REVIEW

The coregulator exchange in transcriptional functions of nuclear receptors

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Nuclear receptors (NR) comprise a family of transcription factors that regulate gene expression in a ligand-dependent manner. Members of the NR superfamily include receptors for steroid hormones, such as estrogens (ER) and glucocorticoids (GR), receptors for nonsteroidal ligands, such as thyroid hormones (TR) and retinoic acid (RAR), as well as receptors that bind diverse products of lipid metabolism, such as fatty acids and prostaglandins (for review, see Beato et al. 1995; Chambon 1995; Mangelsdorf and Evans 1995). The NR superfamily also includes a large number of so-called orphan receptors for which regulatory ligands have not been identified (Mangelsdorf and Evans 1995). Although many orphan receptors are likely to be regulated by small-molecular-weight ligands, other mechanisms of regulation, such as phosphorylation (Hammer et al. 1999; Tremblay et al. 1999) have also proven to be of importance. Remarkably, the sequence of the *Caenorhabditis elegans* genome has revealed the presence of >200 members of the NR family, suggesting a critical role of these proteins in environmental adaptation (Sluder et al. 1999). Although mammalian genomes are unlikely to contain such a large complement of these factors, >24 distinct classes of NR have been identified in humans, and these factors exert diverse roles in the regulation of growth, development, and homeostasis. Based on their importance in biology and medicine, as well as the relatively simple mechanism of regulation, NR represent one of the most intensively studied and best-understood classes of transcription factors at the molecular level.

Members of the NR family regulate transcription by several mechanisms (Fig. 1). Nuclear receptors can activate or repress target genes by binding directly to DNA response elements as homo- or heterodimers or by binding to other classes of DNA-bound transcription factors. A subset of NRs, including TR and RAR, can actively repress target genes in the presence or absence of ligand binding, and many NR have been demonstrated to inhibit transcription in a ligand-dependent manner by antagonizing the transcriptional activities of other classes of transcription factors. These activities have been linked to interactions with general classes of molecules that appear to serve coactivator or corepressor function. In this review, we will discuss recent progress concerning the molecular mechanisms by which NR cofactor interactions serve to activate or repress transcription.

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Coactivators in transcriptional regulation by NRs

The ability to switch a nuclear receptor from an inactive to an active state by simple addition of a small molecule in vitro has dramatically facilitated biochemical approaches to the elucidation of their mechanisms of action, providing instructive insights with respect to mechanisms of transcriptional activation by other classes of signal-dependent transcription factors. The NR ligand-binding domain (LBD) is connected to the DNA-binding domain by a short flexible linker and mediates ligand-dependent transactivation functions. A short conserved helical sequence within the carboxyl terminus of the LBD, referred to as activation function 2 (AF-2), is required for ligand-dependent activation (Danielian et al. 1992; Durand et al. 1994). Biochemical and expression cloning approaches have been used to identify a large number of factors that interact with NRs in either a ligand-independent or ligand-dependent manner. Many of these factors have been demonstrated to be capable of potentiating NR activity in transient cotransfection assays, suggesting their potential to serve as NR coregulators. Many of these proteins also appear to function as components of large, multiprotein complexes. As the number of potential coregulators clearly exceeds the capacity for direct interaction by a single receptor, the most parsimonious hypothesis is that transcriptional activation by NRs involves multiple factors that act in both a sequential and combinatorial manner to reorganize chromatin templates (Pollard and Peterson 1998),

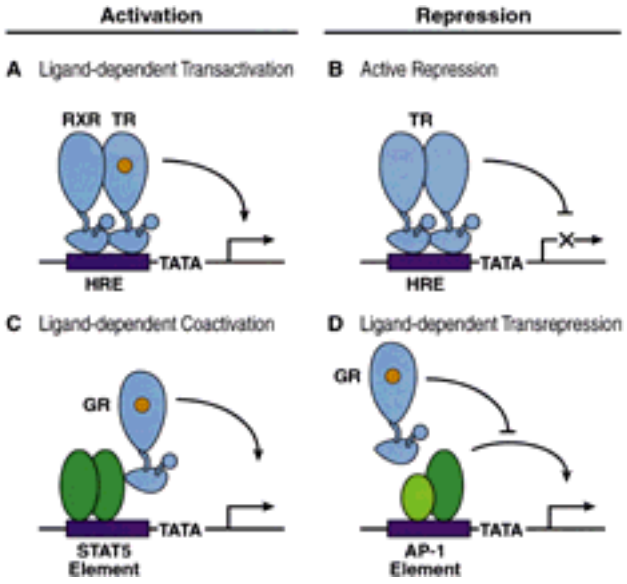

Figure 1. Transcriptional activities of NRs. Members of the NR family can both activate and inhibit gene expression. (*A*) The prototypic activity of NRs is ligand-dependent activation of transcription upon binding to specific hormone-response elements (HREs) in target genes. (*C*) NRs have also been documented to contribute to gene activation by acting as coactivators for other transcription factors, as demonstrated in the case of the glucocorticoid receptor for certain STAT-5-responsive genes. (*E*) A number of orphan NRs, such as CARβ and HNF4, are capable of constitutively activating transcription. Several mechanisms of transcriptional inhibition have also been established. (*B*) A subset of NRs that heterodimerize with the retinoid X receptor, including the thyroid hormone receptor (TR) and retinoic acid receptor (RAR), are capable of actively repressing target genes upon binding to HREs in the absence of ligand. (*D*). In addition, several NRs, exemplified by the glucocorticoid receptor are capable of inhibiting the activities of other classes of transcription factors, such as AP-1, in a ligand-dependent manner. This effect does not require DNA binding by the NR and is referred to as transrepression.

and to modify and recruit basal factors and RNA polymerase II.

A combination of genetic, biochemical, and functional data suggests that several factors, including the Brg (SWI/SNF) complex, CBP/p300, p160 factors, P/CAF, and the TRIP/DRIP/ARC complexes, discussed below, are likely to be critical regulators for at least subsets of NR-regulated genes (Fig. 2). However, by the simple criteria of ligand-dependent binding and the ability to synergize on cotransfection assays, numerous additional proteins have been suggested to exert coactivator roles. These include HMG factors (Verrier et al. 1997; Boonyaratanakornkit et al. 1998; Romine et al. 1998), MEF2 (Lee et al. 1997), cell cycle regulators such as cyclin D (Zwijsen et al. 1997), the ADA coactivators in a yeast context (vom Baur et al. 1998), ARA5Y (Kang et al. 1999), the ring finger SNURF (Moilanen et al. 1998), the vitamin D coactivator NCOA-62 (Baudino et al. 1998), as well as PC2 and PC4 (Fondell et al. 1999). The potential actions of E6-AP (Huibregtse et al. 1995; Imhof and McDonnell 1996; Nawaz et al. 1999) provides intriguing questions regarding proteolytic events. TRIP-1/Sug1 (Lee et al. 1995a,b), identified as a suppressor of Gal4-dependent activation, has been copurified (Rubin et al. 1996) as a component of the 2-MD yeast 26S proteosomal complex, and correlated with reduced ubiquitin-dependent proteolysis in Sug-1 mutants. The potential relationship of proteosomal activity to NR function is tantalizing but unclear; it could ultimately relate to "switching" of receptor-associated complexes.

Swi/Snf/BRG complexes: ATP-dependent chromatin remodeling complexes

As chromatinized transcription units are repressed as compared to naked DNA, a critical aspect of gene activation involves nucleosomal remodeling (for review, see Wu 1997; Wade and Wolffe 1999). Two general classes of chromatin remodeling factors have been identified that appear to play critical roles in transcriptional activation by NRs; ATP-dependent nucleosome remodeling complexes, and factors that contain histone acetyltransferase activity (for review, see Kingston and Narlikar 1999). Recent work has led to the discovery of several activities that use ATP hydrolysis in the remodeling of chromatin templates and appear to be involved in NR function. At least two related remodeling complexes are present in yeast; RSC (remodeling the structure of chromatin) (Cairns et al. 1996; Pollard and Peterson 1998) and the SWI/SNF complex. The *SWI2/SNF2* gene identified encodes a protein that is homologous to DNA-stimulated ATPases/DNA helices (Laurent et al. 1993; Cairns et al. 1996). The yeast SWI/SNF complex (for review, see Peterson 1996; Workman and Kingston 1998) facilitates the binding of sequence-specific transcription factors to nucleosomal DNA and has the ability to cause local changes in chromatin structure in an ATP-dependent manner (Owen-Hughes et al. 1996). Homologs of SWI2/SNF2 are present in flies (Brahma) and mammals (BRG1, hBrm), in each case functioning as components of large multiprotein complexes (Tamkun et al. 1992; Khavari et al. 1993; Muchardt and Yaniv 1993; Dingwall et al. 1995; Tsukiyama and Wu 1995). Remodeling complexes similar to the SWI/SNF complex have been characterized in *Drosophila*, including NURF (nucleosome remodeling factor) (Tamkun et al. 1992), ACF (ATP-utilizing chromatin assembly and remodeling factor) (Ito et al. 1997) and CHRAC (chromatin accessibility complex (Varga-Weisz et al. 1997). All of these complexes contain ISWI (imitation SWI), a member of the SWI2/SNF2 family, suggesting that this protein may serve as the energy-transducing component of chromatin-remodeling machines. These findings indicate that there are a number of chromatin remodeling activities that can generate local modifications in nucleosomes, suggesting a level of combinatorial control even at this step in the activation process.

