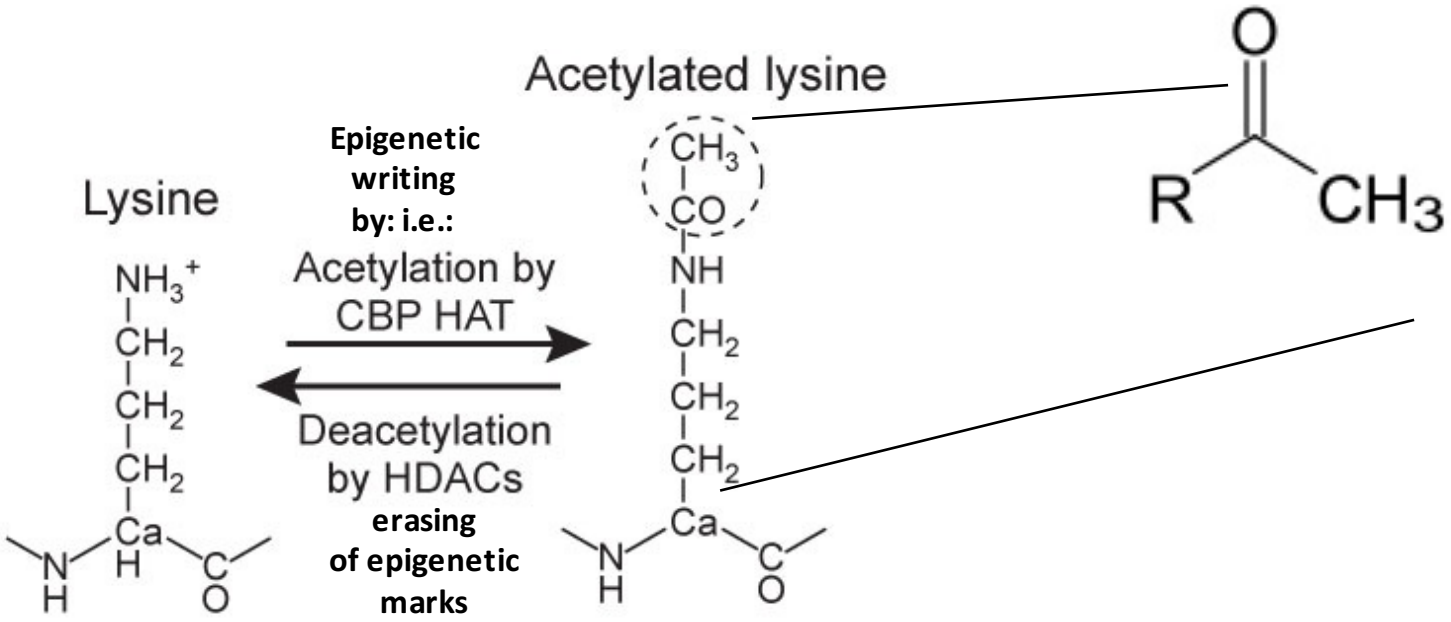
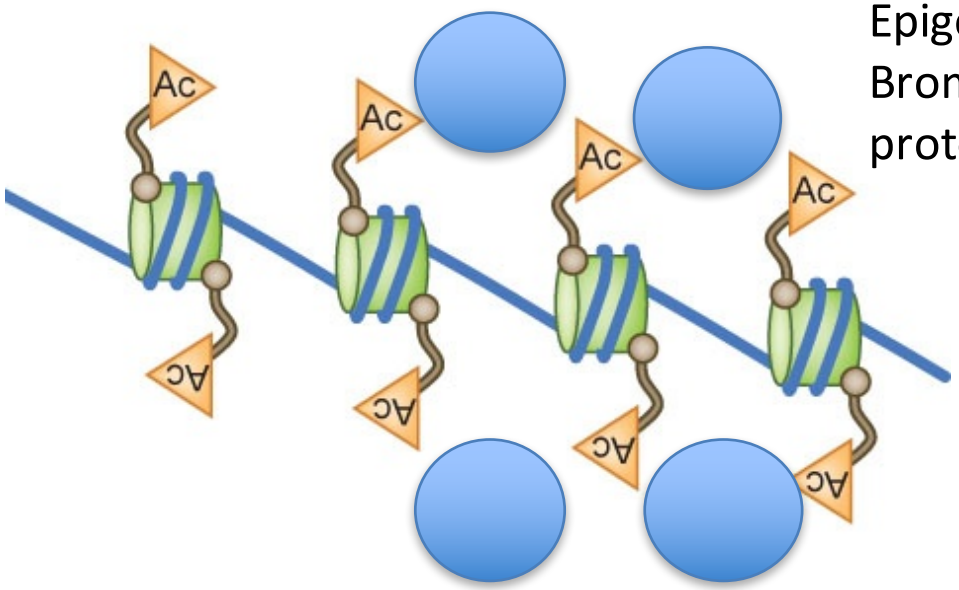
HISTONE ACETYLATION AND DEACETYLATION

Acetylation





Epigenetic reading by Bromo-domain proteins

Transcriptional Activation

Families of Histone acetyltransferases

5 conserved families of histone acetyl transferases (HATs)
also called Lysine acetyltransferases (KATs) → acetylate lysine residues

Table 1. The five major HAT families

Major HAT subfamilies	Prominent members	Key structural and biochemical properties
HAT1	yHat1	Member of the GNAT family Amino- and carboxy-terminal segments used for histone substrate binding Requires the yHat2 regulatory subunit for maximal catalytic activity
Gcn5/PCAF	yGcn5 hGCN5 hPCAF	Member of the GNAT family Uses a ternary complex catalytic mechanism Amino- and carboxy-terminal segments used for histone substrate binding
MYST	yEsa1 ySas2 ySas3 hMOZ dMof hMOF hTIP60 hHBO1	Uses a ping-pong catalytic mechanism Requires autoacetylation of a specific lysine at the active site for cognate histone acetylation
p300/CBP	hp300 hCBP	Metazoan-specific, but shows structural homology with yRtt109 Uses a ternary Theorell–Chance (hit-and-run) catalytic mechanism Contains a substrate-binding loop that participates in AcCoA and lysine binding Contains an autoacetylation loop that requires lysine autoacetylation for maximal catalytic activity
Rtt109	yR11109	Fungal-specific, but shows structural homology with p300 Contains a substrate-binding loop that participates in AcCoA and probably also lysine binding Requires autoacetylation of a lysine residue near the active site for maximal catalytic activity Requires one of two histone chaperone cofactors (Asf1 or Vps75) for maximal catalytic activity and histone substrate specificity

y, yeast; h, human; GNAT, Gcn5-related *N*-acetyltransferase.

Families of Histone acetyltransferases

Best studied HATs

Coding Gene	Site of Histone Modification
HAT 1	H2AK5, H4K5, H4K12
GCN5	H3K9, H3K14, H3K56, H4K5, H4K8, H4K12, H4K16, H4K91 **H3K4**
PCAF	H3K9, H3K14
CBP	H3K14, H3K18, H3K27, H3K56, H4K5, H4K8, H4K12, H4K16
P300	H3K14, H3K18, H3K27, H3K56, H4K5, H4K8, H4K12, H4K16
TAF1	H3K14
TIP60	H2AK5, H4K5, H4K8, H4K12, H4K16
MYST3	H3K9, H3K14
MYST4	
MYST2	H3K14, H4K5, H4K8, H4K12
MYST1	H4K16
ELP3	H3K9, H3K18
GTF3C4	H3K14
NCOA1	H3K14
NCOA3	H3K14
CLOCK	H3K14
CDY1	
CDY2	
CDYL	
MGEA5	H4K8, H3K14
NAT10	

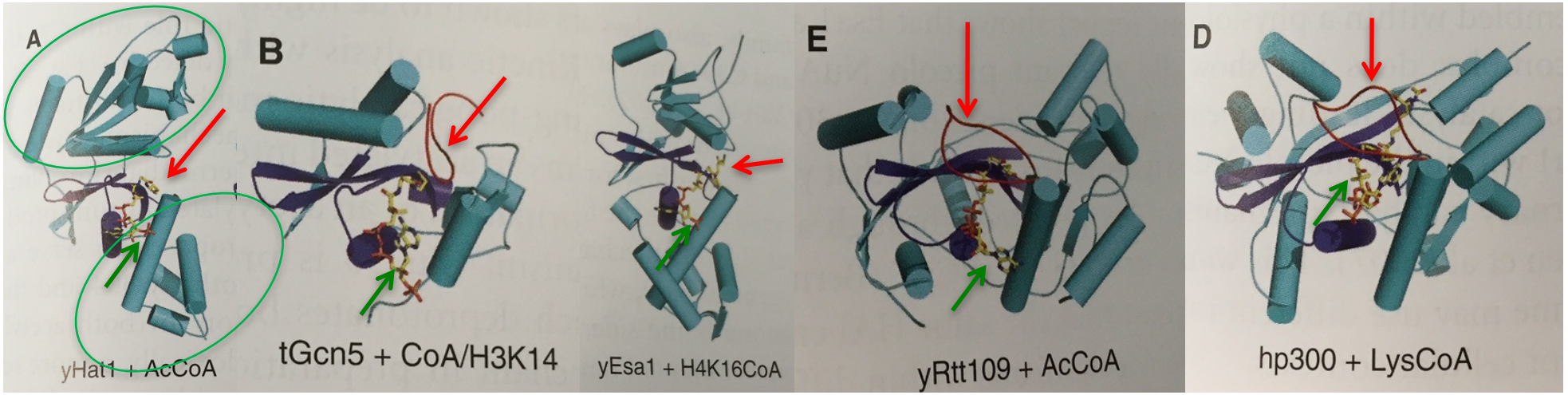
Specificity of HATs

- Many HATs have multiple lysine targets for acetylation
- A subset of lysines can be acetlylated by multiple HATs
- Predominantly located in histone tails
- A subset of HAT target lysines can also be subjected to methylation → competition between epigenetic information

H3K9 → me = silent
H3K9 → ac = active

H3K27 → me = silent
H3K27 → ac = active

Structures of major HAT families



HAT1 Fam.	GCN5/PCAF Fam.	MYST Fam.	P300/CBP Fam.	Rtt109 Fam.

HATs contain a HAT domain and structurally divergent ammino- and carboxy-terminal regions

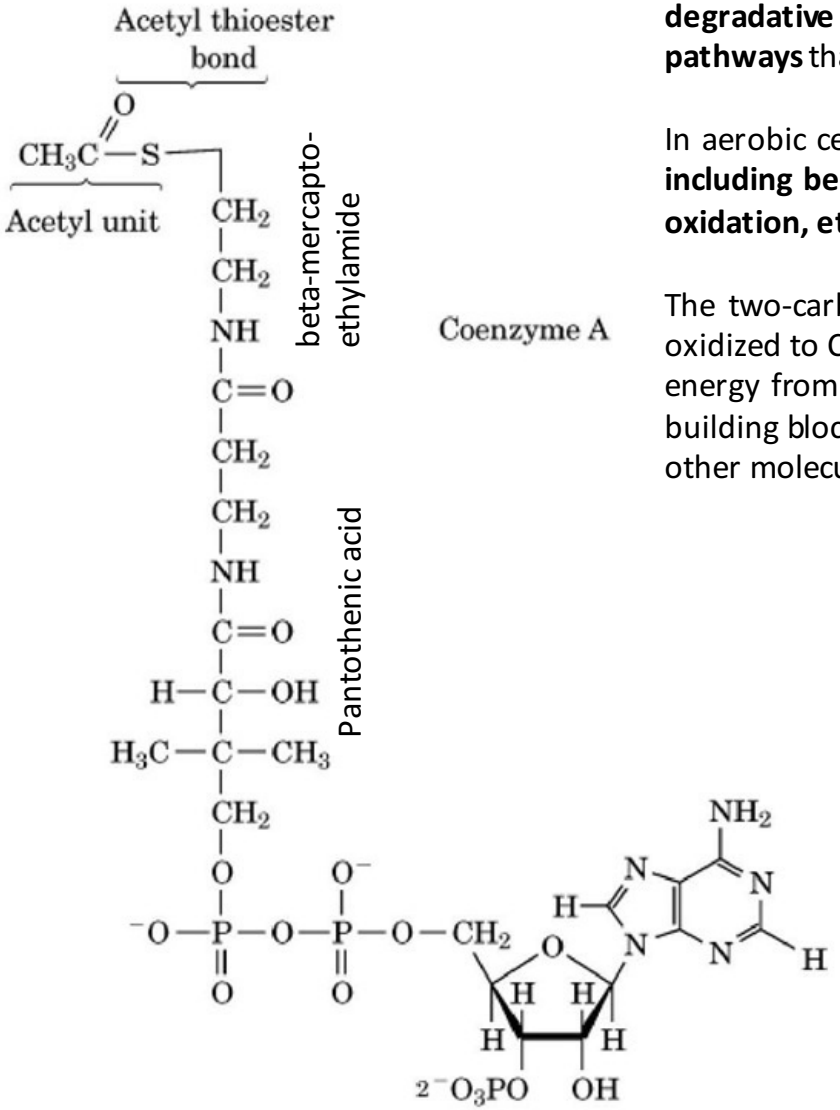
HAT domains are structurally similar and contain: 3 stranded beta-sheet and a long alpha helix
N- and C- terminal domains are divergent between HAT families

HATs contain clefts that allow the histone-substrate to access the central core domain and allow catalysis to occur (red arrow that points on red-line=histone tail)

The central core domain makes interaction with the Acetyl-CoenzymeA (AcCoA) co-factor (green arrow).

Acetyle CoA contains an acetyl group that is transferred by the HAT domain to the histone lysine residue located in the N-terminal histone tail

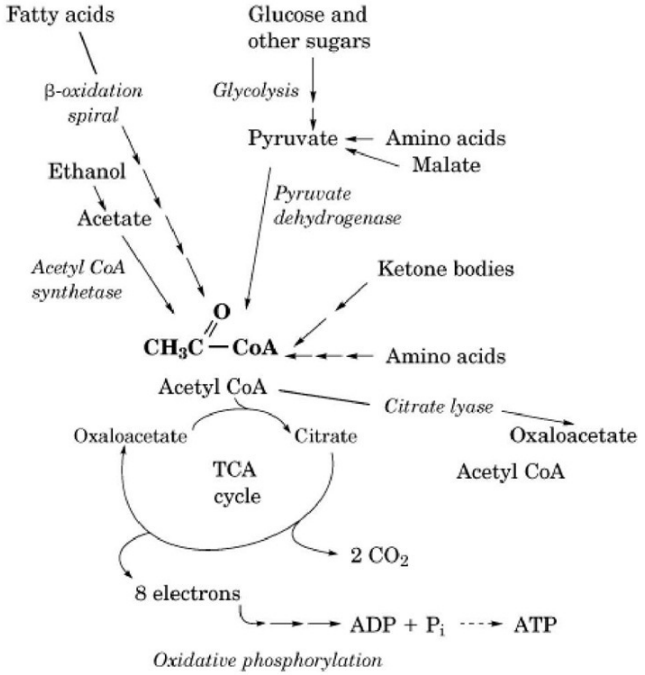
The chemistry of acetyl-transferases



Acetyl coenzyme A (acetylCoA) consists of a two-carbon activated acetyl unit attached to coenzyme A in thioester linkage. AcetylCoA is **central to energy generation from the degradative pathways of oxidative fuel metabolism and to a number of biosynthetic pathways** that utilize the activated two-carbon acetyl unit.

In aerobic cells, it is the **product of all the major catabolic pathways of fuel metabolism, including beta-oxidation of fatty acids, ketone body degradation, glycolysis and pyruvate oxidation, ethanol oxidation, and the oxidative degradation of many amino acids**.

The two-carbon acetyl unit of acetylCoA formed from these pathways can be completely oxidized to CO2 in the **tricarboxylic acid cycle (TCA cycle**), thus providing aerobic cells with energy from the complete oxidation of fuels. The acetyl unit of acetylCoA is also the basic building block of fatty acids, cholesterol, and other compounds, and it can be transferred to other molecules in acetylation reactions (eg, synthesis of N-acetylated sugars).



Catalytic mechanisms of HATs

1. GNAT family HATs

The Gcn5-related N-acetyltransferase (GNAT) family includes **Gcn5, PCAF, Hat1**, Elp3, Hpa2, Hpa3, ATF-2, and Nut1

Ordered sequential wherein both substrates (acetyl-CoA and histone) must bind to form a **ternary complex**

2. MYST family

MOZ, Ybf2 (Sas3), Sas2, and Tip60 [«MYST»], Esa1, **MOF**, MORF, and HBO1

Studies of yeast Esa1 from the MYST family of HATs have revealed a **ping-pong mechanism** involving conserved glutamate and cysteine residue

3. p300/CBP family

p300, CBP

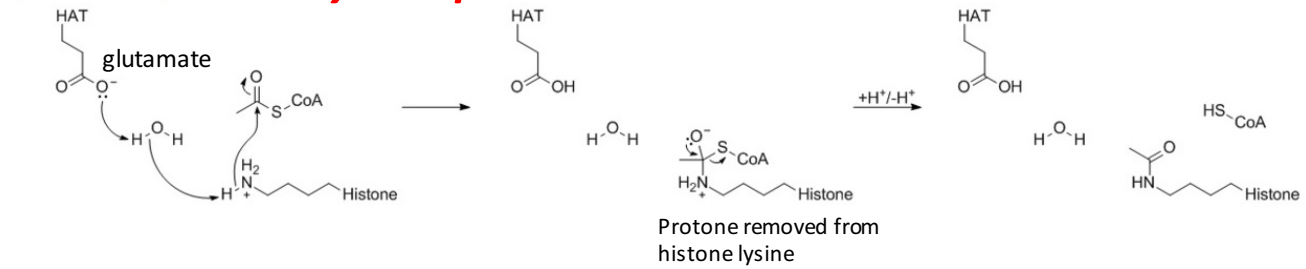
Catalysis by Theorell-Chance or **"hit-and-run"** acetyl transfer mechanism.

4. Rtt109

Not yet understood

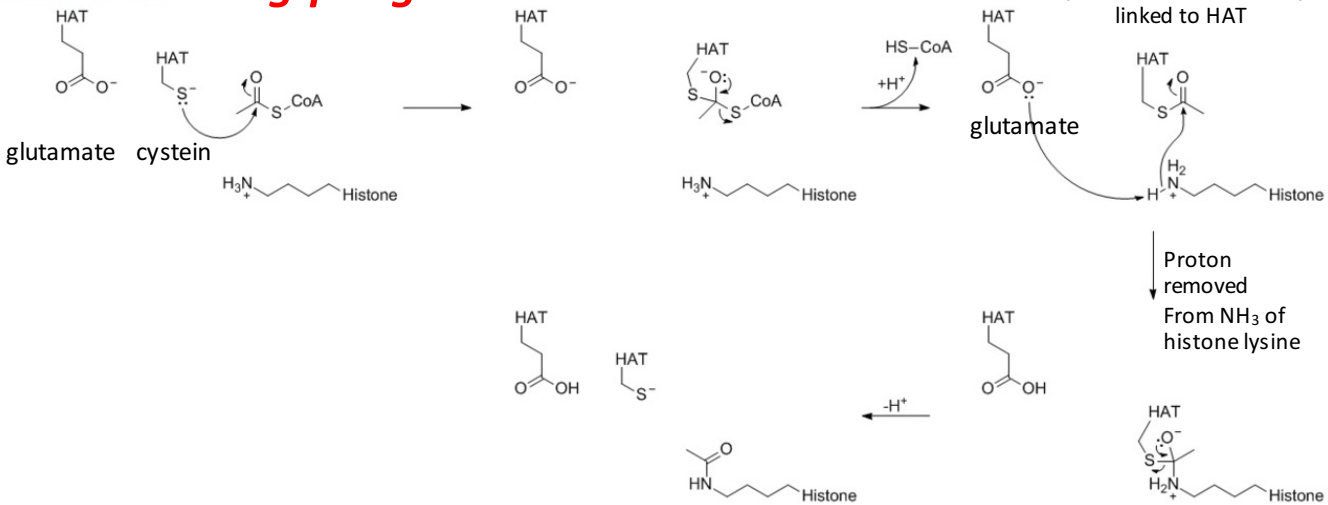
The chemistry of acetyl-transferases

A) GNAT family ***Ternary complex***



Protone removed from histone lysine

B) MYST family ***Ping-pong mechanism***



Nucleophilic attach on carbonyl group of acetyl-CoA
to transfer acetyl group tp lysine

Members of the GNAT family have a **conserved glutamate residue** that acts as a general base for catalyzing the nucleophilic attack of the lysine amine on the acetyl-CoA thioester bond. These HATs use an ordered sequential bi-bi mechanism wherein **both substrates (acetyl-CoA and histone) must bind to form a ternary complex with the enzyme before catalysis can occur. Acetyl-CoA binds first, followed by the histone substrate.** A conserved glutamate residue (**Glu173 in yeast Gcn5) activates a water molecule for removal of a proton from the amine group on lysine,** which activates it for direct **nucleophilic attack on the carbonyl carbon** of enzyme-bound acetyl-CoA. After the reaction, the acetylated histone is released first, followed by CoA.

