Epigenetic regulation by nuclear receptors

Nuclear receptors (NRs) represent a vital class of ligand-activated transcription factors responsible for coordinately regulating the expression of genes involved in numerous biological processes. Transcriptional regulation by NRs is conducted through interactions with multiple coactivator or corepressor complexes that modify the chromatin environment to facilitate or inhibit RNA polymerase II binding and transcription initiation. In recent years, studies have identified specific biological roles for cofactors mediating NR signaling through epigenetic modifications such as acetylation and methylation of histones. Intriguingly, genome-wide analysis of NR and cofactor localization has both confirmed findings from single-gene studies and revealed new insights into the relationships between NRs, cofactors and target genes in determining gene expression. Here, we review recent developments in the understanding of epigenetic regulation by NRs across the genome within the context of the well-established background of cofactor complexes and their roles in histone modification.

KEYWORDS: acetyltransferase ■ coactivator ■ corepressor ■ deacetylase ■ demethylase ■ epigenetics ■ genome wide ■ histone ■ methyltransferase ■ nuclear receptor

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The nuclear receptor (NR) superfamily of transcription factors is responsible for coordinately regulating numerous biological processes such as development, reproduction and metabolic homeostasis. A unique feature of NRs is the ability to activate or repress gene transcription by binding to a NR-specific ligand (e.g., hormones, vitamins, lipid metabolites and xenobiotics), and the design of drugs that mimic NR ligands has become a preferred therapeutic strategy to treat various diseases [1]. Ligand-binding imparts changes in the NR structure and consequently modulates the recruitment of coactivator/corepressor complexes that are necessary for modifying the chromatin environment, altering accessibility of the transcription machinery to DNA. Intriguingly, altered expression and activity of NR cofactors mediating epigenetic changes, such as post-translational histone modifications, have been implicated in the susceptibility to cancer and metabolic disease [2,3].

As NR signaling impacts biological functions relevant to disease, numerous studies have focused on identifying key factors affecting NR-mediated transcription. Epigenetic regulations through DNA methylation and chromatin modifications are well-known mechanisms affecting gene expression. Although much of the understanding of NR signaling comes from gene-specific studies, new insights into the roles of specific modifications and their effectors have come from analyzing the entire genome. Specifically, recent developments combining chromatin immunoprecipitation (ChIP) with tiling arrays (ChIP-chip) and DNA deep sequencing (ChIP-seq) technologies have allowed studies of genome-wide epigenetic modifications and NR localization [4–6]. The findings from these genome-wide studies have challenged the model of NR-mediated transcription and identified new mechanisms determining NR target specificity. Here, we first briefly discuss epigenetic alterations in NR activity from the perspective of NR gene expression itself being regulated by DNA methylation. We then focus on the roles of histone modifications in general and in the context of NR binding and activity with an emphasis on genome-wide studies. We further highlight findings illustrating NR-specific as well as gene-specific cofactor regulation of gene transcription.

Epigenetic modifications mediating nuclear receptor activity

■ DNA methylation

Epigenetic modifications leading to repression of NR gene expression, such as DNA methylation, are anticipated to have dramatic physiological effects, as studies have described how knockdown or overexpression of a NR influences various biological functions and disease susceptibility. Methylation of DNA occurs on the cytosine bases catalyzed by DNA methyltransferases,

and in mammals, this mark primarily resides at CpG dinucleotides [7]. The mechanisms whereby DNA methylation represses genes has been proposed to occur through either directly preventing transcription factor binding or creating a binding site for methyl-binding proteins. Although reports have demonstrated a correlation of DNA methylation with various diseases, only a few studies report DNA methylation of promoters controlling NR expression.

Prenatal dietary restriction is known to increase susceptibility to obesity and diabetes in adults, and changes in DNA methylation are suspected to underlie part of this phenomenon [8]. In an animal model of prenatal protein restriction, CpG island microarray analysis of fetal liver DNA revealed 137 hypermethylated sites [9]. Hypermethylation was identified at the promoter of the NR liver X receptor (LXR) α, a key regulator of cholesterol and fatty acid metabolism, and this correlated with a reduced expression of LXR target genes [9]. Whether these effects correspond to changes in metabolism later in life is not known. In addition to prenatal diet, maternal care of offspring also affects later life phenotypes. The stress-induced hypothalamic–pituitary–adrenal signaling response involves expression of the NRs estrogen receptor (ER)α and glucocorticoid receptor (GR) [10–12]. Elevated ERα and GR gene expression in the brain of offspring receiving high levels of pup-licking/grooming and arched-back nursing by rat mothers correlated with reduced DNA methylation of promoters controlling both receptors [13,14]. More recently, suicide victims with a history of childhood abuse exhibited increased DNA methylation at the GR promoter and reduced GR expression, evidence of a potential link between adult actions and epigenetic changes possibly occurring during childhood experiences in humans [15]. Additional research utilizing global CpG methylation technologies will be necessary to understand how altered DNA methylation status relates to disease susceptibility and the mechanism of gene transcription.

