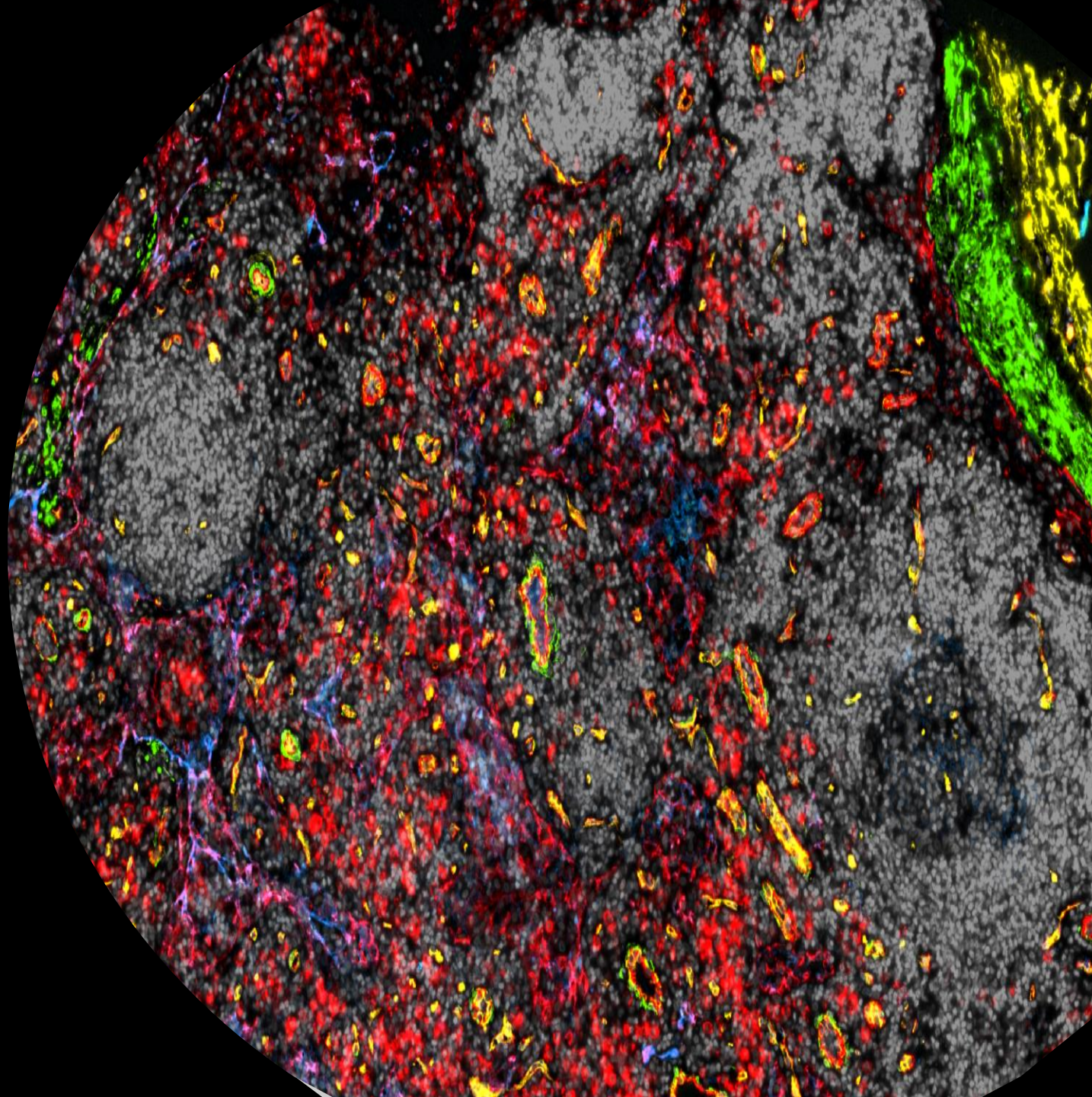
Cancer Biology I

Part-II

Week 10



AGENDA

Nov 3rd: Cancer genomics- mutations

Nov 10th: Cancer genomics-copy number alterations, heterogeneity, tumor evolution

Nov 17th: Cancer Epigenetics- chromatin 3D structure, cell plasticity

Nov 24th: – Major signaling pathways leading to cancer

Dec 1st: Cancer Therapies – chemo and targeted therapies

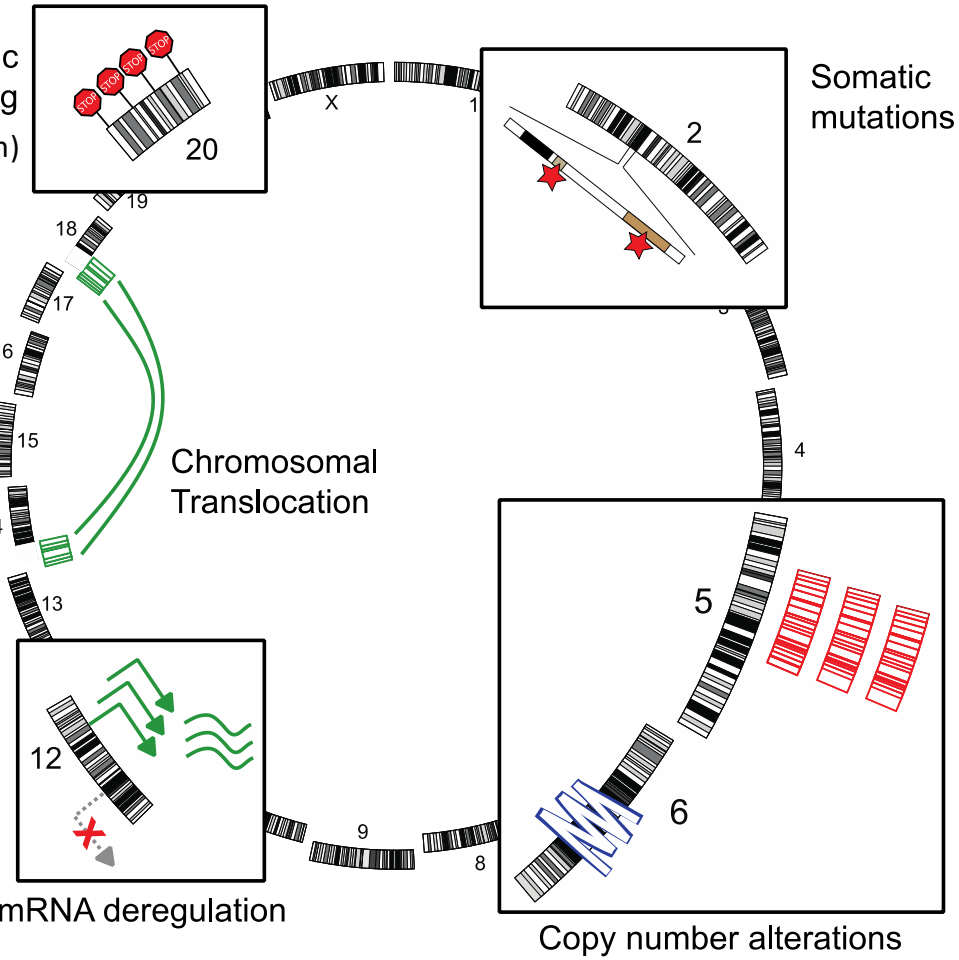
Dec 8th: Introduction to immunotherapies –

Dec 15th: discussion of unclear points and career development discussion towards a PhD

Dec 17th: Exam 2-4 PM (room to be decided)

What are they?

Cancer Genomic Alterations



You like my dogs

You like my dots

You like my dogs

Invert the meaning

my dogs like you

you like my cat

change the meaning

You like my dogs

You like __ dogs

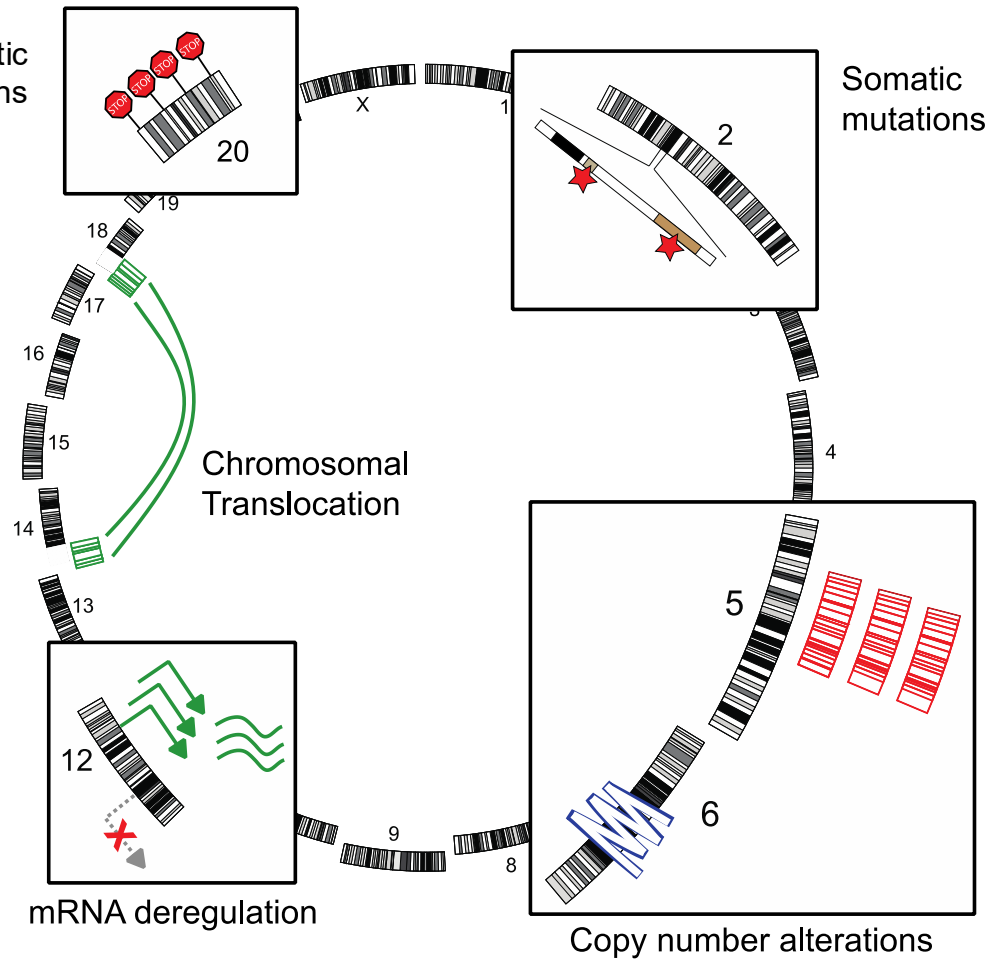
You like like my dogs

What are they?

Cancer EPIGENTICS Alterations

You like my dogs

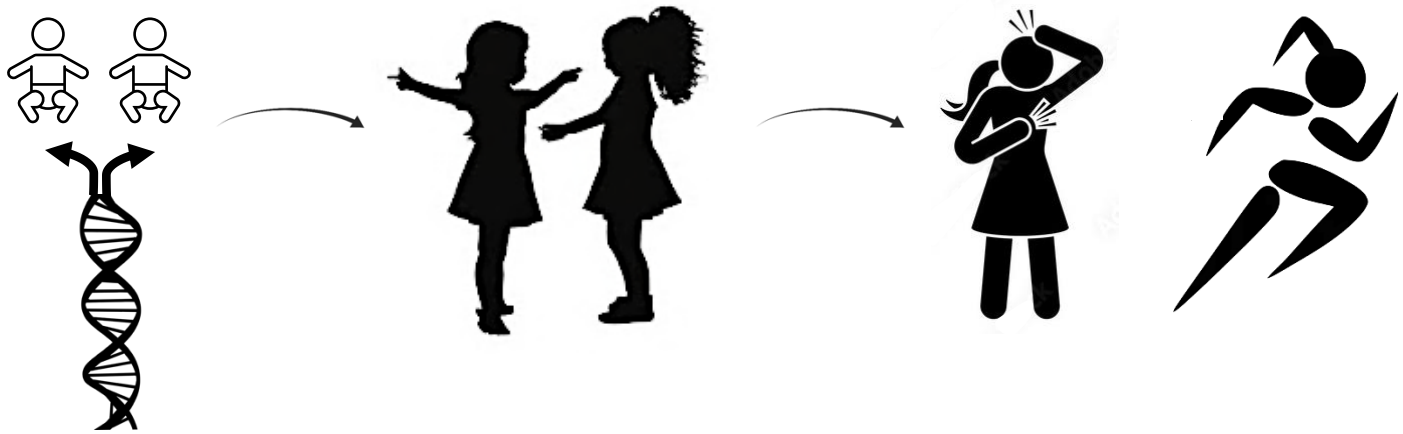
You like my dogs



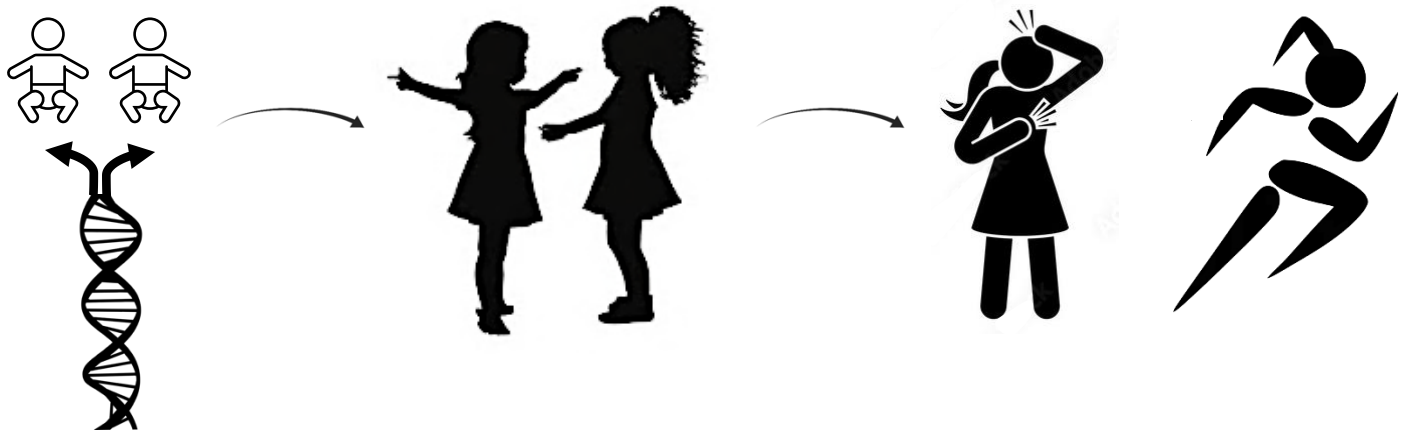
What is epigenetics?

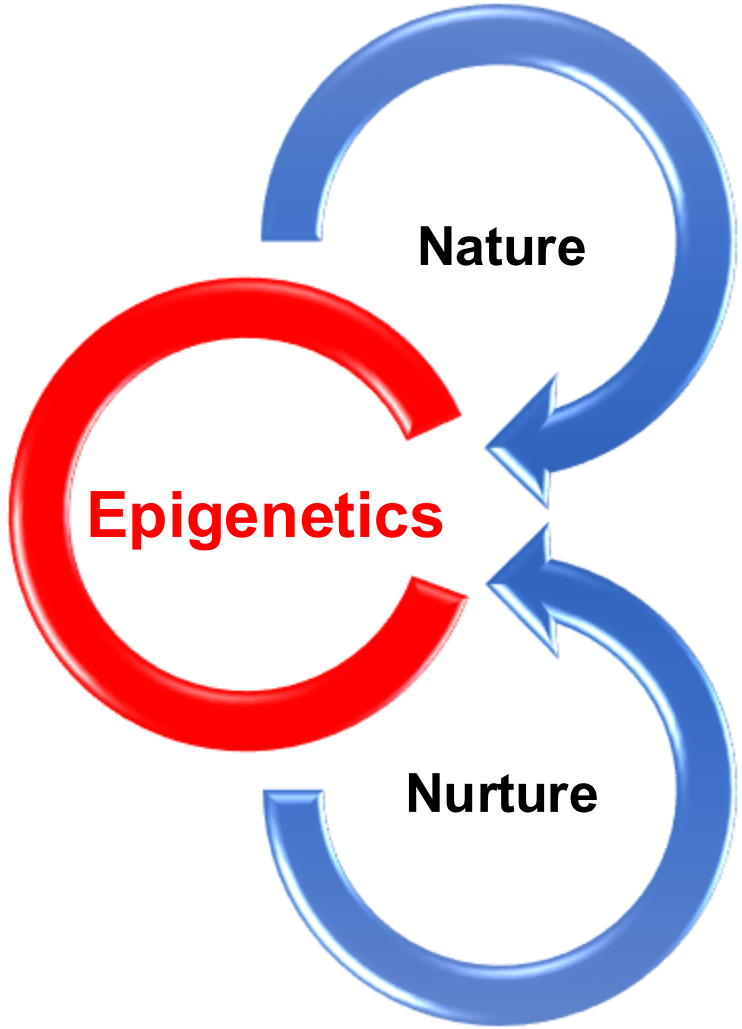
Epigenetics: study of stable heritable traits - *not attributable to alterations in the DNA sequence* - that change gene expression and cellular phenotypes

What is epigenetics?



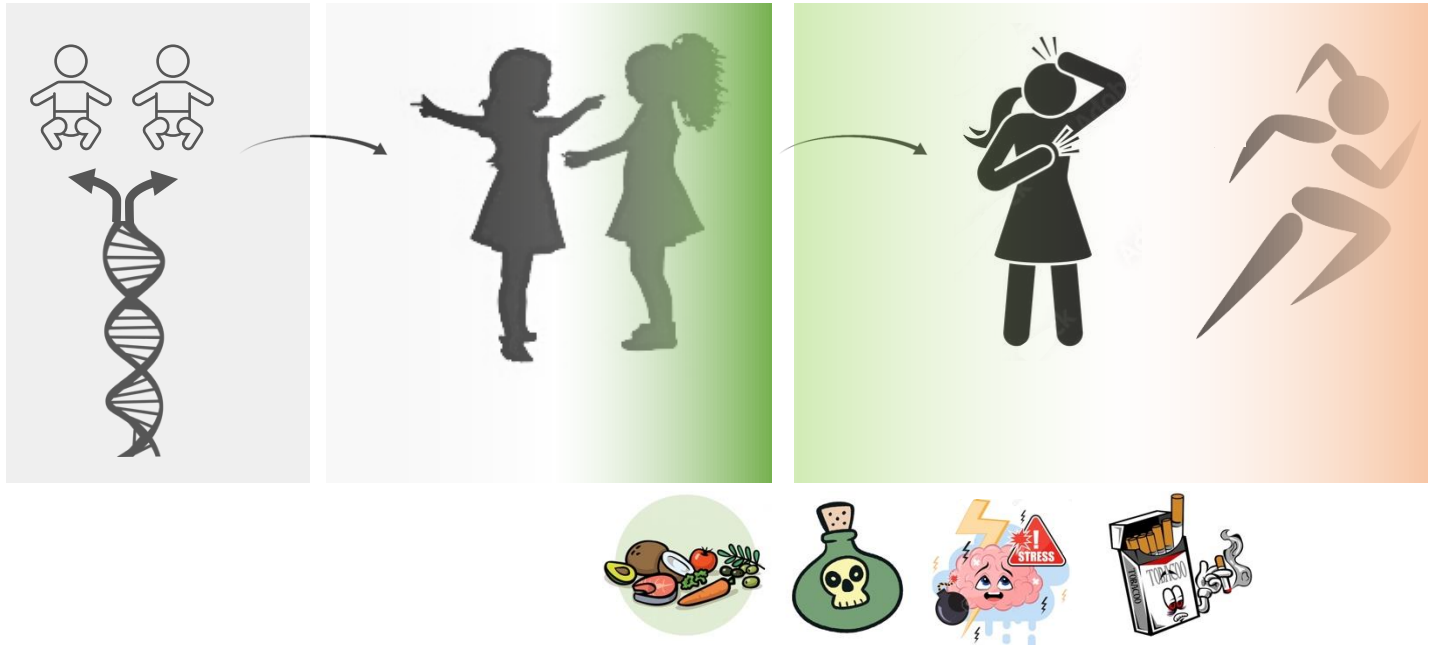
What is epigenetics?

