

# EPIGENETICS

OMICS TECHNIQUES

GABRIEL SANTPERE (GRIB)

# DEFINITIONS



C.H. Waddington

Authors	Epigenetics is the study of:
Waddington	the processes by which the genotype * brings the phenotype into being
Nanney	the systems that regulate the expression of the ' <u>library of specificities</u> ' (that is, the genetic material, which is meant to be the DNA or RNA sequence)
Riggs, Holliday, Martienssen, Russo	mitotically and/or meiotically heritable changes in gene function that <u>cannot be explained by changes in DNA sequence</u>
Bird	structural adaptations of chromosomal regions so as to register, signal <u>or</u> perpetuate altered activity states
Greally, Lappalainen	properties of a cell, mediated by genomic regulators, that confer on the cell the ability to remember a past event.
Nicoglu	various intracellular factors that have an effect on the stability of developmental processes through their action on <u>genome potentialities</u>

Genome potentialities

\* Genetic assimilation

1942

1958

1994

2007

2017

2017

# QUESTIONS

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Epigenetics produces different phenotypes without altering the DNA sequence

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How from one original cell we get a plethora of cell types using the SAME genome?

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How identity is maintained once established?

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Is it reversible? Can a cell be re-programmed?

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Can it be inherited from a cell to its daughter cells?

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If so, can that happen somatically only or also be transmitted across generations?

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Which is the causal direction between epigenetic modifications and transcription activity?

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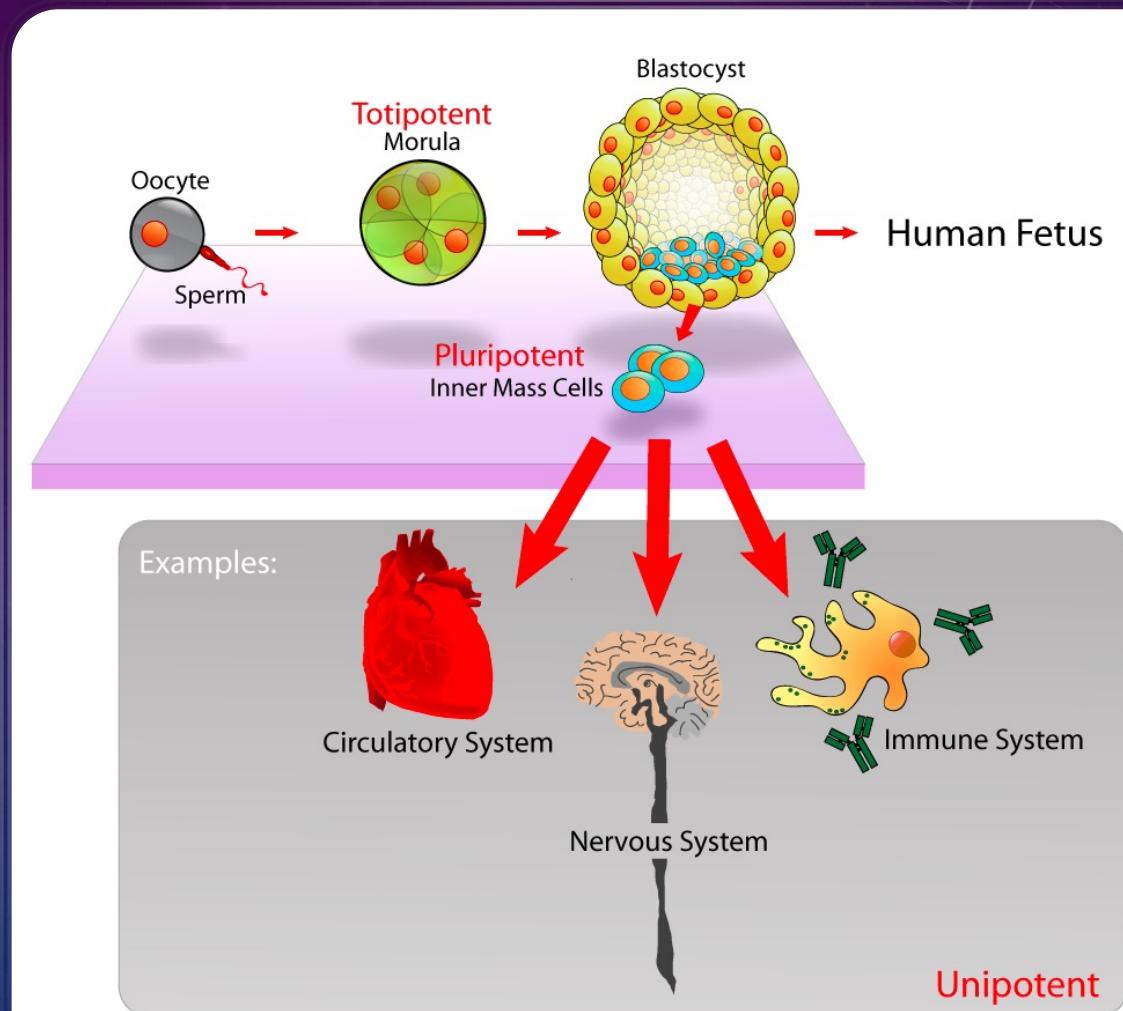
Which are the molecular mechanisms?

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How do transcription factors interact with all that?

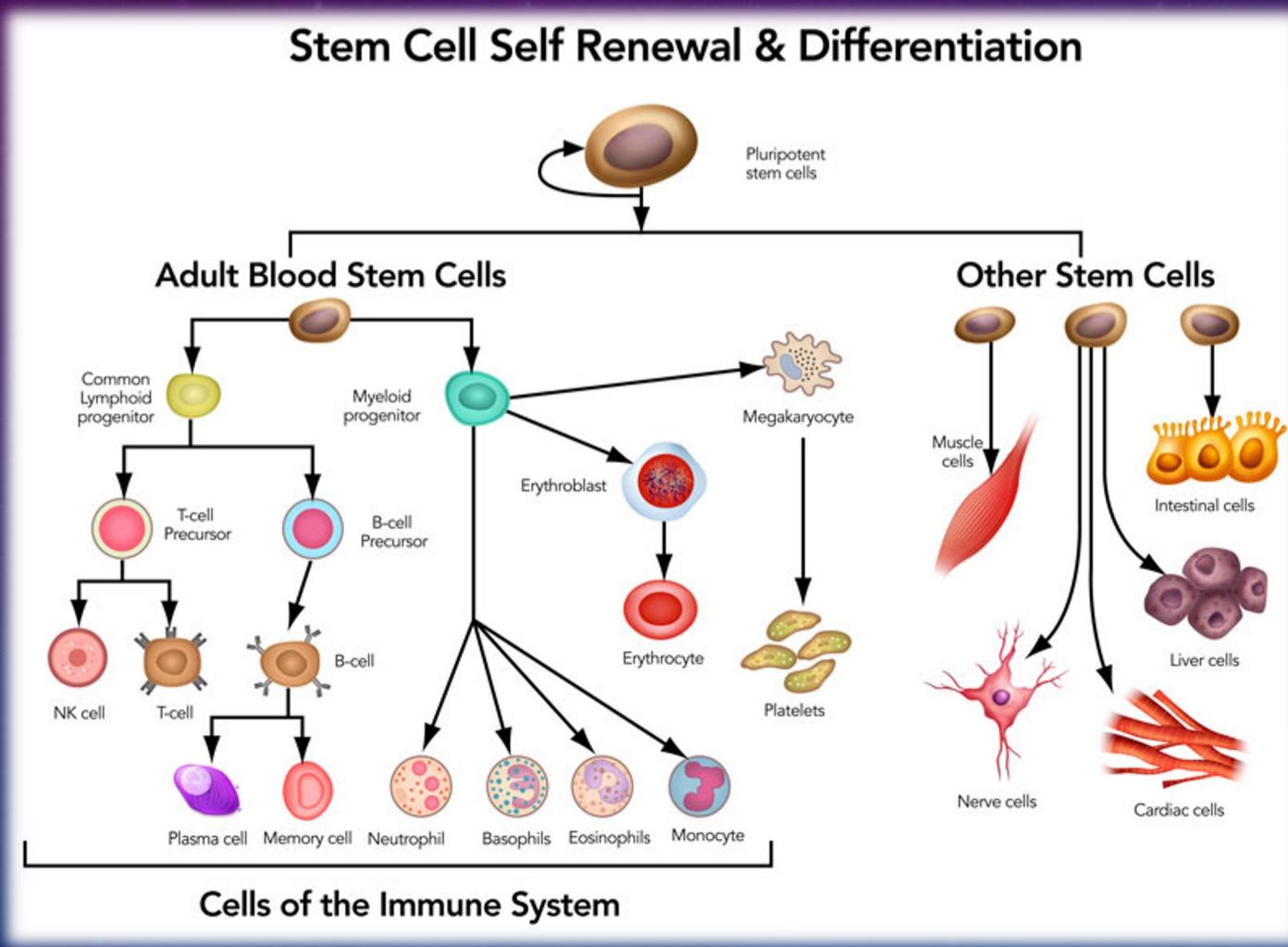
How can we study epigenomes?

- Totipotent cells: Can give rise to all cell types, including extra-embryonal (placental).
- Pluripotent: Can give rise to all cell-types in the body.
- Multipotent: Can give rise to more than one cell-type.
- Unipotent: Can give rise to just one cell-type.



# A GENERAL WORKING DEFINITION

- Molecules and mechanisms that can perpetuate a cellular state.



Establishment and maintenance of cellular identity

# IS GENE EXPRESSION A HERITABLE TRAIT?

We just saw it is across cell division. What about across generations?

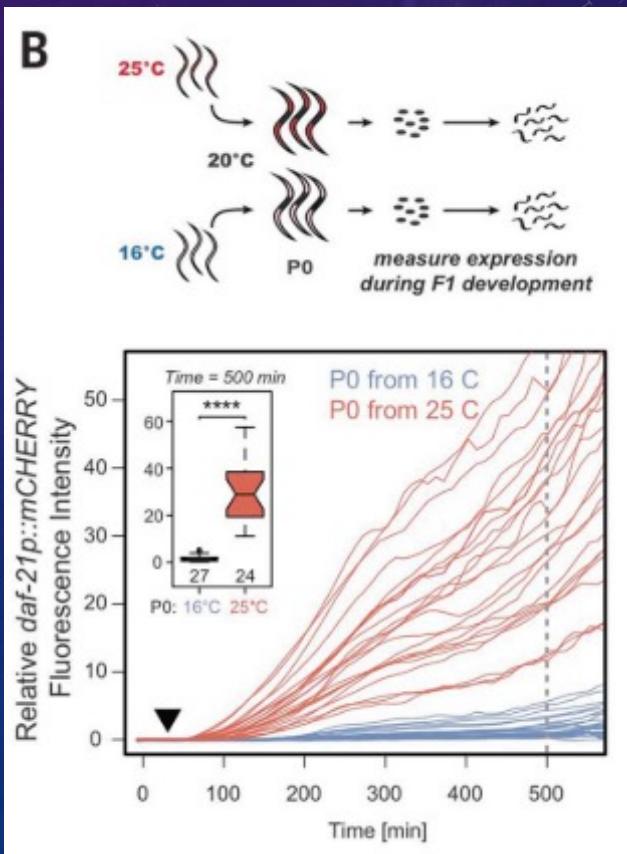
Well... yes, as long as sometimes we observe eQTLs and allele-specific expression and those are heritable variants.  
But is it epigenetically modified gene expression heritable?

Gene expression can be regulated by the environment

## EPIGENETIC INHERITANCE

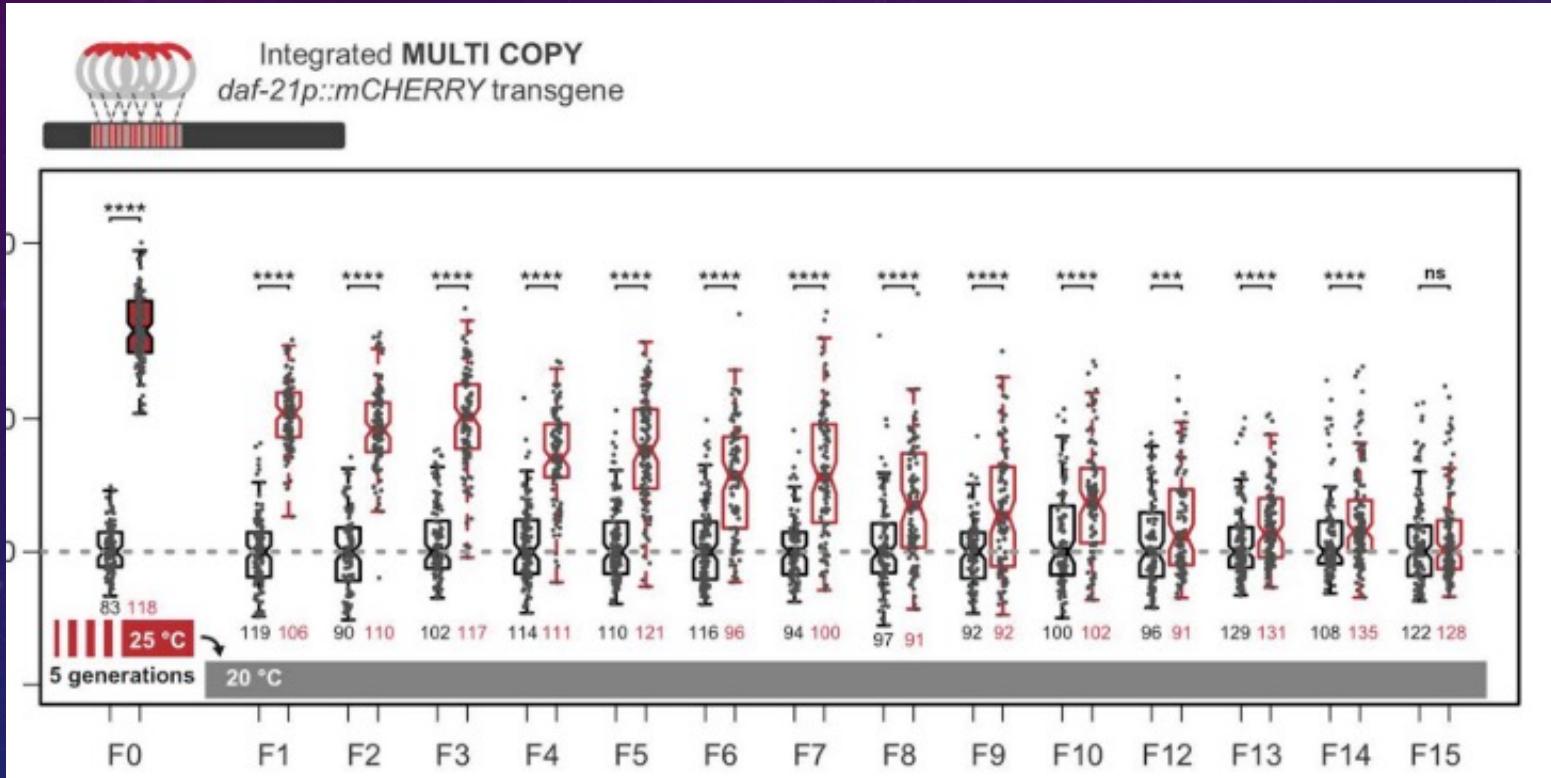
### Transgenerational transmission of environmental information in *C. elegans*

Adam Klosin,<sup>1,2</sup> Eduard Casas,<sup>3</sup> Cristina Hidalgo-Carcedo,<sup>1,2</sup>  
Tanya Vavouri,<sup>3,4\*</sup> Ben Lehner<sup>1,2,5\*</sup>



F1 worms whose parents were exposed to heat have the same genotype, and same environment as F1 worms whose parents were not exposed to heat.

14 generations of heritability for an epigenetic trait



Phenotype = f( genotype , environment , epigenetic factors , random factors )

\* Genetic accommodation

# DNA IS THE CODE BUT YOU CAN READ IT IN MANY WAYS

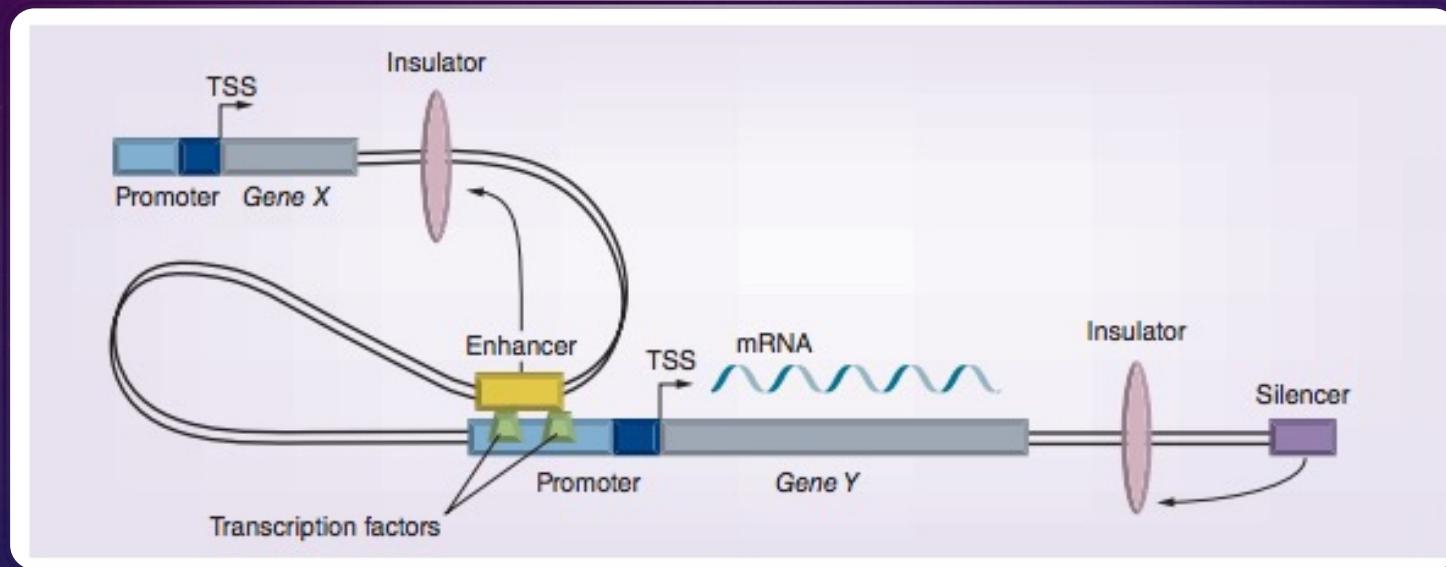
- Many ways means regulation.
- Regulation is dynamic.

IACHATEGTLOVEACCCTGGSUSHIATTGTCA

IACHATEGT**LOVE**ACCCTGGSUSHIATTGTCA

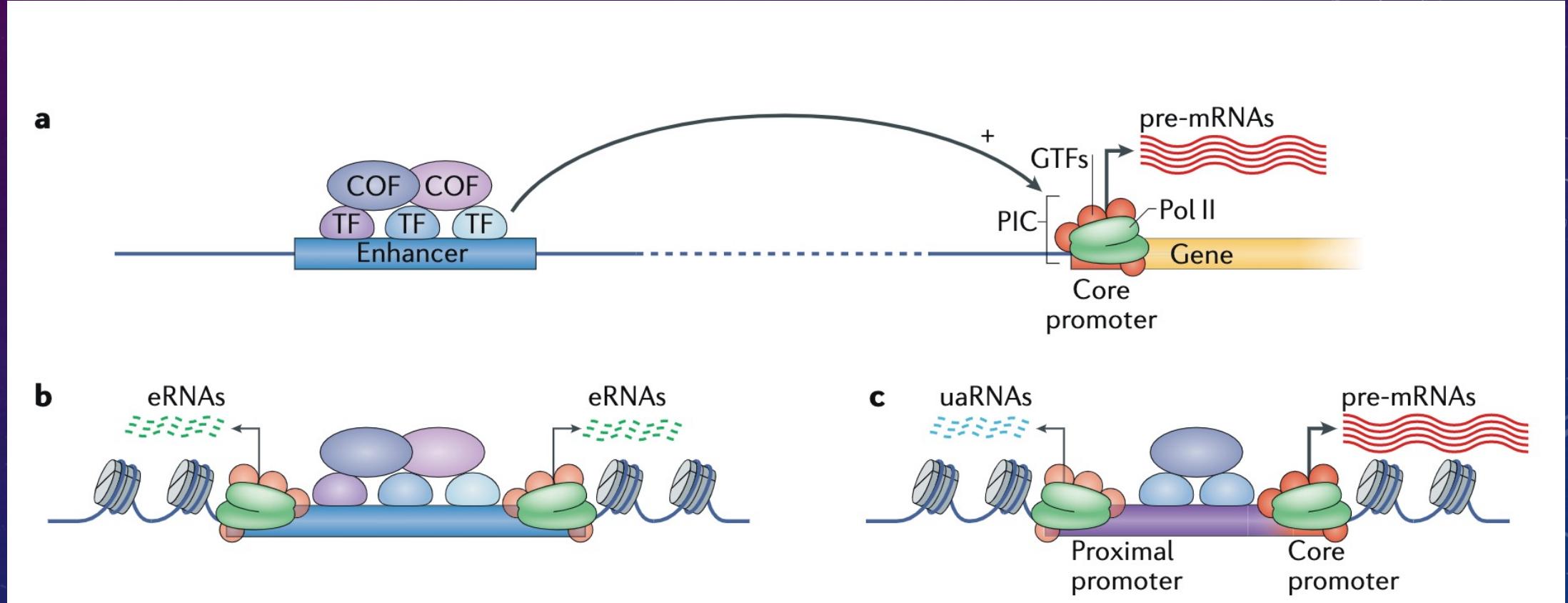
I**ACHATE**GTLOVEACCCTGGSUSHIATTGTCA

# REGULATION OF GENE EXPRESSION



- Luizon and Ahituv (2015)  
10.2217/pgs.15.121

# REGULATION OF GENE EXPRESSION

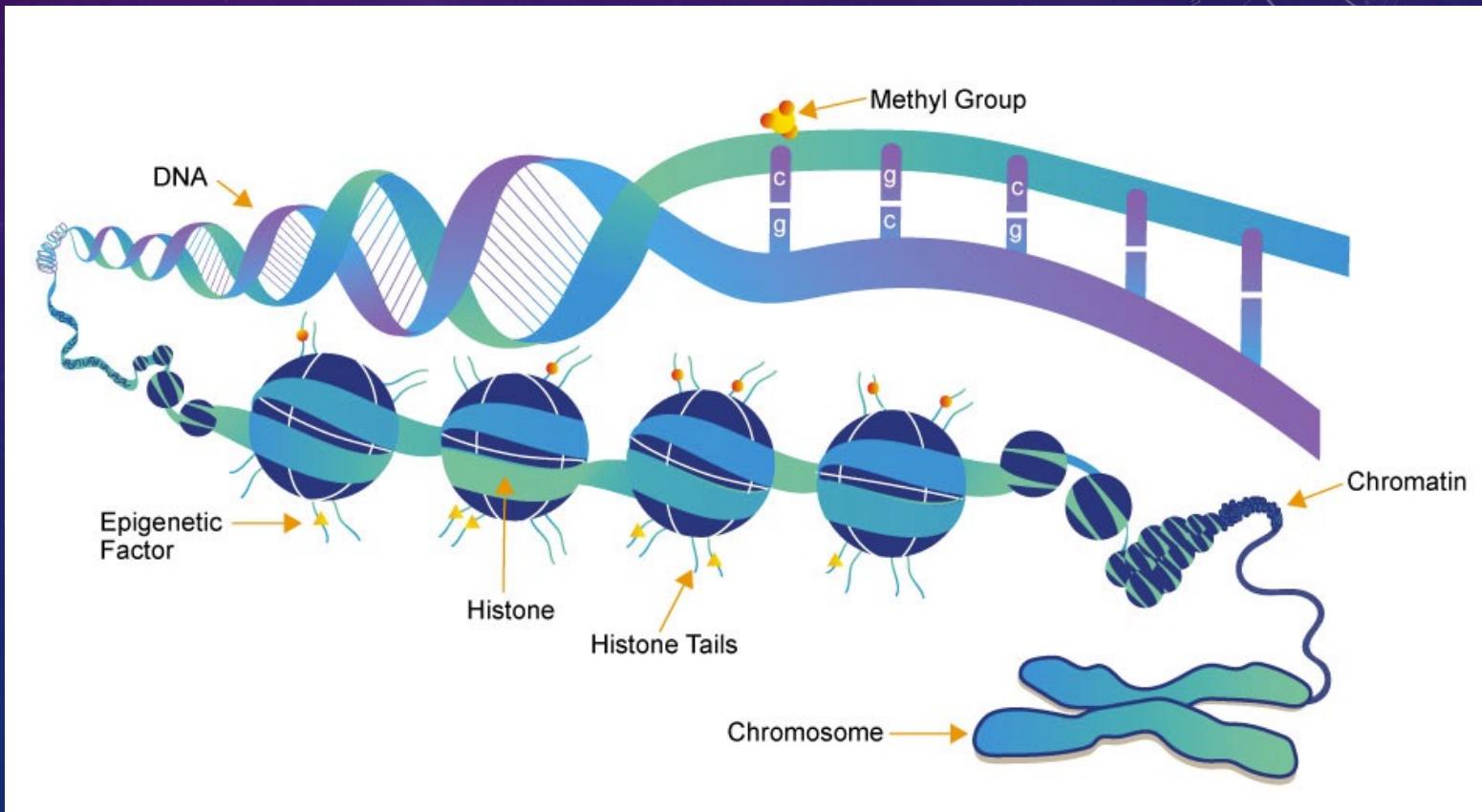


Eukaryotic core promoters and the functional basis of transcription initiation  
Haberle and Stark

