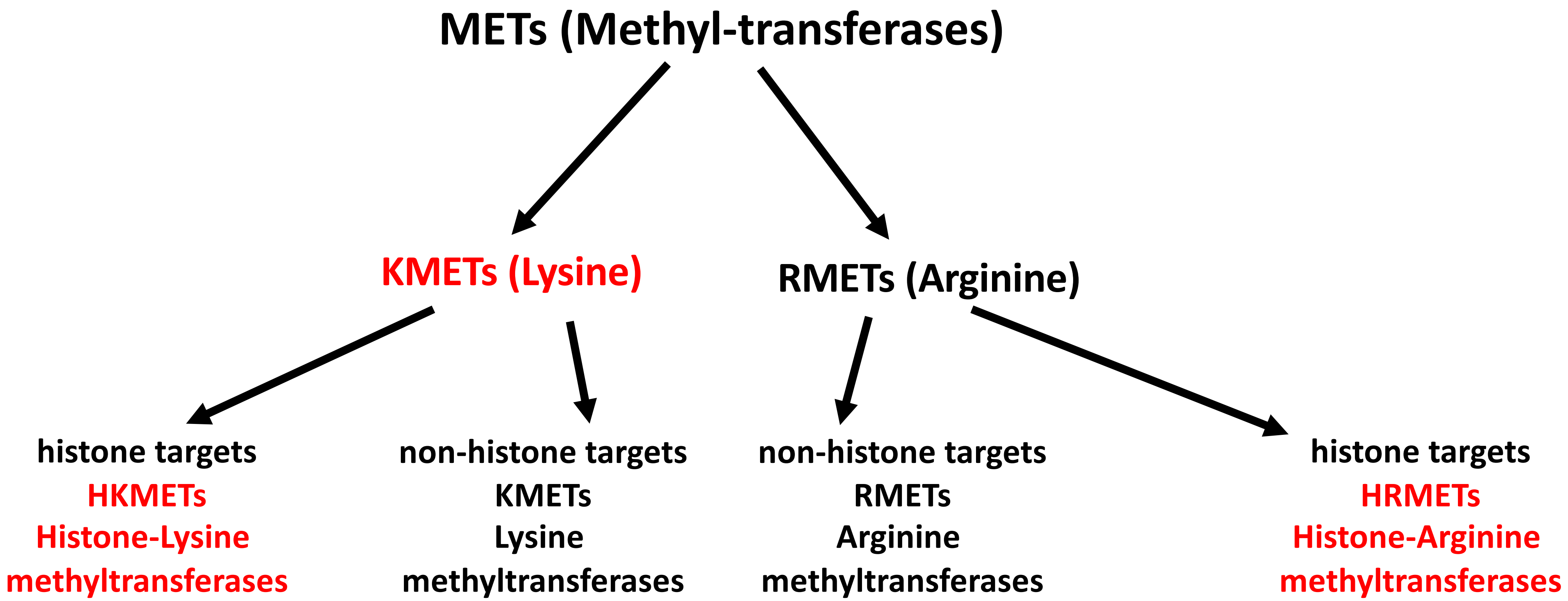
HISTONE METHYALTION

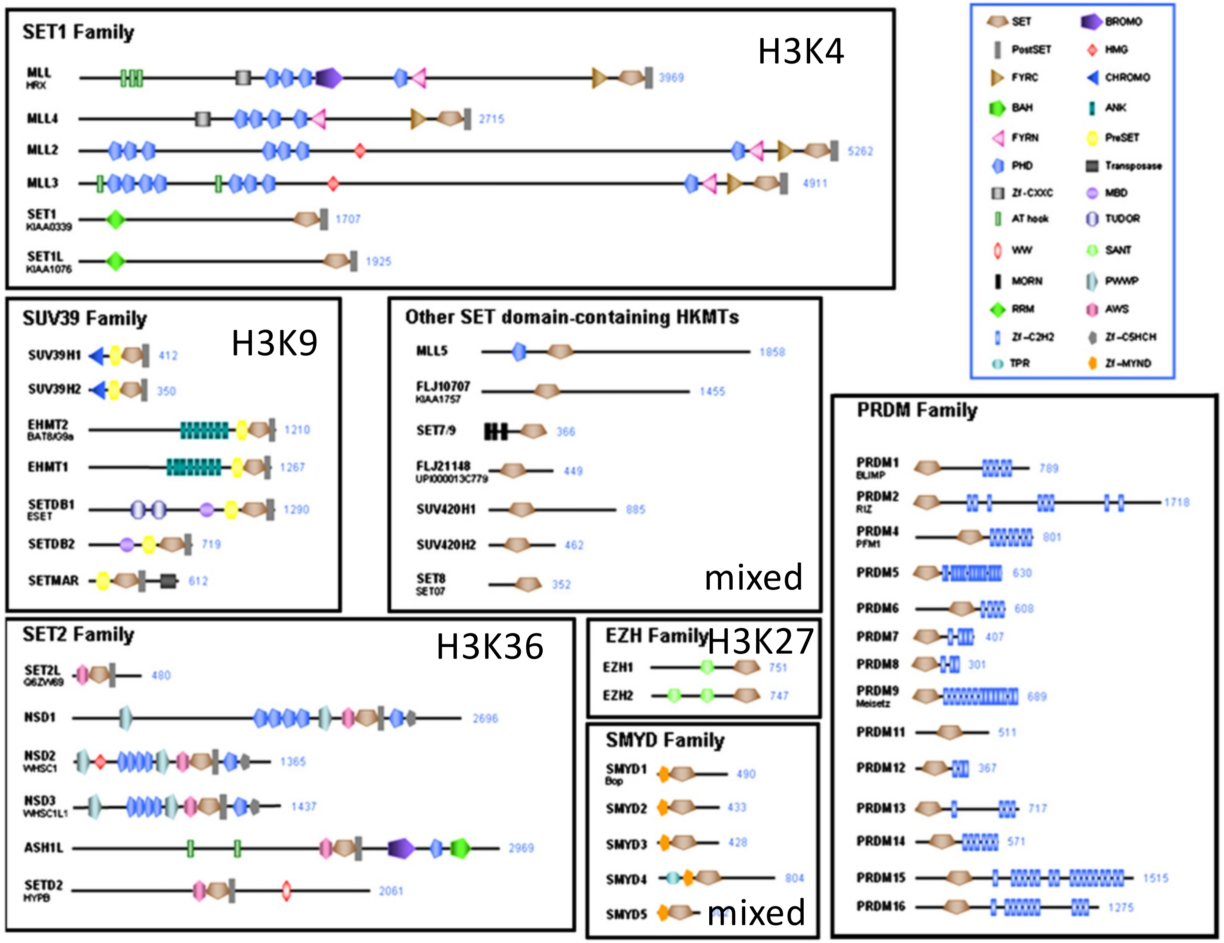
HISTONE METHYALTION - MECHANISMS

HISTONE LYSINE AND ARGININE METHYL TRANSFERASES (HKMETs and HRMETs))



HISTONE LYSINE METHYL TRANSFERASES (HKMETs)

all HKMETs contain a conserved SET domain that catalyzes the methylation of Lysines (K)
(exception Dot1 – no SET domain)



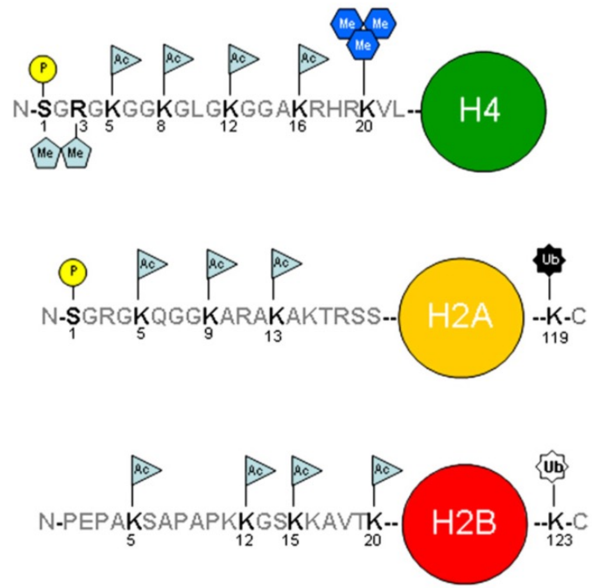
50 SET domain proteins are categorized according to sequence homology into 6 HKMET subfamilies

1. SET1 family
2. SET2 family
3. SUV39 family
4. EZH family
5. SMYD family
6. PRDM family
7. other SET domain HKMETs

50 SET domain proteins contain many other protein domains
→ Interaction with other proteins or DNA

HKMET HRMET SUBSTRATES ON HUMAN HISTONES

A



HKMETS epigenetic writers are substrate specific and can result in gene repression but also gene activation
→→→
The epigenetic reader that binds to the modified histone K residue at the individual histone tail makes the difference

Effect on gene activity: **Best studied examples of histone methylation:**

Effect on gene activity	Substrate	Histone lysine methyltransferases
activation	H3K4	SET9, SET1, MLL, ASH1L, SMYD3, PRDM9, SETMAR
repression	H3K9	SUV39H1, SUV39H2, EHMT1, EHMT2, SETDB1, PRDM2, ASH1L
repression	H3K27	EZH2, EHMT2
activation	H3K36	NSD1, SETD2/HYPB, SETMAR
activation	H3K79	DOT1L
repression	H4K20	SET8, SUV420H1, SUV420H2, NSD1, ASH1L

Fig. 1. Histone modifications. (A) The modifications on human histones include methylation (Me) on arginine and lysine residues, acetylation (Ac) on lysine residues, phosphorylation (P) on serine and threonine residues and ubiquitination (Ub) on lysine residues. (B) The enzymes responsible for methylation of human histone lysine residues are listed according to their target sites. Histone lysine methyltransferases (HKMTs) are very specific but redundant in several cases.