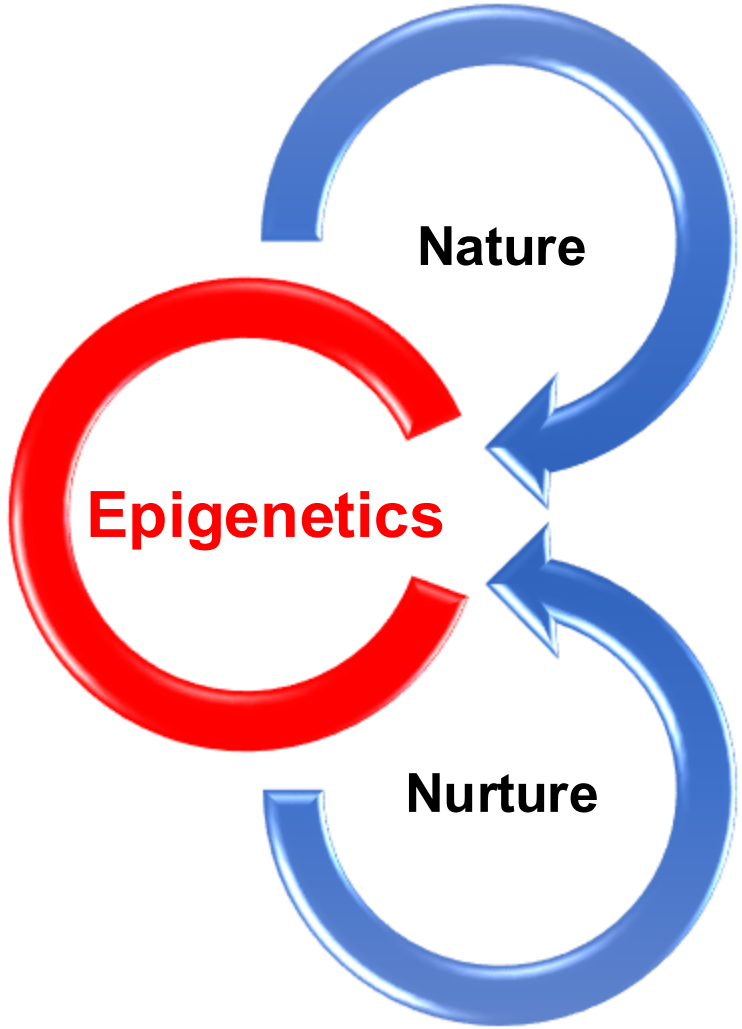




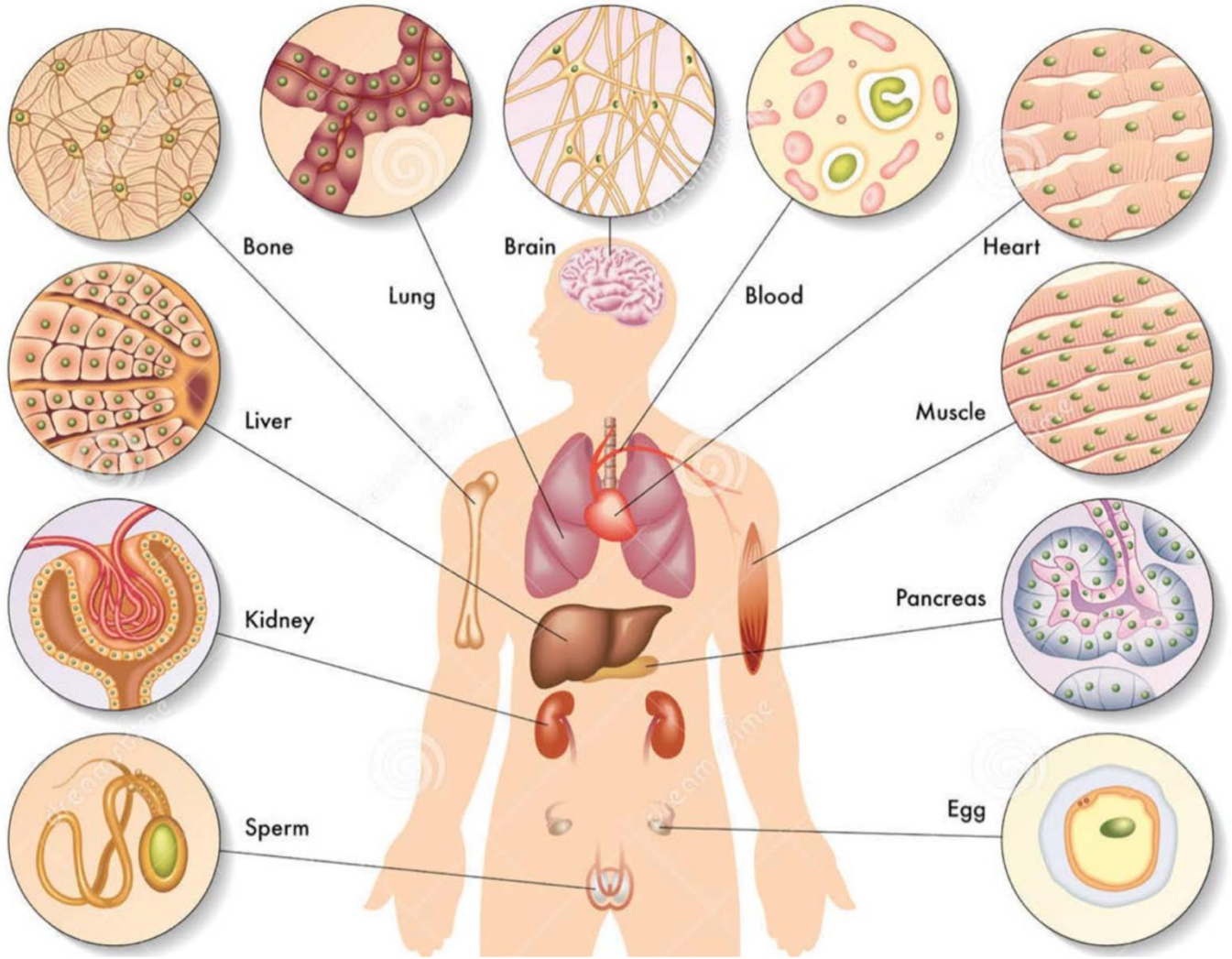
What is epigenetics?

environment

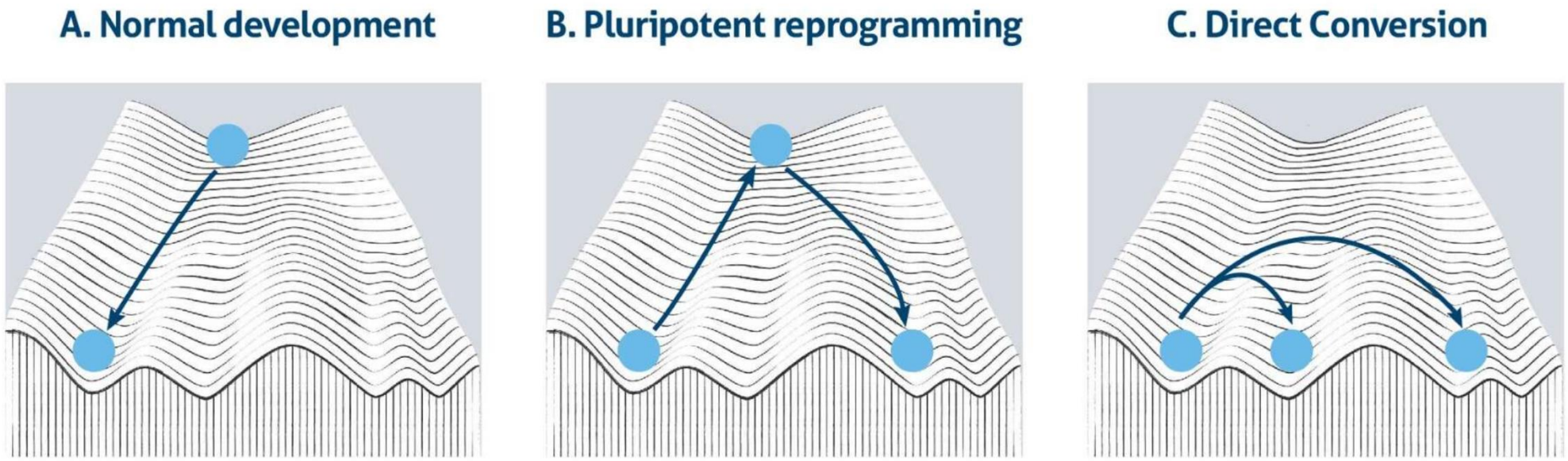




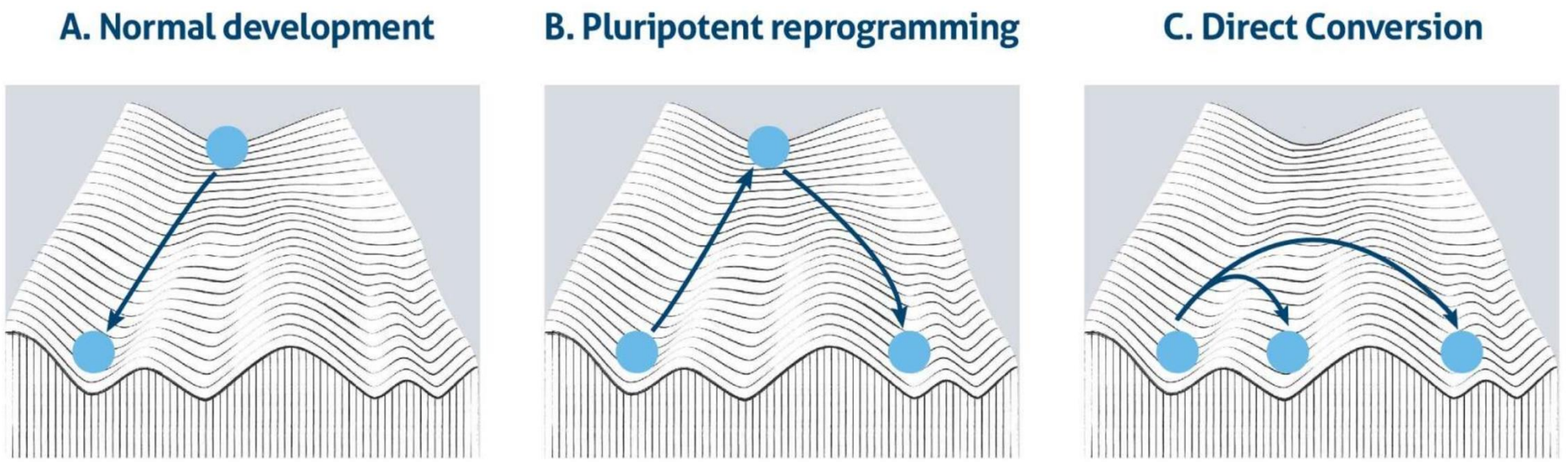
Epigenetic regulation determines cell fate



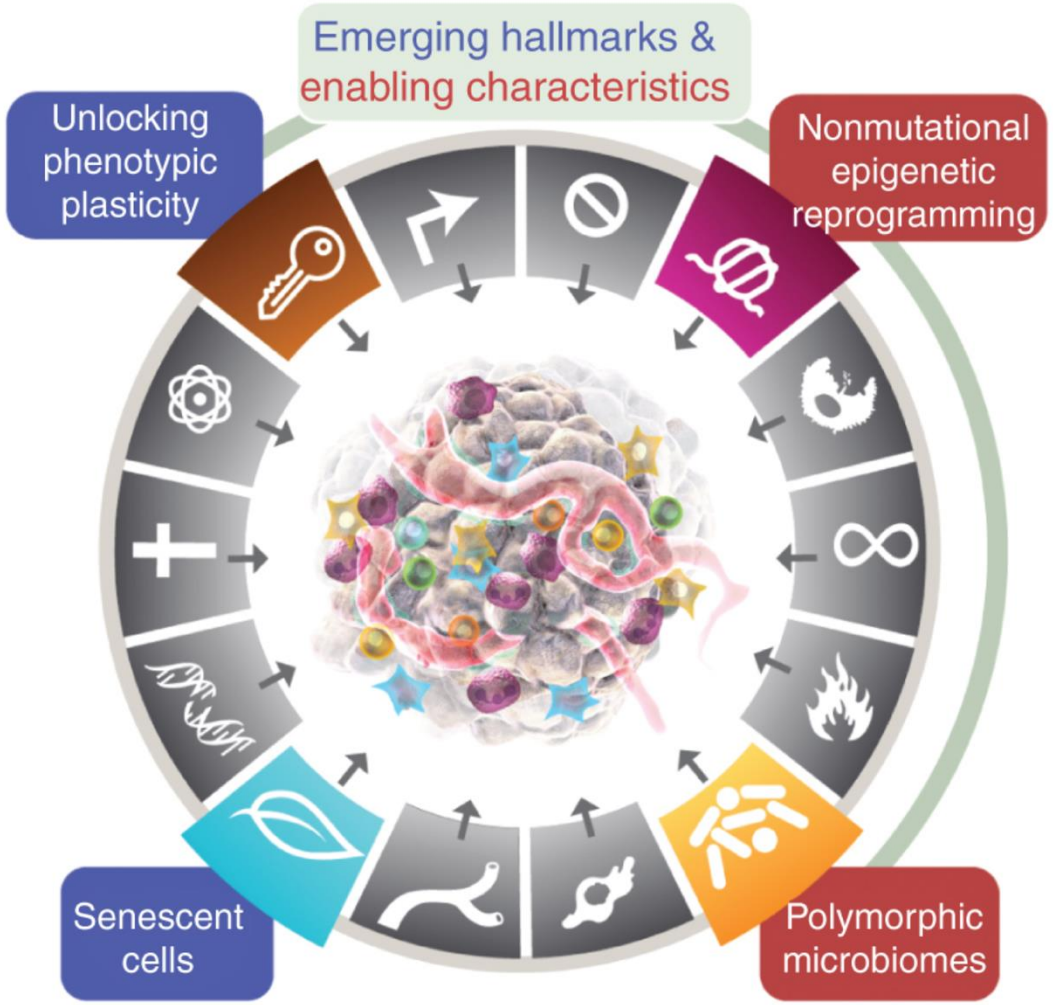
Epigenetic regulation determines cell fate



Epigenetic regulation determines cell fate



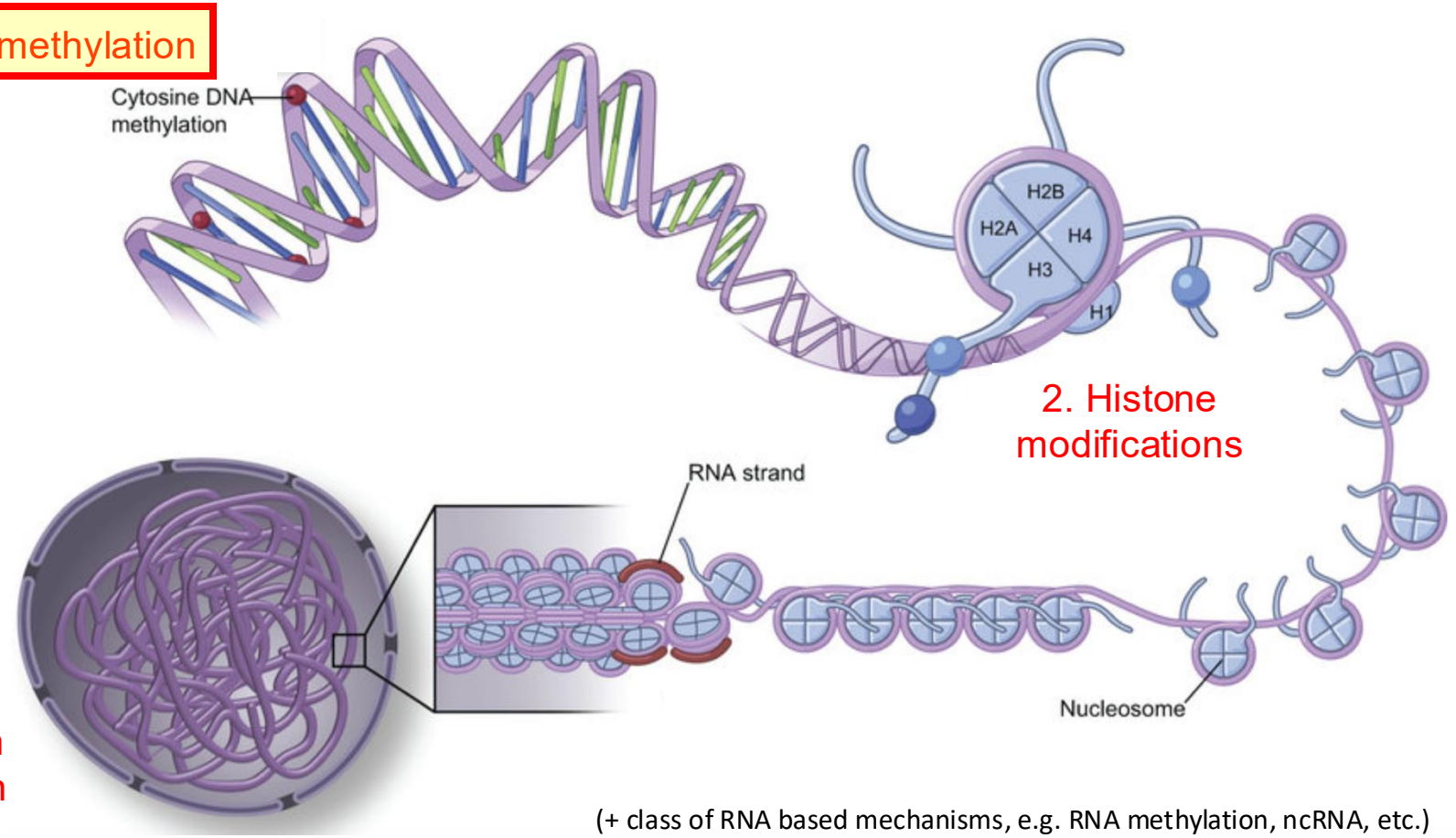
Epigenetic de-regulation is a hallmark of cancer



(Hanahan, 2022)

Which are the main regulators of epigenetics?

1. DNA methylation



2. Histone modifications

3. Chromatin conformation

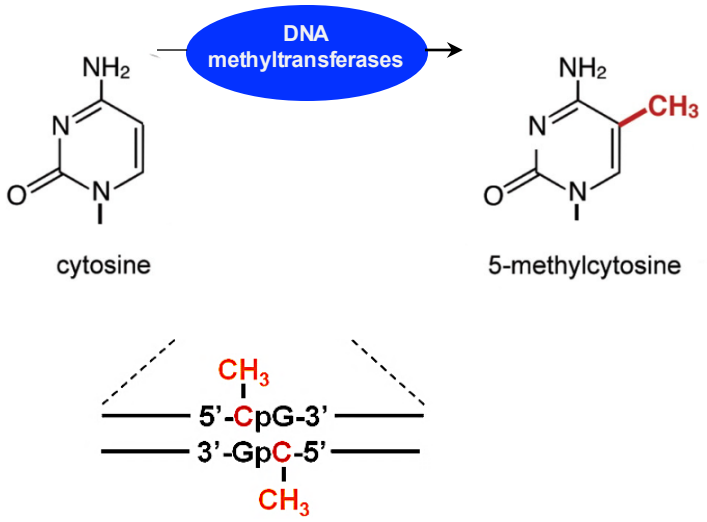
(+ class of RNA based mechanisms, e.g. RNA methylation, ncRNA, etc.)

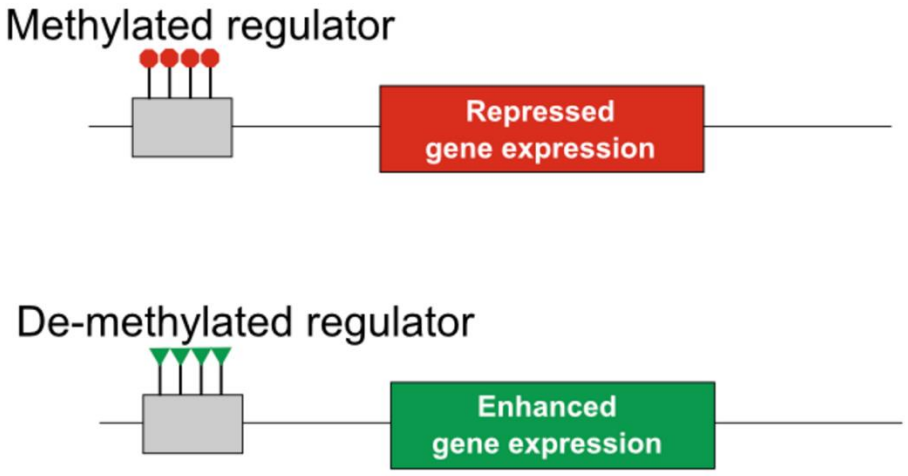
1. DNA methylation

Which residue on the DNA acquires DNA methylation?

1. DNA methylation

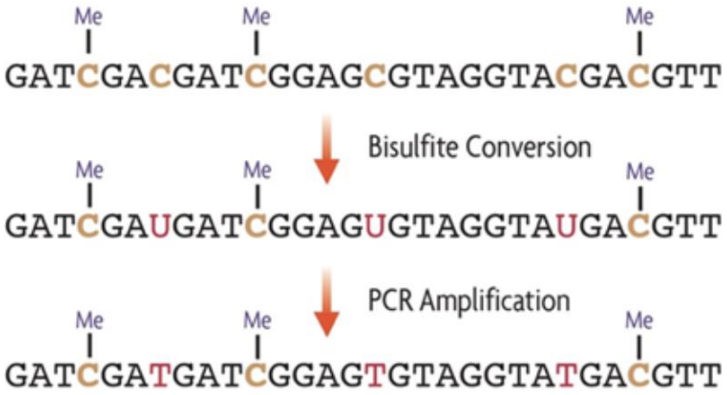
- In higher eukaryotes, DNA methylation is formed by the addition of a methyl group to the 5’ position of cytosine residues within a CpG dinucleotide context.
- CpG sites are highly methylated in the mammalian genome with the exception of CpG islands which are largely unmethylated.
- ~60% of all human genes are dependent on promoters within CpG islands. When these sequences are methylated, promoter activity is silenced.





