Regulation of Epigenomes

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Summary

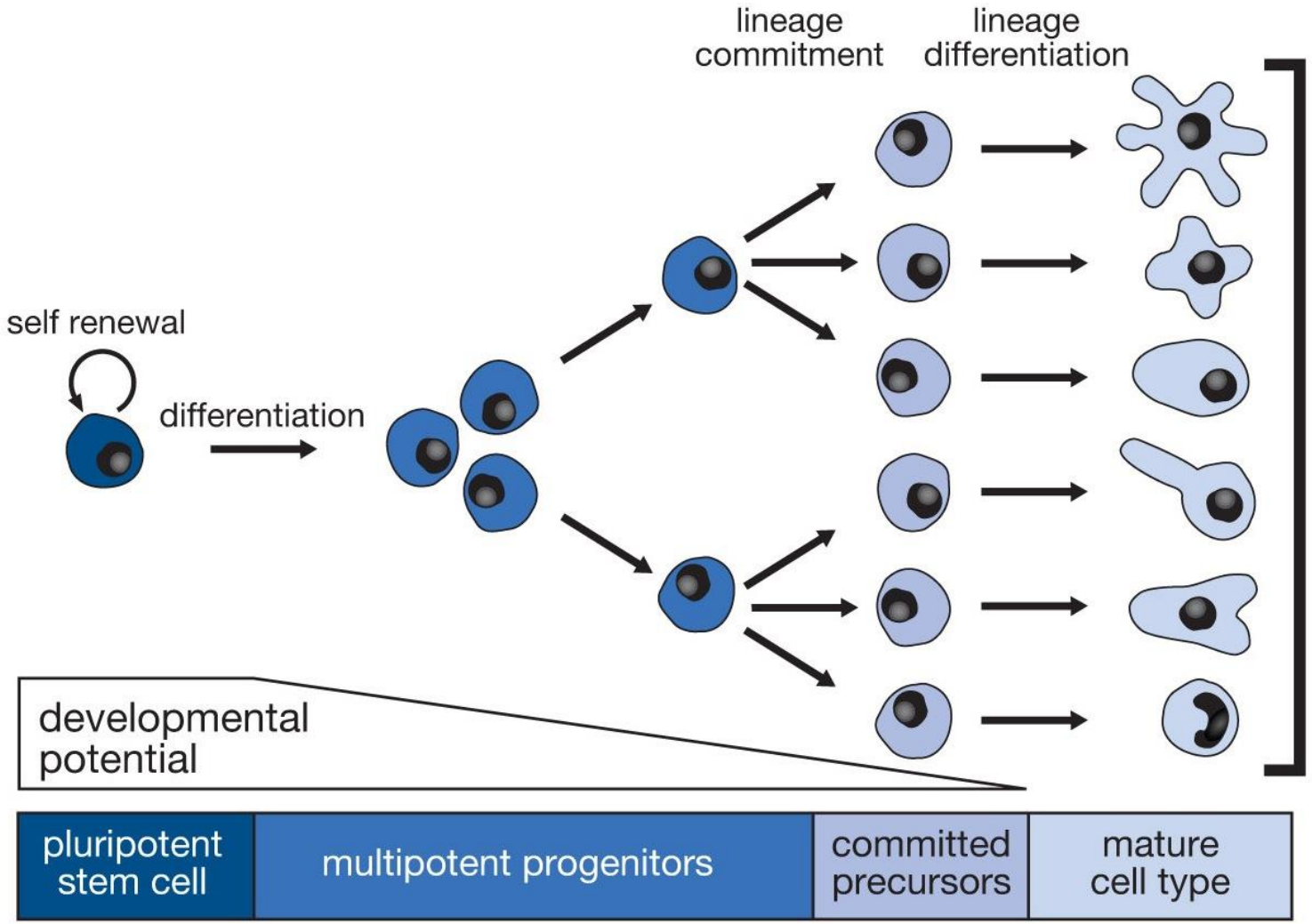
- Classical examples of epigenetic regulations
- Chromatin
- Epigenetic inheritance
- Epigenetic changes in cancer
- Methods to detect epigenetic changes

Epigenetics

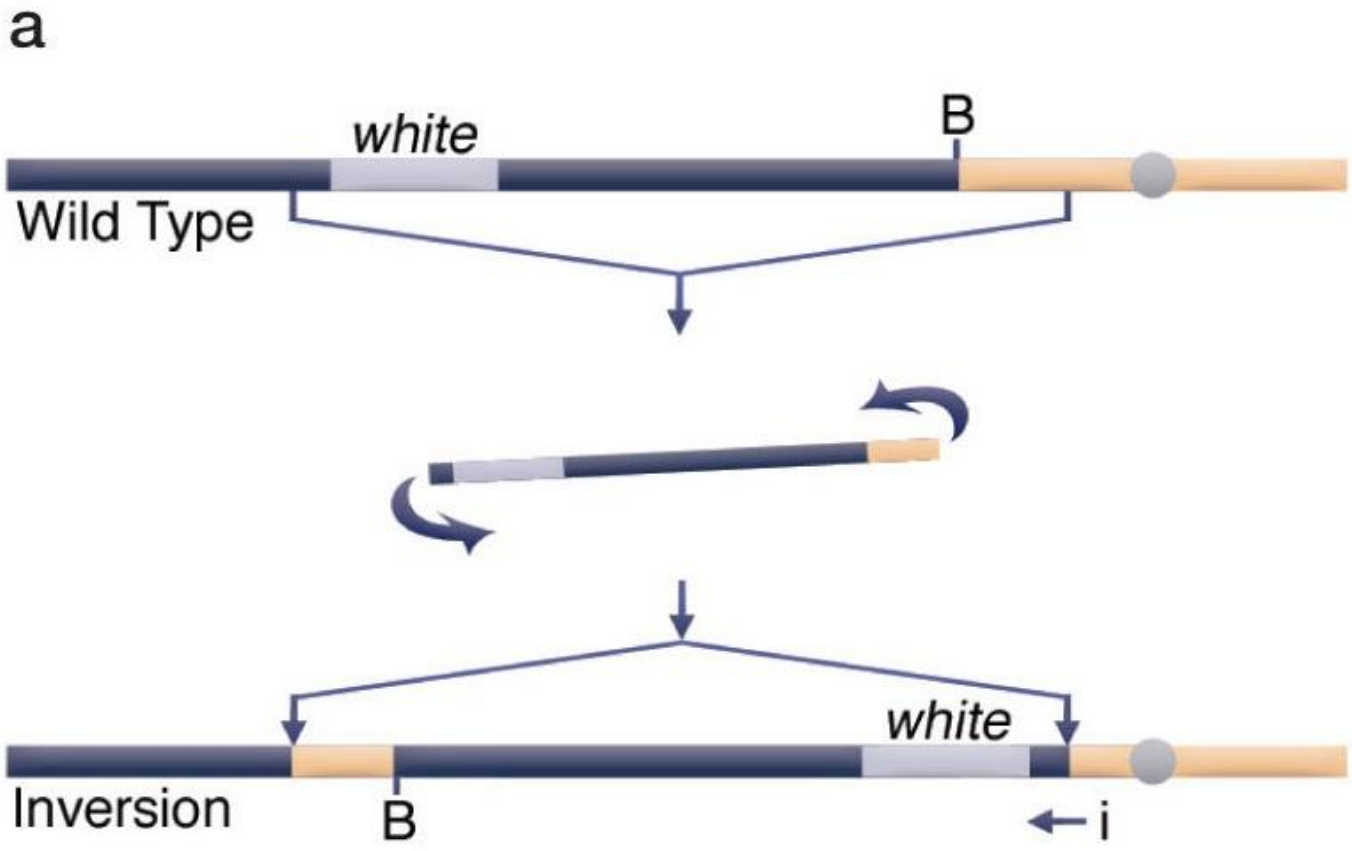
- **Coined by Waddington in 1942 to explain differentiation of cells from one state to another**

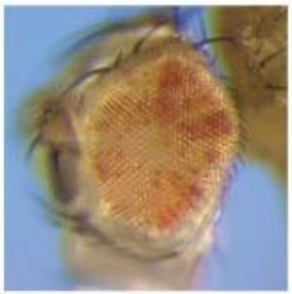
- **Epigenetics: heritable changes in gene expression without changes in DNA sequences.**

Epigenetic regulation of gene expression during development

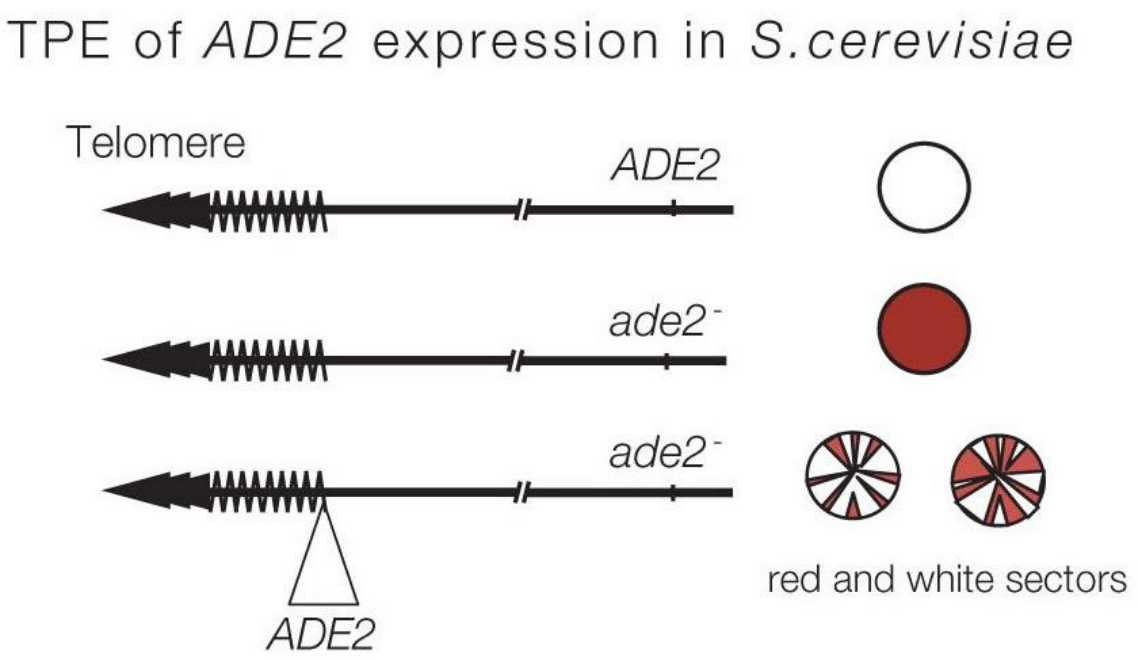


Position effect variegation in *Drosophila*



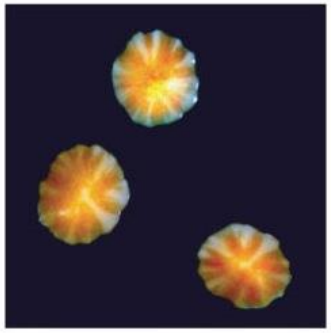


Telomere position effect (TPE): a form of epigenetic silencing



ade2⁻; *ADE2*-TelVR

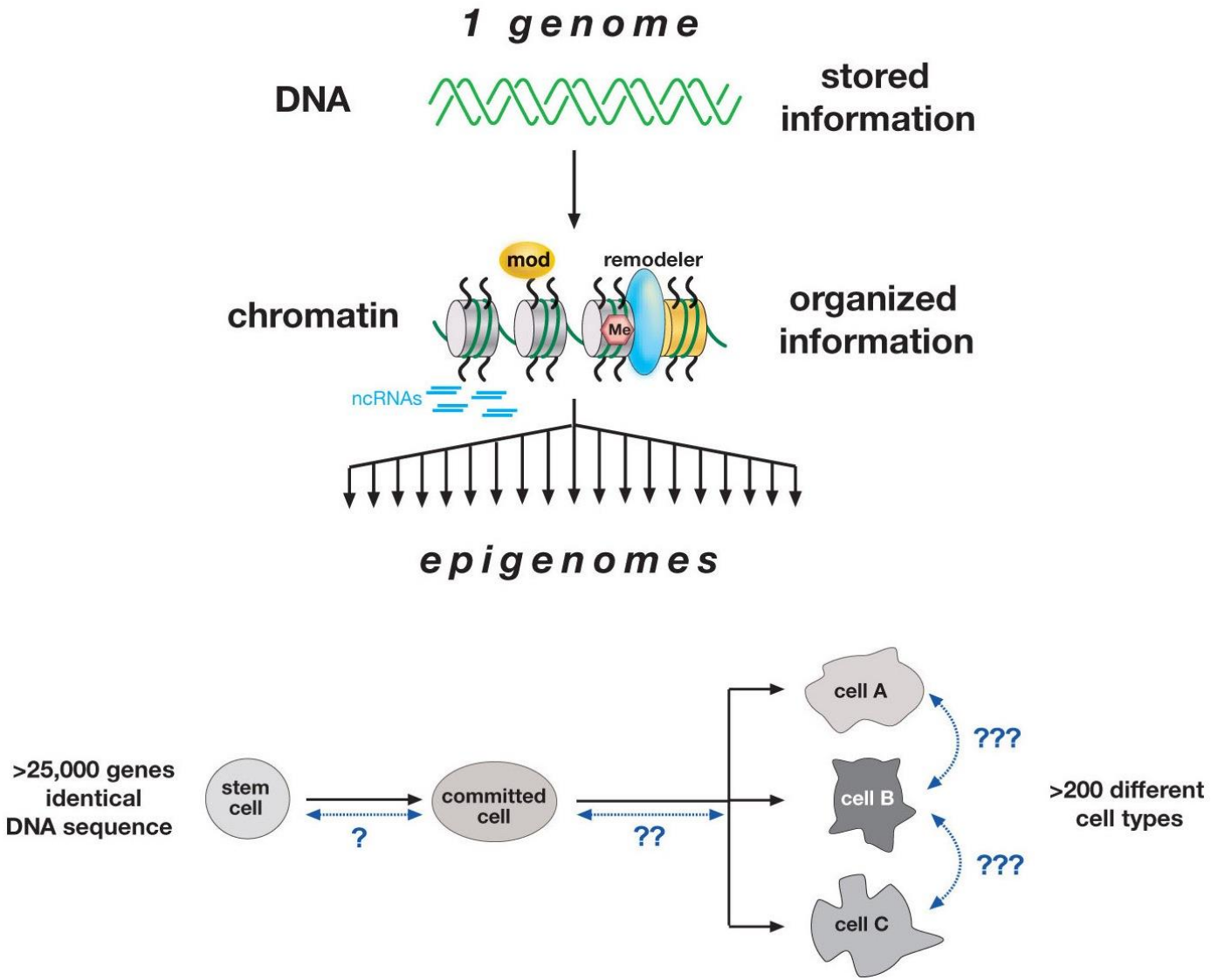
variegated repression



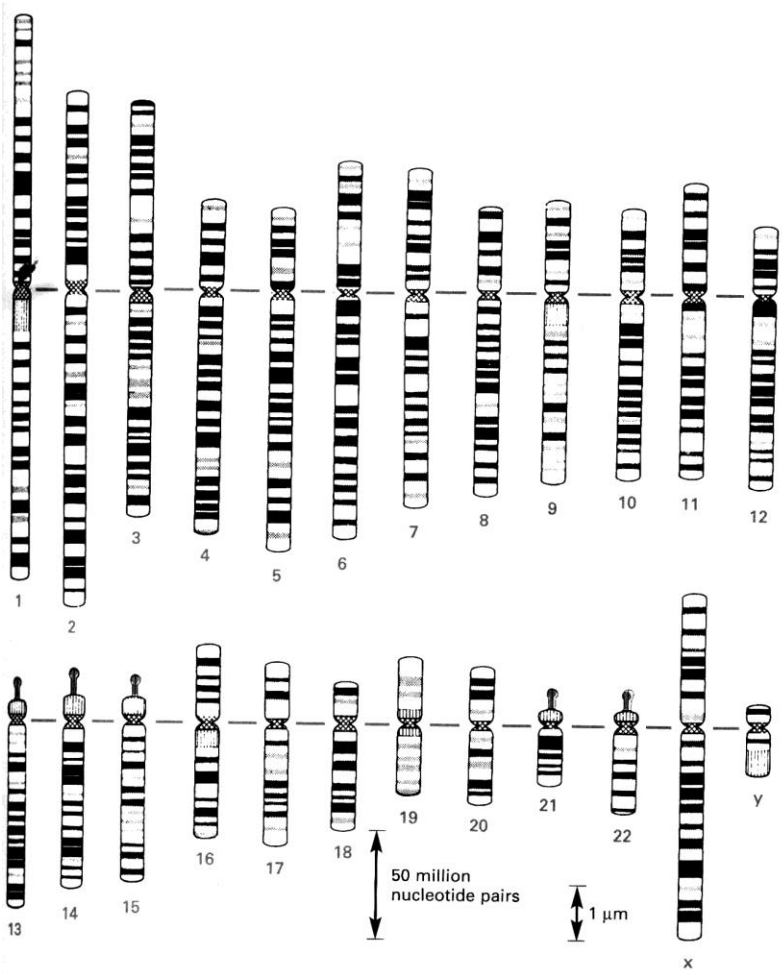
Colors of flowers, animal furs…..

