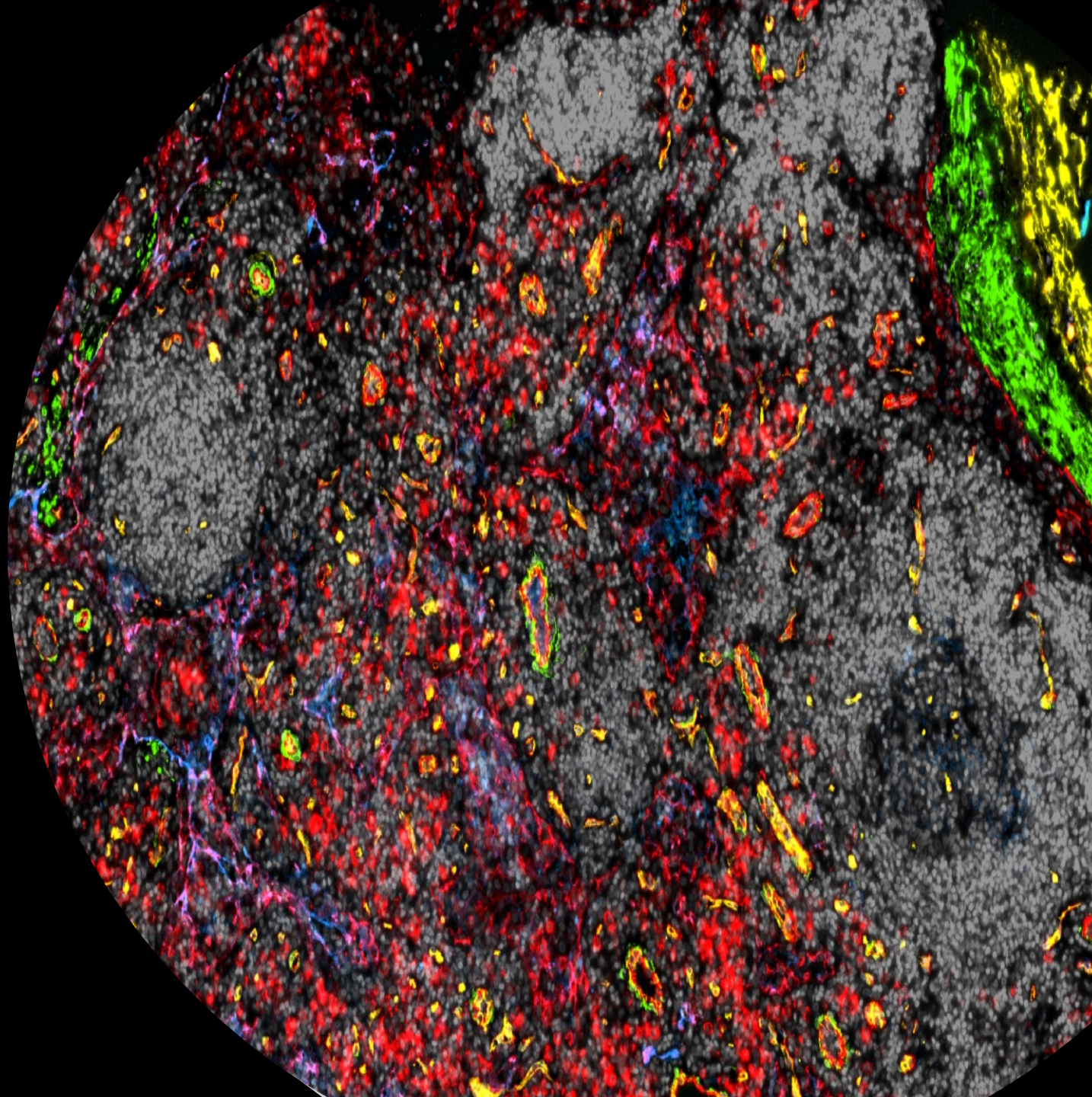
Cancer Biology I

Part-II

Week 10



AGENDA

Nov 5th: Cancer genomics- mutations

Nov 11th: Cancer genomics-copy number alteration, heterogeneity, evolution *(recording)*

Nov 18th: Cancer Epigenetics- chromatin 3D structure, cell plasticity

Nov 25th: – Major signaling pathways leading to cancer

Dec 2th: Cancer Therapies – chemo and targeted therapies

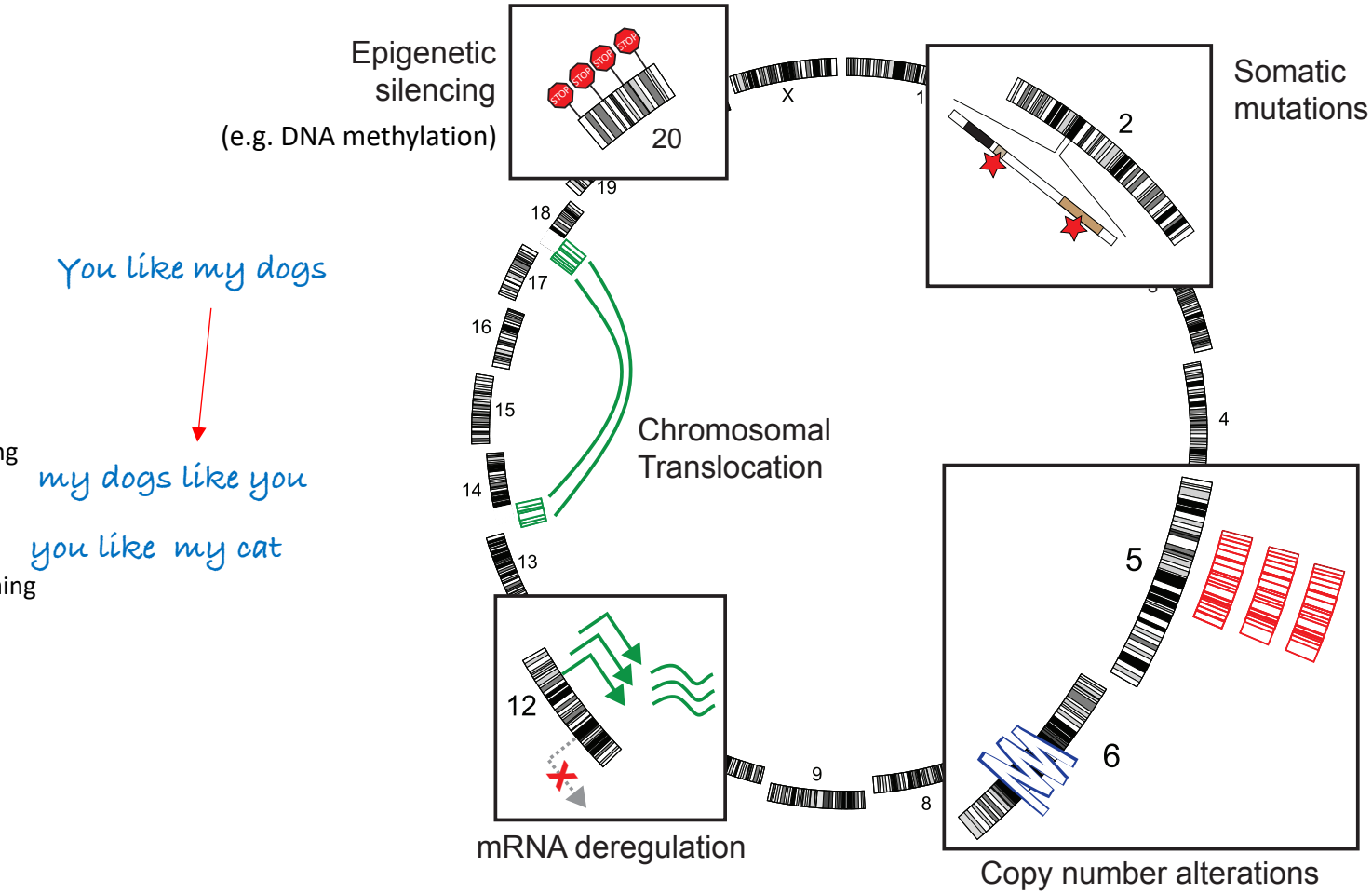
Dec 9th: Introduction to immunotherapies –

Dec 16th: questions for the exam

Dec 18th: Exam 1.30 PM-3.30 PM

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

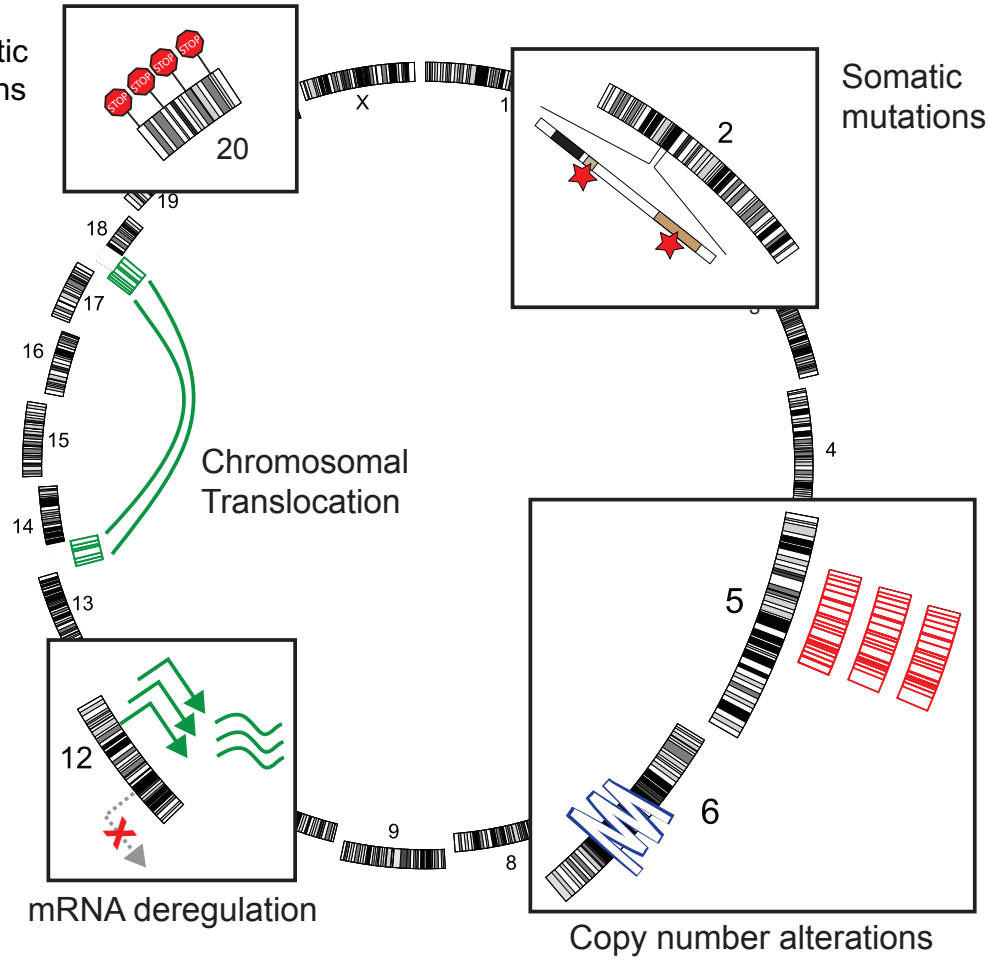
What are they?

Cancer EPIGENTICS Alterations

You like my dogs

↓

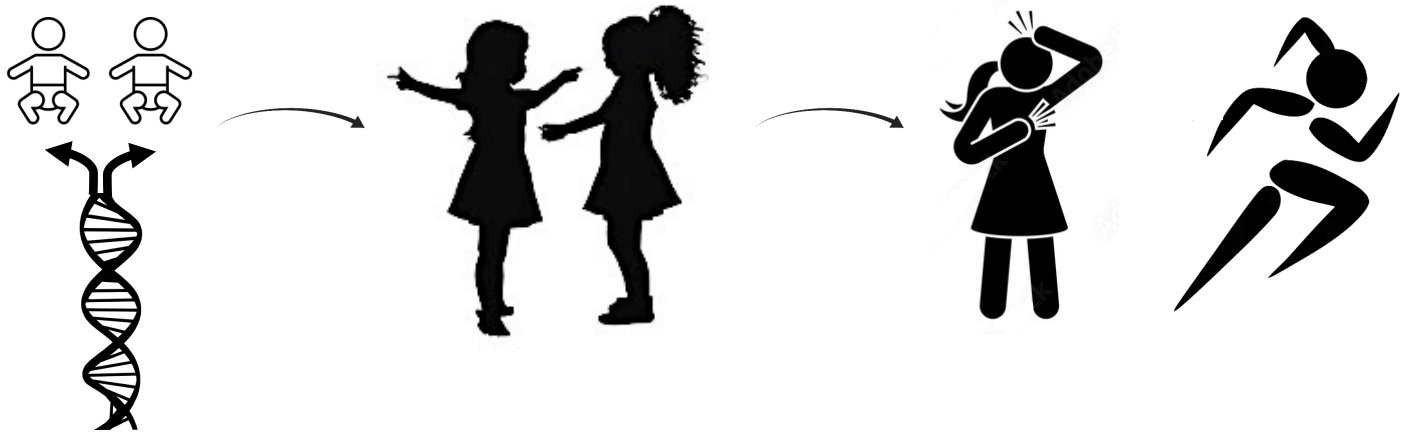
You like my dogs

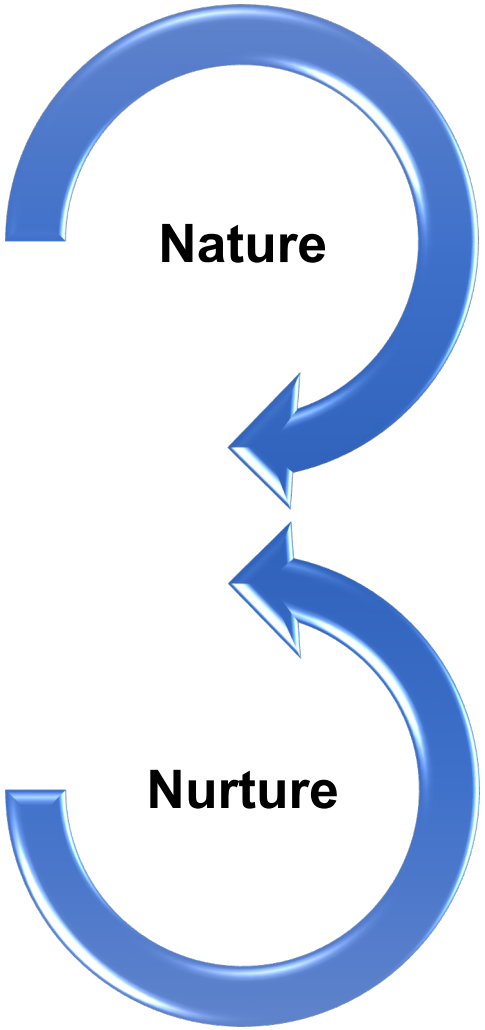


What is epigenetics?

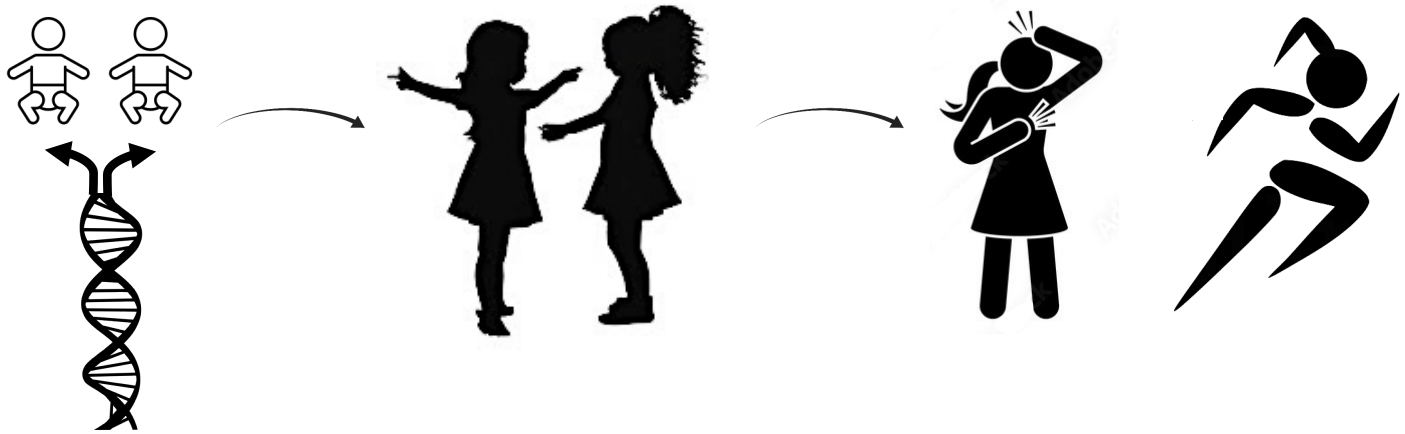
Epigenetics: study of stable heritable traits - *not attributable to alterations in the DNA sequence* - that change gene expression and cellular phenotypes

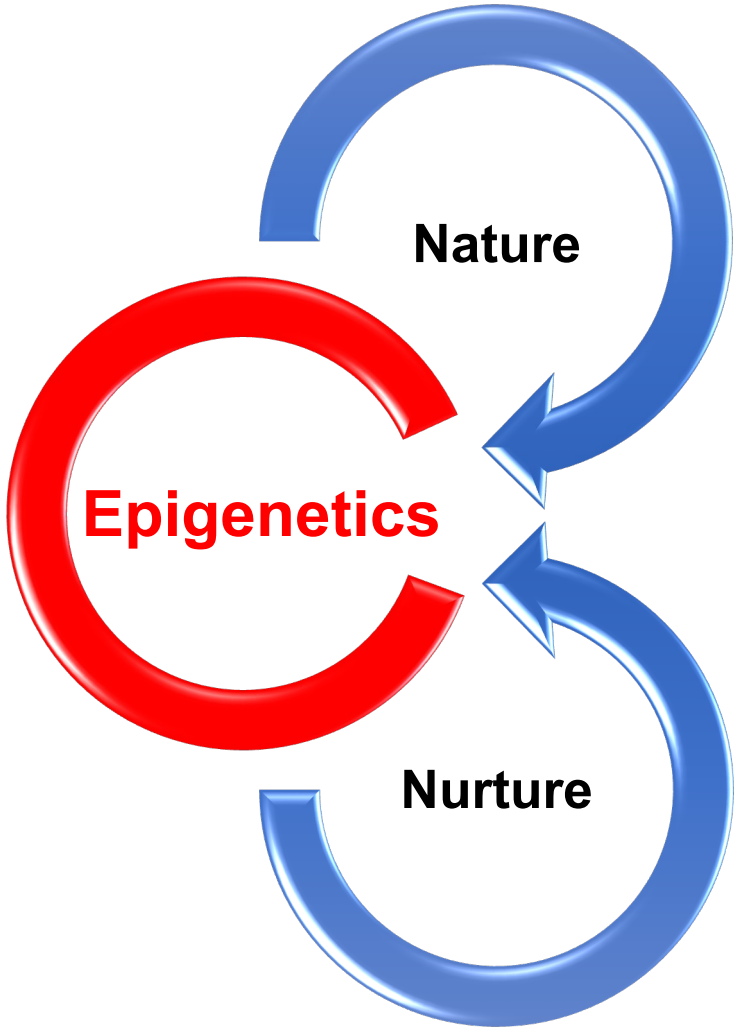
What is epigenetics?





What is epigenetics?