DNA Methylation

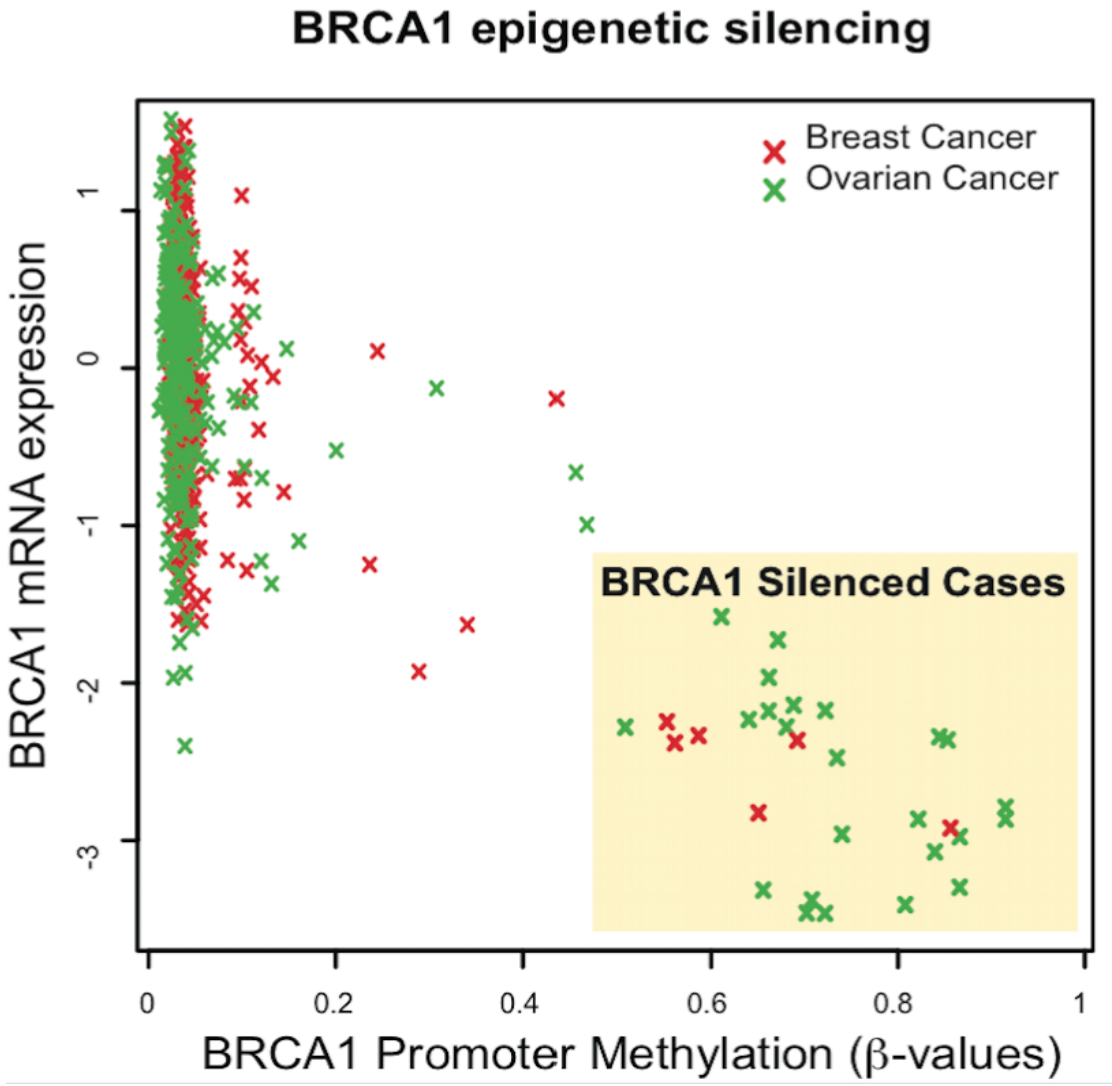
based on bisulfite conversion and sequencing



- Distinguish between methylated and unmethylated cytosine
- Coupled with high-throughput sequencing to know the DNA methylation status if the genome
- Probing DNA methylation preferentially at CpG promoters, but now also gene body / up-downstream gene regions

DNA Methylation

based on bisulfite conversion and sequencing



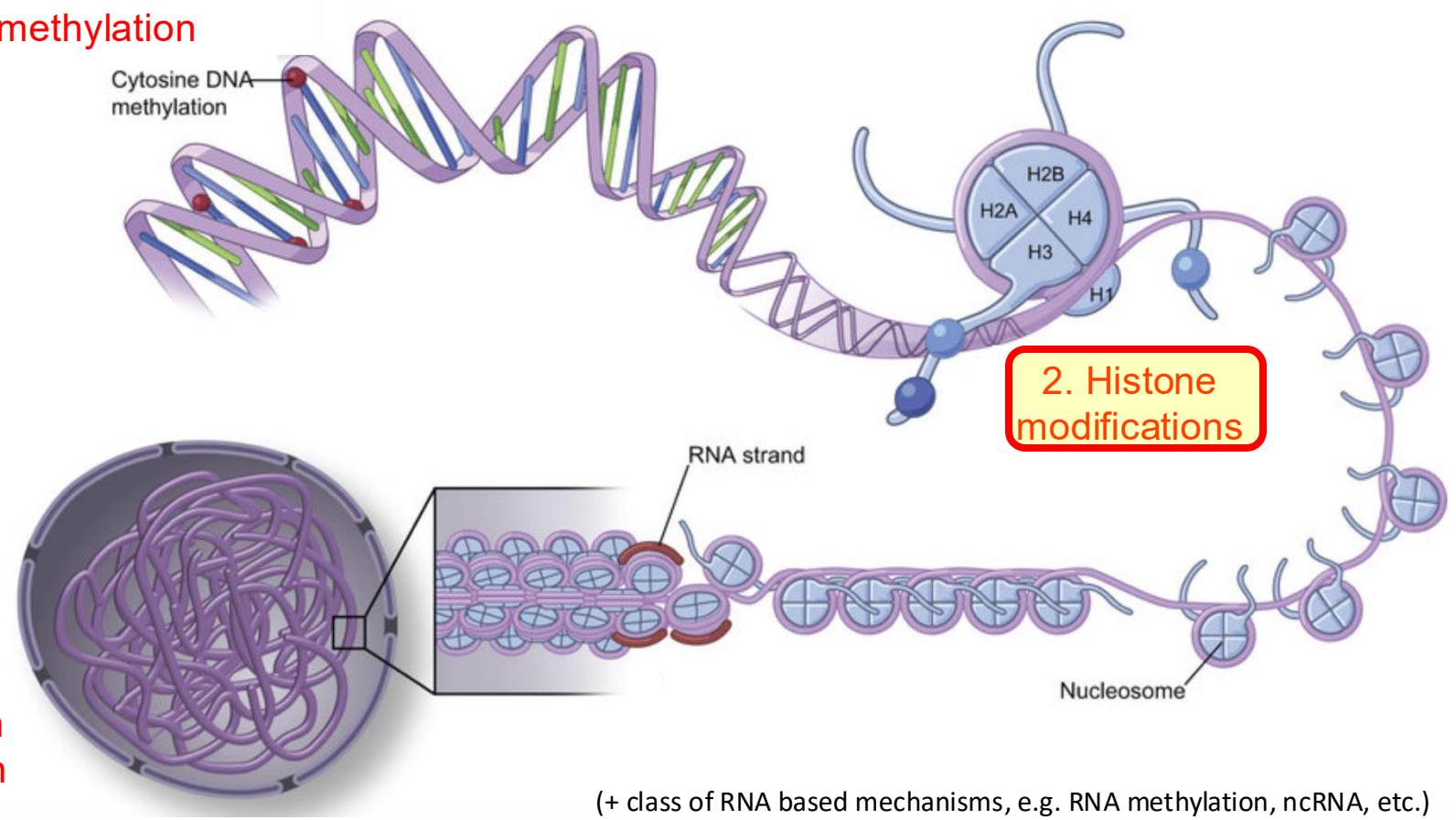
What drives DNA methylation changes?

- Alterations of proteins regulating DNA methylation (DMT, Demethylase)
- Increased cell division leads to DNA methylation loss in late replicating genomic regions (e.g. intergenic repeats) → **Loss of DNA methylation is also an aging phenotype**
- Differences in DNA methylation between cancer and normal cells from the same tissue might reflect the tumor cell of origin (tumors originating from progenitor cells)
- Tumor cells frequently trigger de- and trans-differentiation programs → changes of DNA methylation

How does this happen?

Mechanisms of epigenetic regulations:

1. DNA methylation



2. Histone modifications

3. Chromatin conformation

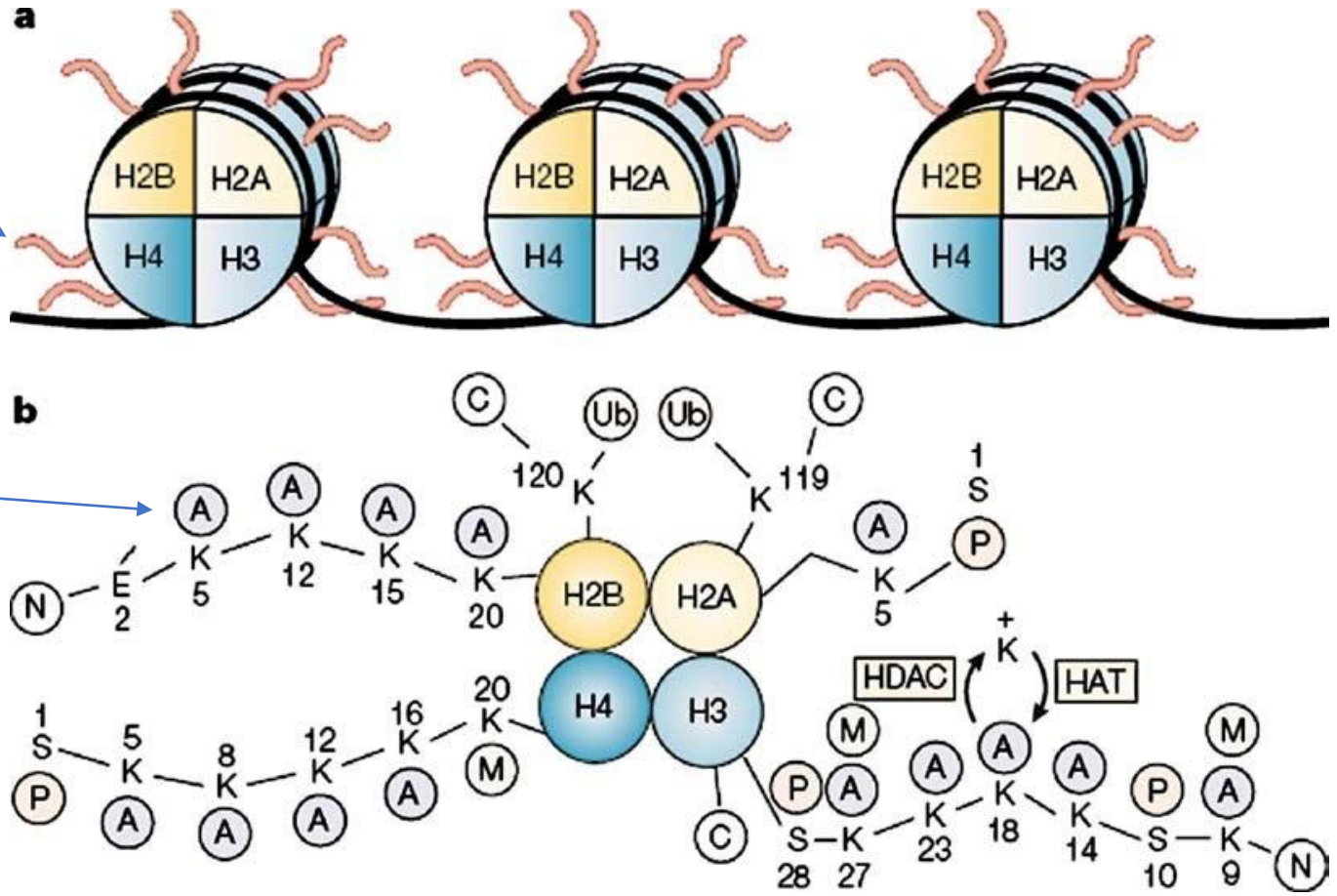
(+ class of RNA based mechanisms, e.g. RNA methylation, ncRNA, etc.)

Histone post-translational modifications

Histone tails are accessible for protein binding

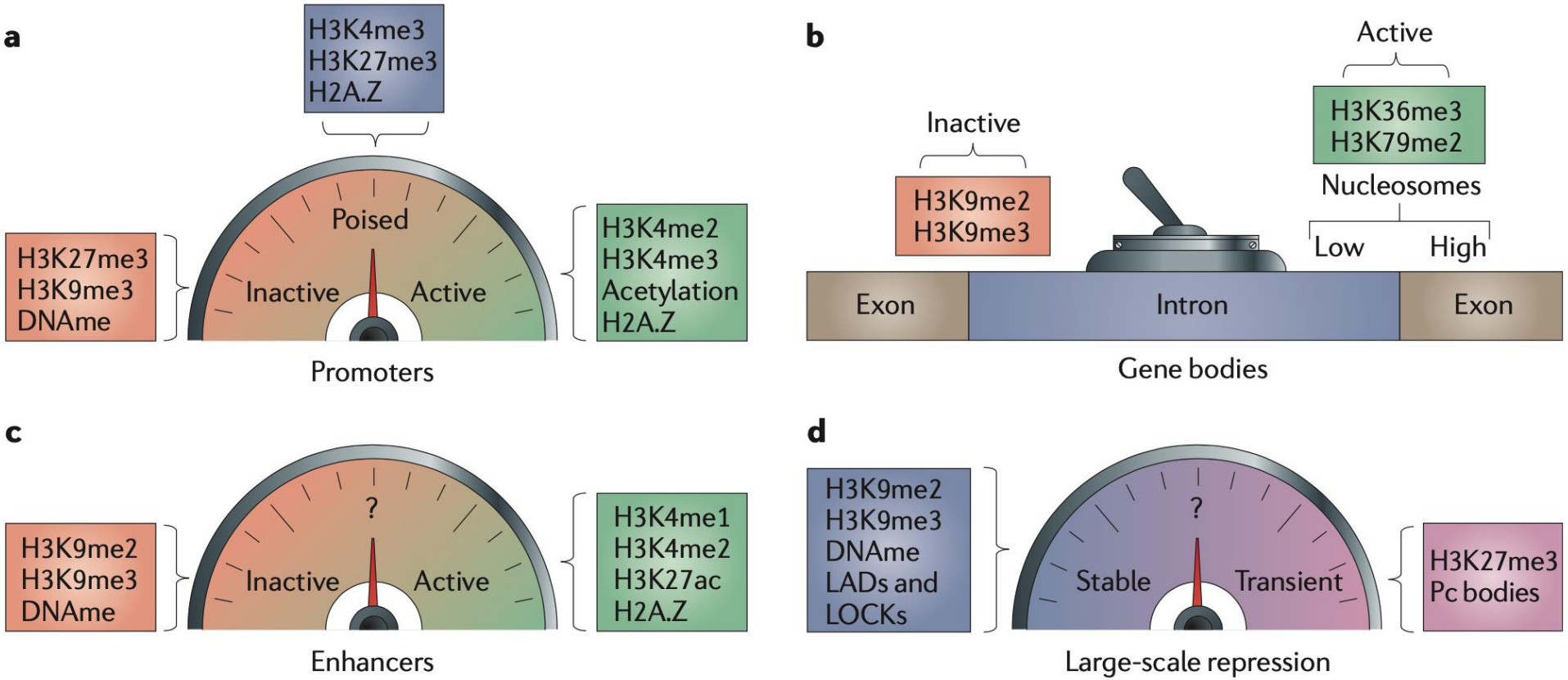
Different type of modifications can be found at lysine tails

Most common:
Acetylation (A)
Methylation (M)



The histone code

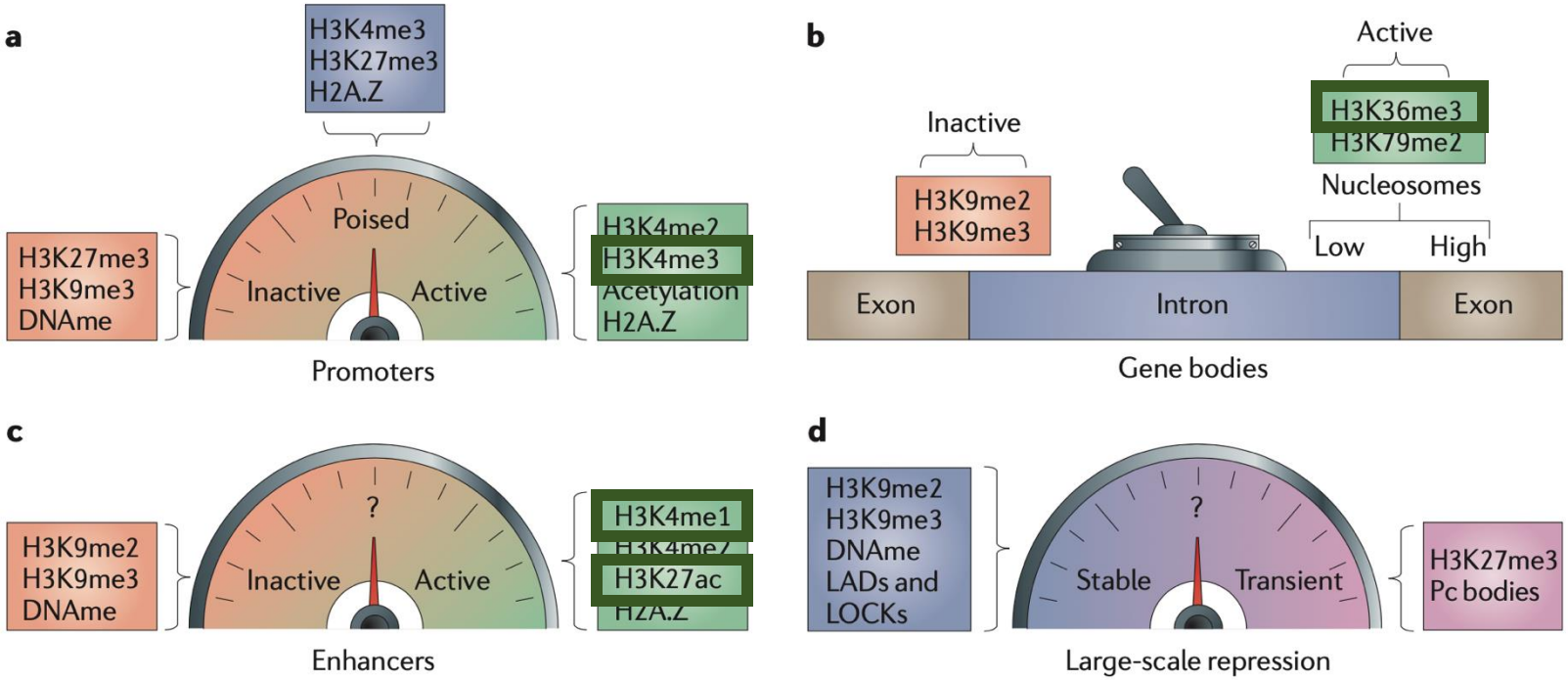
‘Dashboard’ of histone modifications for fine-tuning genomic elements



Histone-<n> **K** (lysine) <n> mono/di/tri-methylation (**me1/2/3**) or acetylation (**ac**)

The histone code

- **H3K4me1/3** marks active promoters and/or enhancers
- **H3K36me3** is present in the body of active genes
- **H3K27ac** specifies the degree of activity of distal regulatory elements (enhancers); whereas **H3K9ac** specifies the degree of activity of proximal regulatory elements (promoters)