HISTONE MODIFICATIONS AND EPIGENTIC READERS

Protein domains that bind to histone modifications

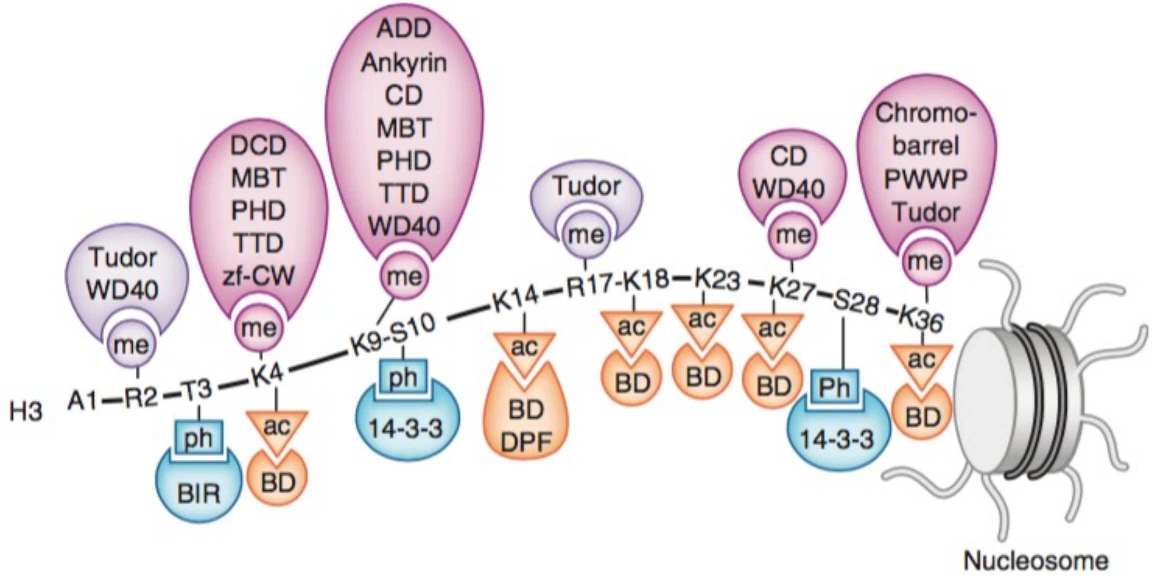


Figure 1 Readers of histone PTMs. Recognition of the methylated (me) lysine, methylated (me) arginine, acetylated (ac) lysine and phosphorylated (ph) serine and threonine residues of the N-terminal histone H3 tail by indicated readers.

Table 1 Histone readers and their target PTMs

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	PWWP	H3K36me3, H4K20me1, H4K20me3, H3K79me3
	TTD	H3K4me3, H3K9me3, H4K20me2
	Tudor	H3K36me3
	WD40	H3K27me3, H3K9me3
	zf-CW	H3K4me3
Methylarginine	ADD	H4R3me2s
	Tudor	H3Rme2, H4Rme2
	WD40	H3R2me2
Acetyllysine	Bromodomain	H3Kac, H4Kac, H2AKac, H2BKac
	DBD	H3KacKac, H4KacKac
	DPF	H3Kac
	Double PH	H3K56ac
Phosphoserine or phosphothreonine	14-3-3	H3S10ph, H3S28ph
	BIR	H3T3ph
	Tandem BRCT	H2AXS139ph
Unmodified histone	ADD	H3un
	PHD	H3un
	WD40	H3un

A large number of proteins contain these protein domains:
→ High complexity in gene regulation that
→ Creation of large numbers of EPIGENOMES

THE BIOCHEMISTRY OF HISTONE LYSINE METHYLATION

The source of the methyl group is S-adenosyl-l-methionine (AdoMet) or (SAM), which is converted to S-adenosyl-l-homocysteine (AdoHcy) in the reaction.

S-Adenosyl methionine is a common co-substrate involved in methyl group transfers, transsulfuration, and aminopropylation.

SAM = enzymatic cofactor

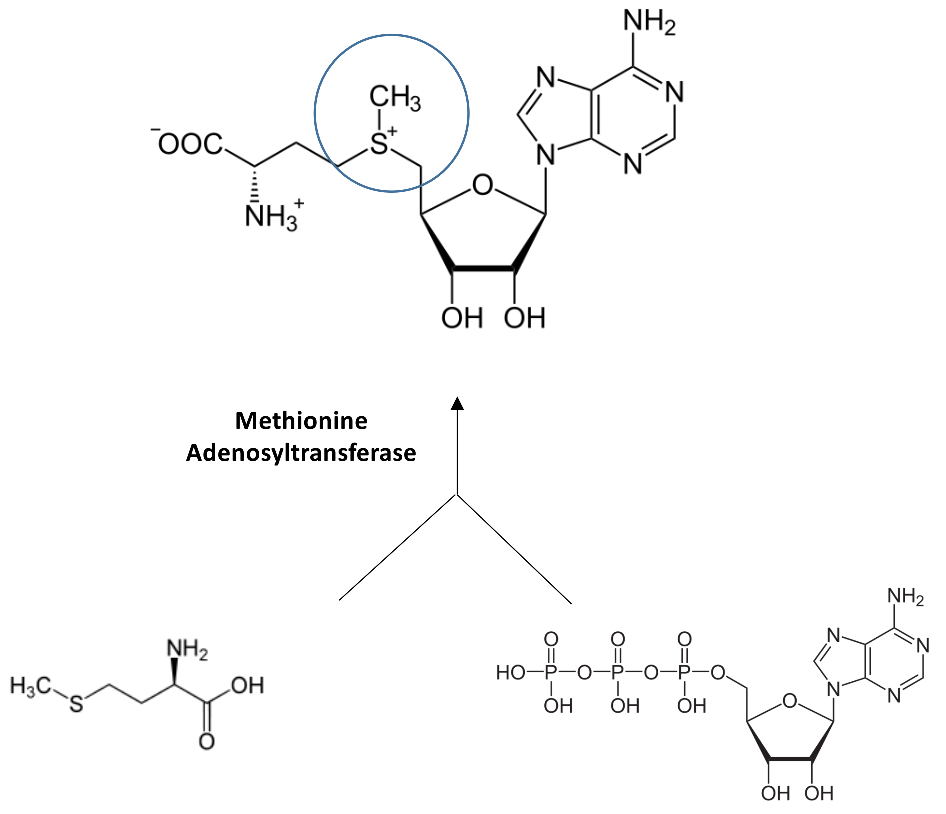
<u>-SAM is after ATP the most commonly used cofactor used by the cell</u>

-Although these anabolic reactions occur throughout the body, most **SAM is produced and consumed in the liver**.

- SAM is made from adenosine triphosphate (ATP) and methionine by methionine adenosyltransferase. SAM was first discovered in Italy by Giulio Cantoni in 1952

- More than 40 methyl transfers from SAM are known, to various substrates such as nucleic acids, proteins, lipids and secondary metabolites.

S-adenosyl-l-methionine (AdoMet) or (SAM),

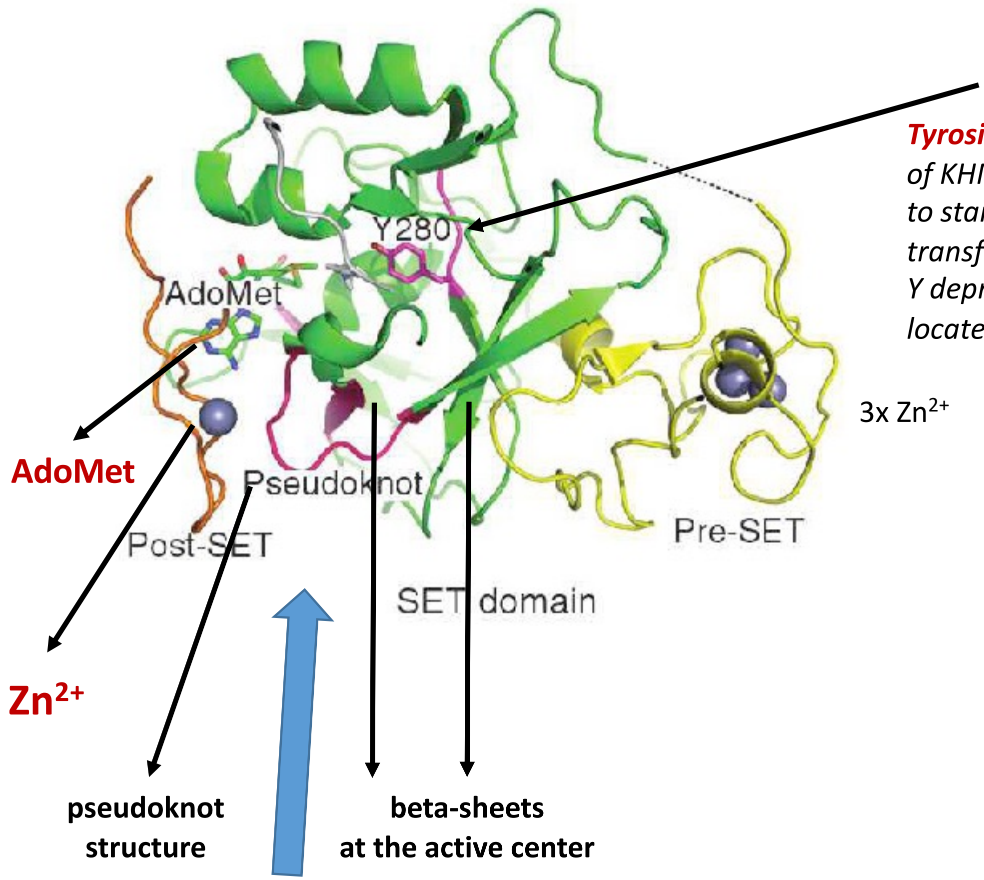


methionin

Adenosin triphosphate (ATP)

