Review Article

Physiological Roles of Class I HDAC Complex and Histone Demethylase

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Epigenetic gene silencing is one of the fundamental mechanisms for ensuring proper gene expression patterns during cellular
differentiation and development. Histone deacetylases (HDACs) are evolutionally conserved enzymes that remove acetyl
modifications from histones and play a central role in epigenetic gene silencing. In cells, HDAC forms a multiprotein complex
(HDAC complex) in which the associated proteins are believed to help HDAC carry out its cellular functions. Though each HDAC
complex contains distinct components, the presence of isoforms for some of the components expands the variety of complexes
and the diversity of their cellular roles. Recent studies have also revealed a functional link between HDAC complexes and specific
histone demethylases. In this paper, we summarize the distinct and cooperative roles of four class I HDAC complexes, Sin3, NuRD,
CoREST, and NCoR/SMRT, with respect to their component diversity and their relationship with specific histone demethylases.

1. Introduction

Eukaryotic chromosomes consist of two cytologically defined structures, euchromatin, and heterochromatin. Euchromatin contains transcribable genes, which are subject to either activation or inactivation depending on the cellular situation, whereas heterochromatin contains transcriptionally inert genes. Structural changes in chromatin are tightly linked with posttranscriptional modifications of the histone tails. The combinations of histone modifications establish local and global patterns of chromatin structure and define specific downstream events [1]. These patterns are highly dynamic and can be altered by multiple extracellular and intracellular stimuli. Thus, chromatin has been proposed to serve as a signaling platform by which various signaling pathways are integrated.

Among the numerous covalent modifications identified so far, acetylation and methylation play central roles in chromatin dynamics [2]. Histone acetylation on lysine residues is primarily associated with gene activation, and its levels are controlled by two counteracting enzymes: histone acetyltransferases (HATs) and histone deacetylases

(HDACs). Many lysine and arginine residues on histones are target sites of methylation. In contrast to acetylation, histone methylation is linked with both gene activation and inactivation and is regulated by histone methyltransferases (HMTs) and histone demethylases (HDMs). In most cases, these histone-modifying enzymes are present in multisubunit protein complexes, in which the other components are thought to regulate enzyme activities, modulate substrate recognition, recruit other cofactors, or carry out other undefined functions.

In metazoans, HDACs are divided into three classes, Class I to Class III, based on their sequence similarity. Class I HDACs, which include HDAC1, HDAC2, and HDAC3, are found in four distinct multiprotein complexes, the Sin3, NuRD, CoREST, and NCoR/SMRT complexes. These complexes are highly conserved and function in distinct cellular processes including cell-cycle regulation [3, 4], maintenance of stem cell pluripotency [5], self-renewal, and cellular differentiation. Recent studies have revealed that all of these complexes are associated with specific HDMs. The HDMs are classified into two distinct enzyme families: the nuclear amine oxidase homologs and the JmjC-domain
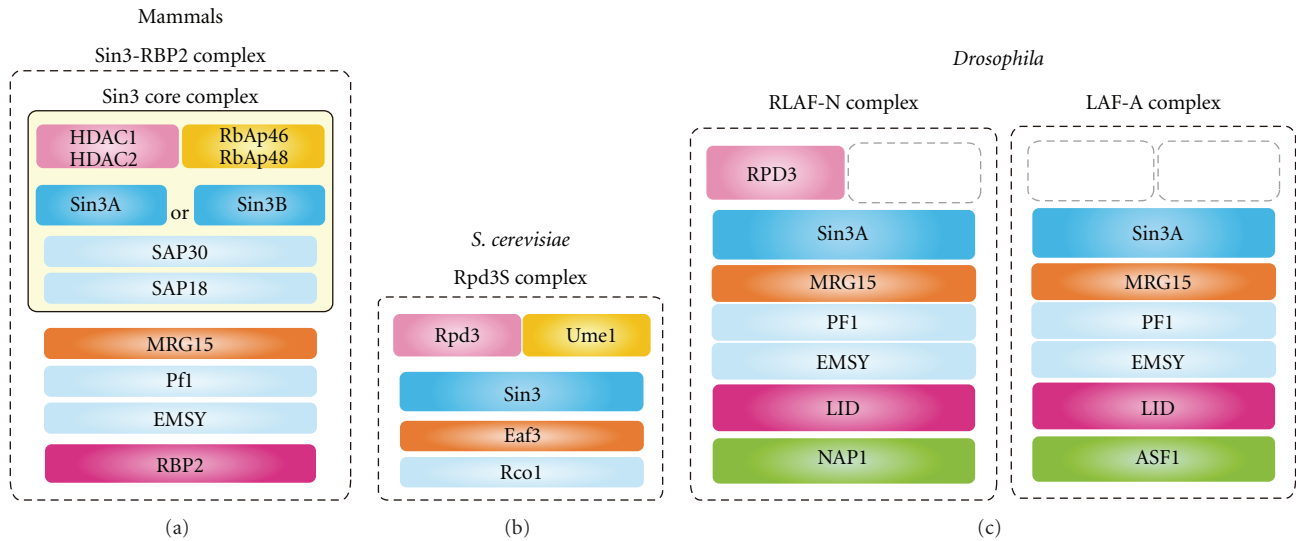
FIGURE 1: *The Sin3 complexes.* Schematic representation of the Sin3-containing HDAC complexes: (a) the Sin3-core and Sin3-RBP2 complexes in mammalian cells; (b) the Rpd3S complex in *S. cerevisiae*; (c) RLAF-N and LAF-A complexes in *Drosophila*. The empty dotted boxes indicate missing subunits compared with the Sin3-core complex.

