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RETINOID RECEPTORS AND THEIR COREGULATORS

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■ **Abstract** Retinoids regulate gene transcription by binding to the nuclear receptors, the retinoic acid (RA) receptors (RARs), and the retinoid X receptors (RXRs). RARs and RXRs are ligand-activated transcription factors for the regulation of RA-responsive genes. The actions of RARs and RXRs on gene transcription require a highly coordinated interaction with a large number of coactivators and corepressors. This review focuses on our current understanding of these coregulators known to act in concert with RARs and RXRs. The mechanisms of action of these coregulators are beginning to be uncovered and include the modification of chromatin and the recruitment of basal transcription factors. Challenges remain to understand the specificity of action of RARs and RXRs and the formation of specific transcription complexes consisting of the receptors, coregulators, and other unknown factors.

INTRODUCTION

Retinoids, a group of small lipophilic molecules, are essential for a variety of biological processes. The indispensability of retinoids in animals was first demonstrated in a controlled study by Hale (1), which showed that deficiency in vitamin A (retinol) induced congenital malformations in pigs. Following this pioneering work, Warkany's group demonstrated congenital abnormalities in vitamin A-deficient (VAD) rat dams, such as those affecting the eyes, myocardium, aorticopulmonary septum, diaphragm, and the respiratory and urogenital systems (2). Subsequent studies showed that vitamin A was also indispensable in postnatal life for survival, reproduction, vision, and maintenance of epithelial tissues (3, 4). The first indication that retinoic acid (RA) was the active ingredient of vitamin A came from the observation that RA could repair most of the defects caused by VAD, except defects in the visual system, which specifically required retinaldehyde (4, 5).

In the late 1980s, the cloning of the nuclear RA receptor α (RARα) (6, 7) opened an avenue for the dissection of the RA signaling pathways. Subsequently, the detection of endogenous RA in animals substantiated the notion that RA could be the physiologically active component of vitamin A (4). This was further supported

by two studies that showed the rescue of VAD embryos by RA at specific stages of embryogenesis (5) and the teratogenic effects of pharmacological doses of RA during embryogenesis (4). It was then proposed that RA could play an essential role in many aspects of life, such as development, growth, reproduction, vision, and cancer prevention. With the help of molecular genetic tools, a direct role for RA in many of these biological processes was unambiguously demonstrated in mutant animals that were deficient in specific functional receptors (8–10). These studies have provided the ultimate proof that RA (specifically all-*trans* and 9-*cis* RA) constitutes the active ingredient of vitamin A and its nuclear receptors are ligand-activated transcription factors responsible for regulating the expression of RA-responsive genes. However, it remains highly challenging to dissect the complex RA signaling pathways because the specificity of the actions of retinoids in different gene systems cannot be accounted for by the actions of the receptors alone. This puzzle began to unravel through the discovery of a large number of receptor coregulators (11–15) that are now known to act in concert with the receptors. Identification of these coregulators marked the beginning of a new era for the dissection of the complicated mechanisms underlying specific actions of RA in different gene systems.

This review focuses on our current understanding of coregulators of the nuclear RA receptors, as well as the working mechanisms underlying their actions. I begin with a summary of the features and functions of these receptors followed by a review of the identification and the properties of their coregulators (coactivators and corepressors). Finally, a discussion is provided on the problems that challenge future studies of the action of vitamin A, their receptors, and their coregulators.

NUCLEAR RECEPTORS FOR RA AND THE PRINCIPLE OF THEIR ACTIONS

Cloning of Nuclear Receptors for RA

It was a long-standing and challenging task to address the pleiotropic effects of a group of structurally simple molecules like retinoids. Biochemical and ligand-binding studies, as well as the demonstration of genome-wide changes triggered by these small molecules, suggested the existence of intracellular receptors for these retinoids (16, 17). It was the discovery and cloning of the RARα from the groups of Chambon (6) and Evans (7) that marked the beginning of an era of molecular biology leading to our understanding of hormone nuclear receptors. It is now known that RARs and retinoid X receptors (RXRs) belong to the superfamily of nuclear receptors that transduce signals to the gene transcription machinery, resulting in changes of gene expression (8, 18–20).

The RA receptor family consists of RARα, RARβ, RARγ, RXRα, RXRβ, and RXRγ, as well as their isoforms. RARs bind to all *trans*- and 9-*cis* RA, whereas RXRs bind specifically to 9-*cis* RA. From molecular and genetic studies, it has become clear that the actions of RA are closely tied to the functions of these receptors. The most convincing evidence comes from combinatorial genetic

studies where mutant animals were made defective in the function of one or two of these receptors. These mutant animals essentially recapitulated the entire spectrum of VAD (10). Single mutant mice lacking the function of a single RAR or RXR gene were viable and displayed one or some of the many aspects of postnatal VAD syndrome. However, double mutants defective in a pair of isotypes such as RARβ/RARγ, RARα/RARβ, and RARα/RARγ (10) all died in utero or at birth, suggesting essential roles for these receptors in animal survival and some functional redundancy of RARs. The physiological functions of RXRs were less conclusive based upon these genetic studies. However, it seemed that RXRα was the most critical receptor, whereas RXRβ and RXRγ appeared to be dispensable. In vitro structural studies as well as molecular biological approaches all suggested an RAR/RXR heterodimer as the physiologically functional unit (21–25). This notion was supported by the genetic studies of compound mutants that were defective in one RXR and one RAR (4, 10).

The complexity of vitamin A signaling pathways was suggested from studies that detected many types and isoforms of these receptors, as well as the discovery of many similar yet distinct RA response elements (RAREs) present in the regulatory regions of RA target genes (20, 26). Most studies have supported the notion that the functional unit of these receptors is a dimer consisting of one RAR and one RXR. Therefore, a large reservoir of distinct receptor dimers can potentially be generated from the different receptor types and their isoforms. The dimeric receptor pair binds to a specific RARE located in the proximity of a gene promoter's regulatory regions. As a result, the activity of vitamin A target genes can be dictated by a combination of various receptor pairs that bind to different RAREs in the context of specific promoters. Questions remain as to what determines the specificity of these receptor dimers for these very similar RAREs of different genes and how the structurally similar molecular entities (receptor dimers) are able to elicit variable actions on different gene promoters. The recent discovery of "coregulators" for RAR and RXR provides a clue for another level of control that can potentially contribute to the diverse actions of these dimeric receptor units.

