

Review

Molecular mechanisms of transcriptional regulation by nuclear receptors

Perspectives for therapeutic implications

Gerasimos P. Sykiotis, Athanasios G. Papavassiliou

Department of Biochemistry, School of Medicine, University of Patras, Patras, Greece

ABSTRACT

Nuclear receptors are ligand-regulated transcription factors that evolved from an ancestral orphan receptor into a highly diverse family present throughout the entire animal kingdom. They encompass receptors for steroid and non-steroid hormones, vitamins and metabolic intermediates. These receptors signal through endocrine, paracrine, autocrine and intracrine networks to regulate multiple aspects of animal physiology, including homeostasis, development and reproduction. They exert genomic effects via direct binding as monomers, homo- or heterodimers on cognate DNA elements (hormone response elements). They also participate in signal transduction cross-talk to indirectly modulate other gene expression programmes. By coordinating expression of genetic programmes, nuclear receptors contribute to cell fate-determining processes, thereby shaping and sustaining the organism. All these actions result from one fundamental interaction: receptor binding of a cognate ligand, which induces a major allosteric change in the ligand-binding domain. This conformational alteration is transformed into cascades of protein-protein recognitions, culminating in the establishment of coregulator/cointegrator complexes on gene promoters. Coregulators induce chromatin remodelling and acetylation, thus enabling the targeted recruitment and activation of the basal transcription machinery. This review discusses the molecular infrastructure of nuclear receptor signalling. Emphasis is given to determinants of signalling specificity, especially since they highlight prominent targets for novel drug discovery.

Key words: transcriptional control, nuclear receptor, coactivator, corepressor, drug design

INTRODUCTION

Nuclear receptors constitute a direct signal transduc-

tion system in which the same polypeptide receives a hormonal stimulus and converts it into a transcriptional response¹. This superfamily of ligand-dependent transcription factors plays crucial roles in controlling reproduction, development and tissue homeostasis². It includes receptors for steroid and thyroid hormones, peroxisomal activators and the hormonal forms of vitamins A and D, as well as a multitude of orphan receptors for which no ligand has yet been identified³. Ligands of nuclear receptors are relatively small and hydrophobic and there-

Address correspondence and requests for reprints to:
Athanasios G. Papavassiliou, MD, PhD, Professor &
Chairman, Department of Biochemistry, School of Medicine,
University of Patras, GR-26110 Patras, Greece, Telephone:
(+3) 0610 996144; Fax: (+3) 0610 996110,
e-mail: papavas@med.upatras.gr

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fore enter target cells mostly by simple diffusion, a slow process generally employed to control gradual changes. Furthermore, this method of signalling lacks the advantage of signal amplification that can be achieved using kinase cascades. However, nuclear receptors can also exert rapid, non-genomic effects⁴.

As a general scheme, activated nuclear receptors attach to cognate hormone response elements (HREs) as monomers, homo- or heterodimers⁵. They associate with coactivator and corepressor proteins that mediate positive and negative effects on transcription, respectively. These coregulators act by affecting chromatin structure, histone acetylation state and RNA polymerase II holoenzyme activity^{6,7}. The two best characterized groups of nuclear receptors are the steroid hormone receptors and the retinoid X receptor (RXR) heterodimers¹. Steroid receptors include the estrogen receptor (ER), progesterone receptor (PR), androgen receptor (AR), glucocorticoid receptor (GR) and mineralocorticoid receptor (MR). The second group consists of factors such as the thyroid hormone receptor (TR) and the all-trans retinoic acid receptor (RAR) which can only bind DNA with high affinity after heterodimerizing with the RXR. RXR does not need to bind a ligand in order to function in such heterodimers. However, RXR can also bind DNA as a homodimer whose ligand is 9-cis retinoic acid.

Mechanisms of specificity and diversity generation in nuclear receptor signalling

Nuclear receptor homodimers recognize palindromic HREs spaced by three nucleotides in a symmetrical way: each receptor molecule binds to one half-site of the response element. Heterodimers recognize diverse HREs in which half-core motifs can be arranged as palindromes, inverse palindromes or direct repeats⁵. The RAR-RXR heterodimer binds with high efficiency to a recognition sequence organized as a direct repeat with either a 5-bp spacing (DR5) or a single nucleotide spacing (DR1) between HRE half-sites. Because DRs are inherently asymmetric, heterodimers may bind onto them with two distinct polarities: on DR5 elements, RXR binds upstream and RAR downstream; retinoic acid induces release of corepressors, recruitment of coactivators and transcriptional activation. On DR1 elements, the heterodimer has the reverse polarity with RAR upstream and RXR downstream⁸. In that configuration corepressors remain bound to RAR even in the presence of retinoic acid, thus prohibiting the initiation of transcription. Corepressors are the dominant influence in this case and DR1 motifs do not confer responsiveness to all-trans retinoic acid; RAR effectively serves as a repressor of RXR at DR1 sites⁸.