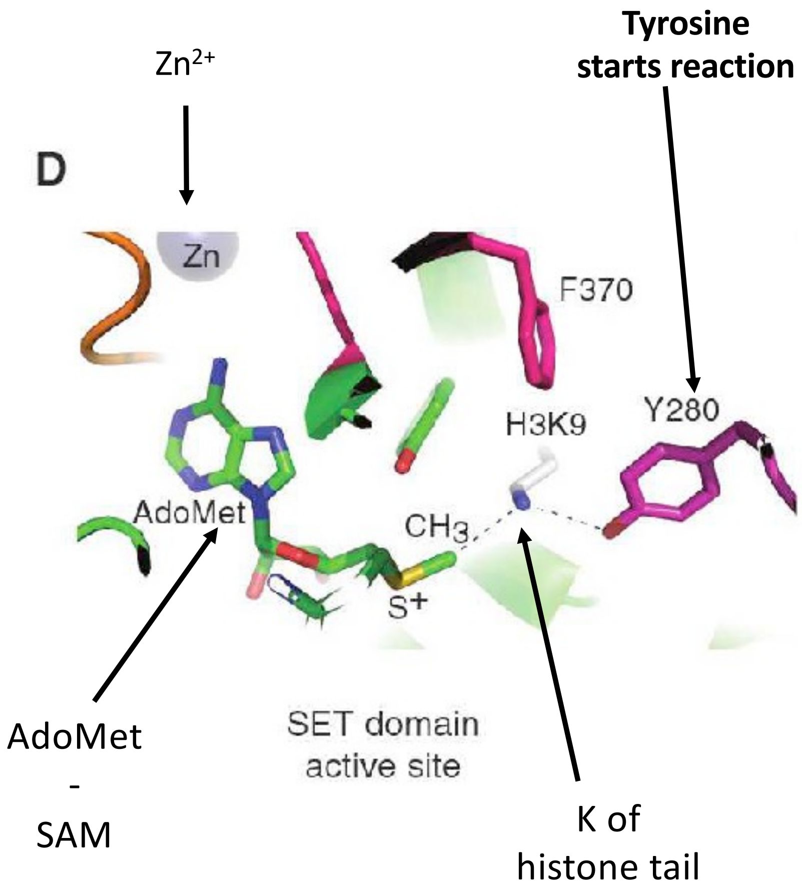
THE SET DOMAIN – EXCLUSIVELY IN KMETS

THE SET DOMAIN



Tyrosine residue
of KHMTs is required to start the methyl-transferase reaction.
Y deprotonates K residue located in the histone tail.

THE ACTIVE SITE IN THE SET DOMAIN



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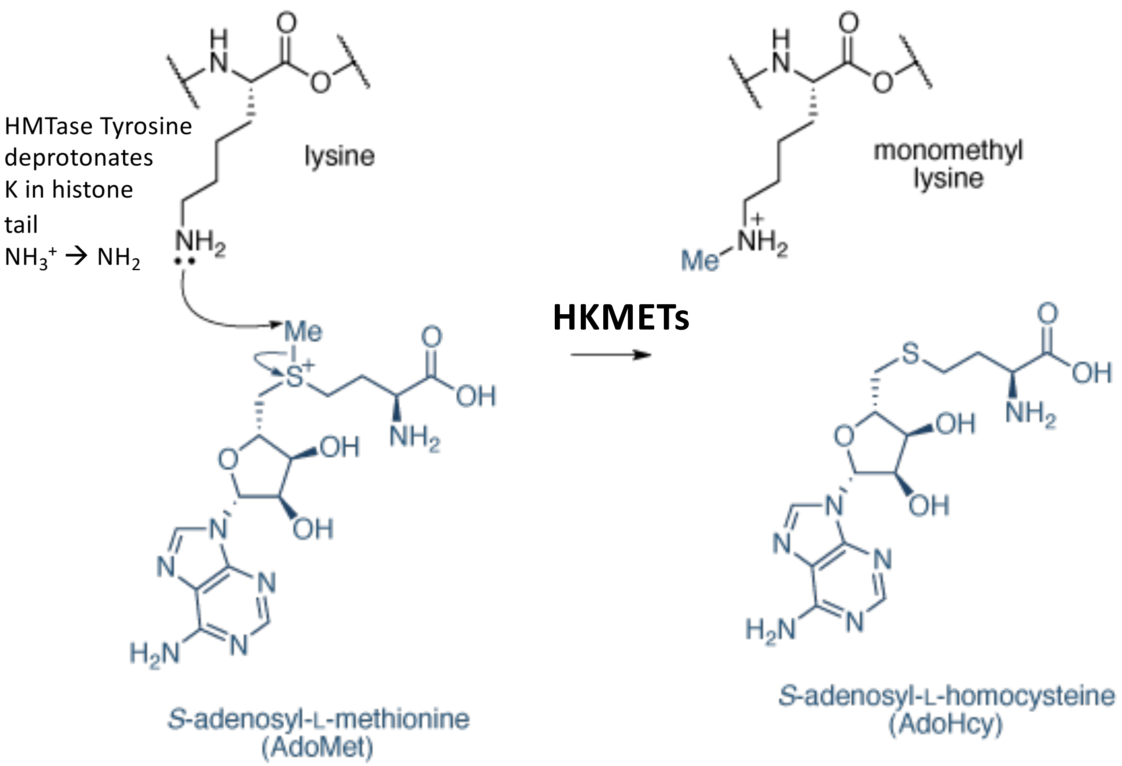


Catalytic mechanism

- In order for the reaction to proceed, S-Adenosyl methionine (SAM) and the lysine residue of the substrate histone tail must first be bound and properly oriented in the catalytic pocket of the SET domain.

- Next, a nearby tyrosine residue deprotonates the ε-amino group of the lysine residue.

- The lysine chain then makes a nucleophilic attack on the methyl group on the sulfur atom of the SAM molecule.

- The methyl-group is transferred to the lysine side chain.

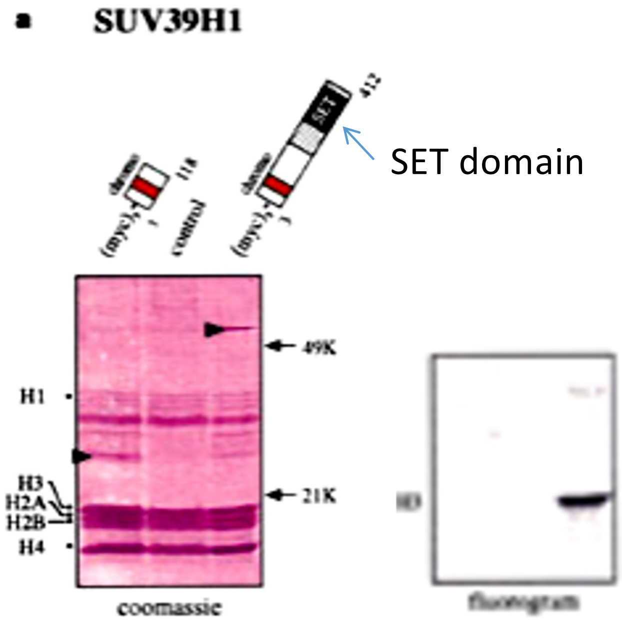
ENZYMATIC ASSAY TO DETECT KMTase ACTIVITY

Experiment:

Overexpression of **myc-tagged-SUV39H1 KMT** in Hela cells

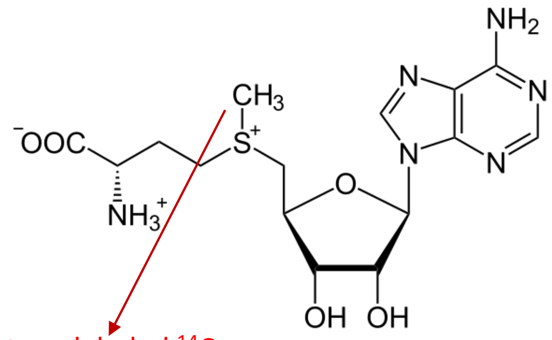
Use an antibody to immunoprecipitate SUV39H1 → high concentration of SUV39H1

Incubate Immunopreciptate with purified histones and S-adenosyl-[methyl-^{14}C]-$_{L}$-methionin as methyl donor