Domain Features of RARs and RXRs

All the nuclear receptors, including RARs and RXRs, share common modular domains (A to F) (27–29) that can be interchanged without loss of function (Figure 1). The N-terminal A/B domains are the least conserved and contain an autonomous activation function, named AF-1. The C domain is the most highly conserved domain and contains two zinc finger modules responsible for DNA binding, and is thus named the DNA-binding domain (DBD). This domain spans approximately 60–70 amino acid residues among which 8 of the 9 conserved cysteines tetrahedrally coordinate two zinc ions, resulting in the formation of two compact finger-like structures that bind DNA. The region at the base of the first finger is named the D box and is responsible for discriminating the DNA sequence, whereas the region at the base of the second finger participates in receptor dimerization.

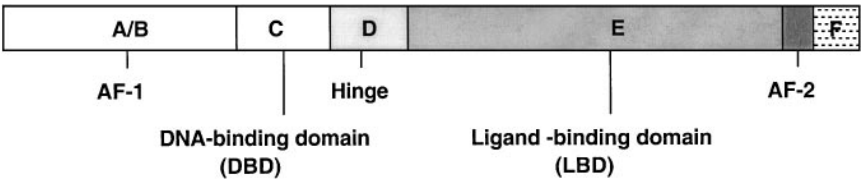

Figure 1 Major functional domains of RAR and RXR. The N-terminal *A/B* segment encodes the activation function-1 (AF-1) domain. The *C* segment encodes the DNA-binding domain (DBD). The *D* segment encodes a flexible hinge region. The *E* segment encodes the ligand-binding domain (LBD) and the activation function-2 (AF-2) domain. The C-terminal tail, *F* domain, is present in some, but not all, of the receptors.

The D domain, or hinge region, is variable and connects the zinc finger DBD to the variable E region, which encompasses the ligand-binding domain (LBD). It is also known that the hinge region contains a nuclear localization signal and is probably responsible for the interaction with certain nuclear receptor coregulators (30, 31). Our recent studies using fluorescence correlation spectroscopy showed a dramatically reduced diffusion rate of a hinge region dissected from orphan receptor TR2, indicating potentially extensive protein interaction involving this region (L.-N. Wei, unpublished observations).

The LBD is a multifunctional domain that is responsible for ligand binding, receptor dimerization, and interaction with other proteins such as coactivators. Crystallization studies have shown a canonical structure for the LBD of all nuclear receptors, including RARs and RXRs (28, 29). This structure is formed by folding 12 conserved α-helical regions named H1 to H12, with a conserved β-turn connecting H5 and H6. These helices are folded into a three-layer antiparallel helical sandwich in which a pocket is created for ligand binding. The cognate agonist makes a stereospecific, high-affinity interaction with the pocket of the LBD, inducing a conformational change in the receptor. The most C-terminal F domain is found in certain members and participates in the action of a second activation domain named AF-2. The AF-2 is composed of an amphipathic α helix that is also highly conserved and is responsible for the agonist-induced conformational change of receptors. Upon agonist binding, the receptor undergoes a conformational change and the structure becomes much more compact. This conformational change results in changes in protein interaction and stimulates the recruitment of coactivators, leading to the activation of gene transcription (see details in the section on Coregulators of RARs and RXRs). It has become apparent that these receptors can also be activated by signals other than the retinoid ligands, such as those involving protein kinases and metabolic products (32, 33). Presumably, modification of receptors by these enzymes also induces a conformational change resulting in the recruitment of coactivators. Evidence has been provided that extracellular signals are able to modulate the activity of nuclear receptors in certain pathways (34–37).

Receptor Dimerization

A large number of studies have demonstrated heterodimerization of one RAR with one RXR (21–25, 38), and it is suggested that the functional receptor unit is a heterodimer. Dimerization is mediated primarily by the interacting surface of the LBD on each receptor. It is believed, at least in the in vitro situation, that receptors are present in oligomeric states and ligand binding induces dissociation of receptor oligomers and facilitates the formation of heterodimers (39–42). It is the heterodimeric receptors that bind a specific RARE with a high affinity. Additionally, occupancy of either receptor by an agonist is sufficient to induce a conformational change of the AF2 domain in the dimeric receptor unit, thereby recruiting associate proteins and leading to the activation of the transcription machinery. The combinations of receptor pairs, as a result of pairing different RAR and RXR types and isoforms, provide a rich reservoir of potential functional receptor dimers. The specificity of transcriptional activation of a gene in a particular cell or tissue type is partially dictated by the action of different receptor pairs that are cell specific. Furthermore, the specific sequence of an RARE and the context of a specific gene regulatory region, i.e., the sequences adjacent to the RARE, can also contribute to specificity of receptor action. Recently, it has become apparent that the regulatory mechanism orchestrated by RAR/RXR is further complicated by the recruitment of receptor associate proteins (coregulators) expressed in specific cells.

COREGULATORS OF RARs AND RXRs

The cloning of numerous proteins that interact with RARs and/or RXRs suggested the existence of a large number of coregulatory proteins for RARs and RXRs. It has become increasingly clear that these associate proteins of RAR and RXR play important roles in the ultimate control of the activity of a gene promoter regulated by RA. Currently, various working models are proposed to address the complexity and specificity of the actions of RA, which are based upon the principle of cofactor recruitment by receptors, i.e., corepressor recruitment by apo-receptors (gene silencing in the absence of hormones) and coactivator recruitment by holo-receptors (gene activation in the presence of hormones) (9, 43–47) (Figure 2). Several coregulators (both coactivators and corepressors) are known to bind to an overlapping surface on the LBD of the receptors. Mutational and structural studies have suggested that in the absence of ligands, or upon binding by antagonists, the receptors recruit corepressors to a hydrophobic surface groove formed by helices 3, 4, 5, and 6 of the LBD. In the presence of an agonist, the AF-2 domain at the C-terminal tail of the receptor changes its position, releasing corepressors and, together with helices 3, 4, and 5, forms a cleft that recruits coactivators onto the receptor pairs. Recent studies have attempted to map the cofactor-interacting surface of the receptors, and have proposed a leucine-rich "LXXLL" signature motif for the p160 coactivator families (48, 49) and a related "L/IXXI/VI" motif for certain corepressors such as N-CoR and SMRT (50–52). It is proposed that these