However, RXR can also bind to DR1 sites as a homodimer and in this situation it is hormone responsive. Crystallographic analysis showed that protein-DNA contacts, dimerization interfaces and DNA curvatures differ between the two dimers⁹. Thus, a difference in recognition motif spacing enables alternative responses to ligand availability. Such subtle control may be delicately employed by retinoids in the coordination of complex developmental programmes.

Whereas RAR-RXR and TR-RXR bind tightly to their HREs in the presence or absence of ligand, unstimulated steroid receptors are associated in the cytoplasm with heat shock proteins, immunophilins and other components of the molecular chaperone machinery¹⁰. These chaperones are important for proper folding and stabilization of steroid receptors in a hormone-accessible conformation. Agonists trigger dissociation of the complex, allowing receptor dimerization and nuclear translocation. Chaperones may restrain receptors by inhibiting their ability to dimerize and recruit coactivators in the absence of cognate stimuli. This can explain the activation by steroids of heterologous signalling proteins fused to nuclear receptors' ligand-binding domains¹¹. The chaperone complex also seems to influence trafficking of steroid receptors into and out of the nucleus. Less-well established is a direct impact of chaperones on transcription activation through effects on chromatin remodelling and coactivator recruitment.

All members of the nuclear receptor superfamily share a similar basic organization (Figure 1)¹². The best conserved region is the DNA-binding domain (DBD), a compact globular structure built around two zinc ions. These zinc ions are coordinated tetrahedrally to four cysteine residues, thus forming two interdependent zinc fingers. The core of the DBD consists of two α -helices that cross each other at right angles. The first one, termed "recognition helix", makes base-specific contacts in the major groove of DNA, thus "reading out" an HRE half-site. High-affinity DNA binding requires homo- or heterodimerization⁵, which is accomplished by appropriate receptor surfaces. The major dimerization interface is located in a carboxy-terminal receptor domain joined to the DBD by a poorly conserved hinge region.

The carboxy-terminal domain is also responsible for ligand binding and contains a ligand-dependent transcription activation function (AF-2)¹. An additional activation function (AF-1) can be found in a poorly conserved region located amino-terminally to the DBD (Figure 1). AF-1 operates autonomously and in a ligand-independent manner when placed outside the receptor.



Figure 1. Structural organization of nuclear receptors. A typical nuclear receptor contains an amino-terminal region harboring a transcription activation function (AF-1). The size of this region can vary substantially between different receptors. This region is followed by the DNA-binding domain (DBD), which is highly conserved between family members. The DBD is connected by a variable hinge region to the ligand-binding domain (LBD). In addition to hormone recognition, this region allows receptor dimerization and bears a transcription activation function (AF-2).

However, in the context of its own receptor, AF-1 activity is also controlled by the cognate ligand¹³. Moreover, AF-1 shows promoter- and cell-specific transcriptional activity¹⁴ which can be significantly altered by its phosphorylation by various signalling pathways¹⁵. Taken together, these data suggest that AF-1 may contribute to the specificity of action among receptor isotypes and/or interact with cell-type specific coregulators. Ligands are classified as agonists or antagonists with respect to a particular receptor-associated activity, but this discrimination is not always clear-cut since a particular ligand can completely or partially antagonize some functions while functioning as agonist for others. Thus, hydroxytamoxifen antagonizes ER α AF-2 but acts as an agonist for AF-1, whereas ICI 164384 antagonizes both activation functions¹⁶. Similarly, certain retinoid receptor antagonists are agonists for repression of cross-talking pathways¹⁷.