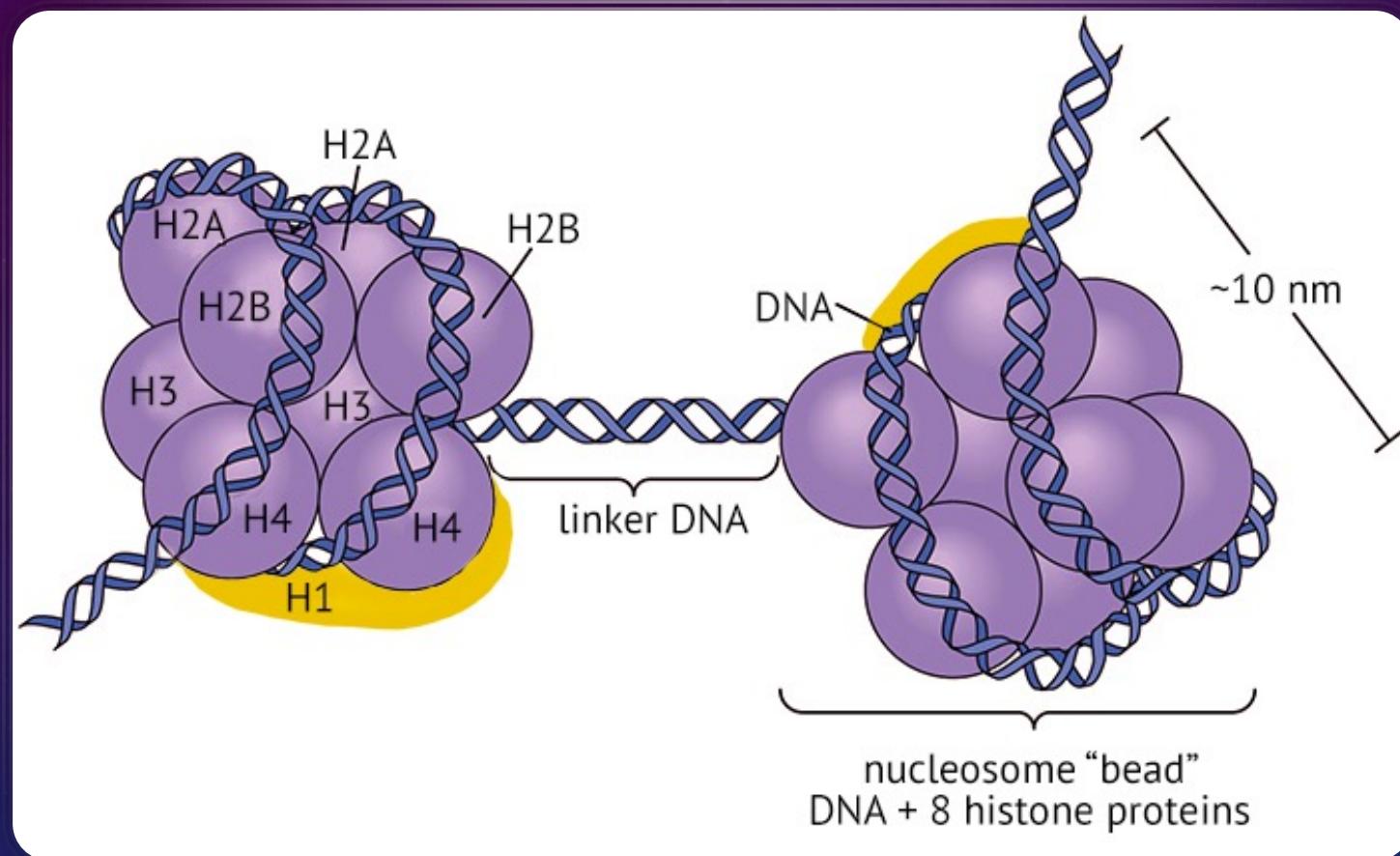
# EPIGENETIC INFORMATION

Chromatin is the compacted product of DNA incorporated around proteins, reducing the length of DNA per chromosome by **a factor of up to  $2 \times 10^{-5}$** . 2m in total.

- Histone modifications
- DNA methylation
- Nucleosome positioning
- Noncoding RNAs
- ...

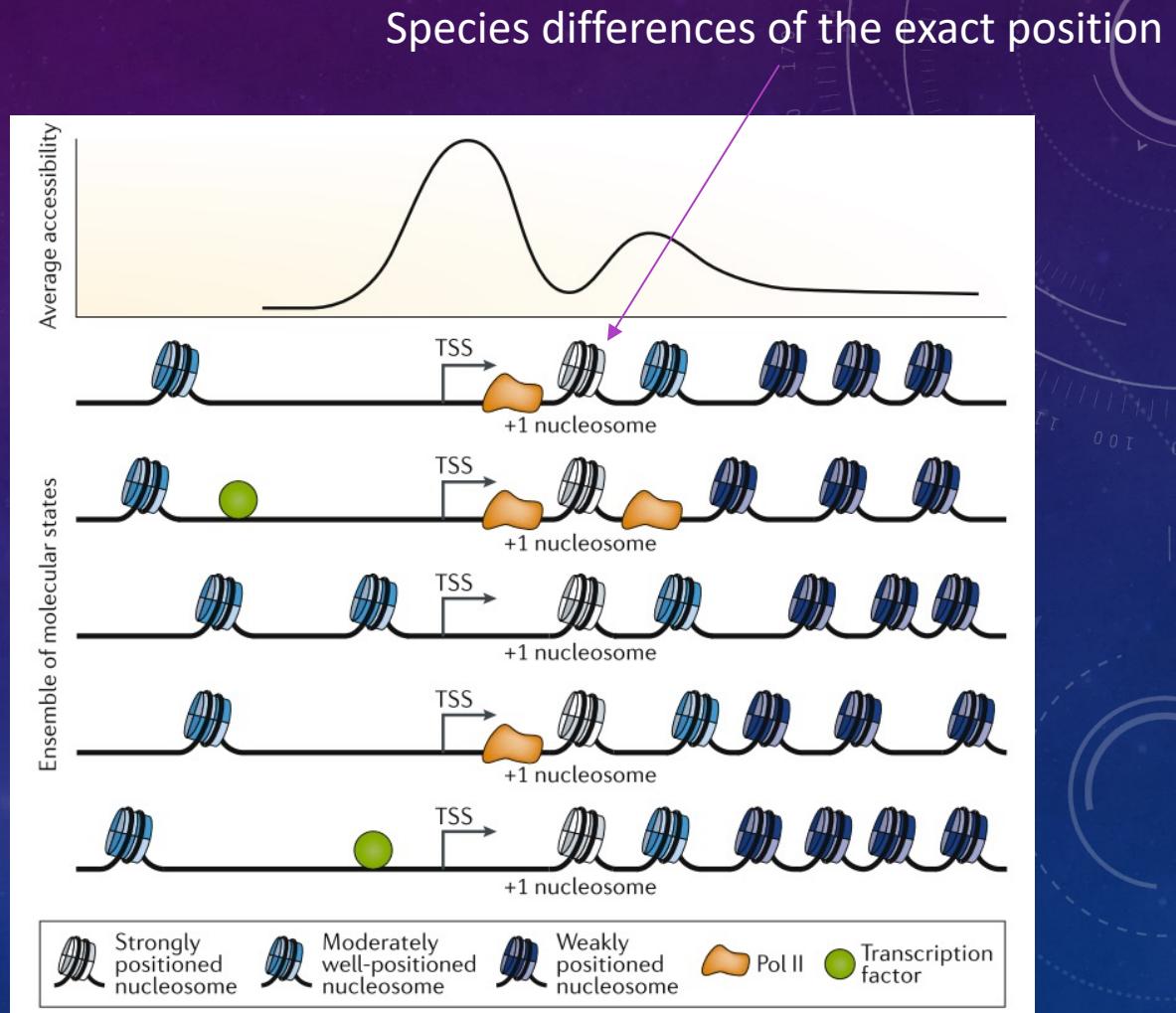
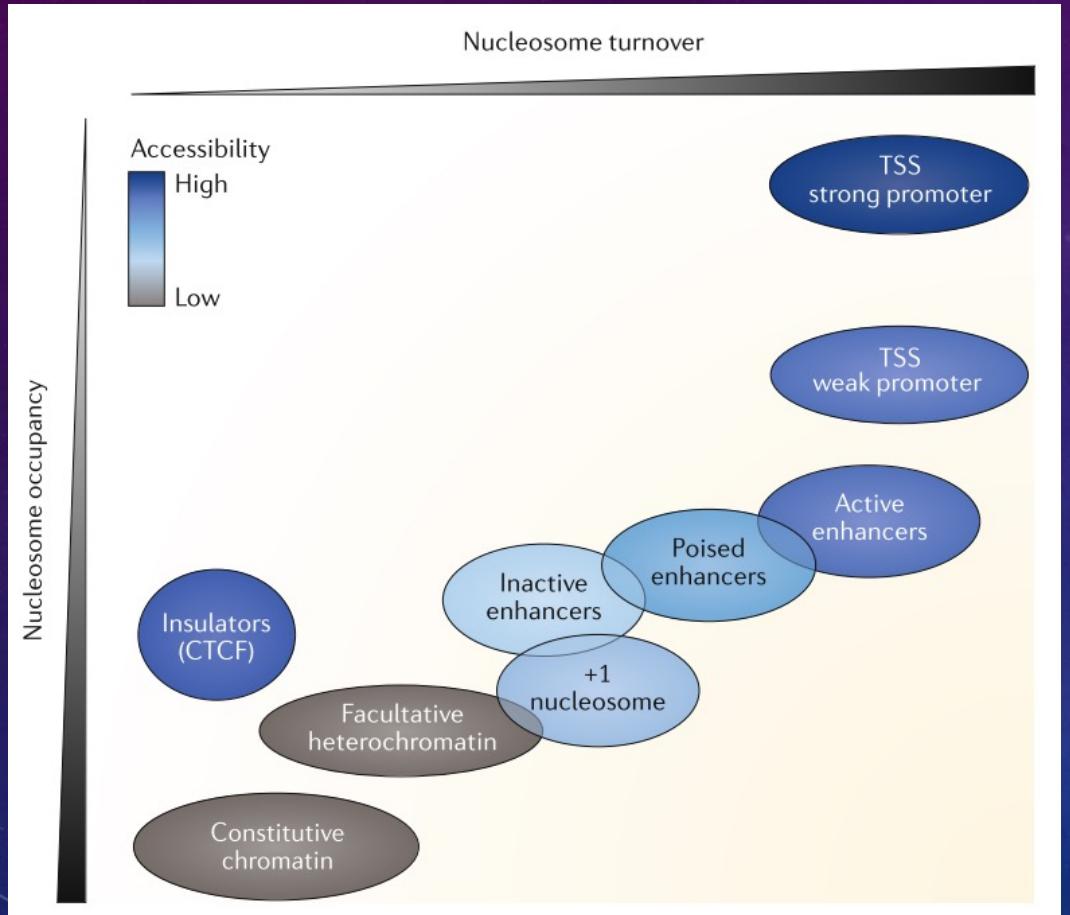


# HISTONE MODIFICATIONS



- Nucleosomes are the fundamental unit of chromatin.
- Composed of histone octamers of core components (H2A, H2B, H3 and H4).
- They are encircled by ~147bp of DNA.

# NUCLEOSOME POSITIONING

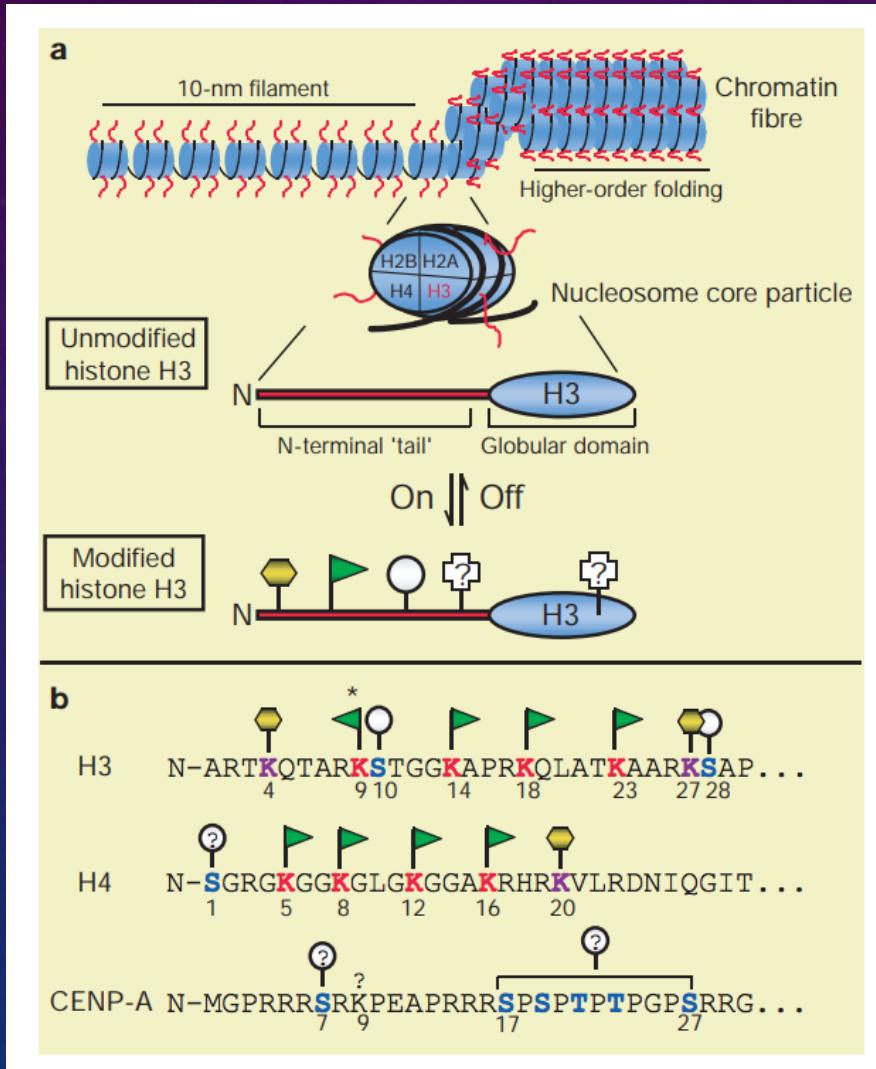


# NUCLEOSOME POSITIONING

- ❖ Chromatin remodellers, each has an effect (lateral displacement or removal)
- ❖ Sequence composition (AT rich)
- ❖ Transcription itself

- They are barriers to transcription as blocks access to activators (TFs) or difficult elongation of transcripts by the polymerase.
- Positioning is particularly important at TSS.
- Loss of nucleosome upstream genes → activation of transcription
- Less nucleosome at 5' and 3' ends to facilitate assembly/disassembly tx machinery.

# HISTONE MODIFICATIONS

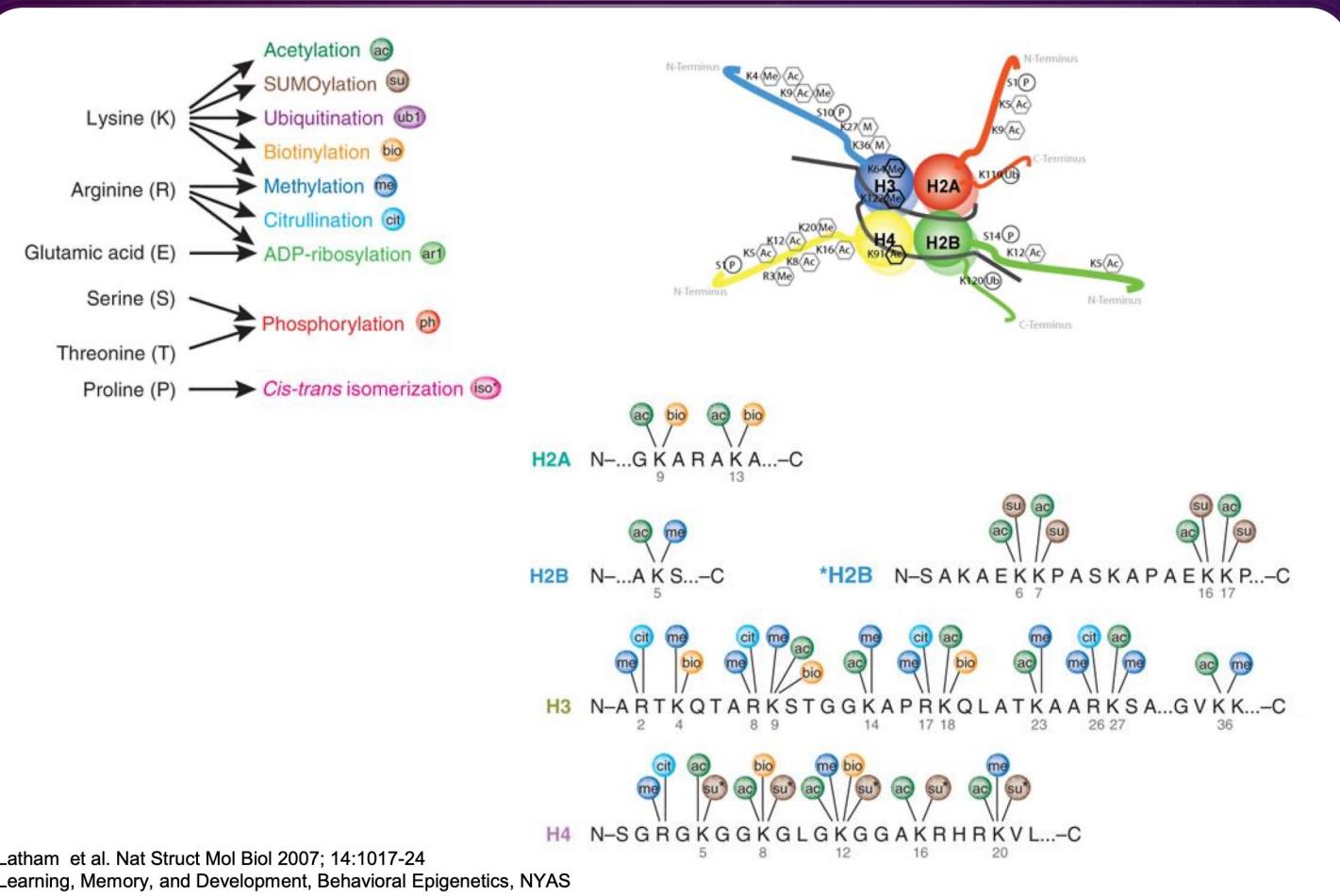


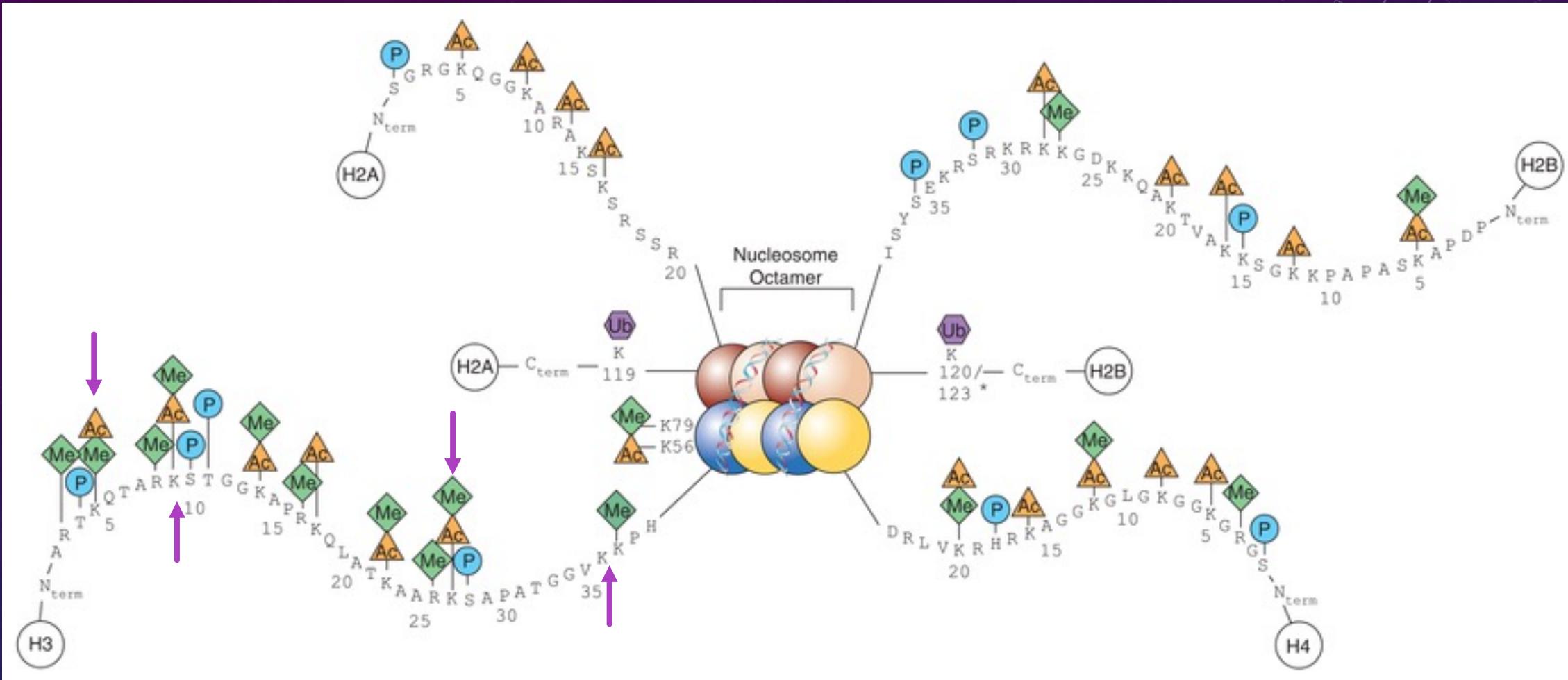
Histone tails unstructured and AA very conserved

Strahl et al, Nature 2000

# HISTONE MODIFICATIONS

- 12 types of chemical modifications on 130 sites.
    - Acetylation, Methylation, Phosphorylation, Ubiquitylation, Sumoylation, GlcNAcylation, Citrullination, Krotonilation, ...
  - High combinatorial complexity.
  - Only a few of them well characterized.





