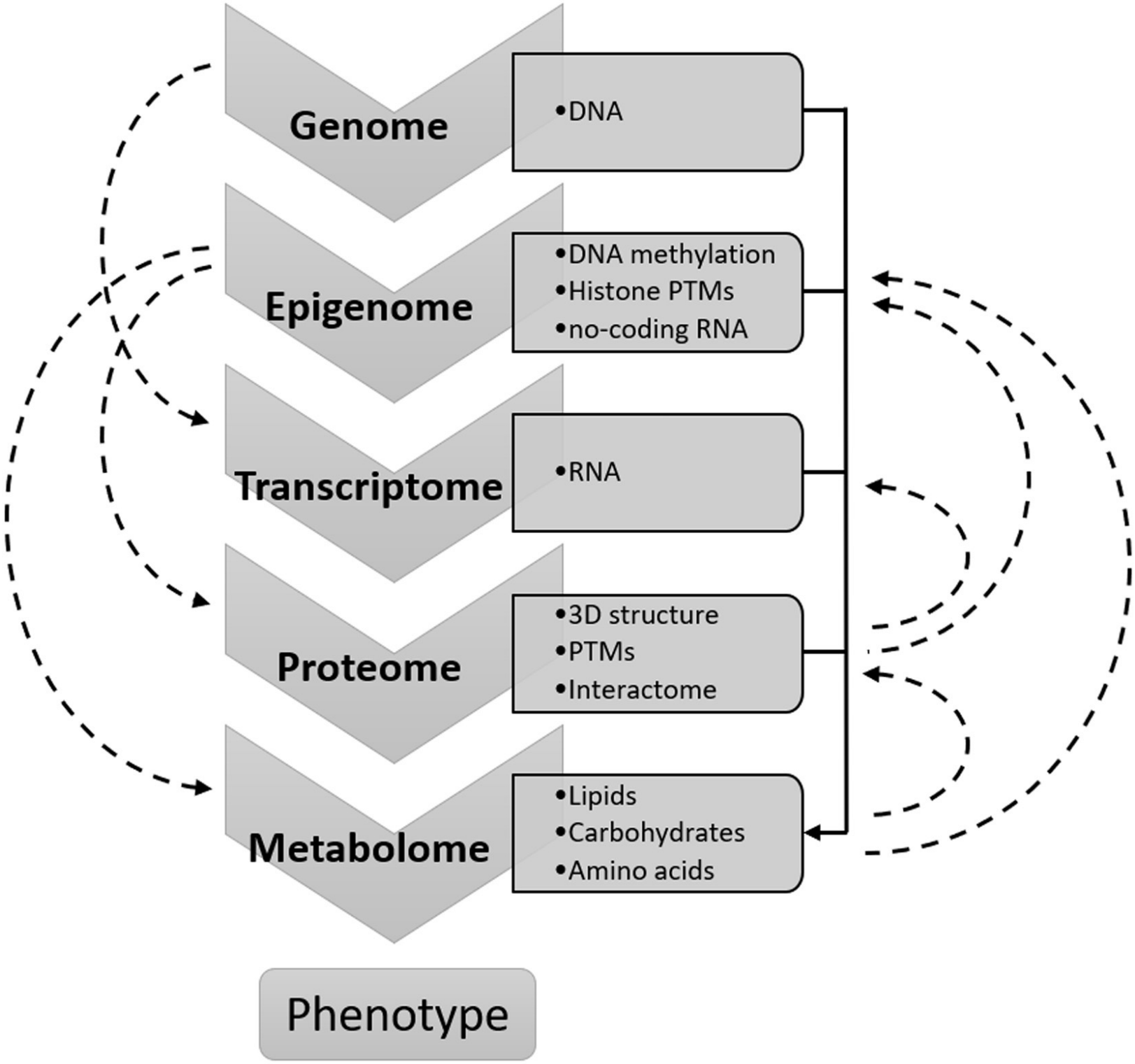
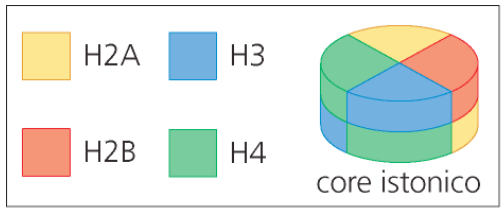
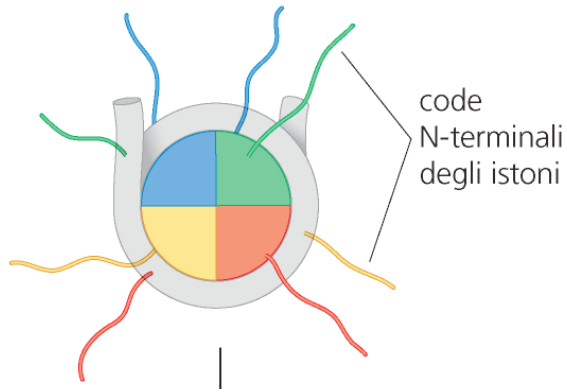

Genome
•DNA
Epigenome
•DNA methylation
•Histone PTMs
•no-coding RNA
Transcriptome
•RNA
Proteome
•3D structure
•PTMs
•Interactome
Metabolome
•Lipids
•Carbohydrates
•Amino acids
Phenotype

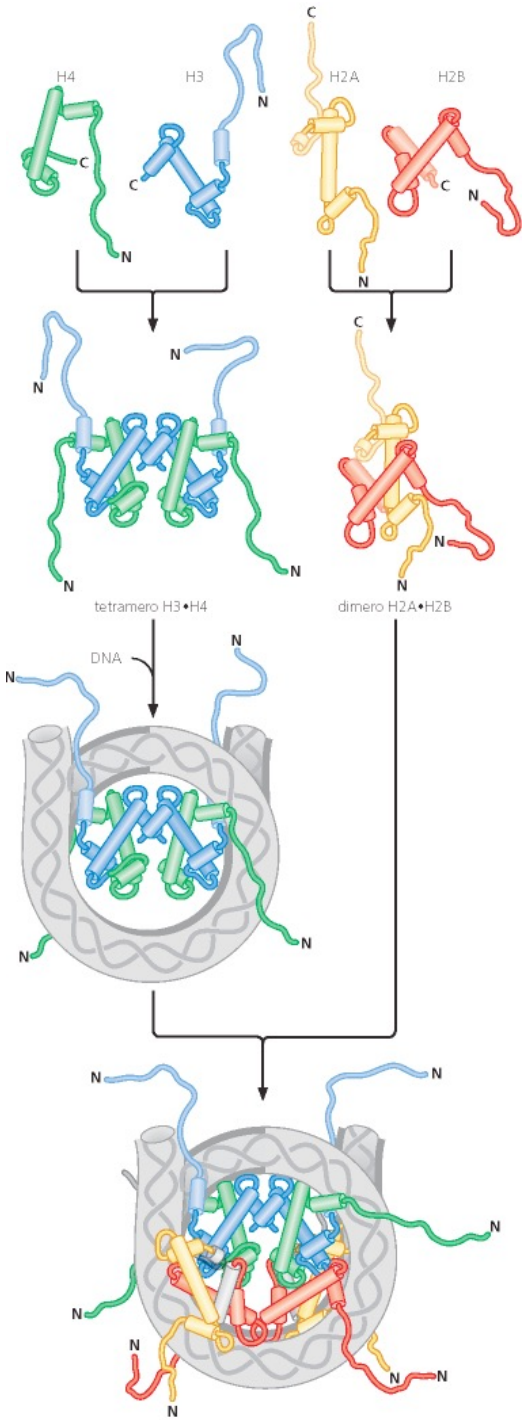
Histone modifications

The nucleosome
- repeat unit of chromatin

- 146 bp of DNA wrapped around a histone octamer core
- so that it forms 1.7 turns of a left-handed superhelix within the nucleosome core particle
- while the histon core is involved in histone-histone packing and DNA-contact, the N-terminal tail point outwards and is available for interaction

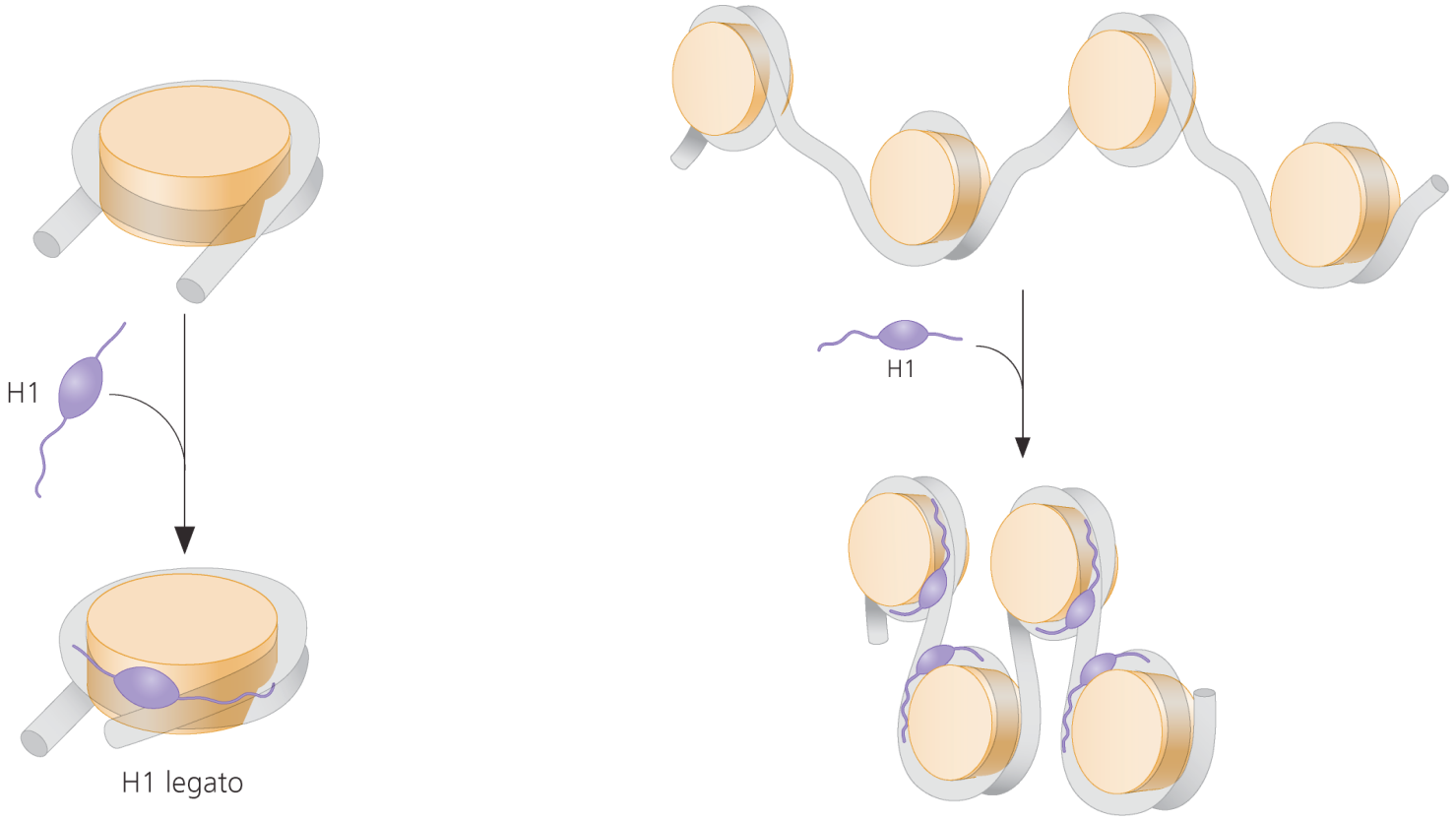


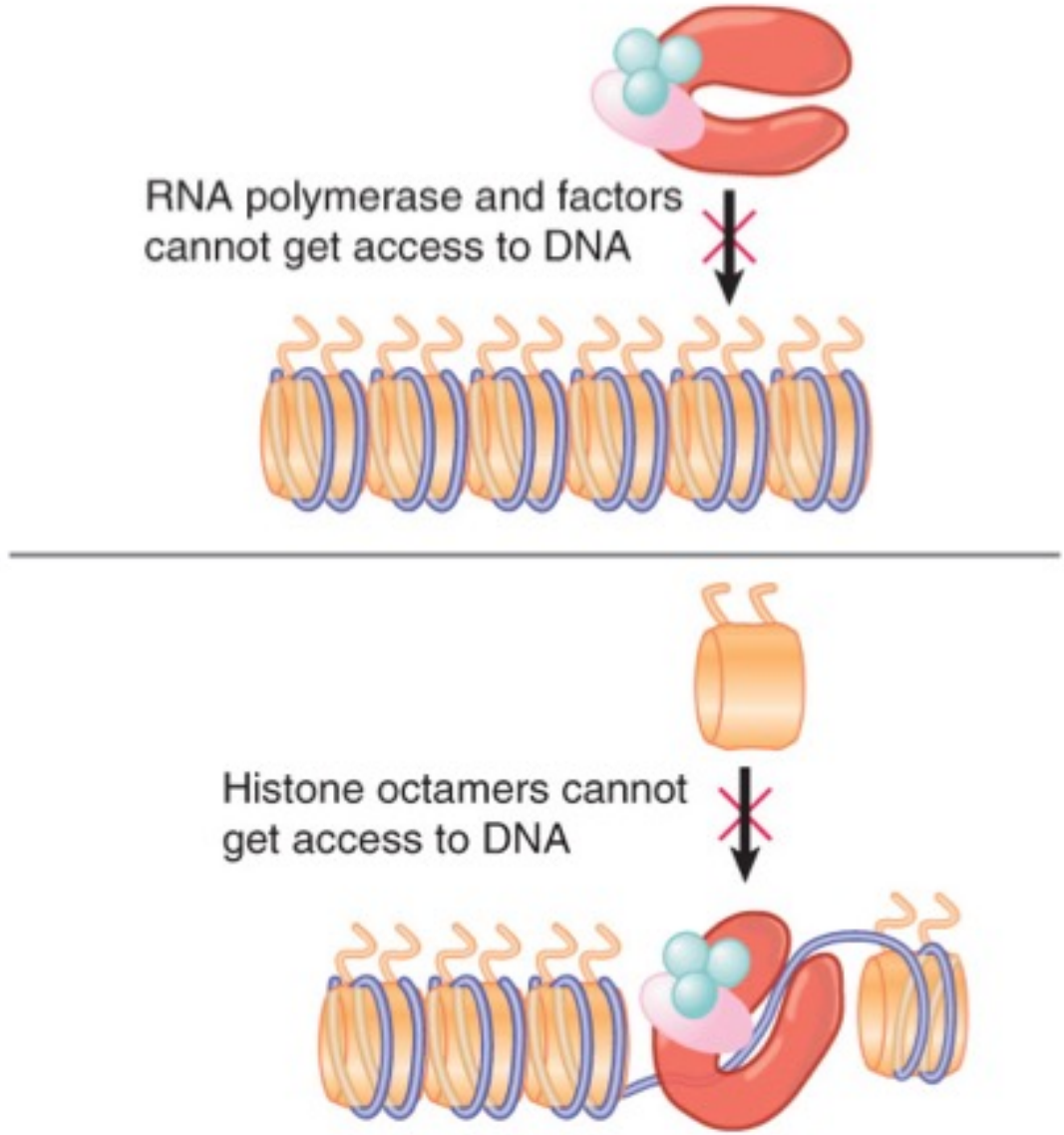




Histone H1 - linker histone

- **H1 linker histone**
  - associated with linker DNA between nucleosomes (about one H1 per nucleosome)
    - Binds DNA at entry/exit
    - stimulates folding 10 nm ➜ 30 nm fiber
    - repressive effect on transcription
  - H1 binds weaker to acetylated nucleosomes




RNA polymerase and factors
cannot get access to DNA
Histone octamers cannot
get access to DNA

Regulation of transcription by chromatin

1. Histone variants

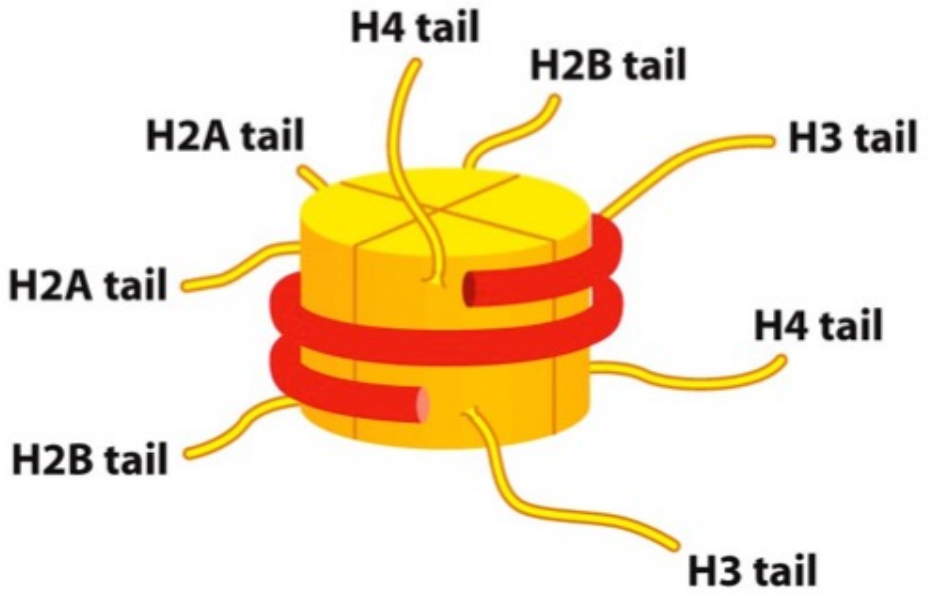
2. Opening of chromatin through directed **modication of histone tails** (e.g. acetylation and methylation)

3. Opening of chromatin through directed **nucleosome mobilization** by **remodeling complexes**

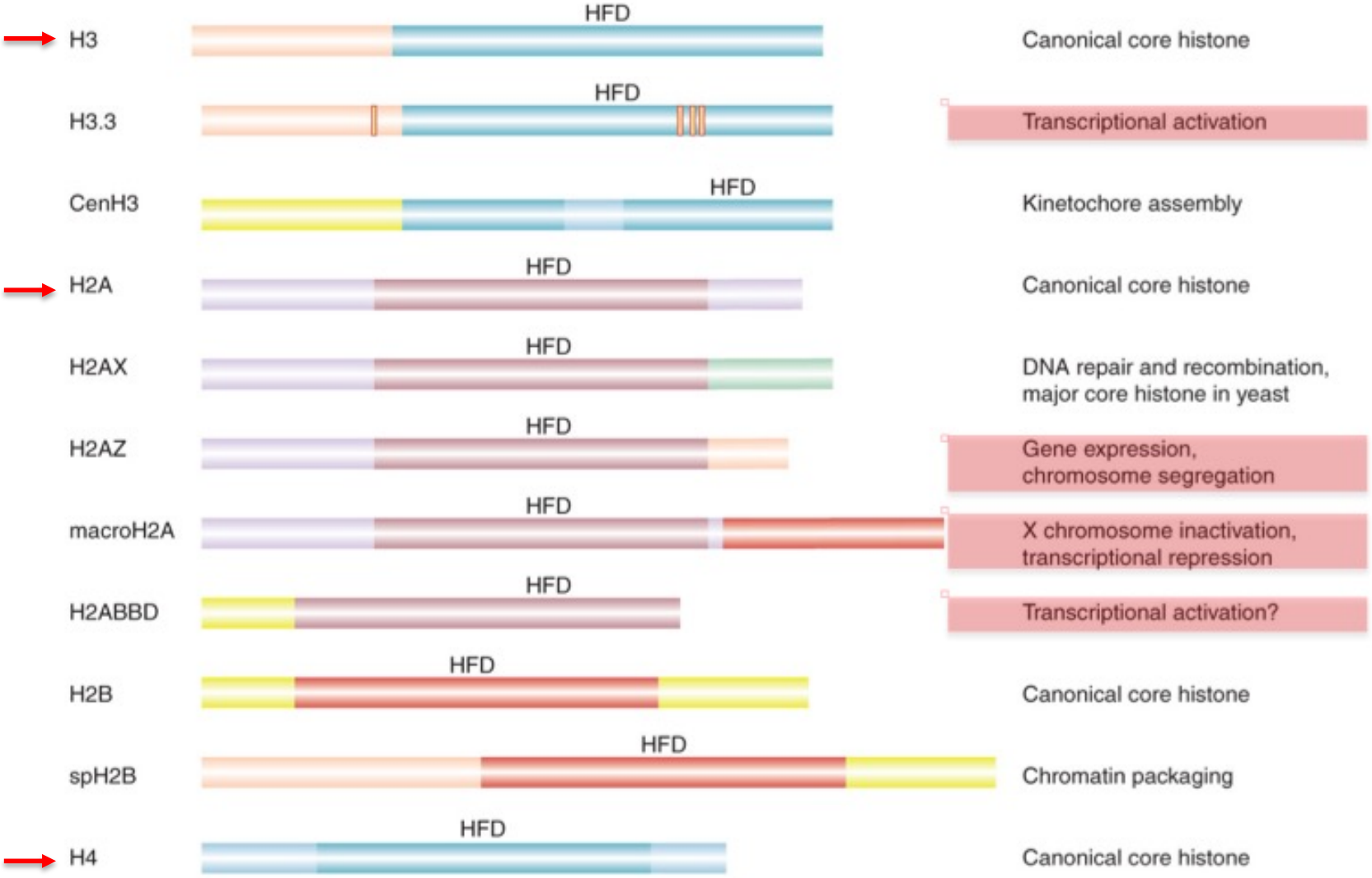
4. **Positioning of nucleosomes** creates promoters with different requirement for remodeling

Histone variants and modifications influence transcriptional activity

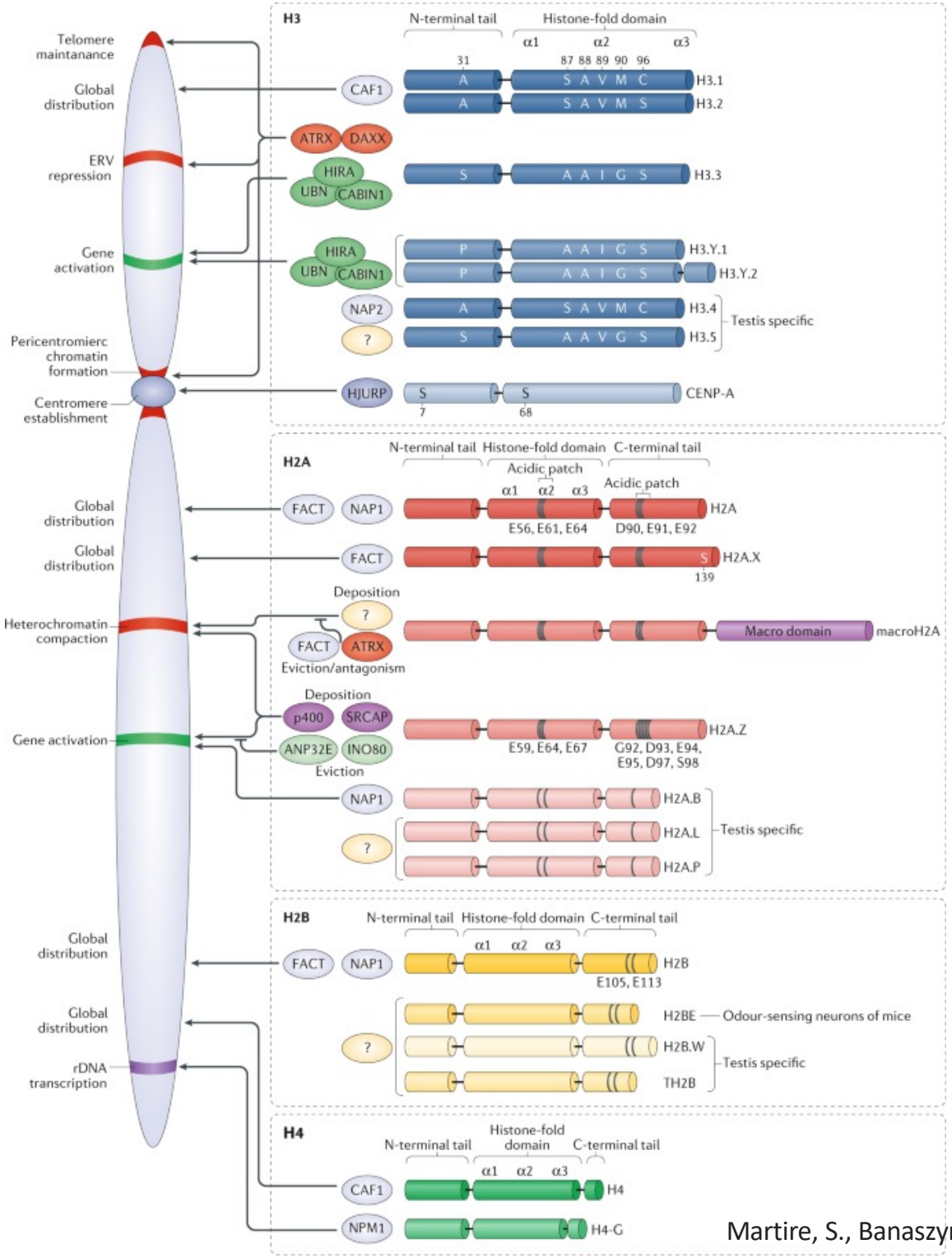
- Histone tails can be covalently modified to facilitate or prevent transcription
- Variants for Histone 2 and 3 mediate transcriptional control



