Eukaryotic Gene Expression: Basics & Benefits

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Lecture 7

Eukaryotic gene regulation: Role of chromatin

Recap……..

- **Eukaryotic RNA polymerases**
- **Core promoter elements**
- **General transcription factors**
- **Enhancers and upstream activation sequences**
- **Transcriptional activators: DNA binding, transactivation**

~~coactivators~~

Chromatin structure

vs

Gene regulation

In eukaryotes, the cellular DNA is tightly wrapped up around histones and is organized in the form of nucleosomes.

DNA

Nucleosome core particle
147 bp DNA + octamer

How do transcription factors gain access to the promoter DNA?

How are histones displaced from the promoter during transcription initiation?

Histone octamer
(H2A, H2B,H3 and H4)2

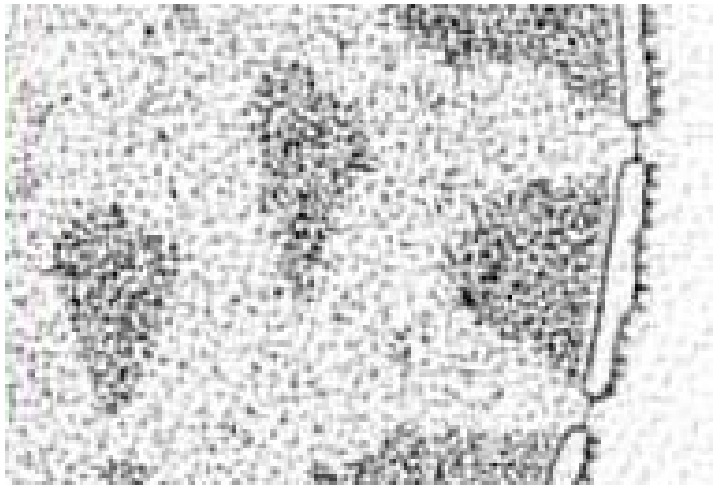
146 bp of DNA wrapped around
a histone octamer core

N-terminal and or C-terminal tails of
histones protrude from the nucleosome
through the minor-groove channels

Chromatin acts a general repressor of transcription

DNA has to be unwrapped for transcription initiation to happen

Chromatin compaction affects transcriptional activity in eukaryotic nuclei



Heterochromatin - transcriptionally silent

Euchromatin – transcriptionally active

Densely packed chromatin will block access of RNA pol II + General transcription factors to core promoters

Chromatin needs to be unpacked, unwrapped or opened before transcription initiation can happen

Transcription Activation in eukaryotes:

step 1: chromatin structure should be modified such that RNA polymerase II machinery can be recruited

step 2: Actual recruitment of RNA polymerase II machinery

Cell-free transcription studies:

- Crude nuclear extracts

- *In vitro* transcription systems reconstituted with RNA polymerase and general transcription factors purified from cell extracts

- *In vitro* transcription systems reconstituted with RNA polymerase and recombinant general transcription factors

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naked DNA templates

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naked DNA + histones = chromatin templates

TBP cannot bind nucleosomal DNA

Nucleosomes prevent binding of TBP to core promoter elements *in vitro*

TBP does not associate with most core promoters *in vivo* in the absence of transcriptional activators

CHROMATIN TEMPLATES NEED TO BE MODIFIED IN ORDER FOR TRANSCRIPTION FACTORS TO BIND

HOW?