Kepler et al 2008 DOI: 10.1517/14728222.12.10.1301

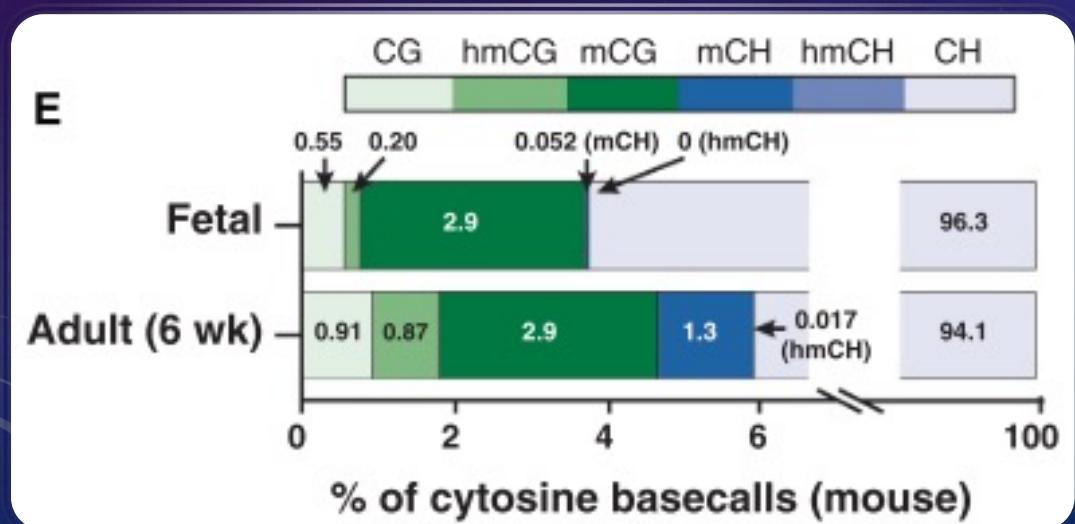
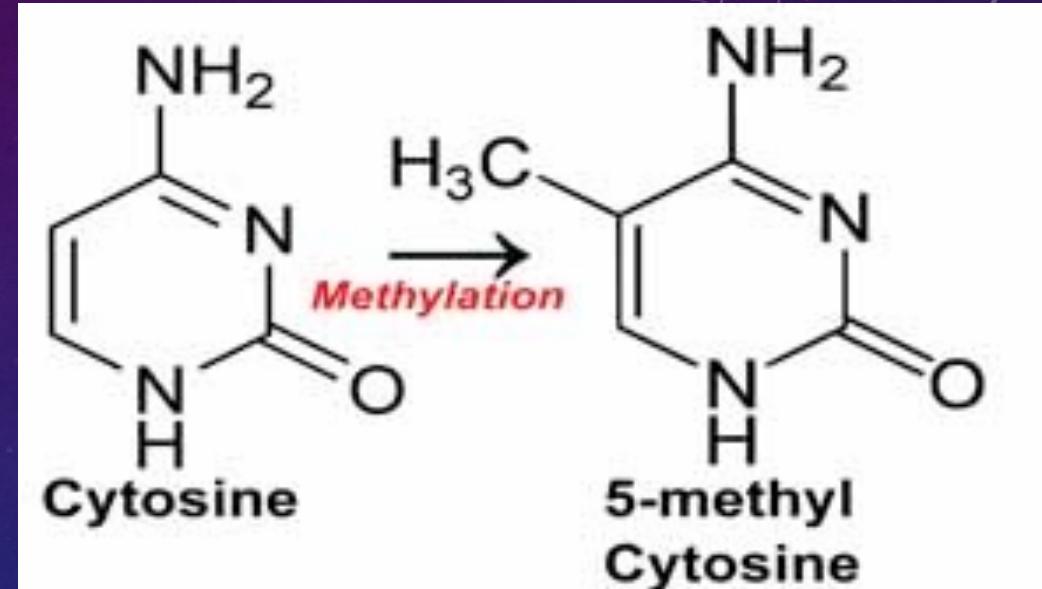
# EFFECT OF SOME HISTONE MODIFICATIONS

- H3K4me3 → Active promoters
- H3K27ac → Active promoters and enhancers
- H3K36me3 → Tx elongation
- H3K9me3 → Heterochromatin
- H3K27me3 → Repressed state

# DNA METHYLATION

- Covalent transfer of methyl group to a Cytosine.
- Frequent in CpGs
- This modification first originated in bacteria, and so it was also present in the first eukaryotes

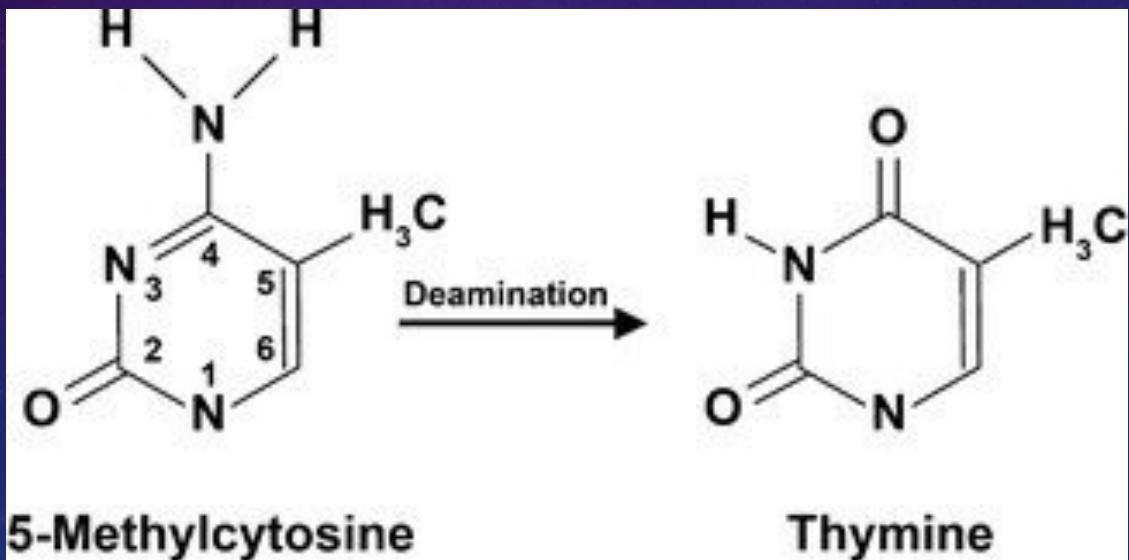
Lost in other lineages (e.g. *C. elegans*, *Drosophila*, yeast)



- Some non-CpG C are also methylated particularly in adult neurons.

# DEAMINATION OF CYTOSINES

- 5fold less CpG proportion than that expected from genome composition. We would expect equal frequency of CA, CT, CC and CG. Why CG are much less represented??
- Because it is mutagenic.
  - Spontaneous



Organisms with no DNA methylation higher content of CpG sites.

# DNA METHYLATION

- ~60M CpG sites (~5% of all Cytosines)
- 70-80% of them are methylated
- ~7% in CpG islands (CGIs) → Majorly unmethylated
- 45% in repetitive elements
- Promoters tend to have stable methylation (usually demethylated)
- Enhancers are more dynamic (non CpG island)
  - CpG density similar to average in the genome

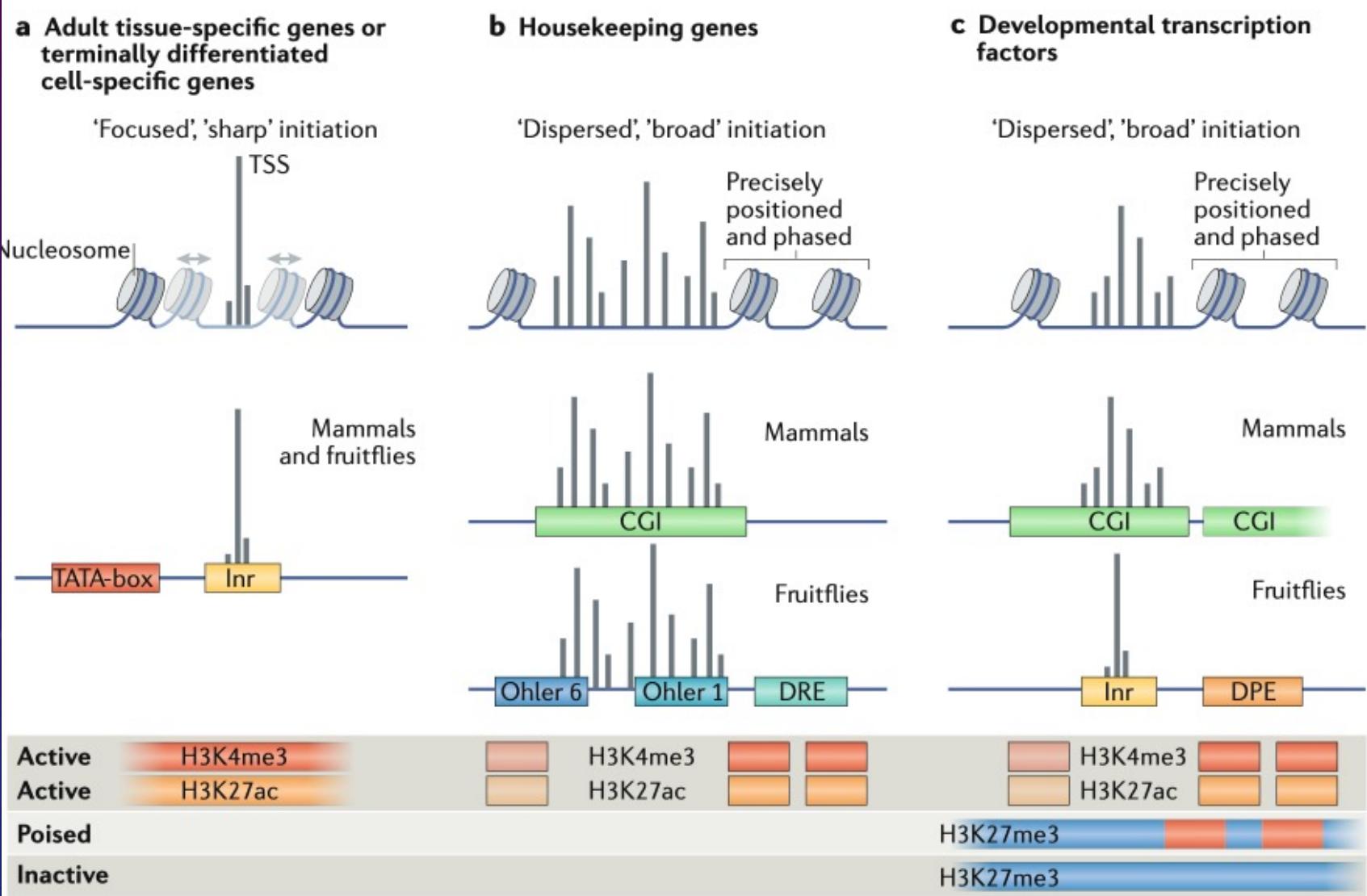
## Which are normally methylated?

- promoter CGI on the inactive X chromosome,
- one allele of imprinted genes,
- tissue-specific genes
- intragenic regions
- Repetitive elements

# DNA METHYLATION

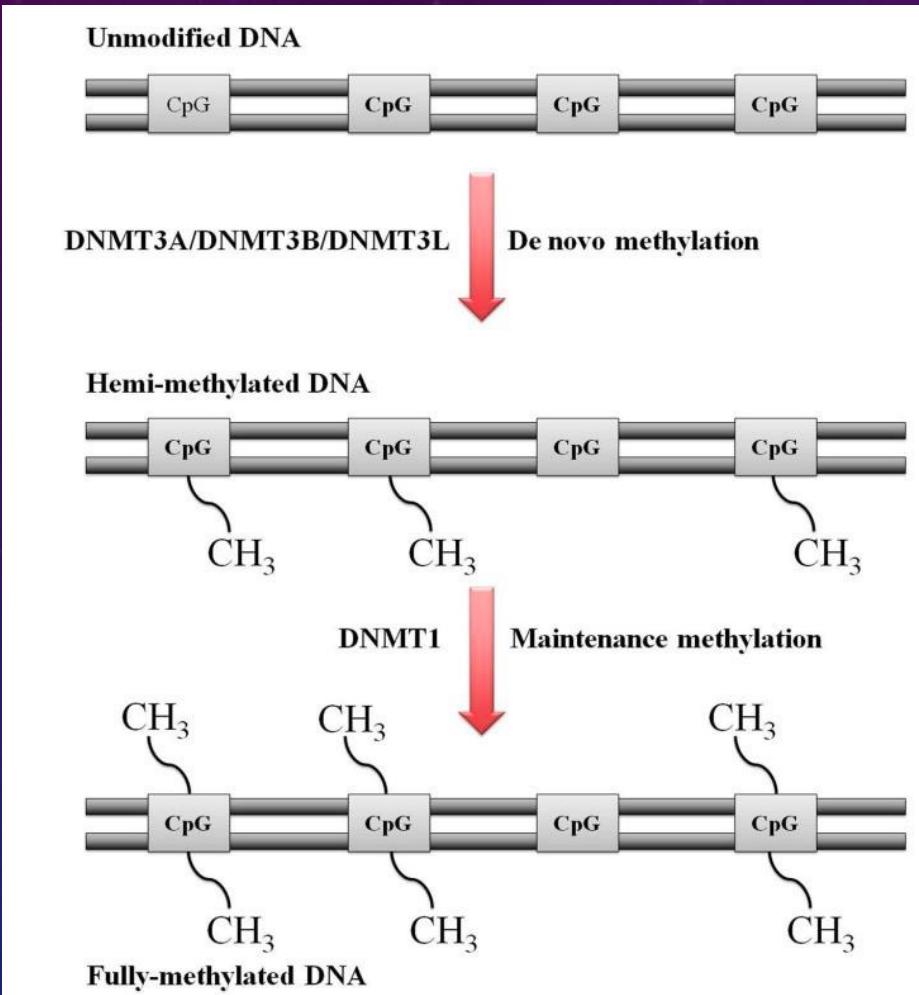
- 60% genes contain CpG Islands
- All housekeeping genes have CGI.
- Methylation in gene body is tissue specific but often related to high expression.
  1. Why is conserved if it is mutagenic? It is enriched in exons compared to introns.
  2. More methylation in constitutive exons than alternative
  3. Repress intragenic promoters
  4. Facilitate transcript elongation/splicing
- Methylation of repetitive sequences such as centromeres is essential to preserve chromosomal integrity.
- Methylation silences TEs (particularly retrotransposons) ---
  - The main target of DNA methylation.
  - 50% of the genome
  - DNA methylation might also difficult non-allelic recombination between copies of TEs.

## TYPES OF PROMOTERS



Eukaryotic core promoters and the functional basis of transcription initiation  
Haberle and Stark

# METHYLATION ENZYMES: DNMTs



DNMT-deficient mice → Embryonic lethality

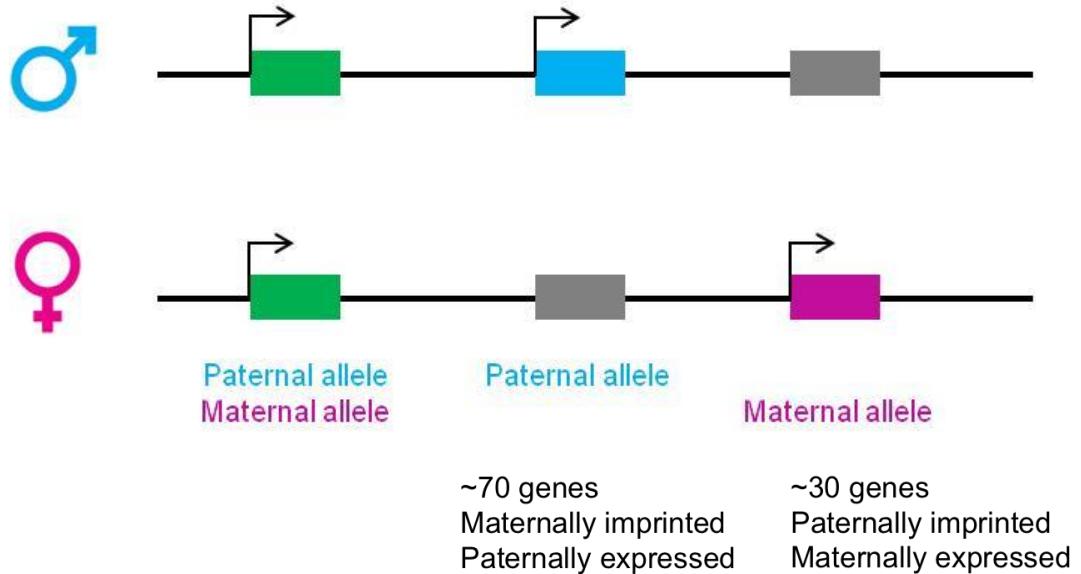
- DNMT3-A, -B, -L : Methylation de novo
- DNMT-1 : Maintenance of methylation (hemi-methylated DNA)

## METHYLATION PHASES

1. Establishment de novo (DNMT3 enzymes)
2. Maintenance (DNMT1)
3. Demethylation (TET enzymes)

# IMPRINTING

Imprinting is the unequal expression of the maternal and paternal alleles of a gene



DNA methylation is a common imprinting mechanism.  
Paternal or maternal alleles may also be expressed in a tissue-specific manner

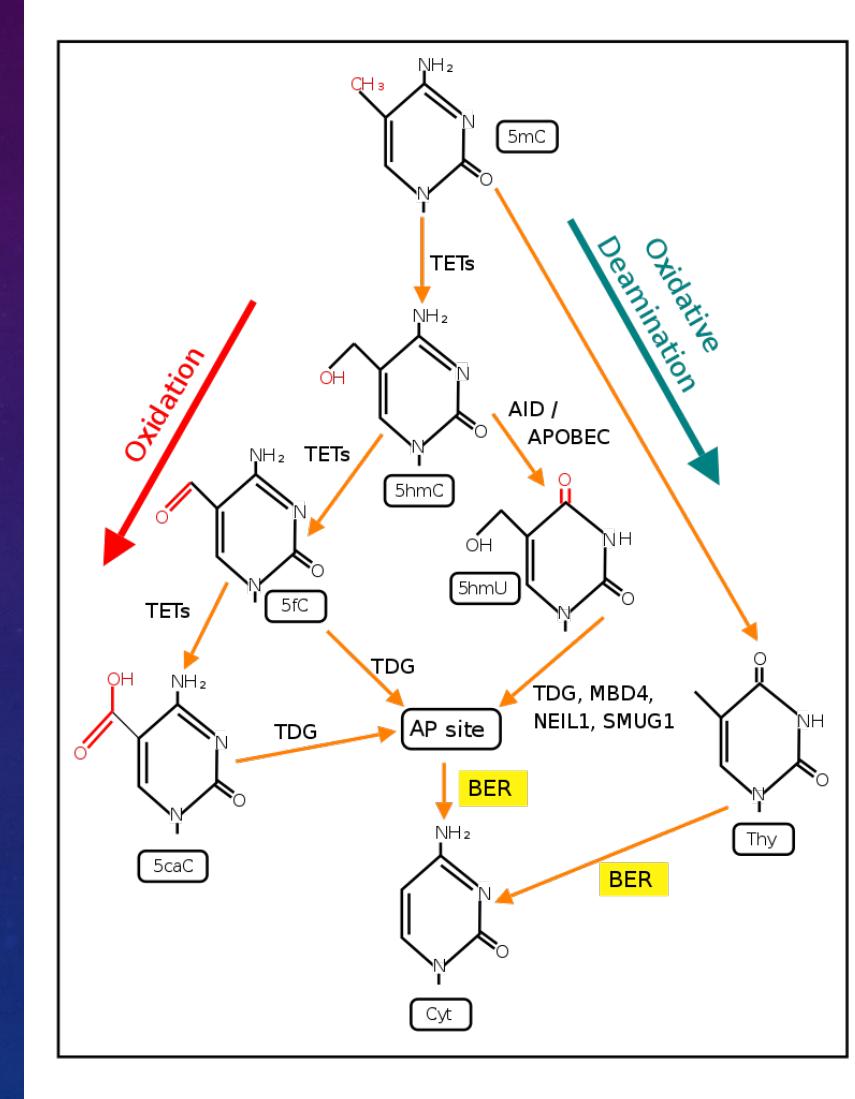
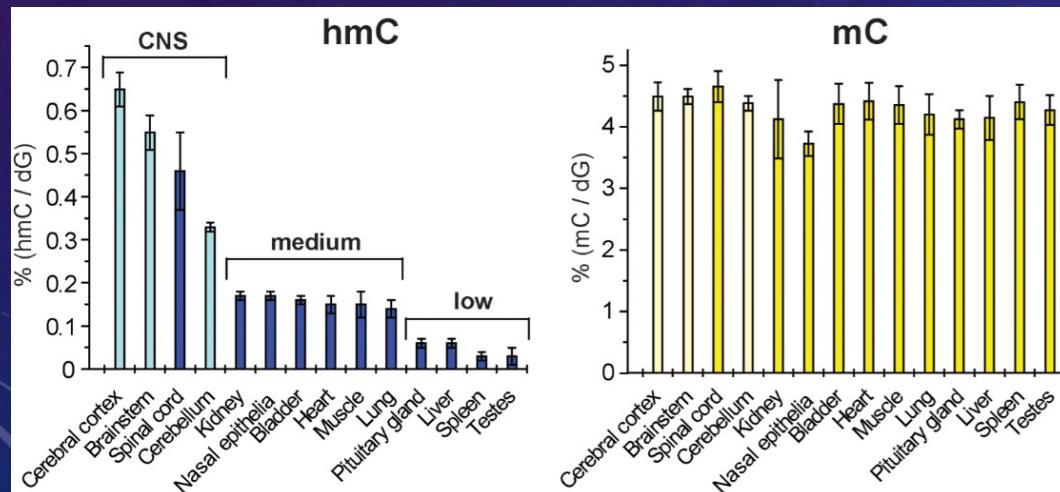
- For some genes, DNA methylation independent imprinting has also been described and associated to H3K27me3.
- Specific motif of CpG methylated sites is recognized by a KRAB-ZF which maintain imprinting.

DNMT3L is required for establishing maternal genomic imprinting. DNMT3L is expressed during gametogenesis when genomic imprinting takes place.