Special functions of histone variants



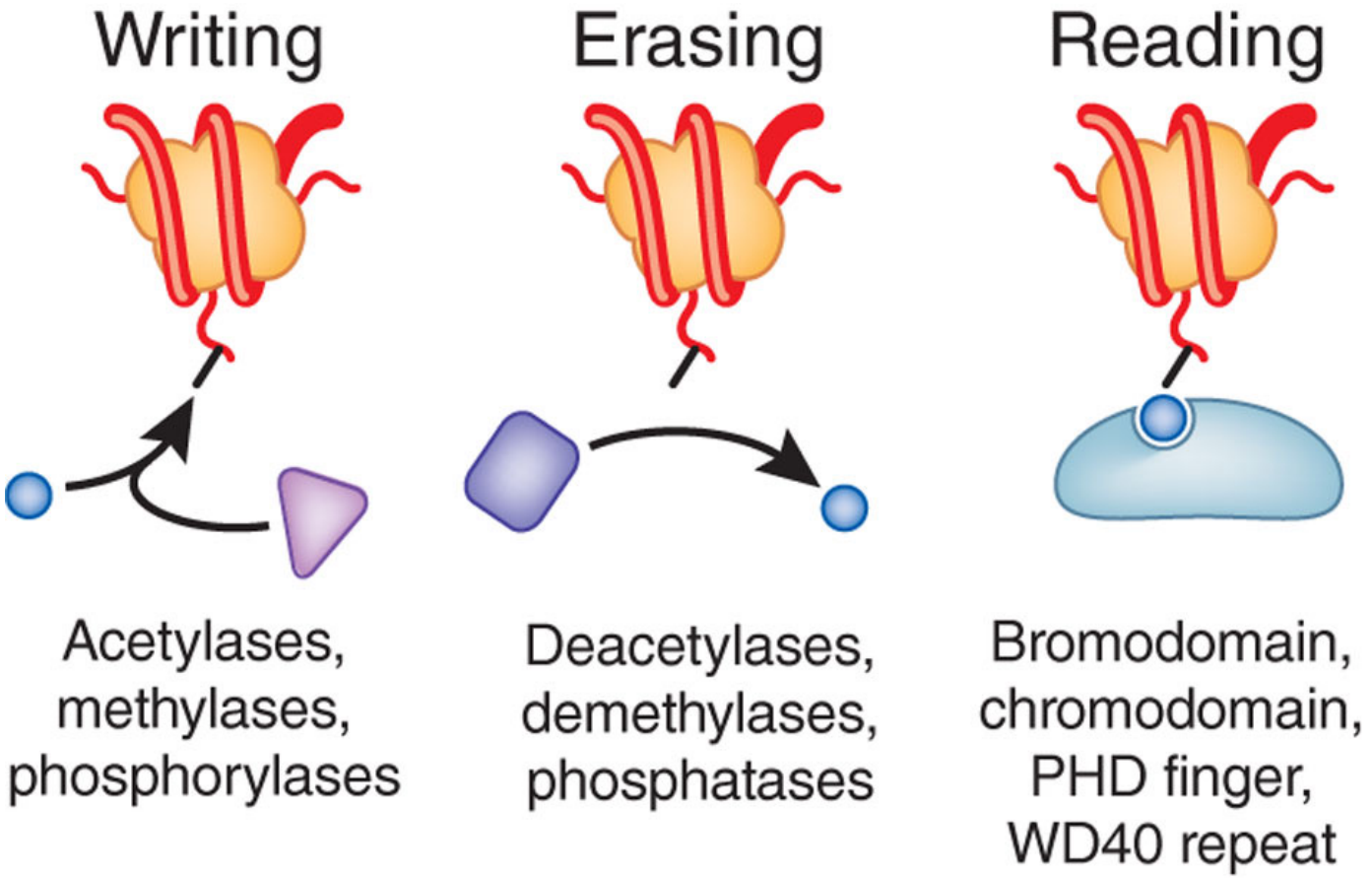
Special functions of histone variants



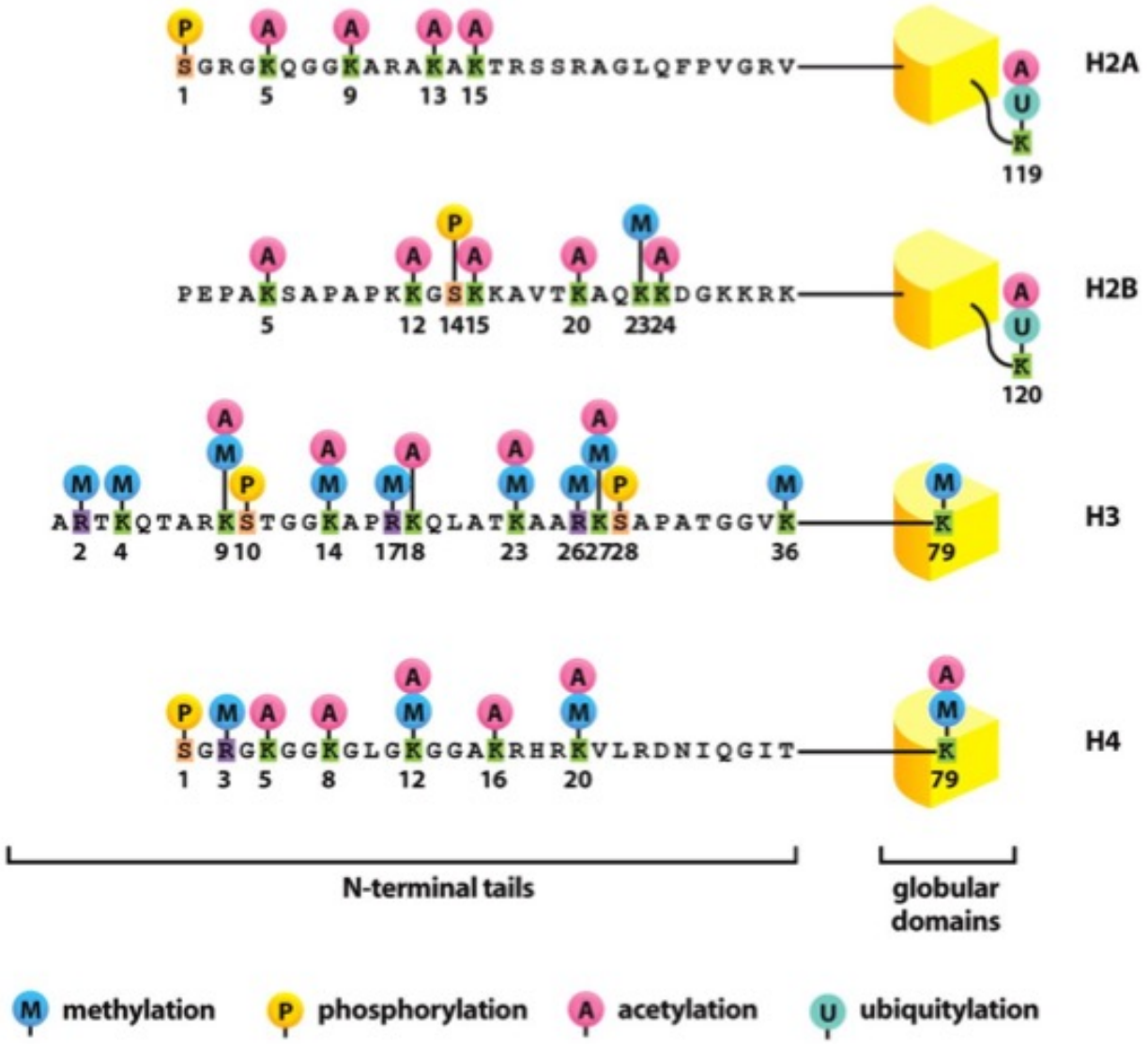
Martire, S., Banaszynski, L.A. *Nat Rev Mol Cell Biol* **21**, 522–541 (2020)

Histone post translational modifications (PTMs)

'**Writers**' introduce histone marks (circles), '**erasers**' (or **editors**) take them out and '**readers**' can recognize a particular form of histone modification.



Histone post translational modifications (PTMs)

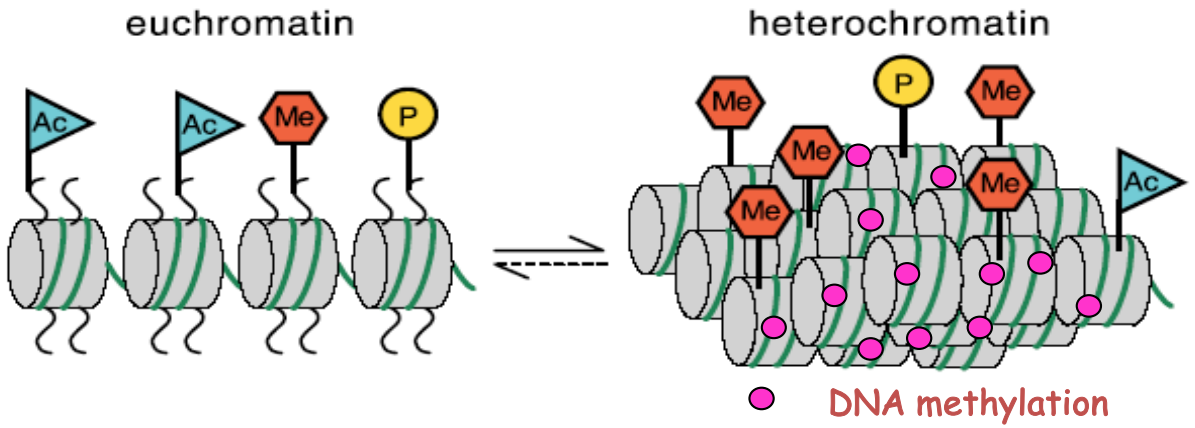


Histone PTMs regulate transcription

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

Histone Modifications Associated with Heterochromatin and Euchromatin



Heterochromatin (inactive/condensed)

H3 ARTKQTARK^{Me_3}STGGKAPRKQLATKAARKSAPAT
9

H3K9Me3

H3 ARTKQTARKSTGGKAPRKQLATKAARK^{Me_3}SAPAT
27

H3K27Me3

Euchromatin (active/open)

H3 ARTKQTARK^{Ac,P}STGGK^{Ac}APRKQLATKAARKSAPAT
9 10 14

H3K9Ac H3S10P H3K14Ac

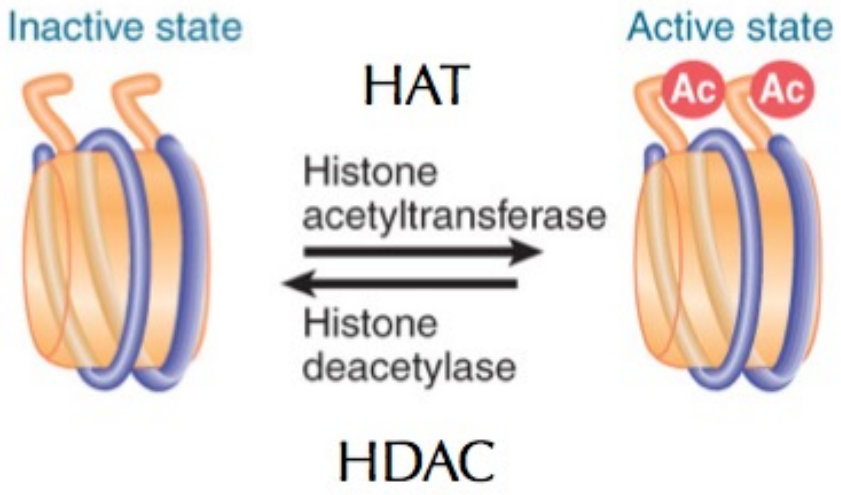
H3 ART^{Me_3}KQTARKSTGGK^{Ac}APRKQLATKAARKSAPAT
4 14

H3K4Me3 H3K14Ac

Histone PTMs regulate transcription

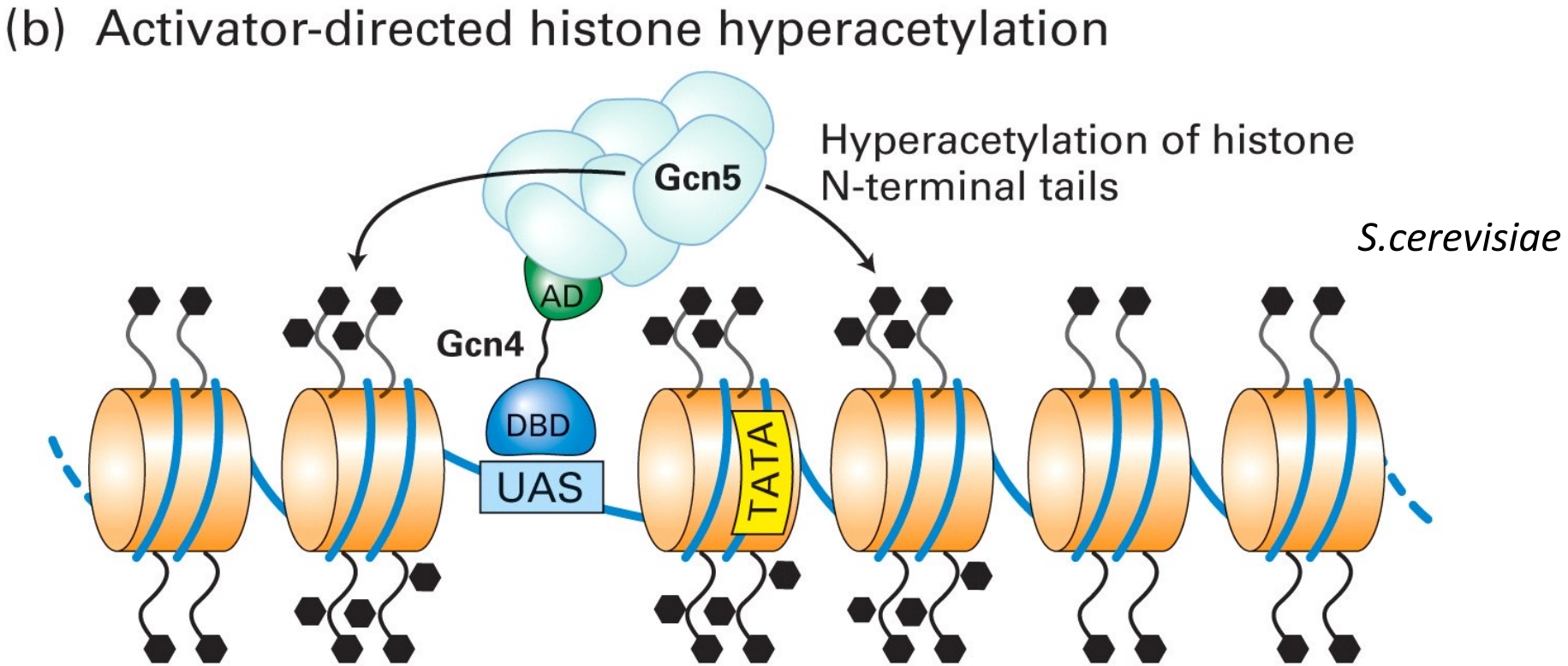
- **Acetylation** is associated with activation of transcription and absent in heterochromatin

- **Histone acetyltransferases (HATs)** and **histone deacetylases (HDACs)** bind to transcription factors or coactivators often forming large regulatory complexes



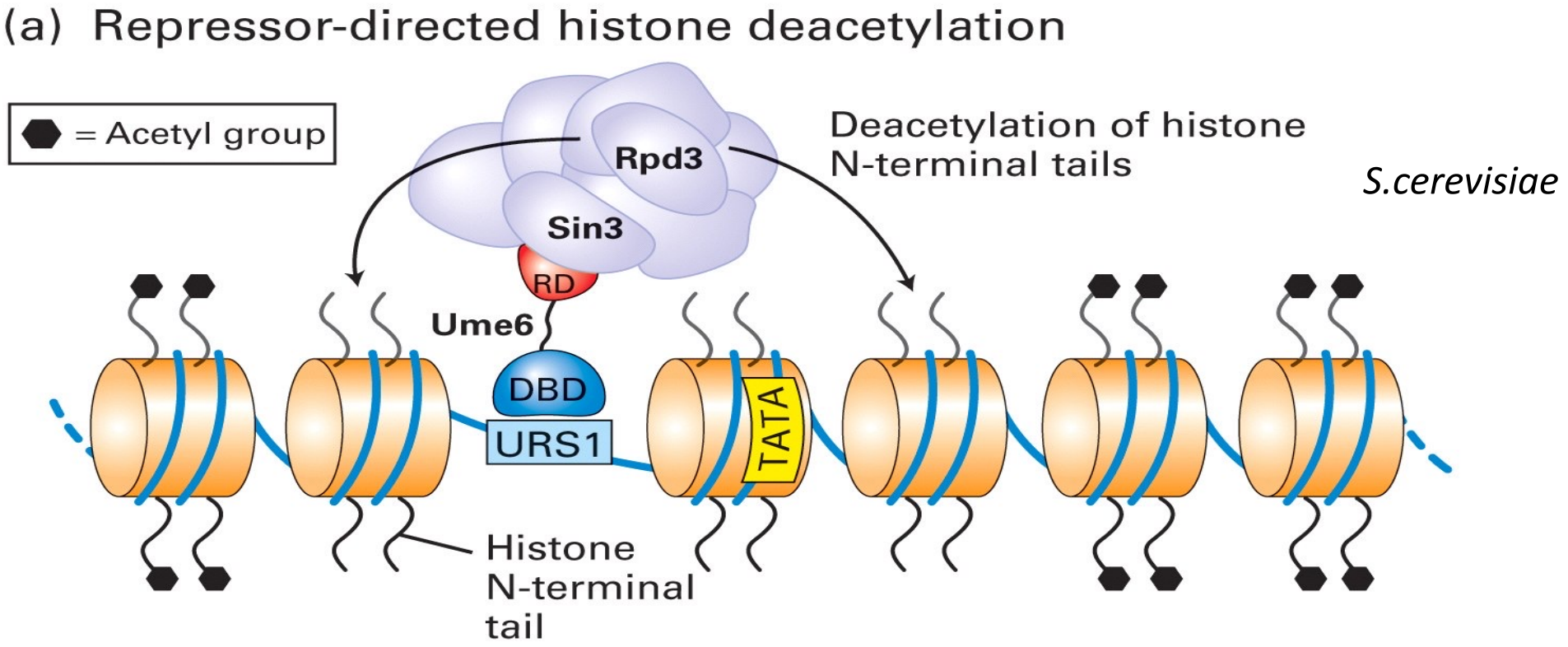
- chemical inhibitors (i.e. trichostatin) that inhibits HDACs resulted in increased gene expression

Activators Regulate Gene Expression by Modulating Chromatin Structure to be Open



- The DBD of Activators like Gcn4 bind their Upstream Activating Sequence (UAS).
- Activation Domain (AD) attracts protein complexes containing **histone acetylases** (Gcn5)
- Subsequent acetylation of histone tails serve to open up chromatin.

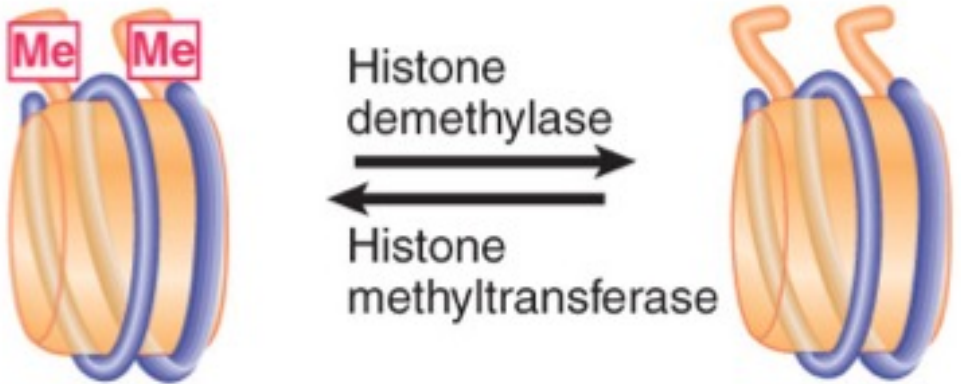
Repressors Regulate Gene Expression by Modulating Chromatin Structure to be Closed



- The DBD of repressors (like Ume6) bind a DNA element (URS1) and the Repression Domain (RD) recruits a protein complex containing a **histone deacetylase** like Rpd 3.
- The subsequent deacetylation of histone N-terminal tails results in chromatin condensation which promotes gene repression.

Histone methylation

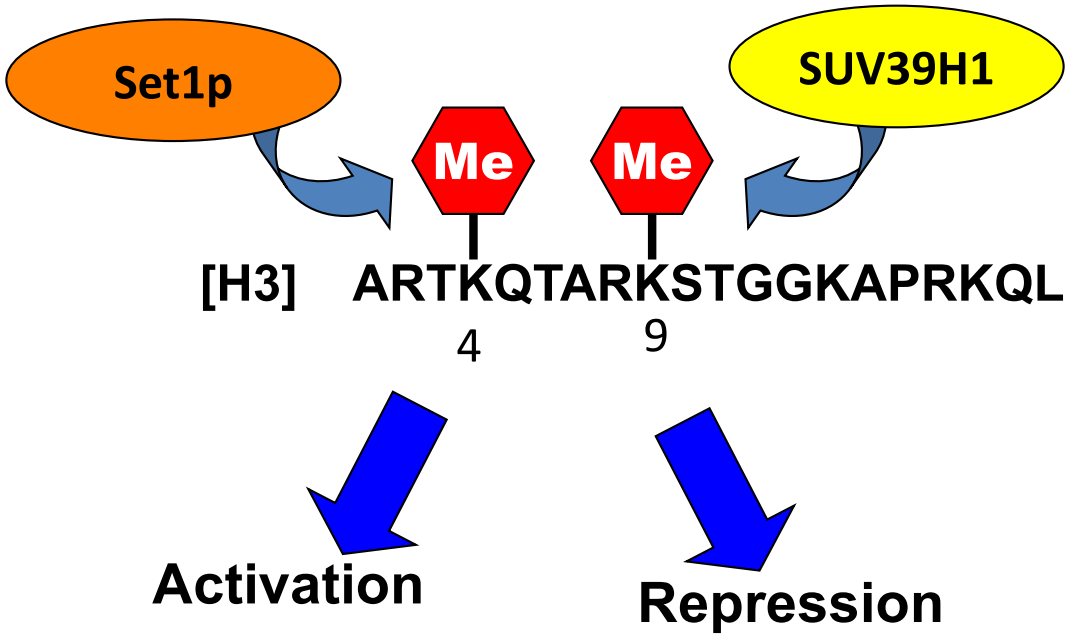
- Both **Lys (K)** and **Arg (R)** can be methylated at more than one methyl-group
- Histone methylation is a relatively stable modification with a slow turnover rate.
- An ideal epigenetic mark for more long-term maintenance of chromatin states.
- Methylated residues are present both in eu- and heterochromatin.