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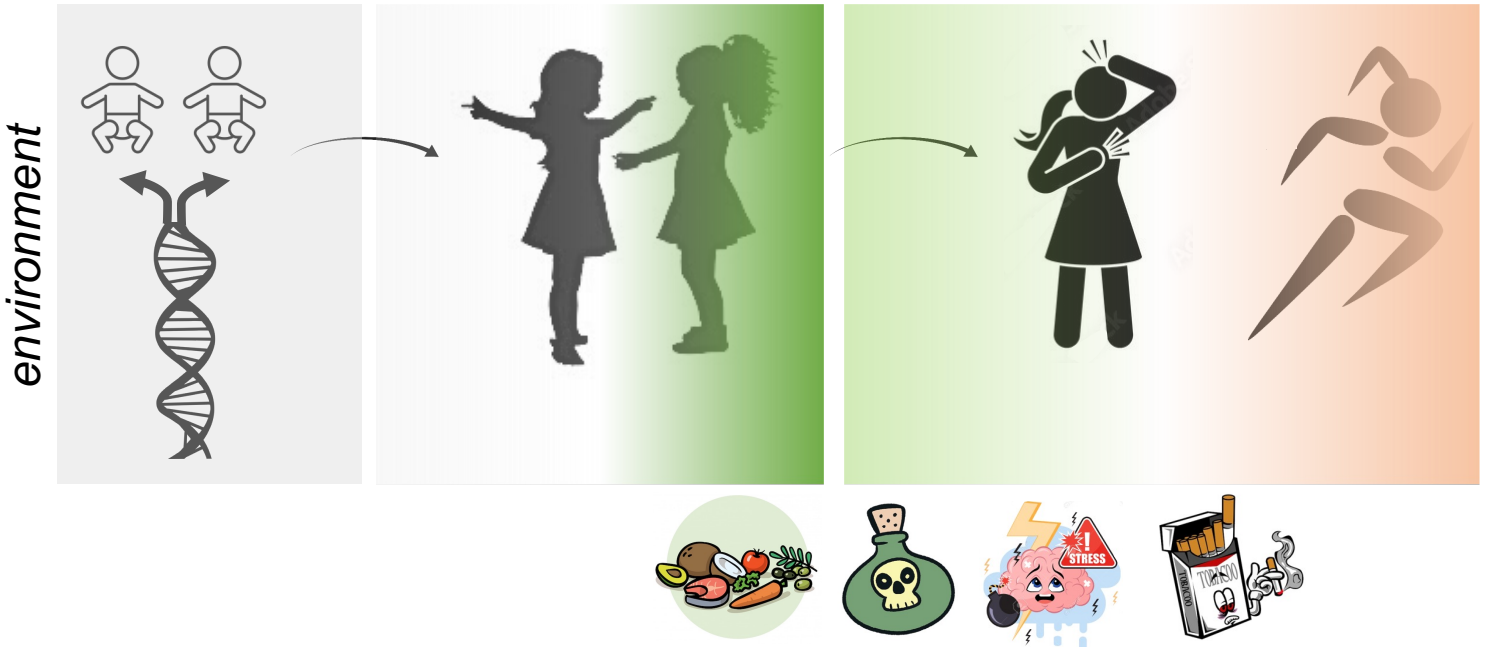
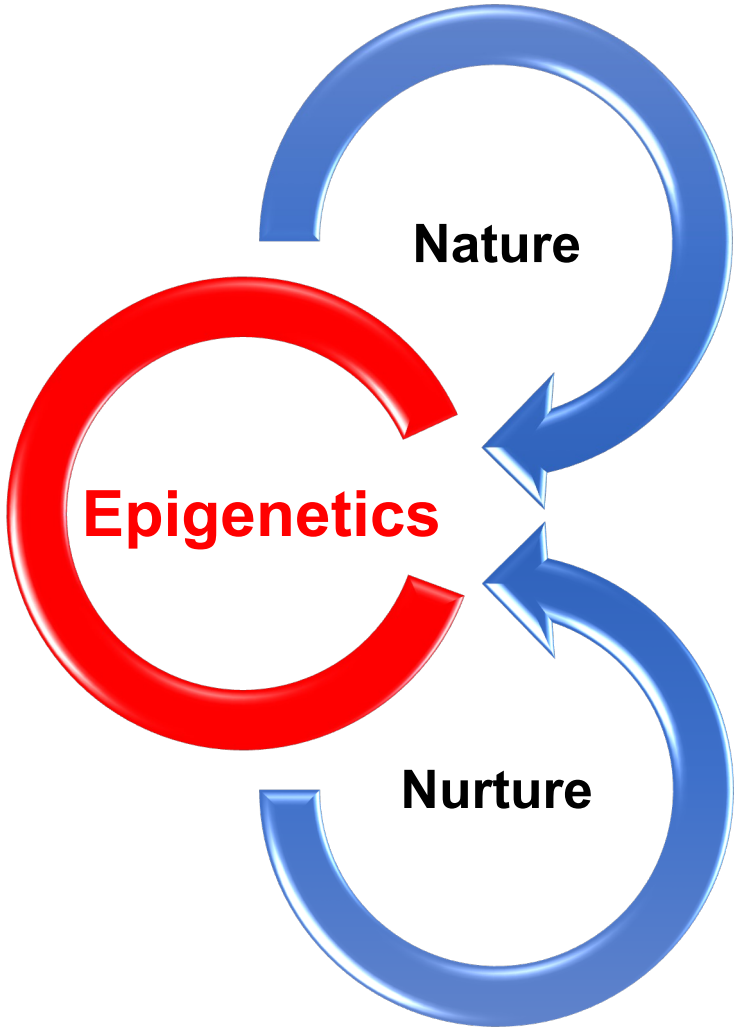
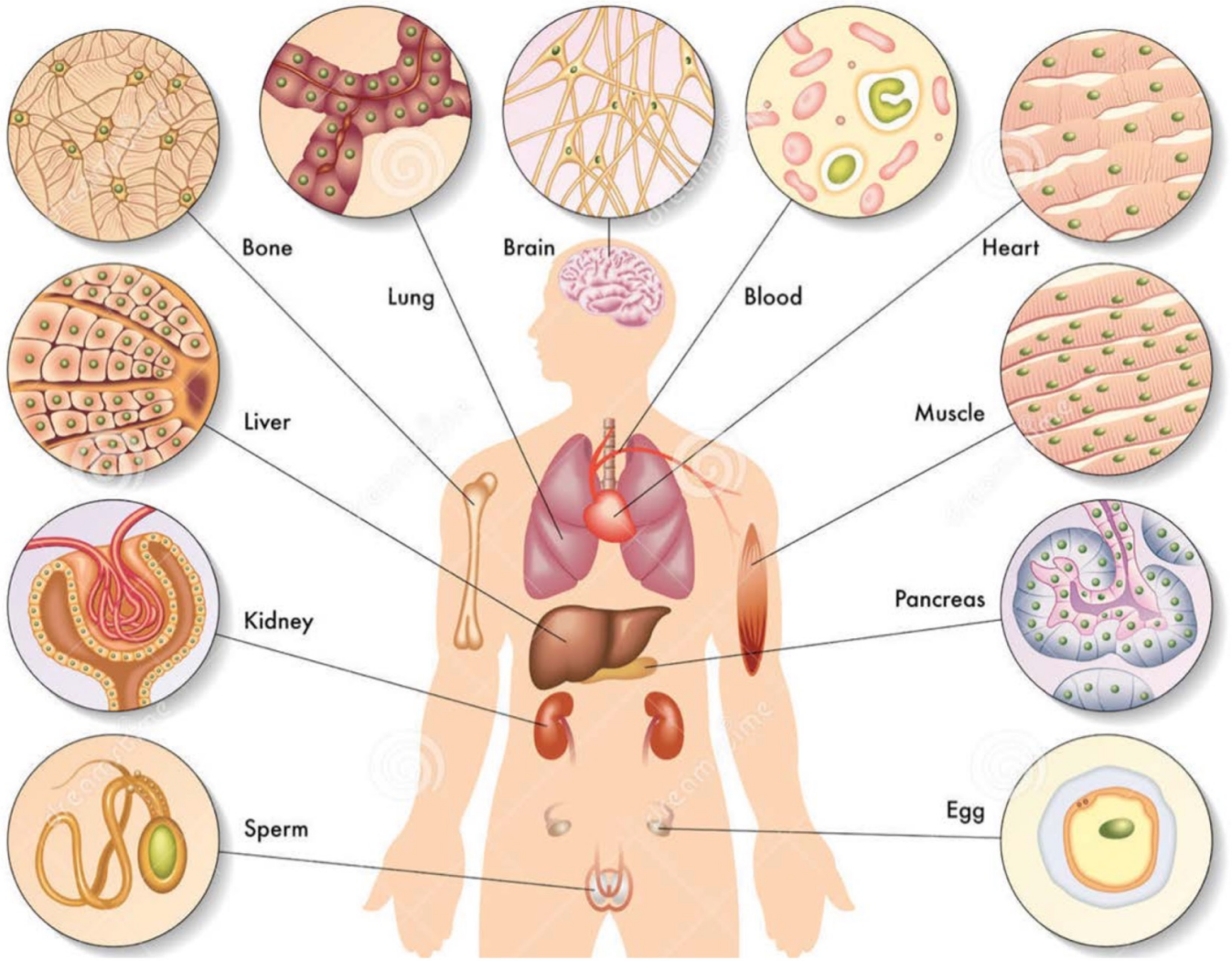
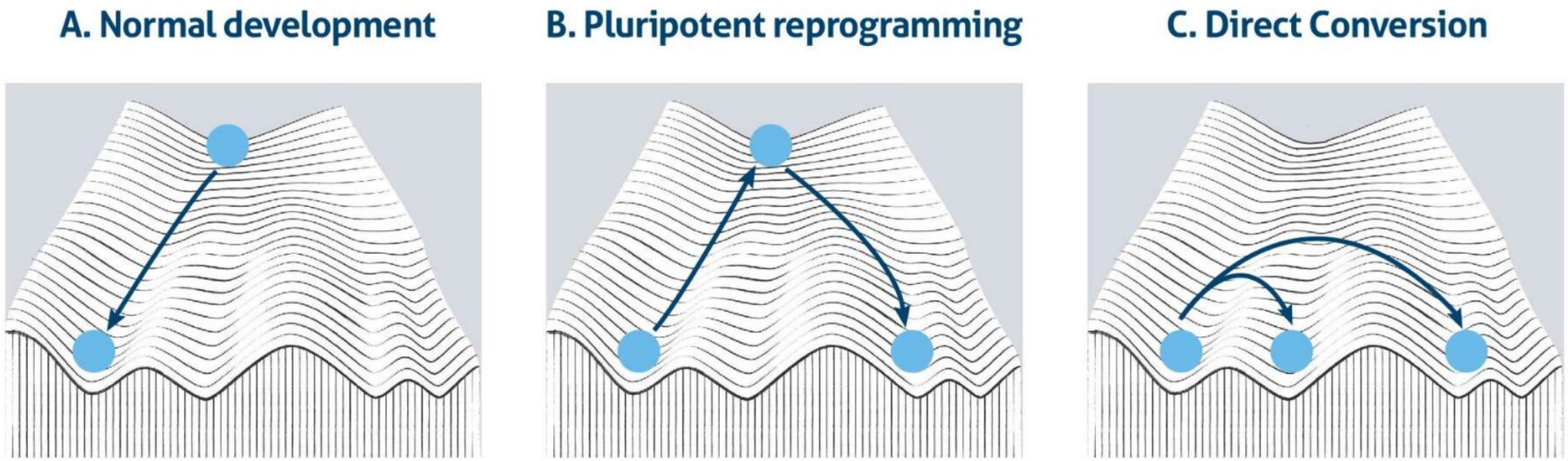




Epigenetic regulation determines cell fate



Epigenetic regulation determines cell fate



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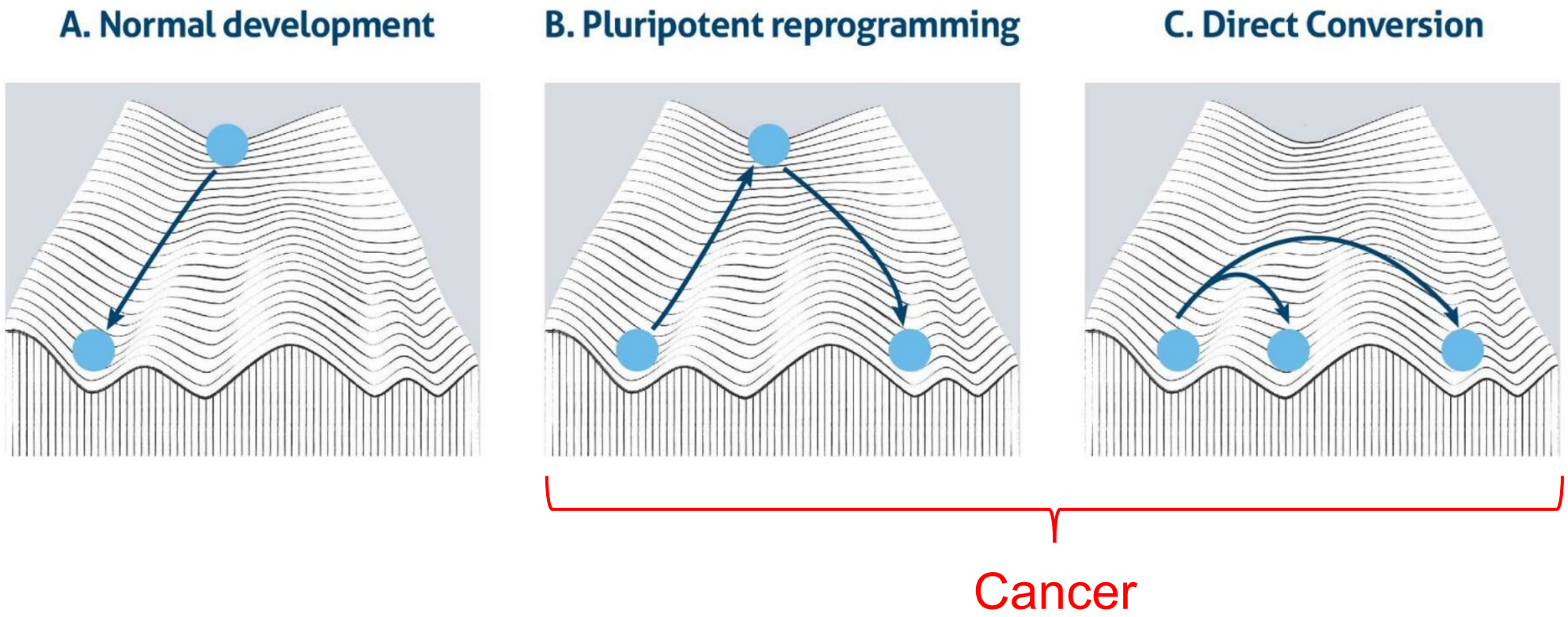
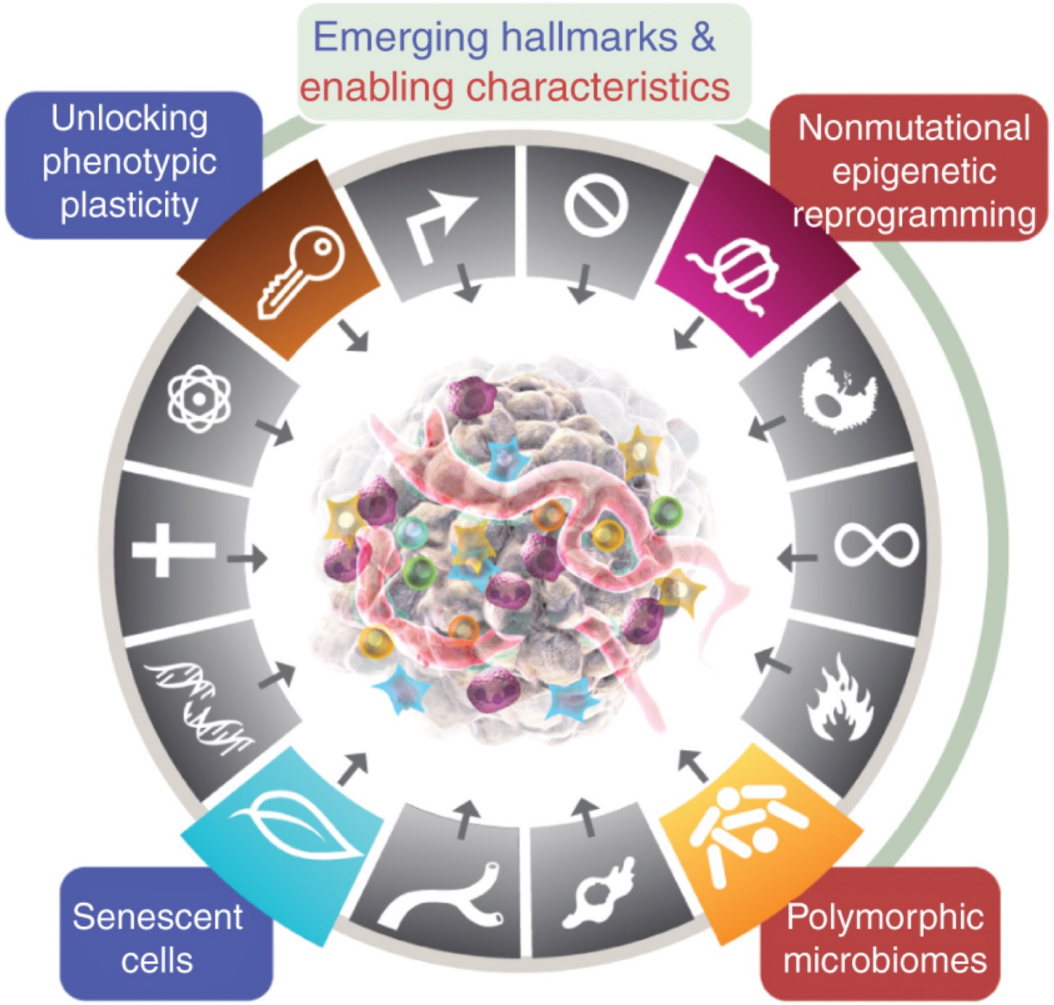


Epigenetic de-regulation is a hallmark of cancer

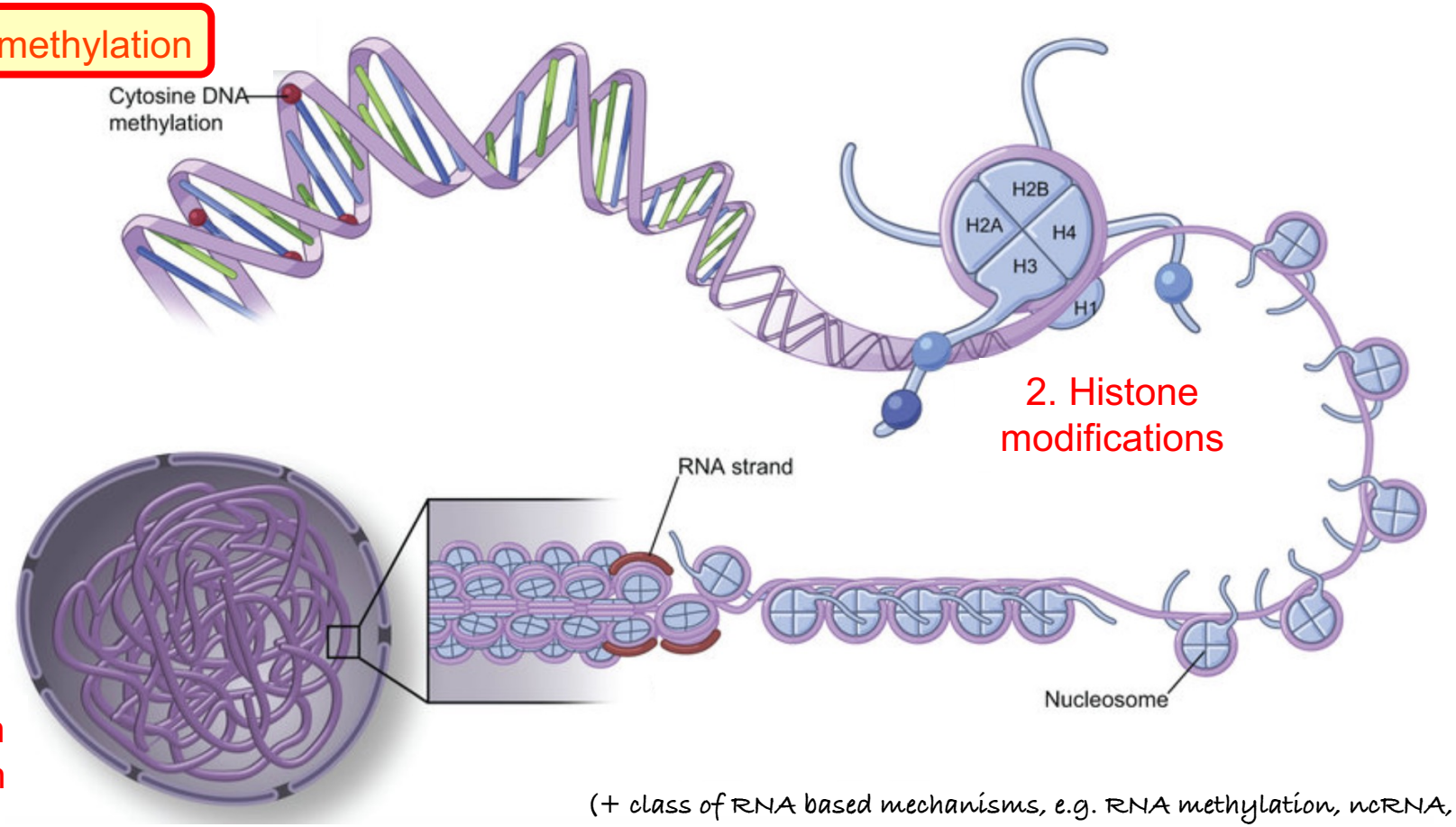


(Hanahan, 2022)

How does this happen?