# DNA METHYLATION IS REVERSIBLE

## 5hmC: The Sixth Nucleotide

- Tet genes catalyze conversion from mC to hmC
- Regions with high hmC in fetal show low mC in adult.
- hmC occurs nearly exclusively in CG context



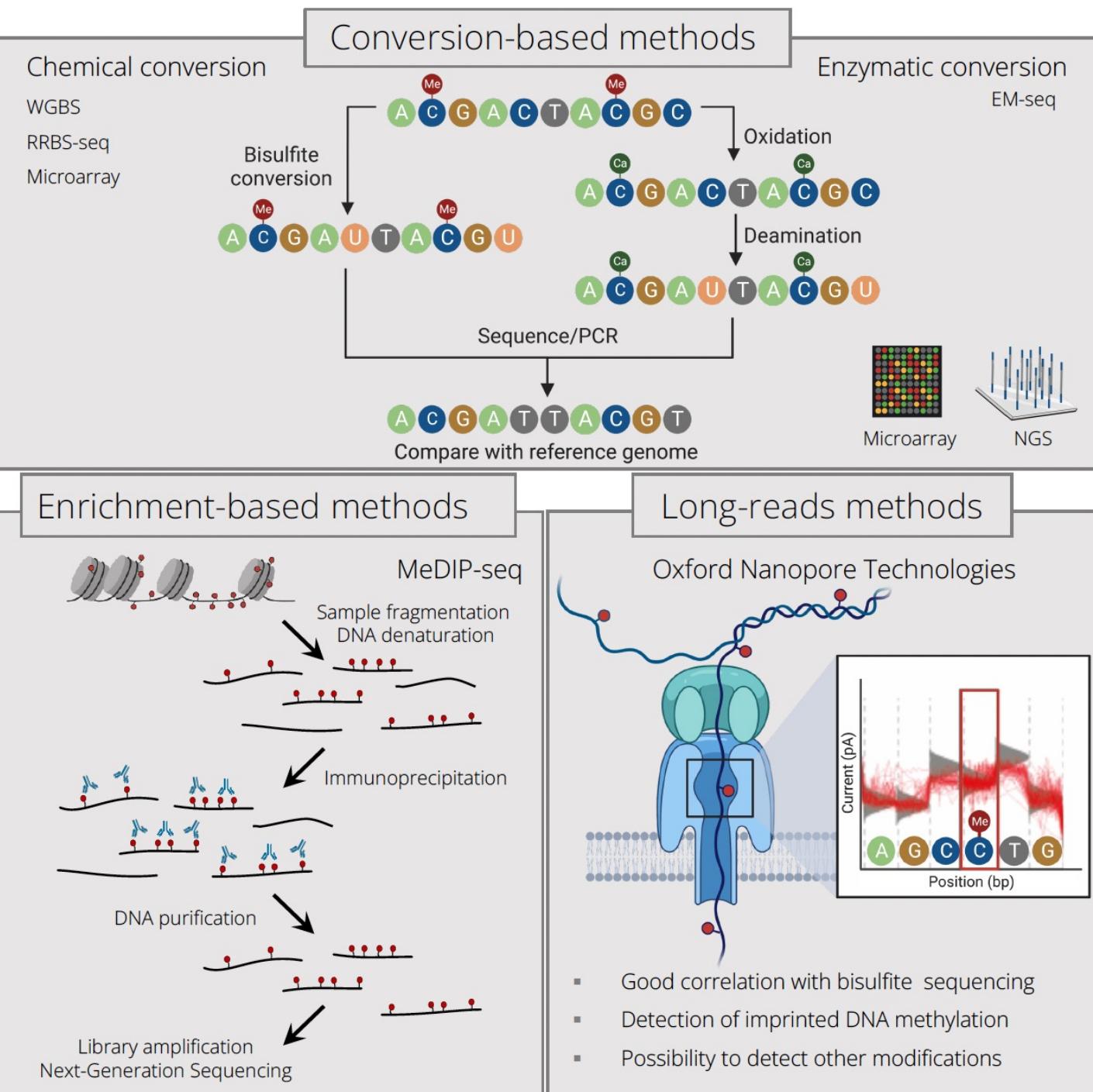
Bayraktar and Kreutz 2018

TAB-Seq :Genome wide maps of 5hmCG

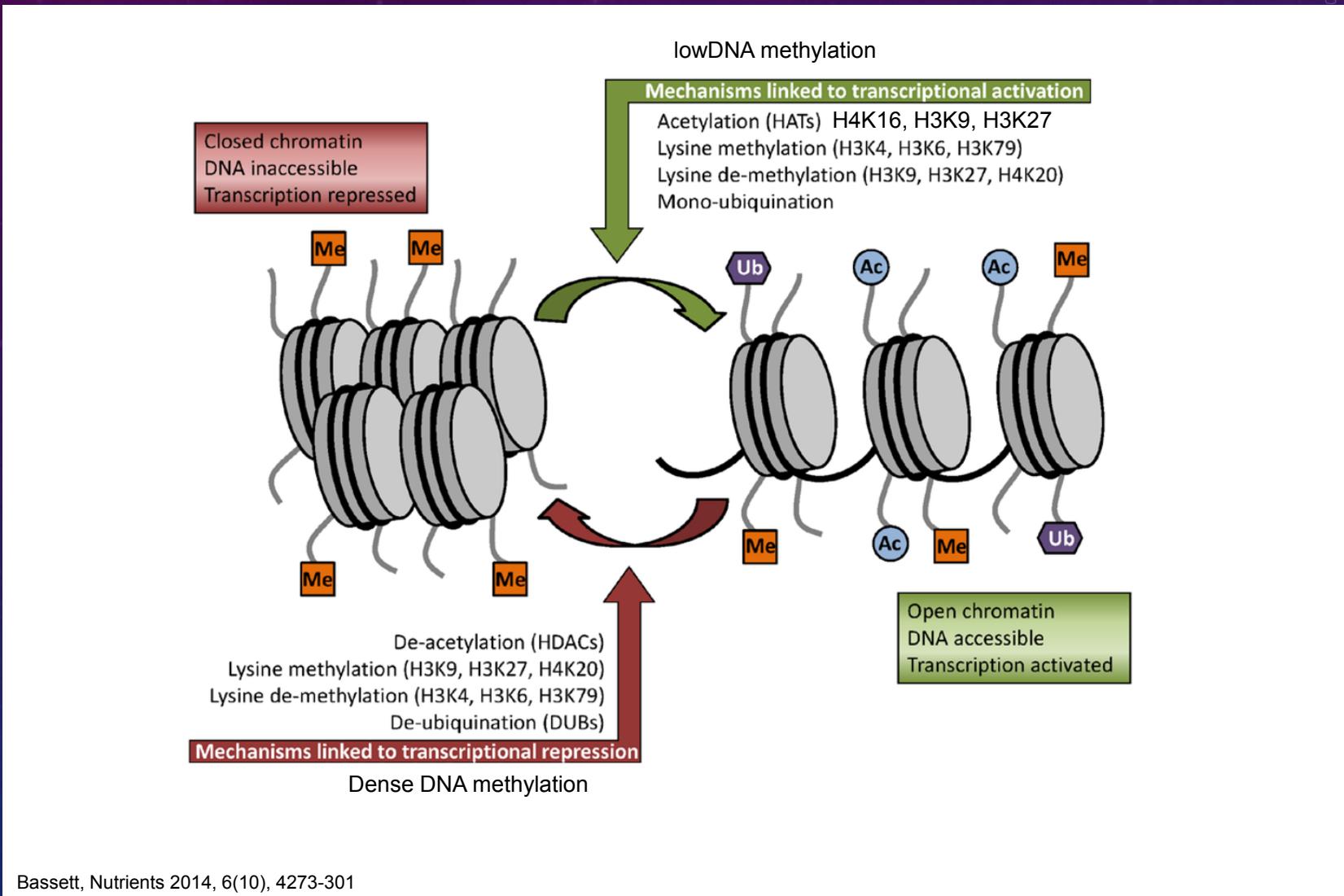
DNMT- deficient mice → Embryonic lethality

## WHY DO DNA METHYLATION- DEFICIENT EMBRYOS DIE AT SUCH AN EARLY STAGE OF DEVELOPMENT?

- Aberrant expression of protein- coding genes
- Massive derepression of transposable elements
- Genomic instability
- A combination all of the above



# EUCHROMATIN VS HETEROCHROMATIN

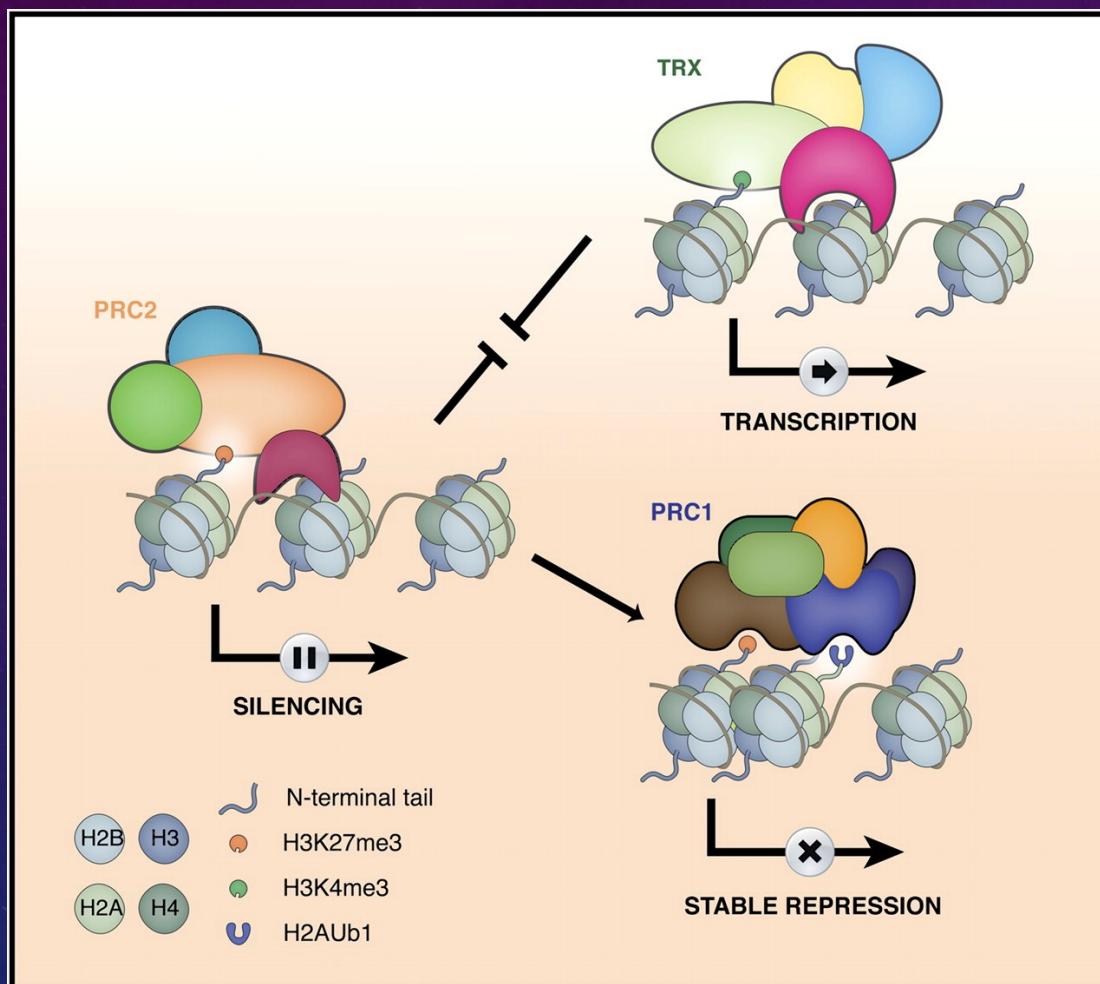


# MOLECULAR AGENTS OF EPIGENETIC INFORMATION

- “Writers” and “readers” is a common but inaccurate metaphor.
  - There is not reading of a code (as in triplets by ribosomes)
  - Binders and modifiers are more descriptive terms
  - The terms ‘activating’ and ‘repressive’ imply causality, and sometimes the opposite effect on transcription is observed.

On top of that one always need to consider the possible combinatorial interactions of these elements

# POLYCOMB AND TRITHORAX



- PRC2 tri-methylates H3K27 and represses
- PRC1 recognizes this and further Ubiquitinises H2A stabilizing repression
- TRX antagonizes PRC2 by tri-methylating H3K4 activating gene expression

Polycomb → OFF  
Trithorax → ON

# EPIGENETIC REGULATORS AND DISEASE

**Table 1** Monogenetic brain disorders associated with DNA methylation and histone-modification defects

Gene	OMIM (gene)	Function	Syndrome(s)	OMIM (phenotype)
<i>ATRX</i> (Xq21.1)	300032	Replication-independent nucleosome remodeling and histone H3.3 incorporation	ATRX; autism <sup>40</sup>	301040
<i>CREBBP</i> (16p13.3); <i>EP300</i> (22q13.2)	600140; 602700	Transcriptional coactivator; histone acetyl-transferase	Rubinstein-Taybi syndrome (RSTS) 1 and 2 (ref. 105)	180849 (RSTS1); 613684 (RSTS2)
<i>DNMT1</i> (19p13.2)	126375	DNA methyltransferase; disease mutations are associated with hypomethylated repeats and promoters	Hereditary sensory and autonomic neuropathy type 1 with adult-onset dementia <sup>5</sup> ; ADCA-DN <sup>6</sup> .	614116
<i>DNMT3B</i> (20q11.21)	602900	DNA methyltransferase; disease mutations are associated with hypomethylation of pericentric repeats	ICF1 mental-retardation syndrome <sup>28,106</sup>	242860
<i>ZBTB24</i> (6q21)	614064	Transcriptional repressor and regulator of DNA methylation at pericentric repeats	ICF2 mental-retardation syndrome <sup>28,29</sup>	614069
<i>KDM5C</i> (Xp11.22)	314690	Histone H3K4 demethylase	X-linked mental retardation <sup>107</sup> ; autism <sup>108</sup>	300534
<i>EHMT1</i> (9q34.3)	607001	Histone H3K9 methyltransferase	Kleefstra syndrome (mental retardation) <sup>109</sup> ; schizophrenia <sup>110</sup> ; nonspecific psychiatric phenotypes and neurodegenerative disease in postadolescence period <sup>111</sup>	610253
<i>NSD1</i> (5q35.2–q35.3)	606681	Histones H3K36 and H4K20 methyltransferase	Sotos syndrome (mental retardation) <sup>112</sup>	117550
<i>PHF8</i> (Xp11.22)	300560	Histone H3K9 demethylase and transcriptional activator	X-linked mental retardation without cleft lip and/or palate (Siderius-Hamel) <sup>113,114</sup>	300263
<i>RPS6KA3</i> (Xp22.12)	300075	Serine/threonine kinase (of both histones and nonhistone proteins)	Coffin-Lowry X-linked mental-retardation syndrome <sup>115</sup>	303600
<i>MECP2</i> (Xq28)	300005	Methyl-CpG-binding protein	RTT and other neurodevelopmental syndromes; autism <sup>116</sup>	312750

# EPIGENETIC REGULATORS AND DISEASE

Factor	Function	Mouse loss-of-function phenotype	Human diseases associated with genetic mutations
DNMT1	Maintenance DNA methyltransferase	<ul style="list-style-type: none"><li>• Low global DNA methylation</li><li>• Derepression of IAP transposons</li><li>• Early embryonic lethality</li></ul>	<ul style="list-style-type: none"><li>• Hereditary sensory autonomic neuropathy 1E (HSAN1E; OMIM 614116)</li><li>• Autosomal-dominant cerebellar ataxia, deafness and narcolepsy (ADAC-DN; OMIM 604121)</li></ul>
UHRF1	DNMT1 cofactor	<ul style="list-style-type: none"><li>• Low global DNA methylation</li><li>• Early embryonic lethality</li></ul>	
DNMT3A	De novo DNA methyltransferase	<ul style="list-style-type: none"><li>• Constitutive knockouts die ~4 weeks after birth<sup>a</sup></li><li>• Sterility in both males and females in germline-specific knockouts</li></ul>	<ul style="list-style-type: none"><li>• Microcephalic dwarfism</li><li>• Tatton-Brown–Rahman syndrome (TBRS; OMIM 602729)</li><li>• Acute myeloid leukaemia (AML; OMIM 601626)</li></ul>
DNMT3B	De novo DNA methyltransferase	Constitutive knockouts die mid-gestation <sup>a</sup> . More important for embryonic DNA methylation than for germline DNA methylation	Immunodeficiency, centromeric instability and facial anomalies syndrome (ICF; OMIM 602900)
DNMT3C	De novo DNA methyltransferase (Muroidea specific)	Males are infertile likely owing to defect in methylating transposon promoters during spermatogenesis	
DNMT3L	De novo DNA methyltransferase cofactor	<ul style="list-style-type: none"><li>• Male germline cells unable to undergo meiosis</li><li>• Females unable to establish maternal imprinting, leading to mid-gestation lethality of progeny</li></ul>	
TET1	DNA demethylation via oxidation of methylcytosine	Loss has subtle effects and the embryos are viable <sup>b,c</sup>	
TET2	DNA demethylation via oxidation of methylcytosine	Increased self-renewal of haematopoietic stem cells <sup>b,c</sup>	<ul style="list-style-type: none"><li>• AML (OMIM 601626)</li><li>• Chronic myelomonocytic leukaemia</li><li>• Lymphomas</li><li>• Myeloproliferative neoplasms</li></ul>
TET3	DNA demethylation via oxidation of methylcytosine	Germline conditional knockout leads to impaired paternal demethylation and reduced fecundity <sup>c</sup>	

# EPIGENETIC MEMORY: HOW IS EPIGENETIC INFORMATION MAINTAINED THROUGH CELL DIVISION?

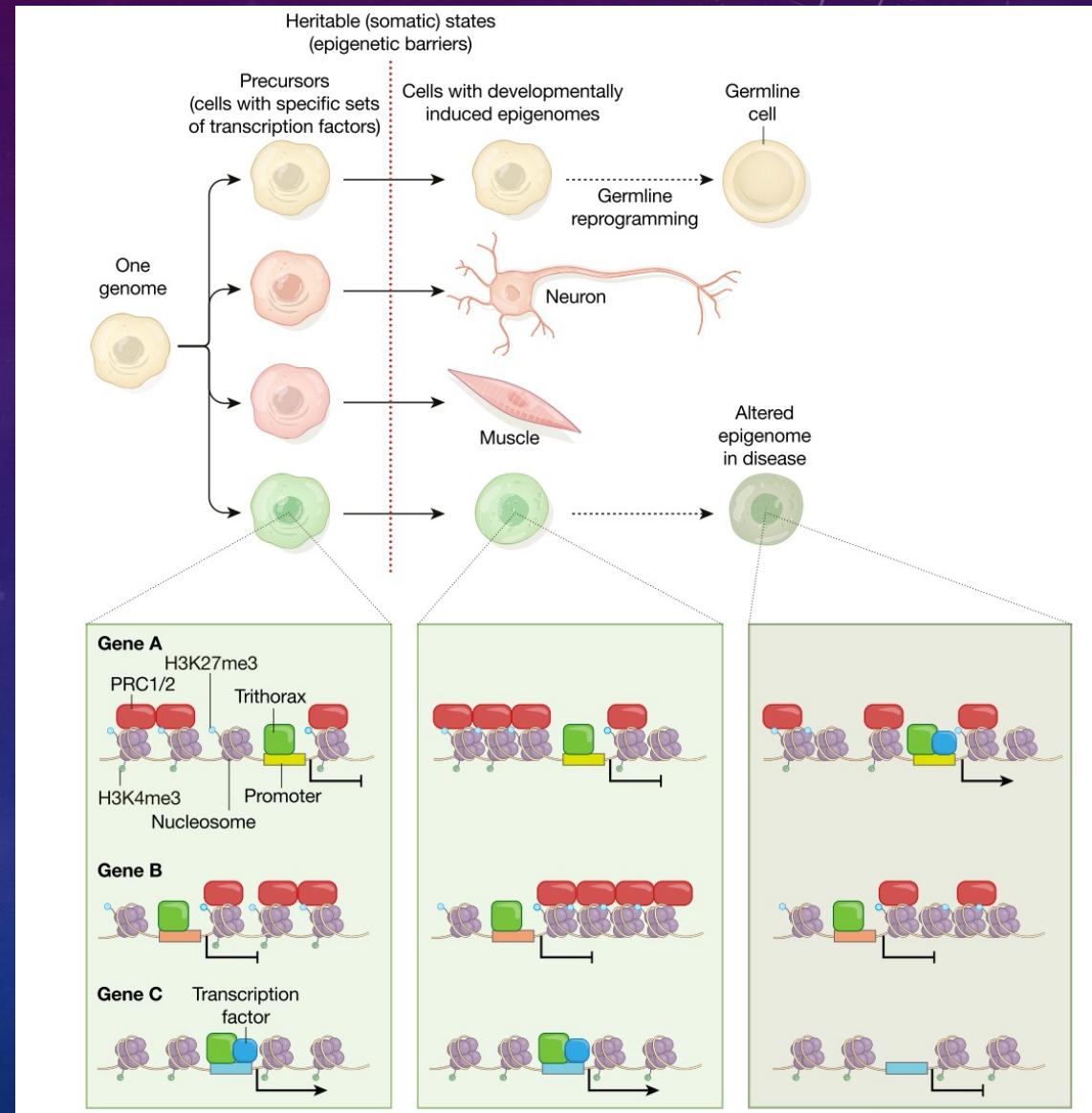
- Epigenetic modification need to survive DNA replication and mitosis.
- Genes need to be kept silenced/activated
- How epigenetic landscape is kept through cell division?

# EPIGENETIC BARRIERS

Epigenetic components maintain ON/OFF states

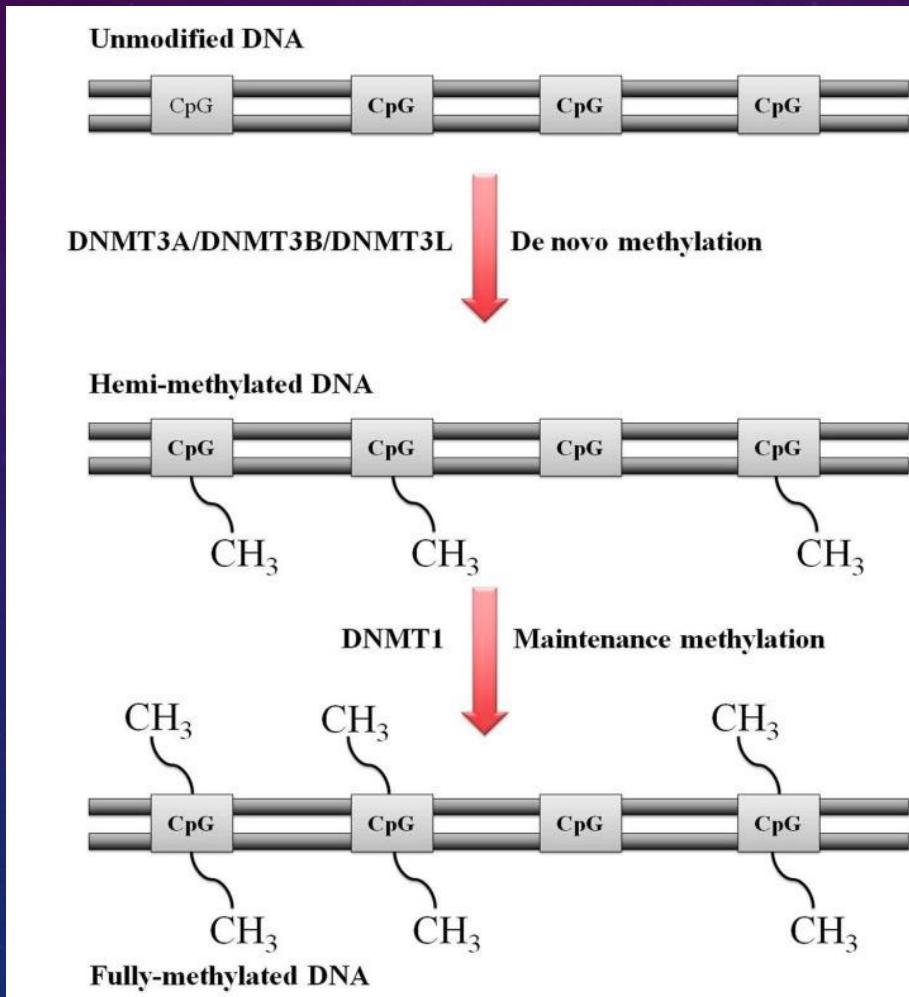
Epigenetic barriers maintain actively somatic states.

Alterations when they are accidental can lead to disease. When programmed they constitute developmental programs.



