Biological roles and mechanistic actions of co-repressor complexes

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Summary

Transcriptional repression, which plays a crucial role in diverse biological processes, is mediated in part by non-DNA-binding co-repressors. The closely related co-repressor proteins N-CoR and SMRT, although originally identified on the basis of their ability to associate with and confer transcriptional repression through nuclear receptors, have been shown to be recruited to many classes of transcription factor and are in fact components of multiple protein complexes containing histone deacetylase proteins. This association with histone deacetylase activity provides an important component of the mechanism that allows DNA-binding proteins interacting with N-CoR or SMRT to repress transcription of specific target genes. Both N-CoR and SMRT are important targets for cell signaling pathways, which influence their expression levels, subcellular localization and association with other proteins. Recently, the biological importance of these proteins has been revealed by studies of genetically engineered mice and human diseases such as acute promyelocytic leukemia (APL) and resistance to thyroid hormone (RTH).

Key words: N-CoR, SMRT, Co-repressor, HDAC

Introduction

The regulation of gene expression by transcriptional control is required for many cellular events and for the proper development of an organism. The essential nature of such control is highlighted by the large number of proteins devoted to regulation of transcription by RNA polymerase II. In eukaryotes, these proteins number in the thousands and include sequence-specific DNA-binding factors, the basal transcriptional machinary, chromatin-remodeling factors, enzymes responsible for covalent modifications of histones and other proteins and cofactors that bridge DNA-binding factors and enzymes. Although activation of transcription has long been recognized as an essential component of gene regulation, the realization that repression of transcription also plays a fundamental role has been appreciated only recently. Repressive factors use several distinct mechanisms, including competition with activator proteins for DNA binding, sequestration of such activators, interaction with the core transcriptional machinery, DNA methylation and recruitment of complexes that have histone deacetylase activity. Here we focus on components of the co-repressor machinery that depend, at least in part, on the actions of histone deacetylases, discussing primarily the nuclear receptor co-repressor (N-CoR) and silencing mediator of retinoic and thyroid hormone receptors (SMRT) (Fig. 1a) (Chen and Evans, 1995; Horlein et al., 1995; Ordentlich et al., 1999; Park et al., 1999).

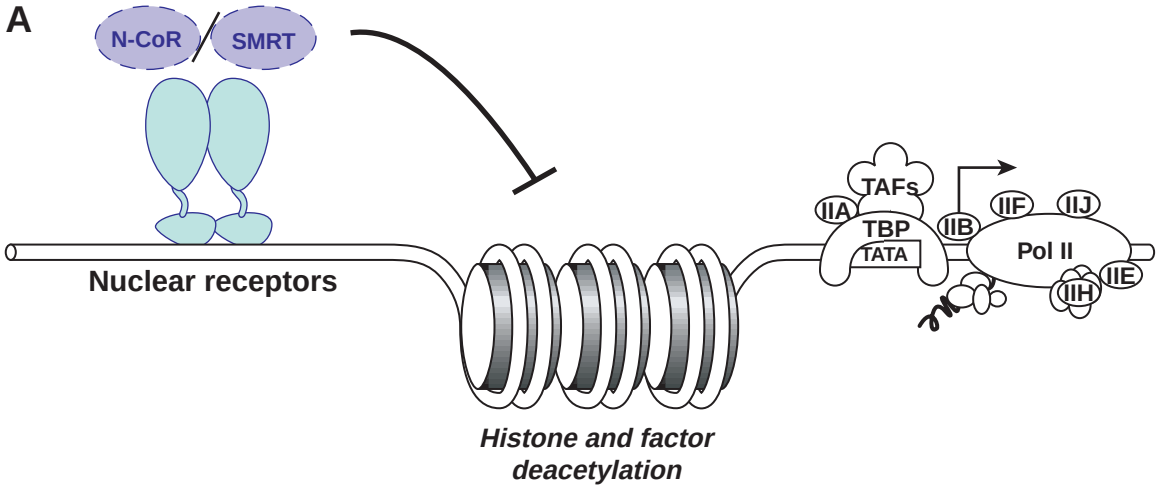
Identification of nuclear receptor co-repressors

