

Eukaryotic Gene Expression: Basics & Benefits

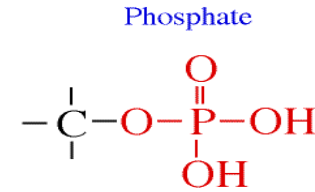
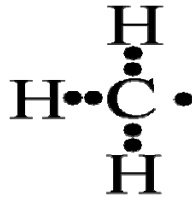
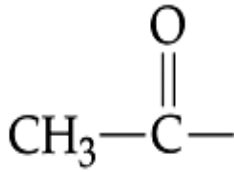
P N RANGARAJAN

Lecture 8

**Eukaryotic gene regulation:
post translational modifications of
histones**

Recap.....

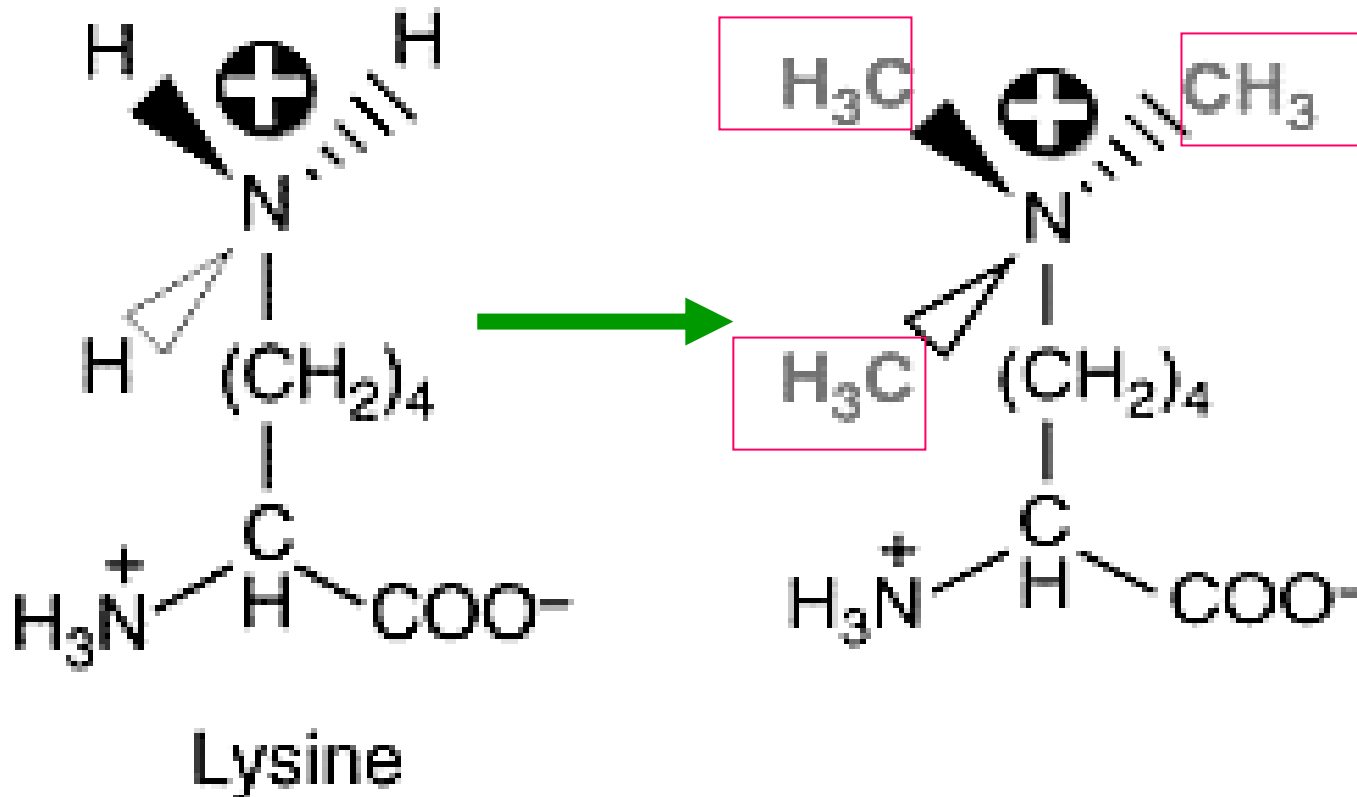
- **Eukaryotic RNA polymerases**
- **Core promoter elements**
- **General transcription factors**
- **Enhancers and upstream activation sequences**
- **Transcriptional activators: DNA binding, transactivation**
- **Role of chromatin: Acetylation & deacetylation of histones**



This class:
**HISTONE METHYLATION &
HISTONE PHOSPHORYLATION**

Histone Methylation

Lysine and arginine residues of histones can be methylated by proteins known as
Histone methyl transferases (HMTs)



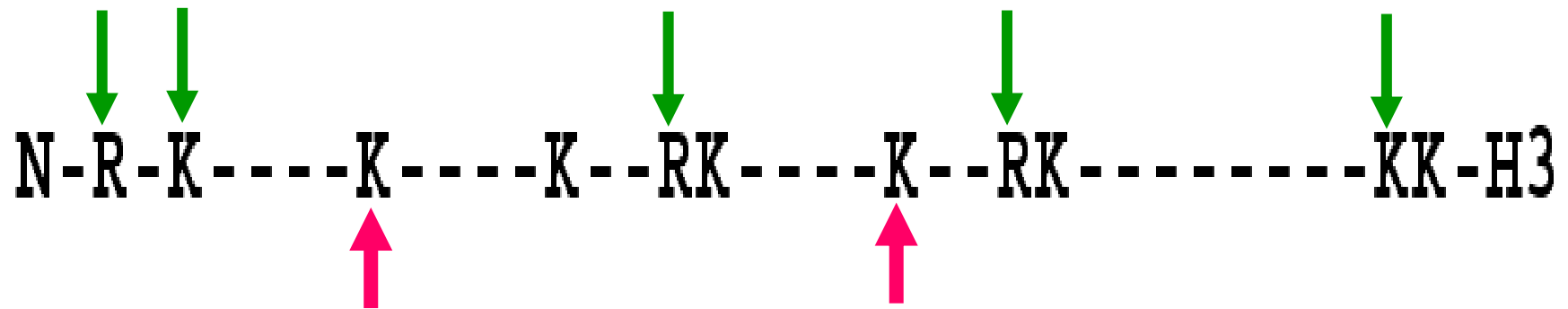
Nomenclature:

H3K4me3: Lysine residue 3 of histone H3 is tri-methylated

H3K9 : Lysine residue 9 of histone H3 is methylated

Unlike histone acetylation, which generally is associated with transcriptional Activation, histone methylation can results in activation or repression

ACTIVATION



REPRESSION

Histone methyl transferases (HMTs)

Histone lysine methyltransferases (HKMTs)

Histone arginine methyltransferases (HRMTs / PRMTs)

Histone lysine methyltransferases (HKMTs)

The first HKMT, SUV39H1 was identified in the year 2000.