General histone modifications

Modifications of the nucleosome core proteins, an octamer of histones H2A, H2B, H3 and H4, of which 146 bp of DNA are wrapped around, are necessary for recruiting cofactors and RNA polymerase II (Pol II) as well as maintaining chromatin stability [16–18]. The majority of the known histone post-translational modification sites associated with transcriptional regulation resides on the N-terminal tails extending from the nucleosome core histones. Post-translational modifications of histone tails include acetylation, methylation, phosphorylation, ubiquitylation, sumoylation and ADP-ribosylation, and the coordinated addition and removal of these modifications modulate the chromatin state [17,19–25]. Compared with acetylation and methylation, less is known regarding the role of other histone modifications in NR-mediated gene transcription. Ubiquitylation of histones H2AK119 and H2BK120 have been associated, respectively, with gene repression by the polycomb complex and gene activation through transcriptional elongation [26–28]. The presence of the large sumoyl moiety, however, blocks both acetylation and ubiquitylation [29].

Genome-wide analyses to date have primarily focused on histone acetylation and methylation as markers for determining the global transcription status. In addition, the relationships between NRs and cofactors capable of these modifications have been studied in detail. Therefore, in the following sections we focus on the roles of histone acetylation and methylation with respect to NR localization and the mechanism of NR signaling.

Histone acetylation

Acetylation of core histones occurs on specific lysine residues, creating a neutral charge that has been proposed to cause a loosening of the DNA–histone interactions and permit factor binding [30]. Actively transcribed genes contain acetylation marks on each of the four core histones. Histone acetyltransferases (HATs) catalyze the transfer of an acetyl moiety to lysine from acetyl-coenzyme A, and HAT coactivators with this capability include GCN5/p/CAF, the CBP/p300 family and steroid receptor coactivators (SRCs). Conversely, acetyl moieties are removed from lysines by histone deacetylases (HDACs). Corepressor complexes targeted to NRs during gene silencing have been found to contain HDAC3 [31], though other HDACs also interact but with a lower affinity. Although histone deacetylation is most often associated with gene silencing by corepressor complexes, HDACs have been found to bind genes that are primed for activation [32]. This suggests that in addition to other mechanisms, such as CpG island content [33], HDACs are involved in maintaining some genes in a state that is poised for rapid activation.

Chromatin regions near actively transcribed genes, including those bound by ligand-activated NRs, contain specific patterns of histone

acetylation and methylation across the genome. In general, histones near promoters, transcription start sites (TSS), and transcribed regions of active genes are hyperacetylated [34–37]. This is exemplified in a study showing acetylation of 18 different lysine residues of the four core histones were all positively associated with gene expression [36]. Indeed, regions within ligand-activated genes and near NR binding sites for androgen receptor (AR), ER, peroxisome proliferator-activated receptor γ (PPARγ), and vitamin D receptor (VDR) correlated with elevated levels of histone H3 and H4 acetylation [38–41].

Histone methylation

Methylation of core histones is more complex than acetylation as the presence of the methyl moiety, in a residue- and context-specific manner, may coincide with either activation or repression of gene transcription. Histone methylation occurs on arginines, in mono- or dimethylated states, and lysines, in mono-, di- or tri-methylated states [17,25]. Arginine methylation on histones H3 and H4 is catalyzed by the protein arginine methyltransferases (PRMTs) and the coactivator arginine methyltransferase (CARM1 or PRMT4) [25]. Unlike arginine methylation, enzymes have been identified for both lysine methylation and demethylation. Lysine methyltransferases, except for Dot1, contain a conserved SET domain, known as *Drosophila melanogaster* Su(var)3–9, enhancer of zeste (E[z]), and trithorax (trx). Mammalian methyltransferases containing a SET domain include ESET, Ezh2, MLL, PR-SET7/SET8, SET7/SET9, SETD2/HYPB, Smyd3 and the SUVs [25,42,43]. Lysine specific demethylase 1 (LSD1) and the jumonji C (JmjC) domain-containing family are capable of demethylating lysines [44–46]. Whereas LSD1 only demethylates mono- and di-methylated lysines, JmjC enzymes additionally act on trimethylated lysines [44,46]. Interestingly, although lysine methyltranferases and demethylases have more restricted substrate specificities compared with HATs and HDACs, recent studies demonstrate that many of these histone methylation and acetylation modifying enzymes are required for specific biological functions [17,18,47,48].