Mechanisms of epigenetic regulations:

1. DNA methylation



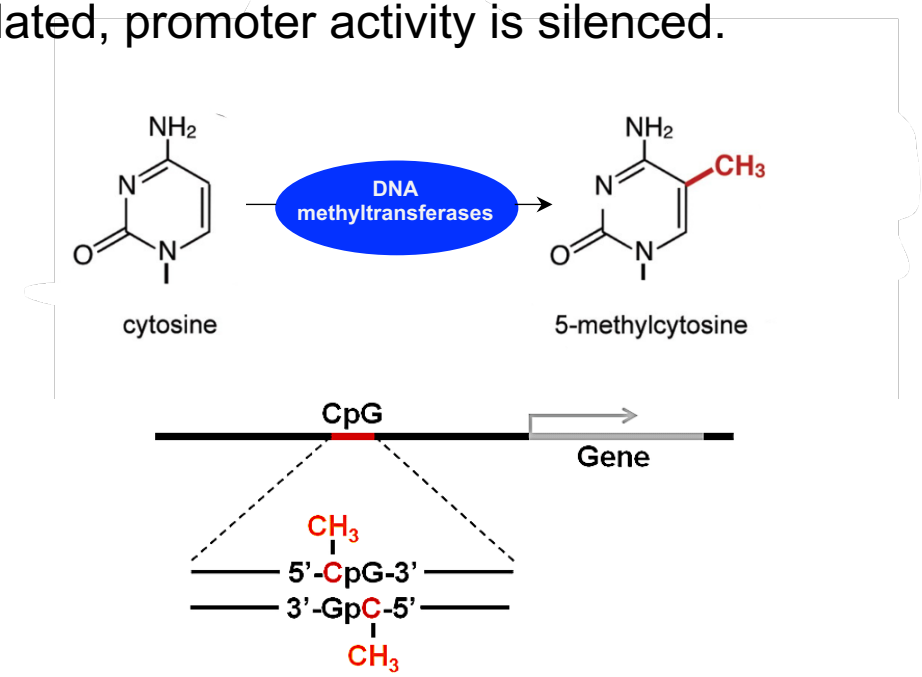
2. Histone modifications

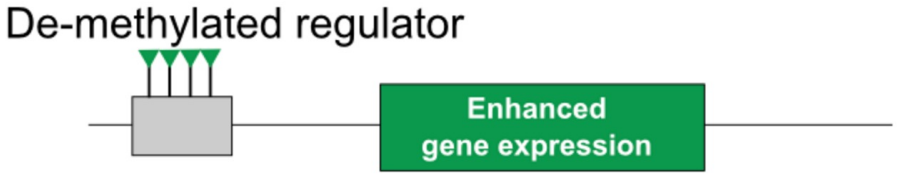
3. Chromatin conformation

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

1. DNA methylation

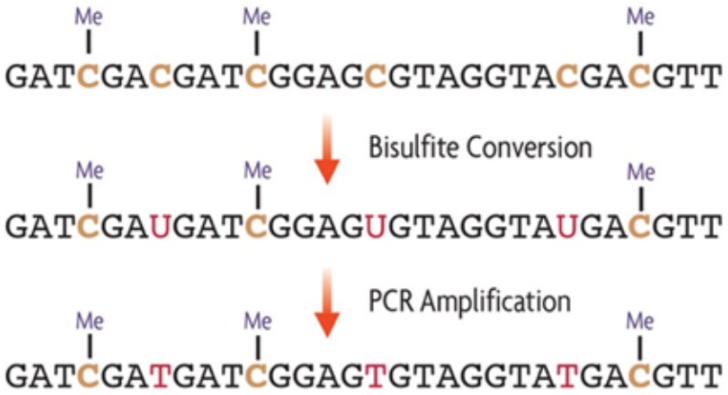
- In higher eukaryotes, DNA methylation is formed by the addition of a methyl group to the 5’ position of cytosine residues within a CpG dinucleotide context.
- CpG sites are highly methylated in the mammalian genome with the exception of CpG islands which are largely unmethylated.
- ~60% of all human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





DNA Methylation

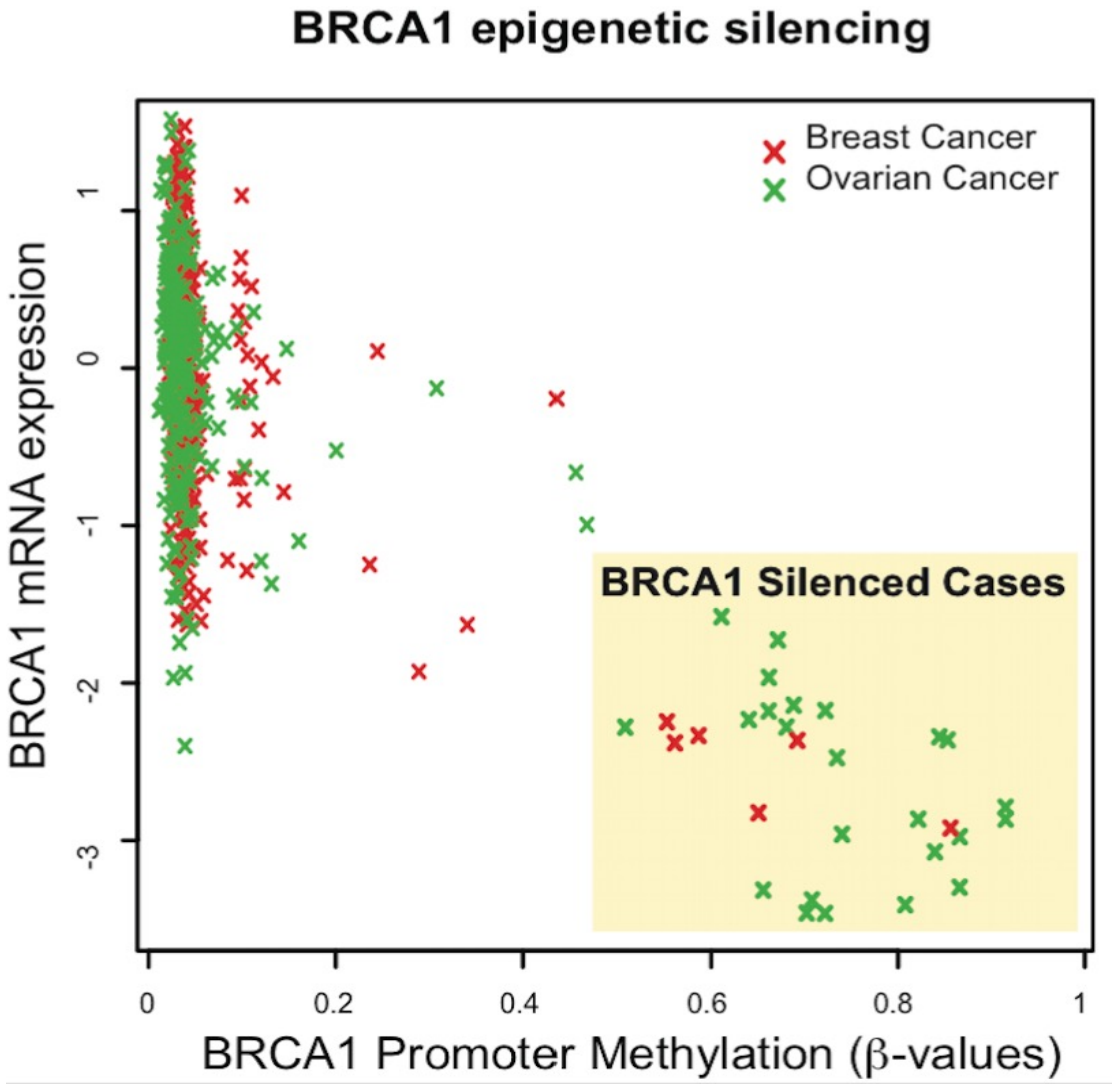
based on bisulfite conversion and sequencing



- 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



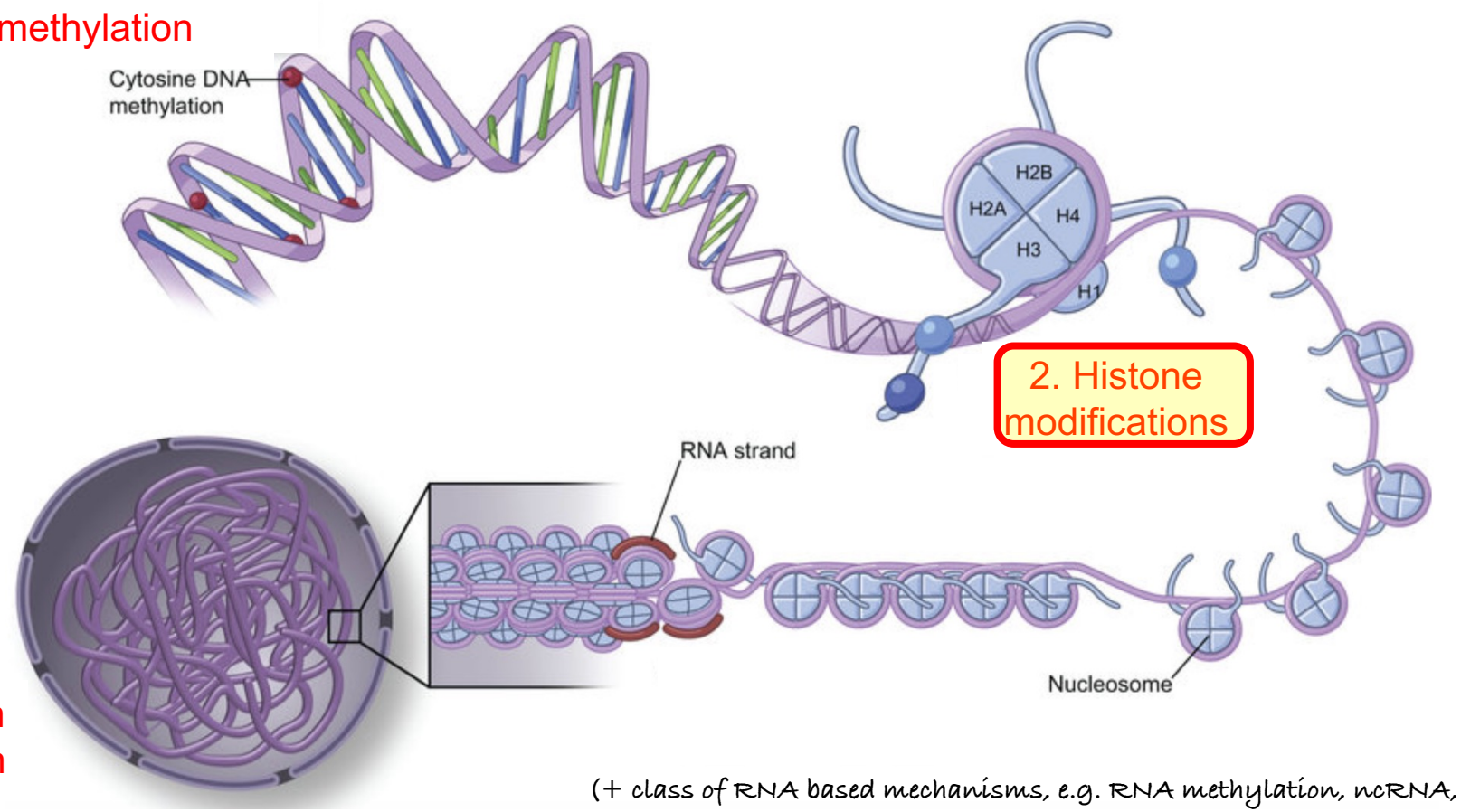
What drives DNA methylation changes?

- Alterations of proteins regulating DNA methylation
- Increased cell division lead to DNA methylation loss in late replicating genomic regions (e.g. intergenic repeats) → loss of DNA methylation is also an aging phenotype
- Differences of DNA methylation between cancer and normal cells from the same tissue might reflect 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

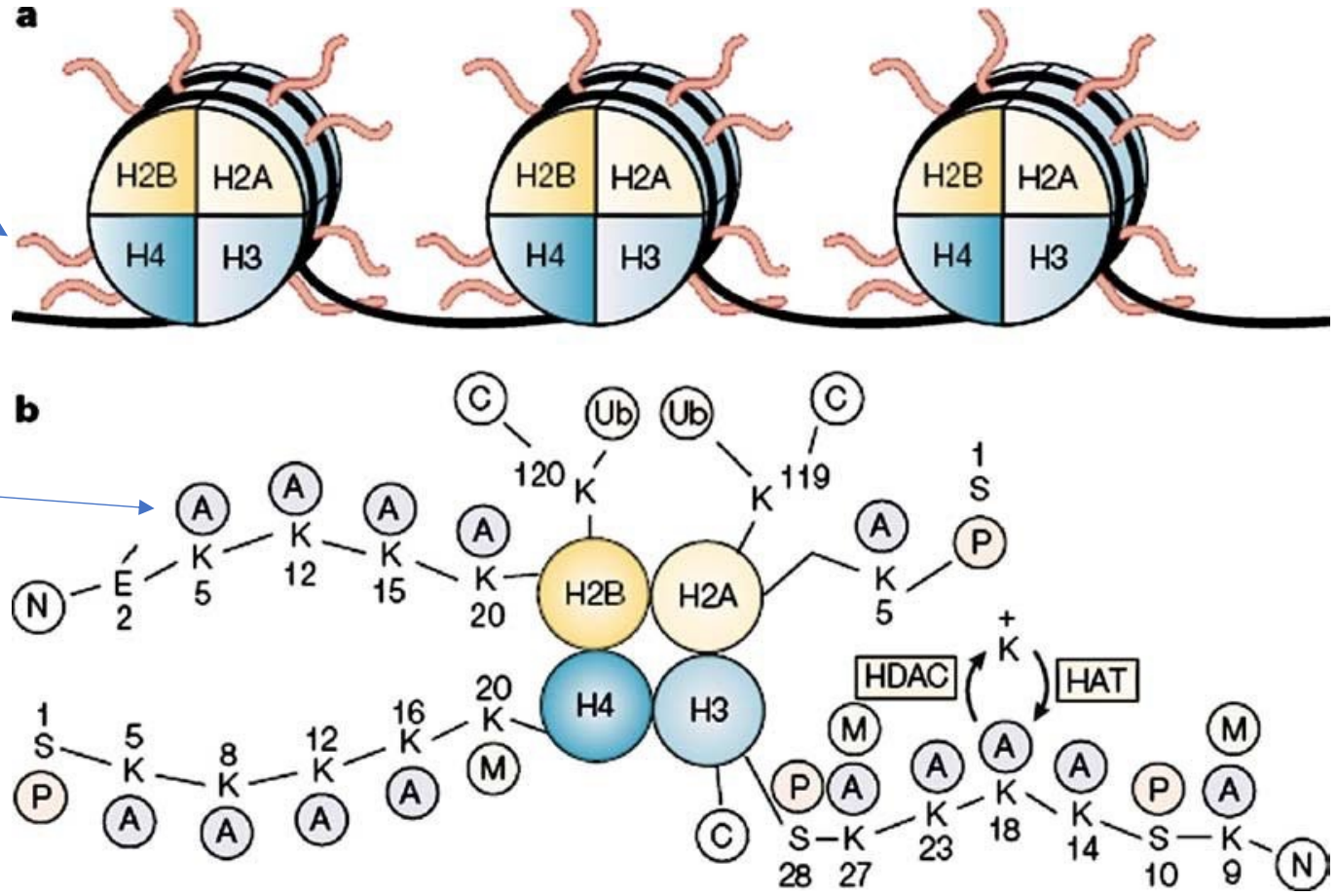
(+ class of RNA based mechanisms, e.g. RNA methylation, ncRNA, etc.)

Histone post-translational modifications

Histone tails are accessible for protein binding

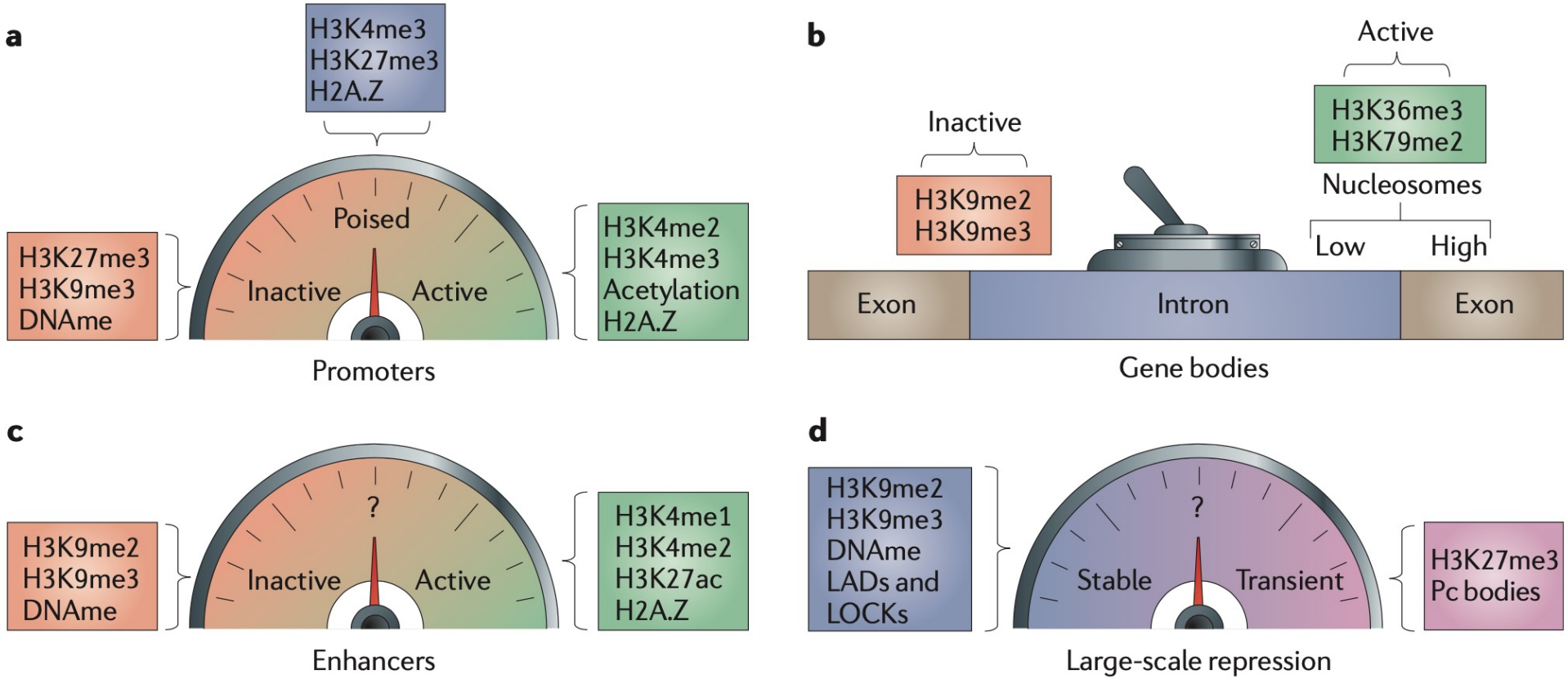
Different type of modifications can be found at lysine tails

Most common:
Acetylation (A)
Methylation (M)



The histone code

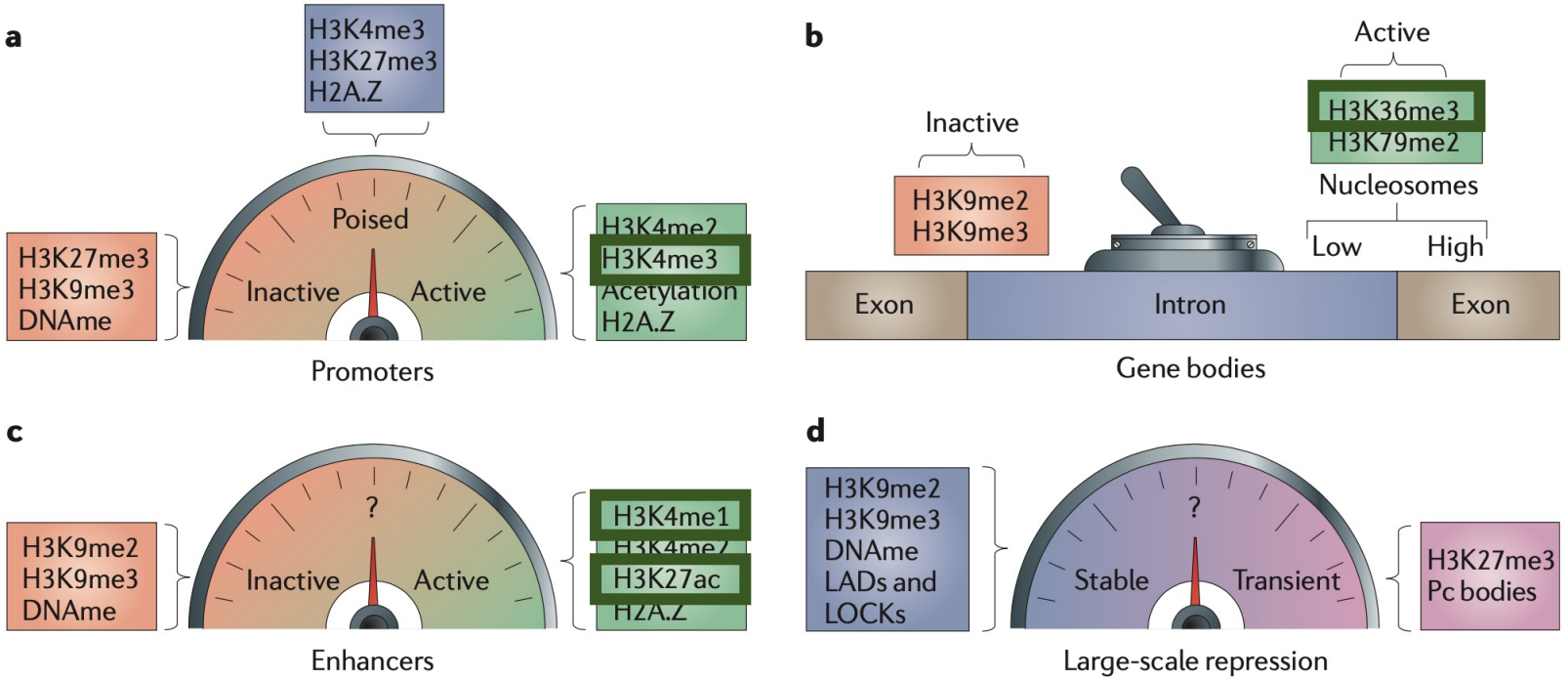
‘Dashboard’ of histone modifications for fine-tuning genomic elements