SET – domain is required for histone methyl transferases activity

S-adenosyl-l-methionine (AdoMet) or (SAM),



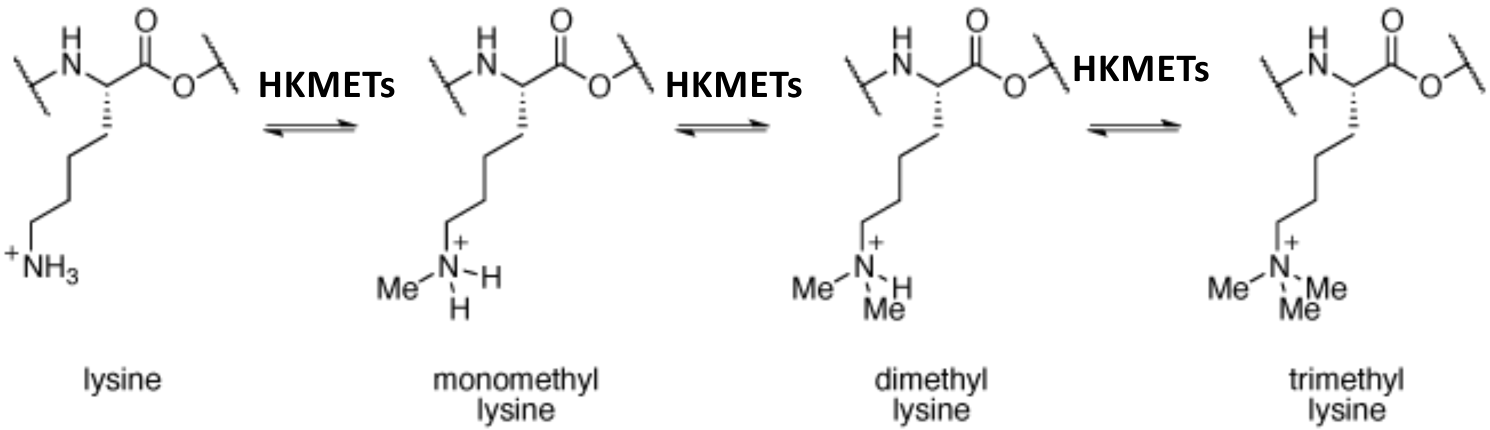
Isotope labeled ^{14}C (radioactive)

- The SET domain of the SUV39H1 is required for histone methyltransferase activity and this enzyme methylates H3 at Lys9

Rea et al, Nature, 2000

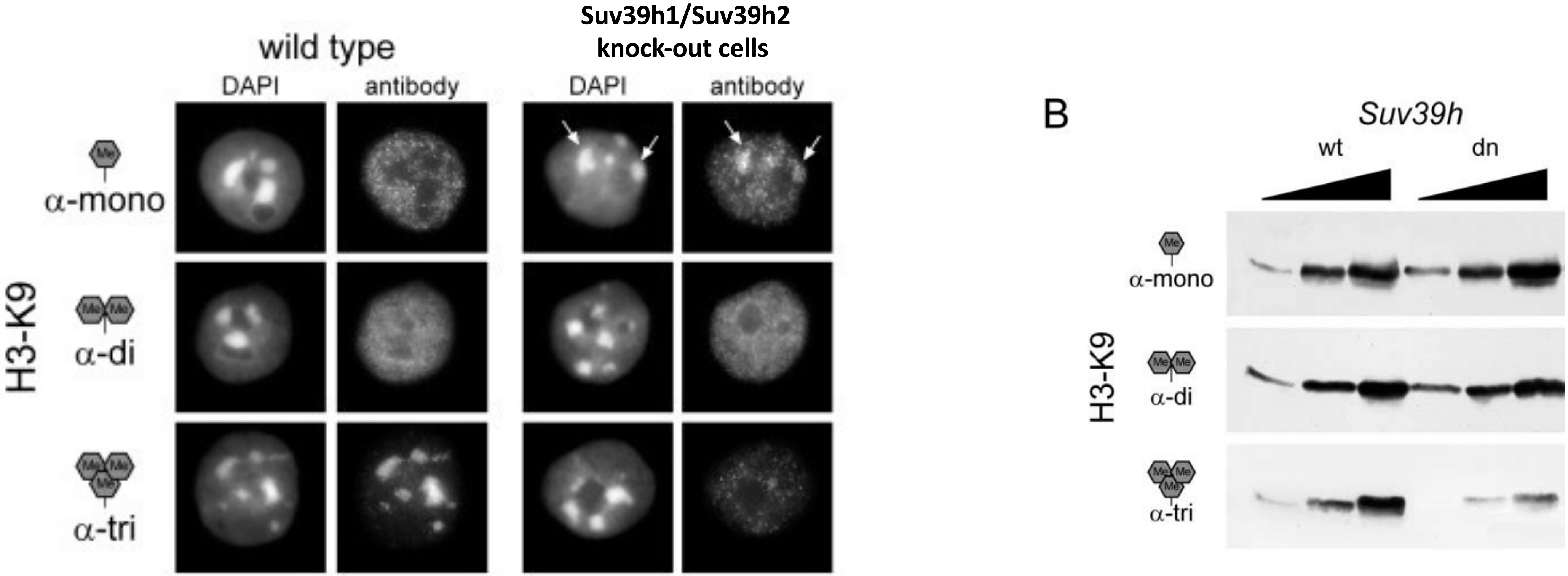
HISTONE LYSINES CAN BE MONO- DI- AND TRI-METHYALTED

lysine methylation



ARE THERE KMTs THAT CREATE SPECIFIC METHYLATION LEVELS (mono-methylation, di-methylation, tri-methylation)?

SUBSTRATE SPECIFICITY OF HISTOME METHYL TRANSFERASES: AN EXAMPLE: THE HKMT SUV39H1



Suv39dn cells

H3K9me1: increased and pattern similar to wt H3K9me3 (chromocenter)
H3K9me2: similar to wt
H3K9me3: strongly reduced; lost at chromocenters

Suv39dn cells

H3K9me1: increased compared to wt
H3K9me2: similar to wt
H3K9me3: strongly reduced

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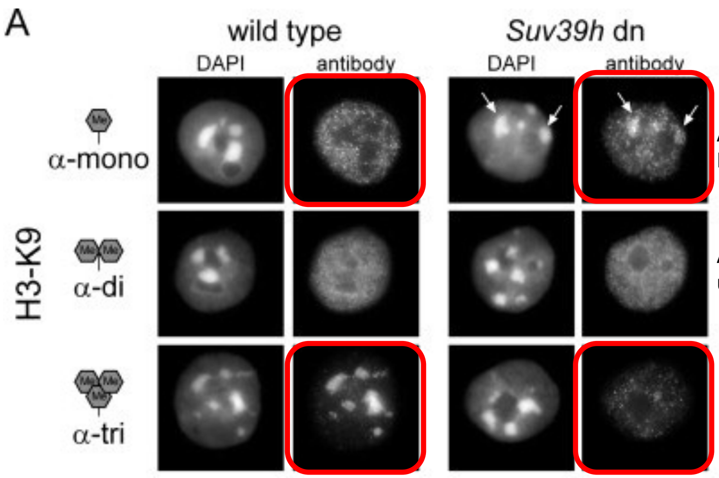
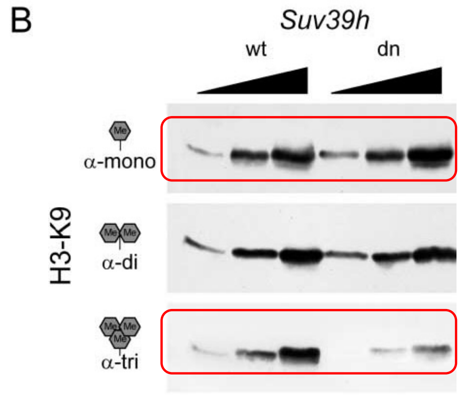


Attention:
Increased of of H3K9me1

Attention:
unchanged H3K9me2

Loss of H3K9me3

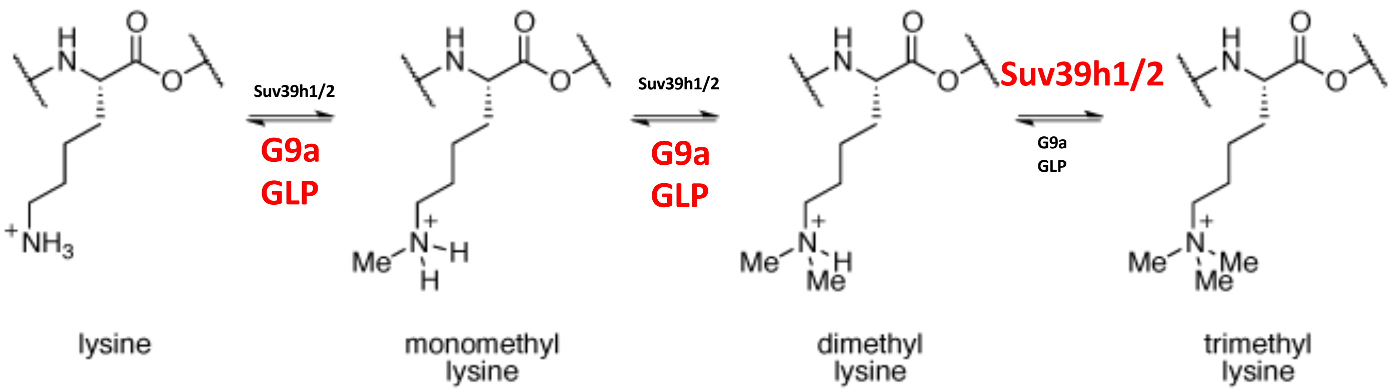


The H3K9 specific KMTs G9a and GLP are the major H3K9me1 and H3K9me2 methyltransferases

