# REVIEW

# Nuclear Receptor Mediated Gene Regulation through Chromatin Remodeling and Histone Modifications

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**Abstract.** Nuclear steroid/thyroid vitamin A/D receptor genes form a gene superfamily and encode DNA-binding transcription factors that control the transcription of target genes in a ligand-dependent manner. It has become clear that chromatin remodeling and the modification of histones, the main components of chromatin, play crucial roles in gene transcription, and many distinct classes of NR-interacting co-regulators have been identified that perform significant roles in gene transcription. Since NR dysfunction can lead to the onset or progression of endocrine disease, elucidation of the mechanisms of gene regulation mediated by NRs, as well as the identification and characterization of co-regulator complexes (especially chromatin remodeling and histone-modifying complexes), is essential not only for better understanding of NR ligand function, but also for pathophysiological studies and the development of therapeutic interventions in humans.

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# I. The Nuclear Receptor Gene Superfamily

# A. Biological functions of nuclear receptors (NRs)

Fat-soluble ligands, such as steroid/thyroid and vitamin A/D, exert a wide variety of biological effects through the transcriptional regulation of target genes via cognate NRs that exhibit specific ligand binding (see Fig. 1). Therefore, NRs are associated with cellular proliferation/differentiation events and are involved in a variety of functions in different cell types. Indeed, NRs are thought to be central to homeostasis as well as the development of clinical pathology in human beings. The physiological importance of the 48 NR family members currently recognized in human beings has been verified in mouse genetic models (Table 1) [1-37]. For instance, peroxisome proliferator-activated receptor (PPAR $\gamma$ ), a principal factor in the regulation of adipocyte differentiation and fat storage, has been shown to control glucose tolerance via the general regulation of insulin sensitivity [38, 39]. Indeed, the PPARy agonist thiazolidinedione has been used successfully in the clinical treatment of Type II diabetes mellitus. Likewise, fibrate, an agonist of the PPARy-

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Fig. 1. Nuclear receptor controls expression of target genes in a ligand-dependent manner.

Lipophilic ligands, such as fat-soluble vitamins A and D, as well as thyroid/steroid hormones, are thought to exert their physiological effects through transcriptional control by the cognate nuclear receptors (NRs). NRs recognize and bind with the specific recognition sites, termed hormone responsive elements (HREs). Ligand binding to NRs induces association with general transcription factors (GTFs) and the target genes are transcribed by RNA polymerase II (RNApol II).

related protein PPAR $\alpha$ , has been used to treat hyperlipidemia, as PPAR $\alpha$  regulates the expression of genes associated with lipid metabolism, including the lipoprotein lipase (LPL) gene that promotes the catabolism of chylomicron, very low density lipoprotein (VLDL), and intermediate density lipoprotein (IDL), with suppression of fatty acid synthesis in the liver and suppression of triglyceride (TG) production [40–42]. Sex hormone antagonists are also effective against sex hormone-dependent tumors of reproductive organs, breast, and prostate [43–45].

Reflecting the significance of NR functions in the biological effects of NR ligands, NR gene mutations underlie a range of genetic diseases. For example, mutations in coding regions of the androgen receptor (AR) can lead to complete loss of the androgen response, resulting in testicular feminization (Tfm) [46], and unusually expanded polyglutamine repeats within the AR A/B domain lead to spinal and bulbar muscular atrophy (SBMA) [47]. Genetic mutations in the vitamin D receptor (VDR) that result in the loss of vitamin D responsiveness cause hereditary vitamin D-resistant rickets type II (HVDRR) [48], while thyroid hormone

resistance syndrome (RTH) is due to mutation of the thyroid hormone receptor (TR)- $\beta$  gene [49]. Genetic disease also results from the malfunction of nuclear orphan receptors (*i.e.* those with unknown ligands). For example, several hepatocyte nuclear factor (HNF)- $4\alpha$  gene mutations are known to cause maturity onset diabetes mellitus of the young (MODY)-1 [50], while photoreceptor-cell specific NR (PNR) mutations lead to enhanced S-cone syndrome [51].