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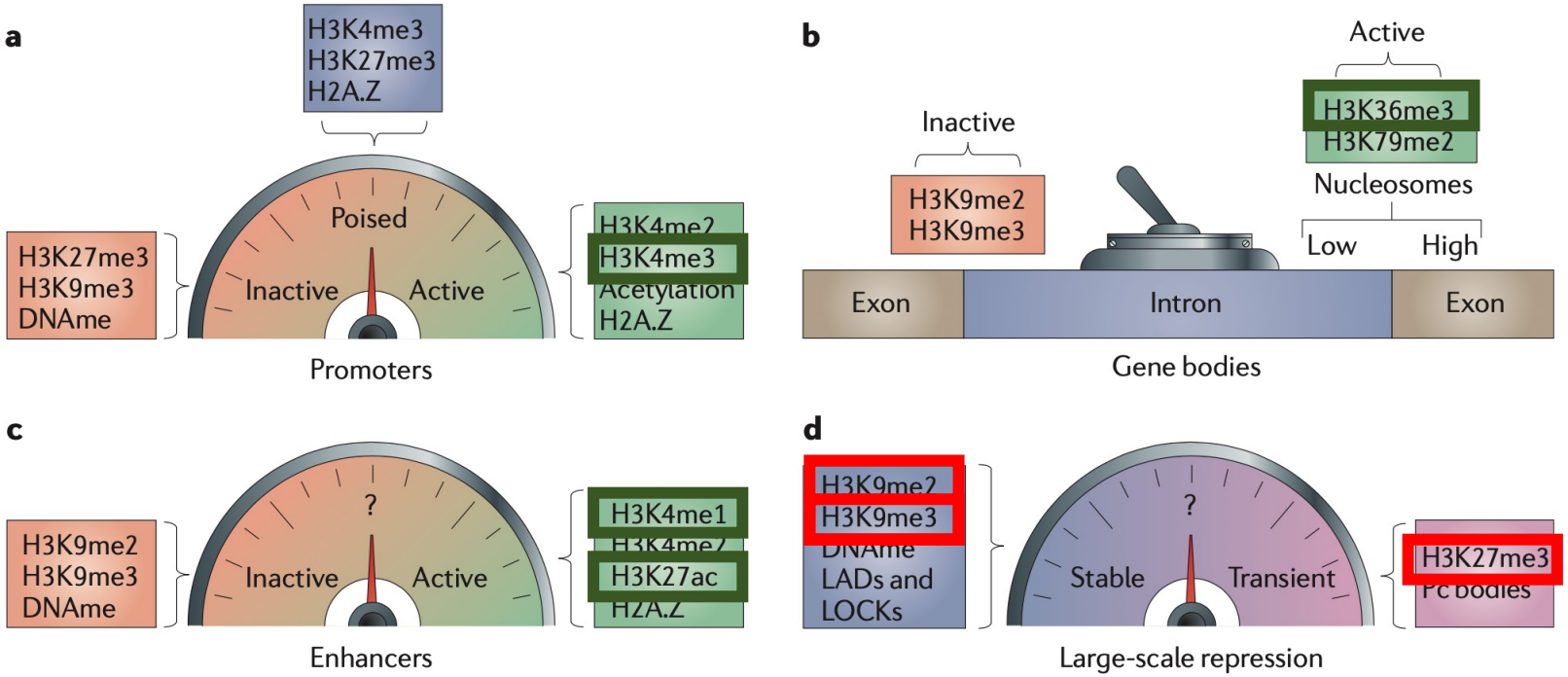
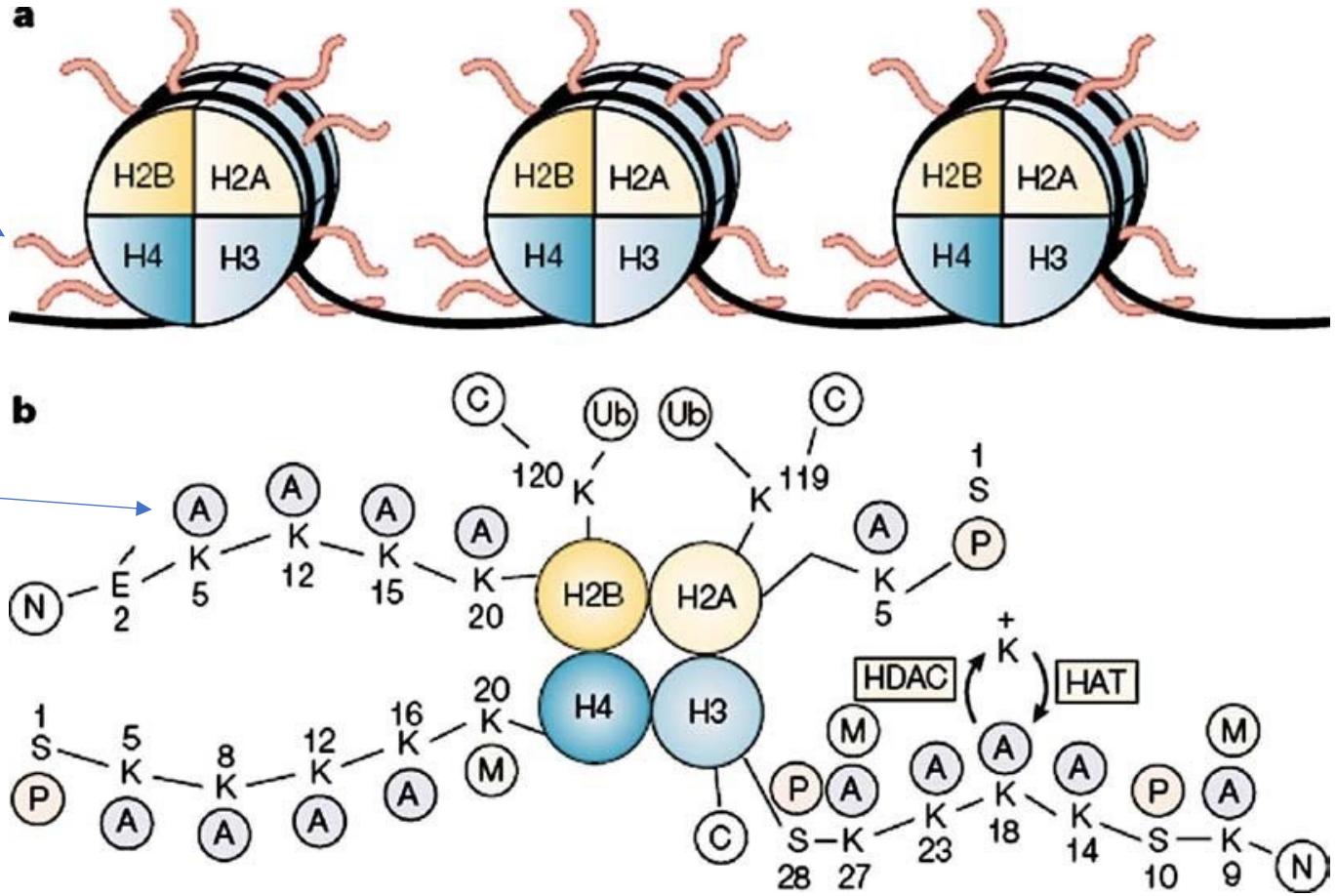
- **H3K9me3** marks the classical heterochromatin (canonical repressive state)
- Wide-spread regions repressed by the Polycomb Group of proteins are enriched in **H3K27me3**
- **H3K9me2/3** mark genomic regions of highly condensed chromatin physically associated with the nuclear lamina



Histone post-translational modifications

Histone tails are accessible for protein binding



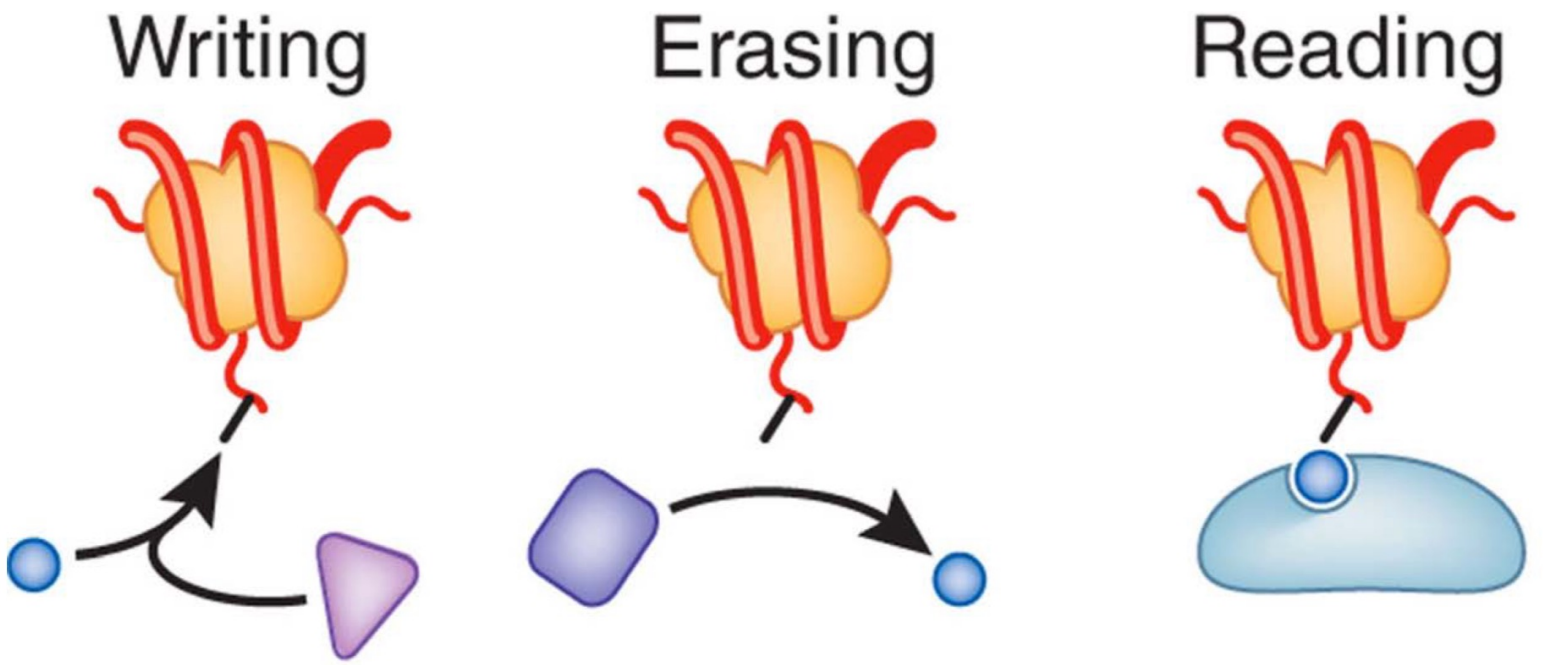
Different type of modifications can be found at lysine tails

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

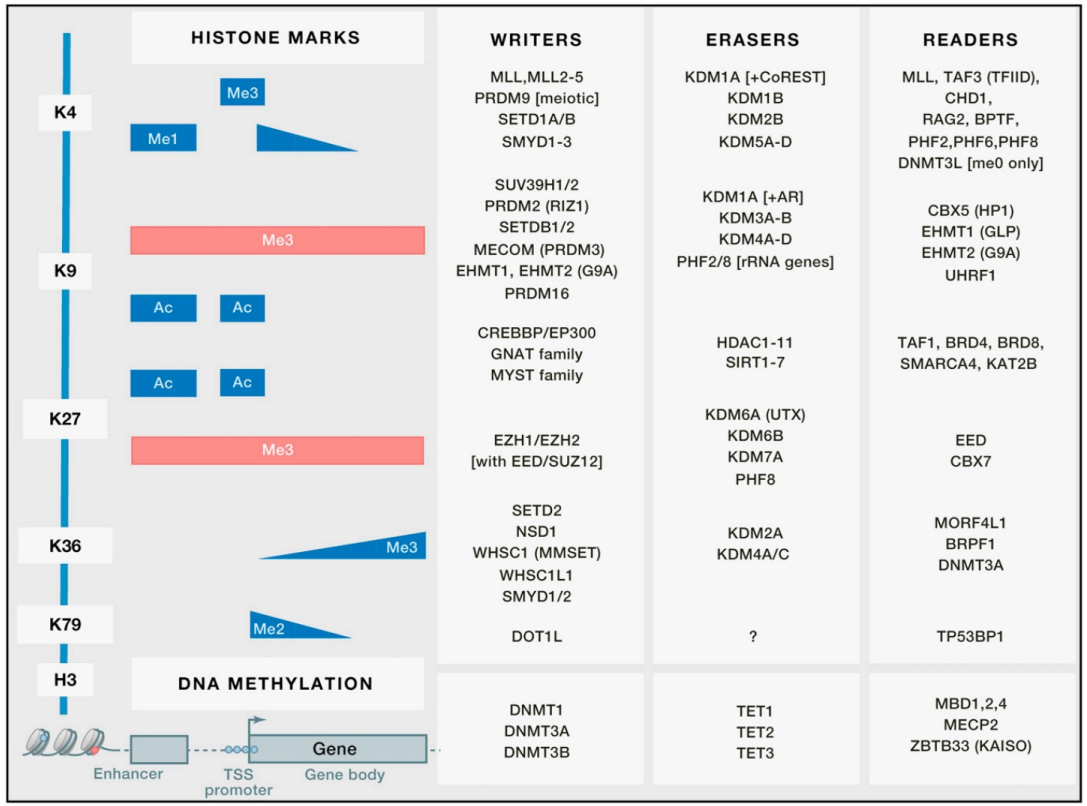


Acetylases,
methylases,
phosphorylases

Deacetylases,
demethylases,
phosphatases

Bromodomain,
chromodomain,
PHD finger,
WD40 repeat

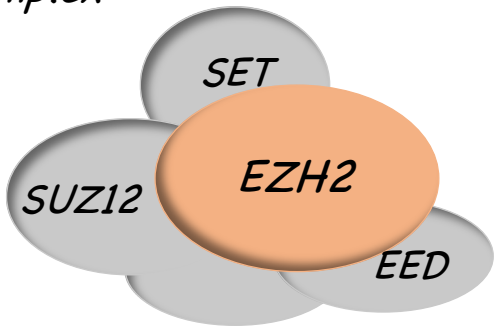
Chromatin regulators are among the most frequently mutated genes in cancer



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

Chromatin regulators: EZH2 (writer)

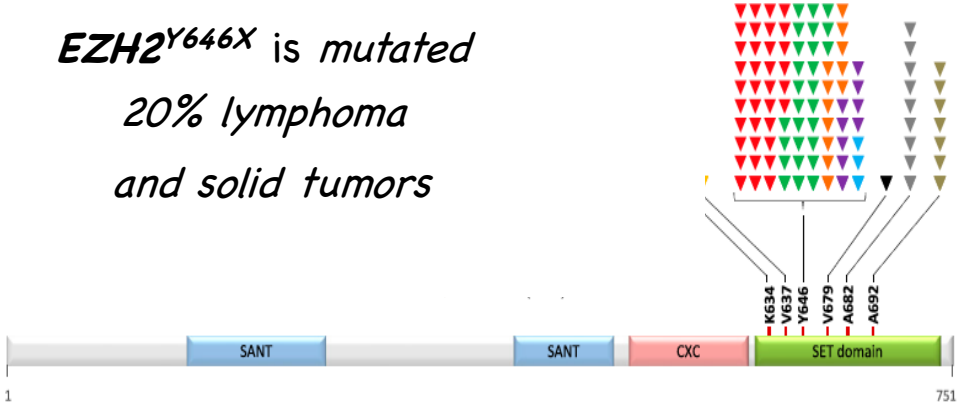
PRC2 complex



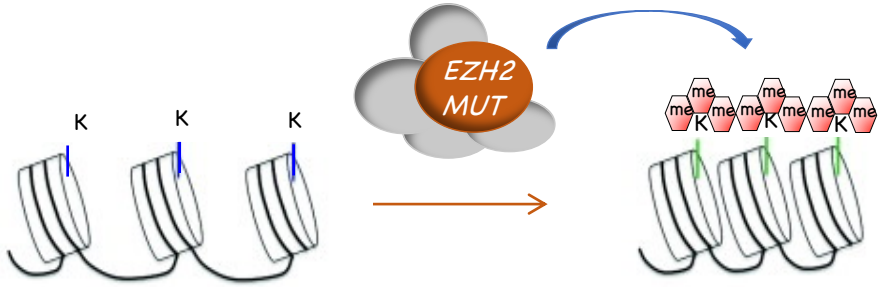
EZH2^{Y646X} is *mutated*

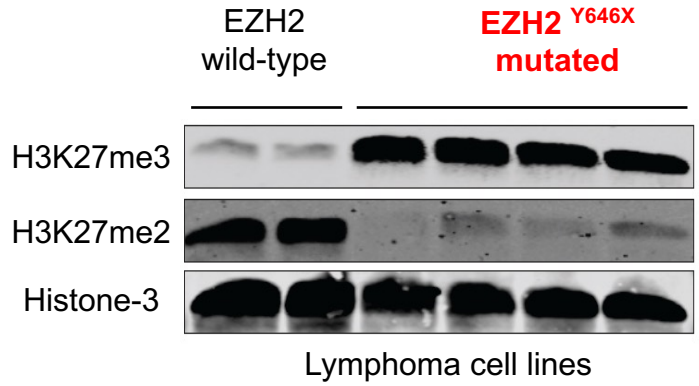
20% lymphoma

and solid tumors

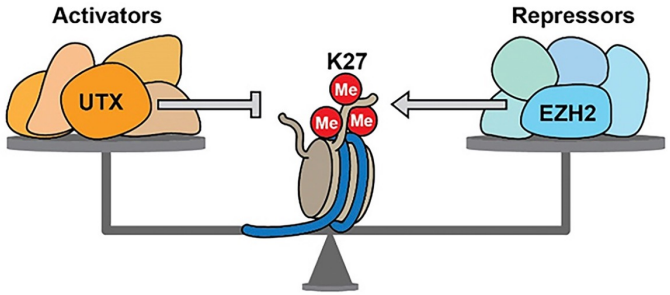


EZH2^{Y646X} increases H3K27 tri-methylation





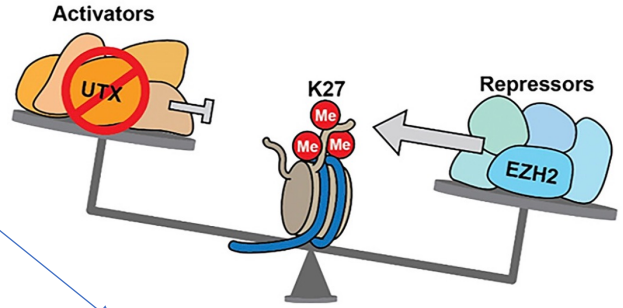
Chromatin regulators: UTX (eraser)



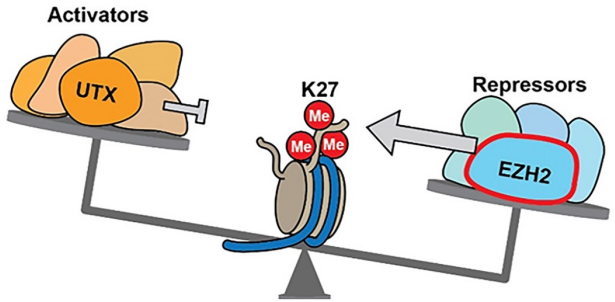
Balanced activated and repressed transcription

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

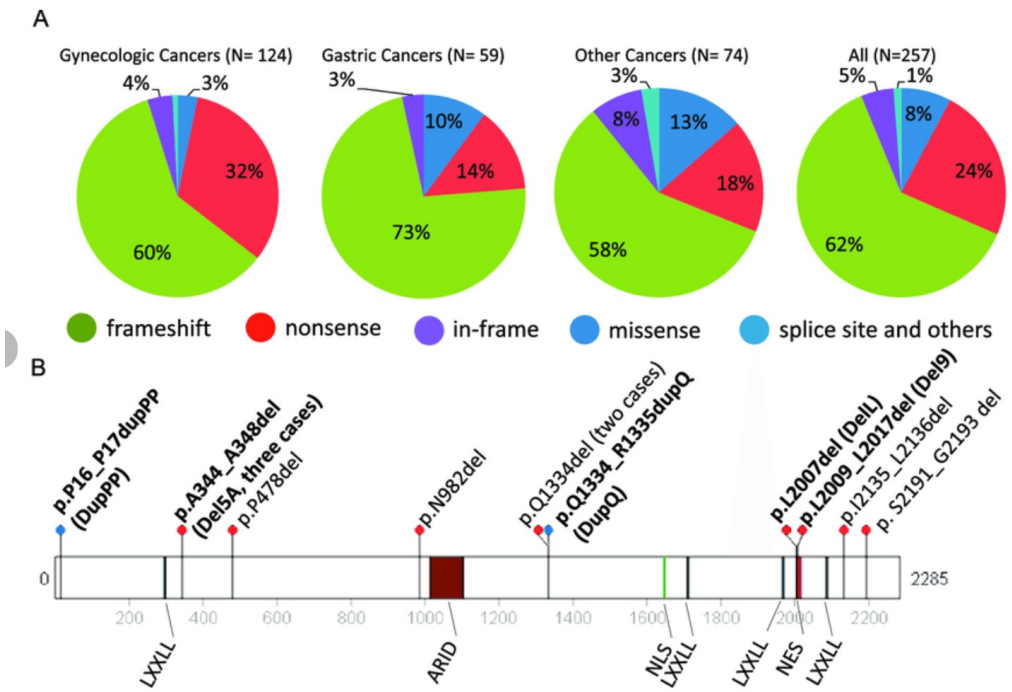


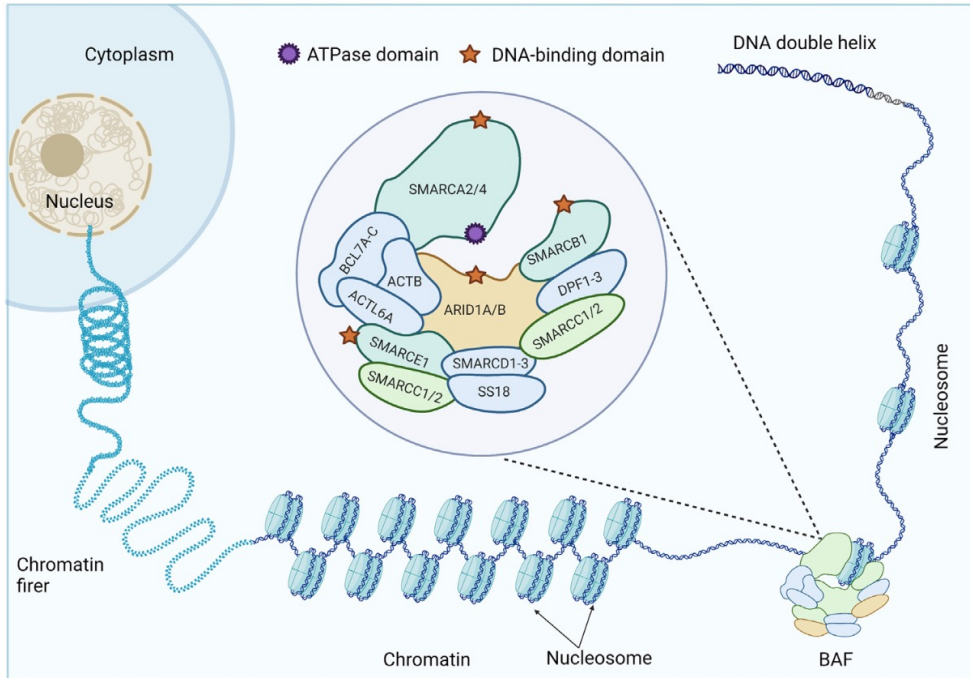
EZH2 gain-of-function mutations

PRC2-regulated transcription repression

Massive increase of H3K27me3

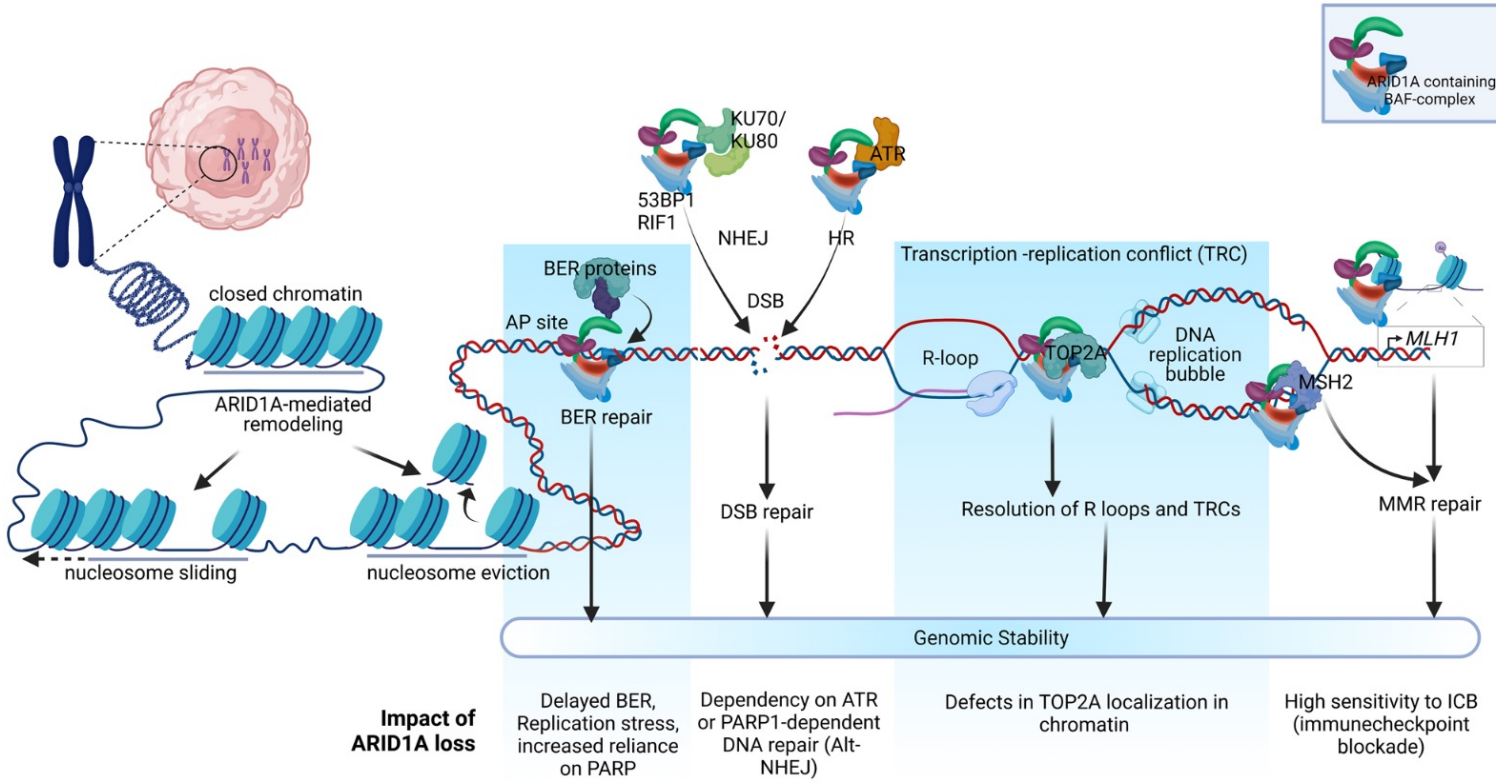
Chromatin regulators: ARID1A (modifier) (chromatin remodeling factors)





