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# Topic 1.1

# Nuclear receptor superfamily: Principles of signaling*

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*Abstract*: Nuclear receptors (NRs) comprise a family of 49 members that share a common structural organization and act as ligand-inducible transcription factors with major (patho)physiological impact. For some NRs ("orphan receptors"), cognate ligands have not yet been identified or may not exist. The principles of DNA recognition and ligand binding are well understood from both biochemical and crystal structure analyses. The 3D structures of several DNA-binding domains (DBDs), in complexes with a variety of cognate response elements, and multiple ligand-binding domains (LBDs), in the absence (apoLBD) and presence (holoLBD) of agonist, have been established and reveal canonical structural organization. Agonist binding induces a structural transition in the LBD whose most striking feature is the relocation of helix H12, which is required for establishing a coactivator complex, through interaction with members of the p160 family (SRC1, TIF2, AIB1) and/or the TRAP/DRIP complex. The p160-dependent coactivator complex is a multiprotein complex that comprises histone acetyltransferases (HATs), such as CBP, methyltransferases, such as CARM1, and other enzymes (SUMO ligase, etc.). The agonist-dependent recruitment of the HAT complex results in chromatin modification in the environment of the target gene promoters, which is requisite to, or may in some cases be sufficient for, transcription activation. In the absence of ligands, or in the presence of some antagonists, certain NRs are bound to distinct multiprotein complexes through the interaction with corepressors, such as NCoR and SMRT. Corepressor complexes comprise histone deacetylases (HDACs) that have the capacity to condense chromatin over target gene promoters. Ligands have been designed that selectively modulate the interaction between NRs and their coregulators. Both HATs and HDACs can also modify the acetylation status of nonhistone proteins, but the significance in the context of NR signaling is unclear. NRs communicate with other intracellular signaling pathways on a mutual basis, and their functionality may be altered, positively or negatively, by post-translational modification. The majority of NRs act as retinoid X receptor (RXR) heterodimers in which RXR cannot a priori respond autonomously to its cognate ligand to activate target gene transcription. This RXR subordination allows signaling pathway identity for the RXR partner. The corresponding mechanism is understood and reveals cell and NR selectivity, indicating that RXR can, under certain conditions, act autonomously. NRs are

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regulators of cell life and death, and NR malfunction can be at the basis of both disease and therapy, as is impressively documented in the case of acute promyelocytic leukemia. Recently, several pathways have been uncovered that link NR action with cell proliferation and apoptosis.

## GENERAL ORGANIZATION OF NUCLEAR RECEPTORS

### Nuclear receptors regulate intra- and intercellular communication in metazoans

Multicellular organisms require specific intercellular communication to properly organize the complex body plan during embryogenesis and maintain its properties and functions during the entire life span. While growth factors, neurotransmitters, and peptide hormones bind to membrane receptors, thereby inducing the activity of intracellular kinase cascades or the JAK-STAT/Smad signaling pathways, other small, hydrophobic signaling molecules such as steroid hormones, certain vitamins, and metabolic intermediates enter, or are generated within, the target cells and bind to cognate members of a large family of nuclear receptors (NRs) (Fig. 1). NRs are of major importance for metazoan intercellular signaling, as they converge different intra- and extracellular signals to initiate and regulate gene expression programs. They act as transcription factors that (i) respond directly through physical association with a large variety of hormonal and other regulatory, as well as metabolic signals, (ii) integrate diverse signaling pathways as they correspond themselves to targets of posttranslational modifications, and (iii) regulate the activities of other signaling cascades (commonly referred to as "signal transduction crosstalk"). The genetic programs that they modulate affect virtually all aspects of the life of a multicellular organism, covering such diverse aspects as, for example, embryogenesis, homeostasis and reproduction, or cell growth and death. Their gene regulatory power and selectivity has prompted intense research on these key factors, which is now starting to decipher a complex network of molecular events accounting for their transcription regulatory capacity. The study of these molecular processes has also shed light on multiple general principles underlying transcription regulation, and it will be a future challenge to uncover the molecular rules that define selective NR-dependent spatial and temporal control of gene expression.

### The family and its ligands

To date, 49 NRs [2,3] have been identified throughout the animal kingdom, ranking from nematodes to humans (Fig. 1). They constitute a family of transcription factors that share a modular structure of five to six conserved domains encoding specific functions [4,5]. The most prominent distinction to other transcription factors is their capacity to specifically bind small hydrophobic molecules. These ligands constitute regulatory signals, which, after binding, change the transcriptional activity of the corresponding NR. For some time, a distinction was made between classic NRs with known ligands and so-called "orphan" receptors, hence receptors without or with unknown ligand. However, recent years have seen the identification of ligands for many of these orphan receptors, making this distinction rather superficial (see, e.g., refs. [6,7–10]). Moreover, the classification of NRs into six to seven phylogenetic subfamilies with groups that comprise both orphan and nonorphan receptors further dismisses such discrimination [1]. The classification of NRs is done by virtue of the homology to other family members, with the DNA- and ligand-binding domains (DBDs and LBDs) having the highest evolutionary conservation.

Interestingly, some recently identified ligands for "orphan" NRs turned out to be metabolic intermediates. It appears therefore that in certain systems the control of built-up, break-down and storage of metabolic active substances is regulated at the level of gene expression, and that in many cases this "intracrine" signaling is brought about by NRs. Furthermore, gene knock-out experiments suggest that metabolic intermediates such as SF1 (NR5A1) or PPARγ (NR1C3) ligands may have regulatory func-

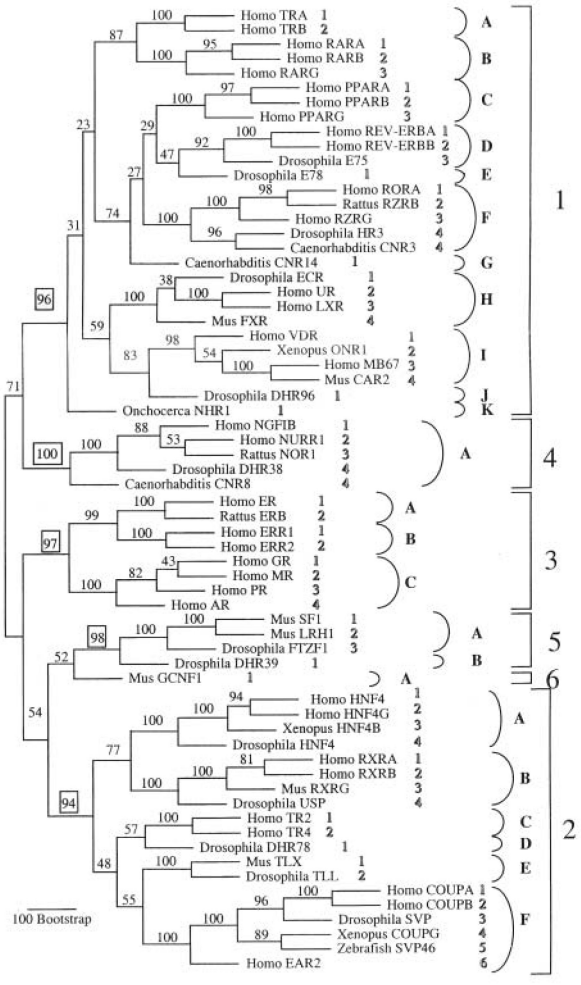


**Fig. 1** Phylogenetic tree of 65 nuclear receptor genes in vertebrates, arthropoids and nematodes. For a detailed description, see Nuclear Receptors Nomenclature Committee (1999) [1] and the regular updates at <http://www.ens-lyon.fr/LBMC/LAUDET/nomenc.html>.

tion in specifying organ development [11,12]. Prominent metabolic ligands are bile and fatty acids, eicosanoides, and oxysterols. The group of steroid hormones encompasses estrogens, progestins, mineralocorticoids, glucocorticoids, androgens, and ecdysterones. Examples for vitamin–derived NR ligands are vitamin $D_3$ (VDR; NR1I1) or the vitamin A derivative, retinoic acid (RARs and RXRs; NR1B and NR2B, respectively). Thus, NRs function in endocrine (steroid hormone receptors), auto/paracrine (retinoid receptors), and intracrine [metabolic receptors such as LXRα (NR1H3), SF-1 (NR5A1), FXR (NR1H4), PXR (NR1I2), PPARs (NR1C), CARβ (NR1I4)] signaling pathways.

## Genetics of nuclear receptors

Genetic programs consist typically of several hundred to thousand genes that are expressed in a spatially and temporally controlled fashion. Nuclear receptors act as master "switches" to initiate specific genetic programs that, for example, lead to cell differentiation, proliferation, or apoptosis or regulate homeostasis. In the context of other programs, these genetic activities support or initiate complex physiological

phenomena, such as reproduction and organ function. Once activated by the cognate ligand, NRs regulate the primary and secondary target gene expressions that make up the corresponding physiological event. Throughout the life cycle of a multicellular organism, the coordinate interplay between programs defining cell fates in different tissues, organs, and finally the entire body is at the foundation of the organisms' development and subsistence. This is fully supported by the analysis of mice bearing mutations or deletions of one or several receptors (a searchable mouse knock-out and mutation database with PubMed can be found at <http://www.biomednet.com/db/mkmd>). Several NR knock-out animals (in particular, compound knock-out animals) die in utero or around birth, displaying severe malformations of organs that render them inviable (for examples, see ref. [13] and refs. therein; for reviews on earlier work, see refs. [14,15]). Others are viable under laboratory conditions, but display a reduced life span and are often infertile (see, e.g., ref. [11]). These knock-out animal models have helped in deciphering the physiology of NR action. Often they provided initial or additional evidence for new, yet undiscovered functions, exerted by the receptor, and thus initiate further research on previously unknown signaling pathways. One example is the involvement of retinoic acid receptor, RARβ (NR1B3), and retinoid X receptor, RXRγ (NR2B3), in long-term memory potentiation and depression [16]. Furthermore, knock-outs have also provided insight into the distinct modes of transcriptional regulation by NRs. An elegant example is the mouse NR3C1 gene encoding the glucocorticoid receptor (GR). $GR^{-/-}$ mice die at birth due to respiratory failure, but replacement of the GR gene by the $GR_{A458T}$ mutant that impairs binding to consensus GR response elements generates mice (termed $GR^{dim/dim}$ with "dim" indicating DBD-mediated dimerization deficiency) that were fully viable [17]. These mice could be used to define direct (cognate response element-mediated) and indirect (e.g., signaling cross-talk-dependent) actions of GR (see Topic 1.5). The indirect actions of the glucocorticoid receptor concern also the transrepression of the activating protein AP1, a phenomenon further discussed below.

An interesting result of studies with NR gene deletion models has been the discovery of redundancy and adaptivity among family members of the same group. In this respect, the interpretation of retinoic acid signaling, which is of remarkable complexity and displays a high degree of apparent retinoic acid receptor redundancy, may serve as an example (Topic 1.6).

## Modular structure and function

### *N-terminal region A/B harbors cell-specific activation function(s) AF-1 of unknown structure*

As schematically depicted in Fig. 2, NRs are composed of five to six regions (A to F; originally defined by [18]) that have modular character. The N-terminal A/B region harbors one (or more) autonomous transcriptional activation function (AF-1), which, when linked to a heterologous DNA-binding domain, can activate transcription in a constitutive manner. Note, however, that in the context of the full-length receptor, AF-1 is silent in the absence of agonist and certain antagonists. When comparing NRs from different subfamilies and groups, the A/B region displays the weakest evolutionary conservation, and the distinction between A and B regions is not always evident. A/B regions differ significantly in their length, ranging from 23 (vitamin D receptor, NR1I1) to 550 (androgen, NR3C4, mineralocorticoid, NR3C2, and glucocorticoid receptors, NR3C1) amino acids. No 3D structure of a nuclear receptor A/B region has been solved up to now, and structure prediction is not straightforward. A/B regions are subject to alternative splicing and differential promoter usage, and the majority of known NR isoforms differ in their N-terminal region. Through alternative splicing and differential promoter usage (PR forms A + B), the absence or presence of different activation functions found in the AB regions can be regulated [for details, see ref. 5]. Moreover, the N-terminus of NRs has reportedly been found as subject of posttranslational events such as phosphorylation (discussed further below). Finally, the activation function(s) AF-1 display cell, DBD and promoter specificity [19–21], the origin of which is still elusive but may be related to the posttranslational modification [22] and cell-specific action and/or expression of AF-1 coactivators [23].

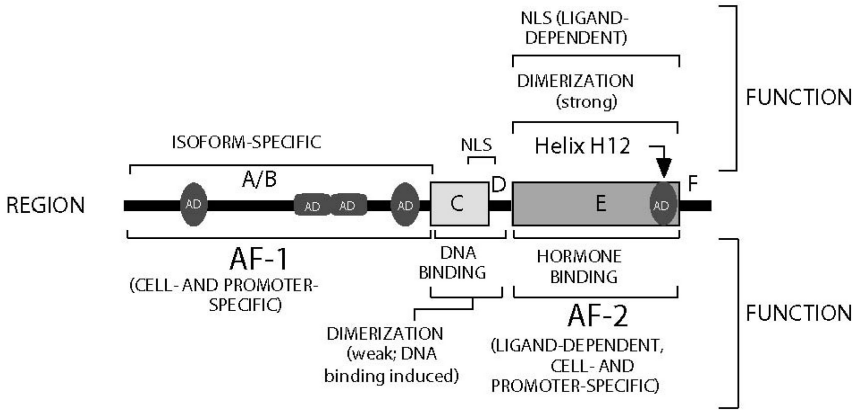


**Fig. 2** Schematic illustration of the structural and functional organization of NRs. The evolutionary conserved regions C and E are indicated as boxes and a black bar represents the divergent regions A/B, D, and F. Note that region F may be absent in some receptors. Domain functions are depicted below and above the scheme. Two transcription activation functions (AFs) have been described in several nuclear receptors, a constitutively active AF-1 in region A/B and a ligand-inducible AF-2 in region E. Within these activation functions, autonomous transactivation domains (ADs) have been defined in the estrogen (ER) and progesterone receptor (PR) N-terminal regions. In the case of the estrogen, retinoid and thyroid hormone receptors an autonomous activation domain (AF-2 AD) encompassing helix H12 has been detected at the C-terminal end of the ligand binding domain E.

### *DNA-binding domain encompasses region C*

The highly conserved domain C harbors the DBD of NRs, which confers sequence-specific DNA recognition. This domain has been extensively investigated, especially with respect to its selective response element recognition and dimerization properties (for details, see below). Several X-ray and NMR data sets are available for different NR C domains in their DNA complexed and uncomplexed forms (see Table 1 for PDB file names). The DBD is mainly composed of two zinc-finger motifs, the N-terminal motif Cys-X2-Cys-X13-Cys-X2-Cys (CI) and the C-terminal motif Cys-X5-Cys-X9-Cys-X2-Cys (CII); in each motif, two cysteine residues chelate one $Zn^{2+}$ ion. Within the C domain, several sequence elements (termed P-, D-, T- and A-boxes) have been characterized that define or contribute to (i) response element specificity, (ii) a dimerization interface within the DBD, and (iii) contacts with the DNA backbone and residues flanking the DNA core recognition sequence. Figure 3 illustrates the three prototypic DNA binding modes of NRs: the estrogen receptor DBD as an example of a homodimer that binds to a palindromic response element (Fig. 3a), the RXR-TR as an example of an anisotropic (5′-RXR-TR-3′) heterodimeric complex on a DR1 direct repeat (Fig 3b), and NGFI-B as an example of a monomer that binds to an extended hexameric motif, the so-called NBRE (Fig. 3c). Note that the so-called A- and T-boxes of TR and NGFI-B are involved in additional minor groove DNA contacts (see below for details).

**Table 1** List of presently reported 3D structures of NR DBDs together with their PDB assignments.

| PDB ID | Receptors | Response element |
|---|---|---|
| *Homodimers* | | |
| 1GLU | GR | GRE |
| 1LAT | GR mutant | non-cognate |
| 1HCQ | ER | ERE |
| 1A6Y | RevErb | DR2 |
| 1GA5 | RevErb | DR2 |
| 1HLZ | RevErb | DR2 |
| 1BY4 | RXR | DR1 |
| 1KB2 | VDR | Osteopontin |
| 1KB4 | VDR | DR3 |
| 1KB6 | VDR | Osteocalcin |
| *Heterodimers* | | |
| 2NLL | 5′-RXR-TR-3′ | DR4 |
| 1DSZ | 5′-RAR-RXR-3′ | DR1 |
| *Monomers* | | |
| 1CIT | NGFI-B | NBRE |
| *NMR structures* | | |
| 2GDA | GR | – |
| 1GDC | GR | – |
| 1RGD | GR | – |
| 1HCP | ER | – |
| 1RXR | RXR | – |
| 1HRA | RARβ | – |
| 1LO1 | ERR2 | – |

### *Region D, a hinge with compartmentalization functions*

The D region of NRs is less conserved than the surrounding regions C and E. This domain appears to correspond to a "hinge" between the highly structured C and E domains. It might allow the DBDs and LBDs to adopt several different conformations without creating steric hindrance problems. Region D contains a nuclear localization signal (NLS), or at least some elements of a functional NLS (see, e.g., ref. [24]). The intracellular localization of NRs is a result of a dynamic equilibrium between nuclear-cytoplasmatic and cytoplasmatic-nuclear shuttling [25]. At equilibrium, the large majority of NRs is nuclear, while some steroid receptors (androgen, glucocorticoid, and mineralocorticoid receptors) apparently reside at cytoplasmic locations in the absence of their cognate ligands and translocate to the nucleus in a ligand-induced fashion [26,27].

### *Region E encompasses the ligand-binding domain and activation function 2*

The hallmark of an NR is its LBD in the E region. This domain is highly structured and encodes a wealth of distinct functions, most of which operate in a ligand-dependent manner. The LBD harbors the ligand-dependant activation function AF-2, a major dimerization interface and often a repression function (for review and references, see ref. [5]). Detailed molecular insights into the structure-function relation of signal integration by NRs have been gained by the elucidation of the crystal structures of the E region alone or in presence of agonists, antagonists and coregulator peptides (see below and Table 2).

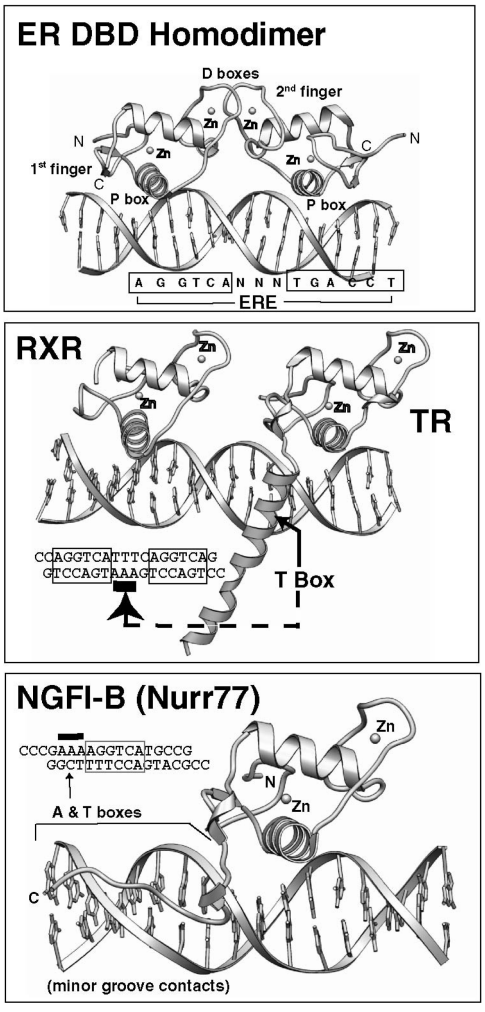


**Fig. 3** 3D structures of the three prototype DBDs of nuclear receptors obtained from X-ray crystal structure analyses of the DBD-DNA response element co-crystals. (a) Structure of the estrogen receptor α (ER) DBD on an ERE; one strand of the canonical ERE DNA sequence is given at the bottom. The various structural elements ($Zn^{++}$ fingers, D and P boxes) are indicated. Note that the D-boxes form a DNA-induced DBD dimerization interface, while the P-box α-helices establish the selective base contacts in the major groove. (b) Crystal structure of the 5′-RXR-TR-3′ heterodimer on a cognate direct repeat response element spaced by four base pairs (DR4), depicted as a double-stranded DNA sequence at the bottom left. Note that the T-box makes minor groove contacts, thus specifying to some degree the DR4 spacer nucleotide sequence (arrow). (c) Crystal structure of the monomeric NGFI-B on its response element (NBRE). The double-stranded NBRE sequence is given at the top left. Note that the A and T-boxes define the 5′ AAA sequence that contacts the minor groove .