Although yeast do not contain NRs, a role for SWI/

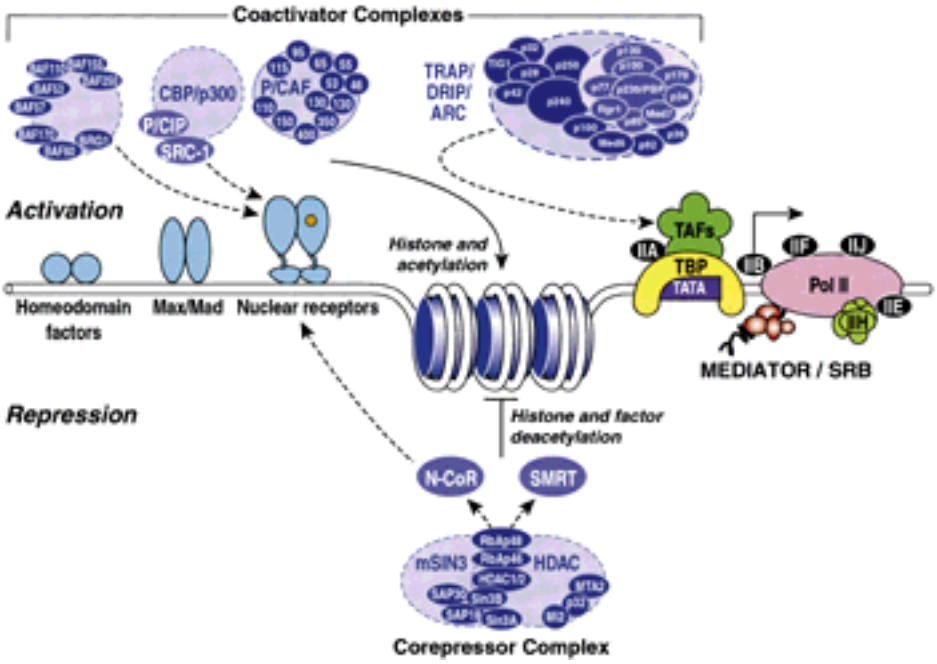

Figure 2. Coactivator and corepressor complexes in NR transcription. Coactivator complexes include SWI/SNF, CBP/SRC-1/p/CAF and TRAP/DRIP/ARC. The SWI/SNF complex possesses ATP-dependent chromatin remodeling activities. The CBP and p/CAF complexes possess histone acetyltransferase activities. These complexes may act in concert to relieve chromatin-mediated repression, with the TRAP/DRIP/ARC complex functioning to recruit core transcription factors. Corepressor complexes include the SIN3/HDAC complex, which has been proposed to be recruited via the NR corepressors NCoR or SMRT. This complex possesses histone deacetylase activity and is thought to reverse actions of histone acetyltransferase-containing complexes.

SNF complexes was initially suggested based on experiments demonstrating that GR activity in yeast required these factors (Yoshinaga et al. 1992). The GR can target the SWI/SNF complex to chromatinized templates containing GR binding sites in yeast, resulting in disruption of local nucleosomal structure (Ostlund Farrants et al. 1997). However, in yeast this action did not appear to require the presence of ligand. Both hBrm and Brg-1 have been shown to interact with the ER in a ligand-dependent fashion using the yeast two-hybrid assay (Ichinose et al. 1997). Transfection of an ATPase and defective allele of either Brg1 or hBrm into several mammalian cell lines leads to a significant decrease in the ability of NRs, including RAR, ER, and GR to activate transcription (Muchardt and Yaniv 1993; Chiba et al. 1994). Studies of the ability of the GR to activate transcription from the mouse mammary tumor virus (MMTV) promoter in mammalian cells indicates that interaction with Brg-1-containing complexes was required when the promoter was integrated stably into chromosomal DNA, but not when the promoter was assessed in transiently transfected cells (Fryer and Archer 1998). ISWI-containing complexes are reported to be targeted by the progesterone receptor to the MMTV promoter, resulting in topographical alterations (Di Croce et al. 1999). These observations are consistent with the possibility that chromatin remodeling at the promoter may represent the first step in the activation of transcription in vivo.

Complexes with histone/protein acetyltransferase activity

The discovery that the *Tetrahymena* histone acetyltransferase A was related to the yeast transcriptional regulator GCN5 (Brownell et al. 1996) suggested a direct link between a histone modification that previously had been correlated with transcriptionally active genes and the function of a transcriptional coactivator. The rates of gene transcription roughly correlate with the degree of histone acetylation, with hyperacetylated regions of the genome being more actively transcribed than hypoacetylated regions (for review, see Pazin and Kadonaga 1997). Thus, the specific recruitment of a complex with histone acetyltransferase (HAT) activity to a promoter may play a critical role in overcoming repressive effects of chromatin structure on transcription (Pazin and Kadonaga 1997; Struhl 1998; Wade et al. 1998). This concept was further supported by the subsequent finding that the mammalian GCN5 ortholog P/CAF (Yang et al. 1996), CREB binding protein (CBP) (Bannister and Kouzarides 1996; Ogryzko et al. 1996), the adenovirus E1A binding protein p300 (Ogryzko et al. 1996), and $TAF_{II}250$ (Mizzen et al. 1996), each possess intrinsic HAT activity. Conversely, the discovery that a mammalian histone deacetylase (HDAC) was a homolog of the yeast corepressor, RPD3 (Taunton et al. 1996), gave rise to the hypothesis that regulated activation events might involve the exchange of complexes containing histone deacety-

lase function with those containing HAT activity (Fig. 2).

However, the significance of the acetylation of lysine residues in the short amino-terminal domains of core histones is not fully elucidated. Possibilities considered include altered nucleosomal placement, for which there is no evidence (Logie et al. 1999). Because hyperacetylation may slightly reduce thermal stability, the binding of some transcription factors may be enhanced. The only argument for altered conformation of nucleosomal core particles comes from slight effects on average linkage number of nucleosomes of a supercoiled plasmid (Barnes et al. 1994). Indeed, in examining ligand-dependent transcription by the T3 receptor in *Xenopus*, there was no significant evidence of nucleosomal rearrangement (Li et al. 1999). A second role involves abolishing the amino-terminal chromatin-dependent intermolecular folding that might reflect chromosomal condensation (for review, see Fletcher and Hansen 1996). The most important role might be to influence binding of other regulatory factors, such as TUP1 in yeast, with specificity provided by sites on histones that are acetylated (Hecht et al. 1995; Edmondson et al. 1996; Kuo et al. 1996; Pollard and Peterson 1998).

P/CAF

P/CAF and GCN5e are homologs of the yeast protein GCN5, which is an essential component of multisubunit coactivator complexes referred to as the ADA or SAGA complexes (Grant et al. 1997, 1998a). The yeast SAGA complex contains at least two groups of gene products; the Ada proteins Gcn5, Ada1, Ada2, Ada3, and Ada5 and the TATA-binging protein (TBP)-related set of Spt proteins, Spt3, Spt7, Spt8 and Spt20. Purification of the SAGA complex has revealed that it also contains a subset of proteins TBP-associated factor) (TAF), including $TAF_{II}90$, $TAF_{II}68/61$, $TAF_{II}60$, $TAF_{II}25/23$, and $TAF_{II}20/17$ (Grant et al. 1998a,b). Tra1, an ATM/PI-3-kinase-related homolog of a human cofactor essential for c-myc and E2F transformation, has recently been identified as an additional component of SAGA (Grant et al. 1998a). The SAGA complex exerts transcriptional activity in vitro only on chromatinized templates and depends on the HAT activity of Gcn5 (Brownell et al. 1996); however the ability of Gcn5 to acetylate nucleosomal histones requires additional components of the SAGA complex.

Using an affinity purification approach, a mammalian core P/CAF complex was recently isolated that contained members of the ADA family as well as TAFs (Ogryzko et al. 1998). This P/CAF complex thus bears clear resemblance to the SAGA complex in yeast, hinting at a link between the P/CAF complex and the core machinery. Other subunits of the complex enable P/CAF to acetylate histones in the context of nucleosomes, which apo-P/CAF alone fails to do, similar to the requirement of yeast Gcn5 for components of the SAGA complex to acetylate nucleosomes (Grant et al. 1997).