#### B. NR structure

All NR genes are thought to have evolutionarily developed from a single ancestor gene, such that in metazoans, the NR gene superfamily is found in all genomes from C. elegans to human. As all NR superfamily members share structural and functional characteristics that reflect this evolutionary relationship, NR proteins contain five functional domains designated A to E [52, 53] (Fig. 2). The A/B domain contains the activation function (AF)-1 region that is constitutively active even without ligand binding. The highlyconserved DNA binding domain (DBD) is located within the C domain, while the D domain contains the nuclear localization signal (NLS) [54]. The moderatelyconserved ligand binding domain (LBD) is mapped to the E domain, and consists of approximately 250 mostly hydrophobic amino acids that form a ligand-binding pocket made up of 12  $\alpha$ -helixes present in most of NRs. This domain plays a critical role in activation function (AF)-2 activity, which is induced by ligand binding, and results in clear shifting of the C terminalmost  $\alpha$ -helix 12 [55]. As the ratio between AF-1 and AF-2 is dependent on the tissue and cell type, AF-1 and AF-2 activities are probably controlled through a diverse range of molecular mechanisms. Of the  $12\alpha$ helixes encoded by the LBD E domain, specific ligands bind to a hydrophobic cave formed by  $\alpha$ -helices 3, 4 and 5. Ligand binding induces a structural alteration in the E domain, mainly in terms of movement of the  $\alpha$ helix 12. For ER $\alpha$  the angle of this  $\alpha$ -helix 12 shift has been reported to vary according to ligand type [56, 57], and appears to define the transactivation function. In contrast, NR AF-1 domains appear to mediate specific intracellular functions as the conservation of A/B domain amino acid sequences between NRs is low. While intramolecular interaction between AF-1 and AF-2 functions in gene regulation has been well described [58, 59], its molecular basis with respect to

glucocorticoid receptor (GR) impaired lung development (most of the mutant mice died during the perinatal period), [increase of corticosterone and ACTH in heterozygous mice] (33)
mineralocorticoid receptor (MR)
hyperkalemia, hyponatremia (pseudohypoaldosteronism) (4)
androgen receptor (AR) testicular feminization (Tfm) and osteopenia in the male mutant mice, abnormal brain masculinization (16, 32)
progesterone receptor (PR)
pietotropic reproductive abnormalities (25)
estrogen receptor (ER) $\alpha$ infertility (22)
ΕR β
reduction in fertility (12, 19)
retinoic acid receptor (RAR) α high postnatal lethality, testis degeneration (23)
retinoic acid receptor (RAR) $\beta$
no abnormality (14)
retinoic acid receptor (RAR) γ
growth deficiency, early lethality, male infertility (21)
thyroid hormone receptor (TR) $\alpha$
reduced linear growth, bone maturation delay, moderate hypothermia, reduced thickness of the intestinal mucosa (9)
thyroid hormone receptor (TR) $\beta$
resistance to thyroid hormone (8), deficit in auditory function (7)
Vitamin D receptor (VDR)
growth retardation, alopoecia, hypocalcemia, impaired bone formation (36)
peroxisome proliferator-activated receptor (PPAR) $\alpha$
lipid accumulation in the livers of fasted or high fat diet mutant mice (17, 20)
peroxisome proliferator-activated receptor (PPAR) $\beta/\delta$
embryonic lethal, growth retardation in surviving mice (30)
peroxisome proliferator-activated receptor (PPAR) $\gamma$
embryonic lethal, [protection from high fat diet induced adipocyte hypertrophy and insulin resistance in heterozygous mice] (38, 39)
liver X receptor (LXR) $\alpha$
loss of normal response to dietary cholesterol (28)
iver X receptor (LXR) b
no apparent abnormal pnenotype (2)
almesoid A receptor (rAK)
requestion of seturit one acting indicatority, and registerizes recentor (SCP)
loss of normal response to venobiotic treatment (18)
retinoid V recentor (RXR) $\alpha$
embryonic lethal forowth deficiency in heterozygous micel (13)
retinoid X recentry (RXR) ß
embryonic lethal male infertility in surviving mice (15)
retinoid X recentor (RXR) v
central resistance to thyroid hormone (5), less weight gain when fed a high fat diet (11)
photoreceptor-specific nuclear receptor (PNR)
retinal degeneration (1)
TLX
reduction in the size of rhinencephalic and limbic structures, including the olfactory, infrarhinal and entorhinal cortex (26, 37)
hepatocyte nuclear factor (HNF) $4\alpha$
embryonic lethal (6)
retinoid-related orphan receptor (ROR) α
cerebellar defects (10)
retinoid-related orphan receptor (ROR) β
retinal degeneration (3)
retinoid-related orphan receptor (ROR) γ
absence of lymph node (35)
adrenal-4 binding protein (Ad4BP)/steroidogenic factor (SF)-1
lack of adrenal glands and gonads, structural and functional abnormalities in spleen (24, 27)
chicken ovalbumin upstream promoter-transcription factor (COUP-TF) I
detects in morphogenesis of the glossopharyngeal ganglion, axonal projection, and arborization (31)
embryonic lethal (29)





