Biochimica et Biophysica Acta 1812 (2011) 824–835

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbadis

Review

Molecular basis for gene-specific transactivation by nuclear receptors ☆

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ARTICLE INFO

Article history:
Received 29 October 2010
Received in revised form 14 December 2010
Accepted 17 December 2010
Available online 28 December 2010

Keywords:
Nuclear receptor specificity
Nuclear receptor structure
DNA binding
Chromatin
Co-factor interaction
DNA looping

ABSTRACT

Nuclear receptors (NRs) are key transcriptional regulators of metazoan physiology and metabolism. Different NRs bind to similar or even identical core response elements; however, they regulate transcription in a highly receptor- and gene-specific manner. These differences in gene activation can most likely be accounted for by mechanisms involving receptor-specific interactions with DNA as well as receptor-specific interactions with protein complexes binding to adjacent and distant DNA sequences. Here, we review key molecular aspects of transactivation by NRs with special emphasis on the recent advances in the molecular mechanisms responsible for receptor- and gene-specific transcriptional activation. This article is part of a Special Issue entitled: Translating nuclear receptors from health to disease.

1. Introduction

Nuclear receptors (NRs) are a family of ligand-activated transcription factors (TFs) that regulate transcription of target genes through binding to specific response elements (REs). NRs regulate several aspects of human physiology and alterations in NR signaling have been implicated in diseases such as type II diabetes mellitus, cancer, and cardiovascular diseases [1,28,32,74,98,138]. The drugability of the NR ligand binding domain combined with the involvement of these receptors in life-threatening diseases have made them subjects of intense pharmacological research. At the same time the ability to easily control the activity of the receptors with lipophilic ligands has made NRs attractive targets for basic research aiming at understanding mechanisms of transcriptional regulation.

NRs can be subdivided into three classes according to the organization of their REs and their dimerization properties [3,91]. Class I NRs bind as homodimers to REs organized as palindromic or inverted palindromic hexanucleotide repeats separated by 3 bp. This group of receptors includes the classical steroid receptors (i.e., estrogen receptors (ERs), androgen receptor (AR), progesterone receptors (PRs), mineralocorticoid receptor (MR), and glucocorticoid receptor (GR)) that are specifically activated by high affinity hormones. Unliganded steroid receptors (except ER) are generally found in the cytoplasm in complex with heat shock proteins. Hormone binding induces a conformational change, which results in dissociation from the cytoplasmic heat shock protein complex and transport into the nucleus, where the receptor can bind to its cognate REs and activate transcription of target genes [6,33,39,60,84,116]. Class II receptors all bind REs consisting of two direct hexanucleotide repeats spaced by 1–5 bp (DR1–5 elements) as obligate heterodimers with retinoid X receptor (RXR). This group of receptors includes endocrine receptors (e.g., thyroid hormone receptors (TRs) and retinoic acid receptors (RARs)) that are activated by hormonal ligands. These receptors are predominantly found in the nucleus even in the absence of ligand [7,90,162], where they associate with corepressors at REs resulting in repression of transcription [20,24,59,61]. Upon ligand binding, a conformational change favors displacement of corepressor complexes and recruitment of coactivator complexes resulting in transcriptional activation of target genes [24,79,110]. Class II NRs also contains adopted orphan receptors such as the peroxisome proliferator-activated receptors (PPARs) and liver X receptors (LXRs). These receptors are found primarily in the nucleus [7,15,90,117], where they are activated by a multitude of different lipophilic metabolites ranging from abundant low-affinity ligands to less abundant high affinity ligands. Thus, this class of receptors functions as metabolic sensors regulating gene programs according to the metabolic status of the cell. Class III NRs (e.g., estrogen receptor-related receptors (ERRs), steroidogenic factor 1 (SF1), and Rev-Erb) bind either as monomers to hexanucleotide sequences or as dimers to composite elements (e.g., DRs). This group consists mostly of orphan receptors for which no endogenous ligand has yet been identified and these receptors have thus not received as much attention as the endocrine and adopted orphan receptors [42].

NRs have a common structural organization consisting of four distinct domains (Fig. 1). The N-terminal domain (NTD, A/B-domain)

☆ This article is part of a Special Issue entitled: Translating nuclear receptors from health to disease.

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doi:10.1016/j.bbadis.2010.12.018

is highly unstructured and contains a constitutive ligand-independent transactivation function, referred to as activation function 1 (AF1). The central part of NRs is the highly conserved DNA binding domain (DBD, C-domain). Following the DBD, a hinge region (D-domain) confers spatial flexibility to the receptors and links the DBD with the second most conserved region of NRs: The ligand binding domain (LBD, E-domain), which contains the ligand-dependent activation function 2 (AF2). In the following we will first review the impact of each of these domains on gene and cell type-specific activation of target genes and then discuss recent findings highlighting the importance of NR crosstalk with other TFs.

2. The DBD and C-terminal extension (CTE) and target site specificity

The core DBD (Fig. 2) consists of approximately 66 residues and is by far the most conserved domain of NRs. The hallmark of the DBD is two highly conserved zinc-finger motifs (Zn-I and Zn-II), in which a zinc ion is tetrahedrally coordinated by four highly conserved cysteine residues [3,35]. The DBD folds into two perpendicular α-helices that constitute the core structure of the DBD. The first α-helix (the recognition helix) forms between the two zinc-finger motifs and interacts directly with the major groove of RE half sites in a DNA sequence-dependent manner [50]. The DNA sequence specificity of NRs was first established for ER and GR [72,109,131,132,144,150]. However, it was soon discovered that several different NRs selectively bind the same DNA sequences [151], raising the question how different NRs can regulate distinct sets of target genes through binding of highly similar REs. The residues critical for sequence-specific DNA binding within the first α-helix are termed the P-box [149]. The second α-helix extends beyond Zn-II and is packed against the first α-helix in a perpendicular manner, which stabilizes the DBD structure and confers non-specific backbone interactions with DNA; however, this second α-helix does not make base-specific contacts with RE half site nucleotides. The second zinc-finger (Zn-II) contains the D-box, which for some NRs is involved in dimerization [160,161].

In addition to the core DBD the C-terminal extension (CTE) constitutes an important determinant of sequence-specific DNA binding for many NRs. The CTE is comprised of the first 10–30 residues following the fully conserved 'GM' (G, glycine; M, methionine) sequence of the core DBD and is, unlike the core DBD, highly variable between the different NRs (Fig. 2). Many NR CTEs have arginine-rich motifs that expand the NR-DNA interaction surface beyond the core RE by minor groove interactions with spacer and/or flanking DNA sequences [19,38,85,94]. Such interactions are highly dependent on the width of the minor groove DNA, which is determined by the DNA sequence [126]. This indicates that the DNA sequences flanking REs contribute to selective DNA binding of a subset of NRs.