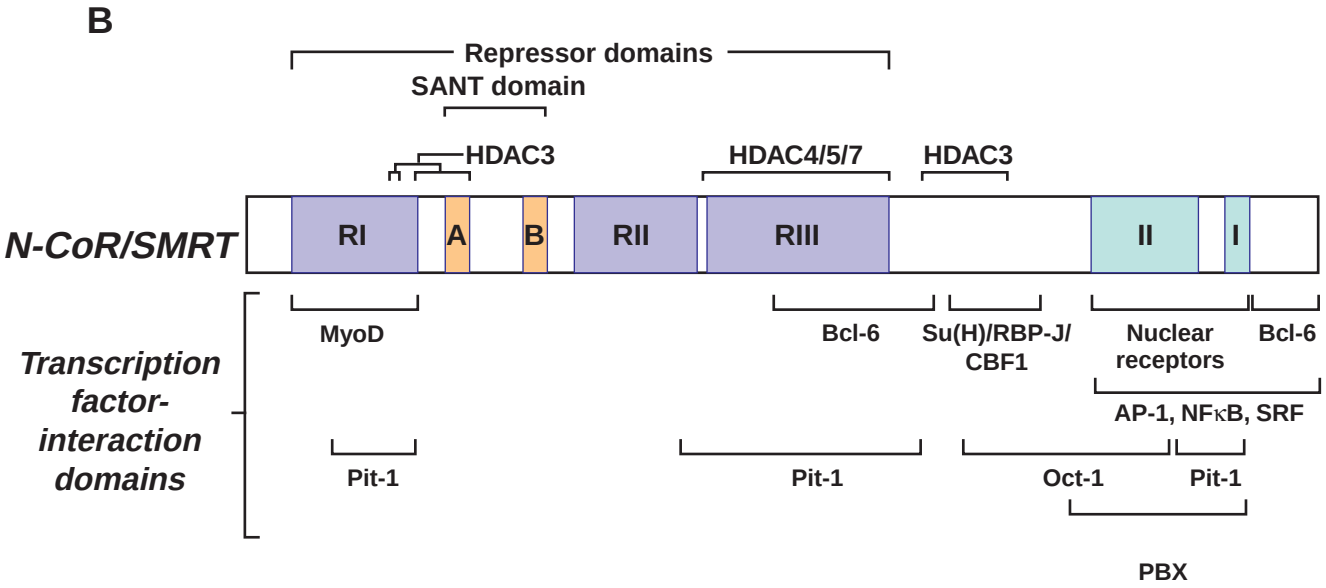
The knowledge that the thyroid hormone and retinoic acid receptors (T_3R and RAR) actively repress transcription in the absence of their cognate ligands through transferable repression domains (Baniahmad et al., 1992) led to the search for factors that might be required for effective gene repression by unliganded nuclear receptors. The identification of a 270 kDa protein associated with unliganded T_3R-RXR heterodimers led to the cloning of N-CoR (Horlein et al., 1995), and a similar approach identified a second, homologous protein SMRT (Chen and Evans, 1995; Ordentlich et al., 1999; Park et al., 1999).

N-CoR and SMRT both contain a conserved bipartite nuclear-receptor-interaction domain (NRID) (Li et al., 1997a; Seol et al., 1996; Zamir et al., 1996) and three independent repressor domains that can actively repress a heterologous DNA-binding domain (Chen and Evans, 1995; Horlein et al., 1995; Ordentlich et al., 1999; Park et al., 1999) (Fig. 1b). Further analysis of the NRIDs revealed that each contains a critical L-X-X-X-I-X-X-X-I/L motif, which includes the L/I-X-X-I/V-I motif termed the CoRNR box (Hu and Lazar, 1999; Nagy et al., 1999; Perissi et al., 1999). This L-X-X-X-I-X-X-X-I/L motif is similar to the L-X-X-L-L recognition motif present in nuclear receptor coactivators (Heery et al., 1997; McInerney et al., 1998) but is predicted to form an extended α-helix one helical turn longer than the coactivator motif. A preference of RAR for SMRT and T_3R for N-CoR (Cohen et al., 2000) is due to specific sequences in the L-X-X-X-I-X-X-X-I/L motif (Hu et al., 2001) as well as to a T_3R-specific interaction domain present in N-CoR but not SMRT (Cohen et al., 2001).

Identification of histone deacetylase proteins

Although co-repressor molecules contributing to the repression of T_3R and RAR had been identified, the mechanism by which N-CoR and SMRT function remained elusive until several groups reported their association with mRpd3 and mSin3A and B, mammalian homologues of the yeast proteins Rpd3p/Histone Deacetylase 1 (msx mit1) and Sin3p (Heinzel


A
N-CoR
SMRT
Nuclear receptors
TAFs
IIA
TBP
TATA
IIB
IIF
IIJ
Pol II
IIE
IIH
Histone and factor deacetylation


B
Repressor domains
SANT domain
HDAC3
HDAC4/5/7
HDAC3
N-CoR/SMRT
RI
A
B
RII
RIII
II
I
Transcription factor-interaction domains
MyoD
Bcl-6
Su(H)/RBP-J/ CBF1
Nuclear receptors
Bcl-6
AP-1, NFκB, SRF
Pit-1
Pit-1
Oct-1
Pit-1
PBX