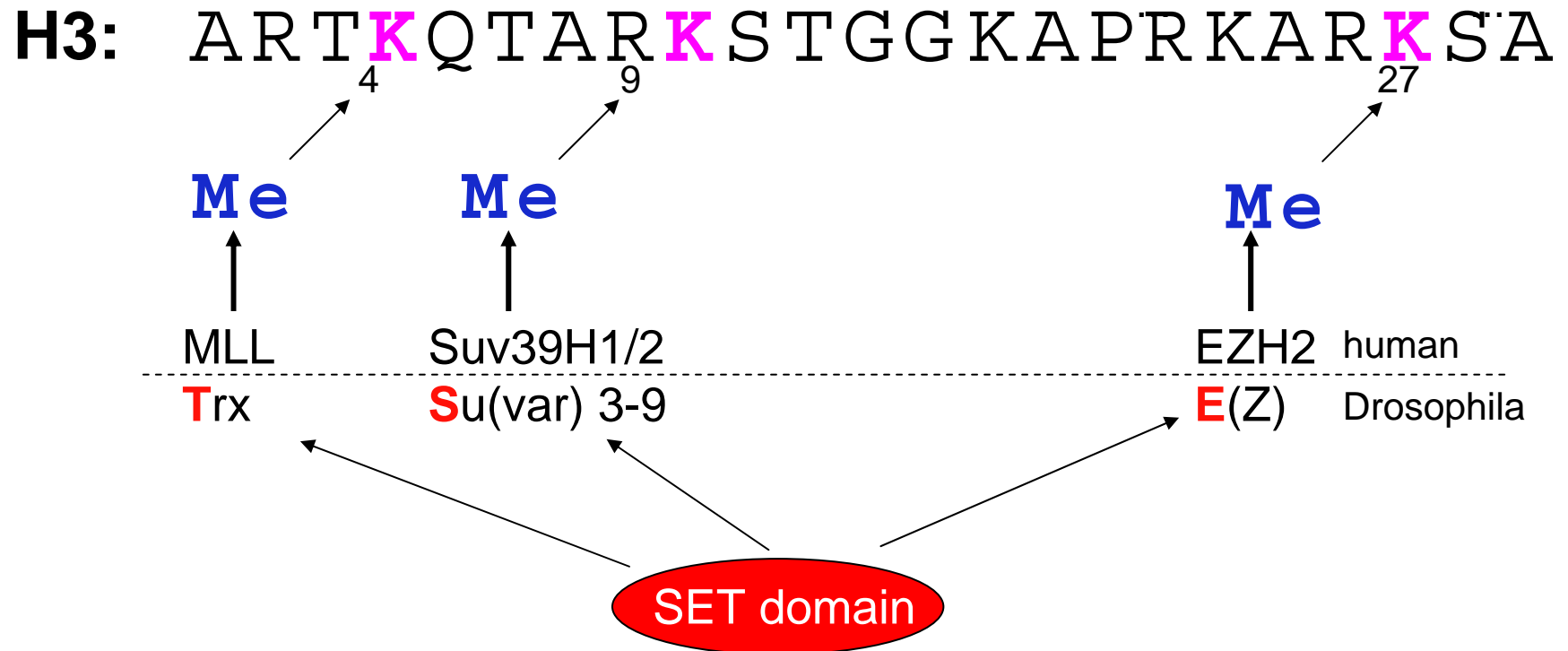
It specifically methylates Lys 9 of histone H3.

Later several other HMTs have been identified

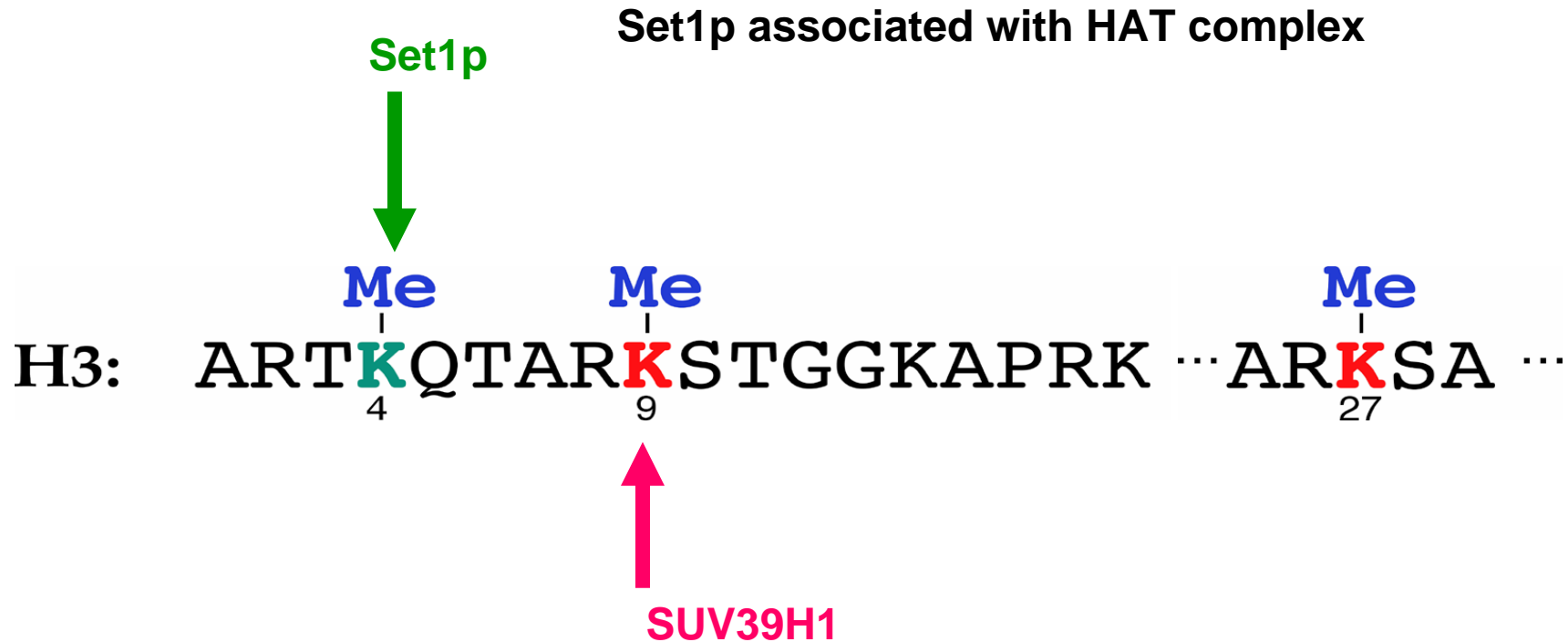
**5 lysines within H3 (K4, K9, K27, K36 and K79) and
1 lysine within H4 (K20) are methylated by specific HKMTs**