Class I NRs bind as homodimers to REs consisting of two hexanucleotide half sites, organized as palindromic or inverted palindromic repeats, separated by an invariant 3 bp spacer. ERs preferentially bind the hexanucleotide half site consensus sequence 5'-AGGTCA-3', whereas the consensus halfsite sequence of all other class I receptors is 5'-AGAACA-3'. Moreover, AR has been shown to bind REs organized as direct repeats [133], which confers AR-specific regulation of target genes in vivo [46,47,130]. The ERs are also functionally unique among the class I NRs, as they are able to function not only as homodimers, but also as ERα/ERβ heterodimers [112]. It has been shown that ER heterodimers are functionally distinct from ER homodimers in their ability to bind REs and to activate transcription of a subset of target genes in vivo [106]. Besides the

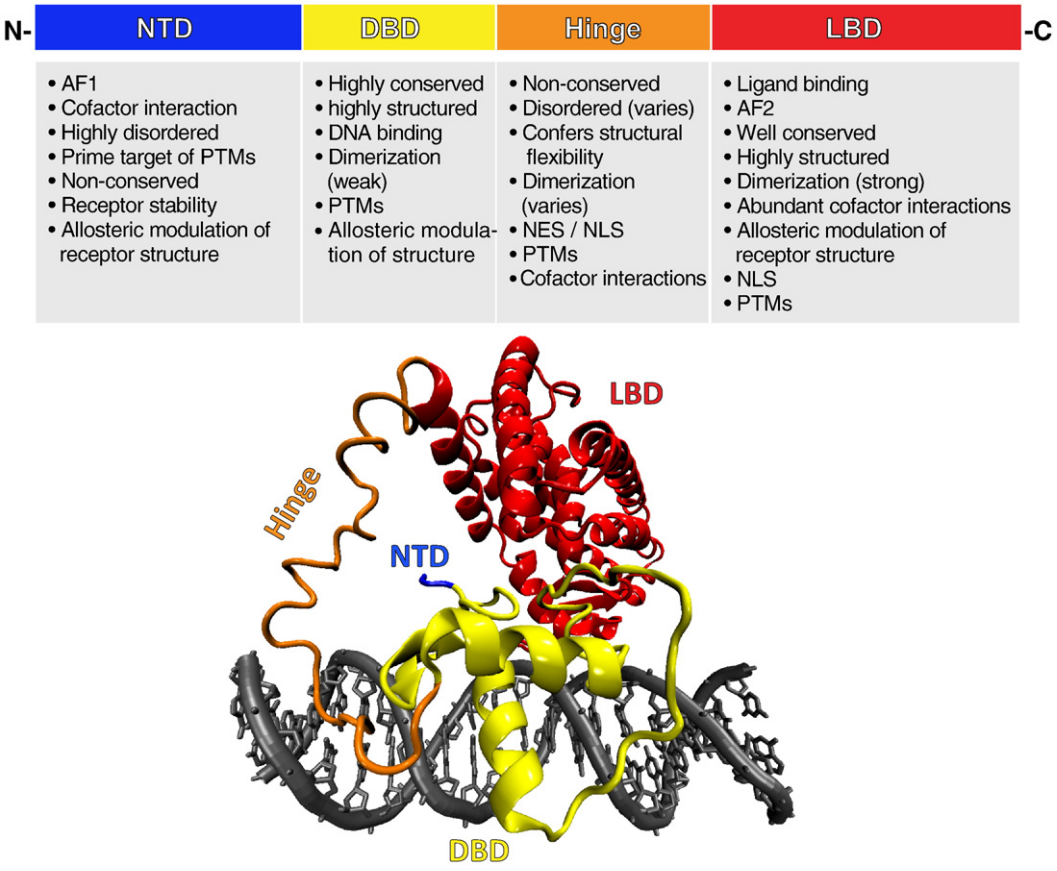

Fig. 1. General structural and functional organization of NRs. Top: Schematic outline of the general structural organization of NRs. The NTD, DBD, hinge and LBD contribute differently to receptor function. Functions that map to the individual domains are summarized as bulletpoints. Not all functions apply to all NRs. Bottom: Structure of the DNA-bound PPARγ DBD, hinge and LBD. The DBD and LBD are structurally highly conserved among NRs, whereas the NTD and hinge region lacks structural and sequence conservation. See text for details. NLS; nuclear localization signal, NES; nuclear export signal, AF; activation function, PTM; posttranslational modification. [PDB ID; 3dzy].

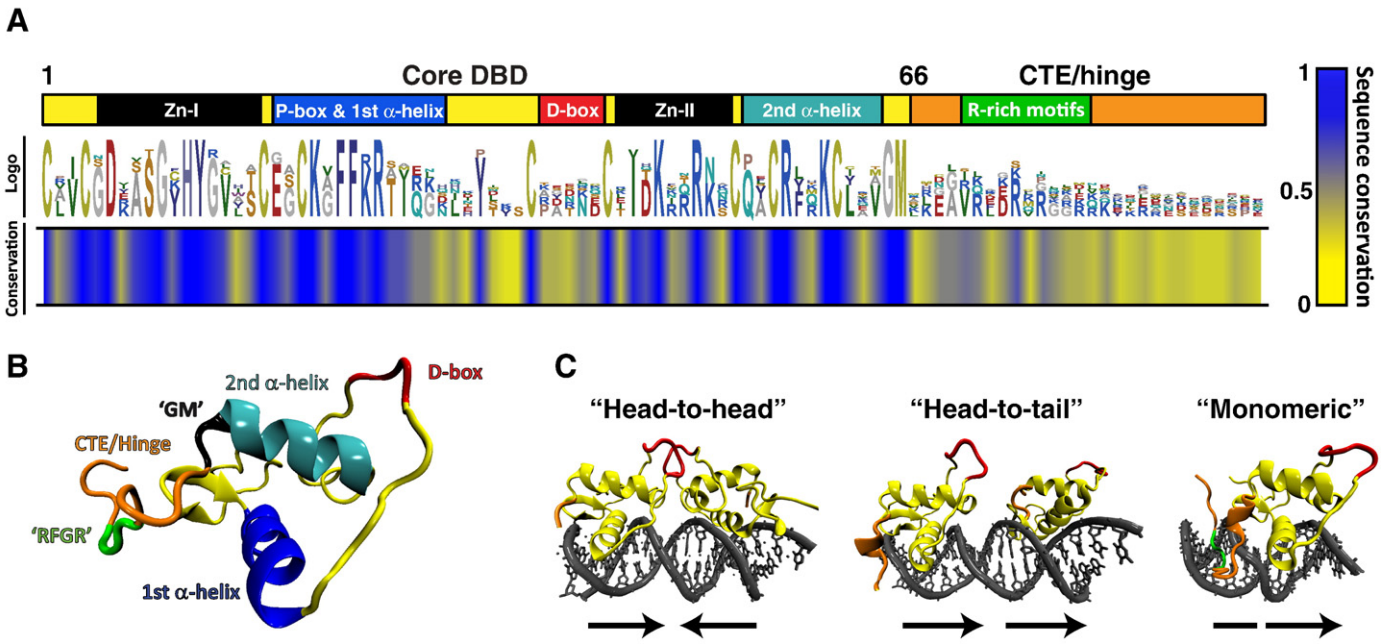

Fig. 2. Organization of the DNA binding domain (DBD) of NRs. (A) Schematic representation of the DBD functional motifs involved in DNA binding and dimerization. The core DBD is highly conserved, whereas conservation is not maintained in the CTE/hinge region. (B) Structural organization of NR core DBDs with location of functional motifs shown. (C) Different classes of NRs preferentially bind DNA as homodimers in a head-to-head fashion (class I NRs), as heterodimers in a head-to-tail fashion (class II NRs) or as monomers (class III NRs). [PDB ID: (B) 3dzy; (C) 1ynw, 1lo1, 1hcq].

apparent AR-specific ability to bind REs organized as direct repeats, an intriguing question is how the different homodimers selectively bind highly similar REs. As mentioned above, the P-box sequence of the recognition helix confers some NR selectivity towards specific REs. For instance, changing a single residue in the P-box sequence of ERs to that of the GR P-box changes the RE selectivity of ER towards GR REs and vice versa [163]. However, this does not explain the differential ability of, e.g., ERα and ERβ to bind REs in vivo [86], because the residues that make base-specific interactions with RE halfsites are fully conserved between the two subtypes.

