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## Biochemistry of Multiprotein HDAC Complexes

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### SUMMARY

Histone deacetylases perform an important role in the regulation of transcription by modifying the histone components of chromatin. This imparts specific restrictions to transcription and contributes to the proper coordination of gene expression. In order to perform these functions and to achieve proper modulation of their activity, HDACs associate with other proteins, and in some cases, even with themselves. The purification and analyses of these complexes during the last few years has changed our view of the functions of these enzymes, as well as how they are regulated and interconnect with other chromatin-related activities. We are starting to understand how a limited number of HDACs can perform such a variety of functions. Here we review all the known HDAC-containing complexes including classes I, II, and III and we

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**Acronyms and Abbreviations**


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Abf-1	Activated B-cell factor-1
ACF1	ATP-utilizing chromatin assembly and remodeling factor 1
ADA3	Transcriptional adapter 3
ALL-1, (same as MLL, HRX, HTRX)	Acute lymphoblastic leukemia 1
AP-1	Activator protein 1
APPL-1,-2	Adaptor protein containing PH domain, PTB domain, and leucine zipper motif 1,2
ASAP	Apoptosis- and splicing-associated protein
BAF57,-60a,-170	BRG1-associated factor 57, 60a, 170
BCH110	BRAF-HDAC component 110
Bcl6	B-cell lymphoma 6
BRG1	Brm/SWI2-related gene 1
BTB/POZ	BR-C, ttk, and bab/poxvirus and zinc finger
CaMK	Ca <sup>2+</sup> /calmodulin-dependent kinase cells
CoREST	Corepressor of REST
Cpr1p	Cyclophilin A peptidyl-prolyl isomerase
C-Ski	Sloan-Kettering virus isolates
CtBP	Carboxyl-terminal binding protein
CTCF	CCCTC-binding factor
CTIP2	Chicken ovalbumin upstream promoter transcription factor-interacting protein 2
DNMT1	DNA (cytosine-5)-methyltransferases
Ebi	Epidermal growth factor receptor regulator
Eto	Eight twenty-one transcription factor
EuHMT	Euchromatic histone-lysine <i>N</i> -methyltransferase
FAD <sup>+</sup>	Flavin adenine dinucleotide
FLO10	Flocculation factor 10
FOXO	Forkhead box
G9a	Euchromatic histone-lysine <i>N</i> -methyltransferase
GSP2	G protein pathway suppressor
HES	Homeobox gene in ES
HML	Silent mating type loci L
HMR	Silent mating type loci R

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summarize the implications of their composition to the function for HDACs *in vivo*.

**Key Words:** *BCH110*, chromatin, CoRest, HDAC1-11, deacetylation, histone tails, *MBD2*, *MeCP1*, *Mi2*, *MTA1*, *MTA2*, *MTA3*, NCoR/SMRT, NURD, RENT complex, repression, *Sin3*, *Sirt1-7*, TEL complex.

## INTRODUCTION

The year 1996 was a momentous one for members of the chromatin community. Two reports provided the long-awaited connection between chromatin and the regulation of gene expression. In one report, Allis and coworkers (1) described a histone acetyltransferase and discovered that it was *GCN5*, whose gene had been previously identified in yeast using a genetic screen that scored for transcriptional regulators. In a second report, Schreiber and coworkers (2), revealed that a histone deacetylase (HDAC) resides in a mammalian homolog of yeast *RPD3*, a gene isolated in genetic screens scoring for transcriptional repressors. These two seminal findings, together with subsequent observations demonstrating that the enzymatic activities of *Gcn5* and *Rpd3* are regulated through their association with other proteins opened the door for chromatin research in the context of transcriptional regulation. We now know that many transcription regulators contain activities that covalently modify the histone tails. In this chapter we describe the different HDACs and their regulation.

HDACs perform an important role in the proper regulation of cellular functions through their connection with chromatin and transcriptional regulation. To perform these functions and to achieve proper modulation of their activity, HDACs associate with other proteins and in some cases even with themselves. The purification and analyses of these complexes during the last nine years has changed our view of the functions of these enzymes, as well as how they are regulated and interconnect with other chromatin-related activities. These endeavors have begun to reveal how a limited number of HDACs can perform such a variety of functions (3,4).

## CLASS I HDACs

Class I HDAC members, which are defined by their homology to the yeast HDAC *Rpd3*, are *HDAC1*, -2, -3, -8, and -11 (5). Complexes have been described for HDAC-1, -2, and -3, whereas little is known about the proteins interacting with *HDAC8* and -11. Class I deacetylases access specific regions of DNA, yet they lack DNA binding activity. Access to DNA is facilitated through the large number of transcription and chromatin-related factors that interact with and recruit class I HDACs to specific chromosomal regions. These include the sequence-specific DNA binding

### Acronyms and Abbreviations (*Continued*)

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Hos2p	High osmolarity sensitivity two
HOXA9	Homeobox protein A9
Hst1	Homolog of Sir2p, 1
HTLV-1	Type I human T-cell leukemia virus
Ini1	Integrase interactor protein 1
IR10	WD-repeat protein
ISWI	Imitation-switch
KAP-1	KRAB-interacting protein 1
KRAB	Krüppel associated box
Ku70	Lupus Ku autoantigen protein p70
Mad/Max	MAX dimerization protein 1/MYC associated factor X
MAPK	Mitogen activated protein kinases
MARK	Microtubule affinity regulating kinase
MBD	Methyl-CpG binding domain
MeCP2	Methyl-CpG binding protein 2 gene
MEF2	Myocyte enhancer factor 2
Mi2	Dermatomyositis-specific autoantigen
MITR	MEF2-interacting transcription repressor
MLL-1	Myeloid/lymphoid leukemia 1
Mnt	MYC antagonist
MTA2	Metastasis-associated protein 2
Mxi1	Max interactor 1
Myb	Avian myeloblastosis virus oncogene
Nan1p	Net1-associated nuclear protein
NCoR	Nuclear receptor corepressor
Net1p	Nucleolar silencing establishing factor and telophase regulator 1
NF-κB	Nuclear factor κ-B
NLS	Nuclear localization signal
Nop1	Nuclear protein one
NoRC	Nucleolar chromatin remodeling complex
NuRD	Nucleosome remodeling and histone deacetylase
ORC1	Origin replication complex 1
PCAF	p300/CBP associated factor

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factors *YY1* (6), *Mad-Max* (7), *Runx2* (8), *RBP-1* (9), and *Sp1* (10), the insulator factor *CTCF* (11), the corepressors *Sin3* (12,13), *SMRT*, and *NCoR* (14,15), the DNA methyltransferase *DNMT1* (16), and the H3 histone methyltransferase *Suv39H1* (17), among others. The retinoblastoma protein *Rb*, a regulator of cell growth, also binds *HDAC1* (18). Additionally, promyelocytic leukemia is caused by an oncoprotein, produced by fusion of the *PML* and *RAR- $\alpha$*  genes, which recruits class I HDACs to repress transcription of specific genes (19). Class I members exhibit very weak activity in isolation and their multiple functions require interactions with specific factors that modulate the response to different stimuli. Thus, class I HDACs are found in vivo as part of protein complexes that provide the appropriate structural, functional, and regulatory environment to elicit their activity.

### ***HDAC1 and HDAC2 Complexes***

#### **THE SIN3 HDAC COMPLEX: FROM YEAST TO HUMANS**

*Sin3* was discovered to be a global repressor of transcription in yeast (20). *Sin3* functions as a suppressor of the transcriptional activator *Swi5*, which is required for expression of the *HO* gene. Null mutations in *Sin3* consistently relieve the requirement for *Swi5* in *HO* expression (21,22), implicating *Sin3* as a repressor of transcription. *Sin3* is a 175-kDa protein that contains four putative, paired amphipathic helix (PAH) domains (23) but is devoid of known DNA binding domains. *Sin3* is directed to target sites through association with other proteins. The *Sin3*-PAH domains have been found to be involved in protein–protein interactions and to mediate interactions with the DNA binding and transcriptional repressors *Mad*, *Mxi1*, and *Mnt*, which are discussed later (12,13,24,25).

#### **Yeast *Rpd3* and *Sin3***

The histone deacetylase that defines class I HDACs is the budding yeast enzyme *Rpd3*. It was shown to be the enzymatic component of a multiprotein complex (2,26). *Rpd3* was originally isolated as a repressor of the same set of genes repressed by *Sin3* (27). This suggested a genetic link between *Sin3* and *Rpd3*, and, as expected, they were shown to exist together in a large multiprotein complex (28). As with *Sin3*, *Rpd3* did not exhibit DNA binding activity, indicating the need for its interaction with DNA binding proteins to facilitate its access to chromatin for transcriptional repression.

