Ch 1 – L 3.2

Histone PTMs
Interacting proteins
Histone variants
CpG methylation

3. Post-translational Modifications to Histones (PTM)

Table 1. Different Classes of Modifications Identified on Histones

Chromatin Modifications	Residues Modified	Functions Regulated
Acetylation	**K**-ac	Transcription, Repair, Replication, Condensation
Methylation (lysines)	**K**-me1 **K**-me2 **K**-me3	Transcription, Repair
Methylation (arginines)	**R**-me1 **R**-me2a **R**-me2s	Transcription
Phosphorylation	**S**-ph **T**-ph	Transcription, Repair, Condensation
Ubiquitylation	**K**-ub	Transcription, Repair
Sumoylation	**K**-su	Transcription
ADP ribosylation	**E**-ar	Transcription
Deimination	**R > Cit**	Transcription
Proline Isomerization	**P-cis > P-trans**	Transcription

Overview of different classes of modification identified on histones. The functions that have been associated with each modification are shown. Each modification is discussed in detail in the text under the heading of the function it regulates.

From: Kouzarides, T. (2007), Cell 128:693-705

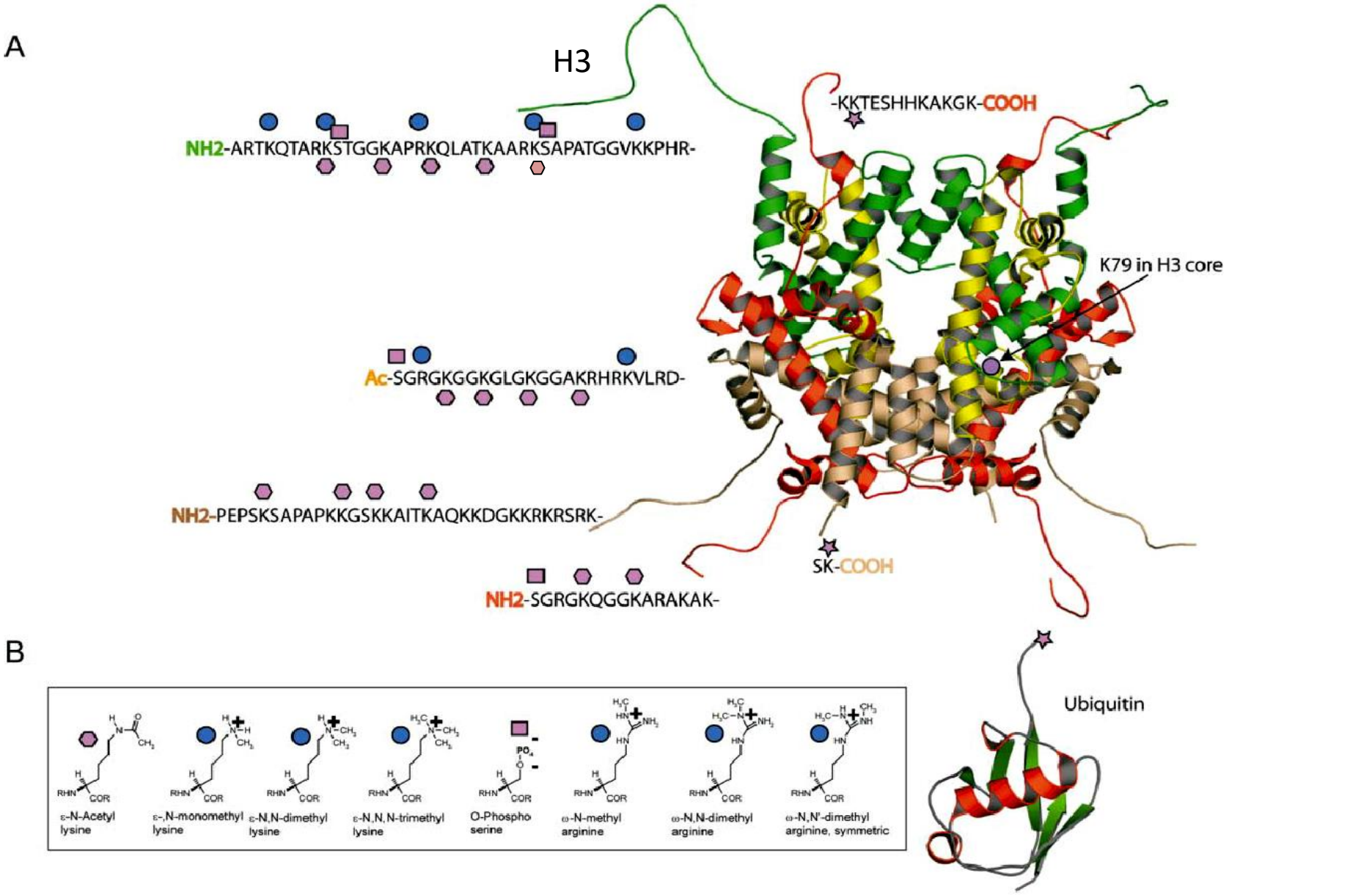
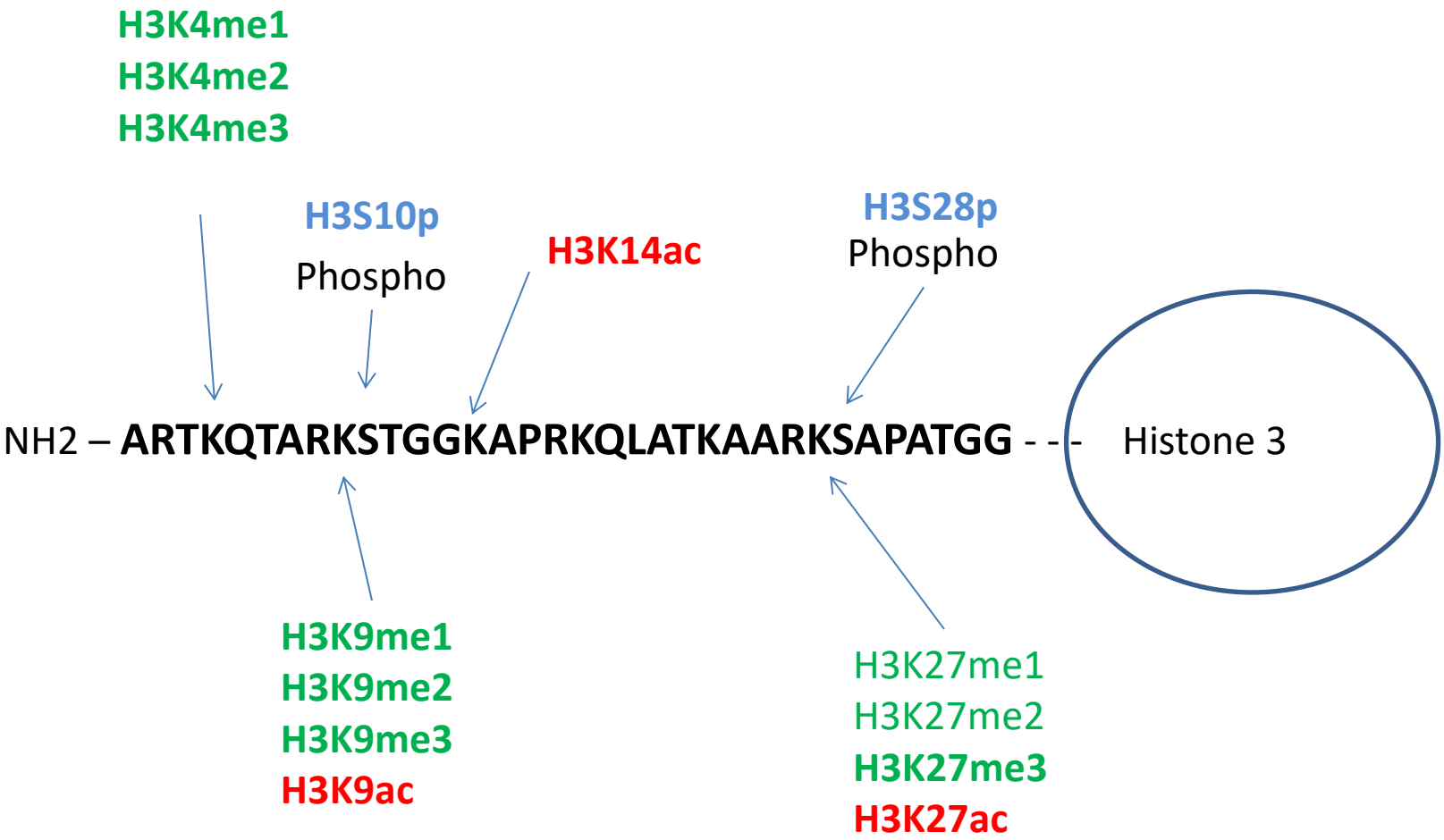


Figure 4. The Types of Posttranslational Modifications Observed on the Core Histones

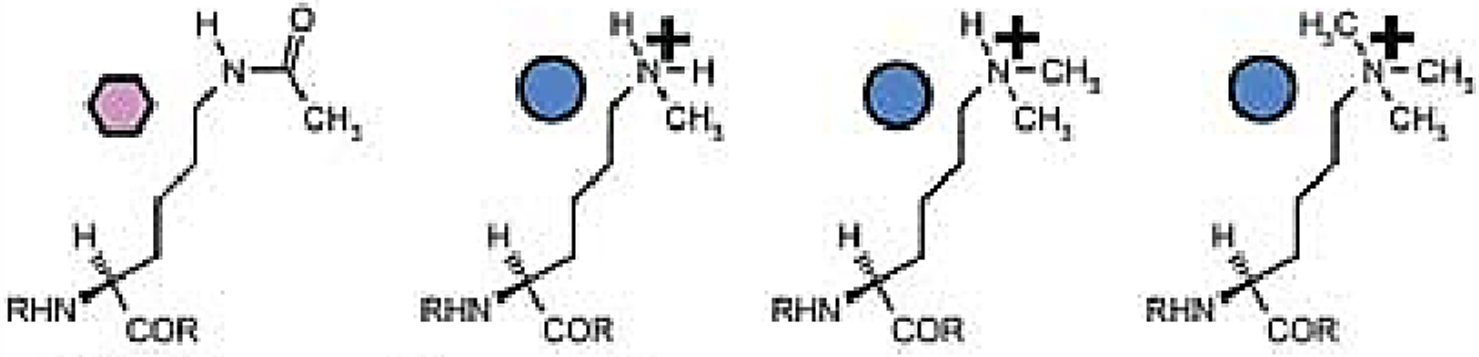
(A) The histone octamer portion of the nucleosome core particle is shown. The sites of modifications on marked. For clarity, the modifications are shown on one copy of each protein.