The stronger DNA binding conferred by ERα has been suggested to be a result of an 'RGGR' motif known as a 'GRIP-Box' located in the CTE of ERα, but not ERβ [94]. This and similar arginine-rich motifs in other NRs map to the same region of the CTE (Fig. 2) and interact directly with the minor groove of DNA upstream from the RE halfsite [38,85]. Such minor groove DNA interactions contribute to, and in some cases are even required for, functional DNA binding by these NRs. Similar regions of, e.g., AR have been shown in vitro to be highly important for DNA binding [130], albeit structural data confirming direct interaction with DNA are lacking. Recently, Tanner et al. identified a region of only five residues (RKLKK) in the CTE of AR which is important for multiple functions, including nuclear localization, DNA binding, receptor protein stability, and transactivation potential [147]. Interestingly, this RKLKK motif maps to the same region of the CTE as the RGGR and RFGR motifs that has been shown for other NRs to interact with minor groove DNA flanking the RE. This suggests that RKLKK may constitute an alternative motif able to interact with minor groove DNA, thereby contributing to AR-selective binding of REs.

The P-box sequence is also fully conserved between AR, GR, MR, and PRs, suggesting that selective binding of these NRs to REs is not conferred by this region of their DBDs. Instead it is becoming increasingly clear that the non-conserved CTE contributes to selective binding of these NRs to REs. PR, MR and GR contain a highly similar RKXXK motif at the same position in the CTE, which could possibly contribute to multiple functions of these NRs in a similar manner as shown for AR. For instance, it has been shown for PR that at least part of this motif directly interacts with minor groove DNA flanking the RE and that this interaction is important for PR binding to REs [125]. Similarly, the RKXXK motif found at the same position in GR has been shown to be directly responsible for interaction with the cofactor Bcl-2 associated Athanogene 1M (Bag-1M), a cofactor reported to reduce GR binding to DNA, and to be involved in recruitment of corepressors such as NCoR and SMRT thereby attenuating GR transactivation potential [57,58]. Moreover, it has been demonstrated that acetylation of a cluster of lysine residues directly flanking this RKXXK motif reduces the ability of GR to bind REs [101]. Taken together, current data suggest that the non-conserved CTEs of class I NRs contribute to selective RE binding directly (e.g., by interaction with minor groove), and indirectly (e.g., by posttranslational modifications (PTMs) and cofactor recruitment that in turn modulates the RE binding properties of the NRs).

Class II NRs bind REs as obligate heterodimers with RXR, and these heterodimers recognize REs organized as two direct repeats of the 5'-AGGTCA-3' consensus half site sequence spaced by 1–5 bp (DR1–5 elements) [119], reviewed in ref. [118]. Generally, different heterodimerizing receptors selectively bind REs with a specific spacing between halfsites. This selectivity of NRs towards one particular type of RE is due to the requirement of extensive protein–protein interactions between the heterodimerizing partners for stable DNA binding. Thus, adding or removing a single base pair in the spacer (e.g., changing from a DR1 to a DR2 element) displaces the heterodimerizing partners by a distance and rotation of approximately 3.4 Å and 35°, respectively, which is incompatible with the protein–protein interactions required for heterodimerization. Consequently, the spacing between half sites is a key determinant of high affinity binding of heterodimers to specific REs.

Most heterodimers bind REs with RXR occupying the 5'-halfsite and the dimerization partner occupying the 3'-halfsite [160,161]. In these cases, the CTE of the dimerization partner will be located at the center of the dimeric complex, due to the head-to-tail configuration of the heterodimer and will contribute to selective binding to REs with the correct spacing, while preventing binding to REs with incorrect spacing, e.g., as shown for the RXR:TR heterodimer [119]. In contrast,

the PPARs and RARs bind DR1 elements with the opposite polarity, which positions the CTE in the periphery of the heterodimer in close proximity to the 5'-extension sequence. The recently solved crystal structure of the PPARγ:RXRα heterodimer complexed to a consensus PPAR RE, clearly shows that the CTE of PPARγ is deeply buried in the DNA minor groove directly upstream of the core DR1 [19]. Interestingly, the region of the PPAR CTE buried in the minor groove ('RFGR') closely resembles the GRIP-box sequence shown to be important for selective RE binding by many NRs, including ERα (described above), and class III NRs (see below).

While most heterodimers are restricted to binding REs with a specific spacer length and polarity, the RXR:RARα heterodimer has been shown to bind both DR1 and DR5 elements with RARα occupying the 5'- and 3'-halfsite, respectively. Intriguingly, shifting the polarity by binding DR1 rather than DR5 elements has been reported to shift the activity of the RARα-RXR heterodimer from an activator to a repressor of RAR target genes, due to differences in the ability of the two types of RARα-RXR heterodimer to recruit coactivators and corepressors [77].

Class III NRs preferentially bind as monomers to hexameric 5'-AGGTCA-3' REs. However, stable monomeric binding requires interaction between the CTE and a trinucleotide sequence flanking the hexameric RE (5'-trinucleotide). The CTE of, e.g., SF1 and the ERRs harbors a 'GRIP-box', similar to ERα, which interacts with minor groove DNA of the 5'-trinucleotide, thereby extending the protein–DNA interaction surface beyond the core hexanucleotide RE [85]. Moreover, the sequence of this 5'-trinucleotide has also been shown to be important in other aspects of class III NR function. For instance, the consensus 5'-trinucleotide sequence of the ERRs is 5'-TNA-3', with N being any nucleotide. However, the nature of the N nucleotide has been shown to dictate whether ERRα binds REs preferentially as a monomer (C/G at the N position) or as a homodimer (A/T at the N position) [5]. Furthermore, it has been reported that ERRα only recruits specific cofactors, such as PGC-1α, as a homodimer [4,5]. This indicates that the sequence of the 5'-trinucleotide sequence may directly dictate the mode of binding and functional outcome of at least a subset of class III NRs. In contrast to ERRα, both ERRβ and SF1 bind REs almost exclusively as monomers. Following RE binding the CTE of ERRβ and SF1 has been shown to loop back on itself and interact with a specific surface of the core DBD, which has been suggested to 'lock' the conformation of the DNA bound receptor thereby contributing to stable monomeric DNA binding [38,85].