BY COVALENT MODIFICATION OF HISTONE TAILS

Histones can undergo several post translational modifications

ACETYLATION

METHYLATION

PHOSPHORYLATION

UBIQUITINYLATION

SUMOYLATION

ADP RIBOSYLATION

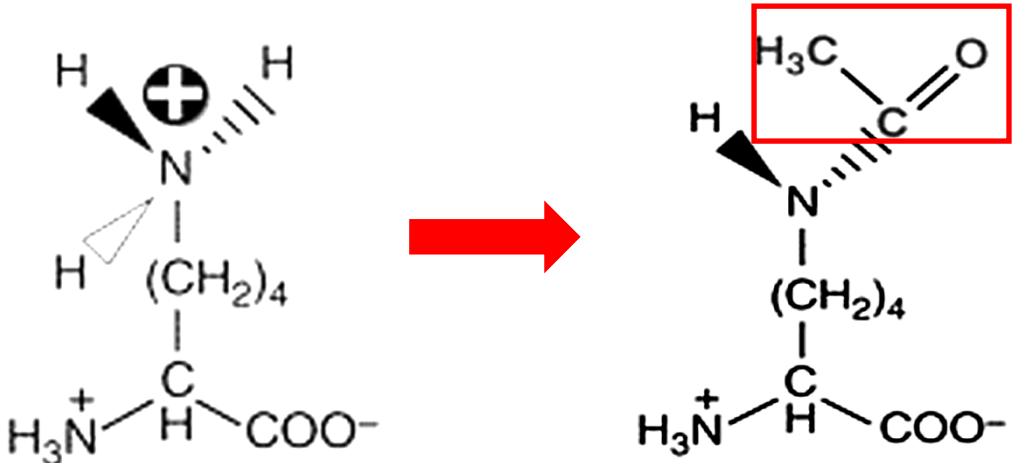
ACETYLATION OF HISTONES

The lysine residues in the N-terminal region of histones H3 and H4 can undergo acetylation in a reversible manner

Acetylation of ε-amino groups in lysines results in reduced positive charge, and weakens histone interaction with DNA

Acetylation changes the conformation of nucleosomes and destabilizes internucleosomal contacts

Acetylation of histones may alter the interaction with other regulatory proteins



Heterochromatin - transcriptionally silent

Euchromatin – transcriptionally active

Acetylated histones are enriched in transcribed chromatin

Acetylation of histones potentiates transcription by making nucleosomal DNA more accessible for transcription factor binding

How are histones acetylated?

Many proteins involved in transcriptional activation

(transcription factors/coactivators/TAFs)

are

histone acetyl transferases

(HATs)

Gcn5p, a well known transcriptional regulator in yeast cells was shown to possess HAT activity

The human homologue of Gcn5p is also a HAT

For the first time, researchers working on histones and chromatin structure got interested in transcriptional regulation

Similarly, researchers working on transcriptional regulation realized that they cannot ignore chromatin any longer

Gcn5, the first nuclear histone acetylase to be identified

Yeast Gcn5 was found in at least two distinct multiprotein complexes, Ada and SAGA, neither of which is tightly associated with TFIID or the Pol II

Brownell et al., 1996 *Cell* 84: 843-851

Histone acetyltransferases (HATs)

HAT- A **HAT-B**

A-Type HATs catalyze transcription-related acetylations

B-Type HATs are cytoplasmic.
They catalyze acetylations linked to transport of newly synthesized histones from the cytoplasm to the nucleus

HAT FAMILIES

GNAT family
Gcn5-related N-acetyltransferase (bromodomains)

MYST family
MOZ, Ybf2/Sas3, Sas2, Tip60 (chromodomains)

