HATs and Disease

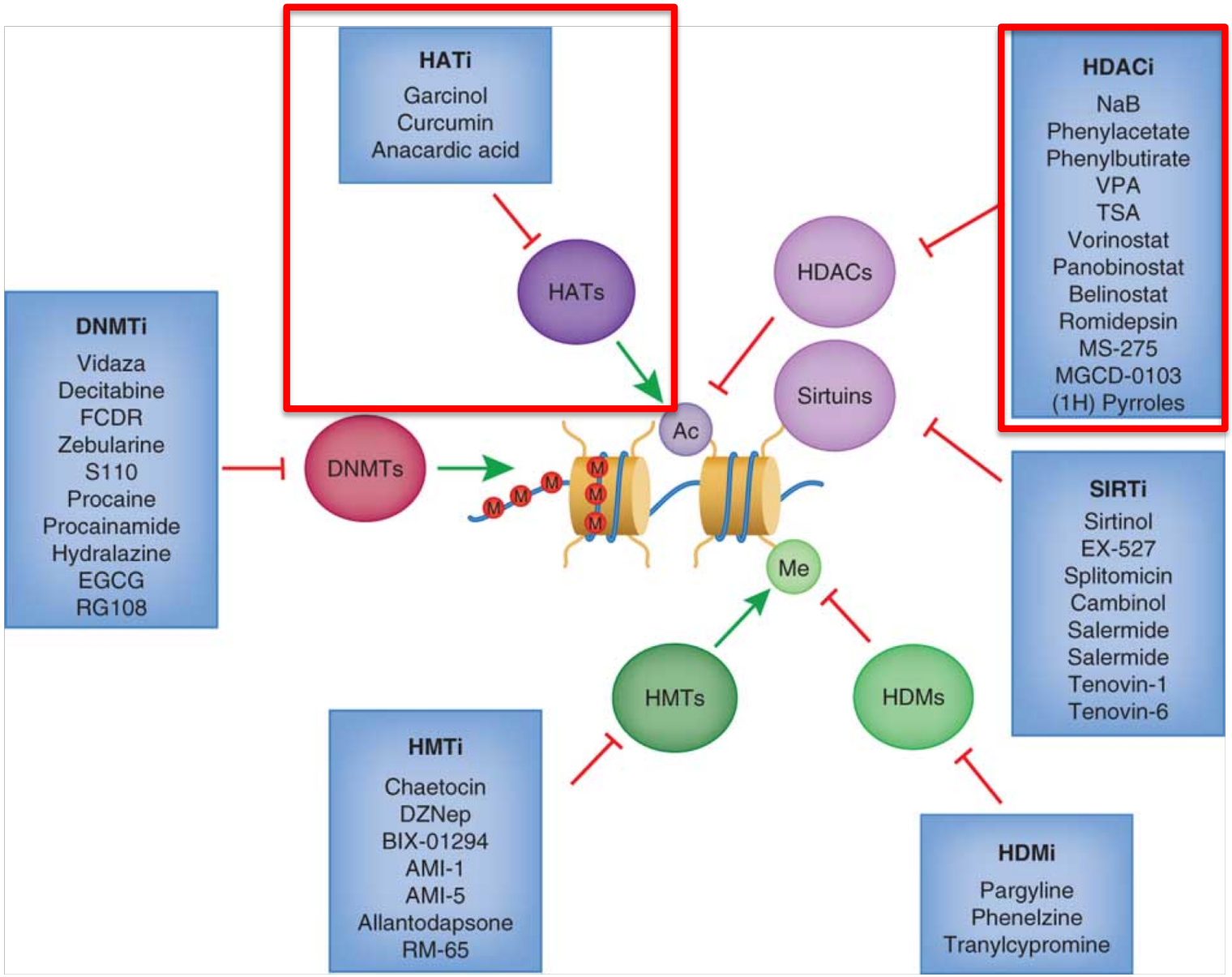
HATs are transcriptional co-activators:

→Abnormal HAT function can cause altered gene expression → leading or driving disease

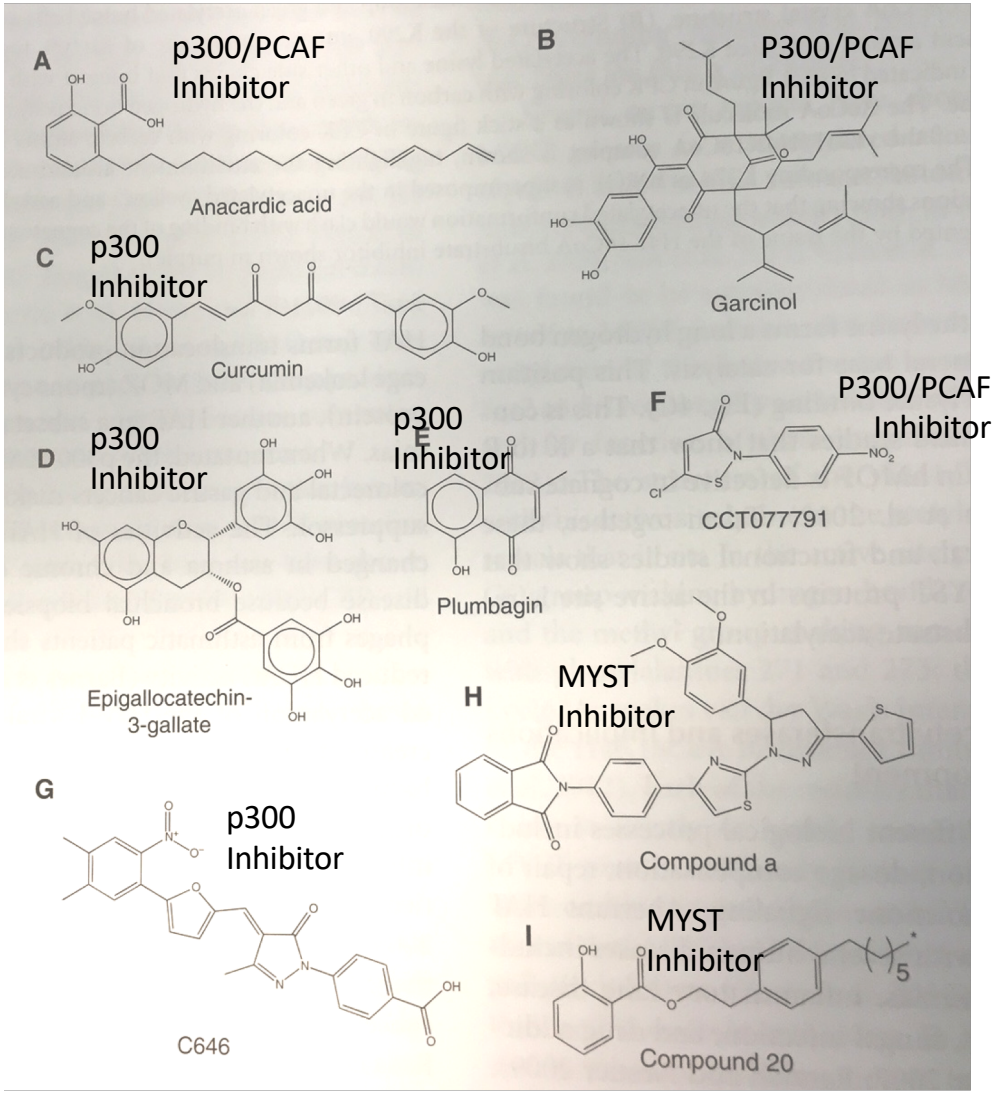
p300/CBP: translocation → cancer
p300 mutations found in colorectal and gastric cacer → p300 is a tumorsuppressor
p300 is involved in diabetes
p300 links drug addiction to histone acetylation status
…

Usefulness of epigenetic drugs?