Crystal structures of ligand-free (apo) and ligand-occupied (holo) ligand-binding domains (LBDs), alone or complexed with coactivator fragments, have provided molecular details of ligand-induced changes and their translation into protein-protein recognitions¹⁸. LBDs generally consist of 12 α -helices and a short β -turn, arranged in three layers to form an anti-parallel α -helical sandwich¹². Superposition of apo- and holo-LBD structures has shown that the lower LBD part is a structurally variable region which contains the hydrophobic ligand-binding pocket (LBP). This region undergoes major transformations after ligand binding, the most striking difference being the repositioning of the carboxy-terminal helices. Core AF-2 activity resides in the carboxy-terminal region of helix 12¹⁸, but this activation domain also comprises dispersed LBD elements, including a conserved nuclear receptor "signature motif" region that encompasses the carboxy-terminal part of helix 3, helix 4 and the loop between them. Upon agonist binding, helix 12 functions as a lid that folds over the LBP in a maneuver that brings together LBD AF-2 elements to generate a defined interaction surface. Coactivators recognize this surface via conserved LXXLL motifs (L = leucine, X =

any amino-acid) called "NR boxes"; this constitutes the molecular basis of AF-2 activity¹⁹.

The LBD effectively serves as a molecular switch that shifts nuclear receptors between active and inactive states¹⁸. Antagonist binding induces a different repositioning of helix 12 due to a steric clash between its holo thesis and the ligand's bulky extension. The new low-energy position of helix 12 enables its interaction with part of the LBD groove that recognizes the LXXLL motif, which precludes coactivator binding. Thus, AF-2 antagonism involves two structural principles: steric impairment of helix 12 holo position and competition between helix 12 and coactivator NR boxes for a common LBD surface¹⁸.

Importantly, complete AF-2 antagonists such as raloxifene and hydroxytamoxifen may still be AF-1 agonists, which can explain the tissue-specific effects of these drugs¹⁶. The molecular mechanism underlying AF-2 partial agonism-antagonism is in accordance with the above model: such ligands generally do not have bulky extensions; thus they do not sterically preclude the agonist position of helix 12 and are similar to agonists in this respect. However, they induce unwinding of helix 11 and subsequent positioning of helix 12 in the antagonist groove. In the presence of such mixed ligands the holo conformation of helix 12 is not firmly stabilized and the position of helix 12 probably depends on the intracellular concentration of coactivators and corepressors¹⁸. Therefore, these agents might act as either AF-2 agonists or antagonists depending on the cellular context.

In addition to CBP [CREB (c-AMP response element binding)-binding protein], p300 and PCAF (p300/CBP-associated factor) cointegrators, which also associate with other classes of transcription factors [e.g. CREB, AP-1 (activator protein-1), STATs (signal transducers and activators of transcription)], a bewildering array of coactivators are more specific to the nuclear receptor superfamily²⁰. The p160 family of coactivators includes proteins generically named SRC-1, (steroid receptor coac-

tivator-1), SRC-2 [called TIF-2 (transcription intermediary factor-2) in humans] and SRC-3²¹. SRC-1 was isolated using the ligand-bound PR as bait in a yeast two-hybrid screen²². SRC-3 or ACTR (activator of TR/RAR) was isolated independently by several approaches, the most interesting involving microdissection of chromosomal regions amplified in breast cancer; hence, an alternative name is amplified in breast cancer (AIB) 1²³. ACTR/AIB1 is overexpressed due to amplification in primary breast tumours and several ER-positive breast and ovarian cancer cell lines²³, suggesting that it may contribute to the development of steroid-dependent cancers. SRC-1, SRC-2 and ACTR display 40% amino-acid identity. They have a modular structure which contains a region with histone acetyltransferase (HAT) activity and two major transactivation domains, the stronger of which also associates with CBP²⁴. Other coactivator families include PPAR γ (peroxisome proliferator-activated receptor gamma; see below) coactivator-1 (PGC-1)²⁵ and even a coactivator composed of RNA²⁶.

Coactivators associate with nuclear hormone receptors in an agonist-dependent manner. After ligand-induced coactivator-mediated histone acetylation, chromatin assumes a more relaxed conformation that facilitates assembly of the transcription machinery⁶. Some coactivators, like PGC-1, bind to AF-1, but most require the AF-2 domain within the LBD. Ligand-induced AF-2 also recruits a cointegrator called TRAP (TR-associated protein) or DRIP (vitamin D receptor-interacting protein) that does not have HAT activity²⁷. The TRAP/DRIP multiprotein resembles the mediator complex which associates with the carboxy-terminus of RNA polymerase II; it may therefore serve to recruit the RNA polymerase II holoenzyme (i.e. RNA polymerase II and associated basal transcription factors) to hormone-responsive promoters. As both CBP/PCAF/p160 and TRAP/DRIP systems bind to the same receptor region they should not bind simultaneously and their precise interaction is presently unclear²⁸. It is, however, established that coactivator complexes initiate an ordered sequence of events²⁹, in which remodelling of chromatin nucleosome structure precedes histone acetylation and subsequent formation of a stable transcription initiation complex (Figure 2)³⁰.