Identification of H3 methyltransferases

- The SET domain is the conserved catalytic core of histone methyltransferases



Histone methylation was traditionally linked to repression, but it can also lead to transcriptional activation



H3 lysine 9 methylation correlates with heterochromatin formation.

Interestingly, methylation of H3-K4 specifically impairs methylation at H3-K9, thereby blocking a major pathway of heterochromatin formation

Di-methyl H3-K4 is associated with euchromatic regions

Tri-methylation of H3-K4 correlates with active transcription

The initiation and maintenance of heterochromatin is carried out by histone H3 lysine 9 methylation.

Mammalian HMTs which can methylate lysine 4 of Histone H3 are:

SET1/ASH2, SET7/9, MLL1/ALL1, MLL2/HRX2, MLL3/HALR and SMYD3.

Methylation of H3K27 is also associated with transcriptional repression. The H3K27 methylation is catalysed by multi-molecular complexes referred to as polycomb repressive complexes (PRCs).

Methylated histones are recognized by chromodomains

Methylated lysines are recognized by proteins containing specific domains known as chromodomains

These are found in several chromatin-associated proteins.

The chromodomain specifically binds to histone tails bearing methyllysine.

Chromodomains have the highest affinity for trimethyllysine and lowest for monomethyllysine.

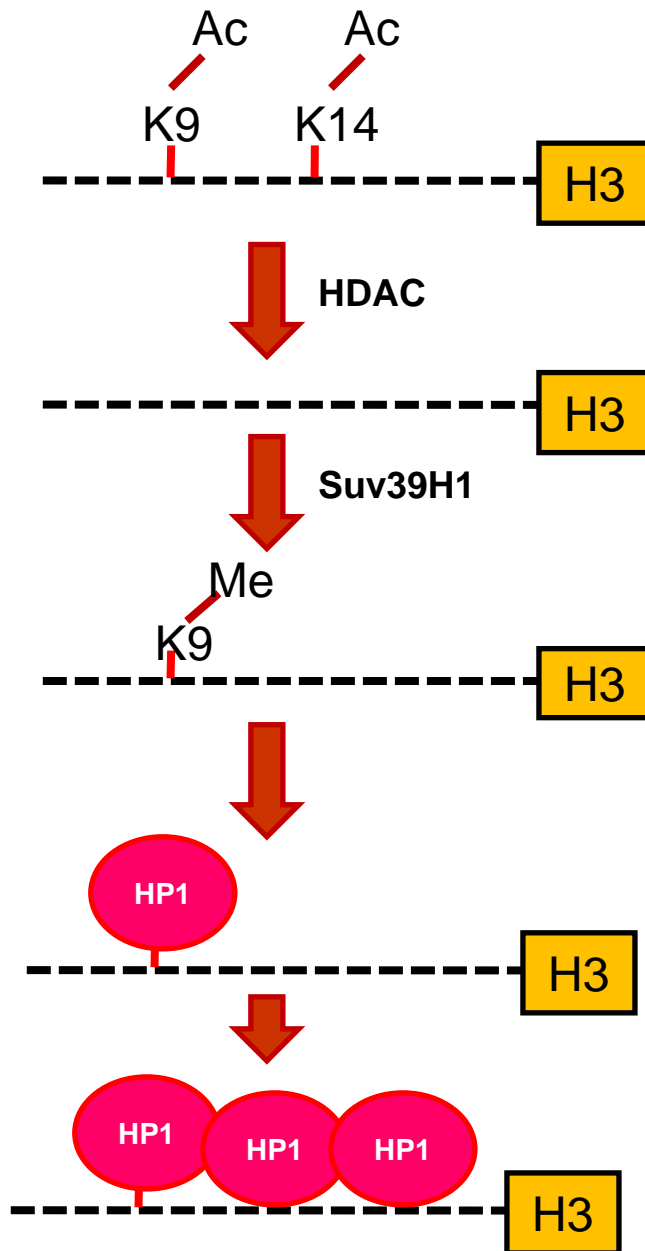
Examples of chromodomain containing proteins:

- The heterochromatin protein 1 (HP1)**
- Drosophila Polycomb (PC) protein**

Both HP1 and PC specifically recognize H3K9 and H3K27.

