0163-769X/99/$03.00/0
Endocrine Reviews 20(3): 321–344

Nuclear Receptor Coregulators: Cellular and Molecular Biology*

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I. Introduction

NUCLEAR receptor coregulators are coactivators or corepressors that are required by nuclear receptors for efficient transcriptional regulation. In this context, we define coactivators, broadly, as molecules that interact with nuclear receptors and enhance their transactivation. Analogously, we refer to nuclear receptor corepressors as factors that interact with nuclear receptors and lower the transcription rate at their target genes. Most coregulators are, by definition, rate limiting for nuclear receptor activation and repression, but do not significantly alter basal transcription. Recent data have indicated multiple modes of action of coregulators, including direct interactions with basal transcription factors and covalent modification of histones and other proteins. Reflecting this functional diversity, many coregulators exist in distinct steady state precomplexes, which are thought to associate in promoter-specific configurations. In addition, these factors may function as molecular gates to enable integration of diverse signal transduction pathways at nuclear receptor-regulated promoters. This review will summarize selected aspects of our current knowledge of the cellular and molecular biology of nuclear receptor coregulators.

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* Research described in this review was supported by a United States Army Breast Cancer Postdoctoral Award (to N.J.McK.); a Swiss National Foundation Fellowship (to R.B.L.); and by NIH and NICHD Grants (to B.W.O.).

A. The nuclear receptor superfamily

Nuclear receptors are ligand-inducible transcription factors that specifically regulate the expression of target genes involved in metabolism, development, and reproduction. Their primary function is to mediate the transcriptional response in target cells to hormones such as the sex steroids (progestins, estrogens, and androgens), adrenal steroids (glucocorticoids and mineralocorticoids), vitamin D_3, and thyroid and retinoid (9-*cis* and all-*trans*) hormones, in addition to a variety of other metabolic ligands. More than 100 nuclear receptors are known to exist, and, together, these proteins comprise the single largest family of metazoan transcription factors, the nuclear receptor superfamily.

Even the briefest consideration of research on the nuclear receptor superfamily affords an appreciation of its global importance in cellular signaling and differentiation. Seminal studies in the 1960s identified the estrogen receptor (ER), and the general pathway for steroid hormone action was subsequently elucidated. Numerous subsequent studies led to the belief that steroid receptors act at the level of DNA to enhance recruitment of the preinitiation complex of general transcription factors (GTFs) at target promoters. The cloning in the mid- to late 1980s of cDNAs encoding many of the receptors prefaced their designation, on the basis of extensive amino acid sequence identity, as an evolutionarily related family of proteins. Phylogenetic analysis has identified several subfamilies within this superfamily: type I ("classical" or "steroid") receptors include those for progestins (PR), estrogens (ER), androgens (AR), glucocorticoids (GR), and mineralocorticoids (MR), whereas type II receptors encompass those for thyroid hormone (TR), all-*trans* retinoic acid (RAR), 9-*cis* retinoic acid (RXR), and vitamin D_3 (VDR). A third subclass contains orphan receptors, for which ligands are only now being characterized. Although they have common structural features, divergence of the steroid and thyroid/retinoid/vitamin D_3 receptor subclasses is supported by differences in their functional characteristics, as well as by their discrepant recognition of *cis*-acting hormone response elements. Type I receptors, in the absence of ligand, are sequestered in nonproductive associations with heat shock proteins and, in this state, are not thought to influence the rate of transcription of their cognate promoters. Conversely, type II receptors are able to bind DNA in the absence of ligand and often exert a repressive effect upon the activity of their subject promoters, a phenomenon referred to as silencing (1). Type I receptors bind to palindromic repeats in a homodimeric head-to-head arrangement only in the presence of ligand, whereas type II

receptors bind constitutively to response elements that contain direct repeats. In addition, type II receptors exhibit promiscuous dimerization patterns, many involving heterodimerization with RXR, and such interactions may serve to modulate the amplitude of the transcriptional response to ligand.

Meticulous domain-mapping experiments have identified a number of functional domains now designated as defining structural features of members of the nuclear receptor superfamily. For a detailed discussion of these domains, the reader is referred to Tsai and O'Malley (1) and references therein. Broadly, the receptor structure is comprised of: an amino-terminal activation function, AF-1 (A/B domain); the DNA-binding domain (DBD) (C); a hinge region (D); and a carboxy-terminal ligand-binding domain (LBD) (E). Mutational analysis of the E domain led to the designation of a second activation function, AF-2, which is indispensable for proper ligand-dependent activation by nuclear receptors (2–4). Other functions have been ascribed to the E domain, including ligand binding (5), heat shock protein (hsp) interactions (6), and nuclear localization (7). These functional domains reflect a intricate, but well characterized, ligand-mediated receptor activation pathway (Fig. 1).This multistep process involves activation of receptor by binding of the cognate hormone, a change in receptor structure and dissociation of several heat shock proteins, nuclear translocation of the activated receptor (in the case of GR, MR, AR, and PR), and dimerization and apposition of the transformed receptor to its DNA response elements. Rather less well characterized though, is the sequence of events by which the activated, DNA-bound receptor achieves transcriptional regulation. While the role of GTFs in mediating basal transcription is well documented (see *Section I.B.* below), it has recently become clear that nuclear receptors recruit a host of ancillary factors (coregulators) that 1) create, depending upon the activation state of the receptor, a transcriptionally permissive, or nonpermissive environment at the promoter and 2) communicate with the GTFs and RNA Pol II.

B. General transcription factors (GTFs)

The entire sequence of events leading to the assembly of a preinitiation complex of GTFs at enhancer-controlled promoters is beyond the scope of this chapter. For a thorough discussion of eukaryotic transcriptional initiation, the reader is referred to selected reviews (8, 9). Steroid and thyroid/retinoid hormones regulate transcription via enhancer elements that may be several kilobases from their target promoters, at which transcription is mediated by RNA polymerase II (Pol II). The initial step is the binding of TFIID to the promoter at a short distance from the transcriptional start site. TFIID functions as a multiprotein complex composed of TATA-binding protein (TBP) and the highly conserved TBP-associated factors (TAF_{II}s). Human TFIID has been shown to be comprised of at least two distinct subpopulations: a core group containing human (h)$TAF_{II}250$, $hTAF_{II}135$, $hTAF_{II}100$, and $hTAF_{II}28$, present in all TFIID complexes; and another group containing promoter-specific $hTAF_{II}$s, such as $hTAF_{II}30$, $hTAF_{II}20$, and $hTAF_{II}18$ (10, 11). After TFIID binding is that of TFIIB, a GTF with affinity for single-stranded DNA, which apposes to sequences adjacent to the TATA box in response to a critical change in DNA topology induced by TBP (12). Recruitment by TFIIB of another GTF, TFIIF-α, is followed by binding of RNA Pol II (13). While this description implies a stepwise accretion of factors, recent evidence suggests that stable, preformed basal transcription complexes may also exist, which contain RNA Pol II in addition to other GTFs (14). Ultimately, it is by influencing the rate of assembly of such complexes that nuclear receptors, in association with their coregulators, achieve transcriptional regulation at hormone-regulated promoters.

II. Nuclear Receptor Coactivators

A. Background

1. Direct interactions between receptors and GTFs. Direct protein-protein interactions, the functionality of which is yet to be determined, have been reported between receptors and GTFs. TBP and several TAF_{II}s interact functionally with specific receptors and are, by our definition, nuclear receptor coactivators. Consistent with the designation of TBP recruitment as a rate-limiting step in transcriptional initiation (15), several interactions between TBP and nuclear receptors have been reported. Protein-protein interaction assays, such as the yeast two-hybrid screen and *in vitro* binding assays with recombinant proteins, have detected an association between a portion of the TBP and the AF-2 function of RXR (16). Similarly, AF-1 and AF-2 of the ER bind TBP *in vitro* (17), and a similar interaction has been documented between PR and the $TAF_{II}110$ subunit of TFIID (18). In addition to those with TBP, contacts of nuclear receptors with other GTFs have been described. Interactions between AR and TFIIF (19) and RAR and TFIIH (20), and the interactions of TFIIB with VDR (21) and other nuclear receptors (22), may be influential in modulating a DNA-bound ternary complex of receptor, TFIIB, and TBP-TAF_{II}s. These interactions suggest that direct inter-

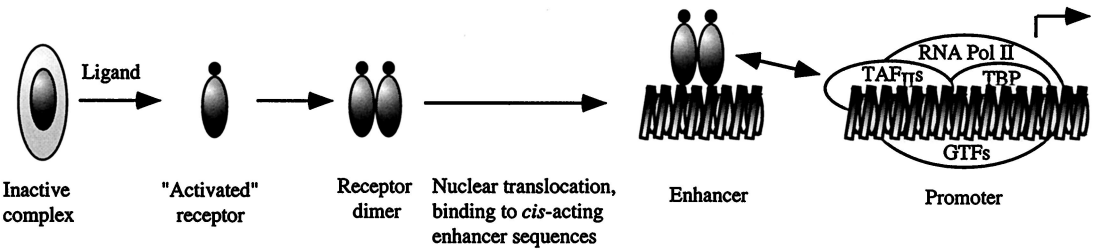

FIG. 1. Model for transactivation by a nuclear receptor. While this model applies generally to type I receptors, type II receptors can bind their response elements in the absence of ligand.

actions between nuclear receptors and GTFs may contribute to the assembly of final transcriptional complexes at their target promoters.

2. Evidence of the existence of coactivators. An early indication of the interaction of activated receptors with factors other than GTFs was the phenomenon of squelching, or transcriptional interference between receptors, in transient receptor/reporter co-transfection assays (23, 24). In the context of activation, squelching defines the reduction in transactivation of a promoter regulated by nuclear receptor A (more specifically, an activation function) in the presence of a distinct, activated receptor B. The clear inference from such experiments was that titration of a cellular pool of factors for which the activation functions competed limited the overall reporter gene activity of the receptors. Such experiments indicated that common cofactors might be an important functional link between the receptor and transcriptional initiation. Supportive of such a notion was the fact that tissue- and promoter specificity were characteristic of the activation functions of the ER (25) and RAR (26). Collectively, these studies suggested a level of control at enhancer-controlled promoters beyond the actual receptor-response element interaction.

B. Receptor-associated proteins and coactivators

1. ER-associated proteins (ERAPs) and RIPs. In a seminal study, Halachmi *et al.* (27) used a purified ligand-bound ER LBD to identify ER-interacting proteins from ^{35}S-radiolabeled MCF-7 cell lysates. Two proteins, ERAP-140 and ERAP-160, were identified in this manner. A potential role for these proteins in ER function was suggested both by the ligand dependence of their interaction with ER and by the fact that transcriptionally defective mutants of ER failed to recruit these factors. Moreover, the estrogen antagonists 4-hydroxytamoxifen (4-HT) and the pure antiestrogen, ICI 164384, uncoupled the ER-ERAP interaction (27). While ERAP-140 and ERAP-160 (subsequently cloned as SRC-1/hSRC-1[1], see *Section II.B.2.a*) exhibited similar associations with RARα and RXRβ, other transcriptional activators, including Rb and Pit-1, did not interact with the ERAPs, indicating a degree of specificity in ERAP binding. Eggert *et al.* (28) biochemically characterized a 170-kDa protein, GRIP-170 (GR-interacting protein 170, postulated to be equivalent to ERAP-160), which interacted with GR in a hormone-dependent manner and which was enriched in a mammalian cellular fraction that potentiated GR activity in an *in vitro* transactivation assay.

Cavaillès *et al.* (29) used far-Western blotting and *in vitro* interaction assays to identify receptor-interacting proteins (RIPs) of 160, 140, and 80 kDa. As with ERAPs, RIPs failed to interact either with antiestrogen-bound ER or with transcriptionally-defective mutants of ER. Subsequently, this group (30) reported the cloning of the cDNA encoding RIP-140 and demonstrated its widespread expression in mammalian tissues. *In vitro* interactions of RIP-140 were demonstrated with wild-type ER, but not with transcriptionally defective ER mutants. Although marginal coactivation of ligand-dependent ER transactivation was exhibited in transient cotransfection in mammalian cells, no interaction of RIP-140 with GTFs such as TBP or TFIIB could be demonstrated. Indeed, recent evidence, while supporting the ligand-dependent interaction of RIP-140 with TR2, suggests that RIP140 acts as a corepressor for this orphan receptor member of the nuclear receptor superfamily (31).