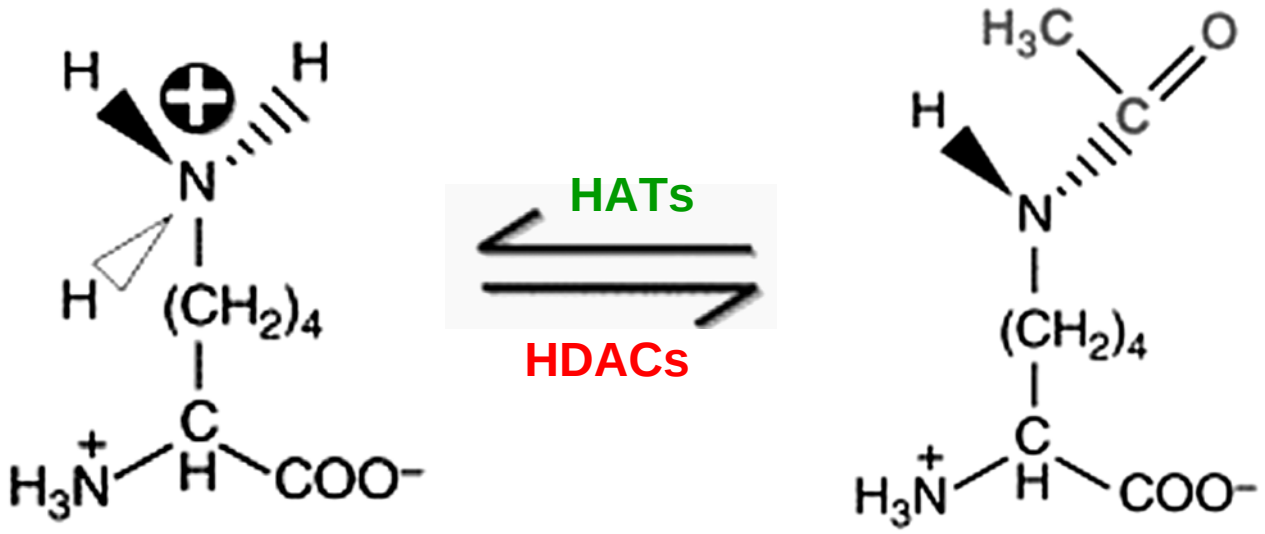
CBP/p300

GTF-HATs
TAFII250

Nuclear receptor linked HATs
SRC1, ACTR

Soon, enzymes which remove acetyl groups from histones were identified

and these were named Histone deacetylases (HDACs)



HATs such as Gcn5p are present as huge multi protein complexes

SAGA (Spt-Ada-Gcn5-Acetyltransferase)

Human Gcn5p was also found in a similar complex STAGA

These complexes were found to Interact with both TBP and acidic activators

The GNAT family of HATs include not only Gcn5p but also
PCAF, TFTC (TBP-free TAF-containing complex) etc.

Free Gcn5 acetylated only free histones,
but as part of the SAGA-complex it could
acetylate nucleosomes