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

Chromatin regulators: ARID1A (modifier)

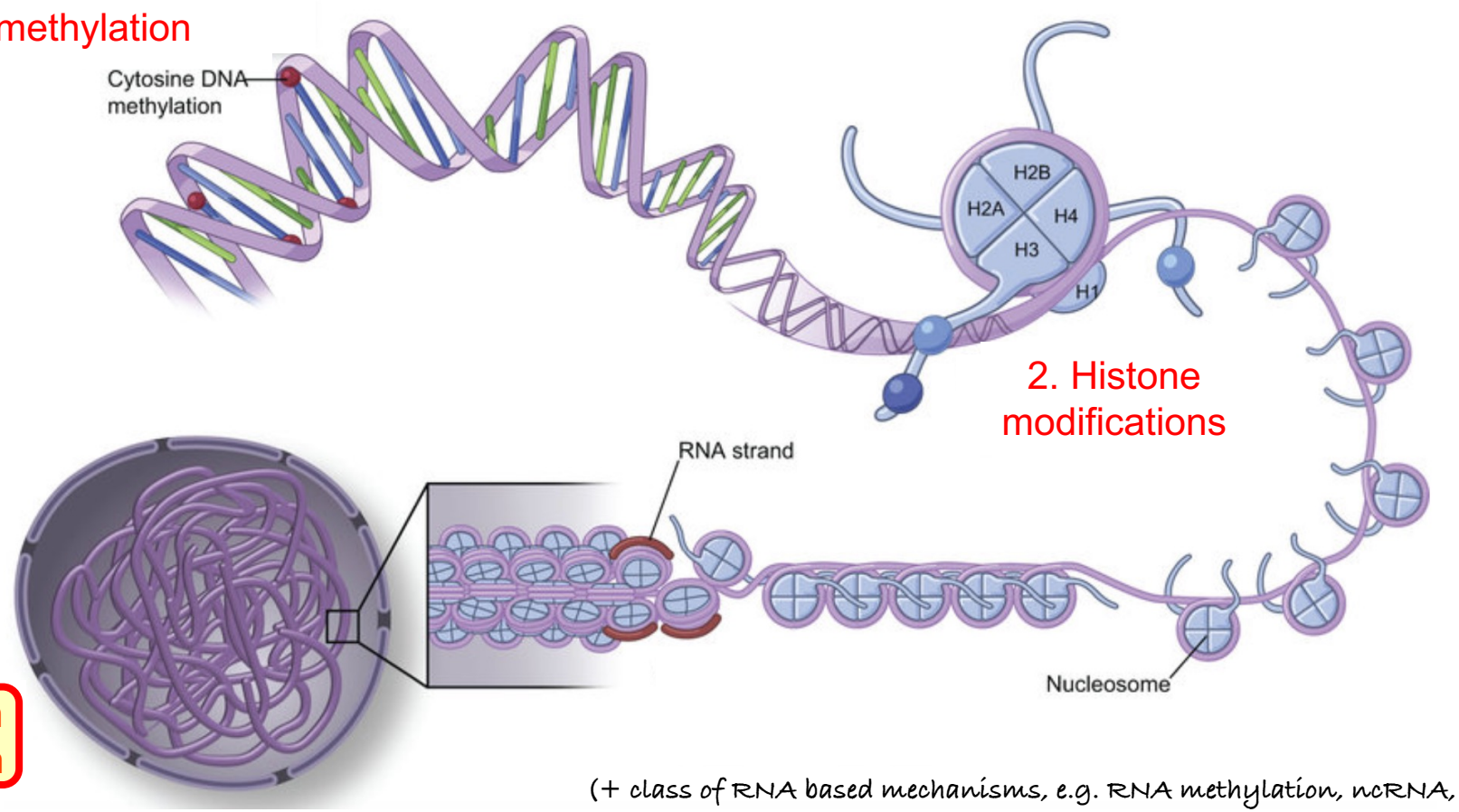


Changes in nucleosome positioning lead to genomic instability

How does this happen?

Mechanisms of epigenetic regulations:

1. DNA methylation

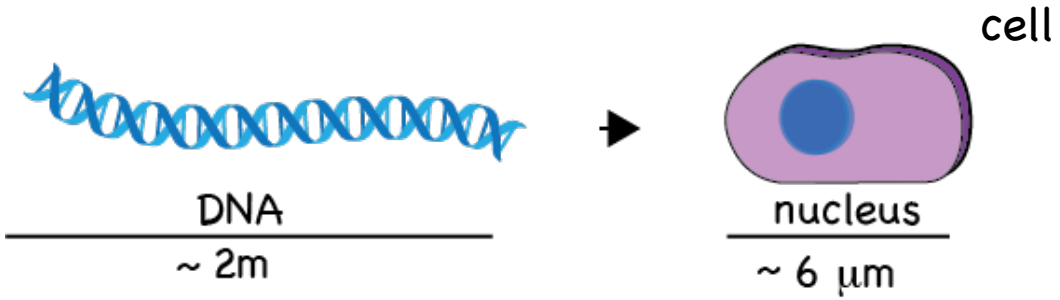


2. Histone modifications

3. Chromatin conformation

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

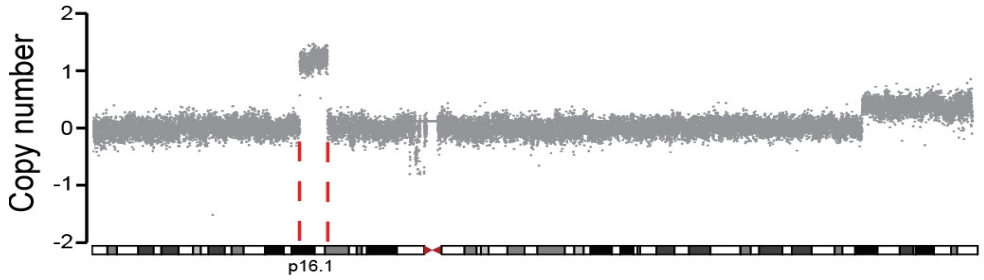
Why chromatin 3D structure?

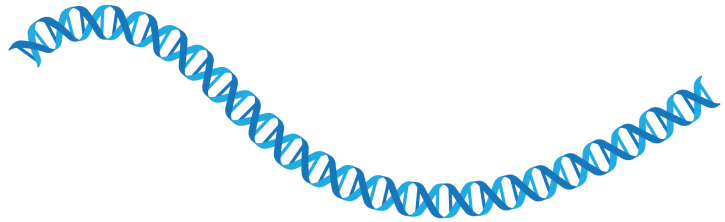


Linear view of the DNA

Compaction in the cell nucleus

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



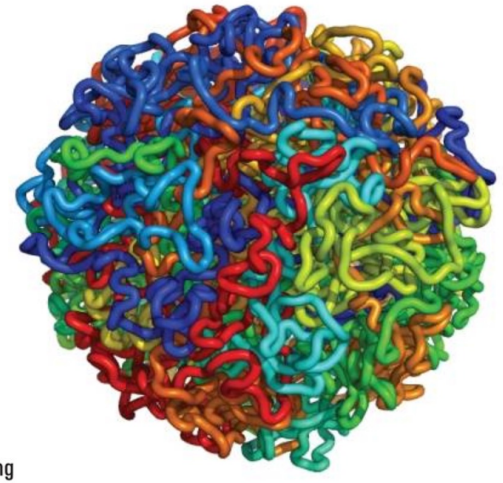


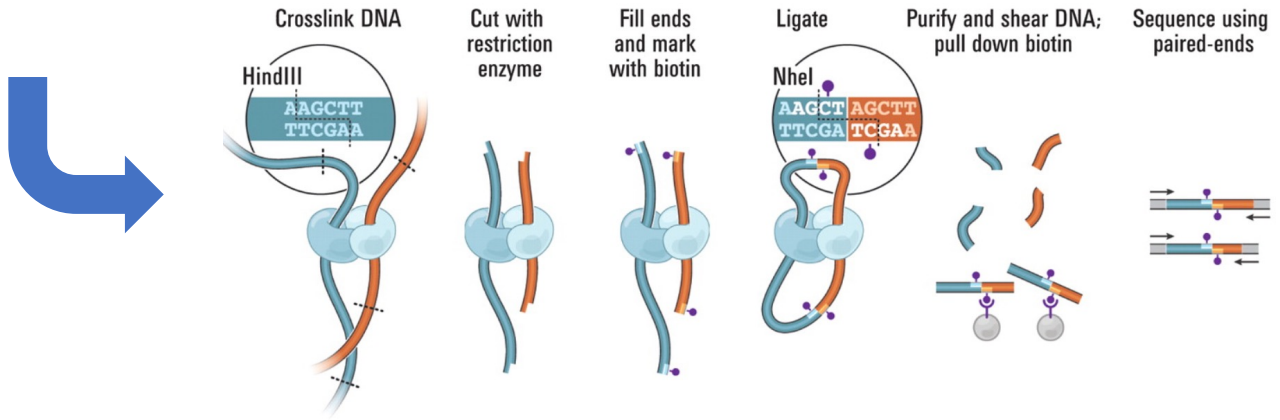
Where is this amplification in the cell nucleus?

Chromatin conformation in 3D

Linear view of the DNA

3D view of the DNA

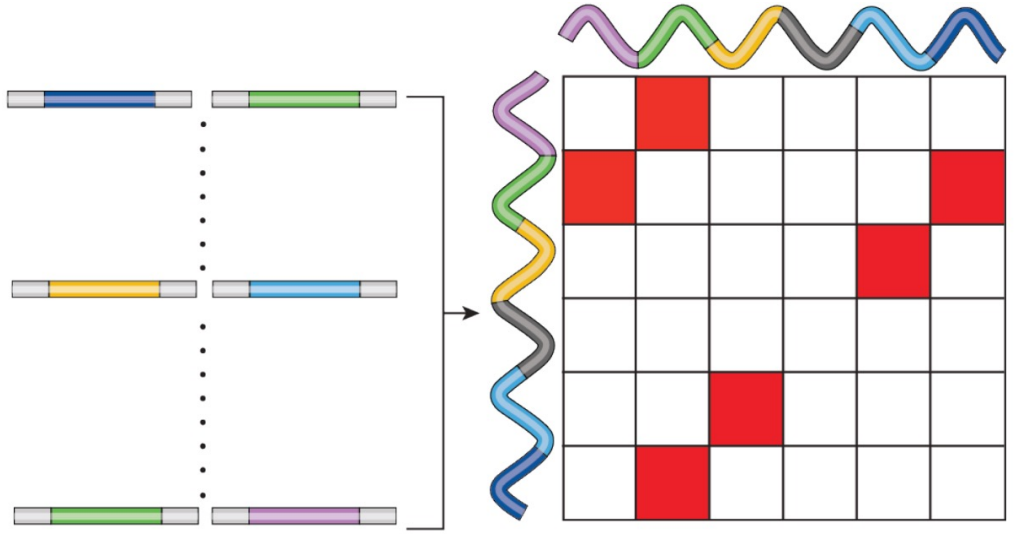




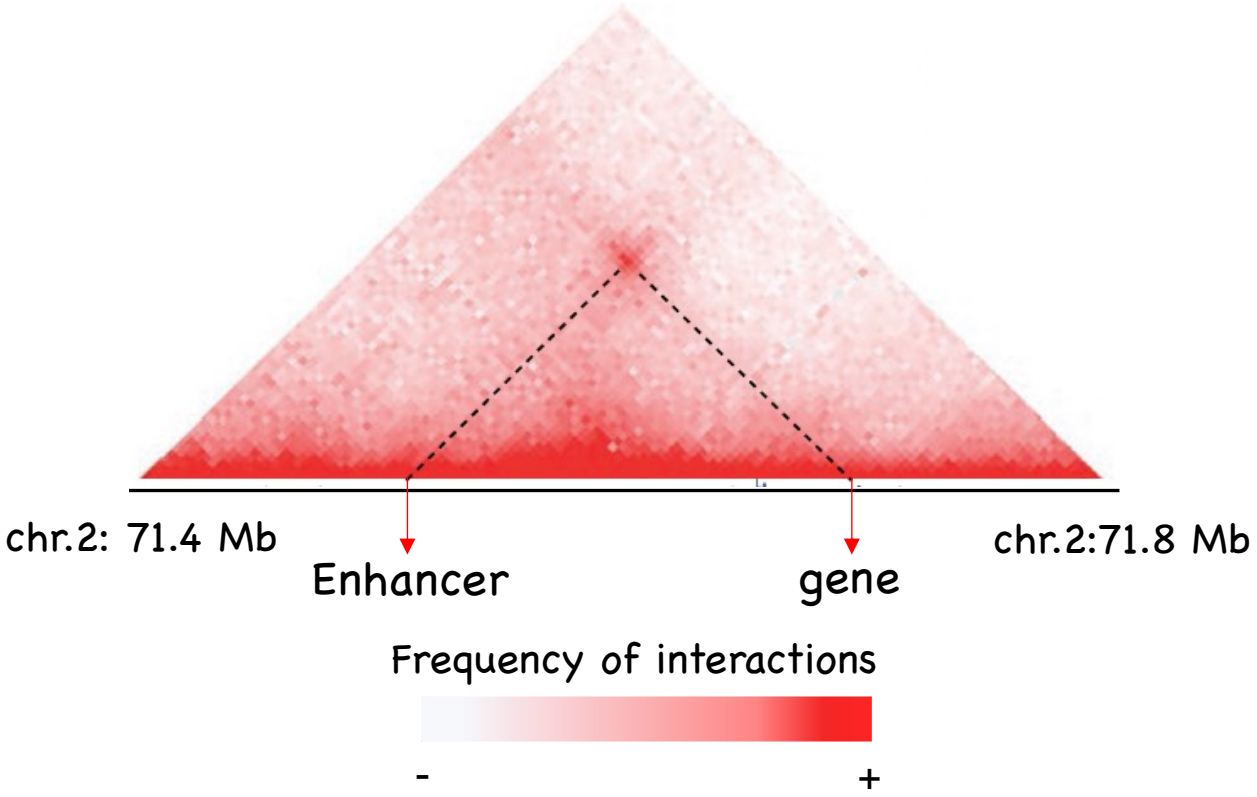
(3C, 4C, 5C, Hi-C, ...)

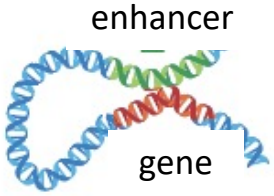
Chromatin conformation capture

How close are these genomic regions in 3D?
interactions (# of reads indicating proximity)



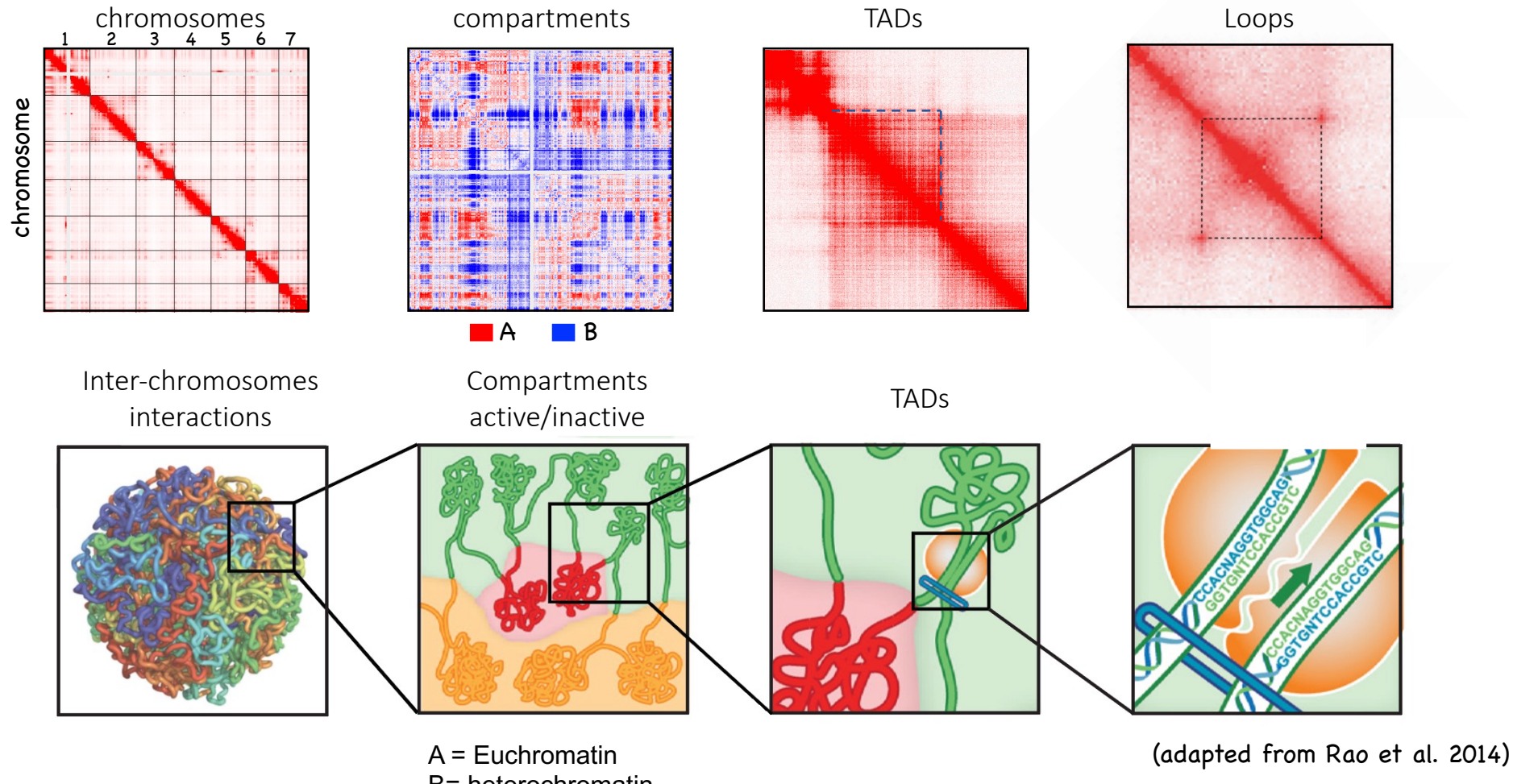
High-throughput chromatin conformation capture (Hi-C):



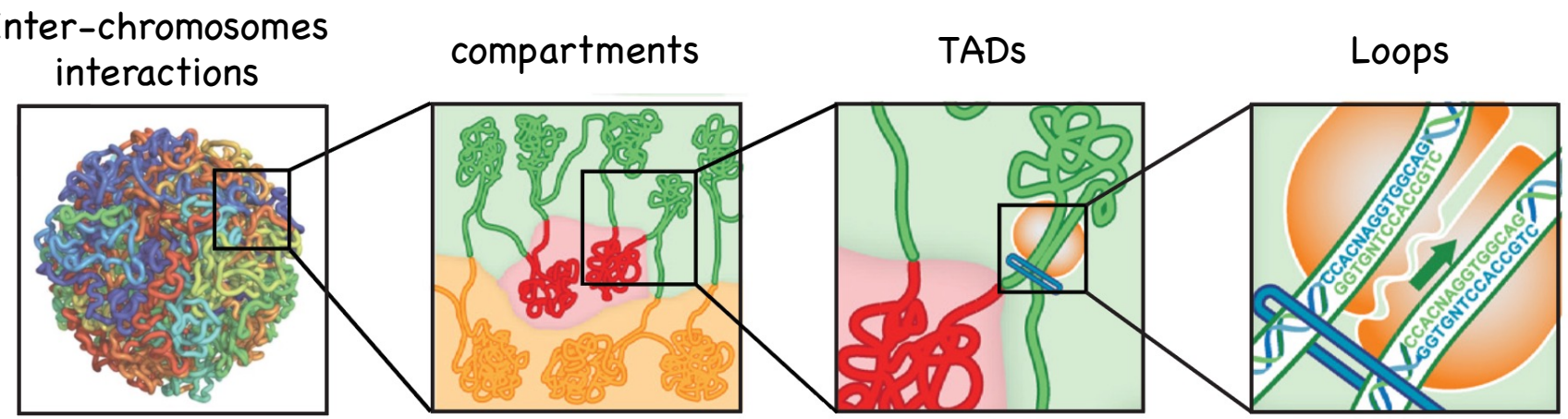


HiC measures the frequency of interactions between distant genomic loci

Hi-C to study chromatin structure



Chromatin organization in the cell nucleus



(adapted from Rao et al. 2014)