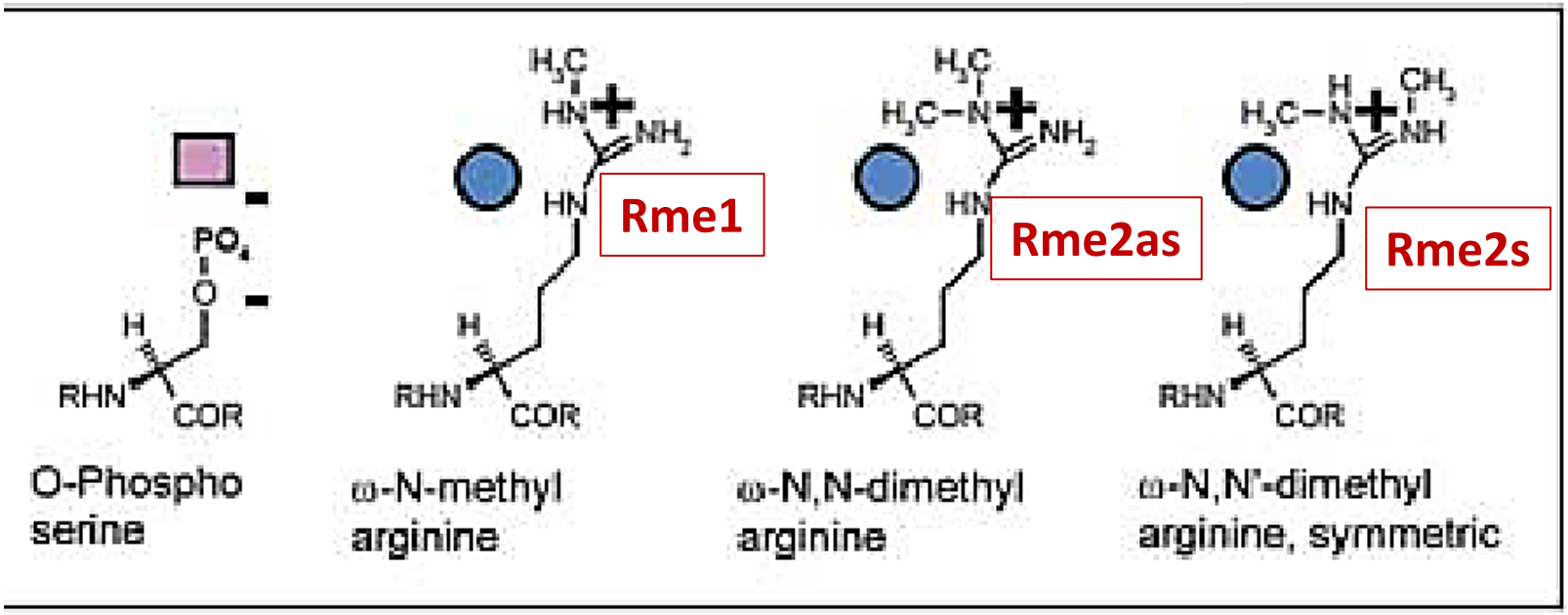
(B) The covalent modifications of the amino acids are shown.

Nomenclature

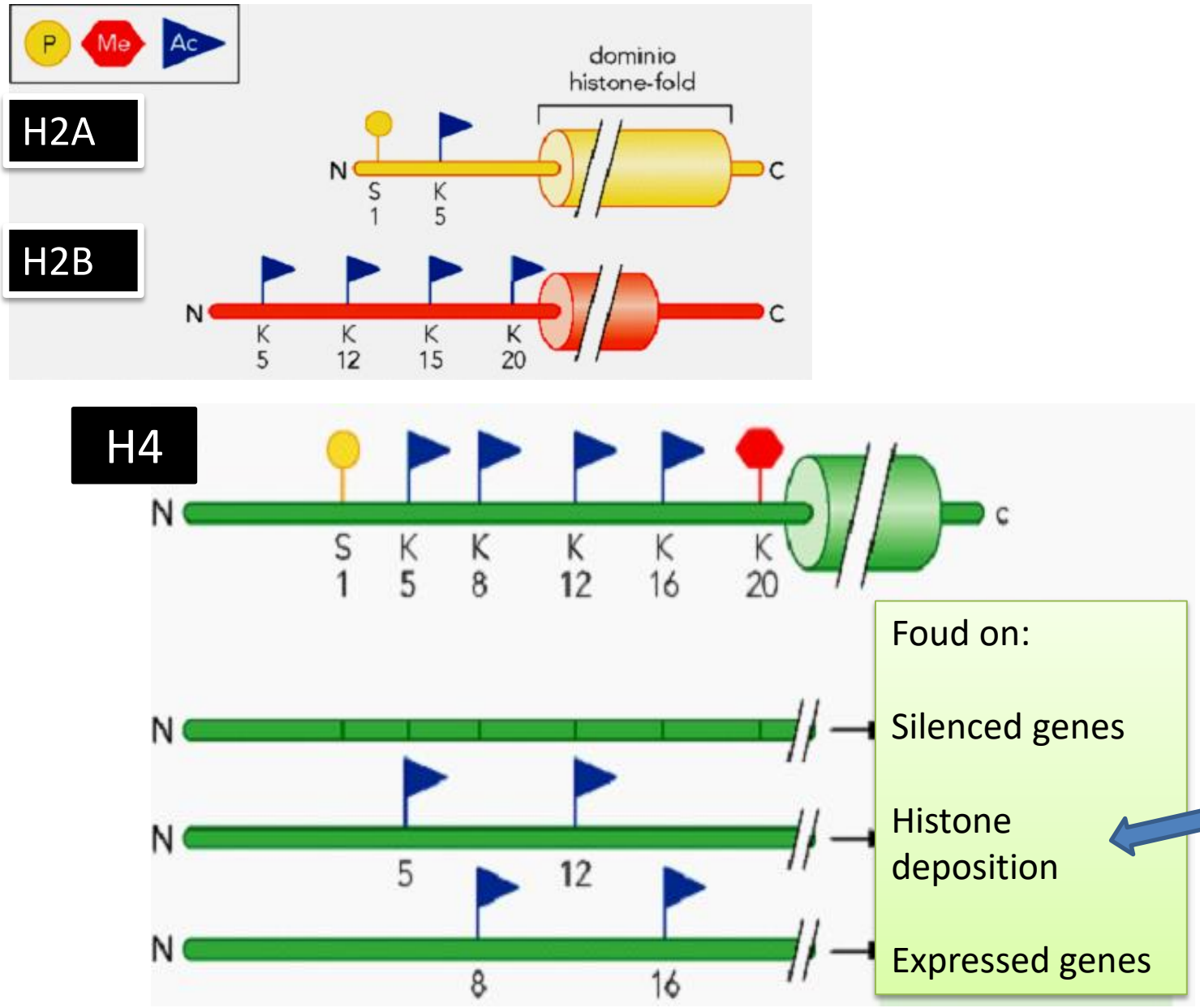


Pay attention to biochemistry !!!