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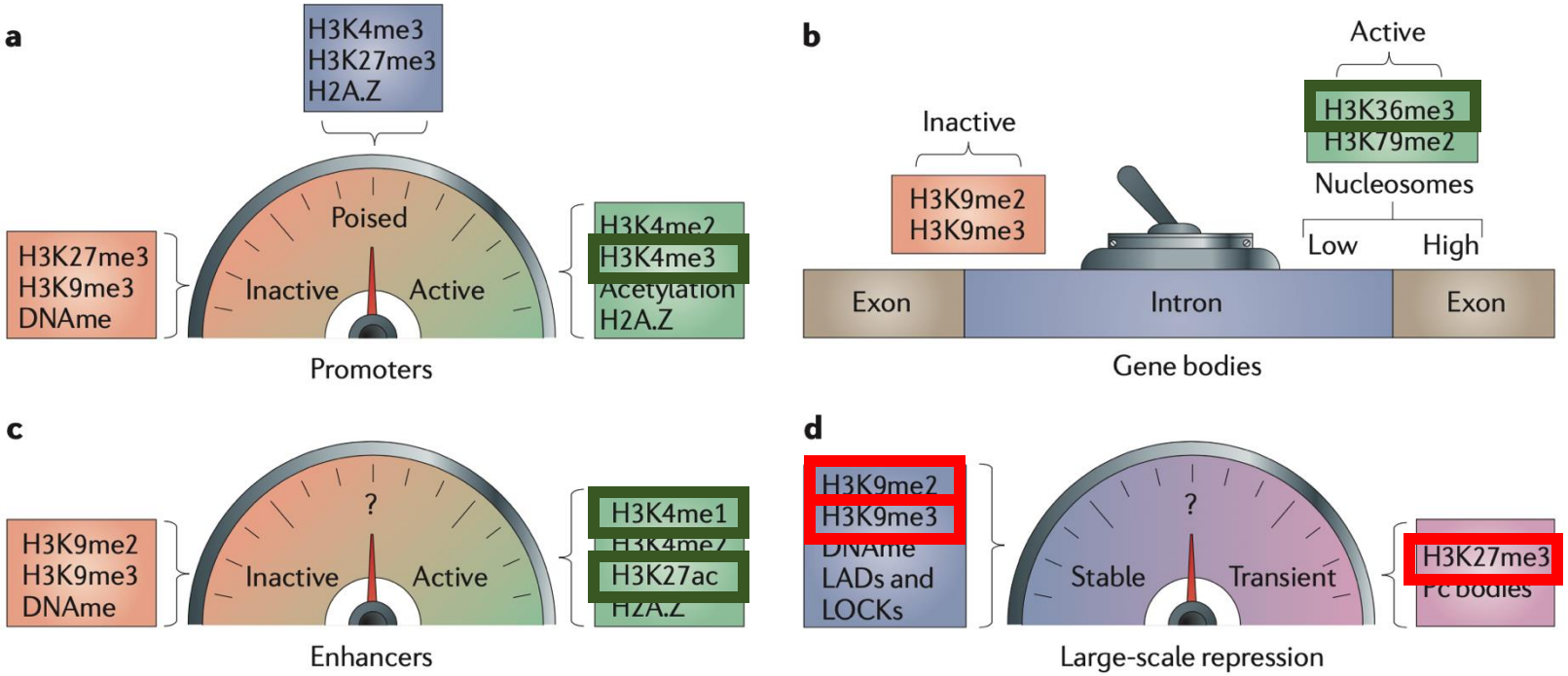
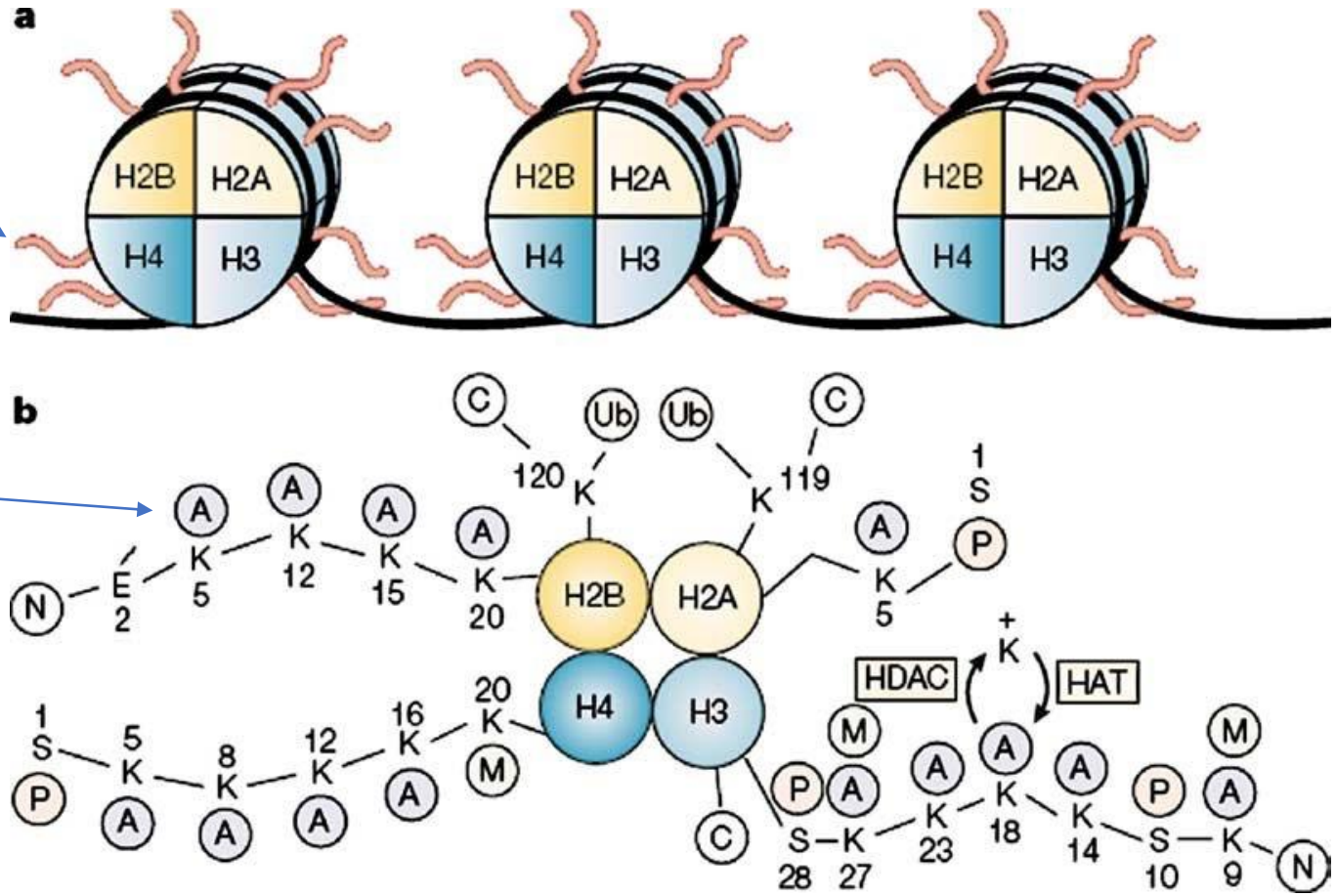
- **H3K9me3** marks the classical heterochromatin (canonical repressive state)
- Wide-spread regions repressed by the Polycomb Group of proteins are enriched in **H3K27me3**
- **H3K9me2/3** mark genomic regions of highly condensed chromatin physically associated with the nuclear lamina



Histone post-translational modifications

Histone tails are accessible for protein binding



Who is regulating histone Modifications?

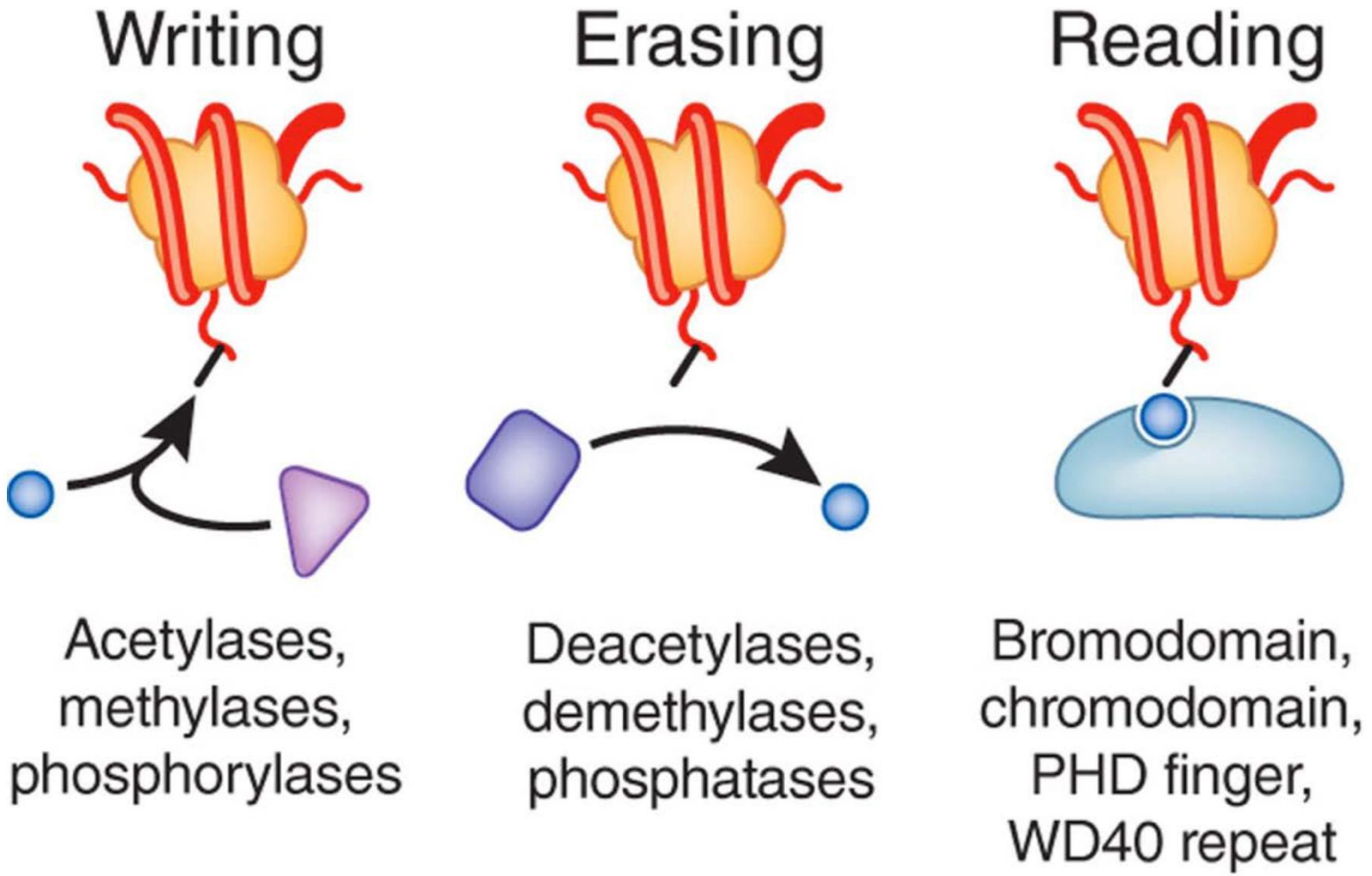
Different type of modifications can be found at lysine tails

Most common:
Acetylation (A)
Methylation (M)

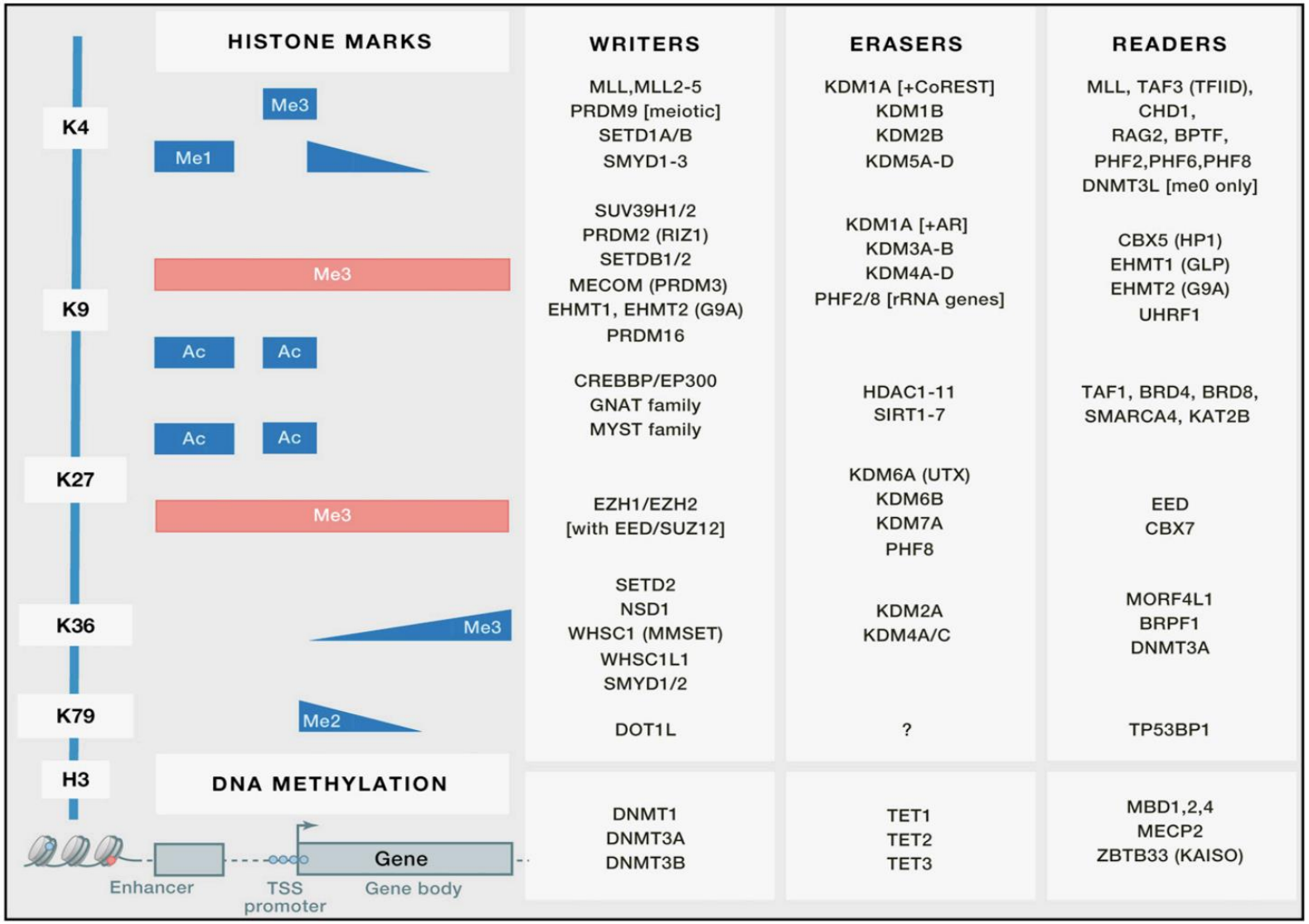
Histone acetylation and methylation is mediated by chromatin regulators:

K-methyl-transferases (KMTs)
K-demethylases (KDMs)
Histone acetyl-transferases (HATs)
Histone deacetylases (HDACs)

Chromatin regulators



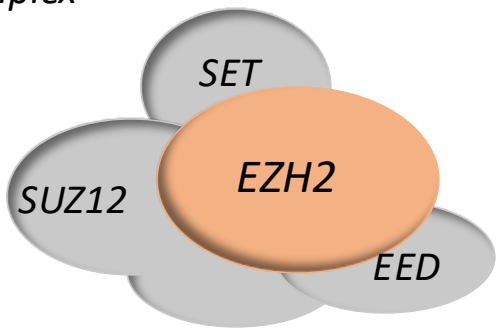
Chromatin regulators are among the most frequently mutated genes in cancer



(Gonzalez-Perez *et al.* Gen. Bio. 2013)

Chromatin regulators: EZH2 (writer) or HMT

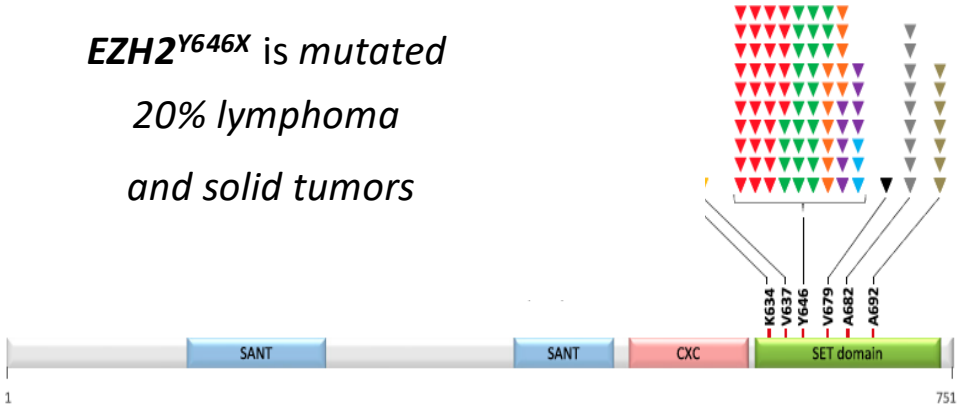
PRC2 complex



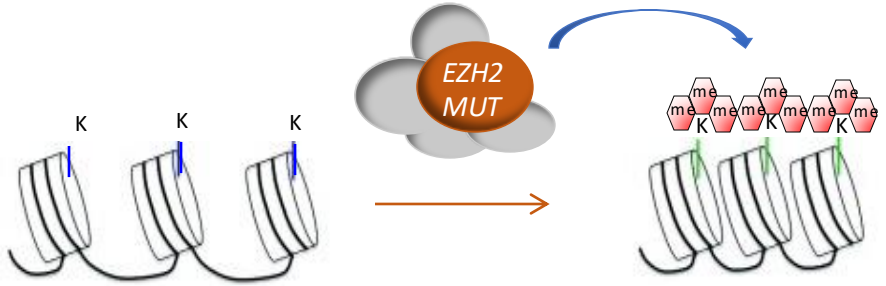
EZH2^{Y646X} *is mutated*

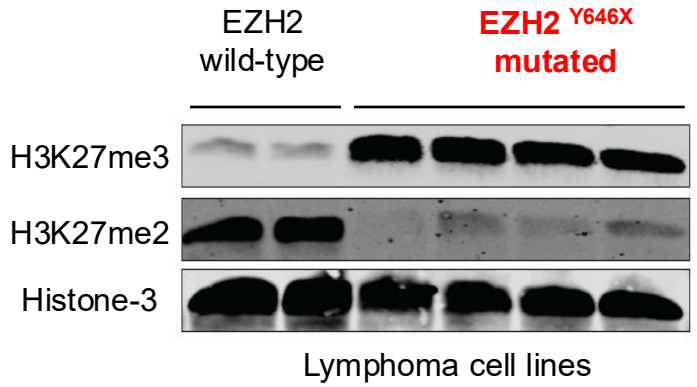
20% lymphoma

and solid tumors

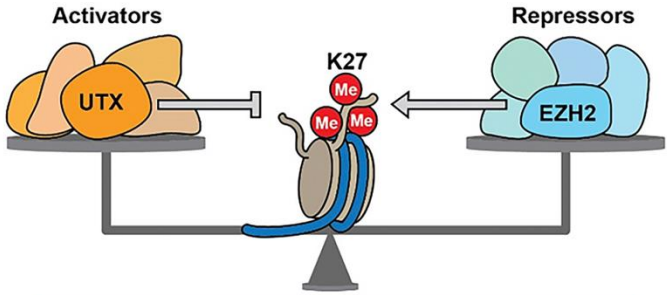


EZH2^{Y646X} increases H3K27 tri-methylation





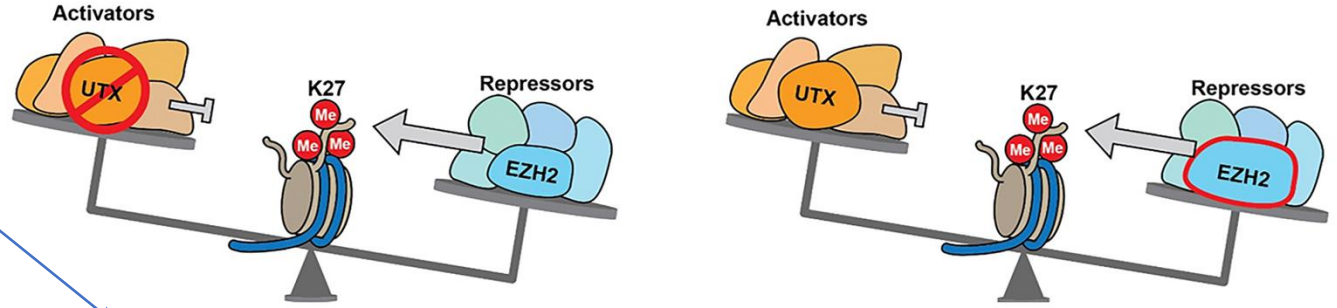
Chromatin regulators: UTX (eraser) or HDM