Heterochromatin formation by deacetylation and methylation of histone H3

Genes Dev (2001) 15: 2343-2360



Deacetylation by HDACs

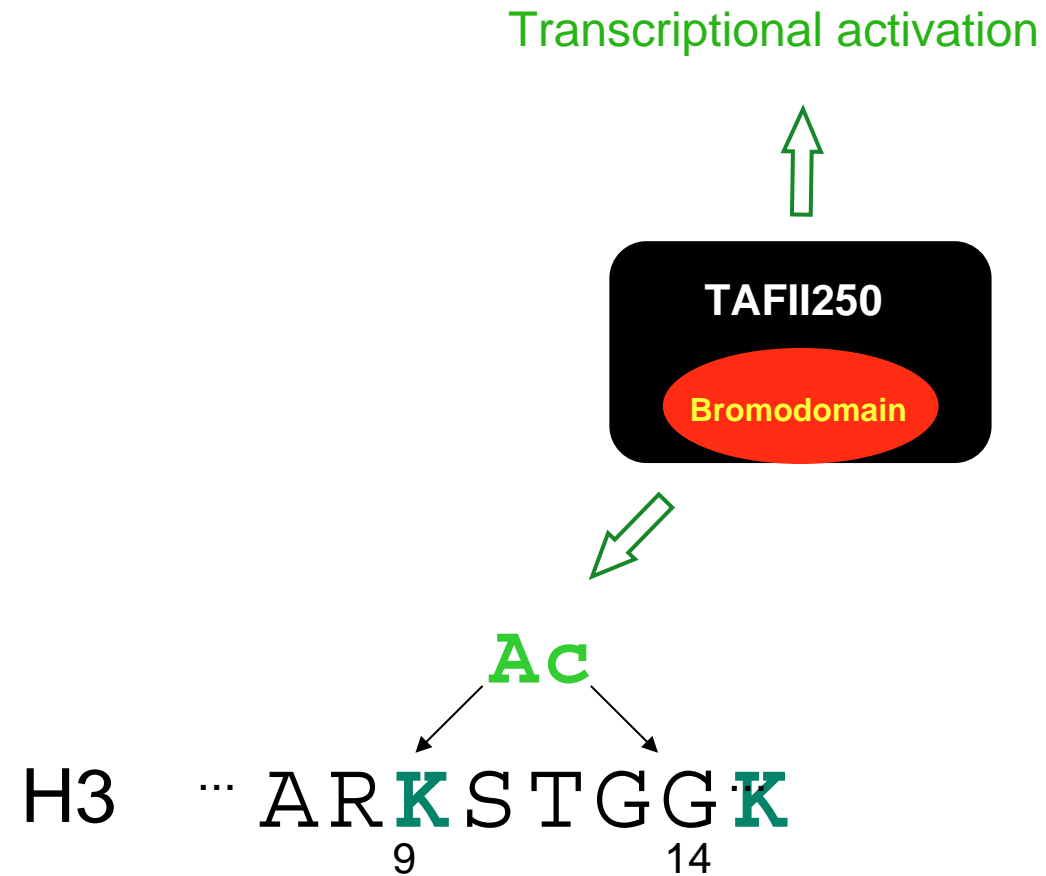
Methylation by Suv39H1

Recognition by the chromodomain of HP1

Initiation of heterochromatinization

Propagation of heterochromatin by self association of HP1

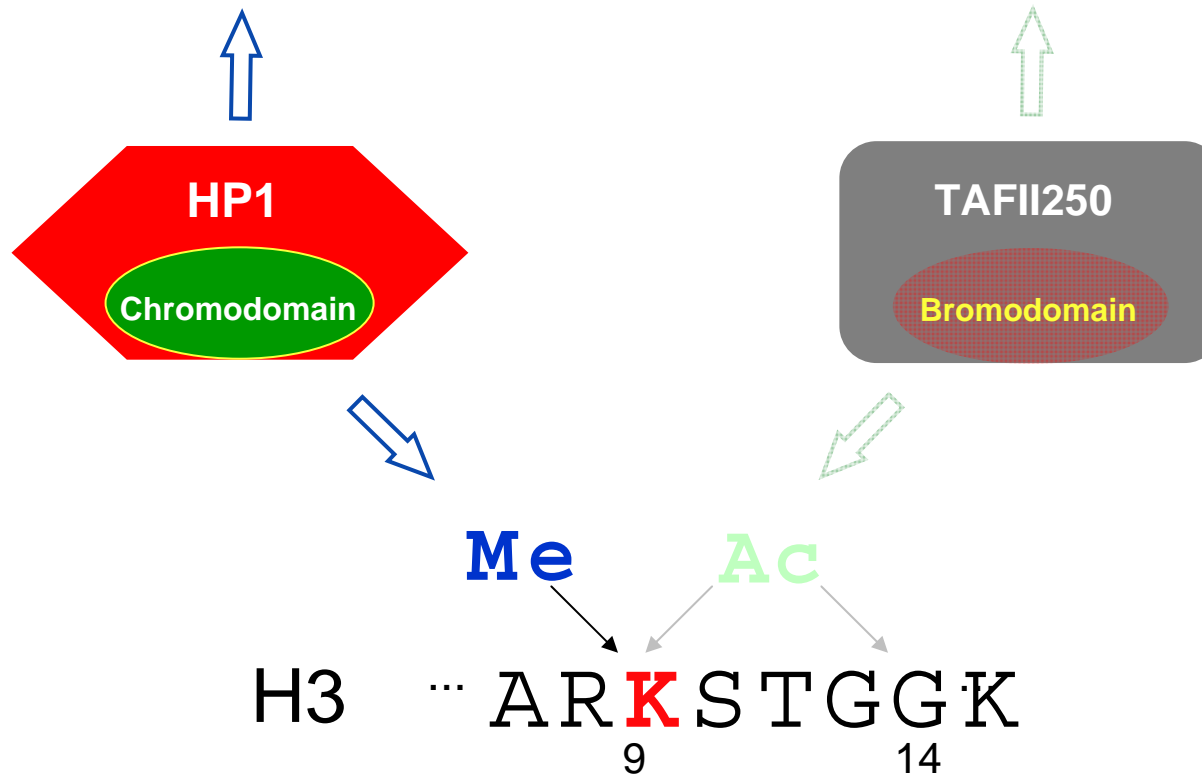
Histone modification-dependent recruitment of proteins



Histone modification-dependent recruitment of proteins

Heterochromatin assembly,
Transcriptional silencing

Transcriptional activation



Histone arginine methyltransferases (HRMTs / PRMTs)

Carm1 (Coactivator-associated arginine methyltransferase).

PRMT1 (protein arginine methyltransferase).

The PRMT(protein arginine methyltransferase) domain

The catalytic module that methylates specific arginines is known as PRMT

The PRMT domain transfers the methyl group from S-adenosyl methionine to the guanidino group of arginines to produce monomethylarginine or dimethylarginine.

Methylation of Arginine 3 of histone H4 facilitates H4 acetylation and enhances transcription activation by nuclear hormone receptors.

Certain HMTs play a key role in early embryogenesis

A mammalian histone methyltransferase (HMT) known as G9a methylates lysine 9 of Histone H3 in vitro.