2. The SRC family. Table 1 shows a summary of the properties of characterized nuclear receptor coactivators. To encourage brevity, consensus, and clarity in discussion of SRC coactivators, we are adopting the proposed nomenclature[1] (32).

a. SRC-1/NCoA-1. The cloning and characterization of steroid receptor-coactivator-1 (SRC-1/hSRC-1) by our laboratory (33) was the first description of an authentic common transcriptional mediator for nuclear receptors. Identified using a yeast two-hybrid screen of a human B-lymphocyte cDNA library with a bait encoding the PR LBD, hSRC-1 exhibits a broad range of specificity in the coactivation of the ligand-dependent transactivation of nuclear receptors, including PR, GR, ER, TR, RXR (33), HNF-4 (hepatocyte nuclear factor 4; Ref. 34), and PPARγ (peroxisome proliferator-activated receptor; Ref. 35). The interaction of hSRC-1 with the PR LBD is ligand dependent (33) and is abolished in the presence of the antiprogestin RU486. Furthermore, hSRC-1 has been shown to be capable of reversing the squelching of PR transactivation by cotransfected ER, indicating that it constitutes a common, limiting factor recruited by the LBDs of ER and PR for efficient transactivation (see *Section II.A*). In addition, a hSRC-1 mutant, containing only the C-terminal receptor-interacting domain (Fig. 2), suppresses PR coactivation by hSRC-1 in a dominant-negative fashion, both in transient transfection (33) and by *in vitro* transcription assay (36). Lee and colleagues have shown that, in addition to nuclear receptors, hSRC-1 modestly coactivates other transcription factors, including AP-1 (37), serum response factor (38), and NF-κB (39).

Several studies have indicated the ability of hSRC-1 to mediate functional interactions between the N-terminal AF-1 and C-terminal AF-2 activation functions of steroid receptors. Individual domains of hSRC-1 are required for full functional synergy between AF-1 and AF-2 of the PR (40), as well as ER (41) and AR (42), indicating that the efficient assembly of a preinitiation complex by steroid receptors is contingent, at least in part, on an SRC-1-assisted interaction between their individual AFs. Because the functional interaction of SRC-1 with receptors appears to be largely dependent on the integrity of a conserved amphipathic helix in the AF-2 region of receptors (43), we suggest that it and other predominantly AF-2 interacting coactivators be referred to as

[1] To resolve the complex issue of nomenclature in this family, we are adopting a unifying system proposed by Li and Chen (32). The prefix "h" will be used for all human clones and the prefix "m" will identify those clones originating in the mouse. The family will be called the SRC coactivator family to acknowledge the initial cloning of SRC-1 (33). The name hSRC-1 will identify SRC-1 (33); and the name mSRC-1 will represent NCoA-1 (45). GRIP1 (48) and NCoA-2 (50) will be referred to as mSRC-2; and hSRC-2 will represent TIF2 (47). RAC3 (54)/ACTR (53)/AIB1 (55)/TRAM-1 (56)/SRC-3 (57) will be referred to as hSRC-3; and p/CIP will be identified as mSRC-3. Throughout this review, discussions of individual clones will refer to original clone name/name under proposed nomenclature, *e.g.*, NCoA-1/mSRC-1.

TABLE 1. Nuclear receptor coactivators

Cofactor	Alternative designations	Related factors	Comments	References
ERAP-160	GRIP-170, p160	ERAP-140	ERAPs bind ER in ligand-dependent manner; mammalian cellular fraction enriched in GRIP-170 coactivates GR.	27, 28
RIP-140		RIP-160	Interacts with and coactivates ER; acts as a corepressor for TR2 orphan receptor.	29, 30, 31
TBP/TAF_{II}s			Interact with and specifically coactivate nuclear receptors.	10, 16–22, 85–86
SRC-1	hSRC-1 NCoA-1/mSRC-1 p160	TIF2/hSRC-2 p/CIP/mSRC-3 hSRC-3	Interacts with and coactivates nuclear receptors; interacts with CBP/p300; contacts basal transcription factors; possesses acetyltransferase activity, interacts with PCAF; contains autonomous activation domains; targeted deletion causes partial hormone insensitivity in mice.	33–46, 58–60, 67, 93, 104, 105, 109
Trip-1	Sug-1	Trips	Substitutes for Sug1 in yeast and interacts with TR, Gal4 and VP16; Trips interact with RXR and show homology to yeast transcriptional activators; Sug1 interacts with RARα in yeast and mammalian cells; contains ATPase domain.	74, 75
TIF1α		TIF1β, γ	Interacts with and coactivates RXR/RAR AF2 in yeast; TIF-1α represses when fused to DNA-binding domain; TIF-1s interact with factors related to chromatin-modifying proteins.	187–194
ARA-70			ARA70 interacts with and coactivates AR in prostate cells.	94–95
TRAPs	DRIPs		Biochemically-identified protein complexes; interact with liganded TR/VDR; enhance TR and VDR-mediated transcription *in vitro*. TRAP/DRIP220 contains NR boxes; share components with SMCC complex.	76–80
CBP		p300	Interacts with and coactivates multiple activators, including nuclear receptors; acetyltransferase; interacts with PCAF, SRC-1, TIF2/hSRC-2 and p/CIP mSRC-3; mutated in Rubinstein/Taybi syndrome.	45, 50, 51, 58, 60, 81, 99, 100, 103–106, 165, 171–173, 175
p300		CBP	Broad functional similarity to CBP; interacts with and coactivates nuclear receptors; possesses acetyltransferase activity; interacts with PCAF, SRC-1 and p/CIP/mSRC-3.	100–102, 107–112, 165, 174
PCAF	hGCN5		Possesses intrinsic acetyltransferase activity; interacts with PR and SRC-1; interacts with TR and ACTR; present in 2 MDa complex containing histone-like TAF_{II}s.	36, 58, 165, 167–169
TIF2/hSRC-2	GRIP-1/mSRC-2 NCoA-2, p160	hSRC-1 mSRC-3/hSRC-3	Interacts with and coactivates nuclear receptors; interacts with CBP.	47–52, 58
TRIP230			Rb-binding protein that selectively coactivates TR in specific cell types.	96
L7/SPA			Interacts with RU486-bound PR and enhances partial agonist activity of RU486 with PR.	71
p/CIP/mSRC-3	ACTR/hSRC-3 RAC3/hSRC-3 AIB-1/hSRC TRAM-1/hSRC-3 p160, SRC-3	SRC-1 TIF2/hSRC-2 GRIP-1/mSRC-2	p/CIP coactivates CBP-mediated signaling pathways; interacts with CBP and p300. ACTR interacts with and coactivates TR; possesses acetyltransferase activity; interacts with CBP/p300 and PCAF. RAC3 interacts with nuclear receptors, coactivates PR & RAR and contains autonomous activation domain. AIB-1 interacts with, coactivates ER; overexpressed in breast tumors and breast cancer cell lines. TRAM-1 interacts with and coactivates TR. SRC-3 preferentially coactivates ERα over ERβ	50 53 54 55 56 57

TABLE 1. Nuclear receptor coactivators—*continued*

Cofactor	Alternative designations	Related factors	Comments	References
E6-AP		RPF-1 (hRSP5)	E3 ubiquitin-protein ligases; E6-AP interacts with and coactivates AR, ER, PR and GR; E3 ubiquitin ligase; E6-AP and RPF-1 synergistically enhance PR activation.	58, 68–70
BRG-1	SWI2/SNF2	Brahma	Interacts with GR and ER; required by GR for chromatin remodeling.	176–186
NSD-1			SET domain-containing protein; interacts with liganded and unliganded nuclear receptor LBDs.	63
PGC-1			Expressed in brown adipose tissue and skeletal muscle; interacts with PPAR-γ in a ligand-independent manner; induced at low temperatures, enhances PPAR-γ activation during adaptive thermogenesis.	97
HMG-1		HMG-2	Coactivation specific for steroid receptors; promotes DNA binding by steroid receptors.	98
NCoA-62		BX42	Interacts with VDR, RAR and RXR; coactivates VDR strongly, GR, RAR and ER weakly.	89
TSC-2	Tuberin		Interacts with RXR; coactivates PPARγ and VDR; gene located in a locus, 16p13, associated with familial tuberous sclerosis.	90–92
PBP	TRAP 220, TRIP2, mPIP9		PBP binds to PPARγ, RARα, RXR and TRβ-1 and coactivates PPARγ; originally isolated as a TR-binding protein, TRIP2.	77, 79, 87, 88
Positive cofactors	PC2, PC4		Synergistically activate TR-TRAP activation in an *in vitro* transcription assay.	82
ADA			Yeast acetylase complex which mediates AF-2 dependent activation by RXR and ER.	166
SMCC		TRAPs/DRIPs	Human SRB/mediator-containing complex; contains components homologous to TRAPs/DRIPs.	80
SRA			Functions as RNA transcript; selectively coactivates AF-1 of steroid receptors; present in SRC-1 complex.	93
SNURF			Interacts with AR in yeast and mammalian cells; coactivates AR, PR, GR, Sp1 and AP-1 and basal activity; contains RING finger domain.	213
ARIP3		Miz1, PIAS3, GBP	Interacts with and modestly coactivates AR; expression specific to the testis in humans.	214

SRC family members are referred to by original clone names/names according to proposed nomenclature (Ref. 32; see footnote 1).

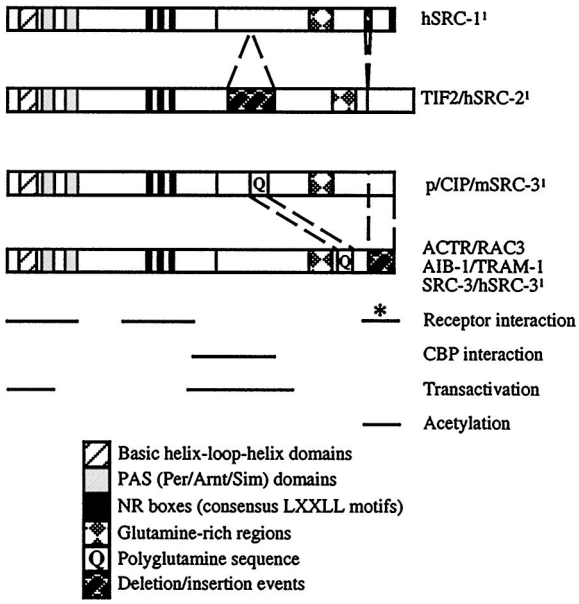

FIG. 2. Multiple members of the SRC family. Proteins have been aligned according to major structural similarities and to emphasize both the structural divergence of the carboxy termini and the conservation of the amino-terminal domains of SRC family members. Regions to which specific functions of individual coactivators have been assigned are indicated. We have adopted the unifying nomenclature of Li and Chen (32). m, Mouse; h, human. *, Only SRC-1 contains a consensus LXXLL/NR box motif in this region.

AF-2 coactivators, to distinguish them from non-AF-2 interacting factors, such as steroid receptor RNA activator (SRA; *Section II.B.4.a*) and PPARγ coactivator-1 (PGC-1, *Section II.B.4.d*).

