Published in final edited form as:

Toxicol Appl Pharmacol. 2007 September 15; 223(3): 288–298.

Corepressors of Agonist-Bound Nuclear Receptors

Igor Gurevich, Anthony M. Flores, and Brian J. Aneskievich

Graduate Program in Pharmacology/Toxicology, Department of Pharmaceutical Sciences, School of Pharmacy, University of Connecticut, Storrs, CT USA (IG) Department of Pharmaceutical Sciences, School of Pharmacy, University of Connecticut, Storrs, CT USA (AMF, BJA)

Abstract

Nuclear receptors (NRs) rely on coregulator proteins to modulate transcription of target genes. NR coregulators can be broadly subdivided into coactivators which potentiate transcription and corepressors which silence gene expression. The prevailing view of coregulator action holds that in the absence of agonist the receptor interacts with a corepressor via the corepressor nuclear receptor (CoNR, “corner”) box motifs within the corepressor. Upon agonist binding, a conformational change in the receptor causes the shedding of corepressor and the binding of a coactivator which interacts with the receptor via NR boxes within the coregulator. This view was challenged with the discovery of RIP140 which acts as a NR corepressor in the presence of agonist and utilizes NR boxes. Since then a number of other corepressors of agonist-bound NRs have been discovered. Among them are LCoR, PRAME, REA, MTA1, NSD1, and COPR1. Although they exhibit a great diversity of structure, mechanism of repression and pathophysiological function, these corepressors frequently have one or more NR boxes and often recruit histone deacetylases to exert their repressive effects. This review highlights these more recently discovered corepressors and addresses their potential functions in transcription regulation, disease pharmacologic responses and xenobiotic metabolism.

Keywords

Nuclear receptor; Coregulator; Corepressor

Introduction

Nuclear Receptors (NRs) mediate the transcriptional regulatory effects of numerous small, lipophilic compounds. These compounds have wide-ranging effects on human physiology subsequent to their endogenous production (steroid hormones), intake via foodstuffs (fatty acids, phytochemicals), use as therapeutic drugs (insulin-sensitizing thiazolidinediones), and occurrence among xenobiotics. Deciphering the molecular determinants controlling NRs provides an opportunity to improve human health via directed regulation of their activity through numerous available NR agonists and antagonists. Similarly, this improved understanding could help prevent or ameliorate the adverse effects of pharmaceuticals or toxic effects of certain environmental pollutants which work through NRs. These potential benefits have driven the investigation of NR activity which has conclusively demonstrated that in addition to ligand, NRs are regulated by various factors within the cellular milieu including interactions with coregulator proteins leading to receptor activation or repression and

Corresponding author: Brian J. Aneskievich, Department of Pharmaceutical Sciences, University of Connecticut, 69 North Eagleville Road, U-3092, Storrs, CT 06269, Phone: 860-486-3053; Fax: 860-486-5792, Email: brian.aneskievich@uconn.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

subsequent expression or silencing of target genes (Perissi and Rosenfeld, 2005). NR coregulators can be broadly subdivided into coactivators and corepressors. Coactivators include SRC-1, SRC-2 also known as GRIP-1, SRC-3, p300/CBP and pCAF (McKenna *et al.*, 1999). For corepressors, the two best characterized are the earliest discovered, NCoR and SMRT (Perissi and Rosenfeld, 2005). Coregulator proteins modulate the transcription of NR target genes by participating in chromatin remodeling or interacting with general transcription machinery to affect the formation of the preinitiation complex (Perissi and Rosenfeld, 2005). The prevailing view of coregulator action holds that in the absence of bound agonist, NRs bound to their response elements on DNA interact with a corepressor via the so called corepressor NR (CoRNR “corner”) box (Perissi and Rosenfeld, 2005), typically having a sequence of L/V-X-X-I/V-I (where L is leucine, V is valine, I is isoleucine, and X is any amino acid) or alternatively LXXXI/LXXXI/L (Perissi *et al.*, 1999) which interacts with the corepressor docking surface on helices 3 through 5 of the receptor ligand binding domain (LBD) (Privalsky, 2004). For steroid receptors and peroxisome proliferator-activated receptors (PPARs) translocation to the nucleus occurs after ligand binding. In the absence of bound agonist, these receptors are held in the cytoplasm in a complex with heat shock protein 90 (steroid receptors) or 72 (PPAR α) by multiple chaperone proteins (Huang *et al.*, 1994; Pratt *et al.*, 2004). While coactivators and corepressors share several interaction sites in the receptor LBD, helix 12 (also referred to as activation function 2 [AF-2]) of the NR is critical in determining which coregulator docks: a corepressor or coactivator (Fig. 1A). Unliganded NRs have helix 12 in an extended conformation providing access to the corepressor binding site. Upon agonist binding, helix 12/AF-2 is repositioned close to the LBD. With this three-dimensional change in the NR, the three turn α -helix of the CoRNR box no longer fits into the corepressor binding area. In contrast, this helix 12/AF-2 repositioning favors interaction with the two-turn α -helix of a coactivator NR box, typically having a sequence of LXXLL (Fig. 1B). Once coactivators interact with the NR, helix 12 forms a charge clamp with helix 3 thus locking the coactivator into place on the agonist-bound receptor (Privalsky, 2004). Therefore, typical corepressors lack the structural means to repress an agonist-bound NR; this review will build on the corollary that atypical corepressors, i.e., those with LXXLL NR boxes, could inhibit agonist-activated NRs. Thus, receptor conformation and amino acid motifs in both proteins are key to their interaction.

The progression of aporeceptor association with corepressor and then holoreceptor association with coactivator following agonist binding was initially challenged with discovery of receptor interacting protein 140 (RIP140). Though first characterized as a coactivator, RIP140 acts as a corepressor of agonist-bound NRs (Augereau *et al.*, 2006 and references therein). For several years, this seemingly anomalous corepressor has been the lone known coregulator capable of acting as a corepressor of agonist-bound NRs. Recently, a few more such coregulators have been described giving rise to a small family of agonist-dependent corepressors of NR activity. RIP140 has been the subject of a number of recent reviews (White *et al.*, 2004; Augereau *et al.*, 2006) and therefore will not be individually considered here. Rather we will focus this review on the more recently isolated members of the expanding class of agonist-bound NR corepressors which exhibit diversity in structure, mechanisms of repression, physiological and pathological function. Nevertheless, a critical analysis shows some important similarities do emerge, specifically the presence of one or more LXXLL motifs and association with histone deacetylases (HDACs) as a means of mediating repression.