#### **HDAC1 and HDAC2**

Whether it be a single cell of budding yeast or multicellular organisms, the regulation of gene expression requires the same basic components, such as HDACs.

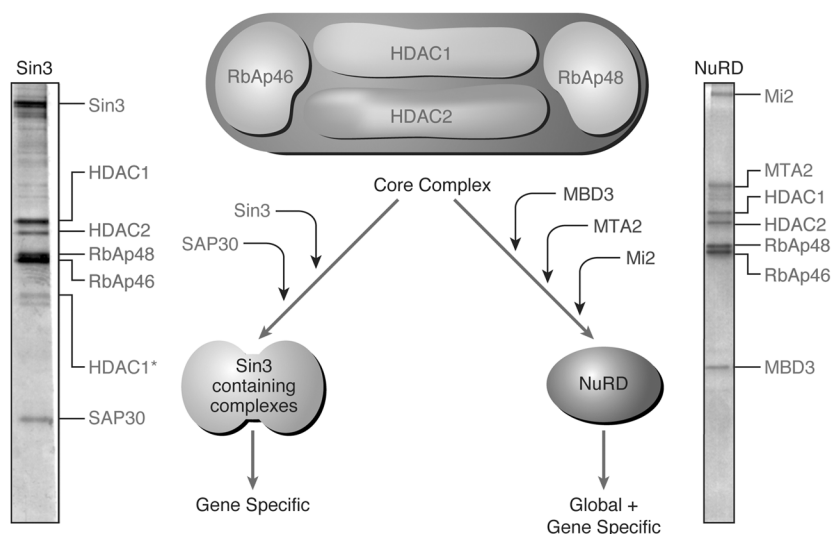
### Acronyms and Abbreviations (*Continued*)

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PHD	Plant homeodomain
PML	Promyelocytic leukemia
PPAR- $\gamma$	Peroxisome proliferator activated receptor, gamma
Pyr	Pyrimidin-rich binding, SW1/SNF related complex
Rab5	
RAD21	Radiation-sensitive mutant 21
Rap1	Repressor/activator protein 1
RAR- $\alpha$	Retinoic acid receptor $\alpha$
RAS	Harvey sarcoma virus transforming gene
RbAp	Retinoblastoma-associated protein
RBP-1	Retinoblastoma binding protein 1
RENT	Regulator of nucleolar silencing and telophase
REST/NRSF	RE1-silencing transcription factor/Neuronal restricted silencing factor
ROR $\gamma$	Retinoid-related orphan receptor $\gamma$
Rpd3	Reduced potassium dependency three
RunX2	Runt-related transcription factor 2
SA1/SA2	Stromal antigen 1/2
SANT	SWI3, ADA2, NCoR, and TFIIB B
Sap	Sin3 associated protein
SBE	Smad-binding element
SET	SU(VAR)3-9, enhancer of Zeste, Trithorax
SFL1p	Suppressor gene for flocculation 1
SID	Sin3 interacting domain
Sif2p	SIR4 interacting factor 2
Sin3	Switch-independent three
Sir2p	Silent information regulator 2
siRNA	Small interfering RNA
SirT1	Sir2-like (Sirtuin)1
SMC	Structural maintenance of chromosomes factors
SMRT	Silencing mediator for retinoid and thyroid hormone receptors
SNF2h	Sucrose nonfermenting 2 homolog
Sntp	Two SANT domains

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**Fig. 1.** Polypeptides composing the Sin3 and NuRD corepressor complexes. The scheme in the middle of the figure illustrates the core complex of the Sin3 and NuRD repressor complexes, composed of histone deacetylases 1 and 2 (*HDAC1/HDAC2*) and the histone binding proteins *RbAp 46* and *48*. This core complex can interact with *SAP30* and *Sin3* or with *MBD3*, *MTA2*, and *Mi2*, forming the Sin3 and NuRD corepressor complexes, respectively. The Sin3 complex interacts directly or indirectly with sequence-specific DNA binding proteins repressing expression of specific genes. The NuRD complex represses transcription more globally but also interacts with gene-specific transcription factors (see Fig. 2). For abbreviations, see Acronyms and Abbreviations table. See Color Plate 1 following p. 180.

Affinity pull-down experiments using the HDAC inhibitor trapoxin resulted in the isolation of human *HDAC1* and *RbAp48* and the novel finding that *HDAC1* contains the enzymatic activity (2). A multiprotein complex was demonstrated to exist in human cells upon the isolation of a human Sin3-HDAC complex (25,29,30). The complex contained the class I *HDAC1* and *HDAC2*, *hSin3*, and the histone chaperones retinoblastoma-associated proteins (*RbAp*) 48 and 46, as well as two novel proteins termed *Sap18* and *Sap30* (Sin3-associated proteins of 18 and 30 kDa, respectively; Fig. 1; see Color Plate 1 following p. 180). These components function together to impart specificity to the complex with respect to its localization to certain regions of the genome as well as to regulate its transient activity. Although initial studies described *Sap18* as part of the Sin3 complex (25), the majority of *Sap18* isolated from HeLa cells was actually found in a complex that did not contain *Sin3* or *HDAC1/2*; it appears to function during apoptosis and in the regulation of splicing (ASAP) (31).

Acronyms and Abbreviations (*Continued*)

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Sp1	Specificity protein-1
Srg3	SWI3-related gene product 3
Sum1p	Suppressor of uncontrolled mitosis
Suv39H1	Suppressor of position-effect variegation 3-9 homolog 1
Swi/Snf	Switch/sucrose nonfermenting
SWI3	Matting-type switching defective mutant 3
TAFI68	TBP-associated factors Pol I 68
Tax	HTLV-1 trans-acting transcriptional activator
TBL1	Transducin $\beta$ -like protein 1
TBLR1	Transducin $\beta$ -like related protein 1
TEL complex	Telomere complex
TFIIIB	RNA polymerase III transcription factor B
TGF- $\beta$	Transforming growth factor $\beta$
TSA	Trichostatin A
Tup1	Deoxythymidine monophosphate uptake factor 1
UbcH5	E2 ubiquitin conjugating enzyme H5
Ume6p	UAS <sub>PHR1</sub> multi copy enhancer six
WCRF180 (same as ACF1)	Williams syndrome transcription factor-related chromatin remodeling factor 180
XFIM	X-linked mental retardation, zinc finger protein 261
YIL112w	Ankyrin repeats-containing protein
YY1	Yin-yang 1

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*Sap30* was also shown to be present and genetically linked to a Sin3 complex in budding yeast, suggesting that the Sin3 complex performs conserved functions in all eukaryotes in terms of gene repression (32). Analyses of *Sap30* resulted in the isolation of two Sin3-containing complexes from human cells, the Sin3 complex described above and a complex containing the p53 binding candidate tumor suppressor *p33<sup>ING1</sup>* (33). The interaction of *p33<sup>ING1</sup>* with *Sin3* is mediated through *Sap30*. Interestingly, our studies (33) as well as those of others (34) revealed that the Sin3-HDAC complex can also interact with the Swi/Snf chromatin remodeling complex. Again, *Sap30* was found to link the Sin3-HDAC complex to Swi/Snf (33). *Sap18* was not isolated in these complexes, providing further evidence that it is probably not a member of the Sin3 complex.

It is clear that the class I HDACs present in the complex provide the enzymatic activity, whereas Sin3 appears to function as a switchboard that

coordinates the interaction between HDACs and sequence-specific DNA binding proteins (*see* Fig. 1 and the NurD section). The RbAp proteins appear to have a role in stabilizing the interaction of this complex with the core histones present in nucleosomes. The Sin3-HDAC complex may also impart substrate specificity once it is recruited to chromatin. Its role in vivo was confirmed with chromatin immunoprecipitation (ChIP) experiments performed in a yeast strain in which *Rpd3* was deleted. This strain exhibited increased acetylation of all histone residues, except for H4 lysine-16 (35). In vitro, the human Sin3-HDAC complex was able to deacetylate all of the core histones when in isolation, but not when composing nucleosomes, suggesting that chromatin remodeling precedes deacetylation in vivo (32).

*Sin3* protein from human or yeast is large and capable of multiple interactions. In yeast, the DNA binding and transcriptional repressor of meiotic genes *Ume6p* interacts with the Sin3-HDAC complex and specifically interacts with *Sin3* in a yeast two-hybrid screen (36). Repression by *Ume6p* depends on the enzymatic activity of *Rpd3*.