proteins. LSD1 (KDM1A, also known as AOF2/BHC110) is a member of the former family and was the first histone lysine demethylase identified; it preferentially demethylates methylated histone H3 lysine 4 (H3K4me) [6]. The latter enzyme family, the JmjC-domain proteins, plays important roles in development and cellular differentiation [7–10]. This family has 27 members in mammals, and these members are divided into 11 subgroups based on similarity within the JmjC domain [11]. So far, seven of these subgroups have been shown to have demethylase activity for specific residues on histone H3 or H4.

To achieve strict transcriptional regulation in the complicated chromosomal architecture of the nucleus, it makes sense that eukaryotic organisms developed a system involving combinatorial histone modifications and protein complexes that contain multiple enzymatic activities, such as HDAC and HDM. It is noteworthy that cells produce a variety of HDAC-containing complexes by replacing an integral component with one of its subtypes. These subtype-specific HDAC complexes appear to play distinct roles in particular cellular contexts. In addition, incorporating sequence-specific DNA-binding proteins, such as zinc-finger proteins, can also modulate the function of the HDAC complex. In this paper, we focus on how HDAC-HDM interactions and subtype-specificity regulate functions of the Class I HDAC-containing complexes, in transcriptional regulation and other cellular processes.

2. The Sin3 Complex

The HDAC-containing multiprotein complex, Sin3 complex, is highly conserved from yeast to humans. In mammalian cells, the Sin3 complex consists of six core subunits, HDAC1/2, RbAp46, RbAp48, Sin3A/Sin3B, SAP18, and SAP30 (Figure 1(a)) [12–14]. Mammalian Sin3A and Sin3B (mSin3A and mSin3B) show high sequence similarity and an overlapping expression pattern, and both are essential in mouse development. However, these subtypes play distinct and nonoverlapping roles. mSin3A has a critical role in the early developmental preimplantation stage, since no mSin3A-null zygotes are found at E6.5 [15]. mSin3A-depleted mouse embryonic fibroblasts (MEFs) exhibit the deregulation of genes involved in cell-cycle control, DNA replication, DNA repair, apoptosis, chromatin modification, and mitochondrial metabolism. Transcriptome analysis has revealed that mSin3A modulates the transcriptional network controlled by Myc-Mad, E2F, or p53. On the other hand, mSin3B has an essential function at the late-gestation stage, and its mutant is different from the mSin3A-/- embryo [16]. In particular, erythrocyte and granulocyte differentiation and G0/G1 cell-cycle control are impaired in the mSin3B-/- embryo, and these phenotypes are due to the derepression of E2F target genes.

Transcriptional regulation is tightly coupled with dynamic change of histone acetylation in the promoter regions. However, the Sin3 complex functions not only at promoter regions but also at transcribed regions. In *Saccharomyces cerevisiae*, Sin3 and a Class I HDAC, Rpd3, are involved with two functionally distinct complexes, Rpd3L and Rpd3S [17]. Both complexes contain Sin3, Rpd3, and Ume1. In addition to these core subunits, Rpd3L contains at least six unique components: Rxt1, Rxt2, Dep1, Sds3, Pho23, and Sap30, whereas Rpd3S contains Rco1 and Eaf3 as unique components (Figure 1(b)) [18]. Rpd3L is localized primarily to promoter regions. In contrast, Rpd3S is localized to transcribed regions that are enriched in methylated H3K36 (H3K36me). H3K36me is a mark catalyzed by Set2 (KMT3) and is tightly coupled with the transcriptional elongation processes [19]. Rpd3S recognizes this H3K36me mark via