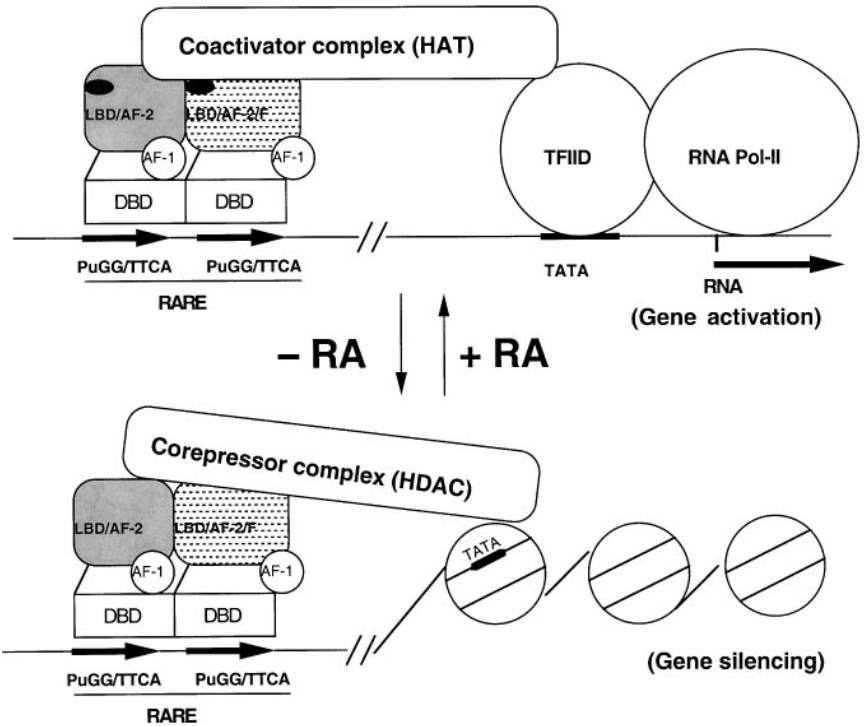

Figure 2 A model for RAR/RXR acting in concert with coactivator or corepressor complex for gene activation or silencing. In the presence of ligands (+RA), the holo-receptor pair binds to the RA response element (RARE) and recruits coactivator complex, which encodes histone acetyl transferase (HAT) activity. HAT acetylates histone proteins, opens up the chromatin, and allows the transcription machinery to act on the promoter for active gene transcription. In the absence of ligands (−RA), the apo-receptor pair binds to the RARE and recruits corepressor that encodes histone deacetylase (HDAC) activity, inducing histone deacetylation, chromatin condensation, and gene silencing.

complexes occupy an overlapping hydrophobic groove on the receptor surface. Although this seemingly simple change in receptor conformation is able to explain the mutually exclusive nature of receptor interaction with either coactivators or corepressors, it is not sufficient to account for the complexity of gene regulation, such as the variable level of induction and silencing, or the combinatorial control of gene expression by many signals acting in concert. As such, it is tempting to speculate that further highly specific mechanisms are adopted by different cells for the same hormonal signals to be variably transduced in a wide variety of tissue/gene systems, most likely through orchestrating extensive protein-protein interactions and multiprotein complex formation in a very specific manner.

An increasing number of coregulators of transcription factors, including that of nuclear receptors, are being identified. Several of these coregulators are shared by a large number of transcription factors and some are highly specific to certain members. By a strict definition, some of these coregulators do not fall into the category of either coactivator or corepressor. For the sake of simplicity, the coregulators reviewed here are categorized as coactivators or corepressors according to the outcome of their actions on the RAR- and RXR-systems.

Coactivators of RARs and RXRs

The ultimate target of RA is the genome of the cells, and the principle of its action is to induce a conformational change of the receptor pairs that interact with the transcription machinery, resulting in altered gene transcription rates. As such, components of the transcription machinery, including the enzymes and the substrates, can all potentially be involved in the interaction with RARs and RXRs. Broadly speaking, coactivators of a transcription factor can be divided into two major classes: adapters that recruit transcriptional apparatus and components that are involved in chromatin-remodeling or modification. Although some of the coactivators of RARs and RXRs are known for their apparent functions, the roles of many remain to be determined or are complicated by their presence in overlapping complexes. By definition, a coactivator must fulfill several criteria: an agonist-dependent direct interaction with holo-receptors and the ability to interact with the components of the basal transcription machinery and enhance the basal transcriptional activity. Because of the ambiguity of several members that participate in gene activation by RARs and RXRs, the coactivators are not categorized by using this stringent definition. Rather, the coactivators of RAR and RXR, as reviewed here, are grouped based upon their effects on RAR- and RXR-mediated gene expression and the most commonly adopted nomenclature and/or their apparent molecular features.

SRC1/p160, CBP/p300, AND p/CAF COMPLEXES The steroid receptor coactivator (SRC) family consists of three subfamilies, i.e., SRC-1, TIFII/GRIP1/p160, and RAC3/ACTR/pCIP/AIB-1 (49) (Figure 3*A*). SRC-1 was first cloned in a yeast two-hybrid screening with the progesterone receptor (PR) as the bait (53), and was demonstrated as a common coactivator for all steroid receptors tested. It was later shown to encode a new member of the basic helix-loop-helix-PAS domain family and interact with the RARs and the p300/Phospho-CREB binding protein (CBP)/p300/CBP-associated factor (P/CAF) complex, as discussed later. A coactivator complex containing SRC-1/p300/CBP/PCAF was then proposed as a component that mediates the activating function of RARs (54). Subsequently, evidence was presented that SRC-1 contained intrinsic histone acetyltransferase (HAT) activity specific to H3 and H4 histones, an activity encoded by the C-terminal region of SRC-1 (55). This provided the first evidence for a role of a hormone nuclear receptor's coactivator in modifying chromatin by facilitating the