Function - Activation or repression?
2 sites - 2 HKMTs - 2 effects

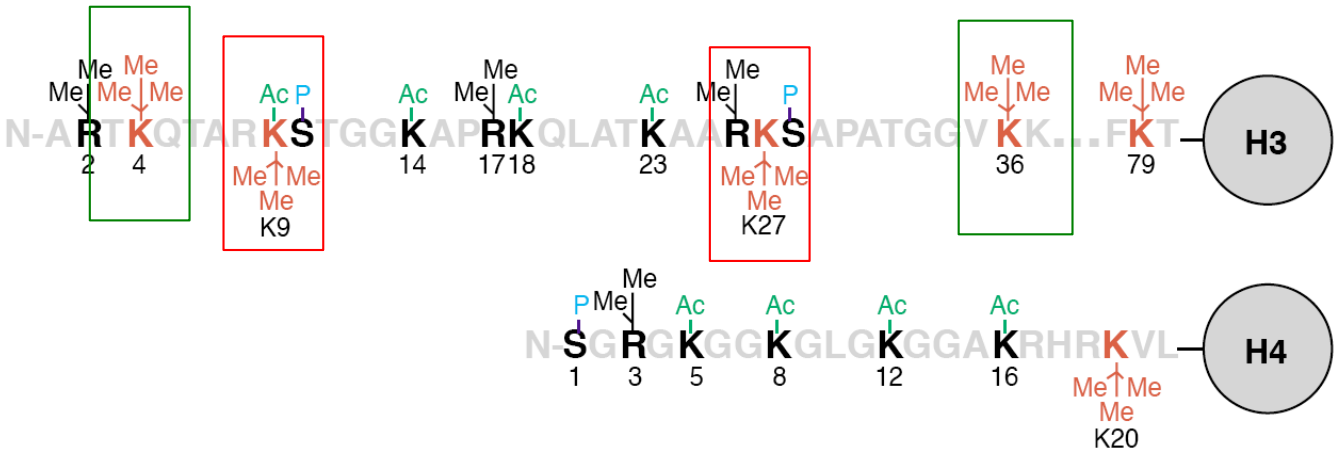
- Histone methylation was traditionally linked to repression, but turns out to be linked also to activation

  - **H3 K9-Methylation** correlates with heterochromatin formation.
  - **H3-K4-Methylation** correlates with TRX activation
  - Set1p, responsible for H3K4 methylation, associated with HAT complex



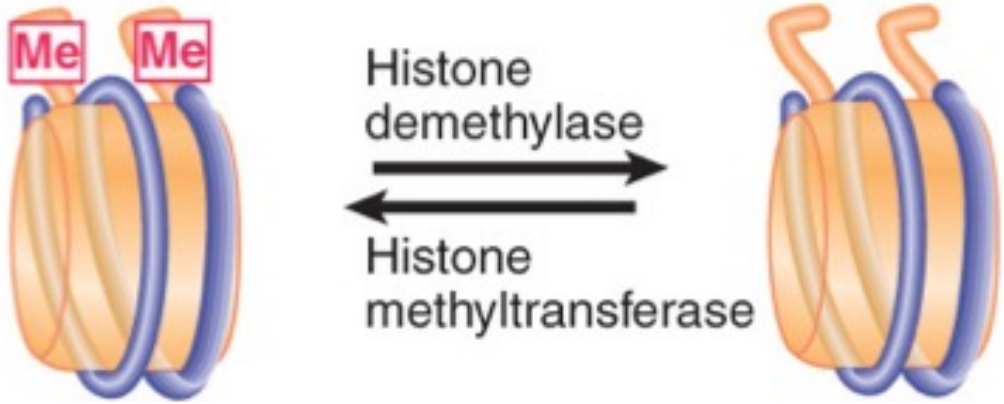
The subtrates:
Histone tails - multiple methylations

N-term tails reversible methylated in **Arginine** (**R**) and **Lysine** (K), particularly in H3 and H4.



- Above ≈ activation
- Below ≈ repression

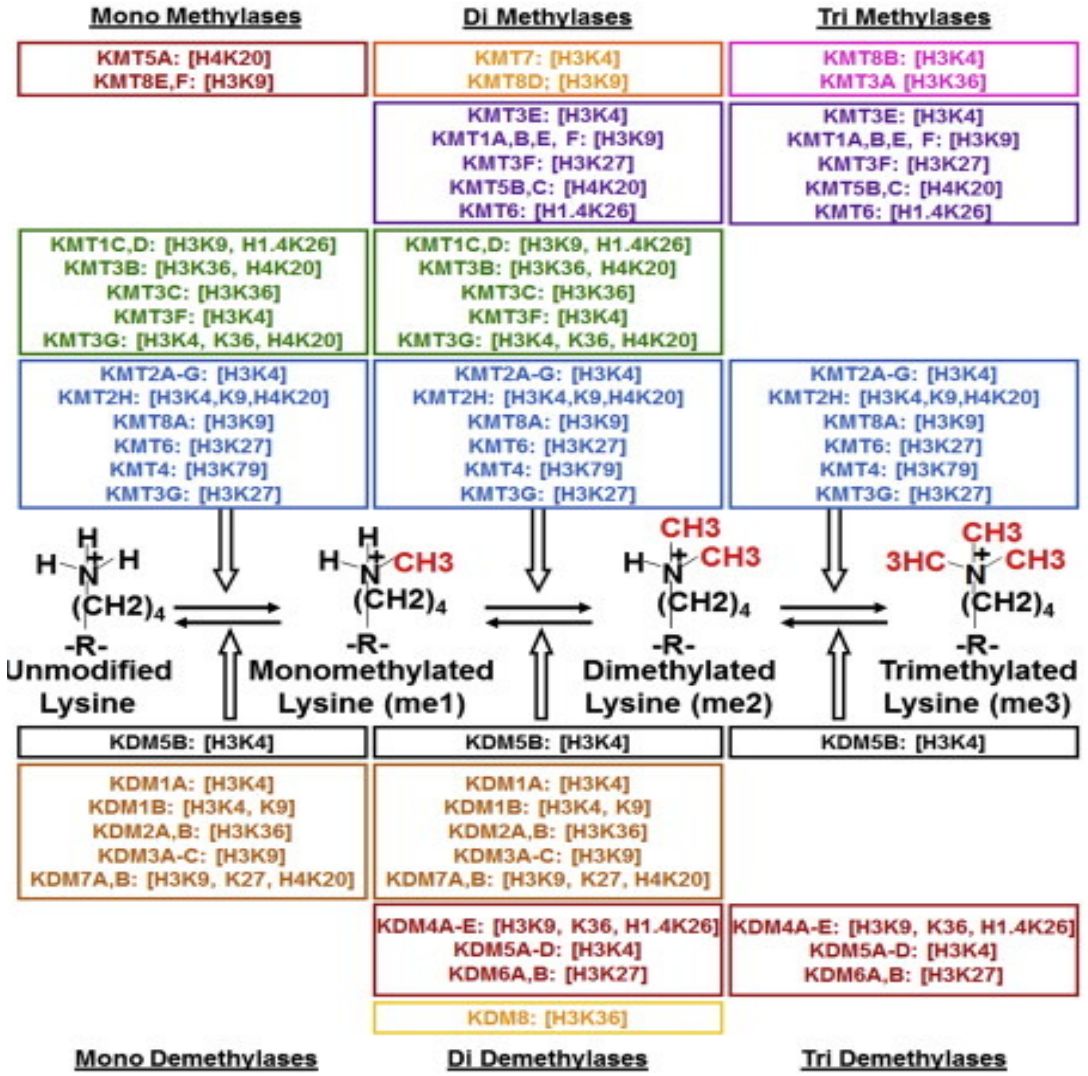
Histone PTMs regulate transcription



	Transcription
H3K4me1	–/+
H3K4me2	+
H3K4me3	+
H3K36me2	–/+
H3K36me3	+
H3K79me	–/+
H3K9me	–/+
H3K27me	–

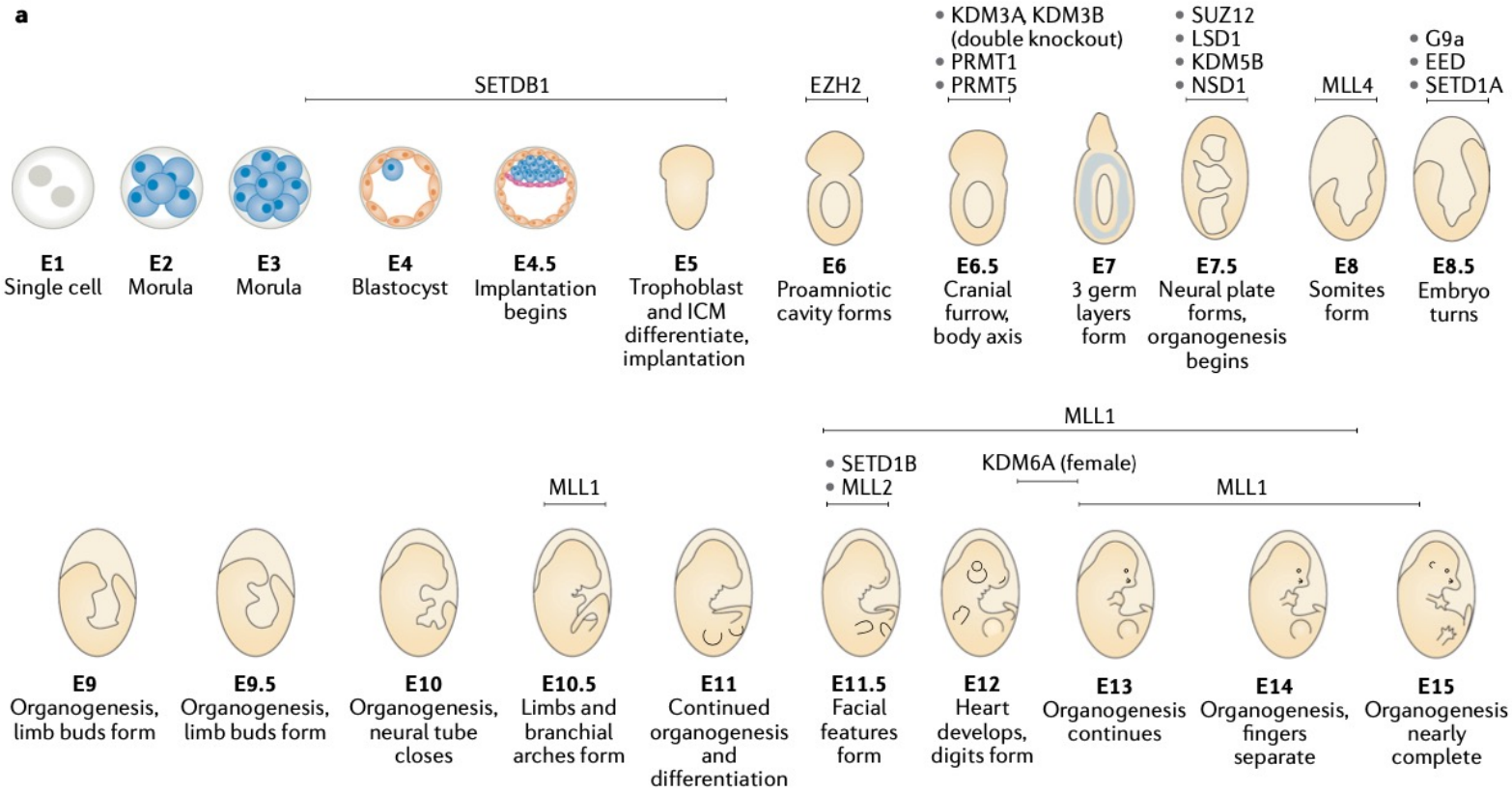
Activation/silencing determined by the counteracting action of KMTs and KDMs

- **KMT**s (histone lysine methylase) and **KDM**s (histone lysine demethylases) have a high degree of specificity for particular lysine residues and the degree of methylation.



The importance of histone methylation regulators in mammalian development

Loss of some histone methylation regulators causes very early lethality, before or during implantation (for example, SETDB1), whereas other regulators are required at later stages of organogenesis, with the majority exhibiting lethality between embryonic day 7 (E7) and E12. For some regulators (MLL1, SETDB1), lethality was observed at different stages, depending on the report.



Polycomb and MLL/Trithorax Complexes

The ON and OFF states of key developmental genes are maintained by the **MLL/Trithorax (Trx)** and **polycomb group (PcG)** proteins, which mediate trimethylation of histone H3 lysine 4 (H3K4me3) to activate genes or lysine 27 (H3K27me3) to repress genes.

H3K27me3

Polycomb-group Proteins

- Maintains a silenced state
- Prevents chromatin remodelling

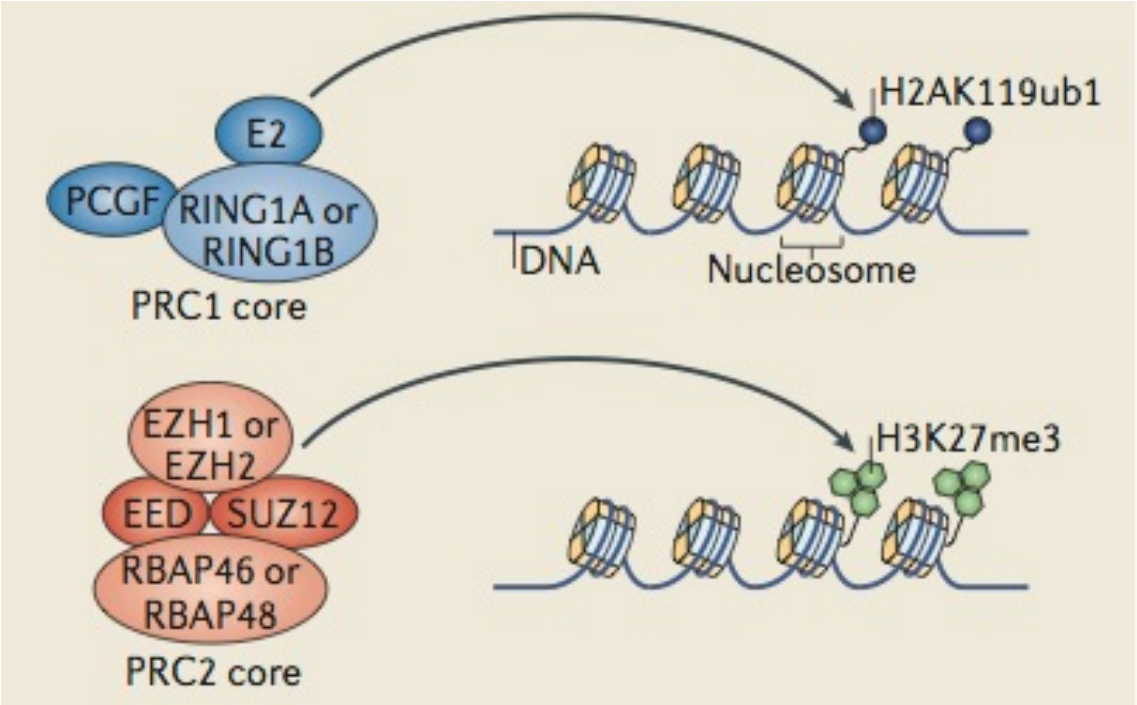
H3K4me3

Trithorax-group Proteins

- Maintains an active state
- Counteracts the action of PcG proteins

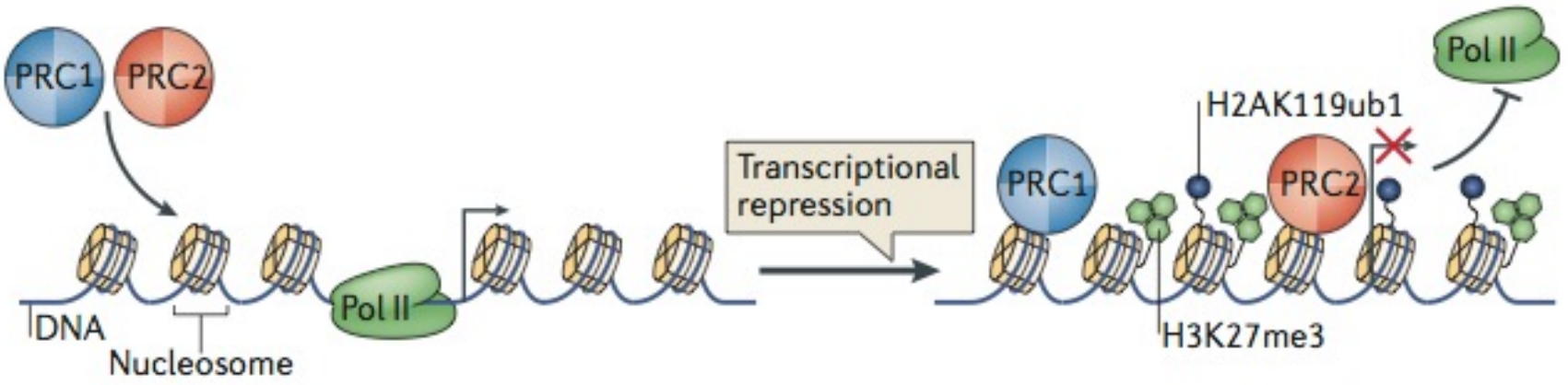
Core PRCs and their chromatin-modifying activities

•**Polycomb repressive complexes** (PRCs) repress transcription by a mechanism that involves the modification of chromatin.

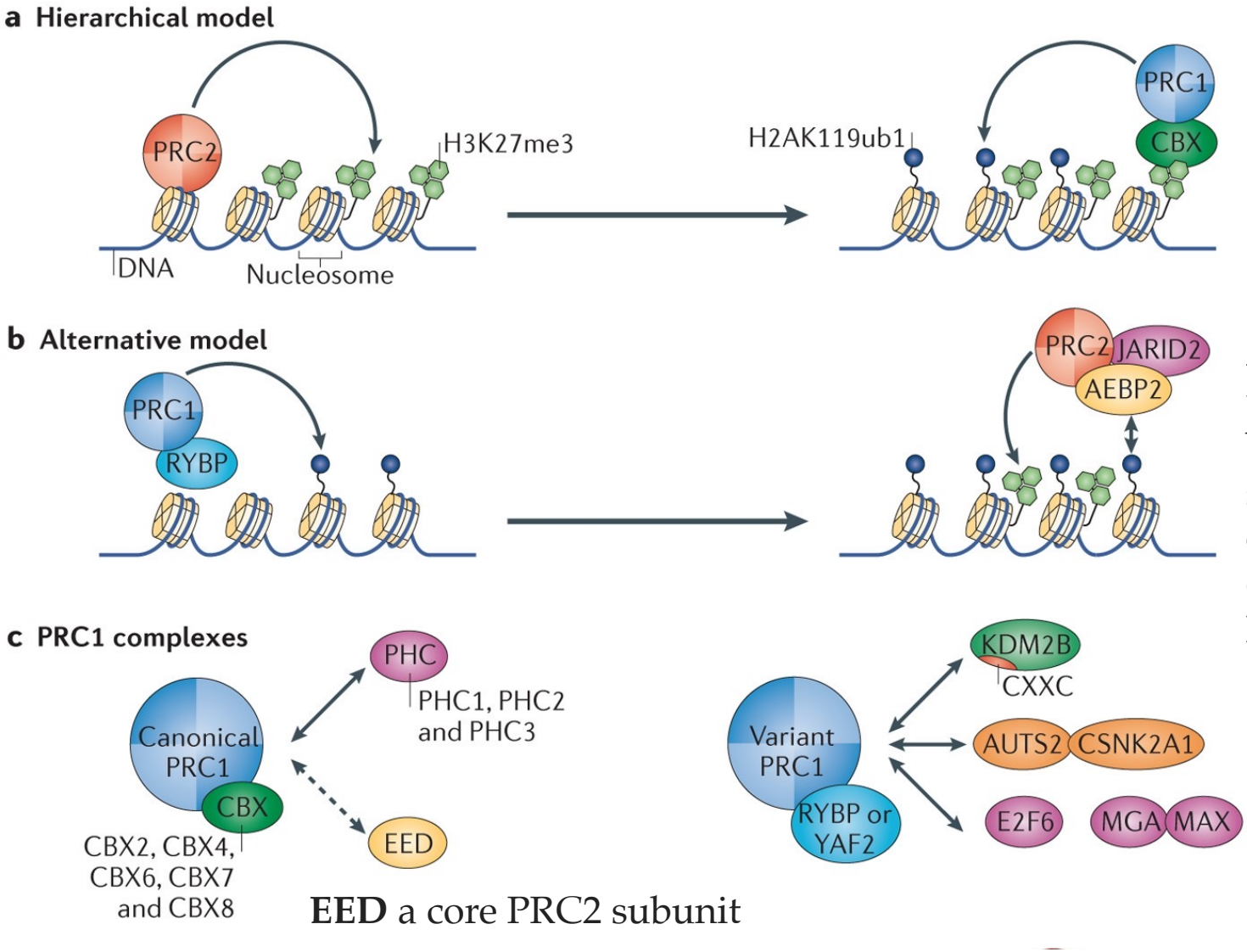


Polycomb systems and gene regulation

Polycomb complexes lead to Polycomb chromatin domain formation, which then directs repression of transcription by RNA polymerase II (Pol II) at the associated gene.