Like Gcn5, P/CAF contains a carboxy-terminal region that contains protein–protein interaction motifs. In addition, P/CAF contains an amino-terminal extension not conserved in yeast GCN5 that appears to mediate additional protein–protein interactions. Although initially identified as a factor that interacts with the C/H3 domains of p300 and CBP (Yang et al. 1996), studies have subsequently demonstrated that P/CAF is capable of interacting with other domains, CBP/p300 with other NR coactivators (Chen et al. 1997; Blanco et al. 1998; Korzus et al. 1998), and with the LBD of the RAR, in a ligand-independent fashion (Blanco et al. 1998). P/CAF has thus been suggested to exert roles in mammalian cells in NR activation events.

CBP/p300

CBP and p300 serve essential coactivator roles for many classes of sequence-specific transcription factors (for review, see Torchia et al. 1997; McKenna et al. 1999), functioning in part by acting as molecular scaffolds, and in part by acetylating diverse substrates. The intrinsic acetyltransferase activity of CBP and p300 was demonstrated initially using histones as substrates. In vitro, CBP and p300 not only acetylate free histones but also histones assembled into nucleosomal complexes, suggesting that nucleosomes can be similarly modified in vivo (Ogryzko et al. 1996).

Evidence for important roles of CBP/p300 in NR function in cells has been provided by the results of gene-deletion experiments, nuclear injection of blocking antibodies, and transfection assays (Chen and Okayama 1987; Chakravarti et al. 1996; Hanstein et al. 1996; Kamei et al. 1996; Yao et al. 1996, 1998; Kawasaki et al. 1998). Cell-free transcription assays of ER activity are consistent with a role of p300 in overcoming chromatin-mediated repression (Kraus and Kadonaga 1998). When ER activity is assessed on naked DNA templates, high rates of transcription are observed that are relatively ligand-independent. When assessed on chromatinized templates in the presence of defined core transcription factors, both basal and ligand-dependent transcription are markedly repressed. Addition of p300 does not significantly influence basal transcription, but markedly stimulates ligand-dependent activity. Evidence for differences in p300 and CBP function has been suggested by analysis of in vivo gene deletion experiments, genetic diseases, which reveal distinct phenotypes (Miller and Rubinstein 1995; Petrij et al. 1995; Tanaka 1997; Yao et al. 1998; Oike et al. 1999a,b), and by results using specific hammerhead ribozymes (Kawasaki et al. 1998).

CBP and p300 acetylate a range of substrates, including sequence-specific transcription factors. For example, the carboxy terminus of unmodified p53 inhibits its ability to bind to DNA, but is efficiently acetylated by CBP and p300 (Gu et al. 1999). Upon acetylation, this inhibitory effect is relieved, allowing high affinity DNA binding. Additional nonhistone proteins that have been identified as substrates of p300 and CBP include HMG I/Y (Munshi et al. 1998). Intriguingly, it has been proposed that acetylation of HMG I/Y may play a role in termination of transcriptional activation. In the case of some

interactions, acetylation appears to serve as a negative transcriptional signal such as in the case of TCF (Waltzer and Bienz 1998). In turn, it has been proposed that covalent modifications and association with other factors, such as E1A, modulate CBP HAT activity. In vivo, E1A has been suggested to enhance histone acetylation (Ait-Si-Ali et al. 1998), whereas in biochemical assays using either specific histones or HMG proteins as substrates (Chakravarti et al. 1999; Hamamori et al. 1999; Perissi et al. 1999a), E1A at high levels can be inhibitory to both CBP and P/CAF acetyltransferase function. However EIA can inhibit the acetylation of other substrates (Li et al. 1999). This inhibition has been alternatively ascribed to interactions with the HAT domain itself (Chakravarti et al. 1999), or rather to be dependent upon specific interactions with the C/H3 domain (Perissi et al. 1999). Although the biological significance of these observations is not at all clear, they raise the possibility that the HAT activity of CBP, and potentially other HAT-containing factors, may be regulated.

The ability of CBP to coordinate the assembly of additional protein complexes appears to be an essential aspect of its function as a coactivator. Biochemical studies suggest that CBP serves as a coactivator of CREB in part by recruitment of RNA polymerase II (pol II) complexes (Nakajima et al. 1996). Transcriptional activation by CREB is strongly inhibited by E1A, which binds to CBP and p300 via the C/H3 domain (Arany et al. 1994; Eckner et al. 1994), as well as to amino-terminal and carboxy-terminal sites (Kurokawa et al. 1998). The C/H3 region interacts with several proteins, including RNA helicase A, which is a component of RNA pol II complexes (Nakajima et al. 1997). Indeed, immunoprecipitation experiments indicate that a fraction of RNA pol II in the cell can be coprecipitated with antibodies specific for CBP. These findings suggest that E1A inhibits the function of CREB by preventing the assembly of CBP–coactivator complexes that contain RNA pol II. In the case of the RAR, interaction of CBP with SRC-1 appeared to be critical for ligand-dependent transcription, with the C/H3 domain of CBP not being required. Intriguingly, the interaction of E1A with the carboxyl terminus of CBP prevents the assembly of CBP/SRC-1 coactivator complexes, indicating that there may be factor-specific mechanisms of transcriptional inhibition by this factor (Kurokawa et al. 1998).

p160/SRC

Proteins of ~160 kDa molecular mass were among the first factors identified that interact with NRs in a highly ligand-dependent, both in solution (Cavailles et al. 1994; Halachmi et al. 1994) or on DNA (Kurokawa et al. 1995). These biochemically identified factors could themselves associate with CBP (Kamei et al. 1996; Yao et al. 1996). Expression cloning and yeast two-hybrid screening approaches led to the identification of three related genes that encode the p160 factors, referred to as SRC-1/NcoA-1, p160, TIF2/GRIP-2/NcoA-1 and p/CIP/AIB-1/ACTR/RAC/TRAM-1 (Onate et al. 1995; Kamei et al. 1996; Anzick et al. 1997; Chen et al. 1997; Hong et al. 1997; Li et al. 1997a; Takeshita et al. 1997; Torchia et al. 1997). Members of the p160 family of NR coactivators contain a highly conserved amino-terminal basic helix-loop-helix (bHLH) PAS domain that is also present in members of the Per/Arnt/Sim family of transcription factors and mediates protein–protein interactions (see Fig. 5A, below). Several lines of evidence support the idea that p160 factors play important roles as NR coactivators, however the extent of their role is not proven. Consistent with potential redundancy, the knockout of the *SRC-1* gene in mice results in relatively subtle defects in the development of estrogen-dependent tissues, including the uterus and breast, which may be explained by the observed compensatory increases in TIF-2 expression (Xu et al. 1998). No apparent defects in PPARγ-function have been documented in SRC-1-deficient mice (Qi et al. 1999).

The carboxyl terminus of SRC-1 (Spencer et al. 1997) and ACTR (Chen et al. 1997) have been reported to possess HAT activity. This activity is much weaker than the HAT activity of CBP/p300, and p/CAF, and the carboxyl terminus of SRC-1 and ACTR do not appear to contain features that correspond to the acetyl CoA binding site of the P/CAF (Clements et al. 1999), or GCN5 HAT domains (Trievel et al. 1999). The crystal structures of GCN5 and P/CAF are conserved in the CBP HAT domain (Martinez-Balbas et al. 1998), suggesting that the ACTR/SRC-1 HAT activities reflect entirely novel structures. Additional factors may be recruited to specific domains of the p160 factor; for example, a novel protein methyltransferase may be recruited to the p160 carboxyl terminus. This coactivator-associated arginine methyltransferase, CARM1, has selective functional effects on transcriptional activation by NRs, and can methylate histone H3 in vitro (Chen et al. 1999a), thus the platform assembly functions of p160 factors includes recruitment of factors with methyltransferase functions.

Studies also suggest that the same coactivator complexes can be utilized differentially by different classes of signal-dependent transcription factors, perhaps altering factor-specific acetyltransferase requirements (Woloshin et al. 1995; Puri et al. 1997; Korzus et al. 1998; Xu et al. 1998), consistent with the idea that HAT activity is not the only functional property of CBP/p300 and P/CAF.