In higher organisms, a number of transcriptional regulators were shown to bind the Sin3 complex and direct it to specific genes. These are the zinc finger DNA binding proteins *Ikaros* and *Aiolos* (37), the helix-loop-helix heterodimers of the Mad family *Mad/Max* and *Mxi1/Max*, and the Sin3-interacting domain (SID) containing the protein Mnt, which interacts with *Max* (38). *C-Ski* was also found to interact with *Sin3*. *C-Ski* is part of a complex containing *Smad3/4* that binds to the Smad DNA binding element (SBE) and is involved in transforming growth factor (TGF)- $\beta$  signaling (39). NoRC, an *SNF2h*-containing nucleolar chromatin remodeling complex that represses ribosomal gene transcription, has also been shown to recruit *Sin3* (40). *MeCP2*, a methyl binding domain (MBD)-containing protein that binds to and represses promoters containing methylated CpG, also appears to recruit the Sin3 complex specifically (41). The corepressors NCoR/SMRT bind to unliganded nuclear hormone receptors and recruit the Sin3 complexes to repress transcription at the receptor-targeted genes (14,15,20). The interaction with NCoR is mediated through both *Sin3* and *Sap30*, as *Sap30* appears to stabilize the interaction with NCoR/SMRT (42).

Interaction between the class I HDACs and the corepressor COOH-terminal binding protein (*CtBP*) has been reported. *CtBP* was originally discovered based on its interaction with the C terminus of the adenovirus *E1A* protein and was later found to bind the *Drosophila* DNA binding proteins *Hairy*, *Snail*, *Krüppel*, and mammalian *Krüppel-like factor 3* (*BKLF/KLF3*). Interestingly, CtBP-mediated repression of transcription has been reported to be sensitive to the HDAC inhibitor TSA at some promoters and insensitive at others (43). *CtBP* was found to retain transcriptional repression

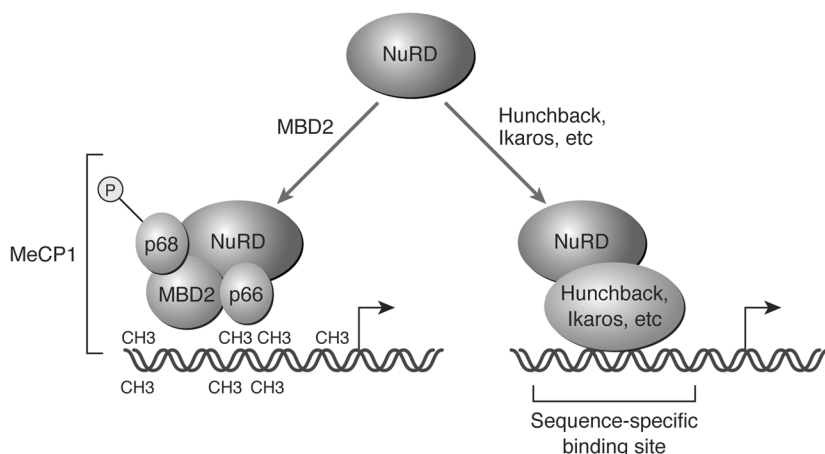
in *Drosophila* embryos deficient in *Rpd3*, suggesting that *CtBP* can repress transcription through alternate means (43).

Class I HDACs are thus highly regulated by virtue of their association with Sin3. This is evident by Sin3-mediated interaction with disparate sequence-specific DNA binding proteins and also through *Sin3* interaction with other corepressors such as *CtBP* and NCor/SMRT that interact with other sequence-specific DNA binding proteins. HDACs are thus directed to target genes and participate in their regulation during differentiation and development and in response to specific environmental stimuli.

## NuRD

In higher eukaryotes, there is another group of complexes comparable in abundance and diversity of function to Sin3-containing complexes. The term nucleosome remodeling and deacetylase complex (NuRD; also NURD or NRD) encompasses a group of complexes from *Caenorhabditis elegans* to mammals that are involved in gene silencing, cell cycle progression, and development (44–48). NuRD contains the core components of the Sin3 complex (Fig. 1), but associates with a different set of polypeptides (*Mi2*, *MTA2*, and *MBD3*), which target the complex to different sites in the genome. Additionally, NuRD can associate with different polypeptides, resulting in the formation of “supra”-NuRD complexes exhibiting diverse NuRD-dependent functions. NuRD imparts a new strategy to the functioning of HDAC complexes as it contains two different types of chromatin-modifying activities: histone deacetylation (regulation through covalent modification of histones) and ATP-dependent chromatin remodeling activities (nucleosome mobilization/alteration). Thus, in the NuRD context, optimal deacetylation involves the ATP-remodeling machinery such that the histone tails are presented in the proper configuration (49). The presence of an activity that mobilizes/alters nucleosome structure in NuRD is compatible with its having a more general function in the maintenance of global chromatin structure.

As in the case of the Sin3-HDAC complexes, NuRD complexes must be brought to the chromatin vicinity to perform their function. This is accomplished in different ways (Fig. 2; see Color Plate 2 following p. 180). First, specific DNA binding transcription factors can recruit NuRD to specific genes, as in the case of the HOX genes (50,51). Second, certain factors with broader DNA binding capacity, such as the CpG-methyl binding protein *MBD2*, recruit NuRD to certain genomic regions containing methylated-DNA (Fig. 2), such that the target may outreach a single gene (52). Third, some studies have shown that NuRD complexes can perform house-keeping roles in the general regulation of chromatin, through a constitutive association that is independent of recruiters (53). This may contribute to the more general dynamics of chromatin structure through the processes



**Fig. 2.** The NuRD complex. This complex can interact with sequence-specific DNA binding proteins and thus be directed to specific genes, as illustrated on the right side. NuRD can also interact with *MBD2* and a polypeptide that exist in phosphorylated (p68) and unphosphorylated (p66) forms. This arrangement of polypeptides composes the MeCP1 complex. The *MBD2* subunit tethers the complex to CpG-methylated DNA. For abbreviations, see Acronyms and Abbreviations table. See Color Plate 2 following p. 180.

of acetylation and deacetylation. This function is probably related to the capacity of NuRD to bind to the histone H3 tails (54,55).

That NuRD can be recruited to sites independent of sequence-specific DNA binding proteins may be related to its association with an activity that alters/mobilizes nucleosomes. This is in contrast to the Sin3 complex that is recruited to specific promoters through interactions with sequence-specific DNA binding proteins, although, in general, these proteins can also recruit factors that alter the structure of nucleosomes. Although the NuRD complex can target specific genes through interaction with sequence-specific DNA binding proteins, this appears to be much more restricted relative to the case of Sin3. NuRD appears to function in a more global manner, based on its recruitment to the histone H3 tail and CpG-methylated DNA.

### Components of the NuRD Complexes

All NuRD complexes share a similar organization that includes a core complex identical to that of Sin3, containing *HDAC1* and *HDAC2* in mammals (or *Rpd3* in lower organisms) and *RbAp46/48*. In the case of NuRD, however, the complex also contains *MBD3* and *MTA1/2* and the ATP-dependent chromatin-remodeling protein Mi2 (Fig. 1). In fact, many

NuRD complexes with different properties and specificities have been isolated (56).

This heterogeneity is one of the defining traits of NuRD complexes. It is evidenced not only by the presence or absence of certain factors but also by the variability among family members of the components that are integral. The most logical explanation of this phenomenon is an evolutionary one: the proteins that were unique in lower organisms diversified to perform new specific functions in a progressively more complex environment within the context of these complexes (48).

With regard to the NuRD-specific proteins, *Mi2* (the dermatomyositis-specific autoantigen) exhibits the least variability. *Mi2* exists in two forms, *Mi2 $\alpha$*  (or *CHD3*) and *Mi2 $\beta$*  (or *CHD4*) (57). Although the predominant form in the NuRD complexes is *Mi2 $\beta$* , reports indicate the presence of *Mi2 $\alpha$*  in some of the complexes (46,56). *Mi2* is important for NuRD enzymatic activity and for recruiting NuRD to multiple targets. Thus, *Mi2* can interact with multiple factors like the corepressor *KAP-1* through its KRAB domain (58), the retinoid-related orphan receptor (*ROR $\gamma$* ) (59), the zinc finger transcription factor *Ikaros* in lymphocytes (51), and the *Drosophila* transcriptional repressors *Tramtrack69* (60) and *Hunchback* (50).

Probably the most important paradigm of variability in proteins composing NuRD is the MTA family of metastasis-associated proteins (or MTAs). These have three members in vertebrates, *MTA1–3*, that have been implicated in cancer progression, metastasis, cell differentiation, and cell type-specific transcription (56, 61). *MTA1* and *MTA3* are present in different isoforms, bringing the total number of proteins to six (*MTA1*, *MTA1s*, *MTA1-ZG29p*, *MTA2*, *MTA3*, and *MTA3L*) (62). All three MTAs have been found in NuRD complexes and, based on their distinctive patterns of expression and performance, they may be responsible for one level of specificity in the functioning of the NuRD complexes. For instance, *MTA1* and *MTA3* expression is cell type specific, in contrast to the ubiquitous *MTA2* (56,61,63). This suggests that *MTA2* may be involved in the housekeeping functions of NuRD, whereas the other two components may participate in specialized repression. *MTA2*, for example, interacts with the multifunctional transcription factor *YY1*, whereas *MTA1* does not (61).