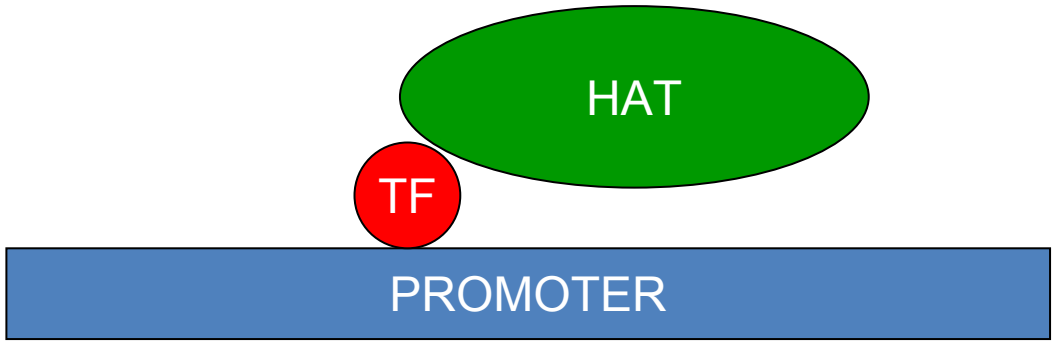
Several coactivators have HAT activity

hTAF250, dTAF230, yTAF130
p300/CBP
ACTR and SRC-1
P/CAF

MICE IN WHICH THESE HATs ARE KNOCKED OUT
EXHIBITED SEVERAL DEVELOPMENTAL DEFECTS

Each of these HATs acetylated specific lysine residues of H3 and H4

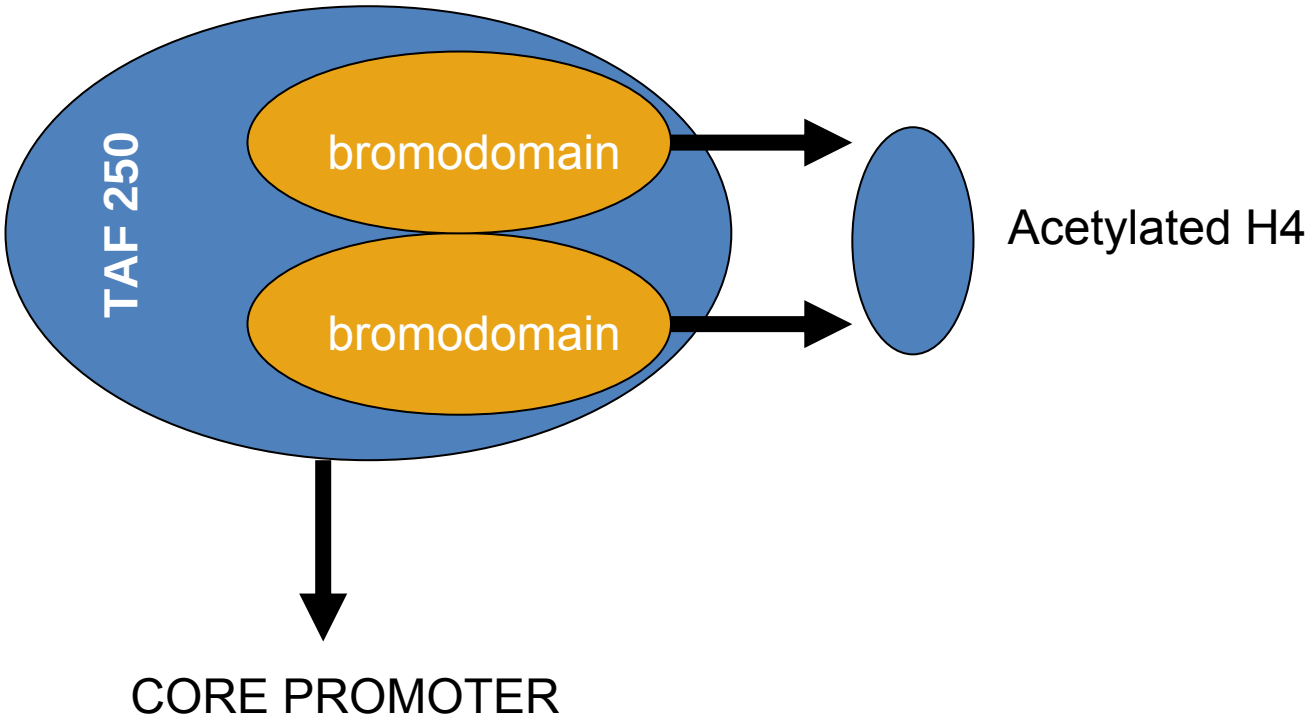
HAT-activity is recruited to promoters through specfic interactions with activators, resulting in local acetylation of nucleosomes around promoters.



Activation or repression is determined by the equilibrium between HATs and HDACs

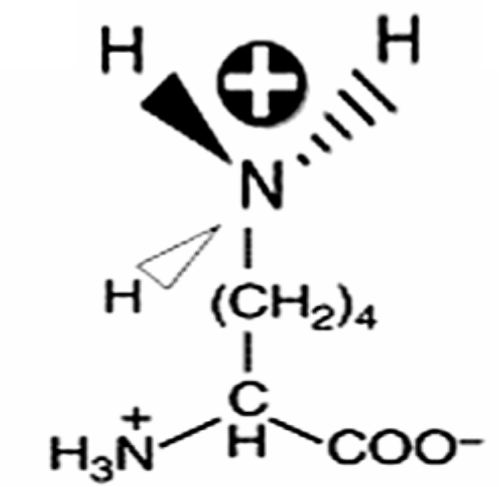
On average, 13 of the 30 lysine residues in a histone octamer are acetylated and this steady-state level of acetylation is maintained by the opposing actions of HAT and HDAC complexes.