In the absence of agonist, some nuclear receptors, including RAR and TR, function as transcriptional silencers^{7,31}. This repression function resides in the LBD, but is separable from AF-2. Corepressors possess motifs called "CoRNR boxes" by which they interact with unliganded nuclear receptors³¹. The best characterized corepressors are N-CoR (nuclear receptor corepressor) and

SMRT (silencing mediator for RXR and TR), which were isolated using two-hybrid screens in yeast⁷. These corepressors possess histone deacetylase activity (HDAC) through which they condense the chromatin environment at the promoter region³². They can also directly interact with the basal transcription apparatus³³. Upon ligand binding, the allosteric change of the LBD induces dissociation of corepressors and recruitment of coactivator complexes⁷. Corepressors also mediate transcriptional suspension by antagonists; for example, they associate with ER and PR bound to tamoxifen and RU-486, respectively³⁴. When the ligand is a partial agonist-antagonist, then the relative expression of coactivators and corepressors may determine the direction of transcription³⁵. Since coregulators may be expressed in a cell-specific manner³⁶, they can partly account for the varying transcriptional effects of such drugs on different tissues.

Apart from direct actions on the chromatin environment and the transcription machinery¹, nuclear recep-

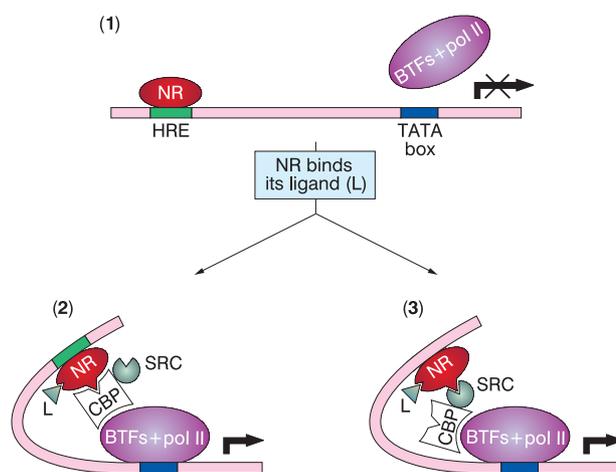


Figure 2. Models for activation of a nuclear receptor-regulated gene. (1) Without its ligand a nuclear receptor (NR) is bound to its hormone response element (HRE), but it cannot contact the basal transcription apparatus [basal transcription factors (BTFs) and RNA polymerase II (pol II)], so the linked gene is not activated. Depending on the type, the NR could also be dissociated from its DNA target in the absence of its ligand. (2) The NR has bound to its ligand (L) and is now able to interact with CBP, which binds to at least one component of the basal transcription apparatus, recruiting it to the promoter (including the TATA box) and activating transcription. (3) The NR, bound to its ligand, can also interact with a member of the p160 family of steroid receptor coactivators (SRC), which is complexed with CBP. CBP, in turn, contacts the basal transcription apparatus, recruiting it to the promoter and activating transcription. In both (2) and (3) a chromatin remodelling/histone acetylation step precedes formation of a stable transcription initiation complex.

tors also modulate gene expression by mechanisms independent of binding to an HRE, through positive or negative interference with the activity of other transcription factors³⁷. In addition to this transcriptional cross-talk, other signalling pathways can phosphorylate nuclear receptors to "fine-tune" their action or even cause activation of unliganded receptors¹⁵. The promoter context of target genes, the cell-specific expression/function of coregulators and non-nuclear transcription factors and the temporal order of incoming signals on a particular promoter probably adjust the transcriptional outcome of nuclear receptors to each particular situation¹. Taken together with several non-genomic effects⁴, these actions demonstrate that nuclear receptors serve as platforms that coordinate cognate and heterologous signals, thereby integrating nuclear receptor signalling into the functional context of cellular state and activity.

Implications for drug design

Given the major impact of nuclear receptor signalling on animal physiology², it is no surprise that aberrant nuclear receptor function underlies a wide gamut of human pathologies which, whether synthetic, complete or partial, agonists and antagonists can tackle. Contraceptive pills, pregnancy terminators and hormone replacement treatments (HRTs) have had a major impact on social life. Thiazolidinediones (TZDs) are PPAR γ agonists used as insulin sensitizers in the treatment of type 2 diabetes³⁸. Breast and prostate cancers are treated with ER and AR antagonists respectively, whereas retinoids constitute a differentiation therapy for acute promyelocytic leukemia (PML), and various agents are considered for treatment and prevention of other types of cancer.