PRCs recruitment



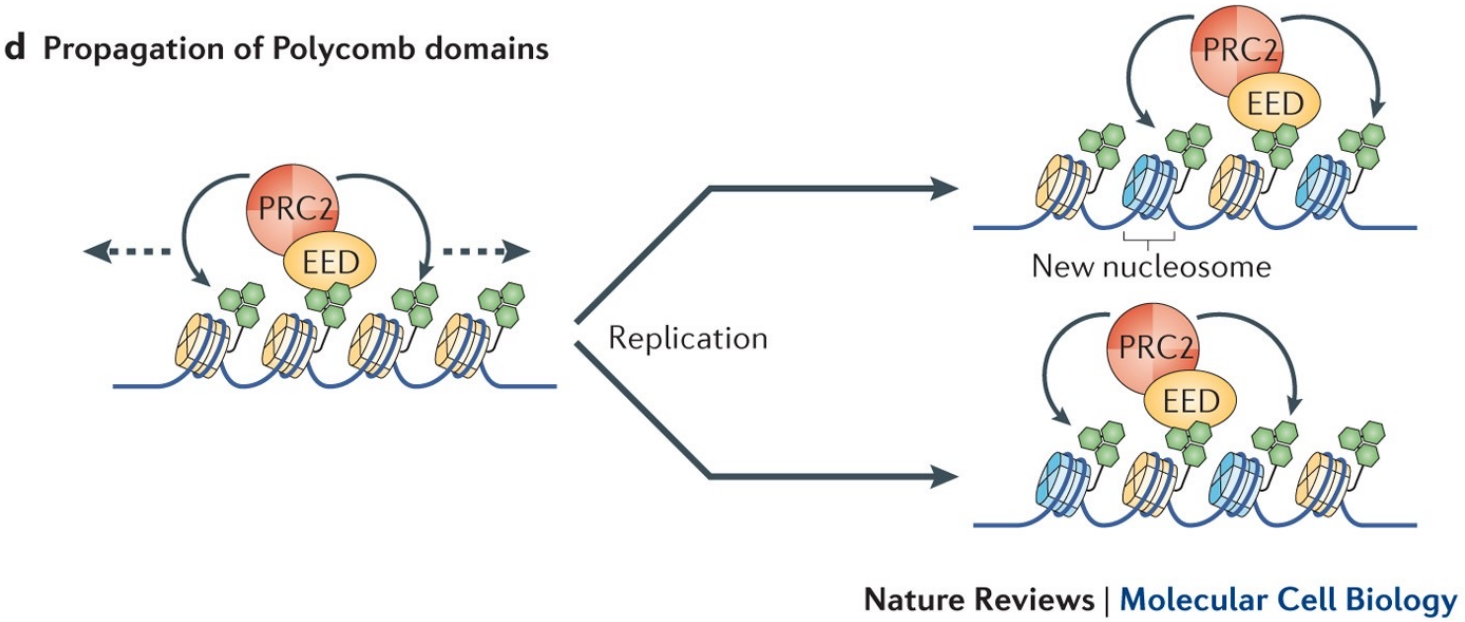
H3K27me3 recognised by **CBX**

AEBP2 and **JARID2** that preferentially binds to and stimulates catalysis of H3K27me3 on chromatin containing H2AK119ub1

KDM2B recognoses non-methylated CpG dinucleotides

EED a core PRC2 subunit

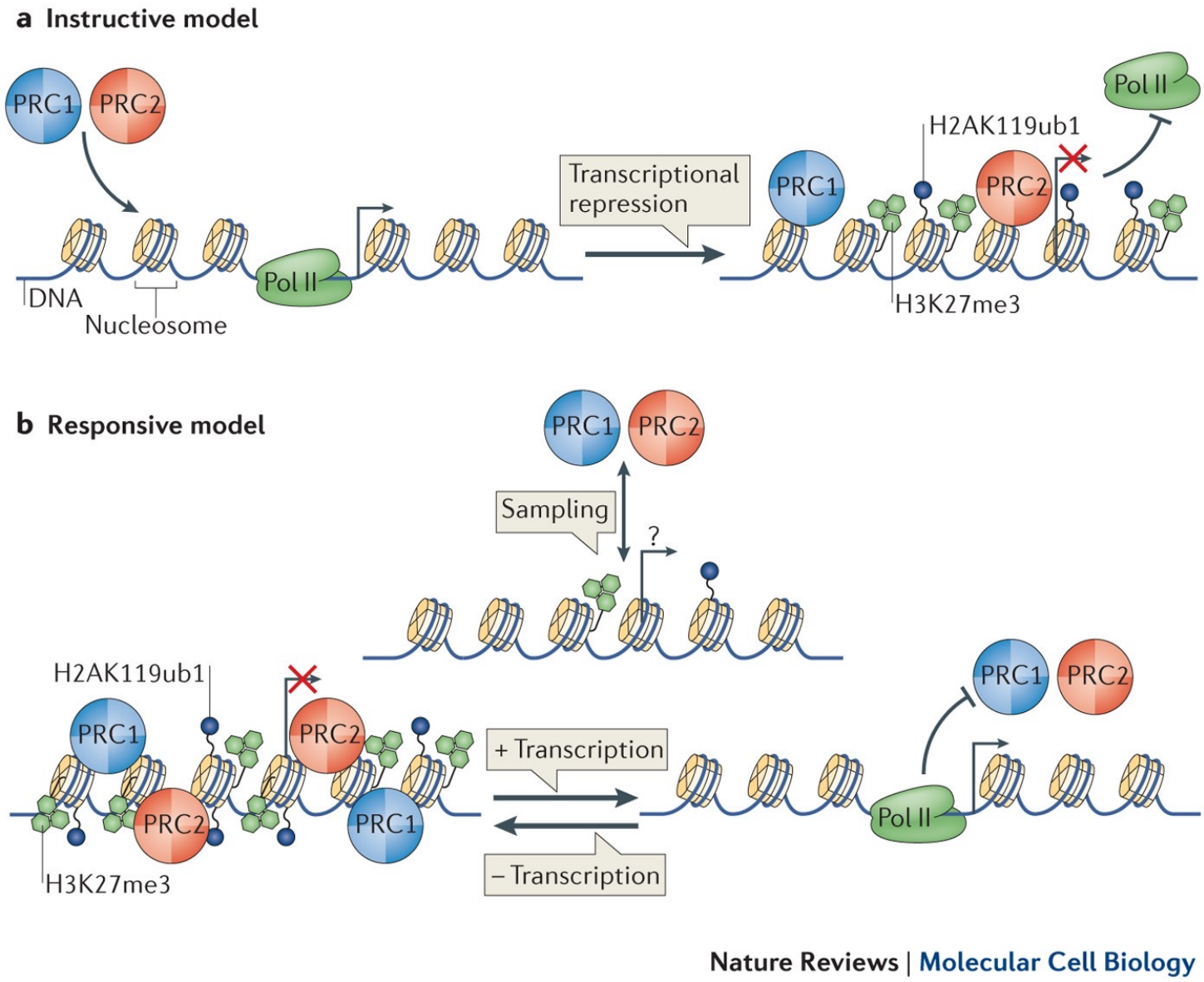
PRCs recruitment



EED subunit of the PRC2 core complex binds to H3K27me3 through its WD40 repeat, and this interaction seems to stimulate the catalytic activity of PRC2, to form an activity-based feedback loop.

It has been proposed that this could promote the spreading of H3K27me3 along chromatin and ensure the propagation of H3K27me3 on newly replicated chromatin

Polycomb systems and gene regulation.



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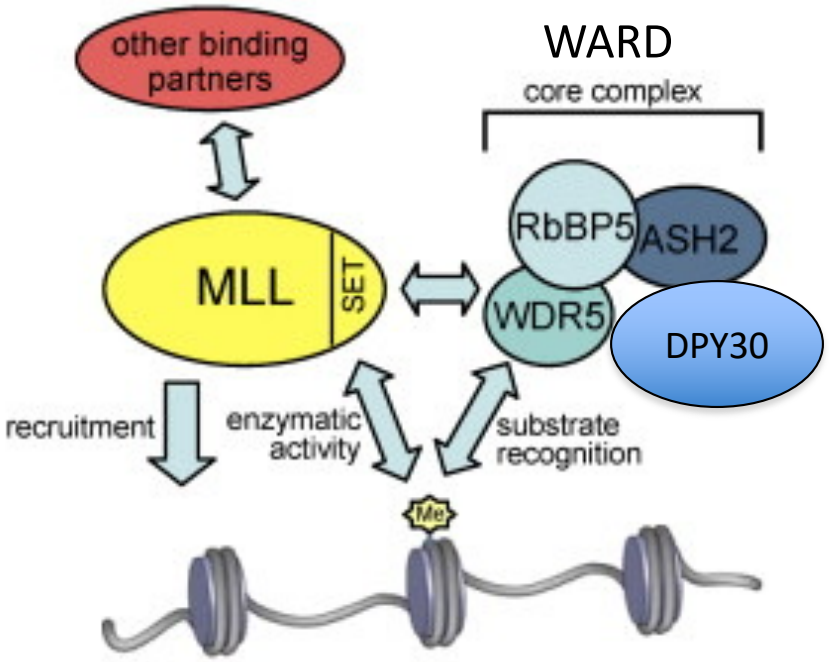
Writing the H3K4 Methylation Mark

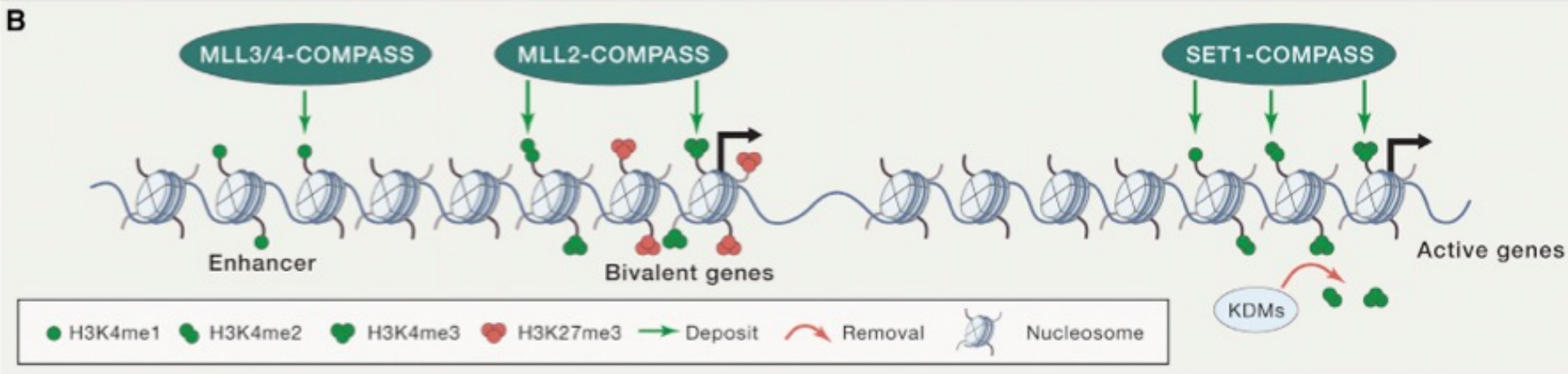
- **Trithorax** (MLL or COMPASS in mammals) methylates H3K4 and recruits HAT and remodeling complexes



MLL-family HMTs associate with the core complex containing, **WDR5**, and **ASH2, RbBP5** and **DPY30 (**abbreviated in **WARD)**. The core complex cooperates with the catalytic SET domain to methylate H3K4, whereas other regions of the MLL protein are involved in association with other protein partners and in recruitment of the MLL complex to the target genes. **WDR5** plays a role in substrate recognition and presentation, with preferential, but not exclusive, binding to the H3K4me2 substrate.

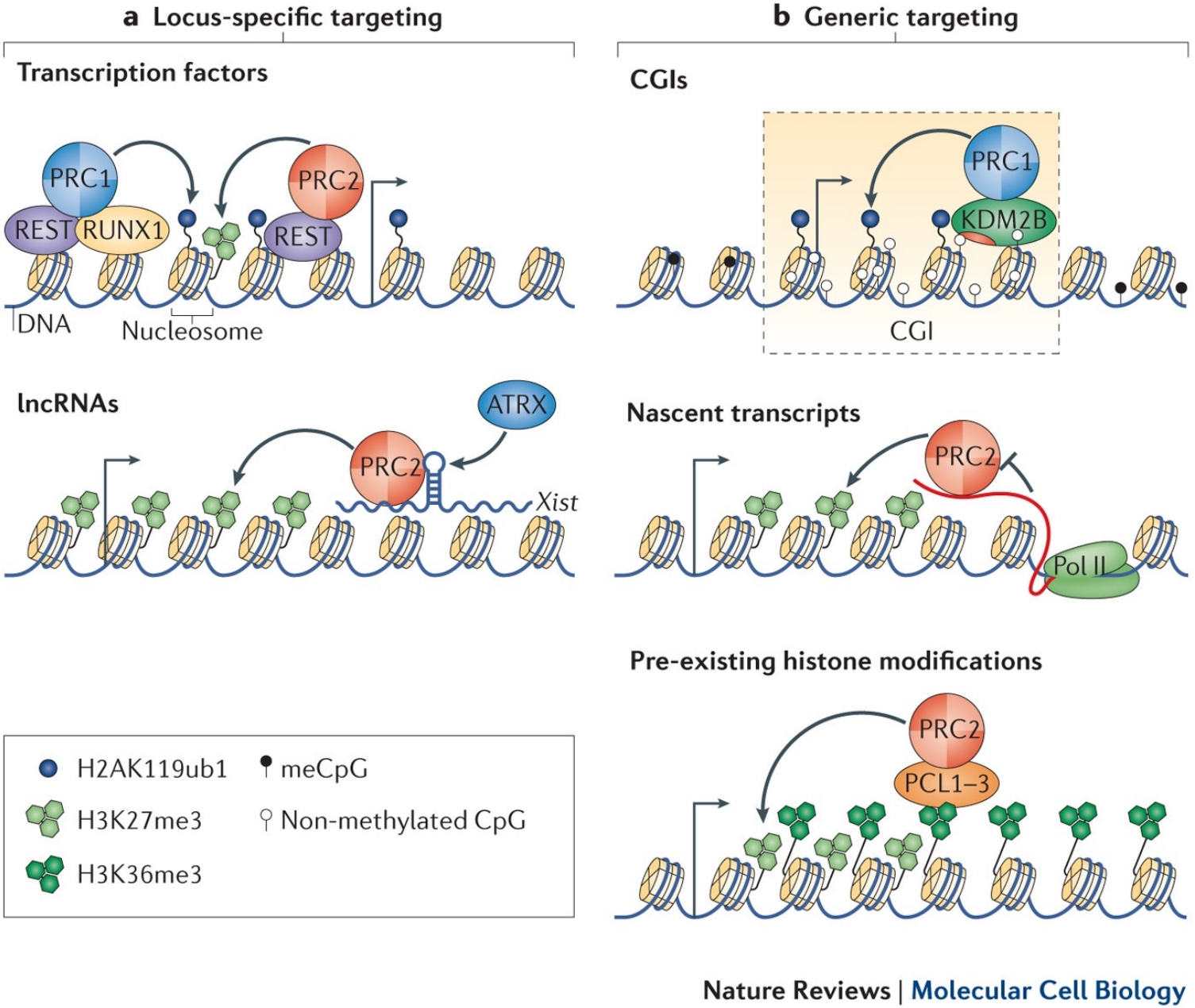
Writing the H3K4 Methylation Mark

Set1A/B COMPASS complexes catalyze mono-, di-, and trimethylation on H3K4 at active promoters. The activity of the partially redundant COMPASS complexes containing MLL3/KMT2C and MLL4/KMT2D leads to the deposition of H3K4me1 at enhancers, facilitating the recruitment of other activators such as CBP/p300. The deposition of methyl marks on H3K4 at bivalent regions is performed by MLL2/COMPASS. Multiple histone demethylases, including members of the KDM1/LSD, KDM2A/FBXL11, and KDM5/JARID families, are implicated in the removal of methyl groups on H3K4.



Recruitment of histone modifying complexes

Recruitment of Polycomb group proteins to target genes



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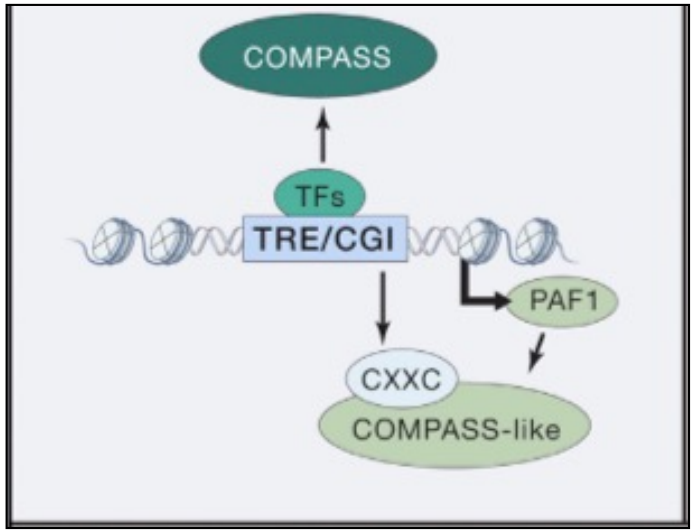
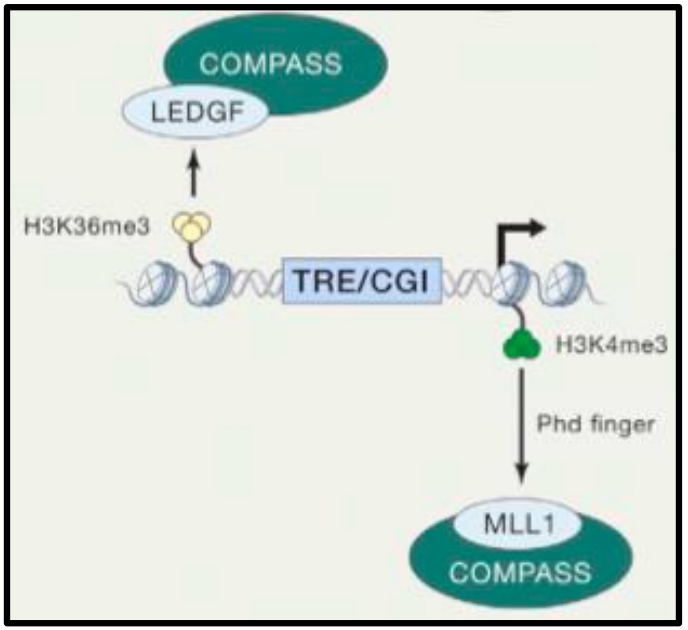
Recruitment of MLL to target genes

- MLL (COMPASS) complexes can bind sequence specifically to their target sites via the CxxC domain of MLL1/2 or can be recruited by TFs such as CFP1 or FOXA1 or via their interaction with PAF1.



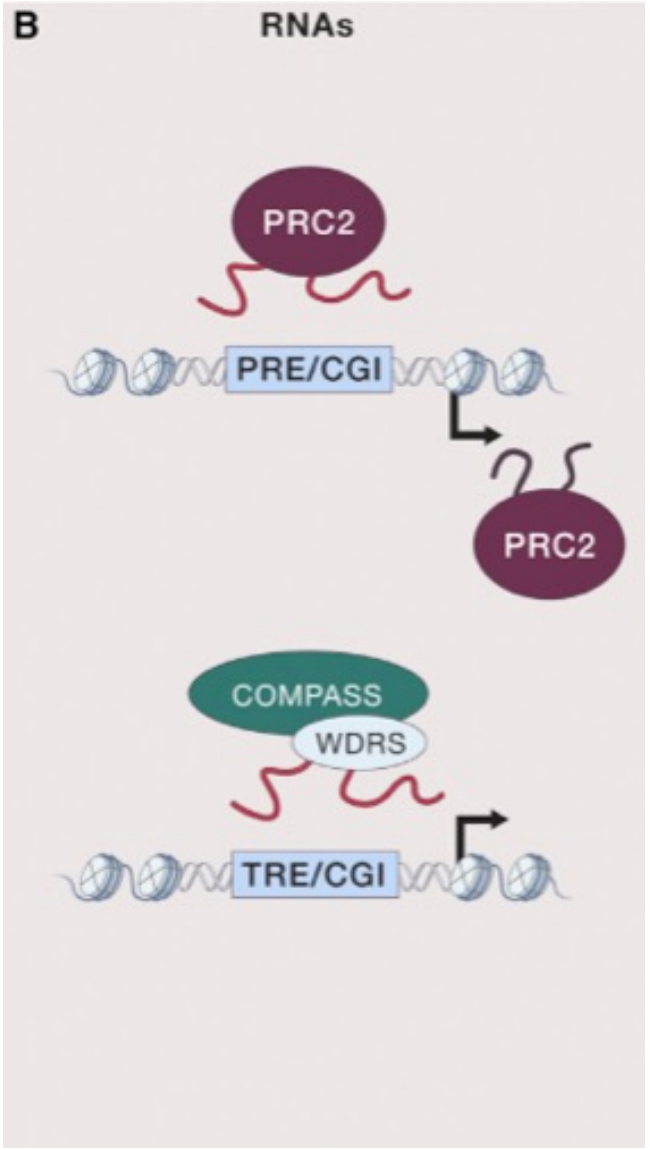
- Tritorax complexes containing MLL1 can be recruited to their target sites via their interaction of the PHD finger with H3K4me3. H3K36me2, deposited by ASH1L, can promote binding of COMPASS complexes via the epigenetic reader protein LEDGF.



RNA-mediated recruitment

- RNA-mediated recruitment. ncRNAs (like XIST, HOTAIR, or KCNQ1, shown in red), or short RNAs transcribed from repressed genes that form stem- loop structures (in black), can induce recruitment of PcG complexes.

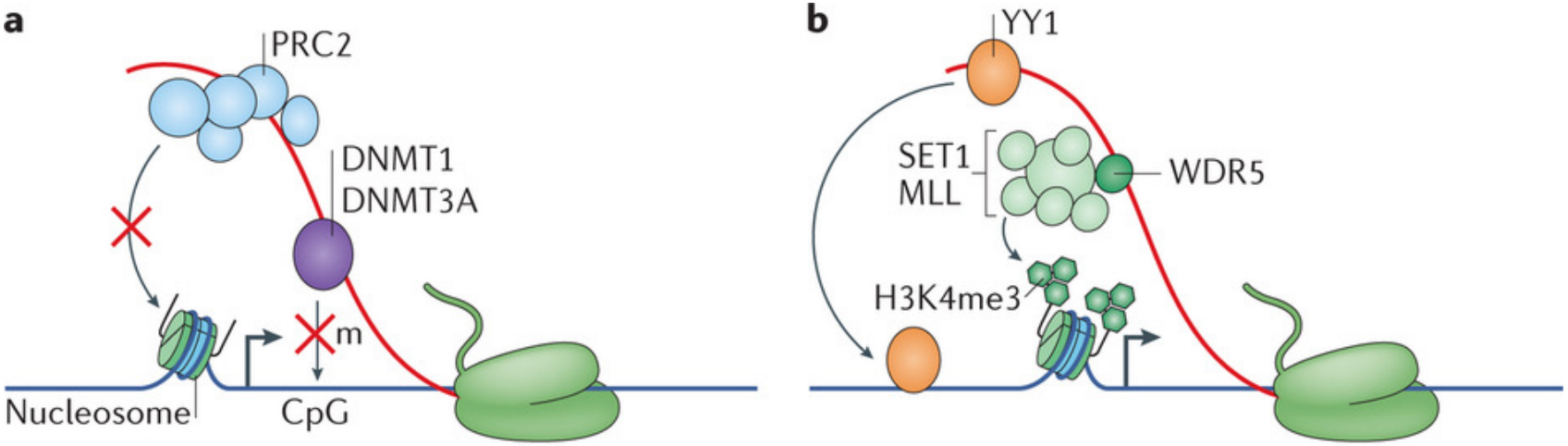
- In a similar manner, HOTTIP ncRNA interacts with the WDR5 subunit of COMPASS to recruit it to the HOXA locus.



Nascent RNA modulates the association of regulatory factors with chromatin to maintain gene activity

a) **Nascent RNA can compete** with chromatin for binding of repressive chromatin modifiers, such as PRC2, and DNMT1 and DNMT3A, which primarily methylate the DNA at CpG dinucleotides.

b) Interaction of the transcription factor YY1 with **nascent RNA facilitates** its transfer to chromatin. Similarly, the interaction of WDR5, which is a component of MLL, with nascent RNA facilitates their transfer to chromatin and H3K4me3, thereby forming a positive-feedback loop that promotes gene expression.