Morita and Hoshino, Breeding Science, 2018

One genome and many epigenomes



Chromatin encodes epigenetic information



- Chromatin is a complex of DNA and proteins
- Genetic information refers to DNA sequences
- Epigenetic information is “stored” in and regulated by chromatin structures

Euchromatin and heterochromatin

Euchromatin

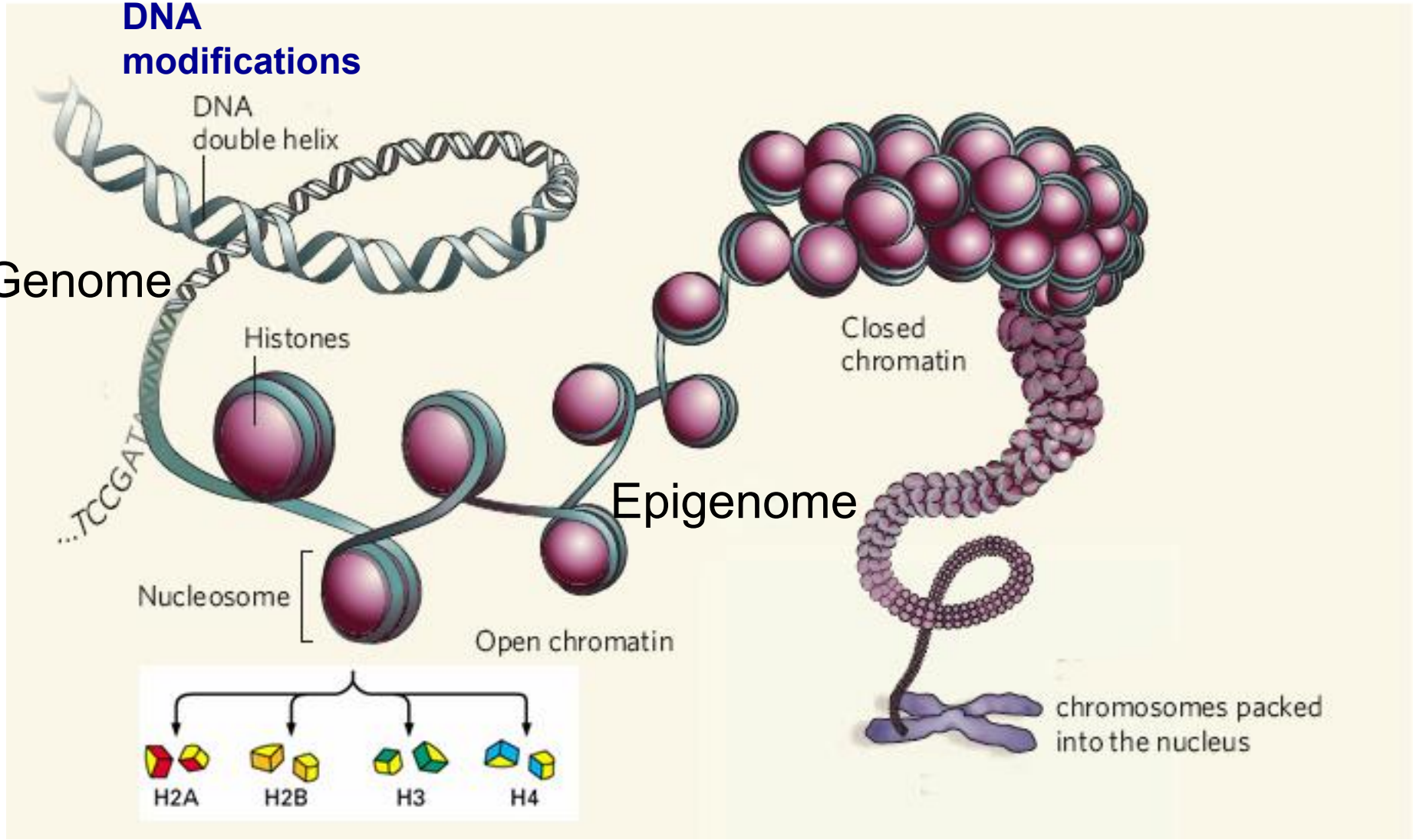
- Regions “rich” in genes
- Regions active in transcription
- Hyperacetylated histones
- Replicate early in S phase of the cell cycle

Heterochromatin

- Regions “poor” in genes
- Regions that silence transcription
- Hypoacetylated histones
- Replicate late S phase

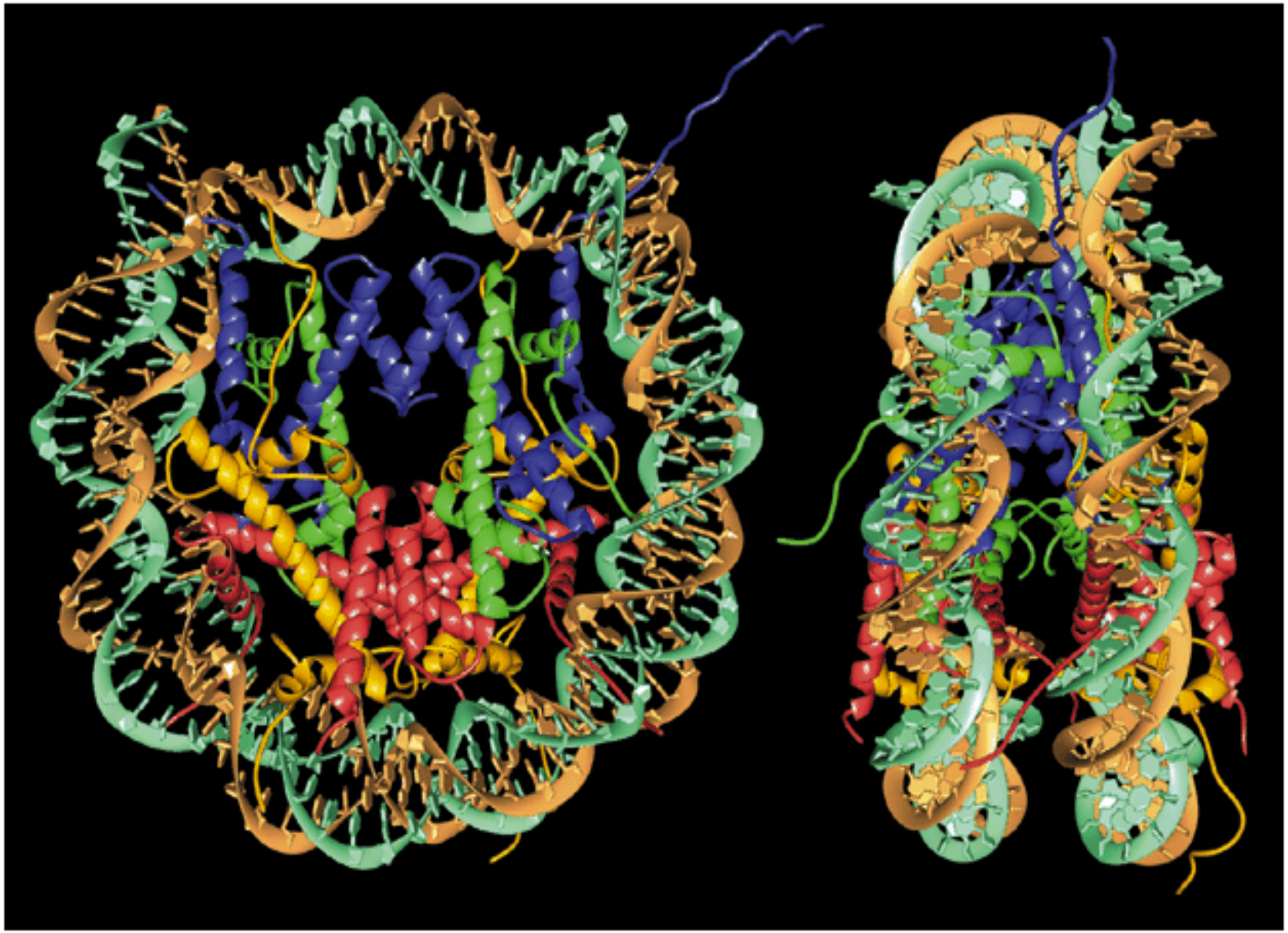
At molecular levels, chromatin domains can be classified based on modifications on histones as well as chromatin binding proteins

DNA is packaged into chromatin in eukaryotes



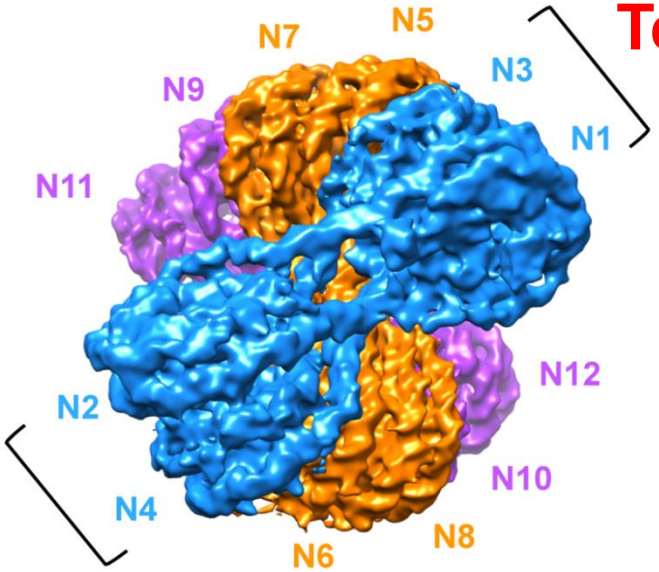
Modified from Baylin and Schuebel, Nature (2007)

Nucleosome

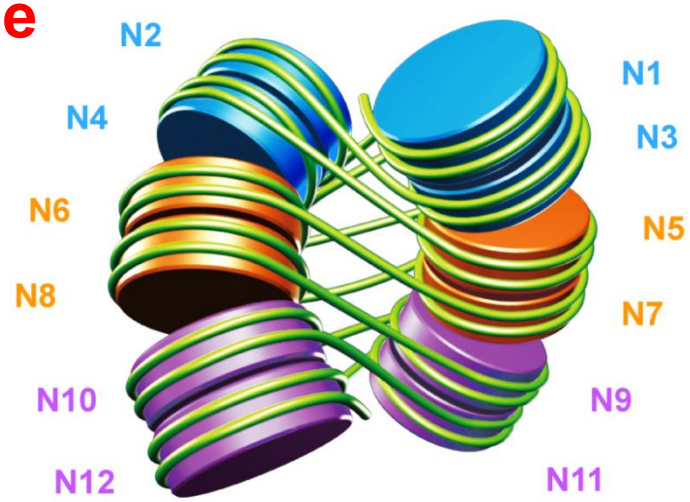


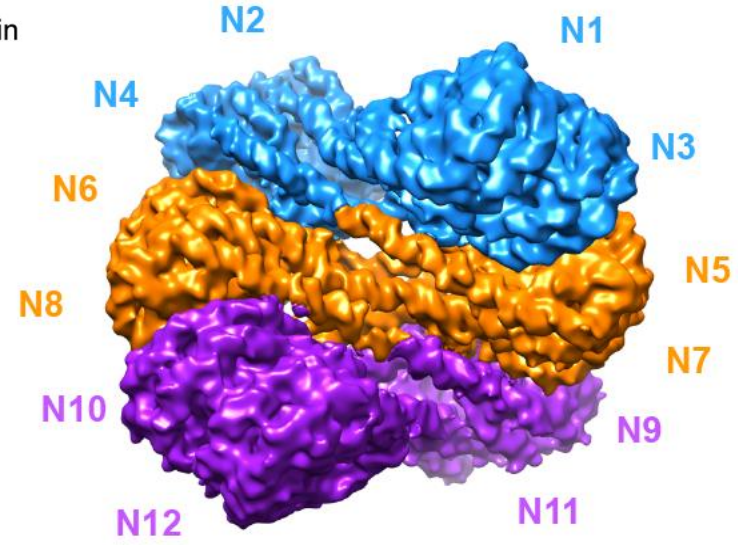
“Tetranucleosome” is the structural unit of chromatin fiber

Tetranucleosome



12×187 bp chromatin



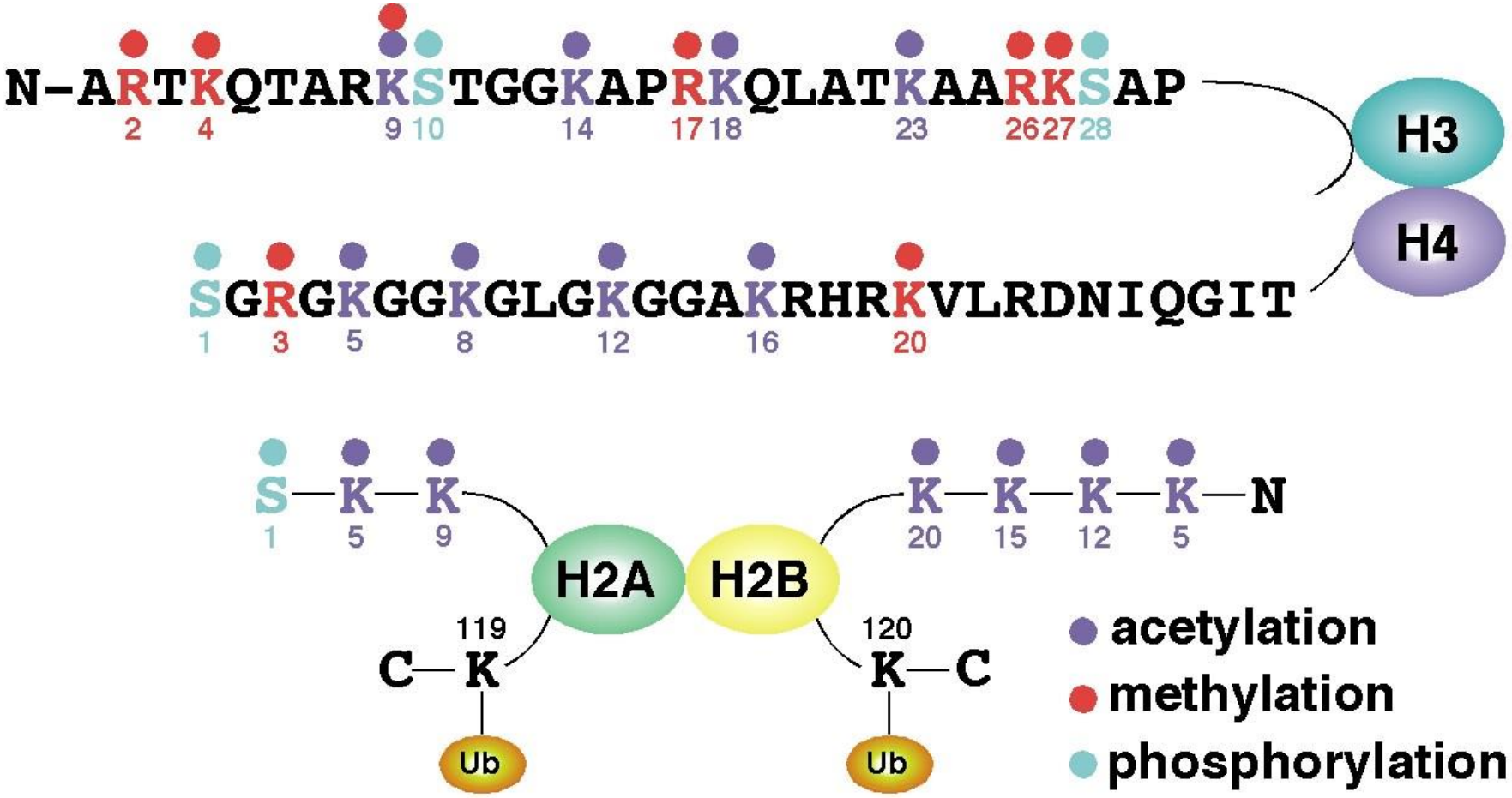


Left-handed twist of tetrnucleosomal unit

Epigenetic Marks

- **DNA methylation, hydroxylmethylation**
- **Histone modifications**
- **Non-coding RNAs?**