Many co-activators possess specific domains known as
BROMODOMAINS
which can recognize the acetylated residues of histones



HISTONE DEACETYLASES (HDACs)

HDAC



The best described HDACs are members of a common family that includes the founding member from human, HDAC1 and yeast Rpd3.

The HAT–HDAC system functions as a key regulatory switch of gene expression.

Human HDACs belong to four classes based on homology to the yeast HDACs:

Rpd3 (class I—HDAC1, HDAC2, HDAC3 and HDAC8),

Hda1 (class II—HDAC4, HDAC5, HDAC6, HDAC7, HDAC9 and HDAC10) and

Sir2 (class III—human sirtuin proteins SIRT1-7)

HDAC11 (class IV HDAC)

HDAC1: Over expressed in prostate cancers (hormone-refractory), gastric, colorectal cancers.

HDAC2: Over expressed in colorectal and gastric cancers.

HDAC3: Over expressed in lung cancer and several solid tumors.

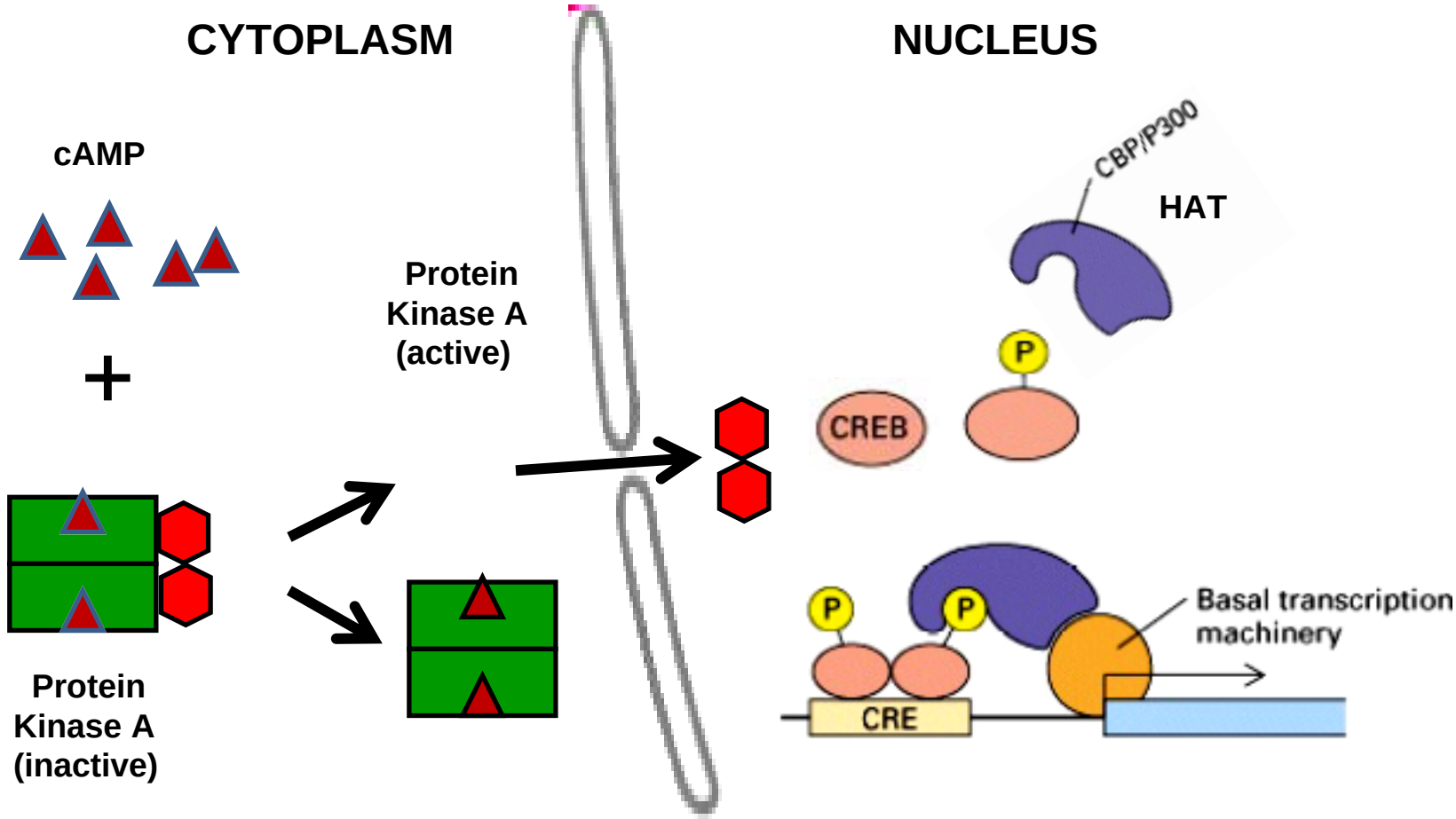
HDAC8: Knock down inhibits cell growth in several human tumor cells