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Model for Role of H3K9 Methylation in Heterochromatin Formation

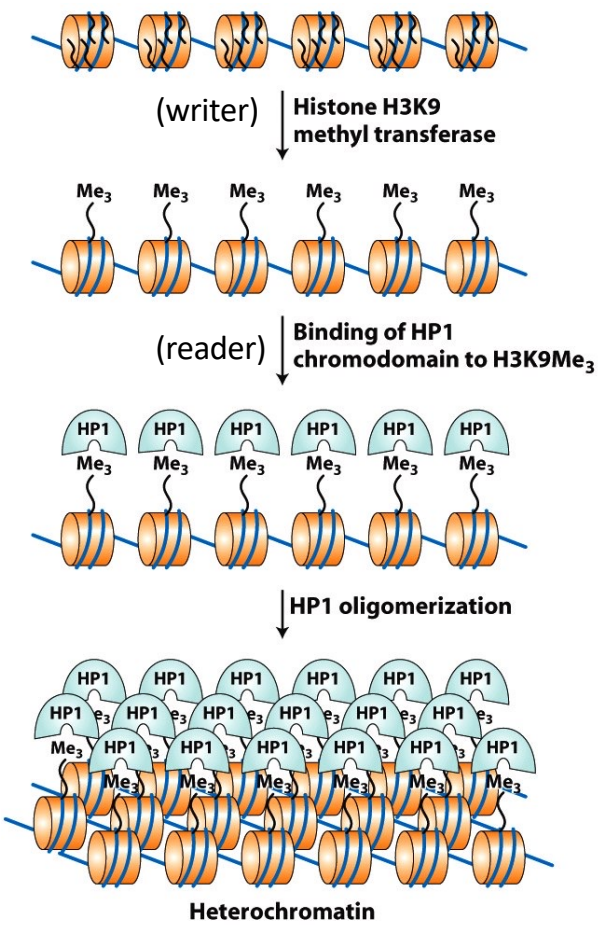


Figure 6-34a
Molecular Cell Biology, Sixth Edition

- HP1 (heterochromatin protein 1) binds to H3K9-Me_3
- HP1 oligomerization
- Spreads along chromatin
- Condenses chromatin into heterochromatin

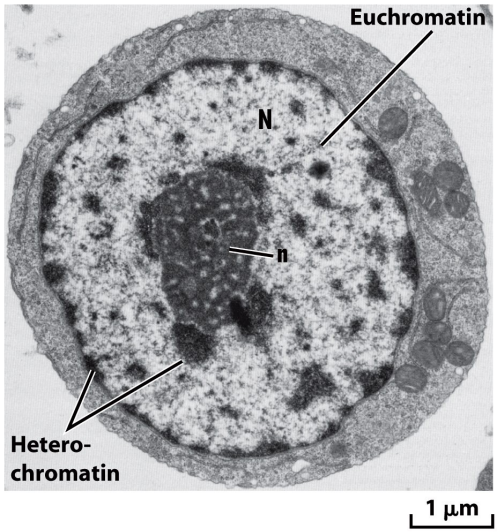
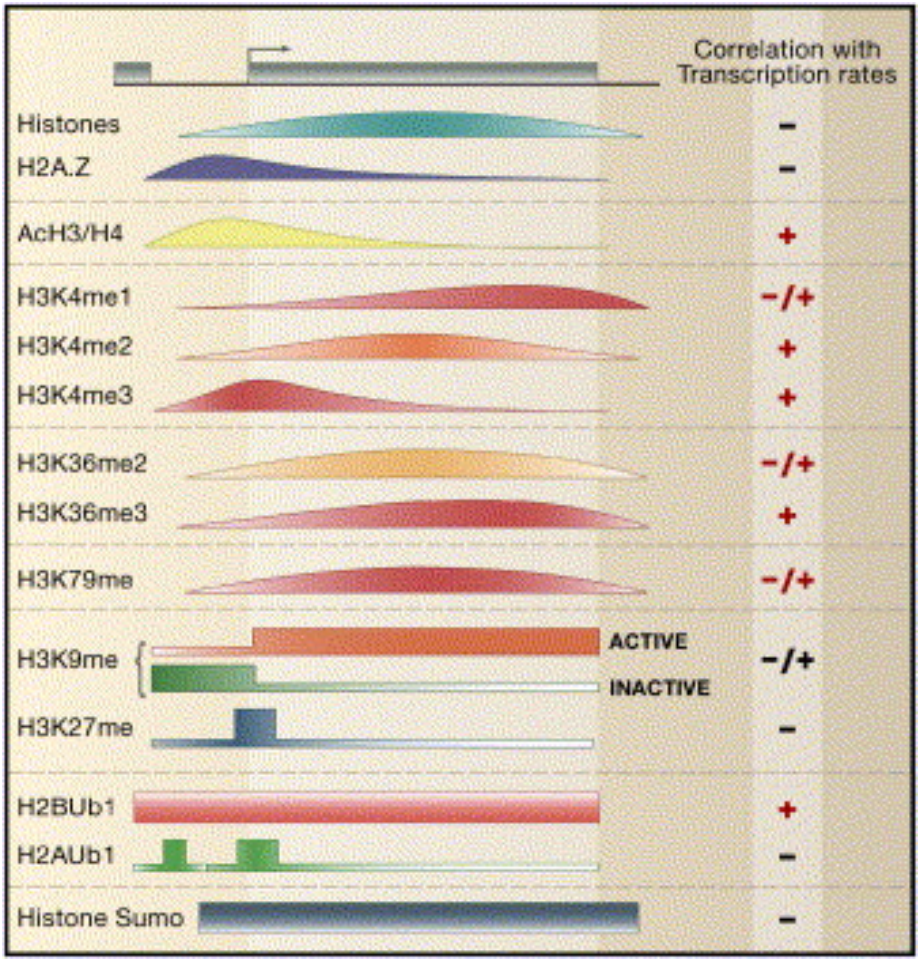


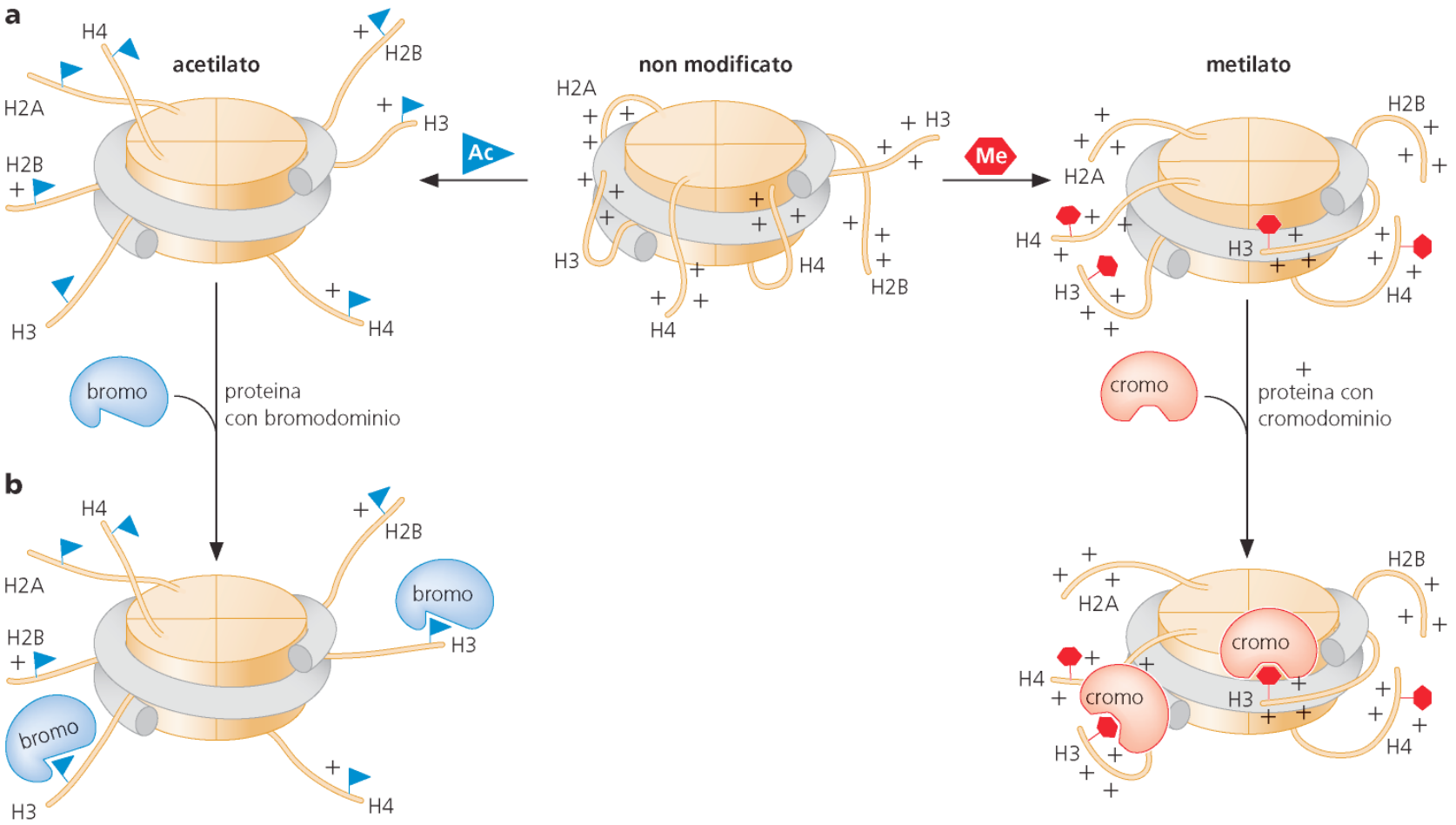
Figure 6-33a
Molecular Cell Biology, Sixth Edition

Histone PTMs regulate transcription

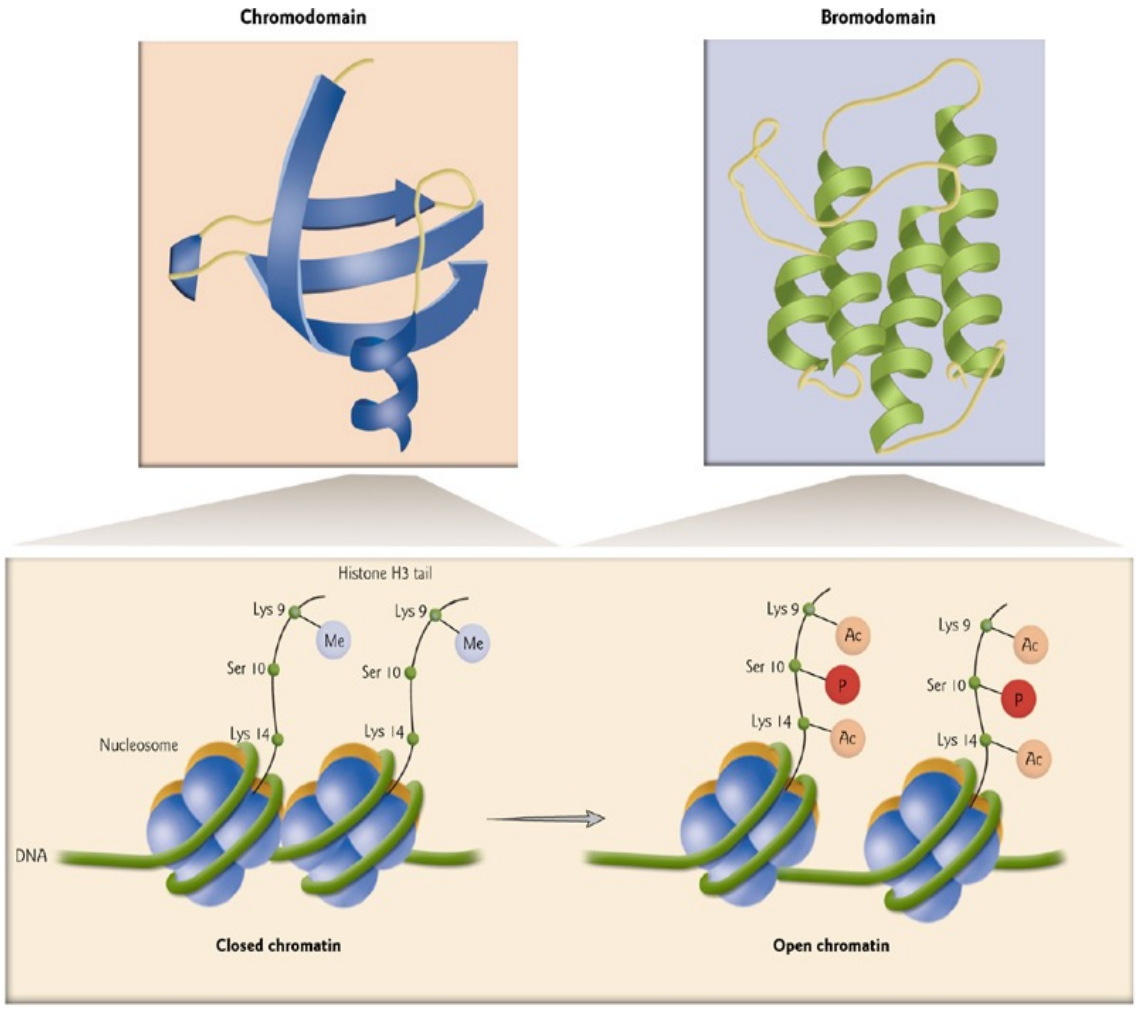


- Histone modifying enzymes (**writers**) generate "the histone code". They usally act in large complexes.
- PTMs dictate chromatin structure and serve as a scaffold for additional regulatory proteins (**readers**).
- Most modifications have been found to be dynamic, and enzymes that remove the modification have been identified (**erasers**).

Histone Modifications are recognized by specific regulative proteins (readers)



Landmarks for Chromatin-binding Proteins



<u>Chromodomain</u>

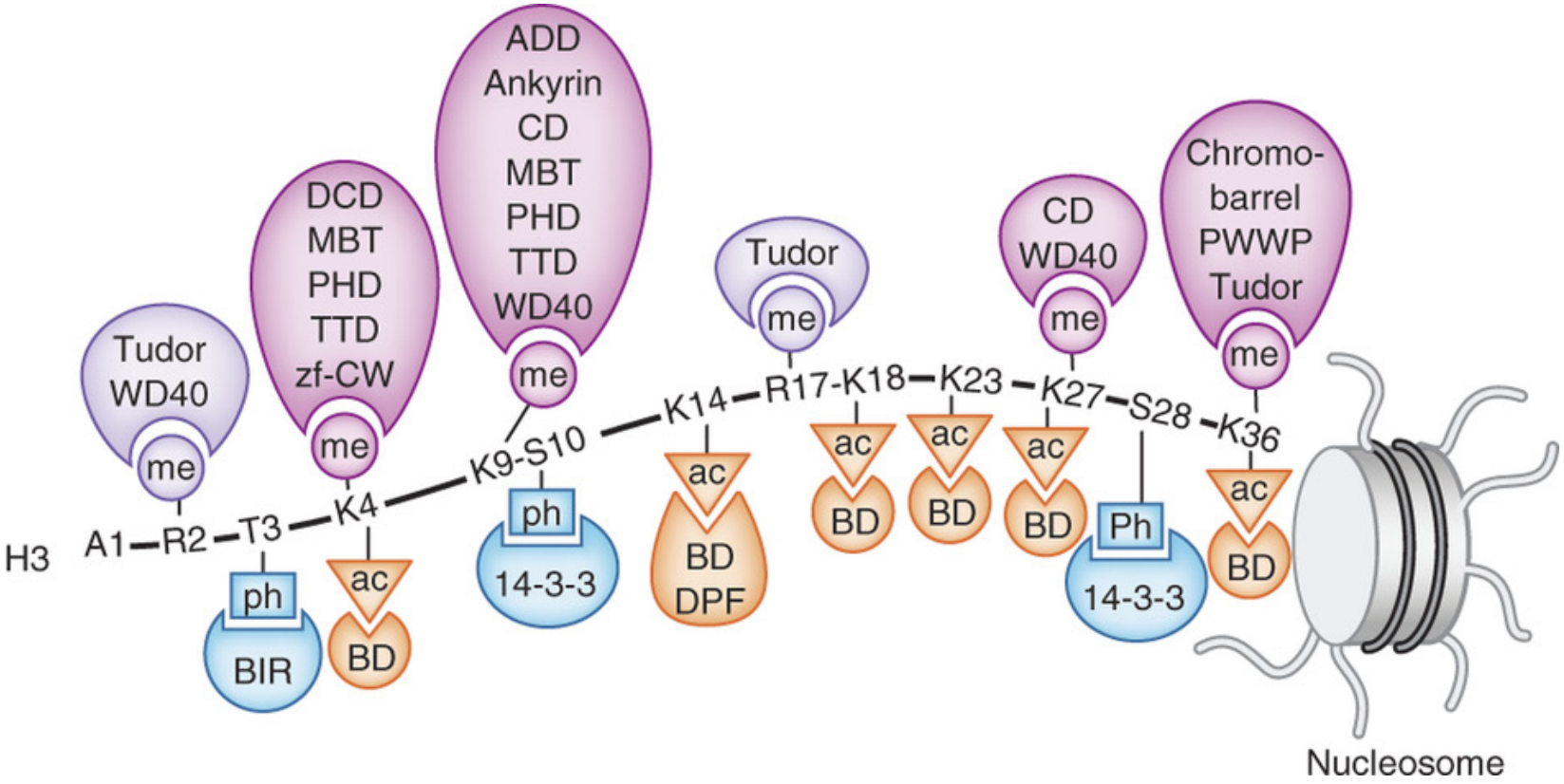
- CH_3(**Me**thyl)- recognition domain
- Targets to **Me-lys**

Bromodomain

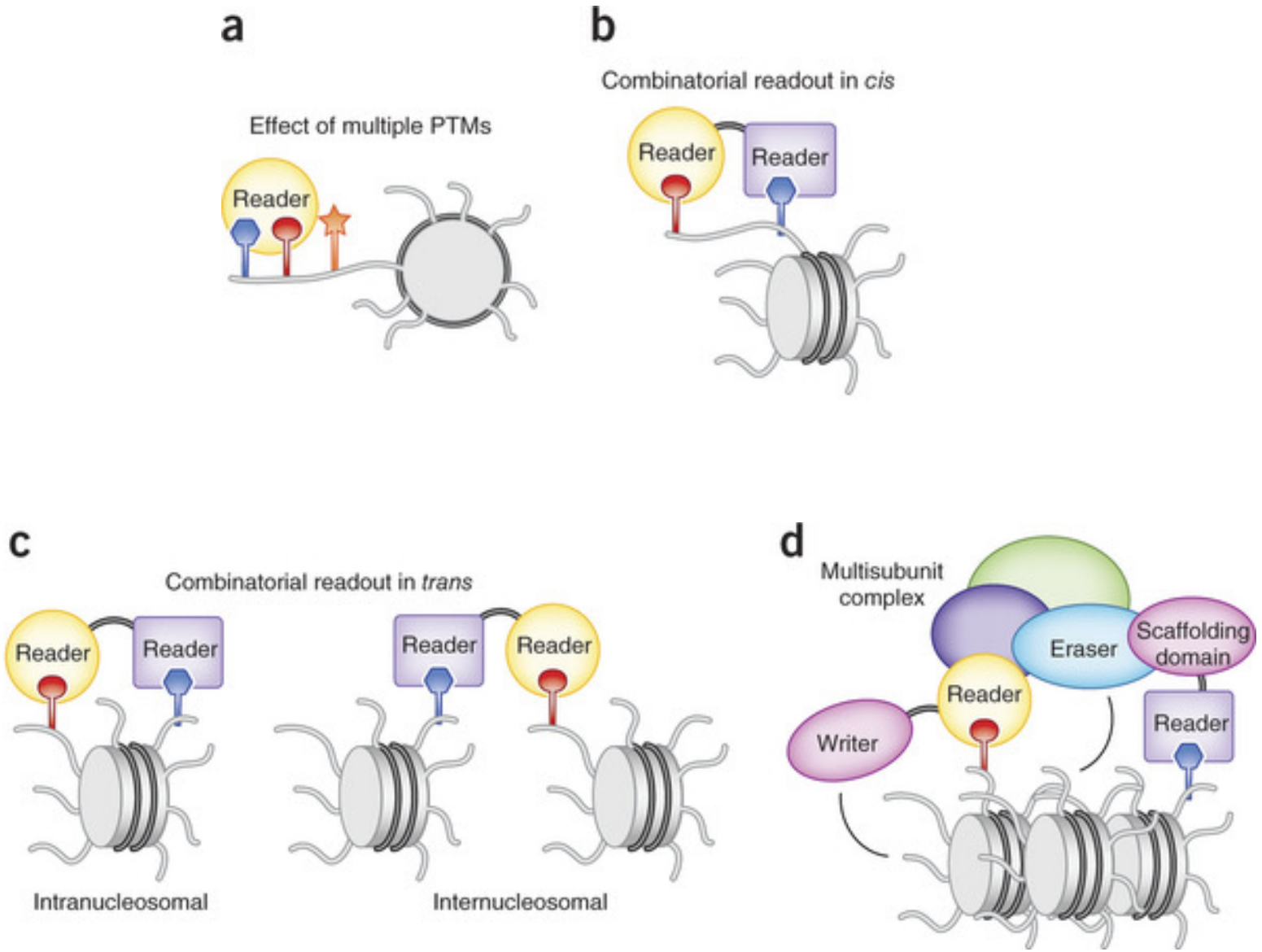
- Binds to acetylated lysines

The list of newly identified histone readers has grown rapidly

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**.



Combinatorial readout of PTMs

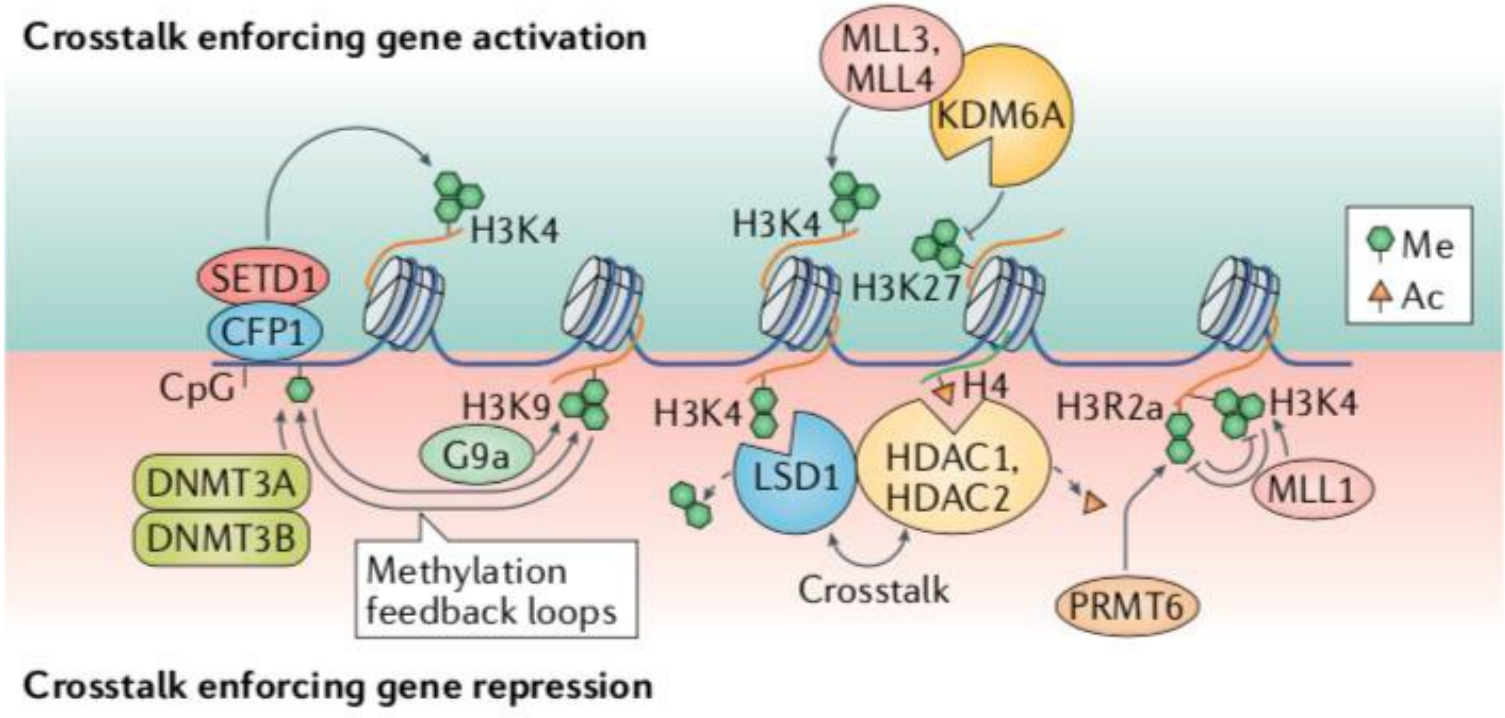


Crosstalk between chromatin marks

- Chromatin is typically marked by multiple modifications, and it is essential that these marks work in concert to achieve a coordinated cellular response. In some cases, the presence or absence of one modification can stimulate or inhibit deposition of another.