TRAP/DRIP/ARC

In a pioneering study, epitope-tagged TR expressed in a permanent cell line was used to affinity purify TR-associated proteins (TRAPs) (Fondell et al. 1996a). Several proteins were copurified in an apparent stoichiometric ratio, suggesting the presence of a complex (Fig. 3). This putative TRAP complex enhanced in vitro transcriptional activation of TR in a chromatin-free system (Fondell et al. 1996b, 1999). A very similar complex of vitamin D receptor (VDR) interacting proteins (DRIPs) was isolated, using VDR (Rachez et al. 1998). Purified DRIP complex substantially potentiated ligand-dependent transactivation function of VDR on a chromatinized template in vitro (Rachez et al. 1999). In each complex,

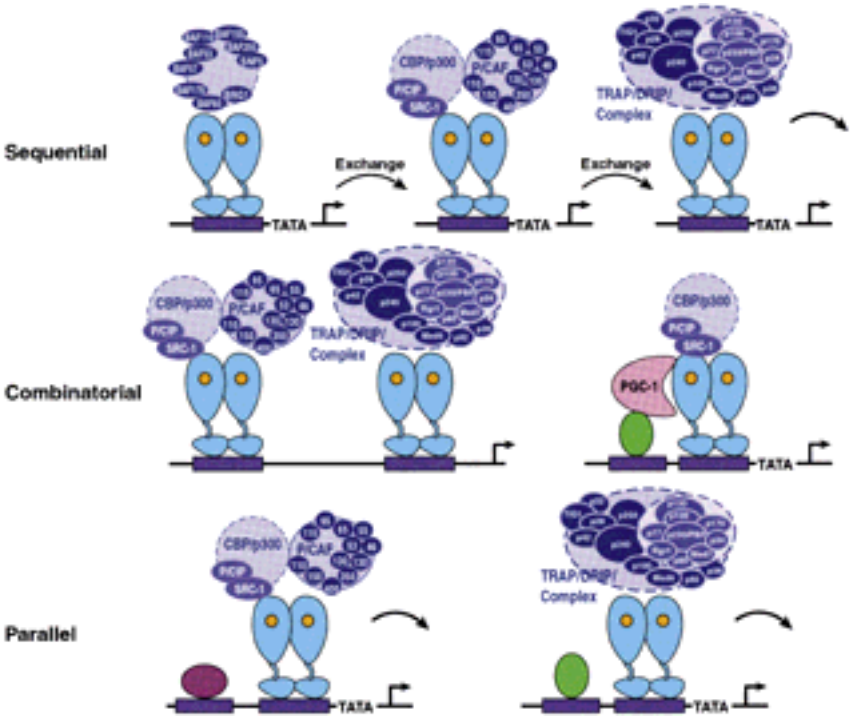

Figure 3. Utilization of multiple coactivator complexes. In the first model, one complex is recruited to carry out acetyltransferase reactions and recruit additional proteins. Upon completion of these functions, this complex leaves the promoter and is replaced by a second complex, that performs a distinct set of steps required for recruitment of core factors and transactivation. Alternatively, an overlap of combinatorial utilization of multiple coactivator complexes may be required for physiologic levels of expression on specific promoters. For example, the CBP/SRC-1/pCAF and TRAP/GRIP/ARC complexes may synergize on a subset of promoters, whereas on another set of promoters the utilization of gene or cell-specific coactivators such as PGC-1 may be required. Finally, activation of the same gene by different coactivator complexes, permitting different responses to specific signaling pathways.

at least nine proteins, ranging from ~70 kD to ~230 kD, were noted. Surprisingly, several constituents of the TRAP/DRIP/ARC (activator-recruited cofactor) complex are similar if not identical to protein components of the recently identified CRSP, NAT, and SMCC complexes (Fig. 3) (Hampsey and Reinberg 1999). These complexes, which were isolated independently, have been found to be required for in vitro transcriptional activation from chromatin templates by a number of other transcription factors, including SREBP, NFκB, and VP16 (Sun et al. 1998; Gu et al. 1999; Ito et al. 1999; Naar et al. 1999; Ryu et al. 1999). Thus, TRAP/DRIP/ARC is a large composite coactivator that belongs to a family of related cofactors and is targeted by different classes of activator to mediate transcriptional stimulation.

Although the TRAP/DRIP/ARC complex has stimulating activity on chromatinized templates (Rachez et al. 1999) it does not contain intrinsic HAT activity (Yuan et al. 1998). Of note is that many components of this complex are also present in a mammalian complex corresponding to the yeast mediator (Gu et al. 1999). The striking similarity in a number of components of the mediator complex and the DRIP/TRAP/ARC complex raises interesting issues of the level at which this complex functions. The TRAP/DRIP/ARC complex has subsequently been shown to contain a factor (TRAP 220/DRIP 205/TRIP2/mPIP1) identical to a PPARγ-interacting protein, PBP (Lee et al. 1995a; Zhu et al. 1997; Rachez et al. 1998; Yuan et al. 1998). This factor appears to mediate the interaction of the TRAP/DRIP/ARC complexes with NRs in response to activating ligands (Yuan et al. 1998; Treuter et al. 1999).

Sequential and combinatorial actions of coactivator complexes

The extraordinary number of factors that appear to be involved in transcriptional activation by NRs represents a formidable challenge to understanding how so many different proteins cooperate in gene activation. For example: are the chromatin remodeling factors recruited to specific promoters? Why are there multiple complexes with HAT activity? Is there a temporal order to the recruitment of CBP/SRC and TRAP/DRIP/ARC complexes to ligand-activated receptors, or do these complexes define parallel pathways for transcriptional activation? These alternative possibilities are schematically indicated in Fig. 3.

Kinetically, transcription may be viewed as a multistep procedure, a derepression process followed by transcriptional initiation. The former refers to relief of the repression imposed by high order chromatin structure; and the latter is assembly of the core RNA polII machinery and the initiation of transcription. Recent studies of mechanisms regulating transcription of the *HO* gene in yeast may providing insights into temporal aspects of gene activation by NRs. *HO* regulates mating type switching and is subject to complex combinatorial control. During budding, *HO* is transiently expressed in late G1 of the cell cycle of the mother cell but at no stage of the daughter cell (actually a small percentage of cells can express *HO*). Transcription of *HO* is regulated by factors binding to *URS-1* and *URS-2* (cell cycle-dependent regulatory regions) (Fig. 4B). Using the chromatin immuno precipitaion (CHIP) assay in a series of genetic back-