A. Classes of ligand-dependent NRs. NRs are subdivided into subfamilies in terms of their partnership, *e.g.* homodimer (Type-I NRs), and RXR heterodimer (Type-II NRs). B. Functional domains of the NR superfamily. Total number of amino acids (a. a.) and the ligand for each receptor are shown on the right. The receptors are specific for estrogens (ER), androgens (AR), glucocorticoids (GR), thyroid hormone (TR), vitamin D (VDR), fatty acid metabolite (PPAR), and retinoic acid derivatives (RAR, RXR).

structure alterations of the entire NR molecule following ligand binding remains largely unknown.

Nevertheless, it is hoped that an understanding of the molecular mechanisms that regulate AF-1 and AF-2 activities will facilitate the development of new therapies targeted to NRs, especially as it may be possible to minimize the risk of side effects of endogenous ligands through the development of synthetic ligands. For example, estrogen replacement therapy for postmenopausal women can cause adverse effects such as uterine bleeding, mastodynia, and weight gain, as well as increased risk of endometrial cancer, breast cancer, and coronary heart disease [60]. Thus, the development of a selective estrogen modulator (SERM) with beneficial effects on bone and the cardiovascular system, but without adverse effects on the uterine tract or mammary glands, would be highly desirable. For example, tamoxifen is a SERM originally designated as a pure estrogen antagonist in the treatment of estrogendependent breast cancer. However, it became obvious from clinical applications over 30 years that tamoxifen in fact served not only as an AF-1 agonist in bone, lipid metabolism, and the cardiovascular system, but also as an AF-2 antagonist in mammary glands and female reproductive organs [61]. Raloxifen, a SERM now marketed in Japan, has been reported to be very effective in improving osteoporosis in post-menopausal women [62, 63], but cannot prevent hot flashes. Thus, based on detailed knowledge of the mechanisms of hER $\alpha$  AF-1 and AF-2 functions, it is anticipated that the desirable effects of SERMs can be further improved.

# **II. Decoding of Histone Signals and the Regulation of Gene Expression**

# A. Chromatin structure and histone modifications

Each human cell contains approximately 2 meters of DNA. As DNA is acidic (*i.e.* negatively charged), chromatin structures are maintained in an electrically neutral state through association with histone proteins that are basic (*i.e.* positively charged). Two molecules of each of the four histone types (H2A, H2B, H3, and H4) interact to form a histone octamer. DNA is coiled around this octamer, which forms a nucleosome, considered to be the minimum and basic structure of chromatin. One nucleosome subunit contains approximately 146 base pairs of DNA. However, not all DNA is coiled around histone octamers, as stretches of protein-free DNA serve as linker DNA between regions of coiled nucleosomal DNA. Repeated nucleosome units then form chromatin structures.

With regard to gene regulation, DNA regions contained within histone octamers are thought to be transcriptionally repressed. In contrast, linker DNA regions may play a leading role in gene activation, as these areas are easily accessible to transcription factors. To decode genetic information within the chromosome via transcription, it is now thought that histone octamers have to slide along the chromosomal DNA. However, the signals encoded on the chromosome that guide this process have long remained a mystery.

Recently, a revolutionary hypothesis was proposed that these chromatin signals are in fact related to posttranslational modifications on the histones. This breakthrough was based on observations from the crystal structure of the nucleosome, that the N-terminal tails of histones extended out from the regions of coiled DNA (see Fig. 3) [64, 65]. It has since become clear that these histone N-terminal tails can be post-translationally modified by processes such as acetylation, deacetylation, methylation, phosphorylation, ubiquitination, and sumovlation, that are targeted to specific amino acid residues [66, 67]. Furthermore, it has been shown biochemically that enzymes associated with these post-translational histone modifications exist as large protein complexes in the nucleus [68, 69]. It appears that each histone octamer is uniquely modified according to different combinations of post-translational modifications. In other words, the nucleosome, long thought to have a relatively simple repetitive structure, may actually contain arrangements of modifications that reflect specific signals. Thus, the nucleosomal array may contain information on chromosomal position, such that the decoding of specific DNA sequences can only occur when certain chromosomal nucleosome arrays are reorganized.