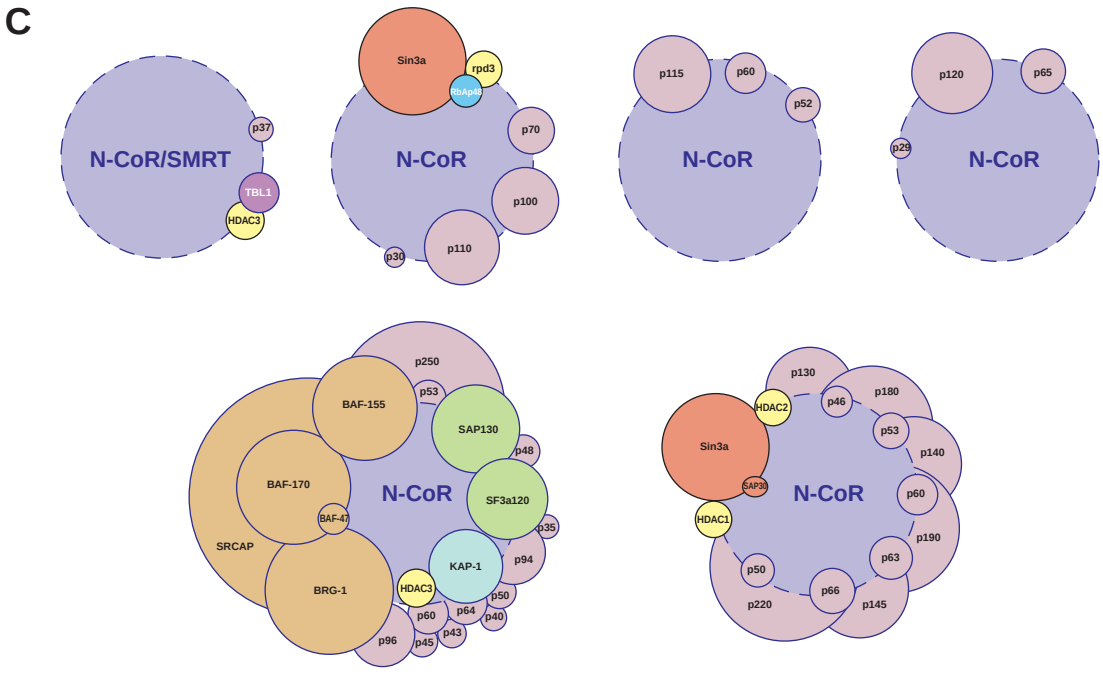

C
N-CoR/SMRT
p37
TBL1
HDAC3
Sin3a
rpd3
RbAp48
N-CoR
p70
p100
p110
p30
p115
p60
p52
N-CoR
p120
p65
p29
N-CoR
p250
BAF-155
p53
SAP130
p48
BAF-170
N-CoR
SF3a120
BAF-47
p35
SRCAP
KAP-1
p94
BRG-1
HDAC3
p50
p60
p64
p40
p96
p45
p43
p130
HDAC2
p46
p180
p53
p140
Sin3a
SAP30
N-CoR
p60
HDAC1
p190
p63
p50
p66
p145
p220

Fig. 1. (A) Transcriptional repression by nuclear receptors is regulated by recruitment of the co-repressors N-CoR and/or SMRT. (B) The domains of N-CoR/SMRT. Repression domains (RI, RII, RIII) and SANT domains (A and B) are indicated, as are interaction domains for HDACs, nuclear receptors (I and II) and other transcription factors. (C) N-CoR–SMRT compexes. Biochemical purification techniques have revealed several different complexes recruited by N-CoR and/or SMRT (Jones et al., 2001; Li et al., 2000; Underhill et al., 2000; Wen et al., 2000; Guenther et al., 2000).

et al., 1997; Nagy et al., 1997). Several years previously, the observations that acetylation of specific lysine residues in the N-termini of histones correlates with increased transcription and that heterochromatic regions are generally hypoacetylated (Grunstein, 1990; Turner, 1993) had led to studies of the role of histone deactylase complex HDAC proteins in transcriptional repression. Experiments in yeast identified two HDAC complexes, HDA and HDB (Rundlett et al., 1996). Further characterization of these complexes identified two closely related proteins, Hda1p and the previously cloned transcriptional regulator, Rpd3p, (Vidal and Gaber, 1991), respectively, as the proteins essential for histone deacetylase activity (Rundlett et al., 1996). These proteins were the first members of what are now known to be large families of histone deacetylases, which are classified on the basis of their homology to Rpd3p or Hda1p as class I or class II HDACs, respectively (Gray and Ekstrom, 2001).

Rpd3p is linked by epistasis to Sin3p (Rpd1) (Bowdish and Mitchell, 1993; McKenzie et al., 1993; Vidal and Gaber, 1991), which was initially identified in genetic screens for mutations allowing expression of *HO* (*homothallic* gene) in the absence of its activating protein, Swi5 (Nasmyth et al., 1987). Although an enzymatic function of Sin3p has not been demonstrated, the protein can be linked through the SWI proteins to chromatin-remodeling events (Winston and Carlson, 1992). Sin3p and Rpd3p are both required for full repression and full activation of transcription of several target genes in yeast (Vannier et al., 1996; Vidal and Gaber, 1991; Vidal et al., 1991), which suggests that, in common with histone acetylation, histone deacetylation is a major player in the regulation of transcription.

To address the relationship between histone deacetylation and transcription at the most basic level, Kadonaga and colleagues used purified recombinant *Drosophila* HDAC1 in in vitro transcription assays on chromatinized templates (Huang and Kadonaga, 2001). Their experiments demonstrate that HDAC1 alone can mediate histone deacetylation and that a Gal4-dHDAC1 fusion protein can repress transcription by ~60% on chromatinized templates but not on naked DNA templates. Their data also suggest that HDAC activity blocks the initiation step of transcription. In some systems, N-CoR and SMRT can function as activating cofactors of HDAC3 (Guenther et al., 2001; Wen et al., 2000). This activation of HDAC3 is mediated by a domain of N-CoR and SMRT that overlaps with the SANT domain (named for its presence in Swi3, Ada2, N-CoR, and TFIIB) (Aasland et al., 1996; Wen et al., 2000; Guenther et al., 2001).

In addition to the class I and class II HDAC families, which are themselves related, a distinct class of HDAC proteins (class III HDACs) exists: silent information regulator 2 (SIR2)-like proteins (Gottschling, 2000). The *SIR2* genes were initially identified by analysis of mutations that result in expression of regions of the yeast genome that are normally transcriptionally silenced (Nasmyth, 1982; Rine et al., 1979). Indeed, Braunstein et al., prior to the identification of the class I and class II HDAC proteins, noted that overexpression of Sir2p in yeast cells produces histones that are under acetylated (Braunstein et al., 1993). This suggested, although in the absence of any biochemical evidence, that Sir2p has a role in histone deacetylation. Later studies identified nicotinamide adenine dinucleotide (NAD)-dependent ADP-ribosyltransferase activity associated with the human Sir2p homologue (Tsang and Escalante-Semerena, 1998), and extension of these studies revealed the surprising fact that Sir2p is in fact an NAD-dependent histone deacetylase (Imai et al., 2000; Landry et al., 2000; Smith et al., 2000). Interestingly, mammalian Sir2 can interact with and deacetylate the p53 protein, reducing the transcriptional activity of p53 (Luo et al., 2001; Vaziri et al., 2001). The potential ability of this and other classes of HDACs to deacetylate proteins other than histones raises interesting possibilities for their ability to regulate gene expression.