SRC-1 contains two activation domains that retain their activity when transferred to a heterologous DBD (40) and, interestingly, Takeshita *et al*. (44) have demonstrated the interaction of hSRC-1 *in vitro* with TFIIB and TBP. When a longer form of SRC-1 (45) was cloned in the mouse [NCoA-1 (nuclear receptor coactivator 1)/mSRC-1], it was found to contain an additional 380 amino-terminal residues relative to the initial SRC-1 clone (33), which might have represented either a partial clone or a splice variant of the full length protein. Sequence analysis of the amino-terminal region has identified tandem bHLH (for basic helix-loop-helix) and PAS (for Per/Arnt/Sim homology) domains. The bHLH/PAS domains mediate homodimeric and heterodimeric interactions between proteins containing these motifs (46), and their conservation in the SRC family (see *Section II.B.2.d*, Fig. 2) suggests that functional cross-talk between nuclear receptor-mediated pathways and other PAS-containing factors might occur (45). On the basis of differences in the deduced encoded amino acid sequences of cDNA clones isolated during screens, the existence of splicing variants of NCoA-1/mSRC-1 has been conjectured (45), but their biological role, if any, is unknown at present.

b. GRIP1/TIF2/NCoA-2/SRC-2.[1] Characterization of cDNAs encoding GRIP1 (GR-interacting protein 1), TIF2 (transcription intermediary factor 2), and NCoA-2, 160-kDa nuclear receptor-interacting proteins with considerable sequence and functional similarity to SRC-1 (47–50), established the existence of what is now termed the SRC family (Fig. 2), also referred to previously as the p160 family (45). GRIP1 (mSRC-2) and TIF2 (hSRC-2) associate in a ligand-dependent manner *in vitro* with several receptor LBDs (47) and, *in vivo*, with RARα, ER, and PR in the presence of hormone, but not hormonal antagonists (47–49). In addition, GRIP1/mSRC-2 and TIF2/hSRC-2 contain two autonomous activation domains capable of stimulating transcription when tethered to a heterologous DBD in yeast (48) and in mammalian cells (47, 48, 51). Furthermore, overexpression of TIF2, like SRC-1/hSRC-1, is capable of relieving squelching by ER (47). Furthermore, a truncated GRIP1/mSRC-2 inhibits hormone-dependent expression from the mouse mammary tumor virus (MMTV) promoter, a property reminiscent of the dominant-negative properties of the receptor-interacting domain of SRC-1/hSRC-1 in relation to PR transactivation (48). GRIP1/mSRC-2 is also capable of enhancing transactivation in yeast of fusions of type I and type II receptors with the DBD of the yeast Gal4 activator (49, 52).

c. p/CIP/RAC3/ACTR/AIB-1/TRAM-1/SRC-3.[1] The identification of a third member of the SRC/p160 family, a highly polymorphic protein isolated independently as p/CIP [p300/CBP cointegrator-associated protein (50)], ACTR [activator of thyroid receptor (53)], RAC-3 [receptor-associated coactivator 3 (54)], AIB-1 [amplified in breast cancer-1 (55)], TRAM-1 [thyroid receptor activator molecule 1 (56)] and SRC-3 (57), serves to illustrate the growing complexity of nomenclature in the SRC family. For clarity, the unifying term "SRC-3" has been proposed for this member of the SRC family. hSRC-3 interacts with and coactivates a wide variety of nuclear receptors in a ligand-dependent manner, including RAR, TR, RXR, GR (53), PR (54), and ER (55). p/CIP/mSRC-3, however, exhibits greater promiscuity than other SRC family members by enhancing the transcriptional activity of a number of different activators, including interferon-α and cAMP regulatory element binding protein (CREB; Ref. 50), which were previously shown to be primarily dependent upon the transcriptional cointegrator CREB-binding protein (CBP; *Section II.B.5*) for efficient activation. Furthermore, SRC-3 selectively enhances the transcriptional activity of ERα over that of ERβ, possibly reflecting a 60% difference in homology between the LBDs of these isoforms (57). Li *et al.* (54) have demonstrated a feed-forward mechanism for regulation of RAC3/hSRC-3 expression by retinoid treatment in HL-60 cells, adding an additional level of control to nuclear receptor action.

d. Redundancy and diversity in the SRC family. The SRC family (Fig. 2) is defined by an overall sequence similarity of 40% between the three proteins, distinguishing its members from other coregulator classes, such as the p300/CBP cointegrators (*Section II.B.5*), E3 ubiquitin-protein ligases (*Section II.B.3.a*), TRAPs (TR-associated proteins, *Section II.B.3.e*), and the TIF-1 family (transcriptional intermediary factor-1, *Section IV.D.2*). The extent of sequence conservation between individual members is most apparent in their N-terminal domains, in which the bHLH/PAS domains exhibit a high degree of similarity. The extensive homology among SRC family members in this region is unique among PAS-containing proteins (54), identifying these proteins as a distinct subfamily of PAS factors. Like other PAS proteins, evidence

suggests that SRC family members are capable of forming heteromultimeric and homomultimeric complexes *in vivo* (58), although the requirement of the PAS domain for such interactions, as well as their functional significance, is unclear.

Redundancy within the SRC family is indicated by the phenotype arising from targeted deletion of the murine *SRC-1* locus. In this study, our laboratory provided the first *in vivo* data for the biological role of mSRC-1 expression in hormone-responsive pathways involved in adult sexual maturation. The phenotype of the SRC-1 null mutant is characterized by viability and fertility of both sexes against a background of significantly decreased growth of steroid target organs such as prostate, testis, and mammary gland in response to hormonal stimulation (59). We believe that the phenotype of the mSRC-1 null mutant arises in part from the compensatory overexpression of GRIP1/mTIF2 in certain tissues in the mutant, providing *in vivo* evidence of partial functional redundancy between mSRC-1 and GRIP1/mTIF2.

While the considerable sequence similarity between SRC family members indicates some redundancy of function, there is sufficient sequence divergence within the family to indicate functional autonomy. The liberal use of putative splice junctions in the C-termini of SRC members gives rise to considerable sequence complexity between each member in these regions. For example, SRC-1 and TIF2/hSRC-2 are distinguished by a 65-amino acid deletion in TIF2/hSRC-2 with respect to SRC-1 (Fig. 2). Such structural anomalies between the members of the SRC family are reflected by differences both in their immunoreactivity and in their functional characteristics. Microinjection into cells of anti-NCoA-1/mSRC-1 antibodies, but not anti-NCoA-2/mSRC-2 antibodies, prevented RAR-dependent transactivation of a retinoic acid response element (RARE)-linked reporter gene. Coinjection of NCoA-1/mSRC-1, NCoA-2/mSRC-2, or p/CIP/mSRC-3 expression vectors showed, however, that either NCoA-1/mSRC-1 *or* NCoA-2/mSRC-2, but not p/CIP/mSRC-3, could rescue transactivation of this reporter gene (50). This result correlates with the compensatory overexpression of GRIP1/mTIF2 (SRC-2) in the SRC-1 null mutant. and indicates the functional distinction between the SRC-1/SRC-2 and SRC-3 subfamilies. In addition, immunodepletion with anti-NCoA-1/mSRC-1 antibodies had no effect on cAMP- or interferon-α dependent reporters, indicating the dispensability of NCoA-1/mSRC-1 in classic CBP-mediated signaling pathways. While the functional importance of the sequence variations between hSRC-3 isoforms and p/CIP/mSRC-3 is unclear, p/CIP, unlike the hSRC-3 isoforms, preferentially enhanced interferon-α stimulation of a reporter gene, suggesting a closer functional similarity of p/CIP/mSRC-3 to CBP than the hSRC-3 isoforms. Furthermore, while p/CIP/mSRC-3 failed to significantly enhance RAR function (50), the hSRC-3 isoforms ACTR (53), RAC3 (54), and TRAM-1 (56) markedly enhance transactivation by RAR/RXR. These conflicting results are quite possibly a consequence of the C-terminal anomalies between p/CIP/mSRC-3 and the hSRC-3 isoforms. Comparison of the sequences of SRC-3 members indicates that they are encoded by the same gene in different species and are distinguishable by the length of their polyglutamine tract and the presence of a lengthy unrelated C-terminal sequence present only in the p/CIP/mSRC-3 isoform (Fig. 2).

Another piece of evidence indicating a degree of autonomy of the SRC-3 subfamily is the overexpression of AIB-1/hSRC-3 in primary breast tumors (55) against a background of relatively low expression levels of SRC-1 and TIF2/SRC-2. These results indicate that overexpression of AIB-1/hSRC-3 is a factor in the genesis and/or progression of these tumors, and the stimulus for growth that it may afford is not limited by the comparatively low levels in these tumors of SRC-1 and TIF2/mSRC-2. In their totality, the structural discrepancies between SRC family members indicate functional diversity that may determine their interaction with nuclear receptors, with other promoter-specific transcription factors, and with other transcriptional coregulators.

e. The LXXLL/NR box motif. Detailed scrutiny of the receptor-interacting domains of RIP-140 (*Section II.B.1*) and SRC family members (50, 60) identified a conserved motif, L*XX*LL (where L is leucine, *X* is any amino acid), termed the nuclear receptor (NR) box (Ref. 61; Fig. 2), which is necessary and sufficient to mediate binding of the coactivators to liganded nuclear receptors. Three such motifs are conserved in SRC family members, and an additional NR box is present in the extreme C terminus of h/m (human/mouse) SRC-1 (Fig. 2). Secondary structure analysis of these motifs has indicated that they form amphipathic α-helices and that the conserved leucines form a hydrophobic surface on one face of the helix. The role of the NR box in mediating ligand-dependent receptor-coactivator interactions is signified by its conservation in the central portions of all three SRC family members (Fig. 2), to which domains mediating interactions with nuclear receptors have been localized (33, 50, 51, 54). Furthermore, the nonconserved NR box motif of h/mSRC-1 is present in its C terminus, which mediates the hormone-dependent interaction of hSRC-1 with PR (33). The mutation of key residues in the four NR boxes of hSRC-1, (I–III in the central portion of the protein and IV in the extreme carboxyl terminus) has been shown to abolish interaction with AF-2 of the ER but does not affect the interaction of hSRC-1 with CBP. In addition, this hSRC-1 mutant fails to coactivate the ligand-dependent activity of ER (60). In a broader context, the importance of the NR box motif is indicated by its presence in a wide variety of nuclear receptor coregulators, including E3 ubiqutin-protein ligases (*Section II.B.3.a*), TRAPs (*Section II.B.3.e*), p300/CBP (*Section II.B.5*), and TIF-1s (*Section IV.D.2*). A detailed approach to the question of the significance of multiple NR boxes in receptor-coactivator interactions (61) suggests that distinct NR box motifs exhibit differential binding to different receptors. It has become apparent that sequence anomalies around individual NR boxes might determine their binding affinity for the AF-2 ligand-induced hydrophobic groove of nuclear receptors (62). Indeed, the notion that the L*XX*LL motif is an immutable requirement for interaction with receptor LBDs has been challenged by the ability of the F*XX*LL motif of NSD-1 (nuclear receptor-binding SET domain-containing protein 1, *Section IV.D.3*) to mediate its interaction with nuclear receptor LBDs (63).

Recent studies have shed light on the series of events that accompany ligand interpretation and coactivator interaction

with the AF-2 of nuclear receptors. Feng *et al.* (64) have dissected the interaction between the TR AF-2 and GRIP1/mSRC-2 and have described the appearance of a hydrophobic groove in the ligand-bound AF-2 of TR (and ER), the interactive surface of which is highly conserved. A peptide modeled upon a GRIP1/mSRC-2 NR box recognizes a hydrophobic groove in the TR LBD lined by a series of residues, the deletion of any of which abrogates GRIP1/mSRC-2 peptide binding and TR transactivation (64). The critical role in AF-2 activity of an agonist-induced conformational change in the region of helix 12 of nuclear receptors has been well documented (65). By presenting crystallographic evidence that implicates helix 12 of tamoxifen-bound ER as a steric impediment to the binding of GRIP1/mSRC-2 to the ER, Shiau *et al.* (66) have shed light on the differential affinity of agonist and antagonist-bound receptor for coactivator. As is the case with TR (64), the NR box peptide occupies a hydrophobic groove fashioned by helices 3, 4, 5, and 12 of ligand-bound ER. Conversely, antagonist-induced apposition of helix 12 to the hydrophobic groove does not form part of an interactive surface, but rather occludes residues critical for the interaction between ER and the NR box peptide (66). The 2.2 Å resolution crystal structure of the ligand-bound PPARγ-SRC-1 complex (67) has highlighted the role of a "charge clamp" of conserved glutamate and lysine residues in the PPARγ-LBD that make contact with backbone atoms of the NR boxes of SRC-1. In addition, tandem NR boxes of the SRC-1 moiety were shown to contact with both members of a PPARγ homodimer, hinting at a possible further role of multiple NR box motifs in coregulators.