G9a was found to repress the transcription of certain genes involved in early embryonic development.

G9a-knockout mice display severe growth retardation and early lethality.

G9a-deficient ES cells exhibit reduced H3-K9 methylation compared to wild-type cells, indicating that G9a is a dominant H3-K9 HMT in vivo.

In the absence of G9a, H3-K9 methylation was drastically reduced in euchromatic regions indicating that euchromatic H3-K9 methylation regulated by G9a is essential for early embryogenesis.

Tachibana M et al., Genes Dev 2002 Jul 15;16(14):1779-91

Just as we have HDACs, are there demethylases?

Histone methylation was thought to be an extremely stable modification.

Histone demethylases were not discovered for a long time.

LSD1 (lysine-specific demethylase)

Demethylation by LSD1 is limited to mono- or dimethylated H3K4

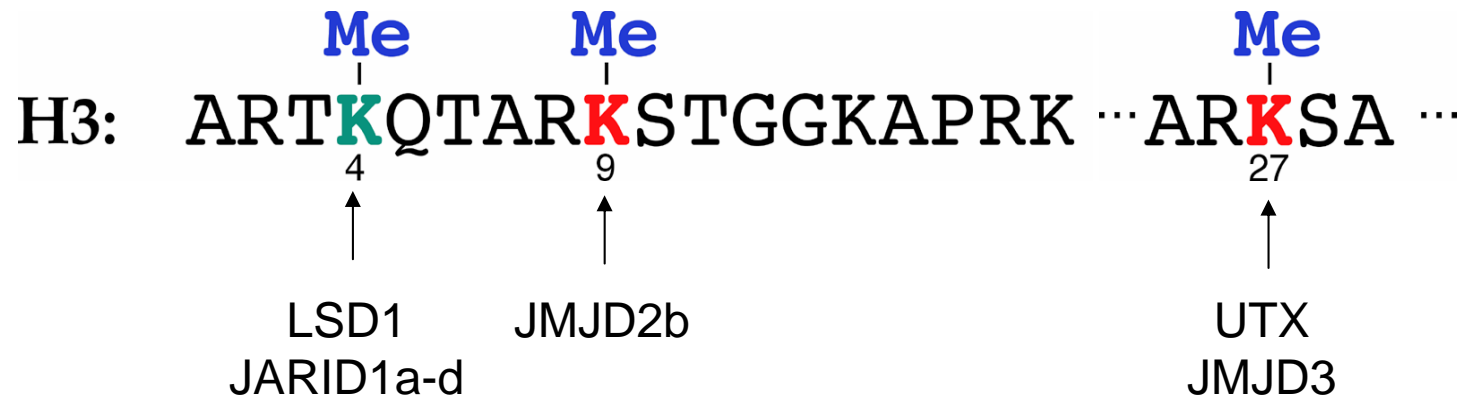
LSD1 cannot demethylate tri-methylated H3K4.

The androgen receptor alters the specificity of LSD1 from H3K4 to H3K9, and thereby converts the demethylase from a repressor to an activator of transcription.

Another class of histone demethylase enzymes containing the **Jumonji domain (Jmjc)** has now been identified, which include at least 15 distinct proteins that have been reported to demethylate specific lysine or arginine of histone H3

JHDM1 (Jmjc domain-containing histone demethylase 1) specifically demethylates histone H3 at lysine 36

Another Jmjc domain containing demethylase called **MJD2A** is a lysine trimethyl specific demethylase that converts trimethylated H3-K9/K36 to di- but not mono- or unmethylated products.



HISTONE PHOSPHORYLATION

**SPECIFIC SERINE AND THREONINE RESIDUES OF HISTONES ARE
PHOSPHORYLATED BY SPECIFIC HISTONE KINASES**

H2A: S1, T119

H2B: S14, S32

H3: S3, S10, T11, S28, Y41, T45

H4: S1, H18, H75

In developing *Drosophila* embryo, T119 phosphorylation of Histone H2A was shown to occur during mitosis but not in S-phase of the cell cycle – Nucleosomal histone kinase 1

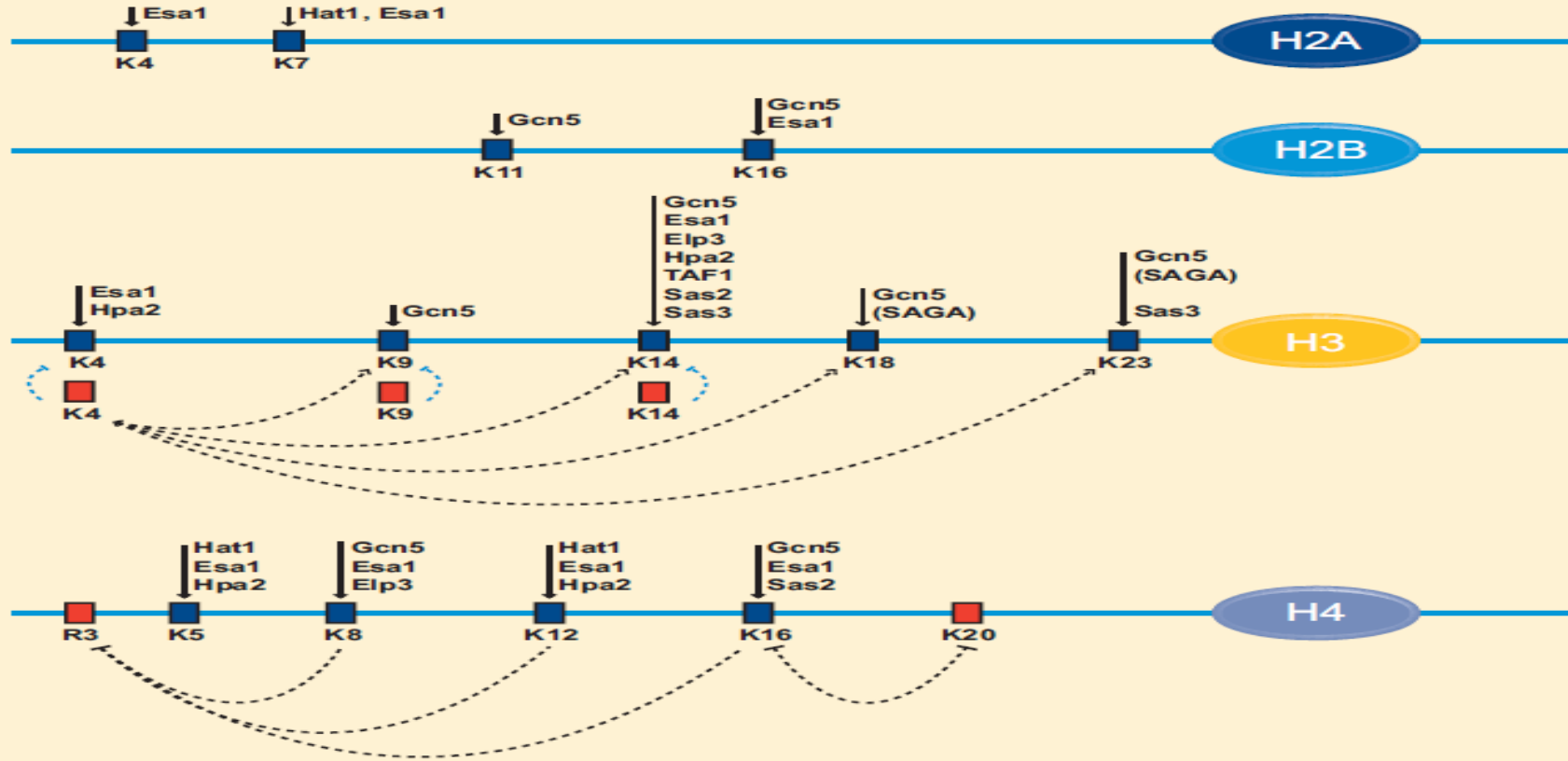
Phosphorylation of histone H3 at T45 occurs after activation of DNA-damage signaling pathways and this is mediated by protein kinase C