The precise roles of histone deacetylation in transcriptional repression are not fully understood. For instance, differential display analysis of cells treated with the histone deacetylase inhibitor trichostatin A (TSA) revealed that the expression of just 2% of cellular genes (8 of 340 genes examined) changed, despite an increase in core histone acetylation (Van Lint et al., 1996). Rpd3p-null strains in yeast have defects in both transcriptional repression and activation (Rundlett et al., 1996; Vidal et al., 1991) and in fact show increased repression at telomeric heterochromatin (Rundlett et al., 1996), revealing the complicated nature of the transcriptional alterations produced by promoter-specific usage of co-regulatory factors. Recent studies examining the acetylation state at various promoters revealed that different transcriptional activators confer distinct patterns of histone acetylation and that activation is not necessarily related to increased acetylation (Deckert and Struhl, 2001). Experiments with Sin3p and Rpd3 mutants, however, did reveal decreased acetylation of histones (Deckert and Struhl, 2001). Thus many questions relating to the complex nature of transcriptional repression versus activation with regards to the acetylation state of histones remain, although the finding that the class III HDAC Sir2 can deacetylate p53 (Luo et al., 2001; Vaziri et al., 2001) suggests that HDAC proteins have multiple roles in the cell.

Purification of co-repressor complexes

Recent biochemical evidence demonstrates that HDACs are associated with known repression complexes, building a circumstantial case for the involvement of HDACs in transcriptional repression (Fig. 2). HDAC1 and HDAC2 have been found in several complexes, including the Sin-associated protein (SAP) complex (Zhang et al., 1997; Zhang et al., 1998c) and the nucleosome remodeling and histone deacetylation (NURD) complex (Tong et al., 1998; Xue et al., 1998; Zhang et al., 1998b; Zhang et al., 1999), which has ATP-dependent chromatin remodeling activity (Fig. 2) (Knoepfler and Eisenman, 1999). Both complexes also contain retinoblastoma protein (Rb)-associated proteins RbAp-46 and RbAp-48, along with several components specific for either

complex. This suggests that a core HDAC complex differentially recruits additional proteins that impart distinct functional roles to each complex. Complexes purified by use of anti-HDAC1 or anti-HDAC2 antibodies share common components with the NURD complex, including MTA 2, the ATPase Mi-2, RbAp-46 and RbAp-48 and methyl-CpG-binding domain proteins MBD 2 and/or MBD3 (Fig. 2) (Humphrey et al., 2001). The presence of proteins involved in binding to methylated CpG dinucleotides is particularly interesting given the association between DNA methylation and gene silencing and is consistent with studies that show that the closely related methyl-CpG-binding protein MeCP2 not only interacts with HDACs and mSin3A but has TSA-dependent repression abilities (Jones et al., 1998; Nan et al., 1998). Although MeCP2 has not been identified in complexes with N-CoR or SMRT, it has been shown to bind N-CoR (Kokura et al., 2001). Thus a picture begins to emerge in which previously distinct methods of transcriptional repression, including ATP-dependent chromatin remodeling, histone deacetylation and DNA methylation, have overlapping roles and influence one another's distinct enzymatic activities.

Depending on the purification strategy, different HDAC proteins, including HDAC1, HDAC2 and HDAC3, have also been identified in both N-CoR and SMRT complexes (Fig. 1b) (Guenther, 2000; Jones et al., 2001; Li et al., 2000; Underhill et al., 2000; Wen et al., 2000). A subset of N-CoR complexes share common components with the SAP complex and contain HDAC1, HDAC2 and mSin3 (Zhang et al., 1997; Zhang et al., 1998c). Biochemical purification of complexes using anti-N-CoR or anti-HDAC3 antibodies also revealed a distinct complex that contains HDAC3, N-CoR or SMRT, and transducin (beta)-like protein 1(TBL1) (Fig. 1b) (Guenther et al., 2000; Li et al., 2000; Underhill et al., 2000; Wen et al., 2000). TBL-1 has six WD-40 repeats (Bassi et al., 1999), a motif also present in the Tup1 and Groucho co-repressors, and is homologous to the *Drosophila* protein ebi, which is involved in epidermal growth factor receptor signaling pathways (Dong et al., 1999). Under different conditions, an N-CoR–SMRT–HDAC3 complex can also contain Krab-associated protein 1 (KAP-1), a TSA-sensitive co-repressor that interacts with members of the heterochromatin protein 1 (HP1) family, and several members of the Swi/Snf ATP-dependent chromatin-remodeling complex family, which is reminiscent of the ATP-dependent chromatin-remodeling proteins found in the NURD complex (Fig. 1b; Fig. 2) (Underhill et al., 2000). Several groups have also shown that the third repressor domain of N-CoR and SMRT can directly interact in vitro with class II HDACs, including HDAC4, HDAC5 and HDAC7 (Huang et al., 2000; Kao et al., 2000), suggesting the full range of complexes has yet to be purified.