**Table 2** List of all presently reported 3D structures of NR LBDs.

| Receptors | Ligands | Remarks | PDB ID |
|---|---|---|---|
| *Monomers* | | | |
| AR | R1881, DHT | agonists | 1e3g, 1i37 |
| AR (T877A) | DHT | agonist | 1i38 |
| AR (« ccr » mutant) | 9α-fluorocortisol | agonist | 1gs4 |
| DHR38 | no | holo conformation | 1pdu |
| FXR | 6-ethyl- and 3-deoxy-CDCA | NR box complexes | 1osv |
| | Fexaramine | agonist | 1osh |
| LRH-1 | no | holo conformation | 1pk5 |
| Nurr1 | no | holo conformation | 1ovl |
| PPARα | GW6471 | CoRNR box complex | 1kkq |
| | GW409544 | NR box complex | 1k7l |
| | AZ242 | agonist | 1i7g |
| PPARγ | no | – | 3prg |
| | YPA | agonist | 1knu |
| | AZ242 | agonist | 1i7i |
| | DRF | agonist | 1nyx |
| PPARδ | GW2433, EPA | agonists | 1gwx, 3gwx |
| | no | – | 2gwx |
| PR | Progesterone, R1881 | agonist | 1a28, 1e3k |
| PXR | apo | – | 1ilg |
| | SR12813, hyperforin | agonist | 1ilh, 1m13 |
| RARγ | T-RA | agonist | 2lbd |
| | 9C-RA, BMS961 | agonists | 3lbd, 4lbd |
| | BMS270394, BMS270395 | agonists | 1exa, 1exx |
| | BMS184394, Cd564 | agonists | 1fcx, 1cfy |
| | BMS181156 | agonist | 1cfz |
| | SR11254 | agonist | 1fd0 |
| RXRα | 9C-RA | agonist | 1fby |
| RORα | Cholesterol | agonist | 1n83 |
| RORβ | Stearic acid | NR box complex | 1k4w |
| | T-RA, ALRT1550 | part. antagonist; NR box complex | 1n4h, 1nq7 |
| TRα | T3, Dimit | agonists | – |
| | IH5 | TRβ agonist | 1nav |
| TRβ | T3 | NR box complex | 1bsx |
| | IH5 | agonist | 1nax |
| | PFA | agonist | 1n46 |
| TRβ (A234T); (R243Q) | 4HY | agonist | 1nq0, 1nq1 |
| USP | Phospholipid | antagonist | 1hg4, 1g2n |
| VDR | Vitamin D3 | agonist | 1db1 |
| | KH1060, MC1288 | super-agonist | 1ie8, 1ie9 |
| *Homodimers* | | | |
| ERα | Estradiol | agonist | 1ere, 1qku |
| | RAL | antagonist | 1err |
| | DES | NR box complex | 3erd |
| | OHT | antagonist | 3ert |
| | Estradiol | agonist | 1a52 |
| | THC | NR box complex | 1l2i |
| | Estradiol | NR box complex | 1gwr |
| | RAL « core » | NR box complex | 1gwq |

(*continues on next page*)

**Table 2** (*Continued*).

| Receptors | Ligands | Remarks | PDB ID |
|---|---|---|---|
| *Homodimers* | | | |
| ERα (Cys mut.) | Estradiol | agonist | 1qkt |
| ERβ | RAL, Genistein | antagonist, part agonist | 1qkn, 1qkm |
| | ICI164384 | antagonist | 1hj1 |
| | THC | antagonist | 1l2j |
| ERR3 | no | - | 1kv6 |
| HNF4 | Fatty acid | agonist | 1lv2, 1m7w |
| GR | Dexamethasone | NR box complex | 1m2z, 1p93 |
| | RU486 | antagonist | 1nhz |
| LXRβ | T0901317, Epoxycholesterol | agonists | 1pqc, 1pq9, 1p8d |
| | GW3965 | agonist | 1pq6 |
| PPARγ | no | – | 1prg |
| | Rosiglitazone | NR box complex | 2prg |
| | GW0072 | partial agonist | 4prg |
| RXRα | no | – | 1lbd |
| | no, undefined ATRA metabolite | tetramer | 1g1u, 1g5y |
| | DHA, BMS649 | agonists | 1mv9, 1mzn |
| RXRβ | LG100268 | agonist | 1h9u |
| *Heterodimers* | | | |
| LXRα/RXRβ | T1317/methoprene acid | NR box complex | 1uhl |
| PPARγ/RXRα | Rosiglitazone/9C-RA | NR box complex | 1fm6 |
| | GI262570/9C-RA | NR box complex | 1fm9 |
| | GW409544/9C-RA | NR box complex | 1k74 |
| RARα/RXRα | BMS614/Oleic acid | antagonist/part. agonist | 1dkf |

### *Role of the C-terminal region F is unknown*

Some receptors possess C-terminal of the LBD, a region F, which displays little evolutionary conservation. Note that the LBD is structurally defined as the domain generated by the elements between the beginning of helix H1 and the end of helix H12; this sequence is not necessarily identical to what is commonly referred to as region E from sequence alignments, and also receptors like the progesterone receptor possess some kind of E region. This region is, however, much longer in the cases of, for example, estrogen (NR3A) and retinoic acid (NR1B) receptors. There are no clues as to the function of the C-terminal sequence. Recent literature suggests that the F region might play a role in coactivator recruitment to the E domain and in determining the specificity of the LBD coactivator interface [28,29]. It seems clear that this domain also inherits little structural features. It is tempting to speculate that it sort of fine-tunes the molecular events associated with the transcriptional properties of the E domain, or the entire receptor, as it may affect antagonist action [30,31].

## DNA RECOGNITION BY NUCLEAR RECEPTORS

### Response elements of nuclear receptors

#### *Common principle*

All NRs recognize derivatives of the same hexameric DNA core motif, 5′-PuGGTCA (Pu = A or G). However, mutation, extension, and duplication, and, moreover, distinct relative orientations of repeats of this motifs generate response elements that are selective for a given (class of) receptors. Apparently coevolutionarily, NRs devised mechanisms to optimally interact with these sequences—they either

modified residues, which establish contacts to the nucleotides that specify a given response element or they generated response element-adapted homo- or heterodimerization interfaces.

### *Spacer "rules" derived from synthetic response elements*

To describe the preference of the various DR-recognizing receptors for elements with a certain spacer length, a simplified rule has been proposed [32,33] that is easily memorized.

Albeit a reasonable approximation, this rule should be used with care, since there are numerous exceptions, such as receptors binding to complex, unusual, or noncognate response elements, and different receptors may bind to common elements that are not predicted by the rule.

**Table 3** "Spacer rules" for DNA binding response elements of nuclear receptors.

| Spacer NTs | Systematic name | Acronym | Receptor complex |
|---|---|---|---|
| 1 | DR1 | RXRE, PPARE,.. | RXR-RXR, PPAR-RXR, RAR-RXR, … |
| 2 | DR2 | RARE | RAR-RXR |
| 3 | DR3 | VDRE | RXR-VDR |
| 4 | DR4 | TRE | RXR-TR |
| 5 | DR5 | RARE | RXR-RAR |

### *Variability of the binding motif, spacer sequence, and flanking nucleotides*

It is important to point out that there is considerable degeneration in the sequence of half-site motifs of a given type of natural retinoid response element and that there is a distinct preference of the various receptors for a certain motif. For example, the preference for the half-site motif 5′-PuGGTCA over 5′-GTTCA follows the order TR > RXR > RAR [34].

In addition to a distinct preference for certain nucleotides in the half-site motif, there is also a receptor-specific preference for certain nucleotides in the DR spacer, which is easily rationalized in view of the crystallograhic data (see Table 1 and ref. [35]). See, in this respect, also the NGFI-B DNA complex, which illustrates the binding of A- and T- box residues to the 5′ minor groove of the NBRE (see Table 2 and ref. [36]).

### *Steroid hormone receptor response elements*

Steroid hormone receptors bind to 3 bp-spaced palindromic arrangements (3 bp-spaced inverted repeats; generally termed IR3) of the prototypic recognition motif (see ref. [5] and refs. therein). The mutation of a single nucleotide at position 4 in each motif from T to A (5′-PuGGTCA to 5′-PuGGACA) will convert an estrogen (ERE) into a glucocorticoid response element (GRE). Note, however, that the classic GRE is rather a 5′-PuGAACA, which corresponds to the mutation of two nucleotides.

Although progesterone (PR), androgen (AR), and mineralcorticoid (MR) receptors bind to GREs, differences in their DNA-binding specificities have also been observed. A systematic mutational analysis concluded that GRs and PRs may not distinguish individual target sites, but may use the whole of the response element context differentially [37]. Notably, the chicken PR was shown to replace GR for the activation of the endogenous tyrosine-amino transferase gene, a cognate GR target gene [38]. This could suggest that in some cases, only the distinct expression profiles of some NRs and their ligands suffice to generate specificity.

### *Response elements for retinoid, thyroid, vitamin D, and peroxisome proliferator-activated receptors*

For detailed discussion of this topic, the reader is referred to a number of extensive reviews [39–43]; please compare also the published crystal structure data on complexes between various NR DNA-bind-

ing domains and the cognate DNA-response elements (Table 1 and Fig. 3). The characteristics of the major response elements to which retinoid can potentially contribute, either as RAR-RXR heterodimer or through RXR as the heterodimeric partner, are reviewed below.

### *Retinoid response elements*

The classic retinoic acid response element (RARE), which was found in the P2 promoter of the RARβ gene and gives rise to the RARβ2 mRNA, is a 5 bp-spaced direct repeat (generally referred to as DR5) of the motif 5′-PuGTTCA. In addition, response elements with a DR5 containing the motif 5′-PuGGTCA (also termed DR5G to distinguish it from the DR5T of the RARβ2 promoter) act as perfect RAREs [44–46] as well as direct 5′-PuGGTCA repeats spaced by 1 bp (DR1) or 2 bp (DR2). RAR-RXR heterodimers bind to, and activate transcription from, these three types of RAREs, provided target cells express both RARs and RXRs. DR1 elements bind, in addition to RAR-RXR heterodimers, also RXR homodimers in vitro, and RXRs can transactivate in response to an RXR ligand target genes containing DR1 elements. That DR1 elements can act as functional retinoid X receptor response elements (RXREs) in vivo is supported by their activity in yeast cells [47], in which any contribution of endogenous RAR via heterodimerization with RXR can be excluded. However, no natural RXRE has been found up to now. The only reported natural RXRE is a DR1-related element found in the rat CRBPII promoter [48].

### *Thyroid hormone receptor response elements*

The thyroid response element (TRE) consensus sequence is 5′-AGGTCA, as for RARs, RXRs, PPARs, or VDRs. However, there is evidence for some differences in the natural response element repertoires of these receptors. It has been shown, for example, that TRα is able to bind to both 5′-AGGTCA and 5′-AGGACA motifs [49–51]. Such differences may lead to further specification by cooperative DNA binding with other promoter-bound factors and could contribute to the ability of a given target gene to preferentially respond to a particular signaling pathway.

Like other receptors, TRs are able to bind to a palindromic element (TREpal) [51–53]; but such an element has not yet been found in cellular genes. Furthermore, this element confers no hormonal specificity, since it can be recognized by a large number of other receptors. The most commonly found TREs are either direct repeats (DRs) or everted repeats (ERs). Examples of direct repeat TREs are discussed in ref. [5].

On direct repeats, TRs have a strong preference for DR4, i.e., direct repeats spaced by four nucleotides (for the corresponding crystal structure, see Table 1 and ref. [35]). Nevertheless, TRs are able to bind to direct repeats with a spacing other than four, such as DR5 [54], DR2, or DR0 [55]. TRs can also bind to inverted palindromes with a preferred spacing of six nucleotides [56].

### *Vitamin D response elements*

Only a few natural vitamin D response elements (VDREs) are known; several of them contain DR3 elements. Studies with "optimized" synthetic response elements assembled from 5′-PuGGTCA motifs have confirmed that DR3 elements bind VDR-RXR heterodimers, and that the cognate ligands, vitamin D, and 9C-RA, activate the corresponding promoters. The promoter of the human/rat osteocalcin gene contains a complex VDRE with several possible combinations of the recognition motifs, including that of a DR6. For more extensive discussion on vitamin D response elements and action see recent reviews [57,58].

### *Peroxisome proliferator-activated receptor response elements*

Natural peroxisome proliferator-activated receptor response elements (PPAREs), which have been found in enzymes that catalyze the peroxisomal β-oxidation and microsomal ω-hydroxylation in response to peroxisome proliferators, usually contain (degenerate) DR1 elements, but more complex PPAREs have been reported also (for reviews, see refs. [42,43,59,60]).

## Principles of DNA recognition by nuclear receptor DNA-binding domains

### *Homo- and heterodimerization*

Nuclear receptors can bind their cognate response elements as monomers, homodimers, or heterodimers with another family member (for reviews, see refs. [39,61]). Dimerization is a general mechanism to increase binding site affinity, specificity, and diversity due to (i) cooperative DNA binding (an extreme case of cooperative binding is the existence, in solution, of stable dimers), (ii) the lower frequency of two hexamer binding motifs separated by a defined spacer compared to that of single hexamers, and (iii) heterodimers that may have recognition sites distinct from those of homodimers.

Steroid hormone receptors bind generally as homodimers to their response elements, while RARs, RXRs, TRs, and VDRs can homo- and/or heterodimerize. RXRs play a central role in these various signal transduction pathways, since they can both homodimerize and act as promiscuous heterodimerization partner for RARs, TRs, VDRs and orphan receptors. Heterodimerization has a three-fold effect: it leads to a novel response element repertoire, increases the efficiency of DNA binding relative to the corresponding homodimers, and allows two signaling inputs, that of the ligands of RXR and its partner. Crystal structures of DBD homo- and heterodimers have defined the surfaces involved in dimerization (see ref. [61 and refs. therein, [62]). It is important to point out that the response element repertoire described above for receptor homo- and heterodimers is dictated by the DBD while LBDs stabilizes the dimers, but do not contribute to response element selection.

### *Specificity of DNA recognition (P-box, D-box, T-box, A-box)*

The DNA response element specificity (half-site sequence, spacing, and orientation) is generated by (i) the recognition of the actual "core" or "half-site" motif and (ii) the dimerization characteristics (mono-, homo- or heterodimerization; structure of the actual dimerization interface) of the receptor(s).

Identification of the residues involved in distinguishing the hexameric half-site motives of EREs (5′-AGGTCA) and GREs (5′-AGAACA) was done by a series of refined swapping experiments. Initially, DBD swaps showed that specific half-site recognition depends on DBD identity [63], subsequently the N-terminal finger was found to differentiate between ERE and GRE recognition [64]. Finally, three studies identified two to three residues at the C-terminal "knuckle" of the N-terminal finger; commonly referred to as the P-box (proximal box; Fig. 3a), to be responsible for ERE vs. GRE recognition [65–67].

A second region, the D-box (distal box; N-terminal "knuckle" of the C-terminal finger; see Fig. 3b), was found to be involved in differentiating between the binding to a 3bp- (characteristic for steroid receptor REs) and a 0 bp-spaced (one type of TRE) palindrome [67]. As was later confirmed by the crystal structures of GR and ER DBDs, this region does indeed contribute to the DBD dimerization interface.

Two other boxes have been described within the DBDs of heterodimerizing receptors, the A- and the T-box (Fig. 3c). The A-box was originally described for NGFIB as the sequence responsible for the recognition of two or three additional A nucleotides in the minor groove 5′ of the hexameric core motif, thus generating an NGFIB response element (NBRE; 5′-(A)AAAGGTCA) [68,69]. This A-box was later found to play a similar role in heterodimers such are 5′-RXR-TR on DR4 elements, where it specifies to some extent the spacer 5′ of TR and sets a minimal spacing by steric hindrance phenomena [70,71]. Interestingly, in the 3D structure, the A-box presents as a helix contacting the minor groove and modeling is in keeping with its role in setting a minimal distance between the half-sites [35].

The T-box was originally defined in RXRβ (then H-2RIIBP) as a sequence required for dimerization on a DR1 element [72]. Its role as a RXR homo- and heterodimerization surface has been subsequently confirmed [70,71,73].

### *Three-dimensional structure of nuclear receptor DNA-binding domains*

A significant amount of structural information has been accumulated during the past years, providing information about the solution structure of the GR, ER, RAR and RXR DBDs. Moreover, the 3D crys-

tal structures have been solved of the GR DBD homodimer bound to noncognate DNAs, the crystal structure of ER DBD homodimer bound to consensus and nonconsensus natural EREs, the crystal structure of the RXR homodimer on a DR1 element and the TR-RXR DBD heterodimer bound to its cognate DR4 element, the structures of the NGFIB-NBRE, RevErb-DR2, and RAR-RXR-DR1 complexes, as well as the VDR homodimer on various response elements (for references to the original studies, see Table 1 (PDB accession numbers) and refs. [5,61]).

The 3D structure of the ER DBD-ERE co-crystal is shown in Fig. 3a [74]. The structure consists of a pair of amphipathic α helices packed at right angles and crossing near their midpoints. A zinc-binding pocket lies near the N-terminus of each of the two helices. Hydrophobic side chains form an extensive hydrophobic core between the two helices. The residues' N-terminal to the first helix are folded to form two loops. Hydrophobic residues at the tips of the two loops pack with hydrophobic residues in the core between the two helices.

Two ER DBD molecules bind to adjacent major grooves from one side of the DNA double helix. The protein makes extensive contacts to the phosphate backbone on one side, orienting the DBD such that the recognition helix enters the major groove, allowing surface side chains to make sequence-specific contacts to the base pairs. Although ER or GR DBDs are monomers in solution, they bind cooperatively to the cognate response elements owing to the DNA-induced formation of a dimerization interface which comprises also D-box residues. For further details, see the original publications [5].

## STRUCTURE OF NUCLEAR RECEPTOR LIGAND-BINDING DOMAINS

### Canonical fold of nuclear receptor ligand-binding domains

To date, the crystal structures of monomeric, homodimeric and heterodimeric NR LBDs in the presence of agonists (holo form), antagonists, partial agonists, fragments of coactivators and corepressors, or in the absence of bound hormone (apo form) have been described (Table 3). All these NR LBDs display a common fold, as originally predicted [75], with 12 α-helices (H1 to H12) and one β-turn arranged as an antiparallel α-helical "sandwich" in a three-layer structure (Fig. 4). Note that some variability exists; for example, no helix H2 was found in RARγ [76], while an additional short helix H2′ is present in PPARγ [77].

### Mouse trap model

A comparison of the apo- and holo-LBD structures (Fig. 4) suggested a common mechanism by which the activation function AF-2 becomes transcriptional competent: upon ligand binding, H11 is repositioned in the continuity of H10, and the concomitant swinging of H12 unleashes the Ω-loop which flips over underneath H6, carrying along the N-terminal part of H3. In its final position, H12 seals as a "lid" the ligand-binding cavity and further stabilizes ligand binding (in some, but not all NRs) by contributing additional ligand–protein interactions. It is a general and essential feature of the ligand "activation" of NRs that the transconformation of H12, together with additional structural changes (such as bending of helix H3), creates distinct surface(s) on the apo- and holo-LBD. The novel surfaces generated upon agonist binding allow bona fide coactivators, such as the members of the SRC-1/TIF2 family, to bind and recruit additional transcription factors (see below). Concomitantly, corepressor proteins, which bind to a surface topologically related to that involved in coactivator interaction of the apo-LBD, dissociate upon agonist, but not necessarily antagonist, binding (see below). Notably, as is discussed further below, certain antagonists "force" H12 in a third position, distinct from the holo position whereby it impairs coactivator binding.

For a given receptor, the equilibrium between the apo and holo (or apo and antagonist) conformational states of a NR LBD can be affected through intramolecular interactions of H12, such as a salt bridge (holo LBD of RARγ [76]) or hydrophobic contacts (as suggested for apo-ER; [78]). This implies

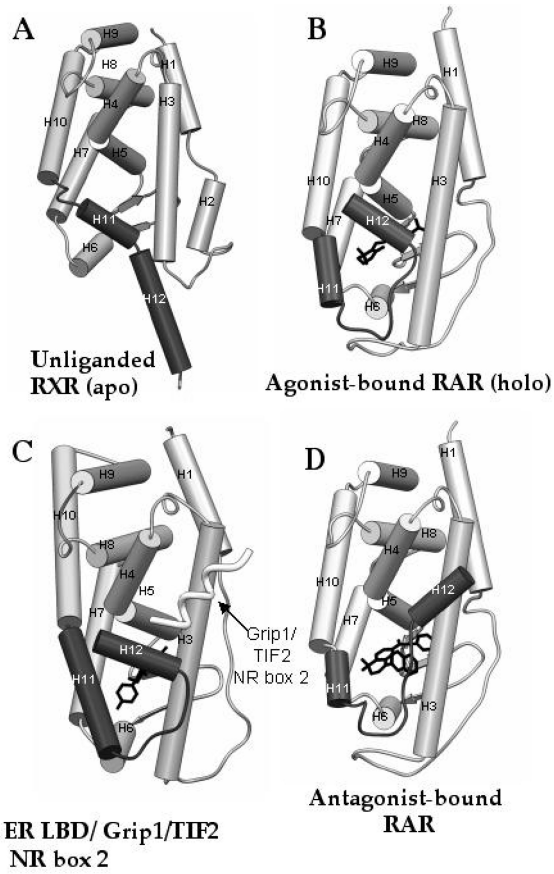


**Fig. 4** Schematic illustration of the canonical 3D structures of NR LBDs. The canonical apo and holo structures of NR LBDs are shown in (A) and (B), respectively. The holo structure allows interaction with the NR box of nuclear receptor coactivators, such as TIF2/Grip1, SRC1, or RAC3/AIB1/ACTR (see text and reviews cited), as depicted in (C). This NR box binding site is occupied by helix H12 in the antagonist-bound LBD illustrated in (D).

that the apo conformation is not necessarily the default state, so that some NRs may be constitutive activators or repressors without possessing a cognate ligand. Moreover, an increase in coactivator concentration can generate a transcriptionally competent RAR under certain conditions [79] and the apo-ER conformation may be destabilized by phosphorylation [78,80]. Thus, overexpression of coactivators or receptor modification may generate ligand-independent receptors. Such scenarios could have significant implications for endocrine cancer therapies.