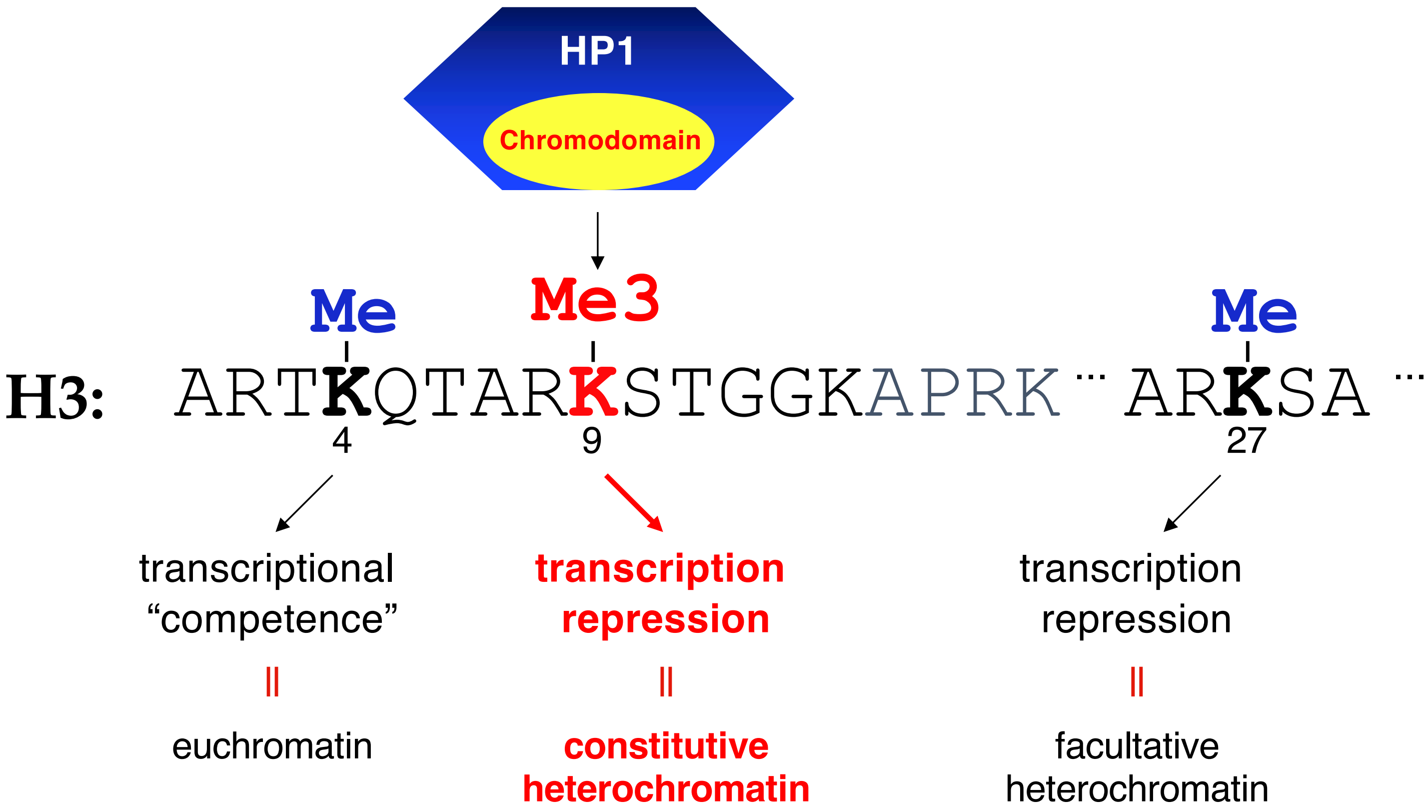
The H3K9 specific KMTases Suv39h1 and Suv39h2 are the major H3K9me3 methyltransferases

Suv39h1 and Suv39h1 work best on H3K9me2

H3K9 methylation



EPIGENTIC READERS
AN EXAMPLE: H3K9me3 and HP1



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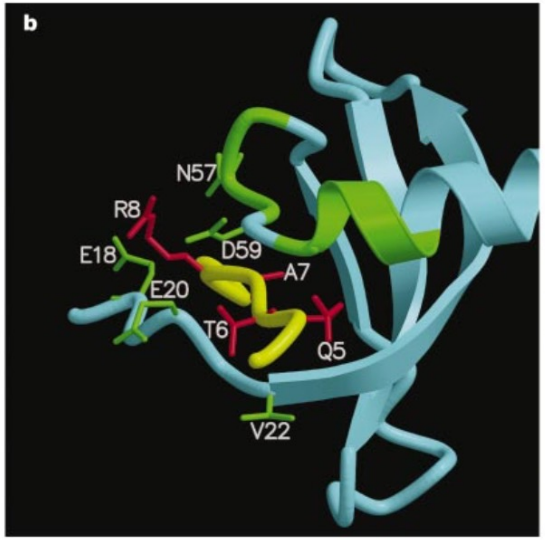
	Tandem BRCT	H2AXS139ph
Unmodified histone	ADD	H3un
	PHD	H3un
	WD40	H3un

EPIGENTIC READERS – IN VIVO EVIDENCE
AN EXAMPLE: HP1 has high affinity for H3K9me3

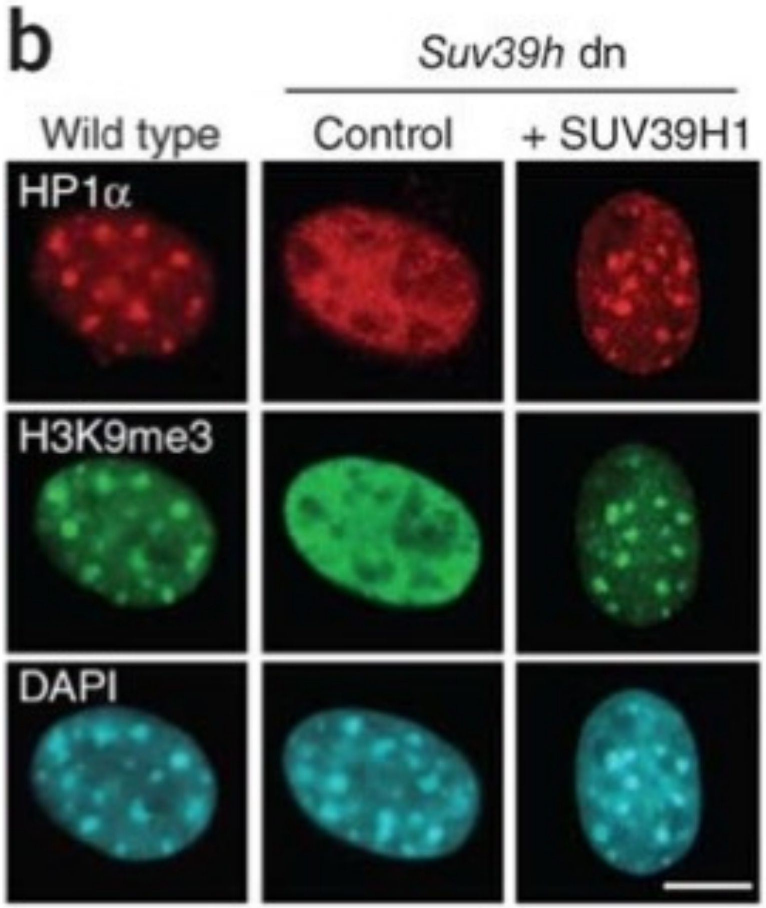


A chromodomain (chromatin organization modifier) is a protein structural domain of about 40-50 amino acid residues commonly found in proteins associated with the remodeling and manipulation of chromatin.

The domain is highly conserved among both plants and animals, and is represented in a large number of different proteins in many genomes, such as that of the mouse.

Chromodomain-containing proteins also bind methylated histones and appear in the RNA-induced transcriptional silencing complex.

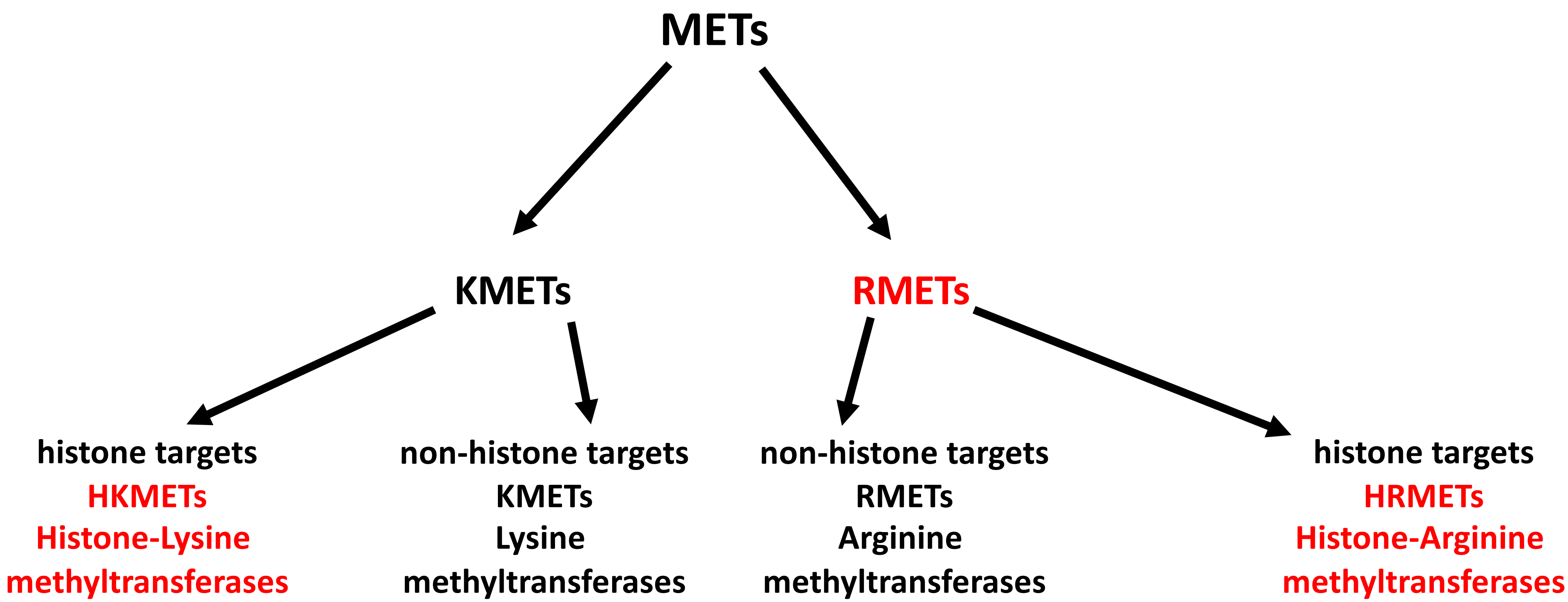
YELLOW: histone tail
RODs: Interacting aminoacids of HP1