HDAC1 and HDAC2 have also been identified as part of a complex containing CoREST and a novel protein homologous to a diverse group of oxidases and dehydrogenases (Fig. 2) (Humphrey et al., 2001; You et al., 2001), which is also present in one version of the NURD complex (Tong et al., 1998). This latter protein is of particular interest because its potential enzymatic function is reminiscent not only of the NAD-

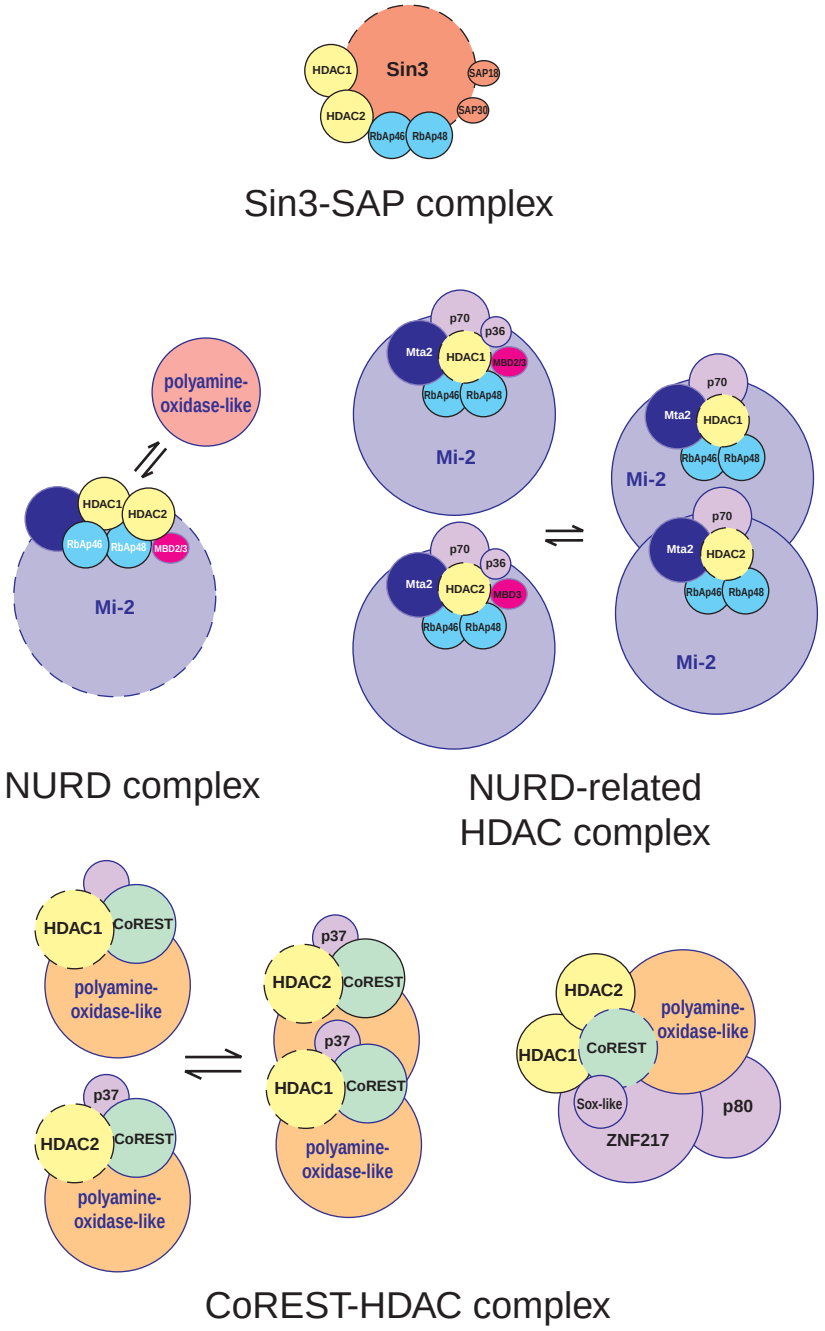


Fig. 2. Other HDAC-containing co-repressor complexes identified by biochemical purification, including the Sin3-SAP (Sin-associated proteins) complex (Zhang et al., 1997; Zhang et al., 1998c), the NURD (nucleosome remodeling and histone deacetylation) complex (Tong et al., 1998; Xue et al., 1998; Zhang et al., 1998b; Zhang et al., 1999), a NURD-related HDAC-complex (Humphrey et al., 2001) and a CoREST-HDAC complex (Humphrey et al., 2001; You et al., 2001). Dashed outlines indicate the molecule that was used to purify each complex.

dependent Sir proteins discussed earlier but of another family of co-repressor proteins, the C-terminal binding proteins (CtBP), which have homology to dehydrogenase enzymes (Nibu et al., 1998; Schaeper et al., 1998). This suggests yet another enzymatic activity may be involved in transcriptional repression. Note that CoREST, which was first identified as a co-repressor for the neural restrictive factor REST (Andres et al., 1999), MTA1, a component of the NURD complex, and N-CoR and SMRT each contains a SANT domain (Aasland et al., 1996). This suggests that this domain is important for some aspects of repression.