As histone methylation is associated with activation or repression, many methylated lysines coincide with active genes, such as histone 3 lysine 4 monomethylation (H3K4me1), H3K4me2, H3K4me3, H3K9me1, H3K27me1, H3K36me3 and H4K20me1 [34,36,37,49]. Arginine methylation of H3R2, H3R17, H3R26 and H4R3 are also implicated in transcriptional activation [50–54], possibly involved in recruiting HATs. The reproducible correlation of gene-expression data with H3 and H4 acetylation and H3K4 methylation has greatly aided in determining functionality of DNA-bound NRs. For example, ER-activated cells showed that the majority of H3K9ac, H3K4me2 and H3K4me3 around genes near the ERα binding sites correlated with Pol II binding and active gene expression [5]. In a similar manner, H3K9ac, H3K4me2 and H3K4me3 levels progressively increased during adipogenesis at genes bound and regulated by PPARγ [40].

In addition, gene repression has been strongly associated with the presence of H3K9me2, H3K9me3, H3K27me2 and H3K27me3, and both H3K9 di- and tri-methylation and H3K27me3 were found in areas of heterochromatin formation [36,37,49,55–58]. In ER-activated cells, levels of H3K9ac increased in parallel with decreased levels of H3K9me3 at active genes [59]. Global analysis of repressive marks also revealed differences in cell type-specific gene regulation. Adipocytes and macrophages were found to share some common PPARγ binding sites, but the majority of PPARγ sites associated with gene expression were unique to one cell type [60]. In this respect, global H3K9me2 and H3K27me3 levels in adipocytes were elevated at macrophage-unique PPARγ binding sites, and overexpression of PPARγ in preadipocytes was found to bind at adipocyte-unique but not macrophage-unique sites [60]. The cell type-specific presence or absence of NR binding likely involves factors such as environmental cues controlling heterochromatin formation during differentiation and the expression of other non-NR transcription factors and their chromatin modifying cofactors that bind nearby. Evidence using ChIP-seq showed that differences in cell type-specific gene regulation by PPARγ were partially determined by which non-NR transcription factor bound nearby [60]. Thus, binding to the existing NR-specific sites in different cell types occurs, in part, through the presence of other transcription factors that alter the chromatin environment to allow NR binding.

Genome-wide nuclear receptor activity

The use of cDNA microarrays has dramatically enhanced our understanding of which genes and biological pathways are activated or repressed during ligand-mediated modulation of NR activity. In addition, ChIP has demonstrated that expression of many genes affected by NRs contain NR-specific DNA sequences to which the NR directly binds. Until recently, however,

knowledge of the totality of genes that are direct targets of NRs had remained elusive. The advent of ChIP-chip and ChIP-seq has significantly furthered the understanding of transcriptional regulation by NRs.

Chromosome- and genome-wide NR localization revealed that AR, ER, GR and PPARγ could bind to intergenic and intronic regions distal to transcription start sites, which represented greater than 60% of genome-wide binding sites [6,61–67]. These observations challenge the classical mechanism of NR binding to receptor response elements in the proximal promoter. The distal localization sites suggest NRs may largely modulate gene transcription by functioning as enhancers. In addition, as many NRs heterodimerize with the retinoid X receptor (RXR), the binding sites of the two factors should overlap. Indeed, PPARγ colocalized with RXR binding sites and this number increased substantially with ligand-mediated PPARγ activation during *in vitro* differentiation of preadipocytes into adipocytes [6,66]. Another interesting finding using computation DNA binding motif scan and ChIP-chip analysis identified non-NR transcription factors, such as c-Myc with ERα and CCAAT/enhancer binding protein with PPARγ, with nearby DNA binding sites that are required for expression of a subset of NR target genes [59,66]. Thus, genome-wide studies have both confirmed previous knowledge and provided new insights into the mechanisms of NR regulation of gene expression.

Many factors interacting directly and indirectly with DNA are necessary for NR activity. A focus for this article is the interactions of cofactors with NRs that are involved in modifying the chromatin environment. In the following sections we describe NR binding with respect to histone modifications and cofactors, and then review evidence of cofactor interactions providing specificity to gene expression.

Histone modification pattern & nuclear receptor binding

Markers of functional regions

Intriguingly, different acetylation and methylation marks tend to appear in specific genomic regions. For example, acetylated lysines such as H2AK9, H2BK5, H3K9, H3K27 and H3K36 were primarily located around the TSS, whereas acetylated H2BK12, H3K4, H4K5, H4K12 and H4K16 lay in the promoter and transcribed regions [36]. Except for levels of H3K36me3 which gradually rise downstream of the TSS until the 3′ end of the coding region [68], most methylation marks of active genes peak either in enhancers, promoters, or near the TSS, which may expand out into the coding region [37,49]. The repressive H3K27me3 mark tends to reside in promoters and the TSS of inactive genes [37,49].