Loss of Suv39h1/2: reduced
(2 slides earlier)
and delocalized
(this slide) **H3K9me3**.

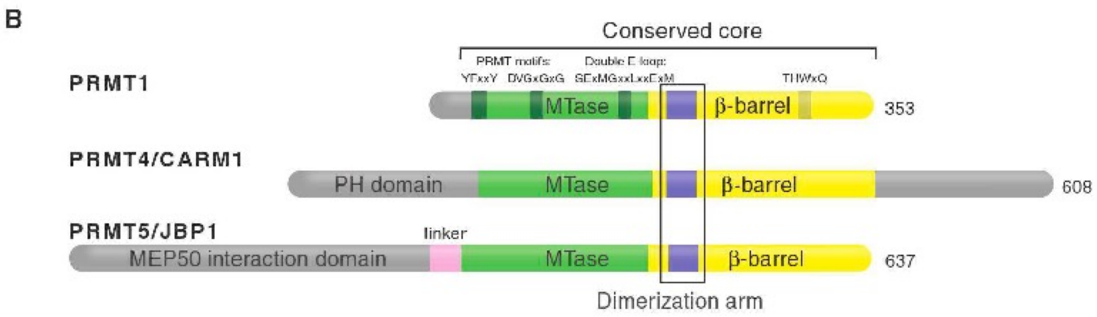
Consequence:
HP1 is also delocalized!!!!
= binding to low-affinity targets

HISTONE LYSINE AND ARGININE METHYL TRANSFERASES (HKMETs and HRMETs))



HISTONE ARGININE METHYL TRANSFERASES (HRMETs)

Family if PRMTs: Protein Arginine (R) methyl-transferases

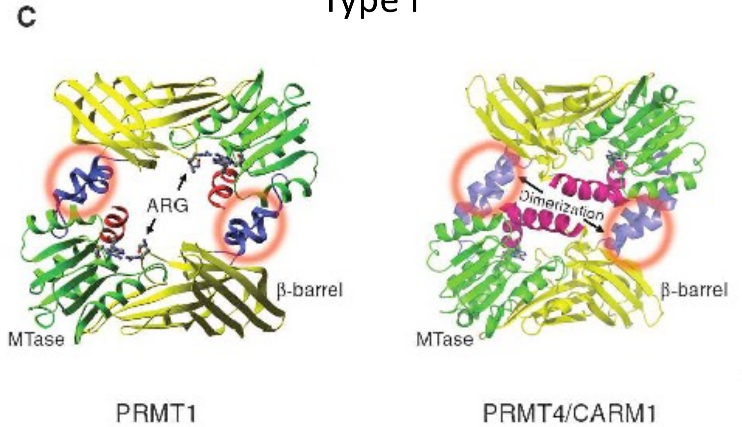


PRMTs have a MTase domain that is
Different from the SET domain!!!

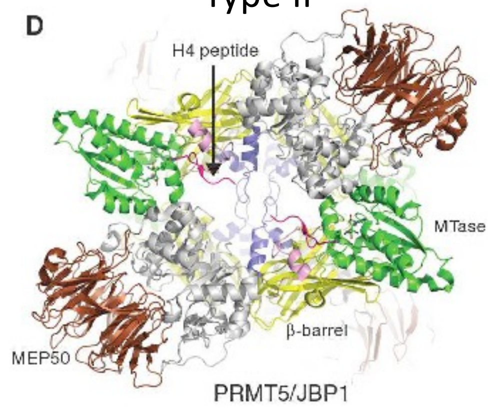
Conserved core:

- MTase domain: catalyzes methylation of Arginine (R)
- Beta barrel domain: Important for dimerization of PRMTs

Type I



Type II



Tetramer: 2 stacked PRMT5 dimers

PRMTs

- Type I PRMTs: need to dimerize to be functional

- Type II PRMTs: form larger complexes – dimers interact to form
tetramers, other proteins can interact

THE BIOCHEMISTRY OF HISTONE ARGININE METHYLATION

Methyl transfer reactions catalyzed by AdoMet-(SAM) dependent PRMTs.

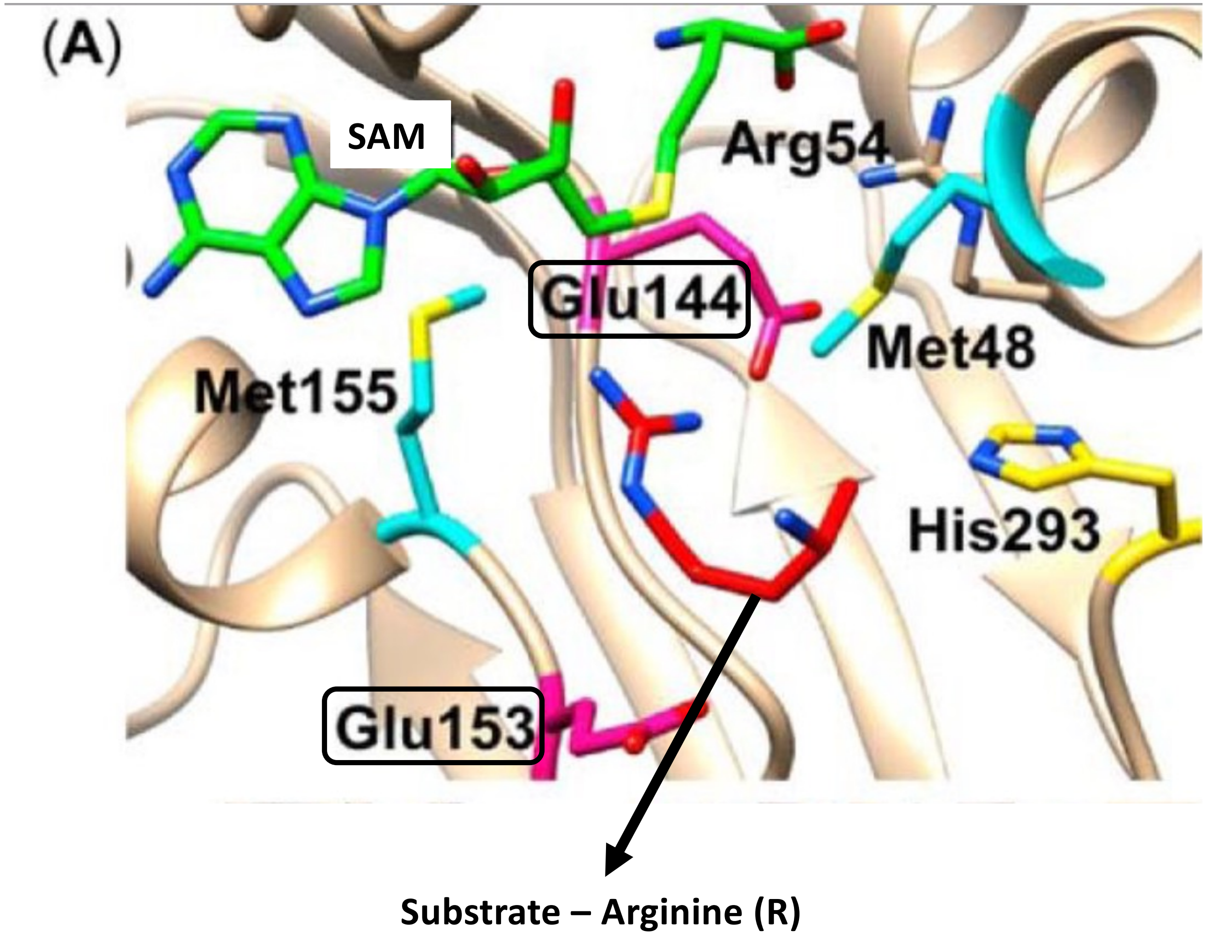
Example: PRMT1

The reacting arginine substrate acts by nucleophil attacking the methyl group present on SAM (S-AdoMet).
The reaction has been proposed to involve 3 key conserved residues in the active site of PRMT1:
Arg-54, Glu-144, and Glu-153.