While direct NR-DNA interactions, with either minor or major groove DNA, are important determinants of selective RE binding for all three classes of NRs, it is becoming clear that local structural features of the DNA helix are also important in this regard (Fig. 3). Recent reports suggest that such local structural features of REs and surrounding DNA sequences may constitute an additional level of DNA binding specificity by NRs and other TFs, by forming local DNA structures that selectively favors binding by specific NRs or NR subfamilies. In other words, RE sequences not expected to form direct interactions with NRs may still be important for selective NR binding and function. For instance, a recent genome-wide investigation of ERα binding to REs revealed that the sequence of the 3 nucleotide spacer is relatively conserved, and that the spacer sequence modulates both the DNA binding and transactivation potential of ERα [137], albeit no direct interaction of ERα with the ERE spacer sequences has been reported. Similarly, the conformation and flexibility conferred by the spacer sequence has for other DNA binding TFs been shown to be highly important for productive high affinity binding [143].

3. The hinge region and NR specificity

The hinge region was until recently thought to serve primarily as a flexible linker that allowed the DBD and LBD to adopt different orientations relative to each other to facilitate RE binding and NR dimerization under different conditions. However, numerous studies have now shown that the hinge of many NRs contributes markedly to receptor function through various molecular mechanisms. Because the hinge region is poorly conserved between NRs (even among subtypes of the same NR), the hinge very likely contributes to cell type, NR subtype- and gene-specific functions of at least a subset of NRs.

The hinge region of many NRs including PPARs [104], AR [64] and GR [58] has been shown to provide interaction surfaces for cofactors, which modulate the transactivation potential of these NRs. Furthermore, multiple PTM sites implicated in several aspects of NR function, including modulation of cofactor recruitment and RE affinity, have been identified in the hinge region of many NRs. A recent study established that SUMOylation of a specific lysine in the PPARα hinge modulates transcriptional activation in a gene-dependent manner [115]. It was furthermore shown that this differential effect on target gene transcription is due at least in part to increased recruitment of the corepressor NCoR in response to SUMOylation. Thus, this study

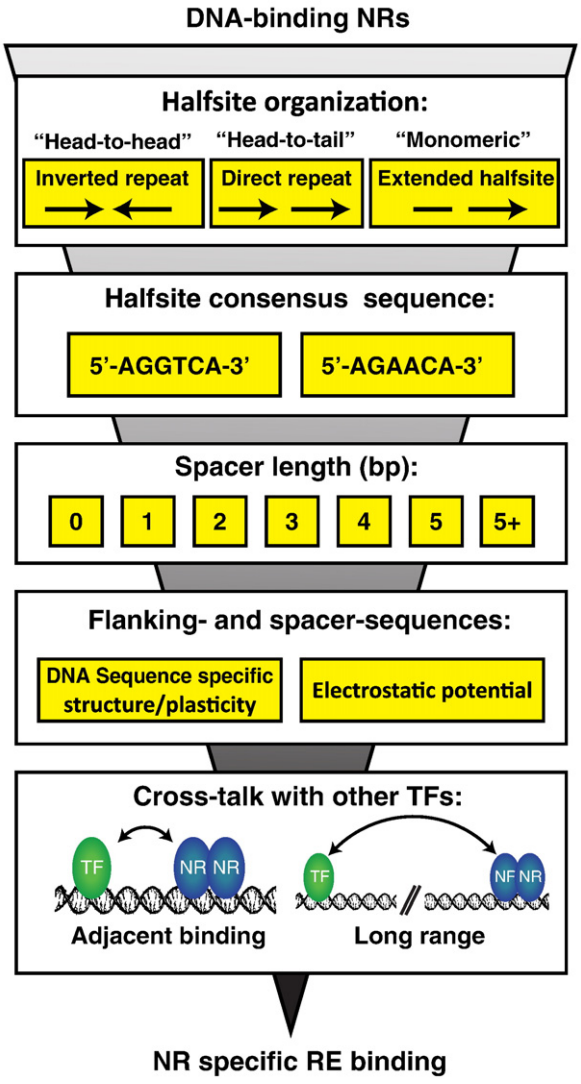

Fig. 3. NRs gain selective RE binding by exploiting multiple sequence and structural features of DNA. Most NRs bind REs with a specific halfsite organization, consensus sequence, and spacer length. Additional RE binding selectivity can be obtained through recognition of structural features of minor groove DNA of flanking and/or spacer sequences. Finally, crosstalk with other TFs contributes to NR-specific binding in a RE-dependent manner.

indicates that expression of some target genes is repressed by NCoR, whereas others are not.

4. The ligand binding domain (LBD)

Following the discovery of the RXRα LBD crystal structure [13], the LBD structure of most NRs has been solved, all sharing a very similar overall architecture. The LBD consists of 11–13 α-helices, and 2–4 β-sheets that adopt the highly conserved tertiary structure of the NR LBD. With almost no variation between different NRs, the LBD folds into a globular domain often described as a '3-layer antiparallel α-helical sandwich'. Activation of NRs by agonist binding involves repositioning of the extreme C-terminal H12 (also referred to as the AF2 helix) of the LBD relative to the rest of the LBD, thereby switching the NR from an 'off' to an 'on' state [14,122,152] by forming a charge-clamped hydrophobic cavity that specifically accommodates a general structural motif present in most coactivators (Fig. 4). This motif has the consensus sequence LXXLL (L, leucine; X, any residue) [26,52], which adopts a two-turn α-helix that binds a cavity formed by specific LBD helices including H3 and the AF2 helix. In response to antagonist binding (or absence of agonist for some NRs) the AF2 helix is repositioned to form an extended binding surface that is selectively recognized by a structural motif, LXXXIXXX(L/I), referred to as a corepressor nuclear receptor box (CoRNR box) present in common corepressors [61]. The binding surfaces of CoRNR boxes and coactivator LXXLL motifs partially overlap, indicating that coactivator and corepressor binding are mutually exclusive. Moreover, the binding surface of CoRNR boxes extends into regions that are usually occupied by the AF2 helix in the active conformation, which supports that corepressor binding (through CoRNR boxes) cannot occur when the LBD is in its active state (Fig. 4) [111,113,129]. In addition, two recent reports revealed that RARs and Rev-Erbs can also form an antiparallel β-sheet with corepressors through a specific corepressor interaction motif in H11 [79,113]. Agonist binding to RARs induces a β-strand to α-helix transition of H11 that provokes corepressor release and facilitates coactivator binding [79]. Besides serving as a prime docking site for cofactors, the LBD is involved in other mechanisms of NR function. The major NR-NR dimerization surface, which for all NRs that function as dimers is imperative to receptor function, resides in the LBD. Moreover, ligand binding has been shown to promote NTD-LBD interactions (e.g., as described for ERα and AR), DNA binding, and NTD folding, for some NRs. In the context of gene-specific transcriptional activation, it has been shown that different types of agonists lead to activation of distinct sets of target genes most likely through differential cofactor recruitment e.g., as shown for ERβ [107]. However, whether the LBD directly contributes to gene-specific transcriptional activation through mechanisms independent of the nature of the ligand bound remains to be shown.