Fig. 3. Representative post-translational modifications of N-terminal tails of histones.

Chromatin is constituted with linker DNA and a nucleosome structure, which forms a complex between histone octamers surrounded by about 150 base pairs of DNA. N-terminal histone tails are protruded from the nucleosome core and are modified by several histone-modifying enzymes. Numerous combinations of post-translational histone modifications generates "histone code" to define the chromatin state and mark the addresses upon chromatin.

#### B. Chromatin remodeling

ATP-dependent chromatin remodeling complexes are primarily responsible for the rearrangement of nucleosomal arrays, according to signals defined by histone modifications [70, 71]. The sliding of histone octamers, around which contacting DNA is coiled, is facilitated by ATP-dependent chromatin remodeling complexes, thereby exposing new naked DNA regions. Chromatin structures are also formed by ATP-depending chromatin remodeling complexes during DNA replication. Histone octamers are transferred to newly synthesized DNA by these complexes, and nucleosomal arrangements adjusted. Thus, chromatin remodeling factors and/or complexes play a major role in tertiary chromatin structure.

# **III. Chromatin Regulation by NRs**

# A. Ligand-induced co-regulator switching by NRs

Unliganded NRs are transcriptionally silent even when bound to specific DNA elements. Upon ligand binding, NR transactivation functions through AF-1 and AF-2 are induced together along with co-regulator switching (Fig. 4). Co-regulators that associate with unliganded or liganded NRs are classified into two



Fig. 4. Co-regulators support ligand-dependent transcriptional controls by NRs through chromatin remodeling and histone modifications.

Ligand binding positively and negatively controls gene expressions of target genes by NRs through switching of co-regulators; most of them form histone modifying enzyme complexes, together with histone remodeling by ATP-dependent chromatin remodeling complexes.

functionally-opposite groups according to their impact on transactivation function. While co-repressors repress the transactivation function of unliganded NRs by physically associating with the LBD, particularly through  $\alpha$ -helix 12, liganded NRs are co-activated by a number of co-activators through physical association with both the AF-1 and AF-2 domains. The switching between co-regulator classes is induced by ligand binding. Most co-regulators appear to form complexes, and their roles in gene regulation are most likely linked to histone modification and chromatin remodeling.

#### B. Chromatin modifications by NRs

Gene regulation is controlled by epigenetic modifications that define the chromatin state, mainly via histone modification. The best characterized of the histone modifications mediated by NRs is histone acetylation and deacetylation. Co-regulator complexes with histone acetyltransferase (HAT) activity activate the transcription of target genes through the acetylation of histones, while histone deacetyltransferase (HDAC) complexes deacetylate histones and serve as co-repressors for unliganded NRs. Histone methylation is also induced by co-regulator complexes that associate with NRs. The methylation of lysine at amino acid position 4 (K4) in histone H3 appears to induce an active state in chromatin that leads to transcriptional activation. In contrast, methylation of K9 in histone H3 is thought to lead to transcriptional suppression by inducing the adjacent chromosomal region to adopt an inactive state [72-75].