Corepressors with Inhibitory Effects Only

Ligand Dependent Corepressor (LCoR)

Aside from RIP140, LCoR is probably the best-known corepressor of agonist-bound NRs. It was isolated in a yeast two-hybrid screen seeking novel proteins that interact with ligand-bound estrogen receptor alpha (ER α) and was subsequently found to interact with a wide variety of

NRs. Western blot data revealed that LCoR is expressed widely in a variety of human fetal and adult tissues. When LCoR was transiently cotransfected into COS-7 cells with an NR construct and cognate reporter gene, it was found to be a repressor of ligand-dependent transcription for all receptors tested. Mutation of the LXXLL domain abolished receptor interaction and subsequent repressive effects of LCoR pointing to the crucial role of the NR box in the protein's corepressor function (Fernandes *et al.*, 2003)

NR corepressors frequently mediate their activity by recruitment of HDACs (White *et al.*, 2004). Indeed, LCoR-induced repression was relieved by treatment with the highly selective HDAC inhibitor trichostatin A (TSA). Physical association between LCoR and HDACs 3 and 6 was confirmed by glutathione S-transferase (GST) pulldown assays and coimmunoprecipitation studies (Fernandes *et al.*, 2003). However, while TSA completely reversed LCoR-mediated repression in ER α and glucocorticoid receptor (GR), no effect was seen with progesterone receptor (PR) or vitamin D receptor (VDR). This study suggested another mechanism for LCoR-induced repression in addition to HDAC recruitment. Examination of LCoR sequence revealed two putative binding sequences for C-terminal binding protein (CtBP) 1 and 2, both previously shown to act as HDAC-independent transcriptional corepressors (White *et al.*, 2004). The binding of CtBP to LCoR was confirmed by GST pulldowns and coimmunoprecipitation and was abolished by mutation of the CtBP binding sites. In addition to ablating CtBP interaction with LCoR, mutation of the CtBP binding sites also greatly relieved LCoR-induced repression of PR (Fernandes *et al.*, 2003). Thus, LCoR's mechanism(s) of NR repression may be additive via recruiting transcriptional repressors e.g., CtBP and/or HDACs depending on the NR and particular cell or tissue type (Fig. 1D). LCoR could be just one component of the combinatorial gene expression mechanisms necessary for a finer degree of control during development and/or differentiation. Given its wide range of expression, LCoR also has the potential for altering the effects of several NRs in numerous cells and tissues. In light of these characteristics, it seems that its physiological function may be to act as an "emergency brake", attenuating inappropriately activated NRs.

Preferentially Expressed Antigen in Melanoma (PRAME)

PRAME is a 509 amino acid protein originally identified as an antigen recognized by cytotoxic T lymphocytes from a melanoma patient. In addition to melanoma, PRAME is expressed at high levels in several malignancies including leukemia, multiple myeloma, lymphoma, head and neck carcinomas and breast cancer where high expression is a prognostic marker of poor clinical outcome. In contrast, it is absent or nearly so from normal human tissues except for testis, ovaries, endometrium, and adrenals (Epping and Bernards, 2006). Despite this expression pattern, there was no function known for PRAME until its identification as a NR coregulator; a role originally suggested by the presence of seven LXXLL motifs (Epping *et al.*, 2005).

Notwithstanding motifs typical of coactivators, PRAME was shown to repress transactivation of a retinoic acid response element by all three isotypes of retinoic acid receptors (RARs) in the presence of retinoic acid (RA). Its NR repression is highly selective for RARs as it had no effect on transcriptional activation mediated by retinoid X receptor (RXR), the heterodimer partner of RARs nor did it repress ligand-induced transactivation of other NRs including ER, PR, and PPAR. Physical interaction of RAR and PRAME was confirmed by GST pulldown, mammalian two-hybrid and coimmunoprecipitation studies. Of note, the interaction was seen only in the presence of RA. Indicative of its selectivity, no interaction was seen in GST pulldown between PRAME and ER or RXR either in the presence or absence of their respective ligands (Epping *et al.*, 2005). The LXXLL motifs as presumptive interaction sites between PRAME and RAR were tested by a series of site-directed mutants altering each of the seven

motifs individually or in combination. The first six LXXLL motifs were found to have little effect on interaction. However, mutating the last LXXLL, LRELL (aa 467-471) to LREVV, nearly abolished the RAR-PRAME interaction in vitro and in vivo. Moreover, mutating the LRELL motif prevented PRAME-induced repression of RAR activity (Epping *et al.*, 2005) confirming the biological importance of interaction via this motif.

Further biological consequences of PRAME repressing ligand-activated RAR were examined in the F9 embryonic teratocarcinoma system where RA induces differentiation, proliferation arrest, and apoptosis (Strickland and Mahdavi, 1978). Overexpression of wild-type PRAME in F9 cells prevented RA-induced effects while cells overexpressing the non-interacting LRELL to LREVV mutant retained full RA responsiveness similar to parental control cells. PRAME's inhibition of RA-induced effects was extended to A375 melanoma cells. Reduced production of endogenous PRAME by RNAi conferred RA sensitivity in vitro to these cells. In the presence of RA, their growth was suppressed and transcription of RAR-mediated gene expression was induced. Significantly, the restored sensitivity to RA was recapitulated when PRAME RNAi treated A375 cells were injected subcutaneously into nude mice (Epping *et al.*, 2005) suggesting PRAME may serve as a target for small molecule control of RAR function in certain malignancies where its overexpression has rendered those cells insensitive to the chemotherapeutic effects of RA. Several important questions remain to be addressed; among them what motif(s) of RAR are responsible for interaction with PRAME and if all retinoid-activated genes or a select set of them are targeted by the PRAME-RAR combination. In light of the absolute dependence of PRAME on a LXXLL motif for interaction with the receptor, it is reasonable to speculate that the AF-2 domain of RAR plays a role in the interaction. Specific target gene sets could help elucidate PRAME's role in oncogenesis.

Treatment with TSA had no effect on PRAME-mediated repression, suggesting an HDAC-independent mechanism. In light of the observation that PRAME expression levels parallel those of EZH2, a member of the polycomb group of proteins that are involved in initiation and maintenance of gene silencing (Kleer *et al.*, 2003), it was hypothesized that EZH2 may be involved in PRAME repression of RAR activity. Indeed, PRAME and EZH2 coimmunoprecipitate. Furthermore, EZH2 knockdown by short hairpin RNA abolished PRAME induced repression (Epping *et al.*, 2005). Similar phenotypic effect of either PRAME or EZH2 knockdown strongly suggests the likelihood of functional cooperation between the two proteins in the same RA-regulated pathway. Thus, as suggested from LCoR's interaction with CtBP and now PRAME's interaction with EZH2, agonist-dependent NR corepressors may serve as the entry site for other repressive proteins into the transcription complex (Fig. 1D).

Hairless (Hr)

Hairless' (Hr) involvement with NRs was discovered in a manner opposite to most NR coregulator proteins. In an effort to characterize its function, Hr was used as "bait" in a yeast two-hybrid screen which identified thyroid hormone receptor (TR) as one of its interacting proteins (Thompson and Bottcher, 1997). This sets the scene for a negative feedback mechanism: *hr* is a thyroid hormone-responsive gene via a thyroid response element in its promoter (Thompson and Bottcher, 1997) and yet the encoded protein, Hr, is a TR corepressor. Loss of Hr expression, whether via retrovirus insertional mutagenesis, the eponymic event responsible for the gene name, or targeted gene knockout, results in a hair loss phenotype. This is accompanied by skin wrinkling, keratinocyte hyperproliferation, and hyperdifferentiation indicating Hr and TR participation in keratinocyte replication and maturation (Zarach *et al.*, 2004). Given that coregulators often interact with more than one NR it was of clear importance to establish Hr's mechanism and range of association with NRs.