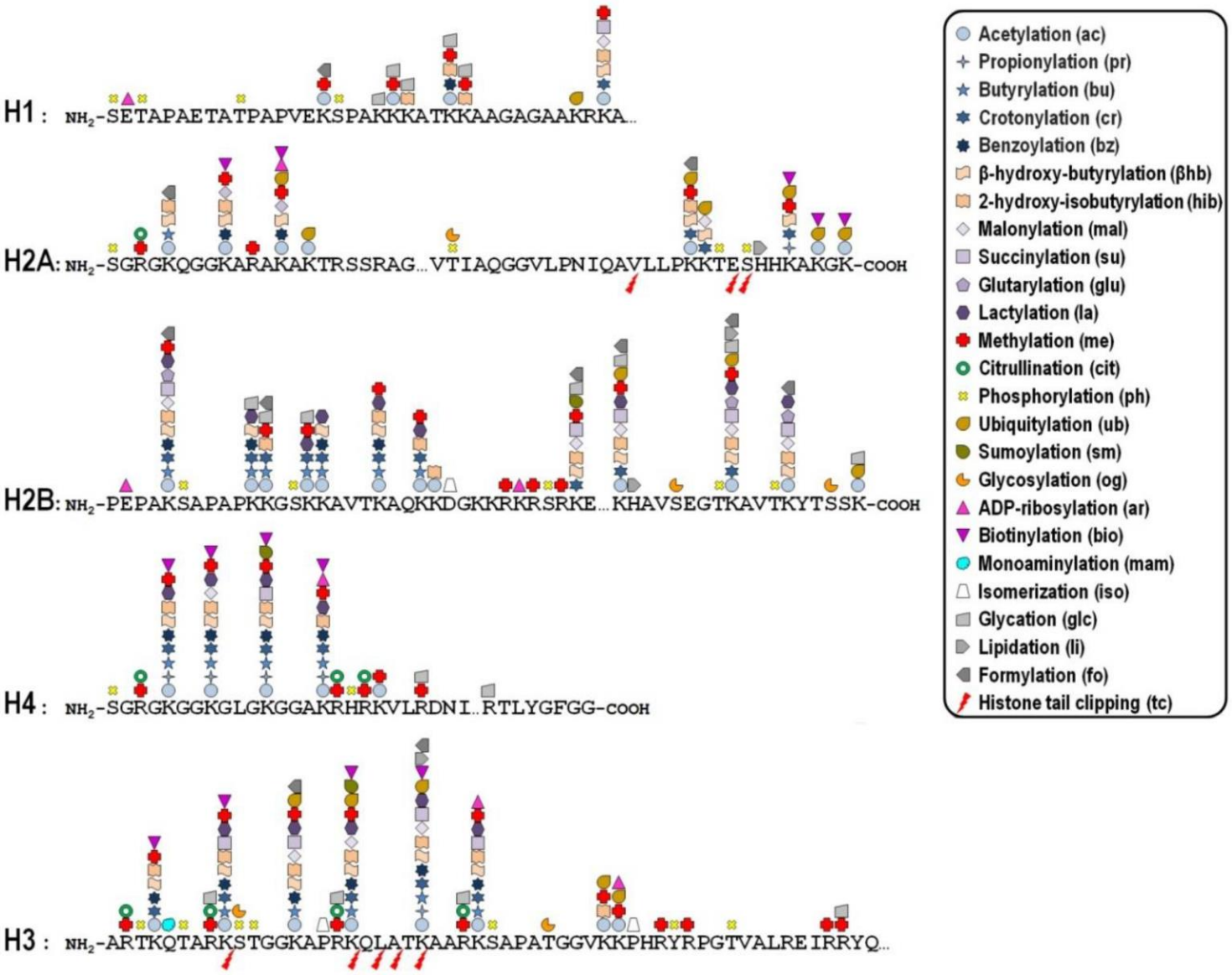
Post-transcriptional modifications of core histones



Adopted from Zhang and Reinberg, Genes & Dev (2001)

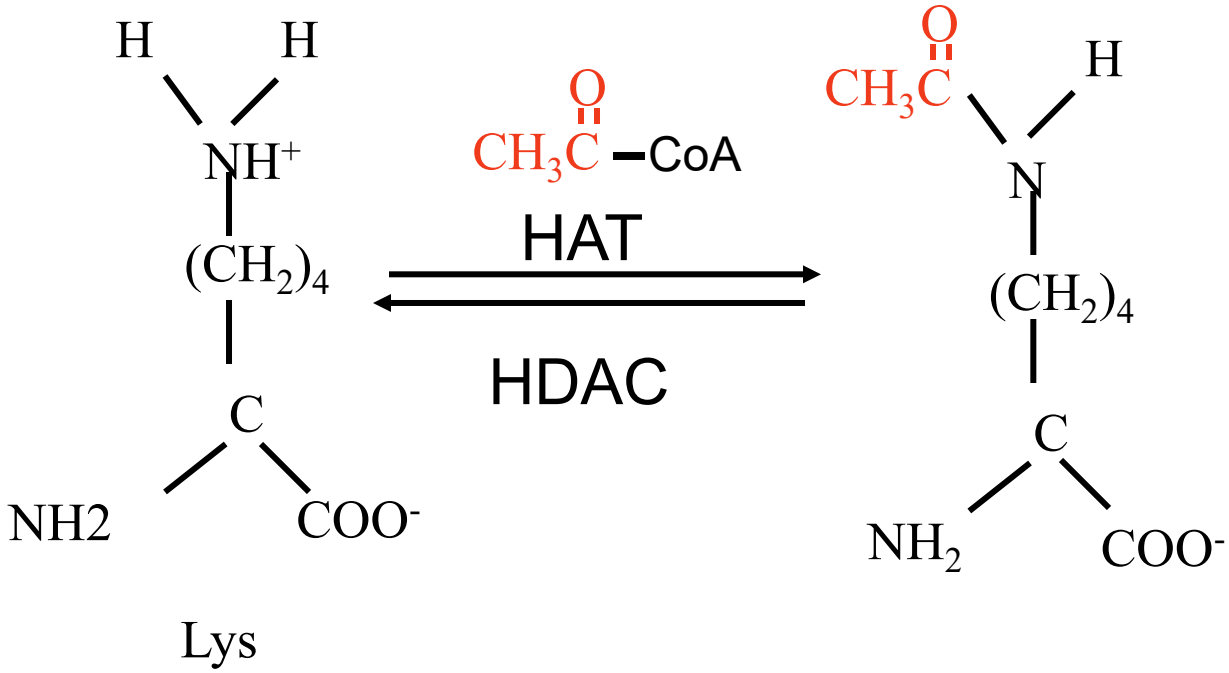
Not all histone modifications are epigenetically inherited.

Post-transcriptional modifications of core histones



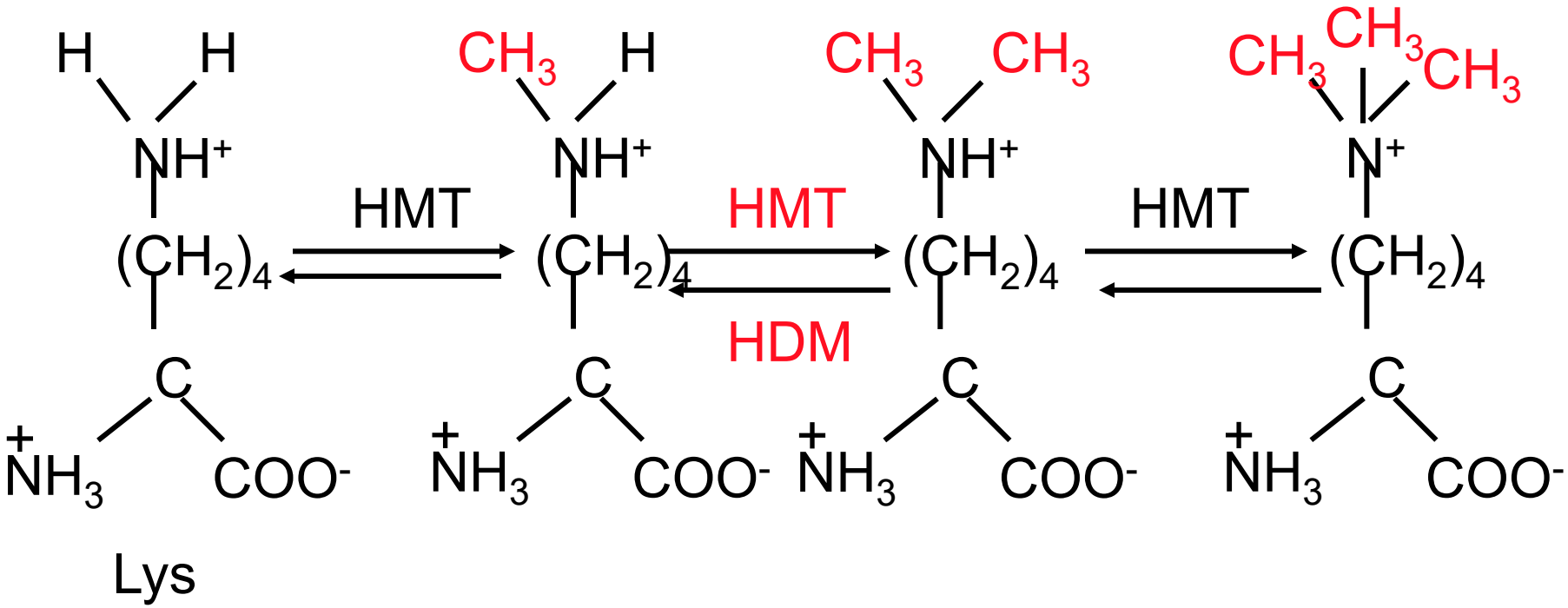
Cavalieri et al Genes 2021

Acetylation of Lysine residue is reversible



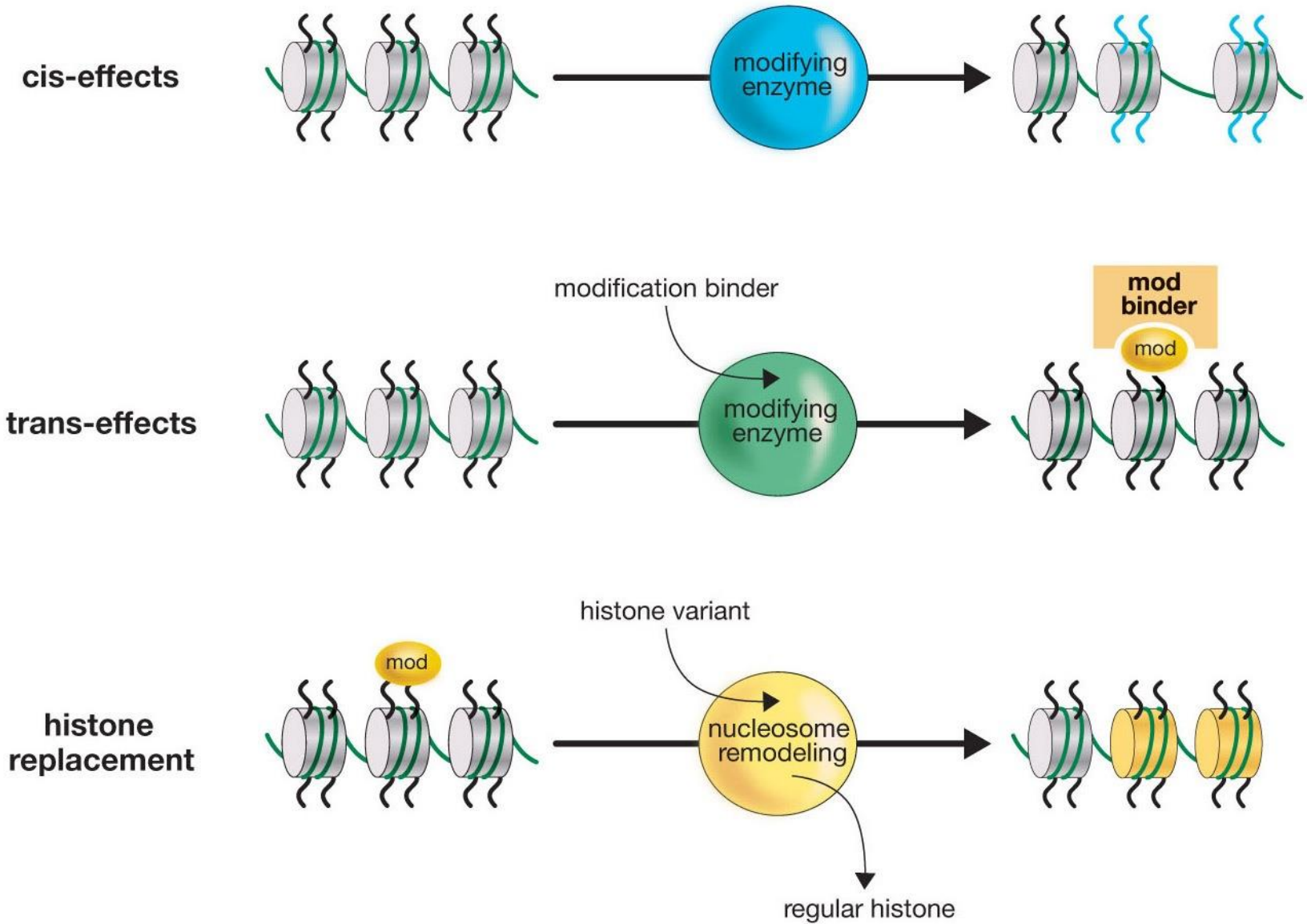
HAT: histone acetyltransferase, more accurately; lysine acetyltransferase
HDAC: histone deacetylases

Lysine methylation is reversible



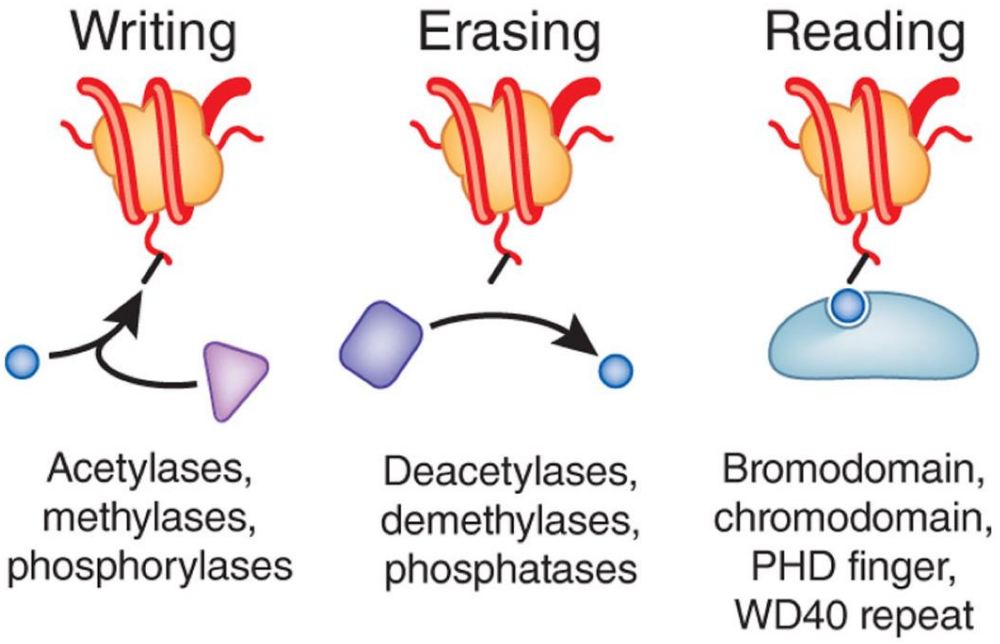
HMT: histone methyltransferase contains SET domain
HDM: JmjC domains

What are the function of histone modifications?