Phosphorylation of Y41 of Histone H3 is mediated by Janus activated kinase (JAK) 2 and this event has implications in hematopoiesis and leukaemia. Decreased Y41 phosphorylation results in the expression of the haematopoietic oncogene, *lmo2*.

Histone H4 is phosphorylated not only on serine 1 but also on histidines 18 and 75.

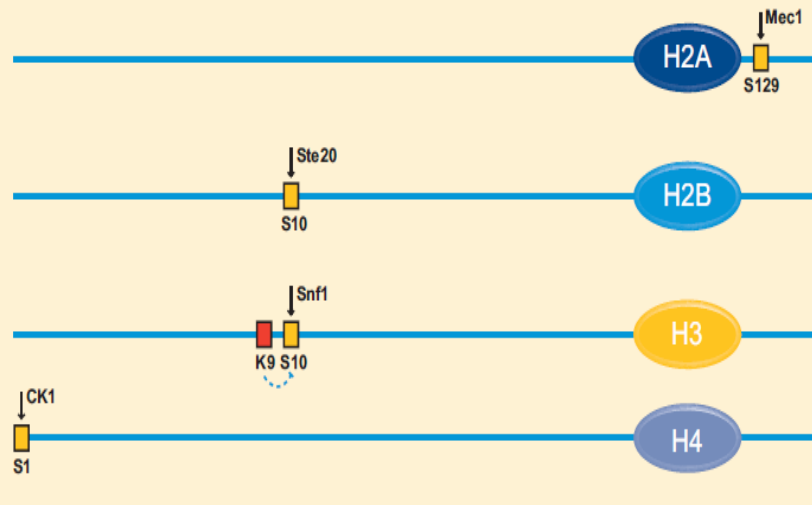
The kinases involved in histone phosphorylation are not well characterized.

Acetylation | Yeast

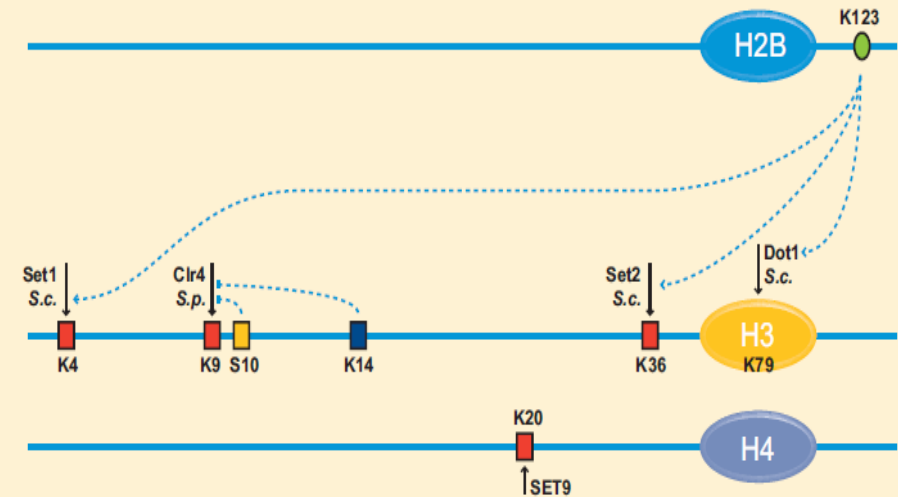


■ Phosphorylated	—————> Adds modification	*** Trimethylase
■ Methylation	- - - - -> Removes modification	** Dimethylase
■ Acetylated> Influences positively	* Monomethylase
● Ubiquitination	-> Influences negatively	