a combined action of chromodomain of Eaf3 and PHD (plant homeobox domain) of Rco1 [20–22]. Deletion of Set2 or one of the Rpd3S-specific components results in spurious transcripts that emerge from incorrect transcription start sites in some transcriptionally active genes [17]. Since the H4 acetylation levels within transcribed regions are increased in these mutant cells, Rpd3S is thought to repress unfavorable transcription by maintaining transcribed regions in a hypoacetylated state. In metazoans, however, the relationship between mSin3 complexes and the emergence of aberrant transcripts remains unclear.

One of the Rpd3S-specific components, Eaf3, is highly conserved from yeast to humans. The human Eaf3 homologue MRG15 was initially identified as a factor closely related to MORF4 (mortality factor on human chromosome 4), whose transient expression induces senescence in a subset of human tumor cell lines [23, 24]. MRG15 is a stable component of mSin3-HDAC complexes, and the MRG15-associated mSin3-HDAC complex also contains Pf1, a component that shares similarity with *S. cerevisiae* Rco1 [25], and a histone H3K4-specific demethylase, RBP2 (KDM5A) [26] (Figure 1(a)). Of note, MRG15 is shared with at least two other complexes: the Tip60 (NuA4)-HAT complex [26–28] and the BRCA2-containing DNA-damage-responsive complexes [29, 30]. Although we will not discuss these complexes further in this paper, the MRG15-associated complexes are implicated in DNA-damage responses [29, 31–33], in addition to transcriptional regulation, suggesting that histone acetylation dynamics is tightly coupled with histone eviction/deposition during DNA-repair processes.

We previously demonstrated that MRG15 recruits RBP2 and controls the H3K4me levels on transcribed regions via the RBP2 activity [26]. In addition, van Oevelen et al. reported that the majority of Sin3 target genes (58%) are bound by RBP2, which spreads over the region immediately downstream of the transcription start site on a subset of E2F target genes during differentiation [34]. These results suggest that the Sin3-HDAC complex and RBP2 play a cooperative role in repressing target genes through deacetylation, demethylation, and, probably, the repositioning of nucleosomes. In *S. cerevisiae*, no evidence has been reported for a physical interaction or functional link between the Sin3 complex and the KDM5 homologue Jhd2. In *Drosophila*, RPD3, MRG15, and PF1, but not SIN3, were identified in an LID-(a KDM5 homologue) containing complex [35]. Intriguingly, the deacetylation activity of RPD3 is inhibited by its interaction with any of these associated proteins, including LID, implying that the relationship between the HDAC and HDM activities is counteractive.

Recent reports have shown that LID associates with SIN3 and functions in Notch silencing [36]. The Notch signaling pathway plays pleiotropic roles during embryonic development and is important for the regulation of cellular self-renewal [37, 38]. Moshkin et al., purified factors associated with histone chaperones (ASF1 and NAP1) from *Drosophila* S2 cells or embryonic nuclear extract, and identified several peptides, including LID, SIN3A, PF1, RPD3, MRG15, and a BRCA2-binding protein, EMSY. Using a reciprocal

purification approach, they further showed that NAP1 binds the RPD3 and LID-Associated Factors (RLAFs) consisting of RPD3, LID, SIN3A, PF1, EMSY, and MRG15, whereas ASF1 binds LAF, a similar complex lacking RPD3 (Figure 1(c)). Both of these complexes, RLAF-NAP1 (RLAF-N) and LAF-ASF1 (LAF-A), are required for the transcriptional repression of Notch-regulated genes. In agreement with the enzymatic activity of LID, the knockdown of ASF1, NAP1, or any of the LAF components results in an accumulation of H3K4me3 at the promoter and enhancer regions. Interestingly, H3K4me3 accumulation is not observed in RPD3 knockdown cells [36]. This implies that LID recruitment to the promoter/enhancer regions of Notch target genes may not depend on the entire RPD3-containing RLAF complex. Considering that the RPD3-containing RLAF complex resembles Rpd3S in *S. cerevisiae* in its subunit composition (Figures 1(b) and 1(c)), it is possible that RLAF acts at the rest of transcribed regions, with or without the association with NAP1. This possibility will be tested by future studies.