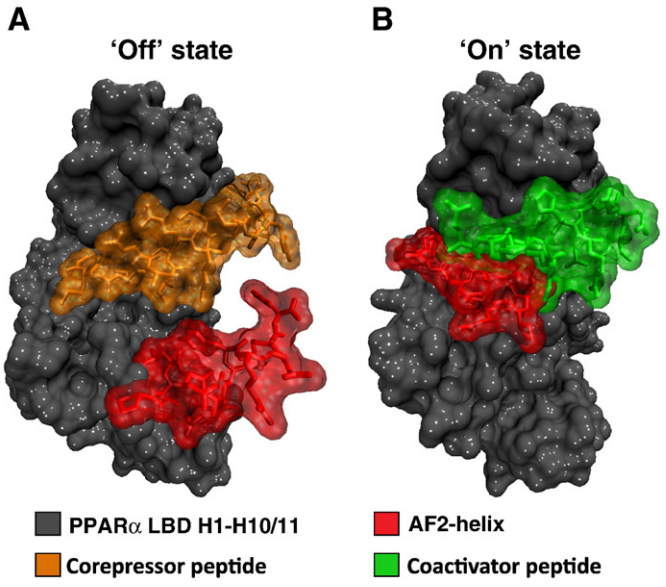

Fig. 4. The AF2-helix is repositioned in response to the nature of the ligand bound. (A) In the presence of antagonist, the position of the AF2-helix allows binding of corepressors through extended CoRNR-box sequences. (B) Binding of agonist switches the AF2-helix into a position that favors binding of coactivators. Moreover, in this position the AF2-helix occupies part of the extended CoRNR-box binding surface and thereby disfavors binding of corepressors. [PDB ID; (A) 1kkq, (B) 1k7l].

5. The NTD confers gene-specific transactivation through receptor-specific protein interactions

The NTD harbors the ligand-independent activation function 1 (AF1) [2,25,45,56,124] and is thought to be highly unfolded in solution but to acquire higher order secondary structure as a result of both inter- and intra-molecular interactions. In vitro studies indicate that direct interaction with specific protein partners induces folding of the NTD of, e.g., AR, ERα and GR [75,76,156]. In general, induced folding of the NTD correlates positively with activation potential of the receptor. Interestingly, the NTD structure of several NRs has been shown to be modulated by allosteric communication as a result of, e.g., DNA binding, ligand binding or protein–protein interactions conferred by other domains in the receptors. For instance, the PR DBD and CTE serve as an interaction surface for specific cofactors, such as Jun dimerization protein 2 (JDP2) [55], which has been shown to promote induced folding of the NTD and stimulate activity of the AF1, thus contributing to PR activity [154,155]. This has led to a model where induced folding (i.e., a general disorder-to-order transition) occurs as a result of interaction with other proteins or DNA (Fig. 5). This model, referred to as the 'induced fit' model, implies that the unfolded NTD can adopt different conformations according to the nature of the binding partner. Thus, the observation that the transactivation profile of a given NR may differ significantly depending on the cellular context may at least in part be explained by differential expression of interaction partners able to modify the structure – and thus the transactivation potential – conferred by the NTD.

The NTD almost completely lacks conservation, even within subfamilies of NRs, such as PPARs (PPARα, PPARβ, PPARγ), ERs (ERα and ERβ) or ERRs (ERRα, ERRβ and ERRγ), which suggests that the functional roles of the NTD may be highly receptor-specific. For instance, the ERα NTD has been shown to confer strong ligand-independent transactivation potential in, e.g., reporter assays, whereas the ERβ NTD under the same conditions does not [27]. Moreover, it has been shown that the ERα NTD is able to engage in interdomain interactions with its own LBD (NTD-LBD interaction, N/C interaction), whereas no such NTD-LBD interaction has been demonstrated for ERβ yet. This type of interdomain communication involving the NTD modulates the transactivation potential of, e.g., ERα by promoting recruitment of specific coactivators such as SRC-1 [96]. Similar functional N/C interactions have been described for AR [9,78], and PR [30]. Studies using NR NTD chimeras and deletion mutants, in which the NTD is either deleted or swapped between NRs demonstrate that the NTD – at least for a subset of NRs – contributes to gene-specific activation of target genes. For instance, swapping the NTD between PPARα and PPARγ revealed that the NTD can act both as an activator or repressor of transactivation in a gene-specific manner [15,62]. Similarly, the use of AR/GR chimeras revealed that the AR NTD is the sole determinant of AR-specific activation via an intragenic RE in the AR gene known to be specifically activated by AR but not GR [44]. This study illustrates that the NTD contributes significantly to gene-specific transactivation by AR. Using a similar strategy, chimeras in which the NTD was swapped between ERα and ERβ revealed that the NTD is an important determinant of ligand-specific transcriptional

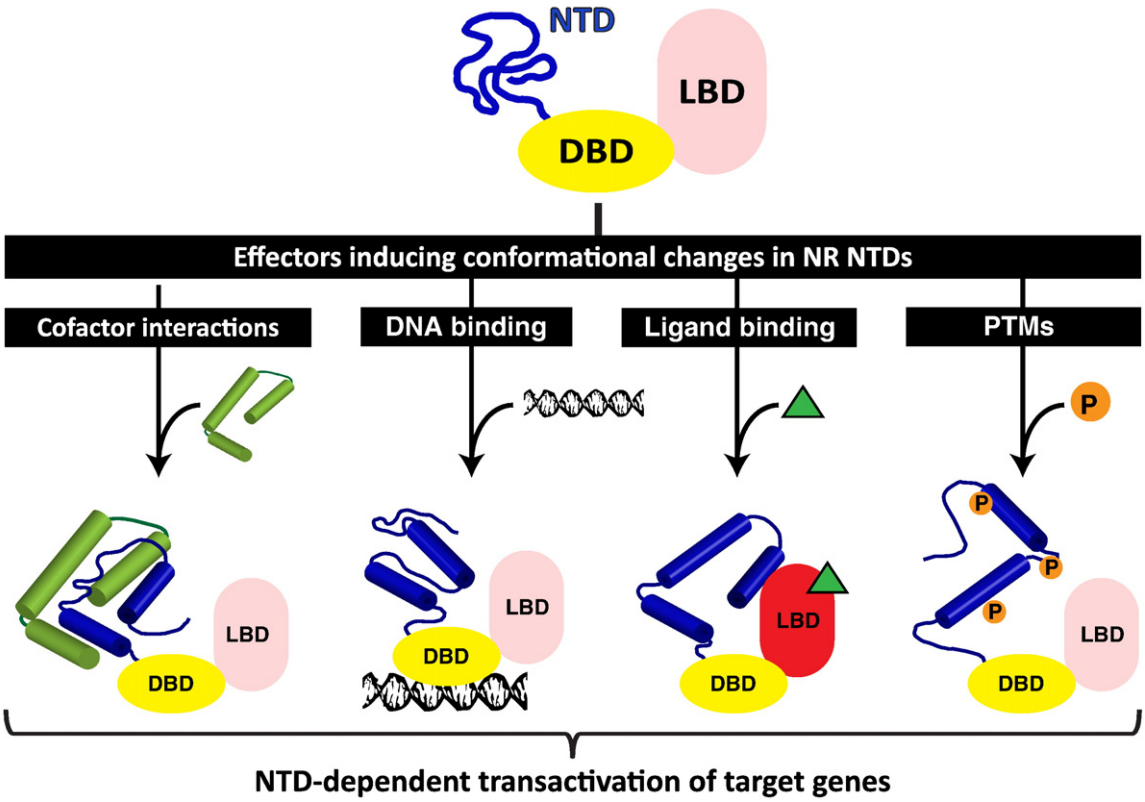

Fig. 5. The non-conserved NTD is disordered in solution but acquires high order structure in response to different cellular effectors, including cofactor interactions, DNA binding, ligand binding to the LBD, and post translational modifications (PTMs). The NTD is thought to adopt a multitude of conformations in response to different effector cues, which ultimately translates into NR-specific NTD-dependent transactivation of target genes.