CoRNR boxes present in Hr mediate interaction with TR in the absence of ligand; the Hr-TR complex represses transcription via recruitment of HDACs (Potter *et al.*, 2002). This would assign Hr, at least for TR, to the typical NCoR/SMRT category of NR corepressor. However, Hr's actions as a corepressor are not limited to TR. It is also a potent repressor of the RAR-related orphan receptor α (ROR α) and its related ROR β and ROR γ isoforms. Unlike its CoRNR box-mediated association with TR, Hr's interaction with ROR is dependent on two LXXLL motifs that are typically characteristic of NR coactivators. Hr demonstrates selectivity in its means for associating with NRs; the Hr region containing the two LXXLL domains that interact with ROR α shows no interaction with TR (Moraitis *et al.*, 2002).

As might be expected of a LXXLL-mediated NR-interacting protein; Hr associates with ROR via the receptor's AF-2 domain. To confirm this, a chimeric RAR α was generated in which the AF-2 domain was changed to that of ROR α . RAR α , while closely related to ROR α , does not interact with Hr. The chimeric receptor retained its ability to activate transcription in response to the RAR ligand (all *trans*-RA) and also gained the ability to interact with Hr in the presence of all *trans*-RA. However, RA-dependent Hr association with the chimeric receptor led to transcriptional repression (Moraitis *et al.*, 2002). A similar mechanism is presumed to apply to ROR whose constitutive activity is attributed to the presence of a ubiquitous, but unknown ligand. Thus, Hr's LXXLL-mediated interaction and repression of select, liganded NRs via their AF-2 domain extends the example provided by LCoR: NR-coactivator interaction mechanisms might be co-opted by NR corepressors as and thus turned into a means of repressing agonist-bound receptors.

As stated previously, mutations in *hr* result in hair loss, a phenotype also observed in vitamin D receptor (VDR) loss-of-function mutations in humans and in VDR-null mice (Malloy *et al.*, 1999). These shared effects led to the hypothesis that VDR and Hr may function in the same pathway, and analogous to its ability to regulate TR and ROR, that Hr may be a VDR coregulator. To test this hypothesis, COS-1 cells were cotransfected with a human VDR expression plasmid and VDR-responsive reporter construct. Activation by either of two agonists, 1,25-dihydroxy (1,25(OH)₂) vitamin D₃ or lithocholic acid, was repressed by coexpression of Hr (Hsieh *et al.*, 2003). This finding adds VDR to those agonist-bound NRs repressed by Hr and suggests that ligand-dependent corepressors may associate with target NRs in the presence of either low (lithocholic acid) or high (1,25(OH)₂ vitamin D₃) affinity ligands. Despite this expanding repertoire, Hr interaction is not a given with all NRs. Neither VDR's heterodimerization partner RXR nor the class I (Mangelsdorf *et al.*, 1995) homodimeric GR had their ligand-induced transcriptional activity repressed by Hr coexpression (Hsieh *et al.*, 2003). Additionally, even those NRs interacting with Hr do not use the same receptor domain. As described earlier for ROR, Hr binds the receptor's helix 12/AF-2 and yet for VDR loss of this receptor region does not compromise interaction with Hr. Instead, helices 3-6 of the VDR LBD mediate association with Hr.

Hr is a multifunctional corepressor. It mediates its repressive effects on three different receptors in distinct ways. It acts as a corepressor of unliganded TR, dissociating upon ligand binding and using two CoRNR box motifs in a manner consistent with the classical corepressors NCoR and SMRT, through HDAC recruitment. Distinct from repression of unliganded TR, Hr represses ROR and VDR in agonist-dependent fashions although by targeting different domains of those NRs. Whereas Hr-ROR α interaction is dictated by the NR's AF-2 domain, this domain of VDR is not important for its interaction with Hr. Also, while the two LXXLL motifs of Hr drive interaction with and repression of ROR α , the minimal Hr region needed for interaction and repression of VDR activity is larger and more complex including both the CoRNR box and LXXLL motifs. Questions remain on the mechanism(s) e.g., HDACs, CtBP or other transcriptional repressors, by which Hr mediates the repression of ROR α and VDR, and to further define the VDR region needed to interact with Hr. Analysis of target genes of

Hr-NR complexes remain to be identified and will likely prove valuable in elucidating the full pharmacological, cell and tissue physiological significance of Hr's corepressor role.

Repressor of Estrogen Activity (REA)

REA was isolated in a yeast two-hybrid screen carried out in an attempt to identify the mechanism of a dominant negative ER α AF-2 (L540Q) mutant using the mutant ER as bait to screen an MCF-7 cDNA library. This dominant negative ER was previously shown to lack transcriptional activity in response to estradiol and could interfere with the wild-type ER activation by forming defective dimers with it (Schodin *et al.*, 1995). AF-2 mutant ERs such as the one used in the isolation of REA cannot activate transcription due to their inability to recruit coactivators (Danielian *et al.*, 1992), suggesting that REA-ER interaction might play a role in the effects of antiestrogens. REA is recruited to, but represses agonist-bound ER. Moreover, REA increased the potency of antiestrogen drugs such as trans-hydroxy-tamoxifen and ICI 182,780 fifty-fold. Effects of REA appear to be ER-specific as it does not alter transcriptional activity of other steroid receptors such as AR, PR (where neither agonist nor antagonist supported interaction), or class II NRs like RAR (Montano *et al.*, 1999).

Protein-protein interaction studies *in vitro* confirmed that ER-REA association was ligand-dependent, promoted by either agonist (estradiol) or antagonist (tamoxifen). As expected from REA's interaction with the dominant negative AF-2 ER mutant, receptor helix 12 is not involved thus distinguishing REA from LCoR. To date, REA interaction sites within the receptor have not been more specifically mapped than to a reliance on the remaining LBD. Nevertheless, given REA's inability to interact with apo-ER, it is reasonable to suggest that helices 3 and 11, which reposition in the presence of ligand (Gronemeyer *et al.*, 2004), might be involved. Conversely, the region of REA required to interact with ER is better characterized and has been located to the end of the middle third of the linear amino acid sequence. Notably, this region contains no motifs with homology to NR boxes or CoRNR boxes suggesting the existence of some third type of NR-interacting motif. ER repression subsequent to interaction mediated by this region required two additional domains from REA: one near the amino terminus and one just before the interaction domain (Montano *et al.*, 1999). Surprisingly, the amino terminal region includes a LXXLL motif. While this region appears to play no role in interaction with ER, leucine to alanine substitutions in it significantly reduced REA-induced repression, indicating that the integrity of the NR box is needed for optimal corepressor function (Delage-Mourroux *et al.*, 2000). In this instance, the LXXLL motif may stabilize interaction primarily mediated by non-LXXLL, non-CoRNR motifs, and thus promote REA's repressive effect. REA, then, employs interaction domains distinct from those of other ligand-dependent corepressors. Moreover, REA uses domains for repression of ER activity distinct from those it uses for interaction with ER.