RBP2/KDM5A was also found to be involved in the Notch pathway in a study using mammalian cells [39]. RBP-J is a nuclear effector of Notch signaling. Upon ligand binding, RBP-J activates the expression of Notch target genes, and, in the absence of Notch signal, RBP-J switches off the target gene expression. Liefke et al. showed that RBP2/KDM5A associates with RBP-J *in vivo*. RBP2/KDM5A is colocalized with RBP-J at the promoter region of Notch target genes and regulates their expression. Although the involvement of HDAC at the target promoter region was not described in this study, it is most likely that RBP2 works together with an HDAC complex, as in the case of the LAF-A complex in *Drosophila*.

Another function of the Sin3 complex was revealed by a genome-wide study. The gene-expression profiles of Sin3-depleted *Drosophila* cells were analyzed by high-density oligonucleotide array [40]. This analysis revealed that the expression of ∼3% of the *Drosophila* genes is altered in Sin3-depleted cells. The affected genes are associated with diverse biological processes, including signal transduction, transcriptional regulation, and cell-cycle control. Interestingly, the list of affected genes also includes a substantial fraction of genes involved in cytosolic and mitochondrial energy-generating pathways. Furthermore, Sin3-deficient *Drosophila* cells exhibit an increase in mitochondrial mass. In accordance with this observation, the genome-wide analysis of RBP2-binding sites using human breast cancer cell lines revealed that RBP2 is enriched at genes encoding proteins that localize to the mitochondrion, including mitochondrial ribosomal proteins [41]. Moreover, the alteration of RBP2 leads to mitochondrial defects in human cells, as in Sin3-depleted *Drosophila* cells.

Overall, the Sin3-HDAC complexes associated with KDM5 regulate the transcription of many genes, in processes such as Notch signaling and mitochondrial functions. Notably, the transcriptional regulation is achieved by dynamic changes in histone modifications not only at promoter regions, but also at transcribed regions. The Sin3-KDM5 complex appears to play an important role at both regions by exchanging its associated cofactors.

3. The Mi-2/NuRD Complex

The NuRD complex was first purified based on its histone deacetylase and nucleosome-remodeling activities [14, 42, 43]. Although the NuRD complex has been identified in mammalian and *Xenopus* cells, its major components have also been found in *Drosophila*, *C. elegans*, and *Arabidopsis*, suggesting that the complex exists widely in animal and plant species. The biochemical association of the histone deacetylase and nucleosome-remodeling activities suggests that these two activities are functionally coupled in this complex action.

The NuRD core complex consists of HDAC1/2, RbAp46, RbAp48, CHD3/CHD4 (Mi-2), MBD2/MBD3, MTA1/MTA2/MTA3, and p66α/p66β (Figure 2(a)). HDAC1/2 and RbAp46/48 are shared with the Sin3 complex. Mi-2 was first identified as an autoantigen in the human connective tissue disease dermatomyositis [44], and is a member of the SWI2/SNF2-related chromatin-remodeling ATPases, which target the chromatin region and unwind the nucleosome structure. MBD2 and MBD3 belong to the MBD (methyl-CpG binding) domain family although MBD3 is unable to bind methyl-CpG [45, 46]. Since the NuRD complex can interact with both MBD2 and MBD3, it was first hypothesized to target methylated DNA through its interaction with MBD2 [47]. However, recent reports have shown that MBD3 and MBD2 are exclusively associated with the NuRD complex and form distinct complexes, MBD3/NuRD and MBD2/NuRD [48]. MBD3 and MBD2 are very similar (70% identical), but only MBD3 is essential for mouse development [49].

The MTA protein family has three members, MTA1, MTA2, and MTA3, and vertebrates have two additional splicing variants, small form of MTA1 (MTA1s) and MTA3L [50, 51]. MTA1s, however, lacks a nuclear localization signal and is found in the cytoplasm [50]; it is therefore unlikely to be a component of nuclear Mi-2/NuRD. Among these MTAs, MTA1 and MTA3 have been clearly shown to be involved in tumor progression. Their expression is upregulated in several types of tumors, and their expression states are closely correlated with the invasive growth properties of tumors [51–53]. MTA1 is induced by the growth factor heregulin and is a potent corepressor of estrogen-receptor element-(ERE-)driven transcription [54]. Similarly, in breast epithelial cells, MTA3 is induced by estrogen, constitutes an estrogen-dependent component of the Mi-2/NuRD complex, and plays a critical role in repressing the expression of Snail, a master regulator of the epithelial-to-mesenchymal transition [51]. These data suggest that MTA proteins are mutually exclusive in the NuRD complex (Figure 2(a)) and that distinct NuRD complexes function as transcriptional repressors in different signaling pathways.