*MTA1* is detected in multiple cancer cells and its presence is associated with the uncontrolled growth and invasive properties of multiple types of tumors (56,61,64). Although its main function is not completely known, it is commonly believed that it may involve the recruitment of the NuRD complex to specific genes.

*MTA3* seems to be highly expressed in breast cancer, and its expression and function seem to be dependent on the activation of the estrogen receptor. In particular, *MTA3* inhibits the expression of the transcription factor *Snail* by bringing NuRD to its promoter (65). *Snail* is a key factor for the



expression of the E-cadherin glycoproteins involved in cell adhesion; thus defects in *Snail* expression would impact on tumor suppression, development, and cell polarity (56,65).

Similar to *Mi2*, in addition to their recruitment function, MTA proteins are also important for NuRD activity. Reconstitution studies showed that the core NuRD complex has a remarkably weak HDAC activity and that its optimal activity in vitro requires the presence of the MTA2 SANT domain (52). SANT domains (from *SWI3*, *ADA3*, *NCoR*, and *TFII-IB*) (66) resemble the DNA binding domains of Myb-related DNA binding proteins and are also present in transcription factors and in proteins associated with HDAC class I complexes like CoREST and SMRT (see sections entitled CoREST Complex and HDAC3 Complexes below).

Finally, another important component of the NuRD complex is a member of the MBD family of methyl-DNA (mCpG) binding proteins found in higher eukaryotes and involved in transcription repression and DNA repair (67). Of the four different MBD proteins (*MBD1–4*) found in mammals, only *MBD3* seems unable to bind to CpG methyl-DNA directly (52). This is in spite of its extensive homology to the well-studied CpG methyl-DNA binding protein *MBD2* and the fact that the *MBD3* orthologs in lower organisms can bind to CpG-methylated DNA (68). *MBD3* is the only member of the family that is present constitutively in the NuRD complexes.

Although *MBD2* is not a component of the NuRD complexes, it can recruit NuRD to CpG-methylated DNA regions (Fig. 2). *MBD2* copurifies with NuRD in the large supercomplex termed MeCP1 (52,69, see that section just below). Notably, the link between DNA methylation and NuRD activity suggests a functional link between histone deacetylation and methylated DNA regions.

Interestingly, the composition of the NuRD complexes found in lower organisms provides a clue to the common origin of *MBD2* and *MBD3*. For instance, the *Drosophila* NuRD complex contains *MBD2/3*, an ortholog to *MBD2* and *MBD3* that possesses characteristics of both proteins (4,48,56,70).

### Supra-NuRD Complexes

In some cases, NuRD has been found as part of larger complexes, or supercomplexes, that incorporate a considerable number of factors and that, in general, seem to be involved in highly specific functions.

**MECP1 COMPLEX.** The MeCP1 complex is able to bind to CpG-methylated DNA containing more than 10 methyl-cytosines and participates in gene repression (71). MeCP1 is formed by an *MBD3*-containing NuRD complex in conjunction with *MBD2* and the two associated proteins *p66* and *p68* (Fig. 2). The *p68* protein is a posttranslationally modified version of *p66*, and both seem to be implicated in the recruitment of MeCP1 to specific loci

(72). MeCP1 can also interact with *APPL1* and *APPL2*, two effectors of the GTPase *Rab5* that are involved in signal transduction and endocytosis (73). Although the functional relevance of these interactions is currently unknown, they may signify a new level of regulation by MeCP1 upon cellular exposure to external stimuli.

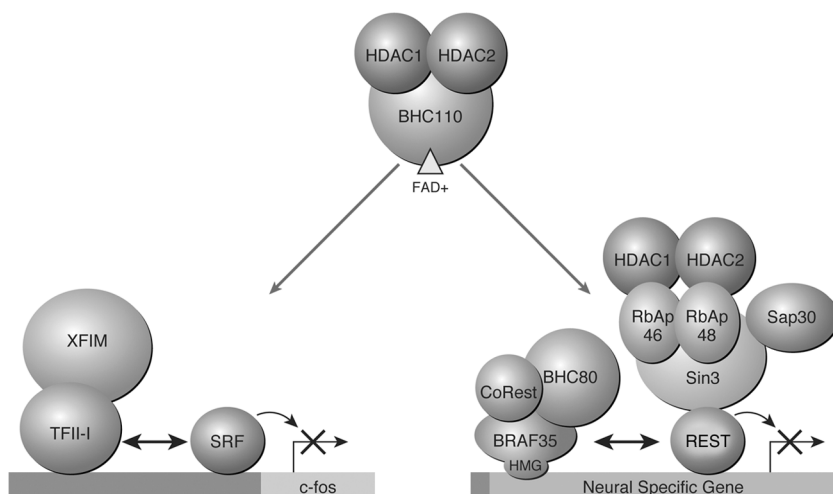
**COHESIN COMPLEX.** Recent studies suggest that NuRD might be involved in functions other than transcriptional silencing, as a cohesin complex was isolated in association with NuRD (74). The isolated complex contains the ISWI-type of ATP-dependent remodeling factor *SNF2h*, the *MBD3*-containing NuRD complex, *MBD2*, and the core-cohesin complex. The core cohesin complex is formed by *SMC1*, *SMC3*, *SA1/SA2*, *RAD21* (75,76), and WCRF180, also known as Acf1, which is the partner of *SNF2h* in the human and *Drosophila* ACF/WCRF complex (77).

ChIP studies identified regions of Alu repeats within the X chromosome that are in association with the isolated NuRD-cohesin complex (74). The role of NuRD in this context has yet to be characterized.

**ALL-1 COMPLEX.** The largest complex described thus far as containing NuRD is ALL-1. This complex is apparently composed of approx 30 polypeptides and is probably greater than 3 Mda in size (78). The trithorax protein *ALL-1* (also known as *MLL*, *HRX*, or *HTRX*) contains a histone lysine methyltransferase activity with specificity for lysine-4 of the histone H3 tail (79). ALL-1 is essential for the development of hematopoietic stem cells (80). Leukemias involving translocation phenomena generate chimeric proteins containing ALL-1 fused to other partners (80).

The putative ALL-1 complex also included subunits of the RNA polymerase II-associated TFIID complex, as well as subunits of the chromatin remodeling complexes *SNF2h*, *Swi/Snf*, *NuRD*, and *Sin3*. Apparently, all these components were found to coexist at the promoter of the *HoxA9* gene in vivo (78). However, it is our belief that ALL-1 may not represent a unique complex. From the data reported thus far, we cannot exclude the possibility that this group of distinct, but functionally related, complexes associate transiently, rather than as a biochemically stable entity in the cell.

The ALL-1/MLL-1 proteins function in transcriptional activation as ALL-1 to methylate lysine-4 of histone H3, which is known to be involved in transcription activity (78). The presence of HDACs is consistent with the need to deacetylate specific residues in histone H3 for their subsequent methylation. However, the exact role of NuRD is unclear given that NuRD is displaced from the histone H3 tail upon methylation of histone H3 lysine-4 (55). Moreover, duplication in activities in this supracomplex is perplexing. For example, the Sin3 complex is also present, and, in addition to *Mi2*, the complex also has two additional ATP remodeling activities, *Swi/Snf* and *SNF2h*. The existence of this supracomplex in vivo would appear to require additional validation.



**Fig. 3.** BHC110-containing complexes. This lesser known group of *HDAC1/2*-containing proteins is formed by the complexes CoREST and XFIM. Both contain a core of *HDAC1/2* and *BHC110*, a  $\text{FAD}^+$  binding protein with unknown function. CoREST participates together with the Sin3-containing complex in repression mediated by the factor *REST*, which is responsible for silencing of the neuronal-specific genes (right). The CoREST complex can bind to DNA through the HMG domain of *BRAF35*. The XFIM complex is involved in the control of basal *c-fos* gene repression (left) and is probably recruited to the *c-Fos* promoter by serum response factor (*SRF*) which interacts with the DNA-binding factor *TFII-I*. For abbreviations, see Acronyms and Abbreviations table. See Color Plate 3 following p. 180.

**PYR.** PYR is a SWI/SNF-related complex that binds to DNA containing pyrimidine-rich sequences located between the human fetal and adult  $\beta$ -globin-like genes (81). Interestingly, this DNA binding activity is specific to adult hematopoietic cells. The complex contains the lymphocyte-specific transcription factor *Ikaros*, the NuRD core complex except for *HDAC1*, and at least five SWI/SNF complex-related proteins—*Brg1*, *Baf57*, *Baf60a*, *Srg3*, *Ini1*, and *Baf170* (82). The function of PYR is not known, but it might be involved in the switch between fetal and adult  $\beta$ -globin expression.