Since its discovery, two complimentary and not necessarily mutually exclusive mechanisms of ER repression by REA have been described (Fig 1C). First is REA's ability to compete with ligand-dependent coactivators, such as SRC-1, for binding to agonist-bound ER (Montano *et al.*, 1999; Delage-Mourroux *et al.*, 2000). Co-expression of REA with the coactivator SRC-1 resulted in inhibition of SRC-1-induced ER activation. The degree of inhibition was dependent on the amount of REA present, suggesting direct competition between the two coregulators. In addition to reducing ER function by occupying receptor sites otherwise bound by coactivators, REA can suppress receptor activity by recruitment of HDACs (Delage-Mourroux *et al.*, 2000). Enzymes from both class I and class II HDACs interact with REA but through different regions within the deacetylase (Kurtev *et al.*, 2004). The ER-repressive function of REA is itself in turn regulated by protein-protein interaction. Prothymosin alpha recruits REA away from ER thus allowing SRC-1 binding to the receptor with a concomitant increase in ER transcriptional activation. Intriguingly, prothymosin alpha expression is upregulated by

agonist-bound ER (Martini *et al.*, 2000). Thus, ER drives the expression of a protein (prothymosin alpha) that inhibits the receptor's repression by siphoning away the protein (REA) responsible for it. These links, ER – REA – prothymosin alpha, in a transcriptional control chain – or perhaps better put, control web – illustrate that regulation of the NR corepressor, both in its production and function, will lend yet another facet in the total combinatorial control over NR activity.

A fundamental biological role for REA is highlighted by the fact that REA knockout mice die before embryonic day 9 suggesting that liganded ER, via interaction with REA, plays a necessary role in active repression of genes during embryogenesis whose expression is otherwise incompatible with continued development. In contrast to the embryonic lethality of REA null mice, the only observed phenotype observed in REA+/- mice was increased body weight of female, but not male, post-puberty mice compared to wild-type controls. Not surprisingly, the examination of estradiol-responsive genes in the uteri of heterozygous animals revealed that genes up-regulated by the hormone are more responsive than in wild-type mice (Park *et al.*, 2005). The increased expression of estrogen-regulated genes in REA+/- animals is evidence of the importance of REA in the downregulation of estrogen effects in post-embryonic physiology.

Metastasis Associated Factor-1 (MTA1)

MTA1 was initially isolated in a differential cDNA library screen using rat mammary adenocarcinoma lines: one nonmetastatic and the other highly metastatic. The screen was carried out in search for new genes involved in breast cancer invasiveness and metastasis. MTA1 is upregulated by a mesenchymal growth factor, heregulin- β 1. Disregulation of the heregulin- β 1 pathway results in the suppression of ER-mediated gene transcription in ER positive breast cancer cells. Furthermore, it has been reported that MTA1 physically interacts with HDACs (Mazumdar *et al.*, 2001 and references therein). In light of this report it was hypothesized that MTA-1 may play a central role in HRG-induced loss of ER-driven transcription. This was confirmed experimentally as transfection of MTA1 led to a decrease of ER-driven reporter activity in the presence of estradiol. Furthermore, physical association between ER and MTA1 was investigated and found to be dependent on the receptor AF-2 domain. Consistent with MTA1 association with HDACs, its repression of ER activity is prevented by addition of TSA (Mazumdar *et al.*, 2001). Moreover, MTA1 interacts with NRIF3 and inhibits its action as an ER coactivator (Talukder *et al.*, 2004). Together these results point to HDAC recruitment and competition with coactivators for ER binding as mechanisms of MTA1-induced repression (Fig. 1D). MCF-7 cells stably overexpressing MTA1 show increased invasiveness and anchorage-independent growth over control MCF-7 cells. Consistent with its function as an ER corepressor, the MTA1 overexpressing cells show significantly lower levels of ER-responsive gene expression (Mazumdar *et al.*, 2001). Adding clinical relevance to these findings is a recent report that in breast cancer patients MTA1 overexpression was found to be closely associated with higher tumor grade and increased tumor angiogenesis (Jang *et al.*, 2006).

Corepressors with Mixed Effects

NR Binding SET Domain Containing Protein 1 (NSD1)

NSD1 was initially isolated in a yeast two-hybrid screen, which used the LBD of RAR α as bait to find novel RAR coregulators. The *NSD1* gene is expressed in a variety of fetal and adult tissues including brain, kidney, skeletal muscle, spleen and thymus. Several mutations in the *NSD1* gene are associated with Sotos syndrome characterized by cardiac and renal abnormalities, seizures, and scoliosis (Tatton-Brown and Rahman, 2006). Moreover, an inappropriate fusion of this gene with the *NUP98* gene encoding a component of the nuclear

pore complex has been implicated in leukemia (Panagopoulos *et al.*, 2003). NSD1 appears to play a crucial role in early post-implantation development, as NSD1-null embryos exhibit a high degree of apoptosis and fail to complete gastrulation (Rayasam *et al.*, 2003)

NSD1 possesses distinct NR interacting domains (NID), one that interacts with TR and RAR only in the absence of ligand (NID^{-L}), and one interacting with several NRs, including TR, RXR, and ER, and, to a lesser degree, RAR, in the presence of ligand (NID^{+L}). Distinct receptor regions drive interaction with the two domains: helix 1 with NID^{-L}, and the AF-2 domain with NID^{+L}. The NID^{+L} contains a FXXLL motif, similar to the NR box, and it was found that point mutations of leucine residues in this motif significantly decreased the ligand-dependent interactions of the receptors with NSD1 (Huang *et al.*, 1998). Just as it possesses distinct domains for interacting with liganded and unliganded receptors, NSD1 also possesses distinct activation and repression domains. The repression domain localized between amino acids 1089 and 1400 was capable of strong repression of hormone induced ER activity (Huang *et al.*, 1998). Given the bipartite interaction between the receptors and NSD1, it seems likely that its activity in regard to modulating the receptor effects depend on the cell type, target gene promoter and other aspects of the cellular environment.

Comodulator of PPAR and RXRa (COPR1)

Research in our laboratory using a yeast two-hybrid screen looking for PPAR α coregulators has discovered another ligand-dependent corepressor. Comodulator of PPAR and RXR α (COPR1) was shown to partially repress the activity of liganded PPARs and RXR α . The interaction between COPR1 and NRs was totally helix 12 dependent and largely LXXLL dependent. The ability of COPR1 to interact with RXR could impact retinoid signaling as well as other class II NRs, such as PPAR, VDR, and TR for which RXR is the heterodimer partner (Flores *et al.*, 2004). Like NSD1, it also possesses an autonomous activation function, with the activation domain localized near the C-terminus. The loss of the activation domain made COPR1 a complete repressor (Flores and Aneskievich, unpublished data).

NRs and Coregulators in Xenobiotic Metabolism

NR Effects on Phase I and Phase II Enzymes

Numerous NRs are directly involved in executing the effects of xenobiotics. NRs can also regulate expression of enzymes involved in their detoxification. Treatment with RAR-specific ligands causes overall lowering of cytochrome P450 (CYP) enzymes in the liver, whereas treatment with RXR-specific ligands causes an overall increase in the CYP levels (Howell *et al.*, 1998). The effects are not universal however. Retinoids have been shown to increase transcription of CYP4B1, as mediated by a retinoid response element found in its promoter (Ashkar *et al.*, 2004). Moreover retinoids have been shown to regulate CYP3A4 via the pregnane X receptor (PXR)/RXR pathway (Wang *et al.*, 2006). CYP induction is subject to other NRs such as liver CYP1A1 via TR (Colombo *et al.*, 2003) and small intestine CYP3A via VDR (Xu *et al.*, 2006). PXR and constitutive androstane receptor (CAR) are also featured heavily in the list of NRs involved in regulating drug metabolizing enzymes (Gibson *et al.*, 2006). CAR is the target of phenobarbital and is responsible for inducing hepatocellular carcinomas in mice (Yamamoto *et al.*, 2004). The PXR promoter is in turn regulated by PPAR α and its ligands, which supports a complex network of xenobiotic-NR signaling (Gibson *et al.*, 2006).