The NuRD complex plays important roles in development. Mice lacking a functional *Mbd3* gene die prior to midgestation [49]. Embryonic stem (ES) cells lacking Mbd3 are viable, but they fail to completely silence genes that are expressed before implantation of the embryo [55] and are unable to commit to developmental lineages [56]. Another NuRD component, p66, was first cloned in *Drosophila* as a genetic modifier of the Wingless signaling pathway. The human paralogs p66α (GATAD2A) and p66β (GATAD2B) function synergistically in transcriptional repression, and both bind to MBD2 [57]. Although p66α is not required for normal blastocyst development or implantation, loss-of-function mutant embryos of p66α die at around day 10 of embryogenesis [58].

ES cells require several key molecules, such as Nanog and Oct4, for their self-renewal and pluripotency [59]. Nanog-containing complexes were recently purified from mouse ES cells, and several NuRD-related components were identified as Nanog-interacting proteins [5]. Interestingly, the Nanog-interacting NuRD-like complex contains most of the NuRD components (Mta1, Mta2, Hdac1/2, p66α, and p66β) but lacks Chd3/4, Mbd3, and RbAp46/Rbbp7 (Figure 2(b)). This NuRD-like unique complex was thus named NODE (for Nanog- and Oct4-associated deacetylase). Without Mbd3, deacetylase activity in the canonical NuRD complex is greatly reduced [47], but the NODE complex contains HDAC activity comparable to that of NuRD complexes. Importantly, Mbd3-knockdown ES cells show different gene-expression profiles than Mta1- or Mta2-knockdown ES cells. Moreover, while Mbd3 loss-of-function mutant ES cells can self-renew, the knockdown of NODE subunits leads to the increased expression of developmentally regulated genes and ES-cell differentiation. These observations support the idea that NODE is functionally distinct from canonical NuRD.

Although the functional interaction between NuRD/NODE and histone demethylases in ES cells is still unknown, cooperative roles of NuRD and LSD1 have been described (Figure 2(a)) [60]. Using HeLa cells, Wang et al. purified the MTA2-containing protein complexes and identified LSD1 in addition to other NuRD components [60]. LSD1 interacts directly with all three MTA proteins and appears to form distinct complexes. The NuRD-LSD1 complexes target distinct, yet overlapping, sets of genes. Transcriptional analyses have revealed that these complexes regulate several cellular signaling pathways, including TGFβ, cell-communication, focal-adhesion, MAPK, and cell-cycle pathways, which are critically involved in cell growth, survival, migration, and invasion. Furthermore, LSD1 inhibits the invasion of breast cancer cells in a Mi-2-dependent manner *in vitro* and suppresses breast cancer metastatic potential *in vivo*. In fact, LSD1 is downregulated in breast carcinomas and negatively correlated with *TGFβ1* expression.

Collectively, the LSD1/NuRD complex is required for the repression of important genes in cellular signaling pathways and suppresses breast cancer metastasis. While LSD1 is a component of NuRD complexes, it has been also observed that LSD1 targets promoter regions independent of NuRD [60]. Therefore, it is likely that LSD1 also functions as a component of currently unidentified protein complexes.

4. The CoREST Complex

CoREST was first identified as a corepressor of REST (RE-1 Silencing Transcription Factor, also known as NRSF) [61] and later demonstrated to be a component of
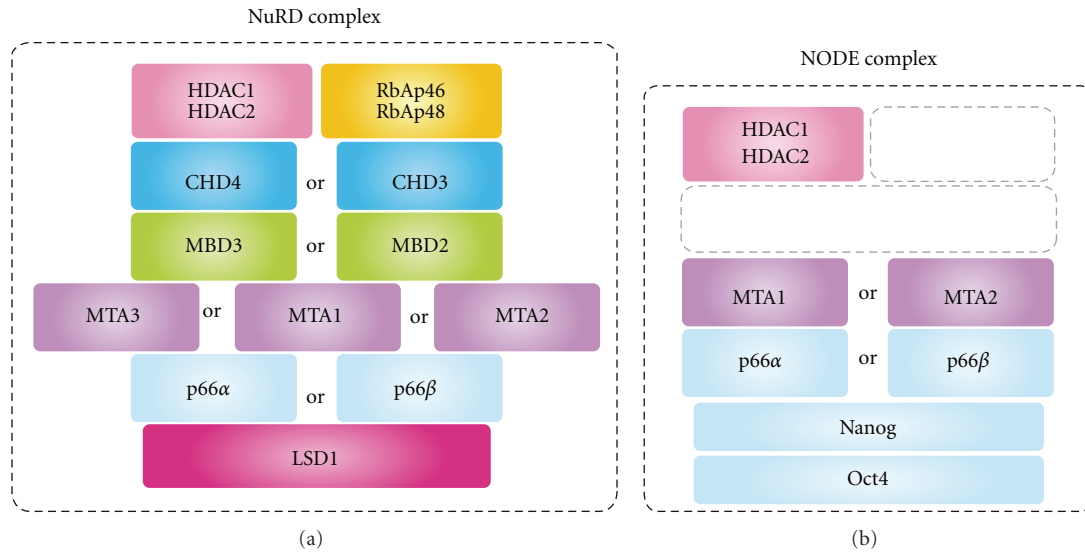
FIGURE 2: *The NuRD complexes.* Schematic representation of the NuRD complex (a) and ES cell-specific NODE complex (b). The empty dotted boxes indicate missing subunits compared with the NuRD complex.

