red and blue, respectively) heterodimer on DNA response element with DR1 (PDB code: 3E00). (B) The HNF-4α homodimer bound to DR1 (PDB code: 4IQR). (C) The LXRβ-RXRα (colored in yellow and magenta, respectively) heterodimer on DR4 (PDB code: 4NQA). (D) The RARβ-RXRα (colored in orange and purple, respectively) heterodimer bond to DR1 (PDB code: 5UAN). The key DBD-interacting region at the H9 and H10 of LBD is marked with capital letters.

LBD and DBD, as well as those between DBD and DNA. In addition, the hinge region can interact with DNA directly and play special roles in stabilizing the formation of the whole complex. (2) Specific response elements, proper ligands and coactivators are needed to obtain a stable complex crystal structure. Therefore, the success of future crystallization efforts on new multi-domain NR complexes would require a lot of trial and error in the combination of these three NR partners above. (3) LBD plays key roles in the formation of homodimers or heterodimers, including the formation of its various interfaces with DBD (Figure 6). These unique interfaces may offer new opportunities for the discovery of NR-targeting drugs with a novel mechanism of action. (4) At present, there is still no clear structural information for the NTD region, which is very flexible and not conserved among NRs.

Structure of NRs in Complex with Other Family Proteins

The homo- or hetero-dimerization of certain NRs is one of the key steps in their transcriptional regulation of downstream genes. Meanwhile, cofactor recruitment also plays important roles in the regulatory function of NRs. For example, the N-terminal AF-1 region of Nur77 can enhance the ability of transcriptional activation by recruiting coactivators such as SRCs, P300, P300/CBP-associated factor (PCAF) [153]. Protein arginine methyltransferases 1 (PRMT1) can enhance the transcriptional activation of Nur77 by cooperating with SRC2 [154]. Nur77 and Nurr1 can also inhibit the transcription of downstream genes by recruiting the repressor FHL2 (four and a half LIM domains protein-2) [155] or repressor cofactor complexes [156,157]. However, the detailed recognition mechanism during the recruitment process is still missing, due to the lack of structures of protein-protein complex between cofactors and NRs.

In addition to the transcriptional regulation, in specific cell lines, NRs can also play other roles through protein-protein interactions. Nur77 directly associates with p65 to block its binding to the κB element [105]. However, this function of Nur77 is countered by the LPS-activated p38a phosphorylation of Nur77. In this process, Nur77 regulates the inflammatory response through direct interactions with p65 and p38a [105]. The direct interaction between 1-(3,4,5-trihydroxyphenyl)nonan-1-one (THPN) and Nur77-LBD helps form a suitable surface that can bind to mitochondria outer protein Nix, and then triggers autophagy in the human melanoma Mel-11, ME4405 and MM200 cells [104]. Therefore, analyzing the complex structures of NRs and other family proteins (especially those identified outside of the classical NR pathways), may provide a new angle for the drug design targeting NRs.

At present, the β-catenin armadillo repeat in complex with the liver receptor homolog-1 (LRH-1) LBD is the only complex structure between NRs and other family proteins that has been solved [112]. As the principal agent of Wnt-dependent effects on cell adhesion, differentiation and cancer, β-catenin engages in multiple protein–protein contacts [158,159], some of which are understood with atomic details [160]. β-Catenin is comprised of a central association region, the armadillo-repeat region (ARM), and N- and C-terminal transactivation domains [161], whereas the N- and C-terminal regions are both intrinsically unstructured [162]. The interaction modes between NRs and β-catenin are complex, but there is evidence that NR LBDs are indispensable for the contact in some cases. Biochemical and genetic evidence reveals that both LRH-1 and AR LBDs bind to the ARM, with AR exhibiting a strong dependence on

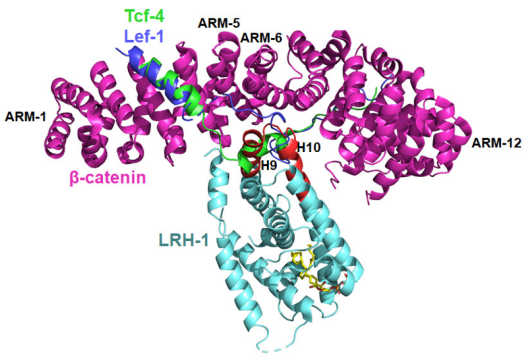

Figure 7. Complex structure of β-catenin and LRH-1 The overall structure of β-catenin and LRH-1 shown as cartoon in magentas and cyan colors, respectively. Peptides of Tcf-4 and Lef-1 colored in green and blue respectively. H9 and H10 of LRH-1 LBD colored in red (PDB code: 3TX7).

the ARM-5 and ARM-6 segments (Figure 7) [163–165]. In this complex structure, the LRH-1 LBD utilizes a novel interaction surface to dock into the positively charged groove at a site that partially overlaps the binding surface for T-cell factor 4 (Tcf-4) and lymphoid enhancer-binding factor 1 (Lef-1) (Figure 7) [166,167]. This structure suggests an interesting mechanism for the assembly of multifactor transcription complexes. NR LBDs are known to engage in multiple protein–protein contacts with co-regulators [168,169], and β-catenin is shown to bind many proteins including a wide variety of transcription factors [164]. The structural information of this complex indicates the possibility to develop new compounds that inhibit NR functions, by binding directly or impacting allosterically towards key protein interaction surfaces. Thus, the β-catenin binding site of LRH-1 (the H9–H10 of LBD) and other NRs could be a new targeting spot for the future drug discovery. Moreover, it is very interesting that NR LBDs use exactly the same "patch" region (H9–H10) to interact with DBDs, as shown in the four multi-domain NR structures (Figure 5) [26]. These findings suggest a potential common mechanism for NR LBDs to mediate the protein-protein interactions.