Studies of yeast Esa1 from the **MYST family** of HATs have revealed a **ping-pong mechanism involving conserved glutamate and cysteine residues.**
The first part of the reaction involves the formation of **a covalent intermediate in which a cysteine residue becomes acetylated following nucleophilic attack of this residue on the carbonyl carbon of acetyl-CoA.** Then, a **glutamate residue acts as a general base to facilitate transfer of the acetyl group from the cysteine to the histone substrate in a manner analogous to the mechanism used by GNATs.**

HATs have the same biochemical function
But can use slightey different chemical reactions to acetylate histones
Reason: reaction is very simple and Acetyl-CoA is very reactive

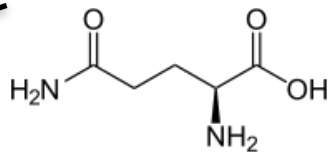
A **ternary complex** is a protein complex containing three different molecules that are bound together. In structural biology, ternary complex can also be used to describe a crystal containing a protein with two small molecules bound, for example cofactor and substrate;

The chemistry of acetyl-transferases – Gcn5/PCAF Family

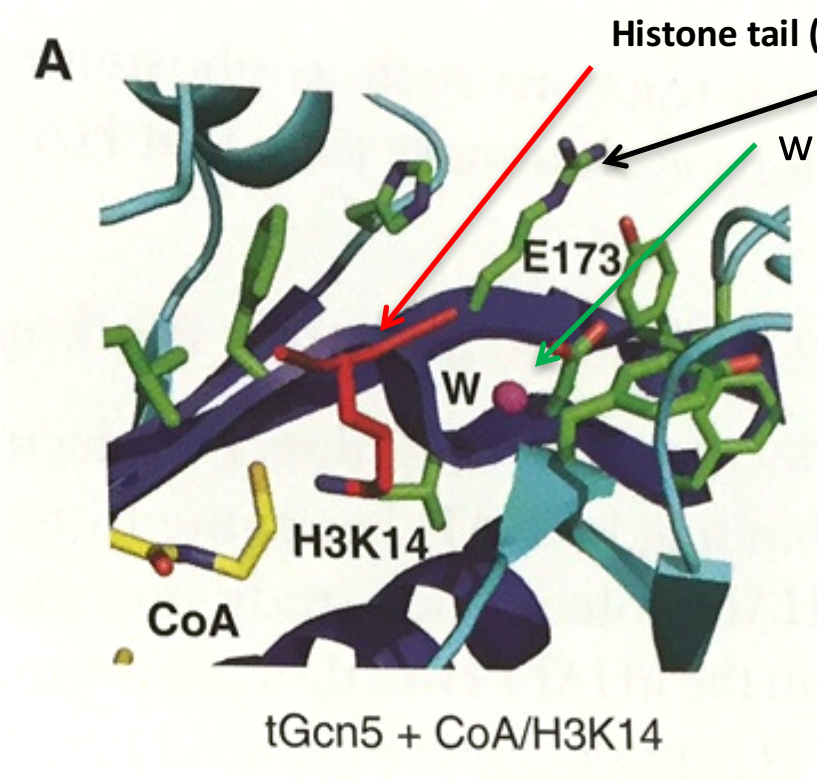
An example:

GNAT family: Gcn5/PCAF/HAT1 – histone H3K14

Glutamate 173



Ternary complex



tGcn5 + CoA/H3K14

Gcn5: glutamate at position 173 (E173) is central for HAT activity.

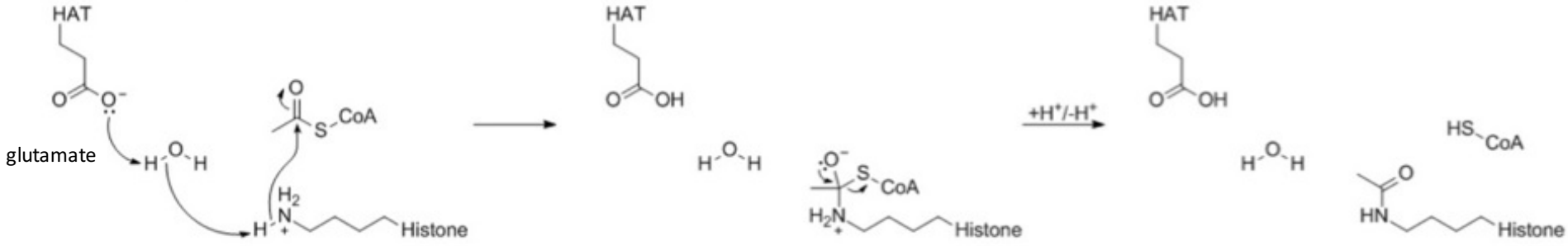
Point mutation at E173 → HAT activity is reduced 300-fold

Glutamate acts as general base for catalysis through a well ordered water molecule in the active site. Glutamate is located in a hydrophobic pocket that supports proton extraction

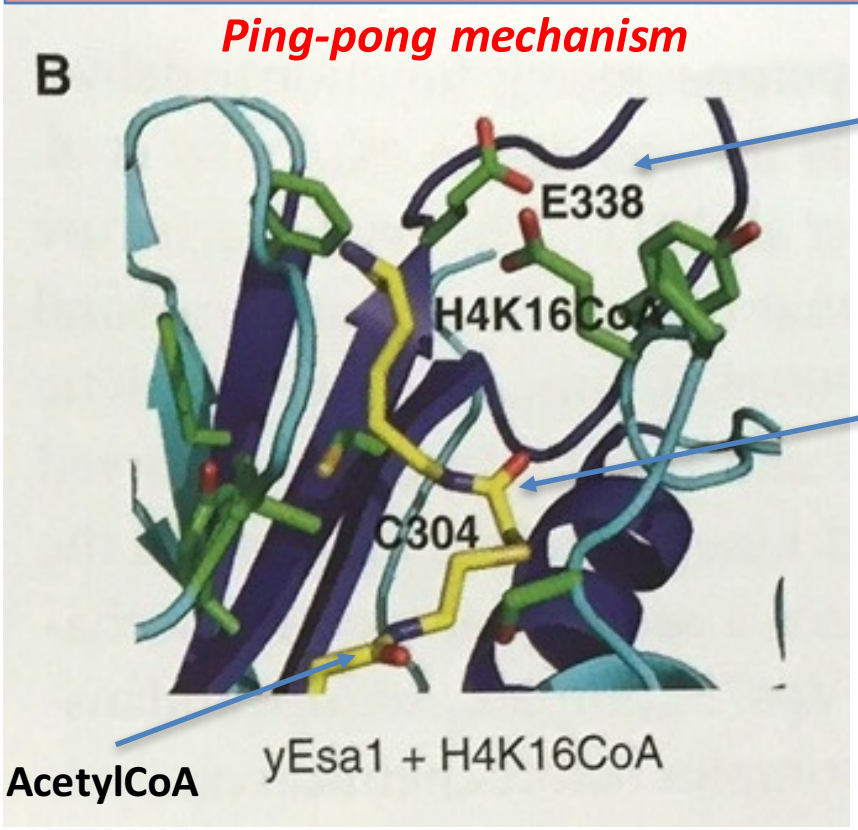
Gcn5 functions through a ternary complex mechanism in which both substrates (lysines and Acetyle-CoA) must be bound to the enzyme before catalysis can occur.

This involves a de-pronotation of the lysine substrate By the glutamate (E173) thus facilitating the direct transfer of the acetyl group from AcCoA to the lysine side chain.

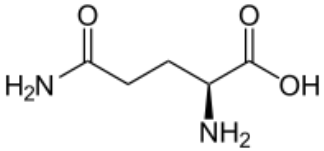
A) GNAT family



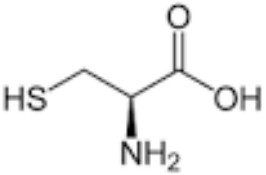
The chemistry of acetyl-transferases – MYST Family



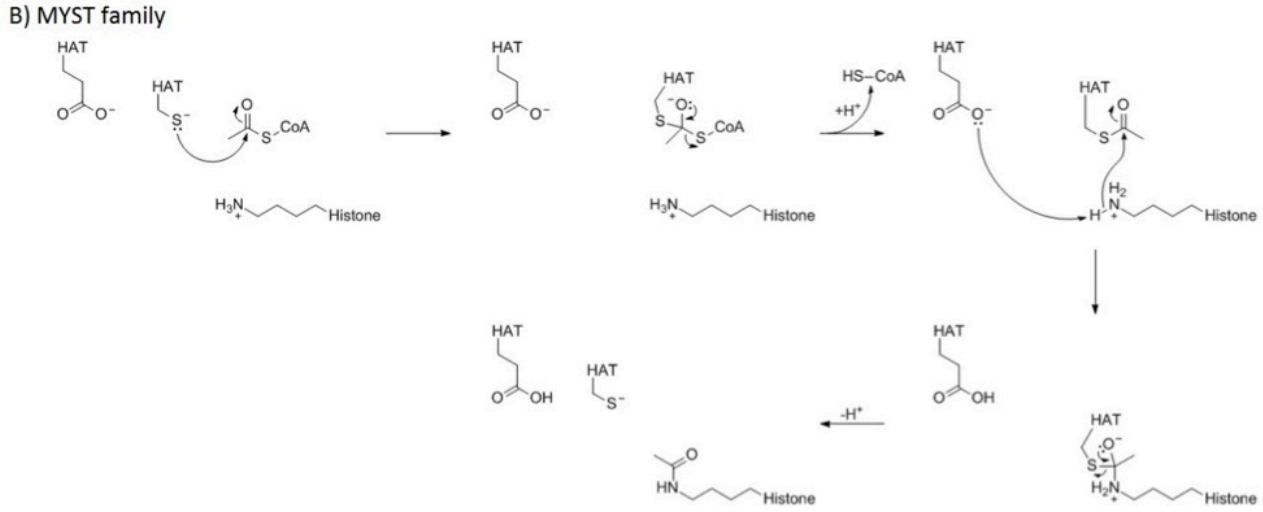
Glutamte (E)



Cystein (C)



MYST family: Important residues: Glu338 and Cys304. Mutations at these residue disrupt HAT activity. Studies of yeast **Esa1 from the MYST family of HATs** have revealed a ping-pong mechanism involving conserved glutamate and cysteine residues. **Glu338 serves as a general base to that protonates Cys304** and the histone lysine residue in preparation for both acetylation events. The first part of the reaction involves **the formation of a covalent intermediate in which a cysteine residue becomes acetylated** following nucleophilic attack of this residue on the carbonyl carbon of acetyl-CoA. Then, a glutamate residue acts as a **general base to facilitate transfer of the acetyl group from the cysteine to the histone substrat**e in a manner analogous to the mechanism used by GNATs.



The chemistry of acetyl-transferases – p300/CBP; Rtt109; HAT1 Family

p300/CBP: ***"Hit and run" mechanism***

- not glutamate residue for driving acetylation reaction;
- One Tyrosine (Tyr) and one Tryptophane (Trp) residues have impact on acetylation
- In human p300, Tyr1467 acts as a general acid and Trp1436 helps orient the target lysine residue of the histone substrate into the active site.

Tyr1467 mutation: 400 fold reduction in catalytic activity: Trp1436 mutation: 50 fold reduction

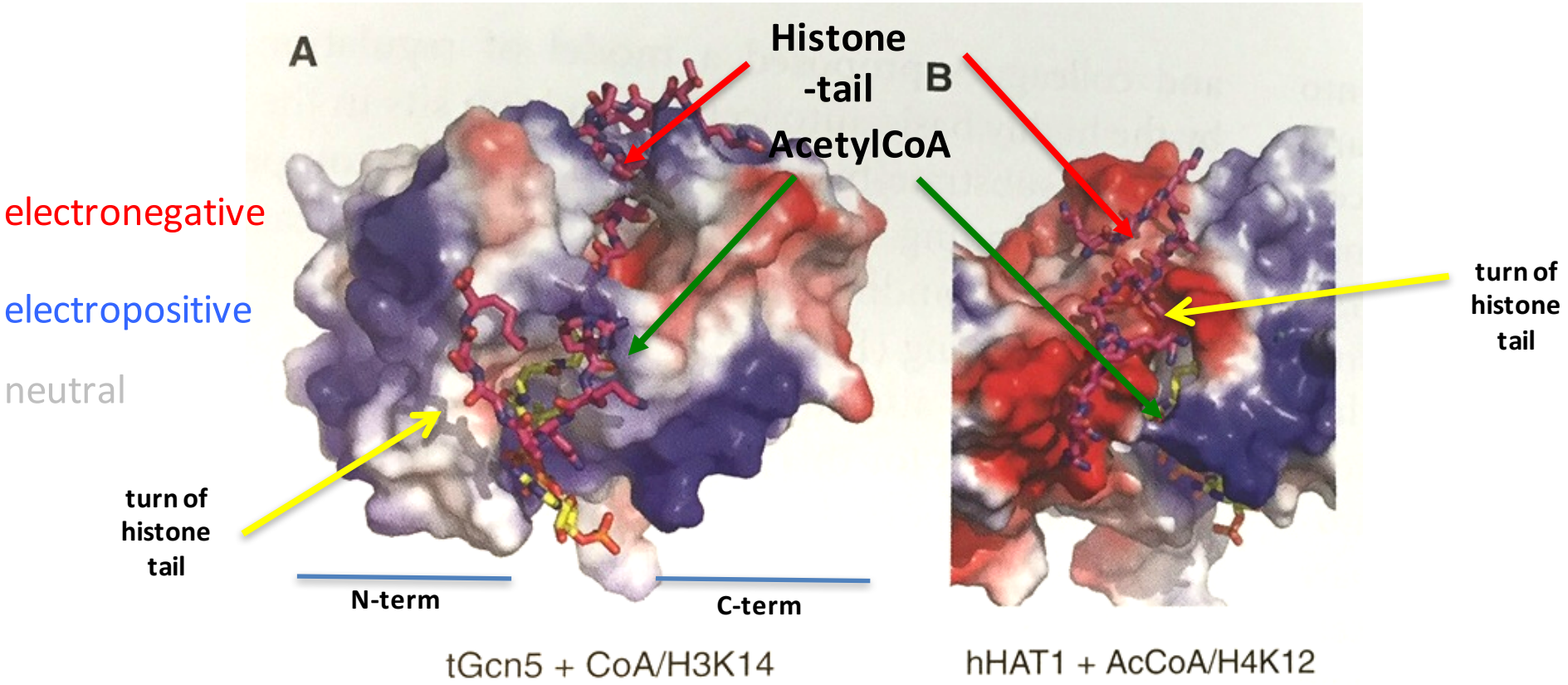
- **Hit and run catalytic mechanism (Theorell Chance)** : distinct from the classic ternary mechanism, Theorell Chance is characterized that the ternary complex never accumulates and the steady-state concentrations of the ternary complex is kinetically insignificant.
- Following the association of Ac-CoA, the protein substrate binds transiently to the p300 surface, allowing the lysine residue to sneak through the enzyme active site to receive the acetyl group, followed by rapid protein dissociation. Note: the detailed molecular mechanism of catalysis remain unanswered

Rtt109;HAT1: less characterized

HATs have the same biochemical function
But can use slightly different chemical reactions to acetylates histones
Reason: reaction is very simple and requires "low chemical input"and Acetyle-CoA is very reactive
Long evolution time allowed to form diverse modes of acetylation

Histone substrate binding

To date information only on the binding of Gcn5 to the H3K14 region and hHAT1 to the H4K12 region



Gcn5: Histone tails are fit into a grove formed by the N- and C-terminal domains. Conserved ammino-acids form **hydrogen bonds and van der Waals interaction with H3 histone tails**. H3 tail adopts an ordered structure → basis for major specificity of Gcn5 family for histone H3 tail (Gcn5 activity towards H4 is low)

hHAT1: Histone H4 tail is fit into a grove and forms a turn structure that normally remains extended.
Two conserved hHAT1 residues (Trp199, Tyr225) interact with Gly9 and Lys8 of the turned histone tail.
Conserved amminoacids accommodate the H4 tail into the groove and Bring the K12 residue in vicinity to the active center. Other histone-tails have other peptide sequence context and to not form specific interactions with hHAT1 → specificity for H3 tails

Regulation by auto-acetylation and protein cofactors

HATs are regulated by AUTOACETYLATION and INTERACTION WITH REGUALTORY PROTEINS

1. REGULATORY PROTEINS

→Purified HATs such as GCN5 or PCAF act efficiently on free histone and histone-tail peptides but are less efficient on prepared nucleosomes

→In vivo, HATs function in *multiprotein complexes (HAT + cofactors)* to acetylate histone tails on nucleosomes. Complexes can contain 10-20 subunits that can also be shared amongst different HAT complexes

EXAMPLES: Gcn5 → SAGA complex (yeast); SLIK complex (human)

PCAF → TCTC complex (yeast); STAGA complex (human)

The role of most complex components is to support HAT specificity and activity

→HATs interact with *cofactors* in HAT complexes to increase processivity

EXAMPLE: Sas2 (MYST family) has to interact with Sas2 and Sas4 to have HAT activity

EXAMPLE: Rtt109 has no or little HAT activity; interaction with Vps75 or Asf1 (histone chaperon) increases HAT acitivity (100x) and mediates H3K9/H3K27 Aacetylation (Vps75) or H3K56 acetylation (Asf1)

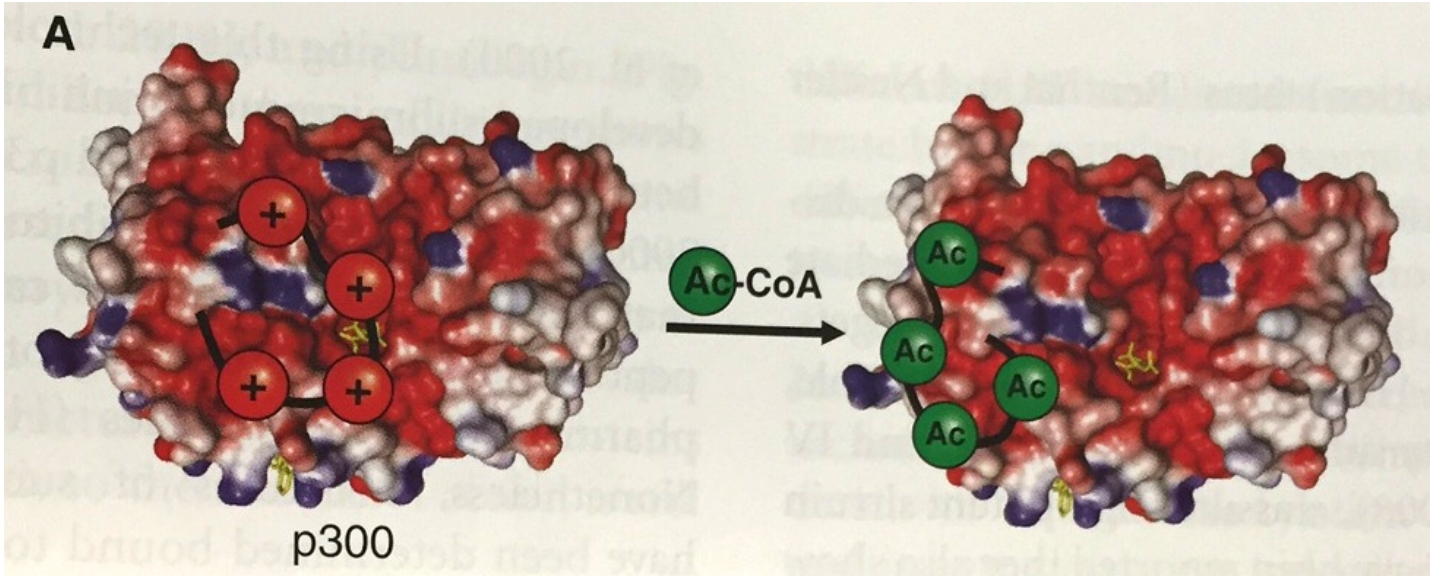
Regulation by autoacetylation and protein cofactors