Steroids also regulate CYP induction. ER induces the transcription of CYP1B1 gene via an estrogen response element in the gene promoter (Tsuchiya *et al.*, 2004). As CYP1B1 is an important enzyme in metabolism of 17- β -estradiol (Hayes *et al.*, 1996), the mutual regulation of ER and CYP1B1 activity appears to play a role in the maintenance of estrogen homeostasis.

As might be expected from a gene family as large as CYPs, numerous transcription factors such as the aryl hydrocarbon receptor, independent from or in combination with steroid receptors have significant control over enzyme expression (Nebert *et al.*, 2004). In light of ER down-regulation by several corepressors reviewed here, e.g. LCoR, REA and MTA1, it is possible that these would also decrease ER-dependent CYP induction. Other transcription factors such as the aryl hydrocarbon receptor might also negatively impact ER-regulated genes (Safe *et al.*, 2000). Members of UDP-glucuronosyltransferase (UGT) family of enzymes have been shown to be targets of PPAR α and PPAR γ (Barbier *et al.*, 2003), and also of ER (Harrington *et al.*, 2006). The effects of ER on both CYP1B1 and UGT are of particular interest here, since they were demonstrated in several breast cancer lines (Tsuchiya *et al.*, 2004; Harrington *et al.*, 2006), where REA and MTA1 are present and exert their corepressor activity.

NR Coregulators and Xenobiotic Metabolism

The role of NRs in responses to and metabolism of xenobiotics taken together with the role of coregulator proteins in mediating the NR activity implies a role for the coregulators in these same processes. This concept is exemplified by PXR, its interaction with coregulators and the consequences on regulation of cytochrome P450 enzyme expression. PXR association with the PPAR γ coactivator 1 α (PGC-1 α) or SRC-1 promotes expression of *CYP2A6* (Itoh *et al.*, 2006) or *CYP3A4* genes (Li and Chiang, 2006), respectively. Nevertheless, PXR interaction with LXXLL-containing proteins can bring about repression as evidenced by the orphan NR short heterodimer partner (SHP) interfering with PXR up-regulation of *CYP3A4* (Li and Chiang, 2006).

The role of ligand-dependent corepressors in xenobiotic-induced metabolism and xenobiotic-binding NRs has yet to be specifically addressed. However, reasonable extrapolations can be made from the current literature of typical NR coregulators and control of gene expression by xenobiotics as mentioned above. For instance, ligand-driven, LXXLL-mediated coactivator interactions participate in acetaminophen (APAP) hepatotoxicity (Jia *et al.*, 2005). A liver-targeted disruption of PPAR binding protein (PBP) significantly reduced hepatotoxic effects and centrilobular necrosis from APAP (Zhang *et al.*, 2002) likely through reduced activity of its namesake target PPAR (Jia *et al.*, 2004). PBP's range of NR interaction extends to CAR, which is dependent on PBP for translocation to the nucleus following exposure to phenobarbital-like CAR ligands (Guo *et al.*, 2006). It is possible that other LXXLL-containing coregulators, such as ligand-dependent corepressors, affect the subcellular localization and therefore activity of NRs involved in response to xenobiotics.

By further analogy to typical NR coregulator proteins, ligand-dependent corepressors might affect NR cross-talk via competition with coactivator proteins. Kemper and colleagues reported CAR interaction with limiting amounts of p160 coactivator led to loss of ER activity (Min *et al.*, 2002). We envision a parallel scenario where successful competition of one NR for a shared ligand-dependent corepressor leads to its reduced activity while the unsuccessful NR may over-activate target genes. Current methods in high throughput screening to identify new NR ligands rely on the candidate compound's ability to enhance interaction of the receptor with a limited number of known coactivator proteins (Nishikawa *et al.*, 1999). While new high affinity agonists identified by high throughput screening would be considered to activate NRs *in vivo*, it should be kept in mind that ligand-dependent corepressors not present in the original screen could decrease the compound's ability to activate the NR and its target genes. Definitive involvement of ligand-dependent NR corepressors in xenobiotic regulation of gene expression awaits direct experimental testing. We expect these investigations will be an important and productive venture both in terms of improving our understanding of xenobiotic effect and this growing family of NR coregulator proteins.

Concluding Remarks

The corepressors of agonist-bound NRs, despite great diversity of structure share several commonalities (see Table 1). They almost always contain one or more LXXLL motifs, which appear to play either a crucial, e.g. LCoR or PRAME or ancillary, e.g. REA, role in the interaction with target receptors and their repressive action. In all cases but one (Hr-VDR interaction) the LXXLL-containing corepressors require the AF-2 domain of the receptor for interaction. In this case, then, the corepressors of agonist-bound NR likely utilize the interaction mechanism typically associated with coactivators: formation of a charge clamp between receptor helices 12 and 3 locking the coregulator NR box into place.

Our examination of amino acid sequences of the ligand-dependent corepressors described above revealed that many of them also contain one or more previously unrecognized CoRNR box sequences. With the exception of Hr where CoRNR boxes have been demonstrated to be important for interaction with TR (Potter *et al.*, 2002) their role has not been examined in other corepressors of this class. LCoR also contains two CoRNR boxes in addition to the NR box and both REA and COPR1 contain a single CoRNR box motif. Within PRAME five of the seven LXXLL motifs also fit the canonical CoRNR box sequence, including the LRELL motif, found critical for interaction with RAR (Epping *et al.*, 2005). It has been shown in the case of SMRT that mutating any of the core amino acid residues within the CoRNR box drastically reduces the corepressor's ability to interact with receptors (Xu *et al.*, 2002). It is unclear then, whether the LRELL motif important in PRAME interaction with RAR is actually a part of functional CoRNR box or is an independent LXXLL motif.

In examining ligand-dependent corepressors and corepressors in general, it is important to differentiate the coregulator interaction domains from the repression domains. In some coregulators described here e.g. LCoR and PRAME, mutation of interaction domains led to loss of the repression. It is likely that this is simply due to loss of interaction, not because these domains possess repressive function. Such dual function, however, can exist as seen in Hr where the region important for interaction with VDR is also crucial for repression (Hsieh *et al.*, 2003). Defining the corepressor domains important for interaction and those important for repression will not only provide better understanding of transcriptional regulation but also may provide pharmacologic targets.

Interestingly, despite acting in a ligand-dependent manner, these corepressors often share a common mechanism of repression with the classical repressors like NCoR and SMRT – the recruitment of HDACs. Not surprisingly, given their structural diversity, these corepressors also exhibit significant differences such as those in specificity of interaction with particular receptors, additional corepressor proteins they recruit, and their physiological roles. Work remains to be done characterizing these novel coregulators, particularly in terms of their roles in homeostatic physiology, hormone-dependent cancers and NR-mediated responses to xenobiotics. Beyond their involvement in attenuation of NRs activated at the wrong time or to too great an extent, misregulation of these proteins may be causally involved in development of cancer. Ligand-dependent corepressors discussed here may be of particular interest in terms of applied pharmacology. This becomes especially clear in view of such findings as PRAME knockdown restoring RA responsiveness in melanoma (Epping *et al.*, 2005) and clinical evidence linking high MTA1 expression levels with poor clinical outcomes in breast cancer patients (Jang *et al.*, 2006).