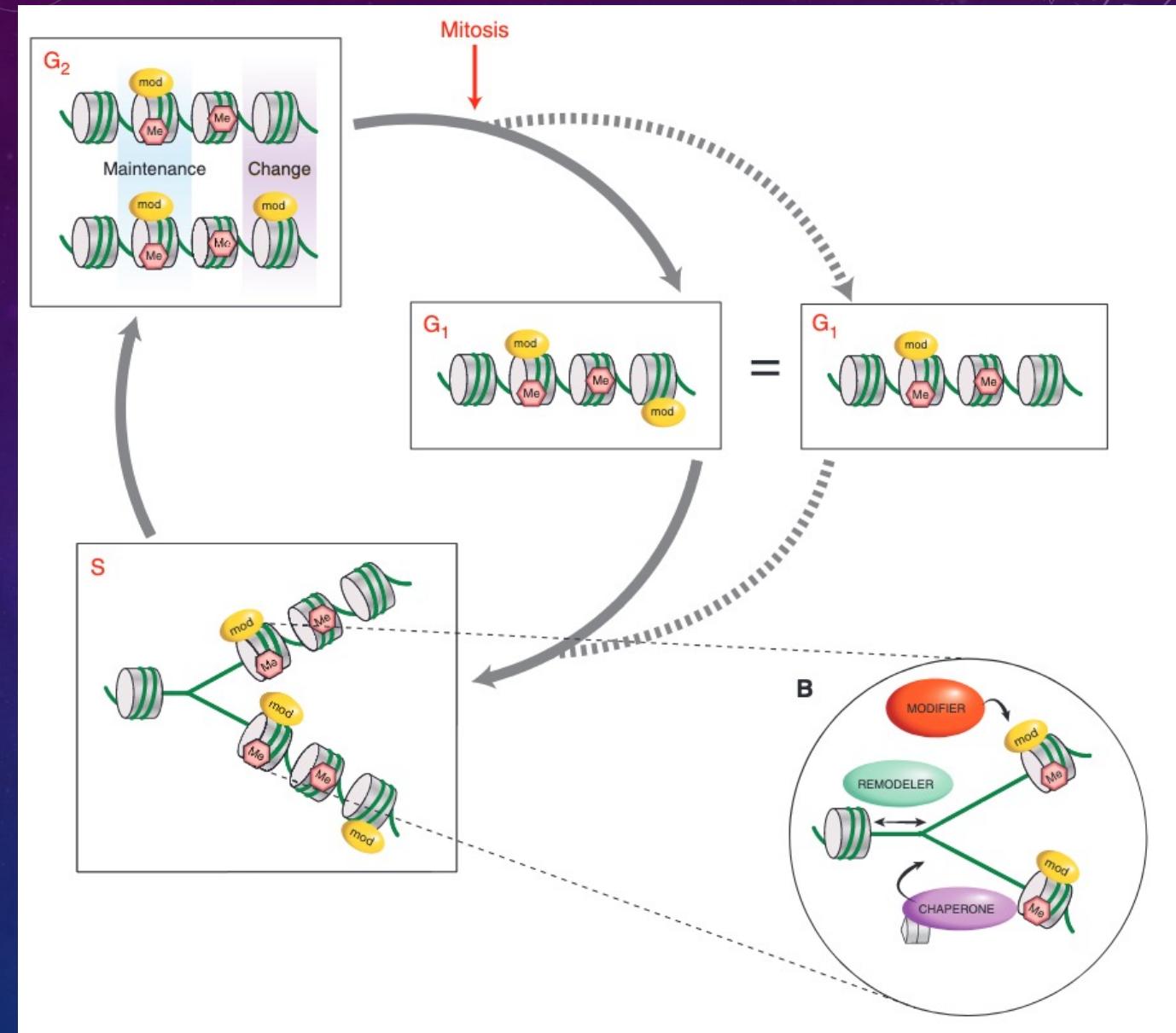
We already know how it works for methylation

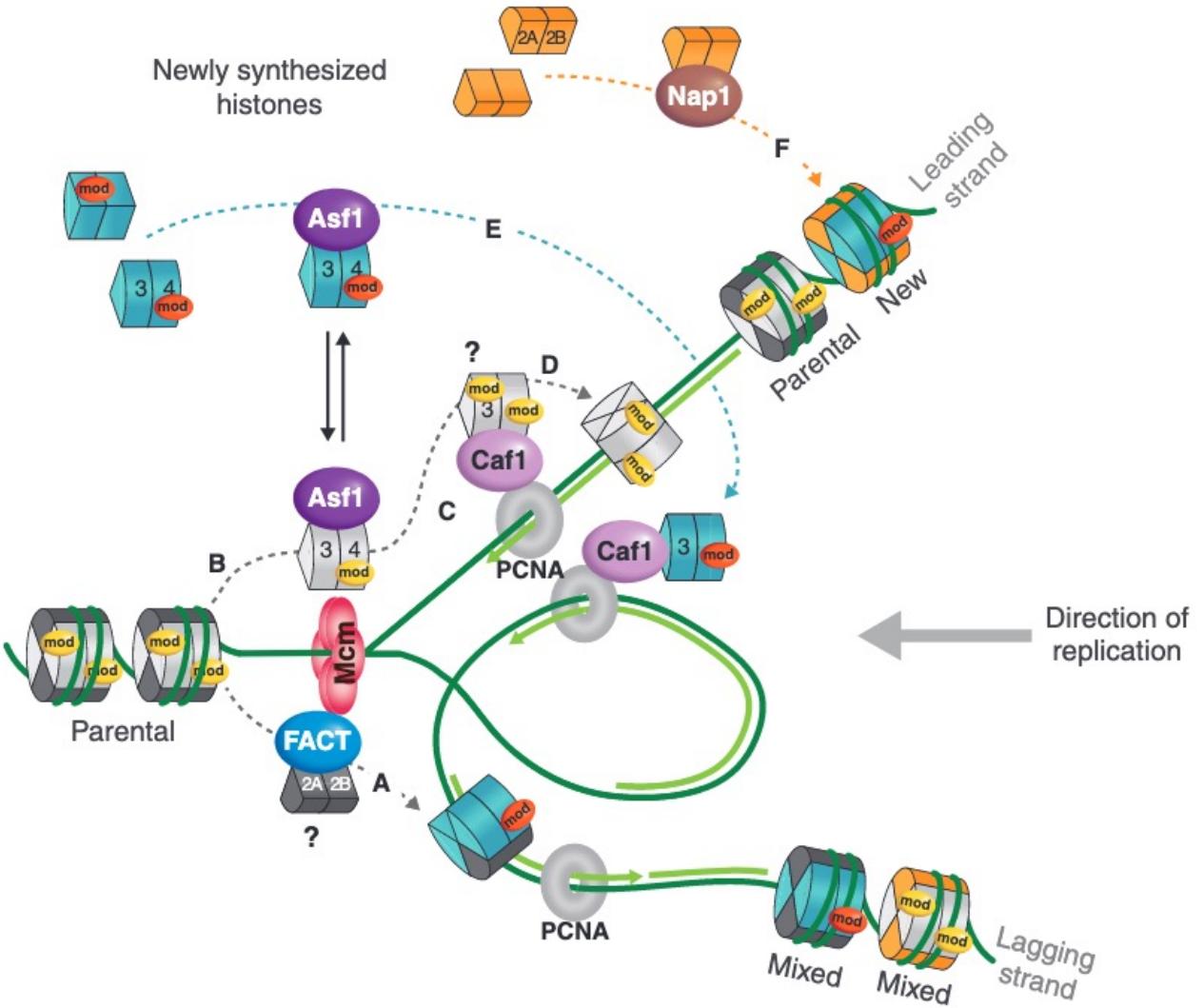
## DNMT1



What about histones?

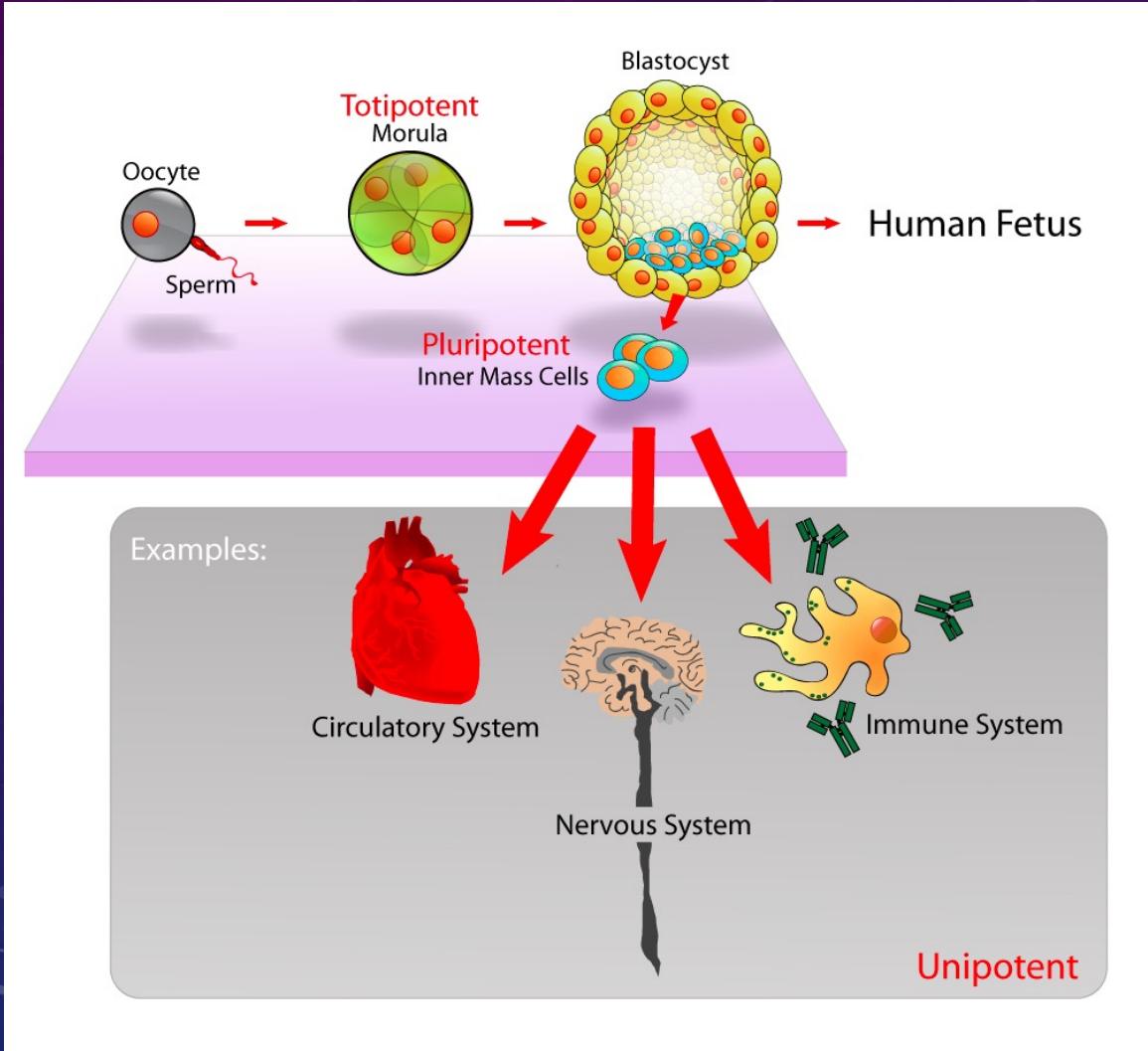
- Chromatin modifiers
- Nucleosome remodellers
- Histone chaperones





Is IDENTITY reversible?  
Can a cell be re-programmed?

# INDUCED PLURIPOTENT STEM CELLS



## Yamaka factors

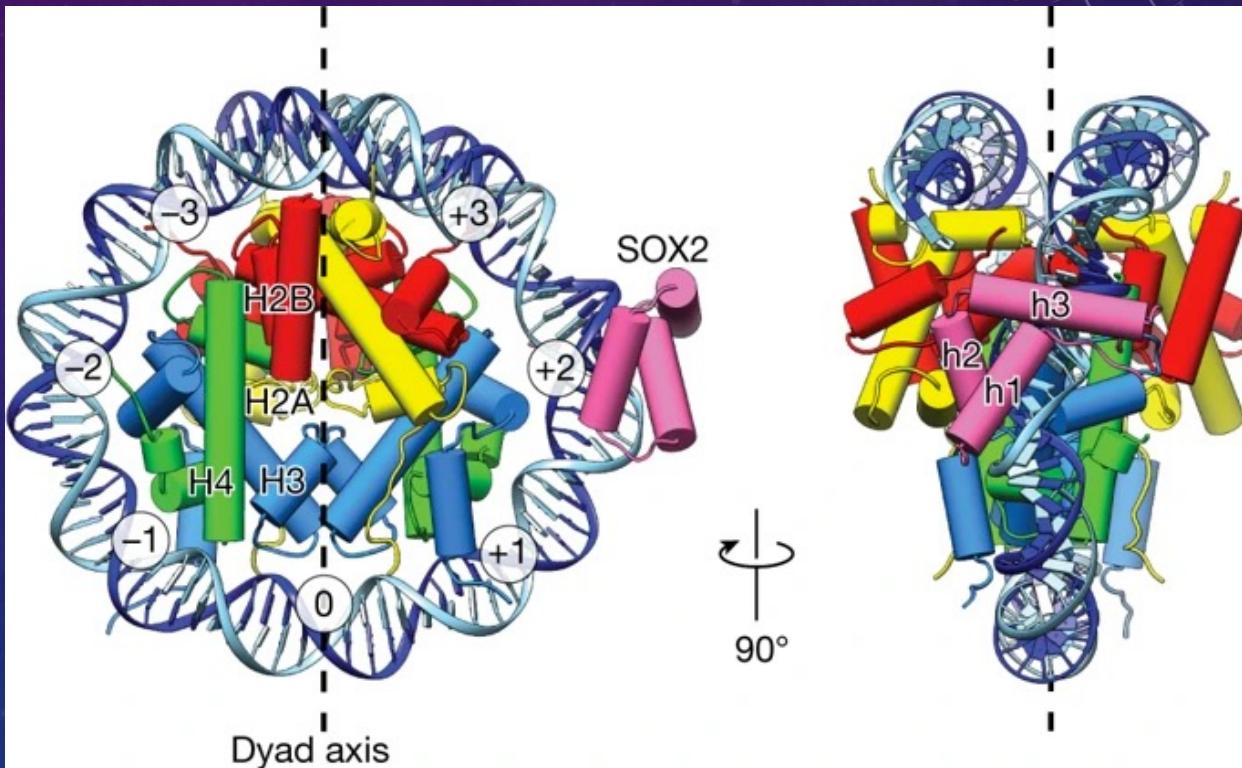
- OCT4
- MYC
- SOX2
- KLF4

“Improved methods to select for iPSCs that have efficiently overcome epigenetic barriers are important to unleash the full potential of iPSC technology.”

Bartoccetti et al. 2020

# PIONEER TRANSCRIPTION FACTORS

- They can bind packaged nucleosome attached DNA
- Example SOX2 and SOX11
- KLF4 and OCT4 for example also bind preferentially to methylated sequences.
- KLF4 recruits TET2 to demethylate DNA.

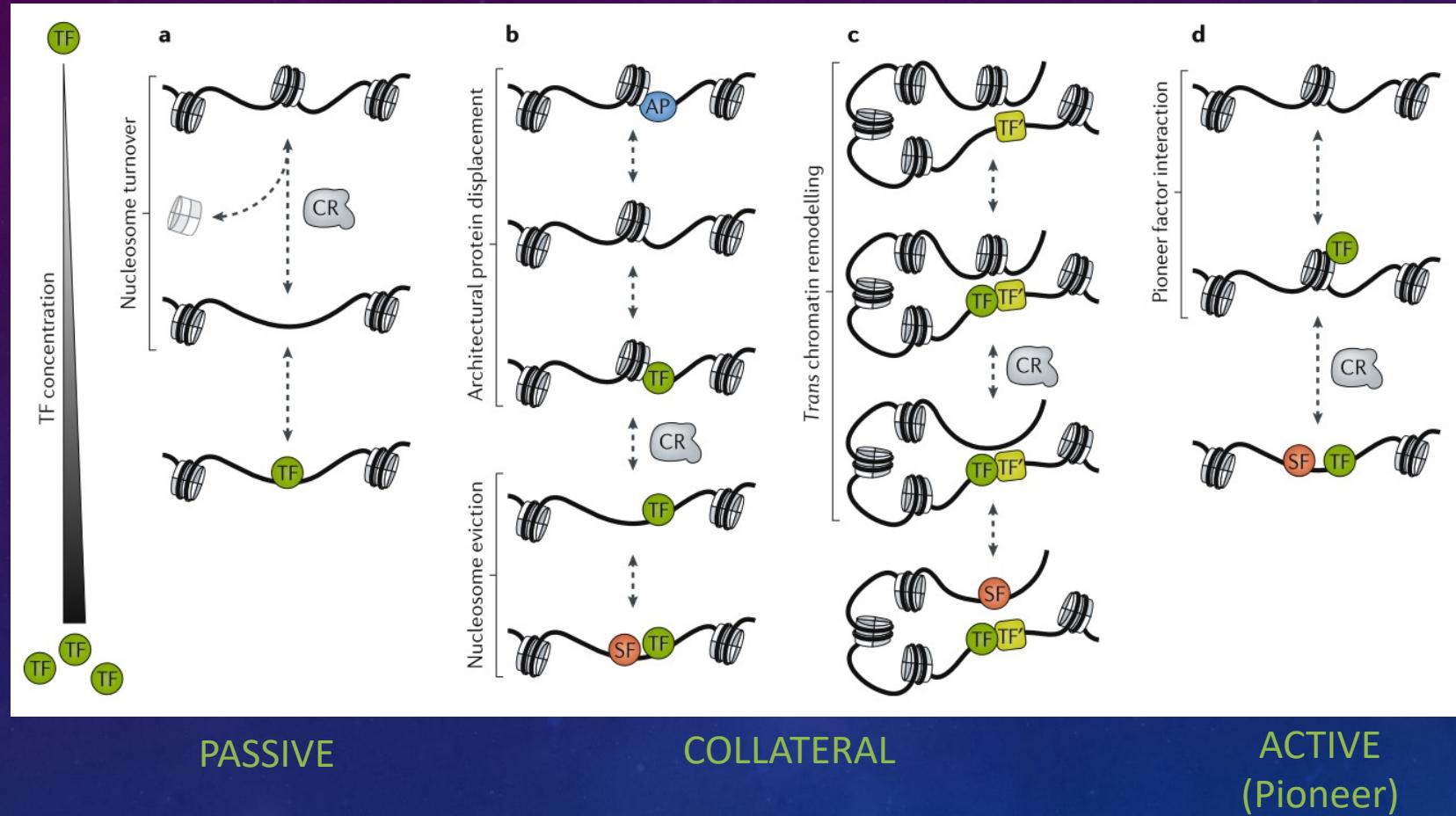


Dodonova et al 2020

- Better reprogramming with e.g. blockers of H3K9me3

- Pioneer TFs: TFs that are first to bind to DNA during an accessibility remodelling process.

Four models:



CR: Chromatin remodeler

TF: Transcription factor

SF: Secondary factor

AP: Architectural protein

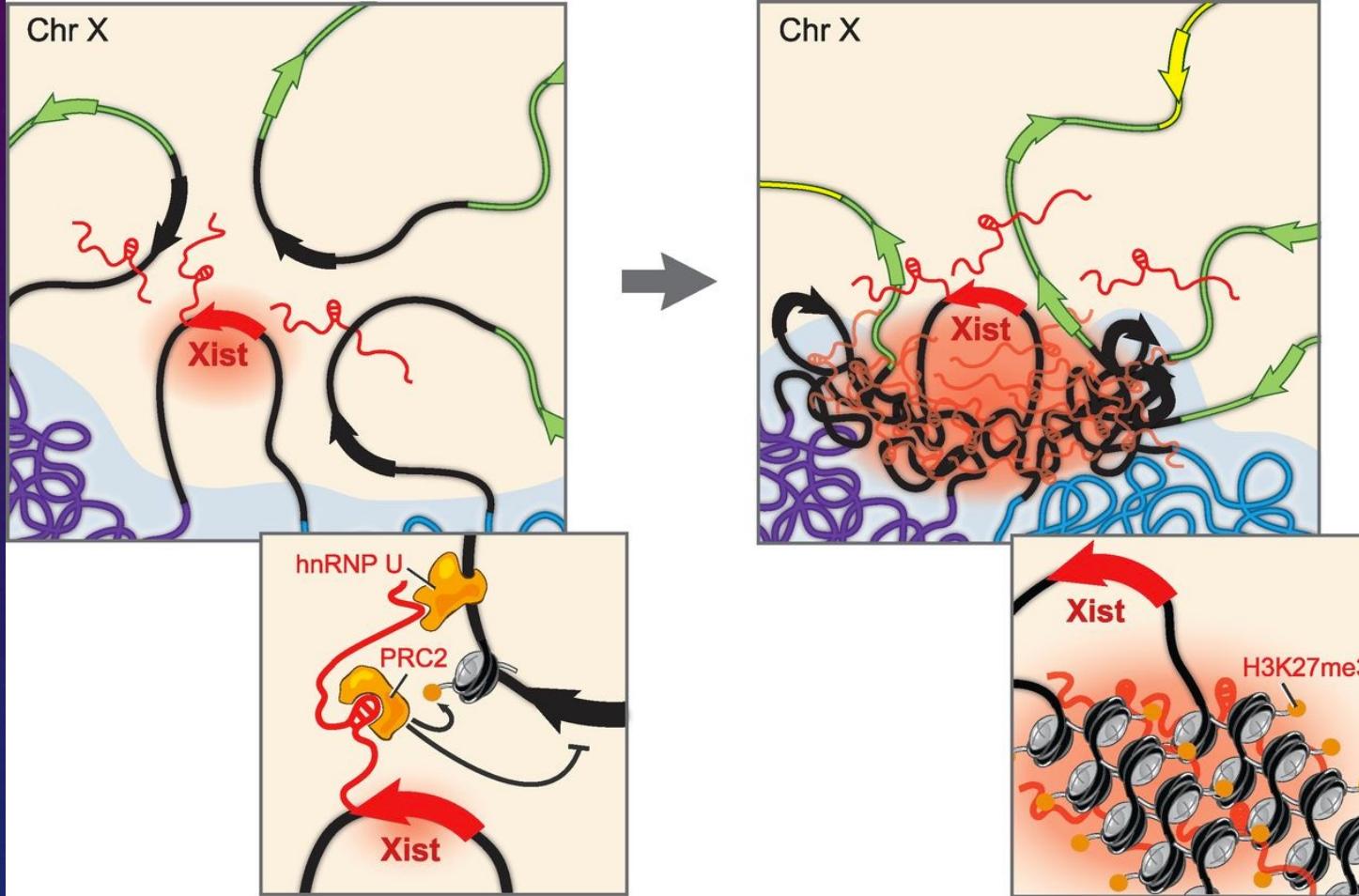
Klemm et al 2019

Search for differentially expressed TFs to understand differentially accessible chromatin.

# CHROMOSOME X INACTIVATION

- One of the female chrX is silenced to avoid gene dosage problems.
- The random choice for an inactivated X-chromosome ( $X_i$ ) (*i.e.*, the paternal or the maternal one) is completed at a very early phase of embryonic development.
- Once it is decided, the copy remains silenced for life in this and all descendent cells.
- Silencing is initiated by XIST. Recruits chromatin remodelling factors to “heterochromotize” one copy.
- 15-20% of genes escape inactivation (recently diverged from ChrY).
- Promoters in  $X_i$  are also methylated.

# CHR X INACTIVATION PART 1: LNCRNA AND HISTONE MODIFICATIONS



XIST interact with Polycomb  
repression complex 2 →  
Histone methyltransferase  
→ H3K27me2

CGI are methylated in a late  
stage vi DNMT3B

# BUILDING EPIGENOMES

- ChIP-Seq
- ATAC-seq
- DNase-sensitive
- Techniques based on eRNA expression
- Methyl-Seq
- Many others
- Large datasets. E.g. Recent human methylome generated 90Gb of reads which took one full week to transfer to NCBI repository.

