REVIEW

Agonist-bound nuclear receptors: not just targets of coactivators

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Abstract

Members of the nuclear receptor superfamily of ligand-regulated transcription factors are targets of a wide range of lipophilic signaling molecules as well as several drugs and xenobiotics that modulate many aspects of physiology and metabolism. Agonist binding to receptors is associated with recruitment of coactivators, which are essential for activation of target gene transcription. However, several biochemical and molecular genetic studies have shown that a full understanding of the function of agonist-bound receptors must also accommodate the recruitment of corepressors. These factors may attenuate agonist-induced transactivation, act more transiently as part of a cycle of cofactors recruited to target promoters by ligand-bound receptors, or function in hormone-dependent repression of target gene expression.

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The nuclear receptor superfamily – primary targets of lipophilic signaling molecules

Nuclear receptors are ligand-regulated transcription factors whose activities are controlled by a range of lipophilic extracellular signals, including steroid and thyroid hormones, metabolites of vitamins A (retinoids) and D, cholesterol metabolites, bile acids, specific prostaglandins, and xenobiotics (Chawla et al. 2001, McKenna & O’Malley 2002). Nuclear receptors have been intensively studied in both academia and the pharmaceutical industry (all 48 members of the human superfamily were identified prior to sequencing of the human genome). Importantly, they are the targets of numerous drugs, including synthetic steroid hormone agonists and antagonists, thiazolidenedione antidiabetics, modifiers of cholesterol and bile acid metabolism, and analogs of vitamins A and D, which, among other actions, have broad potential as anticancer agents. To understand the physiological and pharmacological actions of specific ligands, it is essential to fully characterize the biochemical events induced by their binding to target receptors.

Nuclear receptors contain well-conserved DNA binding domains (DBD) and ligand binding domains (LBD). DBDs control (ligand-dependent) recognition by nuclear receptors of specific DNA sequences found in promoters of target genes that are known collectively as hormone response elements (Sanchez et al. 2002). LBDs are located in the C-terminus of receptors. Crystal structures of agonist- and antagonist-bound LBDs have revealed highly conserved α helical structures (Renaud & Moras 2000). Conformational changes induced by ligand binding control recruitment of specific cofactors required for the complex biochemical events underlying transcriptional regulation. Agonist binding reorients the C-terminal AF-2 helix (helix 12) to create a binding pocket
recognized by signature motifs of coregulatory proteins required for transcriptional regulation.

**Recruitment of coregulatory proteins by nuclear receptors**

Numerous coregulatory proteins have been identified that control transcriptional regulation by nuclear receptors (Glass & Rosenfeld 2000, McKenna & O’Malley 2002). They are classified as coactivators or corepressors depending on whether they promote or inhibit initiation of target gene transcription. The diversity of coactivators suggests that transcriptional activation occurs through recruitment of multiple factors acting sequentially or in combination. The most extensively characterized coactivators are the p160 proteins (Glass & Rosenfeld 2000, McKenna & O’Malley 2002, and references therein). Many coactivators including p160 proteins interact with ligand-bound receptors through LXXLL motifs, known as nuclear receptor (NR) boxes (Voegel et al. 1996, Heery et al. 1997). Alpha-helical NR boxes are oriented within a hydrophobic pocket of LBDs containing the repositioned AF-2 helix by a charge clamp formed by conserved residues in helices 3 and 12 (Renaud & Moras 2000). p160 coactivator binding recruits other factors essential for transactivation, including CREB binding protein (CBP) and its homolog p300 (Glass & Rosenfeld 2000, McKenna & O’Malley 2002). Several coactivators including CBP/p300 and associated factor p/CAF possess histone acetyltransferase (HAT) activity. HAT activity essentially caps positively charged lysine residues and loosens their association with DNA, facilitating chromatin remodeling and subsequent access of the transcriptional machinery to promoters.

The corepressors nuclear receptor corepressor (N-CoR) and silencing mediator for retinoic acid receptor and thyroid hormone receptor (SMRT) were isolated as factors mediating ligand-independent repression by thyroid and retinoic acid receptors (Chen & Evans 1995, Horlein et al. 1995). Unlike p160 proteins, N-CoR and SMRT do not interact with LBDs when the AF-2 helix is in the agonist-bound conformation. Rather, they recognize LBDs in a hormone-free or, in some cases, antagonist-bound conformation through LXXI/HIXXXI/L motifs that resemble extended NR boxes (Perissi et al. 1999). Ligand binding induces movement of the AF-2 helix and displacement of N-CoR and SMRT. N-CoR or SMRT are components of multiprotein complexes implicated in transcriptional repression and histone deacetylation. Histone deacetylases (HDACs) are divided into three classes based on homology, domain structure, subcellular localization, and catalytic properties (Ng & Bird 2001). NCoR and SMRT are components of several different complexes containing distinct combinations of ancillary proteins and class I or class II HDACs (Rosenfeld & Glass 2001), suggesting that their function depends on cell type, combinations of transcription factors bound to specific promoters, and phase of the cell cycle.