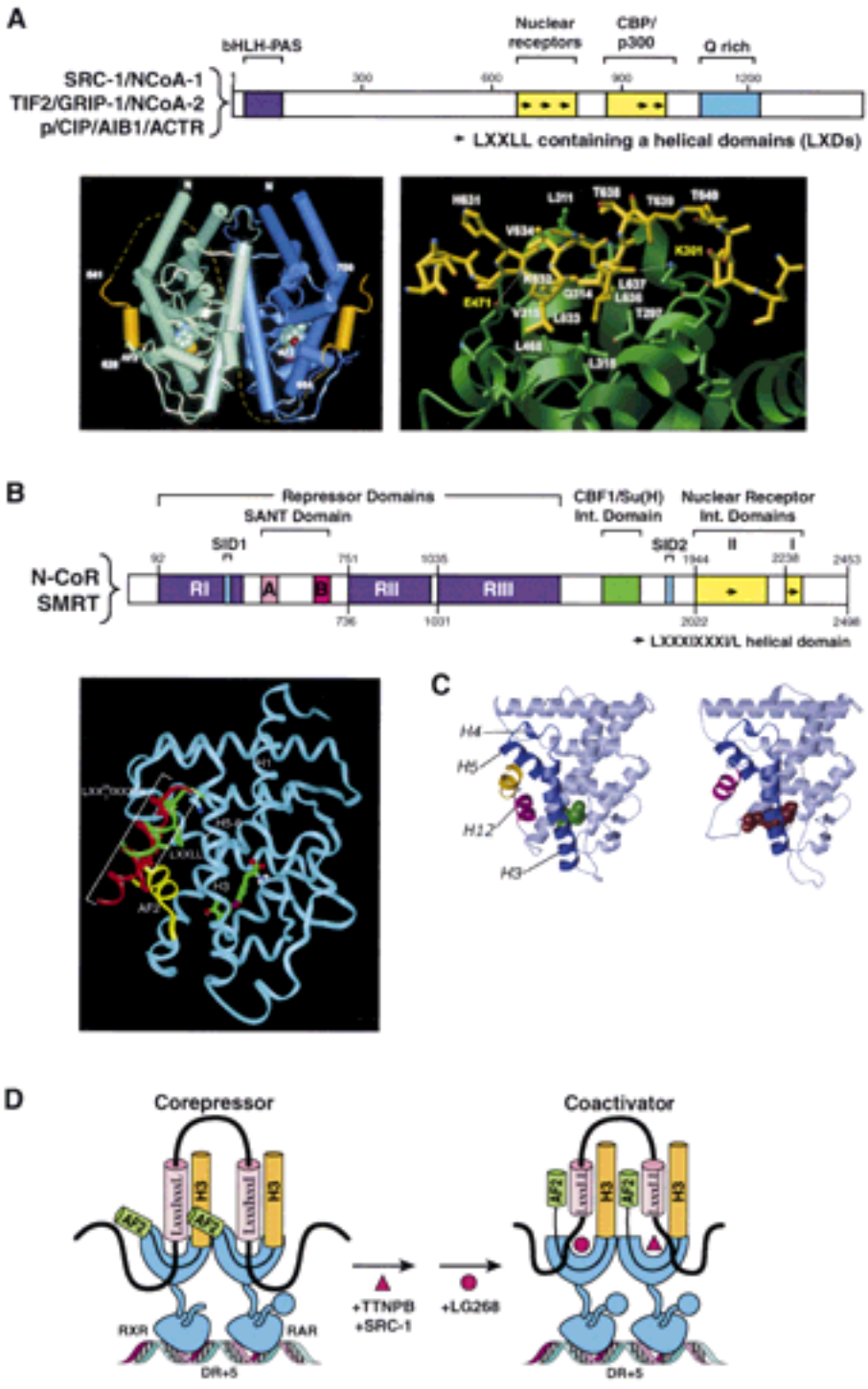

Figure 4. Coactivator and corepressor receptor interaction motifs. (*A*) Domain structures of members of the SRC-1/p160 family. LXXLL motifs within the NR and CBP-interaction domains are indicated by asterisks. (*Left*) Crystal structure of the ternary complex of a PPARγ LBD dimer (blue and cyan) in association with the activating ligand rosiglitazone and a 66-amino acid peptide (yellow) containing LXXLL motifs 1 and 2 (cylinder) from SRC-1. The dotted line represents a relatively disordered linker region of the peptide. The structure illustrates the ability of two LXXLL motifs from the same SRC-1 molecule to dock to each component of the dimer. (*Right*) Interactions of the LXXLL motif with the PPARγ ligand binding domain. The LXXLL helix is presented as a stick model, with the PPARγ LBD shown as a ribbon diagram. Glutamine 471 in the AF-2 domain of PPARγ and lysine 301 in helix 3 form a charge-clamp that grips the ends of the LXXLL helix. The leucine residues of the LXXLL motif packed within a hydrophobic cavity between the charge clamp (Adapted from Nolte et al. 1998 with permission). (*B*) Domain structures of NCoR and SMRT. carboxy-terminal domains I and II mediate NR interactions. RI, RII, and RIII represent domains that harbor intrinsic repression activity when tethered to the DNA binding domain of GAL4. The two interaction domains harbor an extended LXX IXX I/L helix, which binds to the hydrophobic coactivator pocket without need for the change-clamp. (*C*) (*Left*) Structure of the ER LBD in the presence of agonist diethylstilbestrol (DES) or antagonist and an LXXLL peptide derived from GRIP-1 (Le Douarin et al. 1995). DES is buried deep within a central cavity and makes contact with the helix 12 (gold) and contains the AF-2 motif. The LXXLL peptide is bound to a hydrophobic pocket located between helix 12 and the end of helix 3. (*Right*) With the ER LBD bound to OHT, the overall fold of the LBD is similar, with OHT occupying the same binding site as DES. However, the OHT sidechain alters the position of helix 12, such that it occludes the LXXLL binding pocket. (Adapted from Shiau, et al. 1998, with permission. *D* Model for the exchange of a N-CoR with SRC-1/CBP coactivator complex. DNA-bound NRs form ligand-dependent interactions with N-CoR via ligand-independent interactions with two LXXX IXXX I/L motifs. Ligand-dependent binding of coactivator factors depends on recruitment via the LXXLL helix.

grounds, the zinc finger transcription factor Swi5p was found, after transport to the nucleus, to bind to the *URS-1* of the *HO* promoter at the end of anaphase (Cosma et al. 1999). This factor then recruited the SWI/SNF complex, with subsequent recruitment of the SAGA complex, which facilitated binding of the transcription factor SBF (Swi 4/6). Intriguingly, Swi5p remains at the *HO* promoter only briefly, ~5 minutes.

However, the persistence of SWI/SNF and SAGA complexes on *HO* was self sustaining, proposed to constitute an "epigenetic memory" of the transient occupation of *HO* by Swi5p. There is a corresponding cell cycle regulation of histone acetylation at the *HO* locus (Krebs et al. 1999). Interestingly, the corepressor Ash1p (Sil and Herskowitz 1996) selectively accumulates in daughter cells and aborts recruitment of SWI/SNF, SAGA and SBF, providing, at least in part, a molecular explanation for the mating-type switch event.

In considering the various activities of complexes involved in NR function, it is conceivable that chromatin remodeling complexes and complexes containing HAT activities are recruited to the promoter initially. These factors may relieve the repressive actions of chromatin, analogous to the SWI/SNF and SAGA complexes, and set the stage for a second chromatin-dependent step of gene activation requiring factors with HAT activity, HMG proteins, and other factors. Finally, these factors may require the actions of additional complexes that include TRAP/DRIP/ARC. Indeed, a release of CBP and p160s late in RAR-dependent promoter activation has been suggested (Chen et al. 1999b), supporting a potential turnover of complexes during or at the conclusion of activation events.

In broad outline, one could propose a model for transcriptional activation by NRs that closely parallel that for transcriptional activation in yeast. It is pertinent to note, however, that NRs, CBP/p300, and SRC family members are not present in yeast. We suggest that during metazoan development, the developmental and physiologic demands of multicellular organisms required the evolution of other classes of specialized transcription factors and corresponding coactivator complexes. This large number of complexes allows for a combinatorial overlay on the sequential events required for gene activation, in which different complexes are essential for appropriate spatial and temporal patterns of gene expression.

Cell and promoter-specific coactivators

Recent studies suggest that cell-specific coactivators may also play critical roles in gene-specific transcriptional activation. For example, peroxisome proliferator-activated receptor γ (PPARγ) can activate transcription of the uncoupling protein 1 (*UCP-1*) gene in brown fat but not in fibroblasts. Investigation of the basis for this specificity led to the identification of PGC-1 (PPARγ coactivator-1) (Puigserver et al. 1998), which is expressed specifically in brown fat and skeletal muscle and is markedly upregulated by exposure to cold. PGC-1 binds to PPARγ and enables it to activate the *UCP-1* gene in fibroblasts. Although these findings suggest that PGC-1 is a cell-specific coactivator necessary for activation of *UCP-1* and perhaps other genes involved in adaptive thermogenesis, the molecular basis for the requirement of PGC-1 in activation of the *UCP-1* gene is unknown, nor is the basis of its promoter-specificity. As SRC-1 and CBP are also required for activation of this gene by PPARγ, a critical question is why PGC-1 serves promoter-specific activator functions (Fig. 4A).

A number of coactivators have been identified that exhibit relative preferences for a subset of NRs. For example, ARA70 (androgen receptor coactivator 7) is reported to enhance androgen receptor function in transfected cell lines (Yeh and Chang 1996; Miyamoto et al. 1998). Another particularly interesting example is SRA, which activates the AF-1 function of steroid hormone receptors (Lanz et al. 1999). Although identified in a yeast two-hybrid screen, this factor has proven to function as an RNA molecule. SRA can interact and synergize with SRC1 in activation of specific steroid hormone receptors.

Transrepression by NRs

Activation of a NR could alter the expression of a component of a signal transudation pathway that exerts a negative effect. The GR has been demonstrated to inhibit NFκB function in lymphocytes, in part, by upregulating the expression of the inhibitory factor IκBβ (Auphan et al. 1995). Many cases of transrepression are reciprocal, however. One of the best-documented examples of mutual transrepression is the reciprocal antagonistic effect of NRs and AP-1 factors, initially documented for the GR (Fig. 2D) (Jonat et al. 1990) and confirmed for other receptors (Yang-Yen et al. 1990; Schule et al. 1991). Activation of the GR blunts the response of an AP-1-dependent promoter to phorbol ester or Ras stimulation. Conversely, activation of AP-1 inhibits transcriptional responses of GR-dependent genes to glucocorticoids. Intriguingly, transcription units that harbor adjacent DNA binding sites for both factors in promoters/enhancers often exhibit synergy rather than transrepression.

Several lines of evidence suggest that competition for limiting coactivator proteins in the nucleus accounts for at least some aspects of transrepression (Kamei et al. 1996). CBP and p300 appear in the available assays to in some way be limiting and quantitatively important in actions of the NRs and many other signal-dependent transcription factors. Overexpression of CBP or p300 has been shown to alleviate mutual transrepression of NRs and AP-1 in cell-based assays (Kamei et al. 1996), although there may be other limiting components. Genetic evidence further suggests the hypothesis that CBP and p300 are functionally limiting (Tanaka et al. 1997). Animals heterozygous for null alleles of either CBP or p300 exhibit haploinsufficiency phenotypes and compound heterozygotes die in utero (Tanaka et al. 1997; Yao et al. 1998). In contrast to direct activation of target genes, which requires hundreds to thousands of receptors per cell, transrepression is most often observed in situations in which the receptor number is much higher, consistent with the idea that this mechanism of transcriptional inhibition might in some cases reflect sequestration of coactivators, perhaps locally.

The role of transrepression is documented most rigorously by elegant gene replacement experiments in vivo,

which demonstrate that the transrepression activity of the GR appears to account for a significant component of its in vivo biological function. Mice in which the GR has been knocked out die prematurely as a result of a failure of lung maturation (Cole et al. 1995). In contrast, when a point mutation is introduced into the GR DNA-binding domain that abolishes high-affinity recognition of specific glucocorticoid response elements, but did not eliminate transrepression, the animals survived (Reichardt et al. 1998). Thus, the defect in lung maturation was rescued by a GR that is unable to activate direct target genes but retains the ability to transrepress.