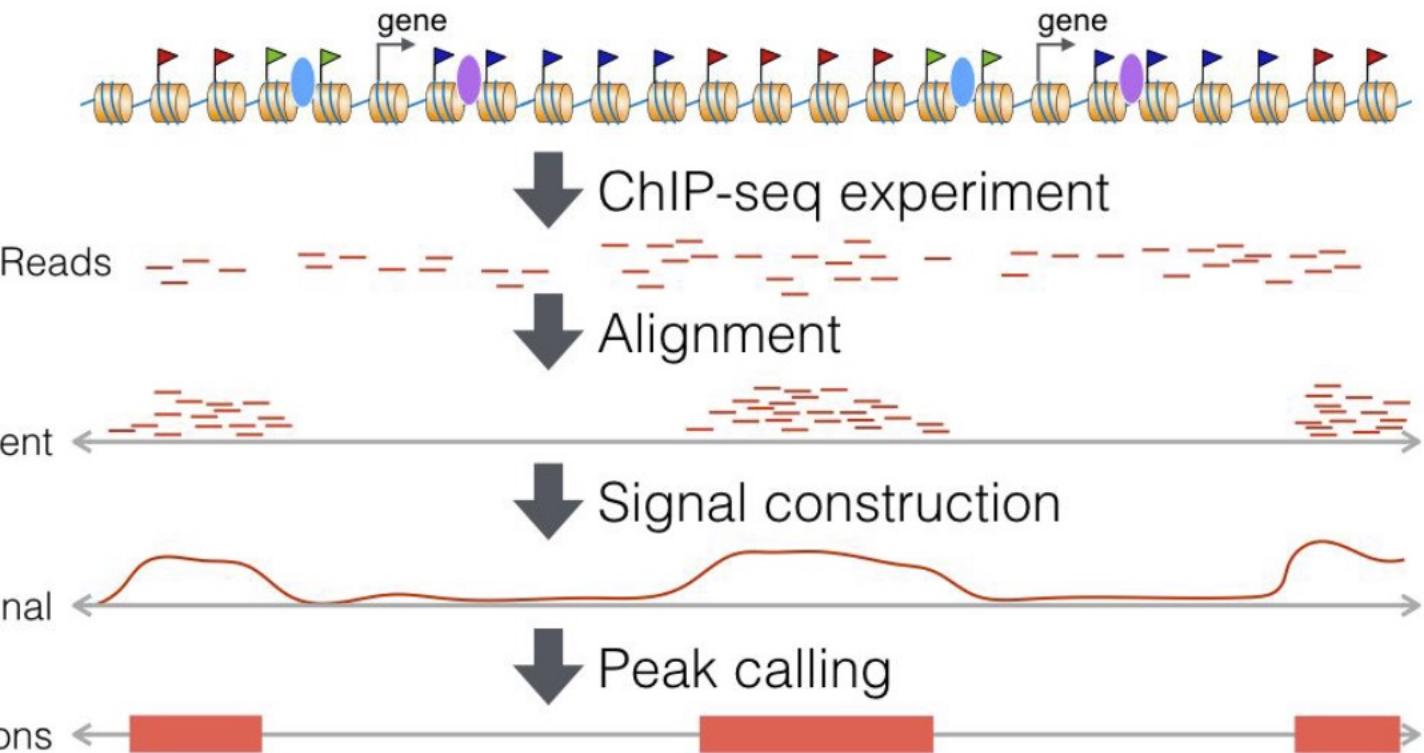
# GROUP ACTIVITY (JUNE 7TH)

- Explain, in a few slides, a technique that uses NGS sequencing to study the function of the genome.
  - How does it work?
  - Which data does it provide?
  - Describe a possible computational pipeline to analyze it
  
- Bisulfite sequencing
- DNaseI hypersensitivity sites
- NOME-seq
- FRAIRE-seq
- CAGE-seq
  
- Mnase-seq
- Chip-seq

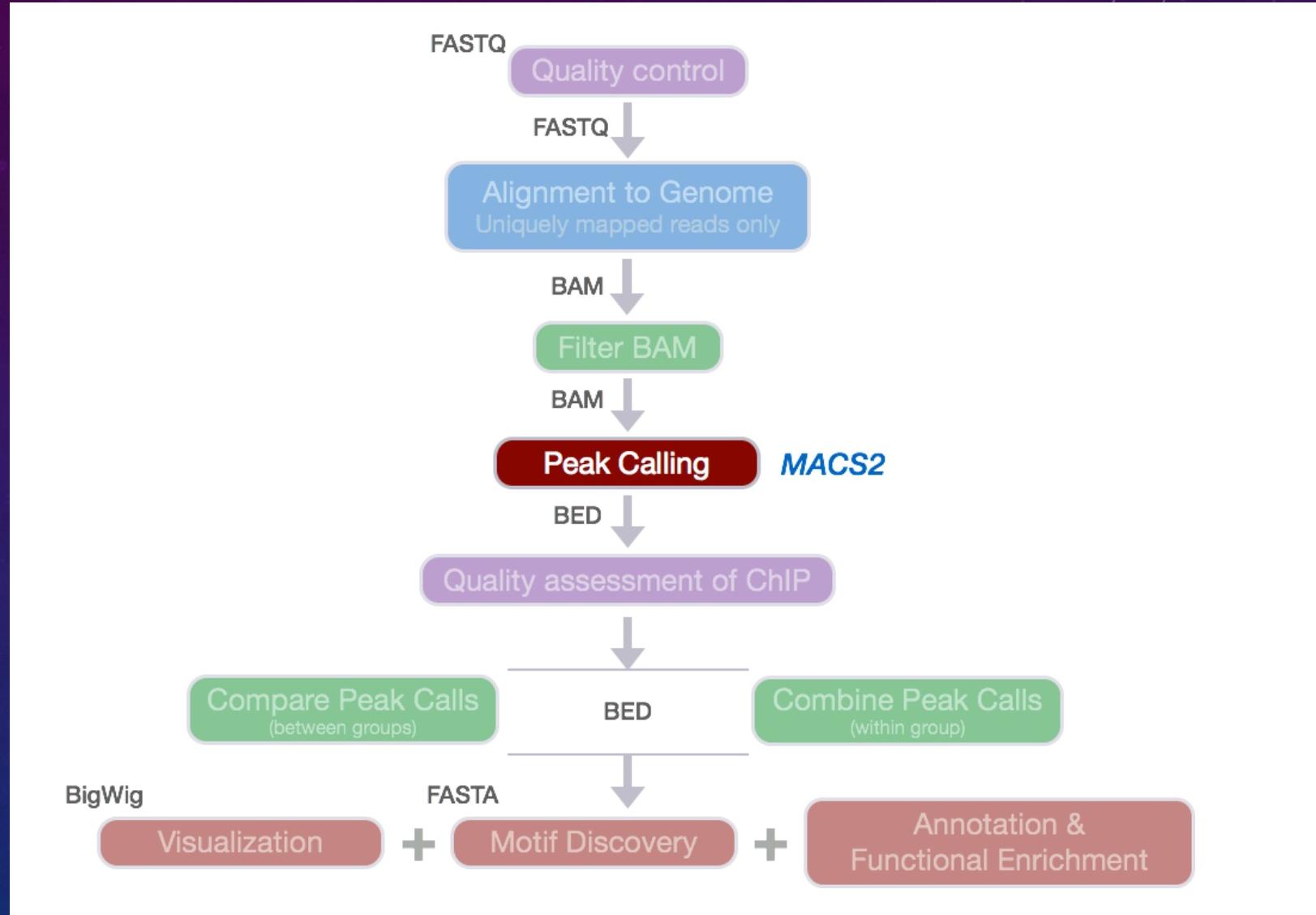
# CHIP-SEQ

**Figure 1**

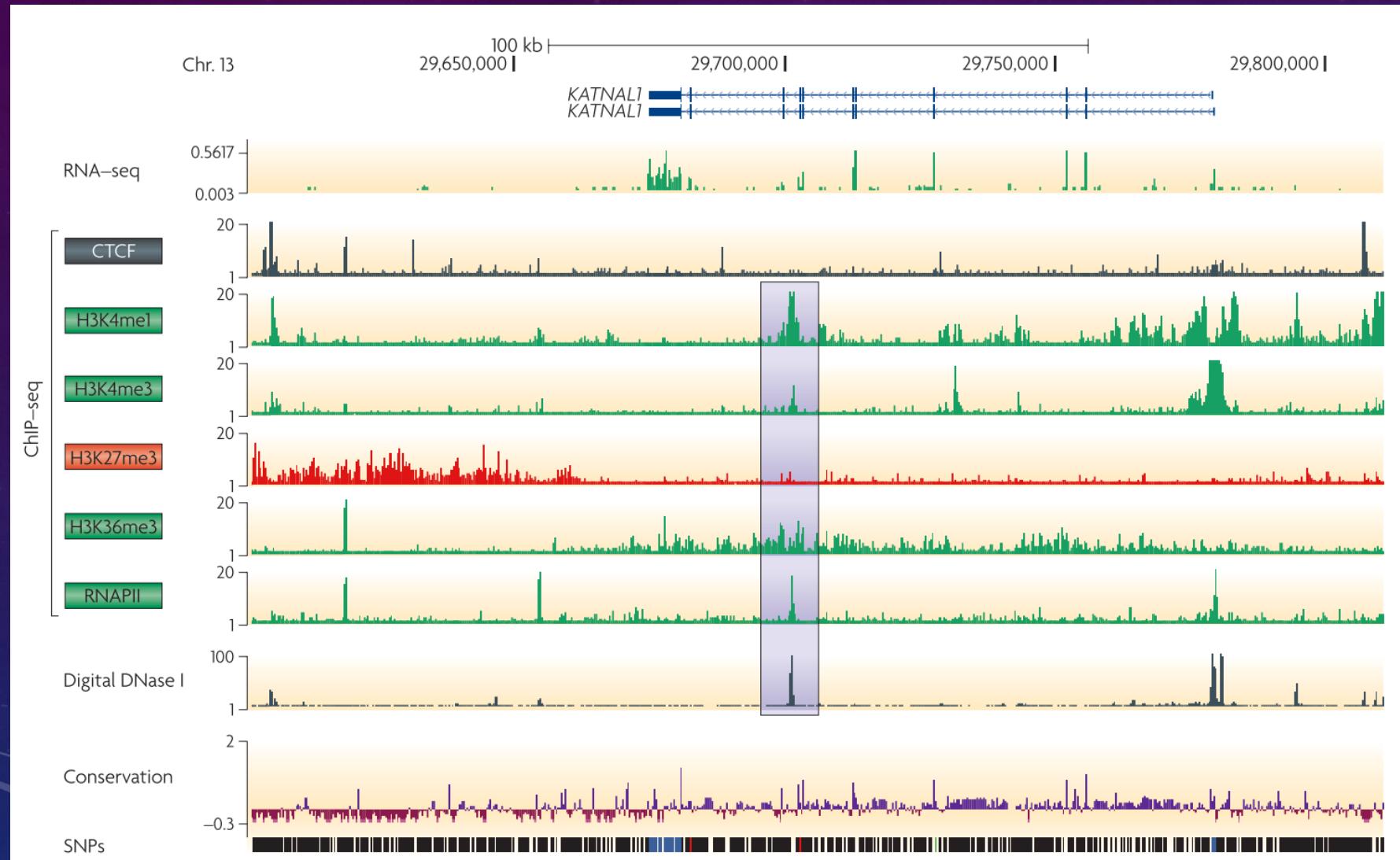
- Histone code
- Transcription (Blue triangle)
- Active Regions (Green triangle)
- Repressed regions (Red triangle)
- TF1 (Blue circle)
- TF2 (Purple circle)



# PEAK CALLING



# VISUALIZE MULTIPLE TRACKS

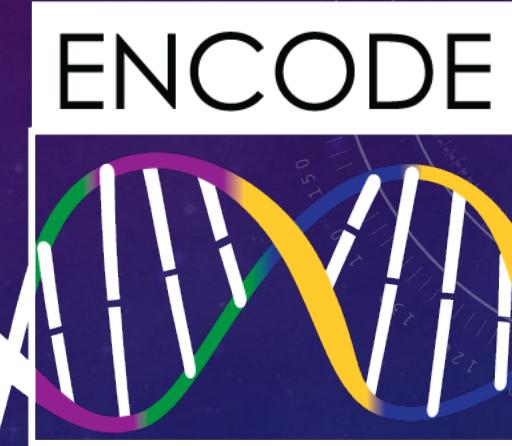


- UCSC Genome Browser
- IGV Broad Institute

The Encyclopedia of DNA Elements

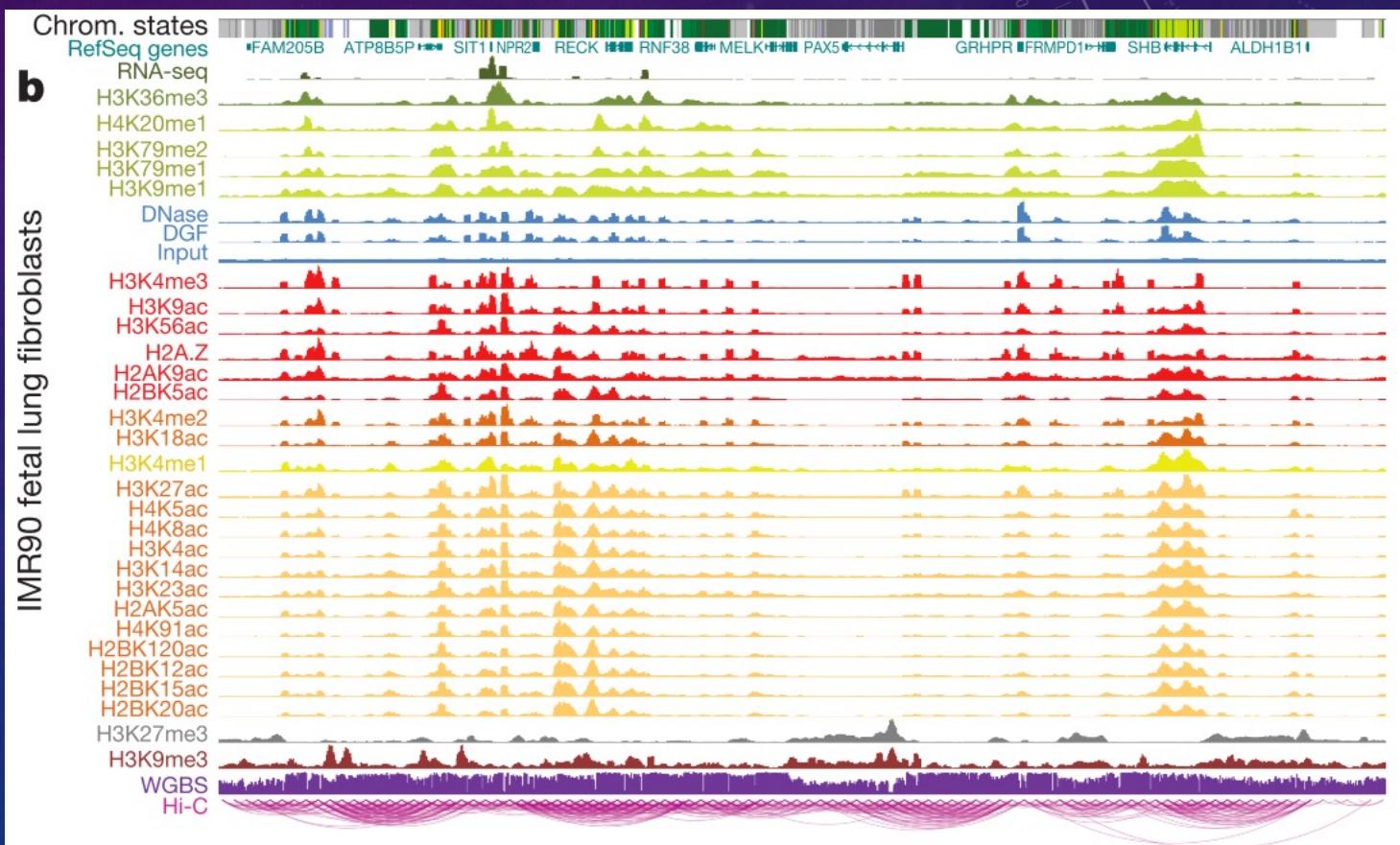
# BUILDING EPIGENOMES -- ENCODE

- 2012
- Variability across cell types in culture
- Methylation correlated with chromatin accessibility
- Multi-OMICS dataset integration better than single marks
- Many are evolutionarily young and currently under selective constraint



# BUILDING EPIGENOMES

- Using human tissues
- Hidden Markov Models with multiple epigenetic modification.
- Up to 31 modifications and chromatin segmentation in up to 18 different states.



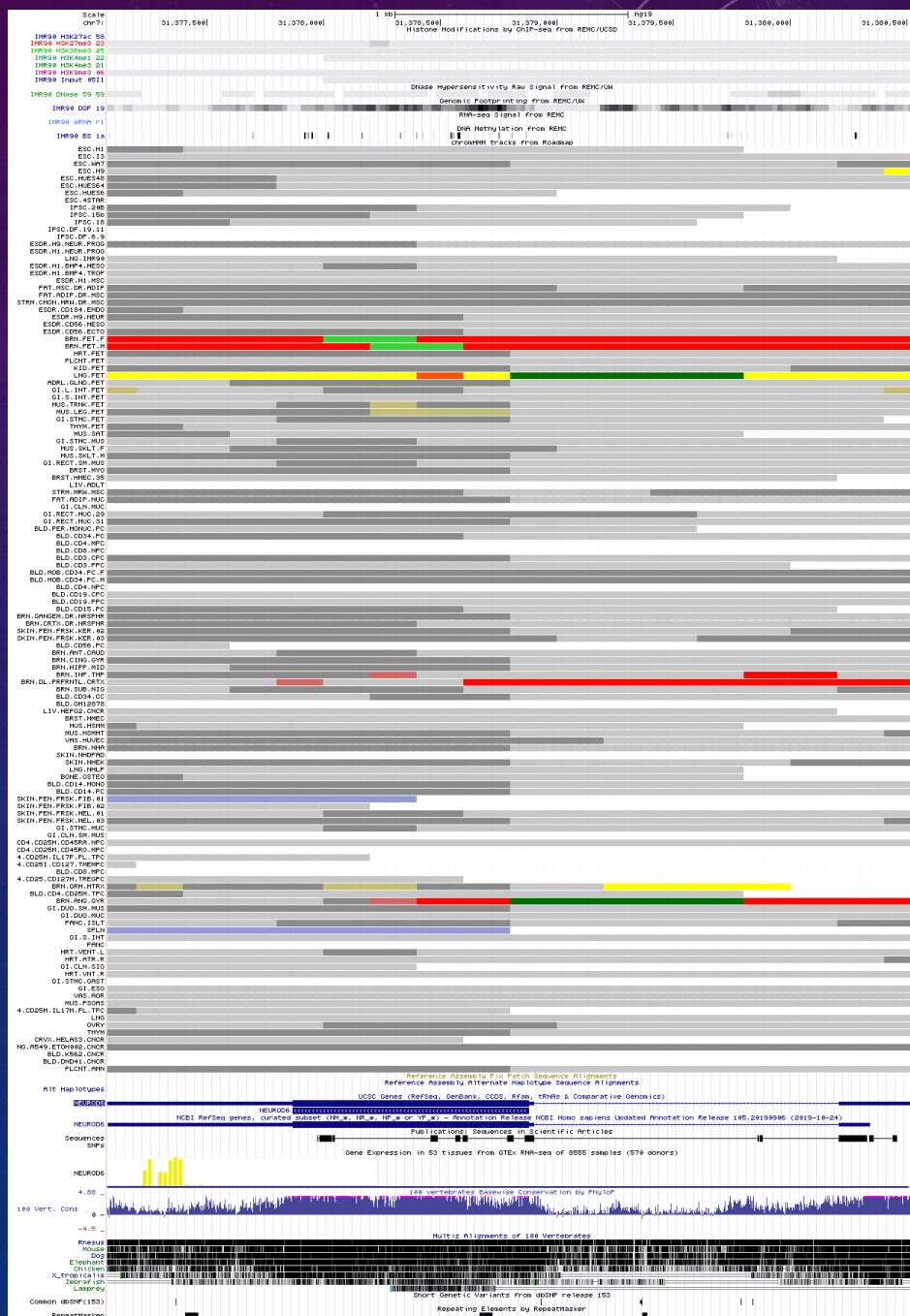
Integrative analysis of 111 reference human epigenomes. Nature. 2015

# DETECTING FUNCTIONAL ELEMENTS

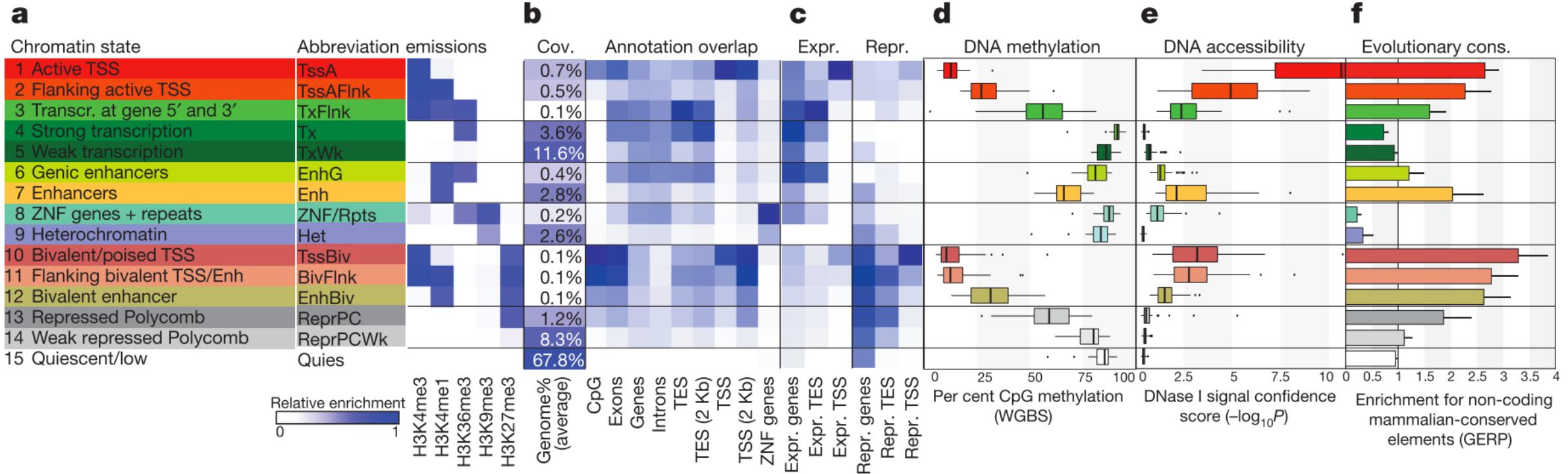
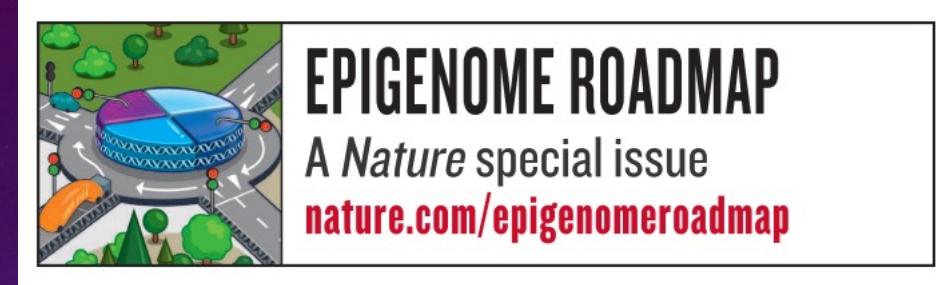
STATE NO.	MNEMONIC	DESCRIPTION
1	TssA	Active TSS
2	TssFlnk	Flanking TSS
3	TssFlnkU	Flanking TSS Upstream
4	TssFlnkD	Flanking TSS Downstream
5	Tx	Strong transcription
6	TxWk	Weak transcription
7	EnhG1	Genic enhancer1
8	EnhG2	Genic enhancer2
9	EnhA1	Active Enhancer 1
10	EnhA2	Active Enhancer 2
11	EnhWk	Weak Enhancer
12	ZNF/Rpts	ZNF genes & repeats
13	Het	Heterochromatin
14	TssBiv	Bivalent/Poised TSS
15	EnhBiv	Bivalent Enhancer
16	ReprPC	Repressed PolyComb
17	ReprPCWk	Weak Repressed PolyComb
18	Quies	Quiescent/Low

→ Segment chromatin →

ChromHMM



# BUILDING EPIGENOMES



Which elements are more/less methylated? Does it match with DNA accessibility?