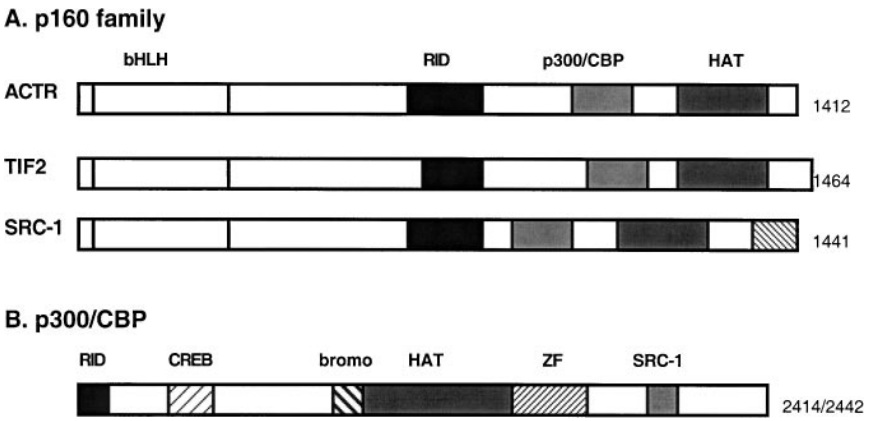

Figure 3 Domain features of the SRC and p300/CBP coactivators. (*A*) The three SRC coactivator families, ACTR, TIF2, and SRC-1, are highly homologous, with characteristic bHLH and HAT domains. The receptor interacting domain (RID) and p300/CBP-interacting domain (p300/CBP) are depicted. (*B*) Domain features of p300/CBP. RID: receptor interacting domain; CREB: CREB-binding domain; bromo: bromo domain; HAT: histone acetyl transfease; ZF: zinc-finger domain; SRC-1: SRC-1 interacting domain.

acetylation of histone proteins, thereby opening the chromatin and enhancing the formation of a stable preinitiation complex.

p300 was first identified as a 300-kDa nuclear protein and a cellular target for the adenovirus E1A oncoprotein transcription factor (56). It was shown to be responsible for a variety of the effects of E1A in host cells (57). The CBP was found to interact with the *trans*-activating domain of CREB and to mediate its activating function in response to cAMP (58, 59). It was also shown that CBP interacted with the basal transcription factor TFIIB and RNA polymerase II itself, and also possessed HAT activity (60), further supporting its role as a coactivator (59). Because of their interchangeable properties in many aspects, p300 and CBP are considered functional homologues. Their connection to the nuclear receptors was first demonstrated in a study where the LBDs of multiple nuclear receptors, including RARs, were shown to interact directly with p300/CBP, which also interacted with the SRC-1/p160 family. In addition, numerous transcription factors were also known to interact with p300/CBP. Structurally, p300/CBP contains discrete domains responsible for their diverse functions, including a bromodomain, a HAT domain, an SRC1 domain, and a receptor interacting domain at the N terminus (Figure 3*B*). Thus, it was proposed that p300/CBP could play a role in coordinating many signaling pathways and serve as a platform for the actions of different transcription factors including nuclear receptors (61–63).

P/CAF was identified that competed with E1A for interaction with p300/CBP. It was also demonstrated that P/CAF possessed intrinsic HAT activity (64). Later,

P/CAF was found to interact directly with liganded RAR/RXR dimer at the LBDs of these receptors (65) and was also found in a complex consisting of more than 20 polypeptides that include TATA-binding protein (TBP)-associated factors (TAFs) and some of the TFIID components. As the binding of P/CAF to RAR/RXR is independent of the interaction of RAR/RXR with p300/CBP, it is suggested that P/CAF and p300/CBP represent two independent activating pathways for nuclear receptors (65), both involving HAT activity to modify the chromatin. The identification of gene-specific coactivator complexes remains a challenge for future studies.

GRIP1/TIF2/p160 With the LBD of glucocorticoid receptor (GR) as the bait, a GR interacting protein (GRIP1) was cloned from a mouse embryo cDNA library and shown to act as a transcription coactivator (66). A 160-kDa human nuclear protein called transcriptional mediators/intermediary factor II (TIFII) was later isolated that appeared to be the homologue of GRIP1 and exhibited typical coactivator properties such as agonist-dependent interaction with receptors and an autonomous transcription activation activity (67). The family of GRIP1/TIFI2/p160 exhibits a partial sequence homology to SRC-1 and possesses a single nuclear receptor-interacting domain that is composed of three LXXLL-containing segments, as well as two autonomous activation domains (AD1 at the N terminus and AD2 at the C terminus). The activity of the AD1 appeared to be mediated by its interaction with the CBP complex, whereas the activity of the AD2 was CBP-independent (68). It was later found that the AD2 of GRIP1/TIFII/p160 acted on the AF-1 of androgen receptor (AR), but the principle of its activation function remains to be determined. Thus, GRIP1/TIFII/p160 can potentially mediate both of the two signal outputs of nuclear receptors, the AF-1 and AF-2.

ACTR/RAC3 AND p/CIP A receptor-associated cofactor 3 (RAC3) was cloned as a coactivator for the liganded receptor LBD (69) and found to be identical to another coactivator named ACTR, which was known to possess intrinsic HAT activity (70). Sequence comparison revealed a homology to the SRC-1 and TIFII members (69); therefore, it was also categorized in the SRC family. A closely related coactivator, the p300/CBP cointegrator associated protein (p/CIP), was found to interact with CBP and was required for the actions of CBP-dependent transcription factors (71). These members represent the third SRC family that functions as coactivators for RARs and RXRs, and possesses HAT activity.

TIF1 The mouse transcriptional intermediary factor I (TIFI) was originally found to interact with RAR/RXR in a ligand-dependent manner and was able to enhance AF-2 mediated activation of receptors (72). A structurally and functionally related protein was later isolated and named TIFIα. TIFI appeared to also interact with heterochromatin protein 1 (HP1) (73), suggesting that the coactivating function of TIF1 was related to its effects on chromatin structure.