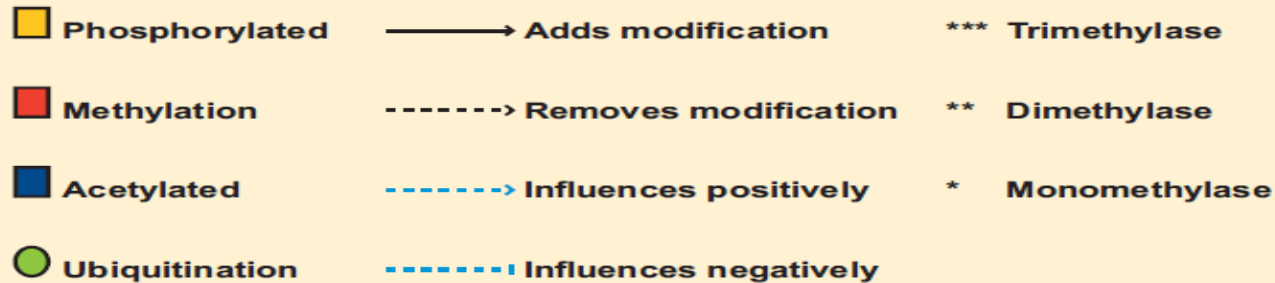
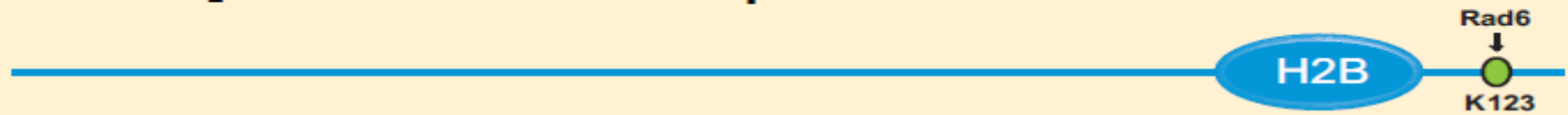
Phosphorylation | Yeast