Active repression by NRs

The ability of the TR and RARs to inhibit gene expression in the absence or in the presence of ligand raises a series of intriguing issues. In some cases, repressive effects may be do to passive inhibition, which can occur due to competition for DNA sites with other, stronger transactivators or formation of heterodimer pairs that are transcriptionally inactive. In addition, many NRs have been demonstrated to inhibit gene expression in a ligand-dependent manner by the previously described phenomenon of transrepression, which in part involves competition for limiting coactivator complexes (Fig. 1). Most consideration has been given to the molecular mechanisms by which unliganded or antagonist-bound NRs mediate active repression on binding to response elements in target genes, or agonist-dependent repression on negative response elements.

Because maximal repression by unliganded TR required domains not involved in inhibitory TFIIB interactions (Baniahmad et al. 1993; Sauer et al. 1995) a search for additional proteins that mediated these effects led to identification of a factor of ~270 kD that associated with unliganded TR and RARs. This led to cloning of N-CoR (NR corepressor) (Hörlein et al. 1995; Kurokawa et al. 1995), a portion of which had been isolated previously in a yeast two-hybrid screen as RIP13 (Lee et al. 1995b), and the highly related factor, SMRT (silencing mediator for retinoic acid and thyroid hormone receptors) (Chen and Evans 1995), or TRAC2 (T3 associated cofactor) (Sande and Privalsky 1996), both ~270 kDa (Ordentlich et al. 1999; Park et al. 1999) (Fig. 5B). Both N-CoR and SMRT contain a conserved bipartite NR interaction domain (Seol et al. 1996; Zamir et al. 1996; Li et al. 1997b). Ligand binding causes decreased interaction of N-CoR to TR and RAR on most DNA sites in both biochemical assays (Horlein et al. 1995; Kurokawa et al. 1995), and intact cells (Perissi et al. 1999b). N-CoR also interacts with, and serves as a corepressor for Rev ERB (Zamir et al. 1996), COUP transcription factors (Shibata et al. 1997), and DAX1 (Crawford et al. 1998). N-CoR has also been proposed to serve as a coactivator on some promoters harboring "negative" T3 repressor elements, such as TSHβ (Tagami et al. 1997), but there is no rigorous evidence for this model.

Both N-CoR and SMRT contain a highly related amino-terminal region and at least three independent repressor domains that are capable of transferring active repression to a heterologous DNA binding domain (Chen and Evans 1995; Horlein et al. 1995). N-CoR and SMRT interact with mammalian homologes of proteins that have been defined genetically in yeast to mediate transcriptional repression (guilt by association). Thyroid hormone-resistance syndromes can be correlated with mutations in the LBD of TR β that enhance ligand-independent interactions anti N-CoR/SMRT (Yoh et al. 1997). Nuclear microinjection of anti-N-CoR antibodies blocks active repression by the TR in cells (Heinzel et al. 1997). Intriguingly, the recruitment of NCoR or SMRT appears to be essential for the antagonist activity of ER and PR antagonists (Jackson et al. 1997; Smith et al. 1997; Lavinsky et al. 1998), potentially by blocking function of the constitutive AF-1 transactivation domain.

There are a few well-documented examples in which the transcriptional responses of the TR to ligand appear to be reversed, that is, the apo receptor activates transcription whereas addition of ligand results in repression. For example, thyroid hormone represses transcription from the thyroid stimulating hormone β (TSHβ) promoter and this effect appears to involve TR binding sites (Darling et al. 1989). Similarly, a thyroid hormone response element (TRE) in the Rous sarcoma virus (RSV) promoter is activated by unliganded TR and this effect is reversed by thyroid hormone (Saatcioglu et al. 1993). In the context of the TSHβ promoter, it has been suggested that the binding of N-CoR to the TR results in transactivation rather than repression (Tagami et al. 1997). Initial insights into the molecular mechanisms for such a switch in ligand-dependent transcriptional function has been obtained by evidence of recruitment of in mSin3 and HDAC2 onto the liganded receptor on a negative TRE, perhaps reflecting binding of receptor homodimers, rather than RAR–RXR heterodimers, on this site (Sasaki et al. 1999).

The potential roles of N-CoR and SMRT as corepressors have been extended to numerous factors unrelated to NRs, suggesting that they play more general roles in the regulation of gene expression. N-CoR has been shown to be essential for transcriptional repression by Mad, a HLH factor that forms heterodimers with Max (Heinzel et al. 1997). SMRT has recently been found to interact with CBF-1/RBP-JK, a mammalian homolog of *suppressor of hairless* (Kao et al. 1998). CBF-1/RBP-JK is a repressor that is converted to an activator by binding to a fragment of the cytoplasmic domain of Notch (Kao et al. 1998). The repressor activity of CBF-1/RBP-JK has recently been suggested to depend on interaction with SMRT, with conversion of CBF-1/RBP-JK from a repressor to an activator postulated to result from displacement of SMRT by the cytoplasmic domain of Notch. N-CoR and SMRT also associate with homeodomain repressors such as Rpx and can bind to other homeodomain factors, such as Pit-1, modulating its activity (Xu et al. 1998). Thus, N-CoR/SMRT may play important roles in the regulation of homeodomain repressor function.

Indeed, in concert with the observation that active repression of gene expression by sequence-specific tran-

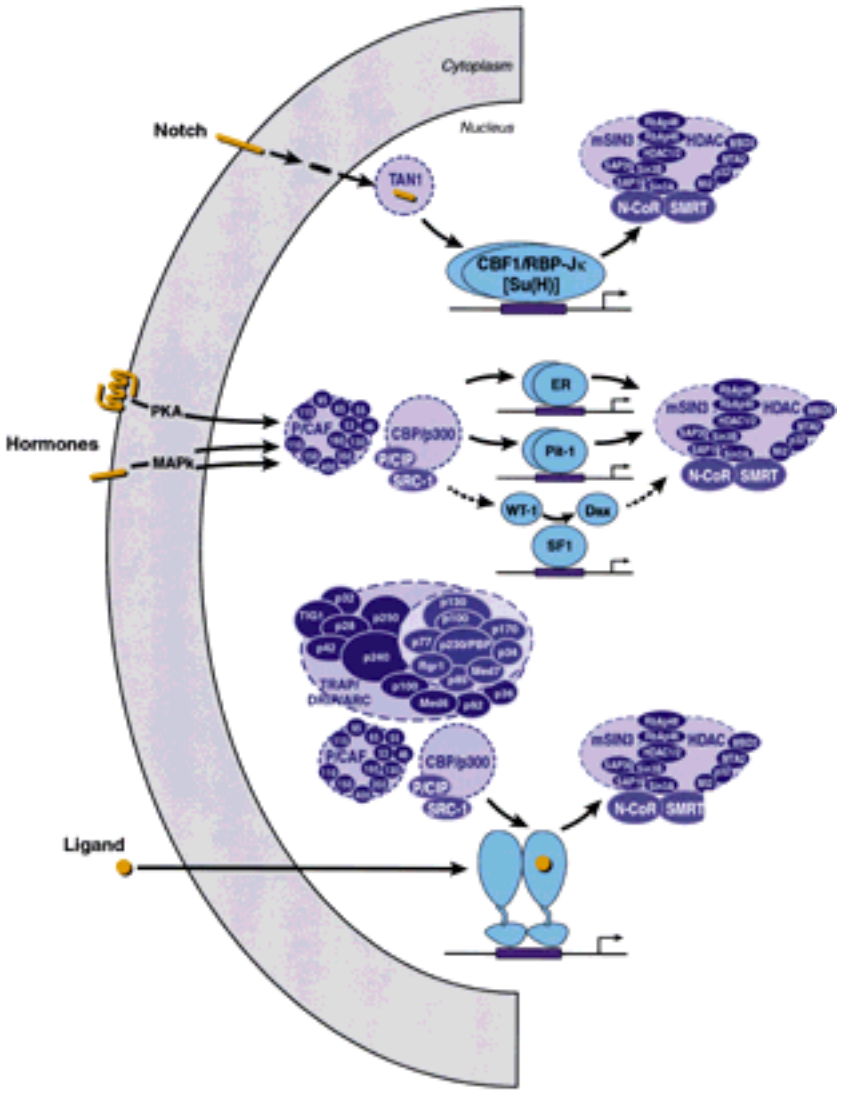

Figure 5. Integration of nuclear signaling events by coactivator and corepressor complexes. At the bottom of the figure, ligands are illustrated to regulate the association of coactivator and corepressor complexes with ligand-dependent nuclear receptors. The interactions of these complexes with nuclear receptors may also be regulated by other SRC-1 signal transduction systems, such as protein A and MAP kinase-dependent signaling events, which may target either the coactivator or corepressor complexes themselves, or the nuclear receptor. This is demonstrated in the case of ERα, ERβ and a similar idea is the switch of WT-1 and Dax-1.

scription factors plays critical roles in the regulation of diverse biological processes, including cell proliferation, development, and homeostasis, there appears to be multiple mediators of active repression. In *Drosophila*, for example, a series of sequence-specific repressors are essential for establishing spatially defined patterns of cell-specific gene expression, referred to as long range or short range repressors, based on the distance over which they exert their effects (Gray and Levine 1996; Sauer et al. 1996; Mannervik et al. 1999). A critical question is whether or not the cofactors mediating these two types of repression are distinct. Several suggestions as to the mechanisms responsible for long and short-range repression have been offered. Based on the identification of Groucho as a repressor for the bHLH factor Hairy required for neurogenesis and segmentation, physical interaction could be demonstrated for many of the *Drosophila* repressors (Aronson et al. 1997; Lee and Young 1998), most of which have been established as long-range repressors (Cai et al. 1996). Because of homology to the yeast repressor, TUP1, which has been postulated to inhibit RNA pol II complex function by nucleosomal positioning over the core promoter (Herschbach et al. 1994; Edmondson et al. 1996; Treitel et al. 1998), a link between transcription factors mediating repression and the recruitment of a complex that can function at core, can be suggested.