Cancer cells

Chromosomal alterations

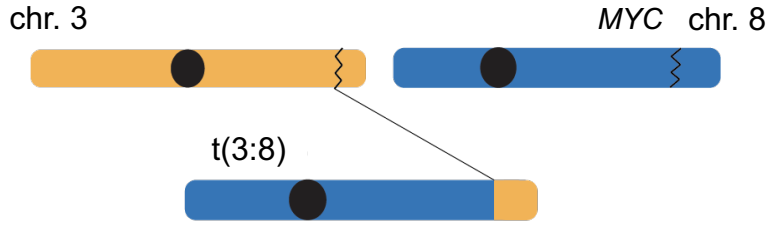
Translocations
Amplifications
Deletions

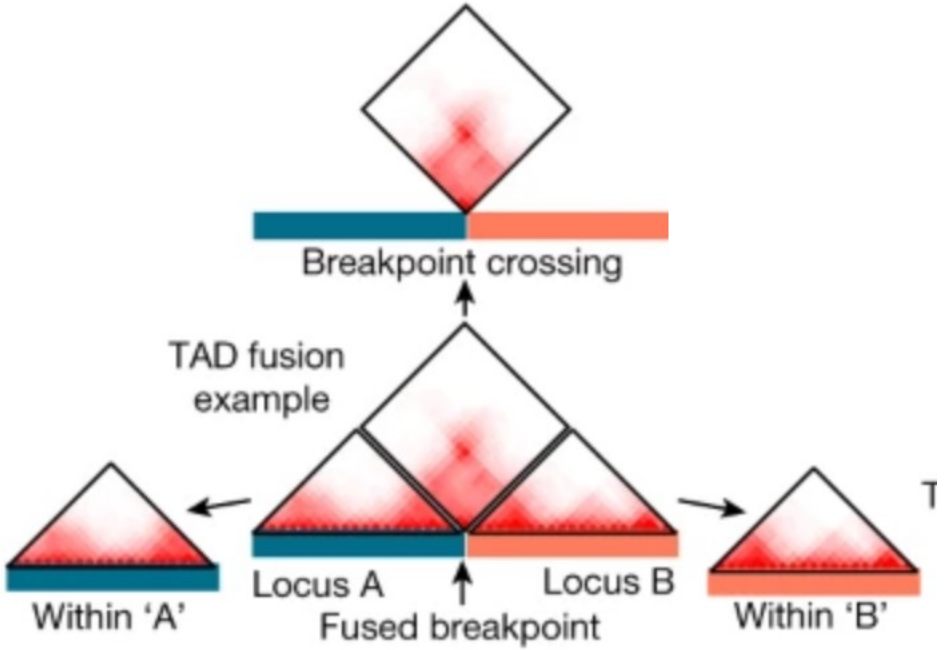
Epigenetic alterations

Histone methylation
acetylation

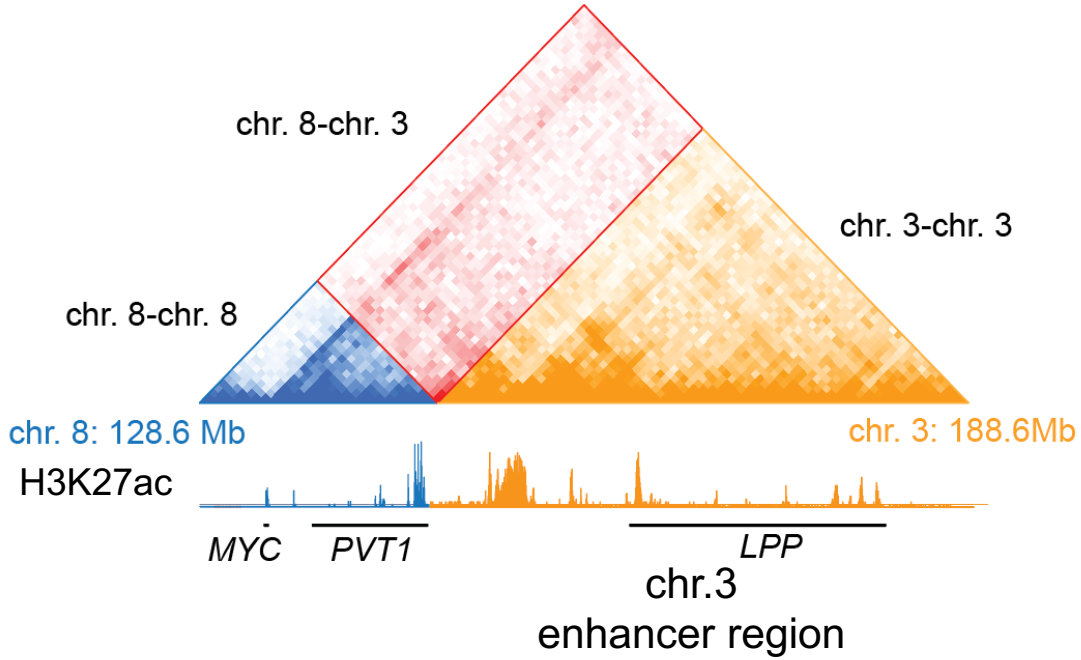
Chromosomal translocation in 3D

Frequent in multiple hit-B-cell aggressive lymphoma





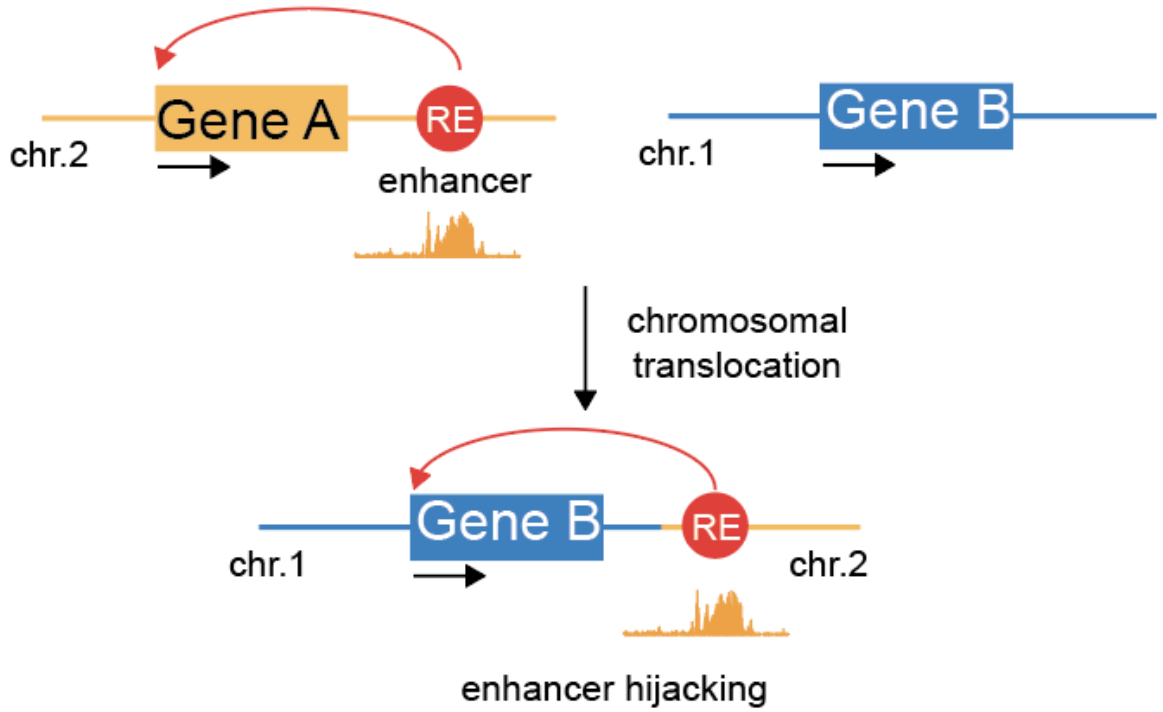
Intra-chromosomal map:



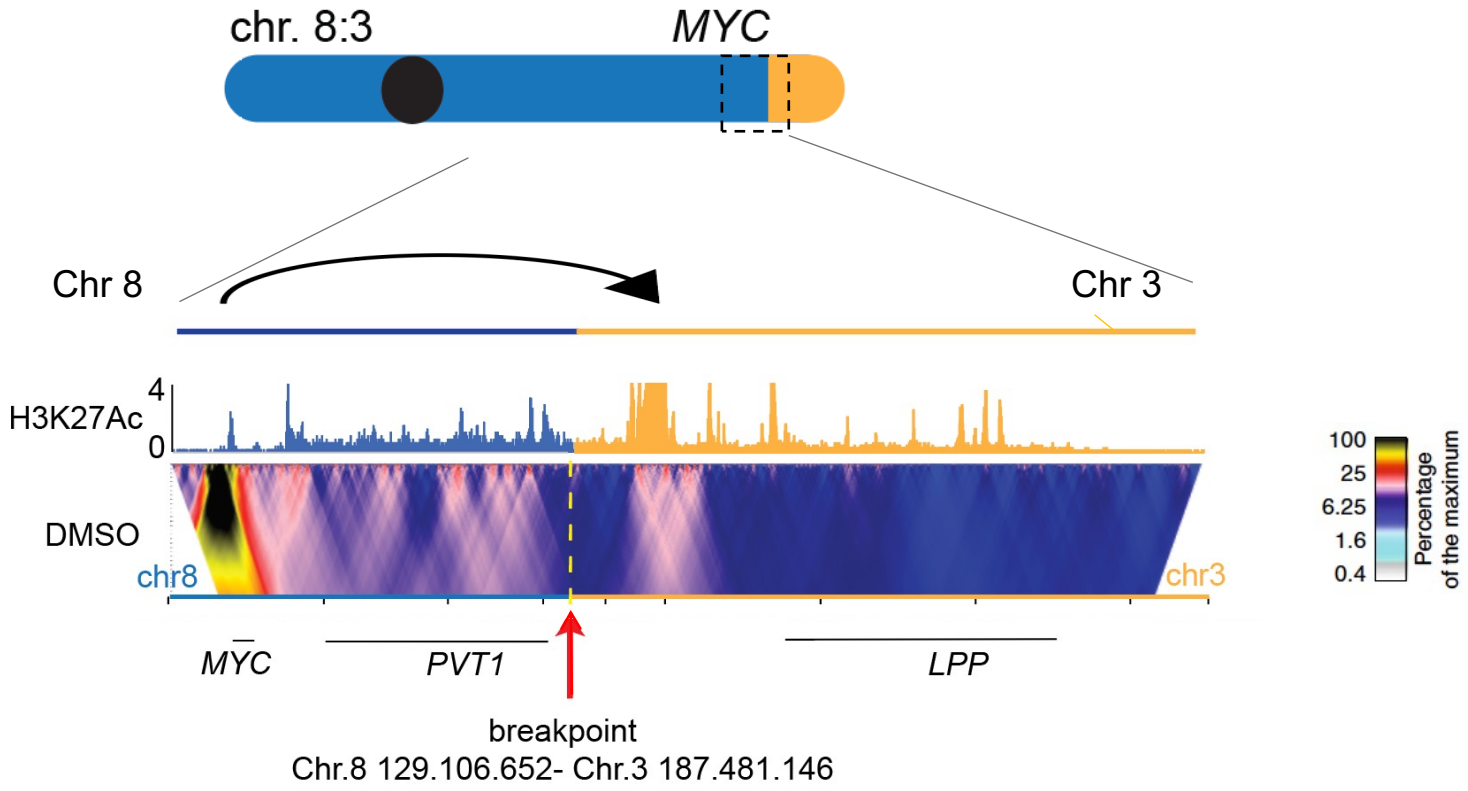
chr.3 enhancer region

Aberrant oncogene expression in cancer

Chromosomal
alterations

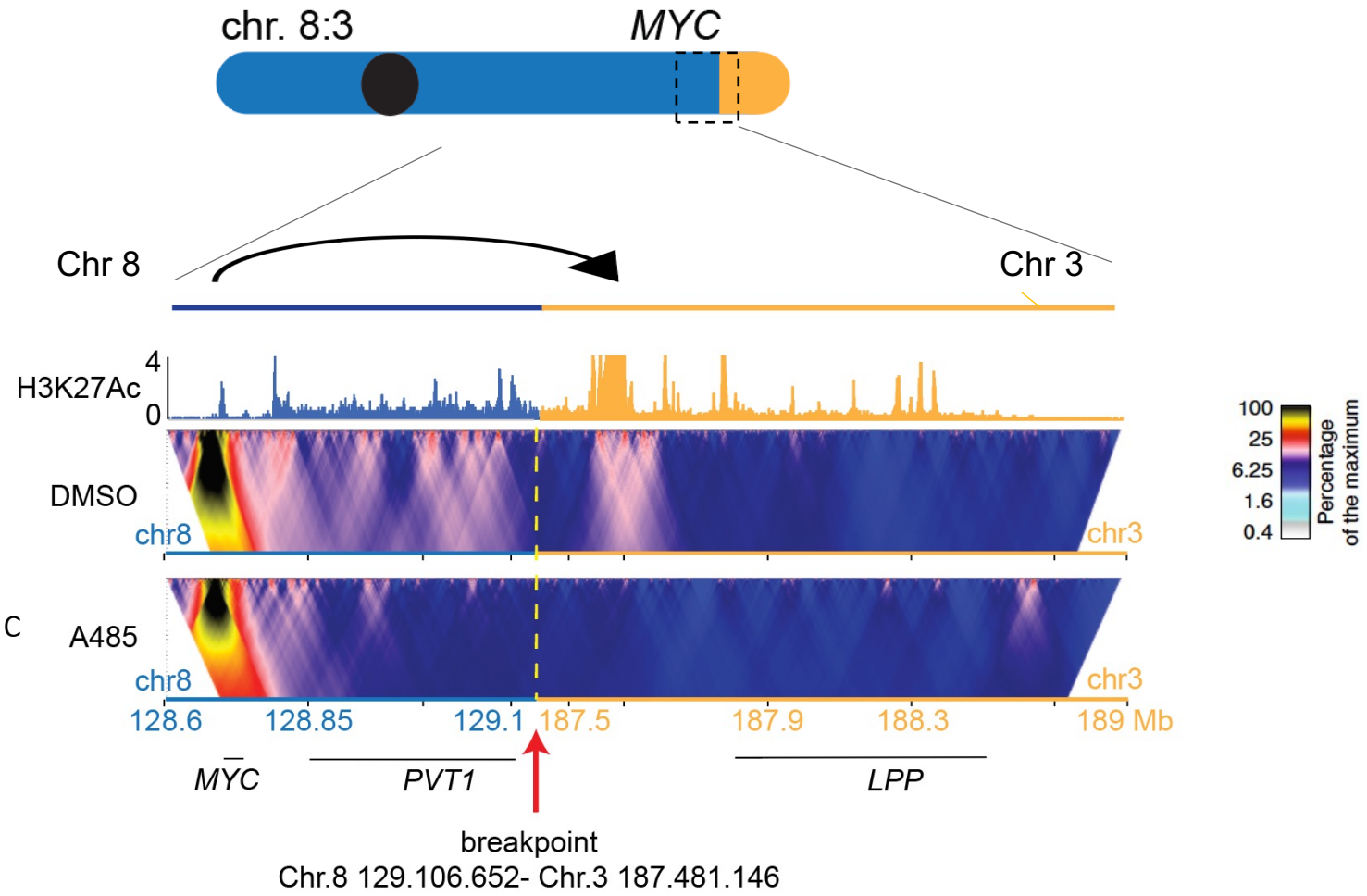


Enhancer promoter interactions and chromosomal translocations

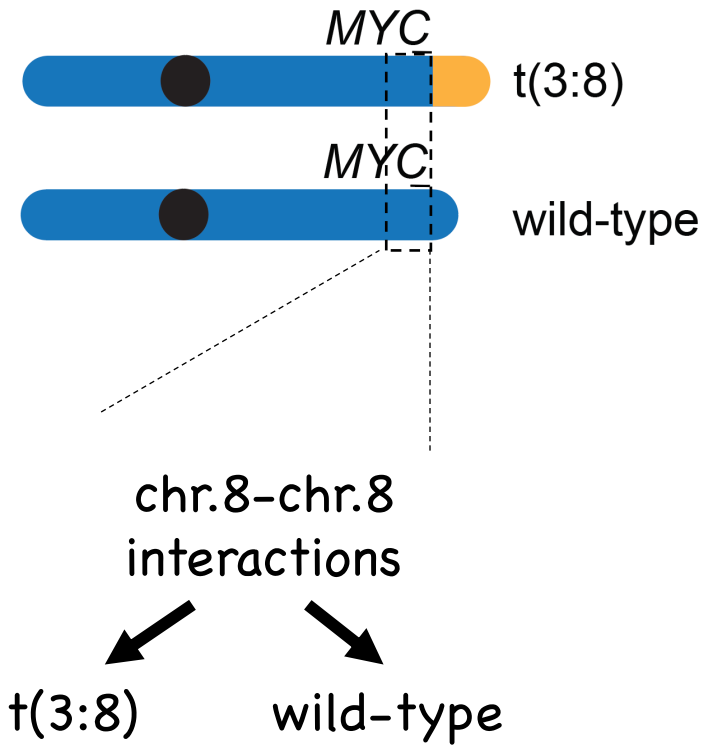


Enhancer promoter interactions and chromosomal translocations

Depletion of H3K27ac
(inhibition of CBP)

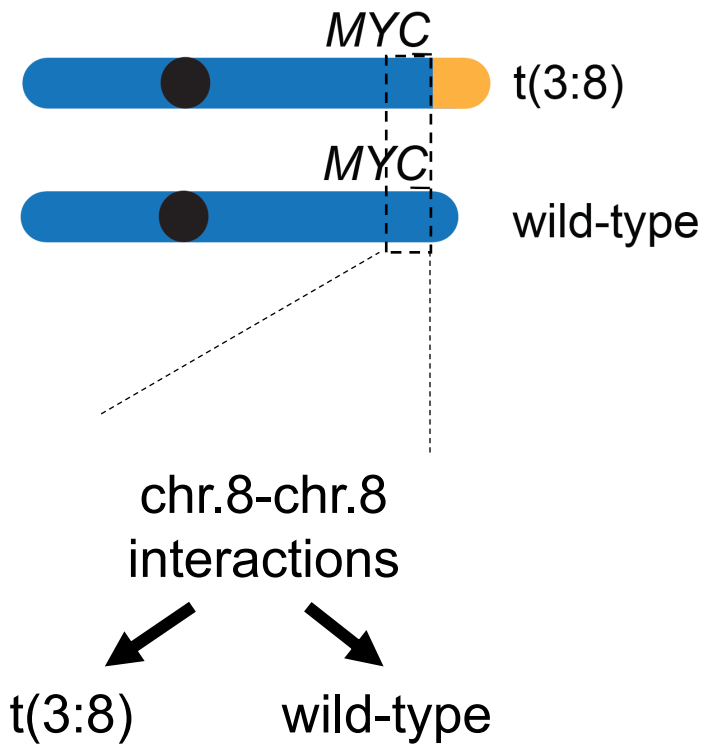


Enhancer promoter interactions and chromosomal translocations



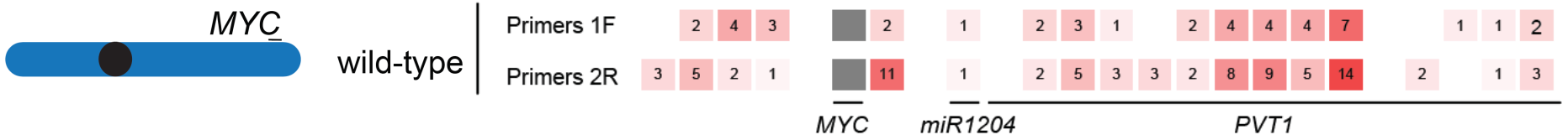
Chromatin conformation in oncogenic loci of two homologous chromosomes

Chromosome 8-3 translocation alters MYC expression

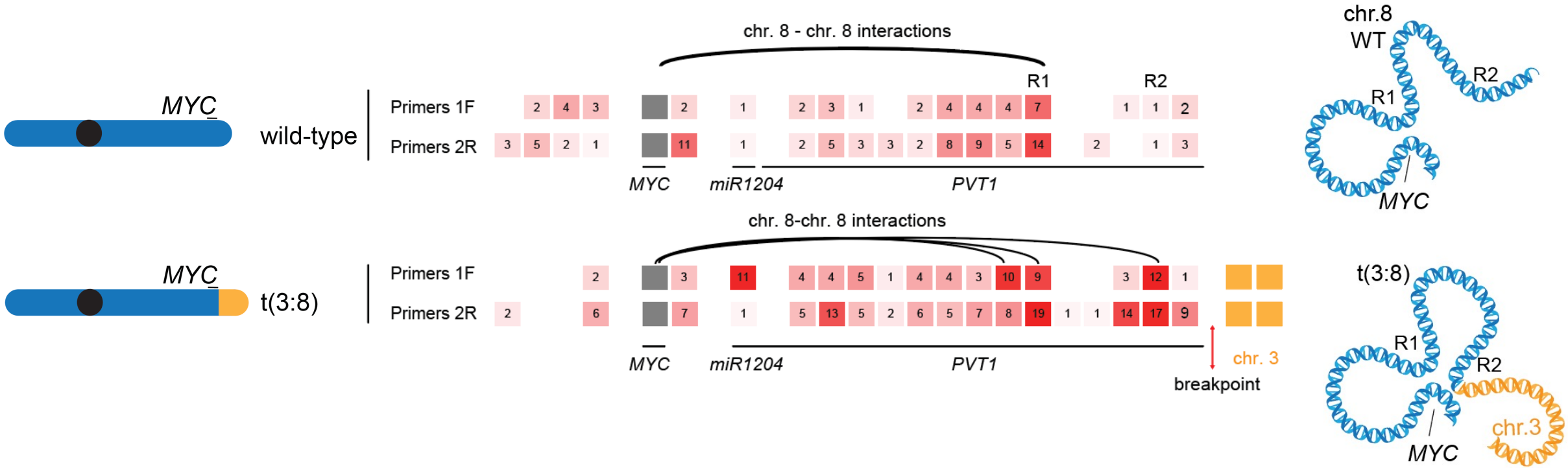


Chromatin conformation in oncogenic loci of two homologous chromosomes

Allele specific chromatin conformation



Allele specific chromatin conformation



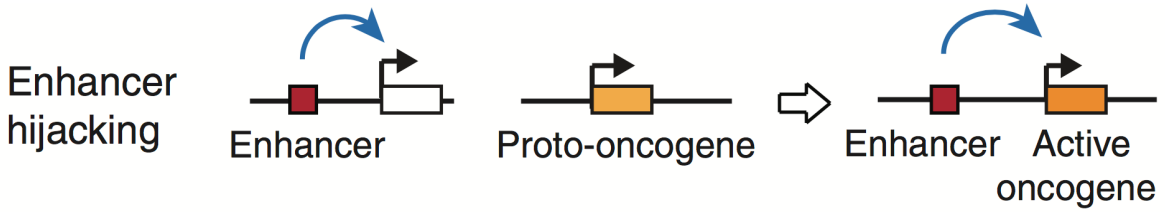
Distinct intra-chromosomal interactions
in the two homologous chromosomes