# C. Chromatin remodeling by ATP-dependent chromatin remodeling complexes and NRs

ATP-dependent chromatin remodeling complexes use ATP hydrolysis to rearrange nucleosomal arrays in a non-covalent manner, thereby rendering chromosomal DNA accessible to DNA-binding transcription factors, including NRs (see Fig. 4). ATP-dependent chromatin remodeling complexes with distinct subunit combinations are classified into three major complex types (SWI/SNF, ISWI, and Mi-2) according to the ATPase that forms the main component of the complex [70]. Some of these complexes are known to physically associate with NRs [76–79]. For example, WINAC, a human multi-protein complex that directly interacts with VDR through the Williams syndrome transcription factor (WSTF), exhibits ATP-dependent chromatin remodeling activity, and contains both SWI/ SNF components and DNA replication-related factors. WSTF is highly homologous to hACF1, which together with hSNF2h are involved in the formation of well-characterized ISWI-based chromatin remodeling complexes. While WINAC mediates the recruitment of VDR to target gene promoters in the absence of ligand, the subsequent binding of co-activators to VDR requires ligand binding (Fig. 5) [80]. WINAC dysfunction seems to be at least partly responsible for some of the phenotypes associated with Williams syndrome, a rare autosomal dominant hereditary disorder with multiple symptoms, typically including congenital vascular lesions, elfin face, mental retardation, growth deficiency, and transient appearance of infantile aberrant vitamin D metabolism, including hypercalcemia [79]. Although some of the biological roles of ATP-dependent chromatin remodeling factors remain to be investigated, defects or mutations in Ini1, hBrg1, or hBrm, which are subunits of the SWI/SNF type ATP-dependent chromatin remodeling complex subtype, have been found in several cancers [81–85]. Furthermore, an SWI2/SNF2-like ATPase motif is present in ATRX, a protein produced by a causative gene for myelodysplasia associated with α-thalassemia (ATMDS) [86], and an SNF2-like domain is present in SMARCAL1, a protein that when defective leads to Schimke immuno-osseous dysplasia [87]. Hence, upon confirmation that these factors do act as chromatin remodelers, their related syndromes could be referred to as "chromatin remodeling factor diseases", and therefore considered as part of the "co-regulator disease" category. As chromatin remodeling is an essential step in gene regulation, ATP-dependent chromatin remodeling complexes presumably directly and indirectly support the ligand-induced transactivation of NRs. However, the functional interplay between NRs and chromatin modifying enzyme complexes remains to be clarified.

# IV. NR Co-regulators and Co-regulator Complexes

# A. NR AF-2 co-activators

It is well understood that ligand-induced transcriptional activation by NRs consists of two activation



Fig. 5. Ligand-induced transrepression by VDR mediates functions of a novel chromatin remodeling complex (WINAC) and coregulator complexes of histone modifying enzymes in the gene promoter of a vitamin D biosynthesis enzyme  $[1\alpha(OH)ase]$ .

steps [88, 89]. The first step is coupled with histone modifications such as acetylation, and is then followed by the formation of multi-complexes with general transcription factors that constitute a transcription initiation complex. Two such complexes, the p160/p300 HAT complex and the DRIP/TRAP complex, have been well characterized. Some components of these complexes have been shown to bind to liganded NR AF-2 domains through consensus LXXLL and related motifs present on some components [90]. p160/p300 complexes harbor one of three p160 family members (SRC-1 [91], TIF2/GRIP-1/SRC-2 [92, 93], pCIP/ RAC3/ACTR/AIB1/TRAM-1/SRC-3 [94-98]) and CBP/p300 [99]. All these components are in fact HAT proteins presumably required by NRs either as single factors or, more likely, as multisubunit complexes with other components in a ligand-dependent manner. HAT activity facilitates transcription by loosening chromatin structures through the acetylation of histone N-terminal tails.

SRC-1, one of the three p160 protein family members, contains three LXXLL motifs essential for ligand-dependent binding to NR AF-2 domains. SRC-1 has been shown to assemble with CARM1, an enzyme with dual histone methyltransferase [100, 101] and HAT activities [102]. From structural analyses, it appears that SRC-1 binds to a groove formed by NR  $\alpha$ -helixes 3, 4, 5, and 12 via the LXXLL motifs upon ligand binding. However, the three SRC-1 LXXLL motifs are not equivalent with respect to NR interactions. Altered amino acid sequences around the LXXLL motifs demonstrated altered NR interaction efficiencies dependent on the NR being used, which suggested that amino acids around the LXXLL motifs are essential for recognition and specific interactions with liganded NRs.

p300 was originally identified as a protein that bound adenovirus E1A [103], while the p300-related CBP protein was initially characterized as a co-activator of cAMP responsive element binding protein (CREB), a transcription factor activated by cAMP signaling [104]. CBP is thought to be the causative gene for the developmental disorder Rubinstein-Taybi syndrome characterized by multiple abnormalities, including broad thumbs and halluces, mental retardation, growth retardation, developmental delay, microcephaly, and craniofacial abnormalities [105]. p300/CBP exhibit high structural homology to each other, and are ubiquitously expressed in a variety of cells and tissues. It is likely that p300/CBP bind to other classes of DNAbinding transcriptional factors in addition to NRs, functioning as common co-activators for these factors [106–109]. Although p300/CBP physically bind in a ligand-dependent manner to NR AF-2 domains to activate transcription [110], they also function as co-activators for AF-1 [58], which suggests that the p300/CBP co-activators bridge AF-1 and AF-2.