HATs and Disease – Epigenetic drugs

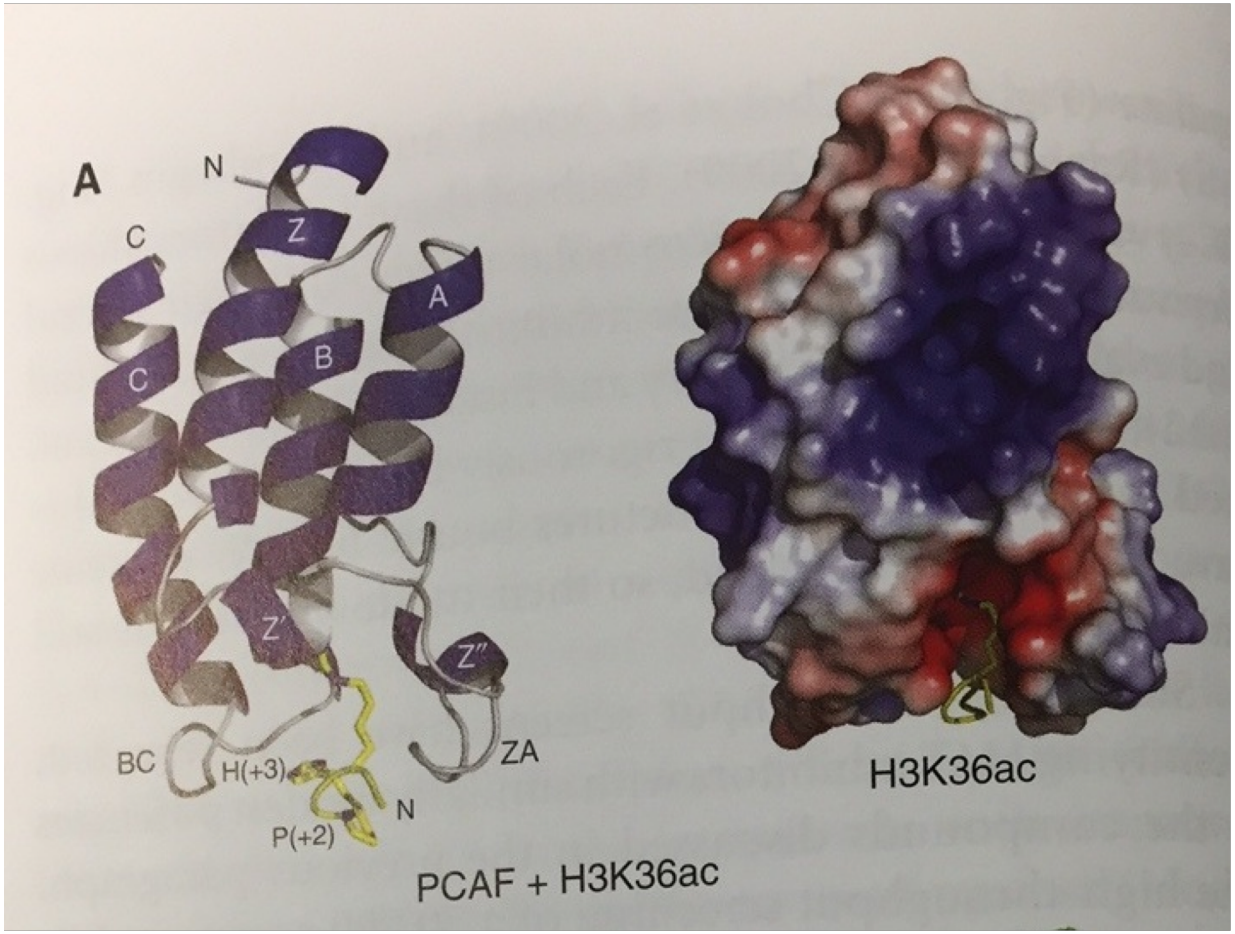


HATs and Disease



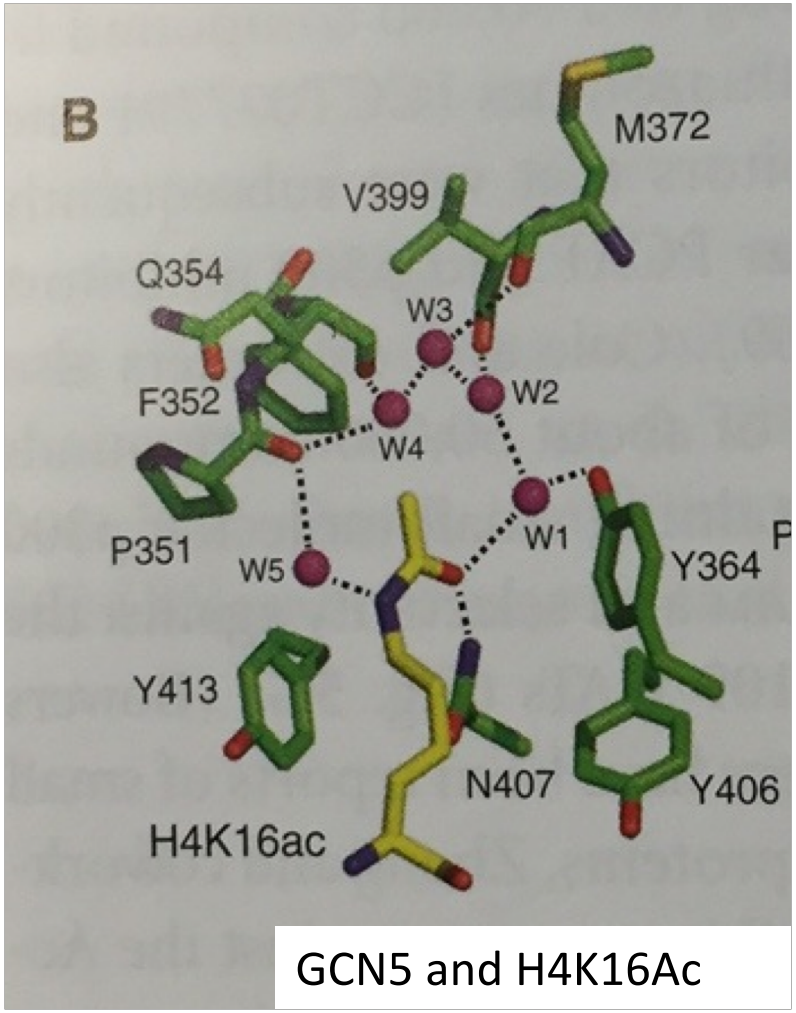
Specific inhbitors to HATs
Have been identified,
However their function in inactivating
HAT activity is MODEST…
→ Better HAT inhibitors need to be developed

READERS OF HISTONE ACETYL TRANSFERASES
BROMO DOMAIN PROTEINS



The bromodomain adopts a distinct structural fold involving a 4 helix bundle termed the BrD fold a hydrophobic pocket recognizes the acetylated histone tail

BROMO DOMAIN PROTEINS



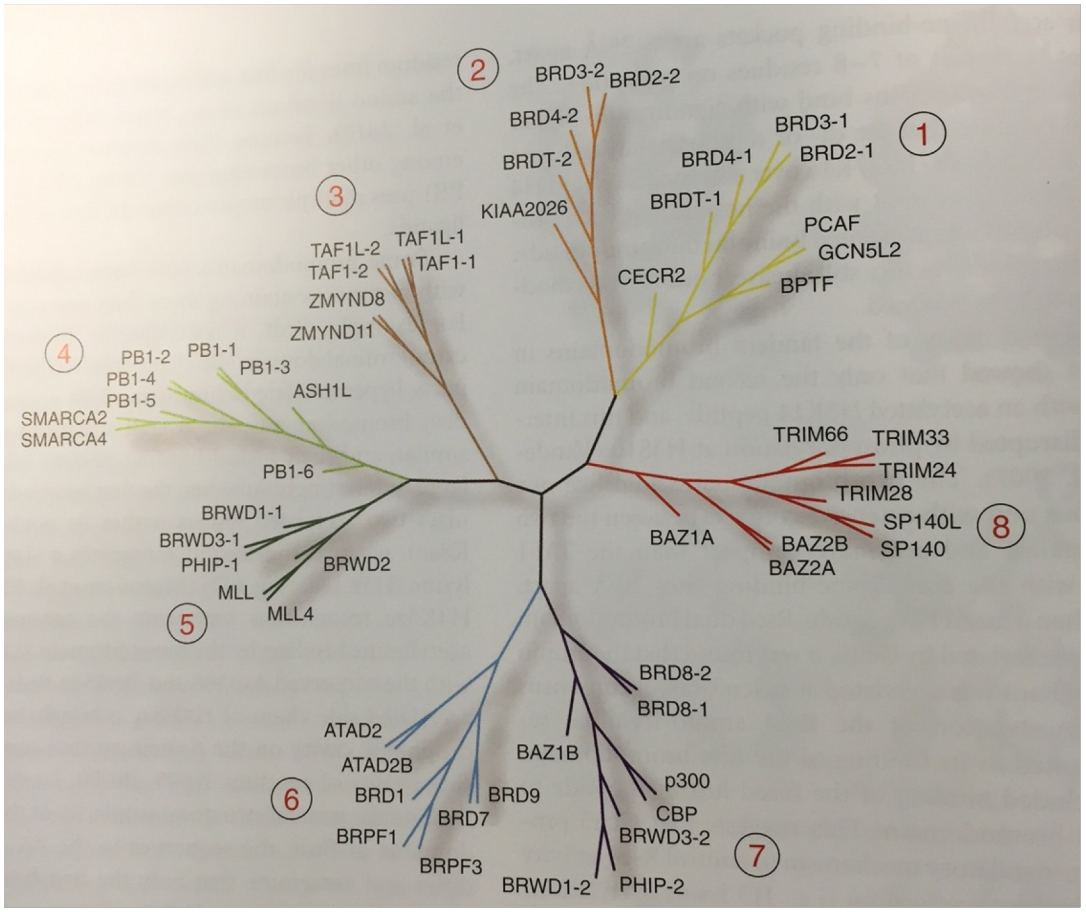
GCN5 and H4K16Ac

The hydrophobic pocket
Binds the acetylated histone tail
via hydrogen bonds

HOWEVER: the affinity of
Bromo domain proteins
for acetylated histone tails
is relatively low

Bromo-domain proteins recruit other factors
that activate transcription/enhance
elongation via different processes

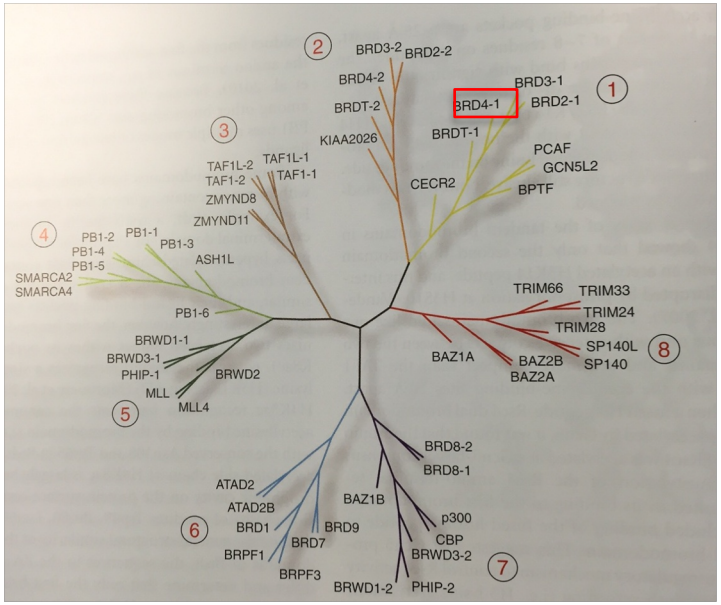
HUMAN BROMO DOMAIN PROTEINS

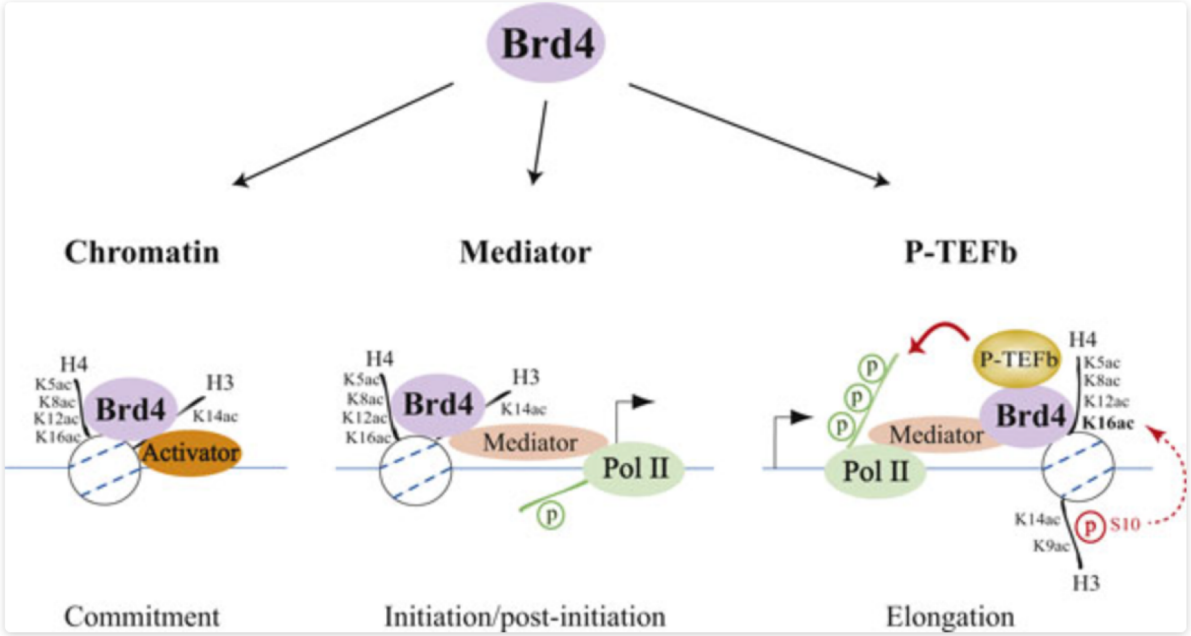


8 subgroups of Bromodomain proteins
total: 42 proteins
Bromodomain proteins cover a wide variety of functionality in CHROMATIN BIOLOGY and GENE TRANSCRIPTION

Lecture 3: ACETYLTRANSFERASES AND DEACETYLASES

BRD4 (bromo domain 4 protein BROMO DOMAIN PROTEINS



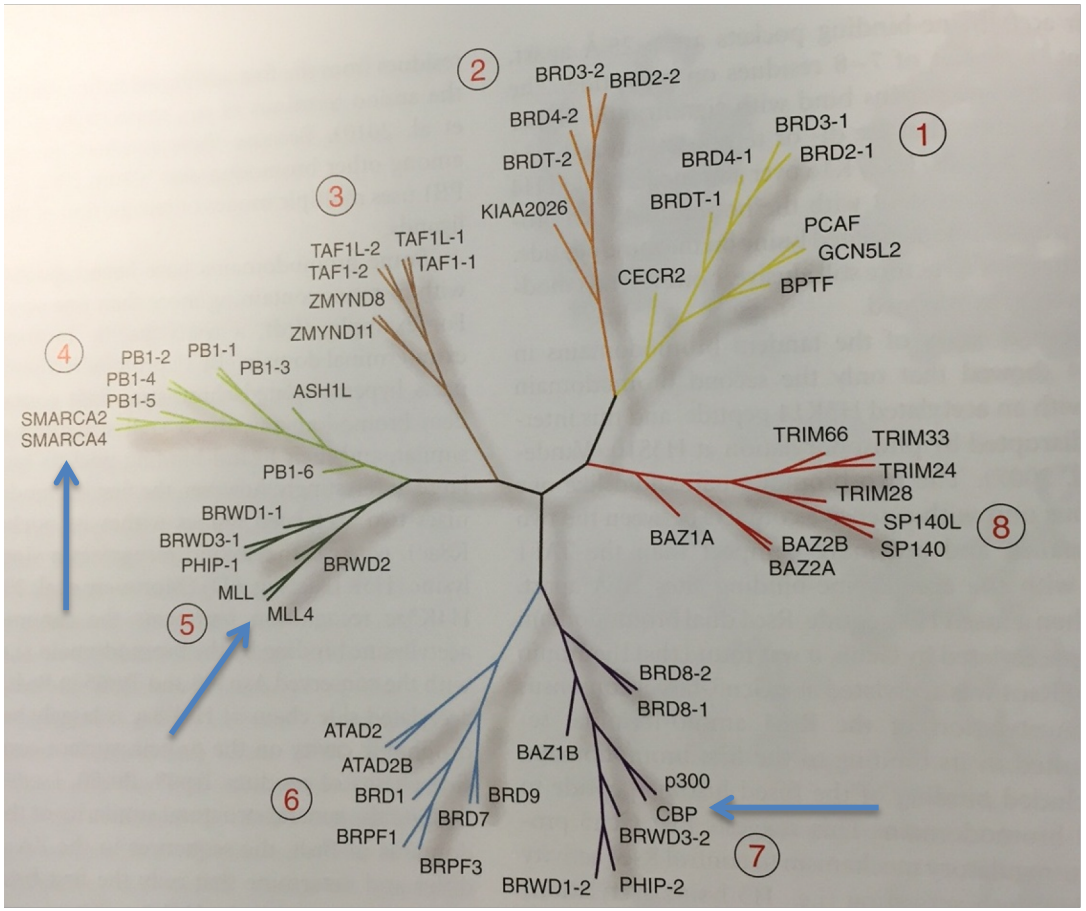


BRD4:
Binds acetylated H3/H4
and activates/anhances
trasncription
via several processes