### BHC110-CONTAINING COMPLEXES

In recent years, a new group of *HDAC1/2*-containing-complexes has been described (83–86). They all contain a core complex formed by *HDAC1/2* and the  $\text{FAD}^+$  binding protein *BHC110* (Fig. 3; see Color Plate 3 following p. 180). The complexes also contain other proteins that confer specificity of function. Two complexes have been described thus far, but preliminary data are suggestive of more to come (86). These complexes are involved in transcriptional repression, although, in contrast to NuRD- and

Sin3-containing complexes, the subset of genes affected is more restricted, with repression seemingly more specialized (85,87,88).

### CoREST Complex

The CoREST/BHC mammalian complex was identified by different investigators as a group of proteins that cofractionated with CoREST, a corepressor of the transcription factor REST/NRSF (83–85). *REST* is responsible for the maintenance of long-term repression of neuronal-specific genes in nonneuronal cells (89). *REST* exerts its function by binding to the Sin3-containing complex through its N-terminal domain (90) and also the CoREST complex through its C-terminal region (91). The CoREST complex is formed by six subunits: the core complex, *CoREST*, BRCA2-associated factor 35 (*BRAF35*), and *BHC80* (Fig. 3) (85).

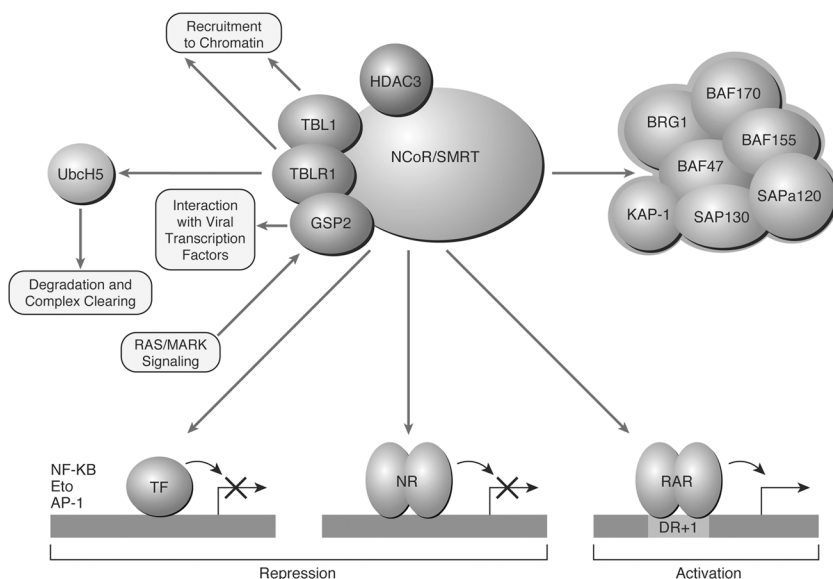
CoREST contains two SANT domains, only the first of which (SANT1) is involved in the interaction with HDACs, being essential for *HDAC1* activation (83). The other component of the CoREST complex, *BRAF35*, was originally discovered as a component of the breast cancer-related factor BRCA2 complex, in which it plays a structural role (92). Its main feature is the presence of an HMG domain that confers an ability to bind DNA, which is critical for the repressive activity of the CoREST complex in vivo (85).

There is not much known about *BHC80*, except that it contains one PHD and two leucine zipper domains (85,93), all of which are involved in protein–protein interactions. However, in contrast to the ubiquitous *BRAF35*, the presence of *BHC80* is highly tissue specific, perhaps reflective of a specialized role (85,93).

Interestingly, a supracomplex that contains the CoREST complex was isolated through affinity purification of the corepressor *CtBP* (94,95). Among the factors comprising this complex are two histone methyltransferases responsible for dimethylation of lysine-9 in the histone H3 tail, i.e., *G9A* and *EuHMT* (96,97). However, as in the case of the ALL-1 complex, there are outstanding issues regarding the existence of such a native complex. These include the fact that the complex seems to contain all of CoREST and that *CtBP* is capable of engaging in interaction with multiple factors; therefore, whether the studies uncovered a supracomplex or a mixed population of *CtBP* complexes remains an open question. Once again, this species may actually be a composite isolated in vitro rather than an entity that exists in vivo. This remains to be clarified.

### XFIM Complex

The XFIM complex contains, in addition to the same core complex as CoREST, the factor *XFIM*, a candidate for X-linked mental retardation, and the DNA binding protein *TFII-I* (Fig. 3) (86). Interestingly, this complex of about 1 MDa contains four molecules of XFIM and is specifically



**Fig. 4.** *HDAC3* complex. On the one hand, the core *HDAC3* complex consisting of NCoR/SMRT and *HDAC3* can form a complex together with *TBL1*, *TBLR1*, and *GSP2* (left part of the figure), although they have not been found in all cases. On the other hand, interaction with SWI/SNF proteins together with KAP1, *SAP130*, and *SAP3a120*, has been described. The role of the first complex is repression of specific genes recruited by transcription factors and by nuclear receptors (NRs). However, reports have also described the involvement of the complex in activation, in the case of retinoic acid receptor (*RAR*) binding to the DR-1 elements. For abbreviations, see Acronyms and Abbreviations table. See Color Plate 4 following p. 180.

recruited to the *c-Fos* promoter by *TFII-I* (98,99). The XFIM complex seems to be involved in the tight control of *c-Fos* gene expression (86). Whereas *c-Fos* levels are tightly repressed, growth factors and other stimuli induce an immediate activation of *c-Fos* gene expression, which then returns to the repressed state soon after the stimulus is gone. ChIP studies have shown that, in vivo, components of the XFIM complex are present at the promoter before and after the stimuli, but not during activation, suggesting an important role for the complex in maintaining a repressed state (86).

### *HDAC3* Complexes

Like *HDAC1* and *HDAC2*, *HDAC3* is involved in transcriptional repression, but *HDAC3* function seems to be less global. In fact, *HDAC3* is involved in repression of a specific group of genes, in particular those connected with nuclear receptor signaling (Fig. 4; see Color Plate 4 following p. 180) (100–102). Interestingly, some reports have also found

a role for *HDAC3* in the transcriptional activation of specific genes responsive to the retinoic acid hormone receptor (103; Fig. 4). In accordance with this, *hos2*, the *HDAC3* homolog in yeast, has been reported to be involved in both repression and activation of specific genes (104).

This more restricted function is also reflected by the nature of the *HDAC3*-containing complexes. So far, different groups have described the purification of several *HDAC3*-containing complexes (102,105–108), and although it is still not clear whether all the subunits reported actually compose the same or disparate complexes, the common constituents are *HDAC3* and the nuclear hormone corepressors NCoR/SMRT (109).

Most of the *HDAC3*-containing complexes described are rather large (1–2 MDa), most of them contain many common subunits, and the combined sizes of the subunits described do not match the complex size observed by gel filtration chromatography (105,108). These observations suggest that there might actually be only a few disparate *HDAC3* complexes, in contrast to the case with NuRD. The most convincingly studied complex (105–108) appeared to contain *HDAC3*, NCoR/SMRT, transducin  $\beta$ -like protein (*TBL1*), transducin  $\beta$ -like related protein (*TBLR1*), and the G-protein pathway suppressor 2 (*GSP2*). Other associated proteins were present in substoichiometric amounts, for example, the coronin-like actin binding protein *IR10* (Fig. 4). Although most reports found *TBL1* as part of the complex (105–108), others did not (102). Discrepancies also involved *GSP2* and *TBLR1*, which were reported by only one (107) and two (107,108) groups, respectively. Additional work is needed to clarify the nature of these complexes.

NCoR and SMRT have been extensively studied because of their wide-ranging role in transcriptional repression from general to cell-type specific, mediated through their interaction with a variety of transcription factors, such as nuclear factor- $\kappa$ B (*NF- $\kappa$ B*) (110), *Eto* (111), *AP-1* (112), homodomain-containing factors (113), and others. However, they also have a particularly important function in hormone receptor signaling (109,114,115). Interestingly, NCoR/SMRT binds to class II HDACs (see below). The enormous implications of the multiple functions associated with NCoR/SMRT fit well with the embryonic lethality observed in *NCoR* knockout mice, with defects in development and cell differentiation (103). In the context of the *HDAC3*-containing complexes, NCoR/SMRT are not only important for the function of the complexes and for interaction with the hormone receptor machinery and other regulators (see above), but are also required for proper *HDAC3* activity. As in the case of the MTA proteins in NuRD and of REST in the CoREST complex, the presence of SANT domains in SMRT is required for full *HDAC3* enzymatic activity (116). However, of the two SANT domains contained in each of these proteins, only the first seems to be involved in this function, in both cases (117).