HDACs are found in large multiprotein complexes that include:

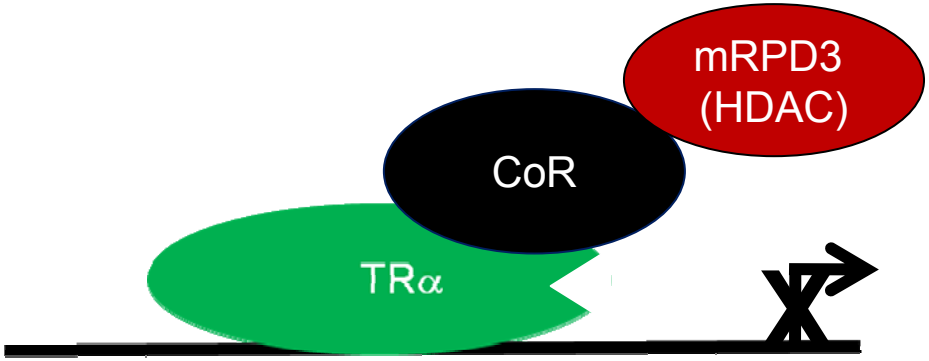
Sin3 corepressor, Transcriptional corepressors such as SMRT and NCoR.

DNA-binding repressors such as Mad, Ume6, YY1 are also associated with HDACs

Transcriptional activation by a transcription factor by recruitment of a HAT



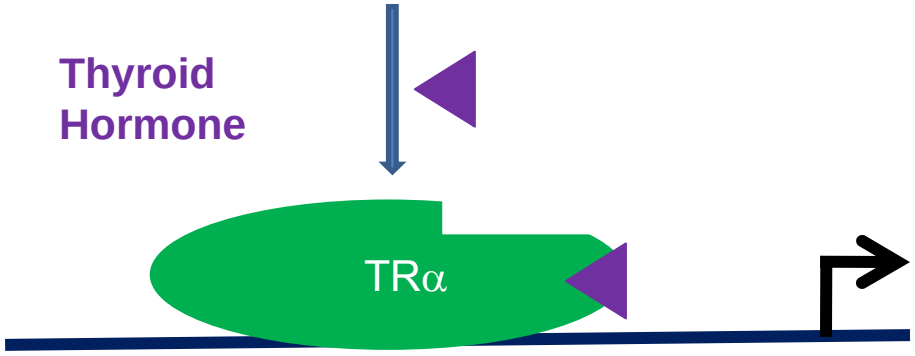
TRα



TRE

mRPD3 causes deacetylation of histones resulting in chromatin condensation and repression of transcription