BRD4 is a prime target for epigenetic therapies

Acetylation of histone H3 and H4 lysine residues modulates Brd4 association with chromatin and the recruitment of Mediator and P-TEFbThree steps for bromodomain-containing protein 4 (Brd4)-regulated chromatin targeting and transcriptional regulation are highlighted. The first step (left) represents a commitment to target gene transcription illustrated by cooperative binding between Brd4 and a transcriptional activator with acetylated chromatin through Brd4-activator interaction, activator-DNA contact, and Brd4 association, via its tandem bromodomains, with acetylated lysine 5 (K5ac), acetylated lysine 8 (K8ac), acetylated lysine 12 (K12ac), and acetylated lysine 16 (K16ac) of histone H4, and/or acetylated lysine 14 (K14ac) of histone H3. The second step (center) is Brd4-mediated recruitment of the initiation cofactor Mediator to the promoter region, which often leads to phosphorylation of the RNA polymerase II (Pol II) carboxyl-terminal domain (CTD) at Ser5 during initiation and post-initiation events. The third step (right) is Brd4-facilitated recruitment of the elongation cofactor P-TEFb (positive transcription elongation factor b) to paused Pol II that results in Ser2 phosphorylation of the CTD, thereby allowing Pol II to resume elongation. The inducible recruitment of Brd4 to an acetylated nucleosome located downstream of the transcription start site (indicated by an arrow) appears to depend on crosstalk between acetylated lysine 9 (K9ac) and phosphorylated serine 10 (S10) of H3 with H4K16ac. http://f1000.com/prime/reports/b/1/98/fig-002

HUMAN BROMO DOMAIN PROTEINS

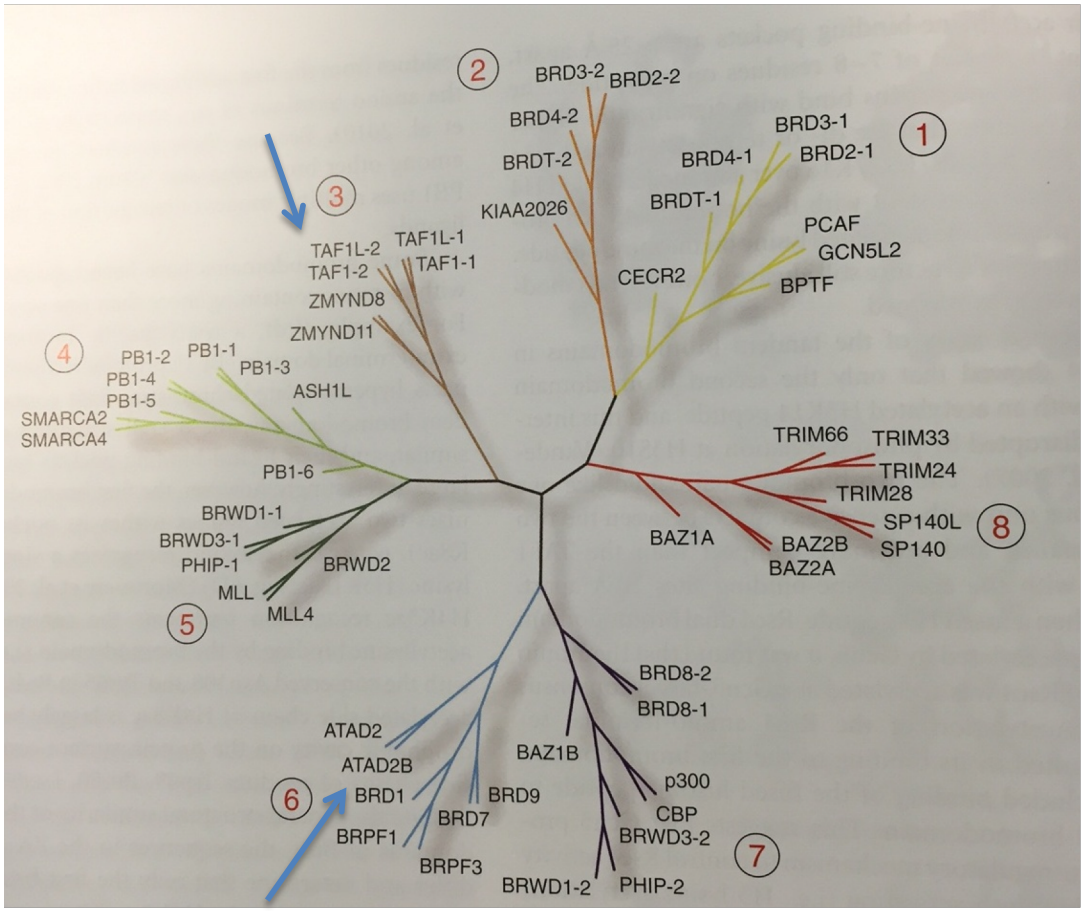


Important Bromo domain proteins:
-**PCAF, Gcn5, p300/CBP !!!**
Bromo domains contribute
to substrate recognition involving
ac. histone and non-histone proteins
→**Acetylation mediated protein-prot**ein
Interaction (complex formation)

-**HMTases such as ASH1L, MLL**
→Trx group members → methylate
H3K4 → <u>transcriptional activation</u>
→→ **link between histone acetylation and methylation during transcriptional Activation**

-**Chromatin remodeling proteins**
SMARC2 (BRM, SNF2/SW12) SMARC4 (BRG1)
→**Acetylation – chromatin remodeling**

HUMAN BROMO DOMAIN PROTEINS



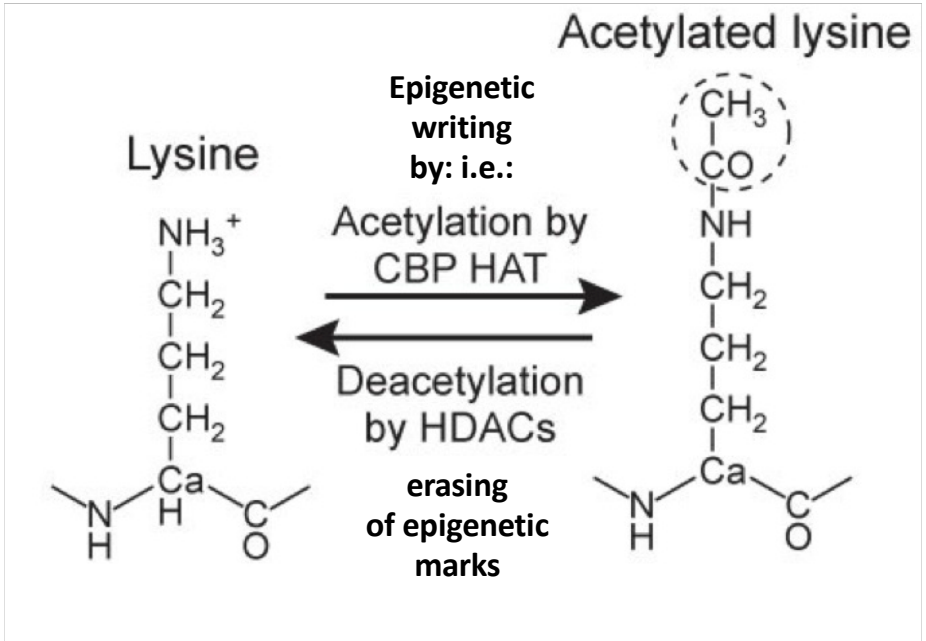
-**ATP-dependent helicases**
ATAD2, ARAD2B
→ **Acetylation and DNA unwinding**

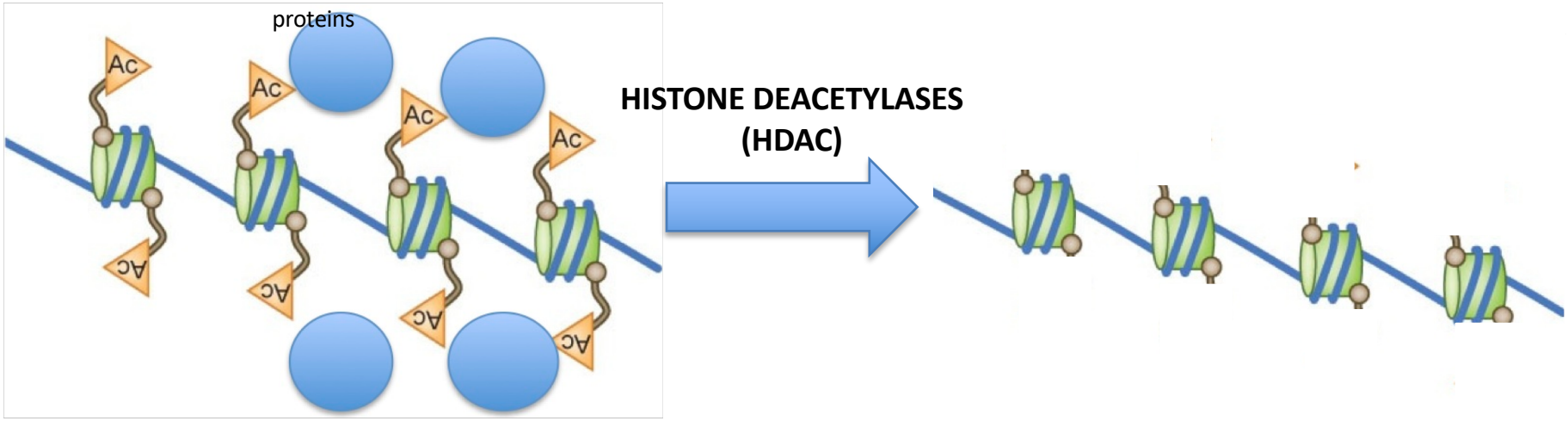
-**Transcription initiation complex components**
TAF1/TAF1L proteins in the TFIID subunit of the transcription initiation complex
→**Acetylation and transcriptional initiation**

-**BET proteins – transcriptional elonation**
BET proteins recruit p-TEFb complex that ensures the processivity of RNA Pol II during transcriptional elongation.
→ Acetylation and elongation

HOWEVER: NEW DATA SUGGEST THAT BROMO DOMAIN PROTEINS CAN ALSO INTERACT WITH NON-HISTONE PROTEINS AND IMPACT ON GENE EXPRESSION

De-Acetylation – De-acetylases





Transcriptional ACTIVATION

Transcriptional REPRESSION

HDAC FAMILIES

Table 1. HDAC classification

Superfamily	Family	Class	Protein (*S. cerevisiae*)	Subclass	Protein (human)
Arginase/deacetylase superfamily	Histone deacetylase family	Class I	Rpd3, Hos1, Hos2, Hos3		HDAC1, HDAC2, HDAC3, HDAC8
		Class II	Hda1	Class IIa	HDAC4, HDAC5, HDAC7, HDAC9
				Class IIb	HDAC6, HDAC10
		Class IV			HDAC11
Deoxyhypusine synthase like NAD/FAD-binding domain superfamily	Sir2 regulator family	Class III	Sir2, Hst1, Hst2, Hst3, Hst4	I	SIRT1, SIRT2, SIRT3
				II	SIRT4
				III	SIRT5
				IV	SIRT6, SIRT7

Families of HDACs:

- Nomenclature according to yeast homologs; HDACs are numbered according to there History of discovery (HDAC 1-10; SIRT1-7)