Other transcription factor partners

Although cloned on the basis of their interactions with unliganded RAR and T_3R, N-CoR and SMRT appear to confer transcriptional repression on many transcription factors. Indeed, they serve as co-repressors for several members of the nuclear receptor superfamily, including v-ErbA, (Busch et al., 2000; Chen and Evans, 1995), RevErb (Zamir et al., 1996), COUP-transcription factors (Shibata et al., 1997), PPARα (Dowell et al., 1999) and DAX1 (Crawford et al., 1998). Although steroid hormone receptors do not appear to interact with N-CoR or SMRT in the absence of ligand (Chen and Evans, 1995; Horlein et al., 1995), both the estrogen receptor (ER) and the progesterone receptor (PR) can interact with these co-repressors in the presence of their respective antagonists to repress transcription (Jackson et al., 1997; Lavinsky et al., 1998; Smith et al., 1997; Xu et al., 1996). In chromatin immunoprecipitation assays (ChIP) performed in the breast-tumor-derived cell line MCF-7, N-CoR and SMRT were present on the estrogen-responsive cathepsin D and pS2 promoters in the presence of the antagonist tamoxifen but not estrogen (Shang et al., 2000). These data suggest a role for N-CoR and SMRT in mediating the antagonist-associated effects of steroid hormone receptors (Chen and Evans, 1995; Horlein et al., 1995) and provide a mechanism for the clinical application of antagonistic ligands.

N-CoR and SMRT have also been implicated as co-repressors for a variety of unrelated transcription factors, which regulate diverse cellular processes (Fig. 1b). SMRT interacts with and can repress transcription by serum response factor (SRF), activator protein-1 (AP-1) and nuclear factor-κB (NFκB), which are all transcription factors involved in stimulation of cell proliferation (Lee et al., 2000). N-CoR and SMRT have both been implicated in abrogation of transcription by the evolutionarily related POU homeodomain factors Pit-1(Xu et al., 1998) and Oct-1 (Kakizawa et al., 2001), which have important developmental roles, and by the homeobox factor PBX (Asahara et al., 1999; Shanmugam et al., 1999), which is an important determiner of cell fate and segment identity. These co-repressors also interact with the Poz/zinc finger transcription factor BCL-6, which may influence apoptosis (Dhordain et al., 1998; Huynh and Bardwell, 1998; Wong and Privalsky, 1998), and with the bHLH proteins MAD (Heinzel et al., 1997), MyoD (Bailey et al., 1999) and HES-related repressor proteins (HERPs) (Iso et al., 2001), to suppress proliferation or induce terminal differentiation, as well as with the Notch-activated adapter protein Su(H)/RBP-J/CBF1 (Kao et al., 1998), which influences differentiation, proliferation and apoptosis in many developmental systems. SMRT has most recently been shown to interact with signal transducers and activators of transcription 5 (STAT5) (Nakajima et al., 2001), which plays a central role in cytokine signaling.

Multiple mechanisms of regulation

Regulation of proteins with such a potentially broad spectrum of activity is likely to be controlled at many levels, and indeed reports suggest that the actions of N-CoR and SMRT are regulated by several mechanisms. The N-terminus of N-CoR interacts with mSiah2, the mammalian homologue of *Drosophila Seven in absentia* (Zhang et al., 1998a). mSiah2 has been implicated in regulation of proteasomal degradation of proteins (Hu et al., 1997; Li et al., 1997b; Tang et al., 1997), and cotransfection of N-CoR and mSiah2 resulted in a dramatic decrease in N-CoR protein levels, an effect that was not seen in the presence of a proteasome inhibitor (Zhang et al., 1998a).

Although association of N-CoR and SMRT with nuclear receptors is clearly controlled at the level of hormone binding, in several systems, cell signaling events seem capable of directly regulating the association of nuclear receptors with N-CoR and SMRT. Treatment of treated MCF-7 or Hela cells with forskolin, which stimulates the PKA pathway, or EGF, which stimulates the ERK MAP kinase and PKC pathways, resulted in decreased association of N-CoR with ER in the presence of the antagonist tamoxifen (Lavinsky et al., 1998). In addition, in microinjection assays, treatment with forskolin or EGF converts tamoxifen from an antagonist to an agonist of ER-mediated transcription (Lavinsky et al., 1998). Activation of the ERK MAP kinase pathway by L-throxine (T_4) results in serine phosphorylation of TRβ1 and dissociation of SMRT in a hormone-independent manner (Davis et al., 2000). Similarly, phosphorylation of SMRT by the MAP kinase kinase MEK-1 and MEK-1 kinase (MEKK-1) can inhibit interactions between SMRT and nuclear receptors or PLZF (Hong and Privalsky, 2000). In contrast, phosphorylation of SMRT by casein kinase II (CK2) stabilizes the SMRT–nuclear-receptor interaction (Zhou et al., 2001). Thus, different cell signaling pathways can effect different transcriptional outcomes.

In addition to their role in modulating protein-protein interactions, cell signaling pathways also cause changes in subcellular distribution, presumably to restrict access of transcription factors to co-repressors. CamKIV phosphorylation of the NFκB p65 subunit results not only in an exchange of SMRT for CBP but also in translocation of SMRT to the cytoplasm (Jang et al., 2001). MEK-1 and MEKK-1 signaling produces a redistribution of SMRT from the nucleus to the perinucleus or cytoplasm (Hong and Privalsky, 2000). Interestingly, co-repressors themselves shuttle associated proteins to the nucleus, as is the case for both Su(H)/RBP-J/CBF1 (Zhou and Hayward, 2001) and certain HDAC proteins (Wu et al., 2001). Intracellular signaling events are also thought to influence the subcellular distribution of HDAC proteins (Grozinger and Schreiber, 2000; McKinsey et al., 2000a; McKinsey et al., 2000b) and thus may be a general mechanism by which co-repressor proteins are regulated.