HDAC1/2-containing complexes [62]. The CoREST complex contains HDAC1/2, p80, Sox-like protein, ZNF217 (p110a), and LSD1 (p110b) (Figure 3(a)). Although p110b was identified in the first purification study, its catalytic activity was later identified and renamed LSD1 [6]. Because the CoREST complex does not associate with mSin3, MTA, CHD4, or RbAp46/48, it is thought to be distinct from the other HDAC-containing complexes. The CoREST protein contains two SANT domains, a conserved domain resembling the DNA-binding domains of Myb-related DNA-binding proteins. This domain was originally identified in SWI3, ADA3, NCoR, and TFIIIB (and thus named SANT) [63], and it is also present in the MTA proteins. Interestingly, the demethylase activity of LSD1 to the nucleosomal substrates requires the CoREST protein [64]. In addition, the CoREST/LSD1 complex shows high demethylase activity for a hypoacetylated nucleosome substrate, and the SANT domain of CoREST preferentially interacts with hypoacetylated histone tails, as was also previously observed for the SANT domain of SMRT [65]. These results suggest that the HDACs in the CoREST/LSD1 complex function upstream of LSD1 and that deacetylated states are recognized by CoREST, which facilitates LSD1's enzymatic activity.

ZNF217, the other CoREST complex component, is a Krüppel-like zinc-finger protein. The ZNF217 complex purified from HeLa cells includes LSD1, CoREST, HDAC2, and CtBP [66]. The zinc-finger motif of ZNF217 specifically binds to the DNA sequence CAGAAY (Y means C or T), and this consensus is highly conserved in the *E-cadherin* promoter. In fact, ZNF217 and other CoREST complex components are found on the *E-cadherin* promoter, which is repressed by ZNF217. As ZNF217 overexpression has been noted in many cancer cell lines [66], aberrant protein levels of ZNF217 may cause unregulated targeting by the CoREST-LSD1 complex, with a profound effect on cancer progression.

The transcriptional corepressor CtBP (C-terminal binding protein) is also implicated in tumorigenesis. Immunoaffinity purification of the CtBP complex revealed that it contains all of the CoREST complex components [67]. In this analysis, many other interactors were also identified (G9a, HuHMT, HPC2, REBB-1, and ZNF516) [67]. As described for ZNF217, CtBP and the complex components, EuHMT and G9a, also repress *E-cadherin* promoter activity. Another report showed that Snail, a master regulator of the epithelial-to-mesenchymal transition, forms a complex with LSD1 and CoREST, and functions to recruit LSD1 activity to the *E-cadherin* promoter [68]. Together, these results suggest that the CoREST complex is a kind of "core complex" that associates with the CtBP complex as an effector module (Figure 3(a)). Considering that CoREST also associates with other corepressors required for hematopoietic differentiation, Gfi-1 and Gfi1-b [69], it is likely that CoREST forms several distinct complexes depending on the cellular situation.