### Dimer interface

Recently, the first structures of heterodimeric LBDs have been solved. One comprises the RARα LBD bound to the α-selective antagonist BMS614 and the constitutively active [81] RXR mutant F318A, which turned out to harbor an oleic acid-like ligand [82]. The other is the LBD of PPARγ bound to rosiglitazone or a synthetic ligand in a heterodimer with 9-*cis* RA-bound RXRα [83]. These structures allowed for the first time to compare the homo- and heterodimerization interfaces of several NR ligand-binding domains. In principle, the overall heterodimeric arrangement closely resembled that of homodimers of RXRα, ERα, and PPARγ [77,84,85]. However, in contrast to the nearly perfect symmetric organization of homodimer interfaces, the heterodimer interfaces are slightly asymmetric. Subsequently,

the heterodimeric interface is described based on the results obtained by Bourguet and colleagues [82]: The interfaces comprises residues from helices H7, H9, H10, and H11, as well as loops L8-9 and L9-10. Within the heterodimer, the two protomers do not equally contribute to the heterodimerization interface. For example, RAR and RXR exhibit different patterns of contact areas, as helix H7 of RXR contributes to the interface four times more surface area than its RAR counterpart. Inversely, the contribution of RARα loop L8-9 to the interface is three times higher than the one of the corresponding loop in RXRαF318A. Recently, RXR homo- and heterodimerization has been separated by mutational analysis (V. Vivat et al., 2003, *Mol. Cell. Biol.*, in press).

Except for side chain rearrangements, the part of the RXR LBD involved in the heterodimeric interface does not differ significantly from that of the RXRα apo-LBD homodimer. In both dimers, helices H9 and H10 contribute to more than 75 % of the total surface and constitute the core of the dimer interfaces. However, some differences in the relative involvement of some RXR structural elements to the homo- or heterodimer interfaces are observed and originate from the nature of the interacting protomer. For example, in the heterodimer, helix H7 contributes twice as much surface area to the interface than in the homodimer. This is due to the different structure of RAR loop L8-9, which makes more extensive contacts with H7 of RXR than its RXR counterpart in the homodimer. The buried surfaces are larger for the RAR-RXR heterodimer (967 $Å^2$ per monomer). The smaller interface in RXR homodimers (915 $Å^2$ per monomer) suggests a weaker link between the promoters that could be related to the promiscuous character of this NR. The moderate stability of RXR homodimeric association may facilitate heterodimerization.

When compared to RAR and RXR, the ERα LBD structural elements generating the dimerization interface are identical. However, helices H8, H9, H10, and the loop L8-9 in ERα are longer and make additional contacts. As a consequence, the buried surfaces are larger for the ERα homodimer (around 1700 $Å^2$), suggesting its higher dimerization affinity when compared to RXR dimers.

In contrast, the recent crystal structure of the GR LBD suggests a novel mode of receptor dimerization [86]. This unique dimer configuration involves residues from the β-turn of strands 3 and 4, the extended strand between helices H1 and H3 as well as the last residue of helix H5. Compared to the dimerization surfaces observed in other NRs, formation of the GR homodimer buries only 623 $Å^2$ of solvent accessible surface, probably reflecting its weaker dimerization affinity (Kd = 1.5 μM).

### Ligand-binding pocket

In all crystal structures presently available, the ligand is embedded within the protein with no clear accessible entry or exit site. PPARγ seems to be the only exception to that rule, since a potential access cleft to the LBP was observed between helix 3 and the β-turn, which could be of sufficient size to allow entry of small ligands without major adaptation. For all other receptors of known structure, significant conformational changes are necessary to generate potential entry sites. The mouse trap model provides an easy solution to the problem: The mobility of H12 opens a channel by removing the "lid" from the ligand pocket.

The ligand-binding pockets are lined with mostly hydrophobic residues. Few polar residues at the deep end of the pocket near the β-turn act as anchoring points for the ligand or play an essential role in the correct positioning and enforce the selectivity of the pocket. Most NRs contain a conserved arginine attached to helix H5, which points into this part of the cavity. These anchoring residues, conserved within a given subfamily, are indicative of the polar group characteristics of each family of ligands (i.e., carboxylate for retinoids and ketones for steroids).

In the case of retinoid receptors, it is the ligand that adapts to a fairly rigid ligand-binding pocket [87]. The ligand-binding pockets of some other NRs are significantly larger and use precise anchoring points for their ligands; in such cases, it is possible to generate ligands, which owing to additional contacts, have higher binding affinities that the natural ligands and may even act as "superagonists". In this context, the recent structure of the human nuclear xenobiotic receptor PXR (NR1I2) is interesting, be-

cause it helps to understand how the ligand-binding pocket of this receptor can accommodate such diverse ligands as dexamethasone, RU486, rifampicin, taxol, and others [88]. In contrast to other NRs, PXR contains a small number of polar residues distributed throughout its large hydrophobic ligand-binding pocket. These unique features allows PXR to bind a diverse set of chemicals containing polar groups capable of hydrogen bonding and to act as chemical sensor.

## MOLECULAR MECHANISMS OF TRANSCRIPTIONAL REGULATION

The expression of a given gene can be regulated at several different levels (transcription, translation or RNA processing, posttranslation) and also transcription itself has multiple levels at which regulation might occur. The transcriptional activity of a gene can be controlled epigenetically via methylation, at the level of its chromatin structure and at the level of the assembly and activity of the initiating and elongating polymerase-complexes. The reported effects of NRs on transcription are so far restricted to the initiation of transcription by RNA polymerase II. Below, we will summarize our current knowledge about the molecular mechanisms/interactions through which NRs can positively or negatively regulate the expression of cognate genes. For further details and references, see recent reviews [89–100].

### Most nuclear receptors contain at least two independent activation functions, AF-1 and AF-2

Nuclear receptors contain at least two distinct regions, termed activation functions AF-1 and AF-2 (Fig. 2), which, when tethered to a (heterologous) DBD, will transactivate transcription from response elements recognized by this DBD. In the context of the wild-type receptor, both AF-1 and AF-2 become active in response to the ligand, but AF-1 can act constitutively in fusion proteins with heterologous DBDs. AF-2 remains ligand-dependent even in such fusion proteins. Within AF-2, at the C-terminal boundary of the LBD, an autonomous constitutively active transactivation function (AF-2 AD) has been identified whose integrity is crucial for AF-2, as mutations in AF-2 AD abolish AF-2 activity structure. This AF-2 AD encompasses in helix H12 in the 3D structure of NR LBDs (see above). Note, however, that the constitutive activity of AF-2 AD is very weak compared with the full ligand-induced activity of AF-2.

Within the AF-1-containing A/B region, shorter regions have been described to display constitutive activation function. In the case of the human PR isoforms, the additional N-terminal sequence, which is unique to the larger ("form B") isoform, was found to be able to squelch and to display an autonomous transactivation activity on its own together with the homologous, but not with a heterologous, DNA-binding domain (for more information and original refs. see ref. [5]).

Several NRs exist as isoforms. As was originally shown for the PR forms A and B, both isoforms exhibit different promoter specificities. For a more detailed discussion on this topic, see the recent review by Conneely and colleagues [101], and for a discussion of the differential antagonist action of the two isoforms and the relevance to endocrine cancer therapy see the corresponding reviews from the Horwitz laboratory [102,103]. Note that the additional N-terminal sequence of PR B may have a peculiar structure [104]. A later study showed that isoform-specific transcription activation is not confined to PR, but can be observed also with the RARs [105].

It is important to point out that the activation functions of NRs act in both promoter context- and cell-specific fashion, as was best documented for ERs and RARs [20,105]. This selectivity may originate from cooperative/synergism with other promoter-bound transcription factors and/or the cell-specific expression of TIFs/coactivators (see below and Topic 1.3).

## Chromatin-modifying nuclear receptor coregulators

### *Coactivators and HATs*

So-called squelching [106] experiments paved the way to predict the existence of factors that would transmit the signal generated by the holo-receptor to the transcription machineries. Squelching occurs if a receptor inhibits the activity "off the DNA" of the same ("autosquelching", [19]) or a different ("heterosquelching", [107,108]) receptor in an agonist- and AF-2 integrity-dependent manner. These squelching data were interpreted as the result of sequestering, by either excess of the same, or addition of another ligand-activated receptor, of so-called transcription intermediary factors (TIFs) that mediate the action of the activation/repression functions of NRs and are limiting constituents of the machineries required for transcription initiation. This concept predicted the existence of TIFs that are shared between, and are critically involved in, the action of different receptors. Indeed, the subsequent cloning and characterization of TIFs, also known as coactivators and corepressors, has fully justified this concept. According to the squelching studies, bona fide coactivators were predicted to fullfill the following criteria: (i) interact directly with NR LBDs in an agonist- and activation function-dependent manner, (ii) enhance NR-dependent reporter gene activity when transiently expressed in mammalian cells, (iii) activate transcription autonomously when tethered to DNA via a heterologous DBD, and (iv) relieve squelching. The development of yeast "two hybrid" and direct cDNA expression library screening approaches has allowed to identify a great number of putative and bona fide of coactivators, corepressors, and other coregulators that are believed to transmit the NR signal to its molecular targets.

The cloning of coregulators was followed by the definition of the coactivator signature LxxLL NR box (where x is any amino acid) motifs embedded in a short α-helical peptide [109–111]. These NR boxes are necessary and sufficient for ligand-dependent direct interaction with a cognate surface in the NR LBD that constitutes the transcriptional activation function AF-2.

Recently, a second contact site for coactivators has been identified in NRs. Proteins from the TIF2 family are able to interact specifically with the A/B domains of estrogen and androgen receptors [114–118]. These interactions result in a stimulation of the transcriptional activity originating form AF-1. Moreover, it appears that simultaneous interaction of coactivators with both the AF-1 and the AF-2 of an NR accounts for the synergy between both transcriptional activation functions [119,120]. The structural features of the A/B domains that are recognized by the coactivator have not yet been defined.

The identification of specific NR coactivators has prompted the question of how they function on a molecular level in transcription. To this end, several observations have been made. It is now generally accepted that NR coactivators possess or recruit enzymatic activities, and that they form large coactivator complexes. CBP, p300, P/CAF, SRC-1, P/CIP, and GCN5 are reported to act as histone acetyltransferases. They are capable of acetylating specific residues in the N-terminal tails of different histones, a process that is believed to play an important role in the opening of chromatin during transcription activation, and also nonhistone targets. Note, however, that the HAT activity of SRC-1 and P/CIP, if real, is negligible compared to that of p300 or CBP. Thus, p160 coactivators may rather recruit such activities by physical association with histone acetyl transferases, such as CBP or p300, or with complexes containing such activities. Specifically, the activation domain AD1 of TIF2 has been demonstrated to function via the recruitment of cointegrator CBP [79], which apparently in turn acetylates TIF2 [121]. Besides HAT activities, also other enzymatic activities have been attributed to NR coactivator complexes. TIF2 proteins are able of interacting functionally via their activation domain AD-2 with a protein methyltransferase [122], via N-terminal region(s) with PIAS proteins that act as SUMO-ligases [123,124], and other factors [125,126]. The roles of these various interactions in epigentic gene regulation are being actively investigated.

In conclusion, bona fide coactivators (i.e., members of the TIF2/SRC-1/RAC3 family), together with the CBP/p300 cointegrators function by rendering the chromatin environment of an NR target gene prone to transcription. This opening of the chromatin environment is achieved by intrinsic or recruited

HAT activity. The HAT activities of different coactivators/coactivator complexes targets (i) the N-termini of histones, which have reduced DNA-binding activity upon acetylation, (ii) certain basal transcription factors, and (iii) at least some bona fide coactivators themselves. The chromatin modification step represents the first of at least two distinct steps in transcription activation by NRs (see Fig. 5 for an illustration of the "derepression" and further below for the second step).

### *Structural basis of coactivator binding to nuclear receptors*

Biochemical [127,128] and structural [77,129,130] studies clearly demonstrated that the holo conformation of NR LBDs harbors an "active" surface that is able to recognize and dock a short α-helix present in the NR interacting domain (NID) of coactivators. This α-helix contains a LxxLL motif (with L being a leucine residue and x any amino acid), called nuclear receptor box (NR box) by Le Douarin and colleagues [109], and is present in several copies in the NID of coactivators [109,110,131]. The NR interaction surface is composed of a static part involving the C-terminal half of helix H3, helix H4, and the loop L3-4 connecting them, and of a mobile part corresponding to the activation helix H12. All together, these structural elements define a hydrophobic groove that can accommodate the coactivator NR boxes. The NR–coactivator interaction is mediated by the leucine residues of the NR box motif, which pack on the hydrophobic cleft but also by two conserved residues of the receptor (a lysine and a glutamic acid residues in helices H3 and H12, respectively) which are hydrogen-bonded to two main-chain carbonyl groups of the helical NR box. This "charge clamp" further stabilizes the interaction and strictly defines the length of the helix that can be docked into the NR recognition groove. Furthermore, biochemical experiments suggest that nonconserved residues adjacent to the LxxLL motif of coactivators make additional contacts with the NR LBD and may determine the specificity of NR–coactivator interaction [129,131].

### *Nuclear receptor corepressors, silencing, and HDACs*

The second class of NR coregulators comprises the corepressors. Early on, it was shown that some NRs do actively repress transcription when in the apo form. This phenomenon had been particularly well established for retinoic acid and thyroid hormone receptors. Soon after the identification of ligand-recruited coactivators, similar approaches identified proteins that recognize the ligand-free NR. To date, several different NR corepressors have been identified; by far, most studies have been performed with NCoR and SMRT. For these two, it was shown recently that a conserved CoRNR box motif interacts with a surface on the ligand-binding domain which is topologically very similar to that recognized by coactivator LXXLL motifs, but does not involve helix H12 [132–135]. Corepressors are believed to reside in, or recruit, high-molecular-weight complexes that display the opposite activity of coactivator complexes. While coactivator complexes acetylate histones, thereby weakening the interaction of the N-terminal histone tails with the nucleosomal DNA, corepressors recruit histone deacetylase activities that reverse this process (illustrated in Fig. 5 as the "repression" step). Deacetylated histones are associated with silent regions of the genome, and it is generally accepted that histone acetylation and deacetylation shuffle nucleosomal targets between condensed and relaxed chromatin, the latter being requisite for transcriptional activation. An unresolved issue is whether all NRs are able of active repression. In concert with this observation, recent evidence has been presented that some steroid hormone receptors also bind to corepresssors in presence of certain antagonists [136–138].

Given this high number of different coregulators for NRs, two principal questions emerge: (i) what defines coactivator selectivity, and (ii) how is the assembly of different coactivator complexes with different intrinsic transcription activities regulated? Future research will have to address such questions, especially also in view of the therapeutic perspectives in disease.

### *Structural basis of corepressor binding to nuclear receptors*

It has been proposed that corepressors interact with RAR and TR LBDs in a region encompassing helix H1 since mutation of residues Ala223, His224, and Thr227 in the so-called CoR box of TRβ abrogated the recruitment of N-CoR [139,140]. However, examination of this portion of NR LBD structures re-

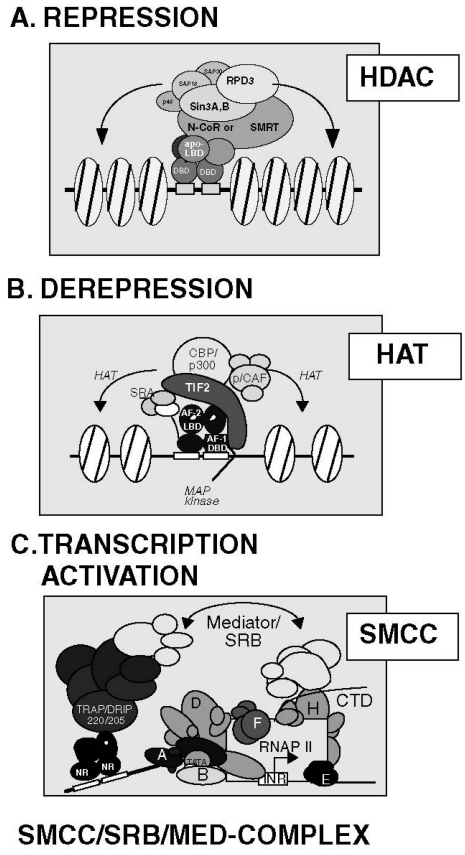


**Fig. 5** Hypothetical 3-step mechanism leading to transcriptional activation by NRs. Some NRs act as silencers of target gene transcription in the absence of ligand (or in the presence of certain antagonists). This "repression" step is due to the recruitment by the apo-NR of a corepressor complex that exerts HDAC activity. Ligand binding dissociates this complex and recruits the coactivator complex that displays HAT activity. The subsequent chromatin decondensation ("derepression") is believed to be necessary, but not sufficient, for target gene activation. At the third step, the HAT complex dissociates, possibly due to acetylation of the coactivator which decreases its ability to interact with the receptor, and the SMCC/DRIP/TRAP complex is assembled through its TRAP220/DRIP205 subunit. The SMCC complex is able to establish contacts with the basal transcription machinery, resulting in transcription. Note however, that it has been proposed that for some receptors p160 coactivator-mediated transactivation can occur without contribution of SMCC factors [112] initiation. This illustration is modified from ref. [113]. Note that the involvement of ATP-dependent chromatin remodeling machineries in NR actions is not considered in this cartoon.

veals that these residues cannot be part of a corepressor interacting surface. Indeed, they are engaged in intramolecular interactions with other structural elements (H3, H8,...) and are not solvent exposed. Most probably, these mutations destabilize the positioning of helix H1, which in turn can affect the structural stability of other parts of the LBD. Another possibility is that the opposite face of helix H1 contains residues important for corepressor binding. However, residues at the surface of helix H1 are not conserved among NRs that were shown to interact with SMRT and N-CoR. Therefore, helix H1 is probably not the major anchoring point of corepressors on NRs. Recent correlative analyses of biochemical data and sequences provided evidence that coactivator and corepressor recruitment share similar molecular basis. Evaluating corepressor binding to mutants in the coactivator binding site of TR$\alpha$, Hu and Lazar (1999) demonstrated that mutations that impared activation and coactivator recruitment, also de-

creased repression and corepressor binding, indicating that corepressors N-CoR and SMRT bind to a NR surface topologically related to that involved in coactivator interaction. Mapping of NR determinants of N-CoR binding using a series of mutations introduced into TRβ indicated that the groove formed by residues from helices H3 and H4 corresponds to the primary corepressor binding site, but that H11 or H12 are not involved [133,134]. Using a similar approach, a parallel study was reported on the interaction between N-CoR and the orphan NRs Rev-erbA/RVR that are known to funtion as transcriptional silencers. In that particular case, the LBD interacting surface with corepressors would encompass H3–H4 and H11, which was correlated to the fact that Rev-erbA/RVR lack the C-terminal activation helix H12 [141]. The C-terminus of SMRT and N-CoR contain a region interacting with NRs composed of two independent interacting subdomains ID1 and ID2 [142,143]. Examination of the two interaction domains ID1 and ID2 in SMRT and N-CoR revealed sequences (CoRNR box 1 and CoRNR box 2 by analogy with NR boxes of coactivators) similar but not identical to the LxxLL motif of coactivators, which were also predicted to adopt an amphipathic helical conformation [132–134]. Chimaeric CoRNR box peptides were designed to show that although the consensus hydrophobic core ΦxxΦΦ is necessary and sufficient for corepressor binding, sequences flanking the CoRNR box strengthen the interaction and determine NR specificity. In addition, within a corepressor, the two CoRNR boxes are not equivalent. CoRNR box 1 interacts almost exclusively with RAR/TR, and CoRNR box 2 binds to both heterodimer partners. Perissi and coworkers further extended the analogy with the helical coactivator LxxLL motifs. They proposed a model in which the CoRNR box motif would fold into a significantly longer LxxI/HIxxxI/L helix when compared to the coactivator LxxLL NR box motif [134]. In the absence of agonists, the CoRNR box helix could interact with the H3-H4 hydrophobic groove displayed by apo-receptors. However, in the presence of an agonist, the repositing of the activation helix H12 in its active (holo) conformation would prevent this interaction. Indeed, in contrast to the unliganded NR case, the length of the helix that can be accommodated by the H12-containing groove is strictly defined by the presence of the charge clamp that specifically recognizes helices of the NR box type [77]. In this respect, the recent crystal structure of a ternary complex containing the PPARα LBD bound to the antagonist GW6471 and a SMRT corepressor motif [135] confirms that the corepressor fragment adopts a three-turn helix that binds into the groove which also serves as the coactivator-binding site.