3. Other coactivators.

a. E3 ubiquitin-protein ligases: E6-AP and RPF-1. Using a yeast two-hybrid screen with the hPR as a bait, our laboratory has recently identified a PR-interacting protein that is identical to the E6 papillomavirus-associated protein E6-AP (68). E6-AP, an E3 ubiquitin-proteins ligase that targets proteins for degradation by the ubiquitin pathway, interacts with and coactivates hormone-dependent transactivation by members of the nuclear receptor superfamily. Further supporting its identity as a coactivator, E6-AP reverses squelching between ER and PR and contains an intrinsic activation function in its N-terminal domain. Tandem NR boxes (*Section II.B.2.e*) are present in its C-terminal receptor-interacting region. E6-AP was originally identified through its association with the papillomavirus E6 protein: a complex of E6 and E6-AP was shown to target the p53 tumor-suppressor protein for degradation (69). Interestingly, however, the ubiquitin ligase activity of E6-AP is separable from its coactivation function. E6-AP is closely related to the E3 ubiquitin-protein ligase RPF-1, the human homolog of yeast RSP-5, a protein shown to enhance PR and GR transactivation in mammalian cells (70). Our laboratory has recently shown that E6-AP and RPF-1 synergistically enhance PR transactivation in mammalian cells. In addition, these proteins copurify by gel filtration, indicating that their synergistic coactivation of PR might be related to their presence in a common complex (58).

b. L7/SPA. A two-hybrid screen of a HeLa cDNA library using the PR antagonist RU486-bound PR D/E domain as a bait isolated a 27-kDa protein, L7/SPA, a previously described nuclear protein having no known function (71). L7/SPA increases the partial agonist activity of 4-HT-occupied ER and RU486-occupied PR or GR by 3- to 10-fold *in vitro* but does not influence the activity of the agonist-bound receptor. Interestingly, the antihormonal effects of the pure antiestrogen ICI164384 on ER and the pure antiprogestin ZK98299 on PR could not be offset by coexpression of L7/SPA.

c. TLS. Powers *et al.* (72) used murine RXR to isolate a 65-kDa protein, termed translocated in liposarcoma (TLS), a protein previously identified as a member of the RNP family of nuclear RNA binding proteins. Translocation-induced fusion of this protein to a DNA-binding protein, CHOP, had been previously shown to result in a potent chimeric transactivator. High-affinity binding of TLS to DNA-bound TR was demonstrated, as was the interaction of TLS with TR *in vivo*. It was suggested that TLS may enhance receptor protein or RNA stability, but this is yet to be determined. Intriguingly, TLS bears significant sequence similarity to $hTAF_{II}68$, a TFIID/RNA Pol II-associated protein (73).

d. Trip-1/Sug-1. Lee *et al.* (74) have identified a protein, Trip-1, that interacts with TR and RXR baits in a yeast two-hybrid assay in a ligand-dependent manner. It was identified as a member of the CAD (conserved ATPase domains) family of proteins and exhibits significant sequence identity with the yeast transcriptional coregulator, Sug1, originally identified as a suppressor of a mutation in the transcriptional activation domain of the yeast activator Gal4. Although Sug1 was originally postulated to be a component of the RNA Pol II holoenzyme complex, Rubin *et al*. (75) have reported its copurification with the 2MDa yeast 26 proteosome complex and have correlated this with reduced ubiquitin-dependent proteolysis in *sug1* mutants. Along with the identification of the E3 ubiquitin-ligases, E6-AP and RPF-1, as coactivators of PR transactivation, these results reiterate the importance of protein degradation pathways in receptor action, although the exact role of such pathways is unknown. Trip-1 does not contain consensus L*XX*LL/NR box motifs (*Section II.B.2.e*), evidence of the existence of binding determinants, other than NR boxes, which govern interactions between nuclear receptors and their coactivators.

e. TRAPs/DRIPs. Recent biochemical approaches have permitted the identification and extensive characterization of multiprotein complexes that interact with liganded nuclear receptors. Fondell *et al*. (76) employed one such biochemical purification strategy to isolate TR-associated proteins. They showed that epitope-tagged TR purified from HeLa cells cultured in the presence of thyroid hormone was associated with a group of distinct nuclear proteins termed TRAPs (TR-associated proteins). Supplementation of an *in vitro* transcription system with the TR/TRAP complex enhanced the transcriptional activity of a promoter driven by thyroid hormone response elements on naked, chromatin-free DNA (76). While the TRAPs were shown initially to be immunologically distinct from SRCs (*Section II.B.2*), CBP (*Section II.B.5*), TIF-1s (*Section IV.D.2*), RIP140 (*Section II.B.1*), and TAF_{II}s (*Section II.B.3.g*), it has since been demonstrated that a 220-kDa member of the complex, TRAP 220, is identical to the PPARγ-binding protein, PBP (*Section II.B.3.h*; Ref. 77). Adopting a similar approach, Freedman and colleagues have presented similar data with respect to the VDR (78). Purified VDR

recruited a complex of proteins (DRIPs or VDR-interacting proteins) that is homologous to the TRAP complex. The DRIPs, which range in size from 70–230 kDa, were shown not to contain SRC family members, p300/CBP, or other characterized coactivators. As with TRAPs for TR, DRIPs were shown to modestly enhance the activity of VDR in a cell-free ligand-dependent transcription assay (78).

The role of such morphologically distinct complexes in receptor activation is currently unclear, but a model has been proposed in which these complexes might assume significant roles in repetitive rounds of transcription mediated by TR and VDR. In such a scenario, initial recruitment of chromatin-modifying complexes containing the cointegrators p300/CBP (*Section II.B.5*) and members of the SRC family (*Section II.B.2*), would be followed by displacement of some of these complexes and interaction of receptor with TRAP/DRIP-like complexes to form a link with general initiation factors (*Section I.B*; Ref. 79). While support for such a model arises from the identification of TRAP/DRIP components in a complex, SMCC, containing human homologs of yeast mediator/RNA Pol II holoenzyme factors (80), it should be noted that CBP itself exists in a stable complex with RNA Pol II (81). An alternative model arises from the interesting observation that in the DRIP study, ER-LBD failed to appreciably recruit DRIPs (78), raising the possibility that TRAPs/DRIPs represent a type II receptor-specific complex.

f. Positive cofactors (PCs). Two positive cofactors, PC2 and PC4, derived from the upstream stimulatory activity (USA) cofactor fraction, act synergistically to mediate thyroid hormone-dependent activation either by TR or by a TR-TRAP complex in a reconstituted *in vitro* system comprised of purified factors and naked DNA templates (82). PC4 is a general coactivator that functions cooperatively with TAF_{II}s and mediates functional interactions between enhancer-bound activators and the general transcription machinery of RNA Pol II-transcribed genes (83) and is related to viral immediate-early transcriptional regulators (84). In the absence of TAF_{II}s and TFIIH, PC4 strongly represses transcription initiation, while simultaneously promoting the formation of preinitiation complexes. Upon concerted phosphorylation by TFIIH and distinct TAF_{II}s (*e.g.*, $TAF_{II}250$), PC4 elicits full coactivator potential, indicating a situation reminiscent of that reported for activated transcription at prokaryotic ρ-dependent promoters.

g. TAF_{II}s. The specific functional interaction of the AF-2 of different nuclear receptors with distinct TAF_{II}s has been well documented, and these interactions may serve to determine the specificity of the transcriptional response at a promoter. Sequestration of $TAF_{II}30$ by ER has been shown to be necessary for ligand-dependent activation by the ER (10). In addition, $TAF_{II}28$ selectively coactivates the AF-2 of RXR, an effect not observed for ER or VDR (85). To corroborate a specific role of TAF_{II}s in receptor transactivation, Mengus *et al.* (86) have demonstrated the ability of $TAF_{II}135$ to coactivate RAR, VDR, and TR, but not RXR or ER, and have speculated that $TAF_{II}135$ might enhance recruitment of TFIID by nuclear receptor AF-2s. Viewed in their entirety, these observations indicate that recruitment of distinct TFIID complexes at diverse promoters might be a component of nuclear receptor action.

h. PBP/TRAP220/TRIP2/mPIP9. The PPAR members of the nuclear receptor superfamily regulate the expression of genes involved in lipid metabolism and adipocyte differentiation. A recent study (77) has shown that a 165-kDa PPARγ-binding protein, PBP, binds to and enhances the transcriptional activity of PPARγ. PBP has exact sequence similarity with TRAP220/TRIP2/mPIP9 (79, 87, 88) and has a broad binding specificity for type II receptors, including RARα, RXR, and TRβ1. Furthermore, PBP contains two L*XX*LL motifs and is widely expressed in adult mice tissues. Given the limited binding specificity of PBP/TRAP220/TRIP2/mPIP9, the possibility exists that it represents a type II receptor-specific coactivator, although this has yet to be demonstrated on a functional level.

i. NCoA-62. Baudino *et al.* (89) used a yeast two-hybrid screen with VDR, RAR, and RXR to isolate a coactivator, NCoA-62, which manifested a broad specificity in both its interaction with, and coactivation of, nuclear receptors. NCoA-62 strongly coactivated VDR-mediated transcriptional activation, but more modestly enhanced ligand-dependent transcription from minimal promoters controlled by RAR, ER, and GR. While NCoA-62 lacks perfect L*XX*LL motifs, close inspection of its sequence indicates the presence of the pentapeptides L*XX*FL and L*XX*AL. The hydrophobic character of these peptides resembles that of L*XX*ML, a motif involved in the intramolecular contact of helix 12 with helix 3 and helix 5 in raloxifene- and tamoxifen-bound ER (see *Section II.B.2.e*), interactions thought to act as a steric impediment to NR-box binding (66). These data, while circumstantial, indicate that the imperfect NCoA-62 motifs might suffice to mediate its interaction with the AF-2 region of its nuclear receptor-binding partners.

j. TSC-2 (tuberous sclerosis-2). Tuberous sclerosis is an autosomal dominant disorder characterized by the appearance of benign tumors in a wide variety of tissues, including the eye, kidney, heart, and brain, where they cause epilepsy and mental retardation (90). TSC has been genetically linked to two loci in humans, one located on chromosome 16p13 (91), and the TSC-2 gene product has been characterized as a 200-kDa protein containing a short N-terminal leucine zipper and a C-terminal region homologous to the RAP1 GTPase-activating protein. In a yeast two-hybrid screen using RXR as a bait, Henry *et al.* (92) isolated a gene bearing 98% homology to that encoding TSC-2, and TSC-2 was also shown to interact with RXR in an *in vitro* pull-down assay. In transient transfection assays, TSC-2 was shown to stimulate PPARγ and VDR-mediated transactivation. A mechanism for TSC-2 in nuclear transport and/or cytoplasmic signaling was suggested (92), but its role in nuclear receptor transactivation is yet to be clearly established.

4. Selective coactivators.

a. SRA. Our laboratory has recently isolated and functionally characterized a novel transcriptional coactivator, termed steroid receptor RNA activator, or SRA (93). SRA was originally isolated in a yeast two-hybrid screen using the amino-terminal domain of PR-A. When overexpressed in mammalian cells, recombinant SRA specifically enhances endogenous steroid receptor AF-1-mediated transactivation by 5- to 10-fold without altering the level of

basal transcription. Several pieces of evidence indicate that SRA functions not as a protein but as an RNA transcript, introducing an entirely novel concept not only in nuclear receptor action, but in eukaryotic transcription as a whole. Transactivation analysis of multiple SRA frameshift and stop codon-containing mutants indicates that these mutants retain the capacity to coactivate steroid receptors. Further evidence of the identity of SRA has been provided by transfection experiments in the presence of the *de novo* protein synthesis inhibitor cycloheximide, in which SRA retained its ability to coactivate a reporter gene, whereas protein coregulators such as hSRC-1 and CBP did not. In addition, biochemical analysis has suggested that the SRA transcript is present in an hSRC-1 complex that is recruited by steroid receptors *in vivo*. We have shown that SRA is expressed in a tissue-specific manner, *e.g.*, in brain, where it colocalizes with the expression of certain steroid receptors. Given its evident functional selectivity, we have proposed a model in which SRA, as an AF-1 coactivator, functions to confer specificity upon coactivator complexes to specifically enhance steroid receptor-mediated transcription (93).

b. ARAs. ARA70 (androgen receptor activator-70), a 70-kDa human protein isolated on the basis of ligand-dependent interaction with an AR AF-2 bait in a yeast two-hybrid screen, was reported to enhance AR transactivation in DU145 human prostate cells, but had no effect on transactivation by other nuclear receptors (94). Unlike the SRC family members hSRC-1 and TIF2/hSRC-2, ARA70 has been shown to be capable of enhancing the partial agonist activity of hormonal antagonists (95).