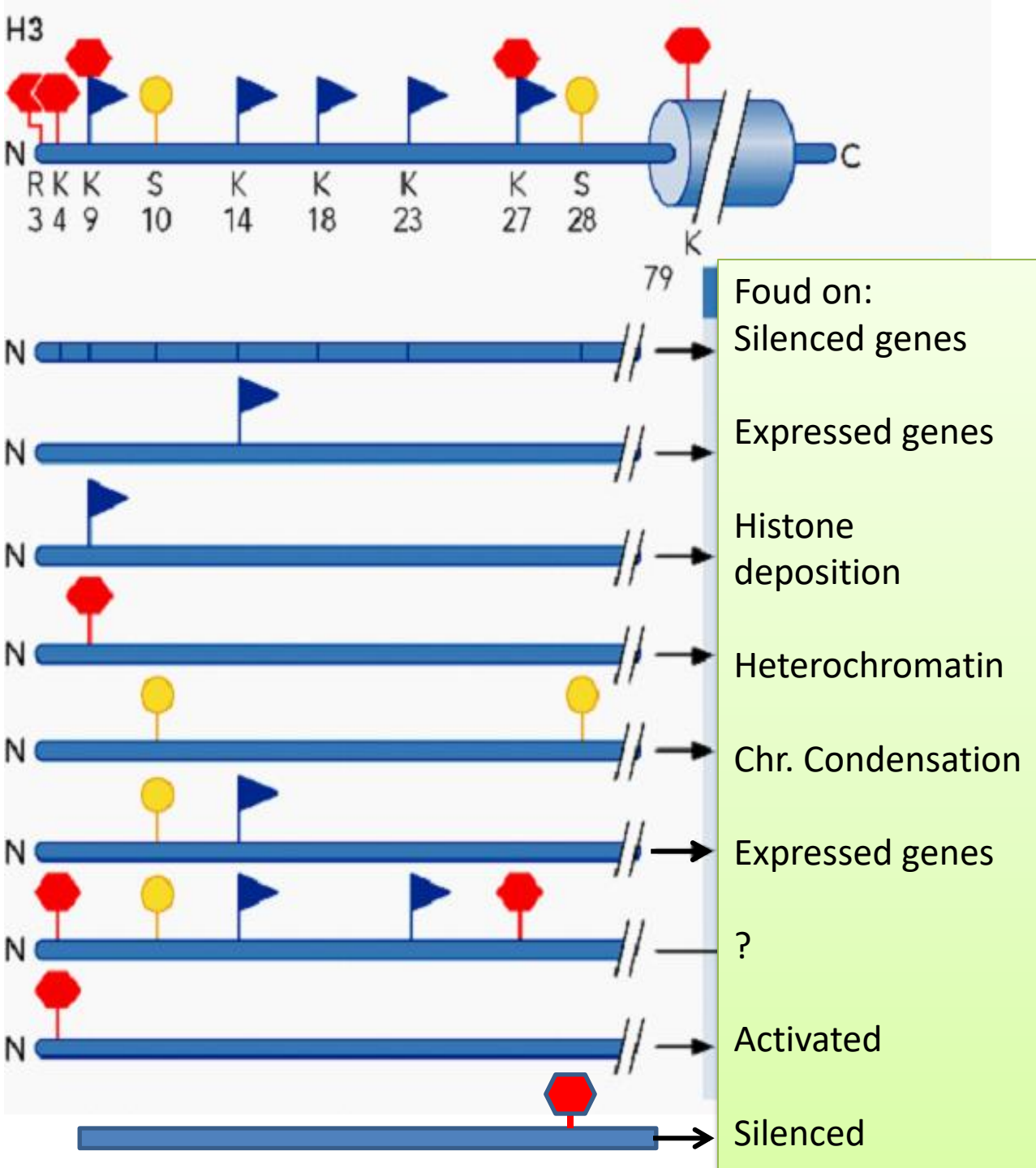


- the histone code

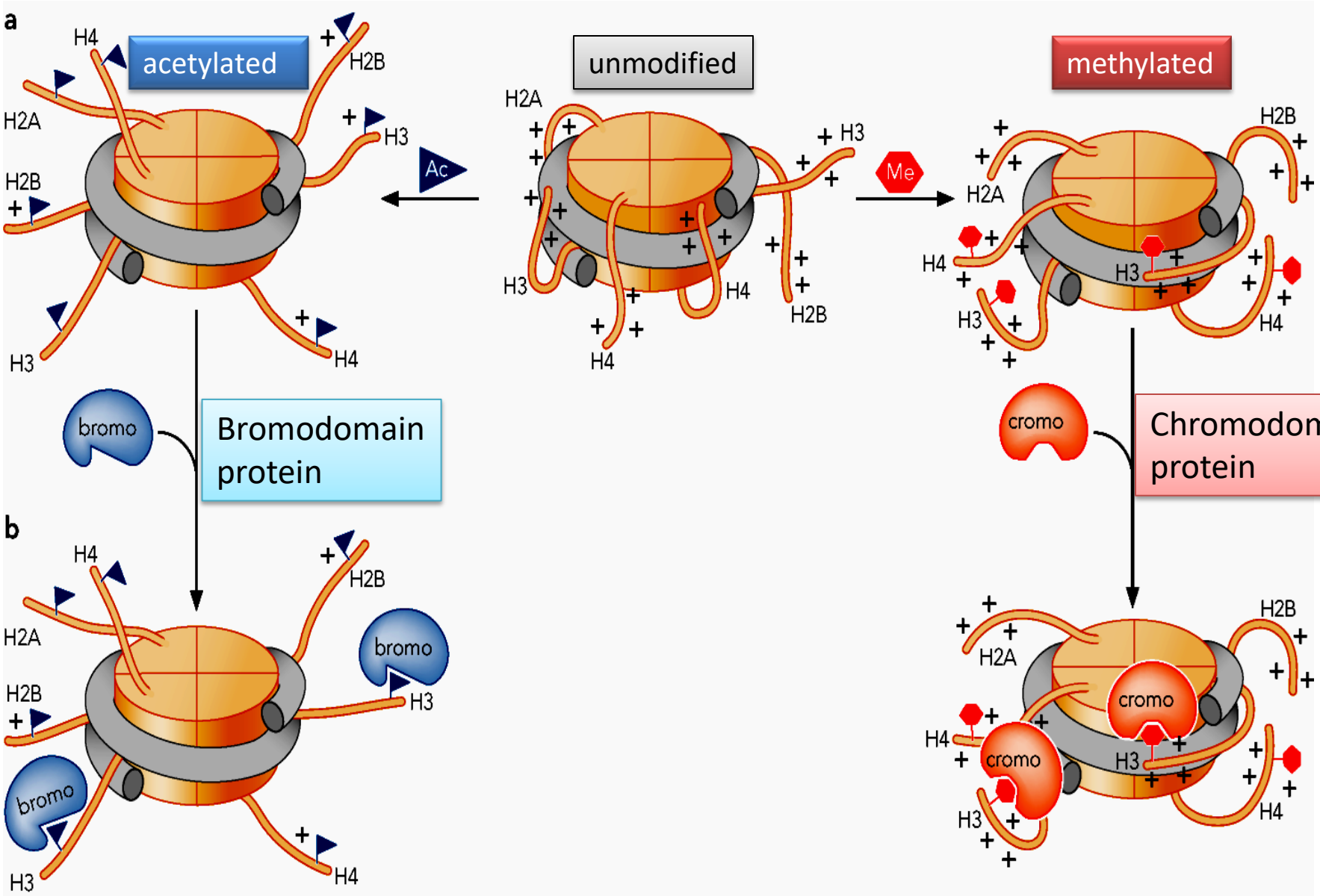


- the histone code

H3



PTMs influence nucleosome stability



This view was derived from single-gene studies.

PTMs are context-specific and quite dynamic

- Some PTMs always go together
- Other PTMs are mutually exclusive (e.g. Kac/Kme)
- Specific PTMs display effects on other PTMs

Question: are the two copies of each histone modified in the same way in the individual nucleosome?

4. Histone-binding proteins and modifying enzymes

Interacting proteins are:

Enzymes introducing modifications	PTM **writers**
Enzymes reversing modifications	PTM **erasers**
Proteins binding to specific PTM	PTM **readers**

histone-modifying enzymes:

HAT- histone acetyltransferases
HDAC – histone deacetylases
HMT – histone methyltransferases
histone demethylases
histone kinases
histone ribosyltrabsferases
ubiquitin-transferases

(ATP-dependent remodelling enzymes)

Modifications are dynamic and reversible

Enzymes that Modify Histones	Residues Modified
Acetyltransferase	
HAT1	H4 (K5, K12)
CBP/P300	H3 (K14, K18) H4 (K5, K8) H2A (K5) H2B (K12, K15)
PCAF/GCN5	H3 (K9, K14, K18)
TIP60	H4 (K5, K8, K12, K16) H3 K14
HB01 (ScESA1, SpMST1)	H4 (K5, K8, K12)
ScSAS3	H3 (K14, K23)
ScSAS2 (SpMST2)	H4 K16
ScRTT109	H3 K56

Other enzymes exist with reduced specificity

From: Kouzarides, T. (2007), Cell 128:693-705

Enzymes that Modify Histones	Residues Modified
Deacetylases	
SirT2 (ScSir2)	H4 K16

histone deacetylases (low specificity)

HDAC: two classes

Class III: NAD-dependent deacetylases (Sir-Sirtuins)

From: Kouzarides, T. (2007), Cell 128:693-705

writers

Lysine Methyltransferase	
SUV39H1	H3K9
SUV39H2	H3K9
G9a	H3K9
ESET/SETDB1	H3K9
EuHMTase/GLP	H3K9
CLL8	H3K9
SpClr4	H3K9
MLL1	H3K4
MLL2	H3K4
MLL3	H3K4
MLL4	H3K4
MLL5	H3K4
SET1A	H3K4
SET1B	H3K4
ASH1	H3K4
Sc/Sp SET1	H3K4
SET2 (Sc/Sp SET2)	H3K36
NSD1	H3K36
SYMD2	H3K36
DOT1	H3K79
Sc/Sp DOT1	H3K79
Pr-SET 7/8	H4K20
SUV4 20H1	H4K20
SUV420H2	H4K20
SpSet 9	H4K20
EZH2	H3K27
RIZ1	H3K9

erasers

Enzymes that Modify Histones	Residues Modified
Lysine Demethylases	
LSD1/BHC110	H3K4
JHDM1a	H3K36
JHDM1b	H3K36
JHDM2a	H3K9
JHDM2b	H3K9
JMJD2A/JHDM3A	H3K9, H3K36
JMJD2B	H3K9
JMJD2C/GASC1	H3K9, H3K36
JMJD2D	H3K9

From: Kouzarides, T. (2007), Cell 128:693-705

writers

Arginine Methlytransferases	
CARM1	H3 (R2, R17, R26)
PRMT4	H4R3
PRMT5	H3R8, H4R3
Serine/Thrionine Kinases	
Haspin	H3T3
MSK1	H3S28
MSK2	H3S28
CKII	H4S1
Mst1	H2BS14
Ubiquitilases	
Bmi/Ring1A	H2AK119
RNF20/RNF40	H2BK120
Proline Isomerases	
ScFPR4	H3P30, H3P38

From: Kouzarides, T. (2007), Cell 128:693-705

readers

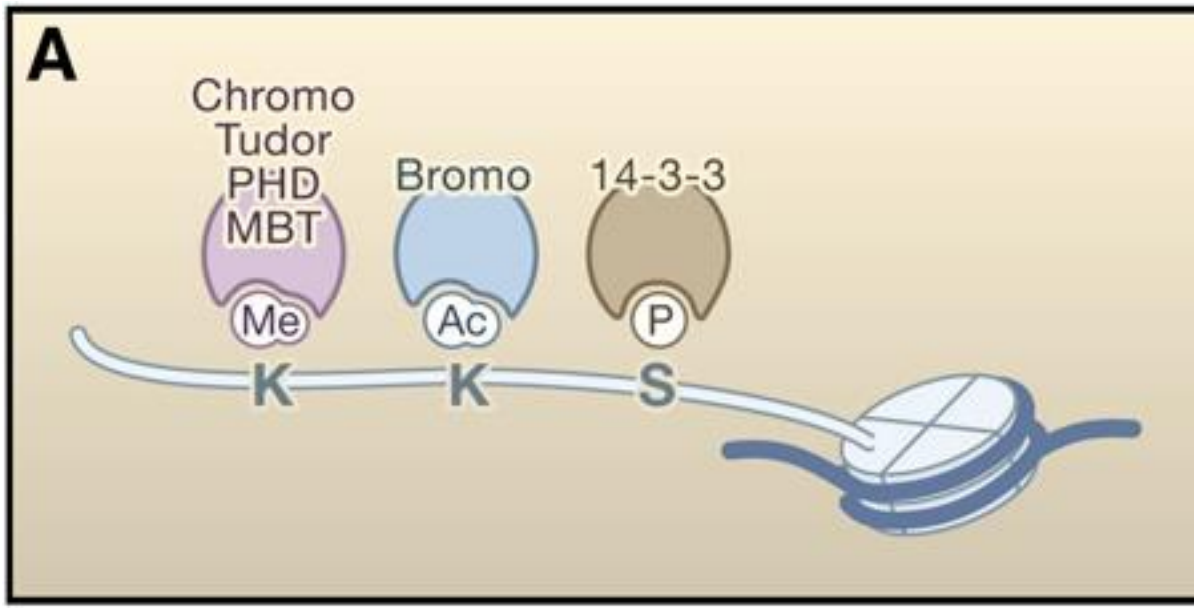


Figure 1. Recruitment of Proteins to Histones
(A) Domains used for the recognition of methylated lysines, acetylated lysines, or phosphorylated serines.