Application of Cro-EM in NR Structure Analysis

While full-length NRs are very difficult to crystallize, the fast developing cryo-EM technology has also been adopted to unravel the structural features of the NR signaling scaffold, as well as the critical roles of inter-domain communications [170]. Orlov *et al.* [171] presented the first cryo-EM structure of a 100-kDa complex of VDR-RXR heterodimer and their cognate DNA response element at the 10–15 Å resolution. Following that, other researchers used cryo-EM to determine the quaternary structure of an active complex of ERα/SRC-3/p300 bound to an ERE-DNA fragment at resolution ~25 Å in 2015 (Figure 8A); the structure of ARE DNA-bound full-length AR at resolution ~12.6 Å, and the structure of ARE DNA-bound AR/SRC-3/p300 complex at resolution ~20 Å in 2020 (Figure 8B) [172,173]. Both cryo-EM structures of ERα and AR in complex with SRC-3 and p300 indicate that the AF-1 in NTD plays a key role in the SRC-3 and p300 recruitment (Figure 8). Especially in the AR complex (Figure 8B), AF-1 in NTD participates in the interactions with SRC-3 and p300 much more than the AF-2 in LBD, which is consistent with previous reports [174,175].

Unfortunately, these current cryo-EM NR structures could not

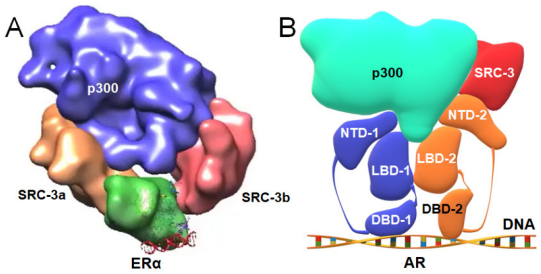

Figure 8. Cryo-EM models of ERα and AR complexes with cofactors and DNA (A) The components of ERα homodimer in complex with SRC-3 and p300. (B) Model of homodimer of AR in complex with p300 and SRC-3.

provide more details at the atom level due to their poor resolutions. To push the resolution limit, a number of factors have to be considered and optimized, especially the intrinsic dynamics between NRs and their co-regulators. For example, the application of specific antibodies targeting certain components of the large NR complex may help increase the overall stability. As the crystal structures of three key components of the NR complex (i.e. LBD, DBD and DNA) have been well defined with their unique sizes and shapes, the model building step in solving NR cryo-EM structures would be relatively straight-forward [170]. With more large NR complex structures available, the discovery of new mechanism drugs targeting NRs will be further accelerated.

Conclusion and Future Direction

Until recently, all previous structural research efforts on NR proteins were more focused on and successful with individual DBDs and LBDs, especially for some classic NRs (Table 1). However, the functions and structures of a considerable number of NRs are still not very clear, especially for TR2, TLL, EAR2 and NOR1. At present, the multi-domain crystal structures of PPARγ-RXRα, HNF4α-HNF4α, LXRβ-RXRα and RARβ-RXRα have been solved. But the structural information about NRs is still not complete, as the N-terminal region with a high flexibility and a low homology still has no high-resolution structure reported yet. Furthermore, no multi-domain structure of orphan NRs has been solved. NRs can be conceptualized as highly dynamic scaffold proteins, where binding of ligand, DNA or transcriptional coregulator proteins can allosterically change the scaffold structure and direct changes in subsequent binding events [170]. Therefore, more full-length NR complex structures are needed to obtain the conformational change information during the transcriptional regulation, which can surely provide a new structural basis for designing better small-molecule drugs. Especially in recent years, a series of major breakthroughs have been made in the development of cryo-EM. It bypasses mysteries in the protein crystallization process, and provides an alternative yet powerful technical approach for the structural analysis of full-length NRs, transcriptional regulatory complexes, as well as the complexes of NRs with other family proteins.

In the past a few years, the induced and targeted protein degradation, based on the PROTAC concept, has gained momentum as a new small-molecule therapeutic strategy [176]. Till now, a large number of PROTAC molecules related to AR and ER have been reported, some of which for the treatment of prostate and breast cancers have entered the phase II clinical stage [177,178]. This technology will bring new opportunities for the research of certain undruggable NRs, especially for some orphan receptors. With the progress of these technologies, new breakthroughs will be achieved in the discovery of novel drugs targeting NRs.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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