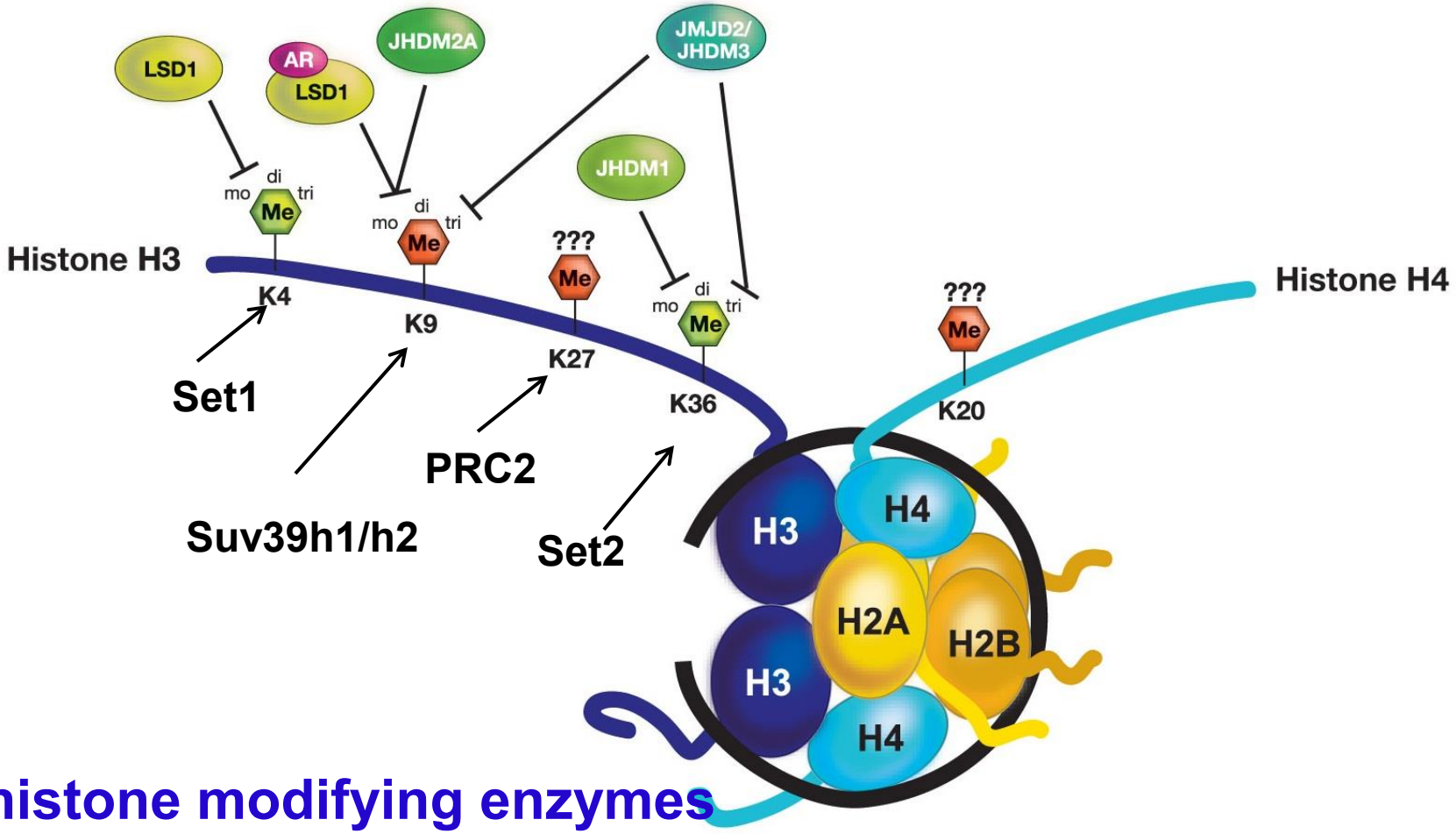
Three classes of proteins working on histone modifications

- Writers: enzymes that add a mark
- Readers: proteins that bind to and “interpret” the mark
- Erasers: enzymes that remove a mark



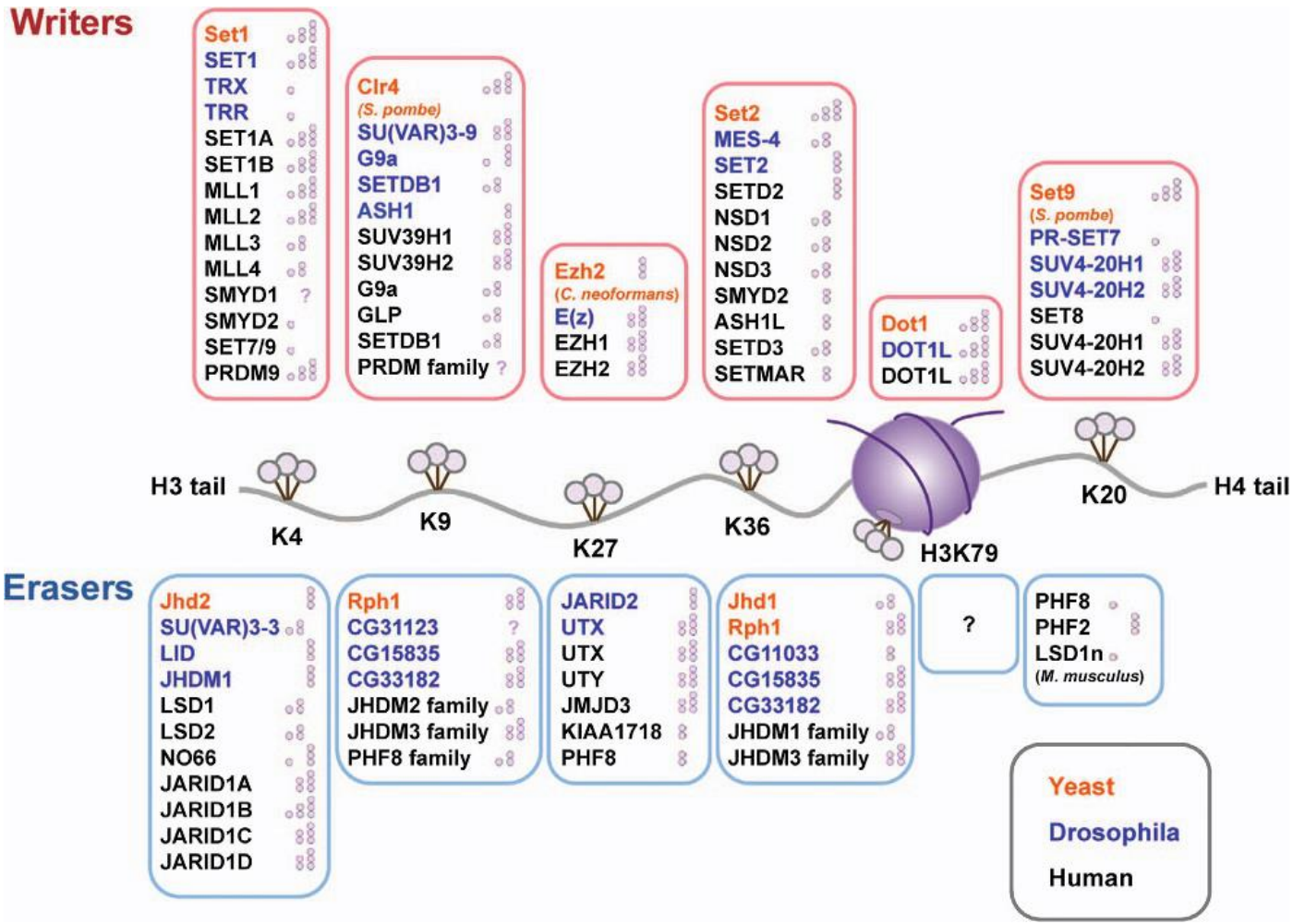
Tarakhovsky, A., Nature Immunology, 2010.

Examples of enzymes (writers) modifying the H3 tails



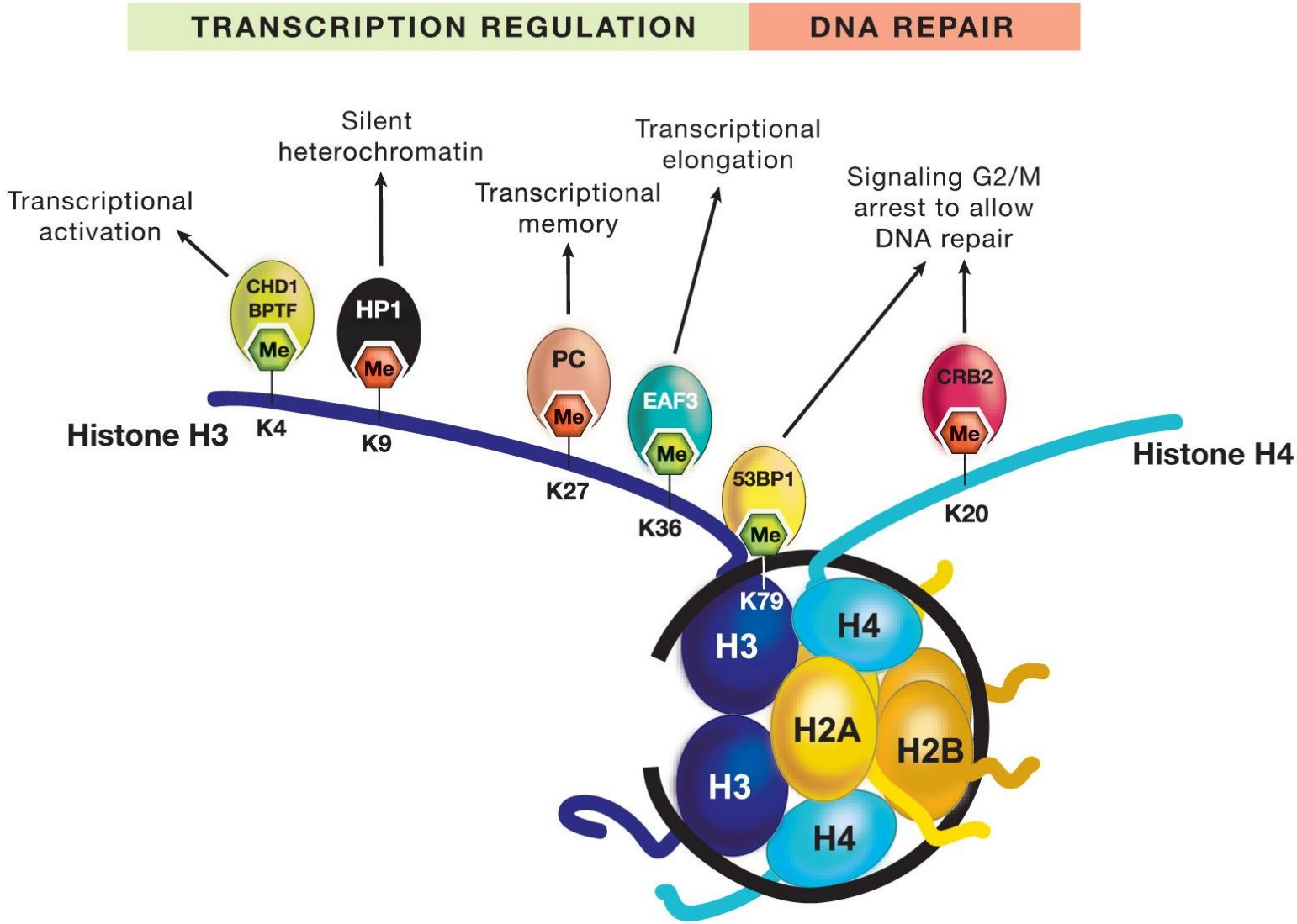
Most histone modifying enzymes have non-histone substrates

Writers and Erasers for methylation of commonly studies lysine residues

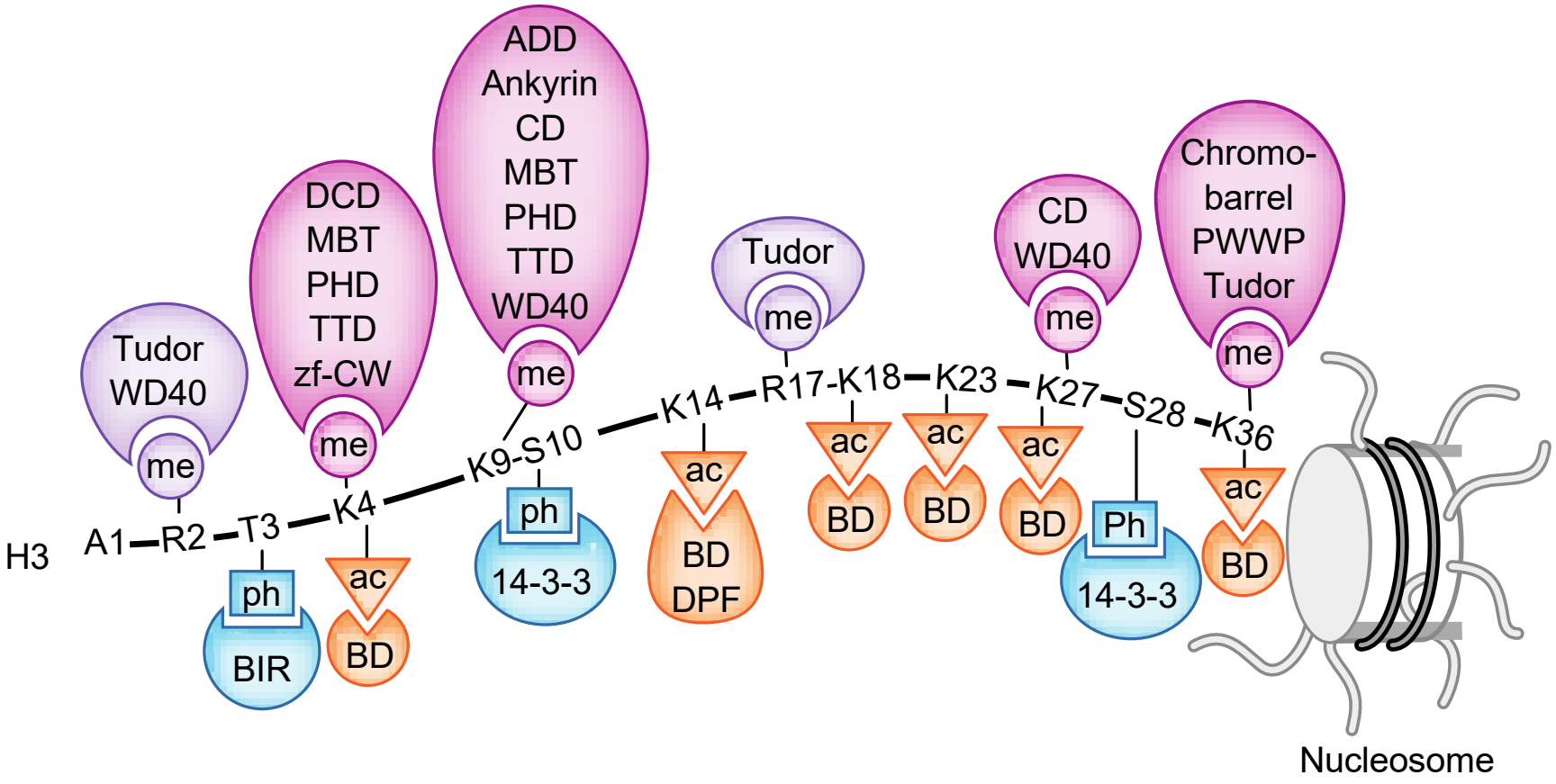


Most histone modifying enzymes have non-histone substrates

Proteins that read modifications on H3 and H4 tails



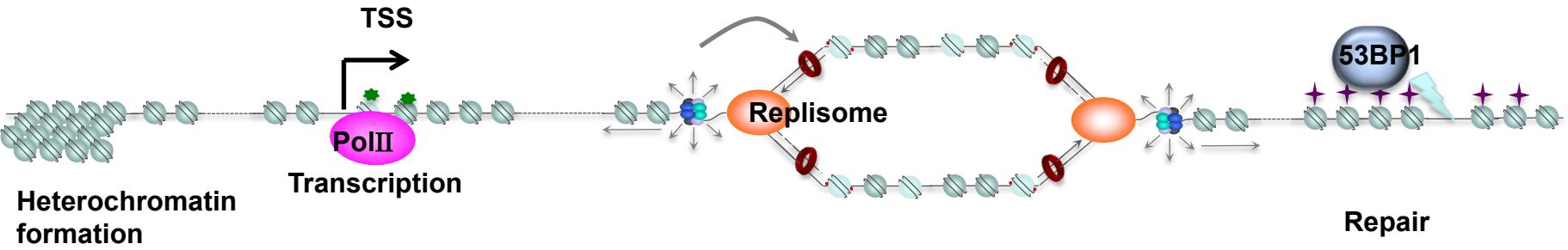
Proteins that read modifications on H3 and H4 tails



Musselman et al, Nature structural and Molecular Biology, 2012

Enormous complexity and intricacy could be generated to regulate human epigenomes