Balanced activated and repressed transcription

UTX (KDM6A) is lost
in ~26% of bladder tumors

EZH2 is hyperactive
(Y646 mutation)
in ~20% of B-cell lymphomas



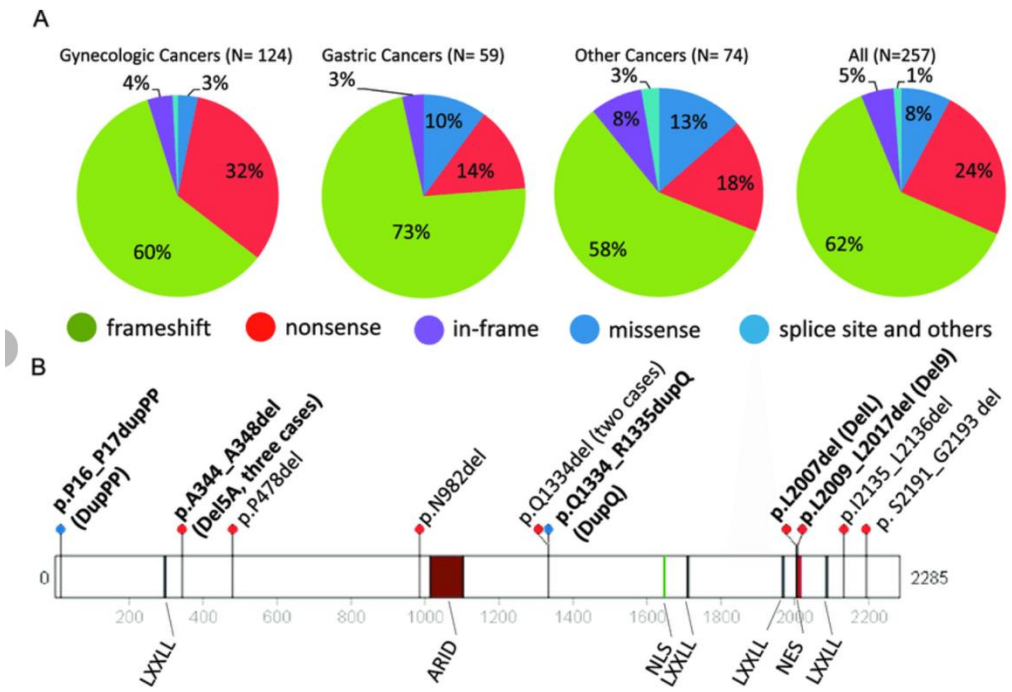
UTX loss-of-function mutations

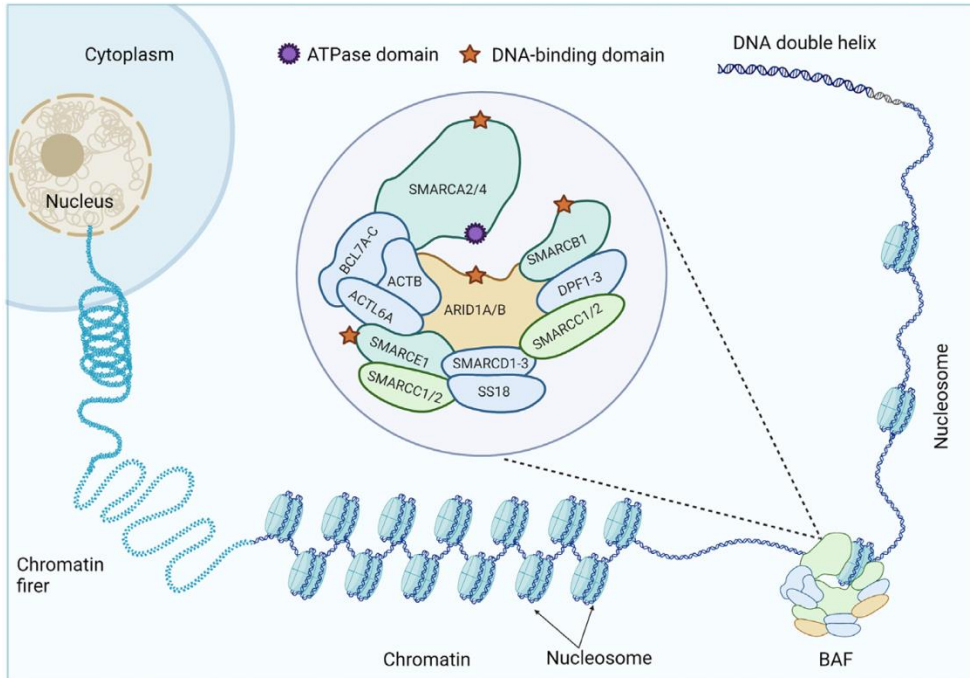
EZH2 gain-of-function mutations

PRC2-regulated transcription repression

Massive increase of H3K27me3

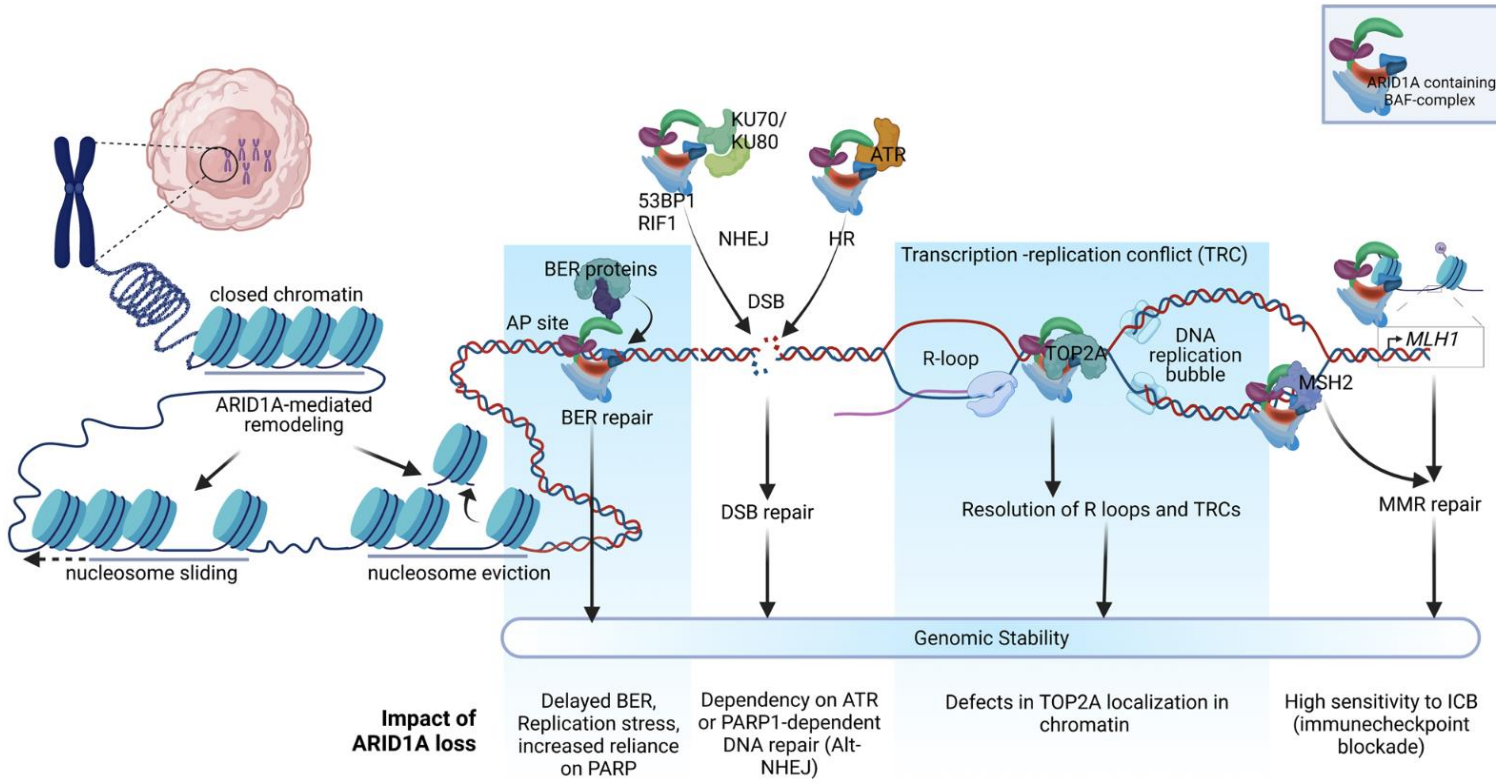
Chromatin regulators: ARID1A (modifier) (chromatin remodeling factors)





Mutations in ARID1A or other components of the SWI/SNF complex alter the nucleosome positioning

Chromatin regulators: ARID1A (modifier)

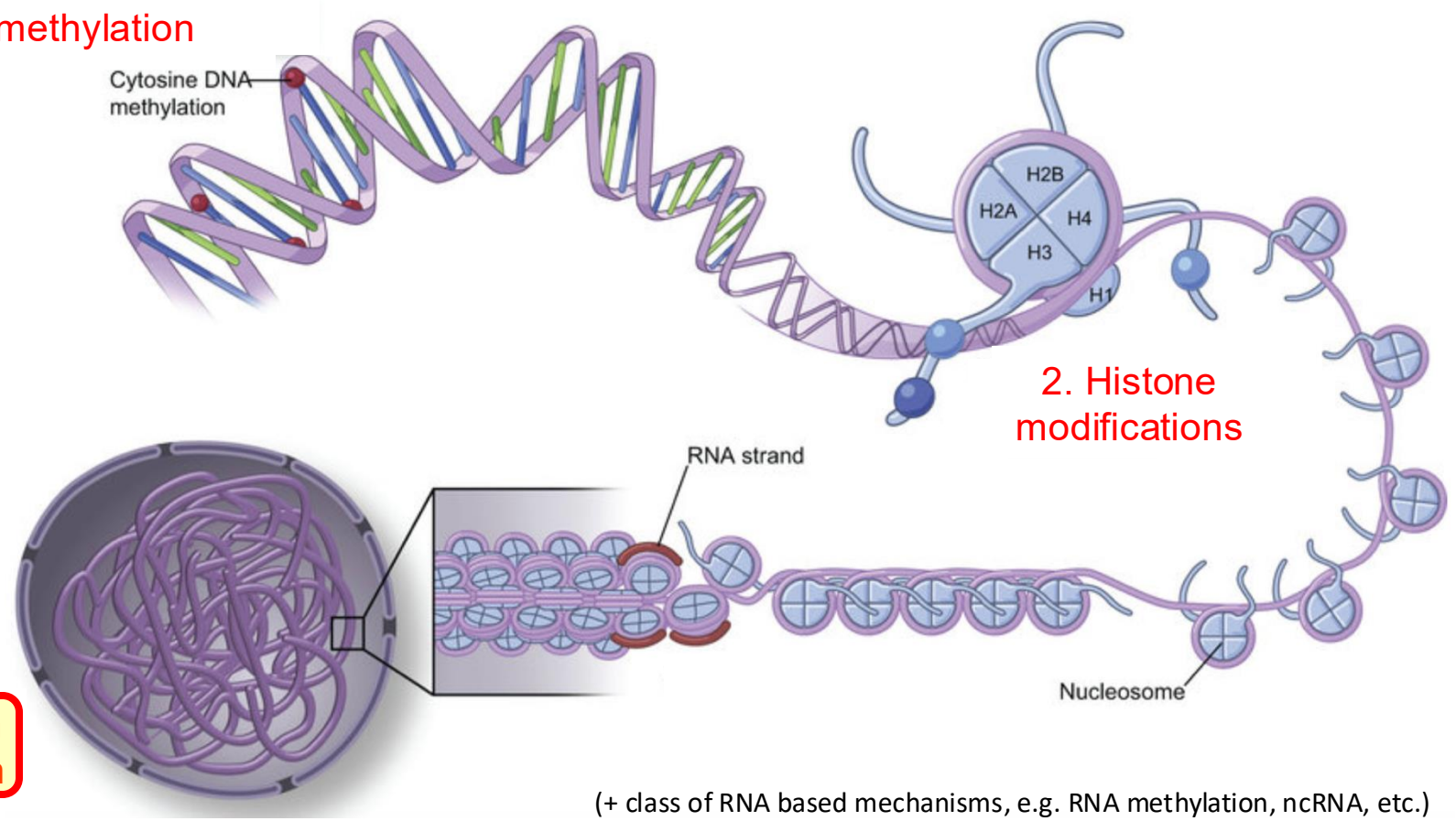


Changes in nucleosome positioning lead to genomic instability

How does this happen?

Mechanisms of epigenetic regulations:

1. DNA methylation

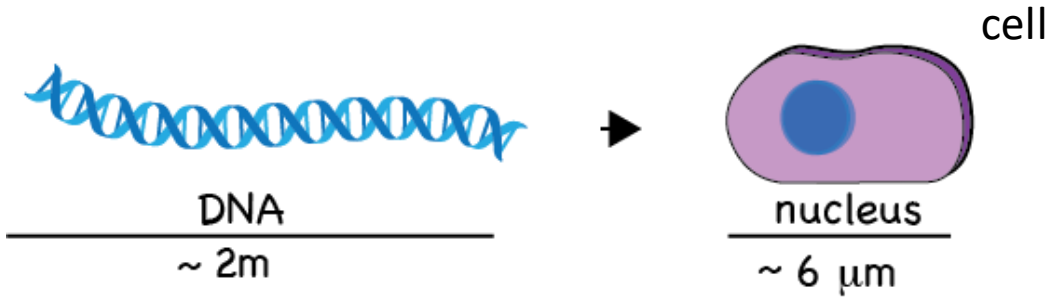


2. Histone modifications

3. Chromatin conformation

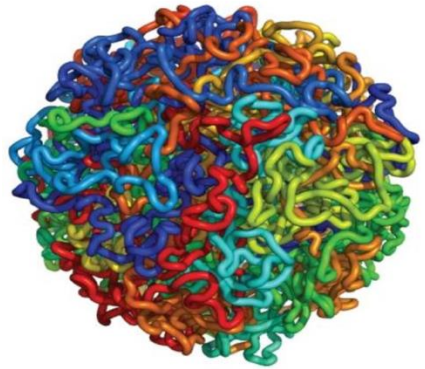
(+ class of RNA based mechanisms, e.g. RNA methylation, ncRNA, etc.)

Why chromatin 3D structure?

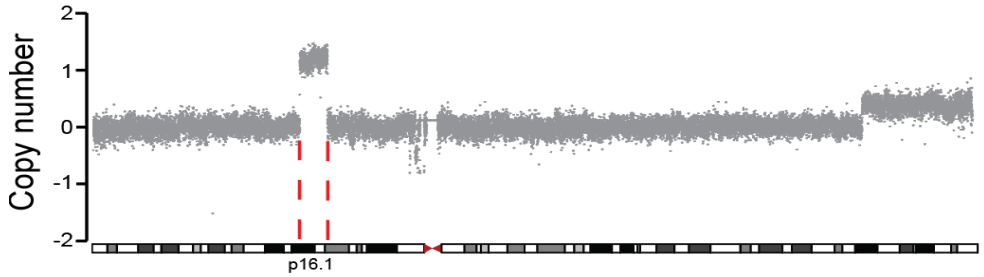


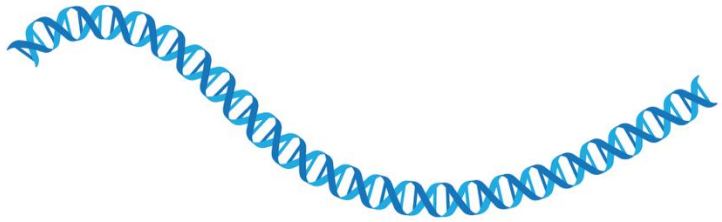
Linear view of the DNA

Compaction in the cell nucleus



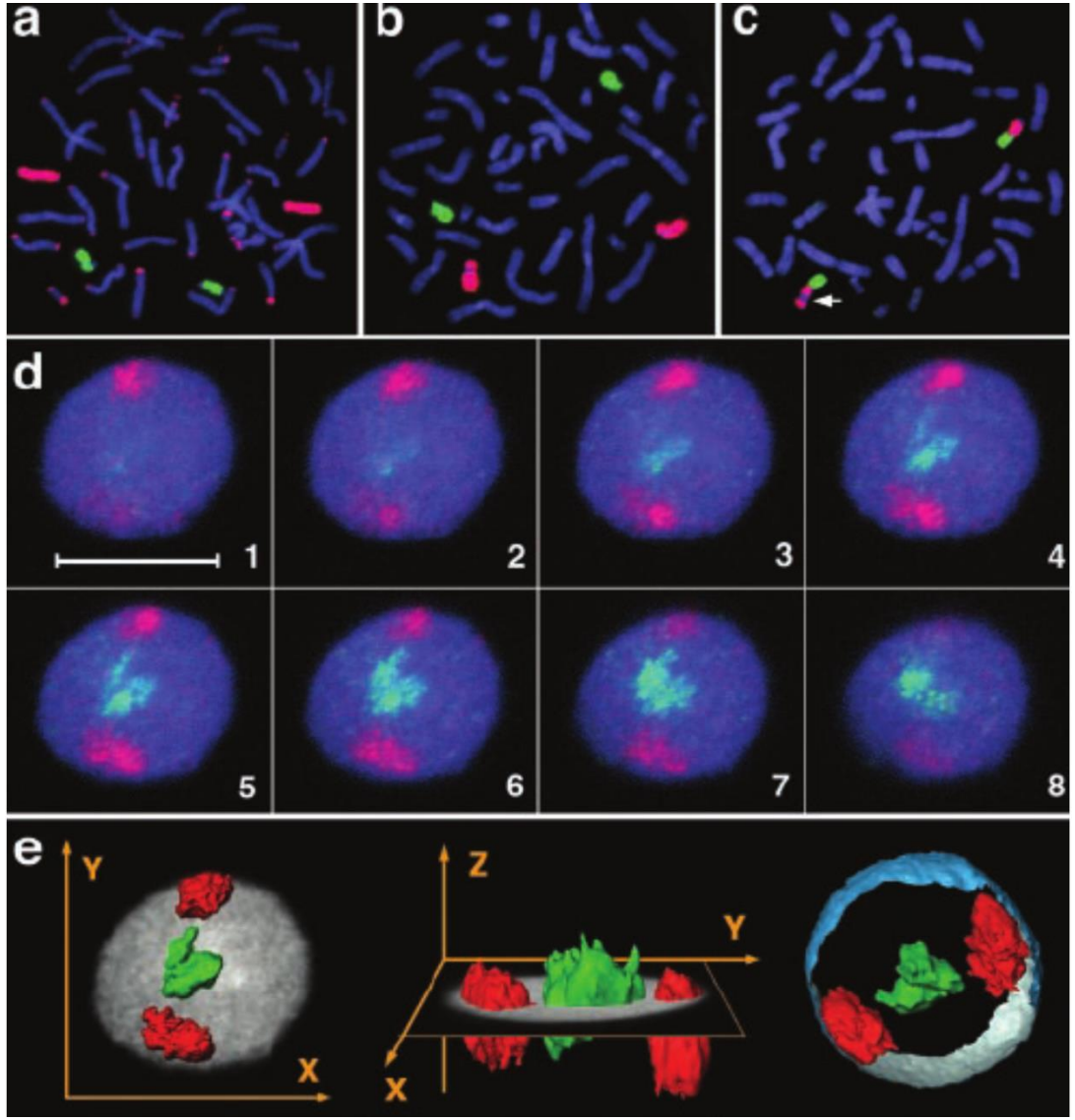
How genomics alterations in cancer cells changes chromatin organization in the nucleus?