activation [92]. Moreover it has been shown that activation of the ERβ-selective target gene TGFβ-inducible early gene-1 (TIEG1) requires the ERβ NTD, as deletion of the ERβ NTD or swapping the ERα NTD into ERβ abolished activation of TIEG1 expression [51]. In this study, it was further shown that both ERα and ERβ are able to bind intronic REs controlling TIEG1 expression, but recruitment of specific coactivators (SRC-1 and SRC-2) to this region required the presence of the ERβ NTD. Interestingly, the ERα NTD has in other studies been shown to interact with both SRC-1 and SRC-2 in vitro [96,157] and to potentiate SRC-1 recruitment to agonist-bound ERα in vivo. This illustrates that the role of specific NR domains in gene-specific transactivation and recruitment of cofactors is dependent on the cellular context.

A role of the NTD in NR subtype- and gene-specific functions has been shown for the PRs (PR-A and PR-B), which are identical except that PR-B contains an additional 164 amino acids in the NTD compared to PR-A. PR-B has a much stronger activation potential than PR-A in reporter assays, and it has been shown that PR-A and PR-B regulate distinct sets of target genes under identical conditions in vivo [123], which is at least in part due to differential ability to recruit cofactors [41]. In this context, it has been suggested that following DNA binding, the unique NTD of PR-B promotes NTD-dependent stabilization of receptor structure as a result of intramolecular interactions between the PR-B NTD and both the DBD (when bound to DNA), and the LBD in response to ligand binding [146], which facilitates recruitment and assembly of transcriptionally competent complexes to PR target genes (Fig. 5).

The structure and function of NR NTDs are also affected by PTMs, which may also contribute to gene-specific transcriptional activation (Fig. 5). Phosphorylation of the NTD of several NRs results in increased higher order secondary structure (e.g., increased α-helical content at the expense of disordered random coil), which positively correlates with the ability of NRs to recruit specific cofactors in an NTD-dependent manner. This was recently demonstrated for GR [37] and ERβ [148]. Moreover, phosphorylation of different sites in the GR NTD was recently reported to differentially affect the ability of GR to bind REs and activate transcription of GR target genes [11,21]. Whether such phosphorylations directly influence the properties of the DBD, or whether the effects on DNA binding is mediated via effects on interacting cofactors remains to be established. However, it is likely that phosphorylations of the NTD of other NRs similarly affect the ability of the respective NRs to bind to specific REs and activate target genes.

Phosphorylation of the NTD has also been shown to modulate other aspects of NR function, albeit it remains unclear whether these mechanisms contribute to gene-specific transactivation by NRs. For instance, phosphorylation of a specific residue in the PPARγ NTD was reported to reduce ligand-affinity of its LBD [135], thereby reducing its transactivation potential. Moreover, NRs are known to be modified by many other types of PTMs, including acetylation, ubiquitylation, methylation, and glycosylation but so far the role of these modifications in gene-specific transactivation remains elusive.

Additionally, SUMOylation of the AR NTD has been shown to correlate with reduced transactivation of target genes [114]. Interestingly, SUMO-specific proteases (SENPs), promote activity of AR by de-conjugation of SUMO in the AR NTD in an RE-dependent manner [68]. SENPs only de-SUMOylate (and thus activate) AR bound to composite REs, consisting of multiple (i.e., two or more) REs in close proximity of each other, whereas the activity of AR bound to single-copy REs is not potentiated by SENPs. The number and location of NR REs found in regulatory modules controlling specific genes varies considerably. In this context, the ability of SENPs to selectively modulate the activity of AR, when bound to regulatory modules containing multiple REs, strongly indicates that this represents another mechanism contributing to gene-specific activation of target genes by AR.

Taken together, the intrinsically unstructured nature of the unique NTD allows it to adopt a multitude of conformations, and to acquire higher order structure by PTMs, interaction with cofactors, and/or allosteric mechanisms (e.g., as a result of NTD PTMs or DNA or ligand binding to the DBD or LBD, respectively). Therefore, the highly receptor- and context-specific functions of the NTD indicate that this non-conserved domain is an important determinant of subtype-, cell type-, and gene-specific functions of many NRs.

6. Dynamic association between NRs and chromatin

Genome-wide studies of NR binding have revealed approximately 3,500-40,000 binding sites depending on the NR and the species under investigation [18,99,103,128]. Furthermore, NRs typically bind to composite elements separated by 1–5 nucleotides, which gives a core RE of 13–17 bp. As the human genome consists of approximately 3 billion base pairs, this suggests that the REs for a given NR comprise roughly 0.1‰ of the genome. The question is then, how do NRs locate their cognate REs in this sea of DNA sequences?

Several studies have now demonstrated that the interactions between TFs including NRs and the chromatin template in living cells are highly dynamic [10,48]. Chromatin binding proteins such as TFs rapidly traverse the nucleus by energy-independent passive diffusion, where they transiently and non-specifically interact with the chromatin template [48,100]. This allows TFs such as NRs to rapidly scan the genome in three dimensions to locate their sparsely distributed binding sites. When the receptor encounters a cognate RE, it may form a productive interaction with the chromatin template, which is typically accompanied by local remodeling of the chromatin structure [54,65,158]. The formation of a productive binding event has previously been assumed to stably tether the TF to the chromatin template based on in vitro studies with naked DNA. Surprisingly however, GR binding to the mouse mammary tumor virus (MMTV) tandem array, containing 800 to 1200 GR binding sites, in living cells was also demonstrated to be highly dynamic in nature (residence time in the time scale of seconds) by fluorescence recovery after photobleaching (FRAP) analyses [93]. This highly dynamic association with chromatin embedded target sites has subsequently been demonstrated for several other TFs such as NF-κB [12], PR [121], ER [136], AR [73], and CUP1 [71] as well as cofactors such as Brahma (BRM) and Brahma related gene 1 (BRG1) [66]. Thus, despite a slight increase in residence time upon formation of a productive binding event [73,140], interactions between NRs and their target REs are highly dynamic. Interestingly, rapid dissociation of NRs from productive binding events in chromatin has been linked to ATP-dependent chromatin remodeling activities [34,102,121], suggesting that eviction of NRs from binding sites is an active process. This mechanism of active release may allow NRs to rapidly respond to changing levels in ligand and may thus be important for the physiological function of NRs. The proteasome system has also been shown to be important for the rapid exchange of GR at the MMTV array, suggesting that proteasomal degradation of GR and/or GR-associated factors is also coupled to the eviction of this receptor from its target sites [140].