Many other co-activators have been identified that may be important in NR functions. PGC-1 was initially described as a PPARy co-activator [111], and reported to dock with p160 member co-activators to NRs. PGC-1 has more recently been shown to be important for energy homeostasis. Indeed, a single nucleotide polymorphism (SNP) of the PGC-1 gene (Gly482Ser) is associated with the conversion from impaired glucose tolerance to Type II diabetes [112, 113]. Other NR co-activators include PRIP/ASC-2/ AIB3/RAP250/NRC that contains a single LXXLL motif and may act as a bridging factor between p300/ CBP and DRIP130, as well as being a component of the DRIP complex. Interestingly, its gene is known to be amplified in breast cancer [114-117]. Another example is GT-198. While its gene is localized to a breast cancer susceptibility locus, GT-198 protein exhibits kinase activity and acts as a tissue-specific NR co-activator through interaction with NR DNA-binding domains [118]. Hydrogen peroxide-inducible clone-5 (Hic-5), which belongs to the group III LIM domain protein family, contains four carboxyl-terminal LIM domains (LIM1-LIM4) and acts in the nucleus as a co-activator for steroid hormone receptors such as GR and AR [119, 120].

Following histone modification and chromatin remodeling, a mediator-like complex that forms a bridge between the NR-associated histone modifying complexes and the RNA polymerase II/transcription initiation complex is believed to be recruited to NRs. One such mediator-like complex is the DRIP/TRAP complex. This complex appears to contain no HAT activity, and was identified independently by two groups as a protein complex that interacted with VDR and TR $\alpha$ in a ligand-dependent manner [121, 122]. The DRIP/ TRAP complex enhances the transcriptional activity of NRs on naked DNA templates in cell-free, liganddependent transcription assays [123, 124], and also appears to activate transcription mediated by several transcriptional factor classes in addition to NRs. The complex component DRIP205/TRAP220 exhibits ligand-dependent binding to NRs via two LXXLL motifs, NR1 and NR2 [125]. In the presence of thyroid hormone, the TR-RXR heterodimer recruits the DRIP/

TRAP complex through the binding of RXR and TR to the NR1 and NR2 motifs, respectively. Mice heterozygous for a defective TRAP220 gene display pituitary hypothyroidism, whereas humans with TRAP230 abnormalities develop hypothyroidism [126]. Such conditions might also be classified as "co-regulator diseases" as might Rubinstein-Taybi syndrome that is caused by abnormal CBP function [105].

It is noteworthy that a third class of NR co-activator complex, the TFTC-type HAT complex, has also been identified [127]. This co-activator complex class harbors HAT activity, like p160/p300 complexes, but functionally resembles the DRIP/TRAP complex as a mediator complex. The TFTC-type HAT complex contains GCN5 HAT, the c-Myc interacting protein TRRAP/PAF400, and TAFII30, which are common factors shared with HAT complex subclass members including hTFTC, hPCAF, and hSTAGA HAT coactivator complexes. Three LXXLL motifs located in the central region of the TRRAP protein serve as the direct ligand-dependent surface for several NRs, including ERa. Surprisingly, antisense mRNA molecules for TRRAP inhibit the estrogen-dependent cell growth of breast cancer cells, which indicates that TRRAP might represent a new therapeutic target in the treatment of estrogen-dependent breast cancer [126, 127].