c. Trip230. Like AIB-1/hSRC-3 (*Section II.B.2.c*), the thyroid receptor coactivator Trip230 highlights the potential role of coactivators in disease states (96). Trip230 was isolated as a partner of the Rb gene product, the interaction being mediated by the N terminus of the Rb protein. 14q31, The chromosomal locale of the Trip230 gene, is a locus to which several abnormalities of thyroid hormone response, including Graves' disease and congenital hyperthyroidism, have been linked, implicating Trip230 as a factor involved in the thyroid hormone response. While Trip230 binds TR in a thyroid hormone-dependent manner and enhances TR-dependent transactivation, thyroid hormone has no effect on its interaction with Rb. Coexpression of Rb abolishes the enhancement of TR transactivation effected by Trip230, indicating a functional antagonism between Rb- and TR-mediated pathways.

d. PGC-1. Puigserver *et al.* (97) have identified a novel coactivator, PGC-1 (PPARγ coactivator-1), which is preferentially expressed in brown fat and skeletal muscle and which enhances transactivation by TR and PPARγ on the uncoupling promoter-1 (UCP-1). In contrast to AF-2 coactivators, PGC-1 was shown to bind preferentially to a region outside the AF-2 domain of PPARγ-1, in the hinge (D) region. Overexpression of PGC-1 in white adipose tissue activates UCP-1 and key mitochondrial enzymes. In addition, exposure to low temperatures enhances expression of the PGC-1 gene, and this has been suggested to be a key mechanism underlying adaptive thermogenesis in mammals. PGC-1 is a striking example of the control over coactivator function exerted by environmental stimuli and is an intriguing insight into the mechanism whereby selective regulation of coactivator expression mediates a specific and isolated transcriptional response *in vivo.*

e. HMGs. The HMG-1 (high-mobility group) and HMG-2 proteins occupy a unique niche among characterized nuclear coactivators by selectively enhancing the DNA-binding activity of the type I steroid receptor subfamily (98). Transient transfection assays showed that cotransfection of HMGs with different steroid receptors resulted in enhancement of PR, GR, and AR transactivation, but not that of VDR. While HMG-1 and -2 interacted only transiently with purified PR in solution, and had no affinity for PRE (progesterone response element) *per se*, PRE binding by PR resulted in the formation of a stable PR-HMG-PRE complex. These results suggested that DNA binding by PR is concomitant with its interaction with HMGs, which serve to stabilize the association of PR with its response element.

5. Cointegrators: CBP/p300. CREB-binding protein (CBP) was initially characterized as a coactivator required for efficient activation of cAMP-regulated promoters by the transcriptional activator cAMP-response element-binding protein (CREB, Ref. 99). Several studies implicate CBP as a coactivator of multiple transcriptional activators, including p53 (100), NF-κB (101), and nuclear receptors (45, 102, 103). In addition, direct interactions between CBP and RXR, TR, and ER are mediated by the N-terminal domain of CBP (45), which contains an NR-box indispensable for receptor interaction (60).

In addition to its interactions with nuclear receptors, CBP interacts with members of the SRC family, including mSRC-1/NCoA-1 (45), TIF2/hSRC-2 (51), and p/CIP/mSRC-3 (50), indicating that it may form a ternary complex with SRC family members and nuclear receptors. Functional evidence suggests such a complex may exist, since CBP synergizes with hSRC-1 in the transactivation of ER and PR transactivation (104). Biochemical evidence suggests, however, that CBP does not form a stable complex with hSRC-1 (58), and it has been shown that the interactions of liganded ER (105) and PR (58) with CBP are relatively weak in comparison with the recruitment by these receptors of hSRC-1-containing complexes. We have proposed (58) that an initial receptor/hSRC-1 complex recruits other functionally diverse complexes containing coactivators such as CBP. In support of this, CBP is ineffective in restoring activity to an RARE-linked reporter gene after immunodepletion of NCoA-1/mSRC-1 (50), suggesting that CBP might require SRC-1 complexes as a platform to effect its coactivation of nuclear receptors. An overall model of CBP action (45) suggests that, as a common limiting cofactor for diverse transcriptional activators and coactivators, it acts as a cellular cointegrator to collate multiple afferent signals into an integrated response at promoters containing multiple *cis*-acting elements. A critical physiological role of CBP is indicated by the fact that Rubinstein-Taybi syndrome, a rare disorder characterized by mental retardation and numerous physical deformities, is associated with mutation of *CBP* in humans (106).

p300 (107) Shares many of the functional properties of CBP, including transcriptional enhancement of diverse tran-

scription factors such as MyoD (108), p53 (100), and nuclear receptors (102). In addition, p300 associates with mSRC-1 (109) and interacts with ER in a ligand-dependent manner (110). This functional redundancy is not complete however: targeted deletion of the p300 locus (111) indicates that functional CBP in such animals is insufficient to prevent defects due to loss of p300 in neurulation, cell proliferation, and heart development, as well as embryonic lethality. Kraus and Kadonaga (112), observing that p300 and ER synergistically activate cell-free transcription in the presence of chromatin, have postulated a cooperative "fire and reload" mechanism, in which p300 and ER cooperatively enhance transcription during a single round of transcription ("fire"), but only ER is required for reassembly of the transcriptional preinitiation complex ("reload"). Their results highlight the functional distinction between different events at a transcriptionally active promoter and the individual role of diverse factors in the fluid and intricate process of transcriptional activation.

III. Nuclear Receptor Corepressors

A. Background

1. Repression of basal transcription by nuclear receptors. Transcriptional repression, or silencing, refers to the ability of type II receptors to lower basal promoter activity in the absence of ligand. A number of different mechanisms have been proposed for silencing by nuclear receptors. Passive repression refers to competition either for DNA binding or for dimerization partners, both mechanisms resulting in steric hindrance by the unliganded receptor on the promoter, blocking access of activators or basal factors (113). In addition, inactive heterodimer formation with other type II receptors has been documented for TR (114, 115). Conversely, in the active silencing model, unliganded receptor either directly affects transcription initiation, or recruits an array of factors, the function of which is to create an environment that is incompatible with proper assembly of a preinitiation complex, a mechanism also known as transrepression. Data supporting direct contacts between nuclear receptors and general transcription factors that result in transcriptional silencing are limited. Baniahmad *et al.* (116) sketched a possible mechanism for silencing by TR, envisaging sequestration of the basal transcription factor TFIIB by domains in the C terminus of the unliganded TR. Maximal repression by TR, however, was contingent upon other more N-terminal domains that did not interact with TFIIB.

2. Evidence for the existence of corepressors. In contrast to cellular TR, its viral counterpart, the oncogene product v-*erb*A fails to bind hormone and is a constitutive repressor of transcription of thyroid hormone-responsive genes (117). Baniahmad *et al.* (118) demonstrated the existence of active silencing domains in TR and showed that these domains functioned as repressors when fused to a heterologous DBD. In experiments symmetrical to those that implied the existence of nuclear receptor coactivators (*Section II.A*), our laboratory showed that the silencing activity of TR could be greatly reduced (squelched) in transient cotransfection assays by coexpression of either the C terminus of v-*erb*A or the unliganded TR-LBD. Such interference predicted the existence of soluble corepressors for TR and other type II receptors, present in limiting cellular concentrations (119, 120). Table 2 summarizes the functional properties of characterized nuclear receptor corepressors.

B. Corepressors

1. NCoR/RIP-13. Biochemical studies of cellular proteins associated with unliganded TR and RAR have identified a 270-kDa protein termed NCoR (nuclear receptor corepressor; Ref. 121), also isolated as RIP-13 (122). While Horlein *et al.* (121) reported that NCoR was specifically recruited by unliganded TRα and RARα, and that little or no interaction was observed between NCoR and RXR, VDR, ER, or GR, Seol *et al.* (122) isolated RIP13 using RXR as a bait. Mutational analysis of the TR LBD has identified a domain, termed the NCoR box, which is indispensable for the interaction of receptor and NCoR. Loss of the NCoR box attenuates repression by the unliganded TRα, strongly suggesting that interaction

TABLE 2. Nuclear receptor corepressors

Factor	Alternative designations	Related proteins	Comments	References
NCoR	RIP-13	SMRT	Interacts with and corepresses unliganded TRα, RARα & COUP-TF1, RevErb and DAX-1; contains autonomous repression domains; reduces RU486/PR partial agonist activity; specifically degraded by 26S proteosome after binding Siah; associates with PML-RARα fusion proteins in acute promyelocytic anemia.	71, 121–127, 131, 133, 134, 138, 140–142, 144, 149–151, 206, 207
SMRT	TRAC2	NCoR	Interacts with and corepresses unliganded TR and RAR; significant sequence similarity with NCoR; contains an autonomous repression domains; reduces tamoxifen/ER and RU486/PR partial agonist activity; associates with PML-RAR fusion proteins in acute promyelocytic anemia.	71, 125, 128–142, 148, 150, 151, 208
TRUP	SURF-3, PLA-X, L7a		Reduces transactivation by TR and RAR; decreases DNA binding by RXR heterodimers.	143
SUNCoR			Contains autonomous repression domain; corepresses TR and RevErb.	144
NURD			Couples ATPase activity to histone deacetylation; immunodepletion decreases repression by TR; contains NCoR-related subunit.	212

with NCoR is required for efficient TRα and RARα-mediated transcriptional repression (121). As is the case with several nuclear receptor coactivators, the existence of NCoR isoforms has been postulated based upon the isolation of cDNAs encoding putative splice variants, although no direct evidence of their existence has been obtained.

To substantiate the identification of NCoR as a mediator of ligand-independent repression, fusion of NCoR to the Gal4DBD effects potent repression at a promoter bearing Gal4DBD-binding sites (121). Deletion mutations of NCoR have identified two receptor-interacting domains (RIDs) in the C-terminal portion of the protein that are required for nuclear receptor interaction (122). Further N-terminal in the NCoR molecule are three repression domains, one at the extreme N terminus (RI) and two more centrally located (RII and RIII), to which the intrinsic repressive functions characteristic of NCoR have been ascribed. Analogous to the dominant negative activity of the C-terminal receptor-interacting domain of the coactivator hSRC-1 (*Section II.B.2.a*), coexpression of the RIDs-bearing domain of NCoR abolishes repression effected by unliganded TR and RAR (123). More recently, studies in our laboratory and others have indicated the role of NCoR in mediating the transcriptional silencing properties of members of the orphan receptor subfamily, including Rev-Erb (124), chicken ovalbumin upstream promoter transcription factors (COUP-TFs; Ref. 125) and DAX-1 (126). Moreover, Muscat *et al.* (127) showed the ability of NCoR and its variants RIP-13α and RIP-13δ1 to directly interact with TFIIB, $TAF_{II}32$, and $TAF_{II}70$, indicating that corepressors may function, at least in part, by mediating repressive interactions of unliganded receptors with components of the basal transcription apparatus (transrepression).

2. SMRT/TRAC2. SMRT (silencing mediator for retinoid and thyroid hormone receptor) was isolated by a yeast two-hybrid screen of a human lymphocyte cDNA library using RXR as a bait (128). SMRT was also identified as TRAC2 (T_3 receptor-associating cofactor 2), a protein isolated on the basis of its interaction with RAR, RXR, and TR (129). While significant sequence similarity exists between the N- and C termini of SMRT and NCoR (130), the N terminus of NCoR contains two repressor domains that are not present in SMRT. RAR and TR interact strongly with SMRT and RXR in a far-Western analysis, and addition of ligand to these receptors induces dissociation from SMRT, but not from RXR. Furthermore, in a yeast two-hybrid assay, a strong ligand-reversible interaction with SMRT has been observed for the LBDs of TR and RAR (128). In addition, direct recruitment of SMRT to a promoter by fusion with a heterologous DBD results in substantial repression of the basal promoter activity (128). SMRT/TRAC2 contains two C-terminal receptor-interacting domains, RID-1 and RID-2, which, analogous to the selective recruitment of receptors by distinct NR boxes (*Section II.B.2.e*), interact differently with individual receptors. RARα, for example, binds RID-1 exclusively, whereas TR binds both domains with equal affinity (131). Sande and Privalsky (129) have described the ability of an amino-terminal truncation of SMRT/TRAC2, named TRAC1, to act as a dominant-negative inhibitor of TRAC2, but the biological significance of this is yet to be determined.

SMRT reverses the squelching of Gal4DBD-RAR silencing by RAR 403, a RAR mutant lacking the RAR C terminus and a robust repressor of the basal activity of RARE-containing promoters (132). In contrast to the ligand-reversible association of full-length RAR and RAR-LBD with SMRT, RAR 403 retains the interaction with SMRT in the presence of ligand. Similarly, the association of the constitutively silencing TR-derived oncogene product v-*erb*A with SMRT is unaffected by ligand, and ectopic expression of SMRT reverses the squelching of Gal4DBD-TR silencing by overexpressed v-*erb*A. The physiological significance of these results has been illuminated somewhat by Yoh *et al.* (133), who have demonstrated that a variety of mutations in the TRα gene, which are associated with general resistance to thyroid hormone, result in strong constitutive retention of the corepressors SMRT and NCoR by the mutant receptors.