HATs are regulated by AUTOACETYLATION and INTERACTION WITH REGUALTORY PROTEINS

2. AUTOACETYLATION

→Rtt109, p300/CBP and MYST HAT family members are controlled by auto-acetylation
HYPOACETYLATED HAT: INACTIVE
HYPERACETYLATED HAT: ACTIVE

EXAMPLE: p300 activity is controlled by acetylation of a 40 aa basic loop = autoacetylation loop



Under-acetylated "autoacetylation loop"
blocks substrate (histone tail) binding site of p300

hyper-acetylated "autoacetylation loop"
enhances substrate (histone tail binding site of p300

Regulation by autoacetylation and protein cofactors

HATs are regulated by AUTOACETYLATION and INTERACTION WITH REGUALTORY PROTEINS

2. AUTOACETYLATION

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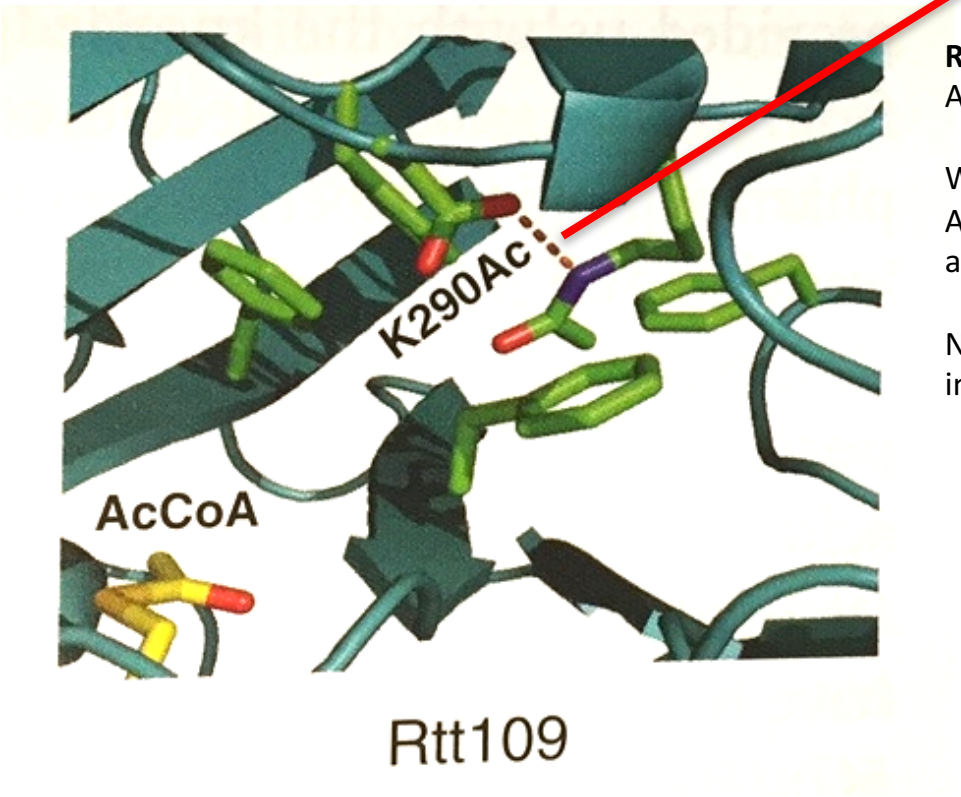
→HYPOACETYLATED HAT: INACTIVE
HYPERACETYLATED HAT: ACTIVE



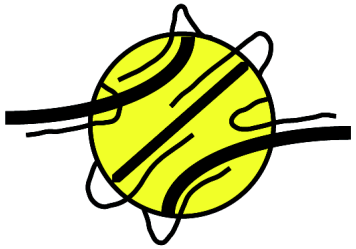
Rtt109:
Acetylation of Lys290 is required for full HAT activity.

WHY?
Acetylation of Lys290 disrupts hydrogen bonds between Lys290 and Asp288. This improves incorporation of Acetyl-CoA

Note: mutations in Asp288 increase HAT activity → presumably improved Acetyl-CoA binding

Impact of HATs on gene expression: ACTIVATORS OF GENE EXPRESSION

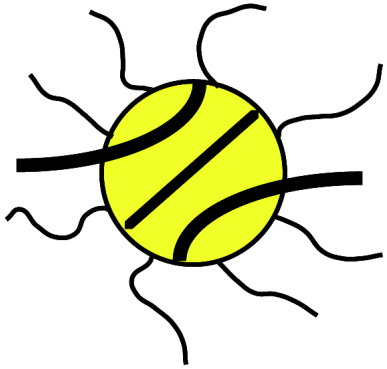
Acetylation induces a conformational change in the core histones



REPRESSED

EXAMPLE

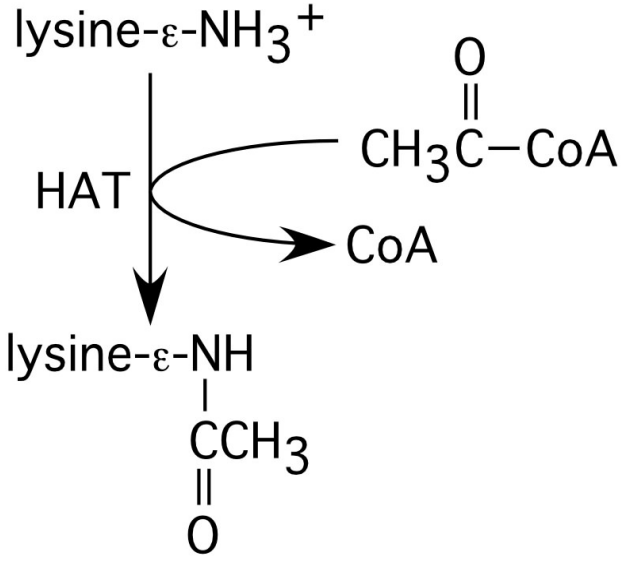
Lysine-rich tails bind tightly to DNA and repress transcription by blocking access of factors to the DNA template.



ACTIVE/COMPETENT

Acetylation of lysine residues alters chromatin structure and allows binding of transcription factors.

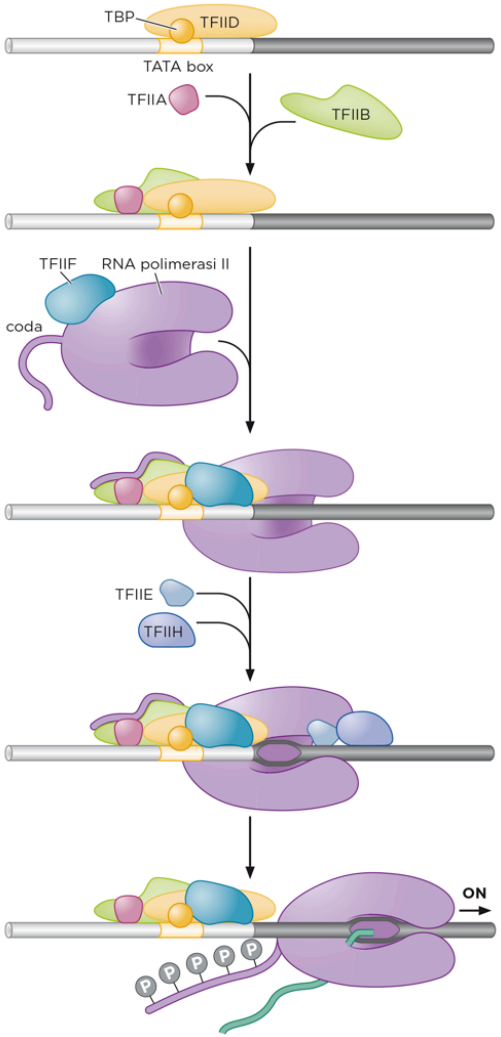
Note: acetylation neutralizes the positive charge of lysine

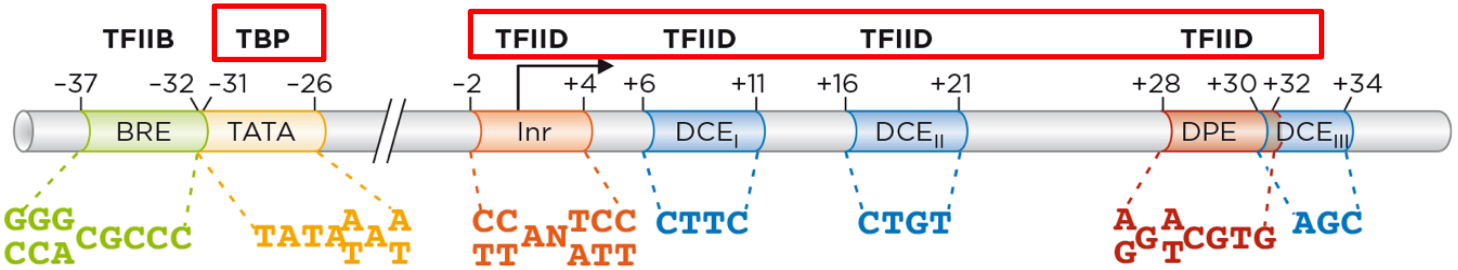


HAT: Histone Acetyltransferase

Transcription by RNA Polymerase II (RNAPII)
The RNAP II core promoter

CENTRAL PROMOTER ELEMNETS + GENERAL TRASNCRIPTION FACTORS
ca 60 nt





BRE TFIIB recognition element

TATA-box Bound by the TATA-box binding protein (TBP)

Inr Initiator (l'iniziatore)

DCE downstream core element

DPE downstream promoter element

GTFs:

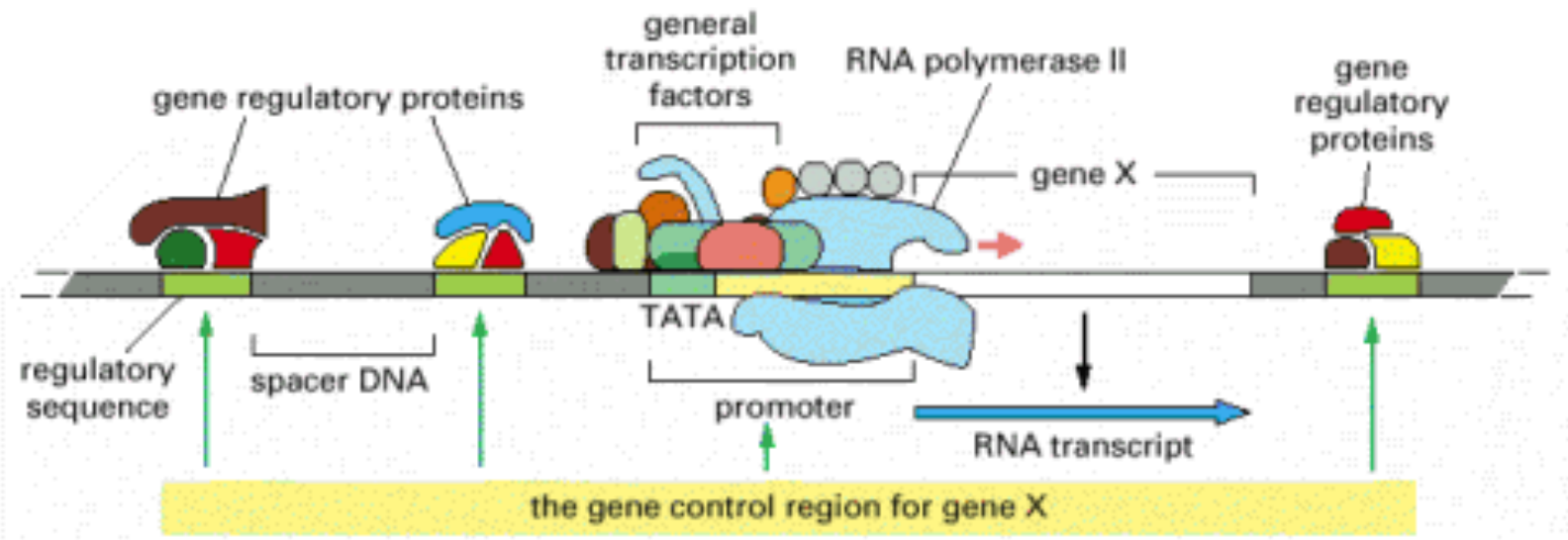
→**Help RNA Pol II to bind promoter**

→**Promoter melting**

(→→→ **same function like sigma factor in bacterial gene activation)**

→ **Pass on to elongation phase of transcription**

A complex interplay of regualtory sequences and transcription factors control the basal transcription complex

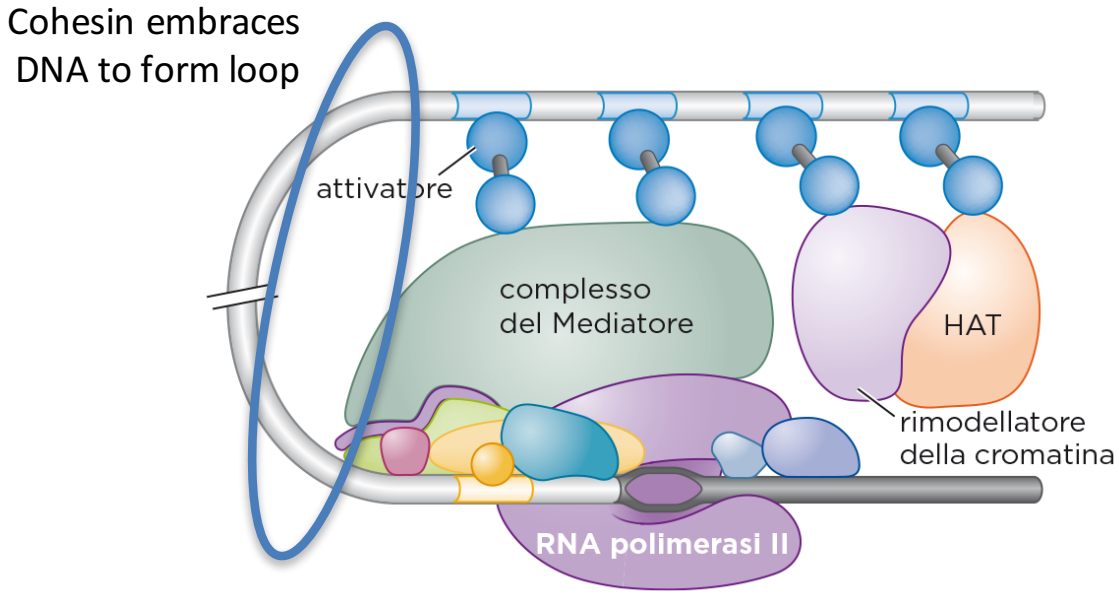


The gene control region of a typical eucaryotic gene.
The ***promoter*** is the DNA sequence where the general transcription factors and the polymerase assemble. The ***regulatory sequences*** serve as **binding sites** for **gene regulatory proteins**, whose presence on the DNA affects the rate of transcription initiation.

These sequences can be located **adjacent** to the promoter, far **upstream** of it, or even **within introns** or **downstream** of the gene. These sites can be bound by cell type specific factors that define promoter specificty or enhance specificity **DNA looping** is thought to allow gene regulatory proteins bound at any of these positions to interact with the proteins that assemble at the promoter.

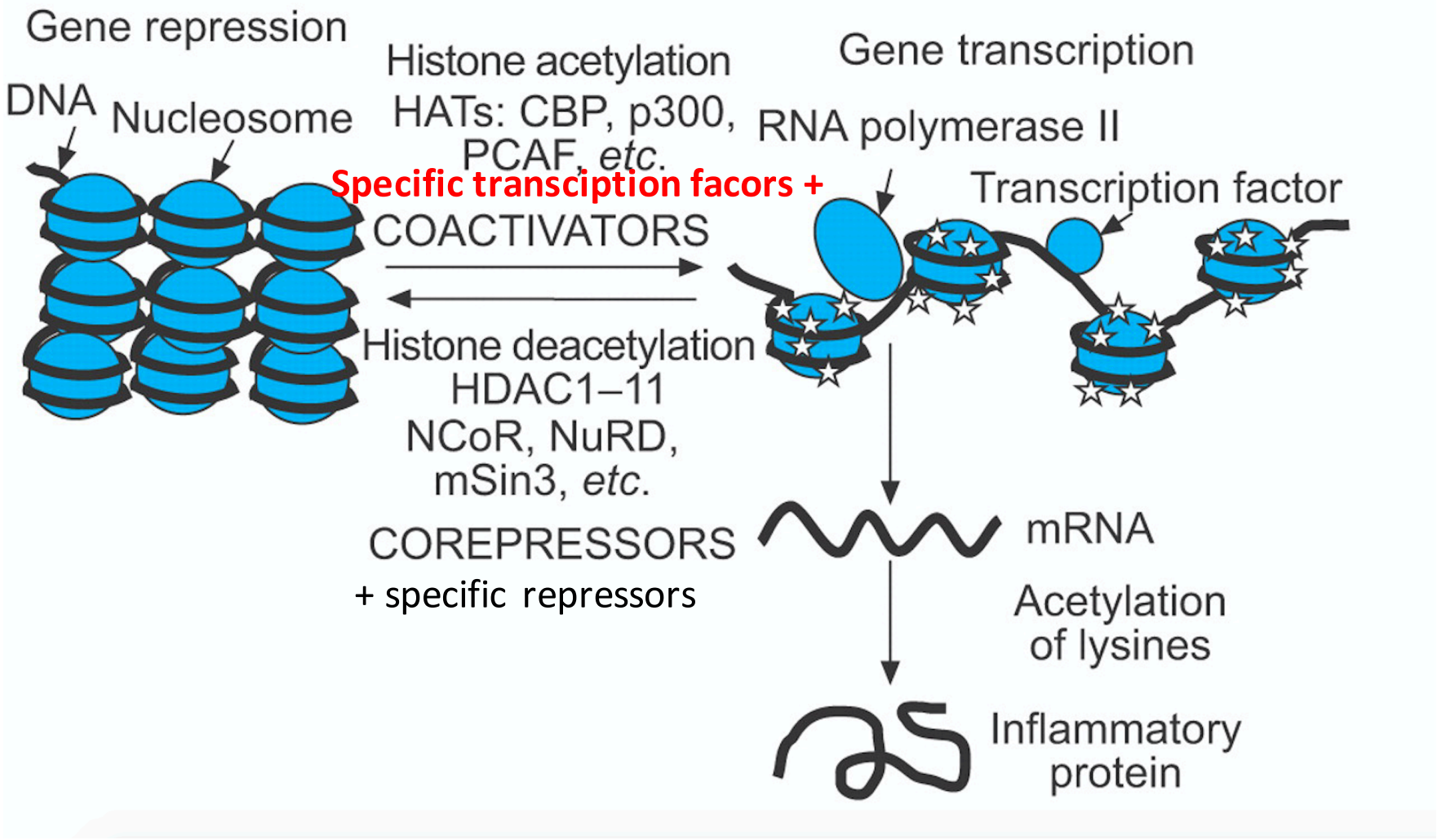
Whereas the **general transcription factors** that assemble at the promoter are **similar for all** polymerase II transcribed **genes**, the **gene regulatory proteins** and the **locations** of their binding sites relative to the promoter are **different for each gene**.