**Agonist-dependent recruitment of corepressors**

Hormone binding, particularly by steroid hormone receptors, is widely associated with activation of target gene transcription. However, a model of receptor action where only coactivators are recruited to agonist-bound receptors cannot account fully for nuclear receptor action, or for all of the coregulatory proteins identified to date. Several NR box-containing coregulators have been identified that function as corepressors or have mixed coactivator–corepressor functions. One of the first coregulatory proteins identified, transcriptional intermediary protein 1/afii9825 (TIF1/afii9825), is an NR box-containing protein that was isolated from a two-hybrid screen for ligand-dependent cofactors of nuclear receptors (Le Douarin et al. 1995). Fusion of TIF1α to the DBD of GAL4 generates a powerful repressor whose activity can be blocked by the HDAC inhibitor trichostatin A (TSA) (Nielsen et al. 1999). However, unlike its homolog TIFβ, TIF1α does not associate strongly with factors such as heterochromatin protein 1 (HP1) that are implicated in heterochromatin formation (Nielsen et al. 1999). A similar two-hybrid screen yielded nuclear receptor-binding SET domain-containing protein (NSD1), a 2588 amino acid protein that contains both NR boxes and LXXI/HIXXXI/L motifs similar to those in N-CoR and SMRT. These motifs control its interaction with multiple nuclear receptors in either a ligand-independent or –dependent fashion (Huang et al. 1998). NSD1 also
contains several distinct coactivation or corepression domains, raising the possibility that their functions may be selectively modulated by secondary signal transduction pathways, thus controlling whether NSD1 acts as a coactivator or a corepressor.

Receptor interacting protein of 140 kDa (RIP140) was initially characterized as a coactivator (Cavaillès et al. 1995) that interacted with agonist-bound receptors through multiple NR boxes (Heery et al. 1997). However, subsequent work showed that RIP140 functioned as a corepressor that competes with p160s for binding to agonist-bound LBDs, blocking coactivation in vivo (Eng et al. 1998, Lee et al. 1998, Miyata et al. 1998, Treuter et al. 1998). Recently, a ligand-dependent corepressor, LCoR, was identified in a screen for proteins that interacted with the estrogen receptor β (ERβ) LBD in an estradiol-dependent manner (Fernandes et al. 2003). LCoR corepressed ligand-dependent transactivation of several receptors and functioned as a repressor when fused to the GAL4 DBD. LCoR contains a single LXXLL motif and, like RIP140, interacts with a number of receptors in the presence of agonist, but not antagonist. LCoR binds to the same coactivator-binding pocket as p160s (Fernandes et al. 2003). However, mutagenesis studies have suggested that LCoR recognizes a distinct and extended portion of helix 3, suggesting that it may be possible to develop synthetic agonists that promote recruitment of LCoR and not p160 proteins.

**Figure 1** Schematic representation of the primary structures of RIP140 and LCoR. The NR boxes of RIP140 and the LCoR NR box are represented by black bars. Positions of CtBP binding motifs are indicated by white boxes. HDAC binding domains are overlined. See text for details.

**LCoR and RIP140 are molecular scaffolds for several repressors of transcription**

LCoR and RIP140 recruit similar cofactors, revealing remarkable parallels in their mechanisms of action in spite of very limited homology (Fig. 1). Corepression by LCoR and RIP140 can be blocked by the HDAC inhibitor TSA, and both LCoR and RIP140 interact directly with HDACs. LCoR was found to interact directly with HDACs 3 and 6, but not HDACs 1 and 4 in vitro, and endogenous LCoR coimmunoprecipitated with HDACs 3 and 6 (Fernandes et al. 2003). Mutagenesis studies have shown that HDACs 3 and 6 interact with distinct domains of LCoR (Fig. 1 and our unpublished results). Similarly, RIP140 has been shown to interact directly with HDACs 1 and 3 (Wei et al. 2000).

RIP140 is also a target of C-terminal binding protein (CtBP) corepressors (Vo et al. 2001), and mutation of the CtBP binding motif of RIP140 severely attenuates its corepressor function. CtBP1 was originally identified as a factor that interacted with the C-terminus of the adenoviral oncoprotein E1A, and mutations in the CtBP binding motif of E1A increase its oncogenicity (Chinnadurai 2002). Remarkably, sequence analysis of LCoR revealed tandem motifs PLDLTVR and VLDLSTK (Fig. 1) that are homologous to the consensus P/VLDLS/TXK/R defined as a binding site for CtBPs (Vo et al. 2001). In vitro binding studies and coimmunoprecipitations revealed that the CtBP binding is
disrupted only upon mutation of both sites (Fernandes et al. 2003, and our unpublished results). Immunocytochemical studies revealed a substantial overlap of CtBPs and LCoR in discrete nuclear bodies.