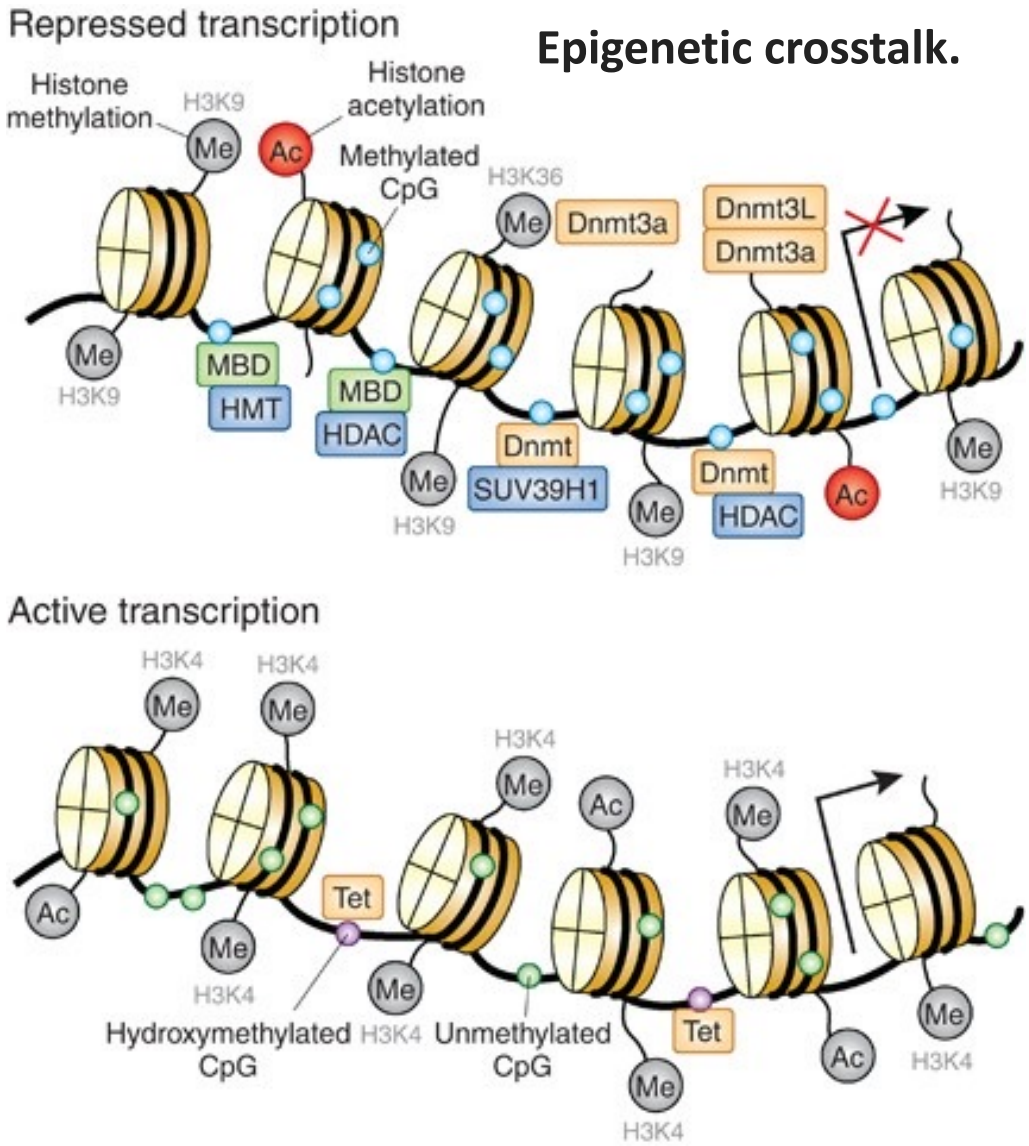
- Several chromatin regulators have important catalytically independent roles in setting chromatin modifications, often by recruiting other enzymes.

- Histone methylations are also highly coordinated with DNA methylation through multiple feedback loops .



Crosstalk of DNA Methylation and Other Epigenetic Mechanisms

Transcription is ultimately regulated by the interaction of multiple epigenetic mechanisms that cooperate to activate or silence gene expression.



Epigenetic crosstalk.

To suppress gene expression, Dnmts target CpG sites and actively methylate DNA. DNA methylation is recognized by methyl-binding proteins such as that along with Dnmts recruit enzymes that modify the histone tails including histone deacetylases (HDACs), which remove acetylation (Ac), and histone methyltransferases (HMTs), which methylate histones (Me) and in conjunction with DNA methylation serve to further repress gene expression.

In regions of DNA with activate transcription, Tet removes DNA methylation, and histone tails in this region often contain H3K4me^3 that inhibits Dnmt binding to unmethylated CpG sites and maintains a permissive environment for transcription.

Regulation of transcription by chromatin

1. Opening of chromatin through directed **modication of histone tails** (e.g. acetylation and methylation)

2. Histone variants

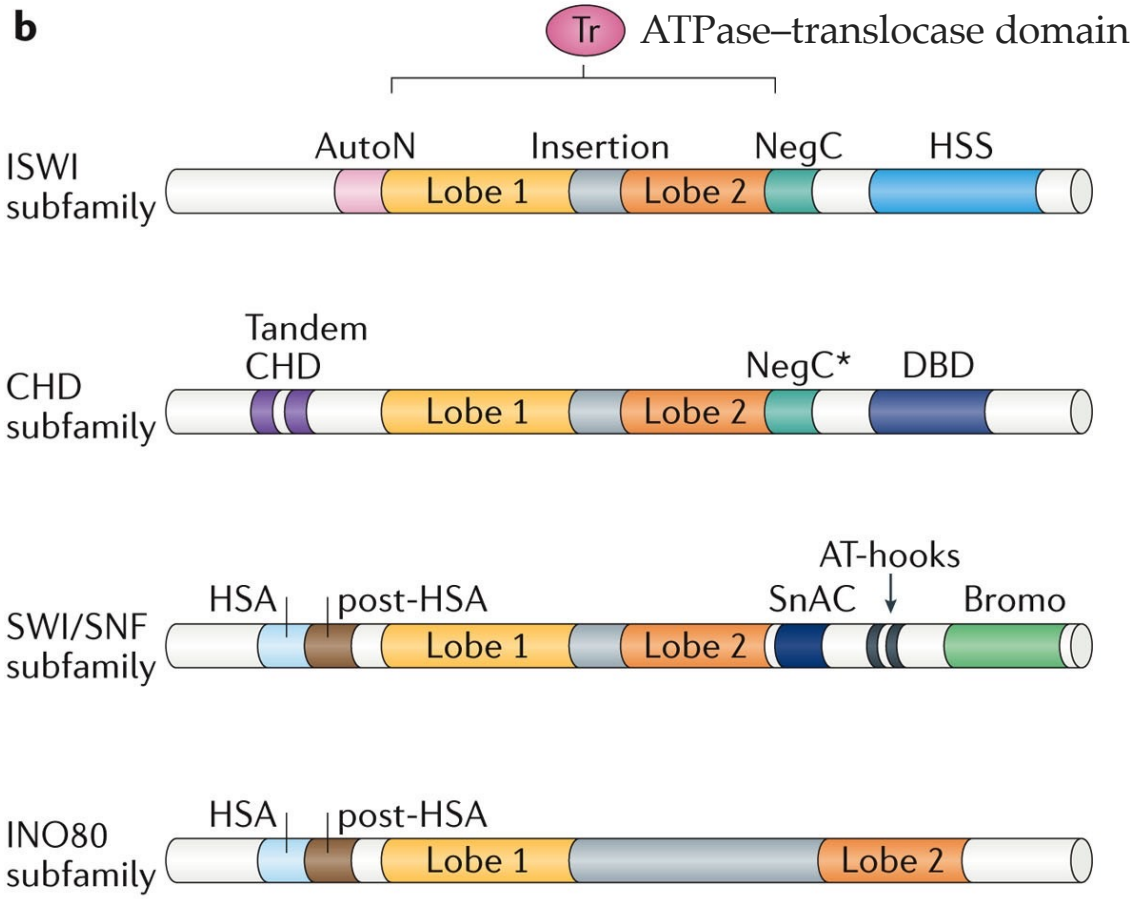
3. Opening of chromatin through directed **nucleosome mobilization** by **remodeling complexes**

4. **Positioning of nucleosomes** creates promoters with different requirement for remodeling

Three families of ATP-driven remodeling factors

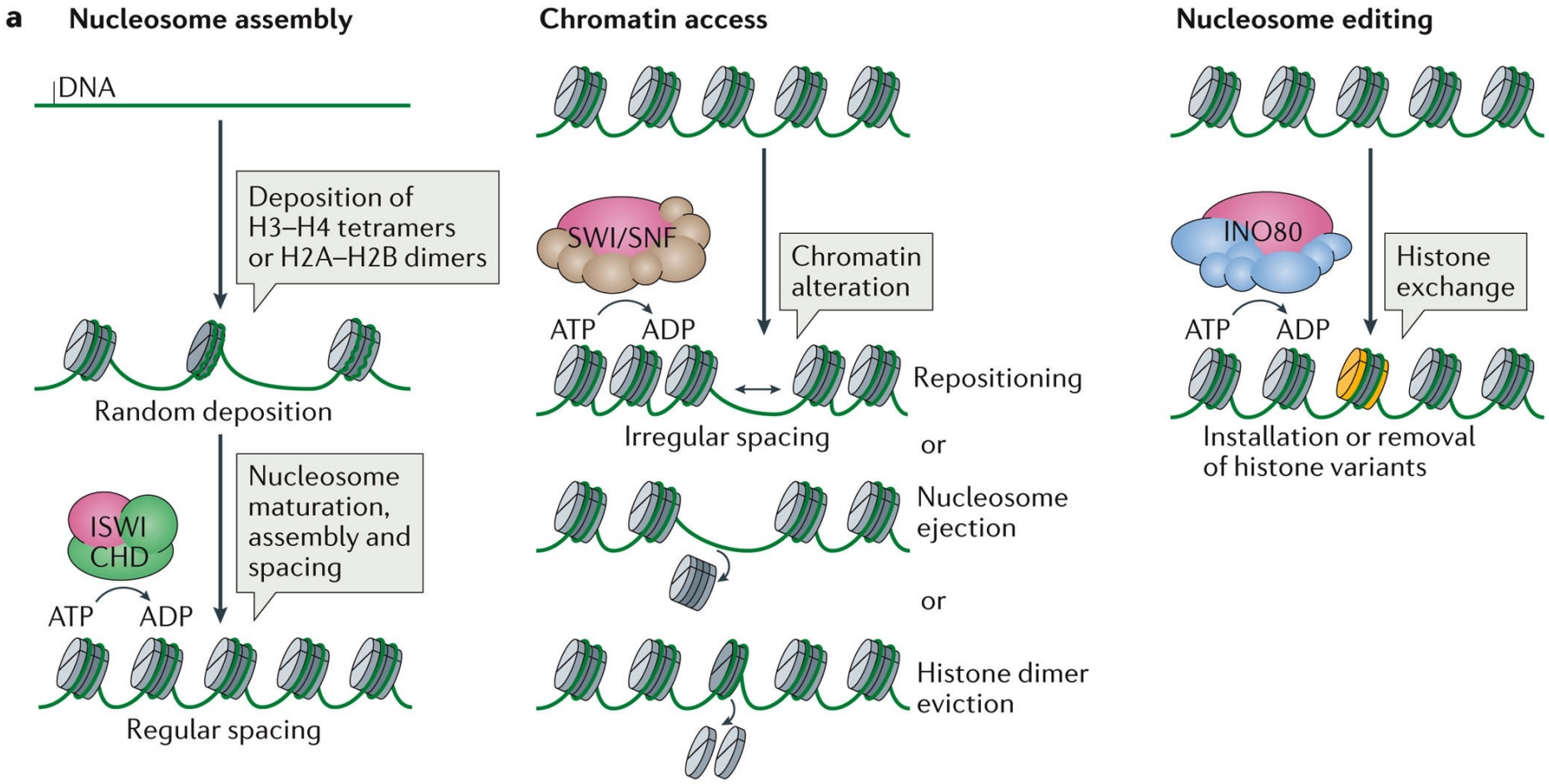
Chromatin remodellers can be classified into four subfamilies: imitation swtich (**ISWI**), chromodomain helicase DNA-binding (**CHD**), switch/sucrose non-fermentable (**SWI/SNF**) and **INO80**, on the basis of the similarities and differences in their catalytic ATPases and associated subunits.

Each subfamily is specialized to preferentially achieve particular chromatin outcomes: assembly, access or editing.



Clapier et sl., 2017 Nature Reviews Molecular Cell Biology

Functions of chromatin remodellers

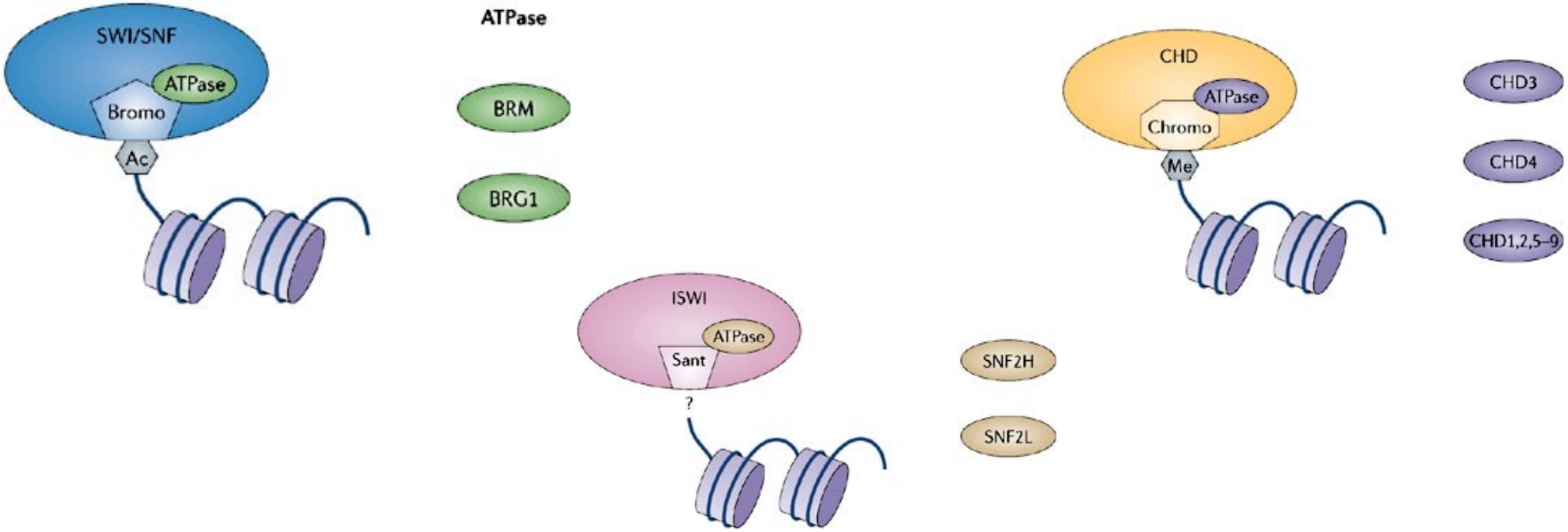


SWI/SNF subfamily remodelers typically facilitate chromatin access, as they slide and eject nucleosomes, and are used for either gene activation or gene repression.

Clapier et sl., 2017 Nature Reviews Molecular Cell Biology

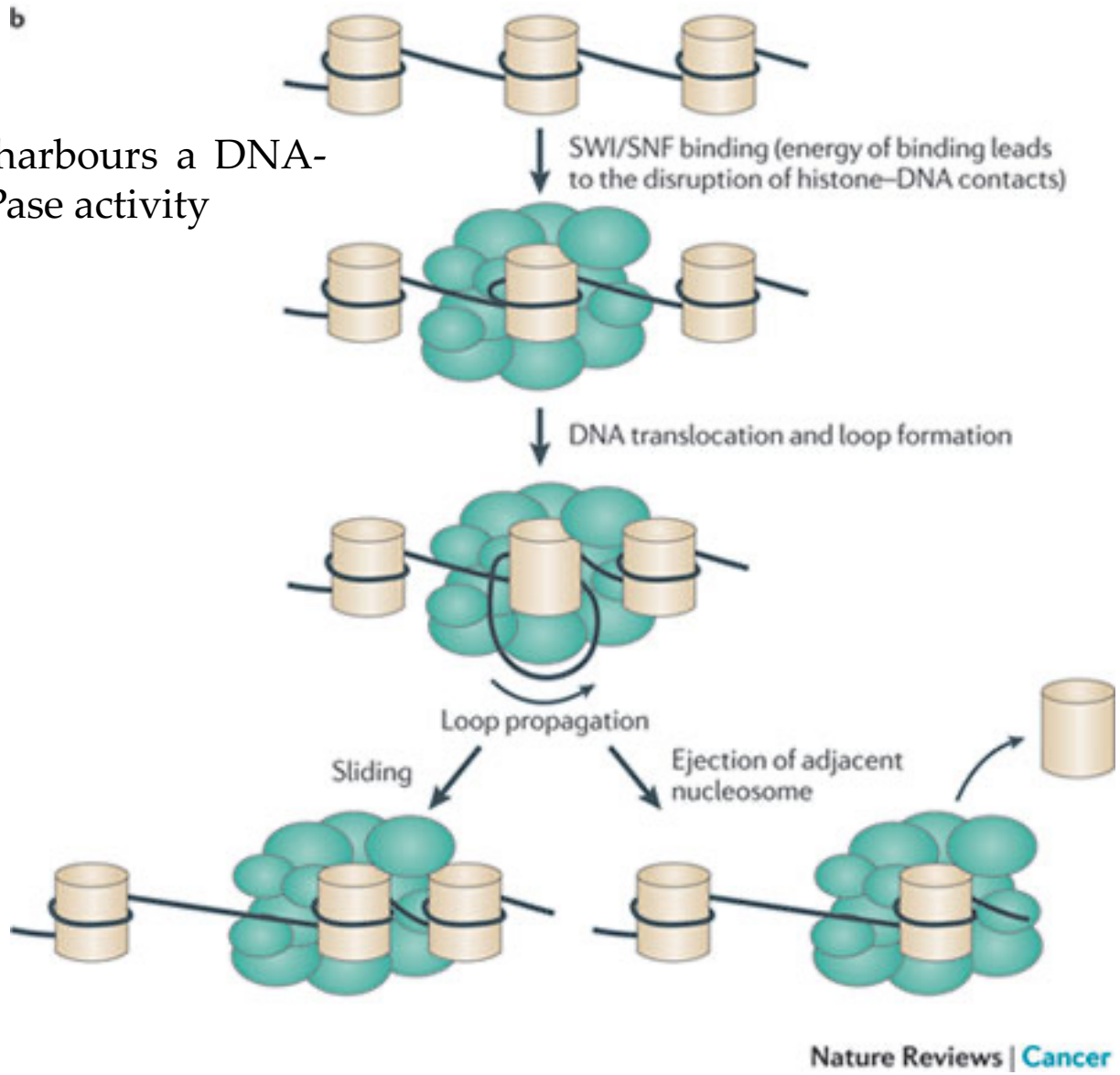
Recruitment

Each remodeliling complex has a unique domain (bromo, chromo and sant), and these are known or thought to interact with specific chromatin substrates. Moreover, they interact only on a subset of genes (Genome-analysis in yeast shows that <5% of all gener require Swi/Snf). Diversity in the protein composition of remodellers enables their specific interaction with particular transcription activators, repressors and histone modifications, which together specify targeting.



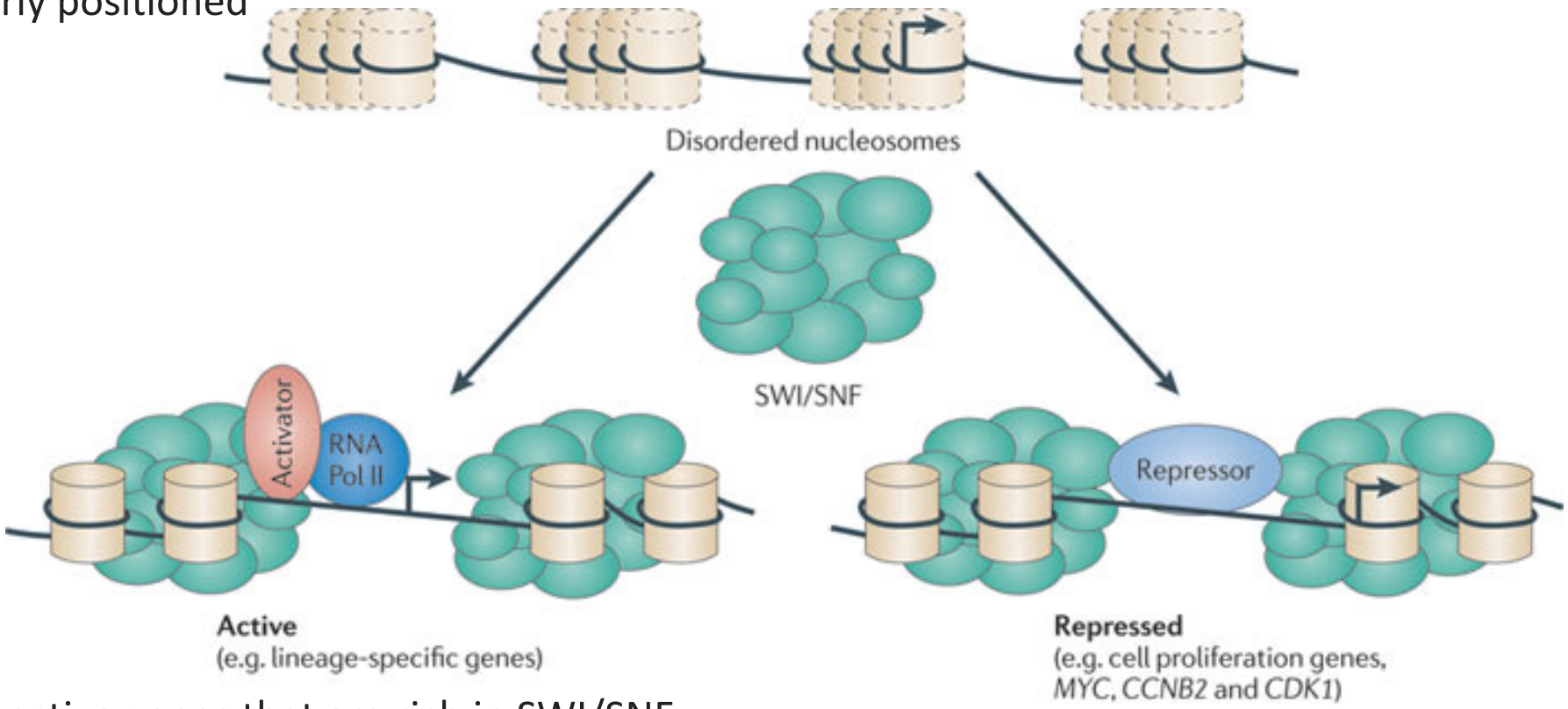
Possible activities of Swi/Snf

SWI2-subunit harbours a DNA-stimulated ATPase activity



Possible activities of Swi/Snf

SWI/SNF complexes are markedly under-represented at silent genes where nucleosomes are poorly positioned



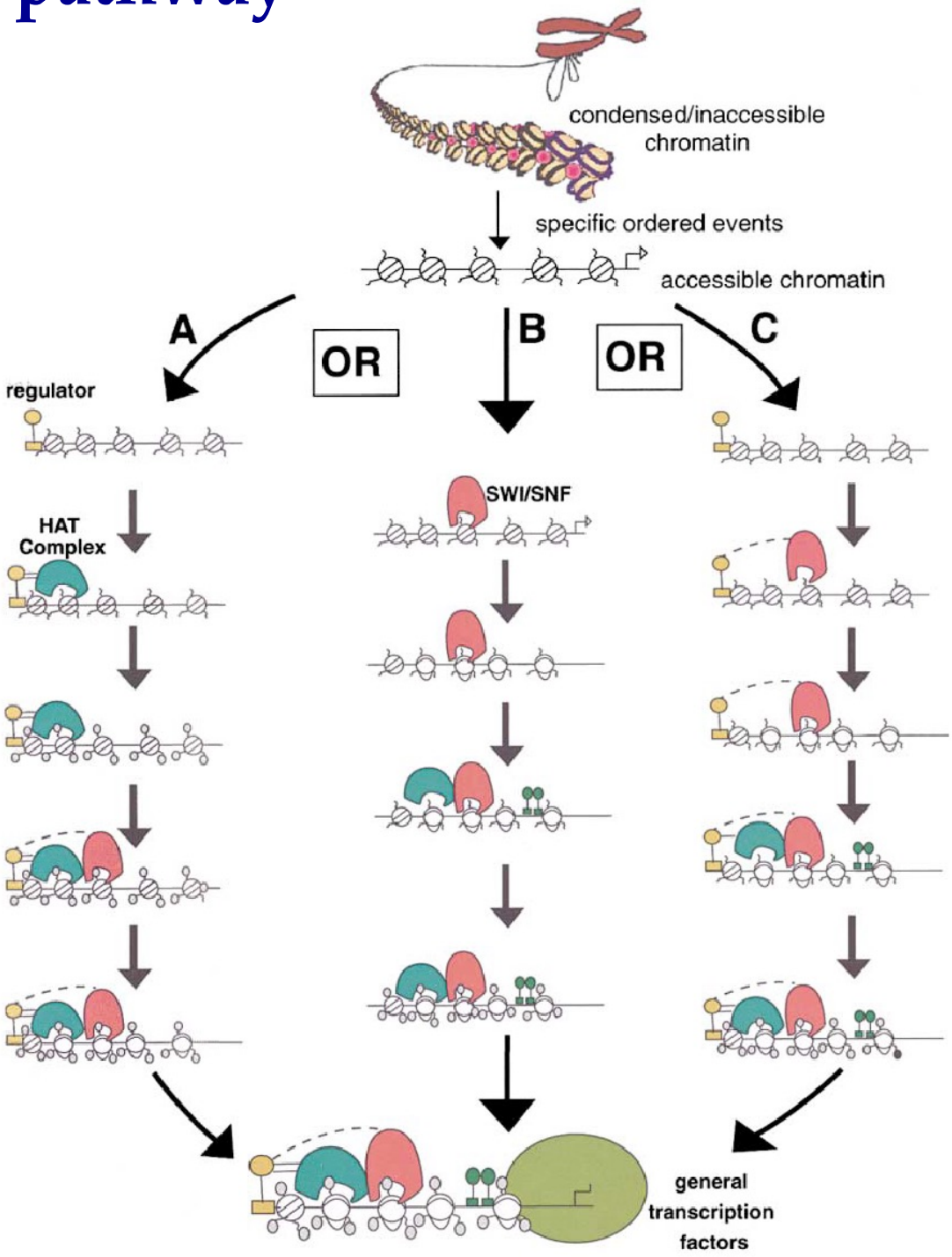
At active genes that are rich in SWI/SNF binding, the transcription start site is flanked by precisely positioned nucleosomes, thus providing access to a nucleosome-depleted region that contains transcription factor binding sites.