TRAP220 The thyroid hormone receptor (TR) associated protein 220 (TRAP220) was identified in immunopurified complexes from thyroid hormone–treated cells and later shown to also interact with many hormone receptors including RARs and RXRs (74). Later, in an RXR/PPARγ heterodimer system, TRAP220 was shown to be selectively recruited by holo-PPARγ, whereas p160 was selectively recruited by holo-RXR, suggesting that cooperative effects of PPARγ and RXR could be due to selective coactivator recruitment by each receptor of the heterodimer (75). TRAP220 also contains multiple LXXLL motifs that mediate its interaction with the LBDs of holo-receptors and is known to stimulate thyroid hormone–activated gene transcription (76).

TRIP1/SUG1 The human TR interacting protein I (TRIP1) was originally cloned in yeast two-hybrid screening experiments with TR and RXR as the baits. TRIP1 is a homologue of a component of yeast transcription mediator, SUG1, which interacts with transcription factor TBP and TFIIB (77). Mouse SUG1 was subsequently isolated with RAR as the bait and was shown to interact with hormone receptors including RAR, RXR, vitamin D receptor (VDR), estrogen receptor (ER), and TR in a ligand-dependent manner. This interaction involved the AF-2 domain of nuclear receptors (78). It was shown that this transcription mediator was able to confer an eightfold greater activity in basal transcription and a 12-fold greater efficiency of phosphorylation of RNA polymerase II (79). Human TRIP1 was also shown to be able to functionally substitute for yeast SUG1. Thus, TRIP1/SUG1 functions as a coactivator for the RAR/RXR heterodimer by facilitating interaction with the basal transcription machinery.

SW1/SNF The yeast SWI/SNF genes were shown to be involved in positive transcriptional regulation of the *HO* and the *SUC2* genes (80, 81). A connection of nuclear receptors to these gene products was first demonstrated for GR (82). In this study, it was found that activation by GR required its interaction with SWI proteins at an early step, prior to the formation of the initiation complex. Later, the human homologue, hSWI/SNF, was shown to mediate the ATP-dependent disruption of nucleosomes (83). A direct ligand-dependent interaction of hormone receptors with SWI2/SNF2 was later demonstrated (84). Therefore, the SWI/SNF coactivators, upon interacting with holo-receptors, remodel chromatin structure to facilitate the binding of transcription factors.

Ada3 A family of transcription factor coregulators, named alteration/deficiency in activation (Ada), was first identified in yeast. In yeast, Ada3 mutation resulted in slower growth and temperature sensitivity. In addition, selection of initiation sites by general transcription machinery in vitro was altered in this mutant, suggesting that Ada3 was a component of the general transcription machinery (85). Later, it was found that the Ada proteins were a part of the yeast Spt-Ada-Gcn5-Acetyltransferase (SAGA) activator complex and that Ada3 protein interacted with the LBDs of RXR and ER in yeast, resulting in holo-receptor activation

(86). Using yeast genetics, a trimeric transcriptional complex consisting of holo-TR/p160/GCN5 was shown to interact with the Ada/SAGA adaptor complex and to mediate activation of target genes by thyroid hormones (87).

PGC-1 A tissue-specific coactivator for PPARγ, named peroxisome proliferator-activated receptor-gamma coactivator-1 (PGC-1) (88), also appeared to be a coactivator for other hormone receptors. PGC-1 was shown to interact with a number of hormone receptors including T3R, RAR, RXR, ER, and GR in a ligand-dependent manner (89, 90), which required the LXXLL motif-containing sequence of PGC-1. A number of studies have suggested a physiological function of PGC-1 in gluconeogenesis by augmenting the activity of phosphoenolpyruvate carboxylase and glucose-6-phosphatase via coactivation of GR and transcription factor HNF-4α (91). Recently, it was demonstrated that PGC-1 and TIF2 synergistically activated RXRα-mediated reporter gene activity (90). It is suggested that PGC-1 plays a role in a wide range of physiological processes that involve nuclear receptors using RXR as the obligate partner.

NSD1 The mouse NR-binding SET-domain-containing protein 1 (NSD1) was isolated in yeast two-hybrid screening experiments and found to encode several distinct domains, including a SET domain and multiple PHD fingers (92). This protein was found to interact with both apo- and holo-forms of many nuclear receptors including RAR and RXR. Interestingly, NSD contains separate activation and repression domains, which may define a new class of bifunctional transcriptional intermediary factors (93). Distinct roles in the presence and absence of hormones were proposed for this protein. Recent genetic studies have identified a gene fusion of the *NSD1* gene to the *NUP98* gene in childhood acute myeloid leukemia (94) and amplification of the NSD gene in human breast cancer cell lines (95), implicating a potential role for NSD1 in human malignancy.

FKHR The human FKHR, a forkhead homologue in rhabdomyosarcoma, was cloned from a tamoxifen-resistant MCF-7 cDNA library by using the LBD of ER as the bait (96). This gene belongs to the hepatocyte nuclear factor 3/forkhead homeotic gene family, and the protein was found to interact with ER ligand dependently but with RAR and TR ligand independently. In transient transfection assays, FKHR stimulated RAR- and TR-mediated gene activation, but it repressed ER-mediated transcription. Therefore, FKHR also appears as a bifunctional intermediary protein that can either activate or repress hormone receptor-mediated gene transcription, depending upon the receptors involved.

PNRC Two proline-rich nuclear receptor coregulatory proteins (PNRC1 and PNRC2) were cloned from a mammary gland cDNA library (97, 98). These proteins are relatively small, with molecular weights of 16 kDa and 35 kDa, respectively. Both PNRCs interacted with hormone receptors in a ligand-dependent manner, mediated by an activation function domain that contains an NR box-like

sequence (LKTLL) and an SH3 domain binding motif (SEPPSPS). These proteins enhanced transcriptional activation of both hormone receptors and orphan receptors, and their interaction with nuclear receptors required a functional AF2 domain in the nuclear receptors. The exact mechanism of their activation functions remains undetermined.