In addition, one of the major questions yet to be addressed is the identification of genes targeted by these corepressors. Use of techniques such as microarray analysis, RNAi, and creation of knockout mice and transgenic animals over-expressing these proteins will be critical for

addressing these issues. Understanding of corepressors of agonist-bound NRs may lead to novel drug targets and identification of new transcriptional networks.

Acknowledgements

Work in the authors' laboratory is supported in part by pre-doctoral fellowships to I.G. from the American Foundation for Pharmaceutical Education and Boehringer Ingelheim Pharmaceuticals, Inc., to A.M.F. from NIAMS, NIH F32AR048483, and to B.J.A from NIAMS, NIH AR048860.

References

- Ashkar S, Mesentsev A, Zhang WX, Mastuygin V, Dunn MW, Laniado-Schwartzman M. Retinoic acid induces corneal epithelial CYP4B1 gene expression and stimulates the synthesis of inflammatory 12-hydroxyeicosanoids. *J Ocul Pharmacol Ther* 2004;20:65–74. [PubMed: 15006160]
- Augereau P, Badia E, Balaguer P, Carascossa S, Castet A, Jalaguier S, Cavaillès V. Negative regulation of hormone signaling by RIP140. *J Steroid Biochem Mol Biol* 2006;102:51–59. [PubMed: 17056252]
- Barbier O, Villeneuve L, Bocher V, Fontaine C, Torra IP, Duhem C, Kosykh V, Fruchart JC, Guillemette C, Staels B. The UDP-glucuronosyltransferase 1A9 enzyme is a peroxisome proliferator-activated receptor alpha and gamma target gene. *J Biol Chem* 2003;278:13975–13983. [PubMed: 12582161]
- Colombo A, Bonfanti P, Orsi F, Camatini M. Differential modulation of cytochrome P-450 1A and P-glycoprotein expression by aryl hydrocarbon receptor agonists and thyroid hormone in *Xenopus laevis* liver and intestine. *Aquat Toxicol* 2003;63:173–186. [PubMed: 12657491]
- Danielian PS, White R, Lees JA, Parker MG. Identification of a conserved region required for hormone dependent transcriptional activation by steroid hormone receptors. *Embo J* 1992;11:1025–1033. [PubMed: 1372244]
- Delage-Mourroux R, Martini PG, Choi I, Kraichely DM, Hoeksema J, Katzenellenbogen BS. Analysis of estrogen receptor interaction with a repressor of estrogen receptor activity (REA) and the regulation of estrogen receptor transcriptional activity by REA. *J Biol Chem* 2000;275:35848–35856. [PubMed: 10960470]
- Epping MT, Bernards R. A causal role for the human tumor antigen preferentially expressed antigen of melanoma in cancer. *Cancer Res* 2006;66:10639–10642. [PubMed: 17108098]
- Epping MT, Wang L, Edel MJ, Carlee L, Hernandez M, Bernards R. The human tumor antigen PRAME is a dominant repressor of retinoic acid receptor signaling. *Cell* 2005;122:835–847. [PubMed: 16179254]
- Fernandes I, Bastien Y, Wai T, Nygard K, Lin R, Cormier O, Lee HS, Eng F, Bertos NR, Pelletier N, Mader S, Han VK, Yang XJ, White JH. Ligand-dependent nuclear receptor corepressor LCoR functions by histone deacetylase-dependent and -independent mechanisms. *Mol Cell* 2003;11:139–150. [PubMed: 12535528]
- Flores AM, Li L, Aneskievich BJ. Isolation and functional analysis of a keratinocyte-derived, ligand-regulated nuclear receptor comodulator. *J Invest Dermatol* 2004;123:1092–1101. [PubMed: 15610520]
- Gibson GG, Phillips A, Aouabdi S, Plant K, Plant N. Transcriptional regulation of the human pregnane-X receptor. *Drug Metab Rev* 2006;38:31–49. [PubMed: 16684647]
- Gronemeyer H, Gustafsson JA, Laudet V. Principles for modulation of the nuclear receptor superfamily. *Nat Rev Drug Discov* 2004;3:950–964. [PubMed: 15520817]
- Guo D, Sarkar J, Ahmed MR, Viswakarma N, Jia Y, Yu S, Sambasiva Rao M, Reddy JK. Peroxisome proliferator-activated receptor (PPAR)-binding protein (PBP) but not PPAR-interacting protein (PRIP) is required for nuclear translocation of constitutive androstane receptor in mouse liver. *Biochem Biophys Res Commun* 2006;347:485–495. [PubMed: 16828057]
- Harrington WR, Sengupta S, Katzenellenbogen BS. Estrogen regulation of the glucuronidation enzyme UGT2B15 in estrogen receptor-positive breast cancer cells. *Endocrinology* 2006;147:3843–3850. [PubMed: 16690804]
- Hayes CL, Spink DC, Spink BC, Cao JQ, Walker NJ, Sutter TR. 17 beta-estradiol hydroxylation catalyzed by human cytochrome P450 1B1. *Proc Natl Acad Sci U S A* 1996;93:9776–9781. [PubMed: 8790407]