# ASSIGNMENT DESCRIPTION

- Download whole epigenomes 18-states Roadmap
- Calculate jaccard index
- Cluster
- MDS
- Compare states

[HandsOn\\_epigenomics.pdf](#)

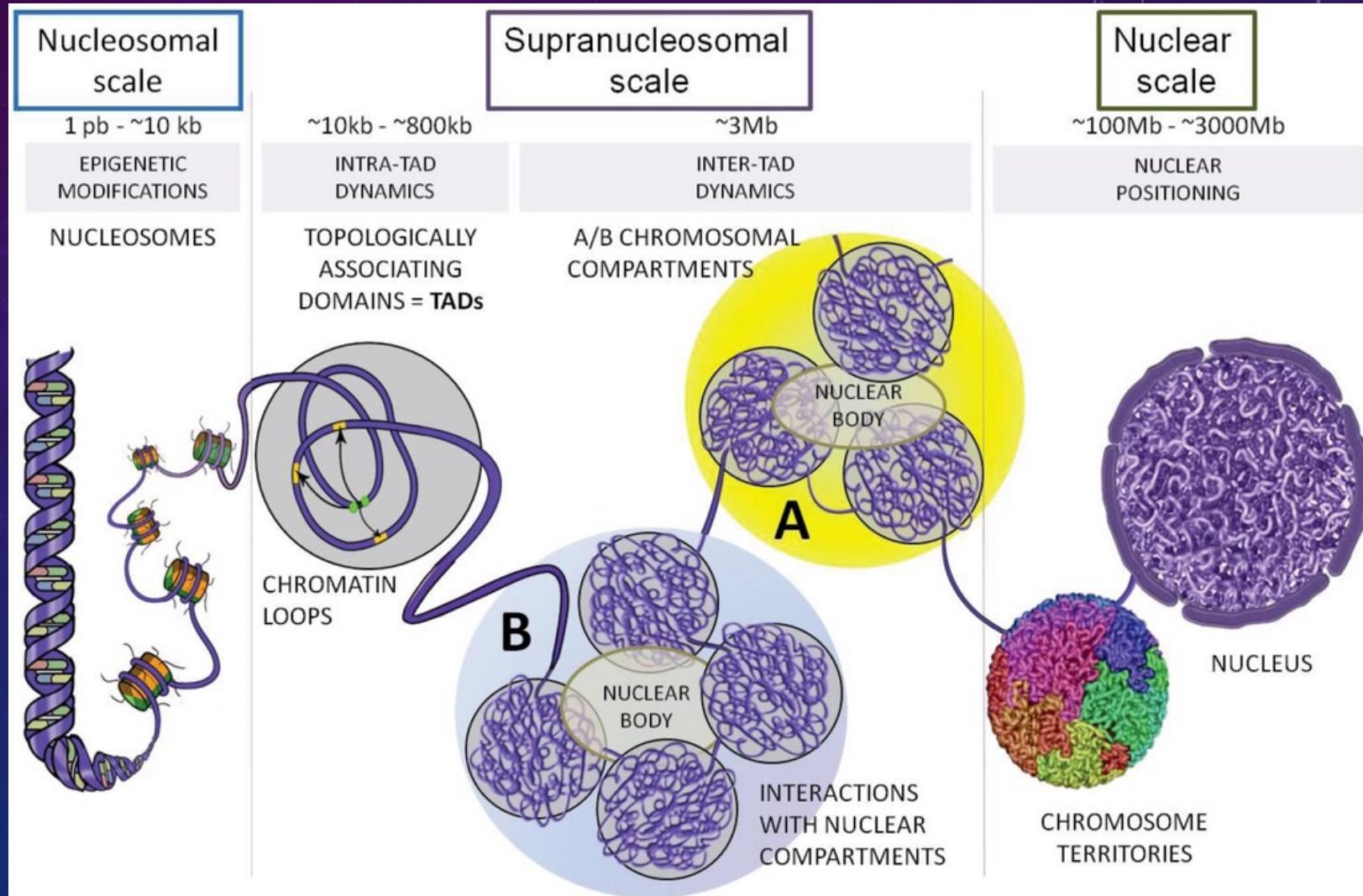
# 3D GENOMIC ARCHITECTURE

OMICS TECHNIQUES

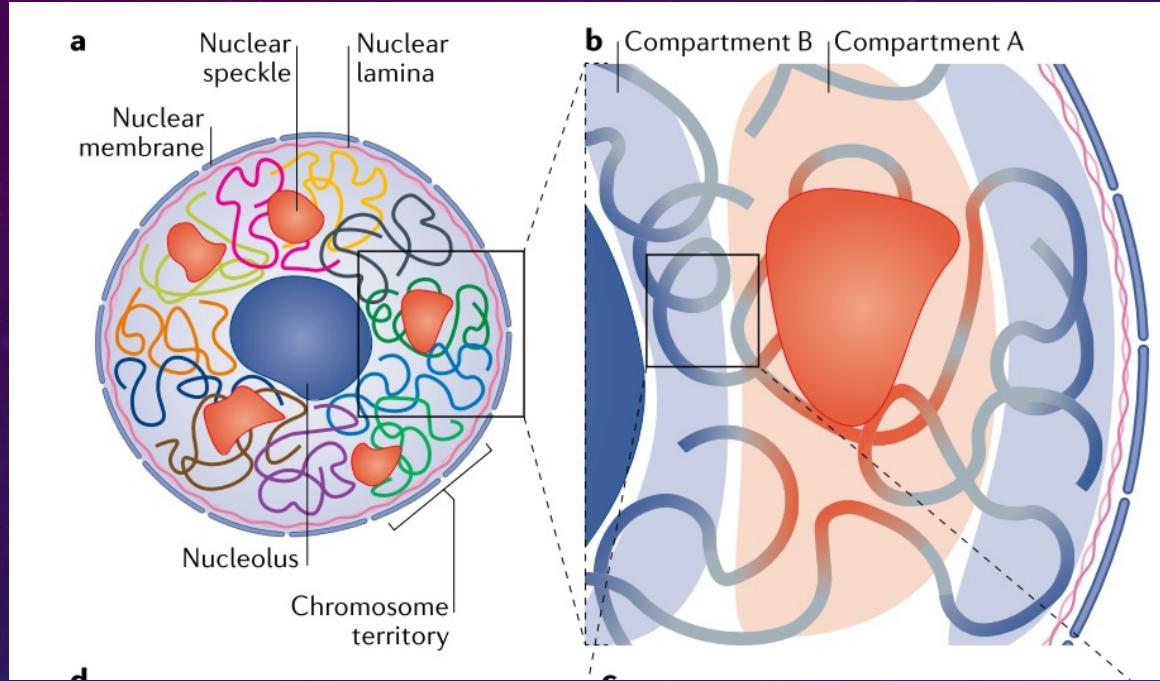
GABRIEL SANTPERE (GRIB)

# 3D STRUCTURE OF THE GENOME

- The genome is not linear but is hierarchically packaged
- Chromosome territories > compartments > TADs > chromatin loops



# TERRITORIES AND COMPARTMENTS

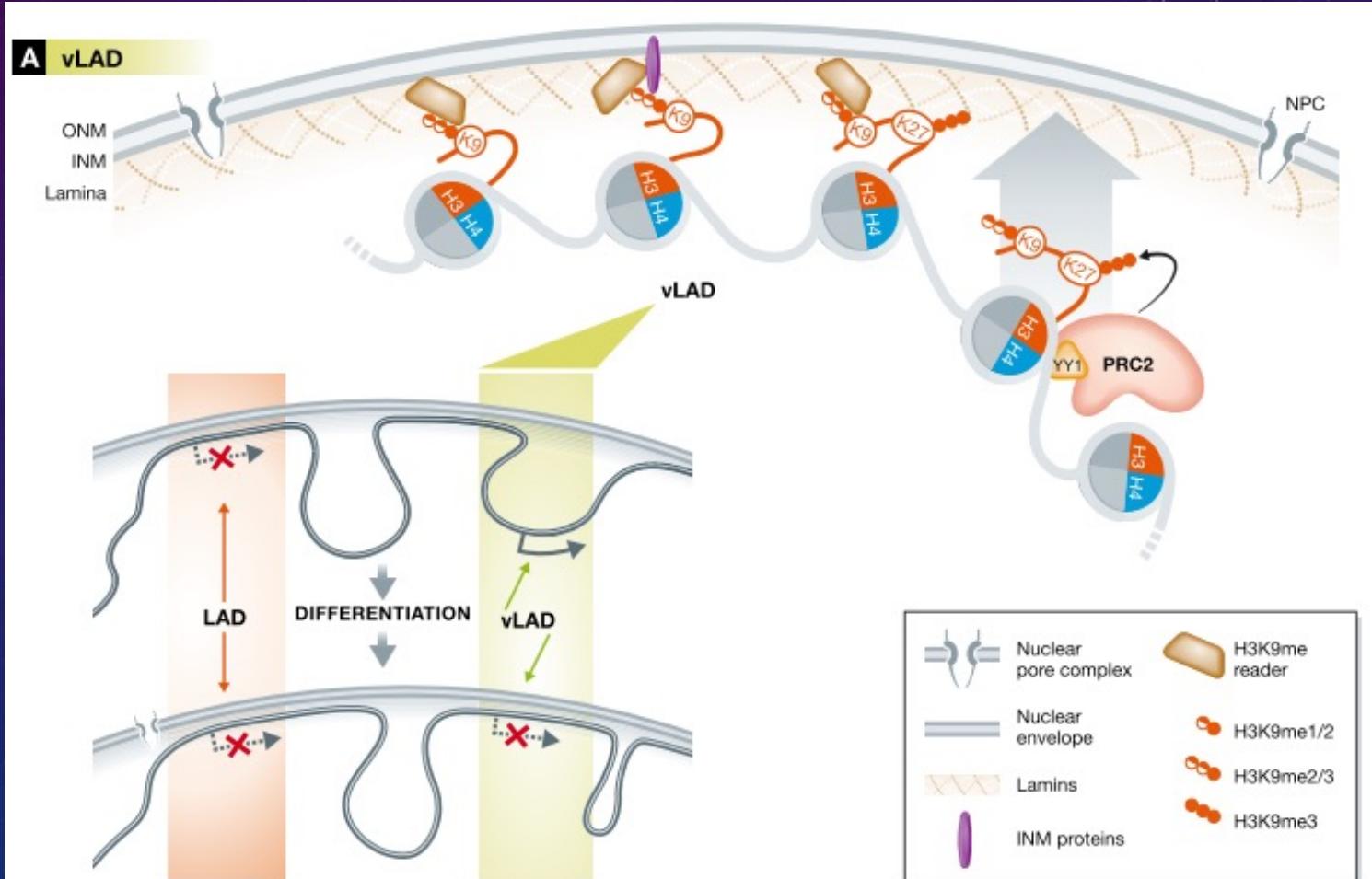


Nuclear speckles = splicing speckles: nuclear domains enriched in pre- mRNA splicing factors and located in interchromatin regions of the nucleoplasm

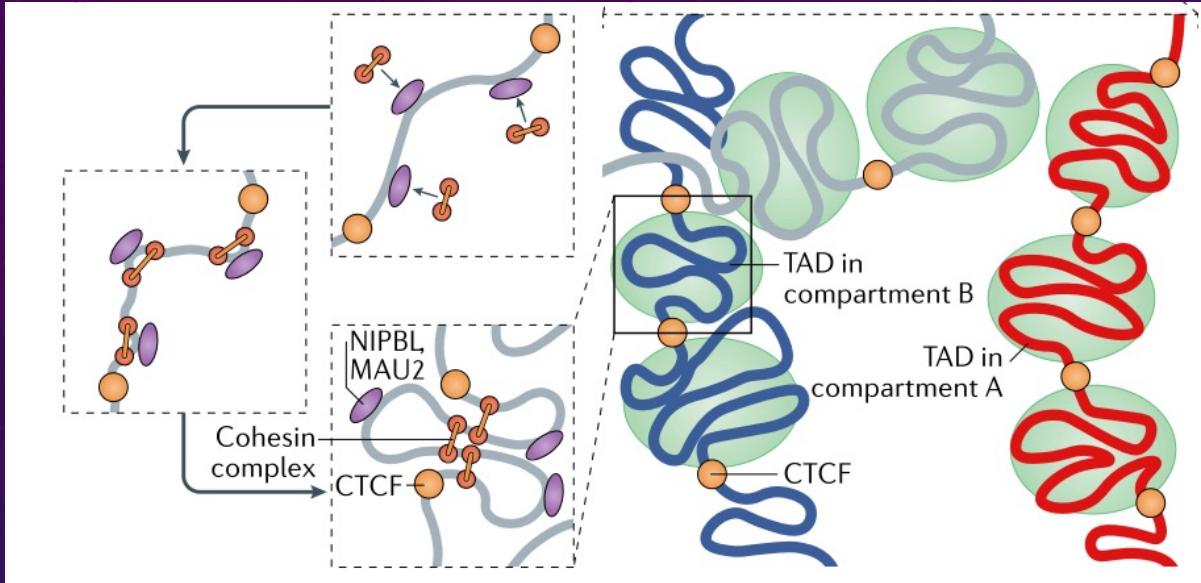
- Within nucleus chromosomes occupy separate **territories**.
- In each territory regions are not randomly distributed and where they are placed correlate with gene expression.
- Regions segregate in compartments A and B (Megabase scale):
  - A → Active expression → Interior nuclear space
    - Around splicing speckles
  - B → Inactive → Nuclear lamina or
    - Around nucleolus (Centromeres, rDNA)
- Compartments are dynamic during differentiation (e.g. ~40% switch in ESC differentiation))
  - Most genes do not change expression
  - Some does (activate when switching to A and vice-versa)
- Interaction with lamina depends on laminin proteins
  - KO laminin → re-localization to interior

# DNA POSITION IN THE NUCLEUS AFFECT GENE EXPRESSION

Some evidence that pulling DNA to the edge of the nucleus can cause silencing



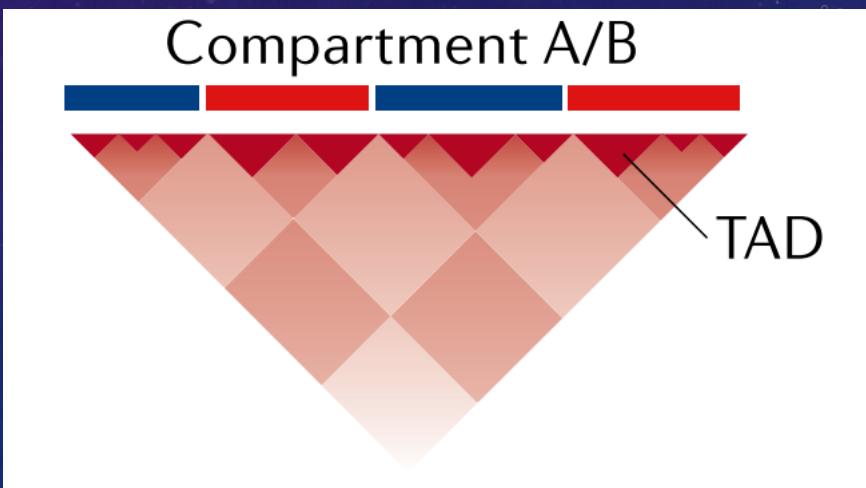
# TADS AND LOOPS



TAD = Topological Associating Domain

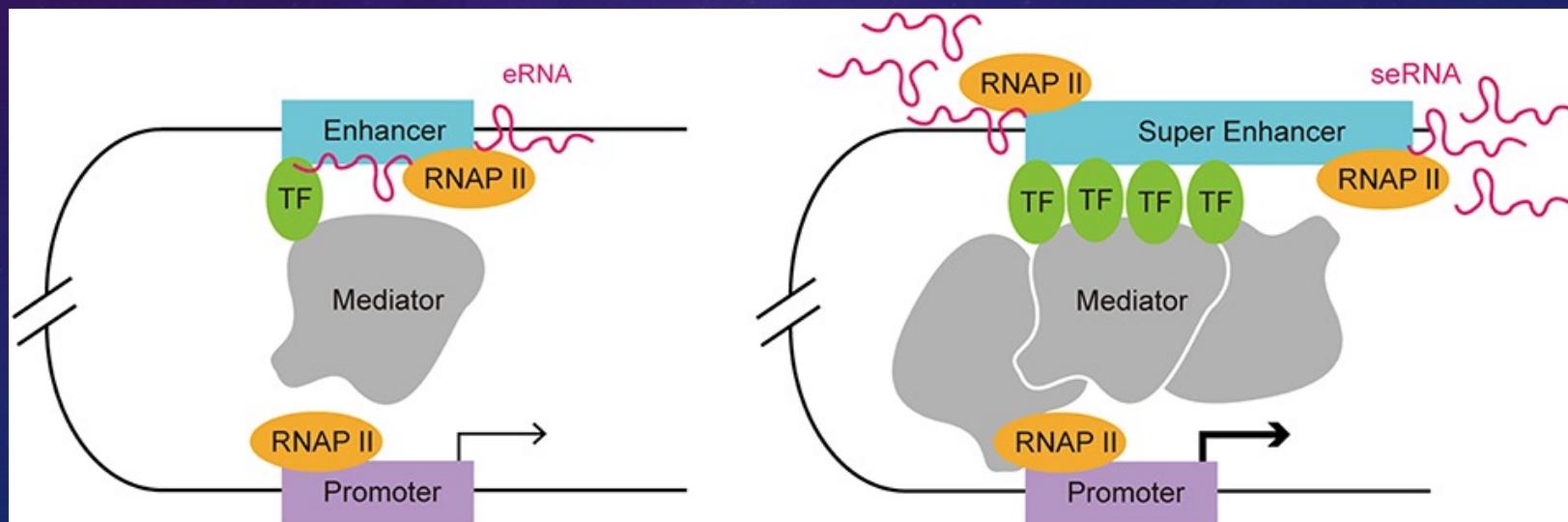
- Restrict enhancer/promoter interactions?
- TAD boundaries conserved across cell types
  - Except small subset
- More stable than compartments in differentiation
- Population level (individual cells show variation)
- Cohesin depletion models still see TAD (CTCF independent)
- Some other KO models loose of TADs and loops but modest impact on gene expression (depend of gene and celltypes?)

- TAD boundaries demarcated by
  1. CTCF
  2. Cohesin
  3. H3K4me3 and H3K36me3 (Active expression)

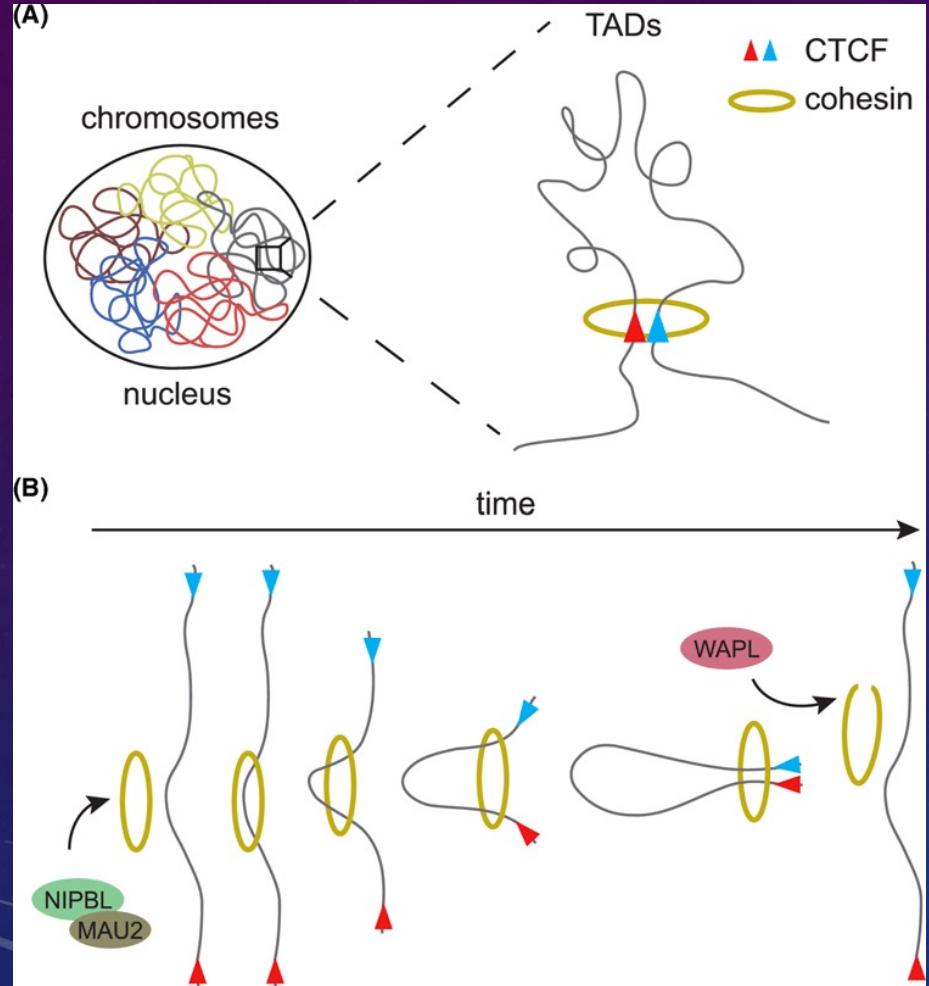


# FIRE: FREQUENTLY INTERACTING REGIONS

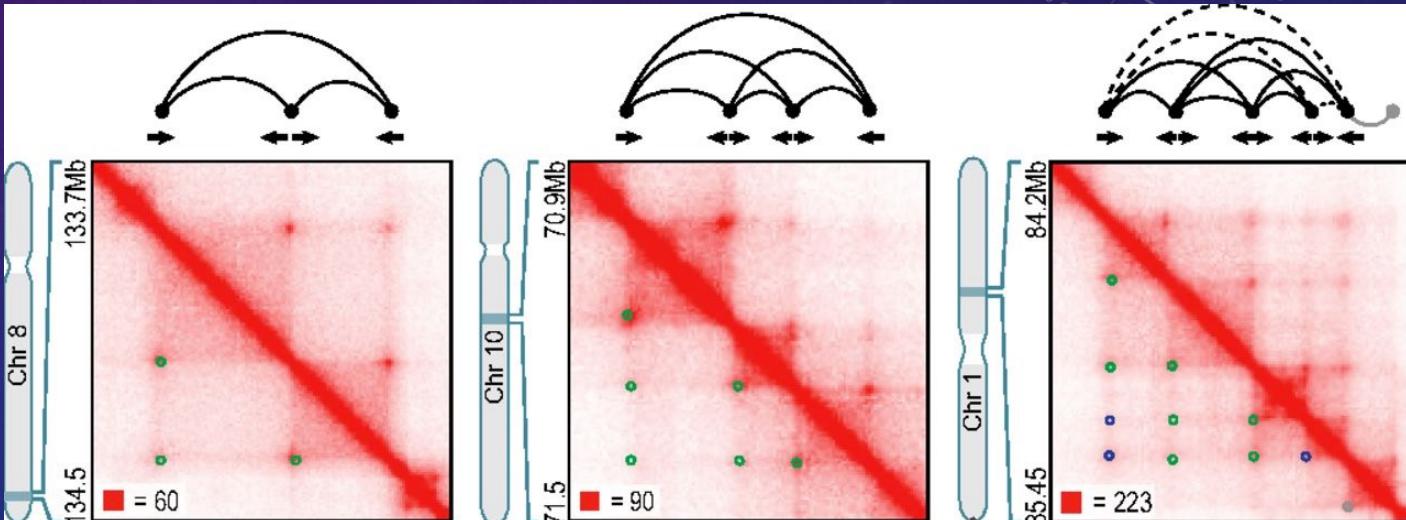
- Regions that interact very frequently.
- Smaller than TADs
- High tissue-specificity
- Enriched in enhancers and super-enhancers



# LOOP EXTRUSION MODEL

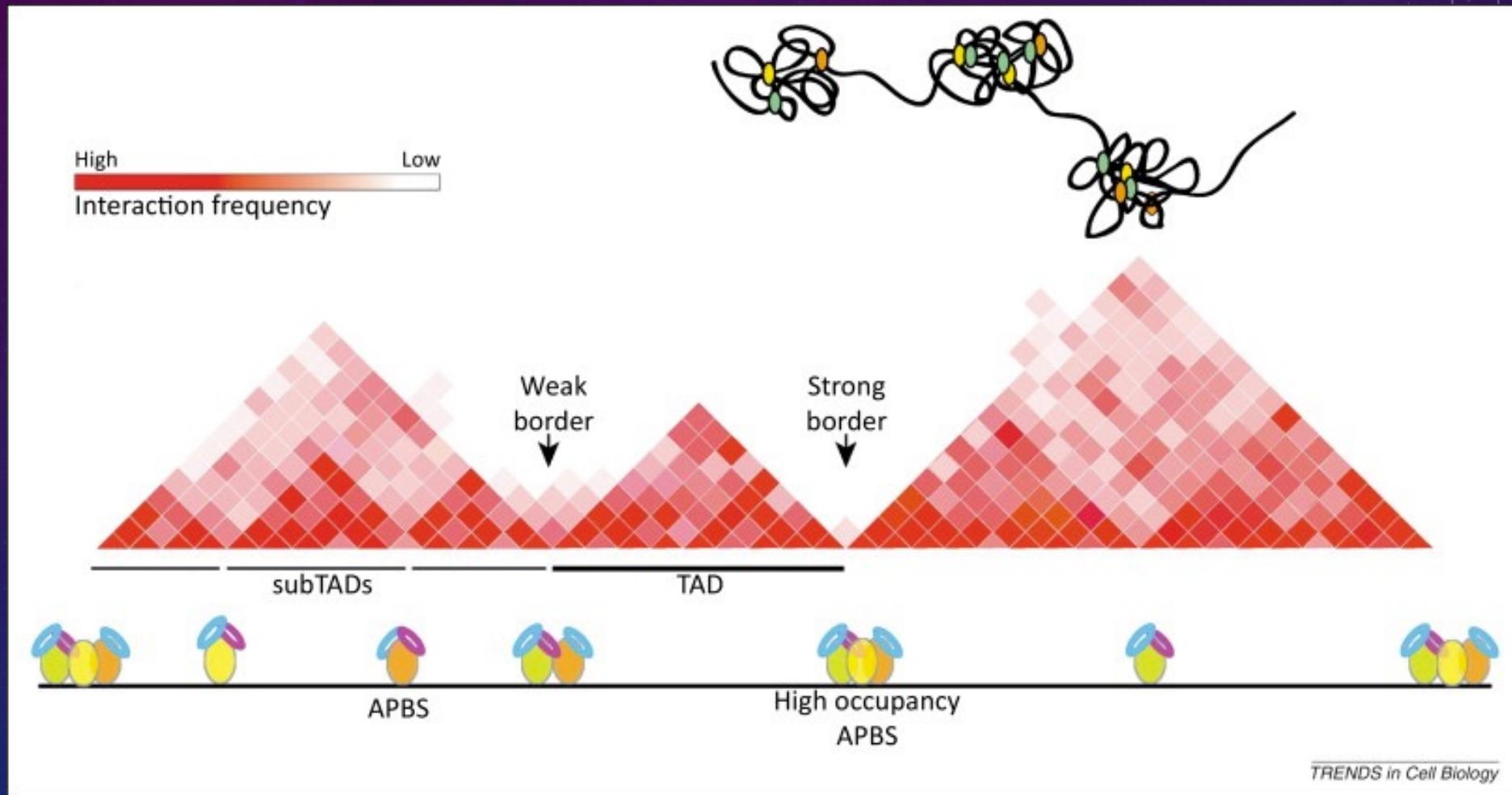


- Orientation of CTCF matters (facing each other)