Histone modifications impact processes linking to DNA transactions



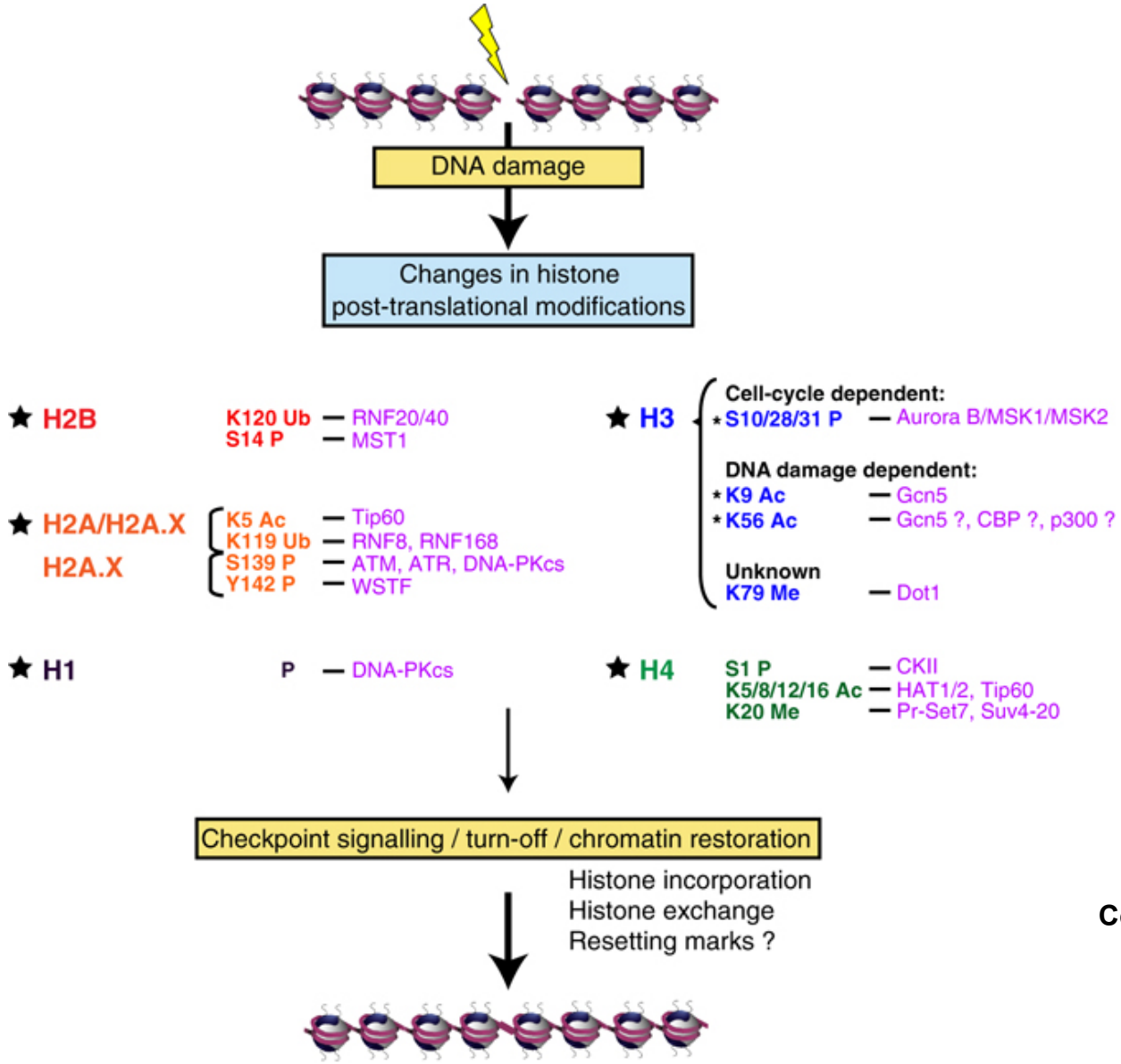
Active and inactive histone marks based on association with gene activity

1) Active marks: histone marks associated with active gene transcription (H3K4me1, H3K4me2, H3K4me3, H3K36me3, H3K9ac, H3K27ac, H4K16ac)

2) Inactive/silent marks (H3K9me2 or H3K9me3, H3K27me3)

3) Bivalent chromatin domains (H3K4me3 and H3K27me3) at developmentally regulated genes

Histone modifications during DNA damage response

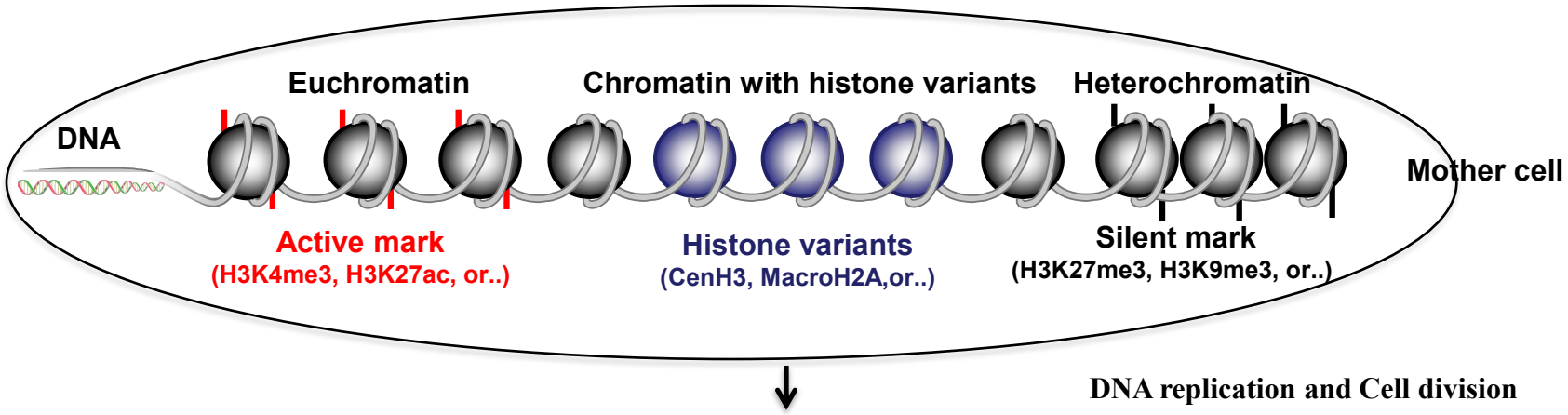


Corpet and Almouzni EMBOJ

Epigenetic inheritance

Epigenome duplication is also a daunting task

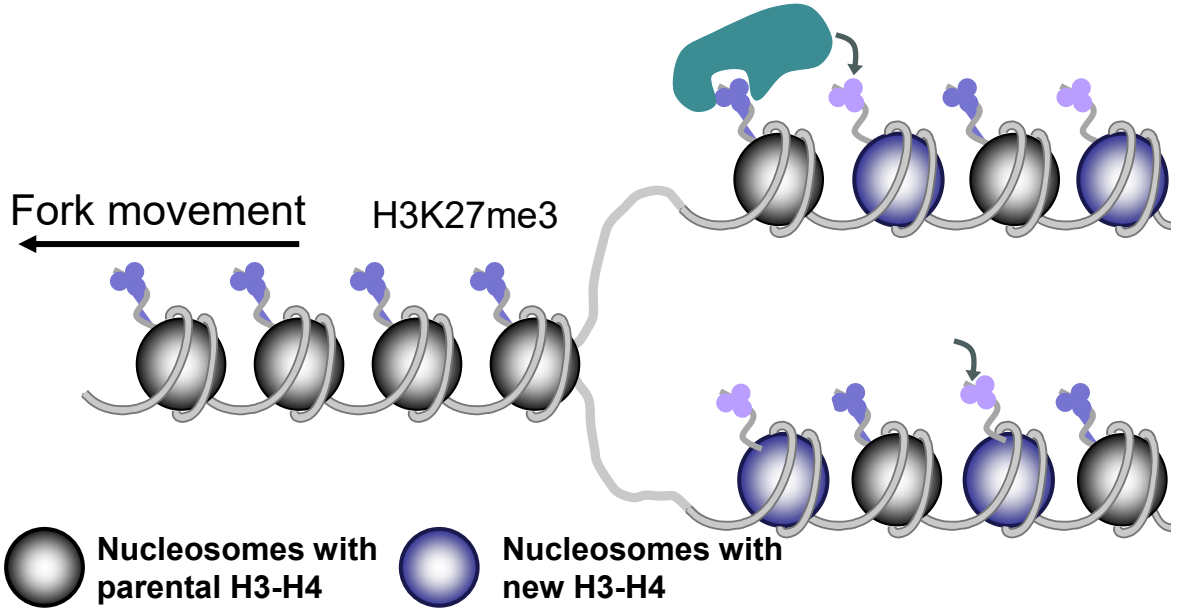
How are epigenetic states inherited during mitotic cell divisions



?

- **Histone and histone variants are assembled at the same places**
- **Histone modification patterns at different chromatin domains are transmitted into daughter cells**
- **Readers for histone marks are recruited to the same places**
- **(Many others…)**

DNA replication-coupled nucleosome assembly and the read and write mechanism for the restoration of histone marks



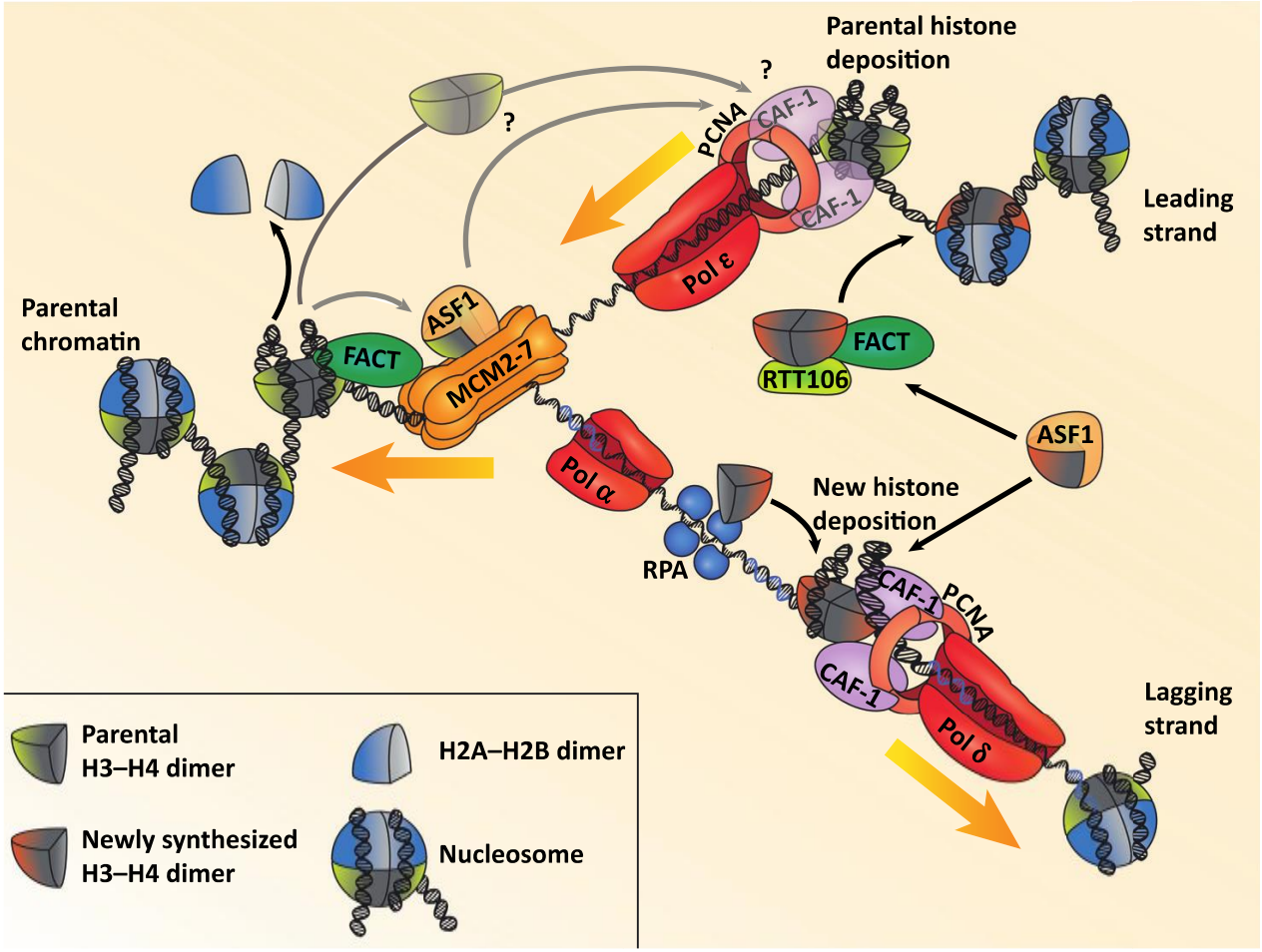
How are nucleosomes formed using newly synthesized H3-H4?

How are nucleosomes formed using parental H3-H4?

Du et al, Sci China Life Sci, 2022

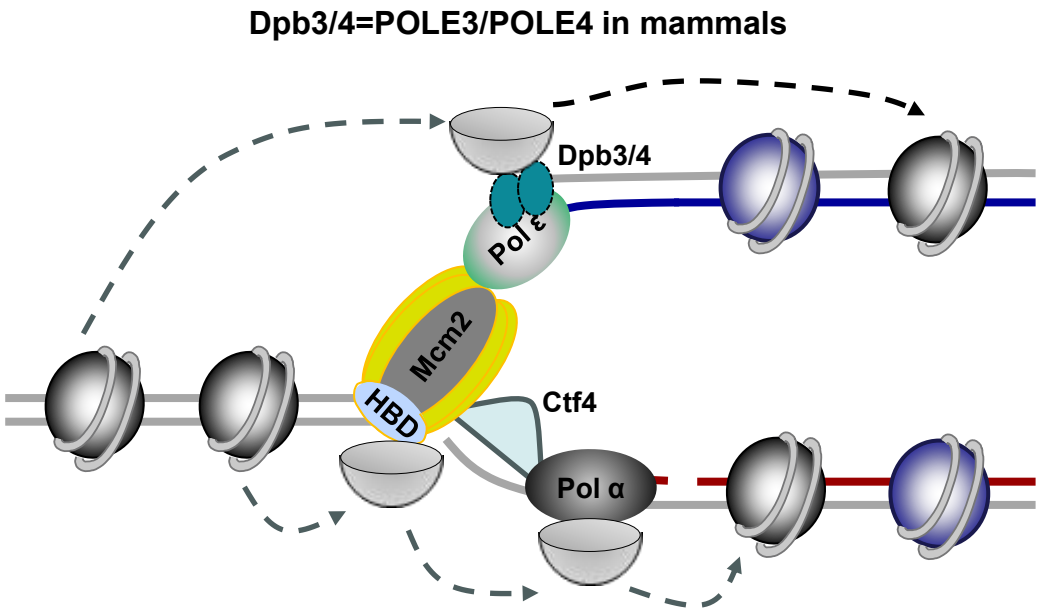
Escobar al, Nature Review Genetics, 2021

Many factors regulate the deposition of new H3-H4



Serra-Cardona and Zhang, TIBS, 2018

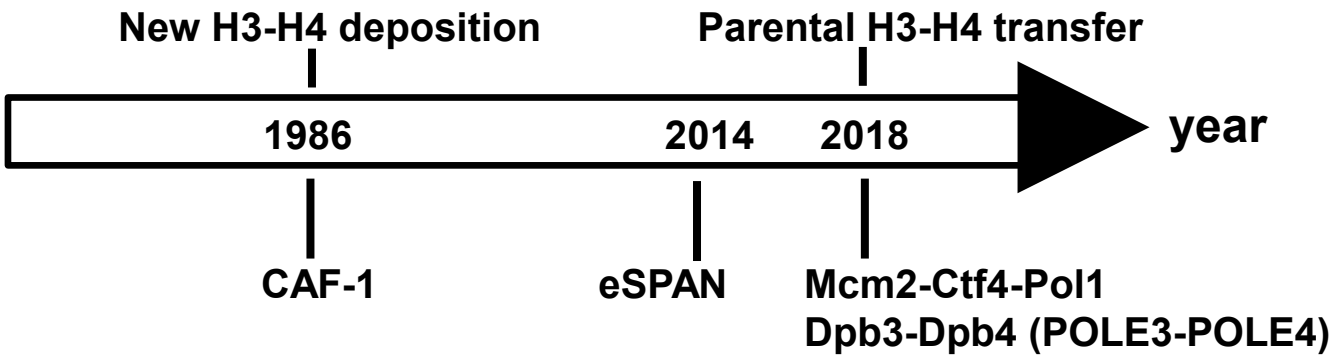
Parental H3-H4 tetramers are transferred to replicating DNA strands via distinct mechanisms



- Parental H3-H4 are transferred almost equally to leading and lagging strand of the DNA replication fork.
- Dpb3-Dpb4, two subunits of leading strand DNA polymerase, facilitates the transfer of parental H3-H4 tetramers to leading strand.
- The Mcm2-Ctf4-Polα facilitates the transfer of parental H3-H4 to lagging strand.
- These pathways are conserved from yeast to mammalian cells.