A complex interplay of regualtory sequences and transcription factors control the basal transcription complex



- → The mediator complex is a large protein complex (<20 proteins) that communicates between the basal transcription factors and activating regulatoy elements.
- → Essential for the initiation of transcription!!
- → Linked with HATs
- → Stabilized by cohesin

Impact of HATs on gene expression:ACTIVATORS OF GENE EXPRESSION

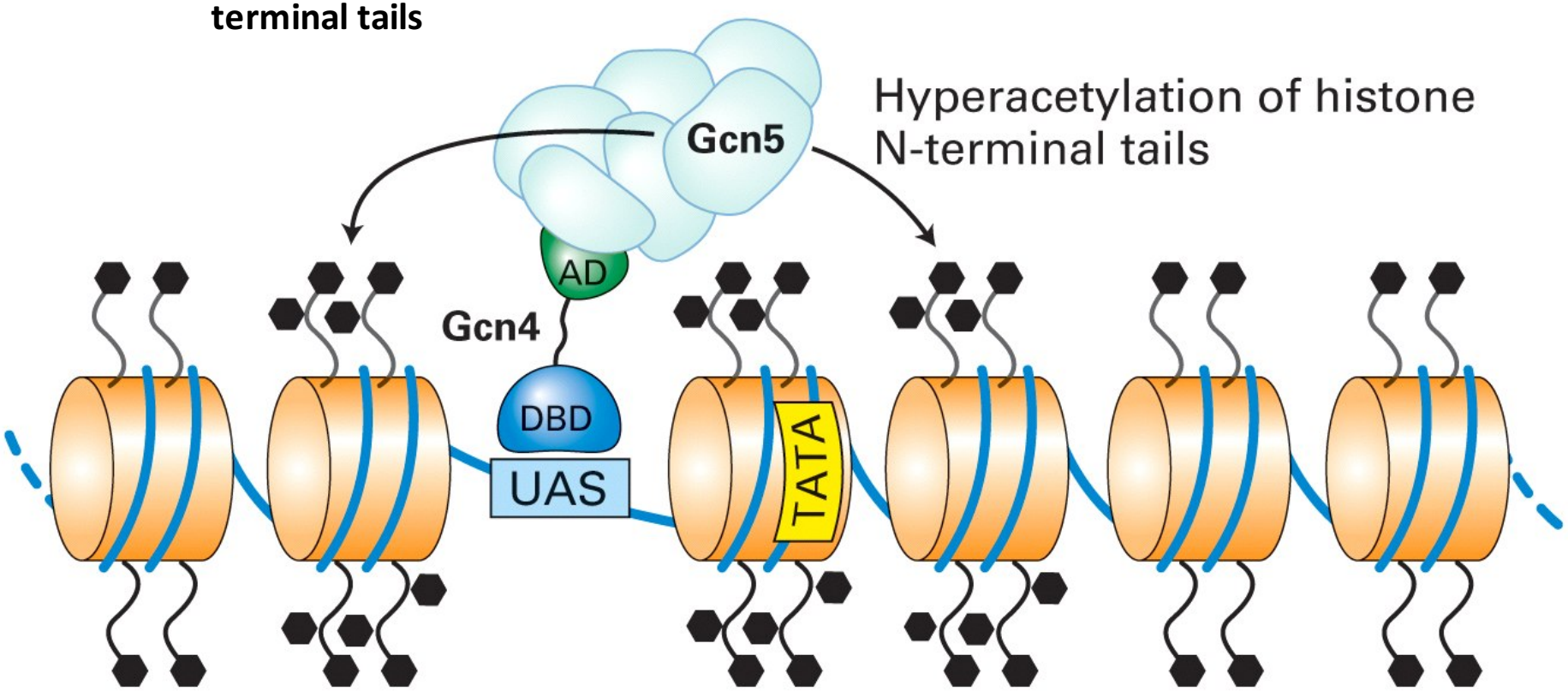


Specific transcription factors bind TF binding sites outside the core-promoter.
Can be in vicinity to the core-promoter, but may be also localized at large disctance from core pomoter (enhancer)

Impact of HATs on gene expression:ACTIVATORS OF GENE EXPRESSION

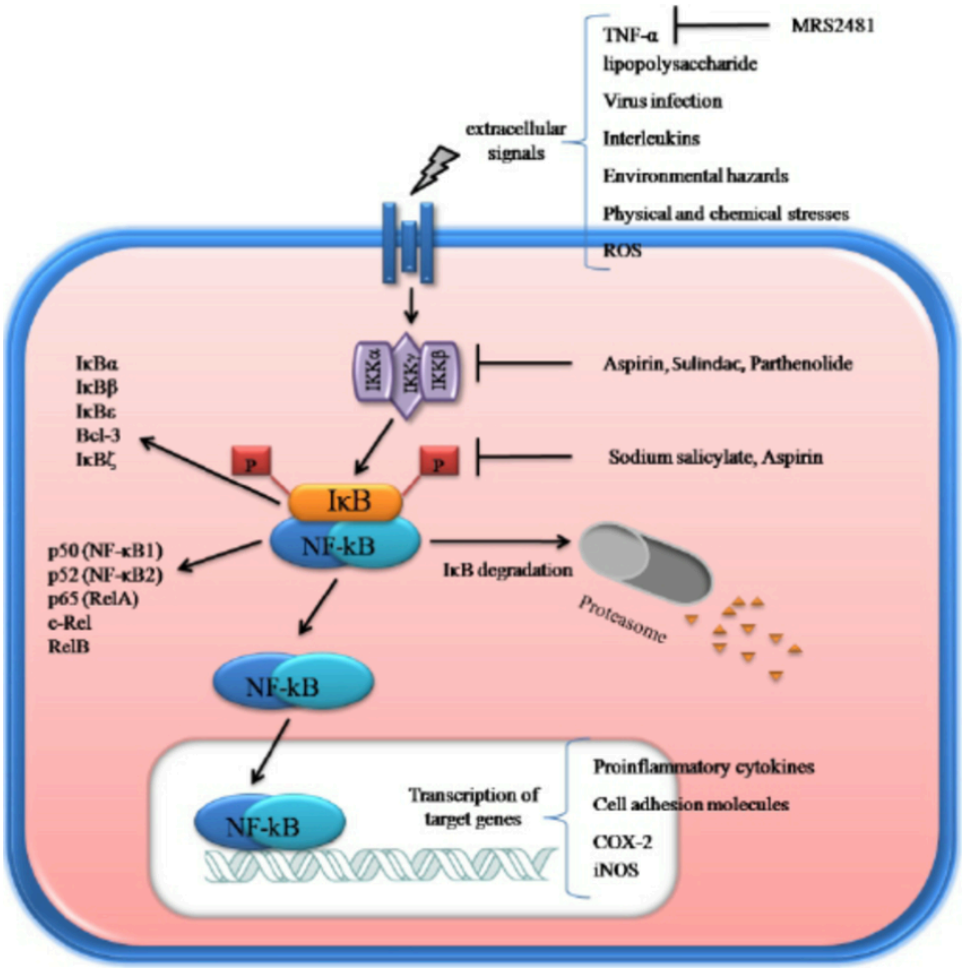
Activator-directed hyperacetylation of histone N-terminal tails

A simple example from YEAST



UAS Upstream activator site is located upstream of core-promoter
UAS is bound by transcriptional co-activator that recruits Gcn5. Gcn5 acetylated Histone tails. This opens chromatin and facilitates the access of general transcription factors required for initiation of transcription.

Impact of HATs on gene expression:ACTIVATORS OF GENE EXPRESSION



NFkappaB is a central regualtor of inflammation that activates pro-inflammatory genes, cell survival and cell proliferation

In unstimulated cells, NF-κB dimers are sequestered in the cytoplasm by a family of inhibitors, called IκBs. IκBs bind NF-κB and cover NLS domain.

Extracelllar stimulus activates the IκB kinase (IKK).

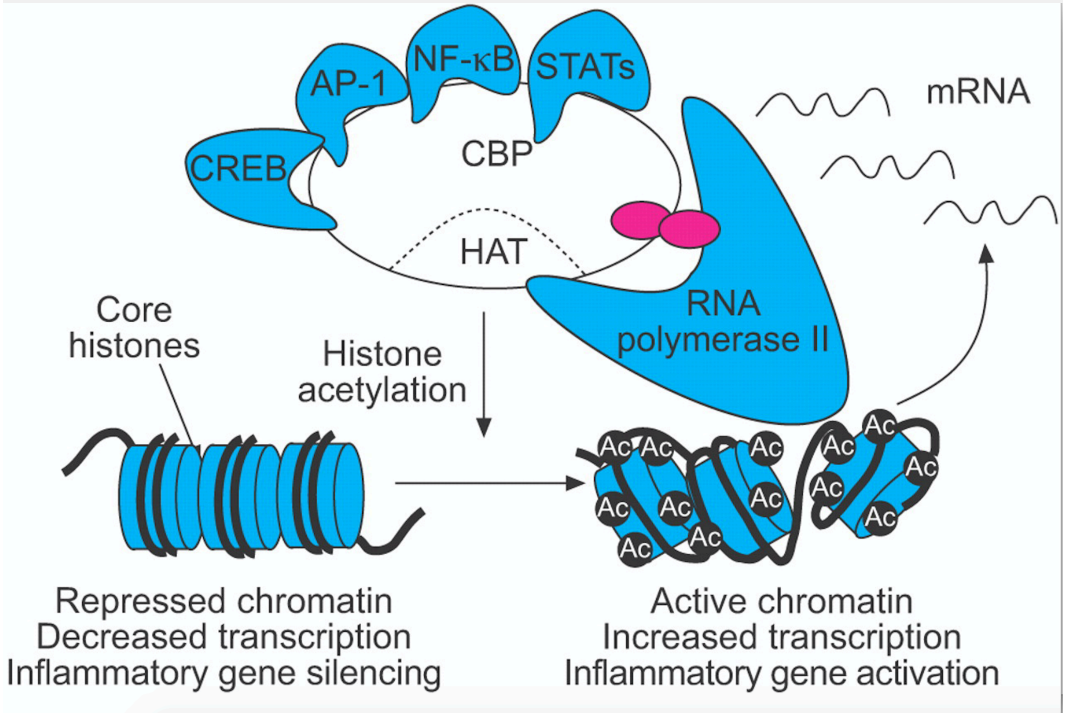
IKK is composed of a heterodimer of the catalytic IKKα and IKKβ subunits and a "master" regulatory protein termed NEMO (NF-κB essential modulator) or IKKγ.

When activated, IκB kinase phosphorylates two serine residues located in an IκB regulatory domain (e.g., serines 32 and 36 in human IκBα); subsequently, the IκB proteins are ubiquitinated, leading to the degradation by the proteasome.

After degradation of IκB, the NF-κB complex can enter the nucleus and 'turn on' the expression of specific genes that have DNA-binding sites for NF-κB at the promoter

Impact of HATs on gene expression:ACTIVATORS OF GENE EXPRESSION

Coactivators, such as CREB-binding protein (CBP), have intrinsic histone acetyltransferase (HAT) activity, resulting in opening up to the chromatin structure, which allows binding of RNA polymerase II and initiation of gene transcription. Several transcription factors interact with CBP, including cyclic AMP response element binding protein (CREB), nuclear factor (NF)-κB, activator protein (AP)-1 and signal transduction activated transcription factors (STATs).

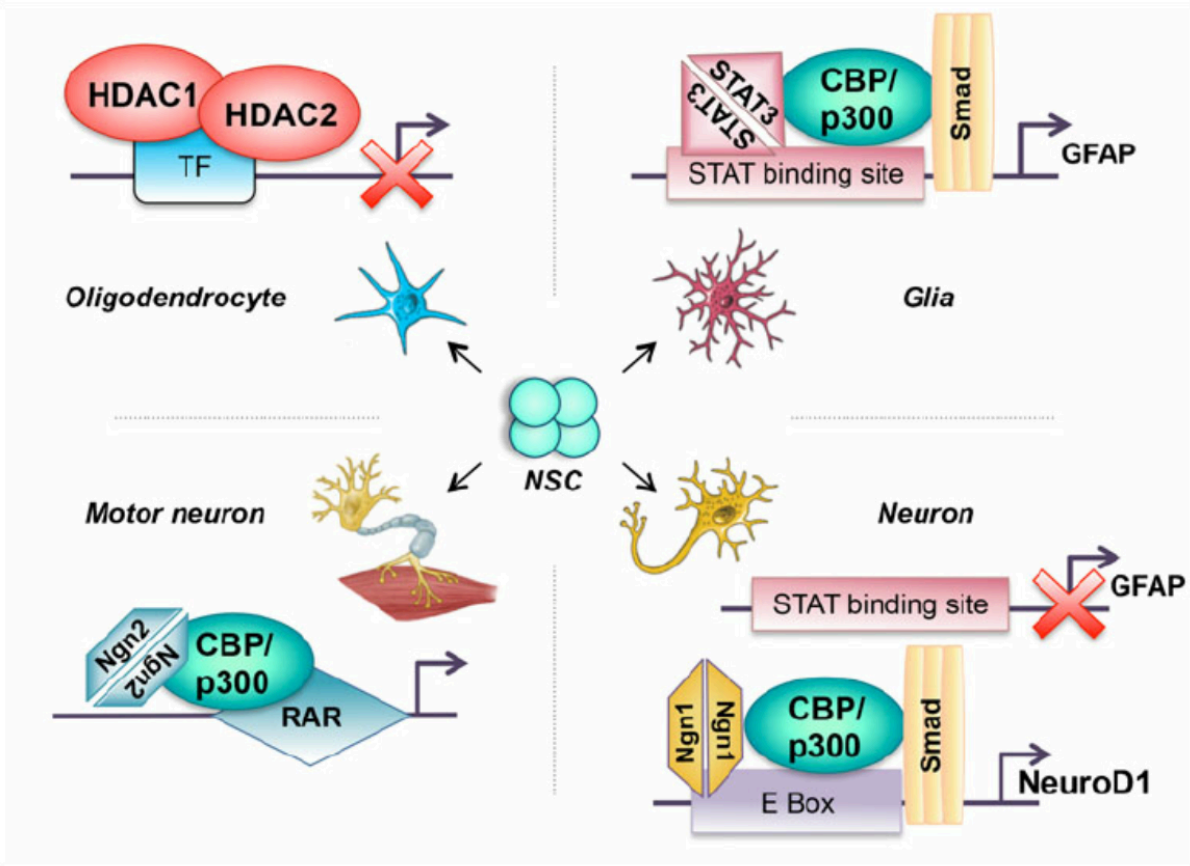


Several strong transcription factors (TF) assemble with NFkB. This fascilitates an efficient activation of transcription.
TFs bind different binding sites in target gene promoters.
The activation complex contains CBP HAT to acetylate histones in target gene promoters

General concept for gene regulation: TFs guide epigentic regualtors

NFkappaB is a central regualtor of inflammation that activates pro-inflammatory genes

Impact of HATs on gene expression:ACTIVATORS OF GENE EXPRESSION



General concept for gene regulation: TFs guide epigentic regualtors

Specific transciription factors recruit HATs to "open" chromatin and to open the promoter

Fig. 2 Role of acetylation in different lineage determination. The neural stem cells (NSCs) exist in a niche, which can be differentially modulated to specific neuronal lineages. A differential recruitment of specific transcription factors (TF) to the same acetyltransferases determine specific neural cell fates from the NSCs. Cyclic adenomonophosphate response element-binding (CREB) binding protein (CBP)/p300 histone acetyletransferases (HATs) interact with STAT and SMAD activating glial fibrillary acidic protein (GFAP) expression, thus specifying the glial lineage. Increased expression of neurogenin (Ngn1) titrates this complex, thus leading to the release of STAT, blocking GFAP expression. The new Ngn1–CBP/p300–SMAD complex subsequently binds to the E box elements, which results in a neuron cell type due to the activation of NeuroD1 expression [53]. CBP/p300 when bound to retinoic acid receptor (RAR) and neurogenin 2 (Ngn2) leads to a differentiation of the motor neuron cells. The deacetylases histone deacetylases (HDACs) HDAC1 and HDAC2 act as a general repressor, blocking the transcription factor and thereby resulting in oligodendrocyte specification

HATs and Disease

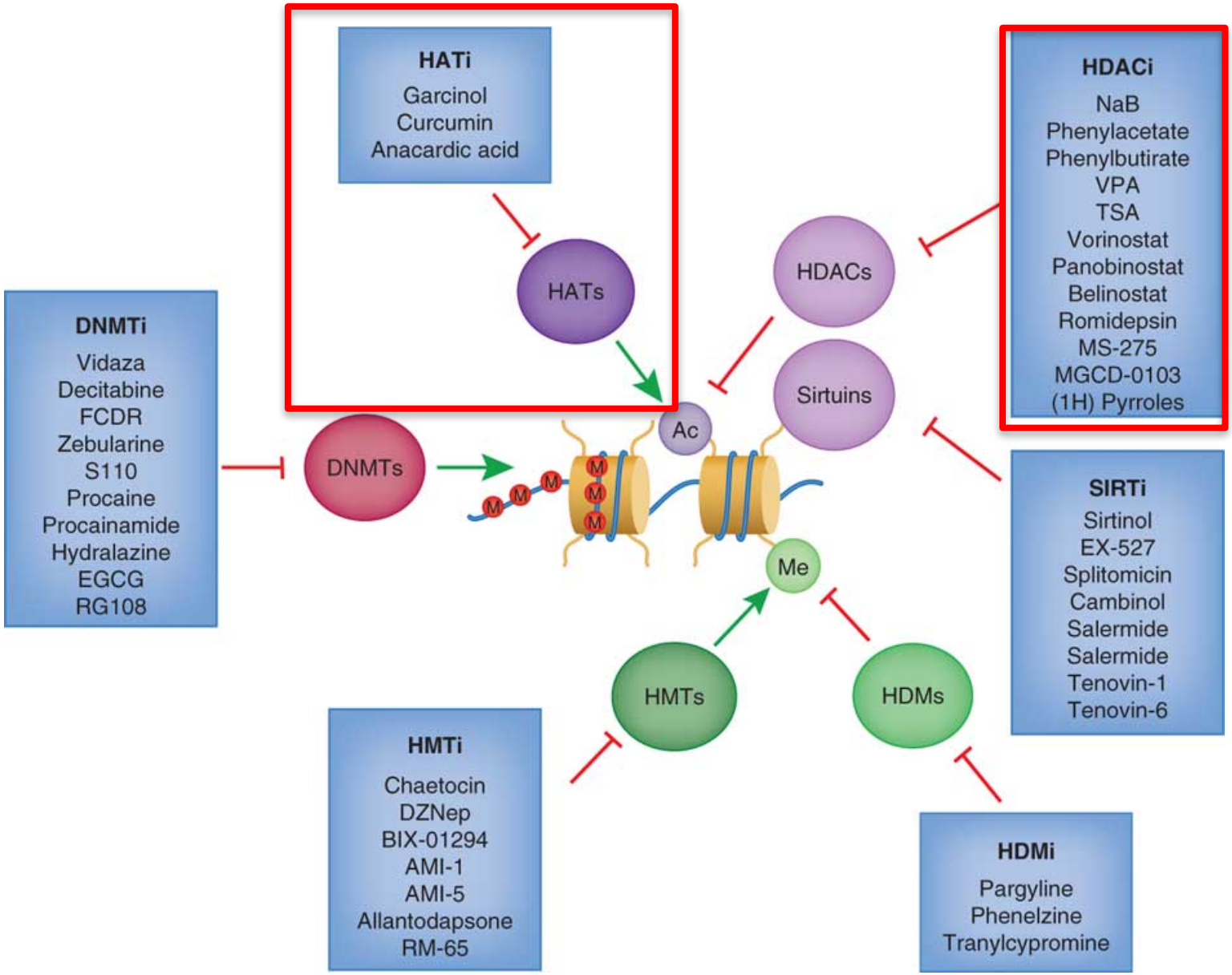
HATs are transcriptional co-activators:

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

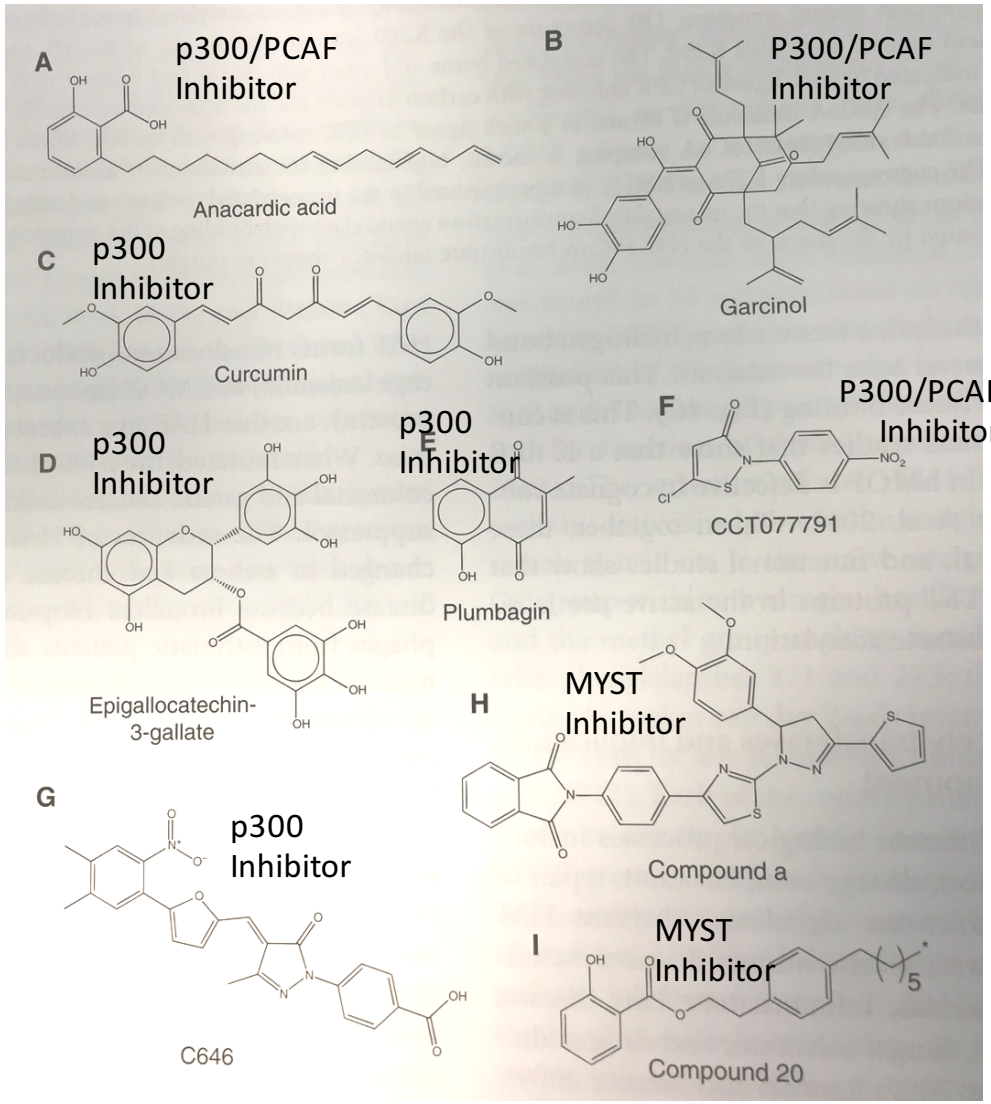
p300/CBP: translocation → cancer
p300 mutations found in colorectal and gastric cacer → p300 is a tumorsuppressor
p300 is involved in diabetes
p300 links drug addiction to histone acetylation status
…

Usefulness of epigenetic drugs?

HATs and Disease – Epigenetic drugs

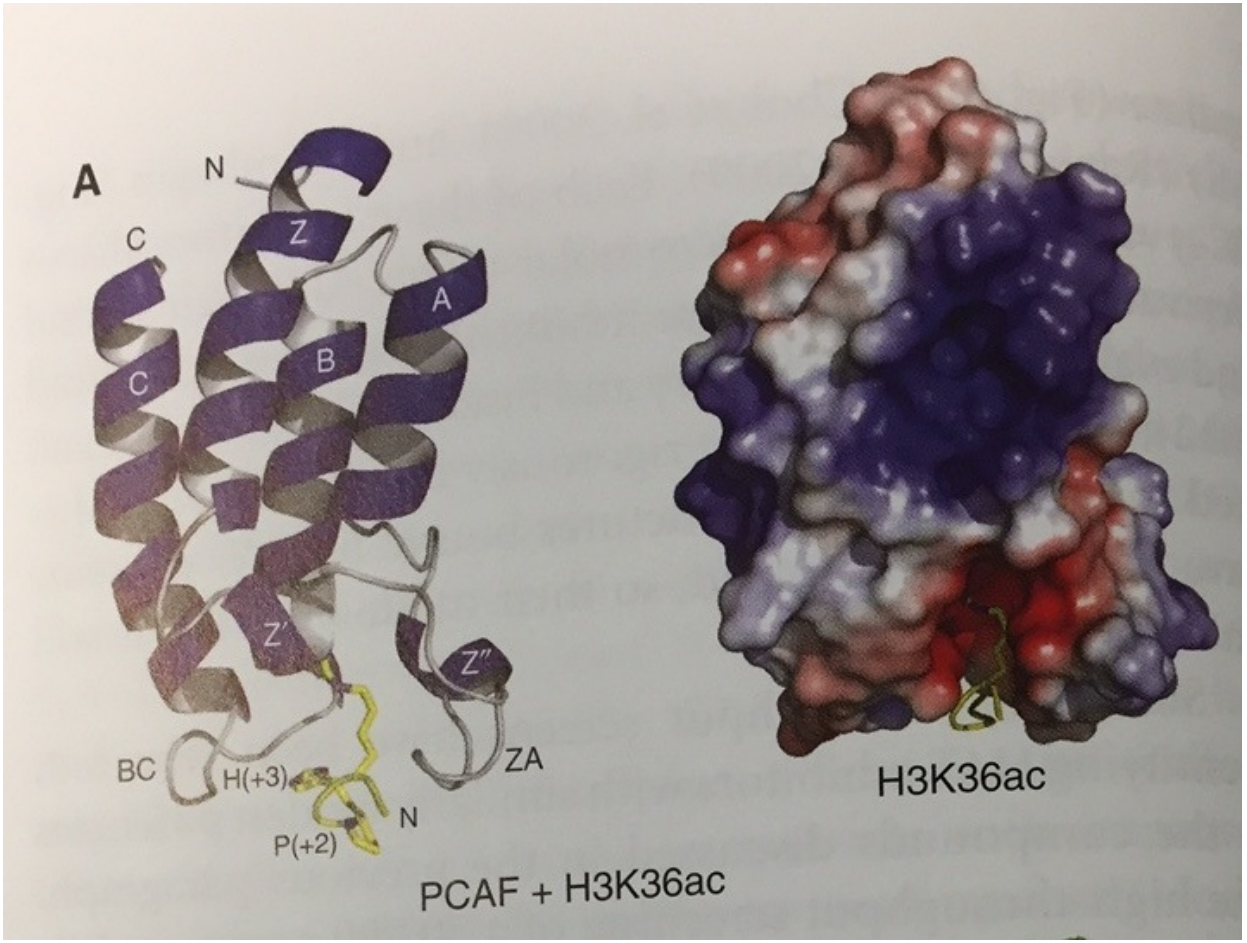


HATs and Disease



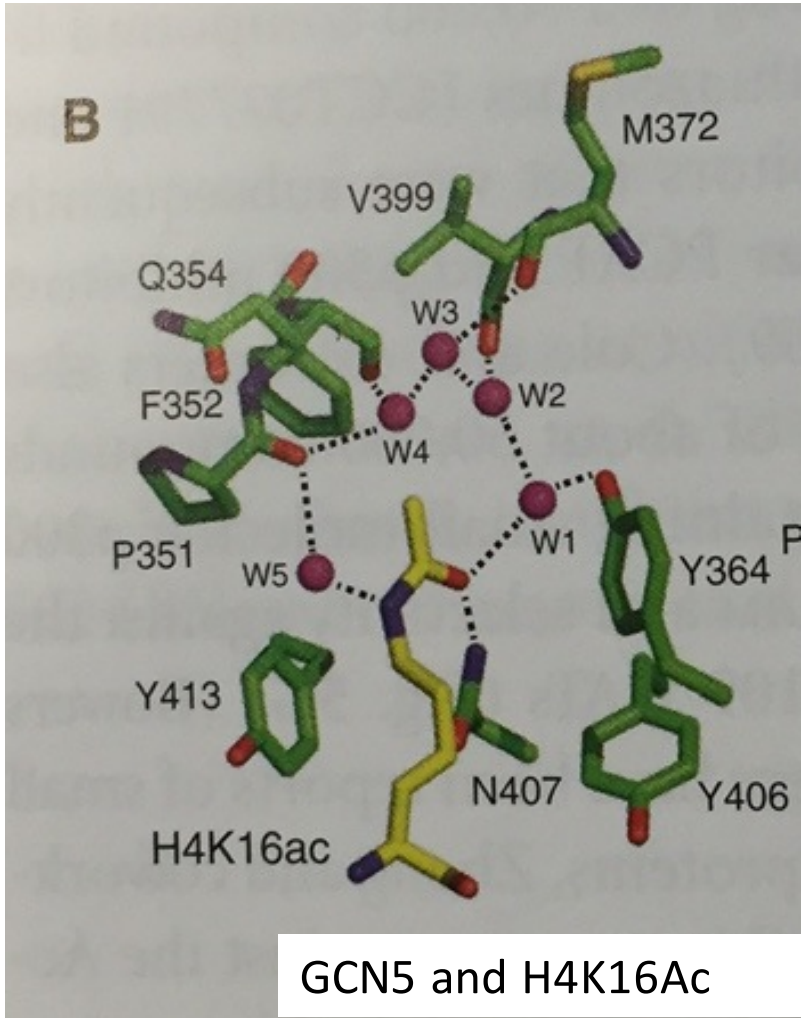
Specific inhbitors to HATs
Have been identified,
However their function in inactivating
HAT activity is MODEST…
→ Better HAT inhibitors need to be developed

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



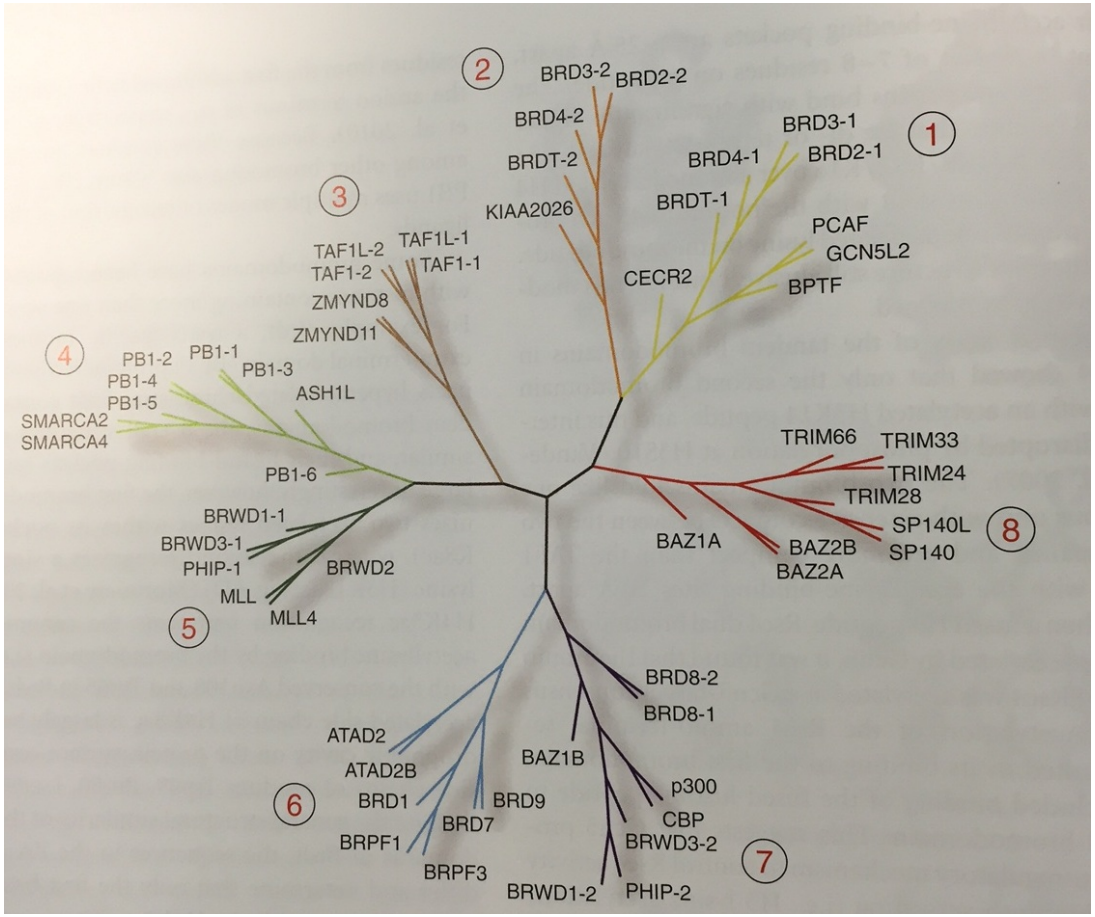
GCN5 and H4K16Ac

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

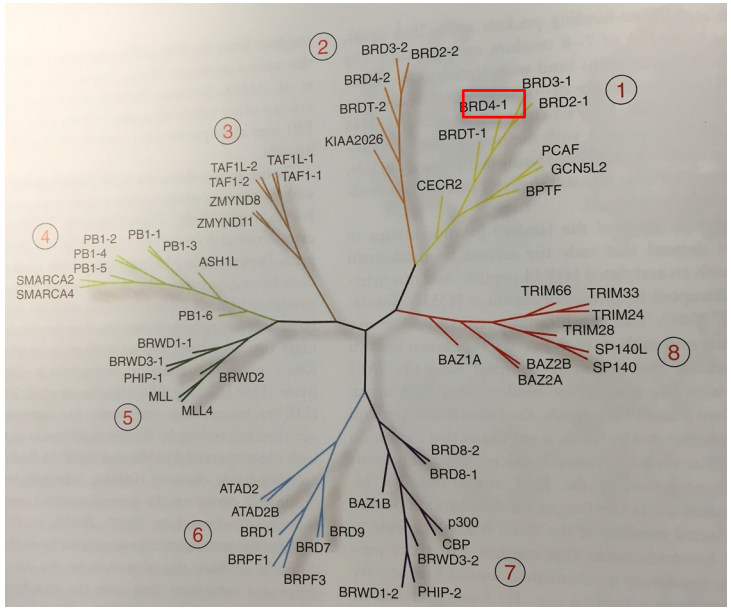
Bromo-domain proteins recruit other factors
that activate transcription/enhance
elongation via different processes

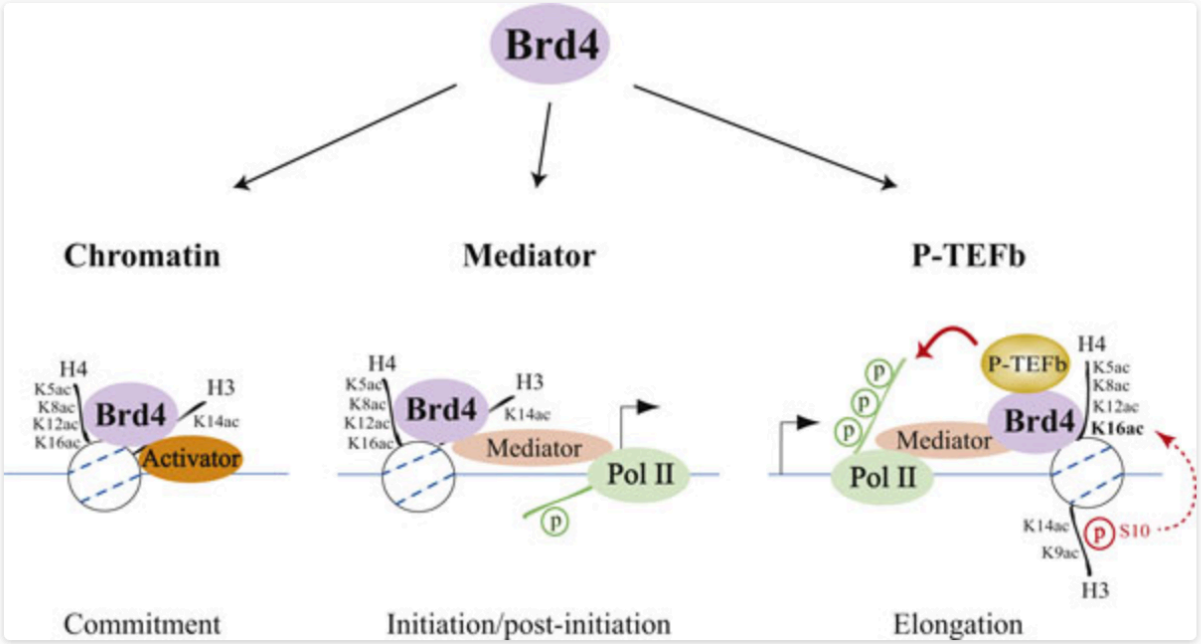
HUMAN BROMO DOMAIN PROTEINS



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

BRD4 (bromo domain 4 protein BROMO DOMAIN PROTEINS



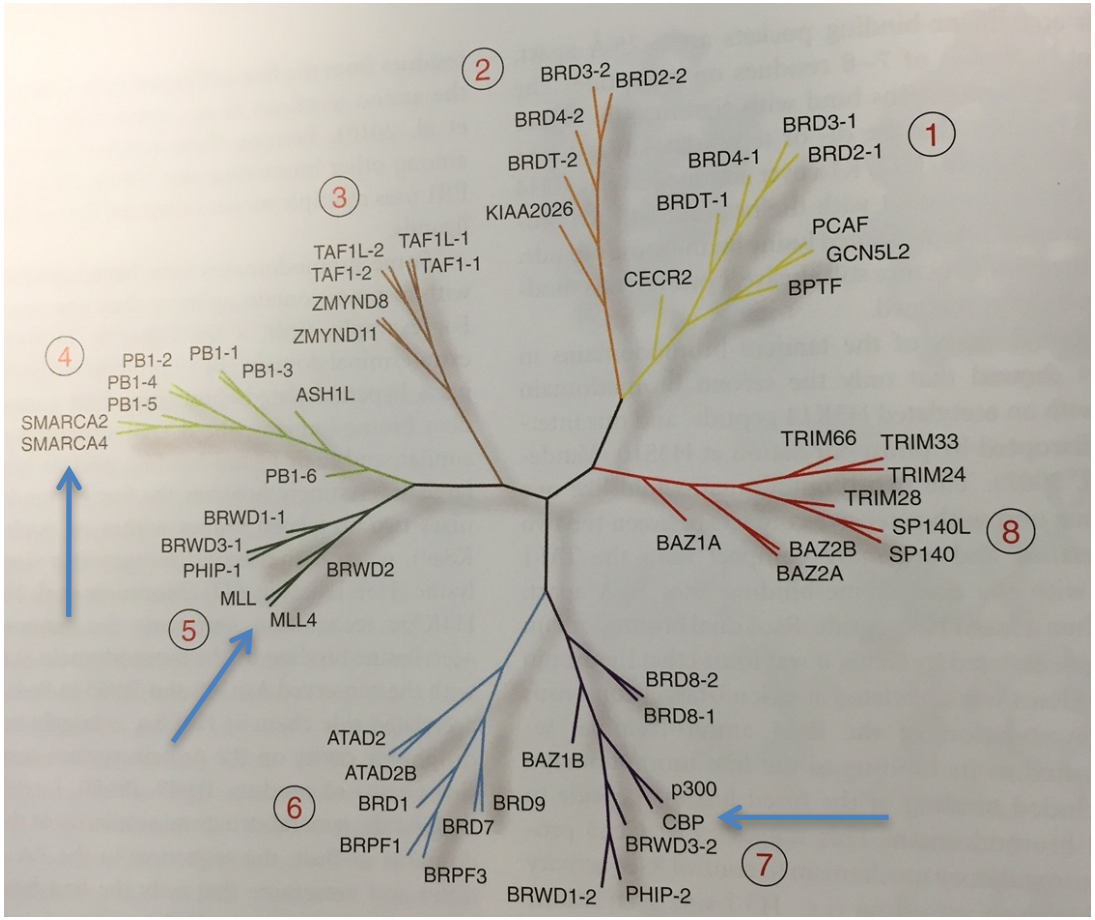


BRD4:
Binds acetylated H3/H4
and activates/anhances
trasncription
via several processes