SWI/SNF complexes also contribute to the dynamic silencing of targets that are required for lineage-specific differentiation and that facilitate the binding of repressors

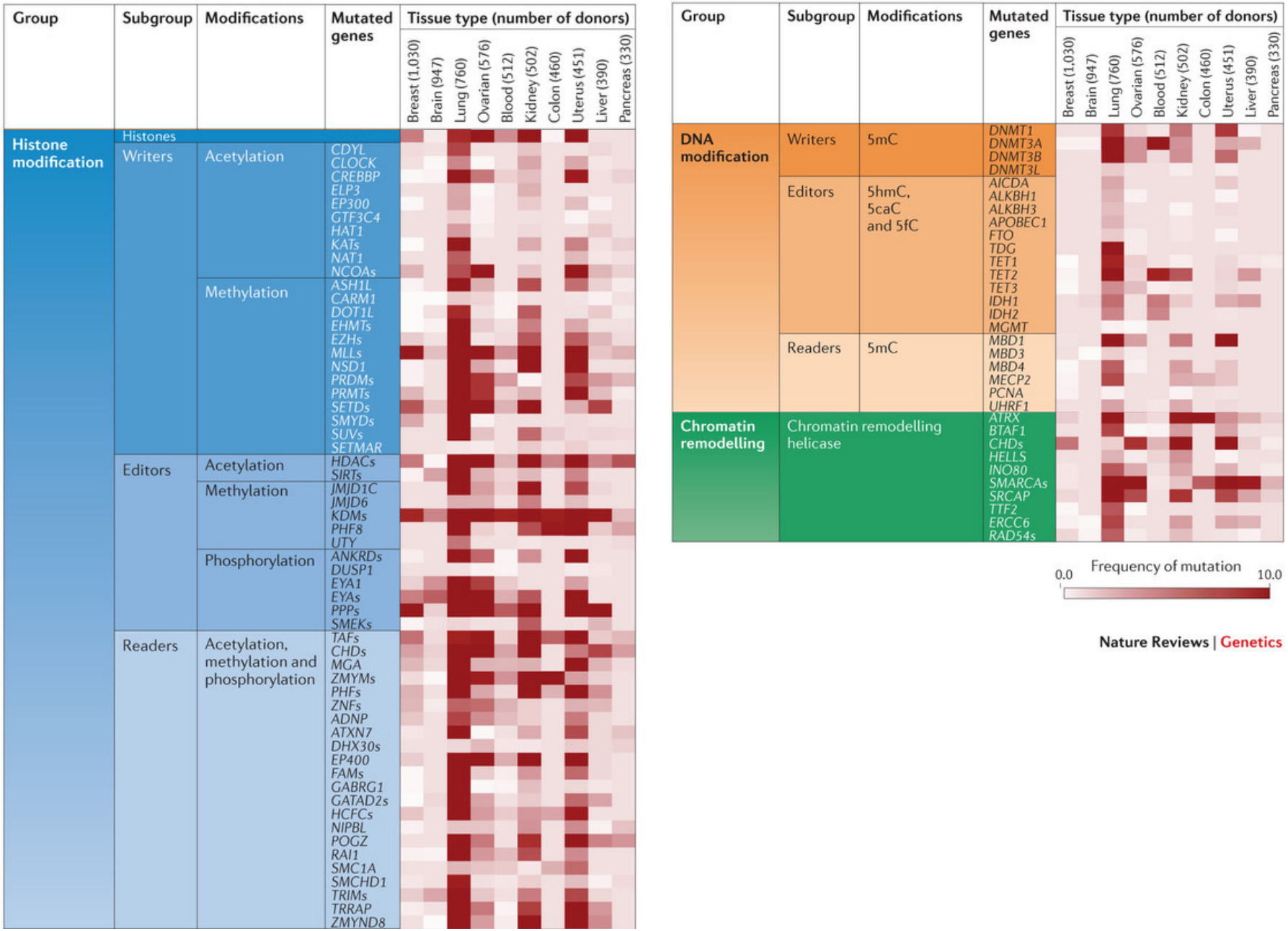
Each gene a specific pathway

- It appears that there is no obligate order for function of ATP-dependent remodelers and covalent modifiers that is general for all promoters.

- Rather, it seems that each individual promoter will work using a set order of action by these complexes that differs from promoter to promoter.



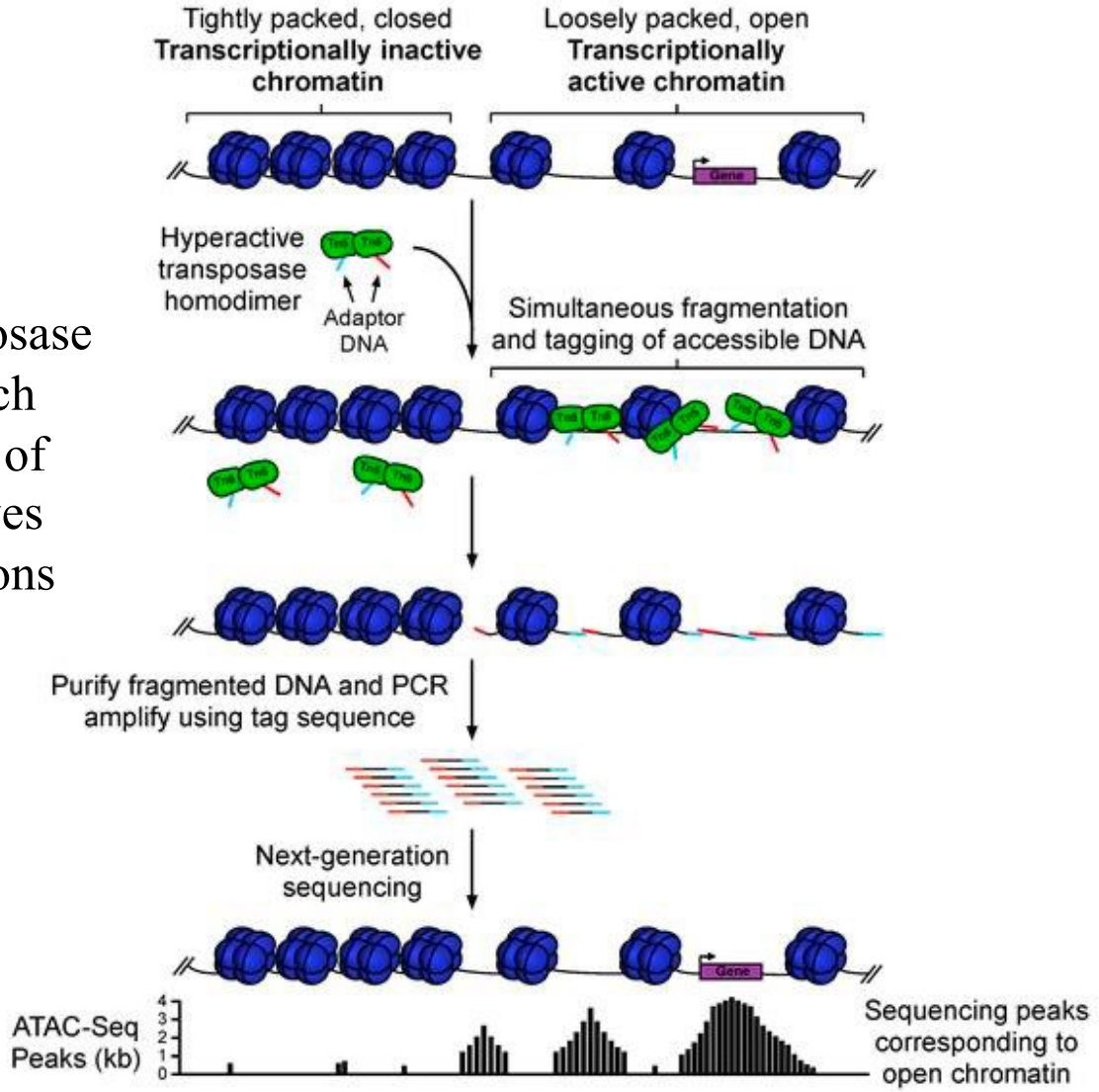
Mutations in regulators of the epigenome identified in cancer



old and novel methodologies for epigenomic studies

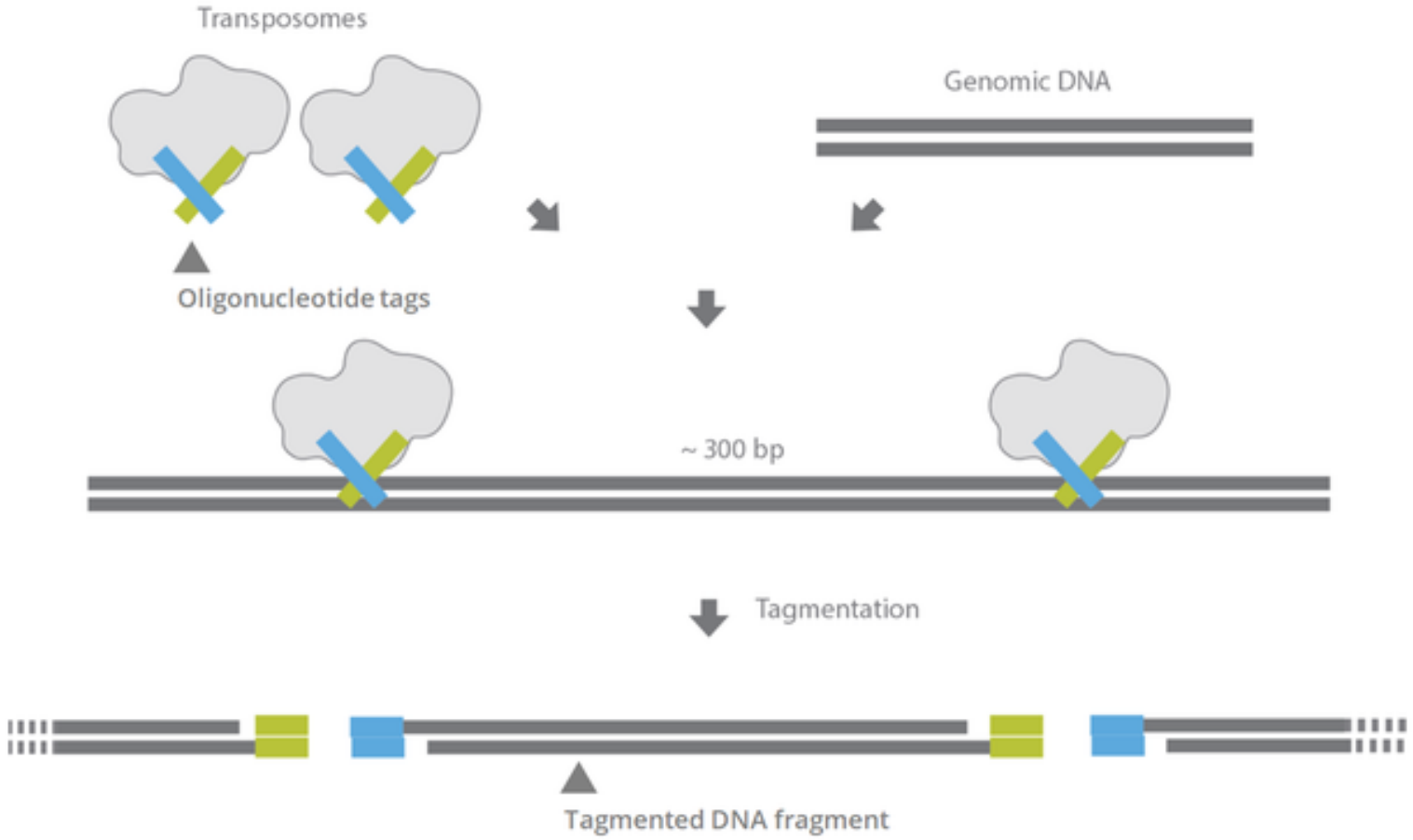
ATAC-seq for chromatin-accessibility studies

Assay for transposase-accessible chromatin sequencing (ATAC-Seq) employs a hyperactive form of Tn5 transposase to identify regions of open chromatin, which are important for global epigenetic control of gene expression. Tn5 simultaneously cleaves and adds adapters to nucleosome-free regions of DNA, priming them for sequencing.



ATAC-seq for chromatin-accessibility studies

Tagmentation is transposase-based methods to prepare high-throughput sequencing libraries, in which a hyperactive transposase is used to simultaneously fragment target DNA and append universal adapter sequences.



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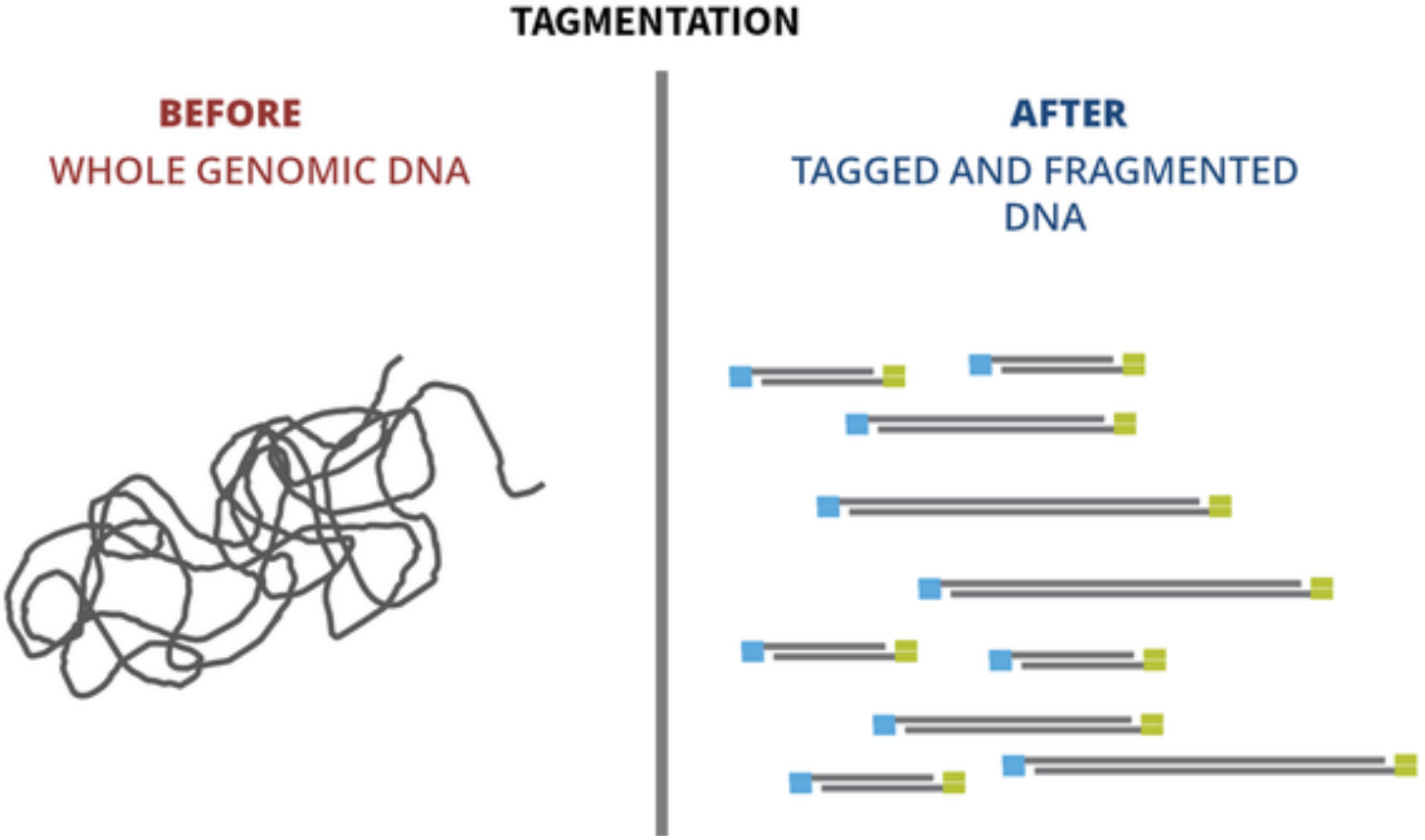
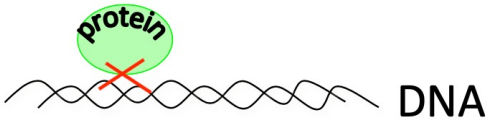


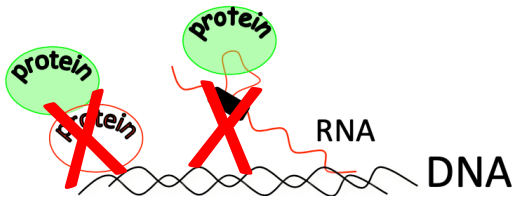
Chromatin Immunoprecipitation (ChIP)

AIM: Identification of the genomic loci bound to a DNA binding protein.

Chromatin Immunoprecipitation (ChIP) is an immunoprecipitation technique used to investigate the interaction between proteins and DNA in the cell after they have been chemically crosslinked. It aims to determine whether specific proteins are associated with specific genomic regions, such as transcription factors on promoters. It can also be utilized for proteins **not directly** bound to DNA but "close" to chromatin.



direct

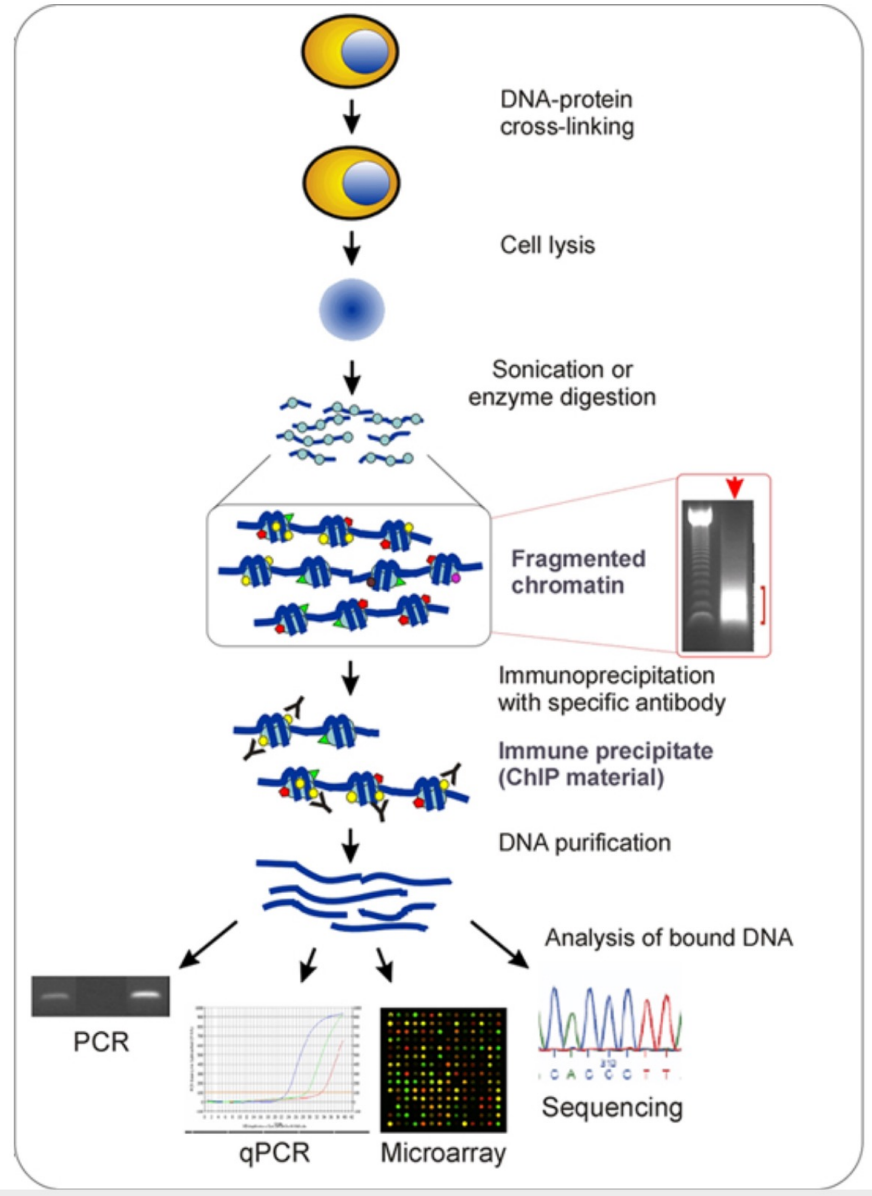


indirect

Chromatin Immunoprecipitation (ChIP)

Work flow:

1. **Cell Crosslinking**
2. **Chromatin Sonication**
3. **Antibody -Extract incubation**
4. **Binding between Beads and Antibody**
5. **Reverse crosslink and DNA purification**
6. **DNA analysis**



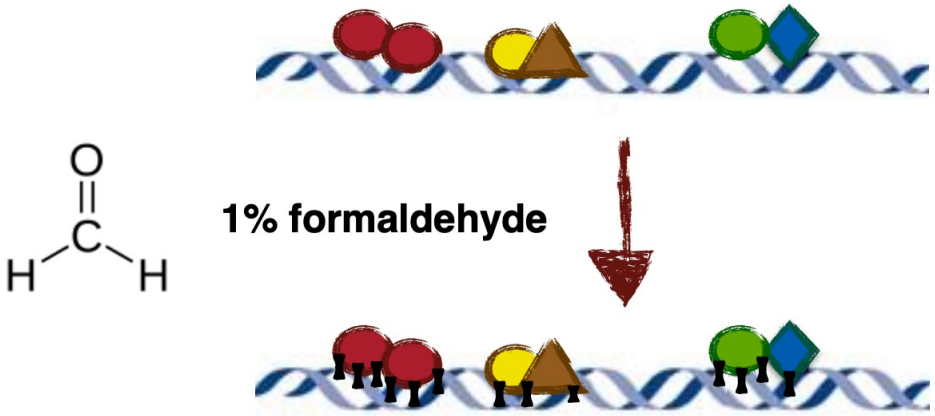
Chromatin Immunoprecipitation (ChIP)

1. **Cell Crosslinking**

The cross-linking is an experimental procedure that convert in **covalent** all the weak and non-covalent interactions between DNA - PROTEINS and PROTEIN-PROTEIN

Crosslinking strategies:

Formaldehyde
Glutaraldehyde



Chromatin Immunoprecipitation (ChIP)

1. Cell Crosslinking

Formaldehyde Cross-linking

- Formaldehyde is an organic compound. It is water soluble and penetrates biological membranes. It targets **primary aminogroups** (i.e. lysine in proteins, side chains of A,C,G in DNA)

- It crosslinks both **protein-nucleic acids**, **nucleic acids- nucleic acids** and **protein-protein**

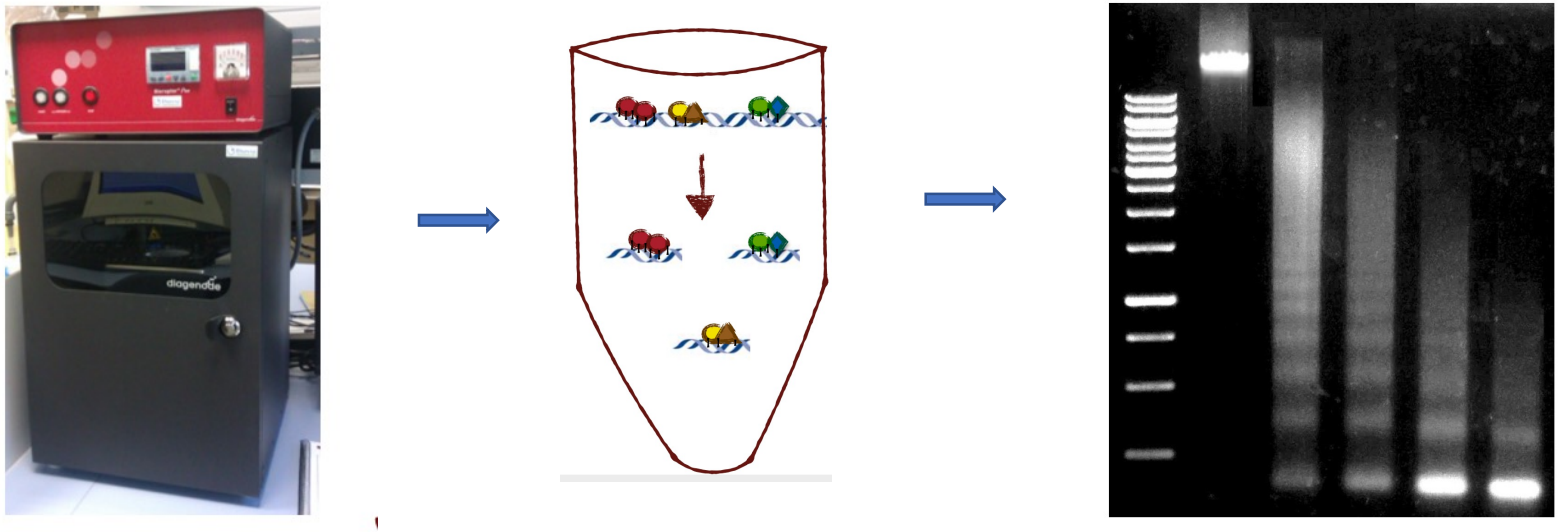
- The crosslinking is reversible (65°C reverse protein-DNA; 100°C reverse protein-protein)

- Reaction is stopped by providing an excess of primary amino groups (0.125M glycine)

Chromatin Immunoprecipitation (ChIP)

2. Chromatin Sonication

The DNA-protein complexes (chromatin-protein) are then sheared into ~500 bp DNA fragments by **sonication** or nuclease digestion.