Combining microarray expression data with global ChIP analyses has been successfully used to locate enhancers by mapping H3 acetylation and H3K4me1 outside of promoters to the nearest TSS of differentially expressed genes [35,69,70]. In the case of NRs, PPARγ binding sites located greater than 10 kb from the nearest TSS were highly enriched in H3K9ac, a known marker of enhancers [66,70]. The H3K9ac signal at most of the potential PPARγ enhancer sites was elevated in adipocytes compared with preadipocytes [66]. Subcloning of PPARγ enhancers into reporter constructs verified the functionality of these sites, as previously reported for enhancer regions containing ER, GR and VDR [61,63,71].

Understanding chromatin states from patterns of modifications

As expected, a single region of chromatin contains multiple combinations of histone modifications. Recently, studies have sought to elucidate how multiple modifications affect the functionality of specific chromatin regions. Extensive computational modeling of datasets for the genome-wide occupancy of 38 histone acetylation and methylation marks, the histone variant H2AZ, Pol II, and the CCCTC-binding protein (CTCF) insulator, demonstrated this approach could be used to systematically annotate chromatin states, such as promoter, transcribed, active intergenic and repressed [72]. Interestingly, another report showed that areas containing both the active H3K4me3 and repressive H3K27me3 marks were found near genes with weak expression in embryonic stem cells [37]. The same regions in differentiated cells contained fewer 'bivalent' sites where genes primarily with H3K4me3 were highly expressed and genes primarily with H3K27me3 were lowly expressed [37,49]. It is proposed that the presence of both of these marks in the same region keep these genes repressed but poised for activation upon signal-dependent differentiation. Besides general activation and repression marks, the relationship between NR binding and combinations of histone modifications in a single region is still not clear. Further studies of how multiple modifications affect the chromatin state will be essential for understanding gene regulation as a whole and in the context of NRs.

Cofactors mediating nuclear receptor activity

Cofactors responsible for mediating epigenetic regulation of NR activity attain access to chromatin through either direct or indirect interactions with NRs. This ability of cofactors to interact with NRs is dependent on the presence or absence of a NR-specific ligand. In the absence of ligand, the configuration of the ligand-binding domain allows a hydrophobic groove of the NR to interact with a corepressor through the motif called LxxH/IIxxxI/L or corepressor NR (CoRNR) box motif [73,74]. Coactivators, however, contain one or more LxxLL motifs which enable interaction with the same site occupied by the corepressor. Ligand binding induces a conformational change in the hydrophobic groove, releasing the corepressor and allowing the LxxLL motif of the coactivator to bind in its place [75]. Once the NR-cofactor interaction occurs, other factors are recruited to carry out activation or repression of the target gene. In the following section, we discuss the key complexes and cofactors associated with regulation of NR activity, highlighting those involved in histone modifications.

Evolving model of nuclear receptor–cofactor interactions

In recent years, the mechanism of NR activation of gene transcription has evolved from the simple replacement of corepressors with coactivators to an intricate model involving ordered and cyclical recruitment of various cofactor complexes (reviewed in [76]). An elegant study of the ER-activated *pS2* promoter using ChIP-quantitative PCR demonstrated the sequential appearance and displacement of HATs and histone methyltransferases (HMTs), intermediary factors and general transcription factors, Pol II and elongation factors, and ATP-dependent chromatin remodeling complexes [77]. Interestingly, these events corresponded with concurrent cycles of H3K14 and H4K16 acetylation and H3R17 and H4R3 dimethylation, with H4 dimethylation remaining longer than other marks [77]. In addition to histone modifications, recent reports demonstrated that cycles of DNA demethylation/methylation occur on promoters of genes transcribed by ligand-activated NRs [78–80]. For example, at the end of each transcriptional cycle of the *pS2* gene, the ERα-binding site exhibited cycling of CpG methylation and DNA methyltransferases that paralleled the occupancy of complexes such as the SWI/SNF ATP-dependent chromatin remodeling complex [79]. Together, these findings, along with similar studies [81–86], emphasize the ordered, dynamic nature of cofactor involvement in gene transcription.

Coactivation complex

Based on the proximity of coactivators with NRs, these coregulators can be described as primary, binding directly to NRs, or secondary, interacting with NRs through an intermediary factor (Figure 1). Of the primary coactivators, the p160/SRC family has been studied extensively and was found to interact with many different NRs in a ligand dependent manner [87–89]. The p160/SRC members include SRC-1/NCoA-1, SRC-2/TIF2/GRIP1 and SRC-3/pCIP/RAC3/ACTR/AIB1/TRAM-1 [87,90–93]. SRCs contain multiple LxxLL motifs for binding to NRs and a domain possessing HAT activity [89,94,95]. The C-terminus of SRCs can interact with the p300 and CREB-binding protein (p300/CBP) HAT homologs, while the N-terminus contains basic helix–loop–helix (bHLH) and PAS domains that interact with other cofactors such as CoCoA, GAC63 and CARM1 [51,96–98]. The p300/CBP HATs are general coactivators that directly interact with NRs through LxxLL motifs [99]. The p300/CBP proteins also share conserved domains such as a bromodomain for recognizing histone acetylation and different regions for interacting with other cofactors [100,101]. In addition, the HAT domain can bind SRCs to reinforce the capacity to acetylate histones during transcription [96,100,102]. Other HATs such as GCN5/p/CAF (p300/CBP-associated factor) and MYST also contain the acetylation recognizing bromodomain and are involved in NR signaling [103]. Although fewer studies have been conducted on the GCN5/p/CAF family, these HATs were shown to be involved in AR and ER steroid hormone signaling [77,103].