The inability of SMRT to interact with constitutively activating TR mutants further hints at its role as a transcriptional corepressor. A TR Pro→Arg mutant, TR-160, devoid of silencing activity but capable of hormone-dependent transactivation, shows little affinity for SMRT in an *in vitro* pull-down assay (128). In addition, a TR-LBD mutant that does not silence but retains its transactivation function, does not interact with the C-terminal RID domain of SMRT (125). Tagami *et al.* (134) have shown that both NCoR and SMRT are capable of functioning as transcriptional activators at negative thyroid response elements (TREs), suggesting that the repressive properties of these corepressors are not intrinsic. Genes regulated by negative TREs are stimulated by unliganded TR and repressed upon the addition of thyroid hormone. In this study, ectopic expression of NCoR and SMRT enhanced basal transcription of a negative TRE in a hormone-dependent manner, whereas a TR mutant, which failed to interact with NCoR, did not activate transcription in this assay (134). These and other results (127) suggest that specific *cis*-acting factors can modulate the function of corepressors and that corepressors may mediate productive, as well as repressive, interactions with general transcription factors.

3. NCoR and SMRT: functional similarities and divergence. NCoR and SMRT appear to be less than discriminate in their binding of repressive transcription factors, suggesting they may have a more general role in transcriptional repression than was initially considered. Dhordain *et al.* (135) have described the interaction of the POZ motif of the non-Hodgkin's lymphoma-associated protein LAZ3/BCL6 with SMRT. The promiscuous interaction of SMRT and NCoR with POZ motif-containing proteins is further illustrated by certain cases of acute promyelocytic leukemia, a disease characterized by incomplete leukocytic differentiation and appearance of leukemic blast cells. Novel fusions of the RARα gene have been identified (136–138) that arise from chromosomal translocations with loci containing the genes encoding the PML (promyelocytic leukemia) and PLZF (promyelocytic leukemia zinc finger) proteins. The resultant proteins, PML-RARα and PLZF-RARα, were shown to retain the RARα DBD and LBD. Clinically, PML-RARα patients achieve complete remission upon administration of pharmacological doses of all-*trans* RA. PLZF-RARα patients, con-

versely, respond poorly to such therapeutic intervention. These phenomena were directly attributed to the constitutive recruitment of NCoR and SMRT by the PLZF protein, an interaction not subject to regulation by binding of ligand by the RARα moiety. The PML-RARα fusion, however, binds NCoR and SMRT only through the RARα LBD, explaining the ability of such patients to eventually respond to RA. To further illustrate its promiscuity, SMRT also interacts with CBF-1/RBP-Jκ, the mammalian homolog of the *Drosophila suppressor of hairless*, which switches from a transcriptional repressor to an activator upon binding of the ligand *notch*. In the absence of ligand, CBF-1/RBP-Jκ is part of a repressor complex containing SMRT, which subsequently dissociates when *notch* binds (139).

While NCoR and SMRT are structurally similar (121, 128), they differ functionally in several respects. The molecular basis of heterogeneity of function among RAR isoforms has been ascribed to their differential interaction with SMRT and NCoR: whereas RARα and RARγ interact with both corepressors, RARβ exhibits no affinity for either SMRT or NCoR (131). The work of Baniahmad *et al.* (140) indicates that the weak repression of basal transcription by TR in CV-1 cells can be amplified by ectopic expression of SMRT, but not NCoR. These corepressors differ most notably, however, in the mediation of transcriptional repression by certain orphan receptors. Crawford *et al.* (126) showed that the orphan receptor DAX-1, which interacts with NCoR, does not recruit SMRT. Similarly, when bound to DNA, the orphan RevErb exclusively recruits NCoR, but does not require SMRT to effect transcriptional repression (141). Zhang *et al.* (142) demonstrated that repression by RevErb is cell line-specific, such that RevErb represses in 293T cells, but not in N18 neuroblastoma cells. They found that while the NCoR transcript is found in both cell types, NCoR protein is greatly reduced in the N18 cells. Yeast two-hybrid screening using the N-terminal portion of NCoR isolated a protein, present in N18 cells but absent in 293T cells, termed Siah (*Seven-in-absentia* homolog), a ring finger protein initially identified as a factor in *Drosophila sevenless* signaling. Siah is a potent mediator of NCoR down-regulation, decreasing the half-life of NCoR by approximately 5-fold. Siah-mediated down-regulation of NCoR has been linked to the 26S proteosome of the ubiquitin pathway: inhibition of the 26S proteosome prevents NCoR degradation and restores repression of RevErb (see also *Sections II.B.3.a* and *II.B.3.d*). Crucially, Siah does not interact with the N-terminal repression domain of SMRT and hence selectively targets NCoR for proteosomal degradation. Unlike RevErb repression, repression by TR is largely unaffected by endogenous Siah, consistent with its ability to recruit SMRT in addition to NCoR (142). The discriminate degradation of NCoR illustrates a mechanism whereby signaling by the function of one receptor type can be selectively abolished and highlights the multiple layers of control over nuclear receptor function.

4. Other corepressors.

a. TRUP/SURF-3/PLA-X. Burris *et al.* (143) have identified a protein, TRUP (thyroid receptor-uncoupling protein), which attenuates hormone-dependent transactivation by TR and RAR, but which has no effect on transactivation by ER or RXR. Sequence comparison of TRUP indicates its complete identity with the nuclear proteins SURF-3 and PLA-X. In addition, TRUP opposes ligand-dependent activation by TR in transient cotransfection. The ability of TRUP to diminish the hormone-dependent transactivation and silencing properties of TR has been attributed to the decreased ability of TR:RXR and RAR:RXR heterodimers to interact with their cognate hormone response elements (HREs). The capacity of TRUP to modulate receptor action in this manner represents another distinct mode of control among coregulators.

b. SUN-CoR. Zamir *et al.* (144) have isolated SUNCoR (small ubiquitous nuclear corepressor), a highly basic 16-kDa corepressor that shows no homology to either NCoR or SMRT. SUN-CoR contains an intrinsic repression domain and enhances silencing of basal transcription by TR and RevErb. The potential role of SUNCoR as an additional functional element in corepressor complexes is evinced by its interaction *in vivo* with NCoR.

5. Steroid hormone receptor repression. Steroid hormone receptors have little DNA-binding activity in the absence of hormone; indeed, steroid receptors, including PR and GR, are sequestered in ternary interactions with hsp90 and hsp70 (1). Recently, considerable effort has been devoted to discerning the mode of action *in vitro* of synthetic steroid hormone antagonists such as RU486 and 4-HT. These ligands induce receptor dimerization and DNA binding, but the resultant receptor dimer is ineffectual in stimulating transactivation. They act either as partial agonists or antagonists, in a manner contingent upon the tissue or promoter context. While the effects of these ligands have been attributed in part to their ability to disrupt interactions of receptor and coactivator (27, 29, 33, 66), recent evidence suggests that they may also induce active repression by nuclear receptors by promoting their association with transcriptional corepressors *in vitro*.

We have investigated the mode of action of RU486 as a PR antagonist and, by providing evidence for the involvement of a cellular corepressor in PR action, have introduced a novel concept in steroid hormone receptor action (145). Observing that PR and GR mutants lacking a short C-terminal portion of the receptor can be specifically activated by RU486 (146, 147), our group postulated the existence of an intrinsic repressor function in this domain that inhibited the transcriptional activity of the RU486-bound receptor. An amino acid sequence was defined in the C terminus of the PR that contained an intrinsic repressive function when fused to a heterologous DBD, indicating that this region interacted with a soluble corepressor (145). Mutations within this amino acid sequence in the full-length protein resulted in a PR that stimulated transcription in the presence of RU486. Competitive overexpression of the putative repressor domain activated the RU486-bound wild-type PR without affecting hormone-dependent transactivation, indicating titration of a cellular corepressor responsible for down-regulating the transcriptional activity of RU486-bound receptor.

A number of studies have since demonstrated the interaction of nuclear receptor corepressors with antagonist-bound steroid receptors. Smith *et al.* (148) demonstrated that SMRT abrogates the ability of mixed antiestrogen to activate transcription of an ER-dependent gene. Furthermore, *in vitro*

interaction assays have indicated an association between ER and SMRT in the presence of 4-HT. It was suggested that tissue-specific variations in corepressor expression might explain the ability of antagonists to evoke an agonist-like response in some tissues but not others. Intriguingly, it has also been demonstrated that SMRT interacts with ligand-bound ER, raising the possibility that corepressors modulate ligand-dependent activation by nuclear receptors (148). Additionally, RU486-bound PR functions as a transcriptional activator in the presence of unliganded TR or 4-HT-bound ER, but loses this ability in the presence of liganded TR or agonist-bound ER (149). In a yeast two-hybrid screen, Jackson *et al.* (71) have shown that NCoR interacted with antagonist-bound PR-LBD and that overexpression of NCoR and SMRT markedly suppressed RU486- and 4-HT-mediated partial agonist activity, an effect reversible, in the case of the PR, by overexpression of the PR LBD. Adding physiological significance to these data, Lavinsky *et al.* (150) have correlated decreased levels of NCoR with acquisition of hormone resistance in a mouse breast cancer model. Wagner *et al.* (151) demonstrated that NCoR and SMRT preferentially associate with antagonist-bound PR and that the partial agonist activity of RU486-bound PR is ablated by overexpression of NCoR and SMRT. In total, these results indicate that steroid receptors occupied by mixed agonists/antagonists such as RU486 or 4-HT are not intrinsically transcriptionally inactive, and that their transactivation functions may be masked by binding of corepressors (149). These observations point to the possible physiological role of mixed agonists/antagonists in steroid receptor action, and pose the question: could similar compounds exist in nature?

IV. Nuclear Receptors and Chromatin

A. Background

Eukaryotic chromosomes (herein referred to loosely as chromatin) are organized in the steady state into a regularly repeating protein DNA unit termed the nucleosome. The basic protein unit of the nucleosome is the histone, a small, highly basic, globular moiety. Solution of the low-resolution structure of the nucleosome core particle indicated a structure comprising a histone octamer, made up of two copies each of histones H2A, H2B, H3, and H4, around which was wrapped 1.7 turns of a left-handed DNA superhelix (152). Higher tiers of organization are thought to involve the assembly of nucleosomes into chromatin domains. The net effect of this arrangement is to create a thermodynamic barrier against the access of transcription factors to their DNA substrate. Recent higher resolution studies have established the fine structure of the nucleosome particle and have highlighted the role of the histone amino-terminal tails in making internucleosomal contacts (153). This arrangement has important consequences for our interpretation of the mode of action of two classes of molecules thought to regulate the access of transcription factors to their cognate DNA elements, namely acetylases (*Section IV.C*) and deacetylases (*Section IV.E*).

A multistep model has been envisaged for transcriptional activation by nuclear receptors (36, 154–156). Binding of the activated receptor to the enhancer region directs modification of the local chromatin structure into a transcriptionally permissive state (derepression), followed by recruitment of GTFs to form a preinitiation complex at the promoter (activation). This section reviews recent findings that substantiate this model, discussing the mechanisms by which coregulators, through intrinsic and recruited chromatin-modifying activities, are thought to manipulate chromatin and facilitate efficient transcriptional regulation by nuclear receptors. Covalent modification of nucleosomal structure is regulated by the diametrically opposed activities of histone acetylation, correlated with gene activation, and histone deacetylation, generally associated with gene repression (Fig. 3). In addition, recruitment of ATPase complexes that effect noncovalent modifications of chromatin domains appears to be important for transcriptional regulation by nuclear receptors.

B. The MMTV and TRβA promoters

The functional consequences of the organization of hormone-responsive promoters in higher order chromatin structures is best illustrated by the MMTV and TRβA gene promoters. These promoters attest to the intimate structural and functional association between the DNA template and the protein component of chromatin.