*TBL1* has intrinsic transcriptional repressive activity and is highly related to *Ebi*, a regulator of epidermal growth receptor signaling in *Drosophila* (118). *TBL1* contains six WD40 repeats that apparently confer chromatin binding ability (105,108). WD40 repeats are also found in eukaryotic corepressors such as *Streptomyces cerevisiae Tup1* (119) and *Drosophila Groucho* (120), and they can recruit HDACs (*Rpd3*) (121). *Tup1* and *Groucho* also exhibit an intrinsic repressive activity, independent of HDACs, which may be related to their interactions with the basal transcription machinery (122,123). The role of *TBL1*, together with *TBLR1*, may be analogous to the histone chaperones *RbAp46/48* in *HDAC1*-containing complexes.

*TBLR1* is highly related to *TBL1* in that it also contains six WD40 repeats and possesses chromatin binding ability (105,108). Using specific siRNA methodology, *TBL1* and *TBLR1* were found to be necessary, but functionally redundant, when tested for repression by unliganded thyroid hormone receptor (108). However, other studies on natural promoters in vivo suggested that *TBLR1* is actually required for clearance of the complex. This entails the recruitment of an ubiquitination complex consisting of the conjugating enzyme *UbcH5* that brings along components of the 19S proteasome degradation system (110,112).

*GSP2*, or *AMF-1*, is involved in the regulation of the RAS/MAPK pathway (124), interacts with the viral transcription factor *Tax* encoded by T-cell lymphotropic virus type I (HTLV-I) (107), and can also repress *JNK1*-activating activity (107,124).

*IR10* contains three WD40 repeats, although it is not highly related to *TBL1* or *TBLR1* (125). *IR10* interacts with *NcoR*, but not *SMRT* (107). It is found in substoichiometric amounts in association with the *HDAC3* core complex formed by *HDAC1/2* and *BCH110* (Fig. 3); its function remains unknown. Interestingly, a related complex has been found in yeast, *SET3C* (126). *SET3C* contains: the SET and PHD domain containing protein *Set3p*, *Hos2p*, the homolog of *HDAC3*, *Sif2p*, a WD40-repeat protein homolog of *TBL1*, *Sntp*, which, like *NCoR/SMRT*, is a SANT domain-containing protein, and *YIL112w*, which contains ankyrin repeats involved in protein-protein interactions. It also contains *Cpr1p* and the class III HDAC *Hst1* (see Class III HDACs section).

One group also reported the purification of another HDAC3-*NCoR/SMRT*-containing complex that included the core of the ATP-dependent chromatin-remodeling SWI/SNF complex, the corepressor *KAP-1*, the splicing-related factor *SAP130*, and the splicing factor *3a120* (127). The SWI/SNF core complex is formed by the SWI/SNF enzyme *BRG1* and associated proteins *Baf170*, *Baf155*, and *Baf47* (Fig. 4) (128). Although functional data are lacking, the presence of the SWI/SNF core complex may facilitate access of the deacetylase to the chromatin substrate, as in the case of *Mi2* in NuRD.

## CLASS II HDACs

The budding yeast *Hda1* is the enzyme that defines this family (24). In humans, class II HDACs are subdivided into classes IIa and IIb (129). As with the class I HDACs, the enzymes of this family perform a wide variety of highly regulated functions. These enzymes also do not contain DNA binding activity, suggesting interactions with other proteins in order to repress transcription (1,3,129). This aspect of class II HDACs will be discussed.

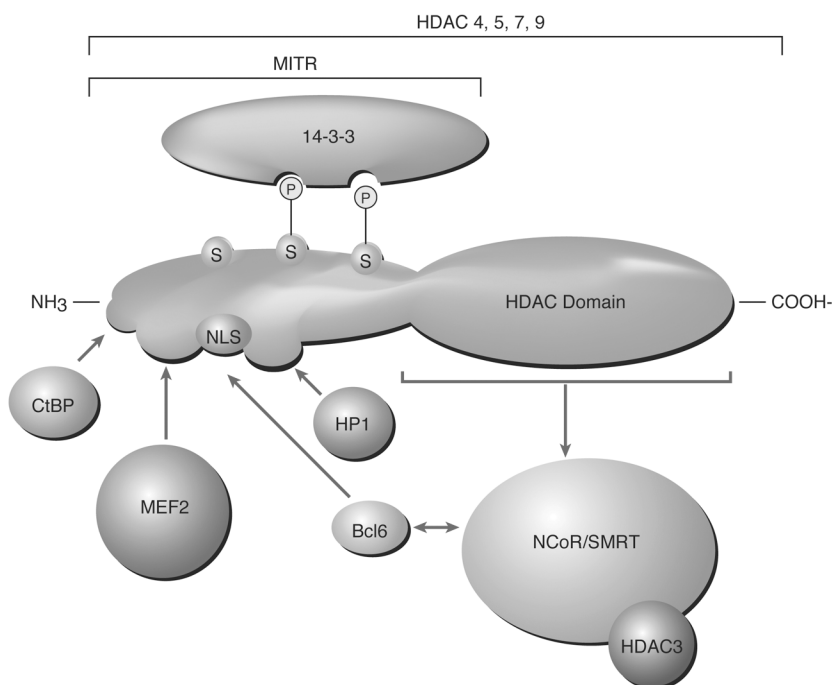
### *HDAC Class IIa*

Class IIa HDACs consist of *HDAC4*, -5, -7, -9, and a splice variant of *HDAC9* that contains only the N-terminus region of the protein, designated myocyte enhancer factor 2 (*MEF2*) interacting transcription repressor (*MITR*) (130). Many interactions regulate the ability of class IIa HDACs to repress transcription (*see* next section). A striking feature of class IIa HDACs, however, is their ability to shuttle between the nucleus and cytoplasm (1,3,129). The cytoplasmic chaperon protein 14-3-3 (131) is responsible for cytoplasmic sequestration of class IIa enzymes. 14-3-3 binds to the HDAC that is phosphorylated on one or two of the three N-terminal serines by calcium calmodulin-dependent protein kinase (*CaMK*), which is activated after  $\text{Ca}^{2+}$  release (132; Fig. 5; *see* Color Plate 5 following p. 180). Once the HDAC reaches the cytoplasm and is thus phosphorylated, complex formation with 14-3-3 sequesters the enzyme in the cytoplasm, thwarting its ability to repress transcription.

### CLASS IIa INTERACTING PROTEINS

Many of the interactions that allow the different class IIa HDACs to repress transcription have been discovered (Fig. 5) (1,3,129,130). Class IIa enzymes bind to the corepressor SMRT/NCoR complex described earlier (which includes *HDAC3*) (116,133). SMRT/NCoR can also be recruited by the human protooncogene *Bcl6* (134). *Bcl6* is a BTB/POZ-zinc finger transcriptional repressor that, upon overexpression, protects B-cell lines from apoptosis induced by DNA damage. In keeping with this activity, recent studies have shown that overexpression of *Bcl6* suppresses *p53* expression (135). However, it has also been reported that *Bcl6* binds to the N-terminus of class IIa HDACs (136). This suggests that *Bcl6* can recruit either the SMRT/NCoR complex, which can then recruit a class IIa HDAC, or the class IIa HDAC directly. Class IIa HDACs do not exhibit enzymatic activity in isolation (129,130) but only in complex with the SMRT/NCoR corepressor complex (137). However, given that this corepressor complex contains the class I *HDAC3*, and that it is enzymatically active without class IIa enzymes, the class IIa HDACs may be redundant.





**Fig. 5.** HDAC IIa interactions. The domain structure of class IIa HDACs is shown. The MITR domain is illustrated. Serines that can be phosphorylated by *CaMK* are represented by S. The phosphorylated proteins (*MITR*, *HDACs 4/5/7/9*) interact with *14-3-3*. Other interaction partners of the class IIa HDACs are illustrated. For abbreviations, see Acronyms and Abbreviations table. See Color Plate 5 following p. 180.

More likely, the enzymatic activity of the full complex (containing both HDACs) may target histone polypeptides as well as nonhistone substrates. This remains to be elucidated.