Based on a more comprehensive understanding of the molecular mechanisms of nuclear receptor signalling, there is an effort to develop new ligands with increased efficacy and reduced side-effects^{39,40}. Such attempts are facilitated by recent developments in drug design, including (i) combinatorial chemistry, (ii) computer-assisted ligand docking based on LBD crystal structure, (iii) ultra-high throughput screening with nuclear receptor-based reporter systems, (iv) ability to dissociate transactivation from cross-talk with other signalling pathways¹⁷, and (v) generation of isotype-selective ligands. Orphan receptors are also targets of such approaches since they are recognized as mediators of intracrine signalling⁴¹.

Given the pharmacological potential of retinoids, the development of novel retinoid receptor ligands has attracted much attention. Isotype-specific ligands and mixed agonists/antagonists are now available for the three

RAR isoforms (α , β and γ), based on the structural background for receptor specificity⁴². Design of RXR isotype- or pathway-selective ligands is another promising task since RXR is a promiscuous heterodimerization partner. For instance, RXR ligands may stimulate insulin action in non insulin-dependent diabetes through a PPAR γ /RXR heterodimer⁴³.

PPAR γ is a nuclear receptor that dimerizes with RXR and plays a key role in adipocyte differentiation¹. It is the target of TZDs, which are used to treat type 2 diabetes by improving the body's sensitivity to insulin³⁸. Rare cases of combined hypertension, insulin resistance and diabetes mellitus have been reported to be associated with mutations in the LBD of PPAR γ ⁴⁴. One kindred harbored a substitution in LBD helix 12, whereas another patient had a mutation which affects the surface that packs against helix 12. In both cases, the orientation of helix 12 is perturbed, thus disrupting ligand binding, AF-2 activity and coactivator recruitment. Consequently, PPAR γ -driven transcription is severely compromised in a dominant negative manner. Moreover, cases of obesity have been attributed to substitutions that impair amino-terminal phosphorylation of PPAR γ by mitogen-activated protein kinases (MAPKs)⁴⁵; these missense mutations have a gain-of-function effect that enhances adipocyte differentiation. These data imply that novel specific PPAR γ ligands could considerably improve the treatment of metabolic disorders like hypertension, obesity, insulin resistance and type 2 diabetes³⁸.

State-of-the-art techniques like real-time imaging in living cells, *in vitro* and *in vivo* chromatin assembly and chromatin immunoprecipitation assays enhanced our understanding of coregulator function at the molecular level⁶. As discussed, coactivators are not entirely promiscuous in their choice of nuclear receptors and alterations in their cellular abundance or altered substrate specificity of coregulator-associated HAT or HDAC activity may determine the cumulative outcome on transcription. This understanding qualifies coactivators and corepressors as attractive pharmacological targets. Indeed, libraries of combinatorial peptides containing the core LXXLL motif have been screened for high-affinity binding to the LBD hydrophobic groove⁴⁶. Such peptides interfere with nuclear receptor function in transfected cells and could be used in probing receptor surfaces differentially generated in the presence of agonists and antagonists. This should facilitate design of small synthetic molecules able to disrupt the interaction between NR/CoRNR boxes and the corresponding LBD region³⁹.

RESPECTIVES

The recognition of nuclear receptors and their interacting proteins as key regulatory molecules in many signalling pathways, together with their implication in a broad spectrum of diseases, establish them as novel pharmacological targets. The ongoing improvement of synthetic ligands with specificity for particular receptor isoforms, activation functions or coregulator interactions, is destined to improve therapeutic potentials while reducing side-effects. Nonetheless, future research on nuclear receptors faces important unanswered questions. What are the constituents of the genetic programmes governed by a given family member? How are nuclear receptor signals matched and complemented with other signalling cascades? What are the precise molecular determinants of the variety and specificity of transcriptional control exerted by these transcription factors? Only when such issues have been adequately addressed will selective interference into these immensely complex systems lead to successful "reprogramming" of an organism's pathophysiology. The plethora of information obtained from the Human Genome Project, complemented by the use of gene microarrays/DNA chip technologies, is expected to have enormous impact towards a detailed understanding of how nuclear receptors regulate general and signalling pathway-specific gene expression events.

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