The long terminal repeat sequences of the MMTV promoter are organized into a series of six positioned nucleosomes (157), directed by the primary nucleotide sequence of the promoter. The array is such that the *cis*-acting elements of the MMTV promoter adopt precise translational and rotational settings on the surface of the histone octamer that favor nuclear receptor binding while precluding the binding of the transactivators NF-1 and Oct-1. Several studies have documented a two-step model of synergistic enhancement of the MMTV promoter by steroid hormones and the NF-1 and Oct-1 transactivators, and the intrinsic role of the nucleosomal structure in this model (Ref. 158 and references therein). After induction by progestins, a rearrangement of the phasing of the nucleosomes exposes the NF-1 and Oct-1 sites and

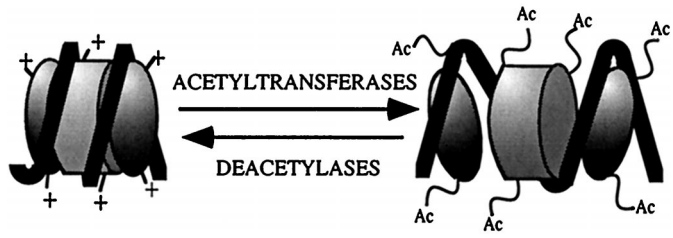

FIG. 3. Simplified model of the biochemical basis of nucleosomal remodeling by histone acetyltransferases and histone deacetylases. Catalytic transfer of acetyl groups to the terminal amino groups of lysine residues of histones H2A, H2B, H3, and H4 histones by histone acetylases (HATs) is thought to result in disruption of interactions between nucleosomes and DNA, between nucleosomes and neighboring nucleosomes, and possibly between nucleosomes and other proteins. The overall loss of compact nucleosomal structure facilitates access of transcriptional activators and coactivators to the promoter template. Conversely, recruitment of histone deacetylases (HDs) is thought to result in loss of the acetyl groups, reestablishing the coherence of the nucleosomal structure, and restricting access of transcription factors to the promoter. Other covalent modifications may also have a role in regulating nucleosome interactions.

primes the promoter for a rapid and synergistic response to these transcription factors.

Another promoter that exemplifies the influence of chromatin on events at hormone-inducible promoters is that of the *Xenopus* TRβA gene. Transcriptional initiation at this promoter is subject to autoregulation by thyroid hormone and TR (159). Wong *et al.* (156, 160) carried out a series of incisive experiments on the TRβA promoter using a *Xenopus* oocyte system, in which heterodimers of TR and RXR bound cognate HREs *in vivo* and were capable of alternately silencing or activating transcription in response to ligand. In the case of repression, simultaneous chromatin assembly and unliganded receptor heterodimer positioning were required for maximal transcriptional silencing, suggesting a synergistic role for chromatin in mediating silencing by the receptor. On the other hand, their results suggested that relief of this transcriptional repression by liganded TR comprises two distinct, independently regulated events: 1) extensive modifications of repressive chromatin structures, which are necessary but not sufficient to effect transcriptional initiation, and 2) interaction with GTFs resulting in assembly of a preinitiation complex (156, 160).

C. Coactivators and acetylation

Historically speaking, increased acetylation of histone tails has been correlated with transcriptional activity, whereas hypoacetylation has been associated with repression (161). The prevailing view has been that the major effect of the reduction of positive charge afforded by hyperacetylation of the amino-terminal histone tails is to uncouple their interaction with the negatively charged DNA, thereby creating an environment more accessible to transcription factors (Fig. 3). This theory has been modified somewhat by the recent solution of the nucleosome particles at 2.8 Å (153), which highlights nucleosomal-nucleosomal contacts made by the amino-terminal tails of histones, and suggests that an additional effect of selective acetylation of lysine residues in these tails may be to disrupt higher order chromatin structures (162).

Brownell *et al*. (163) identified histone acetyltransferase (HAT)-A, a *Tetrahymena* protein that contained acetyltransferase activity and showed close sequence similarity with the yeast transcriptional adaptor protein GCN5 (general control nonrepressed protein 5). Their discovery was the first indication that recruitment of histone acetylation activity by sequence-specific transcription factors might be involved in transcriptional regulation in eukaryotes. This was rapidly followed by the identification of the HAT activity of the general transcription factor $TAF_{II}250$ (164), implying a role for histone acetylation in access of TFIID to the promoter template. Initial indications of the role of acetylation of core histones in transcriptional regulation by nuclear receptors emerged from the identification of the intrinsic HAT activity of p300/CBP-associated factor (PCAF; 165), identified as a mammalian counterpart of yeast GCN5. Interestingly, GCN5 was characterized as a component of the yeast ADA complex, which is known to mediate AF-2-dependent activation by RXR and ER (166). PCAF interacts with p300 and CBP both by *in vitro* pull-down and by *in vivo* coimmunoprecipitation. The HAT activity of PCAF primarily targets histones H3 and H4 as substrates, exhibiting a preference for histone H3. PCAF interacts directly *in vitro* with p300/CBP (165), hSRC-1 (167), ACTR/hSRC-3 (53), and nuclear receptors (36, 168), interactions that may serve to stabilize a functional complex of receptor, SRC family members, PCAF, and p300/CBP on the promoter. Recent evidence suggests that PCAF exists in stable, preformed complexes with histone-like TAF_{II}s (169) in a manner akin to the arrangement of similar TAF_{II}s in the human GCN5 and yeast SAGA acetylase complexes (170). This striking finding raises the possibility of the evolutionary conservation of a mechanism whereby recruited GCN5/PCAF complexes assume the architectural role of local chromosomal histones during transcriptional activation.

HAT activity has also been identified as a property of the transcriptional cointegrators p300 and CBP (171, 172). Unlike PCAF, CBP and p300 can acetylate all four core histone types and, whereas CBP exhibits no substrate specificity, p300 HAT activity is directed primarily toward histone H3. HAT activity is also conserved in members of the SRC family, including ACTR/hSRC-3 (53) and hSRC-1 (167), although no such activity has been identified in TIF2/hSRC-2. The intrinsic histone acetylase activity of hSRC-1 maps to a carboxy-terminal region of SRC-1 and is specific for histones H3 and H4. Korzus *et al*. (173) have suggested that the apparent redundancy of HAT activity among nuclear receptor coregulators may be due to the requirement by diverse promoters of different combinations of HAT activities at different promoters. In support of this, hSRC-1 (58) and SRC-3 (N. J. McKenna, unpublished) complexes are biochemically distinct from those of CBP, p300, and PCAF, suggesting that combinatorial assembly by liganded receptor of these subcomplexes into larger complexes could occur in a cell- or promoter-specific manner (58).

While the discussion to this point has emphasized the well characterized role of cellular acetyltransferases in the catalytic acetylation of nucleosomal histones, it has become apparent recently that the spectrum of substrates for these enzymes extends to nonhistone proteins, implying a broader regulatory role for acetyltransferases in cellular signaling. Acetylation by p300 of p53 enhances the DNA-binding activity of this important sequence-specific activator (174). A recent striking finding showed that acetylation does not necessarily represent a positive impetus for transcription. Acetylation by *Drosophila* CBP of the *wingless* signaling pathway T-cell transcription factor (TCF) acts as a negative stimulus for signaling flux through this pathway (175). Data for the role of acetylation in directly regulating nuclear receptor function are as yet sparse, although our laboratory has shown that the acetyltransferase activity of PCAF targets zinc finger lysine residues in the DBD of PR (M. Burcin, personal communication). Although the functional consequences of this are as yet unclear, it may be that subtle covalent modifications such as these are important determinants of the association of receptor with its response element, and with coregulators, during transcriptional activation.

D. Chromatin-remodeling proteins

Increasing importance is being attached to recruitment by nuclear receptors of protein complexes that mediate chro-

matin remodeling, a term referring to the regulation of the coherence of the higher order chromatin domains into which nucleosomes are organized (*Section IV.A*). This section will summarize several proteins and protein complexes that have been suggested to be recruited by nuclear receptors to effect chromatin remodeling.

1. The SWI/SNF complex. Particularly well characterized in the process of chromatin remodeling are the products of the *swi/snf* genes. These genes were first identified in yeast on the basis of a genetic screen for genes required for regulation of mating type switching (176, 177). Genetic studies and biochemical purification also indicated that SWI/SNF proteins might form a complex that actively disrupted chromatin. Mutations in histone genes alleviate the requirement for functional SWI/SNF genes in yeast (178). Furthermore, SWI2/SNF2 has intrinsic ATPase activity (179), and purified SWI/SNF complex alters nucleosomal structure *in vitro* in a ATP-dependent manner (180, 181).

A wealth of data has implicated members of the SWI/SNF complex in transcriptional regulation by nuclear receptors. Yoshinaga *et al*. (182) showed that a yeast strain bearing mutations in the *swi1*, *swi2*, and *swi3* genes was incapable of transactivating a reporter gene in the presence of cotransfected GR, whereas a wild-type strain was able to support GR-dependent transactivation. In addition, it was shown that GR coimmunoprecipitated with the SWI/SNF complex (182). Purification of the mammalian homolog of the yeast SWI/SNF complex has identified two genes with a high degree of sequence similarity to *swi2* and *snf2*, named *brahma* (*brm*) and *brahma-related gene 1 (brg-1)* for their similarity to the *Drosophila brahma* gene (183). The products of the human *brm* and *brg-1* genes, hBRM and BRG-1, respectively, are reported to interact with ER in a ligand-dependent manner in a yeast two-hybrid assay (184). In addition, GR recruits the ligand-dependent nucleosomal remodeling activity of the SWI/SNF complex in yeast (185). Fryer and Archer (186) identified the dependence of GR regulation of a stably integrated MMTV promoter upon recruitment of BRG-1-containing complexes. A model for the role of HATs and chromatin-modifying enzymes in facilitating recruitment of a preinitiation complex by liganded receptor is shown in Fig. 4.

2. The TIF-1 proteins. Le Douarin *et al*. (187) have isolated a mouse protein, TIF-1α, which interacts with a RAR-LBD bait in a yeast two-hybrid screen. TIF-1α has been shown to complement RXRγ AF-2 activity in yeast in the presence of 9-*cis*-retinoic acid. Functional interactions in yeast have been demonstrated between TIF-1α and VDR, PR, and ER (188, 189). Paradoxically, however, TIF-1α down-regulates RXRα, RAR, and ER transactivation in mammalian transient transfection assays (187). Furthermore, when fused to a heterologous DBD, TIF-1α represses transcription (190).

Recent data suggest that a family of TIF-1 proteins exists, including TIF-1β and TIF-1γ in addition to TIF-1α (189, 191). This family is defined by an N-terminal domain containing a cysteine-histidine cluster (PHD or plant homeodomain), a RING finger, and a B box finger, domains thought to mediate DNA-protein and protein-protein interactions (192). While Le Douarin *et al.* (190) note that TIF-1β, unlike TIF-1α, does not interact with nuclear receptors, Chang *et al.* (193) have provided evidence that it interacts with GR and C/EBPβ to induce expression of the α1-acid glycoprotein gene. Although their function in nuclear receptor action is unclear, the interactions of TIF-1 family members with heterochromatin-associated proteins indicate a potential role in chromatin modification. TIF-1α has been shown to interact with the heterochromatin-associated proteins mHP1α, MOD1 (HP1β), and MOD2 (HP1γ; 189) which in turn interact with mSNF2-β, the mouse homolog of the *Drosophila* brahma protein (*Section IV.D.1*). Intriguingly, TIF-1α (189) and TIF1β (191) associate with the KRAB (Krüppel-associated box) repression domain, a region conserved in many Krüppel-type zinc finger proteins. A model has been suggested for TIF-1s in transcriptional regulation, in which formation of transcriptionally inactive chromatin domains by TIF-1s effects repression, and ligand-dependent association of TIF-1s with receptors mediates formation of transcriptionally primed chromatin domains. An alternative mode of action for TIF-1s is suggested by the observation that TIF-1α is a protein kinase that targets the basal transcription factors TFIIEα, $TAF_{II}28$, and $TAF_{II}55$ for phosphorylation *in vitro*. (194). Our own data suggest that TIF-1α exists *in vivo* as a component of stable preformed multiprotein complexes of approximately 1 megadalton (MDa) in size (N. J. McKenna, unpublished results).