In addition to the rapid dynamics of NR association with chromatin described above, binding of some NRs to their target sites has also been shown to oscillate on a much larger time scale (in the order of minutes and hours). These oscillatory interactions between NRs and target REs were first demonstrated for ER [134] and have subsequently been shown to be accompanied by cycles of coordinated cofactor recruitment [97,134] as well as cycling of histone marks [97] and DNA methylation [70,95]. This suggests that the outcome of productive ER binding events (i.e., epigenetic marks and recruited cofactors) may feedback on the binding of ER itself to create this oscillatory pattern of binding. Cycling at target REs has since been reported for TR [87] and AR [69] suggesting that this may be a general mechanism of NR action at least for the endocrine receptors. The physiological function of this cycling behavior is not fully understood, but it may be important for eliciting the appropriate transcriptional response to changing hormone levels at specific gene promoters.

NR cycling at target sites may also stem from oscillations in hormone levels as has been described for GR. Glucocorticoids are released from the adrenal gland in an ultradian fashion [83] and this leads to oscillations in GR binding to target REs and GR target gene transcription [141]. The net result is a markedly different transcriptional response compared to constant levels of hormone [141], indicating an important physiological function of this oscillation pattern [29].

It is important to distinguish between the rapid exchange of NRs at REs (time scale of seconds, determined by FRAP in living cells) and the slower oscillations described above (time scale of minutes to hours, determined by chromatin immunoprecipitation (ChIP)). The former describes the residence time of NRs at target REs in a single cell and is thus influenced by the local cellular milieu including cofactor availability, chromatin structure etc. The latter describes the equilibrium of the association between the NR and the target RE averaged over a cell population. This oscillation pattern is the result of a complex interplay between hormone, cofactors, epigenetic marks, and the receptor that is still poorly defined.

7. DNA looping is a key aspect of NR transactivation

Hitherto the majority of the identified functional NR regulatory elements map relatively close to and upstream from the transcriptional start site (TSS). However, unbiased global analyses of NR binding have shown that even though NR binding is enriched at gene promoters relative to the rest of the genome, most NR binding sites are found distant from the TSS in introns and intergenic regions [17,81,103,139]. It remains an open question how many of the thousands of binding sites represent direct binding of NRs to DNA. Furthermore, it is unknown to what extent all these sites are involved in transcriptional regulation.

The large distance between most NR binding sites and target gene promoters necessitates studies on the three-dimensional structure of the genome to understand the molecular mechanisms of NR function. Recently, long-range looping between distant regulatory sites occupied by sequence- specific TFs including NRs and gene promoters has been demonstrated for selected gene loci using the chromosome conformation capture (3C) technology [16,40,49,82,105] and for the entire genome using chromatin interaction analysis by paired-end tag sequencing (ChIA-PET) [36]. Ruan and coworkers identified multiple intrachromosomal interactions between distant ERα binding sites and proximal gene promoters, but very few interchromosomal interactions [36]. The distal ERα binding sites involved in intrachromosomal interactions are enriched in RNAPII binding and genes in the proximity of these sites are significantly more activated by estradiol compared to genes in the proximity of non-interacting binding sites. This suggests that these long-range chromatin interactions are functionally important for ERα transactivation. These important data indicate that DNA looping that brings distant enhancers and proximal promoters together is an essential aspect of NR-induced activation of target genes.

8. Crosstalk with other TFs

Genomics studies have demonstrated an extensive overlap between the binding profiles of NRs and other TFs [17,81,89,103,142]. This suggests that NR action can be fine-tuned in a gene- and cell-specific manner by modulating the expression and activity of cooperating TFs (Fig. 3). The molecular mechanisms underlying this crosstalk can be divided into at least four categories as depicted in Fig. 6. In the following,

we will discuss these mechanisms and review the experimental evidence for each of them.

Recently, chromosome-wide profiles of ER binding revealed the enrichment of motifs for Forkhead TFs at ER binding sites [17]. ChIP assays revealed that FoxA1 actually occupied a subset of ER binding sites prior to stimulation with the ER agonist estradiol. Furthermore, these sites mapped to open chromatin regions and depletion of FoxA1 abolished estradiol-induced ER binding and target gene activation [17,31,89]. FoxA1 has also recently been shown to facilitate GR binding to the MMTV array through opening of the chromatin structure [8]. Together with the observation that FoxA1 can bind to histones and open compacted nucleosome arrays in vitro [23], this led to the concept that FoxA1 acts as a pioneering factor for NRs such as ER and GR by locating target sites in closed chromatin and remodeling the chromatin template, thereby assisting NR binding to target REs upon agonist stimulation (Fig. 6A). Pioneering factors such as FoxA1 thereby define the subset of potential REs that have an open chromatin structure and are prone to NR binding, which at least partly explains the discrepancy between the high number of potential REs in the genome and the relatively low number of experimentally identified binding sites [17]. Thus, pioneering factors assist in defining cell-specific binding profiles for NRs by modulating the chromatin landscape of the cell, thereby leading to a cell-specific response to NR activation [31,89].

Crosstalk between NRs and other TFs may also arise from adjacent binding to their respective REs as has been proposed as a mechanism of direct crosstalk between several NRs and cooperating TFs on chromatin, including PPARγ-CCAAT/enhancer binding protein α (C/EBPα), GR-C/EBPβ, and ER-RARα [53,81,103,127,142,153] (Fig. 6B). Alternatively, cooperative interactions may potentially also occur between NRs and TFs bound at distant sites through long-range chromosomal loops (Fig. 6C, D) as described above for enhancer–promoter interactions. Regardless of the relative genomic locations of the direct TF-DNA contacts, juxtaposition of two or more TFs at a given site may facilitate transcription by creating a cofactor interaction surface that results in a more efficient recruitment of coactivators (Fig. 6B–D). Alternatively, different TFs may contact and recruit distinct coactivator complexes [43,88] that work together to induce transcriptional activation. Instead of recruiting a common cofactor complex through simultaneous binding to different REs (Fig. 6B–D), cooperating TFs may also alternate in binding to the same or overlapping REs (Fig. 6E). The short residence time of TFs on individual REs limits competition for the common site and allows dynamic exchange of transcriptional complexes. Efficient binding of cooperating TFs to shared sites has in many cases been shown to be dependent on the presence of both TFs [22,53,127,142], suggesting that efficient recruitment of cofactor complexes by cooperating factors stabilizes TF binding. Alternatively, direct interactions between TFs may stabilize TF binding to DNA as has been reported for c-Myc and C/EBPβ [145]. Taken together, these findings suggest that the expression and activity of cooperating TFs regulate NR function in a gene- and cell-specific manner by directly targeting shared regulatory sites in the genome.