Histone acetyltransferases, especially SRCs and CBP/p300, are known to interact with many different NRs. The global co-occurrence of SRCs and NRs on active genes can be appreciated by a recent SRC-3 localization analysis. In ER-activated cells, ChIP-seq demonstrated an approximate 59% overlap of ERα binding sites with SRC-3, and the majority (83%) of regions bound by both SCR-3 and ERα within -7.5 to +2.5 kb of the TSS correlated with upregulated genes [104]. In addition, SRC localization was also shown to be present in conjunction with histone acetylation and Pol II [38,105]. Global analysis of CBP binding also identified specific genes occupied near ERα bound targets marked with histone acetylation [106].

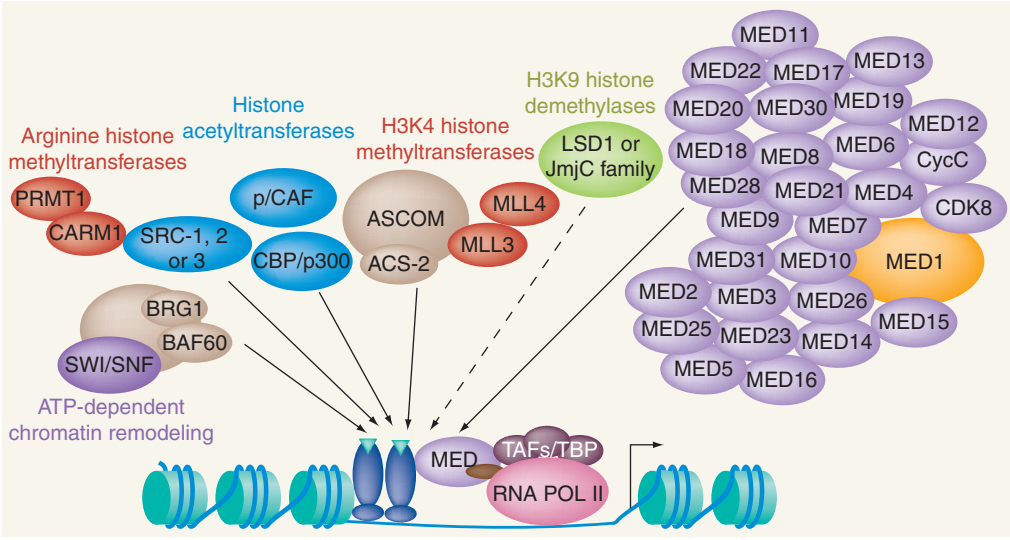

Figure 1. Nuclear receptor-associated coactivation complexes and the respective histone modifying enzymes. Ligand-induced active transcription of nuclear receptor target genes requires either simultaneous or ordered recruitment of cofactors capable of modifying histones and acting as mediators for binding of the RNA polymerase II-containing transcription complex. Histone modifications mediated by coactivators include acetylation (SRC-1, 2 or 3, p/CAF, CBP/p300), methylation (PRMT1, CARM1, MLL3 and MLL4), demethylation (LSD1, JmjC family) and ATP-dependent chromatin remodeling (SWI/SNF). The dashed line represents an unidentified mechanism for cofactor interactions.
MED: Mediator; POL: Polymerase.
MED data based on [147].

Steroid receptor coactivators recruit the arginine methyltransferase CARM1 as a secondary coactivator through the SRC activation domain (AD)2, separate from AD1 that recruits p300/CBPs [51]. CARM1 methylates arginines R2, R17 and R26 of histone 3 at active genes [50–52]. The PRMT1 methyltransferase can function synergistically with CARM1 by methylating H4R3 [53,54], and both CARM1 and PRMT1 were shown to occupy activation complexes of NRs such as AR, ER and thyroid hormone receptor (TR) [54,77,107,108]. During ER regulation of the *pS2* gene, cycling of H3R17 and H4R3 methylation on the promoter was similarly paralleled with the presence of CARM1 and PRMT1, illustrating the ordered co-appearance of cofactor and histone modifications on a NR target [77]. Although lysine di- and tri-methylation of H3K4 is generally associated with transcriptional activation, little information exists as to which methyltransferases possess this function in NR activation complexes. Methylation of H3K4 at LXR target genes was identified for the mixed lineage leukemia (MLL), HMTs MLL3 and MLL4 [109]. These HMTs were found in an activation complex designated ASCOM, which directly interacts with the NR through the ASCOM cofactor activating signal cointegrator-2 (ASC-2, Ncoa6) [109].