From: Kouzarides, T. (2007), Cell 128:693-705

readers

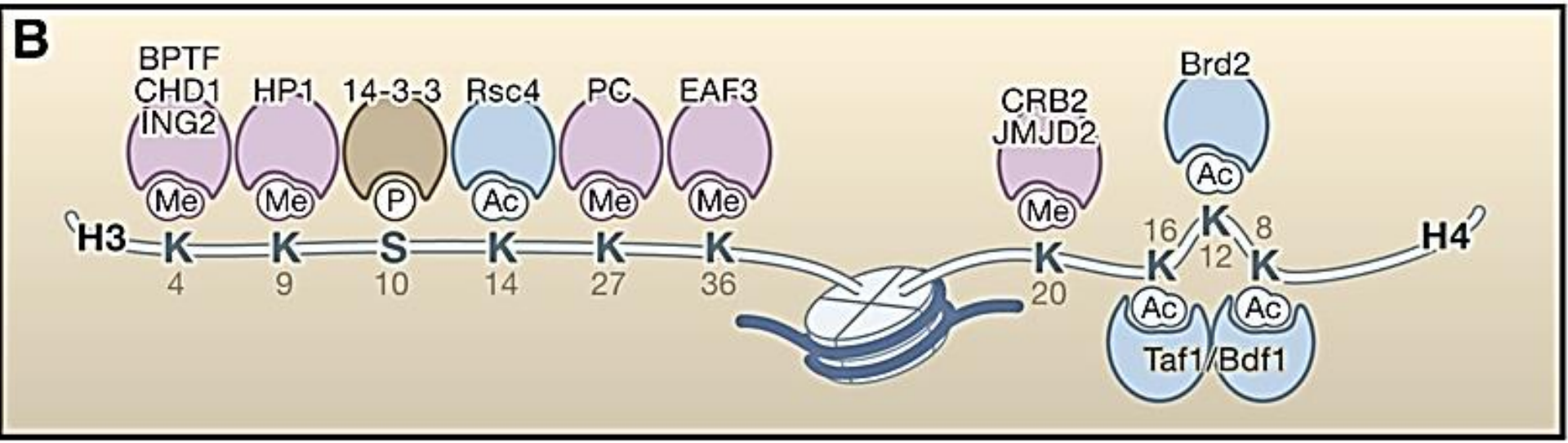


Figure 1. Recruitment of Proteins to Histones
(B) Proteins found that associate preferentially with modified versions of histone H3 and histone H4.

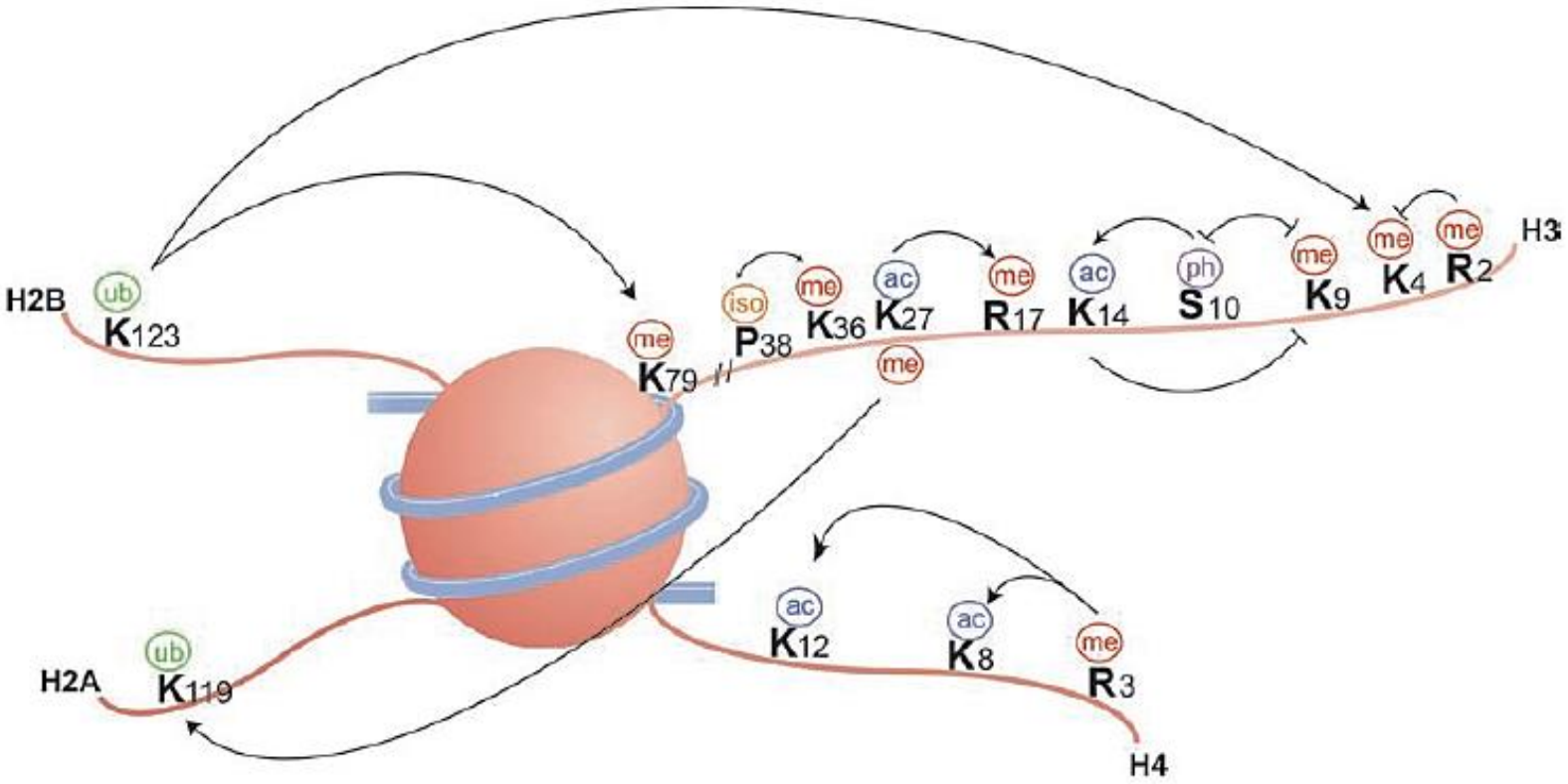
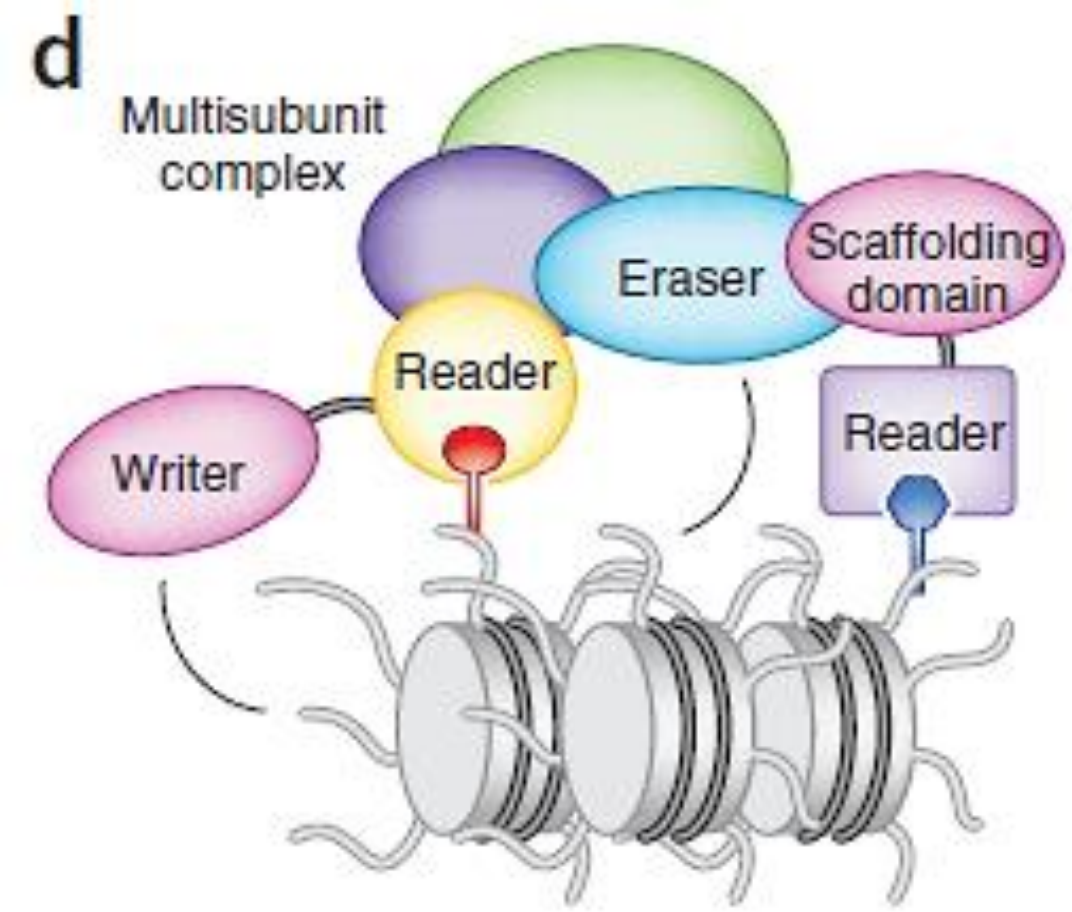


Figure 2 Histone modification cross-talk. Histone modifications can positively or negatively affect other modifications. A positive effect is indicated by an arrowhead and a negative effect is indicated by a flat head (updated from reference [53]).