In addition, regulatory control of Groucho-mediated repression has been suggested in *Drosophila*. The DNA-binding function of dTCF has been shown to be a distal component of the Wingless signaling pathway (Siegfried and Perrimon 1994; Cox and Peifer 1998). dTCF binds Groucho in the absence of the Wg signal, which is displaced upon nuclear translocation of β-catenin (Cavallo et al. 1997, 1998; Chawla et al. 1998). CBP acetylates the amino terminus of dTCF, which is required for target gene activation, and permits the binding of β-catenin (Waltzer and Bienz 1998). A second class of repressors was identified based on binding the E1A carboxyl terminus (Schaeper et al. 1995) referred to as dCtBP (Nibu et al. 1998a,b; Poortinga et al. 1998). Because the patterning defects in dCtBP mutant embryos, resemble those of

Snail and Knirps (Nibu et al. 1998a), which have been described as short-range repressors, dCtBP may interact with a series of *Drosophila* repressors that exert roles as mediators of short-range repression.

Recruitment of Sin3–histone deacetylase complexes

Critical insights into the potential mechanisms of transcriptional repression by NRs were provided by the discovery of mammalian homologs of yeast Sin3 (Ayer et al. 1995; Schreiber-Agus et al. 1995) and the subsequent finding that these proteins interact with N-CoR and SMRT (Nagy et al. 1987; Alland et al. 1997; Heinzel et al. 1997). The yeast and mammalian Sin3 proteins are components of corepressor complexes that also contain histone deacetylases (RPD3 in yeast or HDAC1/HDAC2 in mammals) (Vidal et al. 1991; Taunton et al. 1996; Alland et al. 1997; Hassig et al. 1997; Heinzel et al. 1997; Kadosh and Struhl 1997; Laherty et al. 1997; Nagy et al. 1997; Zhang et al. 1997). These findings therefore suggested a remarkable biochemical and enzymatic symmetry to the transition between repressed and activated states of transcription, in which the recruitment of complexes with HAT activity provides a critical step in the process of transcriptional activation, whereas the recruitment of complexes with HDAE activity provides a critical step in active repression. In this regard, complexes with HAT activity and HDAC activity are conserved from yeast to mammals and appear to play general roles in the control of transcriptional activation. However, like NRs and the SRC-1 family of coactivators, proteins homologous to N-CoR and SMRT are not encoded by the yeast genome. These proteins therefore appear to have arisen during the evolution of metazoan organisms to allow factors such as unliganded NRs and Mad proteins (Alland et al. 1997; Heinzel et al. 1997; Laherty et al. 1997) to mediate transcriptional repression via the Sin3–HDAC pathway. Recently, distinct corepressor domains of N-CoR have been demonstrated to directly interact with other distinct HDAC factors, including HDAC4, HDAC5, and HDAC7, implying a redundant or combinatorial deacetylase-dependent code of repression (Huang et al. 2000; Kao et al. 2000).

Immunohistochemical and biochemical studies suggest that N-CoR and SMRT are not stable stoichiometric components of Sin3 corepressor complexes (Soderstrom et al. 1997; Zhang et al. 1998b). Purification of a murine Sin3 complex resulted in copurification of the histone binding protein RbAp 46, RbAp 48, HDAC1, HDAC2, and two small proteins SAP30 and SAP18 (Zhang et al. 1997; Laherty et al. 1998). Intriguingly, with the isolation of full-length SMRT (Ordentlich et al. 1999; Park et al. 1999), it is now clear that they are likely to share all functional domains, including an amino-terminal domain sequence that interacts with the mammalian homolog of Seven-in-absentia (Siah); this region of N-CoR imparts a decreased half life, and this links its regulation to the 26S proteosome/ubiquitination pathway (Zhang et al. 1998c).

As in the case of coactivators, there are numerous other potential corepressors. Thus, the TR uncoupling protein (Burris et al. 1995) and Sun-CoR (Zamir et al. 1997) may serve specific roles. In addition, the TIF-1 factors (Le Douarin et al. 1996; Moosmann et al. 1996) that interact with NRs via LXXLL (see below) motifs can bind to the Mod-1/Mod-2 factors (Le Douarin et al. 1996), which bind to SNF2-β. Recently, an intrinsic protein kinase function has been suggested for TIF1-α (Fraser et al. 1998). The most perplexing aspect is the biochemical mechanism that allows the assembly of N-CoR/SMRT/HDAC and Sin3 complexes, and potentially other classes of corepressors. Indeed, many DNA-binding transcription factors may bind either a Groucho ortholog, CtBP, or N-CoR/SMRT. Furthermore DNA sequences may recruit corepressors and adjudicate the strength of corepressor action. SAP30, which was isolated independently in a yeast two-hybrid screen with mSin3 (Laherty et al. 1998), is required for a subset of repression events and is conserved from yeast to man. The absence of N-CoR and SMRT as components of these complexes suggests that they either interact only transiently with mSin3 in cells, or require the recruitment of additional factors to become stable components of these complexes.

Determinants of coactivator and corepressor binding

Inspection of the amino acid sequences of the NR interaction domains of p160 factors and RIP140 revealed the presence of leucine-rich motifs of the consensus sequence LXXLL, where L represents leucine and X any amino acid (Fig. 4A). These motifs were also found to be present in a number of other proteins demonstrated to interact with NRs in a ligand-dependent manner. The LXXLL sequence and a short stretch of amino- and carboxy-terminal amino acids is both necessary and sufficient for ligand-dependent interactions of p160 proteins with NR LBDs (Le Douarin et al. 1995; Heery et al. 1997; Torchia et al. 1997; Ding et al. 1998; Voegel et al. 1998). The concept that LXXLL motifs represent a general structure for NR recognition is supported by the observation that nearly all factors that have been cloned by virtue of their ability to interact with NRs in a ligand-dependent manner contain one or more copies of this motif. Additional related motifs are found in the CBP/p300 nuclear receptor interaction domain.

Solving the crystal structures of unliganded and agonist-bound LBDs for several NRs has confirmed the hypothesis that the AF-2 region undergoes a ligand-dependent conformational change (for review, see Moras and Gronemeyer 1998) and permitted evaluation of coactivator and corepressor binding. In the unliganded RXR structure, the AF-2 helix extends away from the ligand-binding domain (Bourguet et al. 1995). In contrast, in the agonist-bound RARγ, TRα, PPARγ, and ER LBD structures, the AF-2 helix is tightly packed against the body of the ligand binding domain and makes direct contacts with ligand (Renaud et al. 1995; Wagner et al. 1995; Brzozowski et al. 1997; Shiau et al. 1998) (Fig. 4A,4C). Taken together, these studies are consistent with the

idea that ligand-dependent changes in the conformation of the AF-2 helix result in the formation of a surface that facilitates coactivator interactions. Intriguingly, the structures of the ER LBD bound to the antagonists raloxifene or dihydroxytamoxifen (OHT) demonstrate a distortion in the position of the AF2 helix (Brzozowski et al. 1997; Shiau et al. 1998) (Fig. 4C). Due to the presence of an additional side chain in these antagonists, the AF-2 helix is unable to pack normally and instead is translated to a position that overlaps with the site of coactivator interaction. These findings suggest that an important aspect of antagonist action is to place the AF-2 helix in a configuration that prevents coactivator binding.

Recent crystal structures indeed demonstrate that the LXXLL motif forms a short α helix (Fig. 4A) (Darimont et al. 1998; Nolte et al. 1998; Shiau et al. 1998). This helix docks to a hydrophobic cleft on the surface of the LBD that is bounded on one side by the AF-2 helix and on the other by the end of helix three. A highly conserved glutamate residue in the AF-2 domain makes contacts with the amino-terminal end of the LXXLL peptide backbone, while the carboxy-terminal end of the LXXLL helix is held by a conserved lysine residue in helix three. Together, the glutamate and lysine residues form a charge clamp that positions the LXXLL helix to allow the leucine side chains to pack into the intervening hydrophobic cavity (Fig. 4A). These structures suggest that the structural basis of ligand-dependent activation is the closure of the AF-2 helix to form the charge clamp. A critical determinant of coactivator interaction is that the charge clamp grips a helix of the specific length specified by the LXXLL motif and capping end-terminal residues. Although the sequence encompassing the LXXLL motifs is sufficient for NR-coactivator interactions, amino acids amino- and carboxyl terminal to the LXXLL motif appear to make additional contacts with the LBD (Darimont et al. 1998; McInerney et al. 1998). These residues are not conserved among different coactivators and may play roles in determining the specificity of NR-coactivator interaction, possibly specifying which coactivators will bind to a particular NR dimer or heterodimer with highest affinity. These interactions are potentially influenced by structurally distinct physiologic ligands for a particular NR that might induce distinct conformations (McInerney et al. 1998).