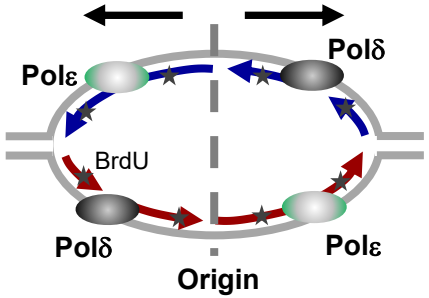
Yu et al, Science 2018
Gan et al, Molecular Cell, 2018
Petryk et al (Groth), Science, 2018

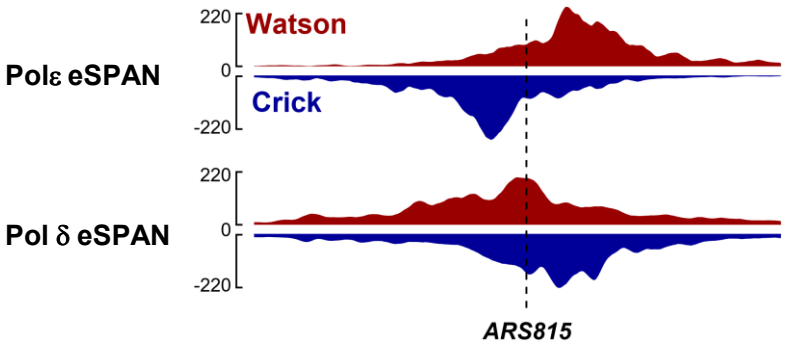
A simplified history of nucleosome assembly



eSPAN can measure the relative amount of proteins at leading or lagging strands of DNA replication forks

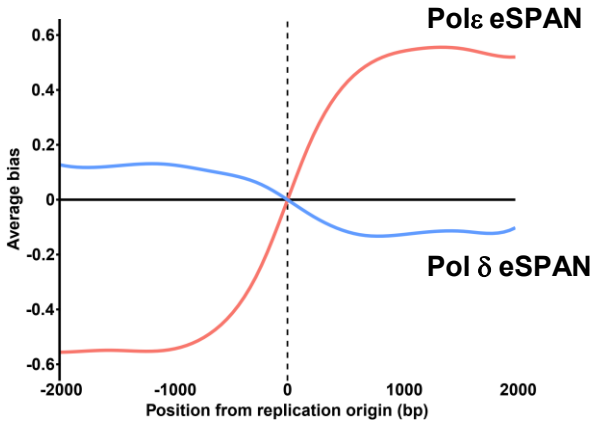
enrichment and
Sequencing of **P**rotein
Associated **N**ascent DNA





Pursell et al (Kunkel), Science 2007
McElhinny et al (Kunkel), Mol Cell, 2008

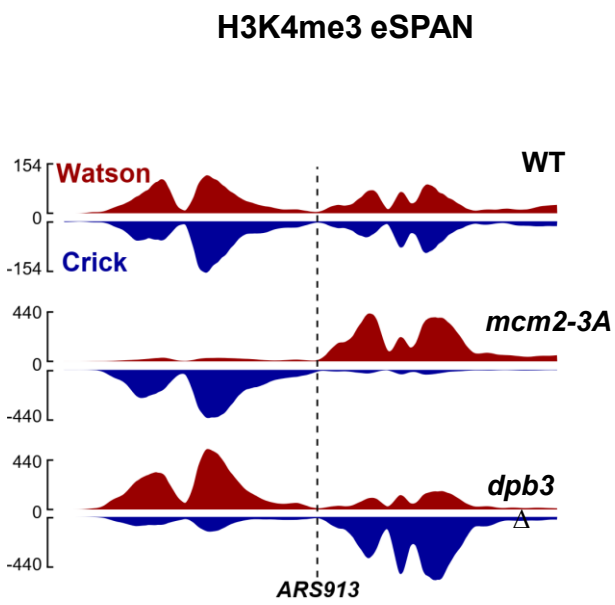
Bias = (W-C)/(W+C)

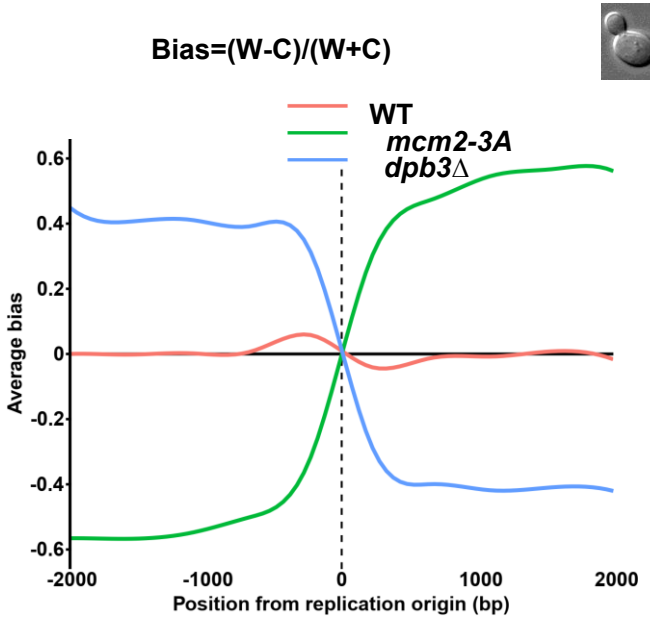


Bias pattern: leading vs lagging bias
Bias ratio: relative amount of a protein at leading/lagging

Yu et al Molecular Cell 2014, PMCID: PMC436266

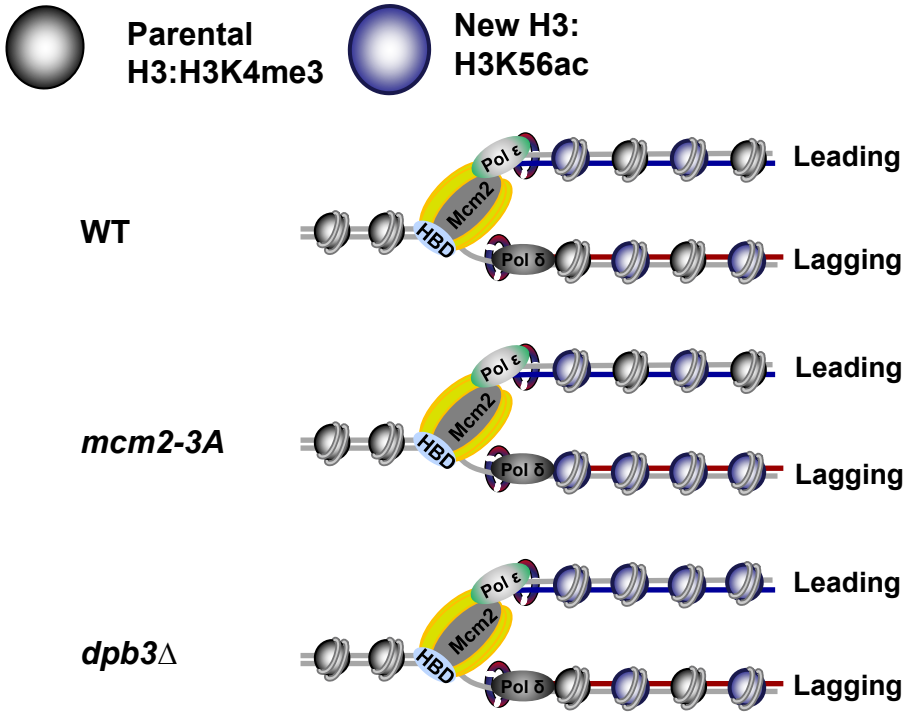
H3K4me3 eSPAN in *mcm2-3A* and *dpb3*Δ mutant cells exhibit a strong leading and lagging strand bias, respectively



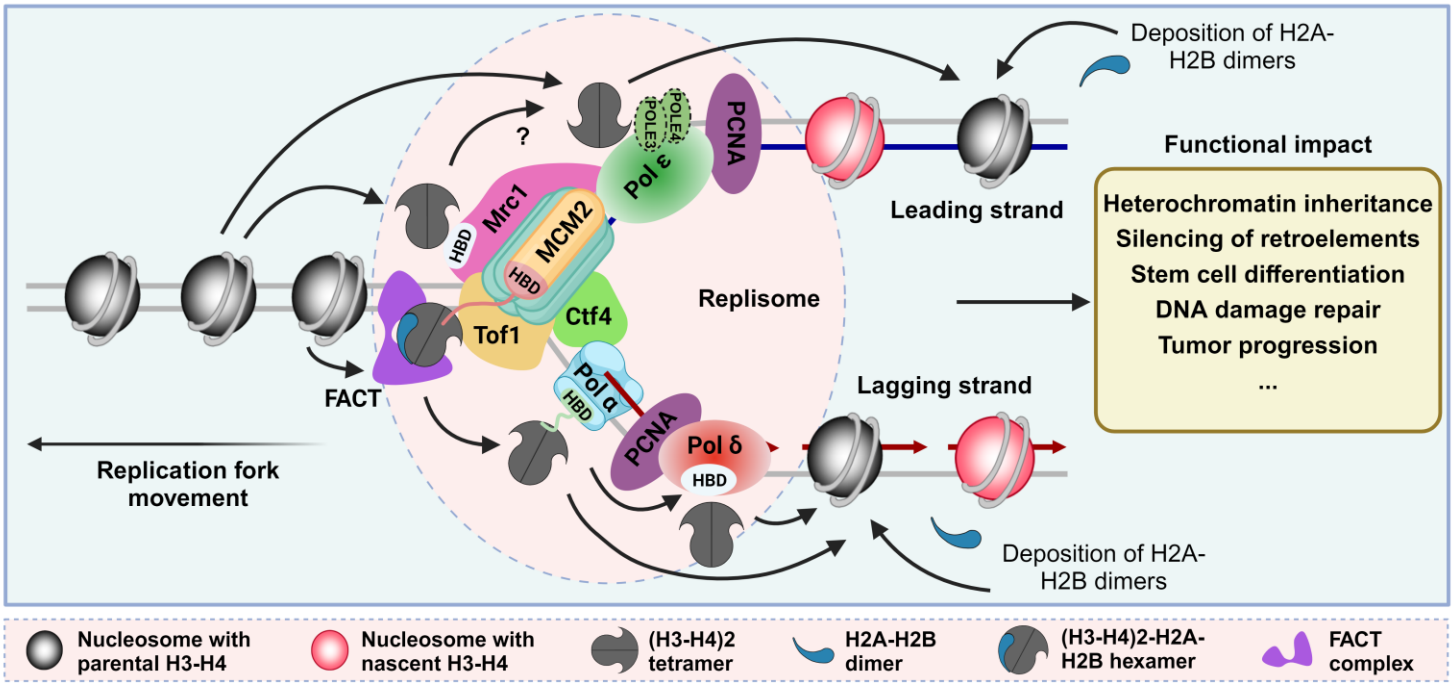


Dpb3 is a subunit of Pol ε.

Yu et al, Science 2018, PMCID: PMC6597248
Gan et al, Molecular Cell, 2018, PMCID: PMC6193272


Parental
H3:H3K4me3
New H3:
H3K56ac
WT
Pol ε
Mcm2
HBD
Pol δ
Leading
Lagging
mcm2-3A
Pol ε
Mcm2
HBD
Pol δ
Leading
Lagging
dpb3Δ
Pol ε
Mcm2
HBD
Pol δ
Leading
Lagging

Multiple replisome components likely work in relay fashion to transfer parental histones to replicating DNA strands



Petryk et al, Science, 2018
Dolce et al, Genes Dev, 2022
Wang et al, NAR 2023
Wen et al, Nature genetics, 2023
Weger et al, Nature Genetics, 2023
Yu et al, Cell, 2024
Toda et al, Mol Cell, 2024
Li et al, Nature, 2024
Charlton et al, Cell, 2024
Tian et al, PNAS, 2024
Shi et al, Science Advances, 2024
Karri et al, NAR, 2024

Yu et al, Science, 2018
Gan et al, Molecular Cell, 2018
Li et al, Science Advances, 2019
Serra-Cardona et al, Science Advances, 2022
Xu et al, eLife 2022
Xu et al, Nature Communications, 2022
Li et al, Nature 2023
Fang et al, Genes Dev, 2024
Serra-Cardona et al, Science Advances, 2024

Replisome components are responsible for faithful duplication of both genetic and epigenetic information

The nucleosome core particle remembers its position through DNA replication and RNA transcription

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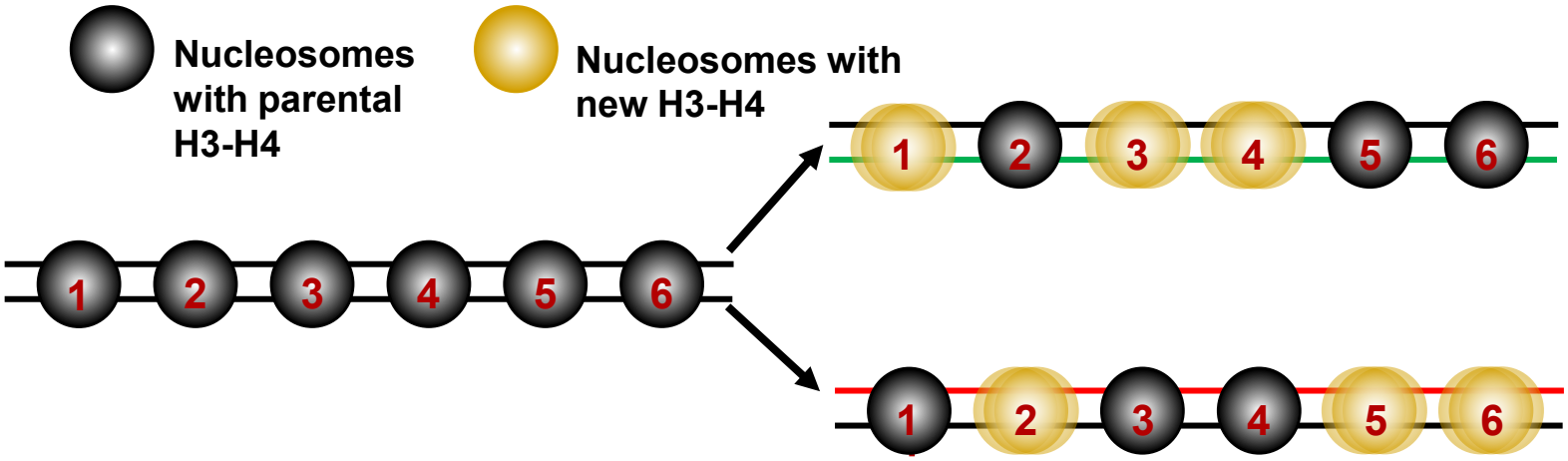
Contributed by Jasper Rine, August 9, 2019 (sent for review July 12, 2019; reviewed by Steven Henikoff and Fred M. Winston)

Article

Cell

Active and Repressed Chromatin Domains Exhibit Distinct Nucleosome Segregation during DNA Replication

Thelma M. Escobar,[1,2] Ozgur Oksuz,[1,2,3] Ricardo Saldaña-Meyer,[1,2] Nicolas Descostes,[1,2,4] Roberto Bonasio,[1,2,5] and Danny Reinberg[1,2,6,*]

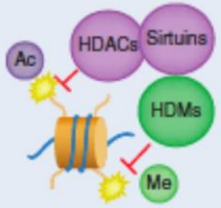


Epigenetics and Cancer

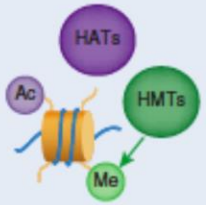
- **Cancer is a disease caused by both genetic and epigenetic changes**

Chromatin regulators are altered in a variety of tumors

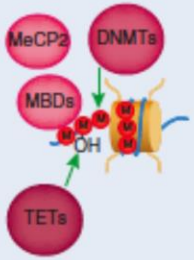
Category	Gene
HDACs	*HDAC1*
	HDAC2
	HDAC6
Sirtuins	*SIRT1*
	SIRT2
	SIRT3
	SIRT7
HDMs	*KDM1A*
	KDM2B
	KDM4C
	KDM5A
	KDM5B
	KDM5C
	KDM6A
	KDM6B



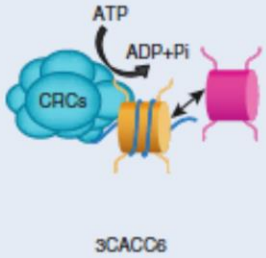
Category	Gene
HATs	*CREBBP*
	EP300
	MYST3
	MYST4
	KAT5
HMTs	*MLL*
	EZH2
	NSD1
	PRDM2
	SMYD3
	WHSC1



Category	Gene
DNMTs	*DNMT1*
	DNMT3A
	DNMT3B
(2OG)-Fe(II)–dependent oxygenases	*TET1*
	TET2
Methyl-CpG binding proteins	*MBD1*
	MBD2
	MBD3
	MBD4
	MECP2



Category	Gene
Histone variants	*H2AFZ*
Chromatin remodeling factors	*ARID1A*
	CHD5
	CHD7
	MTA1
	MTA2
	MTA3
	SMARCA2
	SMARCA4
	SNF5



Category	Gene
Histone modification readers	*ING1*
	ING2
	ING3
	ING4
	ING5

Rodríguez-Paredes and Esteller, Nature Med. 2011

Article

Transient loss of Polycomb components induces an epigenetic cancer fate

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Check for updates

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Although cancer initiation and progression are generally associated with the accumulation of somatic mutations[1,2], substantial epigenomic alterations underlie many aspects of tumorigenesis and cancer susceptibility[3–6], suggesting that genetic mechanisms might not be the only drivers of malignant transformation[7]. However, whether purely non-genetic mechanisms are sufficient to initiate tumorigenesis

Histone modifications and cancer

Many chromatin regulators are frequently mutated in cancer cells

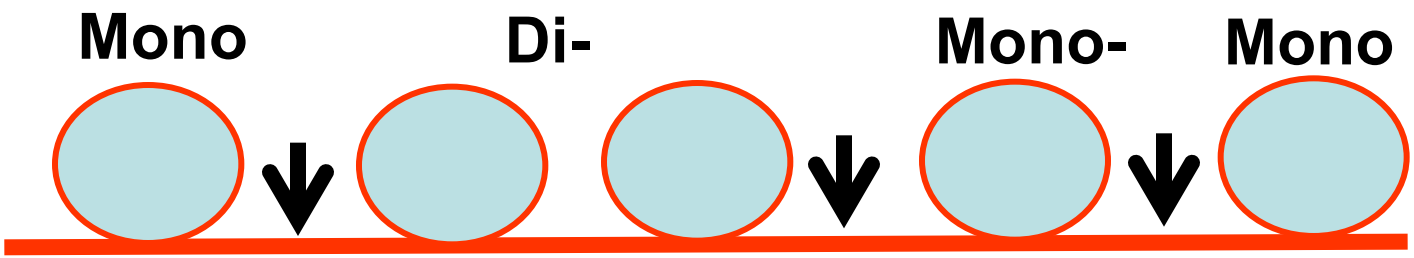
Mutations at gene regulatory elements (enhancers, promoters) are detected in a variety of cancers

A global change in histone modifications has been detected in cancer cells

Expression of histone modifying enzymes is altered in cancer cells

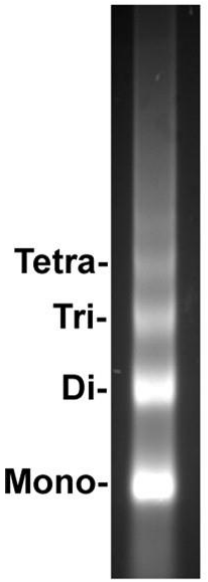
How to probe chromatin and histone modifications?