Sungalee et al. Nature Genetics, 2021

Chromosomal rearrangements in 3D

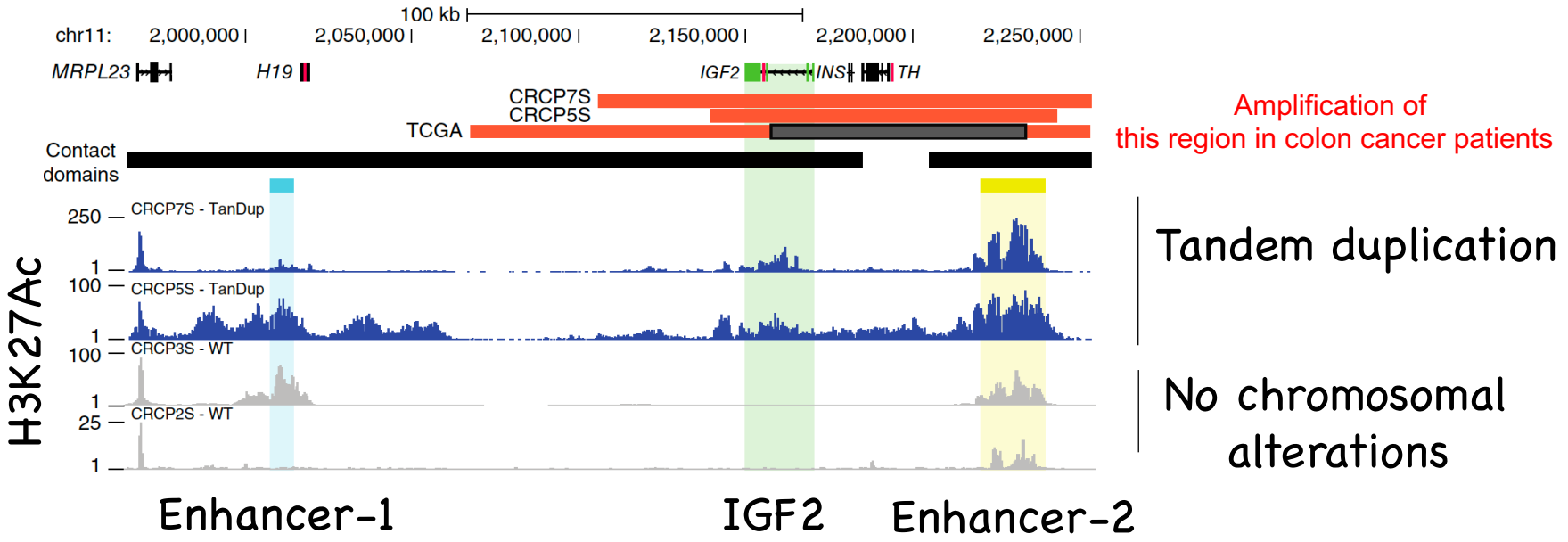
Enhancer Hijacking

Chromosomal Amplifications



Weischenfeldt et al. Nature Genetics, 2016

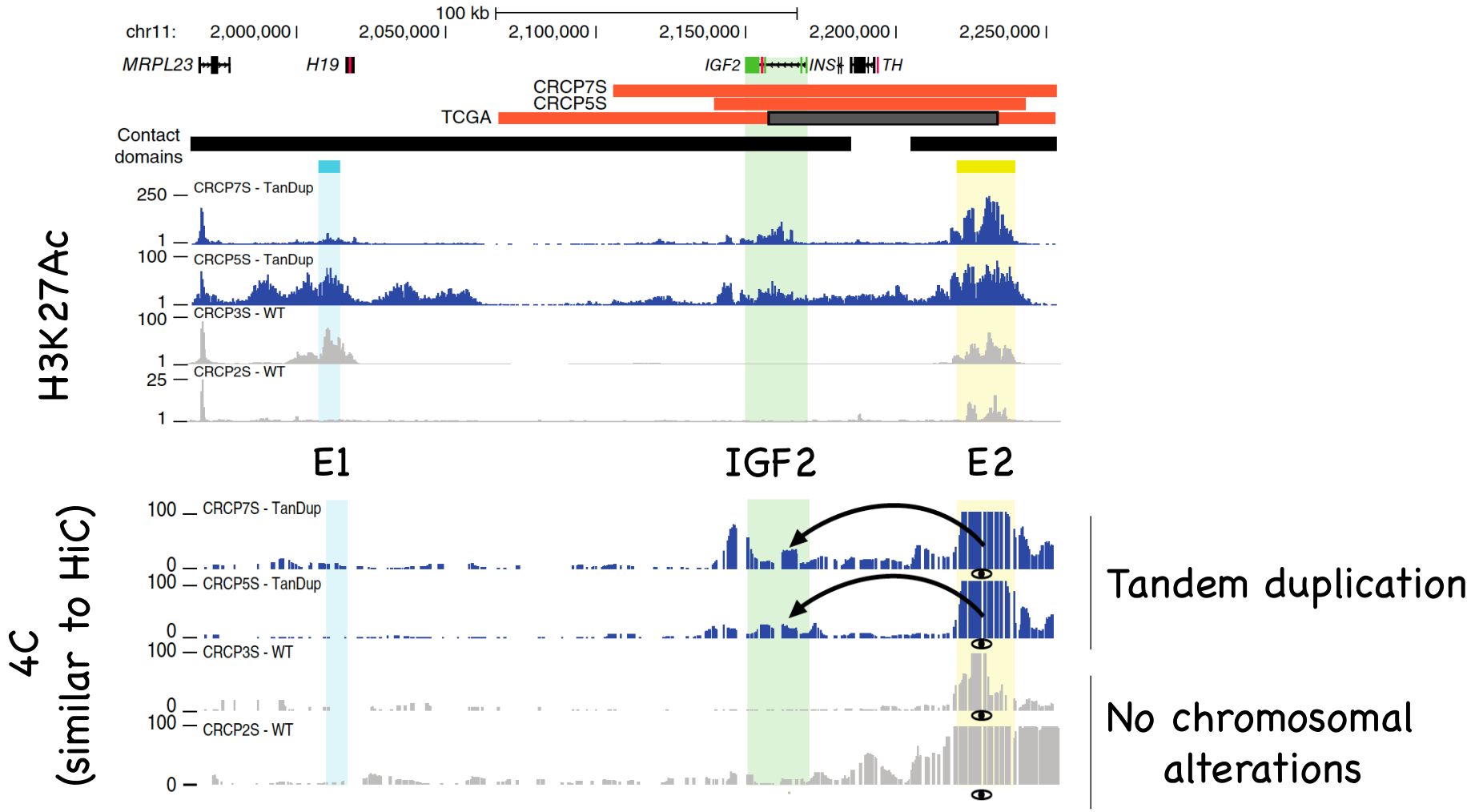
Enhancer Hijacking in colorectal cancer



How does the chromatin 3D structure change?

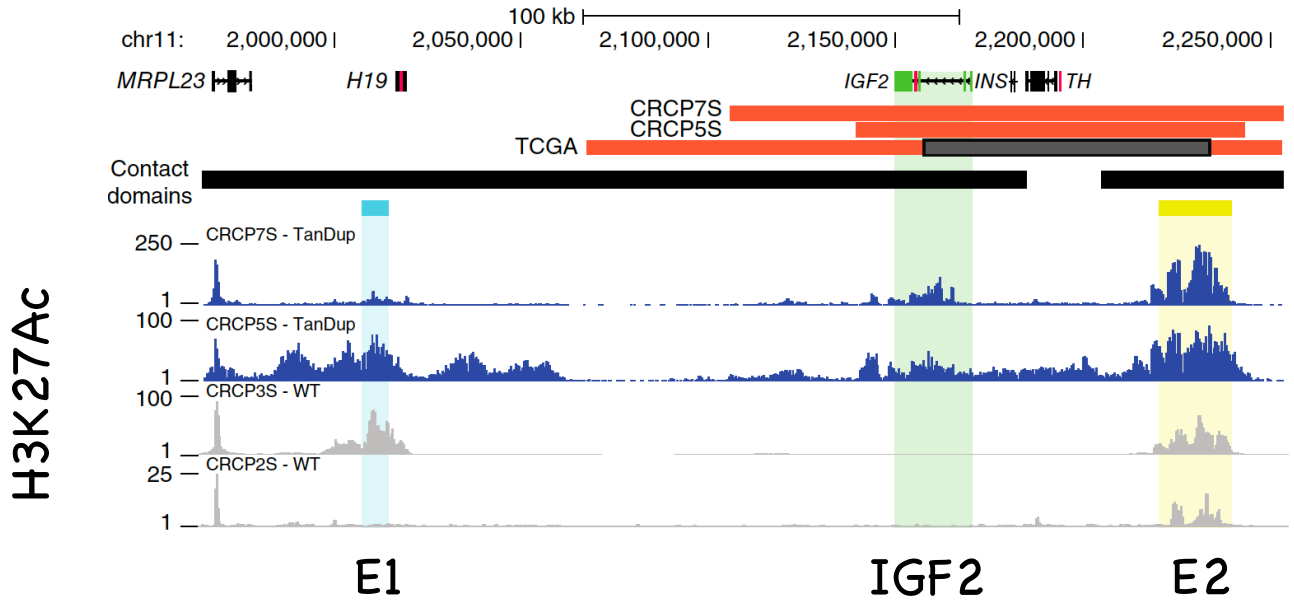
Weischenfeldt et al. Nature Genetics, 2016

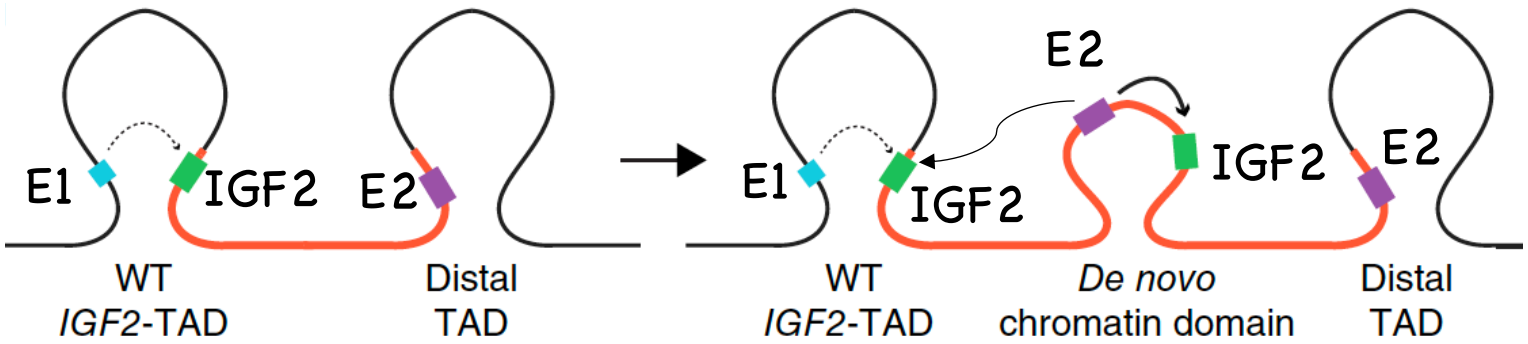
Enhancer Hijacking in colorectal cancer



Weischenfeldt et al. Nature Genetics, 2016

Enhancer Hijacking in colorectal cancer



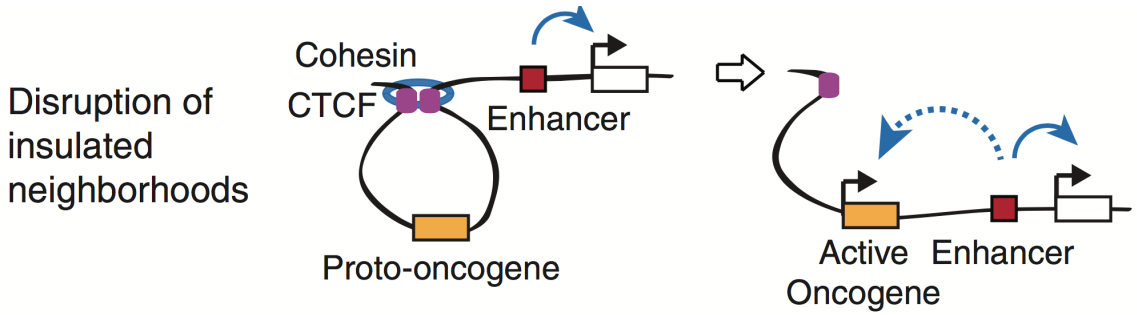


Weischenfeldt et al. Nature Genetics, 2016

Chromosomal rearrangements in 3D

Enhancer Hijacking

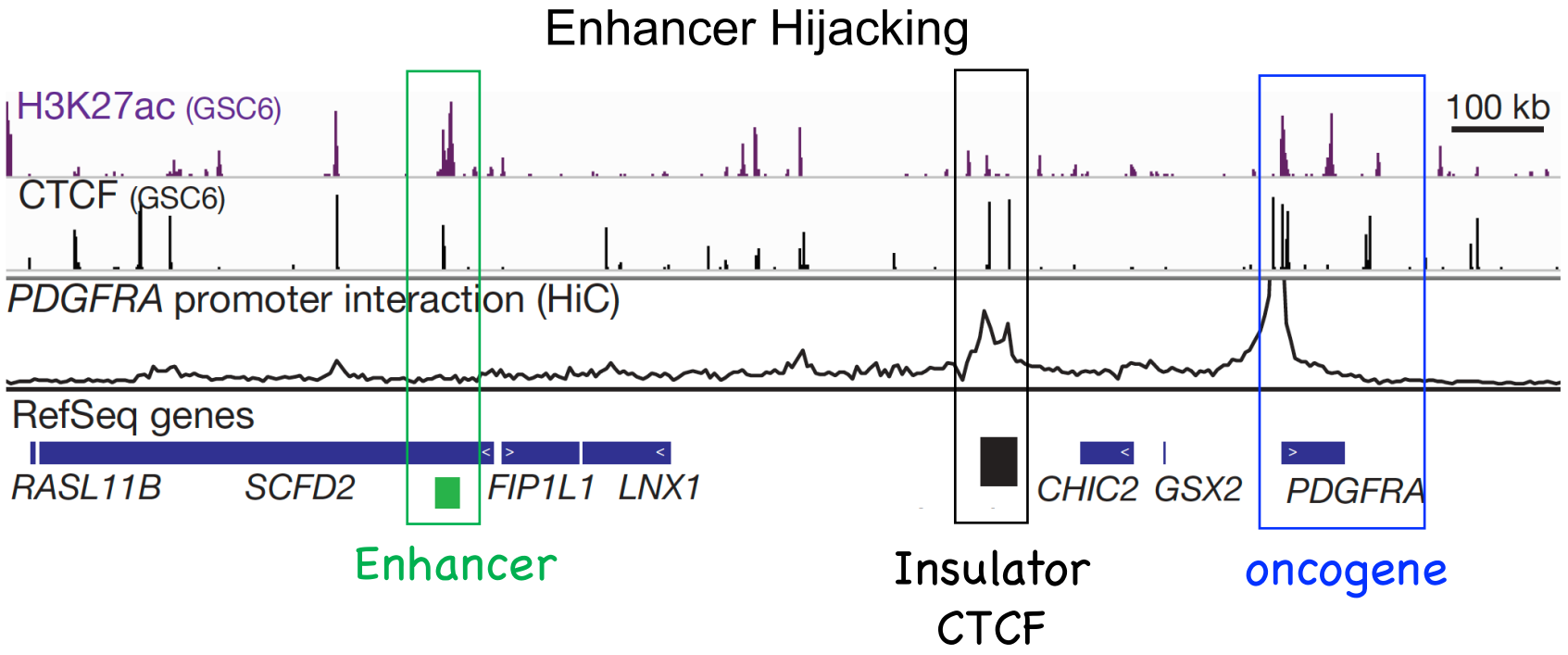
Chromosomal Deletions



Hnisz et al. Science 2016

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

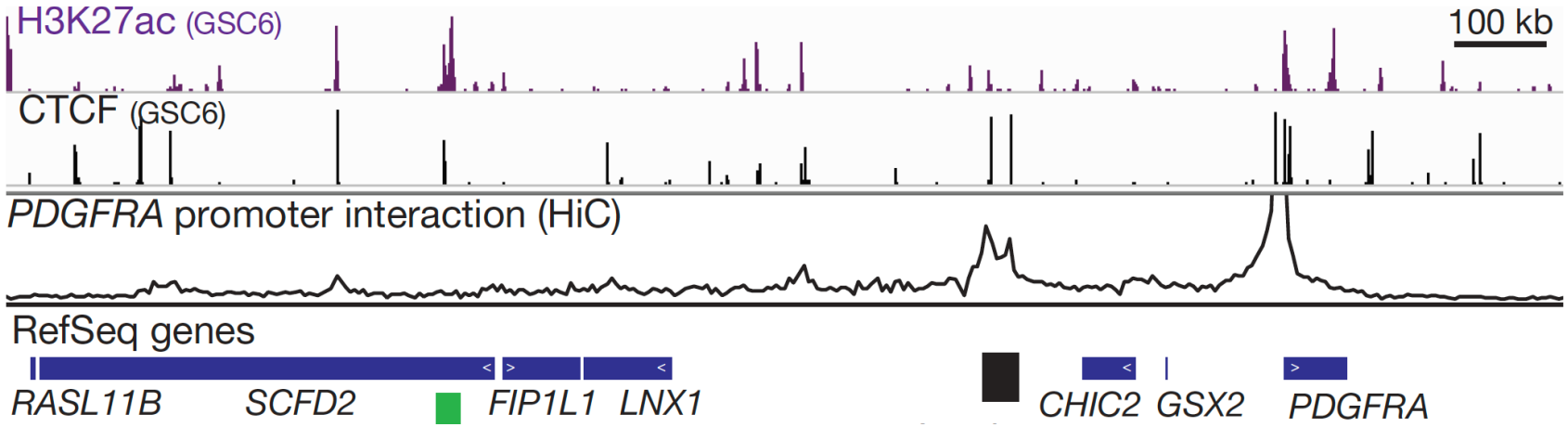
DNA hyper-methylation

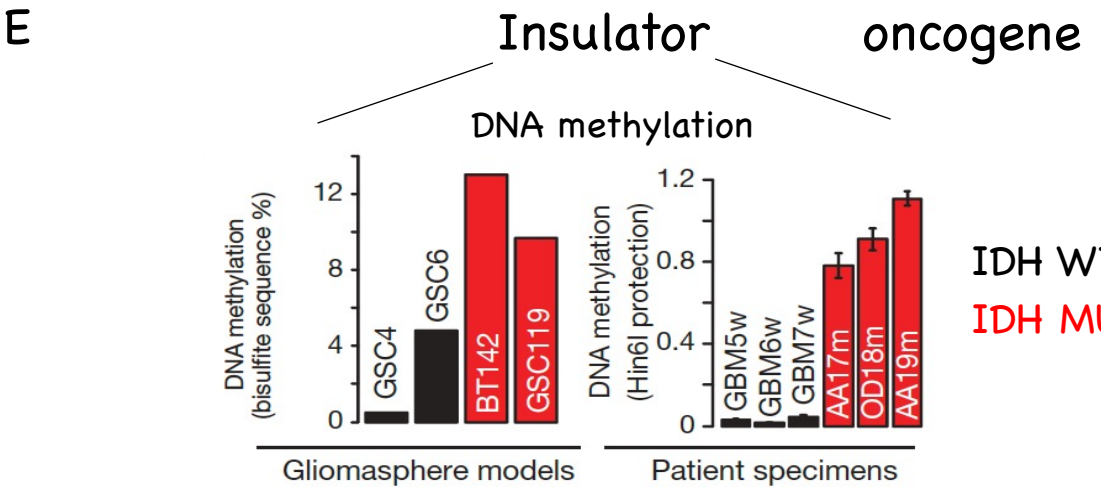


PDGFRA is over-expressed in patients with IDH mutations

Flavahan et al. Nature 2016

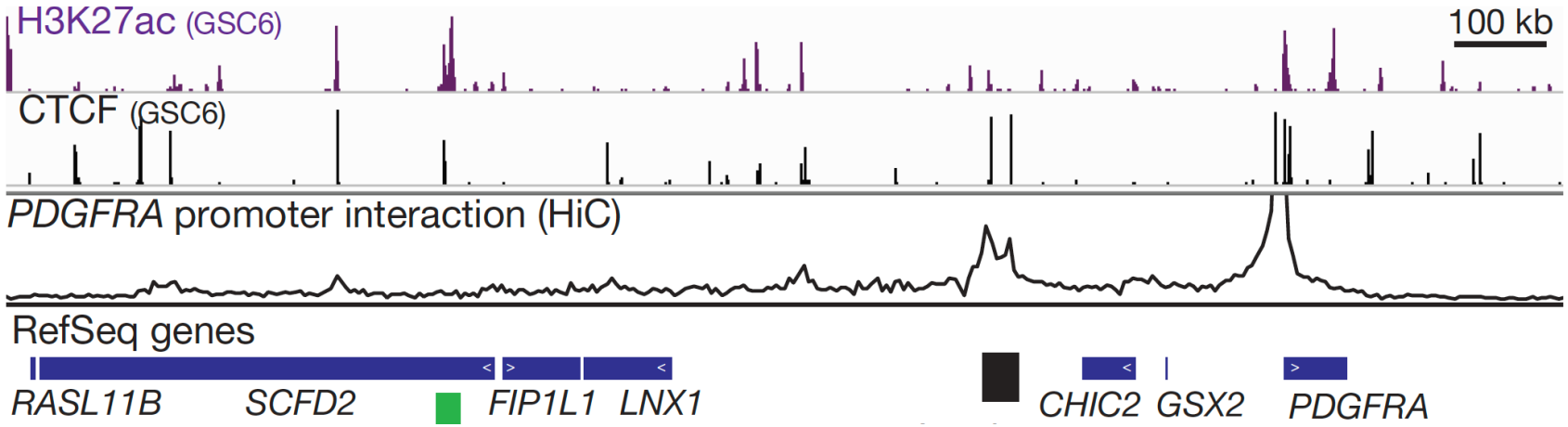
Oncogene activation by hypermethylation of a CTCF locus in IDH mutant glioma