- Howell SR, Shirley MA, Ulm EH. Effects of retinoid treatment of rats on hepatic microsomal metabolism and cytochromes P450. Correlation between retinoic acid receptor/retinoid x receptor selectivity and effects on metabolic enzymes. *Drug Metab Dispos* 1998;26:234–239. [PubMed: 9492386]
- Hsieh JC, Sisk JM, Jurutka PW, Haussler CA, Slater SA, Haussler MR, Thompson CC. Physical and functional interaction between the vitamin D receptor and hairless corepressor, two proteins required for hair cycling. *J Biol Chem* 2003;278:38665–38674. [PubMed: 12847098]
- Huang N, vom Baur E, Garnier JM, Lerouge T, Vonesch JL, Lutz Y, Chambon P, Losson R. Two distinct nuclear receptor interaction domains in NSD1, a novel SET protein that exhibits characteristics of both corepressors and coactivators. *Embo J* 1998;17:3398–3412. [PubMed: 9628876]
- Huang Q, Alvares K, Chu R, Bradfield CA, Reddy JK. Association of peroxisome proliferator-activated receptor and Hsp72. *J Biol Chem* 1994;269:8493–8497. [PubMed: 8132576]
- Itoh M, Nakajima M, Higashi E, Yoshida R, Nagata K, Yamazoe Y, Yokoi T. Induction of human CYP2A6 is mediated by the pregnane X receptor with peroxisome proliferator-activated receptor-gamma coactivator 1alpha. *J Pharmacol Exp Ther* 2006;319:693–702. [PubMed: 16857725]
- Jang KS, Paik SS, Chung H, Oh YH, Kong G. MTA1 overexpression correlates significantly with tumor grade and angiogenesis in human breast cancers. *Cancer Sci* 2006;97:374–379. [PubMed: 16630134]
- Jia Y, Guo GL, Surapureddi S, Sarkar J, Qi C, Guo D, Xia J, Kashireddi P, Yu S, Cho YW, Rao MS, Kemper B, Ge K, Gonzalez FJ, Reddy JK. Transcription coactivator peroxisome proliferator-activated receptor-binding protein/mediator 1 deficiency abrogates acetaminophen hepatotoxicity. *Proc Natl Acad Sci U S A* 2005;102:12531–12536. [PubMed: 16109766]
- Jia Y, Qi C, Kashireddi P, Surapureddi S, Zhu YJ, Rao MS, Le Roith D, Chambon P, Gonzalez FJ, Reddy JK. Transcription coactivator PBP, the peroxisome proliferator-activated receptor (PPAR)-binding protein, is required for PPARalpha-regulated gene expression in liver. *J Biol Chem* 2004;279:24427–24434. [PubMed: 15150259]
- Kleer CG, Cao Q, Varambally S, Shen R, Ota I, Tomlins SA, Ghosh D, Sewalt RG, Otte AP, Hayes DF, Sabel MS, Livant D, Weiss SJ, Rubin MA, Chinnaiyan AM. EZH2 is a marker of aggressive breast cancer and promotes neoplastic transformation of breast epithelial cells. *Proc Natl Acad Sci U S A* 2003;100:11606–11611. [PubMed: 14500907]
- Kurtev V, Margueron R, Kroboth K, Ogris E, Cavailles V, Seiser C. Transcriptional regulation by the repressor of estrogen receptor activity via recruitment of histone deacetylases. *J Biol Chem* 2004;279:24834–24843. [PubMed: 15140878]
- Li T, Chiang JY. Rifampicin induction of CYP3A4 requires pregnane X receptor cross talk with hepatocyte nuclear factor 4alpha and coactivators, and suppression of small heterodimer partner gene expression. *Drug Metab Dispos* 2006;34:756–764. [PubMed: 16455805]
- Malloy PJ, Pike JW, Feldman D. The vitamin D receptor and the syndrome of hereditary 1,25-dihydroxyvitamin D-resistant rickets. *Endocr Rev* 1999;20:156–188. [PubMed: 10204116]
- Mangelsdorf DJ, Thummel C, Beato M, Herrlich P, Schutz G, Umesono K, Blumberg B, Kastner P, Mark M, Chambon P, Evans RM. The nuclear receptor superfamily: the second decade. *Cell* 1995;83:835–839. [PubMed: 8521507]
- Martini PG, Delage-Mourroux R, Kraichely DM, Katzenellenbogen BS. Prothymosin alpha selectively enhances estrogen receptor transcriptional activity by interacting with a repressor of estrogen receptor activity. *Mol Cell Biol* 2000;20:6224–6232. [PubMed: 10938099]
- Mazumdar A, Wang RA, Mishra SK, Adam L, Bagheri-Yarmand R, Mandal M, Vadlamudi RK, Kumar R. Transcriptional repression of oestrogen receptor by metastasis-associated protein 1 corepressor. *Nat Cell Biol* 2001;3:30–37. [PubMed: 11146623]
- McKenna NJ, Lanz RB, O'Malley BW. Nuclear receptor coregulators: cellular and molecular biology. *Endocr Rev* 1999;20:321–344. [PubMed: 10368774]
- Min G, Kim H, Bae Y, Petz L, Kemper JK. Inhibitory cross-talk between estrogen receptor (ER) and constitutively activated androstane receptor (CAR). CAR inhibits ER-mediated signaling pathway by sequestering p160 coactivators. *J Biol Chem* 2002;277:34626–34633. [PubMed: 12114525]
- Montano MM, Ekena K, Delage-Mourroux R, Chang W, Martini P, Katzenellenbogen BS. An estrogen receptor-selective coregulator that potentiates the effectiveness of antiestrogens and represses the activity of estrogens. *Proc Natl Acad Sci U S A* 1999;96:6947–6952. [PubMed: 10359819]

- Moraitis AN, Giguere V, Thompson CC. Novel mechanism of nuclear receptor corepressor interaction dictated by activation function 2 helix determinants. *Mol Cell Biol* 2002;22:6831–6841. [PubMed: 12215540]
- Nebert DW, Dalton TP, Okey AB, Gonzalez FJ. Role of aryl hydrocarbon receptor-mediated induction of the CYP1 enzymes in environmental toxicity and cancer. *J Biol Chem* 2004;279:23847–23850. [PubMed: 15028720]
- Nishikawa J, Saito K, Goto J, Dakeyama F, Matsuo M, Nishihara T. New screening methods for chemicals with hormonal activities using interaction of nuclear hormone receptor with coactivator. *Toxicol Appl Pharmacol* 1999;154:76–83. [PubMed: 9882594]
- Panagopoulos I, Isaksson M, Billstrom R, Strombeck B, Mitelman F, Johansson B. Fusion of the NUP98 gene and the homeobox gene HOXC13 in acute myeloid leukemia with t(11;12)(p15;q13). *Genes Chromosomes Cancer* 2003;36:107–112. [PubMed: 12461755]
- Park SE, Xu J, Frolova A, Liao L, O'Malley BW, Katzenellenbogen BS. Genetic deletion of the repressor of estrogen receptor activity (REA) enhances the response to estrogen in target tissues in vivo. *Mol Cell Biol* 2005;25:1989–1999. [PubMed: 15713652]
- Perissi V, Rosenfeld MG. Controlling nuclear receptors: the circular logic of cofactor cycles. *Nat Rev Mol Cell Biol* 2005;6:542–554. [PubMed: 15957004]
- Perissi V, Staszewski LM, McInerney EM, Kurokawa R, Kronen A, Rose DW, Lambert MH, Milburn MV, Glass CK, Rosenfeld MG. Molecular determinants of nuclear receptor-corepressor interaction. *Genes Dev* 1999;13:3198–3208. [PubMed: 10617569]
- Potter GB, Zarach JM, Sisk JM, Thompson CC. The thyroid hormone-regulated corepressor hairless associates with histone deacetylases in neonatal rat brain. *Mol Endocrinol* 2002;16:2547–2560. [PubMed: 12403844]
- Pratt WB, Galigniana MD, Morishima Y, Murphy PJ. Role of molecular chaperones in steroid receptor action. *Essays Biochem* 2004;40:41–58. [PubMed: 15242338]
- Privalsky ML. The role of corepressors in transcriptional regulation by nuclear hormone receptors. *Annu Rev Physiol* 2004;66:315–360. [PubMed: 14977406]
- Rayasam GV, Wendling O, Angrand PO, Mark M, Niederreither K, Song L, Lerouge T, Hager GL, Chambon P, Losson R. NSD1 is essential for early post-implantation development and has a catalytically active SET domain. *Embo J* 2003;22:3153–3163. [PubMed: 12805229]
- Safe S, Wormke M, Samudio I. Mechanisms of inhibitory aryl hydrocarbon receptor-estrogen receptor crosstalk in human breast cancer cells. *J Mammary Gland Biol Neoplasia* 2000;5:295–306. [PubMed: 14973392]
- Schodin DJ, Zhuang Y, Shapiro DJ, Katzenellenbogen BS. Analysis of mechanisms that determine dominant negative estrogen receptor effectiveness. *J Biol Chem* 1995;270:31163–31171. [PubMed: 8537380]
- Strickland S, Mahdavi V. The induction of differentiation in teratocarcinoma stem cells by retinoic acid. *Cell* 1978;15:393–403. [PubMed: 214238]
- Talukder AH, Gururaj A, Mishra SK, Vadlamudi RK, Kumar R. Metastasis-associated protein 1 interacts with NRIF3, an estrogen-inducible nuclear receptor coregulator. *Mol Cell Biol* 2004;24:6581–6591. [PubMed: 15254226]
- Thompson CC, Bottcher MC. The product of a thyroid hormone-responsive gene interacts with thyroid hormone receptors. *Proc Natl Acad Sci U S A* 1997;94:8527–8532. [PubMed: 9238010]
- Tsuchiya Y, Nakajima M, Kyo S, Kanaya T, Inoue M, Yokoi T. Human CYP1B1 is regulated by estradiol via estrogen receptor. *Cancer Res* 2004;64:3119–3125. [PubMed: 15126349]
- Wang K, Mendy AJ, Dai G, Luo HR, He L, Wan YJ. Retinoids activate the RXR/SXR-mediated pathway and induce the endogenous CYP3A4 activity in Huh7 human hepatoma cells. *Toxicol Sci* 2006;92:51–60. [PubMed: 16632523]
- White JH, Fernandes I, Mader S, Yang XJ. Corepressor recruitment by agonist-bound nuclear receptors. *Vitam Horm* 2004;68:123–143. [PubMed: 15193453]
- Xu HE, Stanley TB, Montana VG, Lambert MH, Shearer BG, Cobb JE, McKee DD, Galardi CM, Plunket KD, Nolte RT, Parks DJ, Moore JT, Kliewer SA, Willson TM, Stimmel JB. Structural basis for antagonist-mediated recruitment of nuclear co-repressors by PPARalpha. *Nature* 2002;415:813–817. [PubMed: 11845213]