Analysis of nucleosome positioning by Mnas-seq



Basic repeating structure can be probed (protect and seq method)

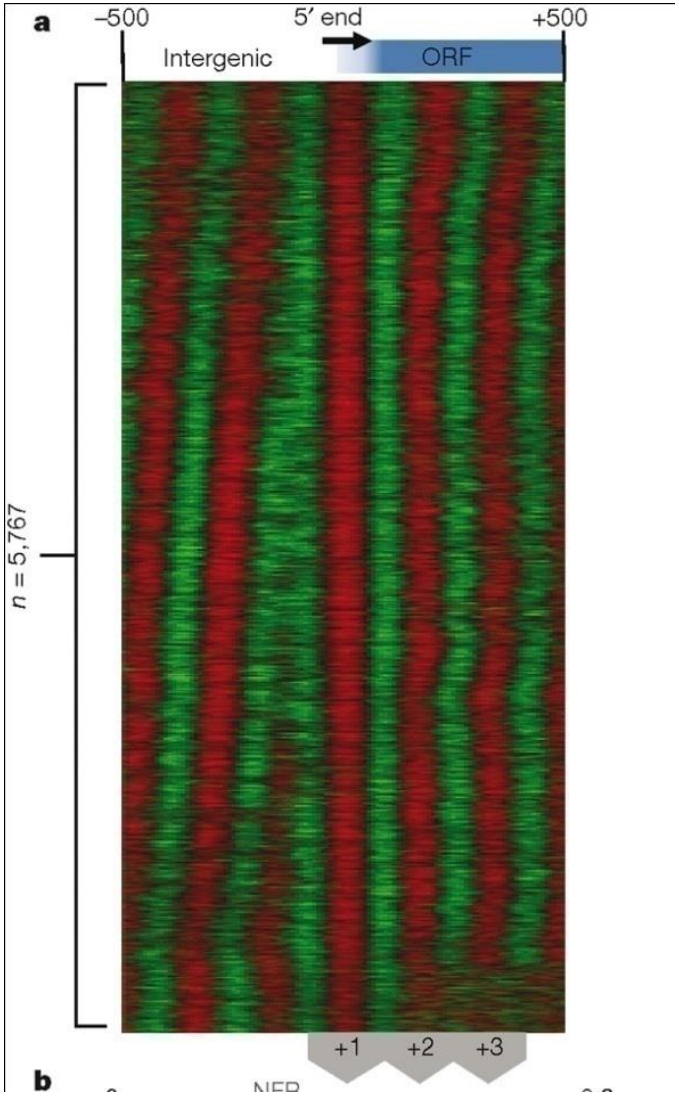
- Digestion enzyme cuts accessible regions of DNA

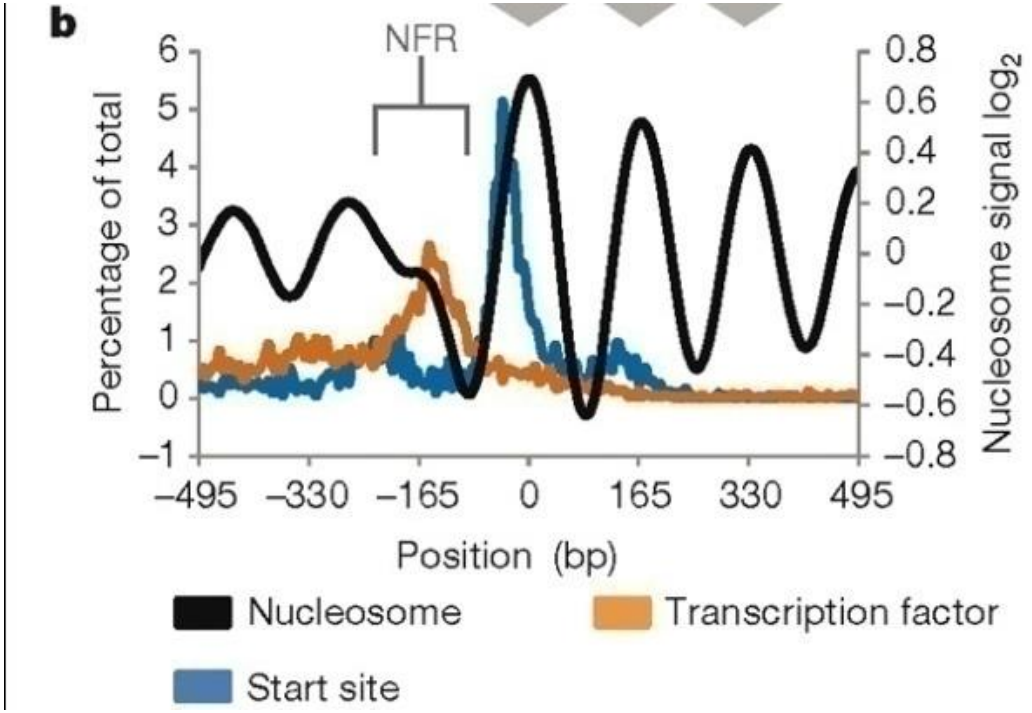


Nucleosome positioning

(Furuyama and Biggins ,PNAS)

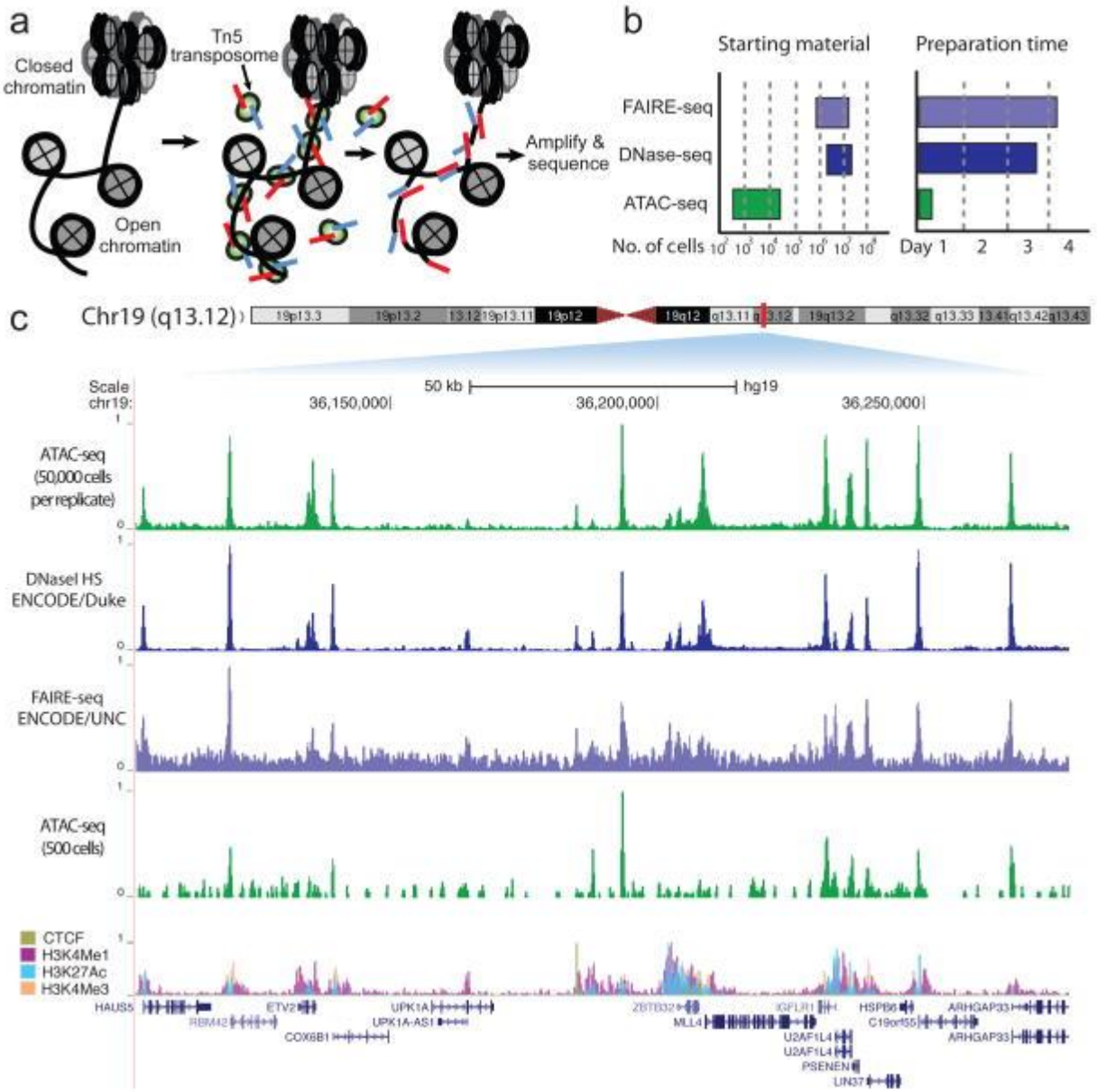
Gene regulatory elements such as promoters and enhancers are at “nucleosome free”





Whitehouse et al., 2007 Nature 450, 1031-1035

Analysis of nucleosome free region/open chromatin by ATAC-seq

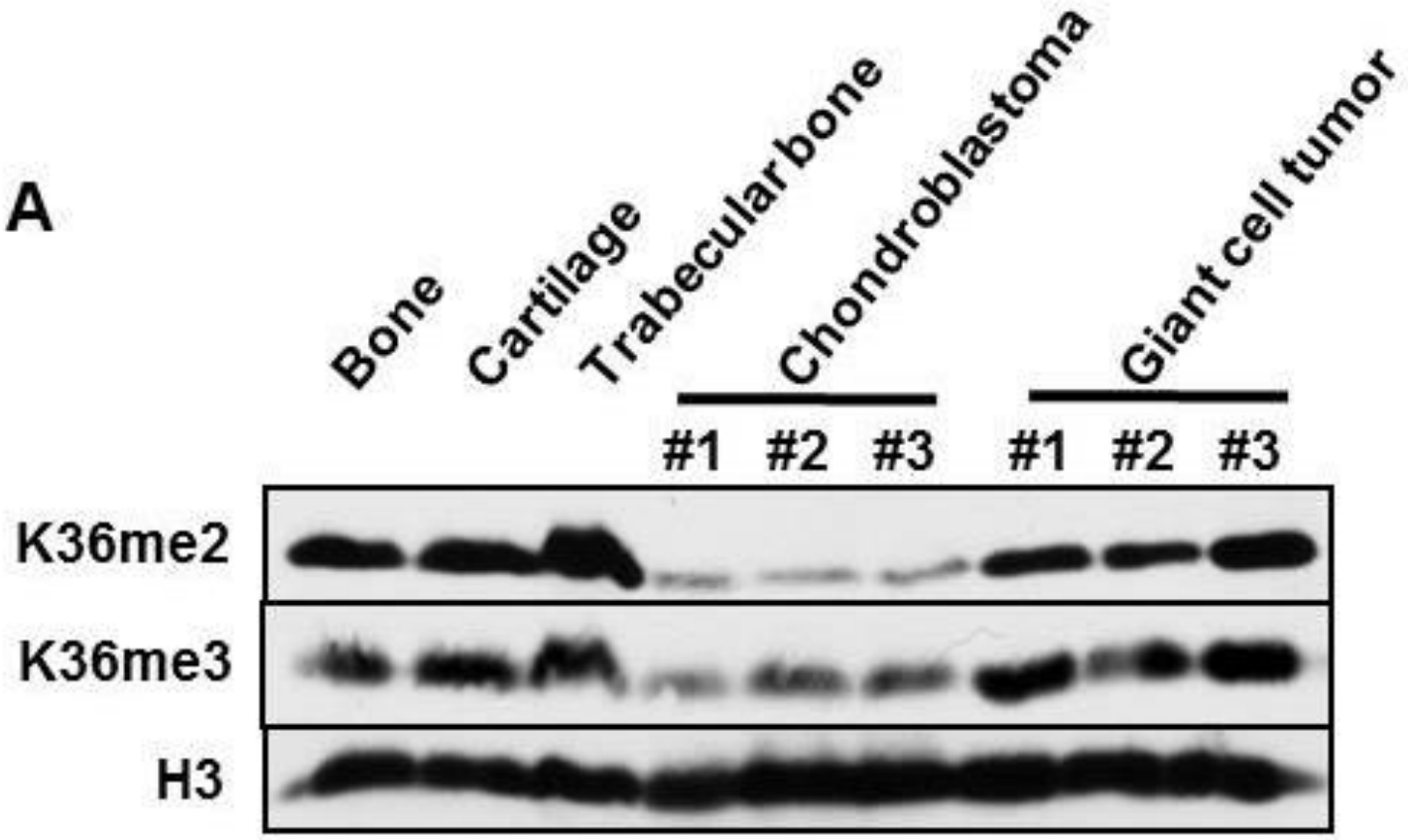


Analysis of histone modification by Western blot and ChIP-seq

Chondroblastoma

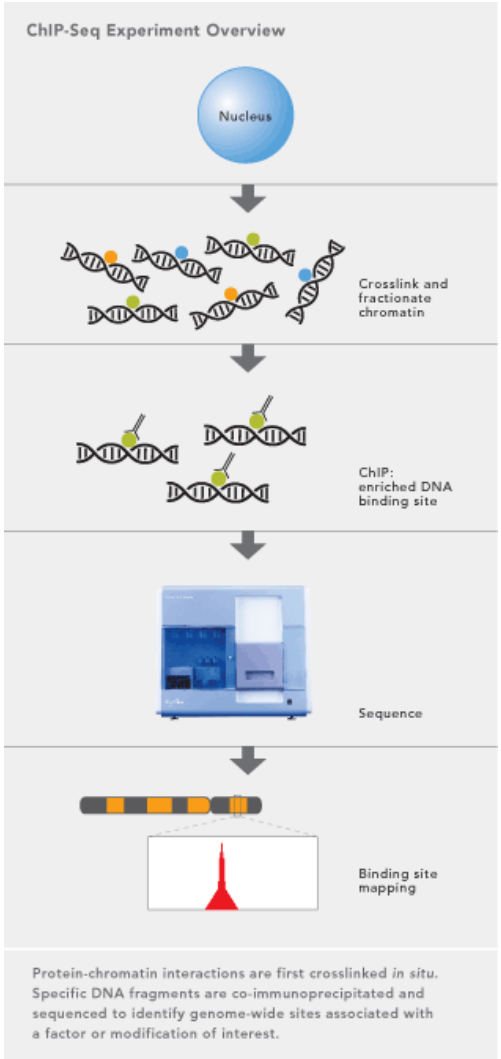
- arises from the epiphysis of the long bones
- Characterized by high cellularity and undifferentiated tissues
- over 90% cases contain H3.3K36M mutation
- The molecular mechanism of tumorigenesis is unknown

H3K36me2/me3 levels are low in chondroblastoma samples compared to normal tissue samples