CRABPII Two forms of cellular retinoic acid binding proteins (CRABPs) have been identified, CRABPI and CRABPII. These are cytosolic binding proteins for RA and are primarily involved in the synthesis and degradation of RA, thereby controlling RA homeostasis (3). Recently, CRABPII was shown to be directly involved in RAR/RXR-mediated gene activation (99–102). CRABPII was able to enter the nuclus and interact directly with RAR/RXR in a ligand-independent manner (99, 101). Activation of RA-responsive reporter was enhanced by CRABPII; thus, CRABPII can act as a specific coactivator for the RAR/RXR families. It is suggested that CRABPII interaction with RAR/RXR facilitates the formation of holo-receptors, thus enhancing transcriptional activity (100).

ASC-2 The activating signal cointegrator-2 (ASC2) was isolated with the LBD of RXR as the bait and was shown to interact with many nuclear receptors in a ligand-dependent manner (103). In addition, it also interacted with a number of other nuclear factors, such as basal transcription factors TFIIA and TBP, coactivators CBP/p300 and SRC-1 (103), serum response factor (SRF), activating protein-1 (AP-1), and nuclear factor-κ B (NF κ B) (104). ASC2, either alone or in conjunction with CBP/p300 and SRC-1, was able to stimulate ligand-dependent activation of nuclear receptors; the AF-2 domain of nuclear receptor was required for this activation. Its interaction with nuclear receptors involved its two NR boxes, whereas its interaction with CBP involved two of its three activation domains. Its activation function was blocked by E1A, a CBP-neutralizing factor. It is proposed that ASC-2 acts by binding to nuclear receptors and recruiting CBP for nuclear receptor-mediated gene activation (105).

p120 p120 encodes 920 amino acids with a molecular mass of 120 kDa. The cDNA of p120 was originally isolated as a cofactor for TR, and its T3-dependent interaction was shown to require the AF-2 domain of TR (106). Recently, p120 was also shown to enhance PPARγ/RXR-mediated transactivation in the presence of either PPARγ- or RXR-specific ligands, but the interaction appeared to be mediated by RXR. It is proposed that p120 can be a specific coactivator for RXR in PPAR/RXR-activated pathways (107).

CARM1 CARM1 (coactivator-associated arginine methyl-transferase I) was shown to methylate nucleosomes and potentiate transcriptional activation by RAR/RXR (108). However, CARM1 was also able to methylate CBP/p300, which blocked CREB activation by disabling the interaction between CBP/p300 and CREB. Therefore, it appears that CARM1 can be a coactivator for hormone

receptors and at the same time a corepressor for transcription factors transducing cAMP signals.

Corepressors of RARs and RXRs

Activation of gene expression by hormones has been the central dogma of a long-standing view on hormonal control of gene regulation. Hormone receptors were believed to function only in the presence of hormones. However, the discovery that basal transcription of a gene could be further repressed in the absence of hormones motivated the field to re-examine the potential roles of hormone receptors in the absence of ligands. A negative role for nuclear receptors in gene expression was first implicated in two early studies of thyroid response element (TRE)-containing promoters. In one study, the v-erb-A oncoprotein abolished thyroid hormone responses of a TRE in the Moloney murine leukemia virus long terminal repeat (109). Another study showed that apo-TR was able to repress the activation of a TRE responsive to RAR-epsilon (110). These observations initially suggested a "passive" repression mechanism, where the apo-receptors repress transcription by competing for DNA binding. Later, a potentially "active" repression, or silencing activity, of receptors was suggested because both RAR and TR were found to contain a transferable, functional silencing domain at the C terminus of the receptor (111). Based upon these observations, the hypothesis was proposed that nuclear receptors could recruit corepressors to actively silence genes. Three major criteria for a corepressor are interaction with apo-receptors and dissociation from receptors upon the binding of agonists, potentiation of repression by receptors, and possession of intrinsic repressor activity. The first two bona fide corepressors were identified in 1995 (112, 113). A number of corepressors were later cloned that were shown to facilitate gene repression and were classified as corepressors (114). However, some of these members were found to interact with both liganded and unliganded receptors. Furthermore, several coregulators function as either a coactivator or a corepressor, depending upon the receptors involved. Studies of the working mechanisms of these dichotomous coregulators are being actively pursued. For the sake of simplicity, the coregulators shown to exert suppressive activities on the RAR/RXR system are categorized here as corepressors of RARs and RXRs.

SMRT AND N-CoR The nuclear receptor corepressor (N-CoR) was identified as a bona fide corepressor of RAR and TR (112). At the same time, another bona fide corepressor was cloned, named silencing mediator for retinoid and thyroid-hormone receptors (SMRT) (113). It was later found that N-CoR and SMRT are related in terms of function, domain structure, and primary sequence (Figure 4*A*). Both N-CoR and SMRT contain multiple repressive domains and receptor interacting domains and they interact with the same components of other nuclear factors such as TFIIB and Sin3 [reviewed in (114–116)].

Their repressive activity was attributed to two mechanisms. One mechanism involved their direct interaction with the corepressor mSin3, originally identified

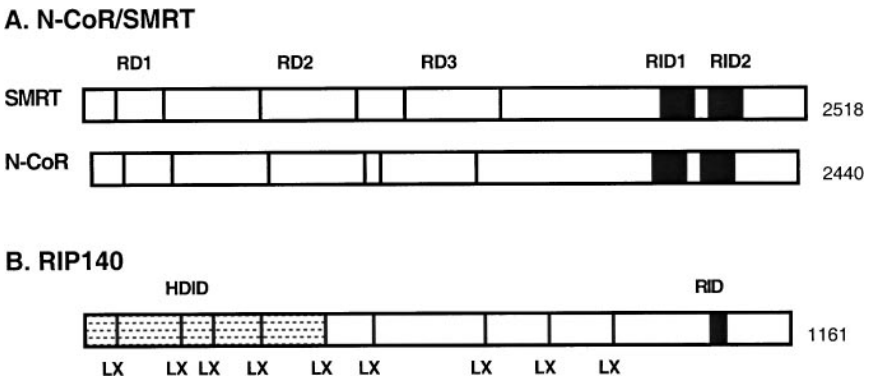

Figure 4 Domain features of N-CoR/SMRT and RIP140 corepressors. (*A*) Alignment of N-CoR and SMRT, with three repressive domains (RD1, RD2, and RD3) and two receptor interacting domains (RID1 and RID2) depicted. (*B*) Nine LXXLL (LX) sequences scattered throughout the entire RIP140 molecule. The histone deacetylase-interacting domain (HDID) is present at the N-terminal portion and a novel LYYML-containing ligand-dependent receptor interacting domain (RID) is present at the C-terminal portion, which is devoid of a canonical LXXLL sequence.