Superfamilies:

Nomenclature according to their functional mechanism:

Class I, II, IV = **HDACs** = *Arginase/deacetylase family*

Class III: SIRTs =**SIRTUINS** = *Deoxyhypusine synthase like NAD/FAD-binding domain superfamily*

HDAC FAMILIES

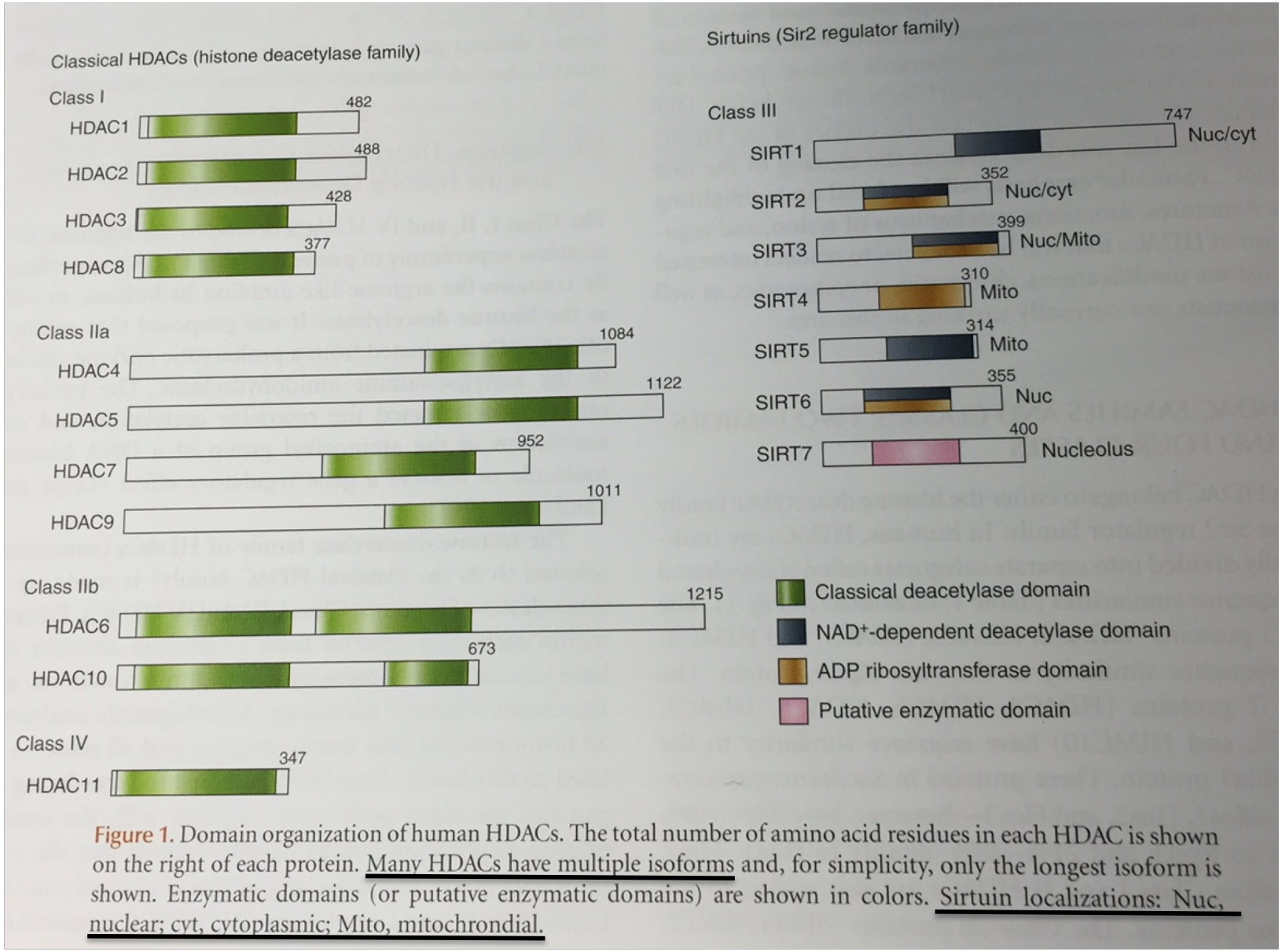
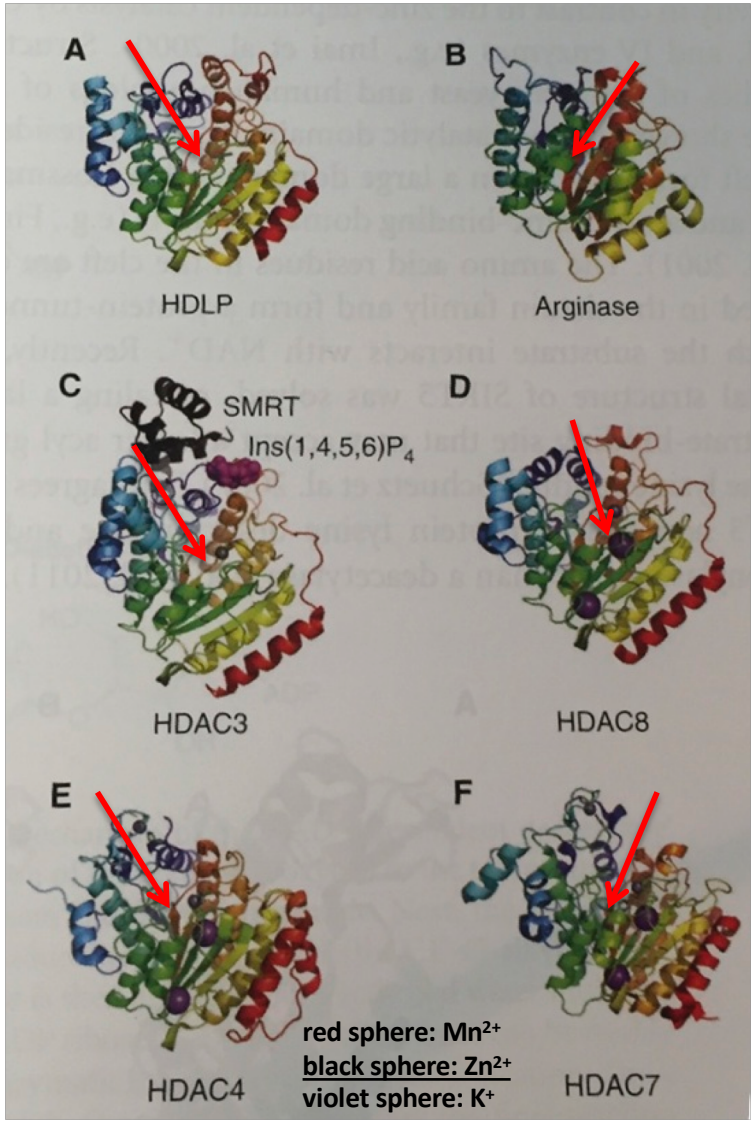


Figure 1. Domain organization of human HDACs. The total number of amino acid residues in each HDAC is shown on the right of each protein. Many HDACs have multiple isoforms and, for simplicity, only the longest isoform is shown. Enzymatic domains (or putative enzymatic domains) are shown in colors. Sirtuin localizations: Nuc, nuclear; cyt, cytoplasmic; Mito, mitochrondial.

STRUCTURE OF DEACETYLASES

Class I and II HDACs

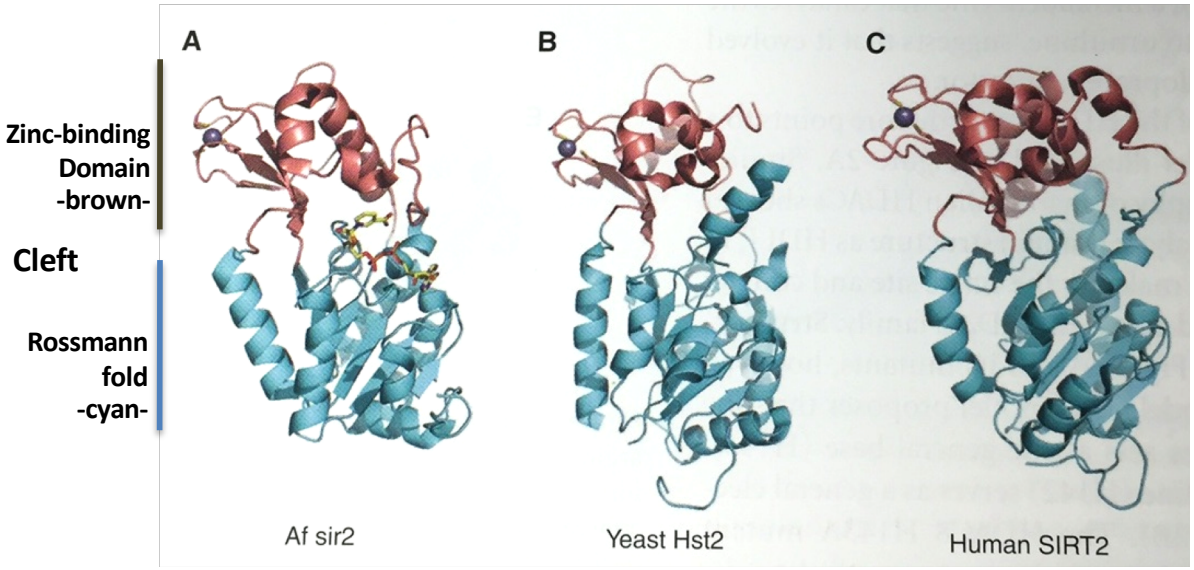
Arginase/deacetylase family



→ **Tubular pocket → catalytic centre (beta sheets)**

Class III HDAC – SIRTUINS

Deoxyhypusine synthase like
NAD/FAD-binding domain superfamily



Cleft: amminoacids on cleft are conserved between Class III HDACs and form a protein tunnel in which the substrate interacts with NAD^+ (nicotinamide adenine dinucleotide

The biochemistry of Class I, II, IV histone deacetylases

HDAC Family

Histone tail – Ac-K

Histidine

water molecule

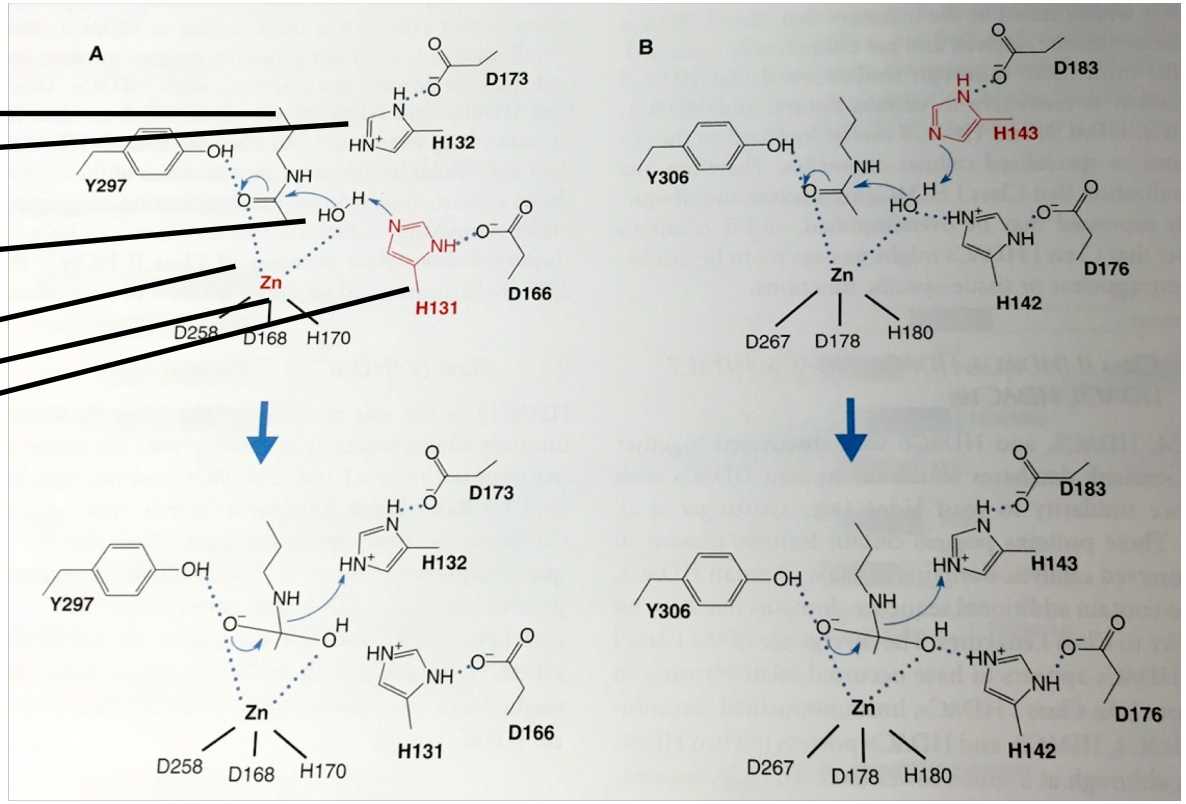
Zn^{2+} ion

Histidine

bold (red and black): active residues

Example_ HDLP HDAC

Example_ HDAC8



VERY SIMILAR STRUCTURE AT CATALYTIC CORE:
Tubular pocket,
Zinc binding site,
Active sites (1xY tyrosine;
2x His that make hydrogen bonds to D aspartic acids)