### *Recruitment of the RNA polymerase II holoenzyme*

The initial chromatin-modifying step carried out by NR coactivators (see above) has to be followed by the actual recruitment of the RNA polymerase II holoenzyme and initiation of transcription (illustrated in Fig. 5 as the "transcription activation" step). Comprehension of the recruitment of the polymerase II holoenzyme by NRs has only become at reach through the identification and cloning of the mammalian mediator complex as a thyroid hormone and vitamin D receptor coactivator [144–146]. The mammalian mediator came in several flavors. It was identified as the so-called SMCC, the Srb and Mediator protein containing complex [147], the TRAP complex, a thyroid hormone receptor associated protein complex [144], or the DRIP complex, a vitamin D receptor interacting protein complex [145]. Furthermore, common subunits are shared with PC2, the so-called positive coactivator 2 [148], the ARC [149], CRSP [150] and NAT [151] complexes. Hereafter, this complex is referred to as "SMCC". It is a large multisubunit protein complex that contains several homologs of the yeast mediator complex (RGR1, MED6, MED7, SRB7, SRB10, SRB11, NUT2, SOH1 [146,147]) as well as additional proteins of unknown function. As expected for a mediator complex, SMCC associates with the RNA polymerase II to form RNA pol II holoenzymes [146]. On the other hand, the SMCC complex is able to interact functionally with different transcription factors such as p53 and VP16 [146]. Furthermore, due to its identification as a thyroid hormone and vitamin D receptor-interacting complex it is believed to function as NR coactivator. This notion is supported by the demonstration that SMCC can enhance thyroid hormone and vitamin D transcription activation in in vitro transcription systems [145,152,153]. The subunit of the complex that is responsible for interaction with the agonist-bound LBD of NRs was identified as DRIP205 [154], which is identical to TRAP220 and contains a functional LxxLL NR box motif [155].

Interestingly, another subunit of the SMCC complex, DRIP150, interacts with the N-terminal region of the glucocorticoid receptor, which harbors the activation function AF-1 [156]. The current working hypothesis is that once the chromatin environment at target gene promoters has been decondensed by coactivator complexes containing members of the TIF2 and CBP families, the NR recruits RNA pol II holoenzymes via its association with the TRAP220/DRIP205 subunit of the SMCC. This switch between coactivators and the SMCC complex might be regulated by the acetylation of coactivators within the HAT complex [121], resulting in their dissociation from the NR, thus allowing the recruitment of factors such as SMCC via the LxxLL motif of the TRAP220/DRIP205 subunit.

The recruitment of the RNA polymerase II holoenzyme might also be enhanced by interactions of NRs with components of the SWI/SNF complex, which is part of RNA pol II holoenzymes.

Whether these various complexes are all necessary for a particular receptor-regulated gene program and, if so in which order, and with which stoichiometry they operate, if they act tissue, receptor, and ligand-selective, if they preexist, or get assembled by signaling events, are all questions that are being actively investigated.

## STRUCTURAL BASIS OF LIGAND ACTION

### Ligand selectivity

As shown in the cases of RARγ and TRβ, the shape of the ligand-binding pocket matches that of the ligand. The accordance of shape and volume maximize the number of mostly hydrophobic contacts, thus contributing to the stability of the complex and the selectivity of the pocket for the cognate ligand.

RAR possesses an interesting LBD, since it can bind equally well two chemically different ligands: all-*trans* retinoic acid and its 9-*cis* isomer. Crystallographic analysis [87] of the two ligands in the RARγ LBD showed that both adapt conformationally to the LBP that acts as matrix. Moreover, the conformation of a RARγ-selective agonist was also shown to closely match that of the natural ligands in their bound state [87]. The adaptation of ligands to the protein leads to an optimal number of interactions for binding and selectivity, and justifies modeling approaches for ligand design.

For steroid receptors, the LBP volume is significantly larger than that of the corresponding ligands, and the rigidity of the ligand does not allow adaptability. Therefore, selectivity cannot be driven by multiple hydrophobic contacts, which could anyway not suffice to discriminate between small structurally similar ligands. In this case, specific key interactions are more important. Note that very large LBP volumes allow for the binding multiple ligands of different stereochemistry such as in the case of PPAR [77], often at the expense of lower binding affinities.

A structure-based sequence alignment revealed that only three residues diverged in the LBPs of RARα, β, and γ, leading to the prediction that these divergent residues were critically involved in differentiating between isotype-selective retinoids [76]. Indeed, swapping of these residues confirmed this hypothesis [157]. Moreover, swapping of these residues not only mediated isotype-selective binding, but also the agonistic/antagonistic response of a cognate ligand onto any other RAR isotype, thus emphasizing the importance of these three residues in triggering the ligand-induced transcriptional response.

### Antagonist action

To define or understand the agonistic/antagonistic features of a ligand, the following aspects have to be considered/elucidated: Ligands may positively or negatively interfere with receptor activities at various levels. In the case of steroid hormone receptors, they may affect: (i) the stability of the so-called hetero-oligomeric 8S complex, which is proposed to exist in hormonally naive cells and comprises, in addition to steroid receptors, the Hsp90 heat-shock protein and additional associated factors (note that certain receptors, such as TR and RAR, do not for complexes with Hsp90); (ii) the homo- or

heterodimerization ability of the receptor; and (iii) its interaction with the cognate DNA response element.

Nuclear receptors harbor two activation functions (AF-1 and AF-2). These activation functions operate in a cell-type and promoter environment-dependent fashion. Thus, a given antagonist may antagonize only one or both AFs, and an AF-2 antagonist can act as an AF-1 agonist.

While the structural basis of AF-1 activity is still unknown, AF-2s correspond to agonist-induced surfaces that can interact with coactivators. Conversely, nonliganded receptors express a surface(s) that can accommodate corepressors. A given ligand may more or less precisely generate these surfaces and lead to different coregulator recruitment efficiencies. Thus, "superagonists" may enhance coactivator binding more efficiently than the natural ligand, while "inverse agonists" may stabilize the receptor–corepressor complex.

Ligands may act at various levels in the sequence of events that leads to transcriptional activation or silencing. Theoretically, the same ligand may stimulate the recruitment of SMCC, but not of the HAT complex. Indeed, initial evidence for the existence of ligands that differently affect HAT and SMCC subunit recruitment has been provided [154].

## Several types of antagonists

Multiple aspects that have to be considered when analyzing the mechanism of action of an antagonist or when the aim is to design an antagonist with certain characteristics. Below we will discuss first some general principles originating from structural studies and then discuss particular mechanisms and individual antagonists. Note that some analyses have to be considered in the context of the experimental setting because some receptor activities, such as DNA-binding, can be ligand-independent in vitro, but are ligand-dependent in vivo (for an example, see refs. [158,159]). Therefore, some of the results/categories described below may have to be reconsidered as more in vivo data accumulate.

### *Structural basis of AF-2 antagonists: Steric hindrance by the ligand precludes holo position of H12*

Agonists are ligands that lock the receptor in the active conformation. In contrast, antagonists should be viewed as molecules that prevent NRs to adopt this conformation. Helix H12 is a crucial component of the NR LBDs, because its ligand-induced repositioning in the holo NR contributes in a critical manner to the surface recognized by the LxxLL NR boxes of coactivators and thereby generates a transcriptional active AF-2 domain. The original structures of apo- and holo-LBDs (Fig. 4) revealed the ligand-induced conformational changes and suggested that the interactions between H12 or residues in its proximity and the ligand was critical for the control of agonist-antagonist properties of NRs [75,76,84]. The crystal structures of the ERα LBD complexes with raloxifen and tamoxifen or the RARα LBD with BMS614 confirmed this hypothesis and showed an alternative stable antagonist "position" for H12 (compare Fig. 4b), nested between H4 and H3 [82,85,130]. Apparently, steric hindrance upon binding of the bulkier ligands (compared with the agonists) prevents the proper positioning of H12 in its "agonistic" site.

In the antagonist conformation, a most important feature is the lengthening of the loop L11-12, resulting from the unwinding of the C-terminus of helix H11. This enables helix H12 to adopt a second low-energy position by binding to the coactivator LxxLL recognition cleft. In contrast to agonists, which stabilize a long H11 helical conformation, different ligand–receptor interactions at the level of H11 and of the surrounding regions (loop L6-7 and H3) most likely explain the antagonist-induced unwinding of the C-terminal part of this helix. Note that these structural features are found in all antagonist-bound LBD complexes crystallized so far. Hence, it appears that the action described above of pure AF-2 antagonists originates from at least two structural principles. The main feature is the presence of a large "antagonistic" ligand extension that sterically prevents helix H12 to adopt the holo position. Without a holo-H12, no LBD-coactivator interface can be formed. The second structural principle is the

unwinding of helix H11, which allows H12 to bind to the binding groove of coactivator NR box LxxLL motifs. Thus, the second feature of antagonism is the competition between H12 and the NR boxes of coactivators for a common LBD surface.

However, there are additional structural principles of antagonism: In a recent report, Pike and colleagues [160] determined the structure of ERβ LBD in complex with the pure antagonist ICI 164,384. In this case, the ligand completely abolishes the association between the transactivation helix H12 and any part of the LBD. The lack of a stabilized interacting surface containing a holo H12 prevents the binding of a coactivator to the NR LBD, and thus accounts for the "complete" antagonism.

### *Structural basis accounting for full and partial AF-2 agonistic activity of a ligand*

In addition to complete antagonists of the AF-2 function (e.g., raloxifen or tamoxifen for ER; BMS614 for RAR; see above), AF-2 partial agonists/antagonists (Table 3) have been crystallized with the corresponding receptors. In contrast to complete AF-2 antagonists, where there was a clear correlation between H12 positioning in the coactivator cleft (also referred to as "antagonist groove"), two crystal structures have been described in which there is an apparent discrepancy between the orientation of the AF-2 helix and the biological activity of the corresponding ligand. The ERβ/genistein and RXRαF318A/oleic acid LBD structures (for refs., see Table 3) show that H12 can adopt the antagonist conformation even though the corresponding ligand elicits a weak but clear transcriptional AF-2 activity. A likely explanation for the discrepancy between the antagonist location of H12 and the transcriptional activity of these complexes is that these ligands display some, but not all, features of pure AF-2 agonists or antagonists. They can thus be classified as partial AF-2 agonists/antagonists. A major difference between pure and partial antagonists lies in their steric properties. In contrast to full antagonists, genistein and oleic acid do not bear a bulky extension. Thus, they do not sterically preclude the agonist position of H12 and are in this respect similar to agonists. However, they induce unwinding of helix H11, which permits the positioning of helix H12 in the antagonist groove; in this respect, these ligands are similar to antagonists. The structure of PPARγ bound to the mixed agonist-antagonist GW0072 suggests that an additional mechanism might account for the particular biological properties of such ligands [161]. In this case, the partial activity of the ligand is attributed to a poor stabilization of the holo position of H12 as a result of a lack of contact between the ligand and the AF-2 helix. In the presence of such mixed ligands, the equilibrium between the agonist holo position of H12 and its antagonist position in the coactivator binding groove is likely to depend on the intracellular concentration of coactivators and corepressors, and these ligands may act as either AF-2 agonists or antagonists depending on the cellular context.

A novel mode of antagonism was recently suggested by the resolution of the crystal structures of ERα and β in complex with THC (5, 11-*cis*-diethyl-5, 6, 11, 12-tetrahydrochrysene-2, 8-diol). Interestingly, THC acts as an ERα agonist and as an ERβ antagonist. Structure comparison of the two ligand-receptor complexes reveals that THC, which lacks the bulky side chain of pure antagonists, antagonizes ERβ by stabilizing the conformation of several residue side chain from helix H11 and loop L11-12 in such a way that they do not create the proper hydrophobic binding surface for the holo helix H12 [162].

### *Inverse agonists: Stabilization of corepressor interaction*

Whereas unliganded steroid hormone receptors do not appear to interact strongly with SMRT and N-CoR, it has been proposed that some antagonists enhance significantly this interaction [136,138,163]. Moreover, it appears that corepressors interact with steroid receptors occupied by partial antagonists, but not with receptors bound to pure antisteroids. Also, some RAR antagonists reinforce corepressor binding to RAR and enhance silencing [164,165]. The structural basis of this stabilization remains to be established. However, such ligands have to induce an alternative position for H12, which does not occlude the hydrophobic groove formed by H3 and H4. Thus, the AF-2 helix may be inhibitory for full corepressor binding, and its deletion or displacement by some antagonists can potentiate the interaction.

In this respect, the antagonist GW6471 binding to PPARα reinforces the corepressor interaction [135]. In contrast to other antagonist-bound NR structures, the AF-2 helix undergoes a rigid body shift toward the N-terminus of helix H3 and is loosely packed against this helix. The third helical turn in the corepressor motif occupies the space that is left by the repositioning of helix H12 and prevents this helix from adopting its agonist-bound conformation.

## RXR SUBORDINATION IN HETERODIMERS

Many NRs use RXR promiscuously as heterodimerization partner. In contrast to homodimerization, heterodimerization allows, in principle, fine-tuning of NR action by using combinatorial sets of ligands. However, whereas RAR agonists can autonomously activate transcription through RAR-RXR heterodimers, RXR is unable to respond to RXR-selective agonists in the absence of a RAR ligand. Consequently, RXR-selective ligands on their own could not trigger RXR-RAR heterodimer-mediated RA-induced events in various cell systems [159,166,167]. Similarly, RXR cannot autonomously respond to its ligand in the corresponding TR and VDR heterodimers, unless those heterodimeric partners are liganded. This phenomenon, referred to as RXR "subordination" or "silencing", may be of utmost biological importance because it avoids confusion between retinoic acid, thyroid hormone, and vitamin D3 signaling pathways. RXR subordination is, however, not due to an inability of the RXR partner to bind its cognate ligand in DNA-bound heterodimers, as has been suggested [168], as RXR ligand binding has been demonstrated to occur in such complexes [158,169–172]. Several studies investigated whether coregulator interactions could account for the inability of RXR to respond to its agonists. Recently, it has been reported that RXR can bind its ligand and recruit coactivators in heterodimer with apoRAR. However, in the usual cellular environment, corepressors do not dissociate and thus compete with coactivators for binding [164]. Corepressor binding to RAR prevents liganded RXR in the context of the holoRXR/apoRAR heterodimer from assembling a transcription-inducing competent complex. Consequently, the only way for RXR to modulate transactivation in response to its ligand in RXR-RAR heterodimers is through synergy with RAR ligands. This synergy results from increased interaction efficiency of a single coactivator molecule with the heterodimer and requires two intact receptor-binding surfaces on the coactivator. Cooperative TIF2 binding was also observed with some RAR antagonists [164]. Together with such RAR antagonists, pure rexinoid agonists can activate transcription of endogenous target genes [159,164]. All these observations do not support the concept that RXR is a priori a transcriptionally "silent" partner in RAR-RXR heterodimers [168,173].

RXR subordination may not apply to all NR partners, as the ligand-induced RXR activity was permissive in heterodimers with FXR, LXR, PPAR, or NGFI-B [173–175]. However, neither the existence of an endogenous NGFI-B ligand nor a weak constitutive activity of the NGFI-B AF-2 can be excluded; both these scenarios would readily explain RXR activity and NGFI-B-RXR synergy due to the absence of RXR silencing.

## POSTTRANSLATIONAL MODIFICATION BY PHOSPHORYLATION

NRs can be subjected to various posttraductional modifications such as phosphorylation, ubiquitylation, or acetylation [176,177], which act in concert to coordinate NR-mediated transcription [178]. Among these modifications, phosphorylation has been more extensively studied than the others and is increasingly recognized as a signaling cross-talk that affects most if not all NRs. Phosphorylation can modify all major domains of NRs, the N-terminal activation function (AF-1), the LBDs and DBDs (Fig. 2). Phosphorylation of NRs by kinases that are associated with general transcription factors (e.g., cdk7 within TFIIH), or are activated in response to a variety of signals (MAPKs, Akt, PKA, PKC), often facilitates the recruitment of coactivators, or of components of the transcription machinery, and therefore, cooperates with the ligand to enhance transcription activation. But phosphorylation can also contribute to termination of the ligand response through inducing DNA dissociation or NR degradation or through

decreasing ligand affinity. These different modes of regulation reveal an unexpected complexity of the dynamics of NR-mediated transcription. Finally, as phosphorylation can occur in the absence of the ligand, deregulation of NR phosphorylation in certain diseases or cancers may lead to apparently ligand-independent activities.

### Phosphorylation targets mainly two nuclear receptor activation functions and the DNA-binding domain

The majority of the NR-phosphorylated residues lie within the N-terminal A/B region. For PRs, phosphorylation of this region is rather complex, with more than 13 phosphorylation sites. For RARs or PPARs, region A/B phosphorylation concerns only one or two residues. In VDRs, this region is apparently not phosphorylated, probably due to its short size. Most of the modified residues are serines surrounded by prolines and therefore correspond to consensus sites for proline-dependent kinases, which include cyclin-dependent kinases (CDKs) [179,180] and MAP kinases [181–183]. Among these sites, many are phosphorylated by CDKs, either "constitutively" (i.e., in the absence of ligand) or in response to the hormone [184]. MAPKs can hormone-independently phosphorylate other sites in response to a variety of signals, such as growth factors, stress, or cytokines. Several serine kinase cascade pathways lead to activation of MAP kinases (Erks, JNKs, p38MAPK) that can enter the nucleus and phosphorylate NRs. Accordingly, the AF-1 function of PR [185], ERα [22,186], ERβ [187,188], AR [189], PPARs [190,191], RARγ [192,193] have been reported to be substrates for p42/p44 or p38 MAPKs, while that of RXRα is targeted by JNKs [194,195].

The N-terminal A/B region also contains consensus phosphorylation sites for the so-called kinase Akt or PKB [196], which plays an important role in cell survival and proliferation. Akt is regulated positively by the phosphoinositol 3-kinase (PI3K) pathway and negatively by a variety of downstream kinases. Upon translocation into the nucleus, it can phosphorylate NRs, such as ERα [197] and AR [198] in their N-terminal A/B region.

In addition to the N-terminal domain, the LBD of NRs is also a target for ligand-independent phosphorylation, involving the same proline-dependent kinases as above. Thus, RXRα can be targeted by stress kinases (JNKs) [194,195]. However, other kinases, such as tyrosine kinases, may phosphorylate ERα [199] and RXRα [195] or PKA for RARs [200,201].

Finally, NRs can be phosphorylated in their DBD. Phosphorylation of this domain involves either PKA in the case of ERα [202] or PKC for RARα [203] and VDR [204].

### Nuclear receptor phosphorylation by cdks

When bound at their response elements, liganded NRs recruit the transcription machinery, including the general transcription factors. One of them is TFIIH, a multiprotein complex mediating transcription activation and nucleotide excision repair [205]. Several NRs, such as ERα [206,207], RARα [208], RARγ [209], and AR [210], have been reported to interact with TFIIH. Notably, the interaction of ERα with TFIIH involves the same surface as that required for the recruitment of coactivators [206] and, therefore, depends on the conformational changes induced by the ligand. In contrast, RARs interact with TFIIH through an alternative surface(s) that is not modified by the ligand [209].

TFIIH is composed of nine subunits, one of them, cdk7, having a cyclin-dependent kinase activity. As a consequence of their interaction with TFIIH, ERα and RARs have been shown to be phosphorylated in their N-terminal A/B region by cdk7 within TFIIH. This phosphorylation process plays a critical role in receptor-dependent transcription activation, because cells from patients bearing mutations in the XPD subunit of TFIIH, which results in an incorrect positioning of the cdk7 kinase relative to its substrate, have hypophosphorylated RARs that diplay a decreased ligand response [211]. As the serine residues targeted by cdk7 lie within surfaces that interact with transcription factors, their phosphorylation would help the recruiment of coactivators and thus would increase the efficiency of chro-

matin derepression. It could also facilitate the recruitment of components of the transcription machinery and therefore stabilize the formation of the NR transcription complex.

The critical role of NR phosphorylation by cdk7 has been further dissected in the case of RARγ, by using F9 cells which represent a cell-autonomous system for analyzing retinoid signaling (for review, see ref. [212]). In these cells, the retinoid-induced events (primitive endodermal differentiation, growth arrest, and the activation of expression of a number of genes) are transduced by RARγ/RXR heterodimers. Consequently, the various RA responses are abolished in RARγ null cells. By reexpressing in these RARγ null cells, RARγ mutated at the cdk7 phosphorylation sites located in the A/B region, it has been demonstrated that the integrity of these phosphorylation sites is indispensible to the activation of certain RA target genes and for RA-induced F9 cell differentiation [213].

## Regulation of nuclear receptor-mediated transcription through phosphorylation

### *Positive regulation of nuclear receptor transactivation*

NRs are substrates for a multitude of kinases activated by a variety of signals, independently of the ligand. For several NRs, phosphorylation of the N-terminal A/B region by MAPKs (Erks, p38MAPK, JNKs) or Akt facilitates the recruitment of coactivators and, thus, transcription activation.