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

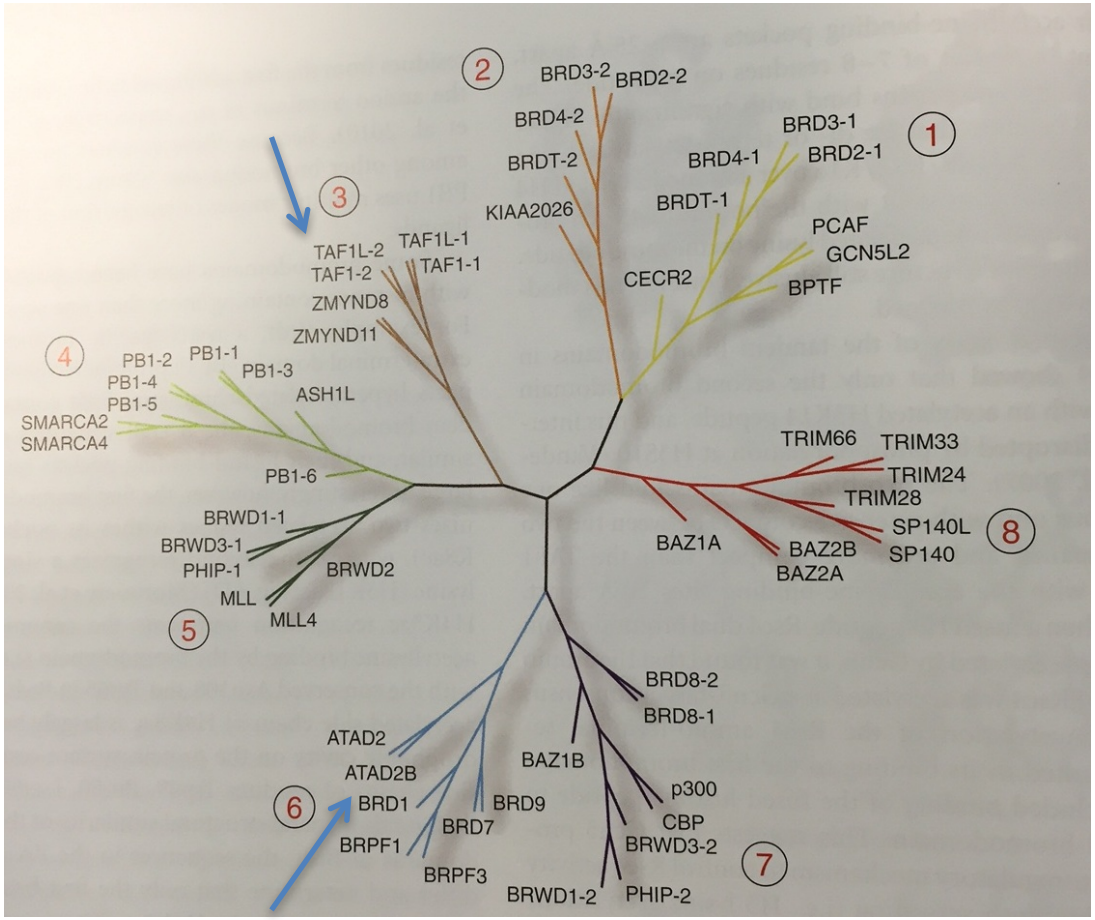


Important Bromo domain proteins:
-**PCAF, Gcn5, p300/CBP !!!**
Bromo domains contribute
to substrate recognition involving
ac. histone and non-histone proteins
→**Acetylation mediated protein-protein**
Interaction (complex formation)

-**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



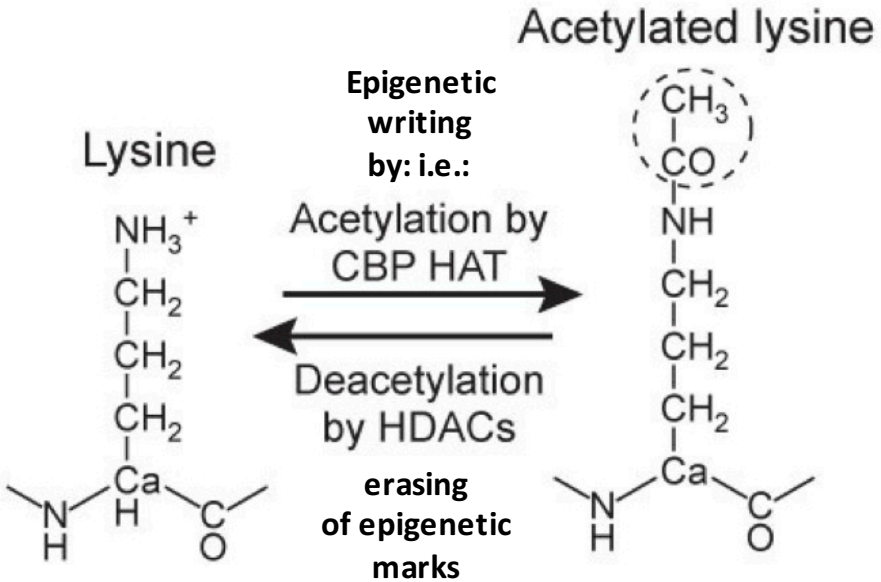
-**ATP-dependent helicases**
ATAD2, ARAD2B
→ **Acetylation and DNA unwinding**

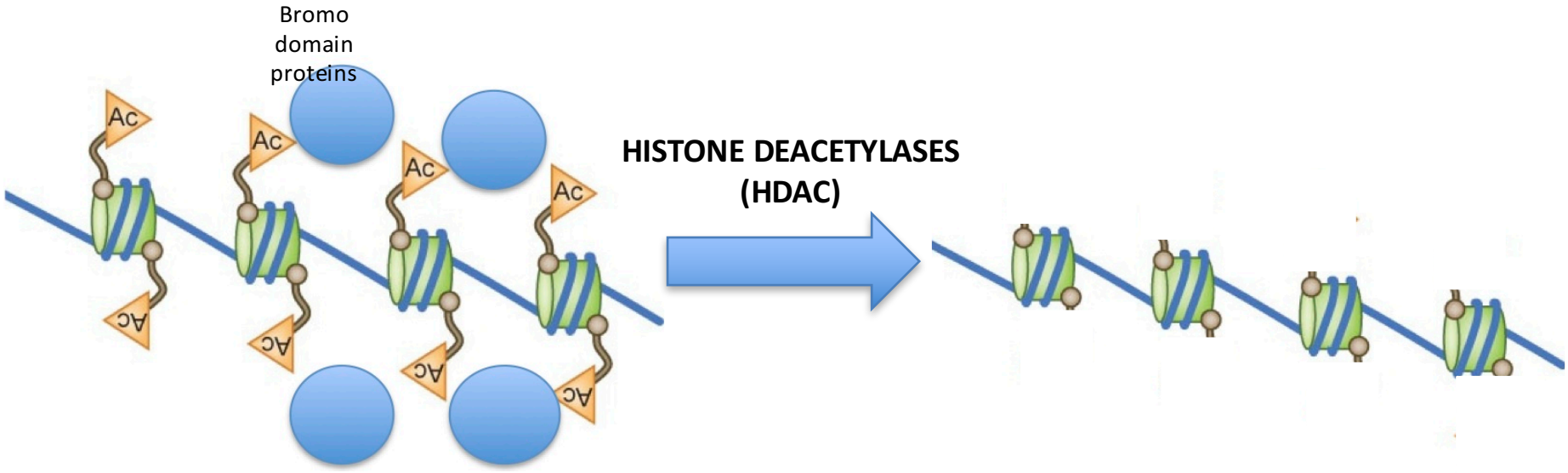
-**Transcription initiation complex components**
TAF1/TAF1L proteins in the TFIID subunit of the transcription initiation complex
→**Acetylation and transcriptional initiation**

-**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 – Histone Deacetylases





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 the 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

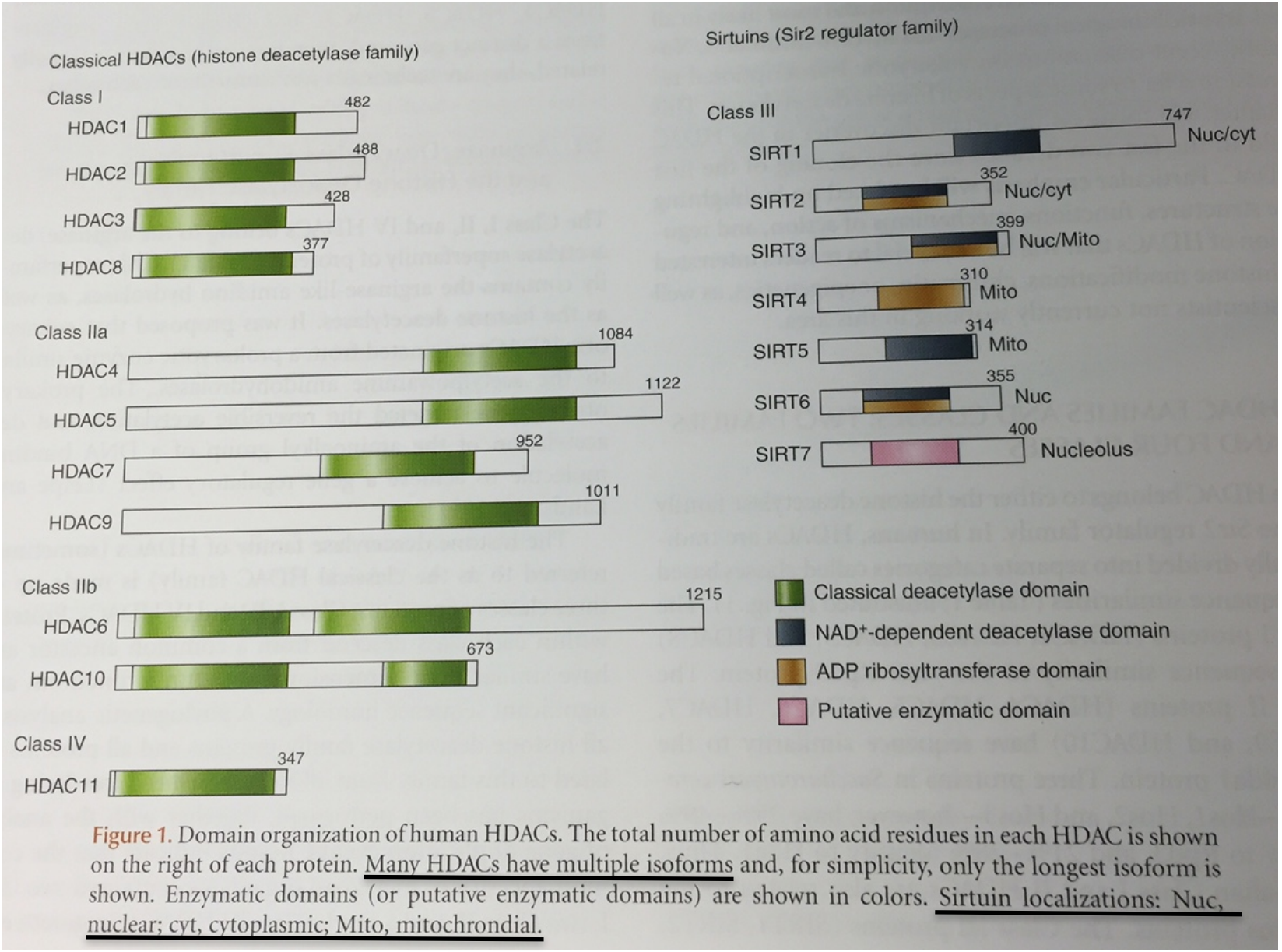
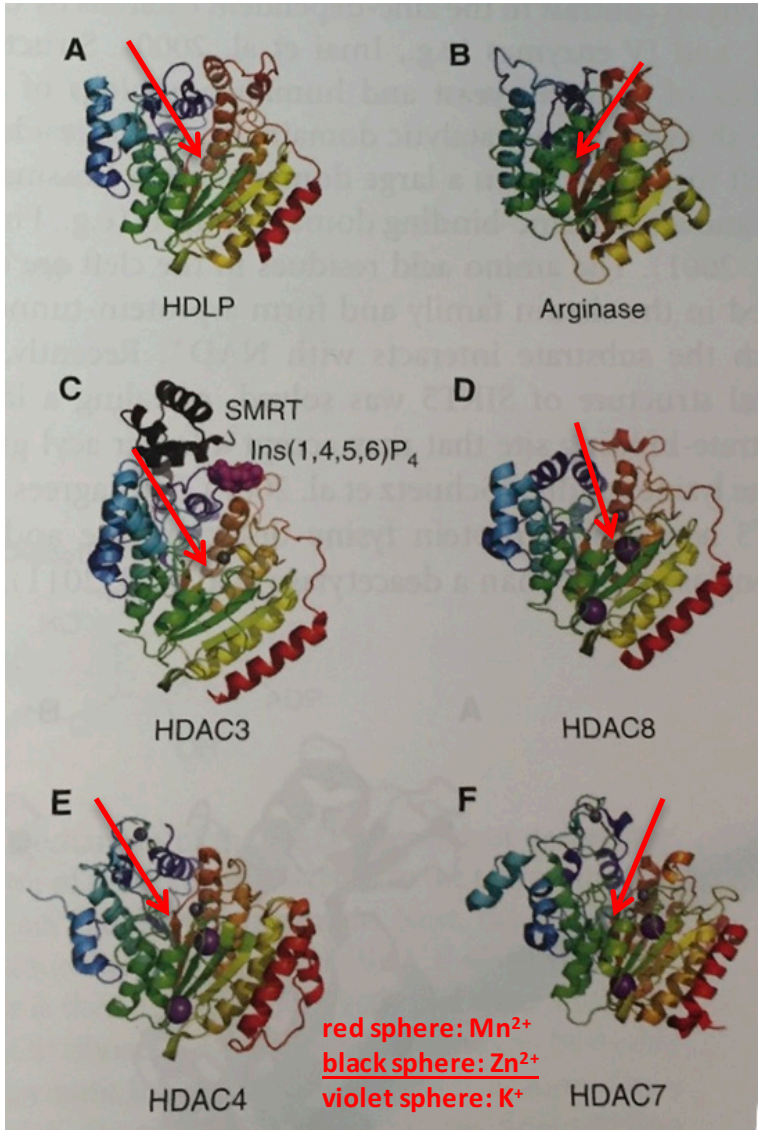


Figure 1. Domain organization of human HDACs. The total number of amino acid residues in each HDAC is shown on the right of each protein. Many HDACs have multiple isoforms and, for simplicity, only the longest isoform is shown. Enzymatic domains (or putative enzymatic domains) are shown in colors. Sirtuin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

STRUCTURE OF DEACETYLASES

Class I and II HDACs

Arginase/deacetylase family

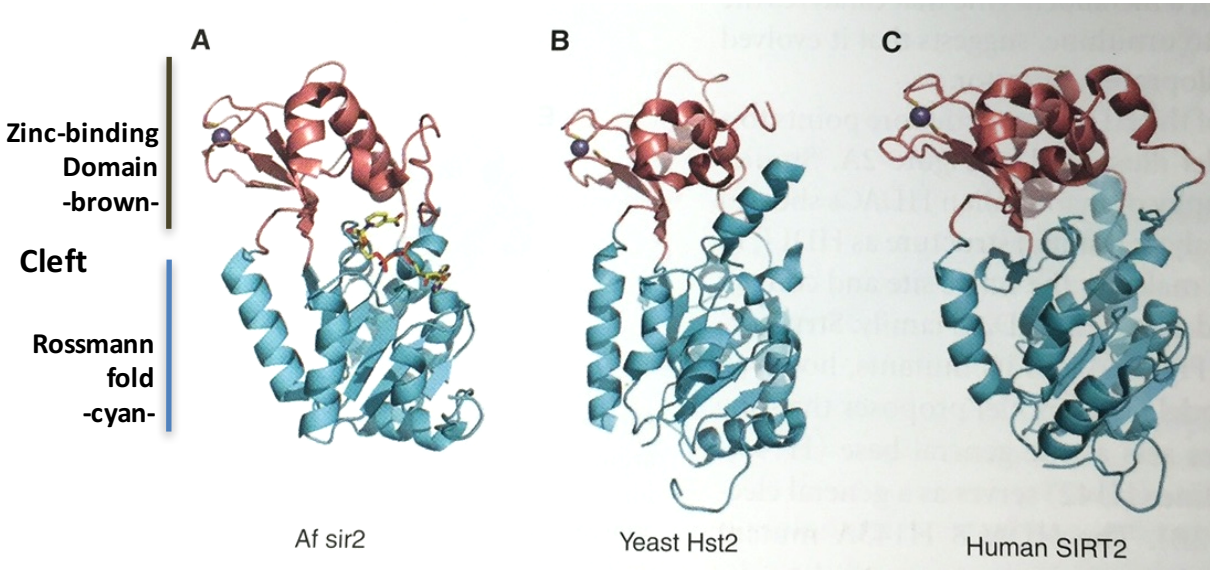


→ Tubular pocket → catalytic centre (beta sheets)

Class III HDAC – SIRTUINS

Deoxyhypusine synthase like

NAD/FAD-binding domain superfamily

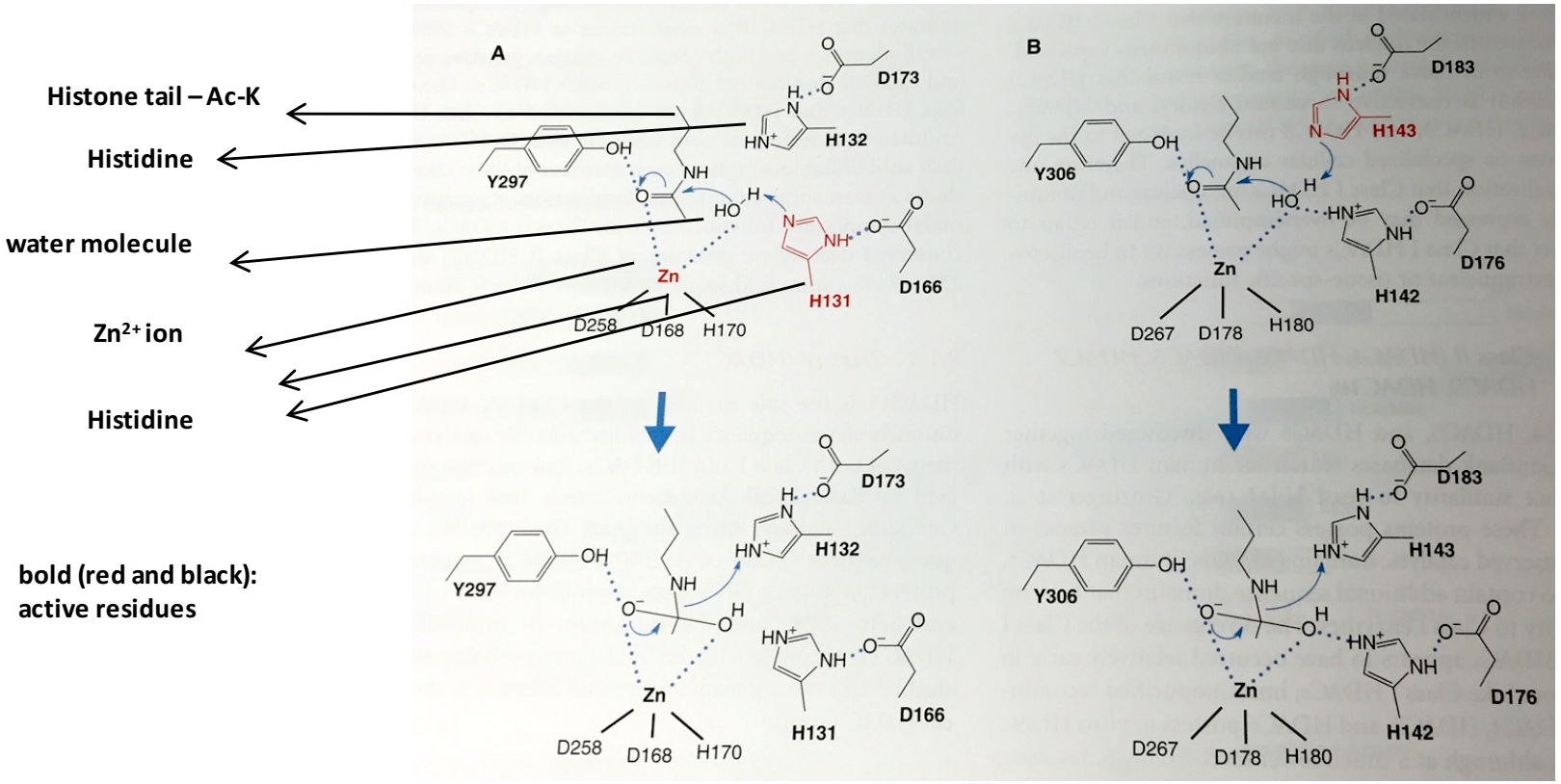


Cleft: amminoacids on cleft are conserved between Class III HDACs and form a protein tunnel in which the substrate interacts with NAD^+ (nicotinamide adenine dinucleotide