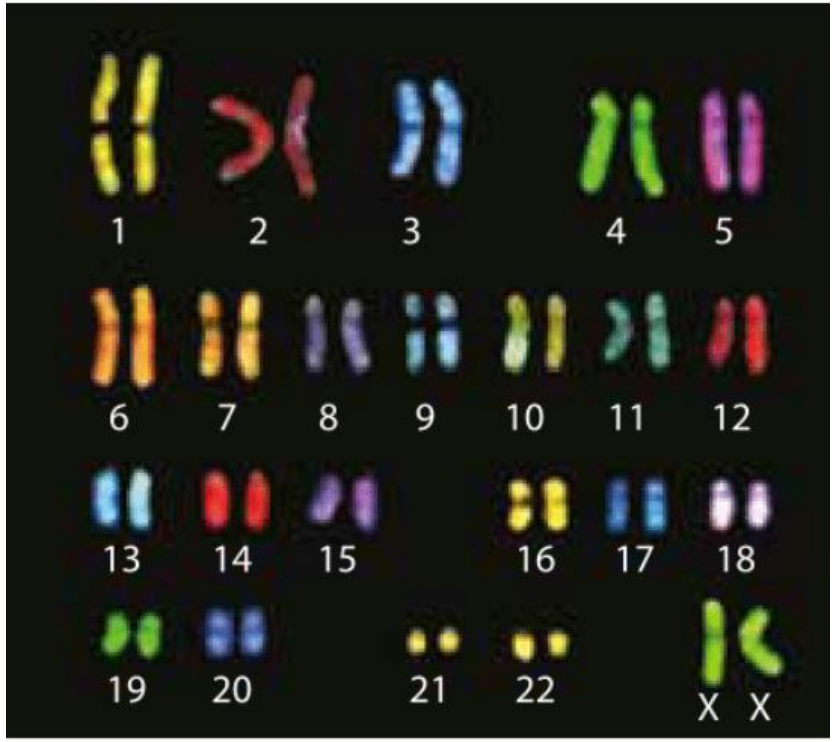


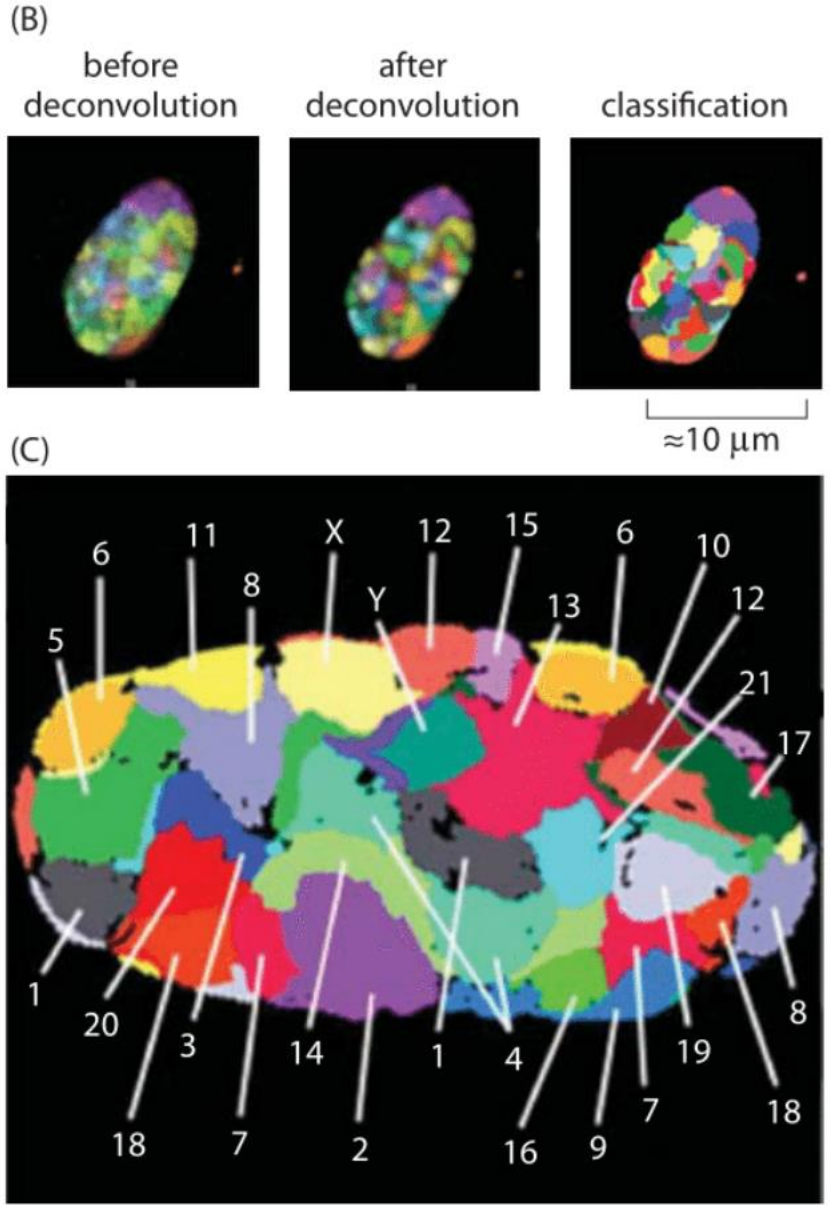
Where is this amplification in the cell nucleus?

Chromatin conformation in 3D

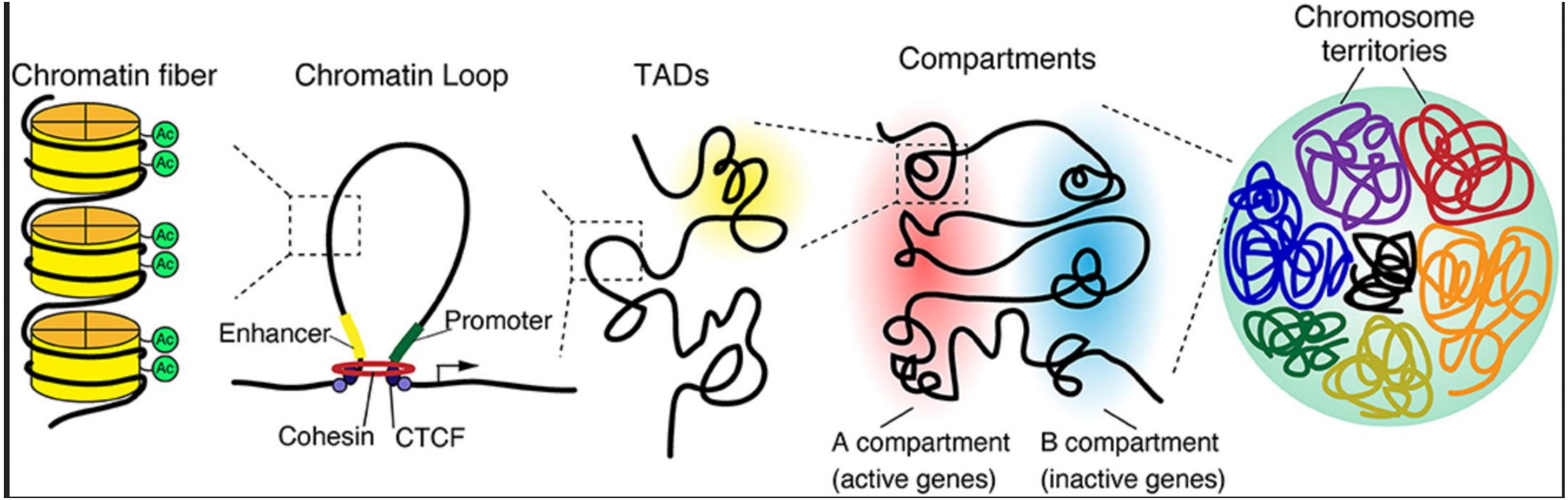


Chromatin conformation in 3D: chromosomes



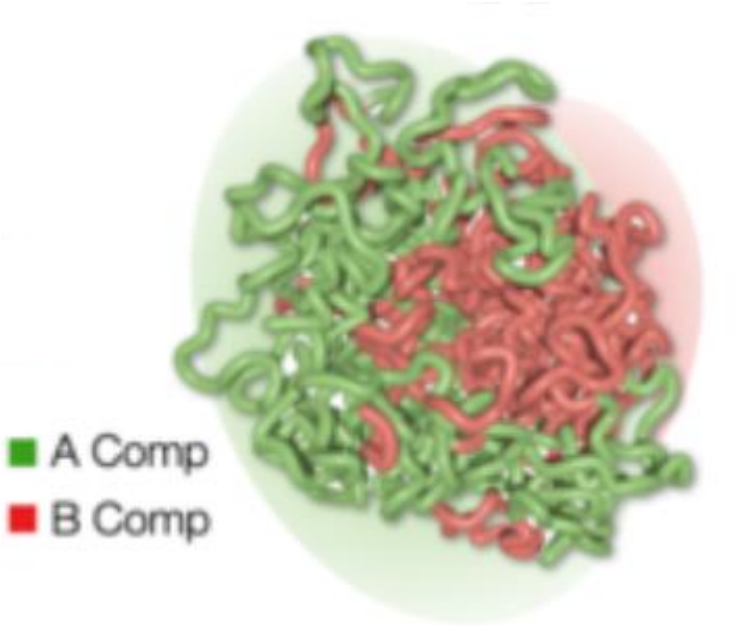


Chromatin conformation in 3D

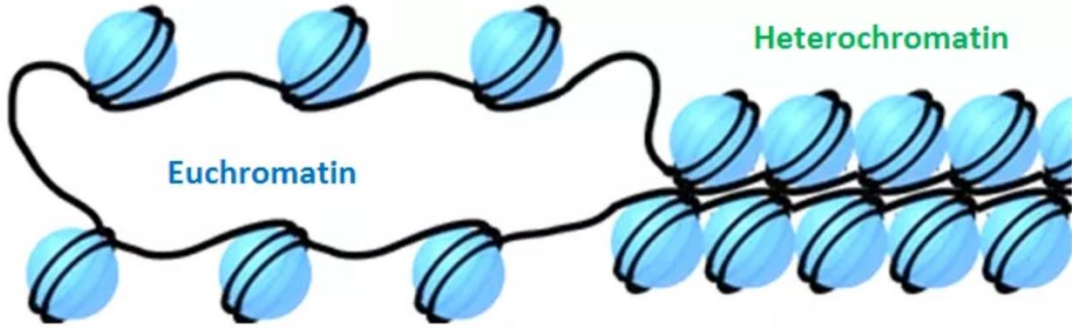


Chromatin conformation in 3D:compartments

Compartment A and B



Euchromatin/heterochromatin



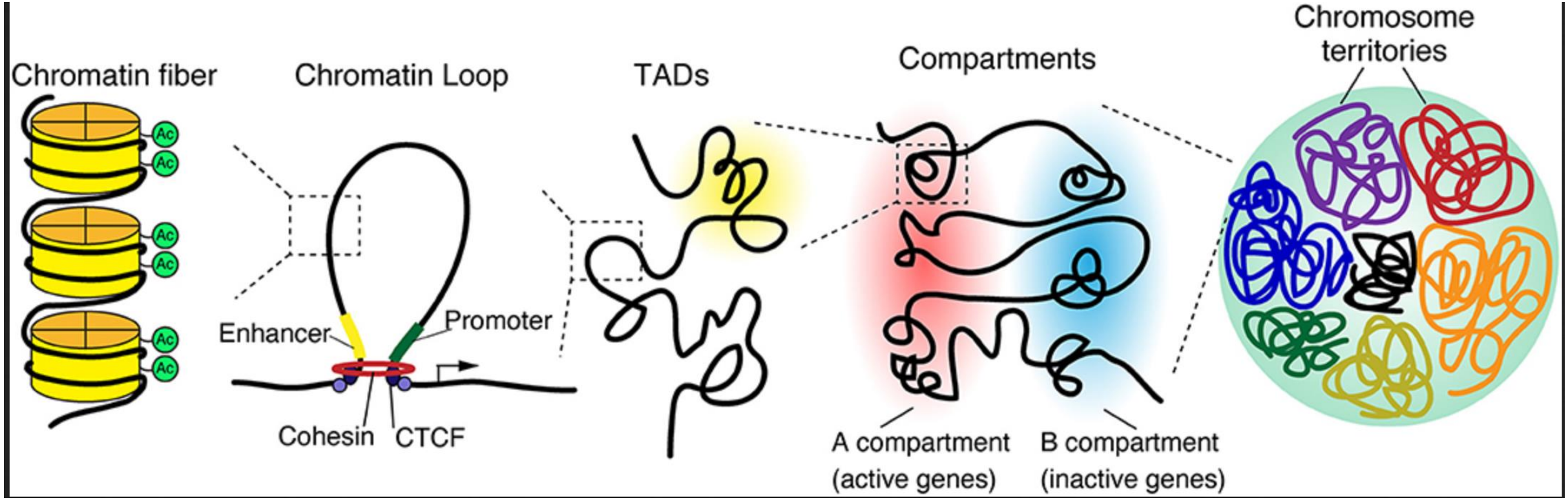
H3K27ac

H3K27me3
H3K9me3

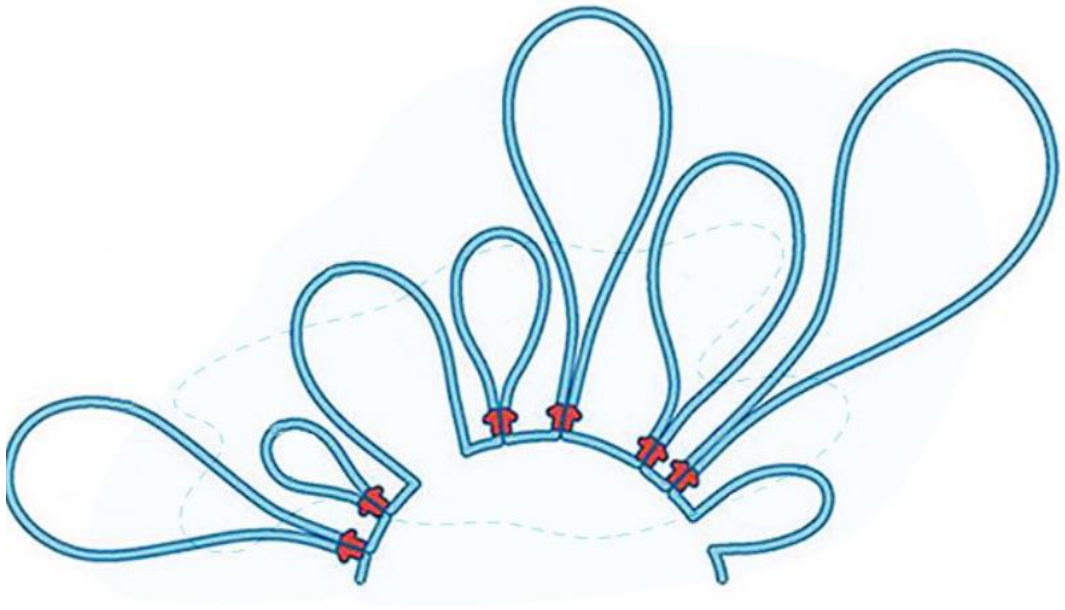
In each chromosome, we can find compartment A (euchromatin) and compartment B (heterochromatin)

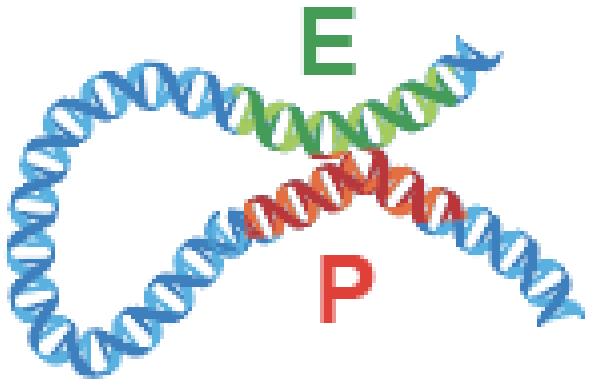
If a gene is expressed in which part of the chromatin should be?

Chromatin conformation in 3D:loops



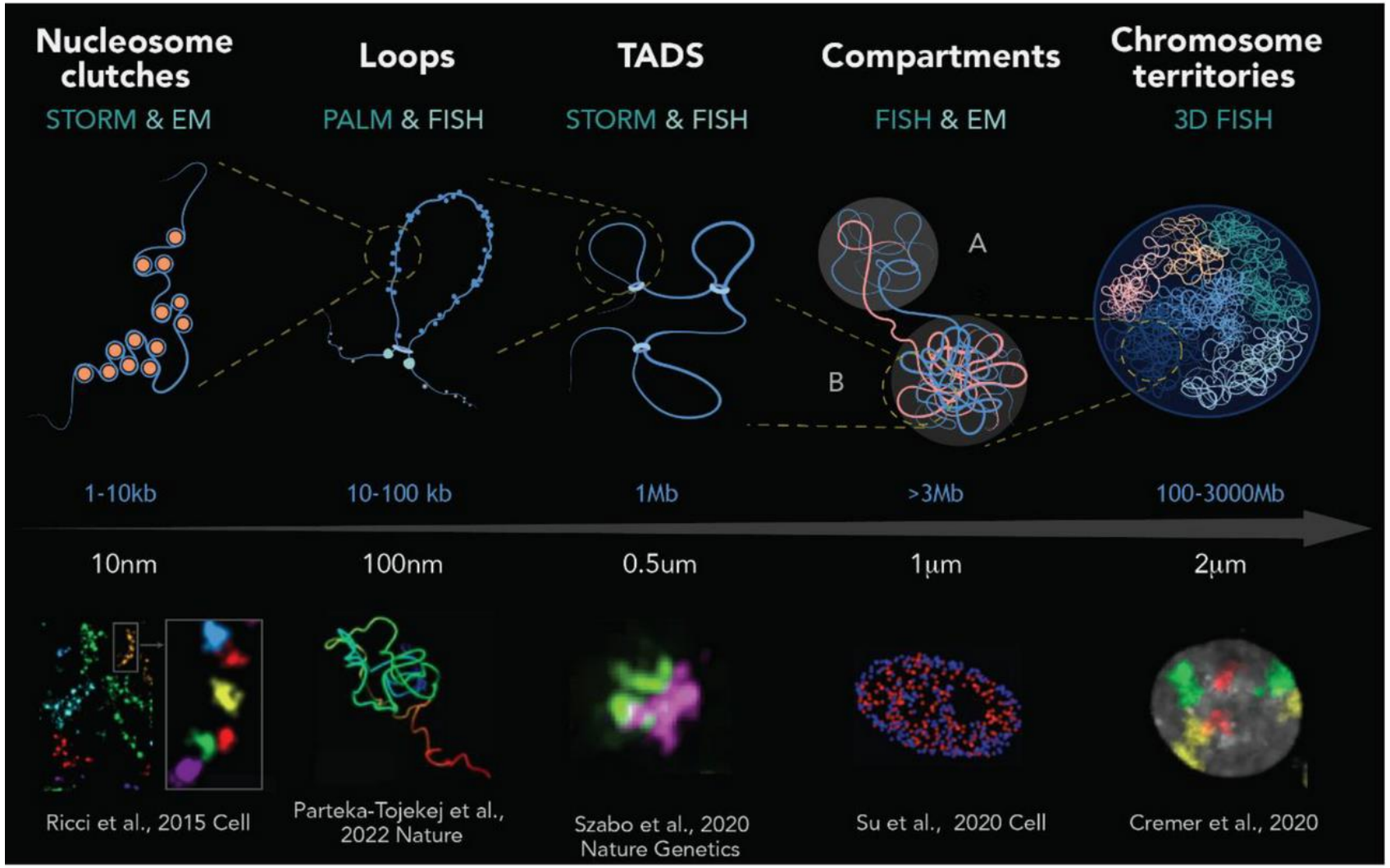
Chromatin conformation in 3D: loops





Bring together regulatory elements in the genome as for example enhancers and promoters

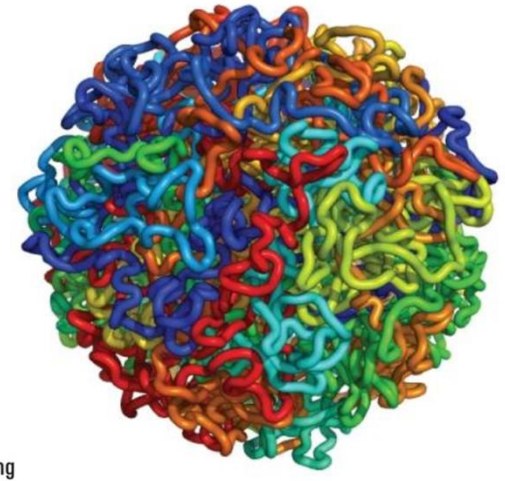
Chromatin conformation in 3D: by imaging