H3K9 di- and tri-methylation is associated with repression, and enzymes capable of demethylating these marks lead to activation. LSD1, identified to demethylate H3K4 and repress gene expression [110], also demethlyates H3K9 to participate in activation of AR and ER gene targets [106,111]. Indeed, global LSD1 localization overlapped with 58% of ERα binding regions, and ERα-activated cells displayed an approximate 84% co-localization of LSD1 with Pol II in promoter regions, the majority of which correlated with gene activation [106]. Interestingly, both LSD1 and the JmjC HDMT JHDM3C/KDM4C were present on an AR-regulated promoter [112]. Members of the JmjC family with H3K9 demethylase activity were found to coactivate genes regulated by AR, GR, PR and PPARγ [3,112]. The mechanism, however, as to how this family interacts with NRs is unclear.

In addition, many cofactors involved in transcriptional activation do not modify histones but possess other essential functions. These include mediators responsible for connecting the NR/coactivator and Pol II transcription complexes (e.g., MED1 and PGC-1) and SWI/SNF chromatin remodeling factors, among others [47]. The functions of these factors are critical to implementing epigenetic regulations by NRs.

Corepression complex

Similar to coactivators, complexes mediating NR repression contain primary corepressors necessary for recruiting other cofactors (FIGURE 2). The absence of ligand allows NRs to interact with the CoRNR box motif of the corepressors nuclear receptor corepressor (NCoR/NCOR1) and silencing mediator of retinoic acid and thyroid hormone receptor (SMRT/NCOR2) [73,74,113,114]. NCoR and SMRT display similar protein structures and regulate many of the same NRs. The core NCoR/SMRT complexes primarily include HDAC3; transducin β-like factors TBL1 and TBLR1, necessary for cofactor ubiquitylation and coactivator/corepressor exchange; and G-protein-pathway suppressor 2 (GPS2), involved in kinase inhibition [115–117]. Other HDACs also bind NCoR/SMRT at lower affinities, some of which require HDAC3 [118–120]. In addition to NCoR/SMRT, the Sin3A and NURD complexes have been associated with NR repression. NURD utilizes HDACs 1, 2 and 7, and cycling of HATs and histone acetylation were anticorrelated with the occupancy of HDACs 1 and 7 on the ERα-regulated *pS2* promoter [77,121,122]. Thus, NR corepression complexes are required for both gene repression and balancing acetylation at active genes.

Although NCoR/SMRT, Sin3A and NURD distinctively contain HDACs, whether HMTs associate with these complexes is uncertain. For example, gene repression by TR in the absence of ligand corresponded with increased levels of H3K9 trimethylation, and an *in vitro* assay demonstrated that SUV39H1, a HMT specific for H3K9 trimethylation, facilitated this TR-mediated repression [123]. However, immunoprecipitation of the NCoR, SMRT, Sin3, and NURD complexes failed to detect any HMT activity [123]. These findings suggest HMTs may either interact with low affinities or are recruited by another cofactor. Other H3K9 HMTs: ERG-associated protein with SET domain (ESET/SETDB1), retinoblastoma-interacting zinc factor (RIZ1), and EuHMTase, were shown to be involved in repression at AR- and ERα-regulated promoters in a ligand-independent manner [106]. ESET also mediates repression of PPARγ targets to control lineage commitment of mesenchymal progenitor cells to osteoblasts or adipocytes, which involved recruiting a complex regulated by the Wnt signaling pathway [124].

Ligand-dependent corepressors

Nuclear receptors are capable of ligand-dependent repression as well. This function requires interactions with corepressors through the LxxLL motif, and cofactors identified with this ability include ligand-dependent corepressor, receptor interaction protein 140 (RIP140), repressor of estrogen receptor activity, and the human tumor antigen PRAME [125–128]. Less is known regarding the histone modifying proteins these cofactors recruit, but association with HDACs has been reported. For example, ligand-dependent corepressor represses ER, GR, VDR and the progesterone receptor (PR), and this involves HDAC3 and HDAC6 [125,129]. The presence of ligand-dependent corepressors further demonstrates the complexity of gene repression, and illustrates the specificity that can be applied to specific NRs and their targets.