Biochemical experiments suggest that two LXXLL motifs from a single p160 protein can interact cooperatively with both subunits of an RAR/RXR or RXR/PPAR heterodimer (Westin et al. 1998). Similarly, a single molecule of SRC-1 containing three LXXLL motifs binds in a highly cooperative manner to the ER LBD (Gee et al. 1999). These findings are supported by the solution of the crystal structure of a dimer of the PPARγ LBD complexed to an SRC-1 peptide containing two LXXLL motifs (Nolte et al. 1998). In this structure, the LXXLL helices interact equivalently with the coactivator binding pockets of both members of the dimer (Fig. 5A). It is also possible that the presence of multiple LXXLL helices within a single coactivator molecule allows for flexibility in complex assembly and/or allows cooperative interactions with adjacent bound transcription factors. Although the amino-terminus of CBP and p300 contains an LXXLL motif and can interact with several NRs in a ligand-dependent manner, effective recruitment of CBP requires additional coactivators, such as p160 factors in the case of DNA-bound RAR/RXR heterodimers (Fig. 4D) (Westin et al. 1998).

Interactions between coactivators and NRs also have recently been suggested to explain the differential responses of permissive and nonpermissive RXR heterodimers to activating ligands. Nonpermissive RXR heterodimers, exemplified by RAR/RXR heterodimers, do not respond to RXR ligands unless the RAR is liganded first (Kurokawa et al. 1994; Forman et al. 1995; Chen et al. 1996). This selective response results from allosteric inhibition of the binding of ligands to RXR that occurs on dimerization and binding to DNA. In contrast, permissive heterodimers, exemplified by PPAR/RXR heterodimers, can be activated independently by RXR or PPAR ligands (Kliewer et al. 1992). Intriguingly, in the case of RAR/RXR heterodimers, an RXR-specific ligand could potentiate only the binding of SRC-1/NCoA-1 in the presence of an RAR-specific ligand (Westin et al. 1998). Molecular modeling of the RAR/RXR heterodimer suggested that in the absence of ligand the AF-2 domain of RXR interact with the LXXLL-binding pocket of RAR. This interaction does not occur when RXR heterodimerizes with a permissive partner, such as PPARγ. As the AF-2 domain of NRs forms part of the ligand-binding pocket, its conformation in the context of an unliganded RAR/RXR heterodimer would be predicted to prevent high-affinity binding of RXR ligands and may also enhance the binding of the corepressors NCoR and SMRT. Recruitment of SRC-1 to RAR in response to RAR ligand is proposed to displace the RXR AF-2 domain, enabling RXR to bind to its own specific ligand and interact with a second LXXLL motif of the same SRC-1 molecule. This model provides an explanation for the requirement by the RAR/RXR heterodimer for an RAR-specific ligand for activation, but which can only then be potentiated by an RXR specific ligand (Westin et al. 1998).

In the case of corepressors, the two interaction domains (Chen and Evans 1995; Horlein et al. 1995; Zamir et al. 1997; Hu and Lazar 1999) have proven to contain a conserved sequence (Perissi et al. 1997b; Nagy et al. 1999; Zhu et al. 1999), referred to as the CoRNR box (Zhu et al. 1999) or as a LXXI/HIXXXI/L helix (Perissi et al. 1999b). These reported motifs are predicted to represent extended helices that are required and sufficient to permit binding to unliganded TR and RARs. Based on mapping of the critical receptor residues, this helix appears to bind in the hydrophobic pocket that is occupied by the coactivator LXXLL helical motifs on addition of ligand, but does not depend on the charge clamp formed by the AF-2 helix and helix 3. Because the ligand-activated charge clamp is specific for the length of the coactivator helix, closure on ligand binding would inhibit the binding of corepressor, potentially representing the mo-

lecular mechanism for ligand-dependent displacement of the corepressor complex. In this model, Ile at position 5 of the extended helix is preferred to Leu, and the presence of an LXXLL motif, in the context of the extended helix abolished interaction. Therefore, it is suggested that the receptor AF-2 helix has evolved to discriminate between the LXXLL helix in coactivators and the extended helix in the N-CoR/SMRT corepressors, permitting the ligand-dependent switch of NR activity (Fig. 4D).

Although the binding of either N-CoR or SMRT to TR and RARs is robust, it is enhanced either by addition of receptor antagonists or the deletion of the AF-2 domain (Chen and Evans 1995; Horlein et al. 1995). Furthermore, whereas unliganded steroid hormone receptors do not appear to interact with N-CoR or SMRT, strong interactions are observed in the presence of antagonists (Vegeto et al. 1992; Lanz and Rusconi 1994; Xu et al. 1996; Jackson et al. 1997; Smith et al. 1997; Cohen et al. 1998; Lavinsky et al. 1998; Wagner et al. 1998; Zhang et al. 1998a). In concert with crystal structures of antagonist-bound ERs, these findings indicate that there is an inhibition of N-CoR and SMRT binding by the activation helix of NRs (Fig. 4C). There are receptor-specific differences in the region of the NR interaction domain preferred by each receptors (Lavinsky et al. 1998; Zhang et al. 1998a).

Coregulators as targets of signal transduction pathways

Signal transduction pathways add another layer of regulation to the functions of coactivator and corepressor complexes (Fig. 5). Phosphorylation events may result in increased or decreased affinity between protein factors, leading to changes in components of the complexes. One example is the SWI/SNF complex, the components of which change at different stages of the cell cycle. In addition, its chromatin remodeling activity depends on the phosphorylation state of some of the subunits, such as Brg1 (Suen et al. 1998), suggesting a mechanism linking cell cycle events and transcription. CBP/p300 can be phosphorylated in vivo (Chrivia et al. 1993) and kinase activities are also found to be associated with CBP/p300 (Nakajima et al. 1996). Thus, it will be informative to investigate if HAT activities or components of CBP/p300, N-CoAs, and P/CAF complexes are also regulated by the cell cycle in a similar manner. CBP also contains a signal-regulated transcriptional activation domain that is controlled by nuclear calcium and calcium/calmodulin-dependent (Cam) protein kinase IV and by cAMP (Chawla et al. 1998). It is conceivable that such a scenario may also apply to corepressor complexes.

Signal transduction pathways may also influence acetyltransferase functions, and substrate preferences. An example of this has recently been provided by the POU homeodomain transcription factor, Pit-1. Pit-1 controls critical aspects of pituitary development and is positively regulated by the cAMP- and MAP kinase-dependent signal transduction pathways. Antibody microinjection experiments indicate that Pit-1 function requires CBP/p300 and P/CAF (Xu et al. 1998). Remarkably, stimulation of Pit-1 activity by cAMP requires the HAT activity of CBP, whereas stimulation of Pit-1 activity via the MAP kinase pathway requires the HAT activity of P/CAF.

Post-transcriptional regulation of coactivation and corepressor complexes will prove to be a critical component of regulated gene transcription. Thus, failure of tamoxifen to mediate regulation of ERα is reversed by growth factors and cAMP that appear to involve decreased interaction with N-CoR/SMRT (Lavinsky et al. 1998) with enhanced coactivator and decreased N-CoR binding; this can be attributed to a single or amino-terminal residue of the ER. Similarly, MAPK-induced phosphorylation of specific residues on the AF1 domain of ERβ (Ser-106 and Ser-184) stimulates SRC-1 recruitment (Tremblay et al. 1999) in the presence of partial agonist/antagonist. The orphan receptor SF-1 responds to MAPK-induced phosphorylation of a single residue (Ser-203), recruiting GRIP1 (Hammer et al. 1999). Negative regulation by amino-terminal phosphorylation has been documented for PPARγ (Hu et al. 1996), apparently decreasing ligand affinity (Shao and Lazar 1999).

Conclusion

The molecular strategies that underlie regulated gene transcription by NRs appear to involve the combinatorial actions of a large number of coregulators. Together, they establish an activity continuum ranging from active repression to strong gene activation. The effects of other adjacent DNA-bound transcription factors, the DNA binding site, and the array of coregulators together seem to generate recruitment events that set the level of gene activation of repression. Because each component is under transcriptional and post-transcriptional control, the complexity of the coregulatory network itself is likely to underlie the gene-specificity required to meet the demands of developmental and homeostatic gene regulation. We can expect many insights into the detailed molecular mechanisms underlying these events in the next ten years.

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