*MEF2* is an important DNA binding transcriptional regulator (138). It is involved in the regulation of myogenesis, in negative selection of developing thymocytes, and in transcriptional regulation of the Epstein-Barr virus (EBV) (139). Class IIa HDACs bind to *MEF2* through a highly conserved 17-amino acid N-terminal motif (Fig. 5) (129,130,140). When a class IIa HDAC is present in the nucleus bound to *MEF2* at a promoter, the gene is repressed (140,141). However, when the HDAC becomes phosphorylated, the interaction is lost and the HDAC becomes sequestered in the cytoplasm, inducing activation of myogenesis in muscle cells, for example (139,141,142). Another protein that was found to interact with *HDAC4*, -5, and *MITR*, through specific regions of their N-termini, is the transcriptional repressor *CtBP* (143), which was discussed earlier toward

the end of the section entitled HDAC1 and HDAC2, as it also interacts with class I HDACs. Heterochromatin protein 1 (*HPI*) has been reported to interact with *HDAC4*, -5, and *MITR* through a distinct region in their N-termini (Fig. 5) (144). *HPI* binds methylated histone H3 lysine-9 and interacts with histone lysine methyltransferase *SUV39H1* (145,146). The interaction between *HPI* and *HDAC4*, -5, and *MITR* is lost following phosphorylation of the class IIa HDACs by *CaMK*. Because silent *MEF2* genes are methylated at lysine-9 of histone H3 (144) and this methylation has been shown to be a chromatin repressive mark, these findings suggest a possible mechanism whereby deacetylation by a class IIa HDAC precedes methylation to allow for repression (144).

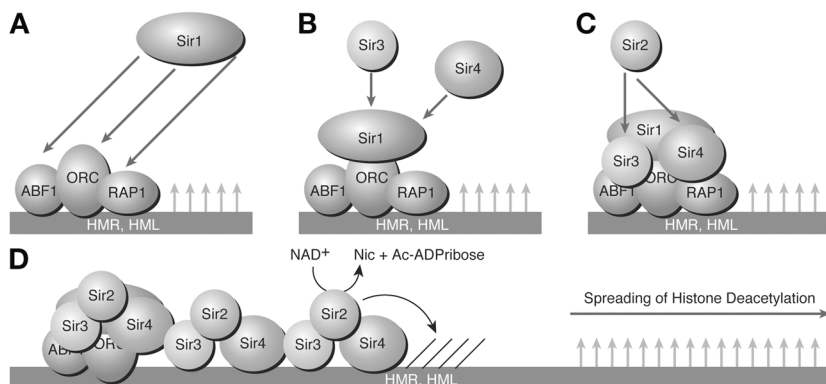
A recent finding illustrates a tissue-specific role for class II HDACs. *HDAC4* gain of function and loss of function mutants display similar phenotypes as the corresponding mutants of *RUNX2*, the DNA binding transcription factor that is involved in bone development; *HDAC4* and *RUNX2* were also shown to interact physically (147).

### **HDAC Class IIb**

Class IIb consists of *HDAC6* and -10. As an *HDAC10* complex has not been described, this section focuses on *HDAC6*. *HDAC6* was found to be a  $\alpha$ -tubulin deacetylase (149,150); it also binds polyubiquitin chains on misfolded proteins. Recently, a link with Parkinson's disease was revealed when *HDAC6* was found to interact with cytoplasmic *dynein*, a microtubule minus end-directed motor protein (148) necessary for the transport of misfolded proteins (148). This evidence suggests that *HDAC6* is an adapter protein that allows aggregated, misfolded, and polyubiquitinated proteins (151) to come together with dynein (152), with subsequent transport to aggresomes. Tubulin hyperacetylation is correlated with more stable microtubules (130). Thus acetylation and deacetylation of tubulin may be important for the movement of such misfolded proteins along the microtubules, highlighting the role of *HDAC6* in this transport. Moreover, *HDAC6* colocalizes with ubiquitin conjugates and  $\alpha$ -synuclein in structures resembling neuronal inclusion bodies, i.e., Lewy bodies, a defining feature of Parkinson's disease (153).

### **CLASS III HDACs**

Class III HDACs are related to the yeast NAD<sup>+</sup>-dependent HDAC silent information regulator 2 (*Sir2p*), which is involved in gene silencing through the generation of heterochromatin-like compacted chromatin that is hypoacetylated in histone H3 and H4 tails. Yeast has four SIR silent information regulator (SIR) proteins, all involved in the formation of specialized repressed chromatin, but only *Sir2p* possesses enzymatic activity on its own (154–156).



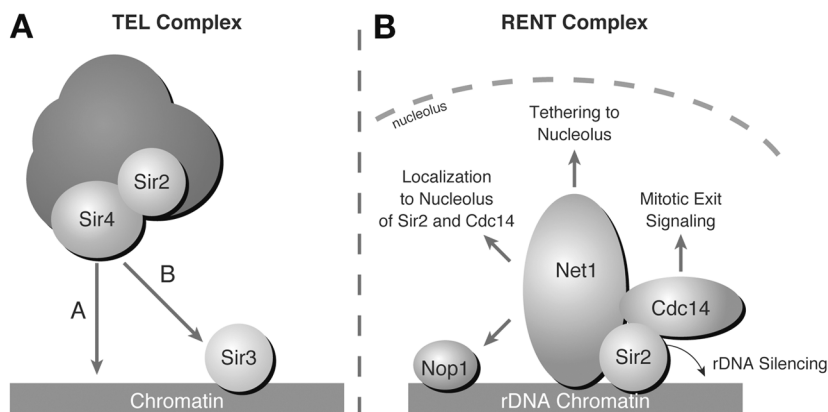
**Fig. 6.** Sequential model of *Sir2p* action at the mating-type Loci. (A) *Sir1p* is responsible for establishment of silencing of the loci, by binding to the factors *Rap1p*, *Abf1p*, and the origin replication complex (ORC). (B) *Sir3p* and *Sir4p* are then recruited and bind directly to histone tails. (C) *Sir3p* and *Sir4p* recruit, in turn, *Sir2p*. (D) Deacetylation and compaction of chromatin occurs in the presence of NAD<sup>+</sup>. The initial assembly of the triplex *Sir2p*-*Sir3p*-*Sir4p* induces the recruitment of more molecules whose spread extends over a few kilobases. For abbreviations, see Acronyms and Abbreviations table. (Adapted from ref. 160.) See Color Plate 6 following p. 180.

Class III HDACs include homologs of *Sir2p* in all higher organisms, including 7 homologs in humans (*SirT1*–*7*) (157,158). In addition, proteins with some similarity to *Sir2p* have been found in bacteria (157). In yeast, a family of four proteins with similarity to *Sir2p* called homologs of *Sir2p* (*Hst1*–*4p*) has also been defined, although not much is known about their function (158). Interestingly, Hstps are probably the true orthologs of the class III members of higher organisms because the SIR machinery is absent in higher eukaryotes. Other evidence supporting this idea is the cellular localization and specificity of these proteins. For instance, *Hst1p* might be the ortholog of *SirT1* and *Hst2p* the ortholog of *SirT2* and *SirT3* (158,159).

### Yeast *Sir2p*

Together with the other Sir proteins, *Sir2p* is involved in the formation of specialized compacted chromatin regions in three specific loci in yeast: telomeres, mating-type (HML and HMR) and rDNA repeats in the nucleolus (160). However, only *Sir2p* is required in all three loci. *Sir3p* and *Sir4p* are involved in mating-type loci regulation and telomeres, whereas *Sir1p* is only involved in mating-type loci (161).

Genetic and biochemical studies suggest a model of sequential recruitment in *Sir2p*-mediated silencing (Fig. 6; see Color Plate 6 following p. 180) (154,155,162). For instance, in the mating-type loci,



**Fig. 7.** *Sir2p*-containing complexes. **(A)** TEL complex components are not fully identified but contain *Sir2p* and *Sir4p* and perhaps partially challenge the sequential model. However, these two models need not be exclusive and together may explain the arrival of the TEL complex at the chromatin. The “A” arrow indicates that TEL could bind independently to chromatin through *Sir4p*, and the “B” arrow shows that *Sir3p* would be responsible for recruitment of the complex to chromatin. **(B)** The RENT complex and its subunits bound to rDNA genes. The functions of the RENT subunits are indicated. For abbreviations, see Acronyms and Abbreviations table. See Color Plate 7 following p. 180.

*Sir1p* is involved in the establishment of silencing by binding to the origin replication complex subunit 1 (*ORC1*), *Rap1*, and *Abf-1*. After *Sir1p* binding, *Sir3p* and *Sir4p* bind to chromatin through interactions with histones H3 and H4 and bring in *Sir2p* (154,155,162).

However, the situation seems to be more complex than this model would suggest. Some studies found that *Sir2p* is essentially present in two large complexes in the cell (163–165). The first one is called the TEL complex (163), a large species of about 800 kDa that contains *Sir2p*, *Sir4p*, and other uncharacterized proteins, but not *Sir1p* or *Sir3p* (Fig. 7; see Color Plate 7 following p. 180). The presence of *Sir2p* and *Sir4p* in the TEL complex suggests that the arrival of *Sir2p* at the chromatin is dependent on the capacity of *Sir4p* to bind chromatin. However, because *Sir3p* binds to *Sir4p* (166,167), it is also possible that *Sir3p* mediates the recruitment of the TEL complex. Additional studies are required to reconcile these observations.