There are also examples of regulation of co-repressor specificity by its association with other co-repressor molecules to form distinct co-repressor complexes. Members of the Ski proto-oncogene family, which includes the proteins Ski and

Table 1. N-CoR/SMRT in disease

	Proposed role for N-CoR/SMRT
Leukemias	
Acute promyelocytic leukemia (APL)	APL is caused by gene rearrangements resulting in fusions of RAR-α with PML or PLZF. Both fusion proteins interact with N-CoR and SMRT.
Acute myeloid leukemia (AML)	AML is caused by gene rearrangements resulting in fusions of AML1 and ETO. The AML1-ETO fusion protein can interact with N-CoR and SMRT.
Common acute lymphoblastic leukemia (cALL)	cALL is caused by chromosomal rearrangements of *TEL*, whose product interacts with SMRT.
Other diseases	
Resistance to thyroid hormone (RTH)	A disease caused by mutations in the *T_3R-β* gene that results in a failure to release N-CoR or SMRT upon hormone treatment.
Huntington's disease	Disease caused by the Huntington's disease gene product, huntingtin, which interacts with N-CoR. N-CoR in the diseased brain is cytoplasmic, whereas in normal brain it is both nuclear and cytoplasmic.

Sno, form complexes with N-CoR, SMRT, HDACs and mSin3 to regulate transcriptional repression by MAD and TRβ (Nomura et al., 1999). A co-repressor complex containing N-CoR and Ski or Sno has also been implicated as a negative regulator of the TGFβ signaling pathway (Luo et al., 1999; Stroschein et al., 1999). Ski or Sno, together with N-CoR, form complexes with the SMAD proteins that positively regulate TGFβ signaling (Moustakas et al., 2001), repressing TGFβ-activated transcription (Luo et al., 1999; Stroschein et al., 1999).

SMRT also interacts with the transcriptional repressor SMRT/HDAC1 associated repressor protein (SHARP) (Shi et al., 2001). Interestingly, SHARP binds the steroid receptor RNA coactivator SRA and suppresses SRA-activated steroid receptor transcription activity, providing a mechanism by which SMRT can modulate liganded nuclear receptors (Shi et al., 2001). As the data collected increase, there will doubtlessly prove to be additional mechanisms by which both the regulation and specificity of N-CoR and SMRT activity are controlled.

Roles in development and disease

Although the biological role of hormonal activation of nuclear receptors is well established, only recently has the biological significance of repression begun to be appreciated, with both N-CoR and SMRT emerging as important players. The role of N-CoR during normal development was revealed by studies of N-CoR-null mice, which die in midgestation and exhibit defects in developmental progression of specific erythrocyte, thymocyte and neural events (Jepsen et al., 2000). Results in erythroblasts were particularly intriguing because the mice had a block in erythroid blast-forming unit (BFU-E) formation that may be related to effects mediated by T_3R. V-ErbA, an oncogenic form of T_3R that cannot bind to hormone, owing to mutations in its C-terminal ligand-binding domain (Munoz et al., 1988; Sap et al., 1986), thus functions as a constitutive repressor of transcription (Damm et al., 1989), induces erythroleukemia and fibrosarcomas in chickens and transforms erythroid cells and fibroblasts in culture (Graf and Beug, 1983). The constitutive repression of v-ErbA target genes, which has been linked to recruitment of N-CoR and HDAC activity, (Busch et al., 2000; Ciana et al., 1998) is thought to contribute to avian erythroblastosis virus (AEV)-induced leukemic transformation. N-CoR$^{-/-}$ erythroblasts have enhanced levels of one v-ErbA target gene that encodes carbonic anhydrase II; this suggests that unliganded T_3R requires N-CoR for repression events critical to expansion of erythroblast progenitors. Studies in primary avian erythroblasts showed that overexpression of T_3R in the absence of T3 resulted in sustained proliferation and tightly arrested differentiation of erythroblasts, whereas addition of T3 caused loss of self-renewal capacity and induced terminal differentiation (Bauer et al., 1998).

A dominant-negative N-CoR protein, lacking the repression domains in the N-terminus but retaining the nuclear receptor interaction domains, has also been used in an attempt to define specific biological roles for co-repressors (Feng et al., 2001). Transgenic mice expressing this construct in hepatocytes showed an increased proliferation of hepatocytes and a derepression of T3-regulated hepatic target genes (Feng et al., 2001). A dominant-negative approach has also been used in *Xenopus* and resulted in embryos that exhibited phenotypes similar to those treated by RA, namely reduction of anterior structures such as forebrain and cement gland (Koide et al., 2001). These data suggest that RAR-mediated repression of target genes is critical for head formation.

Whereas knockout and dominant-negative transgenic animals allow one to assess the consequences of loss of co-repressor function, various disease models have allowed investigation of inappropriate gain of co-repressor function (Table 1). For instance, resistance to thyroid hormone (RTH) is a human genetic disease characterized by an impaired physiological response to thyroid hormone and is associated with mutations in T_3R-β (Kopp et al., 1996) that fail to release N-CoR or SMRT upon hormone treatment (Safer et al., 1998; Yoh et al., 1997). Transcriptional activation by mutant T_3R-β in response to hormone is diminished compared with that of wild-type T_3R-β. Repression might also be involved in normal thyroid hormone physiology because deletion of genes encoding all known thyroid hormone receptors results in a phenotype less severe than that of mice lacking thyroid hormone (Gothe et al., 1999). Thus, there seems to be a role for the unliganded T_3Rs in development, which suggests co-repressor involvement.