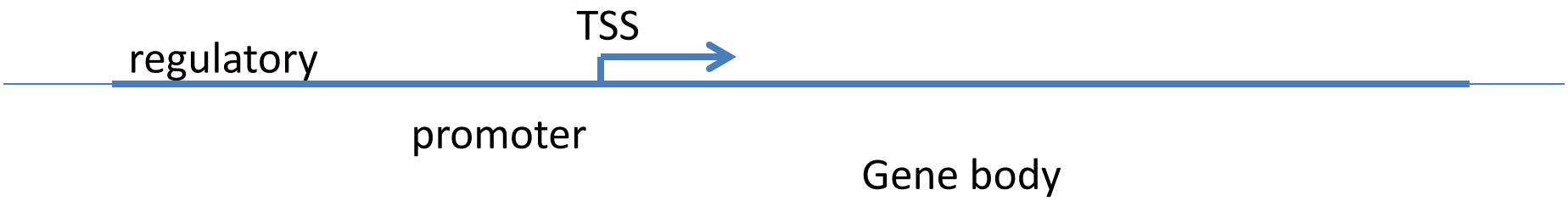
Textbook: Bannister & Kouzarides, 2011

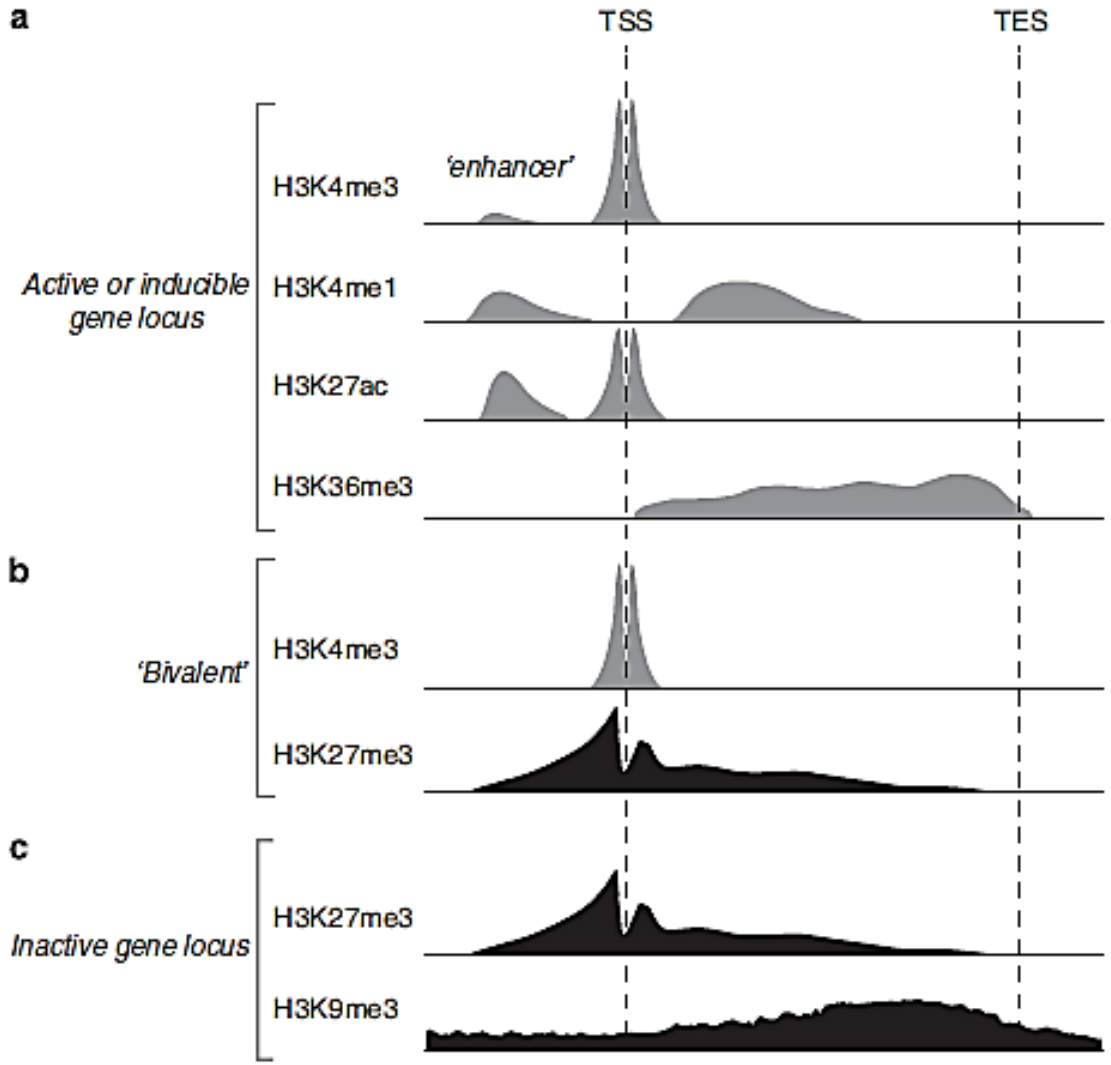
In some cases, the PTM itself guides recruitment of multisubunit complexes, thus explaining «spreading» and maintenance of the chromatic status



PTMs are not only «grossly» distributed to HC/EC

They show finely distinct distributions in functional parts of chromatin, for examples in genes:



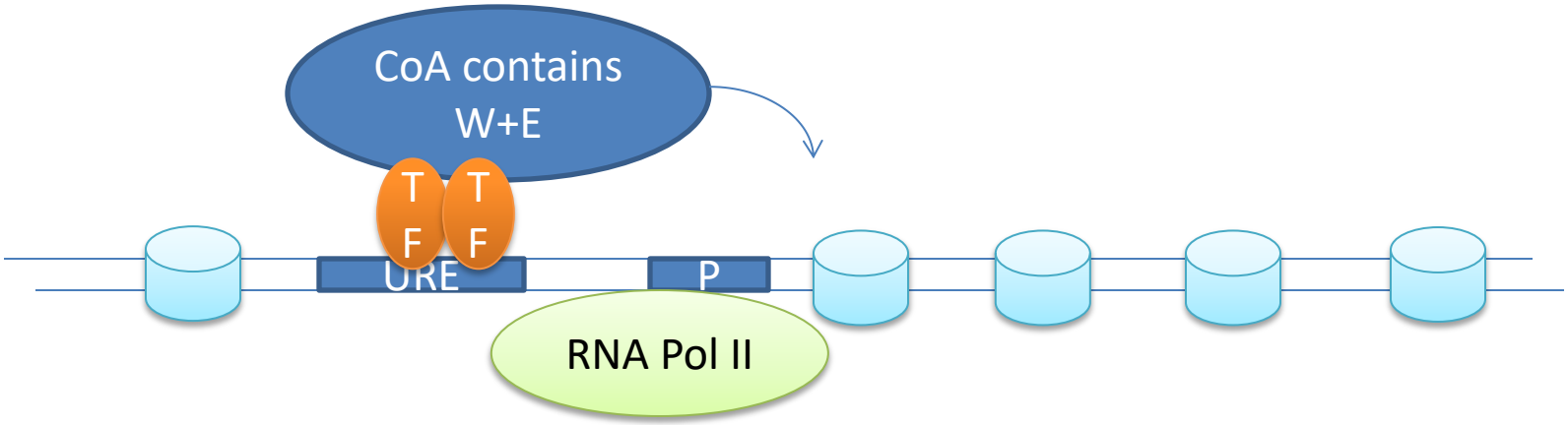


Kimura et al, J Human Genet (2013) 58:439-445

Figure 3 Distribution of histone modifications. Distributions of six modifications with respect to genes are schematically illustrated. TSS, transcription start site; TES, transcription end site. H3K4me3 is enriched around TSSs. H3K4me1 is enriched around enhancers and more downstream. H3K27ac is enriched around active enhancers and TSSs. In undifferentiated stem cells, both H3K4me3 and H3K27me3 (active and inactive marks, respectively) are enriched around TSSs on many genes. H3K27me3 is enriched around inactive TSS in somatic cells. H3K9me3 is broadly distributed on inactive regions. H3K27me3 and H3K9me3 are usually not colocalized. TSSs are generally devoid of nucleosomes.

Causes and effects of PTMs

PTM are introduced in nucleosomes when Transcription Factors bind to regulatory elements through recruitment of Co-activatory complexes



RNA Pol II leads to specific histone methylation

RNA Pol II has a CTD made by repeated peptide.
(52 repeats of the sequence **Tyr-Ser-Pro-Thr-Ser-Pro-Ser**).

S2
Ⓟ in elongating

S5
Ⓟ in initiation

Binds Set2

Binds Set1

Methylates H3K36

H3K36me3 is found in the gene body (active genes)