The second complex is called regulator of nucleolar silencing and telophase exit (RENT) (164,165), which is only present in the nucleolus and contains *Sir2p*, *Cdc14p*, *Net1p*, and *Net1*-associated nucleolar protein (*Nan1p*) (Fig. 7). RENT is involved in mitotic exit control, rDNA silencing, and nucleolar localization of *Nop1*, a factor involved in nucleolar

pre-rRNA processing (168). *Cdc14p* is a protein phosphatase that belongs to the mitotic exit network (MEN) (169). *Net1p* is a key factor in the RENT complex, as it is responsible for tethering the complex to the nucleolus (165). Because of the presence of *Net1p*, *Sir2p* and *Cdc14p* localize to the nucleolus, where they can exert their function. It is possible that *Net1p* is also the factor responsible for bringing *Sir2p* to the rDNA repeats. *Net1p* can also stimulate RNA polymerase I activity by binding directly to the enzyme (170).

### *Hst1 and SirT1*

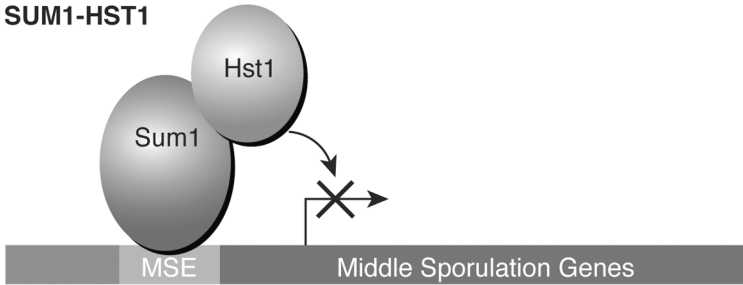
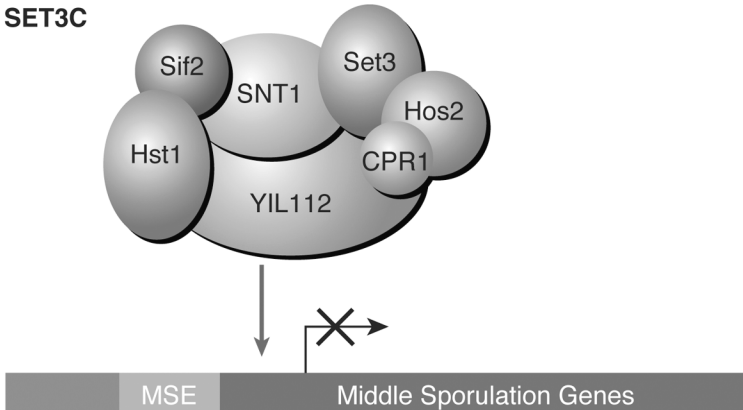
*Hst1p* is probably the best known of the Hst proteins. *Hst1p* is the closest member of the class III HDACs to *Sir2p*, and its main function seems to be related to the repression of middle sporulation genes (171,172).

*Hst1p* forms two different complexes in yeast cells (Fig. 8; see Color Plate 8 following p. 180). One such complex (171,172), together with the DNA binding transcriptional repressor *Sum1p*, participates in middle sporulation gene repression and accounts for almost all the cellular *Hst1p* (173). The second complex that has been described is SET3C (126), the yeast counterpart of HDAC3-containing NCoR/SMRT in higher organisms that also participates in meiotic repression and sporulation. However, *Hst1p* does not seem to be responsible for the activity of the complex, leaving open the possibility that this complex participates in other functions (126). Interestingly, *Hos2p* and *Set3p* have also been found to be involved in the transcriptional activation of certain genes (104). It is unknown whether this function is mediated by SET3C or by an alternative complex that contains both subunits.

### SIRT1

*SirT1* is the member of the human Sir2 family that is closest to *Sir2p*, and it has been reported to deacetylate histones and nonhistone substrates (174). All core histones are substrates in vitro, but *SirT1* exhibits a preference for acetylated lysine-16 of histone H4 and acetylated lysine-9 of histone H3 as well as acetylated lysine-26 of histone H1b (or H1.4) in vitro and in vivo (175). The nonhistone targets are *p53* (176,177), *TAF<sub>i</sub>68* (178), *BCL6* (179), FOXO transcription factors (180,181), *Ku70* (182), and NF- $\kappa$ B (*RelA/p65*) (183).

*SirT1* functions in transcriptional repression and heterochromatin formation, muscle differentiation (184), inhibition of senescence and apoptosis induced by *p53* (176,177), life span extension (182), stress response (180,181), and inhibition of axonal degeneration (185). *SirT1* interacts with multiple factors, most of them transcription factors, which seem to recruit *SirT1* to chromatin specific regions. Among these are *p53* (176,177), *CTIP2* (186), *HES1* and *HES2* (187), *FOXO* (180,181),

**A SUM1-HST1****B SET3C**

**Fig. 8.** *Hst1p* complexes. Two complexes have been described that contain *Hst1p*. (A) *Hst1p* sum1p participates in the repression of middle sporulation genes by the binding of Sum1p to the middle sporulation elements (MSEs). (B) *Hst1p* was found to be part of SET3C, a complex containing *Hos2p* that also participates in middle sporulation gene repression. However, *Hst1p* is not required for the activity of the complex. For abbreviations, see Acronyms and Abbreviations table. See Color Plate 8 following p. 180.

NF- $\kappa$ B (183), NCoR and SMRT (188), PPAR- $\gamma$  (188) and the histone acetyltransferase PCAF (184). Importantly, SirT1 also interacts with histone *H1b* (175), and recent studies have demonstrated that recruitment of *SirT1* to specific genes results in the formation of repressed chromatin (175). This includes the recruitment of histone *H1b* and the modification of the histone H3 and H4 tails with “marks” that are the signature of repressed chromatin.

The native form of *SirT1* is a homomultimer of about 350–400 kDa that most likely corresponds to a trimer (175), although minor amounts of the enzyme may be present in other complexes. This fits well with predictions of trimer formation based on structural studies of other members of the class III family (189).

### *Hst2p, SirT2, and SirT3*

*Hst2p* is a homolog of *Sir2p* in yeast and is part of the class III NAD<sup>+</sup>-dependent HDACs (157). It has been shown to be one of the most enzymatically active of the class III enzymes (190). It is completely sequestered in the cytoplasm, suggesting a substrate other than histones (191). The function of *Hst2p* remains largely unknown, as a multiprotein complex has not been isolated. Some evidence suggests that *Hst2p* is involved in the cell cycle as well as in epigenetic regulation of a select group of genes (174,192). However, given its cytoplasmic localization, it is unclear how and when *Hst2p* could travel to the nuclei to participate in these functions.

#### **SirT2**

In humans, *SirT2* and *SirT3* appear to have the closest homology to *Hst2p* (157,158). *SirT2* may be most homologous, given its almost exclusive localization to the cytoplasm (190,193). *SirT2* was shown to colocalize with tubulin as well as to be able to deacetylate acetylated  $\alpha$ -tubulin (193). Immunoprecipitation experiments with *SirT2* brought down the other known tubulin deacetylase *HDAC6*, suggesting a functional complex between these proteins (193). This is an interesting function that must have been acquired during evolution considering that acetylated tubulin has not been found in budding yeast. This also suggests that human *SirT2* and *Hst2p* may share an even more basic function. Multiprotein complexes containing *SirT2* have not yet been characterized. *SirT2* has been shown to be upregulated during mitosis and can affect the cell cycle when overexpressed (194). Both *Hst2p* and *SirT2* have been shown to inhibit starfish oocyte maturation in microinjection experiments, and *Hst2p* was also shown to delay starfish embryonic cell division in similar experiments using daughter blastomeres (195).

It is also a possibility that *SirT2* and *Hst2p* silence genes. Both *Hst1p* and *Hst2p* are recruited specifically by *Sfl1p* to a specific region of the *FLO10* promoter located near the telomere and are required for silencing of the gene (191). Deletion of *Sir2p* had no effect on this silencing. Both of these findings suggest that *Hst1p* and *Hst2p* (and probably *SirT2*) participate in complex protein interactions yet to be discovered.

#### **SirT3**

*SirT3* is an equally interesting protein. Although it is closely related to *Hst2p* and *SirT2*, it has been reported to be a mitochondrial protein (196,197). *SirT3* has not been found in a multiprotein complex, leaving its role unclarified for the present. *SirT3* has been shown to be cleaved at the N terminus by an interaction with matrix-processing peptidase (*MPP*) once it has reached the mitochondria, resulting in a truncated form of *SirT3* (196,197).

**SIRT4–7**

*Sirt4–7* have the least homology to *Sir2p* and remain virtually unstudied.

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