Supportive evidence for a link between phosphorylation by MAPKs and NR-mediated transcription is the recent finding that the N-terminal AF-1 domain of RARγ can be phosphorylated by p38MAPK [192]. Importantly, this event, which is induced by RA, was found to be also a signal for RARγ ubiquitylation [214]. Moreover, mutations that block phosphorylation and/or ubiquitylation result in defects in the activation of RA target genes controlled by RARγ [192]. It is tempting to speculate that phosphorylation by p38MAPK positively modulates the transcriptional properties of RARγ through the recruitment of the ubiquitylation machinery. However, phosphorylation-dependent ubiquitylation also targets RARγ for degradation by the 26S proteasome [192]. Thus, the modulable equilibrium between transactivation and degradation may represent an efficient mechanism to simultaneously activate RARγ to initiate transcription and tag it for subsequent degradation by the proteasome in a step that attenuates transcription.

Also, phosphorylation of the AF-2 domain can modulate the transcription factor properties of NRs. Phosphorylation by Src kinases of ERα at tyrosine 537 which is close to helix 12, enhances ERα function [215]. Phosphorylation of RARα by PKA at serine 369 also modulates positively the transcriptional activity of the receptor [201]. Phosphorylation by PKA also modulates the parietal endoderm differentiation of F9 cells which occurs subsequently to primitive endodermal differentiation when RA is combined with cAMP and which involves RARα/RXR heterodimers. Accordingly, upon reexpressing in RARα null F9 cells the same receptor mutated at the PKA phosphorylation site, parietal differentiation is delayed [216].

### *Phosphorylation of nuclear receptor coregulators*

The cross-talk between NRs and signal transduction pathways involves not only the phosphorylation of NRs, but also that of their coactivators and corepressors; SRC-1 [217], TIF2 [218], PGC-1 [219], AIB1 [220], and p300/CBP [221,222] are themselves targets for a variety of kinases. Phosphorylation may enhance their interaction with NRs, efficiency to recruit HAT complexes and/or enzymatic activity. In contrast, phosphorylation of corepressors such as SMRT subsequently to the activation of MAPKs cascades, correlates with an inhibition of their interaction with NRs and their redistribution from the nucleus to the cytoplasm [223].

### *Negative regulation of nuclear receptor transactivation by phosphorylation*

Phosphorylation events can also inactivate NRs, possibly to switch off their activity. PKC-dependent phosphorylation of VDRs at the DBD inhibits transcription activation most likely by facilitating promoter escape [204]. Inhibition of the transcriptional activity of other NRs such as ERα and RARα, also

occurs subsequently to phosphorylation of residues located within the DBD dimerization surface, by PKA [202] or PKC [203], respectively.

## NUCLEAR RECEPTORS: PLATFORMS FOR MULTIPLE SIGNAL INTEGRATION

It has become increasingly well documented in the past few years that NR action is not confined to the positive and negative regulation of the expression of cognate target genes. Indeed, these receptors, and most likely also their "downstream" mediators, are targets of other signaling pathways and reciprocally, can modify the activity of such pathways. The best known examples of such a signal transduction "cross-talk" is the mutual repression of NR and AP1 (c-Fos/c-Jun) activities. A distinct type of cross-talk is the modification of NR AF activity by phosphorylation, e.g., by the MAP kinase pathway. The existence of signal transduction "cross-talks" is likely to reflect the integration of NR action in the context of the functional state of the cell in which it is expressed. The importance of signal transduction "cross-talk" in "real life" was recently impressively demonstrated by the observation that GR null mice die at birth, whereas mice harboring a GR mutant ($GR^{dim/dim}$) that can still cross-talk with AP1 but not activate target genes with consensus GR response elements are viable [17]. A particular interesting issue is the possibility to generate NR ligands that can "dissociate" such response element-dependent and cross-talk-dependent gene programming, thus giving rise to the hope of generating ligands with reduced side effects [224].

### Signal transduction cross-talk between nuclear receptors and AP1

In addition to transactivation of their own target genes, certain NRs were shown to cross-talk with other signal transduction pathways. The original observation was made in 1990 when it was observed that GR could inhibit, in a ligand-dependent manner, the ability of AP1 (the heterodimer composed of the proto-oncogene products c-Fos and c-Jun) to transactivate its target gene promoters [225–227]. This trans-repression is mutual and requires an unknown state of the receptor, which can be induced by both agonists and certain, but not all, antagonists. It is important to point out that the nuclear receptor-AP1 cross-talk does not per se imply negative regulation of transcription; several reports show that under certain conditions this cross-talk can lead to positive transcriptional effects [228–230].

The mechanism(s) on nuclear receptor-AP1 cross-talk has remained elusive despite, or because of, several contradictory reports and discrepancies between studies using in vivo and in vitro approaches. For example, the original proposal that AP1 and GR form DNA-abortive complexes (derived from in vitro evidence obtained in gel retardation experiments) appears to represent an in vitro artefact, as in vivo footprinting did not support this concept. Controversially discussed are studies proposing that sequestration of the coactivator CBP (or its homolog p300), which function as mediator of the transcriptional activities of both AP1 and NRs, accounts for the cross-talk phenomenon as transrepression could be relieved when CBP was overexpressed in cells with limiting endogenous levels of this protein [231]. Several other studies, however, have shown that this mechanism can at best only partially account for the observed phenomena [232]. In particular, the use of synthetic ligands that dissociate trans-repression from coactivator recruitment to NRs argue against the involvement of coactivators, since it was shown that antagonists can still transrepress AP1 activity [233]. A second mechanism that has been proposed is based on the observation that estrogen receptors are capable of down-regulating the activity of Jun-kinase, leading to reduced AP1 activity [234]. Thirdly, NR-mediated effects on the dimerization of the AP1 subunits have been observed [235]. Still, further analysis is required to understand the contribution of these various mechanisms to the receptor-AP1 cross-talk. A fourth mechanism proposed to be involved in AP1 nuclear receptor cross-talk is direct physical contact between both factors when bound to so-called composite elements on a promoter [230]. Composite elements are thought to recruit both, AP1 proteins and NRs, bringing them into close physical contact. Depending on the nature

of such response elements, and the activity of the participating proteins, steric effects might lead to differential regulation. It is currently not clear whether this mechanism applies only to promoters that carry composite elements or is of more general significance.

In addition to GR, mutual interference observed between the transcriptional activities of AP1 and RARs and RXRs, ER, TR/v-ErbA, PML-RARα, while MR appears to be insensitive to AP1. In addition, interference between the transactivation abilities of NRs and other transcription factors has been reported. For reviews and references to original work, see refs. [224,236,237].

### NFκB and nuclear receptor cross-talk

The second-best-studied example of transcription factor cross-talk is the mutual interference between GRs and NFκB proteins. This pathway is again of significant importance, since it may also contribute to the anti-inflammative, as well as osteoporotic action of glucocorticoids. Albeit some have suggested that glucocorticoid action can be attributed to the increased production of the NFκB inhibitory molecule IκB, which in turn would sequester active NFκB in the cell nucleus [238], studies with mutant receptors and "dissociated" glucocorticoids showing IκB-independent repression of NFκB activity are incompatible with a simple IκB-mediated mechanism [239]. The underlying molecular events are still elusive, but may be related to those discussed above for the AP1 cross-talk. Note that as in the case of AP1 [233], squelching of limiting amounts of CBP, which also coactivates NFκB, is unlikely to be involved [240] and GR ligands that dissociate transactivation from transrepression still induce transcriptional interference with NFκB signaling [239]. For another NR, PPAR, a positive cross-talk with NFκB signaling pathways due to PPAR response element-independent IκB induction has been observed recently [241].

Other transcription factors that are cross-regulated in their activities by NRs are Oct 2A, RelA (another NFκB family member), STAT5, and Spi-1/PU.1 (for references to original work, see ref. [237]).

## DEREGULATION IN DISEASE AND NOVEL THERAPEUTIC TARGETS

Given the major impact of NR signaling on animal physiology, it is no surprise to find aberrant NR function at the basis of multiple pathologies. Indeed, synthetic agonists and antagonists have been developed and are in clinical use for endocrine therapies of cancer as well as hormone replacement therapies in osteoporosis. Thiazolidinediones (TZDs), known as insulin "sensitizers" in the treatment of noninsulin-dependent diabetes, have been recognized as PPARγ agonists some time ago, and the recent establishment of a link between human type 2 diabetes and PPARγ mutation has proven that PPARγ malfunction can lead to severe insulin resistance, diabetes mellitus, and hypertension [242]. Considering in addition the success story of retinoids in the therapy of acute promyelocytic leukemia [243,244] and the promise of cancer therapy and prevention by NR-derived drugs [245–247], it becomes clear that the NR family is of outstanding importance for both diagnosis and drug design. But this is likely to represent only the top of the iceberg, and novel types of NR-based drugs are expected to be developed based on our increasing knowledge on the structural and molecular details of NR and ligand function, and the elucidation of the signaling pathways involved in (patho)physiological events.

### Nuclear receptor-associated diseases

Nuclear receptors have a major role on human health and disease. Indeed, if their alteration causes pathological syndroms their activity contributes to therapy via agonist or antagonist drugs. Several observations have shown that deregulation of the NR genes leads to specific human diseases. For example, mutations of TRβ have been associated with the syndrome of resistance to thyroid hormone characterized by reduced thyroid hormone action in the presence of high levels of TSH, T3, and T4. Most

commonly, these mutations—located in the LBD of the TRβ—reduce its affinity for thyroid hormones, interfere with the function of the wild-type TR, and impair interaction with cofactors involved in TR action. Affected patients present delayed bone maturation, heart abnormalities, hearing defects, and mental retardation [248–254].

Also, RARs have been associated with several diseases, among which cancer is one of the most important. The prototype of cancer that always involves RARα gene translocation is acute promyelocytic leukemia (APL) [244,245,255–257]. In the vast majority of cases, the origin of APL, according to the French-American-British classification a M3 type of acute myeloid leukemia (AML FAB-M3 or AML3), is a t(15;17)(22; q11.2-12) chromosomal translocation that fuses the PML (promyelocytic leukemia gene) and RARα genes. In rare cases, alternate chromosomal translocations generate RARα fusion proteins in which PML is replaced with PLZF [t(11; 17)(q23; q21)], NUMA PLZF [t(11; 17)(q13; q21)], NPM PLZF [t(5; 17)(q32; q21)], or STAT5b [258]. In contrast to wild-type RARα, only pharmacological doses of ATRA can dissociate the HDAC-containing corepressor complex from PML-RARα. PLZF-RARα binds corepressors through both the apo-RARα and PLZF moieties and ATRA cannot release HDACs. Consequently, PLZF-RARα remains a transcriptional repressor in the presence of ATRA. However, high concentrations of HDAC inhibitor (HDACi) convert also PLZF-RARα into an activator of the retinoic acid signaling pathway [259–261]. PML-RARα exhibits different oligomerization characteristics than RARα and essentially acts as a dominant silencing transcription factor that represses transcription activation mediated by the RAR-RXR heterodimer, which can still originate from the intact RARα allele. PML-RARα interacts with PML and causes nuclear body (NB) disintegration and aberrant localization of PML(-RARα) and other NB constituents. The consequence of the formation of PML-RARα is a block of differentiation at the promyelocytic stage. It is reasonable to assume that the altered functionality of PML in the fusion protein, such as its pro-apoptogenic activity, adds to the growth potential of APL blasts, while HDAC-dependent silencing of "normal" retinoid signaling during myelopoiesis causes the differentiation block.

The molecular analysis of NR action and APL explain also the basis of the retinoid therapy: ATRA, RARα agonists, or RARα agonists/antagonists in synergy with rexinoid agonists [159], bind to the PML-RARα LBD, resulting in allosteric transconformation [262] that dissociates the corepressor complex from the LBD. This event relieves the HDAC-dependent block of differentiation and through association of coactivator complexes, triggers the transcriptional regulation of cognate gene programs normally controlled by the RARα-RXR heterodimer. An exciting observation is originated from the recent analysis of the gene programs induced by ATRA in APL cells. In addition to the induction of anti-apoptotic and survival programs, ATRA induces postmaturation apoptosis through the induction of TRAIL [263], a tumor-selective death ligand [264], as well as caspase 8/10 that mediate TRAIL action through the cognate DR5 receptor.

NR deregulation is also linked with human metabolic disease. The PPARs have been implicated in dyslipidemia, diabetes, obesity, atherosclerosis, and inflammation control, and more recently their role in cancer has been suggested [59]. A mutation of the human PPARγ in the MAP kinase target sequence of the A/B region has been described in obese patients, while two mutations that destabilize helix H12 have been found in the LBD of PPARγ in patients with diabetes mellitus, insulin resistance, and hypertension [242,265,266]. Furthermore, a common polymorphism of PPARγ, Pro12 changed to Ala, has been associated with a decreased risk of type 2 diabetes [267]. The observation that the LBD of the human PPARγ gene was inactivated by point mutations or frame shifts in cases of sporadic colon cancer leads to the proposal that PPARγ is a tumor suppressor gene, and this is in accordance with its antiproliferative effect [268]. These results suggest that PPARγ ligands may have a potential as anticancer agents.

Also, VDR mutations have been linked to human pathology because they were found in hypocalcemic patients [269,270]. Interestingly, a link between VDR gene polymorphism and hyperparathyroidism has been reported [271].

Several studies have addressed the impact of ER mutations on cancer and osteoporosis. A patient that exhibited severe osteoporosis, cardiovascular alterations, with normal genitalia and sperm density, was found to have estrogen insensitivity due to homozygous mutation in the exon 2 of the ERα gene, resulting in a premature stop codon [272]. A large number of studies have shown that ERs can regulate genes implicated in the control of normal and tumoral cell growth. Epidemiological studies suggest that estrogens and ERs are critical in breast cancer etiology. Mutated, truncated, alternatively spliced versions of ERα have been detected in hormone-resistant breast cancer samples, but the precise role of these events in pathogenesis is still unclear [273]. As ER target gene, PR expression is a routine marker for endocrine therapy responsivity and prognosis of breast cancer.

Up to 200 different naturally occurring mutations of the AR gene have been described (see <http://ww2.mcgill.ca/androgendb/>). Mutations found in androgen insensitivity syndrome are located in DBDs and LBDs of the receptor causing androgen insensitivity due to alterations of DNA-binding or impaired ligand-binding activity. Both prostate cancer and spinal/bulbar muscular atrophy have been linked to variation in the number of Gln residues found in the polyglutamine repeat which normally contains 16–39 residues. Expansion of the repeat to 40–65 residues leads to spinal/bulbar muscular atrophy, whereas reduction in the number of repeats confers a higher risk for prostate cancer [274,275]. Furthermore, the amplification of AR is implicated in the androgen resistance of prostate tumors, although other mechanisms have been suggested. Recently, it was shown that two mutations in the LBD of AR allow this gene product to function as a high-affinity GR and reduce its ability to bind androgens [276,277].

Finally, the generalized inherited glucocorticoid resistance or familial glucocorticoid resistance (FGR) is associated with alterations of GR. These patients have mutations or deletions in the GR and exhibit high levels of circulating corticoids. For example, a single amino acid substitution in the GR LBD resulted in reduced binding affinity for glucocorticoids [278], while altered splicing of exon 6 led to FGR in a female patient presenting with hirsutism, menstrual abnormalities, and acne due to ACTH-induced hyper-secretion of androgens [279]. Further information can be found at the glucocorticoid receptor resource (<http://biochem1.basic-sci.georgetown.edu/grr/grr.html>).

## Novel perspectives for nuclear receptor-based therapies

In addition to the well-established endocrine therapies of breast and prostate cancers, and to the more recent differentiation therapy of acute promyelocytic leukemia by retinoids, novel synthetic NR ligands are of considerable interest for the therapy and prevention of different types of cancers [246,247]. The development of novel types of NR ligands is facilitated by recent pharmacological and chemical developments, such as (i) combinatorial chemistry, computer-assisted ligand docking based on LBD crystal structure and ultra-high-throughput screening with NR-based reporter systems, (ii) the possibility to dissociate NR-associated functions such as transactivation and cross-talk with other signaling pathways, and (iii) the possibility to generate receptor and receptor isotype-selective ligands.

### *Nuclear receptor coactivators and cancer*

It is tempting to speculate that coactivators are not entirely promiscuous in their choice of NRs. For example, only AIB1/RAC3 is found to be amplified in breast cancer cells while the expression level of the other two family members remains constant, reflecting estrogen receptor specificity [280]. Furthermore, in some types of acute myeloid leukemia (AML), a chromosomal translocation specifically fuses a monocytic zinc finger protein of unknown function (MOZ) to the C-terminus of TIF2 and not any of the other two TIF2 family members [281–283], again reflecting a bias toward one specific coactivator. Interestingly, the observation has been made that overexpression of coactivators of the TIF2/AIB1 family can lead in some systems to ligand-independent activity under certain conditions [79]. This suggests that transcriptional mediators may possibly be involved in the origin and/or progression of proliferative diseases and may become novel pharmacological targets. Indeed, mutations of the CBP gene have been

implicated in the cause of Rubinstein-Taybi syndrome, and alterations of the p300 gene were found associated with gastric and colorectal carcinomas. Based on these various data, it is tempting to speculate that alterations in the cellular abundance of coregulators, or altered substrate specificity of the associated enzymatic functions may lead to pathological states.

### *Nuclear receptor phosphorylation and cancer*

Several lines of evidence indicate that NR phosphorylation plays a crucial role in the development of certain cancers such as breast, ovarian, and prostate cancers. In most of these tumors, the MAPK and Akt kinase pathways exert increased activity, due to amplification of receptor protein-tyrosine kinases (RPTKs) [284] such as HER-2/neu [285] or deregulated activity of cytoplasmic protein-tyrosine kinases (c-Src, c-Abl, or bcr-Abl). This has been correlated with a ligand-independent transactivation of estrogen and androgen receptors [286]. Evidence is accumulating that this effect results from ligand-independent phosphorylation of the AF-1 domain of AR and ER by the disregulated MAPKs or Akt (see above). Phosphorylation would create receptors that activate transcription independently of the ligand. Such "outlaw" receptors would account for estrogen- or androgen-independent growth of prostate and breast cancer cells and for the failure of androgen ablation or tamoxifen therapy.

### *Toward novel types of synthetic nuclear receptor ligands for therapy*

Synthetic ligands of NRs are classified as agonist and antagonist with respect to a particular receptor-associated function [224]. This discrimination is not always obvious, since a particular ligand might antagonize some activities while functioning as agonist for other activities. Examples are the ERα antagonists hydroxytamoxifen and ICI164.384. Hydroxytamoxifen antagonizes the activation function AF-2, but it acts as agonist for AF-1, whereas ICI164.384 in turn antagonizes both AF-1 and AF-2 [20]. Similarly, certain retinoid receptor antagonist are agonists for AP1 repression [233]. It is, therefore, important to consider, where possible, the molecular basis of the anticipated action of an NR to increase drug efficacy and limit side effects. If the molecular mechanism is unknown, it may be wise to use screening paradigms that consider the multiple dimensions of receptor activities.

Another twist to the classification of synthetic NR ligands results from the availability of isotype-specific ligands. These compounds affect one isotype of NR, but not another. The interspecies conservation of retinoid receptor isoforms, together with results obtained with isotype selective retinoids and gene ablation studies, have established that each of the three retinoic acid receptor genes has a cognate spectrum of functions [15]. Given the pharmacological potential of retinoids, the development of isotype-specific ligands has attracted much attention. Today, a wealth of synthetic retinoids exist, which display either isotype specificity or act as mixed agonists/antagonists for the three retinoic acid receptors [157]. Some of these retinoids were found to display cell specificity, and their pharmacological potential is currently investigated. RXR-specific ligands are also being developed, which is of particular interest in view of the role of retinoid X receptor as the promiscuous heterodimerization partner in a number of signaling pathways (see also "RXR subordination"). A recent report suggests that RXR ligands may stimulate insulin action in noninsulin-dependent diabetes [287] through a PPARγ-RXR heterodimer that is responsive to thiazolidinediones. It is thus conceivable that pathway-specific RXR ligands can be generated.

## CONCLUSIONS

Nuclear receptors are ligand-regulated transcription factors that have evolved from an ancestral orphan receptor into a highly diverse family present throughout the entire animal kingdom and encompassing receptors for steroid and nonsteroid hormones, vitamins, corticoids, and metabolic intermediates. These receptors signal through endocrine, paracrine, autocrine, and intracrine modes of action to regulate multiple aspects of animal physiology, such as homeostasis, development, and reproduction. They regulate target genes that they either bind directly as mono-, homo- or heterodimers at cognate response elements, and have the ability to indirectly modulate other gene expression programs ("signal transduction

cross-talks"). Through the coordinated expression of genetic programs, NRs contribute to cell fate-determining processes, thereby shaping and sustaining the organism. The inducing signal- binding of the ligand induces a major allosteric change in the LBD, which is transformed into cascades of protein-protein recognition paradigms inducing coregulator and cointegrator proteins.