Transcriptional repression by a transcription factor by recruitment of a HDAC



Binding of thyroid hormone causes a conformational change in TRα, which releases CoR leading to activation of transcription

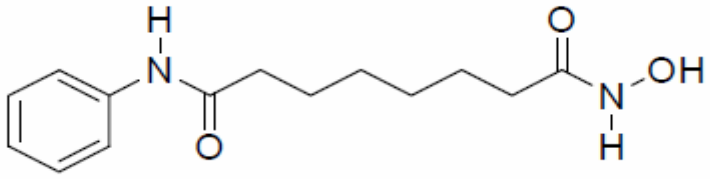
Inhibitors of HDACs, such as:

sodium butyrate,
trichostatinA,
suberoylanilide hydroxamic acid (SAHA),
valproic acid,

induce cell cycle arrest, differentiation, and apoptosis in colon cancer cell lines *in vitro and* have demonstrated anti-cancer efficacy in clinical trials.

A HDAC inhibitor was approved for the treatment of cutaneous T-cell lymphoma

Zolinza (Vorinostat)



SAHA (Suberoylanilide Hydroxamic Acid)

Zolinza was approved on October 6th of 2006 by the United Stated Food and Drug Administration for the treatment of a type of skin cancer, cutaneous T cell lymphoma (CTCL), and Sezary's disease