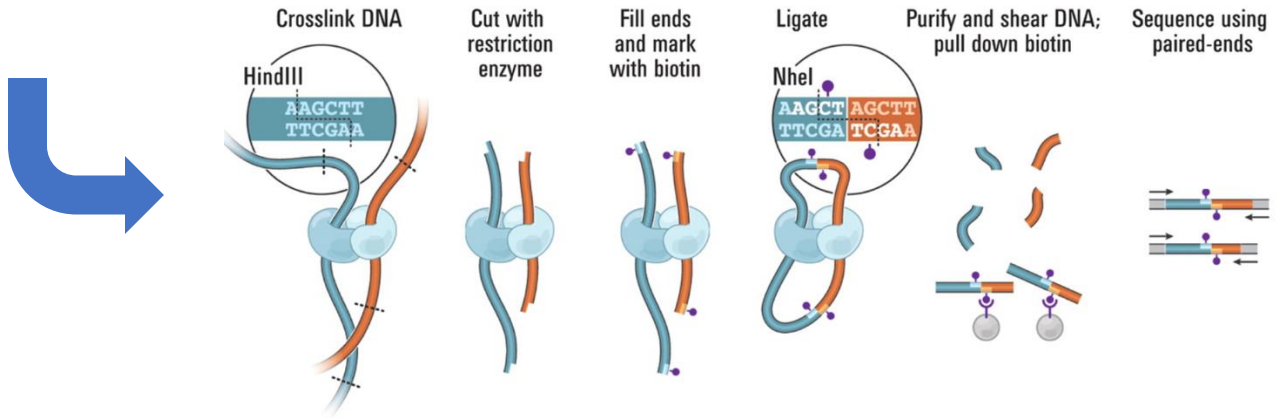


Chromatin conformation in 3D: By sequencing

Linear view of the DNA

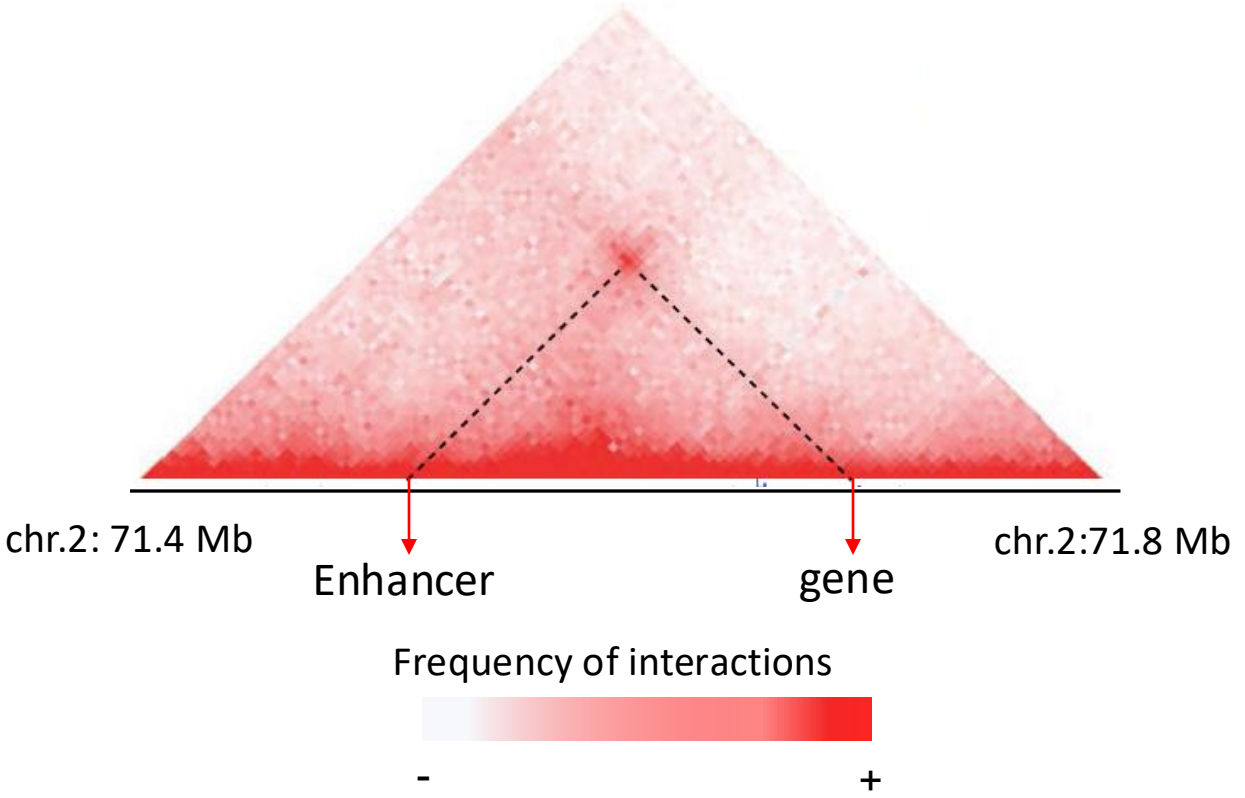
3D view of the DNA

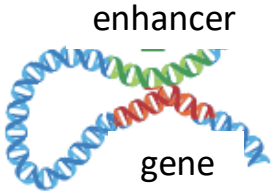




(3C, 4C, 5C, Hi-C, …)

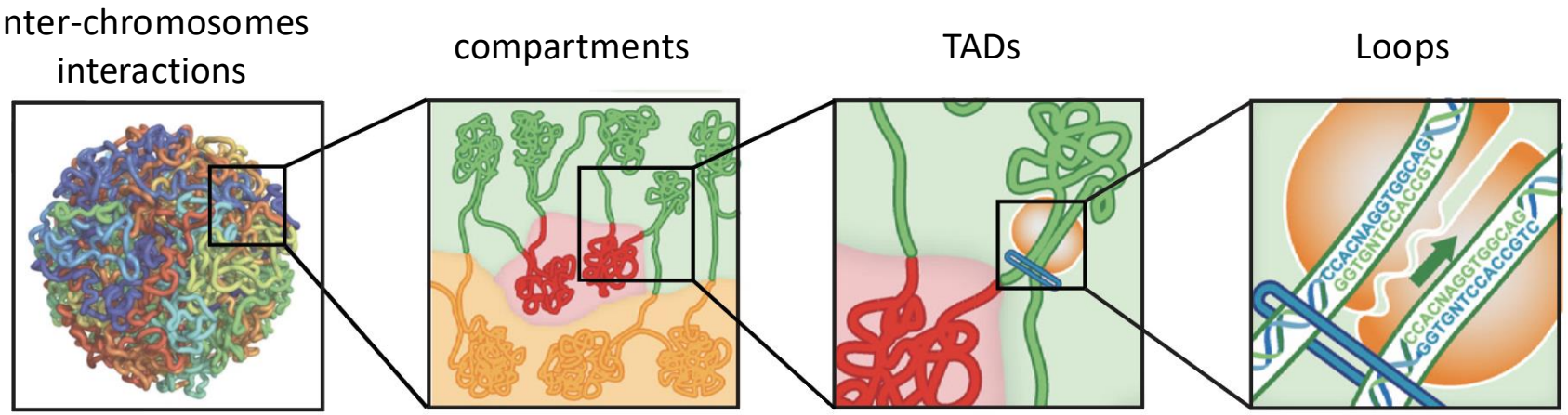
High-throughput chromatin conformation capture (Hi-C):





HiC measures the frequency of interactions between distant genomic loci

Chromatin organization in the cell nucleus



(adapted from Rao et al. 2014)

Cancer cells

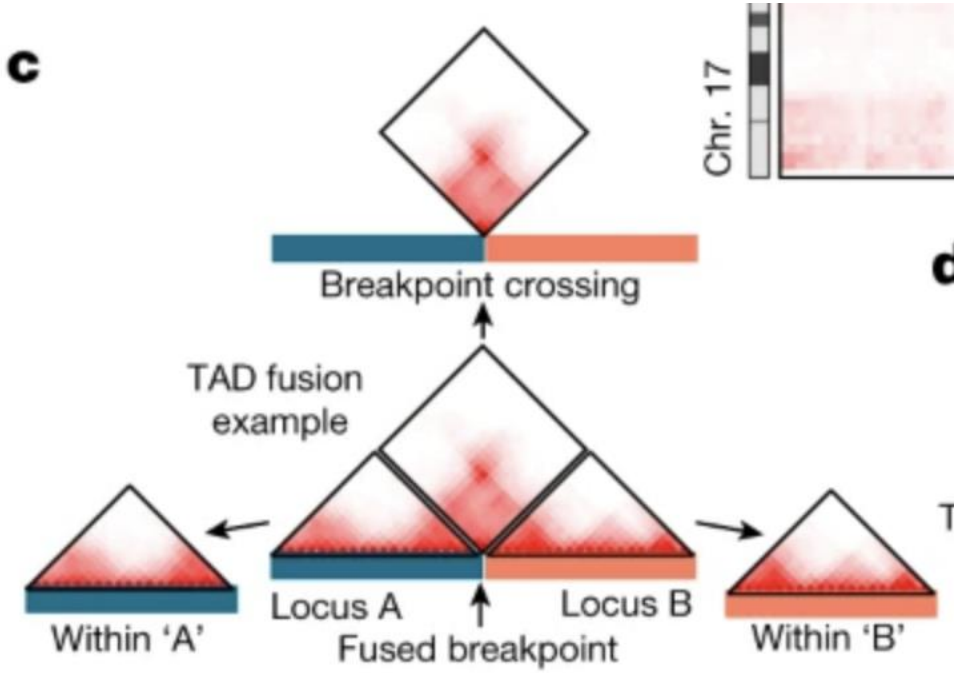
Chromosomal alterations

Translocations
Amplifications
Deletions

Epigenetic alterations

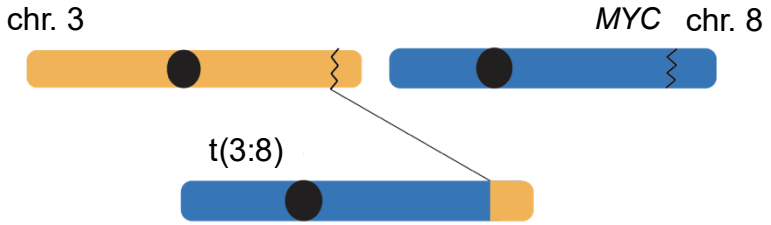
Histone methylation
acetylation

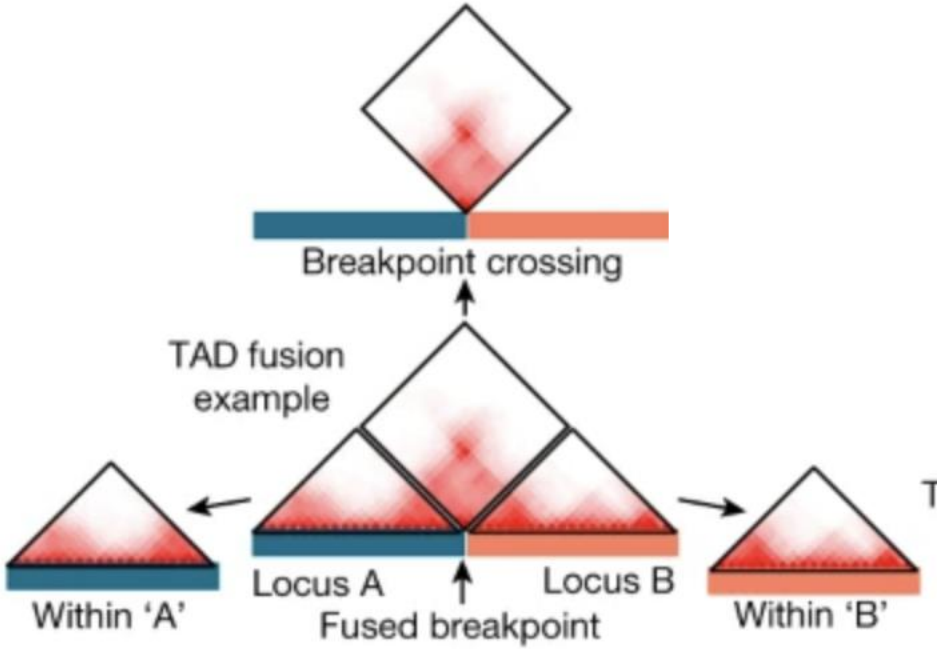
Chromosomal translocation in 3D: Chromosomal translocations



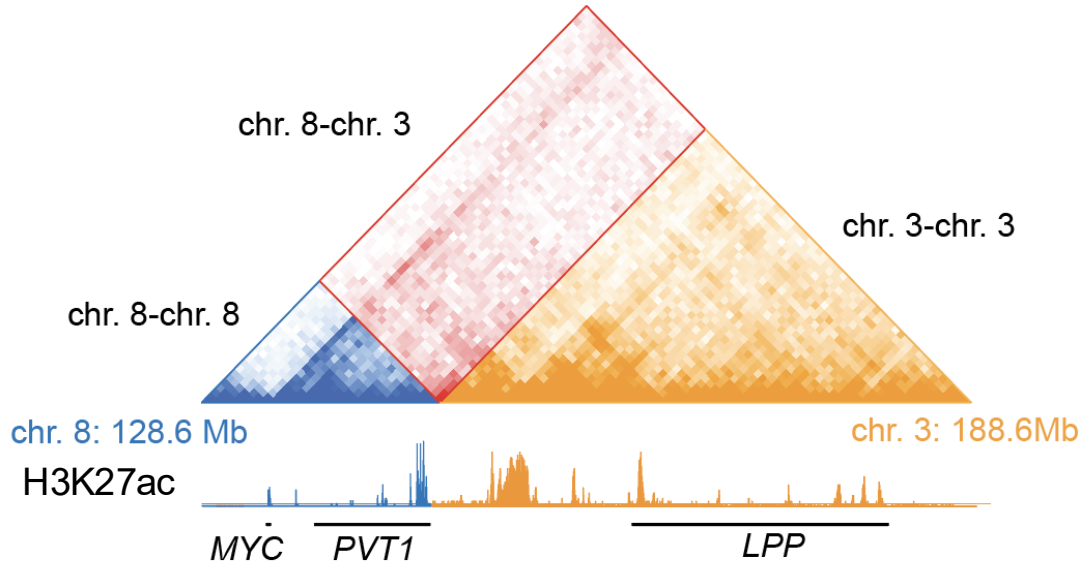
Chromosomal translocation in 3D: Chromosomal translocation

Frequent in multiple hit-B-cell aggressive lymphoma



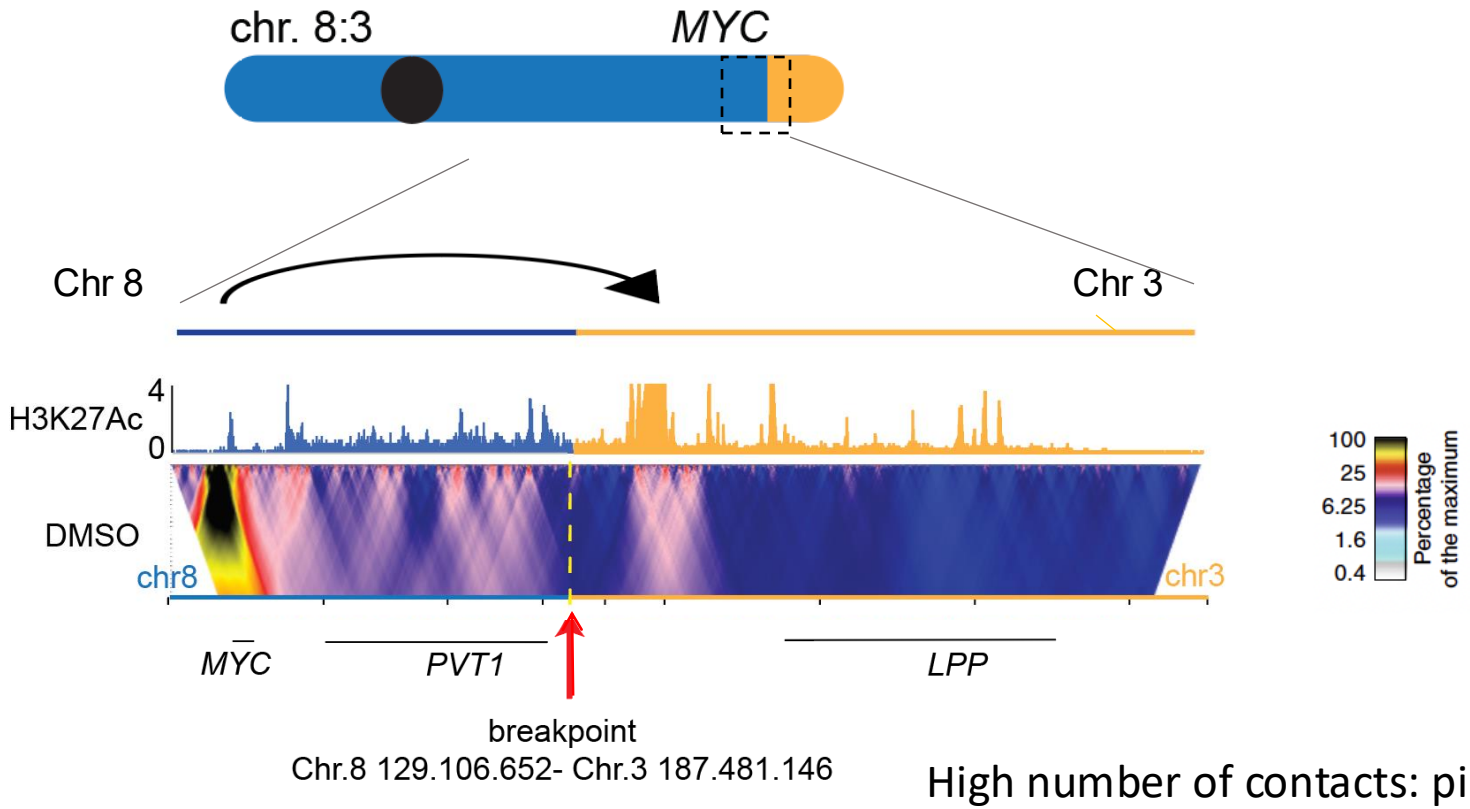


Intra-chromosomal map:



chr.3 enhancer region

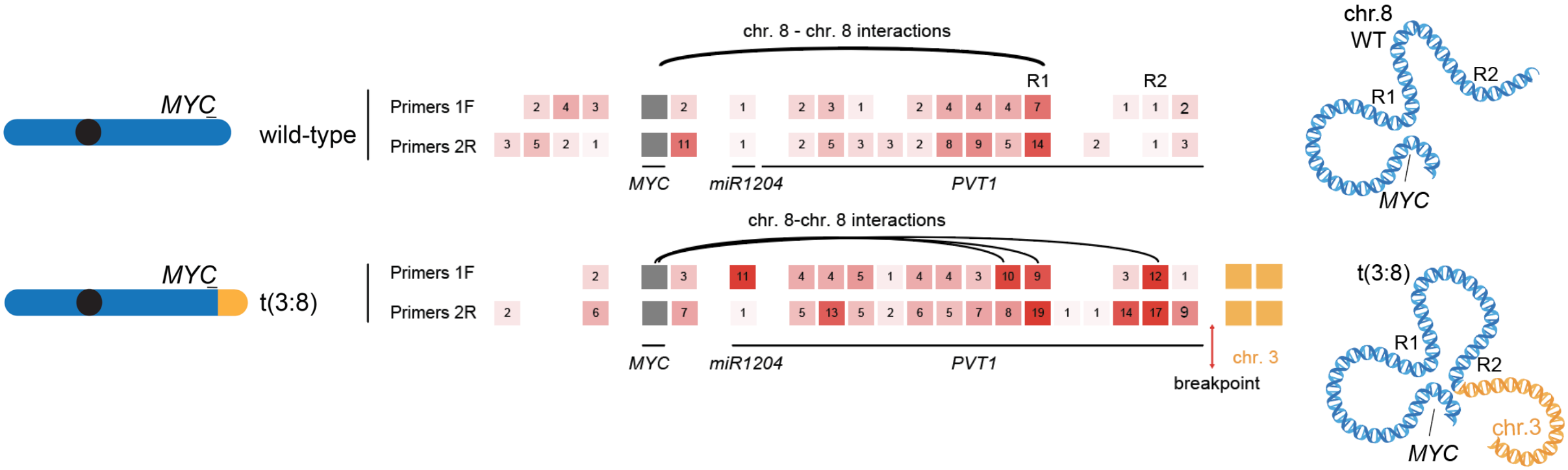
Enhancer promoter interactions and chromosomal translocations



High number of contacts: pink

Low number of contacts: blue

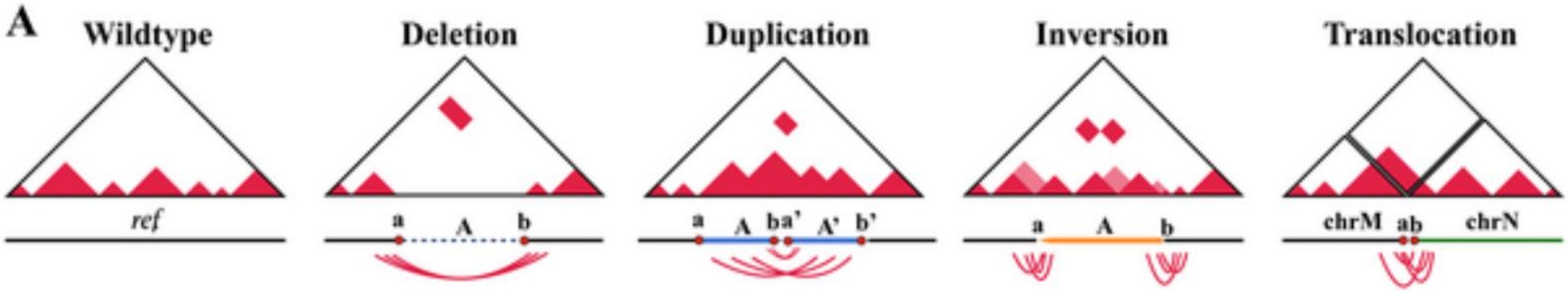
Allele specific chromatin conformation



Distinct intra-chromosomal interactions
in the two homologous chromosomes

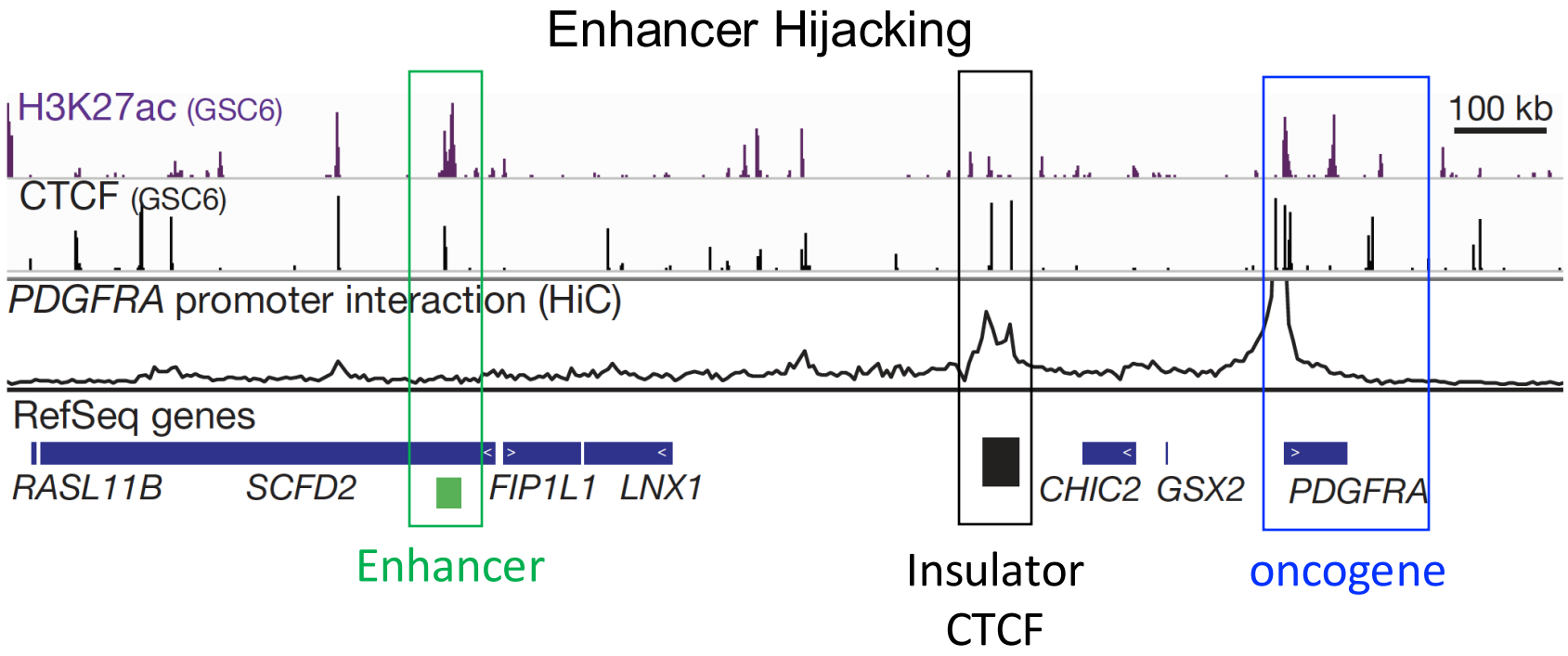
Sungalee et al. Nature Genetics, 2021

Copy number alterations can be studied considering the chromatin 3D structure



Modulation of chromatin 3D structure in absence of chromosomal changes, due to epigenetic alterations