3. NSD-1. The 280-kDa NSD-1 [nuclear receptor-binding, SET domain-containing protein 1 (63)] contains the evolutionarily conserved SET domain, first identified in the *Drosophila* proteins Su (var), E(z), and Trx (195). Certain SET proteins are

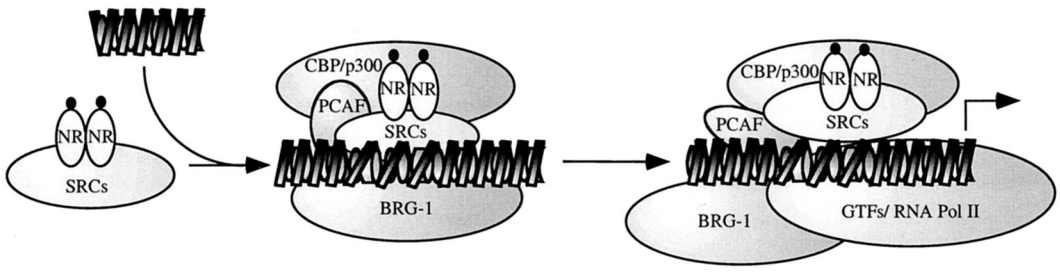

FIG. 4. Two-step model for activation by nuclear receptors at a transcriptionally repressed promoter/enhancer. Liganded receptor recruits SRC family members (denoted SRCs) and other histone acetylases, as well as ATPase-coupled chromatin-modifying enzyme activities to effect local nucleosome disruption around the enhancer/promoter region. Subsequent recruitment of GTFs, RNA Pol II, and other basal factors leads to stabilization of a preinitiation complex. For the sake of clarity, TRAP/DRIP complexes or other less well characterized coregulators have not been represented, nor have direct interactions between receptor and general initiation factors. The reader is referred to the text (*Sections II.A.1* and *II.B.3*) for detailed discussions of these.

thought to be associated with chromatin and commonly function, depending upon the developmental context, either as transcriptional coactivators, as corepressors, or both. For example, while E(z) appears to maintain target genes in a closed chromatin conformation during certain developmental stages, it can act as an activator (196). In contrast, Trx antagonizes the effect of E(z) by maintaining chromatin in a transcriptionally active conformation. These functionally antagonistic properties are also characteristic of NSD-1, which was identified by a two-hybrid screen for RARα-LBD interacting proteins. NSD-1 interacts with the LBD of ER and RARα in the presence of ligand, but its interactions with RXRα and TRα LBDs are reduced in the presence of ligand (63). Moreover, in addition to containing intrinsic activation domains and consensus NR boxes (*Section II.B.2.e*), NSD-1 harbors intrinsic repression domains. While its precise roles are unclear, NSD-1 has been proposed to be a bifunctional coregulator capable of modifying chromatin domains in a developmental stage-specific manner.

E. Corepressors and deacetylation.

Broadly speaking, histone deacetylation opposes the structural incoherence brought to bear upon nucleosomes by histone acetylation (Fig. 3). Extensive genetic studies in yeast have yielded abundant correlative evidence for the global role of hypoacetylation of histones in disabling transcriptional activity and have identified proteins whose mammalian homologs are key factors in transcriptional repression by nuclear receptors.

1. Histone deacetylases and Sin proteins. The product of the yeast *RPD3* gene was isolated as a transcriptional repressor in several independent mutant suppressor screens (197, 198) and was shown to be required for the maximal range of transcriptional efficiency at certain yeast genes. In its absence, both activation and repression of target genes are less efficient (199), indicating a role of RPD-3 in global transcriptional regulation. Rundlett *et al*. (200) demonstrated that a subunit of yeast histone deacetylase activity showed sequence similarity with RPD3. The cloning of a mammalian homolog, histone deacetylase-1 (HD-1/HDAC-1; Ref. 201) established a functional link between histone deacetylation and transcriptional regulation in mammalian cells. HD-1 was isolated by affinity purification using the specific histone deacetylase inhibitor trapoxin. An RPD-3-containing complex, as well as HD-1/HDAC-1, is known to deacetylate core histones *in vivo* (200, 201). Yang *et al*. (202) used a yeast two-hybrid screen to study proteins interacting with the YY-1 transcription factor and identified a cDNA-encoding histone deacetylase-2 (mRPD3/HDAC-2), which bore extensive sequence identity with yeast RPD3. Colinear with the transcriptional regulatory functions of RPD3 are those of another yeast protein, Sin3 (RPD-1), initially identified as a negative regulator of the yeast HO gene (203). The inactivation of the *SIN3* gene, along with other *SIN* genes, was shown to substitute for the requirement of the *SWI5* gene product for *HO* transactivation (204). A model was proposed in which Sin3 effected repression at certain promoters by interaction with specific DNA-binding proteins.

2. Histone deacetylation and nuclear receptor repression. Studies in our laboratory were the first to document the involvement of SIN3 in repression of transcription by nuclear receptors. Nawaz *et al.* (205) demonstrated that SIN3 negatively regulated the transcriptional activity of the PR in a yeast-based promoter system. In addition, yeast strains harboring deletions in the SIN3 gene exhibited increased transactivation of a reporter gene in the presence of liganded PR. A wealth of evidence has since documented the role of mammalian Sin3 homologs and histone deacetylases in repression by nuclear receptors. Anti-NCoR antibodies have been shown to specifically coimmunoprecipitate cellular histone deacetylase activity (206–208). The *in vivo* requirement of Sin3 proteins and histone deacetylase activity by NCoR for repression by TR/RAR heterodimers *in vivo* has been indicated by the ability of anti-mSin3 and anti-mRPD3 antibodies to ablate silencing of a reporter gene by a Gal4DBD-NCoR fusion. Similar results were obtained for a Gal4-TRC′ (Gal4DBD fused to the TRα C-terminal repressor domain) indicating that the repressive effects of TR and NCoR/mSin3-linked histone deacetylase activity are colinear in mammalian cells (206). Laherty *et al*. (209) demonstrated the *in vivo* association of mammalian Sin3 with the two mammalian histone deacetylases, HD-1/HDAC-1 and HDAC-2. In addition, biochemical evidence suggests that Sin3 proteins and histone deacetylases exist in stable preformed complexes in mammalian cells (210, 211). Collectively, these data strongly support the hypothesis that nucleosomal condensation through recruitment of histone deacetylases by corepressors is part of the repertoire by which unliganded type II nuclear receptors inhibit the assembly of a preinitiation complex. An overall model of corepressor/coactivator action (168) envisages unliganded type II receptors maintaining a transcriptionally inactive steady state at the promoter by recruitment of corepressors and their associated histone deacetylase activities. Ligand binding is thought to induce release of corepressors and enable the receptor to recruit PCAF, p300/CBP, and SRC family members to effect local histone acetylation and creation of a transcriptionally permissive environment at the promoter.

3. NURD and Mi-2 ATPase complexes. An interesting footnote to the role of ATPase activity in facilitating transcriptional activation by nuclear receptors (*Section IV.D.1*) is the discovery that ATPase activity may also be harnessed to assist access of nuclear receptor corepressor complexes to promoters (212). The biochemically characterized NURD complex contains a subunit, MTA1, which was shown to contain a region previously identified in NCoR, and immunodepletion of NURD efficiently relieves transcriptional repression by unliganded TR. The coupling by NURD of ATP-dependent nucleosomal remodeling activity to histone deacetylation suggests that nucleosomal disruption may be a key prefatory step in the access of histone deacetylase to its substrate. Wade *et al.* (211) presented similar data with respect to Mi-2, a SNF2-related ATPase (*Section IV.D.1*) that is present in a Sin3/deacetylase complex from *Xenopus laevis*. These results suggest that the acetylation status of histones and their higher order domain structure are not rigidly linked and may be independently manipulated by regulatory proteins.

V. Concluding Remarks

Recent developments in this field have sketched an increasingly complex picture of the functions of nuclear receptor and their associated coregulators. Daunting as it is, however, several strong themes are emerging from the study of this area. A consistently recurrent theme in transcriptional regulation by nuclear receptors is the fluid, multistep nature of the process, in which diverse factors are predicted to have temporally and spatially distinct functions at transcriptionally active promoters. Transcriptional regulation requires the recruitment by receptor of multiple, distinct enzyme activities — acetylases, deacetylases, kinases, ATPases, ligases, and proteases — the concerted action of which, intuitively, must be regulated efficiently to achieve an appropriate transcriptional response to ligand (Table 3). Coregulators are organized into preformed subcomplexes, an arrangement which 1) facilitates their assembly into multiple configurations and 2) makes them readily available to competing pools of transcriptional activators and promoters. Consistent with this level of organization is the notion that efficient activation at different promoters is a function of the assembly of distinct configurations of coregulator complexes at these promoters (172). The requirement of ubiquitin-protein conjugation enzymes for efficient activation by some receptors raises the intriguing possibility that enhancer/promoter clearance of factors may enable the sequential interaction of activated receptor with multiple coregulator complexes. A second theme emerging from the study of coregulators is the multiple layers of control that govern their functional interactions with nuclear receptors. Coregulators appear to be redundant, and no more obvious demonstration than this is provided by the targeted deletion of mSRC-1, a viable phenotype characterized by partial hormone insensitivity and increased, probably compensatory, expression of another SRC family member, mTIF2 (GRIP1/mSRC-2), in many tissues (*Section II.B.2.d*). The functions of nuclear receptor coregulators are governed by factors ranging from tissue-specific patterns of expression (*Section II.B.4.a*) to regulation of their expression by hormone (*Section II.B.2.c*), to environmental stimuli (*II.B.4.d*), to conserved amino acid sequences that determine their physical interaction with liganded receptor (*Section II.B.2.e*). A third theme is the potential of coregulators to act as adaptors to mediate functional interactions of receptors with diverse classes of transcription factors, and integrating receptor-regulated gene networks with a broad spectrum of afferent signals. Implicit in these themes is the prediction that the relative expression level of coactivators and corepressors is an important determinant of an appropriate and graded response to ligand by the target cell.

This review would be incomplete without a brief reference to the clinical and physiological implications of nuclear receptor coregulators. While mechanistic approaches continue to yield essential data, the full impact of these factors on transcriptional biology, and cell biology in general, will be felt as a shift in emphasis from molecular techniques to a more global perspective takes place. Future directions are certain to embrace fundamental questions such as whether (and how) nuclear receptor coregulators contribute to the

TABLE 3. Multifaceted nature of transcriptional regulation by nuclear receptor coregulators: nuclear receptors, through their interactions with coregulators, recruit diverse functional domains and enzyme activities to the promoter to achieve efficient transcriptional regulation *in vivo*

Property	Coregulator	Target	Function	Reference
Activation domain	SRC-1, TIF2/hSRC-2, RAC3/hSRC-3	Basal transcription factors	Stabilization of preinitiation complex	40, 47, 48, 54
Repression domain	NCoR, SMRT, SUNCoR.	Basal transcription factors	Destabilization of preinitiation complex	121, 128
Acetylase	PCAF, CBP/p300, SRC-1, ACTR/hSRC-3	Core histones	Disruption of histone-DNA, histone-histone interactions	53, 165, 167, 171, 172, 173
	p300	p53	Enhances p53 DNA binding	174
	CBP	TCF	Uncouples *Wingless* signaling in *Drosophila*	175
	PCAF	PR	Unknown	–
Deacetylase	HDAC-1/HDAC-2	Core histones, others?	Restoration of DNA-histone, histone-histone interaction	206–209
Protease	Siah2/26S proteosome	NCoR	Protein degradation	142
Ubiquitin ligase	E6-AP	Unknown	Protein degradation	68
ATPase	BRG-1, Trip-1	Chromatin, others?	Domain remodeling during activation	182, 184–186
	SNF2 ATPase	Chromatin, others?	Domain remodeling during repression?	211
Kinase	TIF1α	TFIIEα, $TAF_{II}28$, $TAF_{II}35$	Stabilization/destabilization of target proteins?	194
	ER-associated kinase	?		215
	ANPK (AR-associated kinase)	AR coregulators?		216

etiology of the steroidal cancers of the breast, ovary, uterus, and prostate; their role in the myriad disorders of the endocrine system; and their value as prognostic, diagnostic, or therapeutic targets in such diseases. In addition, targeted deletions of coregulator genes will emerge as a powerful tool in the effort to discern the physiological functions of these factors. We foresee the maturation of these lines of research in the coming years, as this active field strives toward a coherent model of nuclear receptor action.

Acknowledgments

The authors wish to thank Barb Tykal for secretarial assistance. We thank Sophia Y. Tsai, Ming-Jer Tsai, and members of the O'Malley laboratory for valuable discussions during the preparation of the manuscript.

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