Preferential cofactor recruitment

Nuclear receptor-specific cofactors

The ability of NRs to coordinate a diverse range of biological functions requires both redundant and specific mechanisms for defining which cofactors are recruited to NR targets. Primary cofactors such as the p160/SRC family, NCoR and SMRT participate in regulating many of the same NRs due to the conserved LxxLL and CoRNR box motifs. SRCs cooperate with PPARs during gene activation, and in mice null for SRC-1, -2 or -3, the ligand-mediated induction of PPARα regulated genes was not affected by loss of any one of the three genes [48]. By contrast, SRC-1 and -2 act on PPARγ target genes [94,130,131], whereas SRC-3 functions as a coactivator of C/EBP to control the expression of PPARγ [132–134]. In addition, SRC-3 is a key regulator of normal growth, puberty, and mammary development, and its expression has been linked to various cancers [132,135]. Repression of NR activity through NCoR and SMRT appears to be interchangeable. Studies have shown, however, that differences such as a preference of NCoR for TR and SMRT for RAR exist, which occur in part owing to an extended interaction domain of NCoR and additional CoRNR box motifs within isoforms of SMRT [136–138]. The HATs CBP and p300 function in general transcriptional activation of a variety of transcription factors. Although other histone modifying cofactors have been found in a variety of NR complexes, the totality as to which NRs they regulate is not known. Animal models null for various cofactors have identified important biological functions, such as the HDMT JHDM2A/KDM3A with AR and PPARα [3,139], but the mechanisms determining how these NR-cofactor interactions occur is unclear and remains an area for further investigation.

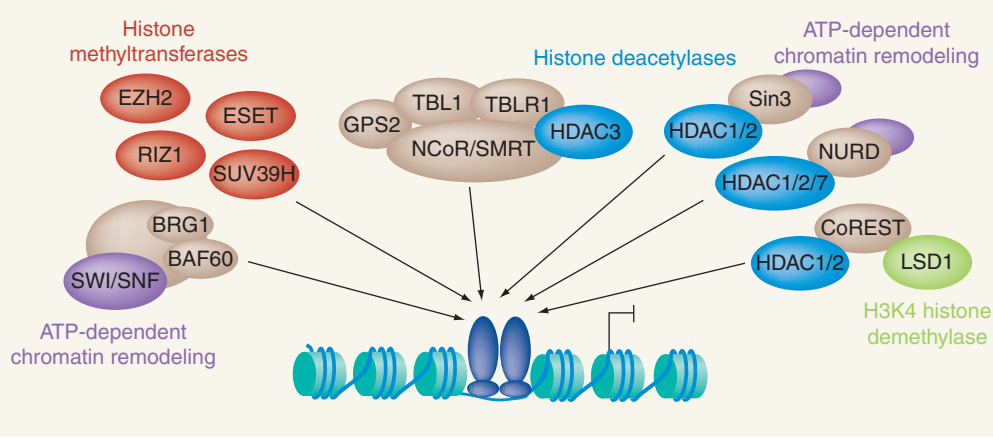

Figure 2. Nuclear receptor-associated corepression complexes and the respective histone modifying enzymes. The absence of ligand allows nuclear receptors to interact with corepressors which prevent coactivator binding and inhibit transcription initiation in part through histone modifications. Corepressors function as multisubunit complexes containing histone modifying activities such as ATP-dependent chromatin remodeling (SWI/SNF), methylation (EZH2, ESET, RIZ1 and SUV39H), demethylation (LSD1) and histone deacetylation (HDACs). The deacetylase HDAC3 is primarily associated with both the NCoR and SMRT complexes and their components GPS2, TBL1 and TBLR1. Sin3, NURD and CoREST also deacetylate histones through HDACs 1, 2, 7 and others with weaker binding affinities.
HDAC: Histone deacetylase.

Target gene-specific cofactors

Recently, accumulating evidence supports a model of cofactor recruitment to NRs in a target gene-specific context. Illustration of this concept was found in reports of SRC-3 and LSD1 localization across the genome with ERα. As described above, SRC-3 and LSD1 have been linked to transcriptional activation and were localized to many ERα targets. However, in ER-activated cells, a large amount of ERα-bound sites (~40%) do not overlap with either SRC-3 or LSD1, suggesting activation of these genes could be specific for other coactivators [104,106]. It would be interesting to combine these results and determine how much overlap exists between SRC-3, LSD1 and ERα at upregulated genes.

Intriguingly, knockdown of LSD1 and another HDMT, JHDM2A/KDM3A, reduced ligand activation of the same and distinct target genes of AR and ER [106]. Furthermore, basal expression of these genes was controlled by three different H3K9 HMTs: RIZ1, ESET and EuHMTase1 [106]. This study demonstrates the distinct as well as overlapping, nonredundant roles of histone methylating/demethylating enzymes working to control gene expression, in some cases possibly being components of the same complex. Of note, a recent global analysis of NCoR and SMRT binding sites involved in ligand-dependent transrepression by LXR revealed corepressor-specific genes, whereas others required both cofactors for functional repression [140]. In addition, the corecruitment of NCoR and SMRT at these specific genes depended on the presence of other transcription factors [140]. Thus, one could speculate the mechanism of gene-specific co-occupancy of similar histone modifying enzymes may involve interactions with complexes of separate transcription factors localized to the same region. This would require gene-specific localization of transcription factor binding sites in a relatively close proximity. Along this line, variations in the DNA sequence of NR binding sites likely contribute to differences in target gene-specific cofactor recruitment. A report of genome-wide GR-response element sequences, for example, found that while the DNA sequence of each individual site was conserved among species, the variations in sequence among all sites correlated with the level of GR occupancy in upregulated but not downregulated genes [141]. These findings suggest that DNA sequence significantly affects the specificity of NR and potentially cofactor recruitment to target genes.