for Mad-Max and Mxi-Max transcription repressors (117, 118). The formation of complexes containing N-CoR/SMRT, mSin3, and HDAC was later demonstrated (119–121). A model was proposed where N-CoR and SMRT served as the platform adapters to recruit Sin3-HDAC complex as well as other Sin3-associated proteins to form a repressive complex, which was then recruited by the apo-receptors to the DNA (116, 122). However, recently it was found that both SMRT and N-CoR could directly interact with the class II HDACs (123), suggesting that a Sin3-independent recruiting pathway could also be adopted by N-CoR and SMRT. For both Sin3-dependent and Sin3-independent pathways, recruitment of histone modifying enzymes appears to be one common underlying mechanism for the corepressive activity of N-CoR and SMRT.

The second mechanism of repression was postulated to be mediated by their direct interaction with certain basal transcription factors, thereby precluding the formation of the basal transcription complex. Before the identification of corepressors, an early study had in fact demonstrated an inhibitory effect of unliganded TR on preinitiation complex assembly (124). Later, unliganded TR was found to directly contact TBP (125). Recently, a direct contact of N-CoR and SMRT with transcription factors such as TFIIB (126, 127), TAFII32, and TAFII70 (127) was demonstrated. Although a growing number of reports have supported the mechanism involving direct or indirect chromatin modification by these repressive complexes, the exact identities of the components present in these complexes remain to be established in more rigorous reconstitution experiments.

RIP140 The human receptor interacting protein 140 (RIP140, or newly renamed as NRIP1) (128) was first cloned with the LBD of ER as the bait (129) and was reported to act as a coactivator for ER functions. Mouse RIP140 was cloned from a testis cDNA library with the LBD of orphan receptor TR2 as the bait (130) and found to function as a corepressor. Most recent studies have shown a repressive role for RIP140 in many other gene systems, including those regulated by nuclear receptors and other transcription factors. For instance, RIP140 was reported as a corepressor for Pit-1, TR, ER (131), PPAR/RXR (132), GR (133), and RAR/RXR (134). In contrast to the first study proposing that the LXXLL motif of RIP140 mediated its ligand-dependent interaction with receptors (135), recent studies demonstrated that ligand-dependent interaction of RIP140 with receptors could be mediated by a region devoid of the LXXLL motifs (136, 137). Furthermore, RIP140 could directly interact with histone deacetylases (HDACs) (138), and tetramolecular complexes consisting of RAR/RXR/RIP140/HDAC were formed in the presence of RA, resulting in the suppression of reporter gene expression in the presence of RA (136). In the presence of RIP140, the RAR/RXR-targeted promoter region was highly deacetylated and the expression of the reporter was also repressed. We have proposed a model where hormone target genes can be selectively suppressed by complexes consisting of ligand-bound RAR/RXR, RIP140, and HDACs. RIP140 was also found to interact with the carboxyl terminal binding protein (CtBP) and to be acetylated by p300/CBP (139), raising the possibility that HDAC recruitment by RIP140 could modify RIP140 itself. Like the studies of many coregulators, the biological activity of RIP140 was examined by either transient transfection of mammalian cells or by yeast reporter assays, where complication by unknown nuclear factors from the mammalian or yeast cells cannot be prevented. Direct evidence for the activity of RIP140 and many coregulators should ensue from studies of in vitro reconstituted systems where defined components are provided in the reactions. We have begun to address the biological activity of RIP140 in RA regulation of its target genes by using assembled chromatin of the reporter as the template for in vitro transcription. Our preliminary results have demonstrated that in the presence of RIP140, RA-induced transcription was inhibited. Furthermore, the inhibition was likely to be a result of the competition of RIP140 with coactivators, like P/CAF, in the binding to holo-receptors (L.-N.Wei, unpublished). We have further employed a quantitative method to obtain kinetic data on these molecular interactions, based upon plasmon surface analyses on a BIAcore machine. In these studies, the real-time kinetic analysis of molecular interaction between RIP140 and liganded RAR/RXR was conducted using highly purified proteins. These kinetic data showed a substantial competition between P/CAF and RIP140 for binding to the RAR/RXR heterodimer, further substantiating the strong competitive nature of RIP140 with other coactivators, such as P/CAF, for interacting with receptors. One indication of this result is the significance of dynamic interactions among these nuclear factors that act in concert to ultimately control specific gene expression. This presents one of the most challenging issues to be addressed in future studies.

Therefore, both in vivo and in vitro transcription studies have supported a corepressor role for RIP140 in nuclear receptor-mediated gene expression. The underlying mechanisms could be its competition with coactivators for binding to holo-receptors and/or its ability to directly recruit HDAC to the gene promoter.

SUN-CoR A small (16 kDa) nuclear protein, small unique nuclear receptor corepressor (SUN-CoR), was isolated by using the unliganded LBD of TR as the bait (140). This protein was found to function as a corepressor for nuclear receptors, including RAR, and to interact with N-CoR and SMRT in vitro. It is suggested that SUN-CoR may be a component of the N-CoR/SMRT complexes. However, the exact mechanism of its repressive activity remains undetermined.

TRUP In a yeast two-hybrid screening experiment, a protein named thyroid hormone receptor uncoupling protein (TRUP) was identified with the bait prepared from the unliganded LBD of TR (141). TRUP was later found to interact with both TR and RAR, but not ER, in a hormone-independent manner through the hinge and N-terminal portions of their LBDs. Its repressive activity is attributed to its interference with DNA binding of TR and RAR.

CALRETICULIN Two studies independently reported that a calcium-binding protein, calreticulin, interacts directly with the DBD of many nuclear receptors including RAR/RXR (142, 143). Although calreticulin was found primarily in the endoplasmic reticulum, it was also found in the nucleus (142). Expression of calreticulin inhibited the activation of RAR and AR by their hormones (143). However, it remains to be determined if blocking receptors' DNA binding via interacting with their DBD is the principal mechanism of the regulatory action of calreticun.