Direct transcriptional repression in the absence of ligand or the presence of certain antagonists by some NRs is mediated by corepressor complexes that are associated with the unliganded receptor and condense the chromatin environment at the promoter region through histone deacetylation. Corepressors interact by virtue of their CoRNR boxes with nonliganded NRs. Upon ligand binding, the allosteric change in the LBD induces corepressor dissociation, and coactivator complexes are recruited. Bona fide coactivators recognize the active NR LBD (AF-2) via conserved LxxLL NR boxes and often the N-terminal activation function AF-1. The NR boxes of coactivators and CoRNR boxes of corepressor bind to topologically similar sites in the LBD, but the surfaces are entirely distinct due to the agonist-induced conformational changes. In particular, the holo-H12 is required for coactivator, but incompatible with corepressor binding. Coactivator complexes reverse the repressive effects of chromatin by specific histone acetylation, and allow access of the basal transcription machinery. In a subsequent step, the mammalian SMCC mediator is recruited to the NR and possibly stabilizes the formation of the preinitiation complex at target gene promoters. SMCC recruitment might be regulated by the acetylation and subsequent dissociations of TIF2 family members allowing thus SMCC-receptor association.

Despite their direct actions on the chromatin environment and the transcription machinery, NRs also regulate transcription by positive and negative interference with other signaling pathways. Different mechanisms for such transcription factor cross-talk have been described, but none of them is fully accepted and can explain all aspects of the particular cross-talk. The activity of NRs is regulated by phosphorylation that may serve to fine-tune the signaling and/or to establish a link to other signaling pathway. Finally, the promoter context, and the temporal order of incoming signals on a particular promoter have the likelihood of adjusting the transcriptional potential of NRs to particular situations. Taken together, NRs serve as platforms to coordinate cognate signals with those emanating from other signaling pathways, thereby integrating the NR signal into the functional context of cellular state and activity.

Nuclear receptors and their coregulators have been implicated in several diseases. Their role as key regulatory molecules in a wide variety of signaling pathways qualifies them as novel pharmacological targets. The ongoing improvement of synthetic NR ligands with altered specificity is likely to improve therapy and reduce side effects.

Future research on NRs still has to answer important questions. What are the constituents of the genetic programs that are governed by a given NR? How are the NR signals matched and complemented with other signaling cascades? What are the precise molecular events leading to the variety of transcriptional effects exerted by NRs? Once these questions have been addressed adequately, specific interference into these immensely complex systems might lead to the successful control and reprogramming of an organism's physiology and pathology. Understanding of NR-controlled transcription will shed light on the general and signaling pathway-selective control of gene expression. In this respect, the use of gene arrays together with the information derived from the genome sequencing will certainly have enormous impact.

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## REFERENCES


1. A unified nomenclature system for the nuclear receptor superfamily [letter]. *Cell* **97**, 161–163 (1999).
2. M. Robinson-Rechavi, A. S. Carpentier, M. Duffraisse, V. Laudet. "How many nuclear hormone receptors are there in the human genome?", *Trends Genet.* **17**, 554–556 (2001).
3. J. M. Maglich et al. "Comparison of complete nuclear receptor sets from the human, Caenorhabditis elegans and Drosophila genomes", *Genome Biol.* **2** (2001).
4. P. A. Chambon. "Decade of molecular biology of retinoic acid receptors", *FASEB J.* **10**, 940–954 (1996).
5. V. Laudet and H. Gronemeyer. *The Nuclear Receptor Facts Book,* Academic Press, San Diego (2002).
6. T. T. Lu, J. J. Repa, D. J. Mangelsdorf. "Orphan nuclear receptors as eLiXiRs and FiXeRs of sterol metabolism", *J. Biol. Chem.* **276**, 37735–37738 (2001).
7. W. Xie and R. M. Evans. "Orphan nuclear receptors: the exotics of xenobiotics", *J. Biol. Chem.* **276**, 37739–37742 (2001).
8. G. B. Tremblay, D. Bergeron, V. Giguere. "4-Hydroxytamoxifen is an isoform-specific inhibitor of orphan estrogen-receptor-related (ERR) nuclear receptors beta and gamma", *Endocrinology* **142**, 4572–4575 (2001).
9. G. B. Tremblay et al. "Diethylstilbestrol regulates trophoblast stem cell differentiation as a ligand of orphan nuclear receptor ERR beta", *Genes Dev*. **15**, 833–838 (2001).
10. T. M. Willson and S. A. Kliewer. "PXR, CAR and drug metabolism", *Nat. Rev. Drug Discov.* **1**, 259–266 (2002).
11. X. Luo, Y. Ikeda, K. L. Parker. "A cell-specific nuclear receptor is essential for adrenal and gonadal development and sexual differentiation", *Cell* **77**, 481–490 (1994).
12. Y. Barak et al. "PPAR gamma is required for placental, cardiac, and adipose tissue development. *Mol. Cell* **4**, 585–595 (1999).
13. B. Mascrez et al. "The RXRalpha ligand-dependent activation function 2 (AF-2) is important for mouse development", *Development* **125**, 4691–4707 (1998).
14. M. Beato, P. Herrlich, G. Schutz. "Steroid hormone receptors: many actors in search of a plot", *Cell* **83**, 851–857 (1995).
15. P. Kastner, M. Mark, P. Chambon. "Nonsteroid nuclear receptors: what are genetic studies telling us about their role in real life?", *Cell* **83**, 859–869 (1995).
16. M. Y. Chiang et al. "An essential role for retinoid receptors RARbeta and RXRgamma in long-term potentiation and depression", *Neuron* **21**, 1353–1361 (1998).
17. H. M. Reichardt et al. "DNA binding of the glucocorticoid receptor is not essential for survival", *Cell* **93**, 531–541 (1998).
18. A. Krust et al. "The chicken oestrogen receptor sequence: homology with v-erbA and the human oestrogen and glucocorticoid receptors", *EMBO J.* **5**, 891–897 (1986).
19. M. T. Bocquel, V. Kumar, C. Stricker, P. Chambon, H. Gronemeyer. "The contribution of the N- and C-terminal regions of steroid receptors to activation of transcription is both receptor and cell-specific", *Nucleic Acids Res.* **17**, 2581–2595 (1989).
20. M. Berry, D. Metzger, P. Chambon. "Role of the two activating domains of the oestrogen receptor in the cell-type and promoter-context dependent agonistic activity of the anti-oestrogen 4-hydroxytamoxifen", *EMBO J.* **9**, 2811–2818 (1990).
21. C. A. Sartorius et al. "A third transactivation function (AF3) of human progesterone receptors located in the unique N-terminal segment of the B-isoform", *Mol. Endocrinol.* **8**, 1347–1360 (1994).
22. S. Kato et al. "Activation of the estrogen receptor through phosphorylation by mitogen- activated protein kinase", *Science* **270**, 1491–1494 (1995).

23. M. Watanabe et al. "A subfamily of RNA-binding DEAD-box proteins acts as an estrogen receptor alpha coactivator through the N-terminal activation domain (AF-1) with an RNA coactivator, SRA", *EMBO J.* **20**, 1341–1352 (2001).
24. T. Ylikomi, M. T. Bocquel, M. Berry, H. Gronemeyer, P. Chambon. "Cooperation of proto-signals for nuclear accumulation of estrogen and progesterone receptors", *EMBO J.* **11**, 3681–3694 (1992).
25. A. Guiochon-Mantel, K. Delabre, P. Lescop, E. Milgrom. "Nuclear localization signals also mediate the outward movement of proteins from the nucleus", *Proc. Natl. Acad. Sci. USA* **91**, 7179–7183 (1994).
26. D. Walker, H. Htun, G. L. Hager. "Using inducible vectors to study intracellular trafficking of GFP- tagged steroid/nuclear receptors in living cells", *Methods* **19**, 386–393 (1999).
27. G. L. Hager, C. S. Lim, C. Elbi, C. T. Baumann. "Trafficking of nuclear receptors in living cells", *J. Steroid Biochem. Mol. Biol.* **74**, 249–254 (2000).
28. G. A. Peters and S. A. Khan. "Estrogen receptor domains E and F: role in dimerization and interaction with coactivator RIP-140", *Mol. Endocrinol.* **13**, 286–296 (1999).
29. F. M. Sladek, M. D. Ruse, Jr., L. Nepomuceno, S. M. Huang, M. R. Stallcup. "Modulation of transcriptional activation and coactivator interaction by a splicing variation in the F domain of nuclear receptor hepatocyte nuclear factor 4alpha1", *Mol. Cell Biol.* **19**, 6509–6522 (1999).
30. M. M. Montano, V. Muller, A. Trobaugh, B. S. Katzenellenbogen. "The carboxy-terminal F domain of the human estrogen receptor: role in the transcriptional activity of the receptor and the effectiveness of antiestrogens as estrogen antagonists", *Mol. Endocrinol.* **9**, 814–825 (1995).
31. M. Nichols, J. M. Rientjes, A. F. Stewart. "Different positioning of the ligand-binding domain helix 12 and the F domain of the estrogen receptor accounts for functional differences between agonists and antagonists", *EMBO J.* **17**, 765–773 (1998).
32. K. Umesono, K. K. Murakami, C. C. Thompson, R. M. Evans. "Direct repeats as selective response elements for the thyroid hormone, retinoic acid, and vitamin D3 receptors", *Cell* **65**, 1255–1266 (1991).
33. S. A. Kliewer, K. Umesono, D. J. Noonan, R. A. Heyman, R. M. Evans. "Convergence of 9-cis retinoic acid and peroxisome proliferator signalling pathways through heterodimer formation of their receptors", *Nature* **358**, 771–774 (1992).
34. S. Mader et al. "The patterns of binding of RAR, RXR and TR homo- and heterodimers to direct repeats are dictated by the binding specificites of the DNA binding domains", *EMBO J.* **12**, 5029–5041 (1993).
35. F. Rastinejad, T. Perlmann, R. M. Evans, P. B. Sigler. "Structural determinants of nuclear receptor assembly on DNA direct repeats", *Nature* **375**, 203–11 (1995).
36. G. Meinke and P. B. Sigler. "DNA-binding mechanism of the monomeric orphan nuclear receptor NGFI-B", *Nat. Struct. Biol.* **6**, 471–477 (1999).
37. V. G. Thackray, B. A. Lieberman, S. K. Nordeen. "Differential gene induction by glucocorticoid and progesterone receptors", *J. Steroid Biochem. Mol. Biol.* **66**, 171–178 (1998).
38. G. Klock, U. Strahle, G. Schutz. "Oestrogen and glucocorticoid responsive elements are closely related but distinct", *Nature* **329**, 734–736 (1987).
39. C. K. Glass. "Differential recognition of target genes by nuclear receptor monomers, dimers, and heterodimers", *Endocr. Rev.* **15**, 391–407 (1994).
40. M. Tsai, and B. O'Malley. "Molecular mechanisms of action of steroid/thyroid receptor superfamily", *Ann. Rev. Biochem.* **63**, 451–486 (1994).
41. H. Gronemeyer and V. Laudet. "Transcription factors 3: Nuclear receptors", *Protein Profile* **2**, 1173–1308 (1995).
42. B. Desvergne and W. Wahli. "Peroxisome proliferator-activated receptors: nuclear control of metabolism", *Endocr. Rev.* **20**, 649–688 (1999).

43. S. A. Kliewer, J. M. Lehmann, M. V. Milburn, T. M. Willson. "The PPARs and PXRs: nuclear xenobiotic receptors that define novel hormone signaling pathways", *Recent Prog. Horm. Res.* **54**, 345–367 (1999).
44. H. de The, M. M. Vivanco-Ruiz, P. Tiollais, H. Stunnenberg, A. Dejean. "Identification of a retinoic acid responsive element in the retinoic acid receptor beta gene", *Nature* **343**, 177–180 (1990).
45. B. Hoffmann et al. "A retinoic acid receptor-specific element controls the retinoic acid receptor-beta promoter", *Mol. Endocrinol.* **4**, 1727–1736 (1990).
46. H. M. Sucov, K. K. Murakami, R. M. Evans. "Characterization of an autoregulated response element in the mouse retinoic acid receptor type beta gene", *Proc. Natl. Acad. Sci. USA* **87**, 5392–5396 (1990).
47. D. M. Heery, B. Pierrat, H. Gronemeyer, P. Chambon, R. Losson. "Homo- and heterodimers of the retinoid X receptor (RXR) activated transcription in yeast", *Nucleic Acids Res.* **22**, 726–731 (1994).
48. D. J. Mangelsdorf et al. "A direct repeat in the cellular retinol-binding protein type II gene confers differential regulation by RXR and RAR", *Cell* **66**, 555–561 (1991).
49. H. Chen, Z. Smit-McBride, S. Lewis, M. Sharif, M. L. Privalsky. "Nuclear hormone receptors involved in neoplasia: erb A exhibits a novel DNA sequence specificity determined by amino acids outside of the zinc- finger domain", *Mol. Cell Biol.* **13**, 2366–2376 (1993).
50. H. W. Chen and M. L. Privalsky. "The erbA oncogene represses the actions of both retinoid X and retinoid A receptors but does so by distinct mechanisms", *Mol. Cell Biol.* **13**, 5970–5980 (1993).
51. B. M. Forman, J. Casanova, B. M. Raaka, J. Ghysdael, H. H. Samuels. "Half-site spacing and orientation determines whether thyroid hormone and retinoic acid receptors and related factors bind to DNA response elements as monomers, homodimers, or heterodimers. *Mol. Endocrinol*. **6**, 429–442 (1992).
52. C. K. Glass et al. "A c-erb-A binding site in rat growth hormone gene mediates trans- activation by thyroid hormone", *Nature* **329**, 738–741 (1987).
53. C. K. Glass, J. M. Holloway, O. V. Devary, M. G. Rosenfeld. "The thyroid hormone receptor binds with opposite transcriptional effects to a common sequence motif in thyroid hormone and estrogen response elements", *Cell* **54**, 313–323 (1988).
54. P. P. Harding and G. Duester. "Retinoic acid activation and thyroid hormone repression of the human alcohol dehydrogenase gene ADH3", *J. Biol. Chem.* **267**, 14145–14150 (1992).
55. J. Burnside, D. S. Darling, F. E. Carr, W. W. Chin. "Thyroid hormone regulation of the rat glycoprotein hormone alpha- subunit gene promoter activity", *J. Biol. Chem.* **264**, 6886–6891 (1989).
56. A. Farsetti, B. Desvergne, P. Hallenbeck, J. Robbins, V. M. Nikodem. "Characterization of myelin basic protein thyroid hormone response element and its function in the context of native and heterologous promoter", *J. Biol. Chem.* **267**, 15784–15788 (1992).
57. M. R. Haussler et al. "The vitamin D hormone and its nuclear receptor: molecular actions and disease states. *J. Endocrinol.* **154 Suppl**, S57–73 (1997).
58. H. F. DeLuca and C. Zierold. "Mechanisms and functions of vitamin D", *Nutr. Rev.* **56**, S4–10; discussion S54–75 (1998).
59. S. Kersten, B. Desvergne, W. Wahli. "Roles of PPARs in health and disease", *Nature* **405**, 421–424 (2000).
60. T. M. Willson, P. J. Brown, D. D. Sternbach, B. R. Henke. "The PPARs: from orphan receptors to drug discovery", *J. Med. Chem.* **43**, 527–550 (2000).
61. S. Khorasanizadeh and F. Rastinejad. "Nuclear-receptor interactions on DNA-response elements", *Trends BioChem. Sci.* **26**, 384–390 (2001).
62. F. Rastinejad, T. Wagner, Q. Zhao, S. Khorasanizadeh. "Structure of the RXR-RAR DNA-binding complex on the retinoic acid response element DR1", *EMBO J.* **19**, 1045–1054 (2000).

63. S. Green and P. Chambon. "Oestradiol induction of a glucocorticoid-responsive gene by a chimaeric receptor", *Nature* **325**, 75–78 (1987).
64. S. Green, V. Kumar, I. Theulaz, W. Wahli, P. Chambon. "The N-terminal DNA-binding 'zinc finger' of the oestrogen and glucocorticoid receptors determines target gene specificity", *EMBO J.* **7**, 3037–3044 (1988).
65. M. Danielsen, L. Hinck, G. M. Ringold. "Two amino acids within the knuckle of the first zinc finger specify DNA response element activation by the glucocorticoid receptor", *Cell* **57**, 1131–1138 (1989).
66. S. Mader, V. Kumar, H. de Verneuil, P. Chambon. "Three amino acids of the oestrogen receptor are essential to its ability to distinguish an oestrogen from a glucocorticoid-responsive element", *Nature* **338**, 271–274 (1989).
67. K. Umesono and R. M. Evans. "Determinants of target gene specificity for steroid/thyroid hormone receptors", *Cell* **57**, 1139–1146 (1989).
68. T. E. Wilson, T. J. Fahrner, M. Johnston, J. Milbrandt. "Identification of the DNA binding site for NGFI-B by genetic selection in yeast", *Science* **252**, 1296–1300 (1991).
69. T. E. Wilson, T. J. Fahrner, J. Milbrandt. "The orphan receptors NGFI-B and steroidogenic factor 1 establish monomer binding as a third paradigm of nuclear receptor-DNA interaction", *Mol. Cell Biol.* **13**, 5794–5804 (1993).
70. C. Zechel et al. "The dimerization interfaces formed between the DNA binding domains of RXR, RAR and TR determine the binding specificity and polarity of the full-length receptors to direct repeats", *EMBO J.* **13**, 1425–1433 (1994).
71. C. Zechel, X. Q. Shen, P. Chambon, H. Gronemeyer. "Dimerization interfaces formed between the DNA binding domains determine the cooperative binding of RXR/RAR and RXR/TR heterodimers to DR5 and DR4 elements", *EMBO J.* **13**, 1414–1424 (1994).
72. T. E. Wilson, R. E. Paulsen, K. A. Padgett, J. Milbrandt. "Participation of non-zinc finger residues in DNA binding by two nuclear orphan receptors", *Science* **256**, 107–110 (1992).
73. M. S. Lee, S. A. Kliewer, J. Provencal, P. E. Wright, R. M. Evans. "Structure of the retinoid X receptor alpha DNA binding domain: a helix required for homodimeric DNA binding", *Science* **260**, 1117–11121 (1993).
74. J. W. Schwabe, L. Chapman, J. T. Finch, D. Rhodes. "The crystal structure of the estrogen receptor DNA-binding domain bound to DNA: how receptors discriminate between their response elements", *Cell* **75**, 567–578 (1993).
75. J. M. Wurtz et al. "A canonical structure for the ligand-binding domain of nuclear receptors", [published erratum appears in *Nat Struct Biol.* Feb. 3 (2), 206 (1996)]. *Nat. Struct. Biol.* **3**, 87–94 (1996).
76. J. P. Renaud et al. "Crystal structure of the RAR-gamma ligand-binding domain bound to all-trans retinoic acid. *Nature* **378**, 681–689 (1995).
77. R. T. Nolte et al. "Ligand binding and co-activator assembly of the peroxisome proliferator- activated receptor-gamma", *Nature* **395**, 137–143 (1998).
78. R. White, M. Sjoberg, E. Kalkhoven, M. G. Parker. "Ligand-independent activation of the oestrogen receptor by mutation of a conserved tyrosine", *EMBO J.* **16**, 1427–1435 (1997).
79. J. J. Voegel et al. "The coactivator TIF2 contains three nuclear receptor-binding motifs and mediates transactivation through CBP binding-dependent and -independent pathways", *EMBO J.* **17**, 507–519 (1998).
80. K. E. Weis, K. Ekena, J. A. Thomas, G. Lazennec, B. S. Katzenellenbogen, "Constitutively active human estrogen receptors containing amino acid substitutions for tyrosine 537 in the receptor protein. *Mol. Endocrinol.* **10**, 1388–1398 (1996).
81. V. Vivat et al. "A mutation mimicking ligand-induced conformational change yields a constitutive RXR that senses allosteric effects in heterodimers", *EMBO J.* **16**, 5697–5709 (1997).