The biochemistry of Class I, II, IV histone deacetylases

HDAC Family = *Arginase/deacetylase family*

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)

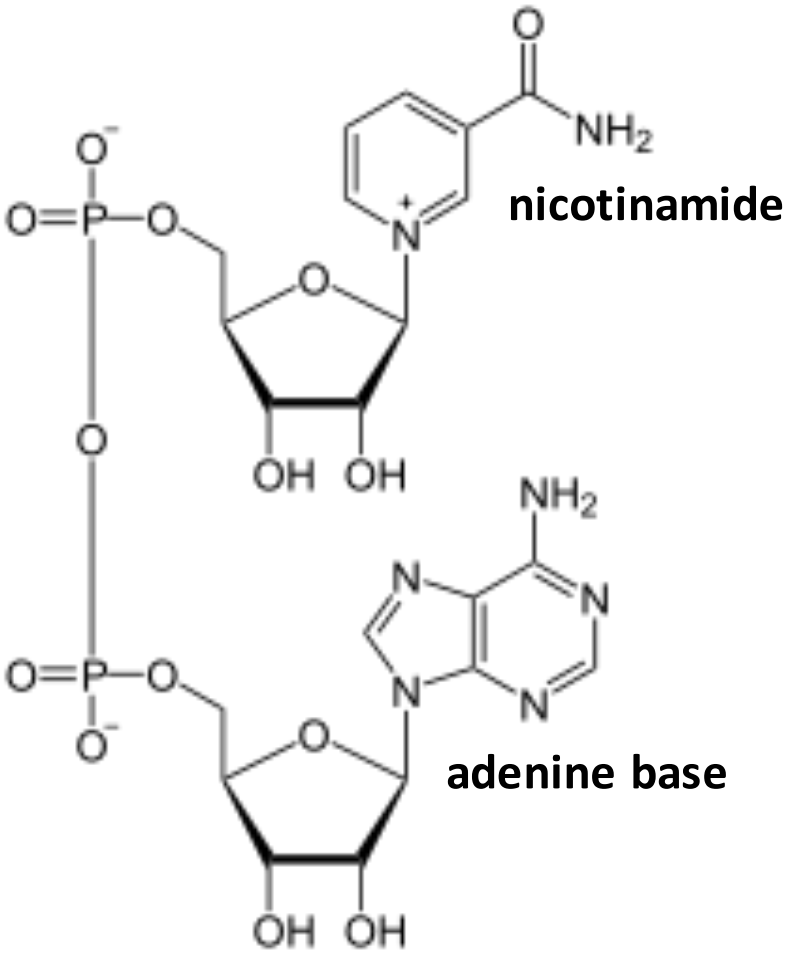
Catalytic His (H131) facilitates a nucleophilic attack at the substrate carbonyl by activating a water molecule coordinated with the Zn2+ ion coordinated aspartic acid (D) and histidine (H)

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)

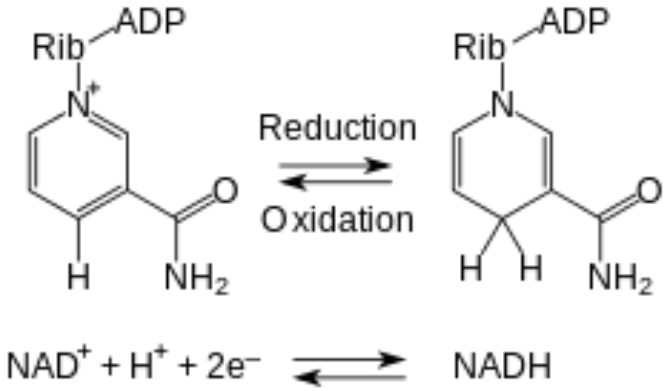
The biochemistry of Class III histone deacetylases

De-acetylation by class III de-acetylases (SIRTUINS)

depend on the coenzyme Nicotinamide adenine dinucleotide (NAD)



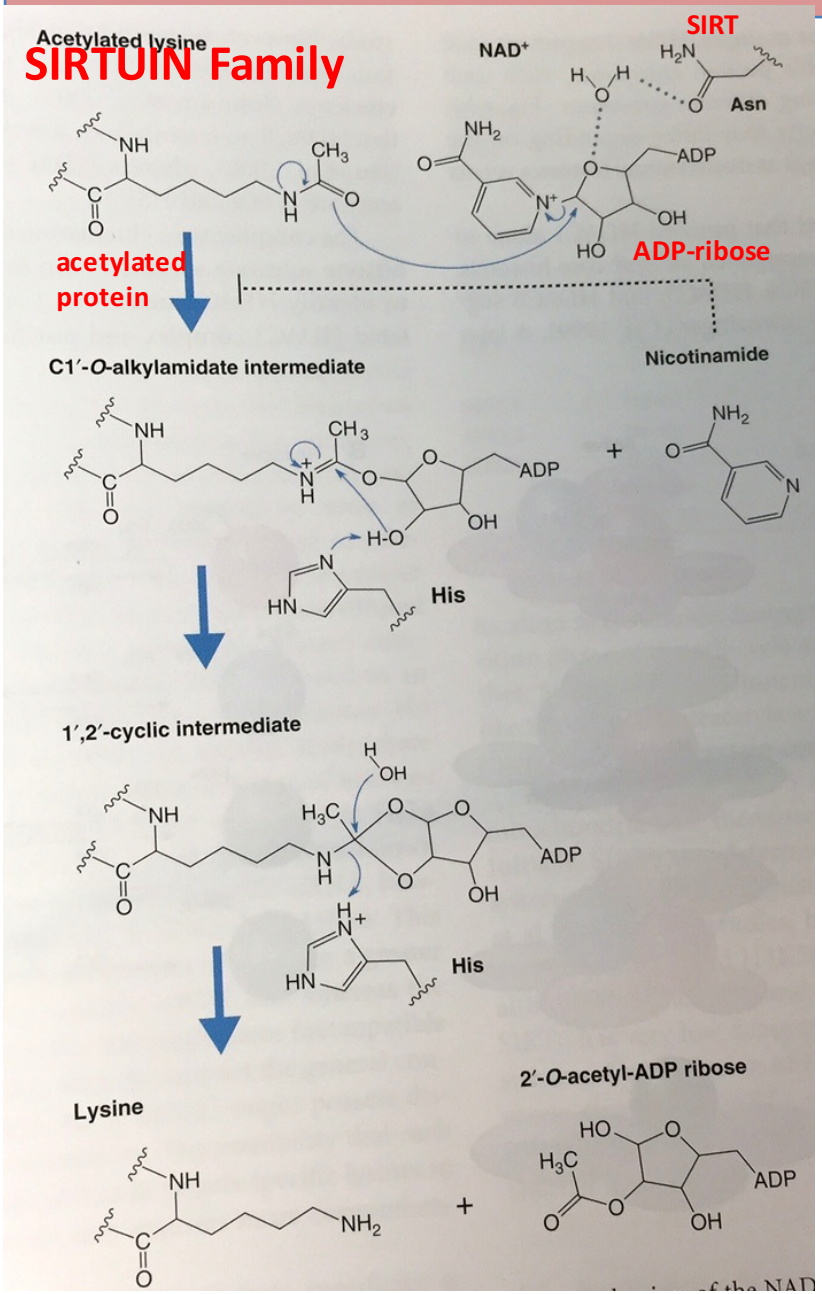
Nicotinamide adenine dinucleotide (NAD)



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**.

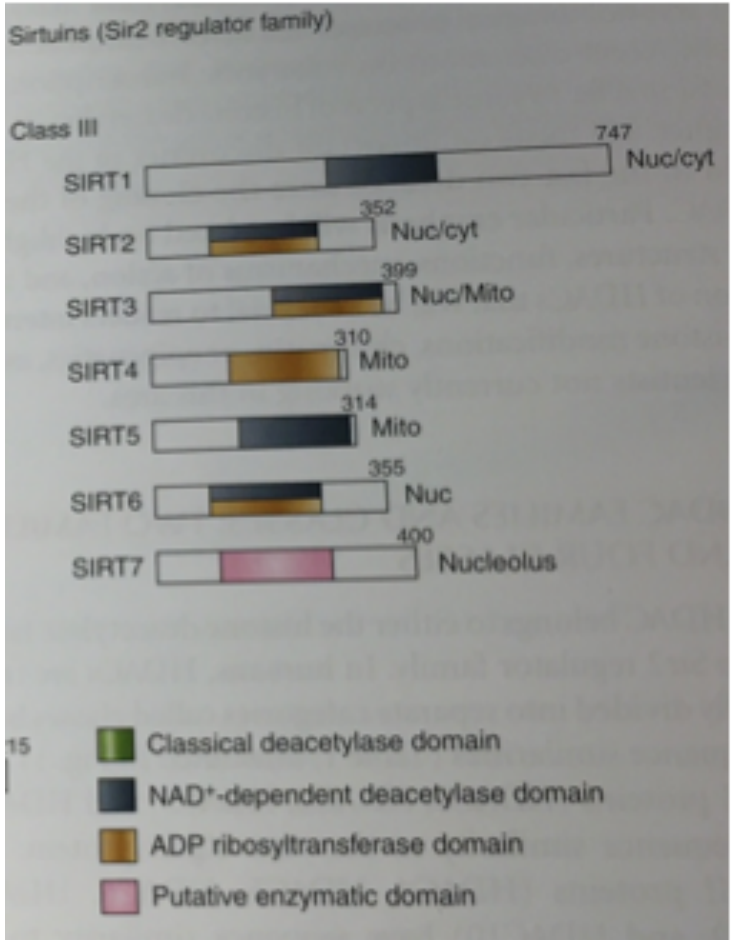
The 1', 2'-cyclic intermediate is then **attacked by an activated water molecule (coordinated by Zn^{2+})** resulting in the formation of **deacetylated lysine** and **2'O-acetyl-ADP ribose**.

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



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

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

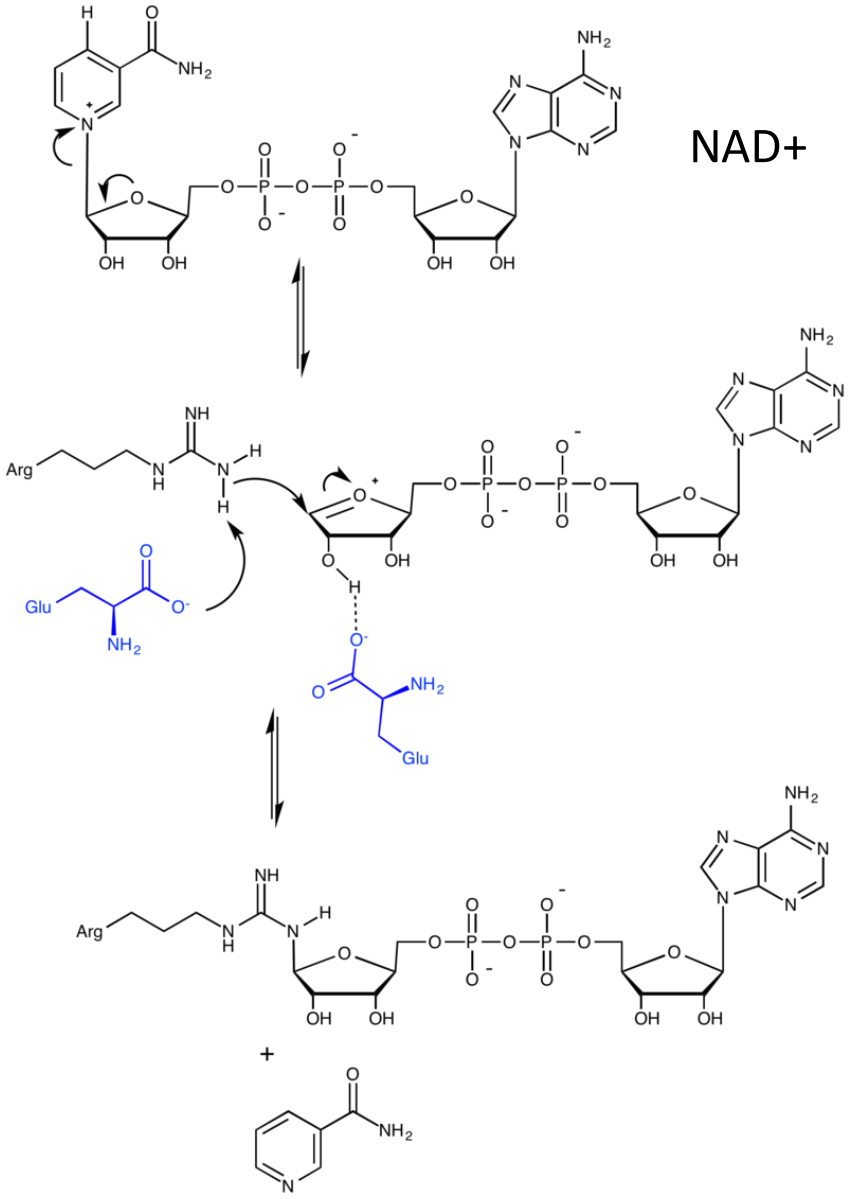
Instead, after nicotinamine cleavage, the remaining ADP-ribose molecule is transferred to the target protein

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

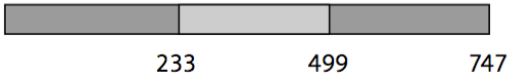
The source of ADP-ribose for most enzymes that perform this modification is the redox cofactor NAD+.
In this transfer reaction, the N-glycosidic bond of NAD+ that bridges the ADP-ribose molecule and the **nicotinamide group is cleaved**, followed by **nucleophilic attack by the target amino acid side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

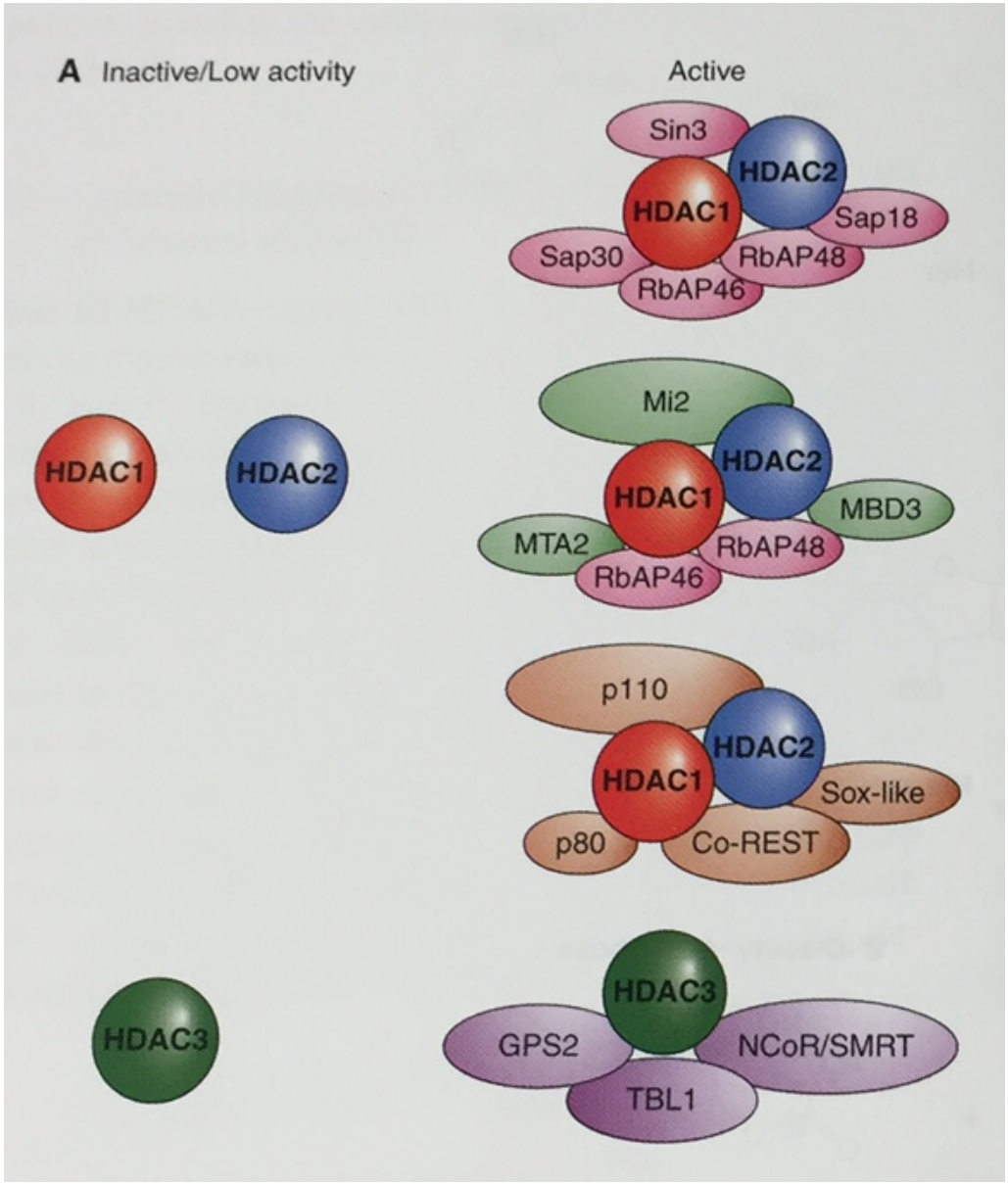
Table 1A - Classification of classic histone deacetylases (HDAC).