In addition to activating target gene transcription from their cognate REs, several NRs have been known for a long time to specifically repress inflammatory signaling through tethering to inflammatory TFs (e.g., Nuclear Factor-κB (NF-κB) and Activator Protein-1 (AP-1)) and recruitment of corepressors (Fig. 6F). Upon glucocorticoid binding, monomeric GR associates with AP-1 and inhibits transactivation of AP-1 target genes by stabilizing formation of a corepressor complex at target regulatory elements [67,120,159]. A similar mechanism has been described for repression of NF-κB signaling by agonist-bound PPARγ [108]. Interestingly, upon binding of agonist, the PPARγ LBD is SUMOylated, and this is required for transrepression, but not transactivation, by this receptor [63,108]. Thus, this SUMOylation has been suggested to constitute a molecular switch between transactivation and transrepression by PPARγ [108].

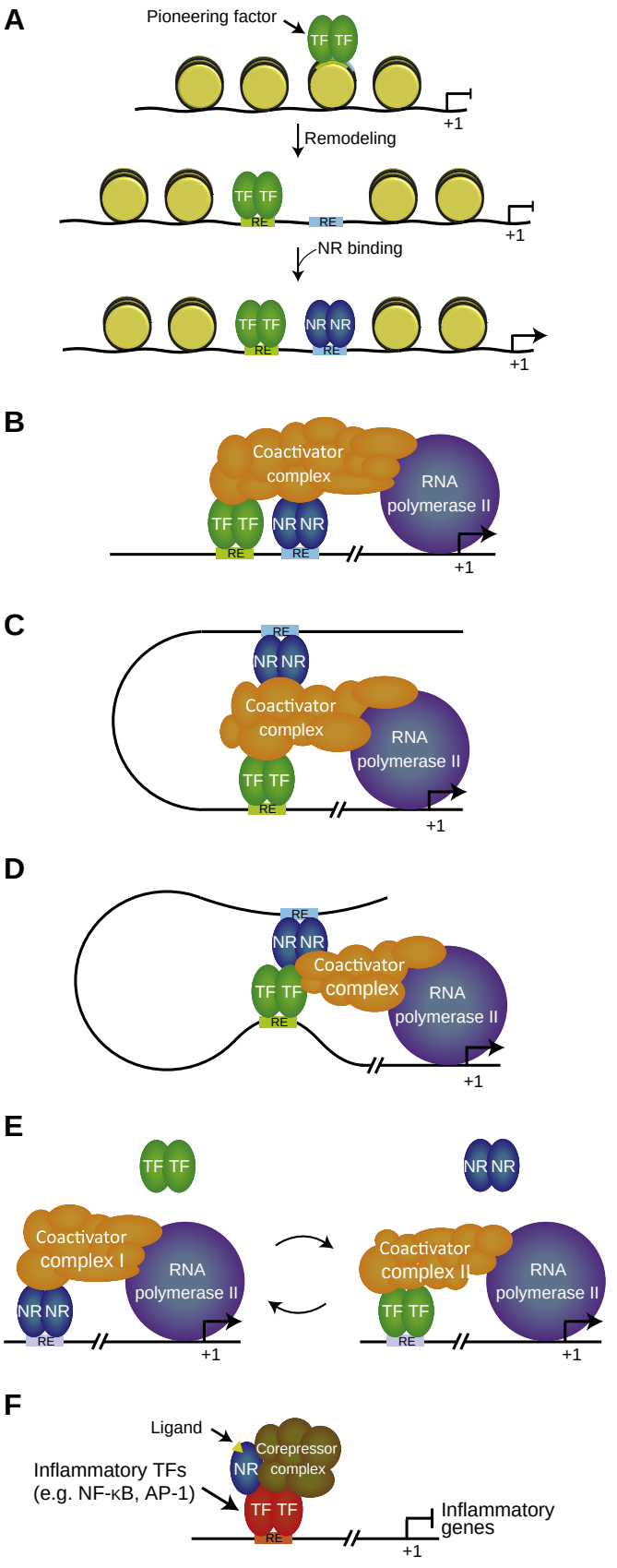

Fig. 6. Molecular mechanisms of crosstalk between NRs and other TFs. (A) Pioneering factors remodel the chromatin template prior to NR binding. (B–D) NR and cooperating TFs interact with different REs and cooperatively recruit coactivator complexes. (E) NRs and cooperating TFs interact with the same or overlapping REs resulting in dynamic exchange of transcriptional complexes. (F) Tethering of ligand-bound NR to inflammatory TFs.

Regardless of the molecular mechanism of crosstalk, the direct interplay between NRs and other TFs on the chromatin template described above allows for gene-specific regulation of NR-mediated transcription by other TFs. As more and more studies describing NR crosstalk with other TFs on a genome-wide level emerge, it is becoming increasingly clear that this is likely to represent an important general mechanism through which other signaling pathways can specifically fine-tune NR signaling in a gene-specific manner. Interestingly, clusters of up to five different TFs that co-occupy specific regulatory elements in the genome have been identified in embryonic stem cells, suggesting a convergence of multiple signaling pathways at specific genomic loci in these cells [22]. This indicates that entire networks of TFs can actually directly cooperate at specific genomic loci to fine-tune transcription of target genes. Furthermore, Glass and coworkers have recently demonstrated that cooperation between hematopoetic TF PU.1 and lineage-specific TFs in macrophages and B-cells results in cell-specific binding profiles of PU.1. Similarly, Lazar and colleagues have recently shown that PU.1 may assist in defining the macrophage-specific PPARγ binding profile, which is distinct from that in adipocytes [80]. This emphasizes the importance of direct crosstalk between TFs on chromatin for specifically regulating TF activity in a gene- and cell type-specific manner. Future studies should aim at extending findings of NR crosstalk with other TFs by identifying TF networks containing NRs that target specific sites in the genome. Furthermore, delineating the molecular mechanisms underlying crosstalk within these networks represents an important challenge as this may reveal a novel avenue for specific targeting of NR signaling by clinical compounds to treat NR-linked diseases such as metabolic diseases and cancer.

Concluding remarks

Gene-specific transactivation by NRs is governed by multiple layers of receptor-specific interactions. First, the non-conserved CTE of the DBD interacts specifically with the DNA sequences surrounding the core RE and thereby directly affects DNA binding for a subset of NRs. Second, the highly unstructured and non-conserved NTD has been shown to affect NR function in a gene-specific manner mainly through cofactor interactions. Finally, recent genome-wide binding profiles have revealed an extensive crosstalk between NRs and other sequence-specific TFs on the chromatin template, which is likely to represent an important mechanism through which NR function can be modulated by other transcriptional pathways in a gene-specific manner. The extent and mechanism of this crosstalk is only starting to emerge; however, it is likely to involve direct protein–protein interactions between TF complexes binding to adjacent as well as distal sequences. In addition, TFs may work together by binding sequentially to overlapping sites. A better understanding of this crosstalk is likely to reveal novel potential therapeutic targets for the treatment of diseases linked to NR malfunction.

Acknowledgements

The authors thank Dr. William Bourguet for valuable comments to the manuscript. The work was supported by grants from the Danish Natural Science Research Council and the Novo Nordisk Foundation.

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