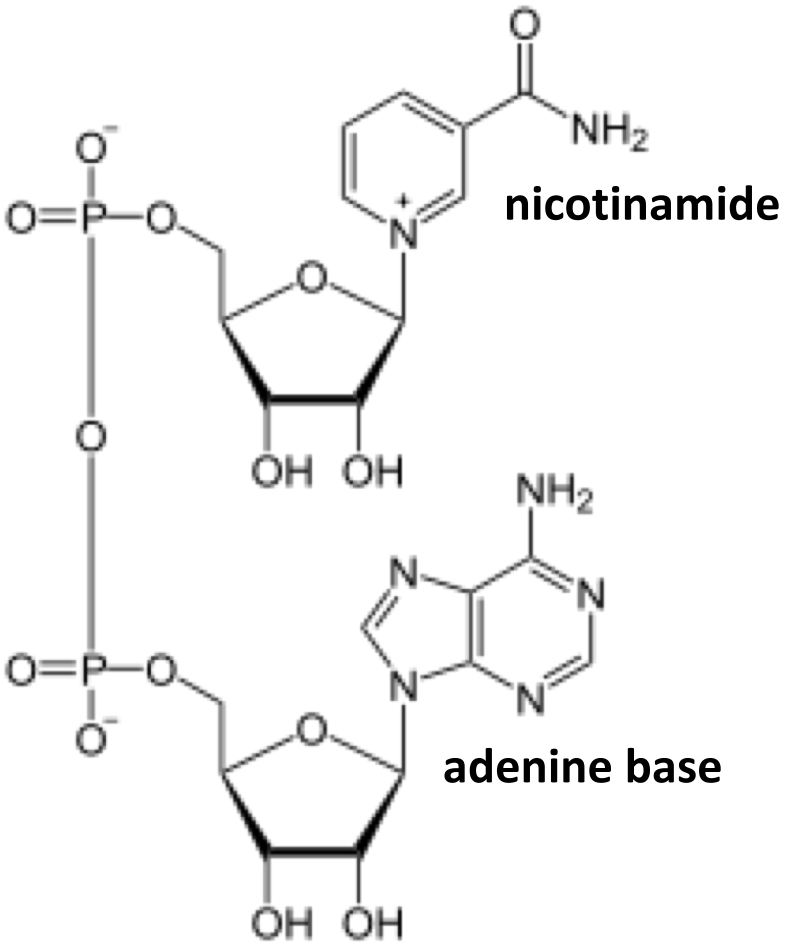
Histone deacetylase like Protein (Aquifex aeolicus)

Catalytic His (H131) facilitates A nucleophilic attack at the substrate carbonyl by activating a water molecule coordinated with the Zn2+ ion coordinated aspartic acid (D) and histidine (H)

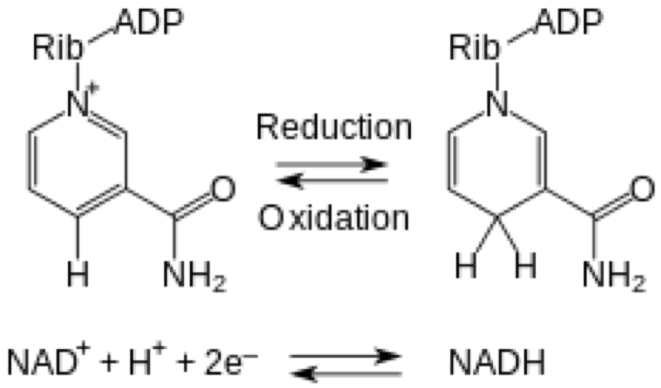
Catalytic His (H143) – !! other position !! --facilitates a nucleophilic attack at the substrate carbonyl by activating a water molecule coordinated with the Zn2+ ion coordinated aspartic acid (D) and histidine (H)

The biochemistry of Class III histone deacetylases

De-acetylation by class III de-acetylases (SIRTUINS) depend on the coenzyme Nicotinamide adenine dinucleotide (NAD)



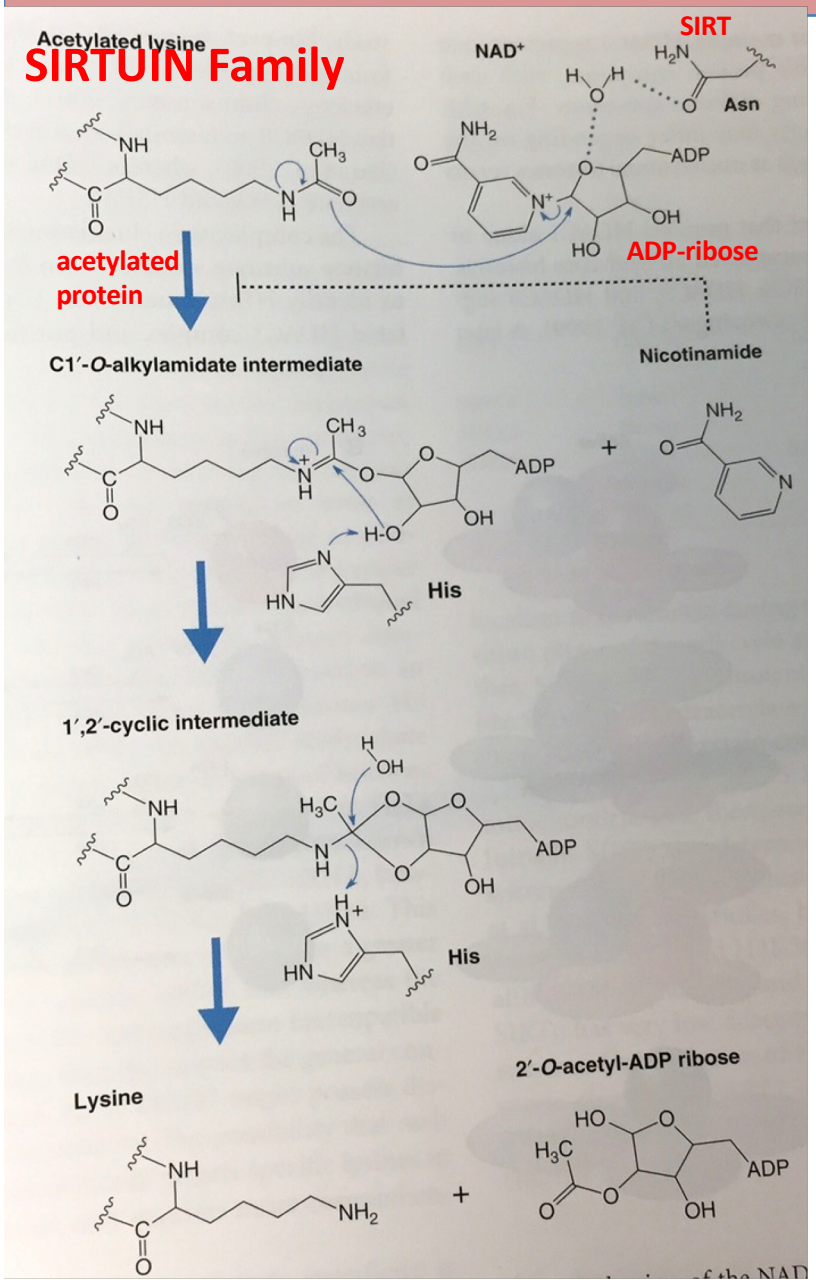
Nicotinamide adenine dinucleotide (NAD)



Nicotinamide adenine dinucleotide (NAD) is a coenzyme found in all living cells. The compound is a **dinucleotide**, because it consists of **two nucleotides joined through their phosphate groups**. One nucleotide contains an **adenine base and the other nicotinamide**. Nicotinamide adenine dinucleotide exists in two forms, an oxidized and reduced form abbreviated as NAD+ and NADH respectively.

In metabolism, **nicotinamide adenine dinucleotide is involved in redox reactions, carrying electrons from one reaction to another.** The coenzyme is, therefore, found in two forms in cells: NAD+ is an oxidizing agent – it accepts electrons from other molecules and becomes reduced. This reaction forms NADH, which can then be used as a reducing agent to donate electrons.

The biochemistry of Class III histone deacetylases



Nucleophilic addition of the **acetamide oxygen** to the C1' position of the **nicotineamide ribose** to form a C1'-O-alkylamidate intermediate and fee **nicotineamide (NAD+ was cleaved to nitotinamide and ADP-ribose)**

Next, the 2'-hydroxy group of the **ADP-ribose is activated by an active site histidine** residue that, in turn, attacks the C1'-O-alkylamidate to form the **1', 2'-cyclic intermediate**.

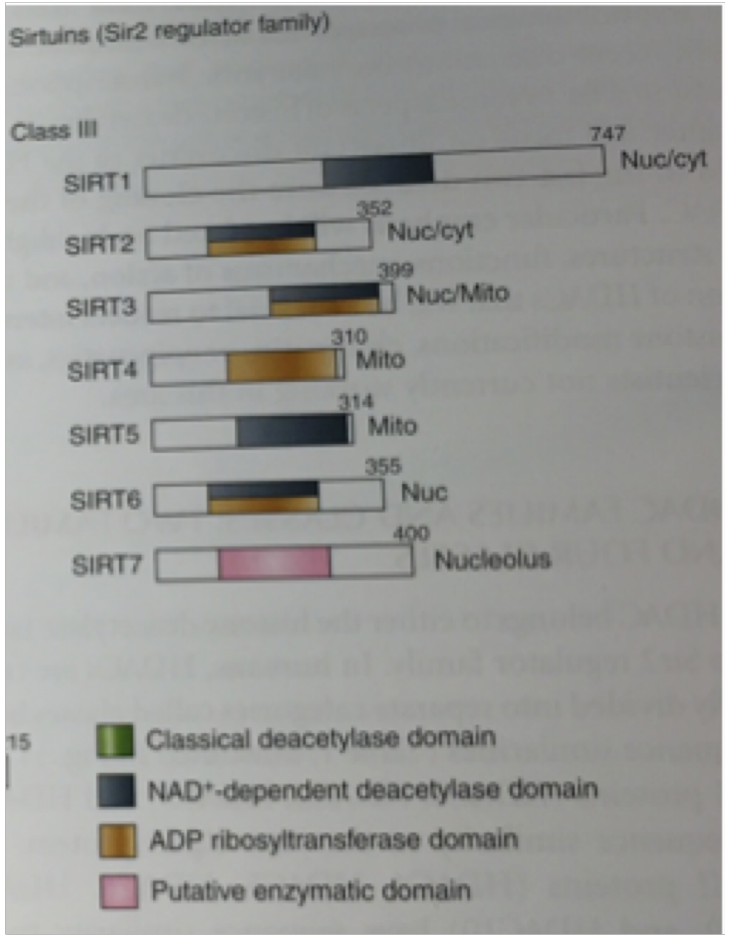
The 1', 2'-cyclic intermediate is then **attacked by an activated water molecule (coordinated by Zn^{2+})** resulting in the formation of **deacetylated lysine** and **2'O-acetyl-ADP ribose**.

2'-O-acetyl-ADP ribose can be easily transformed into 3'-O-acetyl-ADP ribose in aequous solutions by nonenzymatic intramolecular transesterifications.