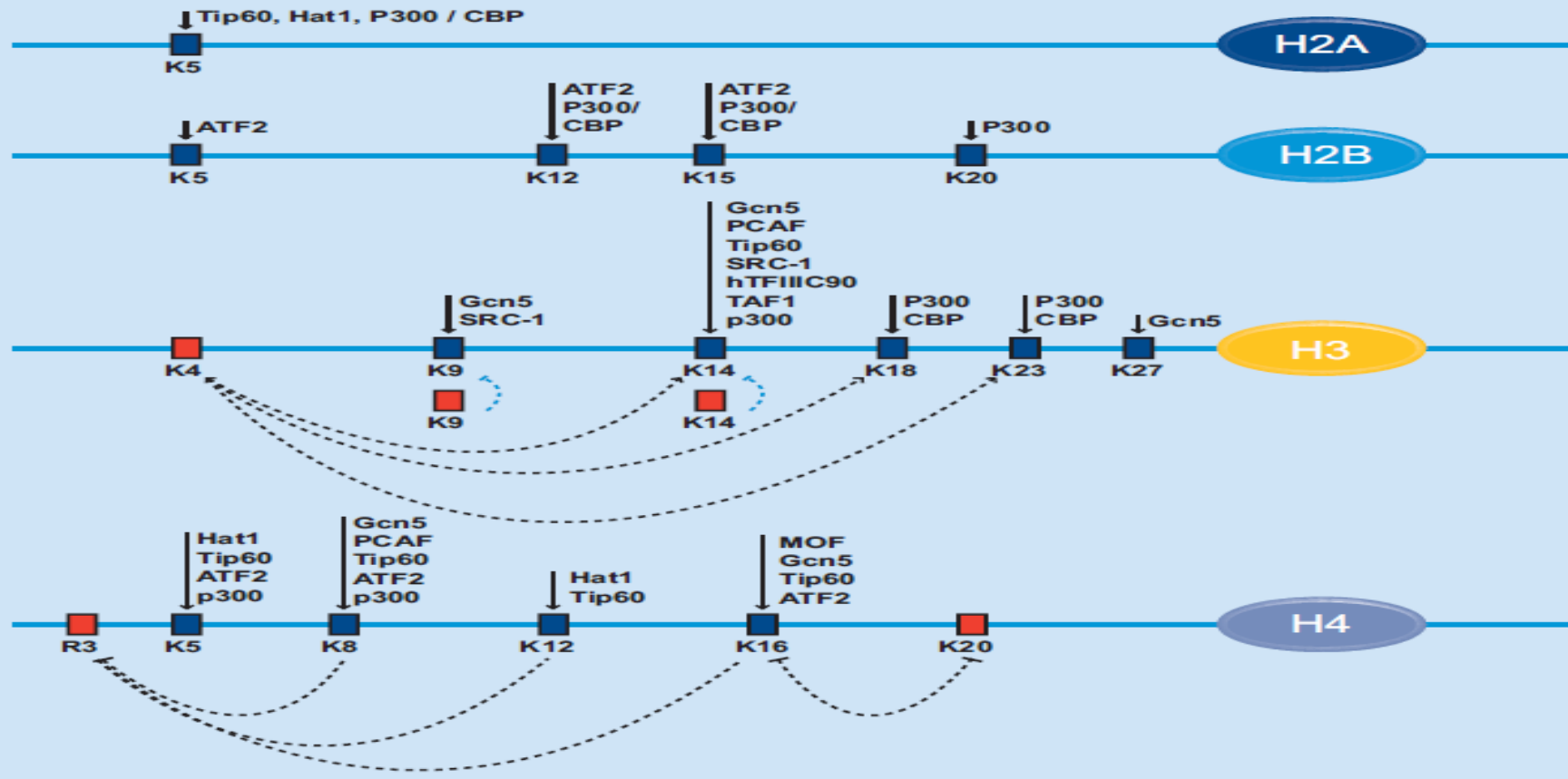
Methylation | Yeast



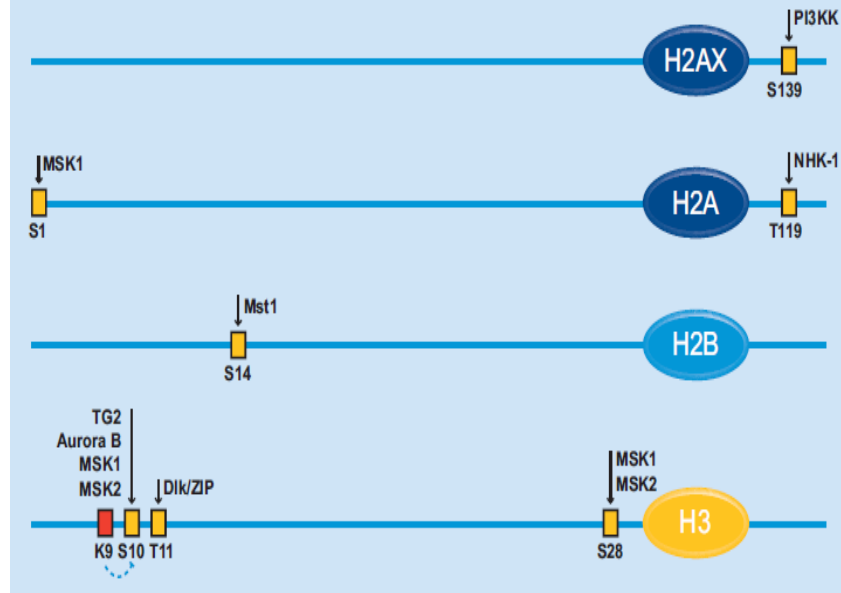
Ubiquitination | Yeast



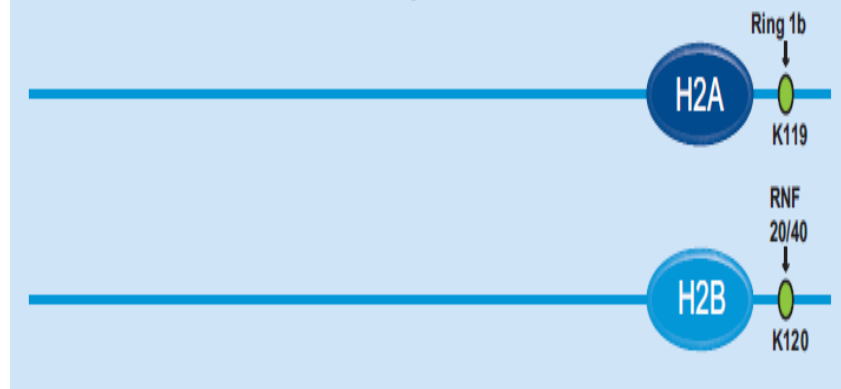
Acetylation | Mammalian



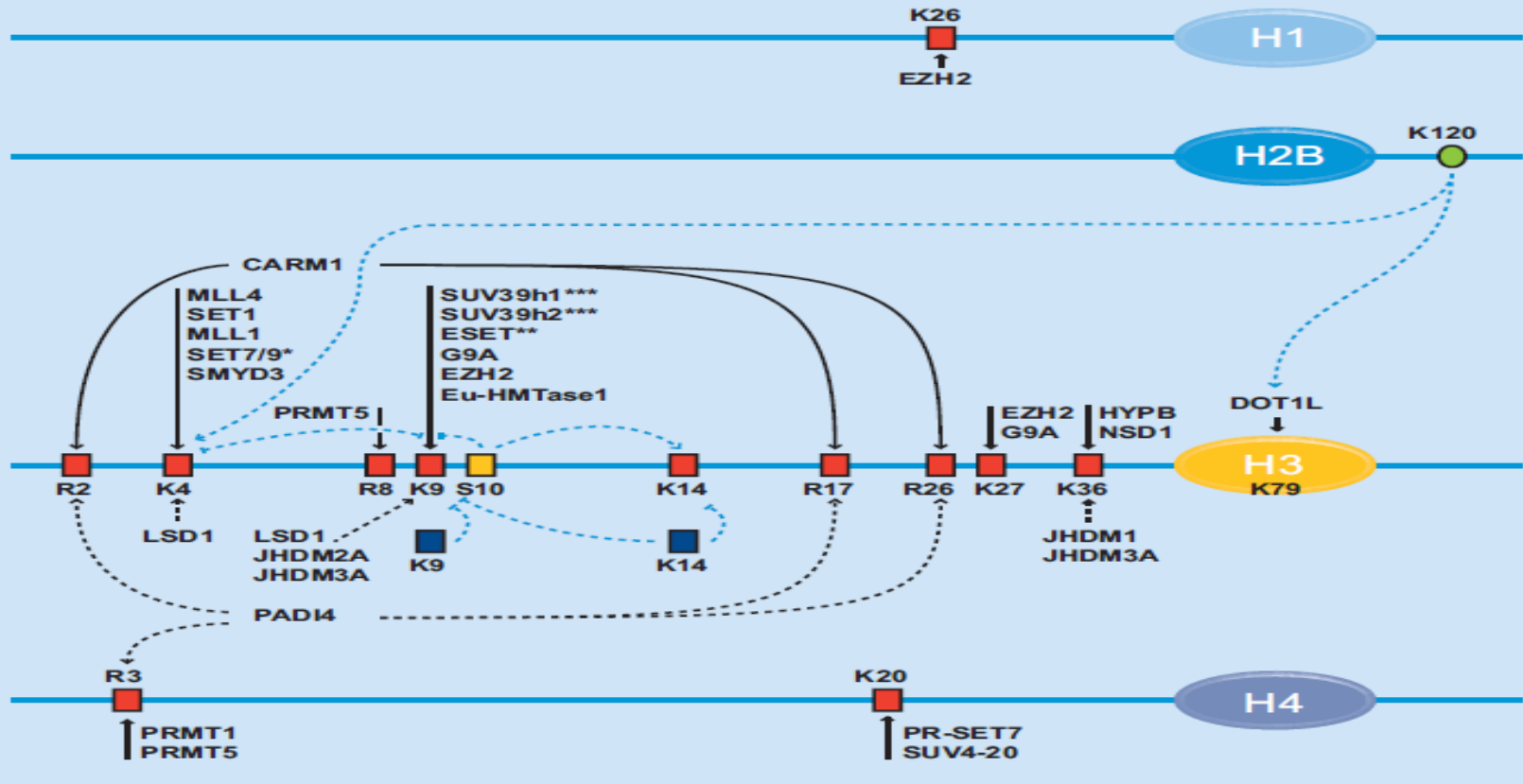
Phosphorylation | Mammalian



Ubiquitination | Mammalian



Methylation | Mammalian



THE HISTONE CODE

Information encoded in DNA (GENETIC CODE)

Information encoded in histones (HISTONE CODE)

Modification of histone tails represents a novel code that is different from that encoded in the DNA sequence

There is an extensive cross talk between histone modifications

One modification can either enhance or suppress another modification

The modified histones serve as binding sites for specific protein domains

For ex., bromodomains bind acetylated histones
While chromodomains bind methylated histones.

Domains recognizing phosphorylated histones ??????

The genetic code is embedded in the DNA sequence

The histone code is written by proteins such as HATs, HDACs, HMTs, etc.

This histone code is read by proteins such as chromo- or bromo-domain containing proteins

The “Histone code hypothesis” has become a key to understand how the eukaryotic transcriptional apparatus interacts with chromatin.