PSF A polypyrimidine tract-binding protein-associated splicing factor (PSF) was isolated by using a biochemical approach to identify proteins interacting with apo-receptors (144). PSF was found to interact with the DBD of apo-receptors and to recruit Sin3A/HDAC complexes. It is suggested that PSF acts as a corepressor through a Sin3A-mediated pathway.

CONCLUSION AND FUTURE DIRECTIONS

The discovery of numerous associate proteins for nuclear receptors, including those for RARs and RXRs, suggests that a complicated regulatory network governs the actions of nuclear receptors. Because many of these associate proteins interact with an overlapping spectrum of target proteins that can be nuclear receptors or other transcription factors, it is likely that different cells have evolved to utilize a rich reservoir of coregulators in order to achieve a high level of cellular

specificity at the transcriptional control of gene expression. Alternatively, some of these coregulators may have been retained, despite their redundant nature, in order to achieve a homeostatic control of a variety of essential physiological processes. As coregulators of nuclear receptors continue to be discovered, a number of issues will have to be addressed in the future.

The first challenge is to address the specificity of these coregulators. To this end, the tissue-, cell-, and stage-specific expression patterns of these coregulators have to be examined and compared to that of RARs and RXRs. Subsequently, the relative affinity of each coregulator to the receptors has to be determined. Theoretically, a high-affinity coregulator would be considered more efficient in terms of its ability to regulate gene transcription. However, these coregulators very often act in concert with other proteins. Therefore, it is tempting to speculate that the ultimate control of transcription would lie in the unique combination of components in a transcription complex consisting of nuclear receptors, coregulators, and other proteins. As such, it would also be critical to determine the exact contents of a specific transcription complex and how a coregulator may affect the formation of a particular complex in the context of a specific gene's regulatory region.

The second challenge is to delineate the pathways and to determine the mechanisms of action of these coregulators. Currently, most of the known actions of coactivators are related to their roles in histone acetylation, and most corepressors are shown to recruit histone deacetylases. However, studies have been presented that reveal substrates of these enzymes, including not only DNA, but also transcription factors themselves. Thus, acetylation/deacetylation of transcription factors could also contribute to the activity of coregulators. More recently, the action of coregulators in another form of covalent DNA modification, methylation, has also been demonstrated (108). However, it remains to be seen whether this is common to different coregulators. Another proposed mechanism of coregulator's action is the sequestration of transcription factors. In theory, this can be commonly adopted by various coregulators. Our recent study of RIP140 competition with P/CAF for interaction with holo-RAR/RXR would support such a hypothesis. To effectively adopt such a mechanism of action, the transcription complexes or subcomplexes are probably more likely to be formed in solution before they are recruited to the target DNA. However, studies to address transcription complexes formed either in solution or on target DNA are scarce. Furthermore, evidence has yet to address this issue in the context of chromatin for many of these coregulators. Toward this end, one is presented with a challenging technical problem that has been vigorously tackled in the field, i.e., the use of appropriate chromatin template for the analysis. Ideally, one would use the chromatin of a natural gene, at least in terms of the nucleosomal organization of the regulatory region of the gene, for the analysis of DNA-protein interaction in order to examine the recruitment of transcription complexes to their targets. However, it is not a simple task to determine the nucleosomal structure of a natural gene, not to mention reconstituting a piece of chromatin that mimics, biochemically and thermodynamically, a natural gene.

The third problem is the ambiguity of ligand-dependency in terms of coregulator interaction with holo-receptors. Although it is demonstrated, in most cases, that coactivators interact with hormone receptors ligand dependently, recently evidence has suggested a complication in the nature of ligand-dependency for the interaction of the coregulators with the receptors. The rule of LXXLL-motif as a ligand-dependent NR signature has been challenged recently by studies of several coactivators and corepressors. For instance, RIP140 was initially demonstrated as a ligand-dependent coactivator and the receptor interaction motif was mapped to the LXXLL-containing regions (134). However, we have demonstrated a suppressive role for RIP140 in genes regulated by the RAR/RXR, and numerous later studies have also shown suppressive effects of RIP140 in many other gene systems. Moreover, we have recently discovered an LYYML motif in the carboxyl terminus of RIP140, which turns out to be a strong ligand-dependent receptor interaction motif for interacting with RAR and RXR (133, 135). This combination of a suppressive role for RIP140 and its ligand-enhanced interaction with RAR/RXR has suggested an unusal but testable hypothesis that retinoid hormones can directly act as a negative signal for the transcription of certain genes, and that it involves negative coregulators such as RIP140. This hypothesis challenges the current dogma of hormonal action. If this hypothesis is substantiated, one important question will be to address the physiological implication of positive versus negative gene regulation that is directly triggered by the hormones. Furthermore, the concept of the LXXLL-motif as a required ligand-dependent NR signature motif has also been challenged during the re-examination of RIP140 interaction with RAR and RXR using different methods. Similar problems have been encountered in the study of other coregulators such as PGC-1, which was initially shown to be a ligand-dependent coactivator for the PPARγ (88, 89) and later demonstrated to interact with receptors in a ligand-independent manner. Therefore, it is tempting to speculate that the effect of ligands, either agonists or antagonists, on receptors' interaction with coregulators may not be as simple as once thought. Currently, most studies rely on assays where complications from unknown cellular factors cannot be avoided. In the future, structural data, as well as interaction studies using more stringent methods like the BIAcore method to allow real-time kinetics to be determined in a highly purified system, will be required to address this issue.

In summary, the cloning of RARs and RXRs has allowed the signaling pathways underlying the action of retinoids to be dissected. The recent discovery and cloning of numerous receptor coregulators, including coactivators and corepressors, has marked the beginning of a new era in the studies of the action of retinoids. In future studies, the specificity of gene expression regulated by the retinoid, which requires highly coordinated protein-protein interaction and variegated complex formation, will present one of the most challenging subjects in this field. The direct connection of the action of these RAR and RXR coregulators to the components of the transcription machinery, including the substrate (chromatin) and the enzymes (transcription complexes), will provide ample opportunities to address the issue of the pleiotropic effects of retinoids.

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