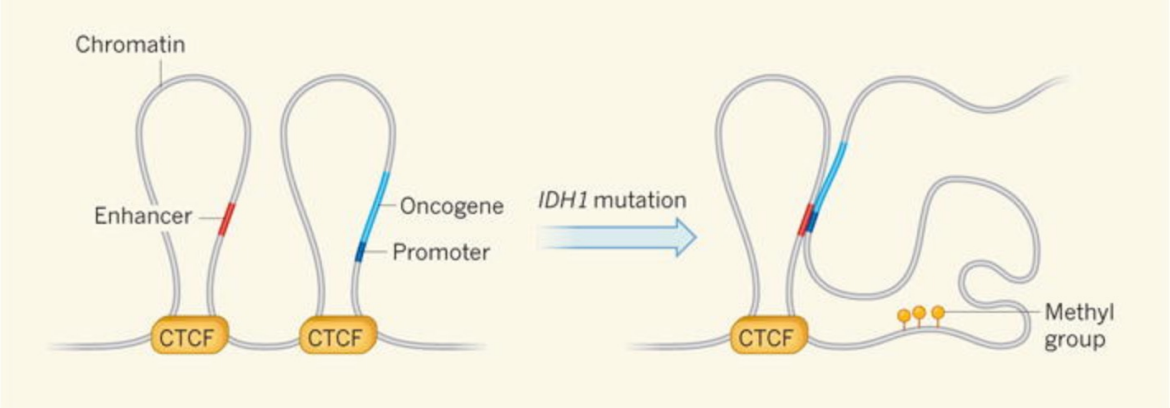


Flavahan et al. Nature 2016

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

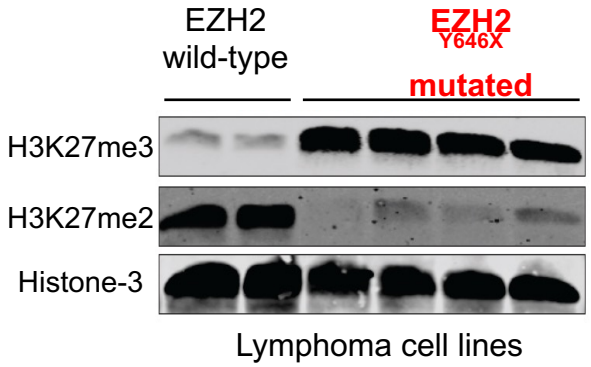


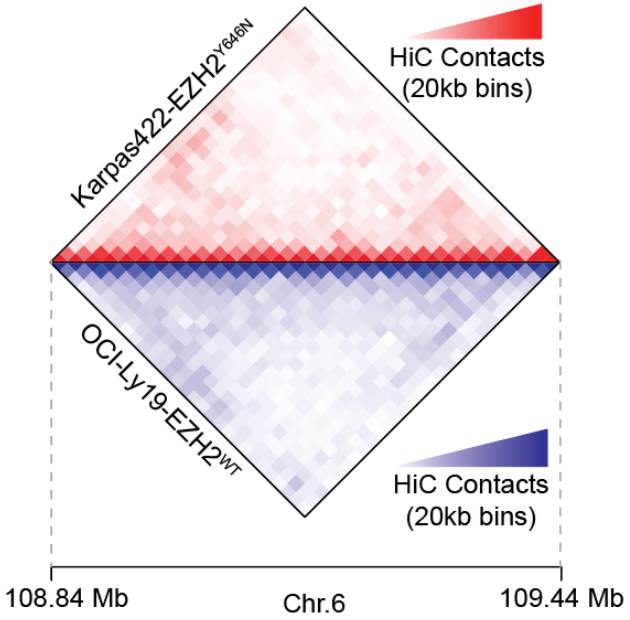
E Insulator oncogene

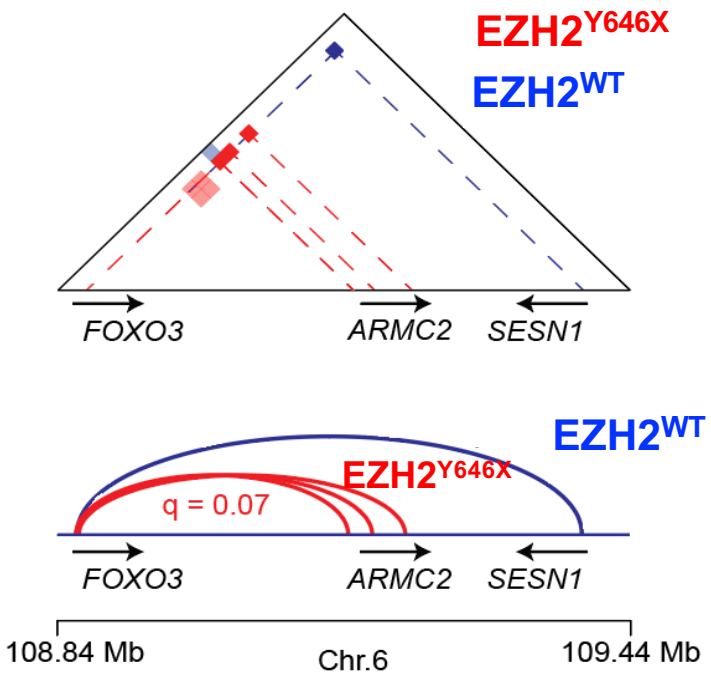


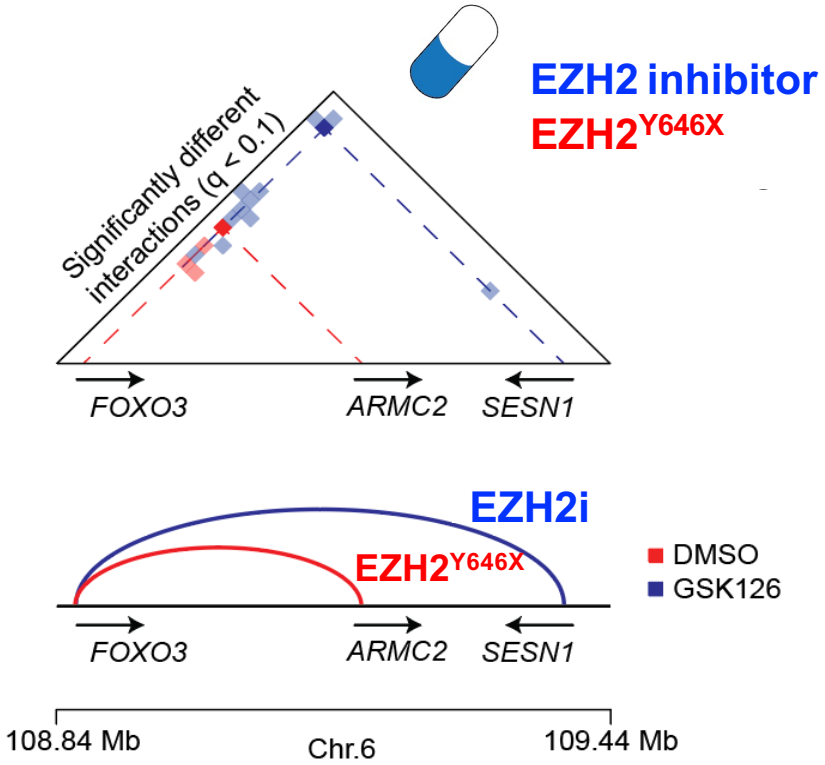
Flavahan et al. Nature 2016

Increases H3K27me3 in EZH2 mutated tumors changes chromatin 3D interactions and expression of tumor suppressor gene





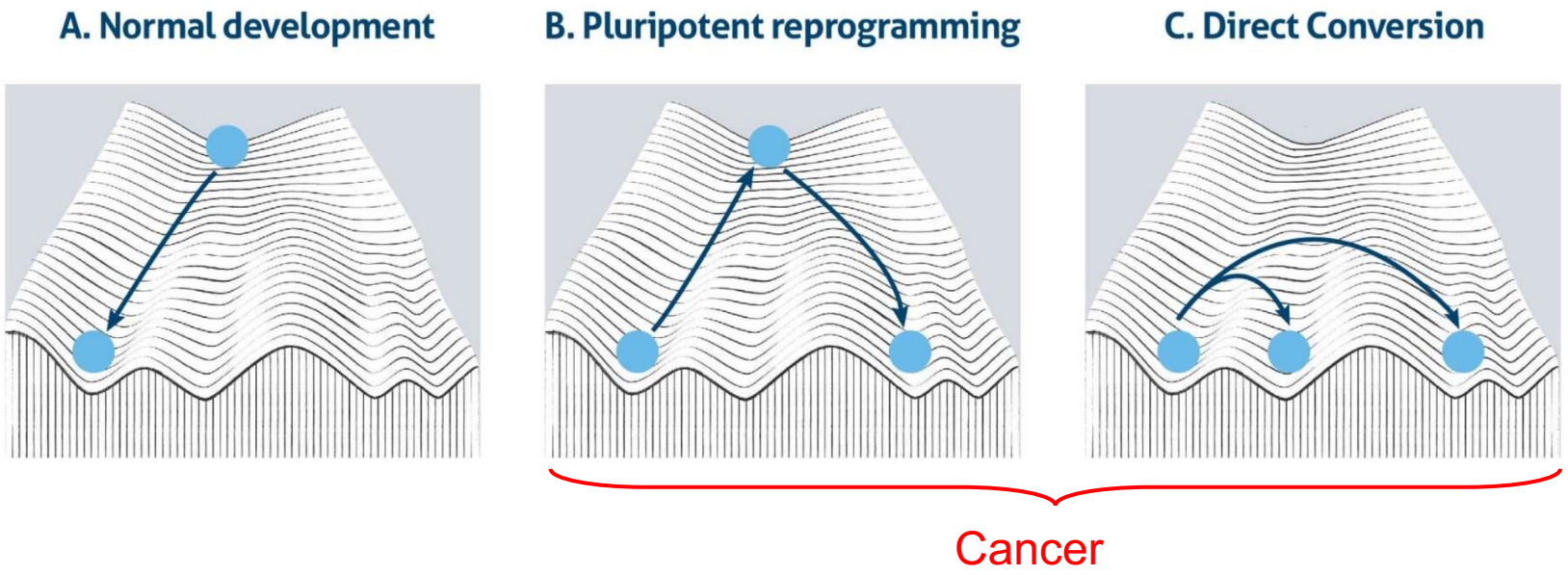


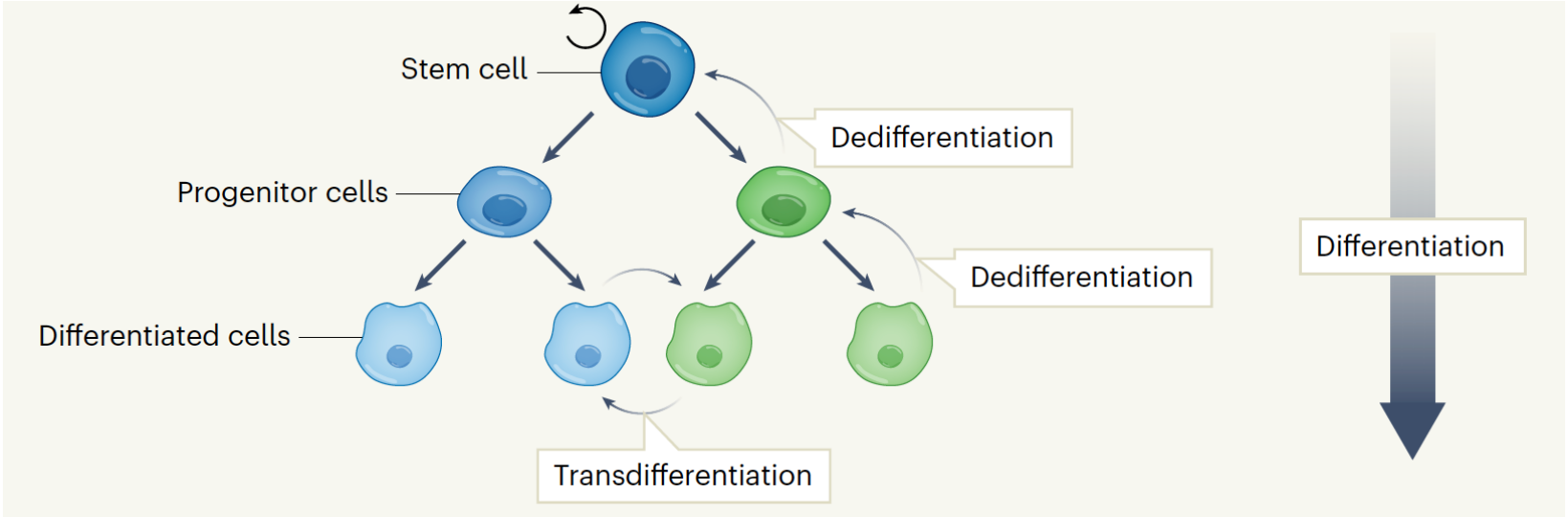


Epigenetic changes influence gene expression allowing cancer cells to adapt in different environments without changing the acquisition of new mutation or copy number change

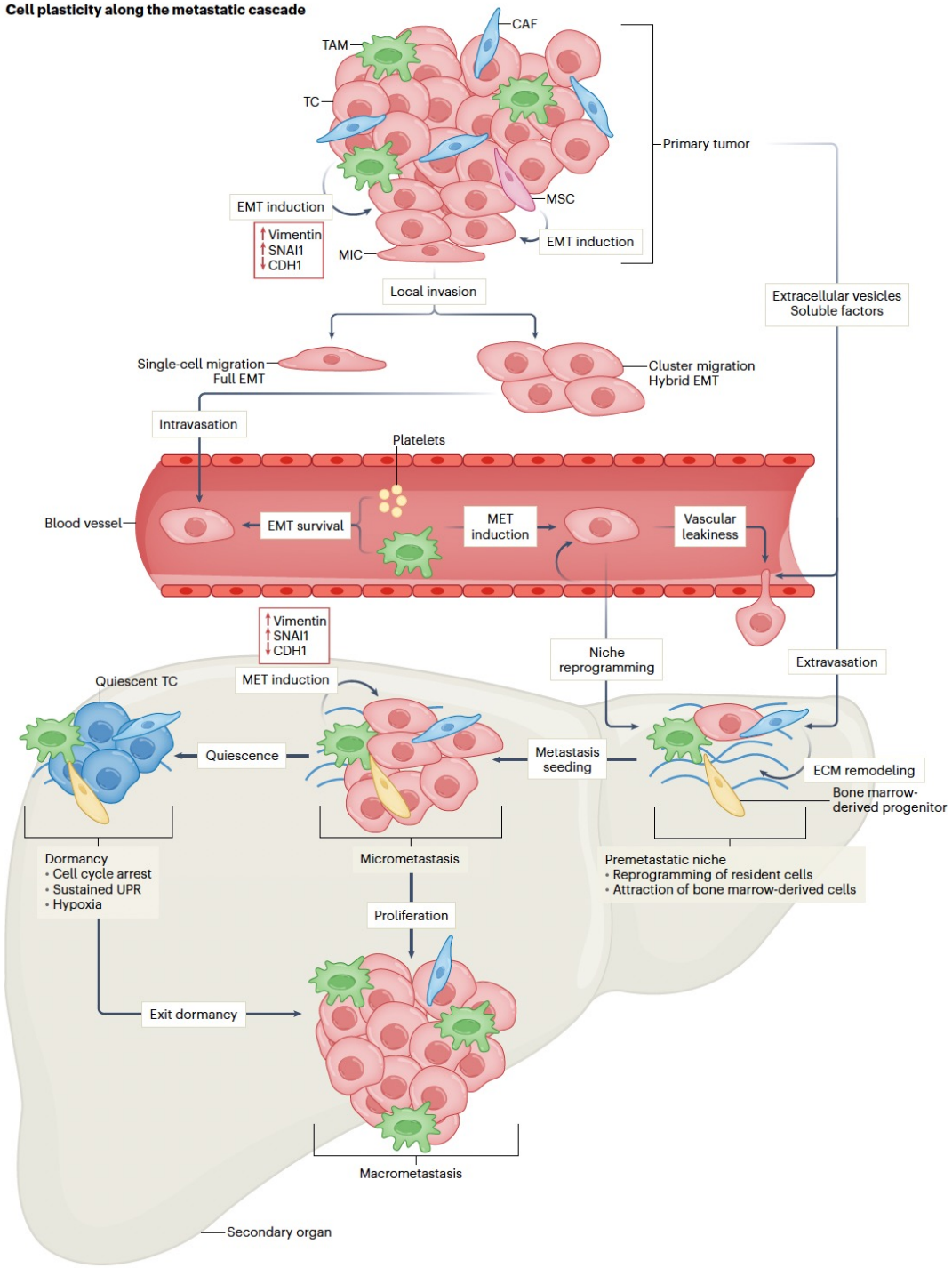
Cancer cell plasticity represents the ability of the cells to change their transcriptional program

Cancer cell plasticity and epigenetic regulation



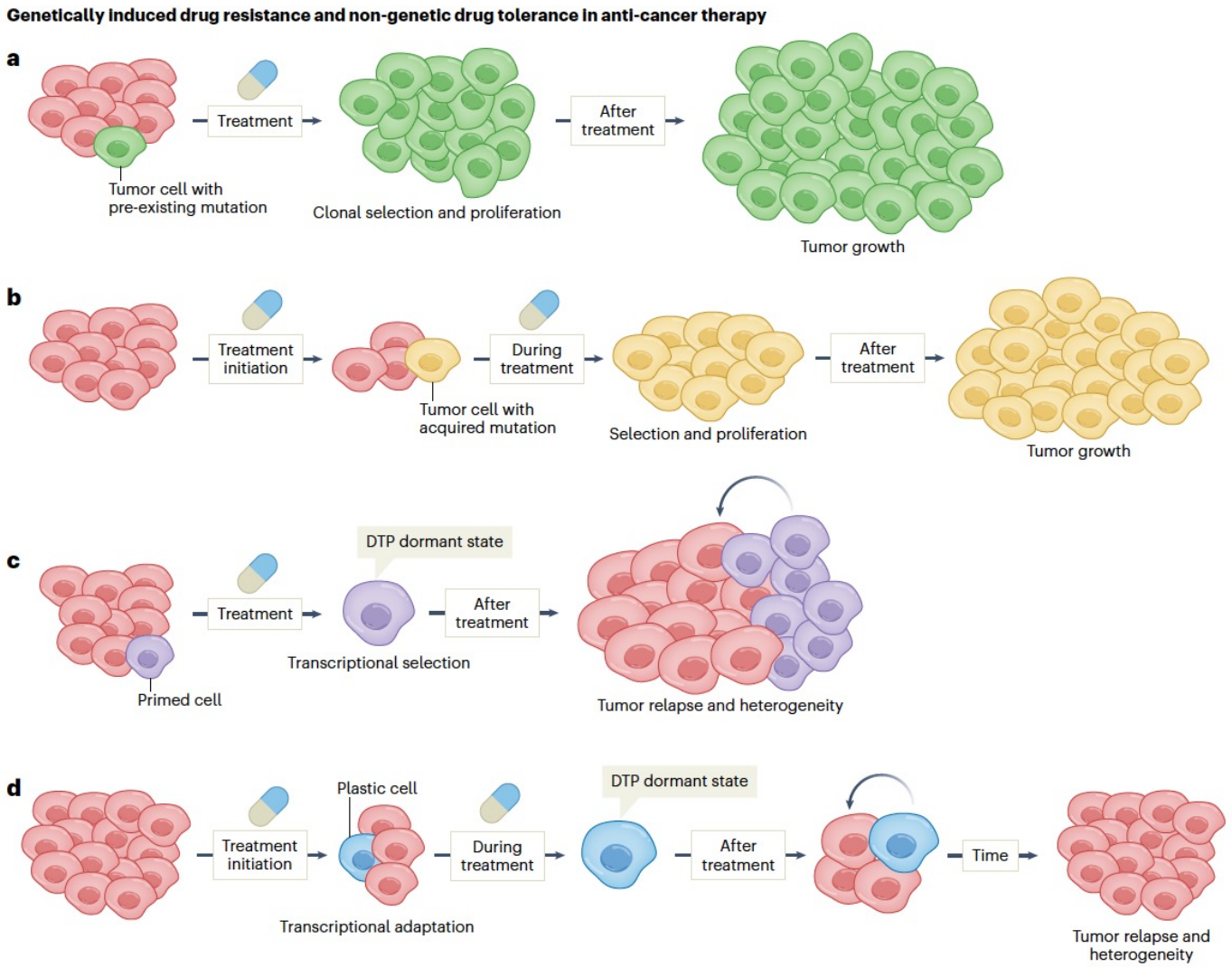


Cell plasticity from primary tumors to metastasis formations



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

Genetic and nongenetic drug resistance



Exercise

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