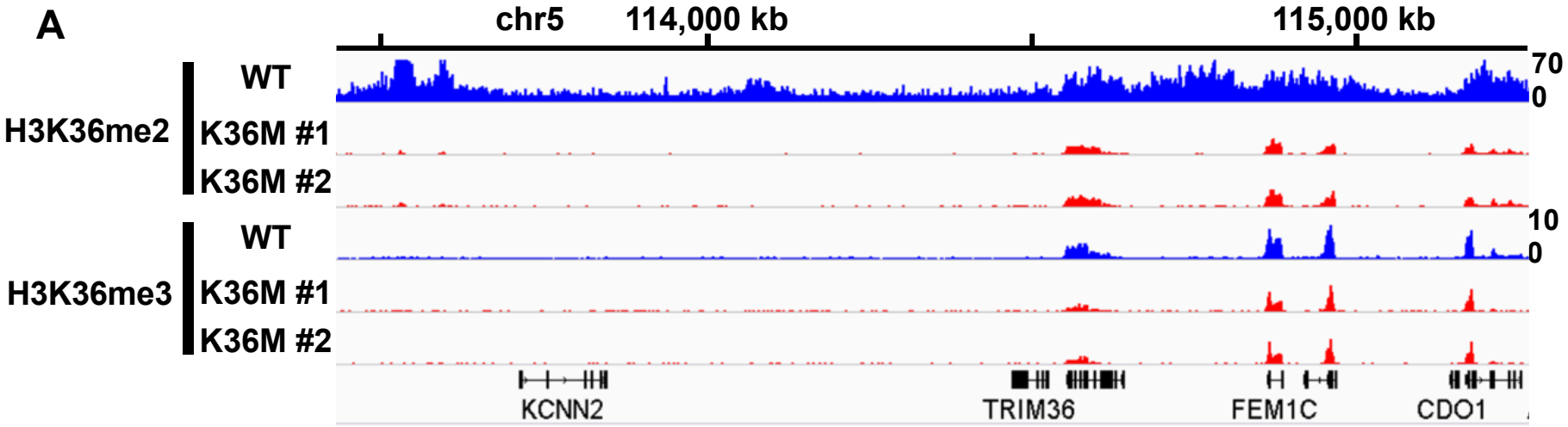


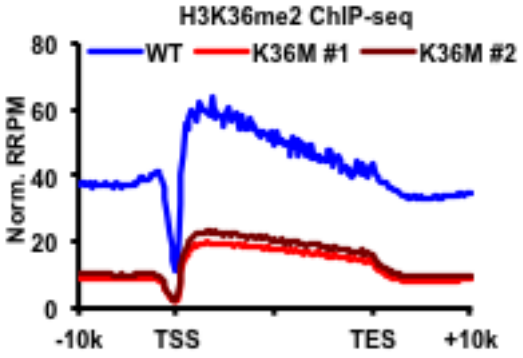
Fang et al Science 2016

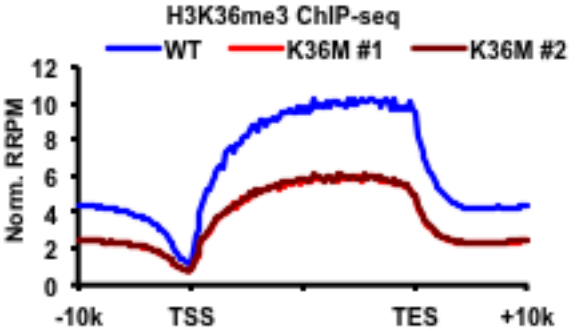
Map histone modifications to a DNA specific sequence by ChIP-seq



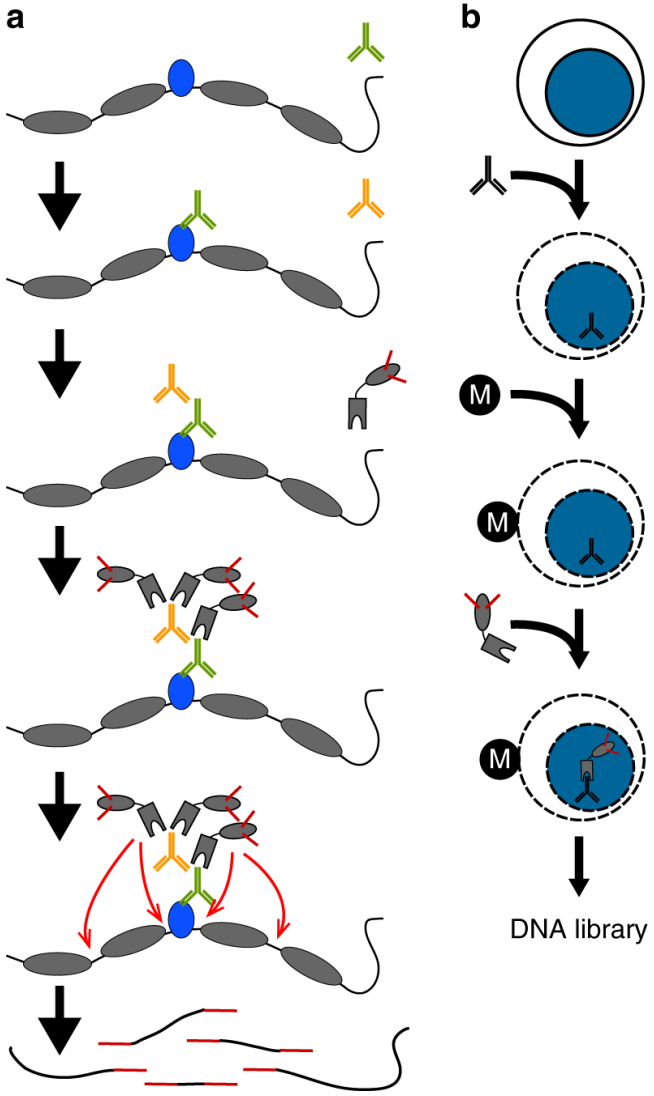
Effect of H3.3K36M mutation on H3K36me2 and H3K36me3 on chromatin in cell lines







Cleavage Under Targets and Tagmentation (CUT&tag): a new method for epigenomic profiling



Advantages compared to ChIP-seq

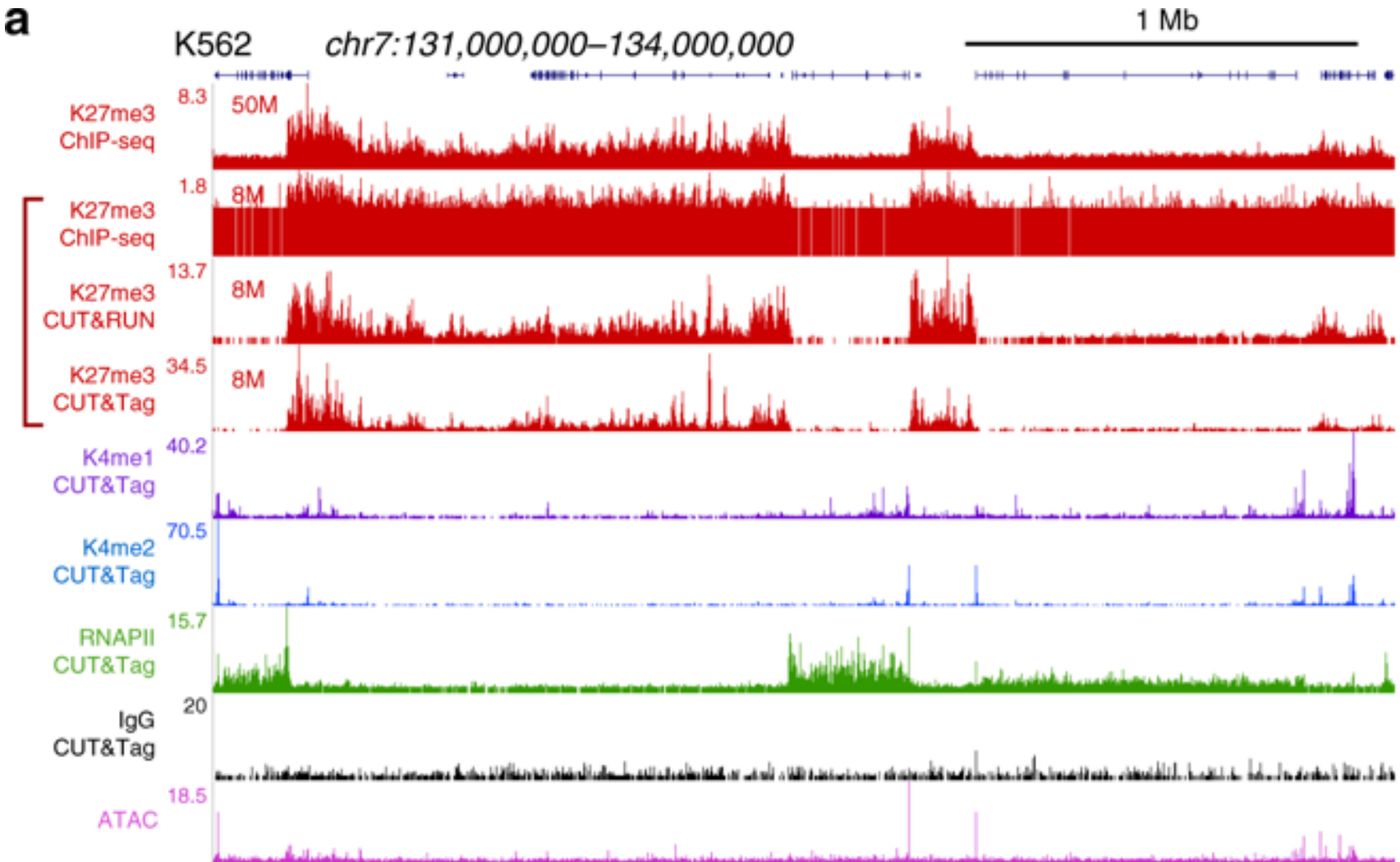
- Fast and efficient
- Low background and therefore less sequence reads
- A dramatic reduction of sequencing cost
- Low cell number

Potential issues:

- complication of ATAC-seq signals

Kaya-Okur et al Nature Communications 2019
Carter et al Nature Communications 2019

Cleavage Under Targets and Tagmentation (CUT&tag) (ACT-seq): a new way for epigenomic profiling



Cleavage Under Targets and Release Using Nuclease (CUT&RUN): a method for epigenomic profiling

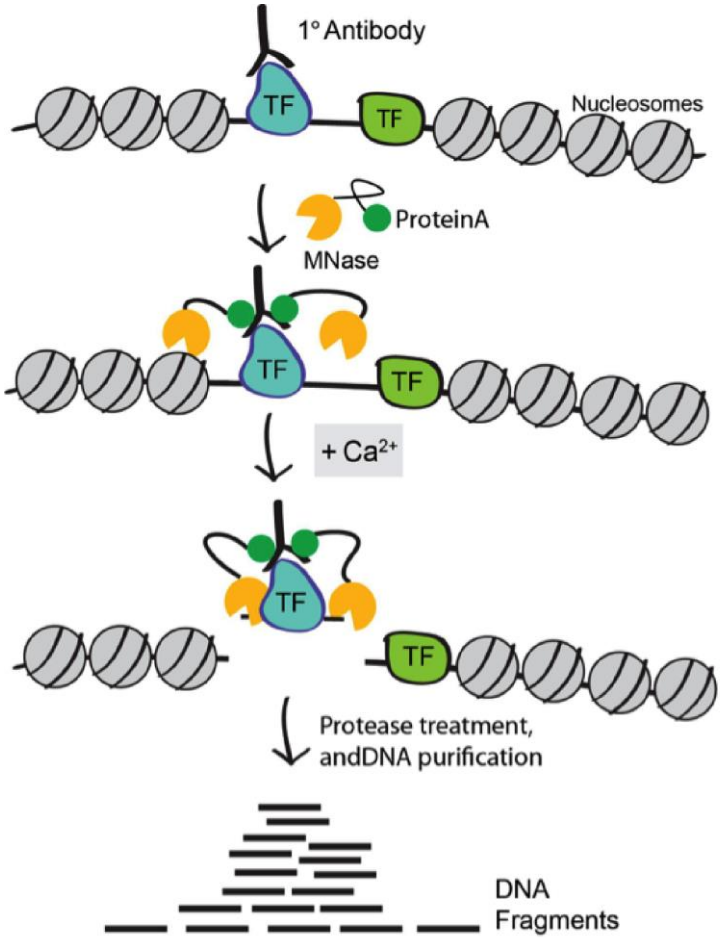


Figure 1. CUT&RUN schematic (see text for details).

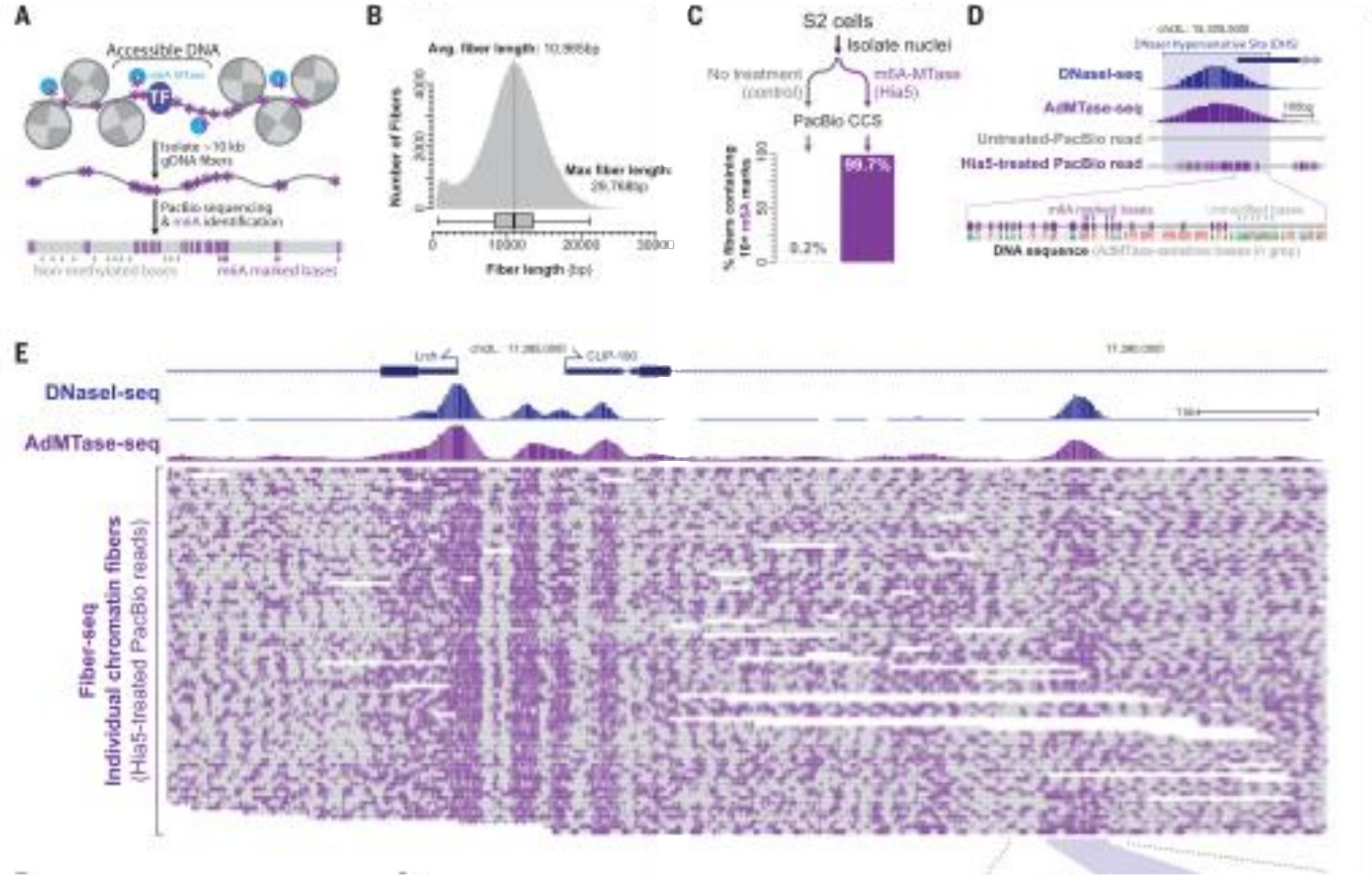
Advantages compared to CUT&tag

- **No potential ATAC-seq signals**
- **Detect transcription factors**

Disadvantages compared to CUT&tag

- **Need a library preparation kits**
- **Maybe better more cells**

Fiber-seq: analysis of chromatin accessibility at single molecule levels



Stergachi et al, Science 2020

Take home messages

- **Epigenetic phenomenon occurs in our daily life.**
- **Mechanisms of epigenetic regulations are complex and evolving.**
- **Basic principles of epigenetic regulations have been defined.**
- **Tools to probe histone modifications and chromatin structures are evolving at single cell and single molecule levels.**