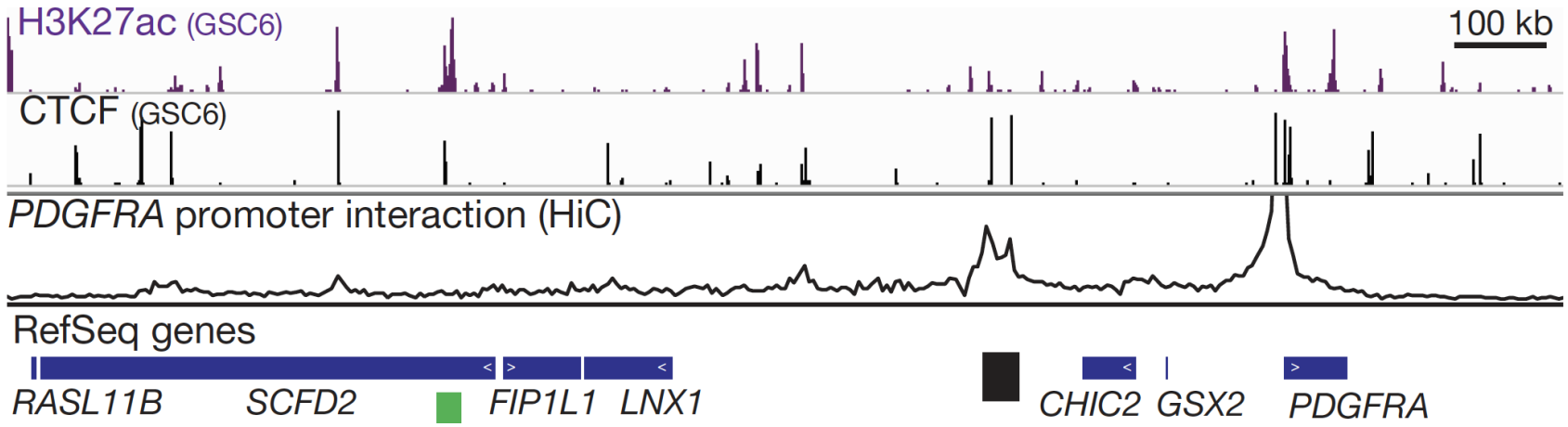
DNA hyper-methylation

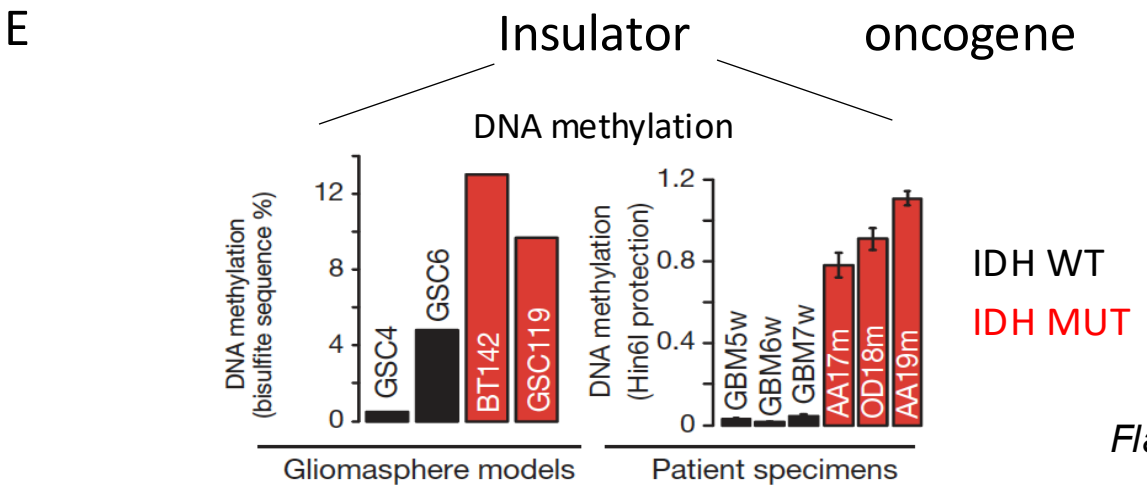


PDGFRA is over-expressed in patients with IDH mutations

Flavahan et al. Nature 2016

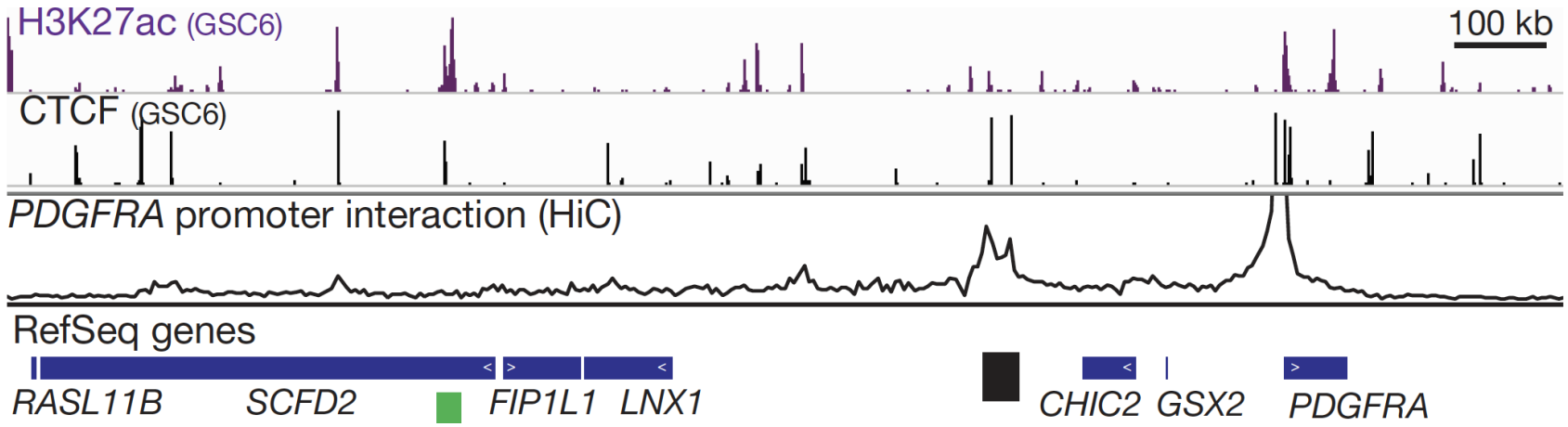
Oncogene activation by hypermethylation of a CTCF locus in IDH mutant glioma



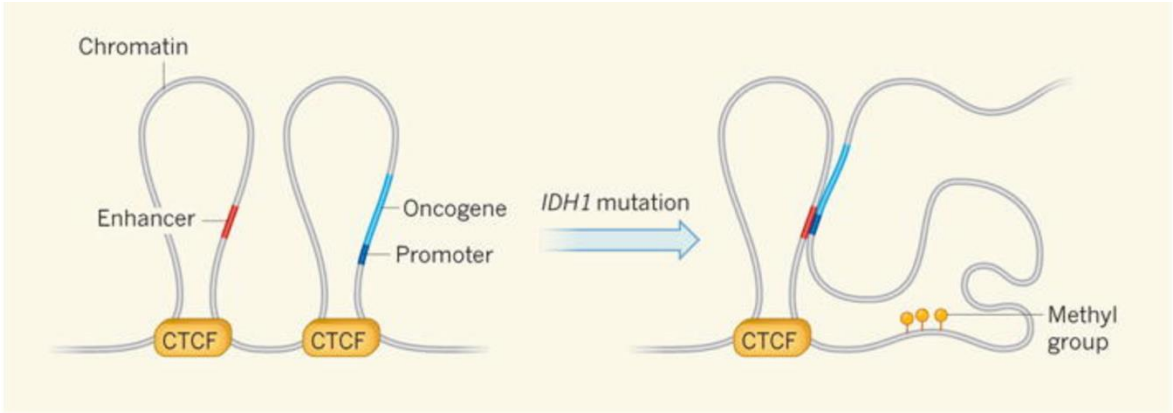


Flavahan et al. Nature 2016

Oncogene activation by hypermethylation of a CTCF locus in IDH mutant glioma



E Insulator oncogene



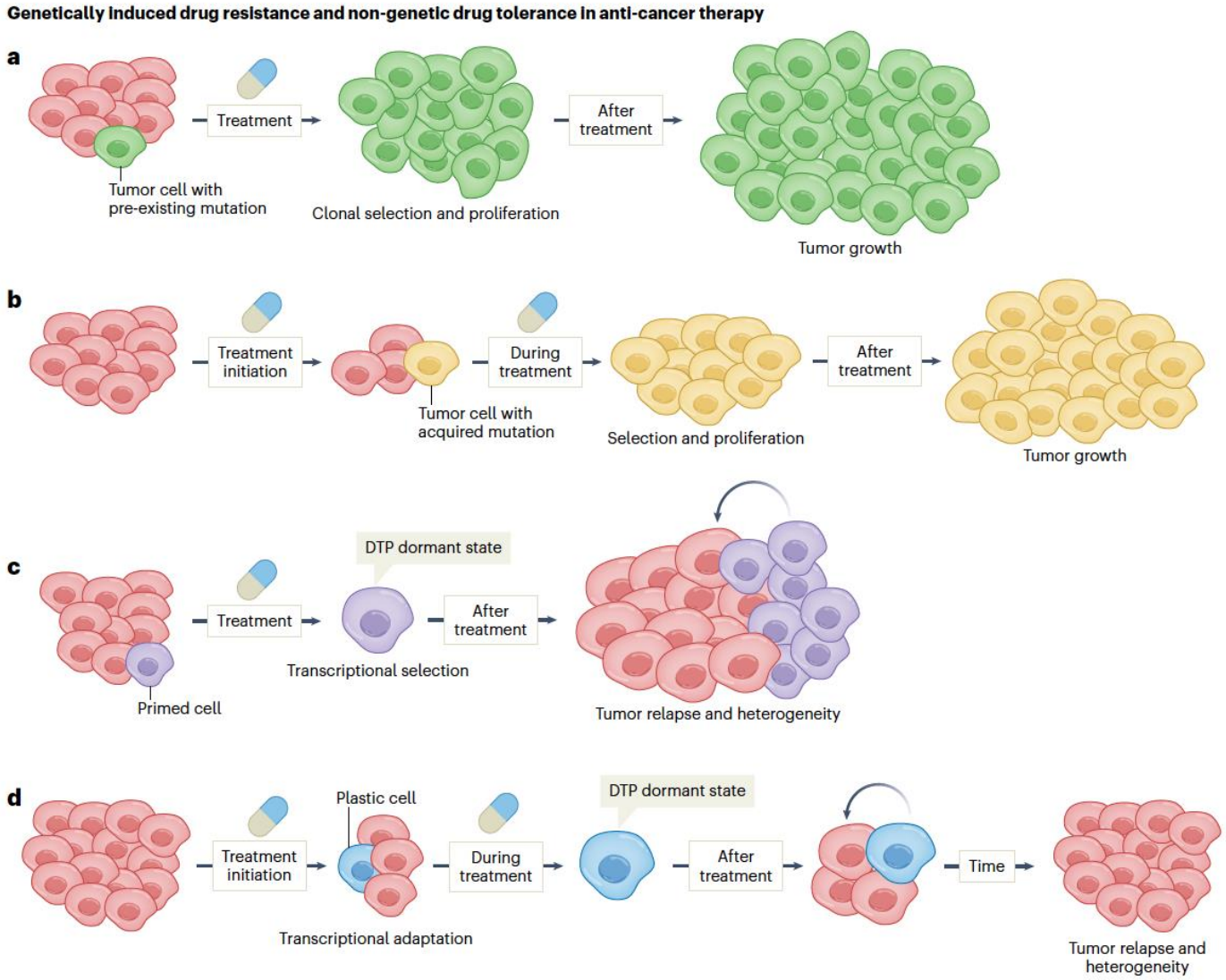
Flavahan et al. Nature 2016

Epigenetic changes influence gene expression allowing cancer cells to adapt in different environments without changing the acquisition of new mutation or copy number change

Cancer cell plasticity represents the ability of the cells to change their transcriptional program

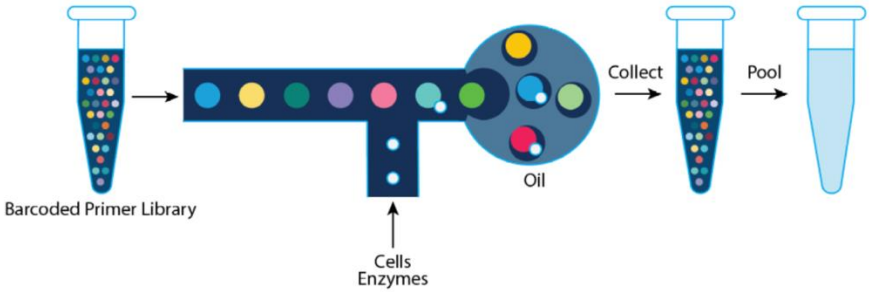
Cancer Cell plasticity can influence tumor evolution and response to cancer treatment

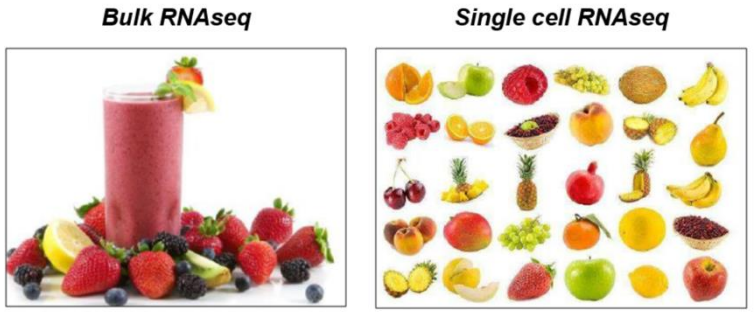
Genetic and nongenetic drug resistance



Single-cell molecular profiles

scRNA: Cell barcoding allows to determine which transcript come from which cells

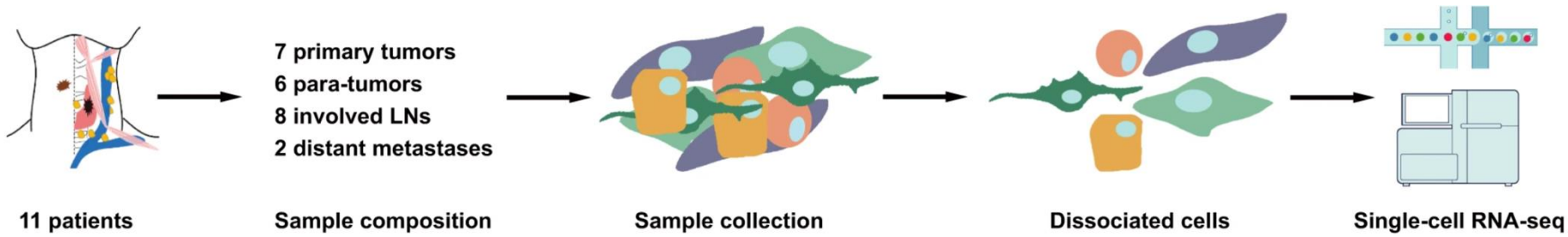




Single-cell molecular profiles

Single cell omics has revolutionized our ability to study **intra-tumor heterogeneity**

- Diversity among difference cancer cells within the same tumor
- Diversity of non-tumor cells (e.g., immune cells) surrounding the tumor (tumor microenvironment)



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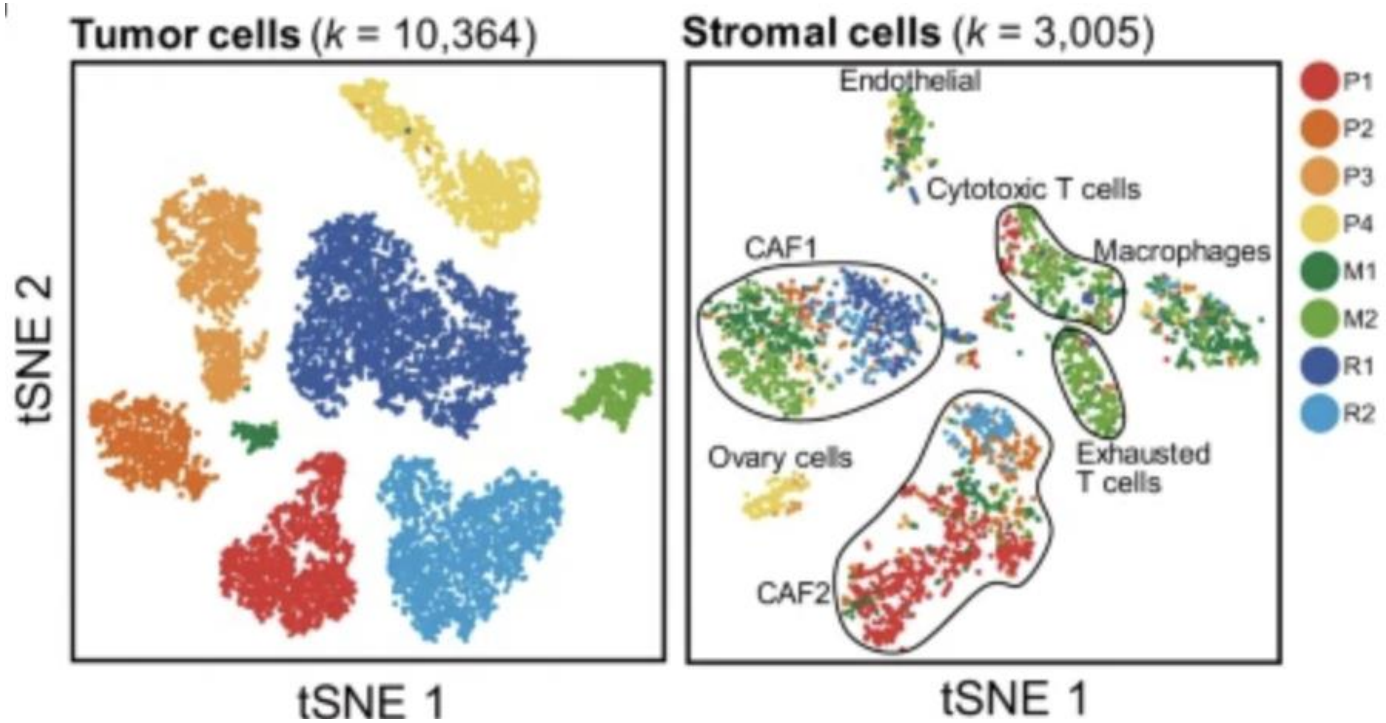
Ovarian cancer



Exercise

https://www.nature.com/articles/s41588-021-00842-x

Skip the first paragraph and start to discuss the paper from paragraph 2. "modulation of EPI"

Skip paragraph H3K27ac, affect EPI and EEI

Exercise: 1.15h-2h30 prepare the paper presentation
2.30h-3h30 presentation of the papers