Roles for N-CoR and SMRT in several types of leukemia are also well characterized. Acute promyelocytic leukemia (APL), caused by a block in myeloid differentiation, is associated with rearrangements of *RAR-α*, which most commonly result in fusions of the *RAR-α* gene with the promyelocytic leukemia gene (*PML*) or the promyelocytic leukemia zinc finger gene (*PLZF*) (Lin et al., 1999). Both RAR-α fusion proteins retain the ability to interact with N-CoR and SMRT (Hong et al., 1997). Interestingly, although retinoic acid (RA) can induce remission in APLs resulting from PML-RAR-α translocations, APLs

resulting from PLZF-RAR-α translocations are insensitive to RA (Lin et al., 1999). Although co-repressor interactions with PML-RAR-α fusions are less sensitive to the effects of RA than are interactions with wild-type RAR-α, pharmacological concentrations of RA do result in the dismissal of the co-repressor complex and in activation of transcription. In contrast, the PLZF-RAR-α fusion protein interacts with the co-repressor even in the presence of RA. These observations correlate co-repressors with the disease state, because the relative RA sensitivity of the interaction between the RAR-α fusions and N-CoR or SMRT correlates with their response to RA treatment. Interestingly, histone deacetylase inhibitors such as TSA, in combination with RA, not only overcome the transcriptional repressor activity of both PML-RAR-α and PLZF-RAR-α but also render PLZF-RAR-α sensitive to RA (He et al., 1998). Together, these results indicate that the presence of a co-repressor is one criterion for disease progression.

N-CoR and SMRT have also been implicated in acute myeloid leukemias (AML). 12-15% of AMLs result from the t(8;21) translocation between *AML1* and *ETO*. AML1 upregulates a number of target genes critical to normal hematopoiesis, and the AML1-ETO fusion protein represses transcription of these target genes. In common with ETO, the AML1-ETO fusion protein can interact with N-CoR and SMRT, and mutations that abolish this interaction affect the ability of AML1-ETO both to repress transcription and to inhibit differentiation of hematopoeitic precursors (Gelmetti et al., 1998; Lutterbach et al., 1998; Wang et al., 1998). The role of N-CoR in AML also appears to be partially due to recruitment of histone deacetylase activity, since recent studies have revealed that the histone deacetylase inhibitors TSA and phenylbutyrate (PB) both partially reverse ETO-mediated transcriptional repression, and PB can induce partial differentiation of an *AML1-ETO* cell line (Wang et al., 1999).

A third class of leukemia results from chromosomal rearrangements of the E26 transforming specific (ETS)-related gene *TEL*, which encodes a strong transcriptional repressor that recruits a co-repressor complex including SMRT, mSin3A and HDAC3 (Chakrabarti and Nucifora, 1999; Wang and Hiebert, 2001). The overall theme for involvement of N-CoR and SMRT in progression of these leukemias thus appears to be the ability of histone-deacetylase-associated repression to block differentiation and allow uncontrolled growth of hematopoietic cells, which ultimately results in the diseased state.

N-CoR has also been implicated in pathologies associated with the nervous system. The C-terminus of N-CoR interacts with the N-terminus of the Huntington's disease gene product, huntingtin, in both yeast two-hybrid screens and pull-down assays (Boutell et al., 1999). Although N-CoR is generally thought to exert its action in the nucleus (Horlein et al., 1995), immunohistochemical studies on Huntington's disease brains and control brains revealed that the localization of N-CoR and mSin3 in the diseased cortex and caudate is exclusively cytoplasmic, whereas in the normal brain they are localized in the nucleus as well as the cytoplasm. This suggests that relocalization of co-repressor proteins in the diseased brain alters transcription and is thus involved in the pathology of this disease. Interestingly, though perhaps counter-intuitively, inhibitors of HDAC activity can arrest neurodegeneration associated with Huntington's disease in a *Drosophila* model (Steffan et al., 2001).

Conclusions

Here, we have focused mainly on the N-CoR/SMRT family of HDAC-associated co-repressors, but there are several repressors that associate with HDACs. These include non-DNA-binding cofactors such as CtBP (Turner and Crossley, 2001) and groucho/TLE proteins (Chen and Courey, 2000), transcription factors that interact directly with HDACs such MEF2, (Lu et al., 2000), which functions in myogenesis, and proteins involved in methylation-induced gene repression such as MeCP2 (Jones et al., 1998; Nan et al., 1998). In fact, it appears that a single transcription factor can frequently utilize more than one family of co-repressor. Hairy, for example, can interact with both CtBP and groucho/TLE proteins (Paroush et al., 1994; Poortinga et al., 1998), and Hesx1 interacts with groucho/TLE proteins and N-CoR (Dasen et al., 2001). On the basis of current studies, it is impossible to determine whether more than one co-repressor is bound to a transcription factor at the same time on a given promoter, but these studies will certainly be done in the near future.

It is intriguing, as mentioned earlier, that a growing number of co-repressor complexes are associated, if circumstantially, with proteins connected to redox pathways. These include CTBP, the FAD-binding protein found in the HDAC1/2 and NURD complexes, and the NAD-dependent class 3 HDACs. Several lines of evidence also point to a connection between DNA methylation and histone deacetylation (Dobosy and Selker, 2001), suggesting that multiple enzymatic activities are recruited to DNA, either simultaneously or sequentially, and subsequently modulate transcriptional repression.

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