Arg-54 and **Glu-144** help to properly position the substrates for the nucleophilic attack.

Glu-153 is hypothesized to play a role in increasing the nucleophilicity of the guanidinium moiety of the substrate via enhanced electronic effects.

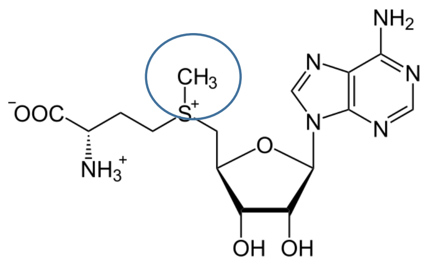
Glu-144 has also been postulated to act as the active site base, abstracting a proton from the reacting arginine.



THE BIOCHEMISTRY OF HISTONE ARGININE METHYLATION

PRMT activity require:

-substrate containing R,
-AdoMet (SAM) as enzymatic cofactor
-PRMT



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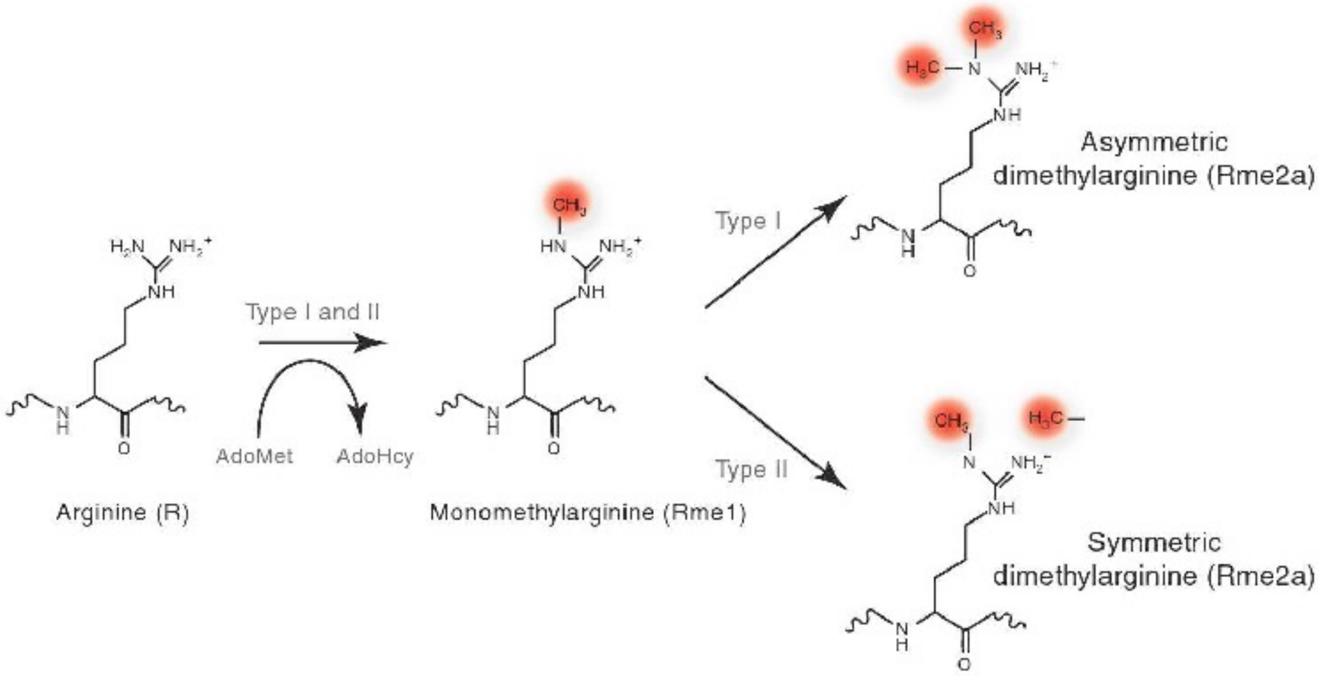
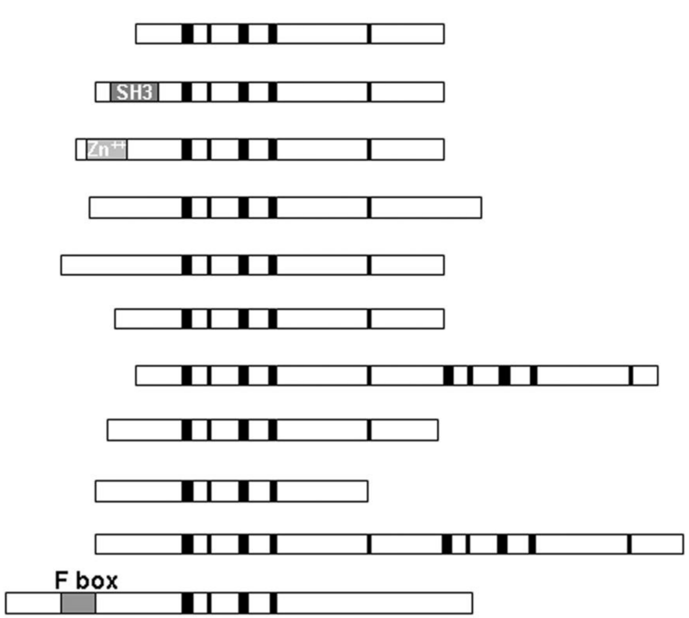


PRMTs CATALYZE MONO and DIMETHYLATION
- Not trimethylation -

PRMT SUBSTRATES AND BIOLOGICAL ACTIVITY

PRMTs can act as activators and repressors of gene expression



PRMTs:	Type	Histone substrate	Biological Function
PRMT1	I	H4R3	NR, chromatin dynamic, transcription activation
PRMT2	?		Coactivator for ER, Cellular proliferation
PRMT3	I		ribosomal biosynthesis
PRMT4	I	H3R2, H3R17 (Rare)	NR, transcription activation, epigenetic reprogram in embryos
PRMT5	II	H4R3; H3R8	Stem cell function, transcription repression, repressive chromatin
PRMT6	I	H3R2	Repressive chromatin, supression of H3K4 methylation
PRMT7	II	H2A, H4R3	Potentiating DNMT3 binding, regulation of imprinting genes
PRMT8	I	H4?	?
PRMT9 Isoform 4	II	H4, H2A	?
PRMT10	?		?
PRMT11	?		?

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→→→

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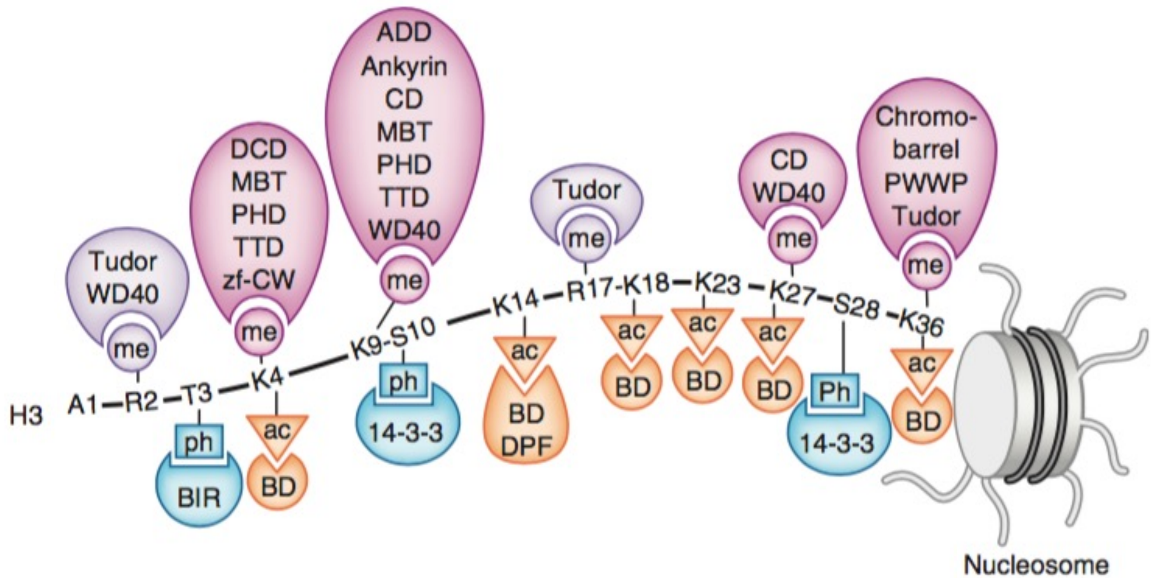
Protein domains that bind to histone modifications