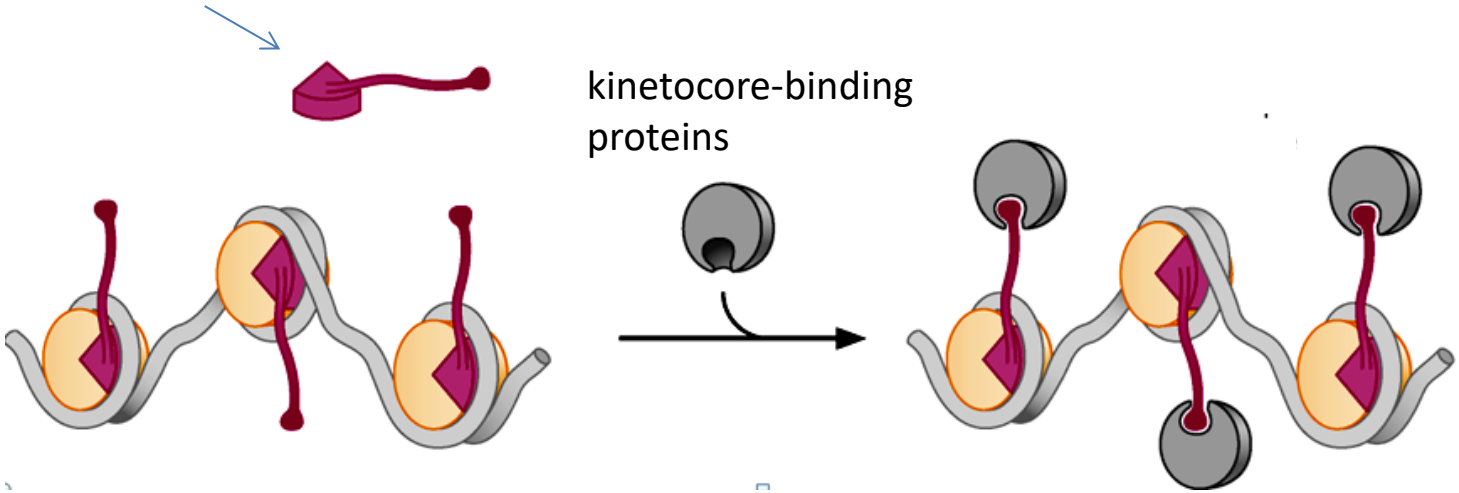
Methylates H3K4

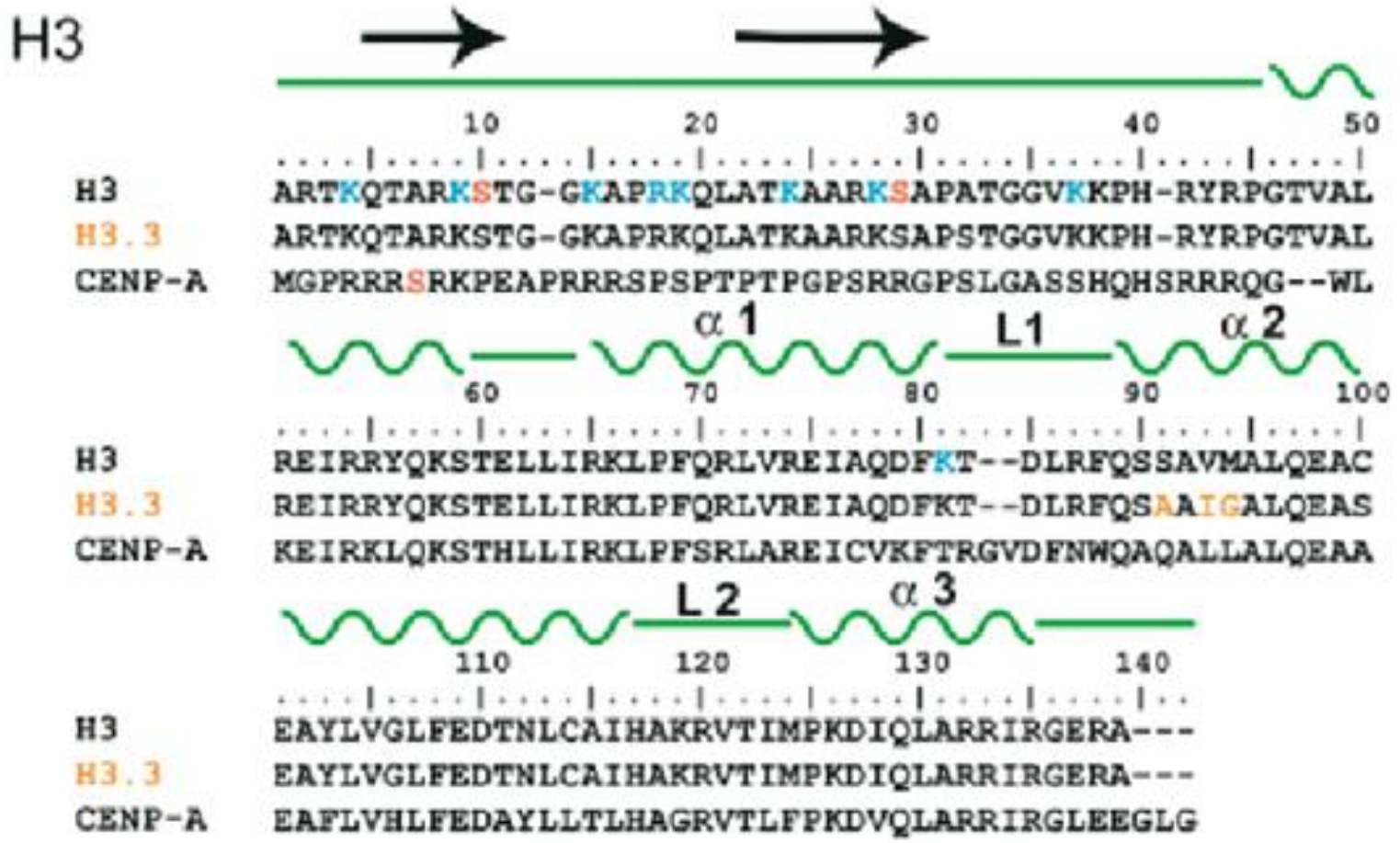
H3K4me3 is found around TSS of active genes.

5. Histone variants

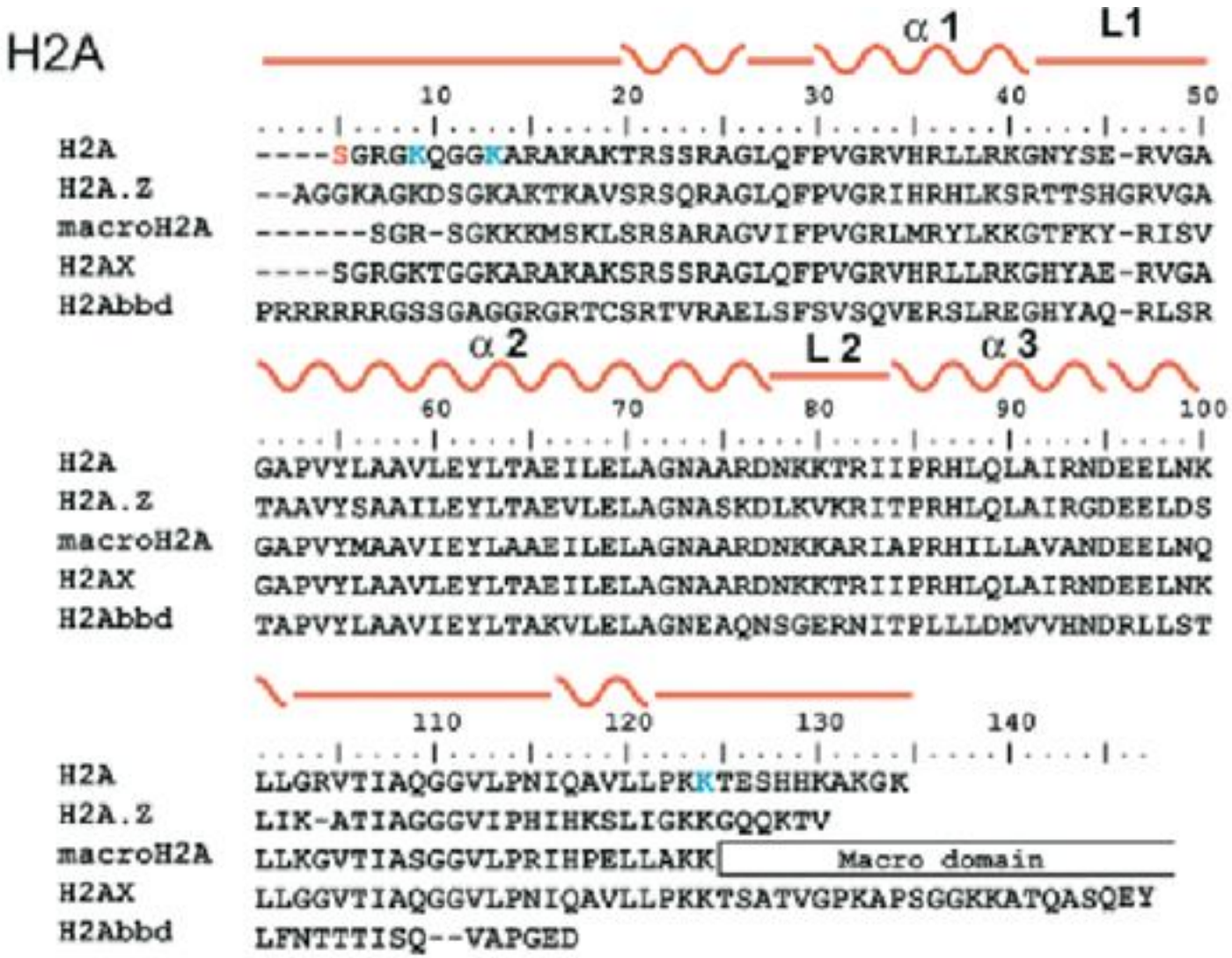
Proteins with extensive similarity with the canonical histones, wich are incorporated in nucleosomes in particular regions with specific functons

Example: CENP-A is a variant of H3 found in centromeres





The sequences of the conserved H3.3 and CENP-A variants. H3.3 differs by only a few residues. The arrows above the H3 N-terminal tail indicate the sites that form strands upon binding to chromodomains



The sequences of the conserved H2A.Z, macroH2A, H2AX, and H2ABbd variants of H2A. The sequence of H2ABbd is most divergent, while others are closely related with some changes in the turn regions connecting the helices.

Info on Histone varaints: best source NCBI

Histone DB 2.0 - https://www.ncbi.nlm.nih.gov/research/HistoneDB2.0/index.fcgi/browse/

Histone variants: if interested, see review by Talbert 2010

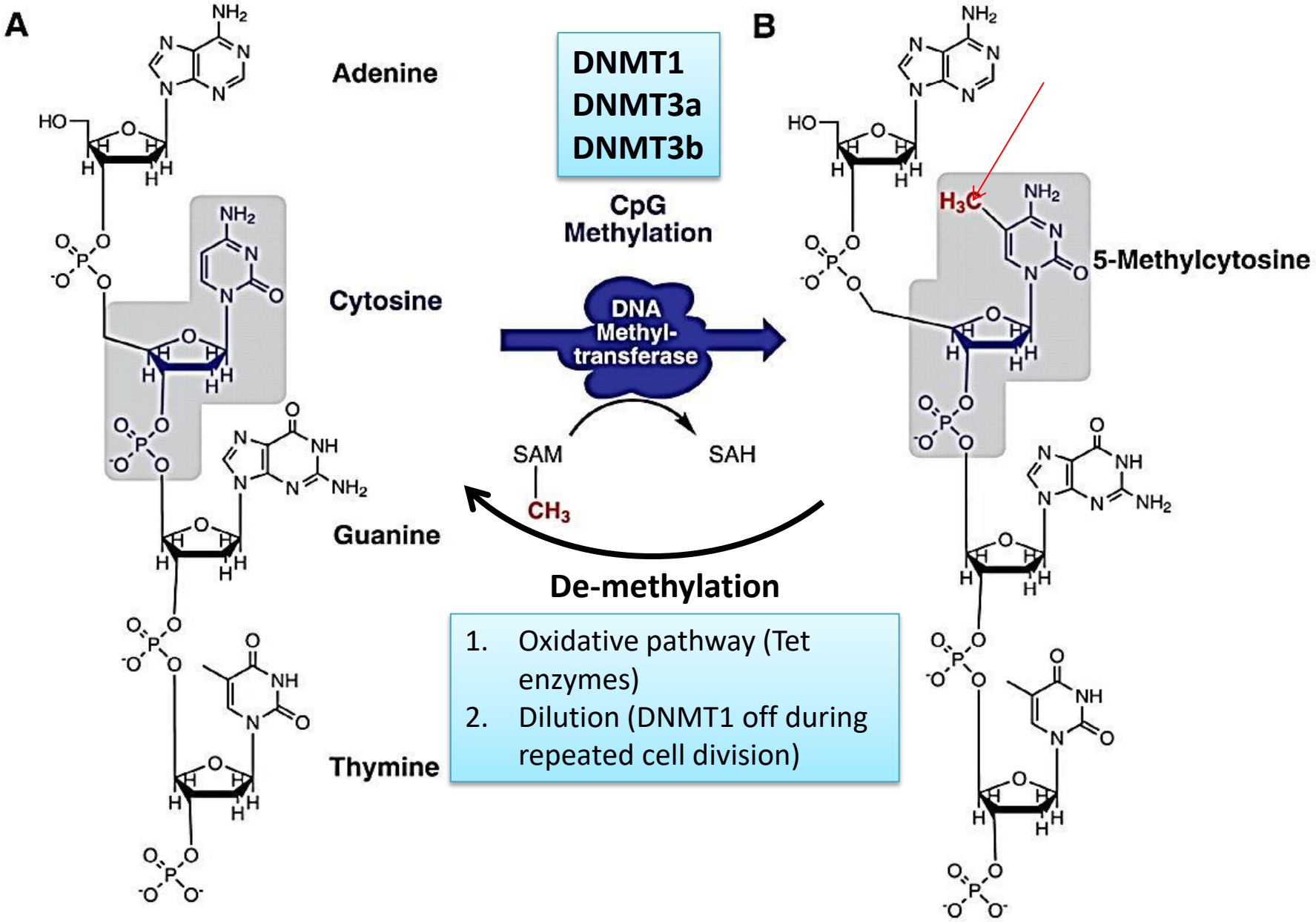
6. DNA methylation

Modification of DNA: 5-methyl-cytosine

a. **Cytonine-5-methylation** (+hydroxymethylation) is the only epigenetic modification concerning the DNA in higher organisms
b. It occurs mostly at **CpG** dinucleotide (also at CpNpG in some cases, e.g. in plants and *see later in next chapter*)
c. Methylation of CpG is observed at regulatory regions of **silenced** genes
d. Hypermethylation is observed through the inactive X chromosome
e. Housekeeping genes (constitutively expressed) show unmethylated CpG islands at promoters