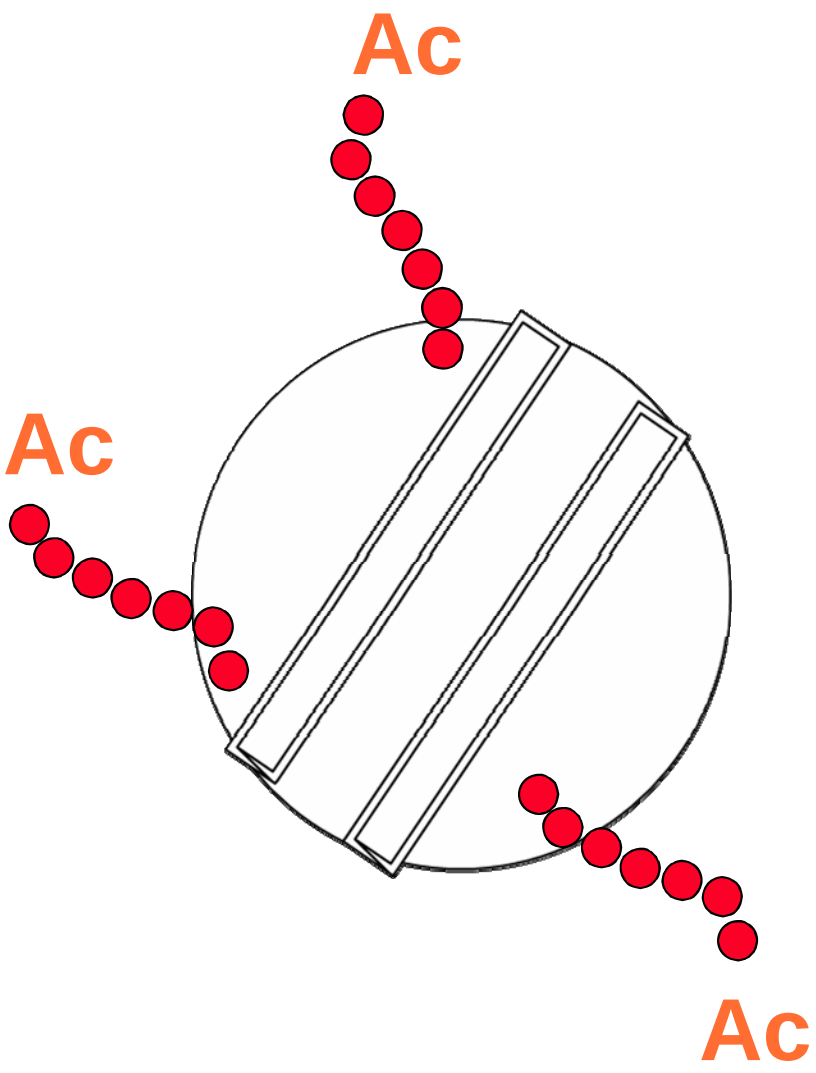
http://www.zolinza.com

In addition to deacetylation of histones, HDACs regulate gene transcription by another mechanism wherein they deacetylate several transcription factors such as p53, E2F, andSp3.

In these cases, deacetylation results in reduced DNA binding or transcriptional activity

Non-histone substrates of HDAC

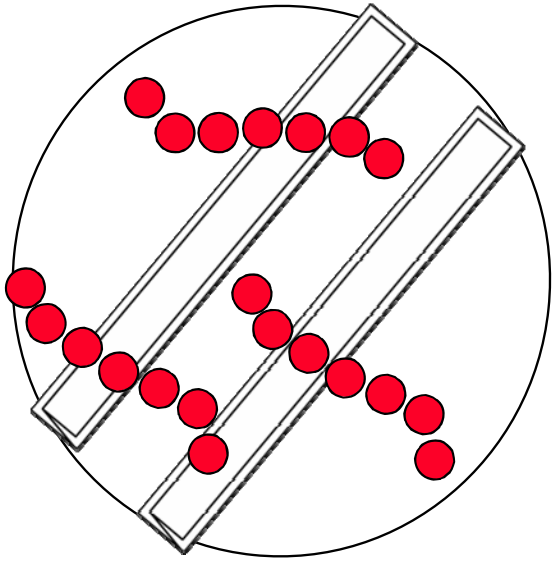
E2F
p53
pRb
Rb
Importin-α
β-catenin
a-Tubulin
Cart-1
Hsp90
TCF
Hmg1(y)
Bcl6
YY1
UBF
P50:relA
HIV-1 Tat



Active

TRANSCRIPTIONAL ACTIVATORS RECRUIT HATs LEADING TO HISTONE ACETYLATION

TRANSCRIPTIONAL REPRESSORS RECRUIT HDACs LEADING TO HISTONE DEACETYLATION



Inactive

HISTONE ACETYLATION / DEACETYLATION

Historical Aspects

Histone acetylation was first linked to transcriptional activation by Allfrey and colleagues (*Proc. Natl Acad. Sci. USA* (1964) **51**, 786-794).

Acetylated core histones were shown to preferentially associate with transcriptionally active chromatin (*Nucleic Acids Res.*(1978) **5:** 1863-1876; *Proc. Natl. Acad. Sci.* (1978) **75:** 2239-2243)

Ingram *et al.* dedmonstrated that *n* -butyrate can alter cellular differentiation by inhibiting histone deacetylation (*Nature(1977)* **268**, 462-464).

Acetylation occurs at lysine residues on the amino-terminal tails of the histones, thereby neutralizing the positive charge of the histone tails and decreasing their affinity for DNA (*J. Biol. Chem.(1993)* **268:** 305-314)

Histone acetylation alters nucleosomal conformation (Norton et al. 1989) so that transcriptional regulatory proteins can access chromatin templates (*EMBO J.* (1996) **15:** 2508-2518).

Chromatin structure and modification can not be viewed as a process that is independent of transcriptional initiation.

Chromatin is not simply a structure that serves to compact DNA in the nucleus.

Histone acetylases and deacetylases provide a critical link between chromatin structure and transcriptional output.

Nature (1997) **389:**349-352;
Cell (1997) **89:**325-328;
Trends Biochem Sci.(1997) **22:** 128-132.

The language of covalent histone modifications
BRIAN D. STRAHL AND C. DAVID ALLIS
Nature **403**, 41 - 45 (2000)