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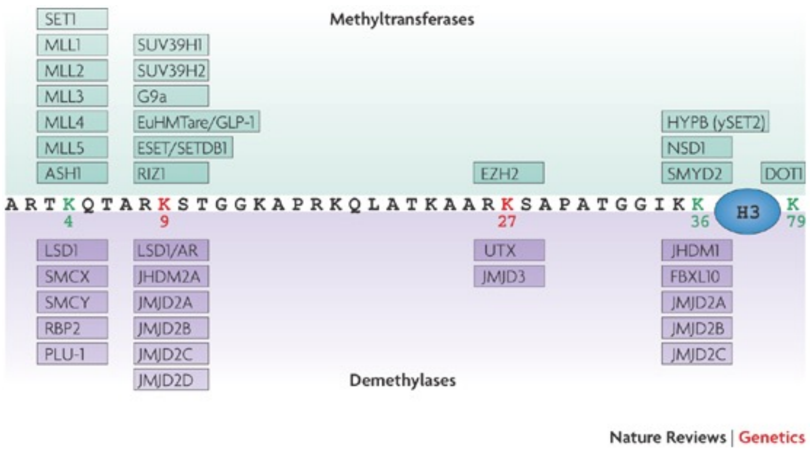
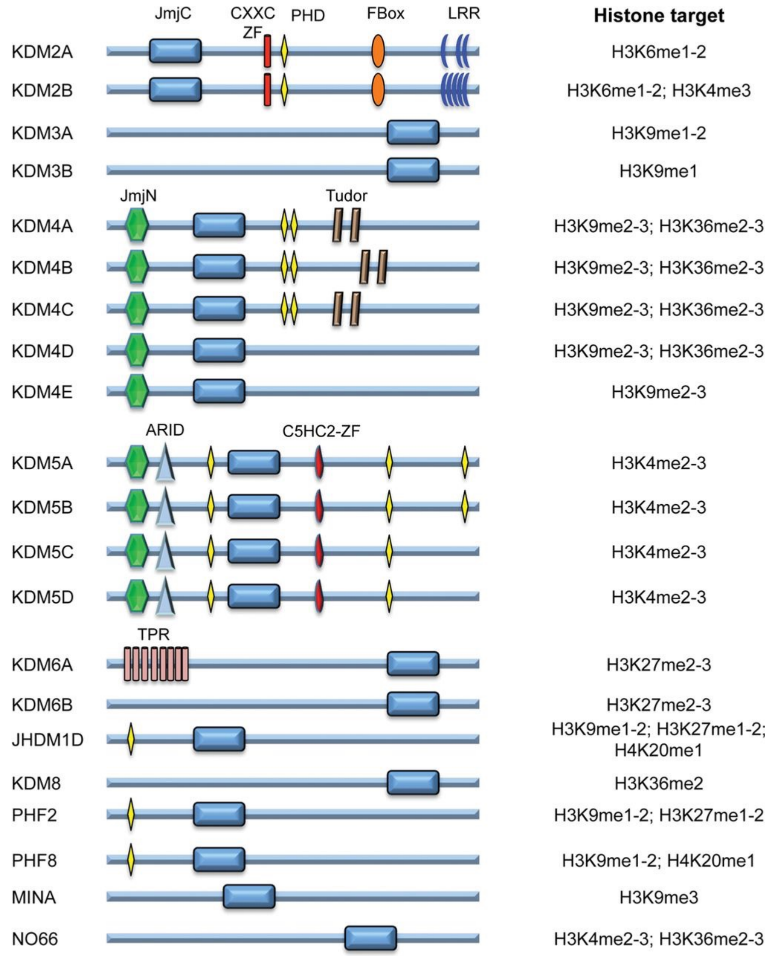
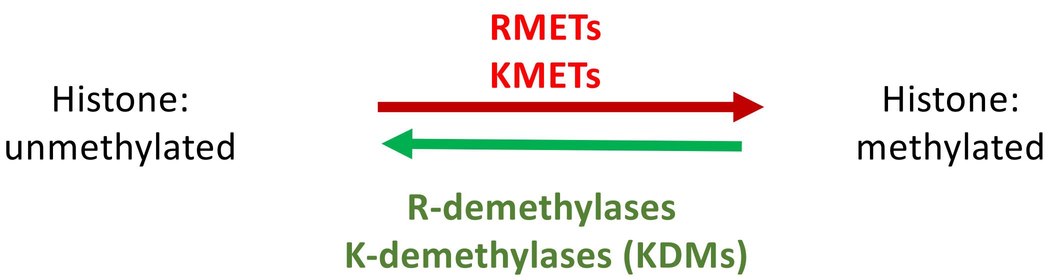
LYSINE AND ARGININE METHYLATION IN HISTONES IS REVERSIBLE



The Jumonji N (JmjN) and Jumonji C (JmjC) domains are two non-adjacent domains which have been identified in the jumonji family of transcription factors. Although it was originally suggested that the JmjN and JmjC domains always co-occur and might form a single functional unit within the folded protein, the JmjC domain was latter found without the JmjN domain in organisms from bacteria to human. The JmjC domain is the best studied domain that mediated histone demethylation - is conserved from yeast to human

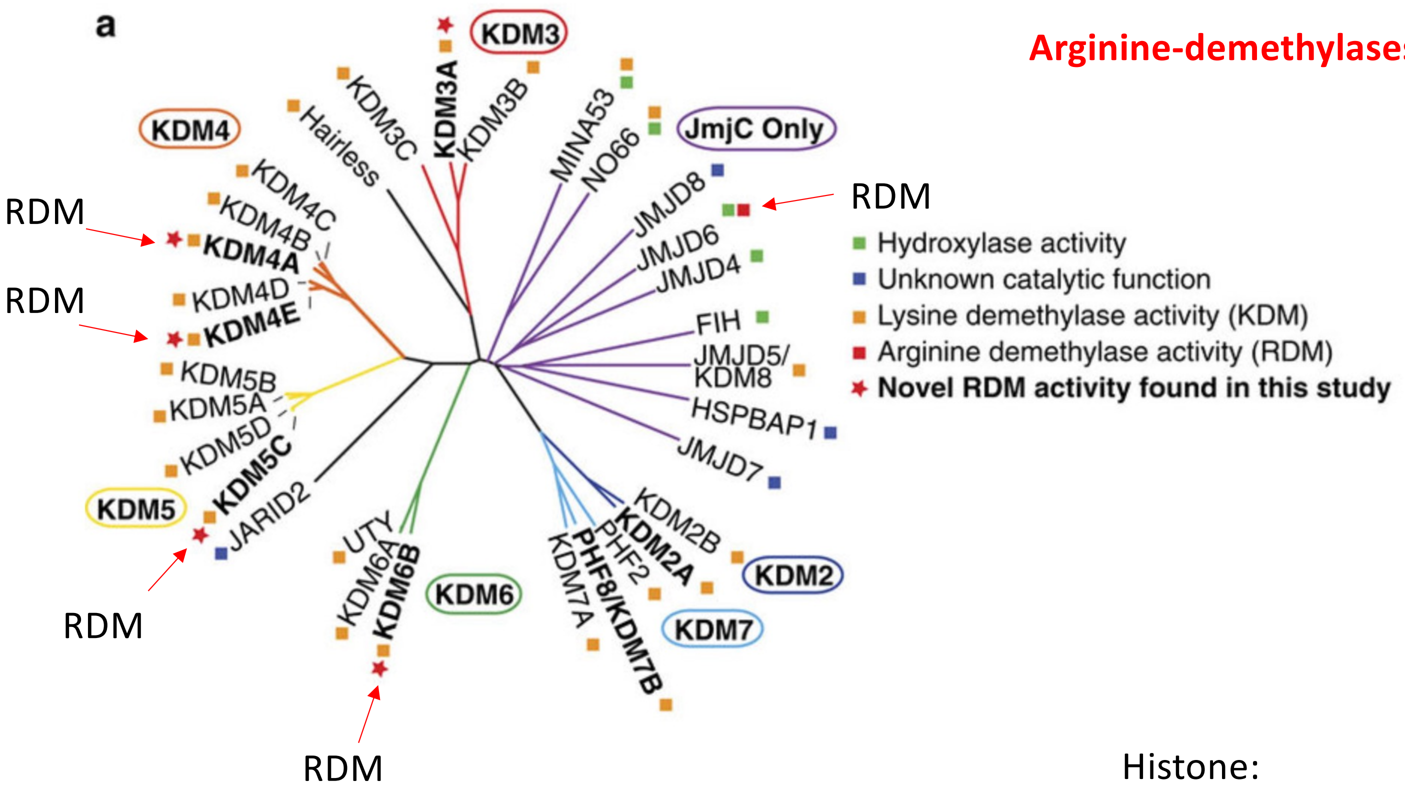


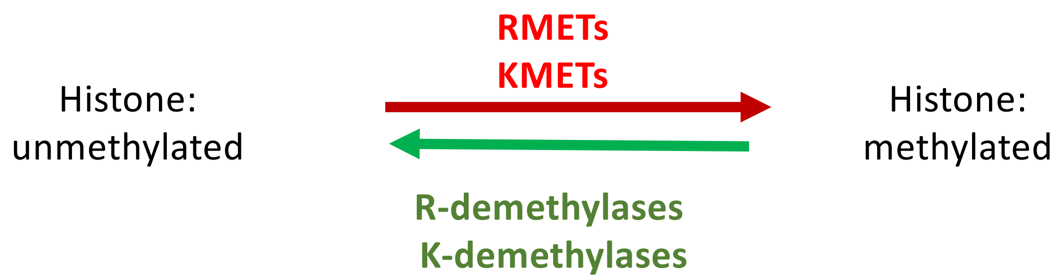
1. LSD1 (KDM1A; Lysine demethylase 1A): demethylation by oxidation
2. Big family of Jumonji domain containing proteins: hydroxylation



LYSINE AND ARGININE METHYLATION IN HISTONES IS REVERSIBLE

Arginine-demethylases are less well studied





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ARTICLE

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Arginine demethylation is catalysed by a subset of JmjC histone lysine demethylases

Louise J. Walport[1], Richard J. Hopkinson[1], Rasheduzzaman Chowdhury[1], Rachel Schiller[1], Wei Ge[1], Akane Kawamura[1,2] & Christopher J. Schofield[1]