The binding of CtBPs may explain the observation that the sensitivity of corepression by LCoR to the HDAC inhibitor TSA is receptor dependent. While inhibition of estrogen- and glucocorticoid-dependent transcription was TSA sensitive, corepression of transactivation by receptors for progesterone (PR) or vitamin D, or repression by GAL-LCoR fusions was largely TSA resistant (Fernandes et al. 2003). Moreover, while corepression of ERα was partially disrupted by mutation of the CtBP motifs of LCoR, corepression of PR function was completely blocked by the same mutations. Like LCoR, the sensitivity of repression by CtBPs to TSA is dependent on the promoter tested, indicative of HDAC-dependent and -independent modes of action (Chinnadurai 2002).

**Potential roles of hormone-dependent corepressors in nuclear receptor function**

While an impressive amount of work has been done to identify nuclear receptor coactivators and corepressors, many important questions remain to be answered. First of all, it remains unclear which signals determine whether an agonist-bound receptor will recruit an NR box containing coactivator or corepressor. In addition, we still do not understand the roles of hormone-dependent recruitment of corepressors in regulating nuclear receptor function. Distinct, non-mutually exclusive models of action can be proposed (Fig. 2). Corepressors may be selectively recruited during hormone-dependent repression of gene transcription (Fig. 2A). Steroid hormone and other nuclear receptors bound to response elements have been widely shown to function as hormone-dependent transcriptional activators, and recruit coactivators. However, recent microarray studies of hormone-regulated gene expression have emphasized the fact that receptors also repress transcription of some target genes, with profiles of regulation mirroring those of activated genes (Inoue et al. 2002, Lin et al. 2002, Lobenhofer et al. 2002, Wan & Nordeen 2002). Target gene repression may entail distinct protein–protein or protein–DNA interactions from those associated with activation, and include hormone-dependent recruitment of corepressors (Fig. 2A). This notion is supported by studies of the recently characterized DEAD box RNA helicase DP97, which has corepressor activity and is recruited to ERα in the presence of estradiol (Rajendran et al. 2003). Knockdown of its expression is associated with attenuation of the repression of genes inhibited by hormone-bound ERα.

Alternatively, ligand-dependent corepressors may be regulated by signals that act to attenuate hormone-dependent transactivation. Several experiments have shown that nuclear receptor function is modulated by signals other than ligand binding (McKenna & O’Malley 2002). For example, there are numerous examples of the effects of phosphorylation on nuclear receptor function (Shao & Lazar 1999). MAP kinase signaling stimulates ERα function by phosphorylation of Ser118 (Kato et al. 1995), and coactivation of ERα by p160 amplified in breast cancer 1 (AIB1) is enhanced by MAP kinase phosphorylation of an AIB1 activation domain, which enhances recruitment of p300 and associated HAT activity (Font de Mora & Brown 2000). Therefore, there may exist signals that counteract the effects of MAP kinase signaling on ERα function and stimulate the recruitment and/or activity of LCoR, RIP140 or other corepressors, thus attenuating receptor activity. These may simply alter the balance between the activities of MAP kinases and MAP kinase phosphatases, or there may be independent signaling events that enhance corepressor function to the detriment of coactivation. Changes in the activities of these pathways (Fig. 2B) would thus modulate competition between ligand-dependent coactivators and corepressors for hormone-bound receptors over relatively long-term periods (several cycles of transcriptional initiation).

It is also possible that ligand-dependent corepressors may intervene more rapidly and transiently to control transcription (Fig. 2C). Current models suggest that multiple factors required for initiation of transcription are recruited by transcription factors sequentially. ERα and p160s associate with promoters within minutes of addition of hormone (Shang et al. 2000, Burakov et al. 2002). Strikingly, studies with fluorescently-tagged receptors have shown that in vivo, even in the continuous presence of hormone, steroid
receptors and recruited factors cycle rapidly (on the order of minutes) on and off target promoters (McNally et al. 2000, Stenoien et al. 2001, Becker et al. 2002). Thus, the relative proportion of hormone-bound receptors complexed with coactivators or corepressors at a given time may

Figure 2 Potential roles of NR box-containing corepressors in hormone-dependent transcription by nuclear receptors. (A) Selective recruitment of corepressors to target genes whose expression is repressed in the presence of hormone. Note that the schematic representation implies direct receptor DNA binding to target promoters. Repression could also occur through receptor–protein interactions with factors bound to the promoter. (B) Inhibition of hormone-dependent transcription due to a change in signaling environment that promotes receptor–corepressor complex binding to a target promoter over receptor–coactivator complex binding. (C) Determination of the degree of hormone-induced target gene expression over the short term due to rapid exchange of receptor–coregulator complexes in a constant signaling environment. CoA, coactivator; CoR, corepressor; H, hormone; S, signal; *, post-translational modification.
determine the degree to which target gene transcription is induced. It will therefore be important to analyze corepressor recruitment to hormone-responsive promoters by chromatin immunoprecipitation (ChIP) assays to determine when they intervene during hormone-dependent transcription. Clearly much work remains to be done to characterize the roles of NR box-containing corepressors in controlling nuclear receptor signaling. However, with the powerful molecular and genetic tools available today, the next few years should see rapid advances in our understanding of their function.

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