Conclusion & future perspective

In this article, we have highlighted the recent developments in the understanding of epigenetic regulation by NRs across the genome within the context of the well-established background of cofactor complexes and their roles in histone modification. Although in its infancy, global analysis of NR localization in relation to cofactors and histone modifications has contributed new insights into important events determining activation or repression of NR targets. It has become

evident that NRs frequently regulate transcription through DNA looping by binding long-range distal enhancers, regions which can be identified by probing sites of histone modifications. In addition, the nature of NR-cofactor interactions is both ordered and dynamic, efficiently cycling on and off gene targets. Accumulating evidence further emphasizes the diversity of gene-specific cofactor recruitment and context-dependent histone modifications in modulating NR signaling.

Nuclear receptor–cofactor interactions are physiologically relevant as reports demonstrate the involvement of chromatin modifying cofactors in diseases such as cancer and the metabolic syndrome. Studies of the SRC coactivators have shown tissue-specific regulation of metabolic pathways, and SRC-3 is an established oncogene that regulates mammary gland metastasis [48,142]. Interestingly, circadian metabolic gene expression is altered by disrupting the interaction of corepressor NCoR1 with HDAC3, which produces a phenotype that is resistant to diet-induced obesity and insulin resistance [143]. With regards to histone methylation, absence of the JmjC demethylase JHDM2A/KDM3A increases susceptibility to obesity and metabolic syndrome, possibly through dysregulation of PPAR signaling, fat storage and glucose transport [3,144]. More recently, regulation of the H3K4/H3K9 methylation status by the LSD1 demethylase and SETDB1 methyltransferase were found to inversely control differentiation of preadipocytes into adipocytes, potentially linking these cofactors to obesity as well [145].

In the coming years, a focus on understanding the mechanisms for how chromatin modifying cofactors interact with NRs and histones will be an active area of research. Future studies are expected to uncover the key features determining context-dependent cofactor recruitment, including DNA sequence variants, DNA/histone modification patterns and components of assembled cofactor complexes, as they have not been fully defined. To address these questions, we anticipate future research to be directed towards genome-wide NR and cofactor localization combined with proteomics and structural analyses, expanding on what has been accomplished in recent studies [104,146]. These approaches will be critical for elucidating where and how specific protein complexes

Executive summary

Epigenetic modifications mediating nuclear receptor activity

- DNA methylation has been associated with gene repression, and in animal models of disease, hypermethylation of a promoter regulating a particular nuclear receptor (NR) has coincided with reduced expression of that NR and its target genes.
- Active sites of histone acetylation correlate with ligand-dependent upregulation of NR target genes.
- Histone methylation marks both gene activation and repression, and active methylation/demethylation of specific residues has been correlated with NR signaling.

Genome-wide nuclear receptor activity

- Global analysis of NR localization revealed that regulation of target gene expression often occurs through binding of NRs to long-range distal enhancers and in regions nearby binding sites for non-NR transcription factors.

Histone modification pattern & nuclear receptor binding

- Specific histone acetylation and methylation modifications have been localized to distinct genomic regions and used to characterize functional NR-binding sites.
- The transcription status of genes is dependent on combinations of different histone modifications, and understanding how these patterns regulate transcription is still unclear.

Cofactors mediating nuclear receptor activity

- The current NR signaling model involves an intricate model of ordered and cyclical recruitment of various cofactor complexes.
- Coactivators and corepressors mediating histone acetylation/deacetylation or methylation/demethylation often interact with NRs as components of multicofactor complexes.

Preferential cofactor recruitment

- Although many cofactors can interact with multiple NRs, studies using gene knockdown approaches have revealed NR-specific functional interactions of cofactors with distinct types of NRs.
- Genome-wide and single-gene analyses of cofactor localization identified target gene-specific cofactors that are recruited to distinct sets of genes irrespective of the NR to which it binds.

Conclusion & future perspective

- NR signaling requires the recruitment of cofactors capable of catalyzing distinct types of histone modifications, often at long-range distal enhancers and in a NR- and target gene-specific context.
- Future research utilizing genome-wide NR and cofactor localization approaches combined with proteomics and structural analyses will be critical in elucidating where and how specific cofactors capable of modifying the chromatin environment come together to mediate transcription.

capable of modifying the chromatin environment come together to mediate transcription. Consequently, knowledge of these events will aid in designing molecules to modulate NR-cofactor interactions with the potential for disease treatments.

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