Important: Cytosine methylation is common in Mammals and Plants, but is not used in the same way in S. cerevisiae and C. elegans

CpG methylation



1. Oxidative pathway (Tet enzymes)
2. Dilution (DNMT1 off during repeated cell division)

The methyl binding domain (MBD) protein family

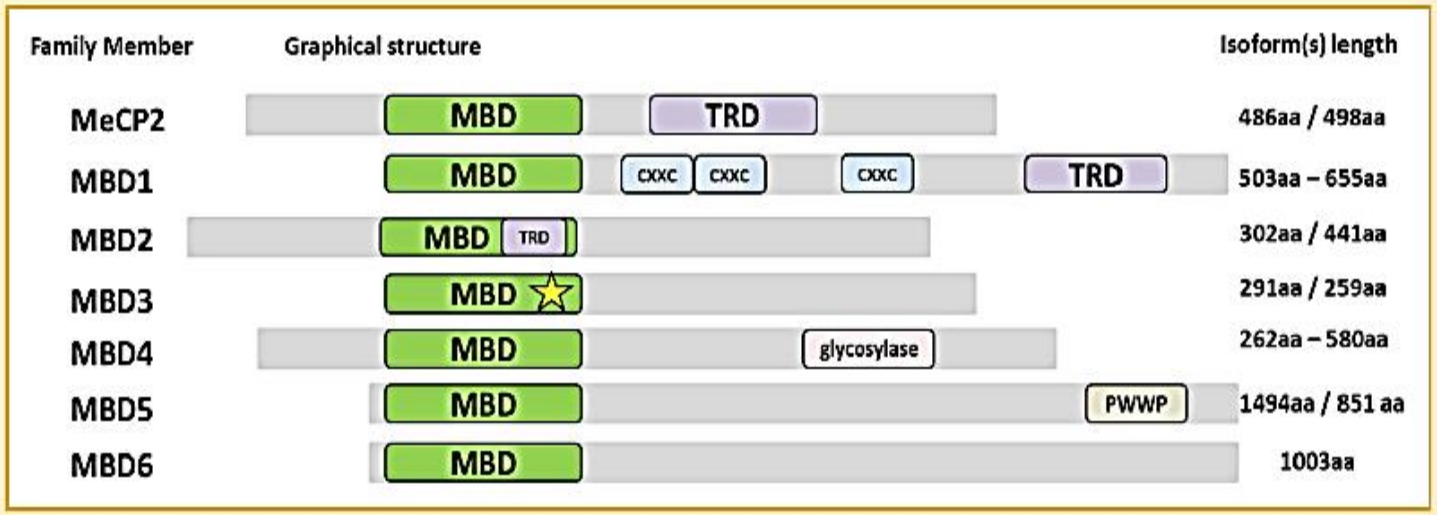
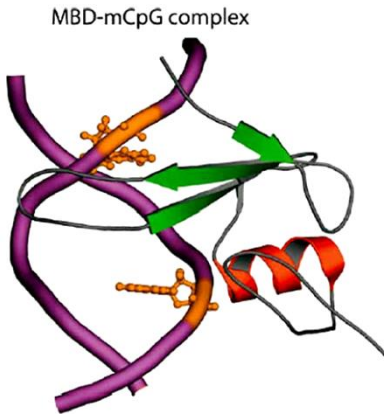


Fig. 1. A scheme of the seven components of the methyl binding domain (MBD) protein family and protein interaction domains. The yellow star represents the two distinct amino acid residues in the MBD domain of MBD3 (MBD, methyl-CpG-binding domain; TRD, transcriptional repression domain; CXXC, Cys-x-x-Cys domain; PWWP, proline-tryptophan-tryptophan-proline domain.

from Oliveira Gigek et al. 2016

MDB4 has a glycosylase domain: important role in **DNA Repair**

MeCP2 – responsible of the Rett syndorme



How to measure CpG methylation

Bisulfite conversion (Methyl-C is not modified , C is converted to U)

1. Enzymatic analysis
2. Cloning alleles and Sanger sequencing

Methylation-sensitive restriction enzymes

1. HpaII*/MspI (CCGG)
2. SmaI*/XmaI (CCCGGG)
3. McrBC – recognize 2 methylated (G/A)pC (50-1000bp apart)

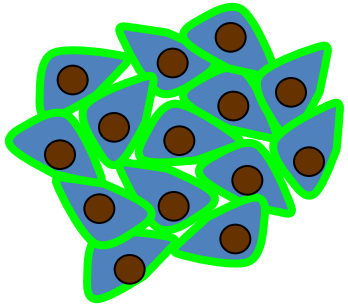
Histone PTMs
chromatin interacting proteins

Chromatin Immunoprecipitation (ChIP)

^{me}CpG-DNA

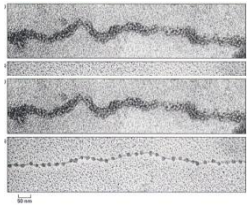
Methyl-cytosine IMPT

Chromatin immunoprecipitation (ChIP)

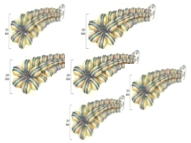


Cross-link with formaldehyde

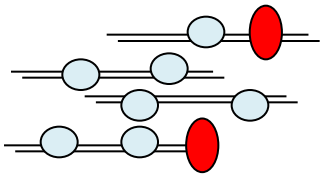
SDS



sonicate

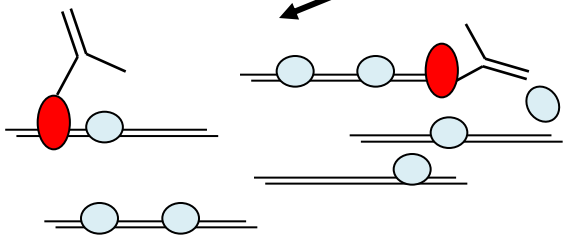


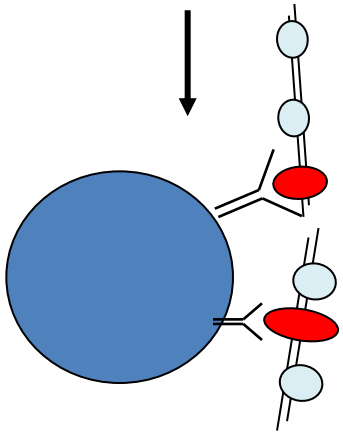
Anti-YFP



Nucleosome carrying your PTM

Other nucleosomes



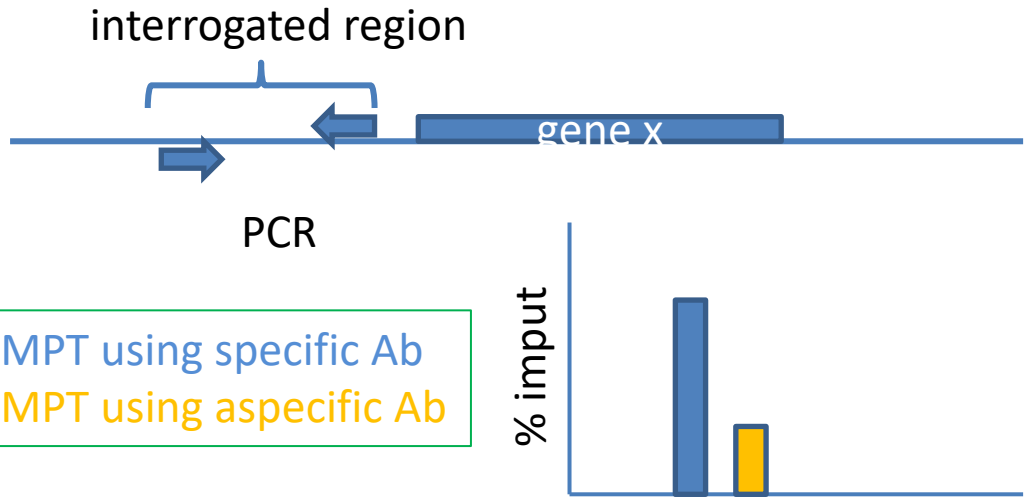


Elute, deproteinize

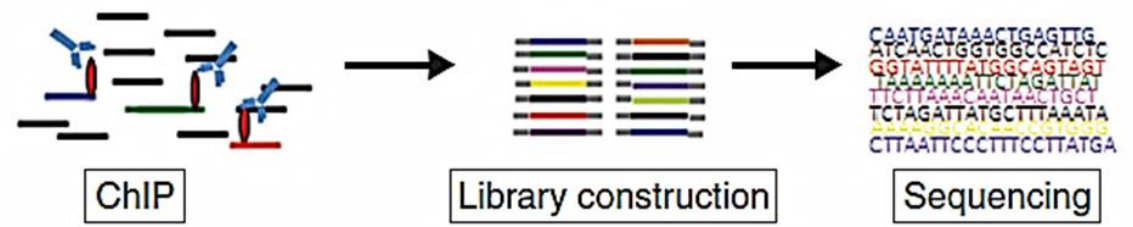
Single locus:
PCR with primers specific for the genome location you wish to interrogate

Genome-wide:
Ligate adapters, PCR-amplify then sequence fragments by NGS

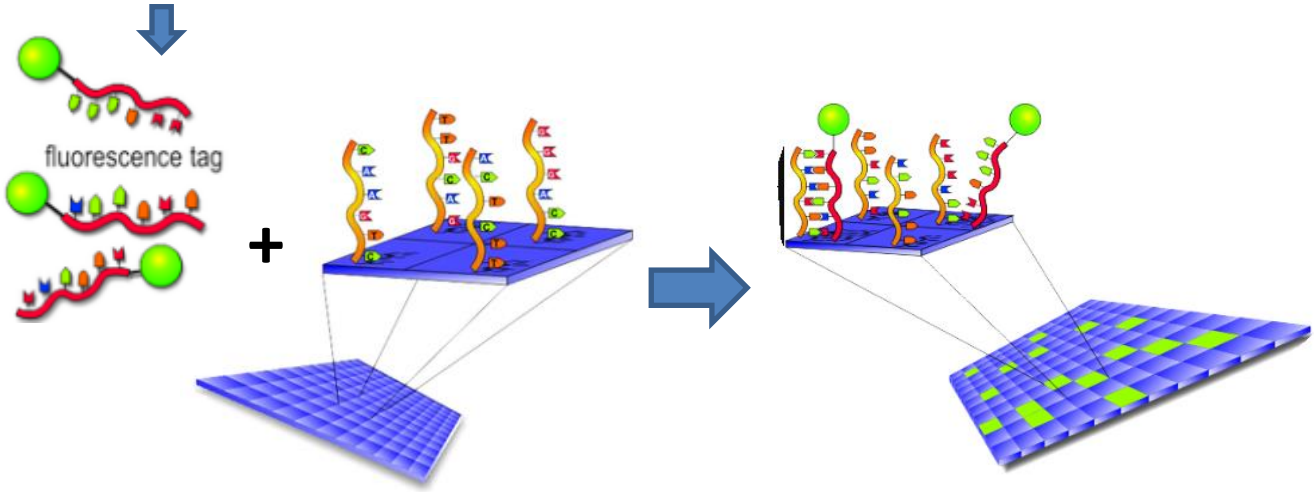
Single locus: **ChIP-qPCR**
PCR with primers specific for the genome location you wish to interrogate