THUS: NICTONE AMIDE, THE DEACETYLATED PEPETIDE AND A MIXTURE OF 2'- and 3'- O-acetyle-ADP ribose
(note: nicotine amide can block deacetylase activity)

The biochemistry of Class III histone deacetylases

SIRTUIN Family proteins can also harbor mono-ADP-ribosylation activity



Protein domains that mediate de-acetylation and mono-ADP-ribosylation of substrate proteins overlap in SIRT2, 3, 6; SIRT4 shows only mono-ADP-ribosylation activity

De-acetylation and mono-ADP-ribosylation depend on the same emzymatic cofactor NAD^+

During evolution the deacetylation of protein substrates and acetyl-transfer to form 2'O-acetyl-ADP ribose was disconnected.

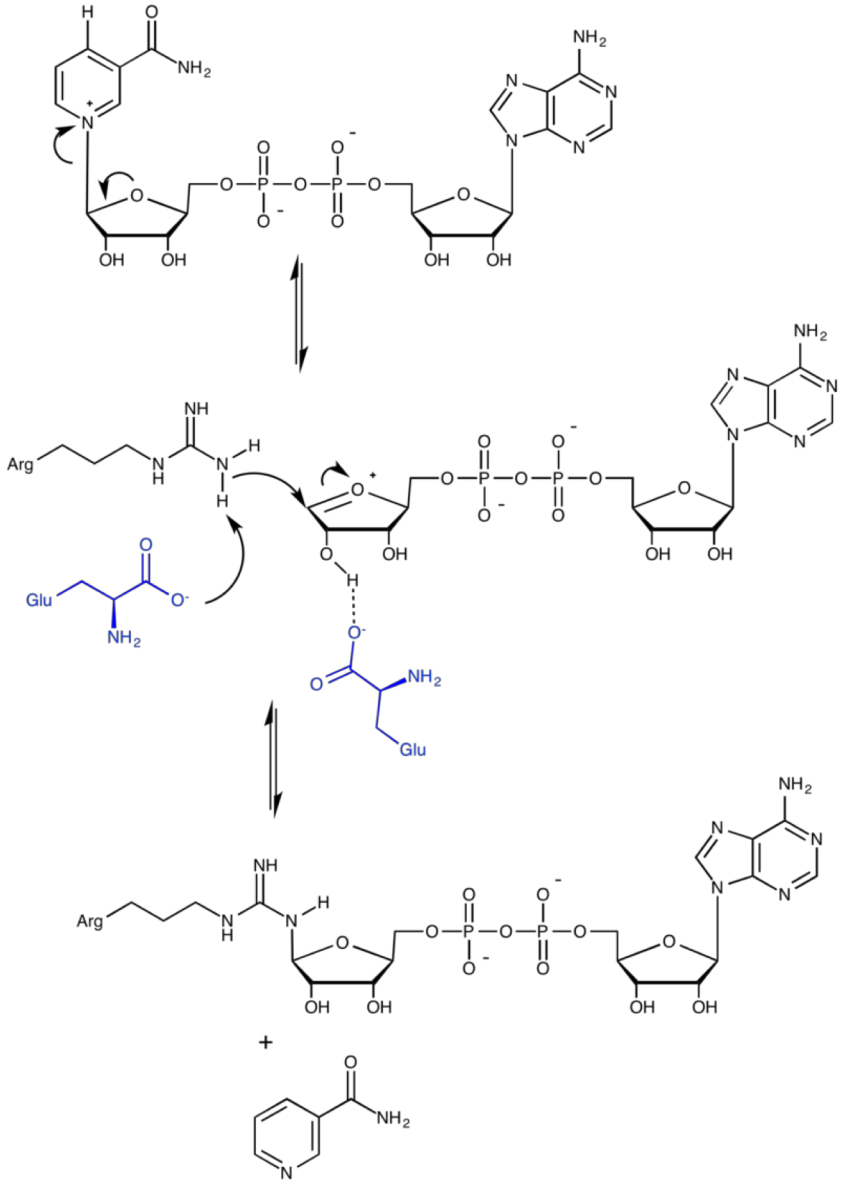
Instead, after nicotidamine cleavage, the remaining ADP-ribose molecule is transferred to the target protein

However: unclear whether de-acetylation and ADP-ribosylation can occur simultaneously

The biochemistry of Class III histone deacetylases

SIRTUIN Family proteins can also harbor mono-ADP-ribosylation activity

The source of ADP-ribose for most enzymes that perform this modification is the redox cofactor NAD+.
In this transfer reaction, the N-glycosidic bond of NAD+ that bridges the ADP-ribose molecule and the **nicotinamide group is cleaved**, followed by **nucleophilic attack by the target amino acid side chain**. ADP-ribosyltransferases can perform two types of modifications: mono-ADP ribosylation and poly-ADP ribosylation.



ADP-ribosylation is another type of post-translational modification that impacts on apoptosis, gene regulation, DNA damage repair and protein degradation.
→Some Sirtuins have 2 parallel enzymatic activities that can impact on epigenetic gene regulation

The biochemistry of Class III histone deacetylases

Table I. Mammalian sirtuin sub-cellular localisation and activities. According to [20,234], modified

	Predicted MW	Primary subcell. localization	Activity	Key targets
SIRT1	80.41; 76.0 kDa[1] 	Nucleus	Deacetylase	p53, FOXO1, 3 & 4, PARP-1; APE1; DNA-PK; RARβ, PGC1α, PPARγ, NFκB, IGF1, histone H1, H3, H4
SIRT2	43.2; 39.5 kDa[2] 	Cytoplasm	Deacetylase	Histone H4, α-tubulin
SIRT3	28.8 kDa; 36.6 kDa[3] ; 43.6 kDa[14] 	Mitochondria	Deacetylase, ADP-ribosyltransferase	Acetyl-coA synthetase, glutamate dehydrogenase, Ku70, isocitrate dehydrogenase
SIRT4	35kDa[15] to 47.3 kDa[4] 	Mitochondria	ADP-ribosyltransferase	Glutamate dehydrogenase
SIRT5	33.8 kDa[5] 	Mitochondria, cytosol[11]	Deacetylase, demalonylase, desuccinylase[10]	Cytochrome c; carbamoyl phosphate synthetase 1; urate oxidase
SIRT6	39.1 kDa[6] 	Nucleus[12], synaptosomes[13]	Deacetylase, ADP-ribosyltransferase	Histone H3; PARP-1; DNA-PK
SIRT7	44.9 kDa[7] 	Nucleus	Deacetylase[9]	RNA Pol I complex; RNA Pol II complex; histone H3[9]; chromatin remodelling proteins[8]

SUBSTRATE SPECIFICITY OF DEACETYLASES

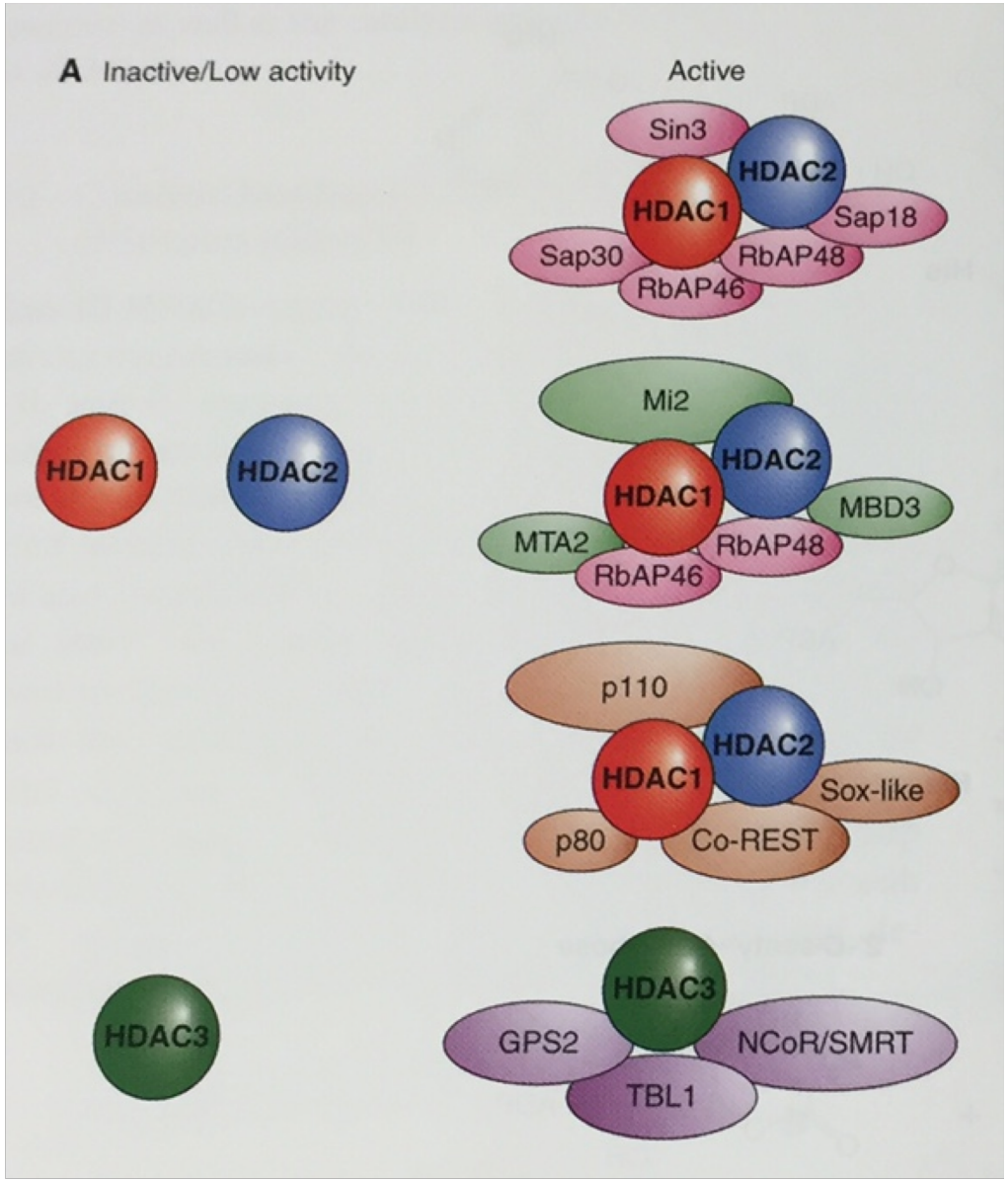
Table 1A - Classification of classic histone deacetylases (HDAC).