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

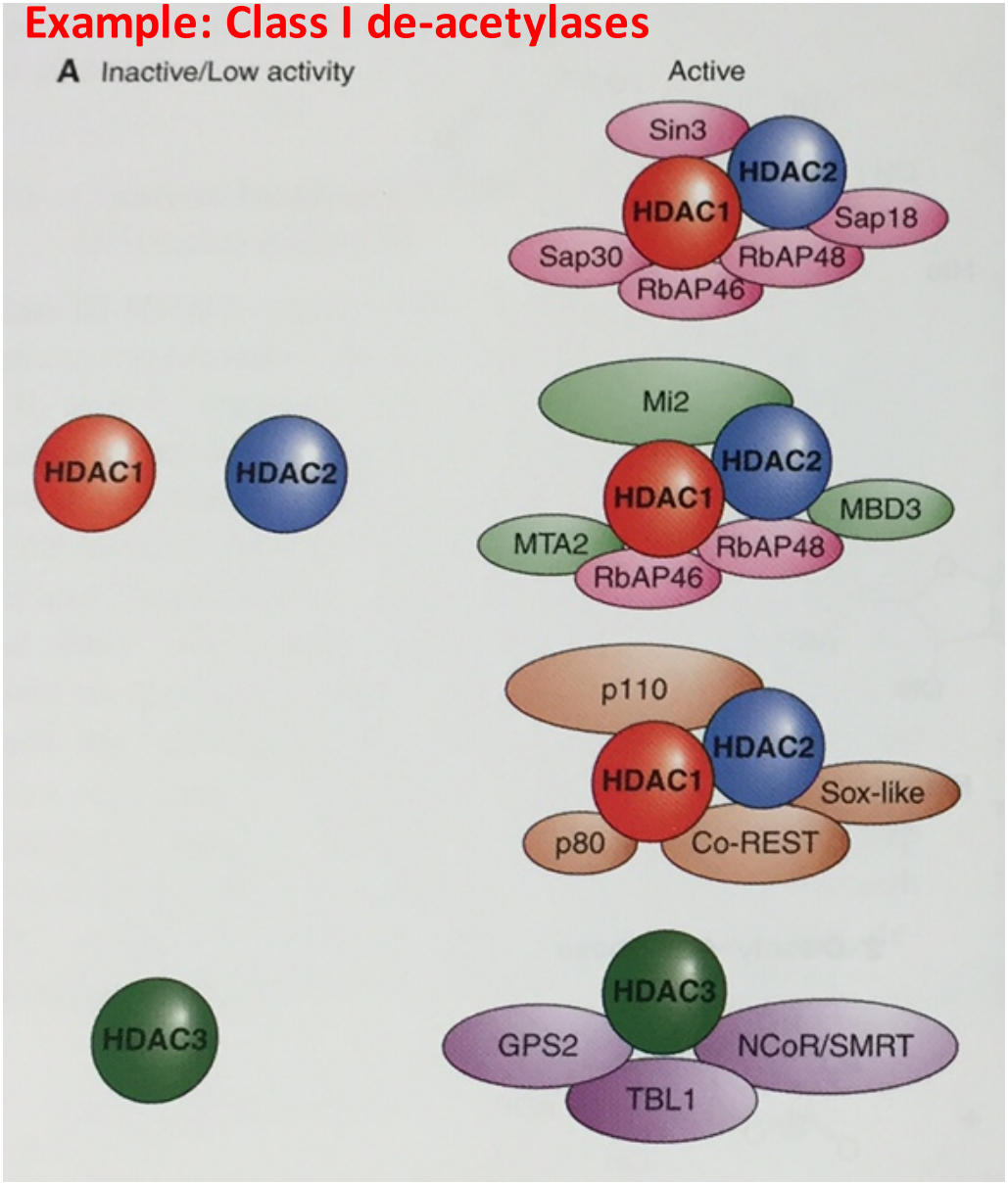
Sirtuin	Histone substrate	Biological relevance
SIRT1	H3K9 H3K14 H3K56 H4K16 H1K26	Chromatin organization, DNA repair/genome stability, cancer
SIRT2	H4K16 H3K56	Chromatin condensation/ mitosis, DNA repair, cancer
SIRT3	H4K16	Chromatin silencing, DNA repair, cellular stress
SIRT4	None	
SIRT5	None	
SIRT6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

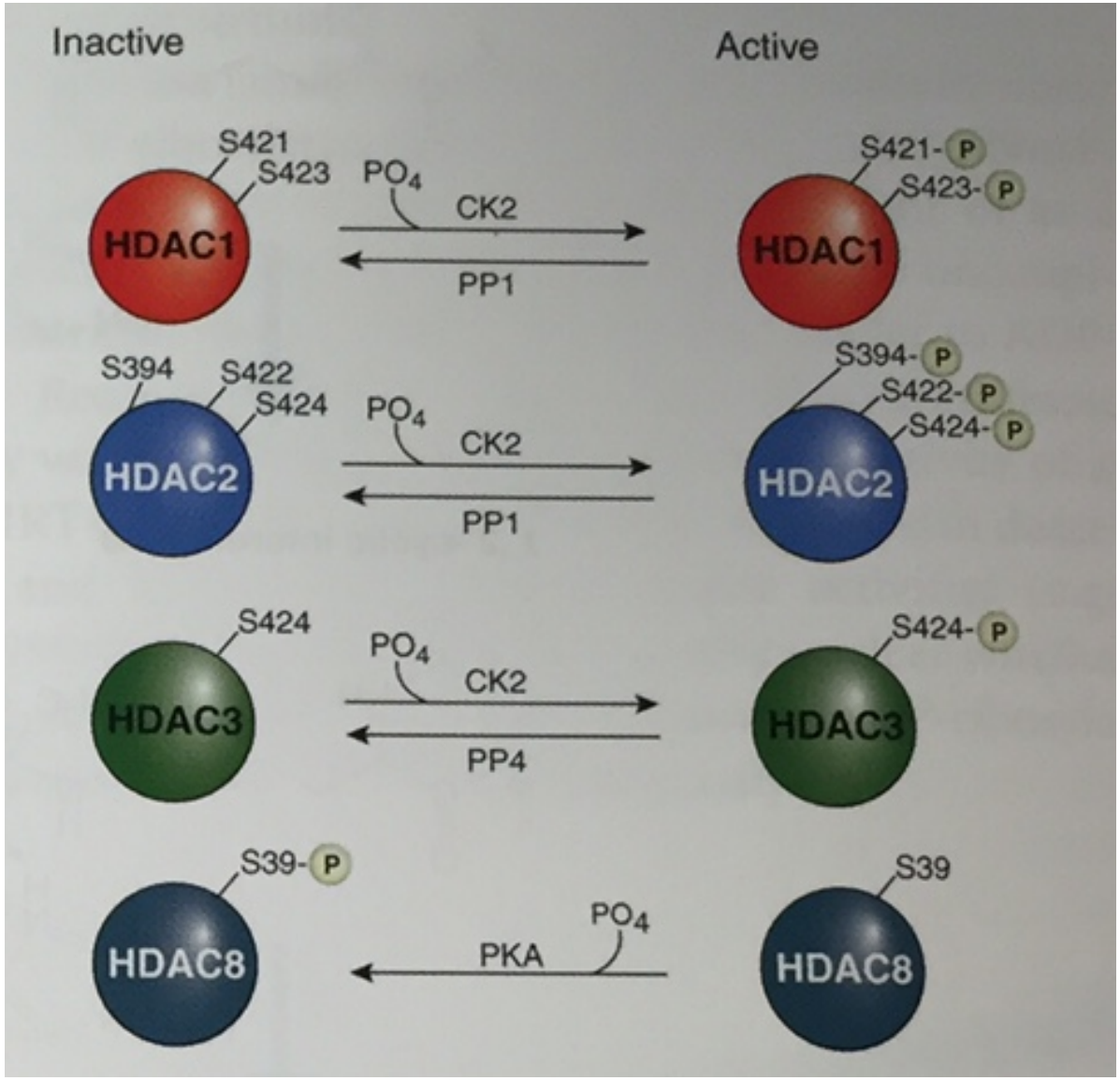
Co-REST Complex

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

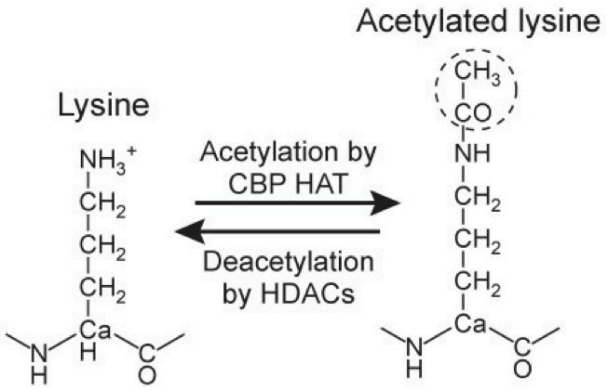
Mutations in phosphorylation sites increase HDAC activity. Phosphorylation disrupts the structure around the active center in HDAC8

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

BIOLOGICAL IMPORTANCE OF HDACs

1. HDACs indirectly regulate many post-translational modifications

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



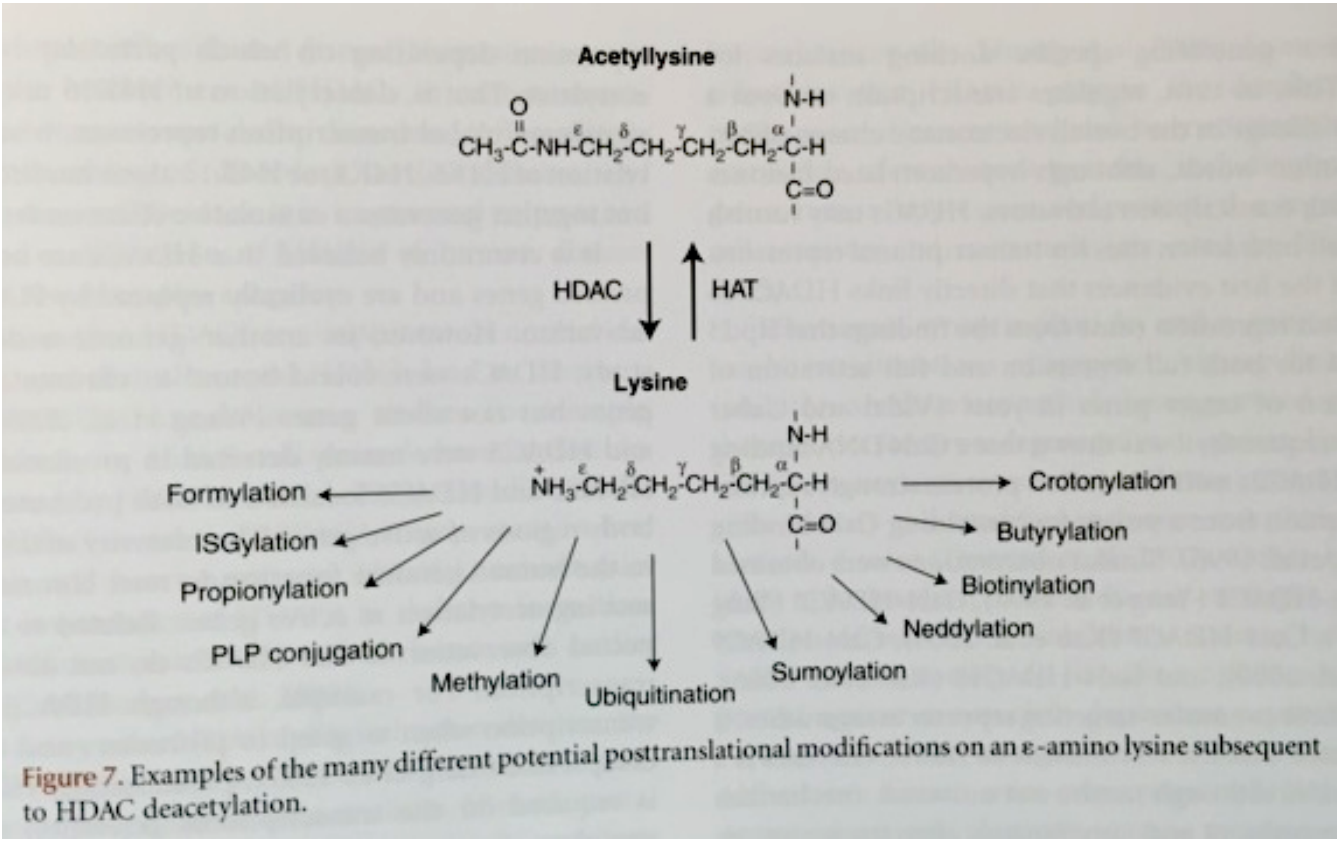


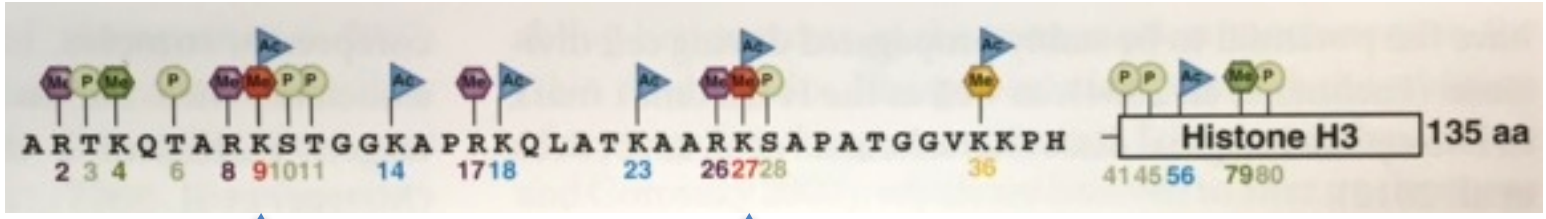
Figure 7. Examples of the many different potential posttranslational modifications on an ε-amino lysine subsequent to HDAC deacetylation.

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

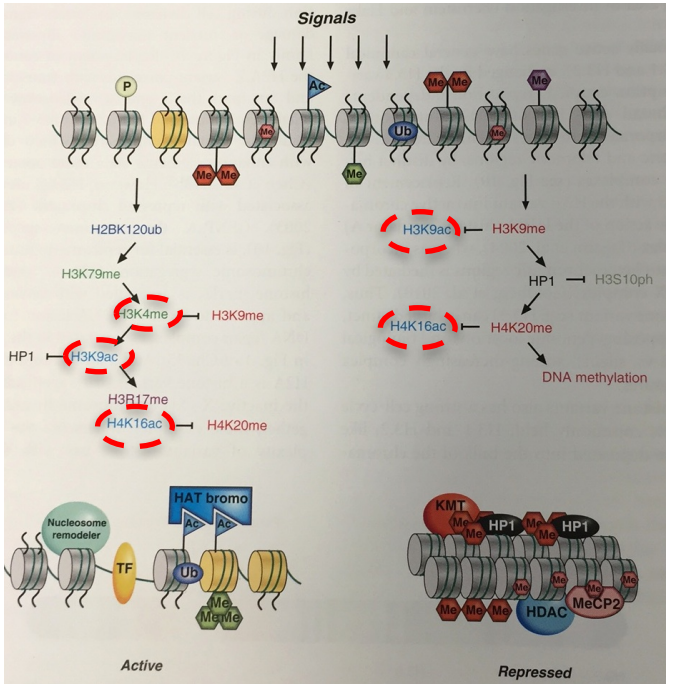
H3K27me: repression
H3K27ac: activation

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

H3K9ac promotes H3K4me → open chromatin and transcription

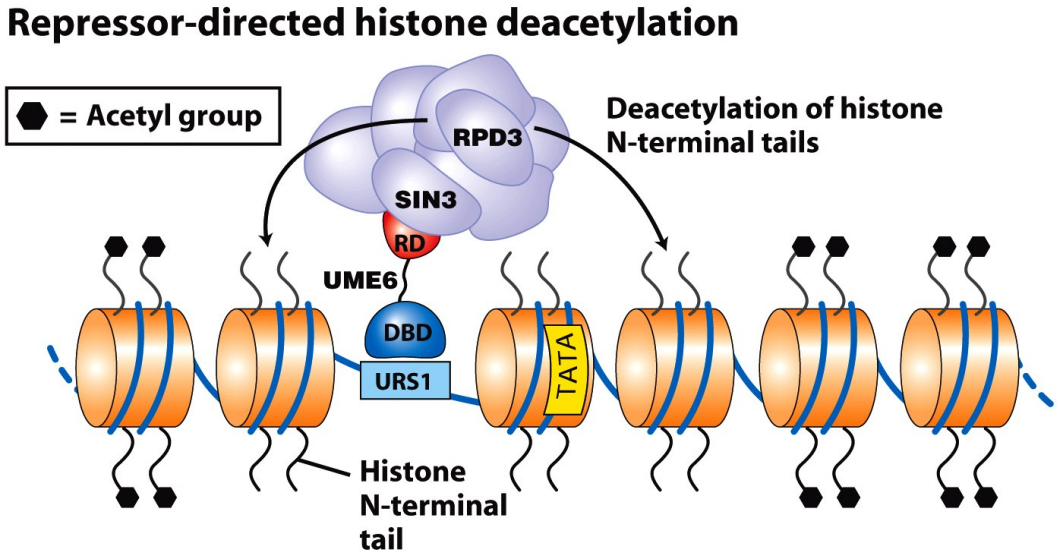
→HDACs deacetylate H3K9 → less H3K4me → repression

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 – combined with gene expression analysis (RNA level):

Acetylation is associated with active gene transcription and found at high levels at the start of genes (promoter region)

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 (recruited by repressor proteins)

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:

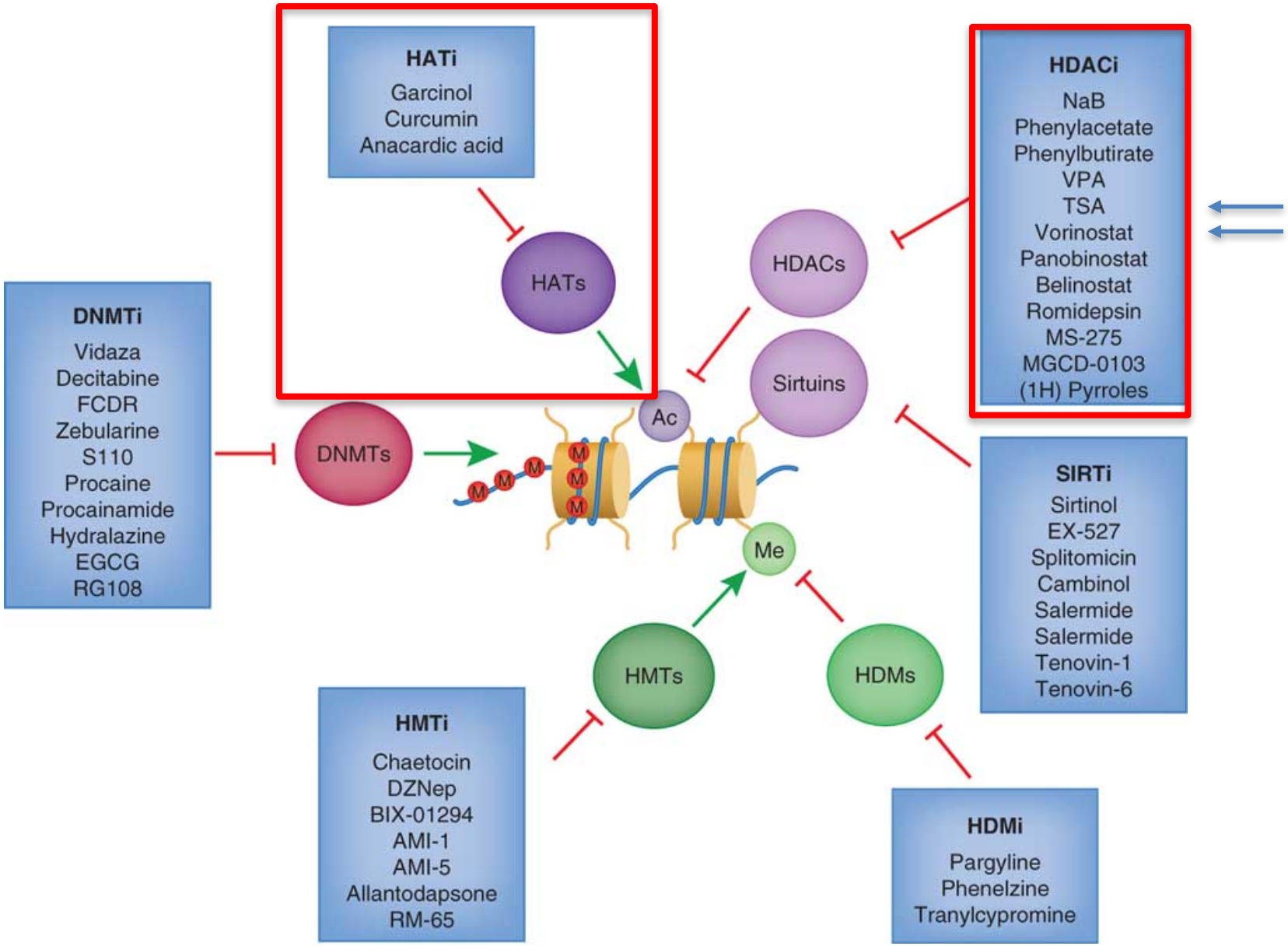
Expectation: overall upregualtion of gene expression

Observed 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.

→ Alteration of epigenetic pathays have DIRECT but also INDRECT effects on gene expression

HATs and Disease – Epigenetic drugs



HATs and Disease – Epigenetic drugs