Genome-wide: **ChIP-Seq**
Ligate adapters, PCR-amplify then sequence fragments by NGS



Genome-wide: **ChIP-on-chip**
Label IP fragments, hybridize to genomic microarray

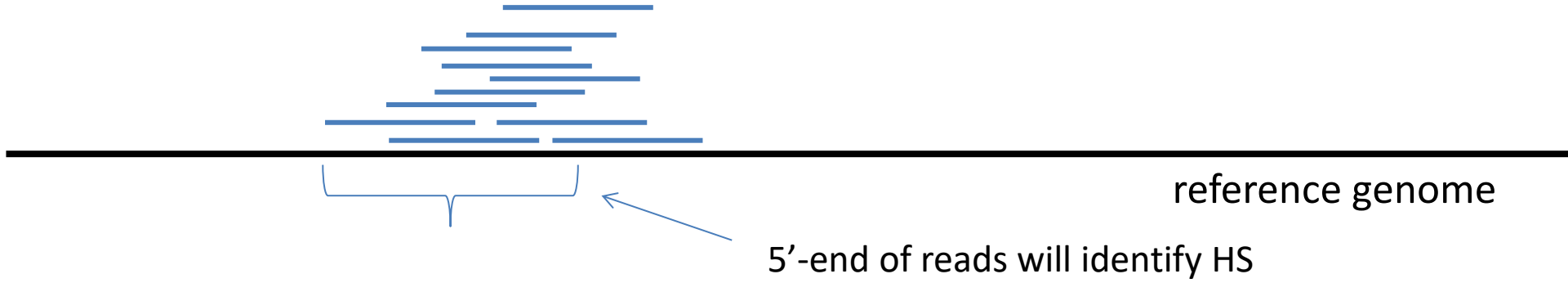


Mapping data.

Results are very different from those obtained from NGS on genomic DNA, which are a «probabilistic» distribution of fragments on the reference genome.

ChIP-Seq

Results of ChIP: fragments cluster to the genome region where the interrogated event is present:



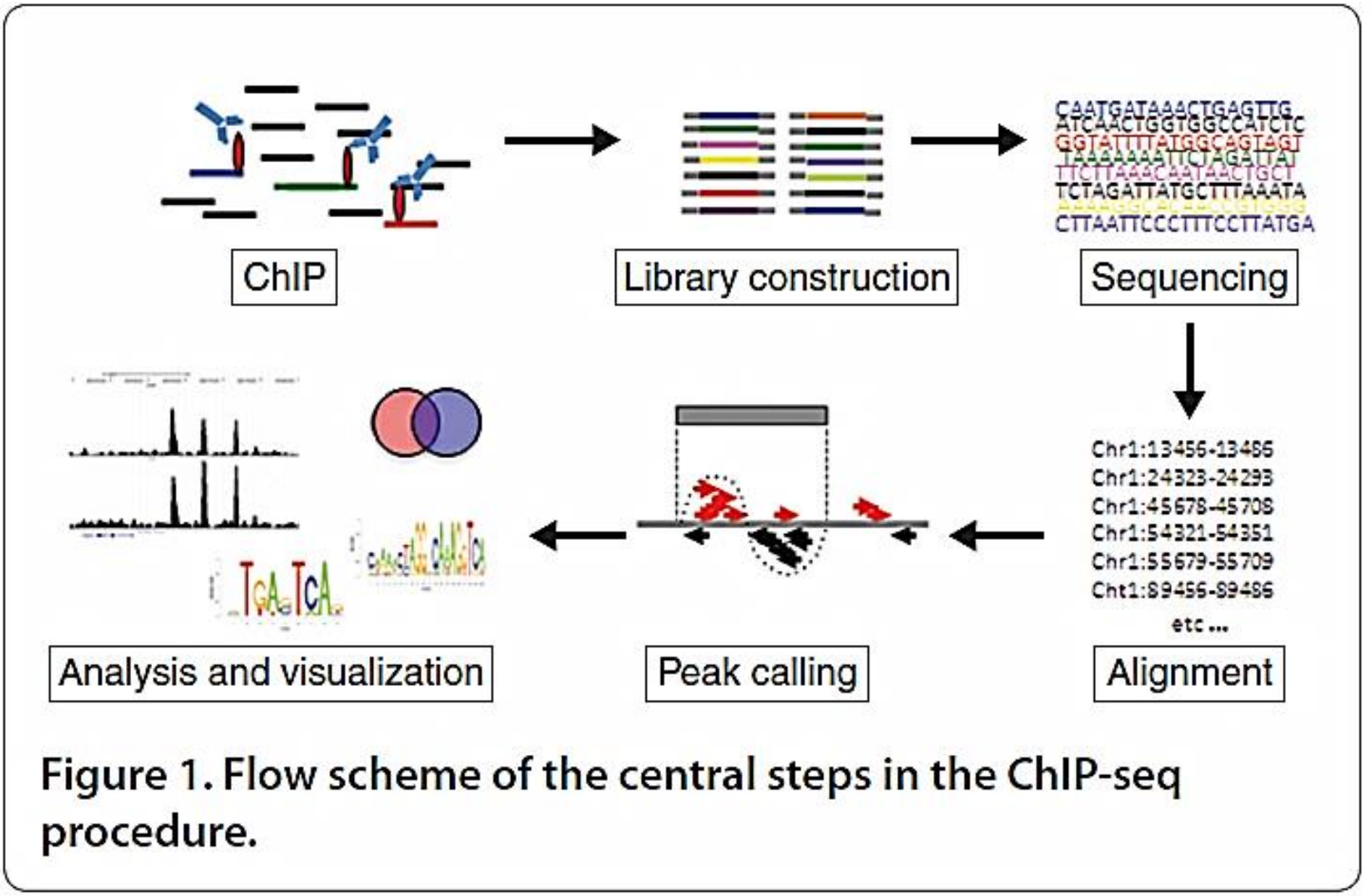
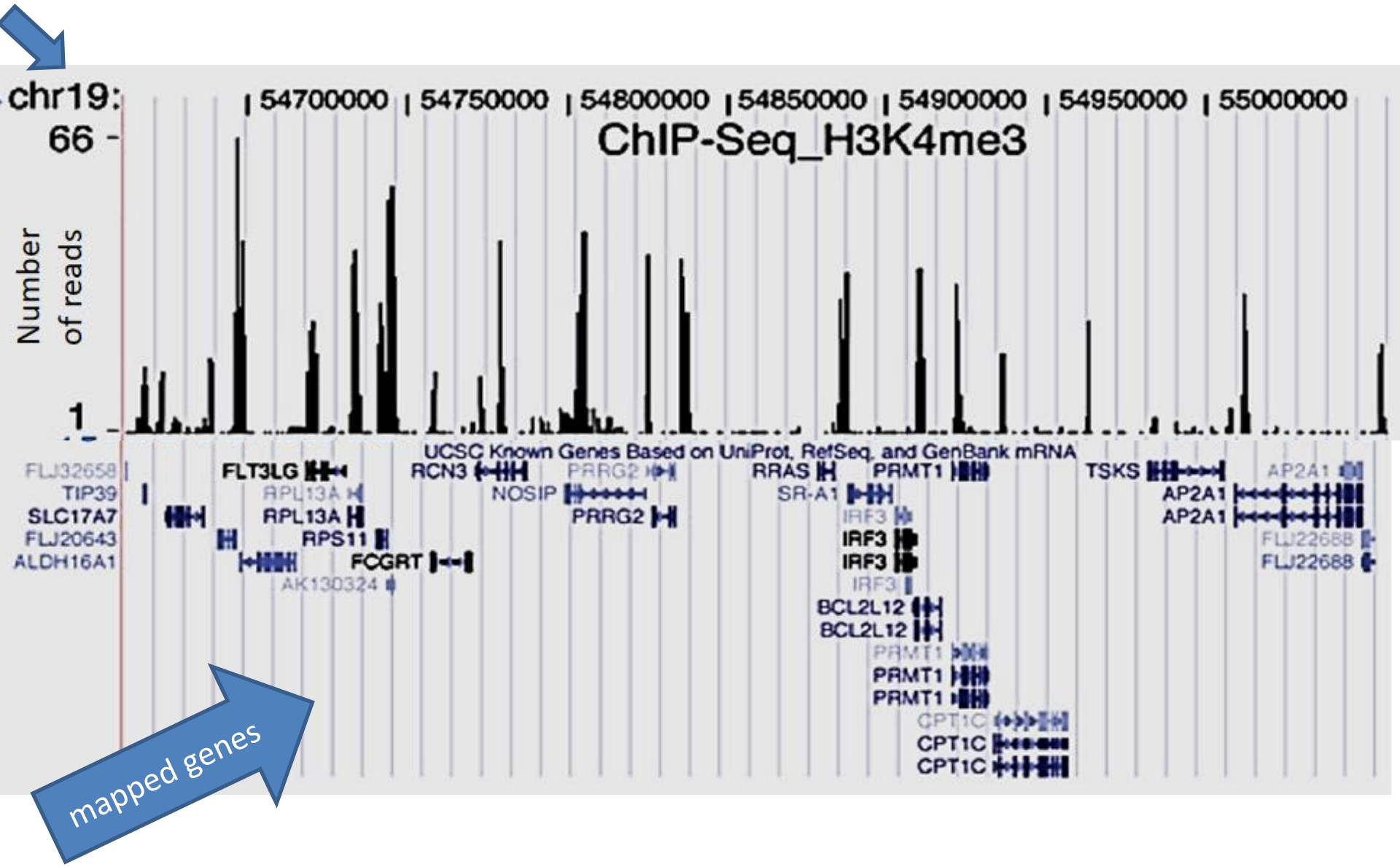


Figure 1. Flow scheme of the central steps in the ChIP-seq procedure.

Liu *et al*. *BMC Biology* 2010, **8**:56

Snapshot of the genome browser Chr. 19: 400 Kb window



If you revise this lesson in order to fix key concepts, we will be able to move on

L3 – Nuclear organization and genome domains

L4 – Analysis of Research Paper one (guided exercise)

Both Lessons will be on Wednesday, as agreed.