5. The NCoR/SMRT Complex

Nuclear hormone receptors are evolutionarily conserved, ligand-dependent transcription factors that influence the biological processes of cell proliferation and differentiation in metazoans. NCoR (Nuclear receptor CoRepressor, also known as NCOR1) and SMRT (Silencing Mediator for Retinoid and Thyroid receptor, also known as NCOR2) bind to nuclear hormone receptors and act as "platform proteins" in recruiting a large protein complex that includes Class I and II HDAC and mSin3A [70]. Of note, proteins homologous to NCoR or SMRT are not found in the yeast genome, as is the case for the nuclear hormone receptors. These proteins appear to have arisen during the evolution of metazoan organisms to allow unliganded nuclear hormone receptors
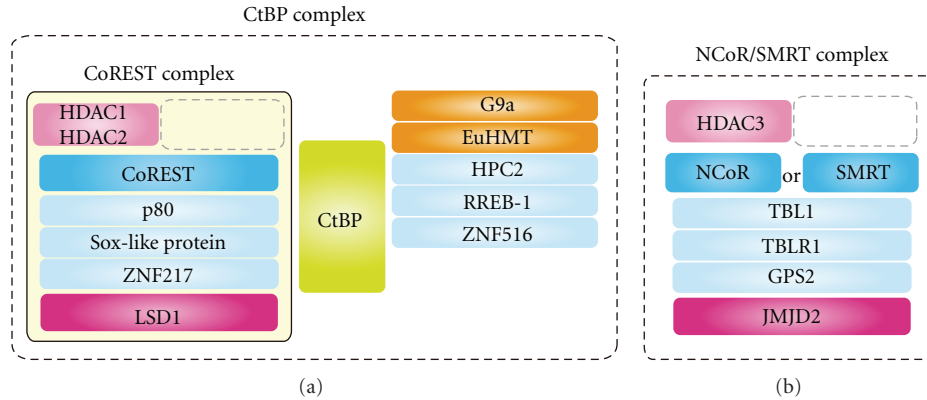
Figure 3: *The CoREST and NCoR/SMRT complexes.* Schematic representation of the CoREST (a) and the NCoR/SMRT (b) complexes. The CoREST complex is included in the CtBP-containing complexes. The empty dotted boxes indicate that both CoREST and NCoR/SMRT complexes lack RbAp46/RbAp48 subunits, which are widely observed in HDAC-containing complexes.

to repress transcription via the Sin3-HDAC pathway [70]. SMRT and NCoR share a similar domain organization and are thought to be paralogues.

NCoR and SMRT are essential for development, but their mutant phenotypes are quite different. NCoR appears to be critical to neural differentiation and the developmental progression of erythrocytes and thymocytes [71, 72], whereas SMRT is required for heart development [72]. The SMRT and NCoR complexes share interacting partners, including HDAC3, TBL1 (transducin β-like 1), TBLR1 (TBL related 1), and GPS2 (G-protein pathway suppressor 2) (Figure 3(b)) (reviewed in [73]). Moreover, 114 and 98 proteins, respectively, are reported to interact physically with NcoR and SMRT (BioGRID). NCoR and SMRT also associate with other HDAC members, HDAC4, HDAC5, and HDAC7, but these enzymatic activities are dependent on the SMRT/NCoR-HDAC3 complex [74]. Previous studies demonstrated that diverse transcription factors, including Mad/Mxi, BCL6/LAZ3, ETO, RBP-J, and REST/NRSF, interact with the NCoR and SMRT complexes [75, 76].

Several demethylases that interact with NCoR have been identified. KDM4A (former name, JMJD2/ JDM3A/JMJD2A/KIAA0677) was identified in an affinity-purified NCoR complex [77]. KDM4A is not a core subunit of the NCoR complex, and it is not required for NCoR-mediated repression by thyroid hormone receptor. However, KDM4A is required for selective repression of the *ASCL2* gene, which is an imprinted gene essential for proper placental development, and this repression requires a functional NCoR complex [78]. KDM4A specifically demethylates tri- and dimethylated lysine-9 and lysine-36 of histone H3 (H3K9/36 me3/2) [79]. A recent study showed that KDM4 also demethylates lysine-26 of histone H1.4 (H1.4K26me), which is one of seven somatic H1 isotypes in humans, and is known to cause transcriptional repression [80]. Considering that both H3K9me3/2 and H1.4K26me are repressive histone marks, it is unclear how KDM4's enzymatic activity is coupled with NCoR-mediated repression. Nevertheless, the substrate specificity of KDM4 may be modulated within N-CoR-containing complexes.

Further studies are required to elucidate the function of the KDM4-associated NCoR complex and the resulting histone-modification dynamics.