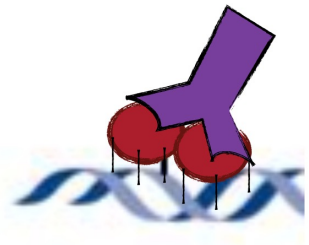


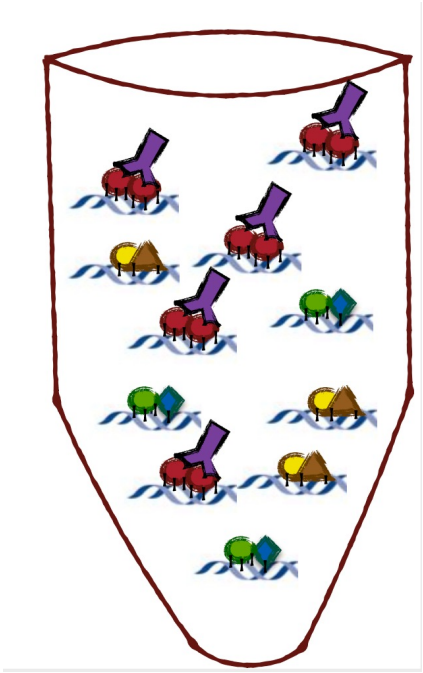
size range: 100-500 bp

Chromatin Immunoprecipitation (ChIP)

3. Antibody -Extract incubation

The antibody specific for the protein of interest, is incubated together with the extract, this step allows the formation of strongly interaction between the antibody and the protein of interest

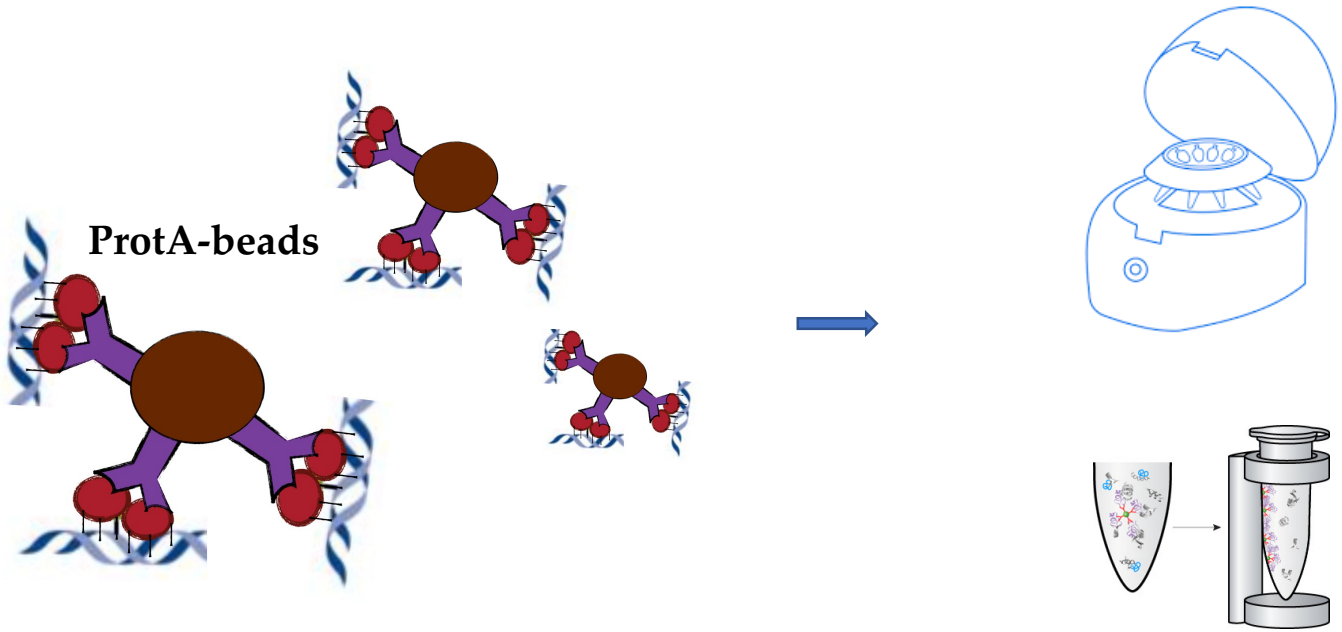




Chromatin Immunoprecipitation (ChIP)

4. Binding between Beads and Antibody

Agarose, Sepharose or Magnetic beads (Protein A or Protein G conjugated) are added to the extract. This step is fundamental for the precipitation of the complex **Bead-Antibody-Protein-DNA**

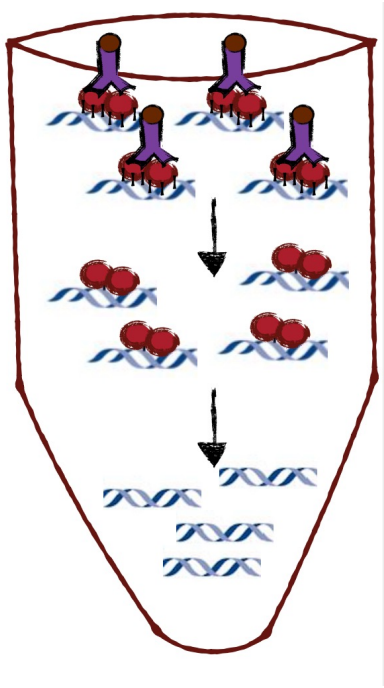


Centrifugation or magnetic recovery step allows the purification of the complexes that are bound to the antibody

Chromatin Immunoprecipitation (ChIP)

5. Reverse crosslink and DNA purification

The cross-linking with formaldehyde is removed through the incubation of the extract at High temperature (70° C for 5 minutes). This step allows the detach of the protein from the DNA that is subsequently purified by Proteinase K digestion and phenol extraction.

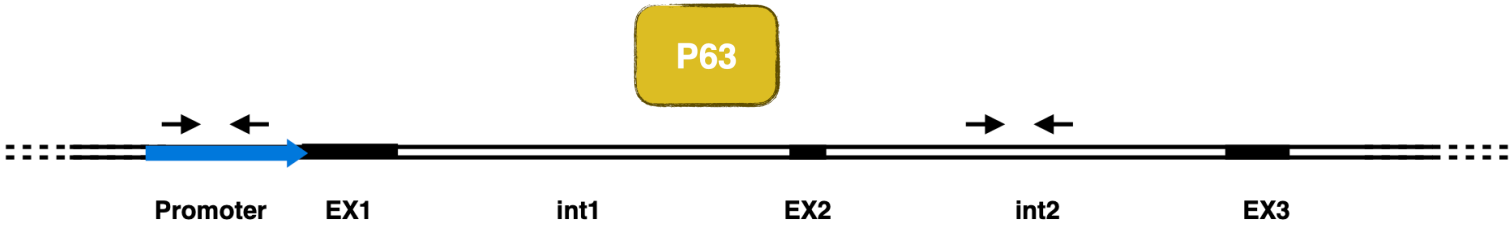


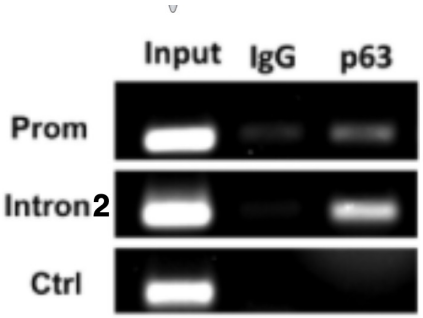
Chromatin Immunoprecipitation (ChIP)

6. DNA analysis

ChIP (PCR)

The isolated DNA can be quantified by PCR using specific probes. This allows the analysis of a specific region in multiple samples.





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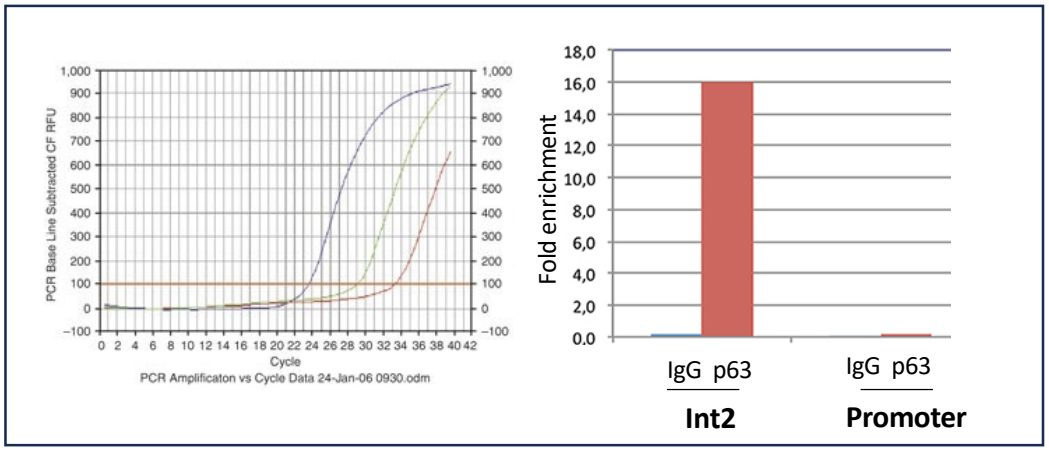
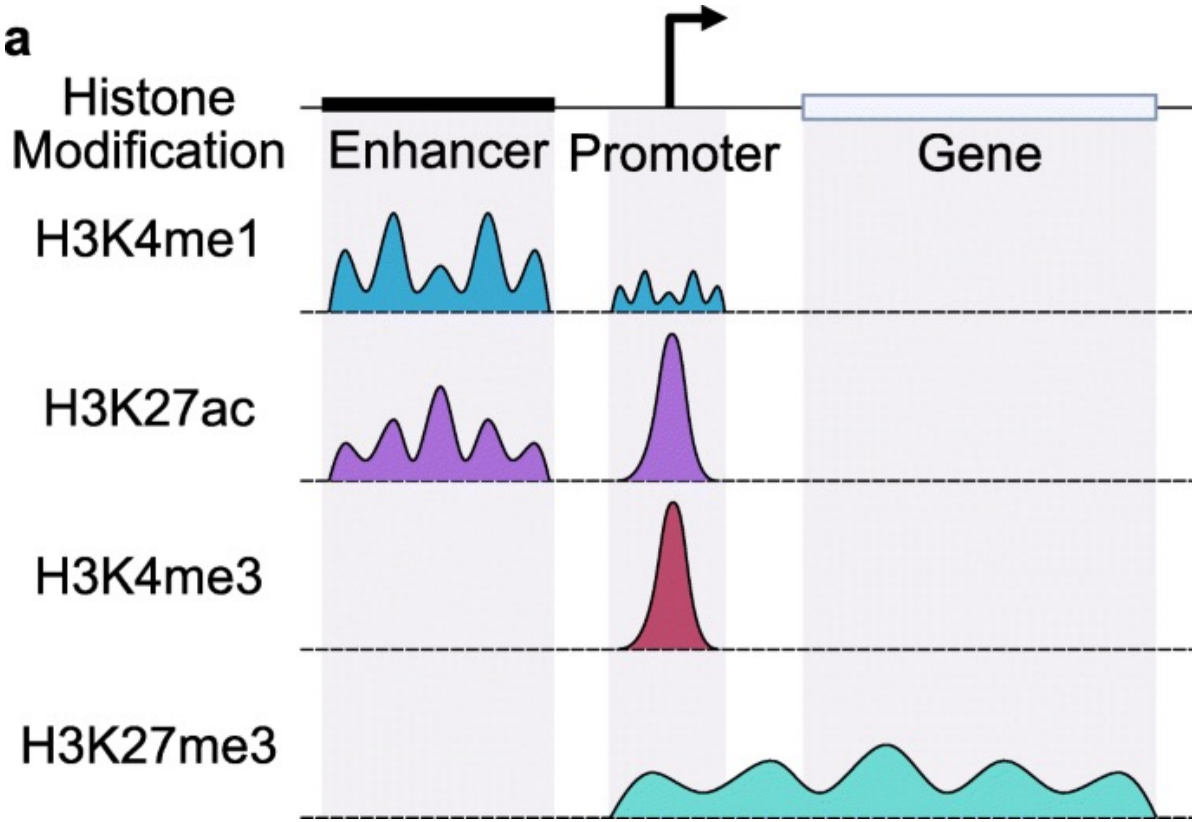


Chromatin Immunoprecipitation (ChIP)

6. DNA analysis

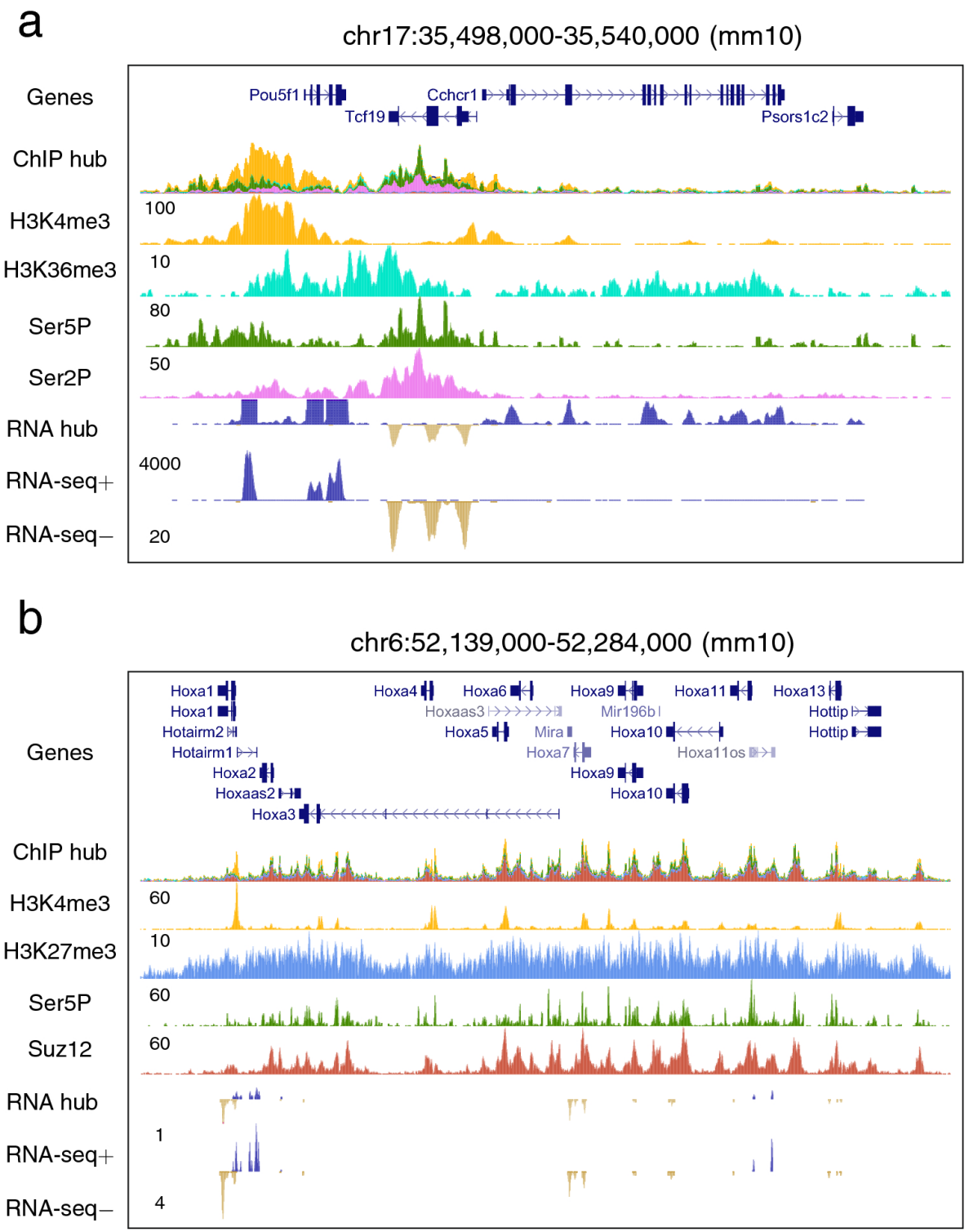
ChIP Seq

Direct sequencing of the DNA isolated, generates genome wide profiles. ChIP-seq combines ChIP and direct sequencing technology for genome-wide analysis of antigen distribution. Immunoprecipitated DNA is sequenced and mapped to the genome



Actively transcribed region containing
the *Oct4/Pou5f1* pluripotency gene.

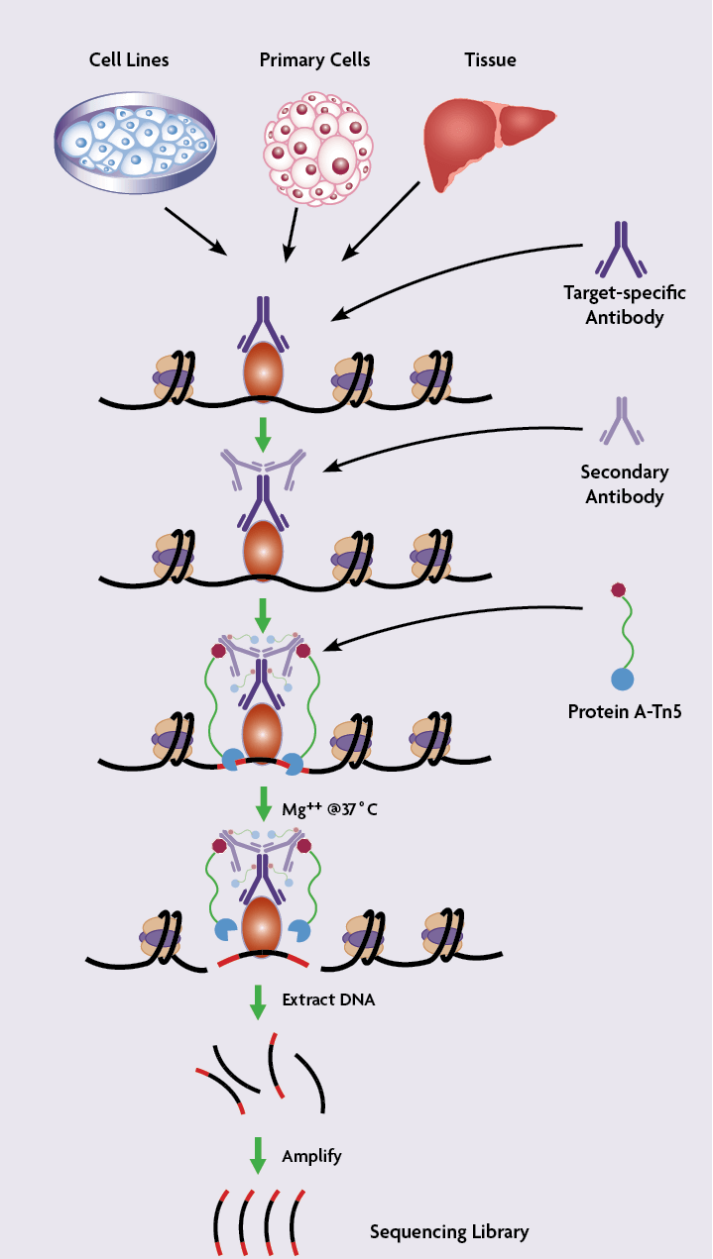
Region repressed for transcription by PcG proteins containing the HoxA complex. Raw data were retrieved from



Cut & Tag: Cleavage Under Targets and Tagmentation

- Cells are first permeabilized and incubated with an antibody immobilized on Concanavalin A-coated magnetic beads to facilitate the subsequent washing steps.
- Next, the cells are incubated with a primary antibody specific for the target protein of interest followed by incubation with a secondary antibody.
- The cells are then incubated with assembled transposomes, which consist of protein A fused to the Tn5 transposase enzyme that is conjugated to NGS adapters.
- After the incubation, unbound transposome is washed away using stringent conditions.

Tn5 is an Mg2+-dependent enzyme so Mg2+ is added to activate the reaction, which results in the chromatin being cut close to the protein binding site and simultaneous addition of the NGS adapter DNA sequences. This leads to chromatin cleavage and library preparation in one single step.



Advantages of Cut & Tag

CUT&Tag is Compatible with Low Cell Numbers

In the publication that first described the CUT&Tag method, the authors went down as low as 60 cells to analyze H3K27me3 profiles across the genome. CUT&Tag is compatible with low cell numbers because pA-Tn5 cleaves DNA directly at the binding site of the antibody and does not require chromatin preparation and sonication steps that can lead to sample loss.

The ability to work with small numbers of cells is an advantage for researchers working on specific cell types, such as rare cell populations.

CUT&Tag Does Not Require Fixation or Sonication

CUT&Tag is performed on native cells or nuclei, avoiding the need for fixation, chromatin preparation, and sonication steps of standard ChIP workflows. Sonication can be quite challenging to set up and requires specialized equipment. Moreover, over-fixation and over- sonication can destroy protein epitopes preventing them from being immunoprecipitated. Some antibodies work better under native conditions.

CUT&Tag is Fast

Relative to ChIP, which is a multi-step process, CUT&Tag is much faster. Cells are immobilized on magnetic beads and the entire protocol happens in a single tube. The tagmentation step involves chromatin shearing and sequencing library adapter insertion at the same time, which is amenable to high-throughput experiments.