Sanborn et al 2015 PNAS

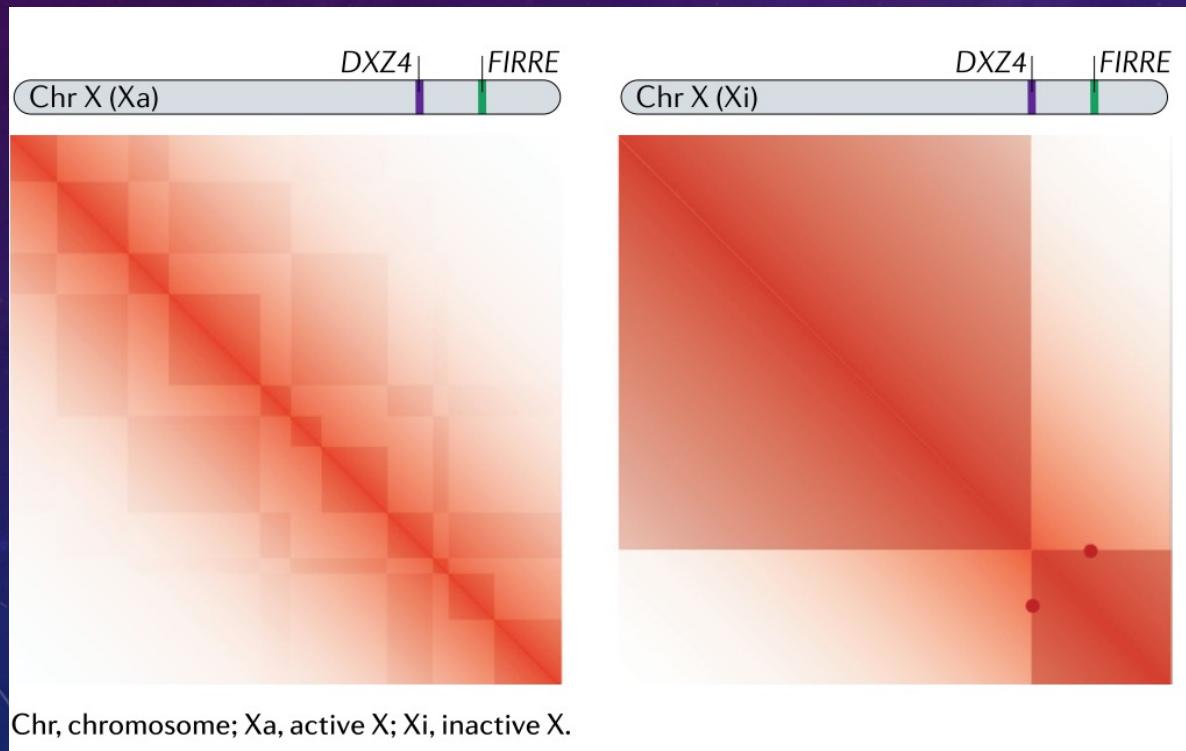
We are measuring/estimating frequencies of the interactions



# CHROMOSOME X INACTIVATION PART 2

## INTERACTION WITH LAMINA

- Recruitment of inactive chrX to lamina important but no sufficient for its inactivation



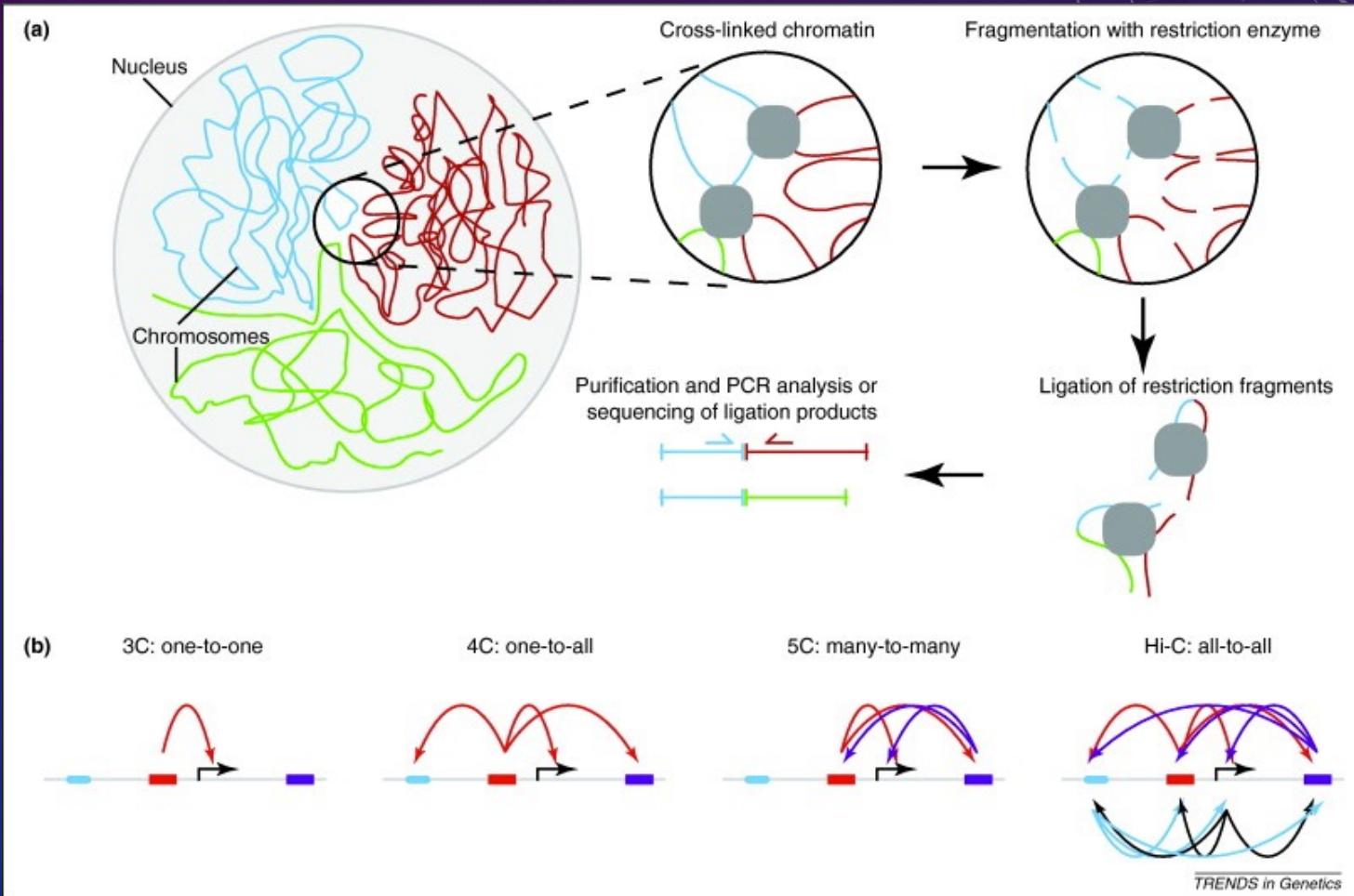
- The inactive does not form TADs
- Except in regions of genes escaping inactivation

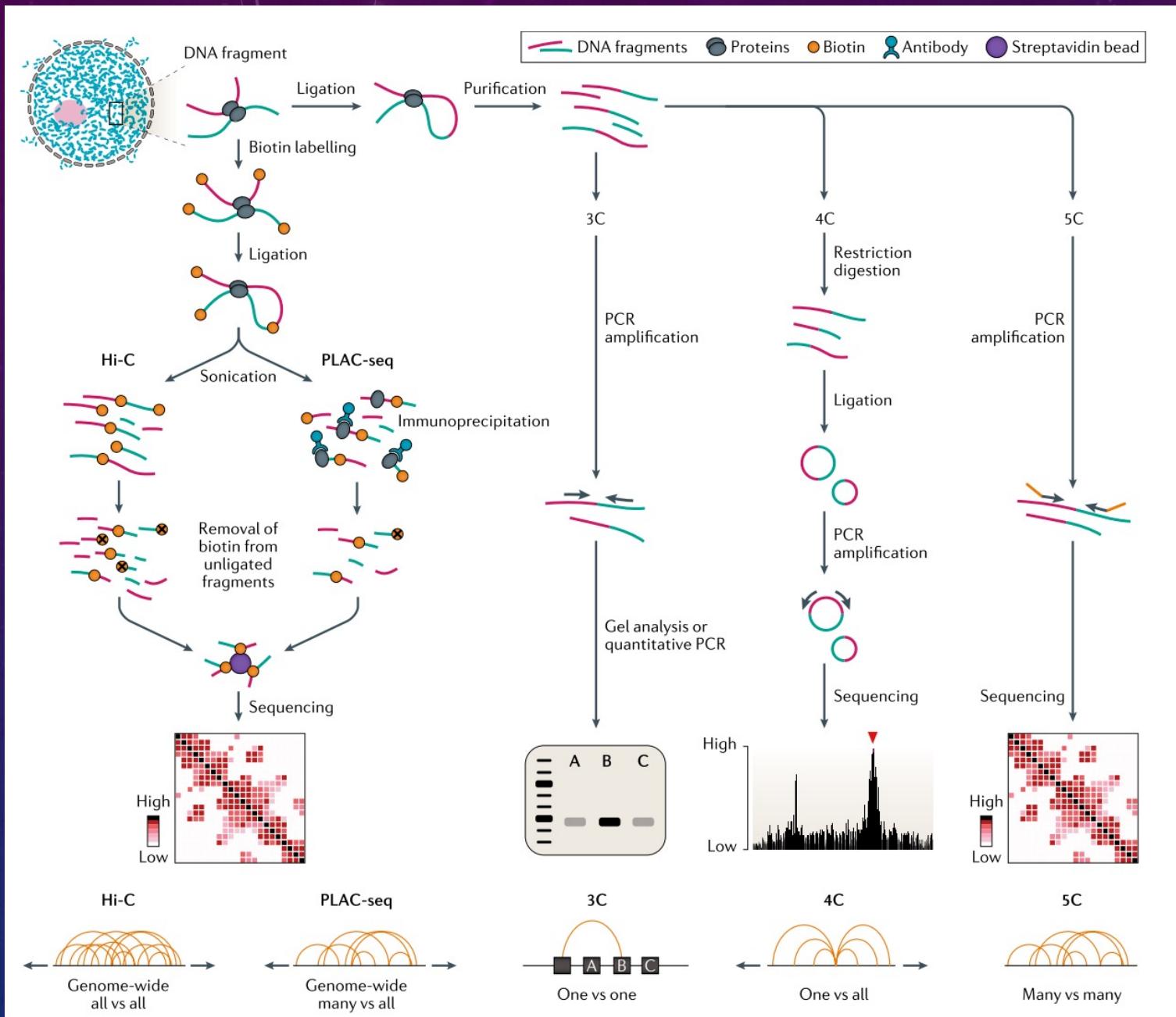
## 3C - Chromatin Conformation Capture methods

Assay	Description	Number of contacts per experiment	Multiplicity of contacts	Single-cell information	Number of cells	Detectable contacts
<b>3C-based methods</b>						
3C	Proximity ligation and selection of target regions with primers, detection by quantitative PCR	One versus one	Pairwise	No	100 million <sup>192</sup>	Protein-mediated
4C	Proximity ligation and enrichment for contacts with one bait region by inverse PCR, detection by sequencing	One versus all	Pairwise	No	Robust: 10 million <sup>193</sup> , low input: 340,000 (REF. <sup>194</sup> )	Protein-mediated
5C	Proximity ligation and enrichment for larger target region with primers, detection by sequencing	Many versus many	Pairwise	No	Robust: 50–70 million <sup>195</sup> , low input: 2 million <sup>196</sup>	Protein-mediated
Hi-C	Proximity ligation and enrichment for all ligated contact pairs, detection by sequencing	All versus all	Pairwise	No	Robust: 2–5 million <sup>64</sup> , low input: 100,000–500,000 (REFS <sup>70,197</sup> )	Protein-mediated
TCC	Tethered proximity ligation and enrichment for all ligated contact pairs, detection by sequencing	All versus all	Pairwise	No	25 million <sup>57</sup>	Protein-mediated
PLAC-seq, ChIA-PET	Proximity ligation and pull-down of specific protein-mediated contacts, detection by sequencing	Many versus many	Pairwise	No	Robust: 100 million <sup>198</sup> , low input: 500,000 (REF. <sup>81</sup> )	Protein-mediated (specific)
Capture-C, C-HiC	Proximity ligation and target enrichment using probes for genomic regions of interest, detection by sequencing	Many versus all	Pairwise	No	Robust: 100,000 (REF. <sup>199</sup> ), low input: 10,000–20,000 (REF. <sup>97</sup> )	Protein-mediated
Single-cell Hi-C	Proximity ligation and enrichment for all ligated contact pairs, detection by sequencing	All versus all	Pairwise	Yes	Hundreds	Protein-mediated

# HOW TO MEASURE 3D CHROMATIN INTERACTION

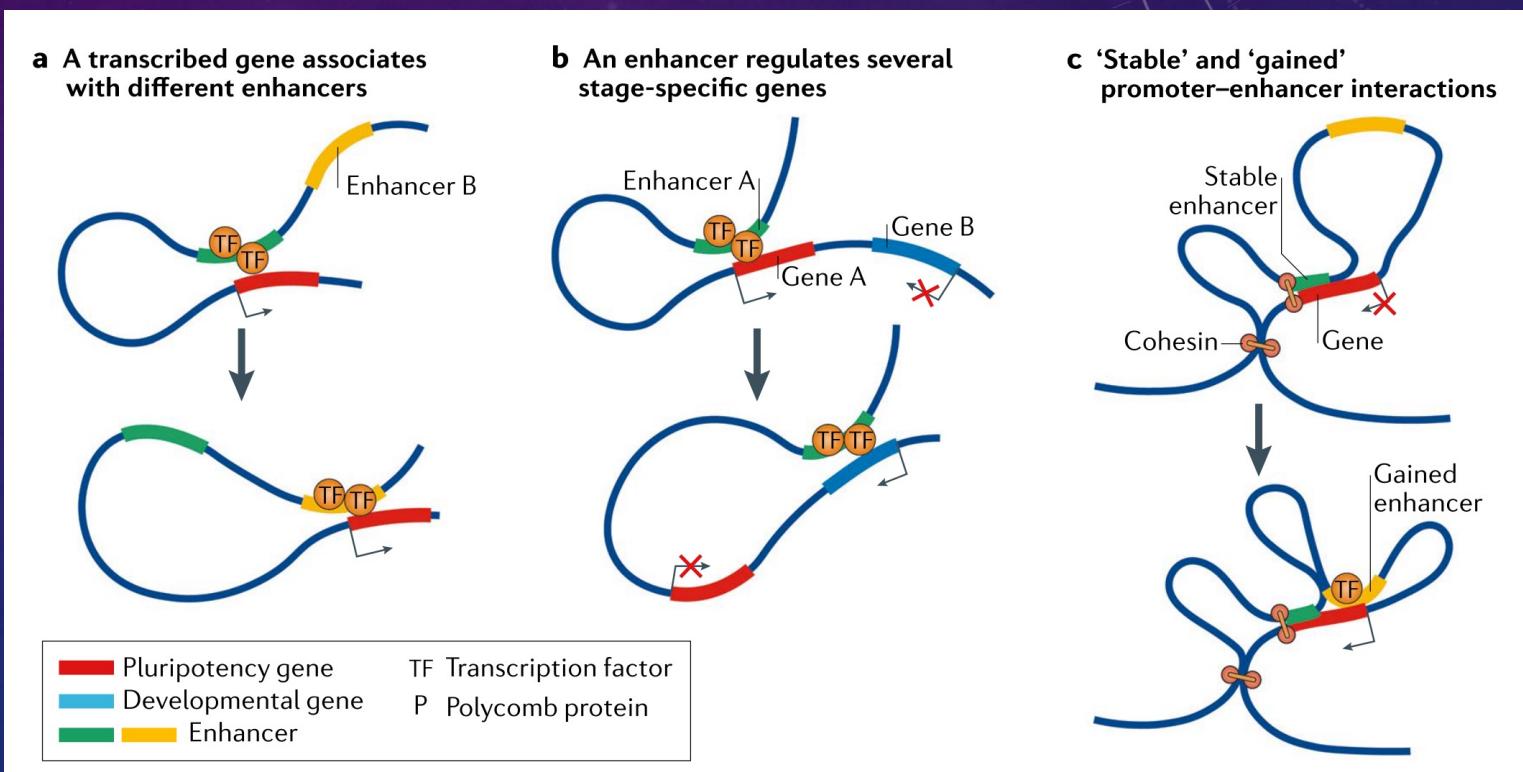
- Fix interactions using formaldehyde
- Digest DNA with restriction enzymes
- Quantify frequency of interactions by next generation sequencing



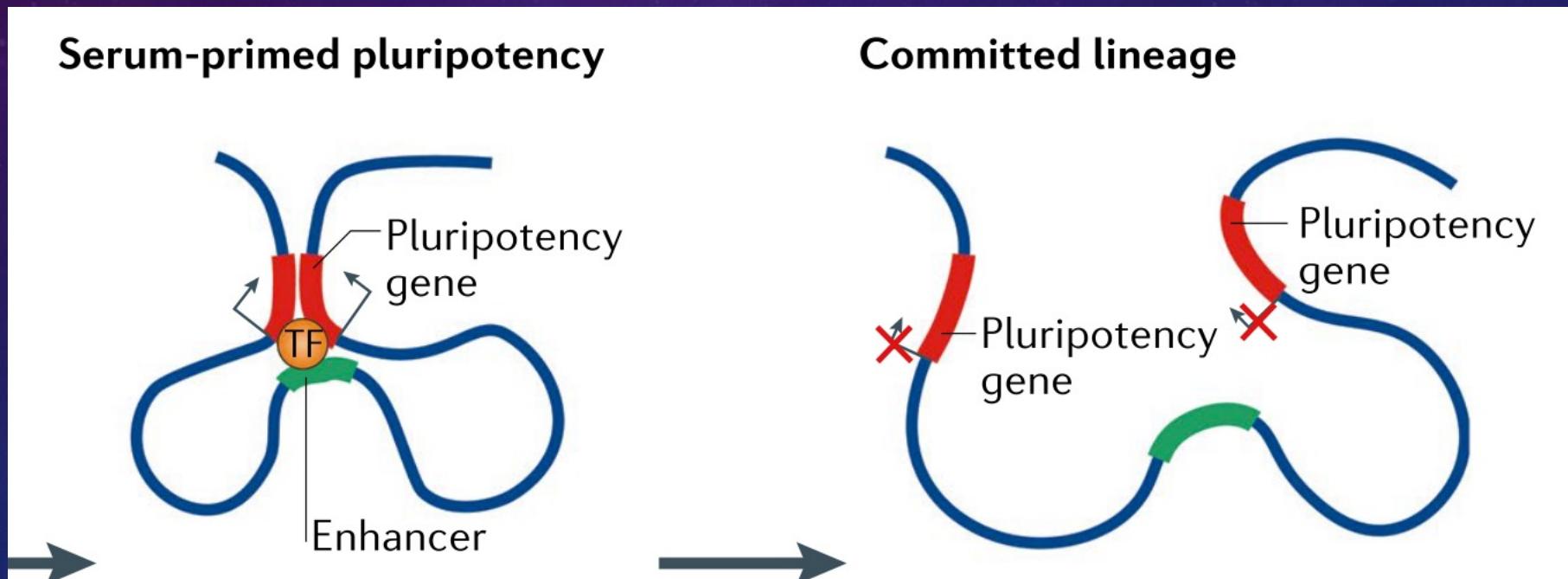


# ENHANCER/PROMOTER INTERACTIONS

- Facilitated within delimited TADs
- Regulated by TFs and/or cohesin and CTCF
- Experimentally forcing a loop can increase expression
- Some E/P interactions occur “in preparation” for gene activation
- Other models proposed: “collision”, increased mobility of CRE within TAD

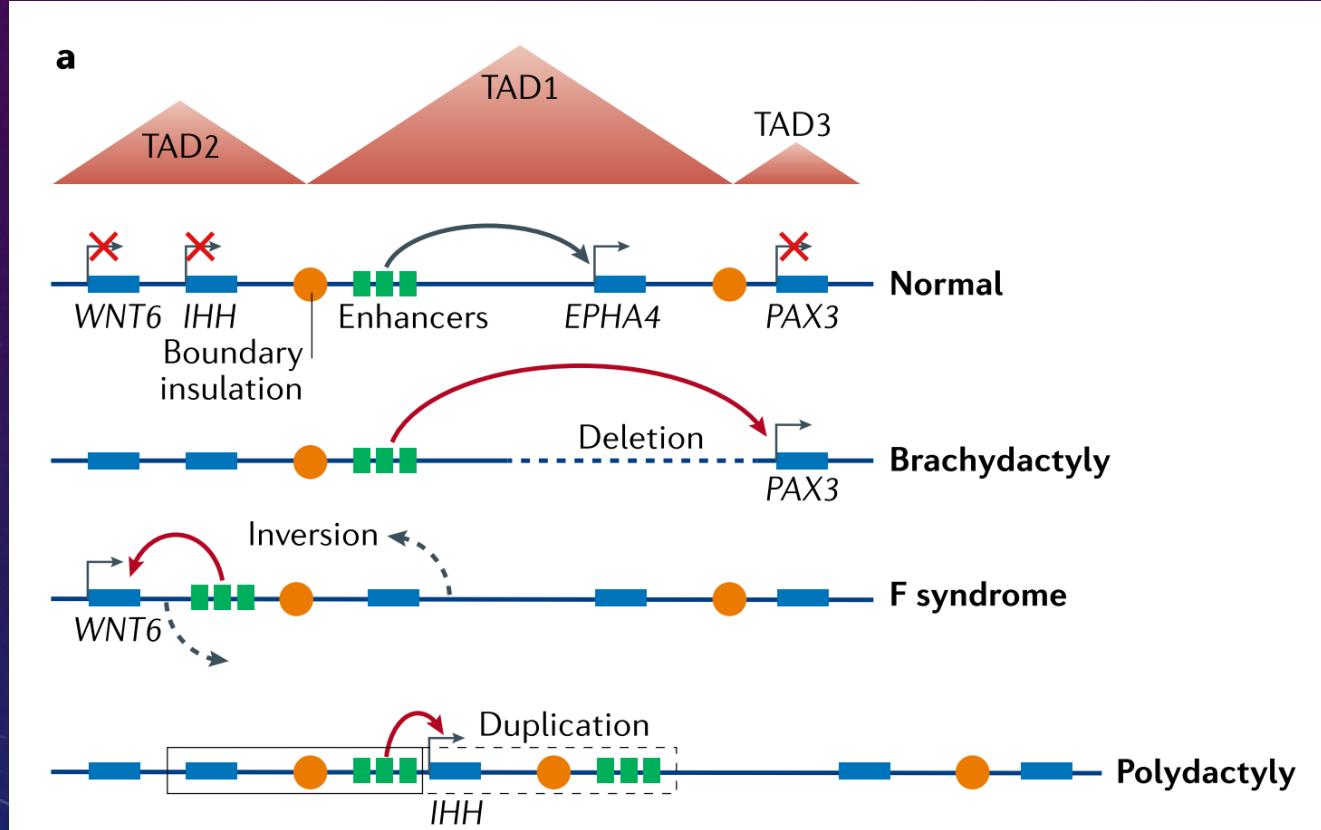


- Promoter-Promoter interactions
  - Co-expressed genes
  - Promoters can behave as enhancers
- Convergent interactions
- Dynamic process



Zheng and Xie; Nature Reviews 2019

# 3D ALTERATIONS AND DISEASE



- Many disease- associated mutations of the linear genomic sequence can only be understood by considering their 3D conformation in nuclear space.
- Disrupting regions containing isolators can alter gene expression.
- Altering CTCF and cohesion can have deleterious effects.