82. W. Bourguet et al. Crystal structure of a heterodimeric complex of RAR and RXR ligand- binding domains", *Mol. Cell* **5**, 289–298 (2000).
83. R. T. Gampe, Jr. et al. "Asymmetry in the PPARgamma/RXRalpha crystal structure reveals the molecular basis of heterodimerization among nuclear receptors", *Mol. Cell* **5**, 545–555 (2000).
84. W. Bourguet, M. Ruff, P. Chambon, H. Gronemeyer, D. Moras. "Crystal structure of the ligand-binding domain of the human nuclear receptor RXR-alpha", *Nature* **375**, 377–382 (1995).
85. A. M. Brzozowski et al. "Molecular basis of agonism and antagonism in the oestrogen receptor", *Nature* **389**, 753–758 (1997).
86. R. K. Bledsoe et al. "Crystal structure of the glucocorticoid receptor ligand binding domain reveals a novel mode of receptor dimerization and coactivator recognition", *Cell* **110**, 93–105 (2002).
87. B. P. Klaholz et al. "Conformational adaptation of agonists to the human nuclear receptor RAR gamma", *Nat. Struct. Biol.* **5**, 199–202 (1998).
88. R. E. Watkins et al. "The human nuclear xenobiotic receptor PXR: structural determinants of directed promiscuity", *Science* **292**, 2329–2333 (2001).
89. J. D. Chen. "Steroid/nuclear receptor coactivators", *Vitam. Horm.* **58**, 391–448 (2000).
90. C. K. Glass and M. G. Rosenfeld. "The coregulator exchange in transcriptional functions of nuclear receptors", *Genes Dev.* **14**, 121–141 (2000).
91. H. Chen, M. Tini, R. M. Evans. "HATs on and beyond chromatin", *Curr. Opin. Cell Biol.* **13**, 218–224 (2001).
92. O. Hermanson, C. K. Glass, M. G. Rosenfeld. "Nuclear receptor coregulators: multiple modes of modification", *Trends Endocrinol. Metab.* **13**, 55–60 (2002).
93. N. J. McKenna and B. W. O'Malley. "Combinatorial control of gene expression by nuclear receptors and coregulators", *Cell* **108**, 465–474 (2002).
94. K. Jepsen and M. G. Rosenfeld. "Biological roles and mechanistic actions of co-repressor complexes", *J. Cell Sci.* **115**, 689–698 (2002).
95. A. Aranda and A. Pascual. "Nuclear hormone receptors and gene expression", *Physiol. Rev.* **81**, 1269–1304 (2001).
96. K. C. Lee and W. Lee Kraus. "Nuclear receptors, coactivators and chromatin: new approaches, new insights", *Trends Endocrinol. Metab.* **12**, 191–197 (2001).
97. M. Ito and R. G. Roeder. The TRAP/SMCC/Mediator complex and thyroid hormone receptor function", *Trends Endocrinol. Metab.* **12**, 127–134 (2001).
98. J. W. Lee, Y. C. Lee, S. Y. Na, D. J. Jung, S. K. Lee. "Transcriptional coregulators of the nuclear receptor superfamily: coactivators and corepressors", *Cell Mol. Life Sci.* **58**, 289–297 (2001).
99. P. Ordentlich, M. Downes, R. M. Evans. "Corepressors and nuclear hormone receptor function", *Curr. Top. Microbiol. Immunol.* **254**, 101–116 (2001).
100. M. G. Rosenfeld and C. K. Glass. Coregulator codes of transcriptional regulation by nuclear receptors", *J. Biol. Chem.* **276**, 36865–36868 (2001).
101. O. M. Conneely, J. P. Lydon, F. De Mayo, B. W. O'Malley. "Reproductive functions of the progesterone receptor", *J. Soc. Gynecol. Investig.* **7**, S25–32 (2000).
102. K. B. Horwitz, L. Tung, G. S. Takimoto. "Novel mechanisms of antiprogestin action", *J. Steroid BioChem. Mol. Biol.* **53**, 9–17 (1995).
103. C. A. Lange, J. K. Richer, K. B. Horwitz. "Hypothesis: Progesterone primes breast cancer cells for cross-talk with proliferative or antiproliferative signals", *Mol. Endocrinol.* **13**, 829–836 (1999).
104. D. L. Bain, M. A. Franden, J. L. McManaman, G. S. Takimoto, K. B. Horwitz. "The N-terminal region of the human progesterone A-receptor. Structural analysis and the influence of the DNA binding domain", *J. Biol. Chem.* **275**, 7313–7320 (2000).
105. S. Nagpal et al. "Promoter context- and response element-dependent specificity of the transcriptional activation and modulating functions of retinoic acid receptors", *Cell* **70**, 1007–1019 (1992).

106. G. Gill and M. Ptashne. "Negative effect of the transcriptional activator GAL4", *Nature* **334**, 721–724 (1988).
107. M. E. Meyer et al. "Steroid hormone receptors compete for factors that mediate their enhancer function", *Cell* **57**, 433–442 (1989).
108. D. Tasset, L. Tora, C. Fromental, E. Scheer, P. Chambon. "Distinct classes of transcriptional activating domains function by different mechanisms", *Cell* **62**, 1177–1187 (1990).
109. B. Le Douarin et al. "A possible involvement of TIF1 alpha and TIF1 beta in the epigenetic control of transcription by nuclear receptors", *EMBO J.* **15**, 6701–6715 (1996).
110. D. M. Heery, E. Kalkhoven, S. Hoare, M. G. Parker. "A signature motif in transcriptional co-activators mediates binding to nuclear receptor", *Nature* **387**, 733–736 (1997).
111. J. Torchia et al. "The transcriptional co-activator p/CIP binds CBP and mediates nuclear- receptor function", *Nature* **387**, 677–684 (1997).
112. Y. Shang, X. Hu, J. DiRenzo, M. A. Lazar, M. Brown. "Cofactor dynamics and sufficiency in estrogen receptor-regulated transcription", *Cell* **103**, 843–852 (2000).
113. L. P. Freedman. "Increasing the complexity of coactivation in nuclear receptor signaling", *Cell* **97**, 5–8 (1999).
114. C. A. Berrevoets, P. Doesburg, K. Steketee, J. Trapman, A. O. Brinkmann. "Functional interactions of the AF-2 activation domain core region of the human androgen receptor with the amino-terminal domain and with the transcriptional coactivator TIF2 (transcriptional intermediary factor2) ", *Mol. Endocrinol.* **12**, 1172–1183 (1998).
115. P. Alen, F. Claessens, G. Verhoeven, W. Rombauts, B. Peeters. "The androgen receptor amino-terminal domain plays a key role in p160 coactivator-stimulated gene transcription", *Mol. Cell Biol.* **19**, 6085–6097 (1999).
116. C. L. Bevan, S. Hoare, F. Claessens, D. M. Heery, M. G. Parker. "The AF1 and AF2 domains of the androgen receptor interact with distinct regions of SRC1", *Mol. Cell Biol.* **19**, 8383–8392 (1999).
117. B. He, J. A. Kemppainen, J. J. Voegel, H. Gronemeyer, E. M. Wilson. "Activation function 2 in the human androgen receptor ligand binding domain mediates interdomain communication with the NH(2)-terminal domain", *J. Biol. Chem.* **274**, 37219–37225 (1999).
118. H. Ma et al. "Multiple signal input and output domains of the 160-kilodalton nuclear receptor coactivator proteins", *Mol. Cell Biol.* **19**, 6164–6173 (1999).
119. E. M. Mcinerney, M. J. Tsai, B. W. Omalley, B. S. Katzenellenbogen. "Analysis of estrogen receptor transcriptional enhancement by a nuclear hormone receptor coactivator", *Proc. Natl. Acad. Sci. USA* **93**, 10069–10073 (1996).
120. A. Benecke, P. Chambon, H. Gronemeyer. "Synergy between estrogen receptor a activation functions AF1 and AF2 mediated by transcription intermediary factor TIF2", *EMBO Reports* **1**, 151–157 (2000).
121. H. Chen, R. J. Lin, W. Xie, D. Wilpitz, R. M. Evans. "Regulation of hormone-induced histone hyperacetylation and gene activation via acetylation of an acetylase", *Cell* **98**, 675–686 (1999).
122. D. Chen et al. "Regulation of transcription by a protein methyltransferase", *Science* **284**, 2174–2177 (1999).
123. N. Kotaja, U. Karvonen, O. A. Janne, J. J. Palvimo. "The nuclear receptor interaction domain of GRIP1 is modulated by covalent attachment of SUMO-1", *J. Biol. Chem.* **277**, 30283–30288 (2002).
124. A. M. Jimenez-Lara, M. J. Heine, H. Gronemeyer. "PIAS3 (protein inhibitor of activated STAT-3) modulates the transcriptional activation mediated by the nuclear receptor Coactivator TIF2", *FEBS Lett.* **526**, 142–146 (2002).
125. A. M. Jimenez-Lara, M. J. Heine, H. Gronemeyer. "Cloning of a mouse glucocorticoid modulatory element binding protein, a new member of the KDWK family", *FEBS Lett.* **468**, 203–210 (2000).

126. S. M. Huang and M. R. Stallcup. "Mouse Zac1, a transcriptional coactivator and repressor for nuclear receptors", *Mol. Cell Biol.* **20**, 1855–1867 (2000).
127. P. M. A. Henttu, E. Kalkhoven, M. G. Parker. "AF-2 activity and recruitment of steroid receptor coactivator 1 to the estrogen receptor depend on a lysine residue conserved in nuclear receptors", *Mol. Cell Biol.* **17**, 1832–1839 (1997).
128. W. Feng et al. "Hormone-dependent coactivator binding to a hydrophobic cleft on nuclear receptors", *Science* **280**, 1747–1749 (1998).
129. B. D. Darimont et al. "Structure and specificity of nuclear receptor-coactivator interactions", *Genes Dev.* **12**, 3343–3356 (1998).
130. A. K. Shiau et al. "The structural basis of estrogen receptor/coactivator recognition and the antagonism of this interaction by tamoxifen. *Cell* **95**, 927–937 (1998).
131. E. M. McInerney et al. "Determinants of coactivator LXXLL motif specificity in nuclear receptor transcriptional activation", *Genes Dev.* **12**, 3357–3368 (1998).
132. X. Hu and M. A. Lazar. "The CoRNR motif controls the recruitment of corepressors by nuclear hormone receptors", *Nature* **402**, 93–96 (1999).
133. L. Nagy et al. "Mechanism of corepressor binding and release from nuclear hormone receptors", *Genes Dev.* **13**, 3209–3216 (1999).
134. V. Perissi et al. "Molecular determinants of nuclear receptor-corepressor interaction", *Genes Dev.* **13**, 3198–3208 (1999).
135. H. E. Xu et al. "Structural basis for antagonist-mediated recruitment of nuclear co- repressors by PPARalpha", *Nature* **415**, 813–817 (2002).
136. T. A. Jackson et al. "The partial agonist activity of antagonist-occupied steroid receptors is controlled by a novel hinge domain-binding coactivator L7/SPA and the corepressors N-CoR or SMRT", *Mol. Endocrinol.* **11**, 693–705 (1997).
137. B. L. Wagner, J. D. Norris, T. A. Knotts, N. L. Weigel, D. P. McDonnell. "The nuclear corepressors NCoR and SMRT are key regulators of both ligand- and 8-bromo-cyclic AMP-dependent transcriptional activity of the human progesterone receptor", *Mol. Cell Biol.* **18**, 1369–1378 (1998).
138. X. Zhang, M. Jeyakumar, S. Petukhov, M. K. Bagchi. "A nuclear receptor corepressor modulates transcriptional activity of antagonist-occupied steroid hormone receptor", *Mol. Endocrinol.* **12**, 513–524 (1998).
139. A. J. Horlein et al. "Ligand-independent repression by the thyroid hormone receptor mediated by a nuclear receptor co-repressor", *Nature* **377**, 397–404 (1995).
140. R. Kurokawa et al. "Differential use of CREB binding protein-coactivator complexes", *Science* **279**, 700–703 (1998).
141. J. P. Renaud, J. M. Harris, M. Downes, L. J. Burke, G. E. Muscat. "Structure–function analysis of the Rev-erbA and RVR ligand-binding domains reveals a large hydrophobic surface that mediates corepressor binding and a ligand cavity occupied by side chains", *Mol. Endocrinol.* **14**, 700–717 (2000).
142. W. Seol, M. J. Mahon, Y. K. Lee, D. D. Moore. "Two receptor interacting domains in the nuclear hormone receptor corepressor RIP13/N-CoR", *Mol. Endocrinol.* **10**, 1646–1655 (1996).
143. I. Zamir et al. "A nuclear hormone receptor corepressor mediates transcriptional silencing by receptors with distinct repression domains", *Mol. Cell Biol.* **16**, 5458–5465 (1996).
144. J. D. Fondell, H. Ge, R. G. Roeder. "Ligand induction of a transcriptionally active thyroid hormone receptor coactivator complex", *Proc. Natl. Acad. Sci. USA* **93**, 8329–8333 (1996).
145. C. Rachez et al. "A novel protein complex that interacts with the vitamin D3 receptor in a ligand-dependent manner and enhances VDR transactivation in a cell- free system", *Genes Dev.* **12**, 1787–1800 (1998).
146. M. Ito et al. "Identity between TRAP and SMCC complexes indicates novel pathways for the function of nuclear receptors and diverse mammalian activators", *Mol. Cell* **3**, 361–370 (1999).

147. W. Gu et al. "A novel human SRB/MED-containing cofactor complex, SMCC, involved in transcription regulation", [published erratum appears in *Mol. Cell* April 3 (4), following 541 (1999)]. *Mol. Cell* **3**, 97–108 (1999).
148. M. Kretzschmar, G. Stelzer, R. G. Roeder, M. Meisterernst. "RNA polymerase II cofactor PC2 facilitates activation of transcription by GAL4-AH in vitro", *Mol. Cell Biol.* **14**, 3927–3937 (1994).
149. A. M. Naar et al. "Composite co-activator ARC mediates chromatin-directed transcriptional activation", *Nature* **398**, 828–832 (1999).
150. S. Ryu and R. Tjian. Purification of transcription cofactor complex CRSP. *Proc. Natl. Acad. Sci. USA* **96**, 7137–7142 (1999).
151. X. Sun et al. "NAT, a human complex containing Srb polypeptides that functions as a negative regulator of activated transcription", *Mol. Cell* **2**, 213–222 (1998).
152. J. D. Fondell, M. Guermah, S. Malik, R. G. Roeder. "Thyroid hormone receptor-associated proteins and general positive cofactors mediate thyroid hormone receptor function in the absence of the TATA box-binding protein-associated factors of TFIID", *Proc. Natl. Acad. Sci. USA* **96**, 1959–1964 (1999).
153. C. Rachez et al. "Ligand-dependent transcription activation by nuclear receptors requires the DRIP complex", *Nature* **398**, 824–828 (1999).
154. W. Yang and L. P. Freedman. "20-Epi analogues of 1,25-dihydroxyvitamin D3 are highly potent inducers of DRIP coactivator complex binding to the vitamin D3 receptor", *J. Biol. Chem.* **274**, 16838–16845 (1999).
155. C. X. Yuan, M. Ito, J. D. Fondell, Z. Y. Fu, R. G. Roeder. "The TRAP220 component of a thyroid hormone receptor- associated protein (TRAP) coactivator complex interacts directly with nuclear receptors in a ligand-dependent fashion", [published erratum appears in *Proc. Natl. Acad. Sci. USA* Nov 24; 95 (24), 14584 (1998)]. *Proc. Natl. Acad. Sci. USA* **95**, 7939–7944 (1998).
156. A. B. Hittelman, D. Burakov, J. A. Iniguez-Lluhi, L. P. Freedman, M. J. Garabedian. "Differential regulation of glucocorticoid receptor transcriptional activation via AF-1-associated proteins", *EMBO J.* **18**, 5380–5388 (1999).
157. M. Gehin et al. "Structural basis for engineering of retinoic acid receptor isotype- selective agonists and antagonists", *Chem. Biol.* **6**, 519–529 (1999).
158. S. Minucci et al. "Retinoid X receptor (RXR) within the RXR-retinoic acid receptor heterodimer binds its ligand and enhances retinoid-dependent gene expression", *Mol. Cell Biol.* **17**, 644–655 (1997).
159. J. Y. Chen et al. "Two distinct actions of retinoid-receptor ligands", *Nature* **382**, 819–822 (1996).
160. A. C. Pike et al. "Structural insights into the mode of action of a pure antiestrogen", *Structure (Camb.)* **9**, 145–153 (2001).
161. J. L. Oberfield et al. "A peroxisome proliferator-activated receptor gamma ligand inhibits adipocyte differentiation", *Proc. Natl. Acad. Sci. USA* **96**, 6102–6106 (1999).
162. A. K. Shiau et al. "Structural characterization of a subtype-selective ligand reveals a novel mode of estrogen receptor antagonism", *Nat. Struct. Biol.* **9**, 359–364 (2002).
163. R. M. Lavinsky et al. "Diverse signaling pathways modulate nuclear receptor recruitment of N-CoR and SMRT complexes", *Proc. Natl. Acad. Sci. USA* **95**, 2920–2925 (1998).
164. P. Germain, J. Iyer, C. Zechel, H. Gronemeyer. "Coregulator recruitment and the mechanism of retinoic acid receptor synergy", *Nature* **415**, 187–192 (2002).
165. E. S. Klein et al. "Identification and functional separation of retinoic acid receptor neutral antagonists and inverse agonists", *J. Biol. Chem.* **271**, 22692–22696 (1996).
166. J. Clifford, H. Chiba, D. Sobieszczuk, D. Metzger, P. Chambon. "RXRalpha-null F9 embryonal carcinoma cells are resistant to the differentiation, anti-proliferative and apoptotic effects of retinoids", *EMBO J.* **15**, 4142–4155 (1996).

167. R. Taneja et al. "Cell-type and promoter-context dependent retinoic acid receptor (RAR) redundancies for RAR beta 2 and Hoxa-1 activation in F9 and P19 cells can be artefactually generated by gene knockouts", *Proc. Natl. Acad. Sci. USA* **93**, 6197–6202 (1996).
168. R. Kurokawa et al. "Regulation of retinoid signalling by receptor polarity and allosteric control of ligand binding", *Nature* **371**, 528–531 (1994).
169. C. M. Apfel et al. "Enhancement of HL-60 differentiation by a new class of retinoids with selective activity on retinoid X receptor", *J. Biol. Chem.* **270**, 30765–30772 (1995).
170. Z. Chen et al. "Ligand- And DNA-induced Dissociation of RXR Tetramers", *J. Mol. Biol.* **275**, 55–65 (1998).
171. S. Kersten, M. I. Dawson, B. A. Lewis, N. Noy. "Individual subunits of heterodimers comprised of retinoic acid and retinoid X receptors interact with their ligands independently", *Biochemistry* **35**, 3816–3824 (1996).
172. H. Li, P. J. Gomes, J. D. Chen. "RAC3, a steroid/nuclear receptor-associated coactivator that is related to SRC-1 and TIF2", *Proc. Natl. Acad. Sci. USA* **94**, 8479–8484 (1997).
173. B. M. Forman, K. Umesono, J. Chen, R. M. Evans. "Unique response pathways are established by allosteric interactions among nuclear hormone receptors", *Cell* **81**, 541–550 (1995).
174. T. Perlmann and L. Jansson. "A novel pathway for vitamin A signaling mediated by RXR heterodimerization with NGFI-B and NURR1", *Genes Dev.* **9**, 769–782 (1995).
175. B. Blumberg and R. M. Evans. "Orphan nuclear receptors—new ligands and new possibilities", *Genes Dev.* **12**, 3149–3155 (1998).
176. M. Fu et al. "Androgen receptor acetylation governs trans activation and MEKK1- induced apoptosis without affecting in vitro sumoylation and trans- repression function", *Mol. Cell Biol.* **22**, 3373–3388 (2002).
177. C. Wang et al. "Direct acetylation of the estrogen receptor alpha hinge region by p300 regulates transactivation and hormone sensitivity", *J. Biol. Chem.* **276**, 18375–18383 (2001).
178. T. Kouzarides. "Acetylation: a regulatory modification to rival phosphorylation?", *EMBO J.* **19**, 1176–1179 (2000).
179. D. O. Morgan. "Principles of CDK regulation", *Nature* **374**, 131–134 (1995).
180. D. O. Morgan. "Cyclin-dependent kinases: Engines, clocks, and microprocessors", *Ann. Rev. Cell Dev. Biol.* **13**, 261–291 (1997).
181. Z. Chen et al. "MAP kinases", *Chem. Rev.* **101**, 2449–2476 (2001).
182. G. Pearson et al. "Mitogen-activated protein (MAP) kinase pathways: regulation and physiological functions", *Endocr. Rev.* **22**, 153–183 (2001).
183. L. Chang and M. Karin. "Mammalian MAP kinase signalling cascades", *Nature* **410**, 37–40 (2001).
184. N. L. Weigel. "Steroid hormone receptors and their regulation by phosphorylation", *BioChem. J.* **319**, 657–667 (1996).
185. T. Shen, K. B. Horwitz, C. A. Lange. "Transcriptional hyperactivity of human progesterone receptors is coupled to their ligand-dependent down-regulation by mitogen-activated protein kinase-dependent phosphorylation of serine 294", *Mol. Cell Biol.* **21**, 6122–31 (2001).
186. G. Bunone, P. A. Briand, R. J. Miksicek, D. Picard. "Activation of the unliganded estrogen receptor by EGF involves the MAP kinase pathway and direct phosphorylation", *EMBO J.* **15**, 2174–2183 (1996).
187. P. H. Driggers, J. H. Segars, D. M. Rubino. "The proto-oncoprotein Brx activates estrogen receptor beta by a p38 mitogen-activated protein kinase pathway", *J. Biol. Chem.* **276**, 46792–46797 (2001).
188. A. Tremblay, G. B. Tremblay, F. Labrie, V. Giguere. "Ligand-independent recruitment of SRC-1 to estrogen receptor beta through phosphorylation of activation function AF-1", *Mol. Cell* **3**, 513–519 (1999).