# B. AF-1 co-activators of NRs

While the above factors and complexes act as NR AF-2 co-activators, a number of NR AF-1 co-activators have also been documented. Amino acid sequences of NR A/B domains, which contain AF-1 activity, vary among NRs, which suggests that tissue-specific AF-1 functions of particular NRs are supported by unique co-activators. For instance,  $ER\alpha$  is phosphorylated by mitogen-activated protein (MAP) kinase activated by growth factor signaling. This phosphorylation occurs at the serine residue at position 118 in the A/B domain, and potentiates hER $\alpha$  AF-1 function [128]. DEAD box helicases p68 and p72 form a p160/ p300 co-activator complex with the RNA co-activator SRA. This complex appears to bind more strongly to phosphorylated than non-phosphorylated ERa A/B domains, and serves as an ERa AF-1 co-activator [129, 130]. Thus, p68 and p72 AF-1 co-activators may mediate cross-talk between the growth factor and estrogen signaling pathways. Besides p68/p72, an RNA splicing complex also appears to be preferentially recruited to phosphorylated Ser<sup>118</sup> in hER $\alpha$  [131]. In this case, the hER $\alpha$  AF-1 domain appears to also serve as an interacting domain for the activated dioxin receptor (AhR), establishing another potential cross-talk between estrogen- and AhR-mediated signals [132]. "Co-regulator diseases" due to AF-1 co-activators abnormalities have also been reported, such as defects in an AR-specific AF1 co-activator that results in Tfm [133], and mice genetically deficient in Cnot 7 (CAF1), an AF-1 co-activator of retinoid X receptor (RXR)  $\beta$ , have recently been reported to exhibit oligoasetheno-teratozoospermia [134].

# C. Co-repressors of NRs

Generally, NRs activate the transcription of target genes through the recruitment of co-activators in a ligand-dependent manner, while in the absence of ligand NRs suppress transcription by recruiting corepressors. These co-repressors can include histone modifying complexes such as the NR-co-repressor (N-CoR) and silencing mediator for retinoic acid receptor and thyroid hormone receptor (SMRT) complexes [135–137]. NCoR and SMRT complexes share a number of proteins such as HDAC 1/2/3, the Mad presumptive co-repressor mSin3, and transducin (beta)-like (TBL) 1, and a WD-40 repeat-containing protein, the gene for which was found to be mutated in human sensorineural deafness [138-142]. These co-repressor complexes deacetylate the N-terminal tails of histones, thereby "locking" the chromatin structure, leading to suppression of target gene transcription.

TBL1 and the homologous TBLR1 are thought to serve as factors that exchange co-repressors for coactivators. The ubiquitin/proteasome system includes the 26S proteasome, a complex composed of a 20S catalytic core involved in protein proteolysis and two ATPase-containing 19S regulatory particles that recognize polyubiquitin-tagged substrates [143]. TBL1 and TBLR1 are thought to function as adaptors for the recruitment of ubiquitin/19S proteasome complexes, thereby mediating the proteasomal degradation of corepressors, and inducing the recruitment of coactivators [144].

The mechanisms of transcriptional repression by nuclear orphan receptors remain largely unknown.

However, it has been reported that the nuclear orphan receptor chicken ovalbumin upstream promoter-transcription factors (COUP-TF) I represses the transcriptional activity of target genes that interact with NCoR and SMRT [145]. Another nuclear orphan receptor, PNR, which is the causative gene for enhanced S-cone syndrome, acts as a sequence-specific repressor that controls neuronal differentiation in the developing retina. A PNR co-repressor complex has been identified that includes E2F/Myb-associated proteins, NCoR/ HDAC complex-related components, TBL3 (part of the same protein family as TBL1), and the DEVH-box co-repressor (Dev-CoR) that belongs to the DEAD/ DEVH protein family. This co-repressor directly interacts with PNR and functions as a platform protein. Notably, the PNR-associated Dev-CoR complex appears to function as a negative cell cycle repressor via inhibition of cell cycle-related gene promoters, indicating that co-repressors may have similar biological importance as co-activators in gene regulation (S. T., H. K., S. K., unpublished results).

# V. Perspectives

NRs require a number of distinct classes of factors and/or complexes for their ligand-independent and -dependent functions in gene regulation. From the most current views on the molecular mechanisms of gene regulation by DNA-binding transcription factors, it appears that a number of complexes and factors associate with a given transcription factor in a sequential and highly regulated manner. However, while NRs appear to recruit a number of factors/complexes, it is still unclear whether particular NR molecules require many factors/complexes or only limited numbers of factors/complexes depending on the promoter/chromatin context. Also, it is likely to become clear in the near future how many of the numerous diseases and pathophysiologies related to NR functions are linked to malfunctions within co-regulators or co-regulator complexes. Such advances in the understanding of molecular mechanisms that underlie NR function in a variety of physiological and pathophysiological situations will contribute to drug discovery and new clinical applications.

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