- Xu Y, Iwanaga K, Zhou C, Cheesman MJ, Farin F, Thummel KE. Selective induction of intestinal CYP3A23 by 1 α ,25-dihydroxyvitamin D3 in rats. *Biochem Pharmacol* 2006;72:385–392. [PubMed: 16769037]
- Yamamoto Y, Moore R, Goldsworthy TL, Negishi M, Maronpot RR. The orphan nuclear receptor constitutive active/androstane receptor is essential for liver tumor promotion by phenobarbital in mice. *Cancer Res* 2004;64:7197–7200. [PubMed: 15492232]
- Zarach JM, Beaudoin GM 3rd, Coulombe PA, Thompson CC. The co-repressor hairless has a role in epithelial cell differentiation in the skin. *Development* 2004;131:4189–4200. [PubMed: 15280217]
- Zhang J, Huang W, Chua SS, Wei P, Moore DD. Modulation of acetaminophen-induced hepatotoxicity by the xenobiotic receptor CAR. *Science* 2002;298:422–424. [PubMed: 12376703]

Abbreviations

aa	amino acid
AF-2	activation function 2
at-RA	all-trans retinoic acid
CAR	constitutive androstane receptor
CBP	CREB binding protein
CtBP	C-terminal binding protein
ER	estrogen receptor
EZH2	enhancer of zeste homolog 2
GR	glucocorticoid receptor
GRIP	glucocorticoid receptor interacting protein
GST	glutathione S-transferase
HDAC	histone deacetylase
LBD	ligand binding domain
NCoR	nuclear receptor corepressor
NR	nuclear receptor

pCAF	p300/CBP-associated factor
PBP	PPAR binding protein
PGC-1α	PPAR γ coactivator 1 α
PPAR	peroxisome proliferator-activated receptor
PR	progesterone receptor
PXR	pregnane X receptor
RA	retinoic acid
RAR	retinoic acid receptor
RIP140	receptor interacting protein 140
RNAi	RNA interference
ROR	RAR-related orphan receptor
RXR	retinoid X receptor
SHP	short heterodimer partner
SMRT	silencing mediator for retinoid and thyroid-hormone receptors
SRC	steroid receptor coactivator
TR	thyroid hormone receptor
TSA	trichostatin A
VDR	vitamin D receptor
UGT	uridine diphosphate glucuronosyltransferase

Figure 1.

Models for corepressor and coactivator regulation of nuclear receptors. Note: for simplicity, only one of the two receptor dimers is illustrated.

A) Corepressors interacting with NR in the absence of ligand. Their interaction with chromatin modifiers, e.g., HDACs leads to histone states unfavorable for transcription and expression of target gene is blocked.

B) Coactivators interacting with NR in the presence of agonist. Agonist occupation of the NR ligand binding domain (LBD) leads to exchange of corepressors for coactivators, and in this scenario, recruitment of chromatin modifiers e.g., histone acetyl transferases (HAT) which acetylate lysine side chains. The remodeled, open chromatin structure allows addition proteins positively regulating transcription (not shown) to be recruited and promoter activity to ensue.

C) Agonist-dependent corepression. REA competes with SRC for the AF-2 of ligand-bound receptor reducing the opportunity for transcription-enhancing proteins, e.g., HAT, to participate in the transcription complex. Further loss of transcription-favorable chromatin structure may be lost by REA's direct association with HDACs and subsequent reduction of acetylated histones.

D) Generalized version of agonist-dependent corepression. Transcriptional repressors in addition to or instead of HDACs may participate in blocking expression. AF-1: activating function 1. AF-2: activating function 2. DBD: DNA binding domain of NR. Lig: ligand. Ac:

acetylated residues on histones comprising nucleosome. CoAct: coactivator. HAT: histone acetyl transferase. Other abbreviations defined in text.

Table 1

Summary of Corepressors of Agonist-Bound Nuclear Receptors

Coregulator (References)	Receptor (s) Affected	Coregulator Motif(s) for Interaction with Receptor	Receptor Domain(s) for Interaction with Coregulator	Coregulator Motif(s) for Repression	Mechanism (s) of Repression
LCoR (Fernandes <i>et al.</i> , 2003)	Class I and II receptors	LXXLL	AF-2	CtBP binding sites near the C-terminus	Recruitment of HDACs and repressor CtBP
PRAME (Epping <i>et al.</i> , 2005)	RARs	7 th of 7 LXXLLs	Not determined	Unlocalized EZH2 binding motifs	Recruitment of repressor EZH2
Hr (Moraitis <i>et al.</i> , 2002; Hsieh <i>et al.</i> , 2003)	ROR, VDR	ROR: LXXLLs VDR: region that includes a CoNR box and a LXXLL	ROR: AF-2 VDR: helices 3-6 of LBD	ROR: LXXLL motifs VDR: same as for interaction	Unknown
REA (Montano <i>et al.</i> , 1999; Delage-Mouroux <i>et al.</i> , 2000)	ER	Region between aa 175-198 with no recognizable motifs	Not determined	Regions between aa 19-49 which includes a LXXLL and aa 150-174	HDAC recruitment; Competition with coactivator SRC-1
MTA (Mazumdar <i>et al.</i> , 2001)	ER	Not determined	AF-2	Not determined	HDAC recruitment; Interference with coactivator NRIF3
NSDI (Huang <i>et al.</i> , 1998)	ER, TR, RXR, RAR	NID ¹ includes a FXXLL motif	Not determined	aa 1089-1400	Unknown
COPR1 (Flores <i>et al.</i> , 2004)	RXR, PPAR	LXXLL	AF-2	Not determined	Unknown

* As per classification system in (Mangelsdorf *et al.*, 1995)