189. S. Yeh et al. "From HER2/Neu signal cascade to androgen receptor and its coactivators: a novel pathway by induction of androgen target genes through MAP kinase in prostate cancer cells", *Proc. Natl. Acad. Sci. USA* **96**, 5458–5463 (1999).
190. E. Hu, J. B. Kim, P. Sarraf, B. M. Spiegelman. "Inhibition of adipogenesis through MAP kinase-mediated phosphorylation of PPARgamma", *Science* **274**, 2100–2103 (1996).
191. C. E. Juge-Aubry et al. "Regulation of the transcriptional activity of the peroxisome proliferator-activated receptor alpha by phosphorylation of a ligand- independent trans-activating domain", *J. Biol. Chem.* **274**, 10505–10510 (1999).
192. M. Gianni, A. Bauer, E. Garattini, P. Chambon, C. Rochette-Egly. "Phosphorylation by p38MAPK and recruitment of SUG-1 are required for RA- induced RARgamma degradation and transactivation", *EMBO J.* **21**, 3760–3769 (2002).
193. M. Gianni et al. "Down-regulation of the phosphatidylinositol 3-kinase/Akt pathway is involved in retinoic acid-induced phosphorylation, degradation, and transcriptional activity of retinoic acid receptor gamma 2", *J. Biol. Chem.* **277**, 24859–24862 (2002).
194. S. Adam-Stitah, L. Penna, P. Chambon, C. Rochette-Egly. "Hyperphosphorylation of the retinoid X receptor alpha by activated c- Jun NH2-terminal kinases", *J. Biol. Chem.* **274**, 18932–18941 (1999).
195. H. Y. Lee et al. "Stress pathway activation induces phosphorylation of retinoid X receptor", *J. Biol. Chem.* **275**, 32193–32199 (2000).
196. M. P. Scheid and J. R. Woodgett. "PKB/AKT: functional insights from genetic models", *Nat. Rev. Mol. Cell Biol.* **2**, 760–768 (2001).
197. R. A. Campbell et al. "Phosphatidylinositol 3-kinase/AKT-mediated activation of estrogen receptor alpha: a new model for anti-estrogen resistance", *J. Biol. Chem.* **276**, 9817–9824 (2001).
198. H. K. Lin, S. Yeh, H. Y. Kang, C. Chang. "Akt suppresses androgen-induced apoptosis by phosphorylating and inhibiting androgen receptor", *Proc. Natl. Acad. Sci. USA* **98**, 7200–7205 (2001).
199. A. Migliaccio, M. Pagano, F. Auricchio. "Immediate and transient stimulation of protein tyrosine phosphorylation by estradiol in MCF-7 cells", *Oncogene* **8**, 2183–2191 (1993).
200. C. Rochette-Egly et al. Phosphorylation of the retinoic acid receptor-alpha by protein kinase A. *Mol. Endocrinol.* **9**, 860–871 (1995).
201. J. I. Huggenvik, M. W. Collard, Y. W. Kim, R. P. Sharma. "Modification of the retinoic acid signaling pathway by the catalytic subunit of protein kinase-A", *Mol. Endocrinol.* **7**, 543–550 (1993).
202. D. Chen, P. E. Pace, R. C. Coombes, S. Ali. "Phosphorylation of human estrogen receptor alpha by protein kinase A regulates dimerization", *Mol. Cell Biol.* **19**, 1002–1015 (1999).
203. M. H. Delmotte, A. Tahayato, P. Formstecher, P. Lefebvre. "Serine 157, a retinoic acid receptor alpha residue phosphorylated by protein kinase C in vitro, is involved in RXR.RARalpha heterodimerization and transcriptional activity", *J. Biol. Chem.* **274**, 38225–38231 (1999).
204. J. C. Hsieh et al. "Phosphorylation of the human vitamin D receptor by protein kinase C. Biochemical and functional evaluation of the serine 51 recognition site. *J. Biol. Chem.* **268**, 15118–15126 (1993).
205. J. M. Egly. "The 14$^{th}$ Datta Lecture. TFIIH: from transcription to clinic", *FEBS Lett.* **498**, 124–128 (2001).
206. D. Chen et al. "Activation of estrogen receptor alpha by S118 phosphorylation involves a ligand-dependent interaction with TFIIH and participation of CDK7", *Mol. Cell* **6**, 127–137 (2000).
207. J. M. Trowbridge, I. Rogatsky, M. J. Garabedian. "Regulation of estrogen receptor transcriptional enhancement by the cyclin A/Cdk2 complex", *Proc. Natl. Acad. Sci. USA* **94**, 10132–10137 (1997).
208. C. Rochette-Egly, S. Adam, M. Rossignol, J. M. Egly, P. Chambon. "Stimulation of RAR alpha activation function AF-1 through binding to the general transcription factor TFIIH and phosphorylation by CDK7", *Cell* **90**, 97–107 (1997).

209. J. Bastien et al. "TFIIH interacts with the retinoic acid receptor gamma and phosphorylates its AF-1-activating domain through cdk7", *J. Biol. Chem.* **275**, 21896–21904 (2000).
210. D. K. Lee, H. O. Duan, C. Chang. "From androgen receptor to the general transcription factor TFIIH. Identification of cdk activating kinase (CAK) as an androgen receptor NH(2)-terminal associated coactivator", *J. Biol. Chem.* **275**, 9308–9313 (2000).
211. A. Keriel, A. Stary, A. Sarasin, C. Rochette-Egly, J. M. Egly. "XPD mutations prevent TFIIH-dependent transactivation by nuclear receptors and phosphorylation of RARalpha", *Cell* **109**, 125–135 (2002).
212. C. Rochette-Egly and P. Chambon. "F9 embryocarcinoma cells: a cell autonomous model to study the functional selectivity of RARs and RXRs in retinoid signaling", *Histol. Histopathol.* **16**, 909–922 (2001).
213. R. Taneja et al. "Phosphorylation of activation functions AF-1 and AF-2 of RAR alpha and RAR gamma is indispensable for differentiation of F9 cells upon retinoic acid and cAMP treatment", *EMBO J.* **16**, 6452–6465 (1997).
214. E. Kopf et al. "Dimerization with retinoid X receptors and phosphorylation modulate the retinoic acid-induced degradation of retinoic acid receptors alpha and gamma through the ubiquitin-proteasome pathway", *J. Biol. Chem.* **275**, 33280–33288 (2000).
215. S. F. Arnold, M. Melamed, D. P. Vorojeikina, A. C. Notides, S. Sasson. "Estradiol-binding mechanism and binding capacity of the human estrogen receptor is regulated by tyrosine phosphorylation", *Mol. Endocrinol.* **11**, 48–53 (1997).
216. C. Rochette-Egly, J. L. Plassat, R. Taneja, P. Chambon. "The AF-1 and AF-2 activating domains of retinoic acid receptor-alpha (RARalpha) and their phosphorylation are differentially involved in parietal endodermal differentiation of F9 cells and retinoid-induced expression of target genes", *Mol. Endocrinol.* **14**, 1398–1410 (2000).
217. B. G. Rowan, N. L. Weigel, B. W. O'Malley. "Phosphorylation of steroid receptor coactivator-1. Identification of the phosphorylation sites and phosphorylation through the mitogen- activated protein kinase pathway", *J. Biol. Chem.* **275**, 4475–4483 (2000).
218. G. N. Lopez, C. W. Turck, F. Schaufele, M. R. Stallcup, P. J. Kushner. "Growth factors signal to steroid receptors through mitogen-activated protein kinase regulation of p160 coactivator activity", *J. Biol. Chem.* **276**, 22177–22182 (2001).
219. D. Knutti, D. Kressler, A. Kralli. "Regulation of the transcriptional coactivator PGC-1 via MAPK-sensitive interaction with a repressor", *Proc. Natl. Acad. Sci. USA* **98**, 9713–9718 (2001).
220. J. Font de Mora and M. Brown. "AIB1 is a conduit for kinase-mediated growth factor signaling to the estrogen receptor", *Mol. Cell Biol.* **20**, 5041–5047 (2000).
221. N. Vo and R. H. Goodman. "CREB-binding protein and p300 in transcriptional regulation", *J. Biol. Chem.* **276**, 13505–13508 (2001).
222. L. W. Yuan and J. E. Gambee. "Phosphorylation of p300 at serine 89 by protein kinase C", *J. Biol. Chem.* **275**, 40946–40951 (2000).
223. S. H. Hong and M. L. Privalsky. "The SMRT corepressor is regulated by a MEK-1 kinase pathway: inhibition of corepressor function is associated with SMRT phosphorylation and nuclear export", *Mol. Cell Biol.* **20**, 6612–6625 (2000).
224. M. Resche-Rigon and H. Gronemeyer. "Therapeutic potential of selective modulators of nuclear receptor action", *Curr. Opin. Chem. Biol.* **2**, 501–507 (1998).
225. M. I. Diamond, J. N. Miner, S. K. Yoshinaga, K. R. Yamamoto. "Transcription factor interactions: selectors of positive or negative regulation from a single DNA element", *Science* **249**, 1266–1272 (1990).
226. C. Jonat et al. "Antitumor promotion and antiinflammation: down-modulation of AP-1 (Fos/Jun) activity by glucocorticoid hormone", *Cell* **62**, 1189–1204 (1990).

227. H. F. Yang-Yen et al. "Transcriptional interference between c-Jun and the glucocorticoid receptor: mutual inhibition of DNA binding due to direct protein-protein interaction", *Cell* **62**, 1205–1215 (1990).
228. L. Shemshedini, R. Knauthe, P. Sassone-Corsi, A. Pornon, H. Gronemeyer. "Cell-specific inhibitory and stimulatory effects of Fos and Jun on transcription activation by nuclear receptors", *EMBO J.* **10**, 3839–3849 (1991).
229. A. Bubulya, S. C. Wise, X. Q. Shen, L. A. Burmeister, L. Shemshedini. "c-Jun can mediate androgen receptor-induced transactivation", *J. Biol. Chem.* **271**, 24583–24589 (1996).
230. D. Pearce, W. Matsui, J. N. Miner, K. R. Yamamoto. "Glucocorticoid receptor transcriptional activity determined by spacing of receptor and nonreceptor DNA sites", *J. Biol. Chem.* **273**, 30081–30085 (1998).
231. Y. Kamei et al. "A CBP integrator complex mediates transcriptional activation and AP-1 inhibition by nuclear receptors", *Cell* **85**, 403–414 (1996).
232. K. De Bosscher, W. Vanden Berghe, G. Haegeman. "Glucocorticoid repression of AP-1 is not mediated by competition for nuclear coactivators", *Mol. Endocrinol.* **15**, 219–227 (2001).
233. J. Y. Chen et al. "RAR-specific agonist/antagonists which dissociate transactivation and AP1 transrepression inhibit anchorage-independent cell proliferation", *EMBO J.* **14**, 1187–97 (1995).
234. C. Caelles, J. M. Gonzalez-Sancho, A. Munoz. "Nuclear hormone receptor antagonism with AP-1 by inhibition of the JNK pathway", *Genes Dev.* **11**, 3351–3364 (1997).
235. X. F. Zhou, X. Q. Shen, L. Shemshedini. Ligand-activated retinoic acid receptor inhibits AP-1 transactivation by disrupting c-Jun/c-Fos dimerization", *Mol. Endocrinol.* **13**, 276–285 (1999).
236. P. Webb, G. N. Lopez, R. M. Uht, P. J. Kushner. "Tamoxifen activation of the estrogen receptor/AP-1 pathway: potential origin for the cell-specific estrogen-like effects of antiestrogens", *Mol. Endocrinol.* **9**, 443–456 (1995).
237. M. Gottlicher, S. Heck, P. Herrlich. "Transcriptional cross-talk, the second mode of steroid hormone receptor action", *J. Mol. Med.* **76**, 480–489 (1998).
238. N. Auphan, J. A. DiDonato, C. Rosette, A. Helmberg, M. Karin. "Immunosuppression by glucocorticoids: inhibition of NF-kappa B activity through induction of I kappa B synthesis", *Science* **270**, 286–290 (1995).
239. S. Heck et al. I kappaB alpha-independent downregulation of NF-kappaB activity by glucocorticoid receptor", *EMBO J.* **16**, 4698–4707 (1997).
240. K. De Bosscher et al. "Glucocorticoids repress NF-kappaB-driven genes by disturbing the interaction of p65 with the basal transcription machinery, irrespective of coactivator levels in the cell", *Proc. Natl. Acad. Sci. USA* **97**, 3919–3924 (2000).
241. P. Delerive et al. "DNA Binding-Independent Induction of IkappaBalpha Gene Transcription by PPARalpha", *Mol. Endocrinol.* **16**, 1029–1039 (2002).
242. I. Barroso et al. "Dominant negative mutations in human PPARgamma associated with severe insulin resistance, diabetes mellitus and hypertension", *Nature* **402**, 880–883 (1999).
243. Z. Wang, G. Sun, Z. Shen, S. Chen, Z. Chen. "Differentiation therapy for acute promyelocytic leukemia with all-trans retinoic acid: 10-year experience of its clinical application", *Chin. Med. J. (Engl.)* **112**, 963–967 (1999).
244. L. Degos and Z. Y. Wang. "All trans retinoic acid in acute promyelocytic leukemia. *Oncogene* **20**, 7140–7145 (2001).
245. L. Altucci and H. Gronemeyer. "The promise of retinoids to fight against cancer", *Nat. Rev. Cancer* **1**, 181–193 (2001).
246. L. Altucci and H. Gronemeyer. "Nuclear receptors in cell life and death", *Trends Endocrinol. Metab.* **12**, 460–468 (2001).
247. M. B. Sporn and N. Suh. "Opinion: Chemoprevention: an essential approach to controlling cancer. *Nat. Rev. Cancer* **2**, 537–543 (2002).

248. S. Refetoff, R. E. Weiss, S. J. Usala. "The syndromes of resistance to thyroid hormone", *Endocr. Rev.* **14**, 348–399 (1993).
249. S. J. Usala et al. "A homozygous deletion in the c-erbA beta thyroid hormone receptor gene in a patient with generalized thyroid hormone resistance: isolation and characterization of the mutant receptor", *Mol. Endocrinol.* **5**, 327–335 (1991).
250. S. J. Usala et al. "A base mutation of the C-erbA beta thyroid hormone receptor in a kindred with generalized thyroid hormone resistance. Molecular heterogeneity in two other kindreds", *J. Clin. Invest.* **85**, 93–100 (1990).
251. T. N. Collingwood et al. "A role for helix 3 of the TRbeta ligand-binding domain in coactivator recruitment identified by characterization of a third cluster of mutations in resistance to thyroid hormone", *EMBO J.* **17**, 4760–4770 (1998).
252. T. N. Collingwood, M. Adams, Y. Tone, V. K. Chatterjee. "Spectrum of transcriptional, dimerization, and dominant negative properties of twenty different mutant thyroid hormone beta-receptors in thyroid hormone resistance syndrome", *Mol. Endocrinol*. **8**, 1262–1277 (1994).
253. R. E. Weiss et al. "Dominant inheritance of resistance to thyroid hormone not linked to defects in the thyroid hormone receptor alpha or beta genes may be due to a defective cofactor", *J. Clin. Endocrinol. Metab.* **81**, 4196–4203 (1996).
254. J. D. Safer, R. N. Cohen, A. N. Hollenberg, F. E. Wondisford. "Defective release of corepressor by hinge mutants of the thyroid hormone receptor found in patients with resistance to thyroid hormone", *J. Biol. Chem.* **273**, 30175–30182 (1998).
255. M. Alcalay et al. "Common themes in the pathogenesis of acute myeloid leukemia", *Oncogene* **20**, 5680–5694 (2001).
256. H. de The and M. K. Chelbi-Alix. "APL, a model disease for cancer therapies?", *Oncogene* **20**, 7136–7139 (2001).
257. F. Piazza, C. Gurrieri, P. P. Pandolfi. "The theory of APL. *Oncogene* **20**, 7216–7122 (2001).
258. E. M. Rego and P. P. Pandolfi. "Reciprocal products of chromosomal translocations in human cancer pathogenesis: key players or innocent bystanders?", *Trends. Mol. Med.* **8**, 396–405 (2002).
259. F. Grignani et al. "Fusion proteins of the retinoic acid receptor-alpha recruit histone deacetylase in promyelocytic leukaemia", *Nature* **391**, 815–818 (1998).
260. L. Z. He et al. "Distinct interactions of PML-RARalpha and PLZF-RARalpha with co- repressors determine differential responses to RA in APL", *Nat. Genet.* **18**, 126–135 (1998).
261. R. J. Lin et al. "Role of the histone deacetylase complex in acute promyelocytic leukaemia", *Nature* **391**, 811–814 (1998).
262. W. Bourguet, P. Germain, H. Gronemeyer. "Nuclear receptor ligand-binding domains: three-dimensional structures, molecular interactions and pharmacological implications", *Trends Pharmacol. Sci.* **21**, 381–388 (2000).
263. L. Altucci et al. "Retinoic acid-induced apoptosis in leukemia cells is mediated by paracrine action of tumor-selective death ligand TRAIL", *Nat. Med.* **7**, 680–686 (2001).
264. L. E. French and J. Tschopp. The TRAIL to selective tumor death [news; comment]. *Nat. Med.* **5**, 146–147 (1999).
265. M. Ristow, D. Muller-Wieland, A. Pfeiffer, W. Krone, C. R. Kahn. "Obesity associated with a mutation in a genetic regulator of adipocyte differentiation", *N. Engl. J. Med.* **339**, 953–959 (1998).
266. M. W. Schwartz and S. E. Kahn. "Insulin resistance and obesity", *Nature* **402**, 860–861 (1999).
267. D. Altshuler et al. "The common PPARgamma Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes", *Nat. Genet.* **26**, 76–80 (2000).
268. P. Sarraf et al. "Loss-of-function mutations in PPAR gamma associated with human colon cancer", *Mol. Cell* **3**, 799–804 (1999).
269. M. R. Hughes et al. "Point mutations in the human vitamin D receptor gene associated with hypocalcemic rickets", *Science* **242**, 1702–1705 (1988).

270. H. H. Ritchie et al. "An ochre mutation in the vitamin D receptor gene causes hereditary 1,25- dihydroxyvitamin D3-resistant rickets in three families", *Proc. Natl. Acad. Sci. USA* **86**, 9783–9787 (1989).
271. T. Carling et al. "Vitamin D receptor genotypes in primary hyperparathyroidism", *Nat. Med.* **1**, 1309–1311 (1995).
272. E. P. Smith et al. "Estrogen resistance caused by a mutation in the estrogen-receptor gene in a man", *N. Engl. J. Med* **331**, 1056–1061 (1994).
273. T. A. Hopp and S. A. Fuqua. "Estrogen receptor variants. *J. Mammary Gland Biol. Neoplasia* **3**, 73–83 (1998).
274. A. R. La Spada, E. M. Wilson, D. B. Lubahn, A. E. Harding, K. H. Fischbeck. "Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy", *Nature* **352**, 77–79 (1991).
275. A. N. Mhatre et al. "Reduced transcriptional regulatory competence of the androgen receptor in X-linked spinal and bulbar muscular atrophy", *Nat. Genet.* **5**, 184–188 (1993).
276. X. Y. Zhao et al. "Glucocorticoids can promote androgen-independent growth of prostate cancer cells through a mutated androgen receptor", *Nat. Med.* **6**, 703–706 (2000).
277. A. O. Brinkmann and J. Trapman. "Prostate cancer schemes for androgen escape", *Nat. Med.* **6**, 628–629 (2000).
278. D. M. Hurley et al. "Point mutation causing a single amino acid substitution in the hormone binding domain of the glucocorticoid receptor in familial glucocorticoid resistance", *J. Clin. Invest.* **87**, 680–686 (1991).
279. M. Karl et al. "Familial glucocorticoid resistance caused by a splice site deletion in the human glucocorticoid receptor gene", *J. Clin. Endocrinol. Metab.* **76**, 683–689 (1993).
280. S. L. Anzick et al. "AIB1, a steroid receptor coactivator amplified in breast and ovarian cancer", *Science* **277**, 965–968 (1997).
281. M. Carapeti, R. C. Aguiar, A. E. Watmore, J. M. Goldman, N. C. Cross. "Consistent fusion of MOZ and TIF2 in AML with inv(8)(p11q13) ", *Cancer Genet. Cytogenet.* **113**, 70–72 (1999).
282. J. Liang, L. Prouty, B. J. Williams, M. A. Dayton, K. L. Blanchard. "Acute mixed lineage leukemia with an inv(8)(p11q13) resulting in fusion of the genes for MOZ and TIF2", *Blood* **92**, 2118–2122 (1998).
283. M. Carapeti, R. C. Aguiar, J. M. Goldman, N. C. Cross. "A novel fusion between MOZ and the nuclear receptor coactivator TIF2 in acute myeloid leukemia", *Blood* **91**, 3127–3133 (1998).
284. P. Blume-Jensen and T. Hunter. "Oncogenic kinase signalling", *Nature* **411**, 355–365 (2001).
285. B. P. Zhou et al. "Cytoplasmic localization of p21Cip1/WAF1 by Akt-induced phosphorylation in HER-2/neu-overexpressing cells", *Nat. Cell Biol.* **3**, 245–252 (2001).
286. B. J. Feldman and D. Feldman. "The development of androgen-independent prostate cancer", *Nat. Rev. Cancer* **1**, 34–45 (2001).
287. R. Mukherjee et al. "Sensitization of diabetic and obese mice to insulin by retinoid X receptor agonists", *Nature* **386**, 407–410 (1997).