KDM5C (also known as SMCX/JARID1C) has histone H3K4-specific demethylase activity and functions as a transcriptional repressor. KDM5C is implicated in X-linked mental retardation, epilepsy, and autism [81–83]. Tahiliani et al., purified a KDM5C-containing complex from HeLa cells and identified NCoR [84]. This complex also contains HDAC1/2, the H3K9-specific methyltransferase G9a, REST (RE-1 Silencing Transcription factor, also known as NRSF), which represses neuronal genes in non-neuronal tissues, along with other proteins that are primarily involved in transcriptional repression. REST not only maintains the transcriptional silencing of a range of neuronal genes in differentiated non-neuronal cells but also plays key roles during lineage commitment in neurogenesis (reviewed in [85]). Tahiliani et al., further showed that KDM5C functions at a subset of REST-regulated neuronal specific genes [84]. It should be noted that NCoR was previously shown to form a stable complex with HDAC3, TBL1, TBLR1, and GPS2 [73], but these proteins were not identified in the KDM5C-containing complex. Thus, it is not clear how NCoR functions in the KDM5C-REST complex. However, a requirement for NCoR in astroglia differentiation was clearly demonstrated [71]. REST modulates not only neuronal, but also glial lineage elaboration [86]. Therefore, it is possible that a unique NCoR-SMCX-REST complex specifically functions in glial development.

Despite its similarity to the NCoR complex, there is no report showing a direct interaction between the SMRT complex and HDMs. However, SMRT appears to control HDM function in trans. SMRT knockout mice exhibit embryonic lethality, with impaired neural development as well as heart defects [72]. SMRT represses the expression of the gene encoding an H3K27-specific demethylase KDM6B (JMJD3), which is also a direct target of the retinoic acid receptor. Retinoic acid induction leads to the release of SMRT from the *KDM6B* gene promoter, and then KDM6B derepresses neurogenic genes in neural stem cells. This finding uncovered

a novel hierarchical relationship between the activities of SMRT and HDM in maintaining the neural stem-cell state.

6. Conclusion

Many HDACs do not function by themselves but act as a component in a multiprotein complex. This complex formation appears to help each HDAC to exert its catalytic activity more effectively and/or specifically. As described above, most HDAC-containing complexes are associated with histone demethylases (HDMs), and the cooperative action of these enzymatic activities enables the complex to act on multiply modified histones at transcriptionally repressive gene regions. Incorporating subtype components gives rise to variations that allow these complexes to repress a wide variety of target genes. Below, we summarize the points raised in this paper.

(1) The two mammalian Sin3 complexes have distinct functions. In embryonic development, mSin3A is required during the preimplantation stage, whereas mSin3B is required in late gestation. In particular, mSin3B is essential for erythrocyte and granulocyte differentiation.

(2) *S. cerevisiae* has two Sin3-containing complexes, Rpd3L and Rpd3S. Rpd3L is required for transcriptional repression at gene promoter regions, whereas Rpd3S is located at transcribed regions and prevents spurious transcription from incorrect transcription start sites.

(3) The *Drosophila* KDM5 protein LID is associated with the SIN3 complex. The LID-SIN3 complex functions to repress the Notch-signaling pathway. Similarly, the human KDM5A protein, RBP2, is required to repress genes of the Notch-signaling pathway.

(4) Two types of MBD protein, MBD2 and MBD3, are distinctively involved in the NuRD complex. Mbd3/NuRD, but not Mbd2/NuRD, is essential for mouse development. Another NuRD component, MTA1/MTA2/MTA3, also contributes to produce distinct complexes. NuRD complexes with each MTA protein bind to specific promoter regions.

(5) Mouse embryonic stem cells contain a specialized NuRD-related complex, NODE. The stem-cell-specific factors Nanog and Oct4 are associated with the NODE complex, which represses several differentiation marker genes.

(6) LSD1 (KDM1A) is associated with the NuRD complex. There are three distinct LSD1-NuRD complexes, LSD1/MTA1/NuRD, LSD1/MTA2/NuRD, and LSD1/MTA3/NuRD. These three different complexes target distinct yet overlapping sets of genes.

(7) LSD1 is also associated with the CoREST complex. Furthermore, all the CoREST complex components are included in the CtBP-containing complex. Many of these components are required for the repression of the *E-cadherin* promoter.

(8) NCoR/SMRT is the transcriptional repression machinery for nuclear-receptor-mediated transcription. NCoR has critical roles in neural differentiation and hematopoiesis, whereas SMRT is required for heart development. KDM4A is associated with the NCoR complex, and its association is required for *ASCL2* gene repression. The NCoR complex also binds SMCX (KDM5C), and the NCoR-SMCX-REST complex functions in glial development.

The transcriptional repression machinery is highly elaborate, and we still lack a complete picture of the transcriptional repression mechanisms. Future studies will continue to uncover these complicated mechanisms.

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