Class	Subclass	HDAC enzymes	Cellular localization
I	Ia	HDAC1	Nucleus
		HDAC2	Nucleus
	Ib	HDAC3	Nucleus and cytoplasm
	Ic	HDAC8	Nucleus
II	IIa	HDAC4	Nucleus and cytoplasm
		HDAC5	Nucleus and cytoplasm
		HDAC7	Nucleus and cytoplasm
		HDAC9	Nucleus and cytoplasm
	IIb	HDAC6	Nucleus and cytoplasm
		HDAC10	Nucleus and cytoplasm
IV	No subclass	HDAC11	Nucleus and cytoplasm

HDACs act in nucleus and cytoplasma

SUBSTRATE SPECIFICITY OF DEACETYLASES

Class I, II, IV HDACs:



Substrate specificity for class I, II, IV HDAC is difficult to define:

- **purified HDACs have very low de-acetylase activity**
-**HDACs purify in large complexes**
-**More than one HDAC can be found in a complex**
-**HDACs can be functionally redundant (→ knock-down of one class of HDAC can be compensated from family member or even by different HDAC class**

Difficult to directly link HDACs to biological activities and pathways to individual Sirtuins family members

SUBSTRATE SPECIFICITY OF DEACETYLASES

Class III De-acetylases - SIRTUINS

Table 2. Sirtuin histone substrates

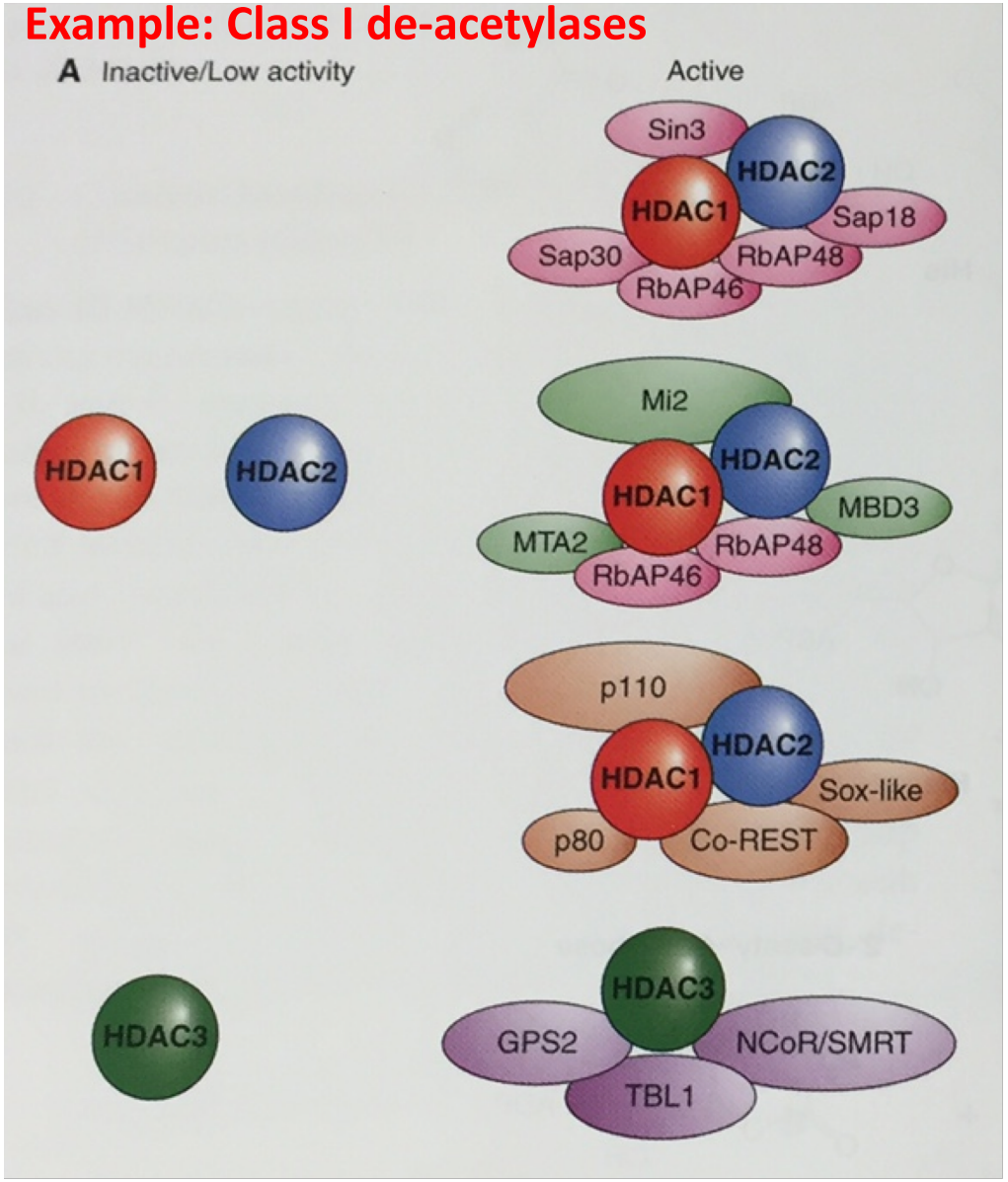
Sirtuin	Histone substrate	Biological relevance
SIRT1	H3K9 H3K14 H3K56 H4K16 H1K26	Chromatin organization, DNA repair/genome stability, cancer
SIRT2	H4K16 H3K56	Chromatin condensation/ mitosis, DNA repair, cancer
SIRT3	H4K16	Chromatin silencing, DNA repair, cellular stress
SIRT4	None	
SIRT5	None	
SIRT6	H3K9 H3K56	Telomeric chromatin/senescence, DNA repair/genome stability
SIRT7	H3K18	Cellular transformation

SIRTUINs have an easy to define substrate specificity. This allow to directly attribute biological activities and pathways to individual Sirtuins family members

REGULATION OF HDAC ACTIVITY

1. PROTEIN INTERACTION

Example: Class I de-acetylases



HDAC1 and HDAC2 reside in 3 different complexes

Sin3 Complex

NuRD Complex

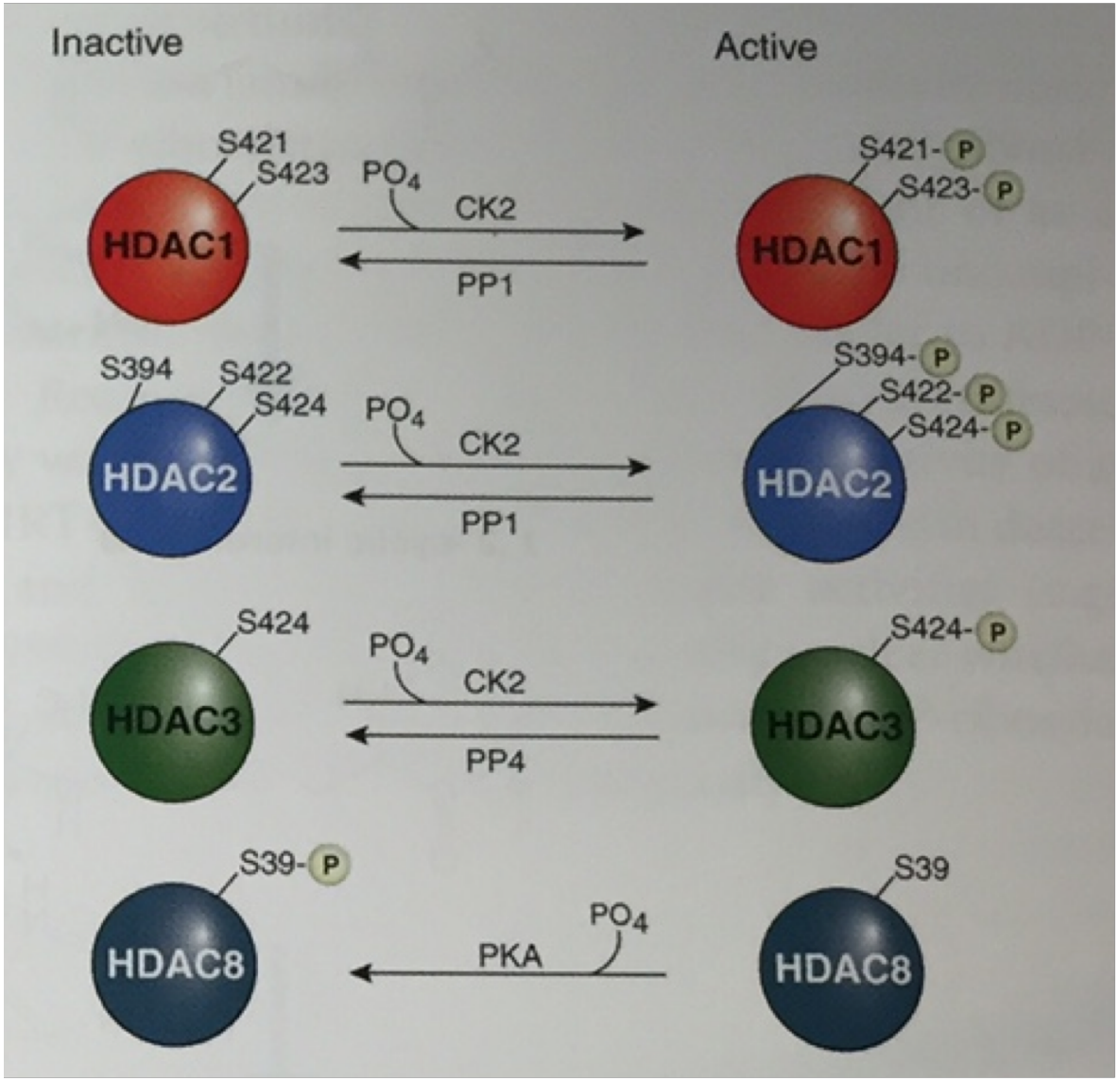
Co-REST Complex

NCoR/SMRT Complex

Depleting complex Components reduces HDAC activity

REGULATION OF HDAC ACTIVITY

2. POST_TRANSLATIONAL MODIFICATIONS → most important



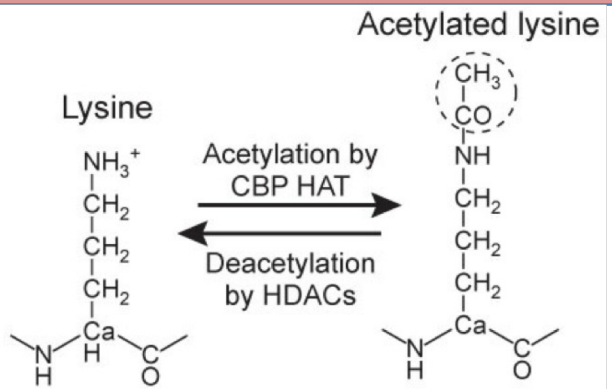
Mutations in phosphorylation sites disrupt HDAC activity and disrupt the Sin3/NuRD/CoREST complex (CK2: protein kinase CK2; PP1: protein phosphatase 1)

Mutations in phosphorylation sites disrupt HDAC activity and disrupt the Sin3/NuRD/CoREST complex

Mutations in phosphorylation sites increase HDAC activity. Phosphorylation disrupts the structure around the active center in HDAC8

Other modifications: acetylation, glycosylation, S-nitrosylation, sumoylation, ubiquitination

BIOLOGICAL IMPORTANCE OF HDACs



1. HDACs indirectly regulate many post-translational modifications

Liberation of lysine residues opens the possibility for numerous post-translational modifications

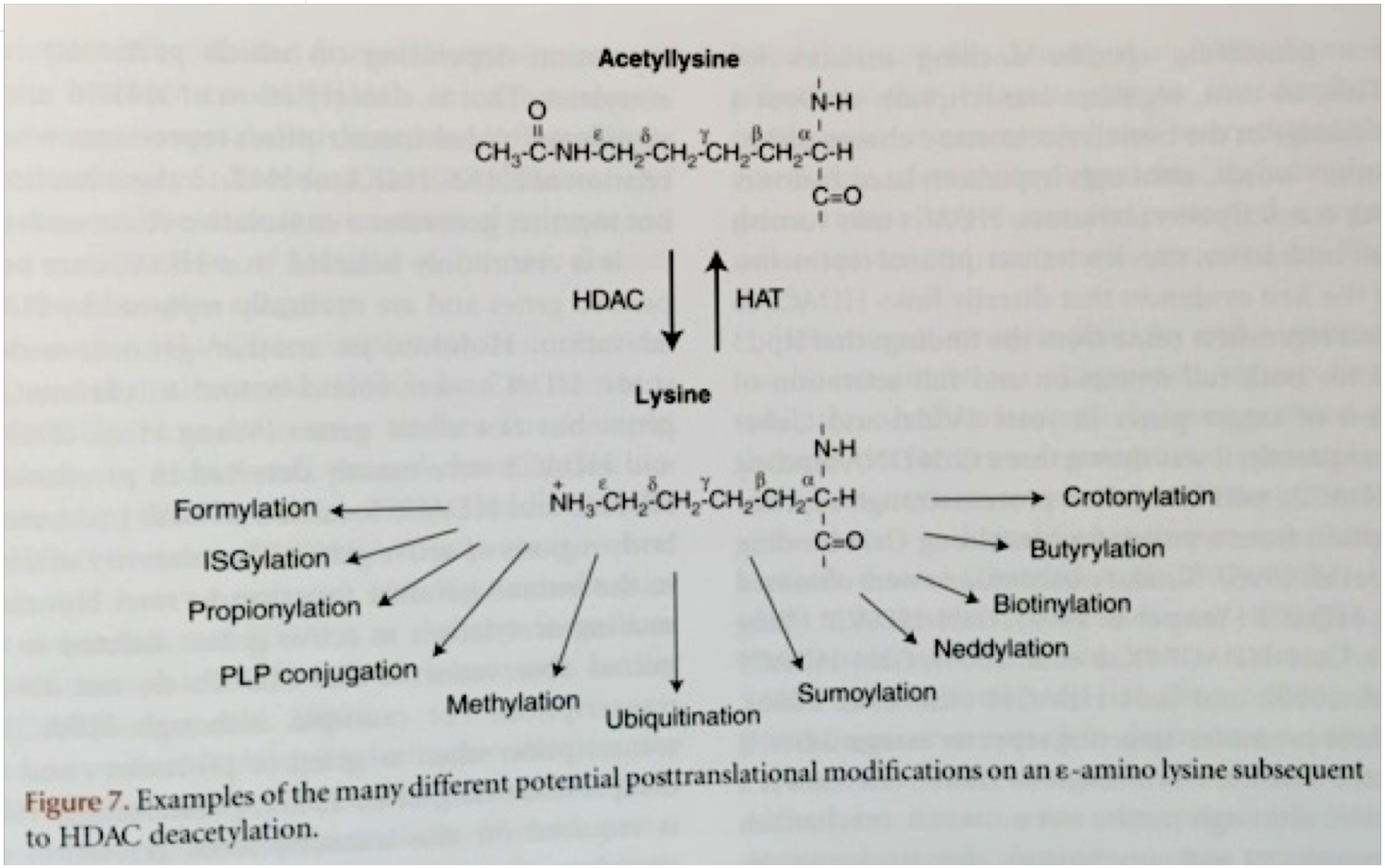


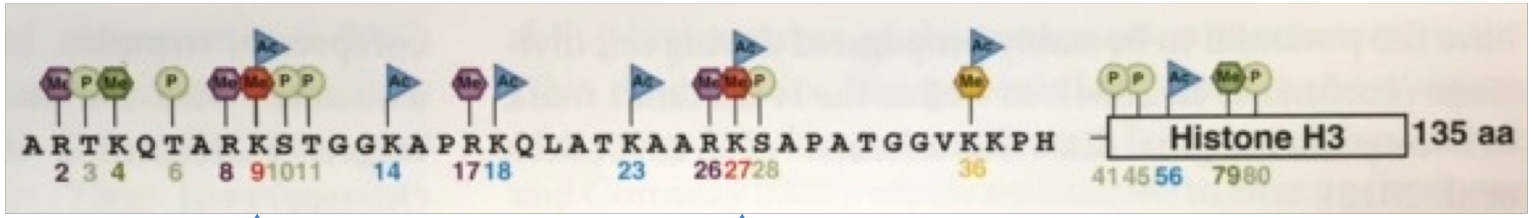
Figure 7. Examples of the many different potential posttranslational modifications on an ε-amino lysine subsequent to HDAC deacetylation.

BIOLOGICAL IMPORTANCE OF HDACs

1. HDACs indirectly regulate many post-translational modifications

- **Actylation of K prevents ubiquitination** (ub is a signal for protein degradation by the proteasome). HDAC inhibitors accelerate protein degradation

-**Acetylation of K interferes with methylation of K in histone tails**



H3K9me: repression
H3K9ac: activation

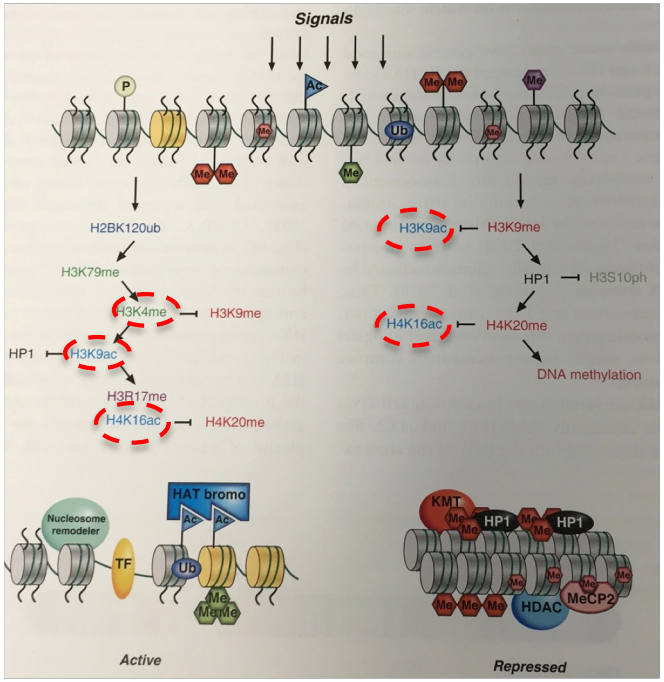
H3K27me: repression
H3K27ac: activation

-**Acetylation of K crosstalks with other histone modifications**

H3K9ac promotes H3K4me → open chromatin and transcription

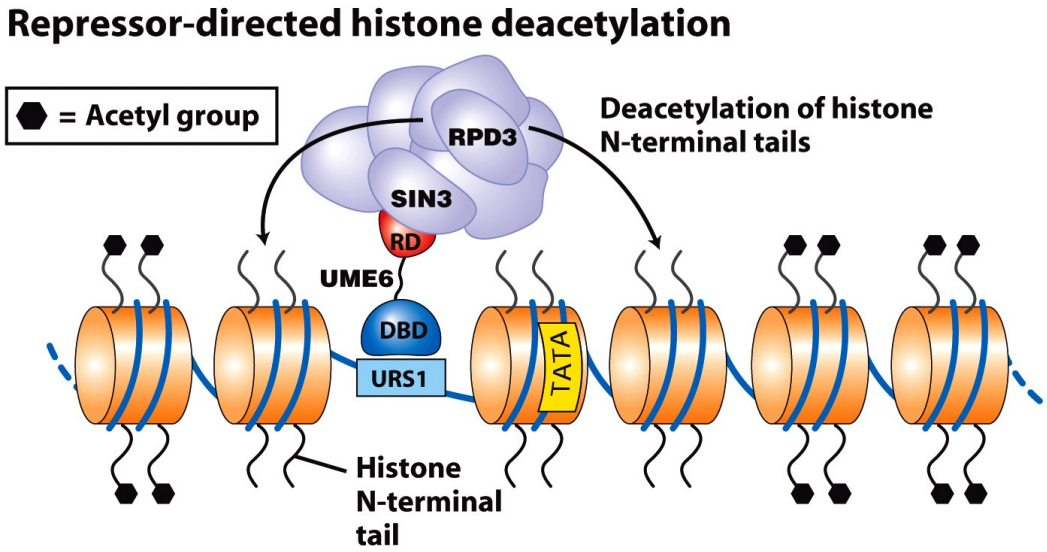
→HDACs deacetylate H3K9 → less H3K4me → repression

Note: HDAC1 and HMTases G9a are in the same complex and collaborate to silence genes



BIOLOGICAL IMPORTANCE OF HDACs

2. HDACs ALTER GENE EXPRESSION – REPRESSION …..BUT ALSO ACTIVATION



1. Transcriptional repressors recruit HDACs:

The UME6 repressor binds to URS1 control elements and recruits a co-repressor complex containing SIN3 and RPD3 to these sites (in yeast). RPD3 is a histone deacetylase, and this enzyme removes acetyl groups from histones in the vicinity of the URS1 sequence. The nucleosomes bound to DNA in this region (which contains a TATA box promoter) subsequently condense, and expression of the gene is repressed.

→ HDAC recruitment is a common mechansims in gene repression

2.ChIP on ChIP using anti-Histone-ac antibodies:

Acetylation is associated with active gene transcription and high at the gene start

3.Mutating H4K16 results in specifically reduced gene transcription (H4K5, 8, 12 are less specific)

4.Direct association of HDACs with genes and gene promoters

HDACs reset gene expression control from an active to a neutral/inactive state.

HDAC1, 3 located by ChIP-Seq / ChIP on ChIP at gene promoters → repression

HDAC2, 6 locate at gene promoter and gene body → repression

BIOLOGICAL IMPORTANCE OF HDACs

2. HDACs ALTER GENE EXPRESSION – REPRESSION …..BUT ALSO ACTIVATION

Variation of the the general theme: the HDAC domain is not always required for gene silencing

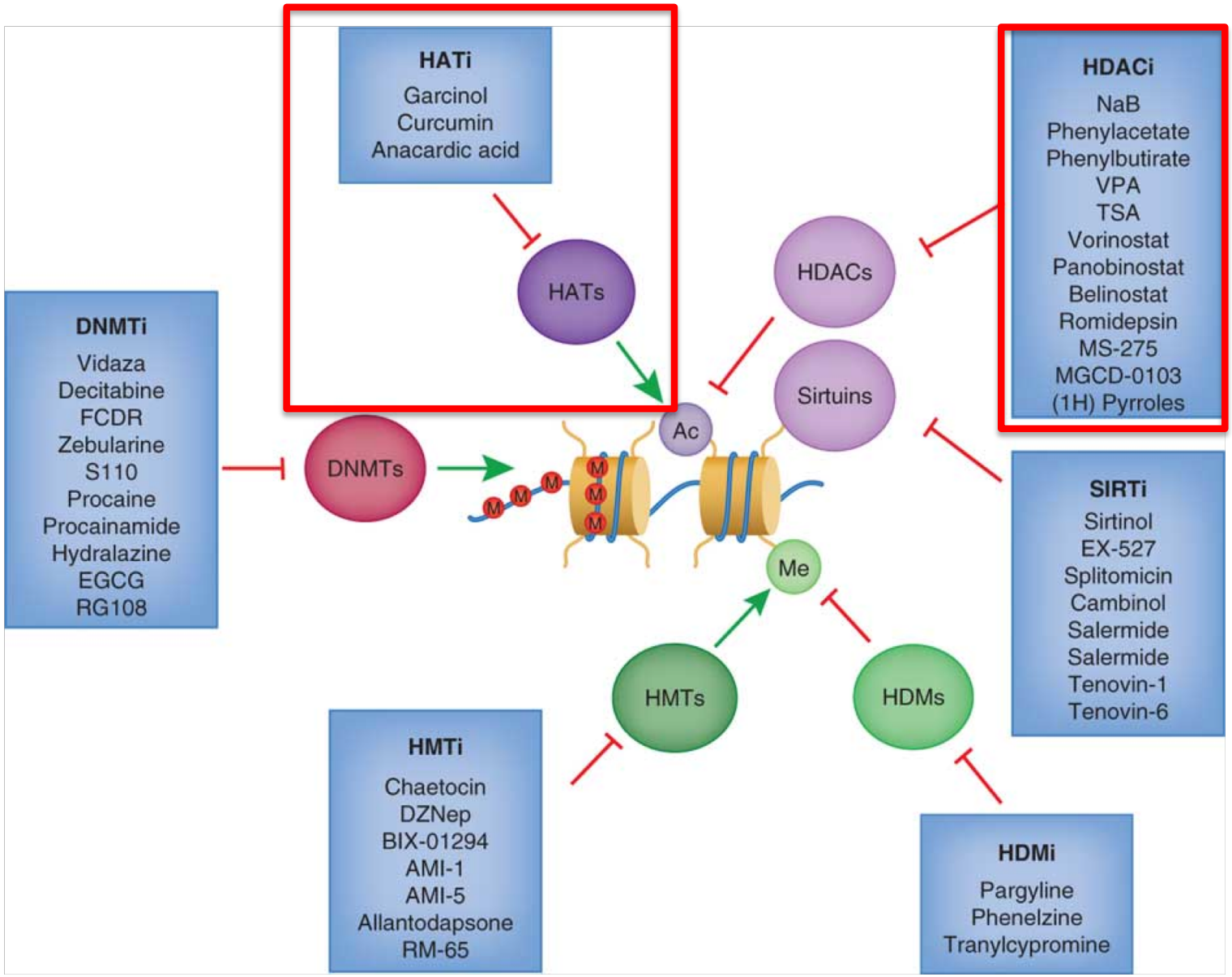
→ HDACs can contribute to gene repression without de-acetylating histones (structural component of a multifunctional repressor complex (for example HDAC5, 7)

→ NOTE: Gene expression experiments in HDAC3 knock-out cells:

Result: Altered gene expression: 50% of genes upregulated, 50% of genes downregulated!!!!!!!!

WHY: HDACs have a global role in gene expression control: loss of HDAC activity also increases the expression of transcriptional repressors that directly act on genes and might recruit other HDACs to drive gene silencing.

HATs and Disease – Epigenetic drugs



HATs and Disease – Epigenetic drugs