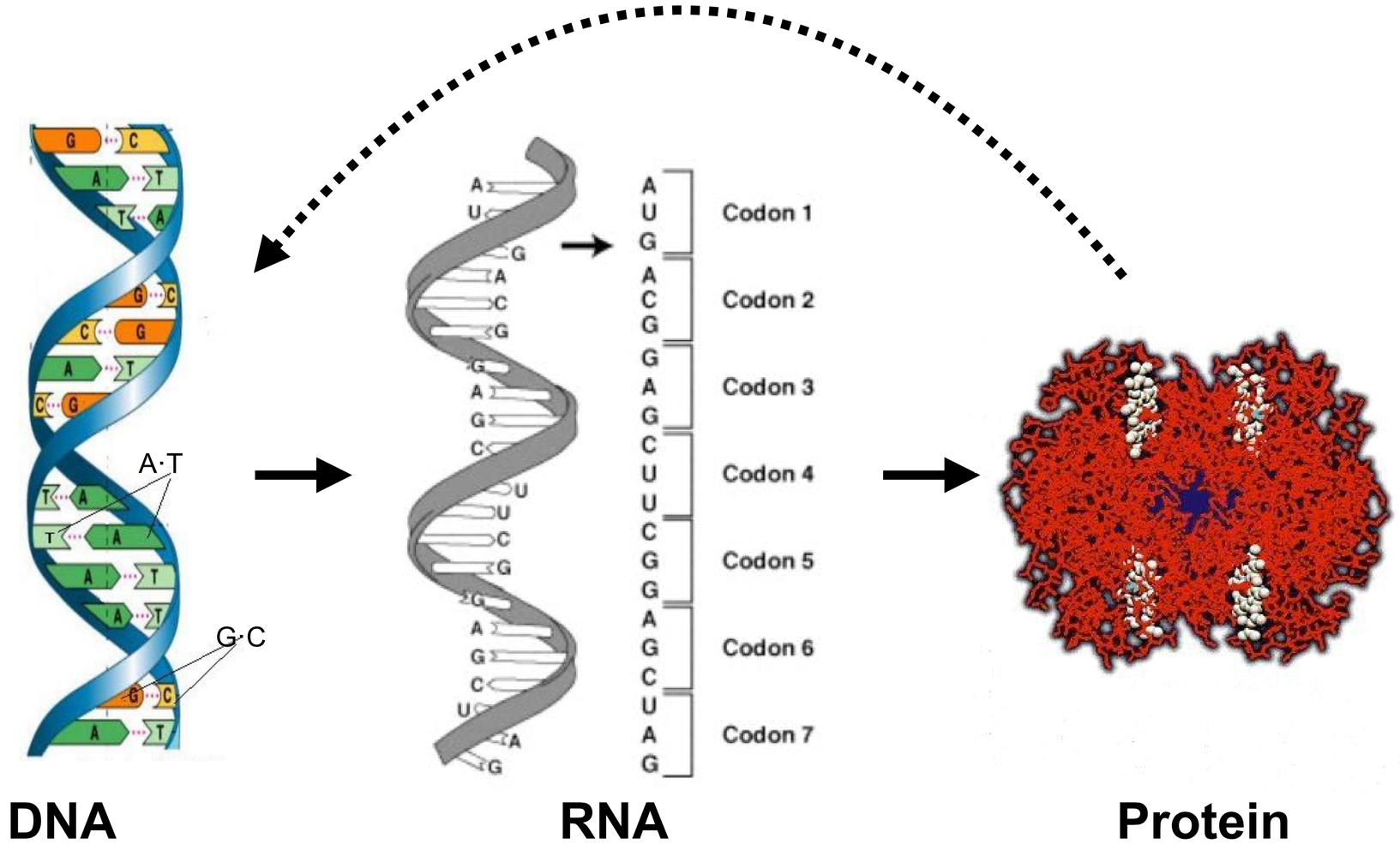
Gene regulation programs in a chromatin context

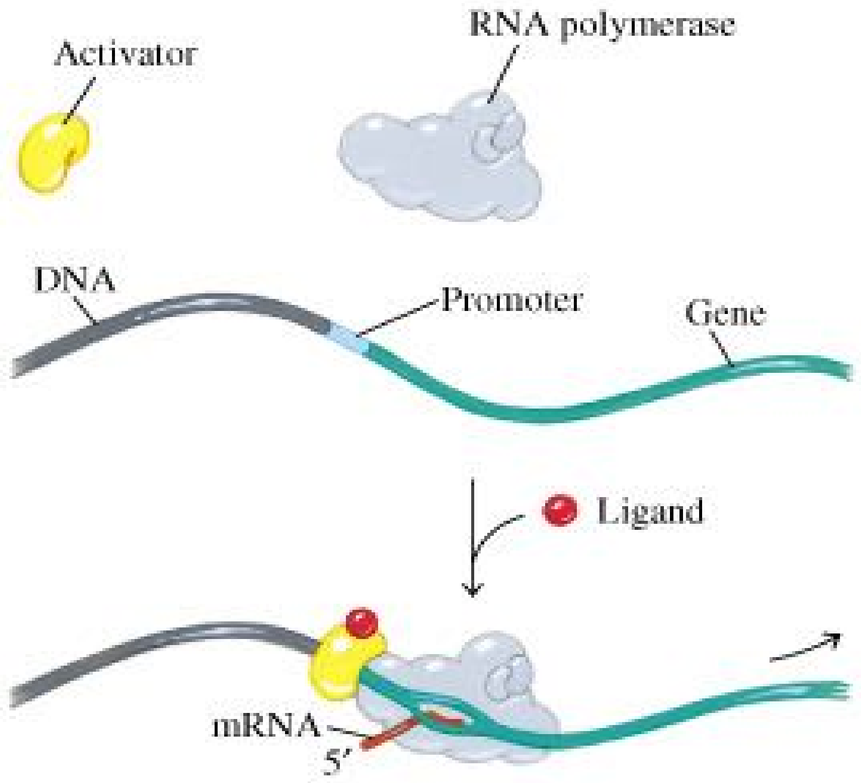
The central dogma in molecular biology



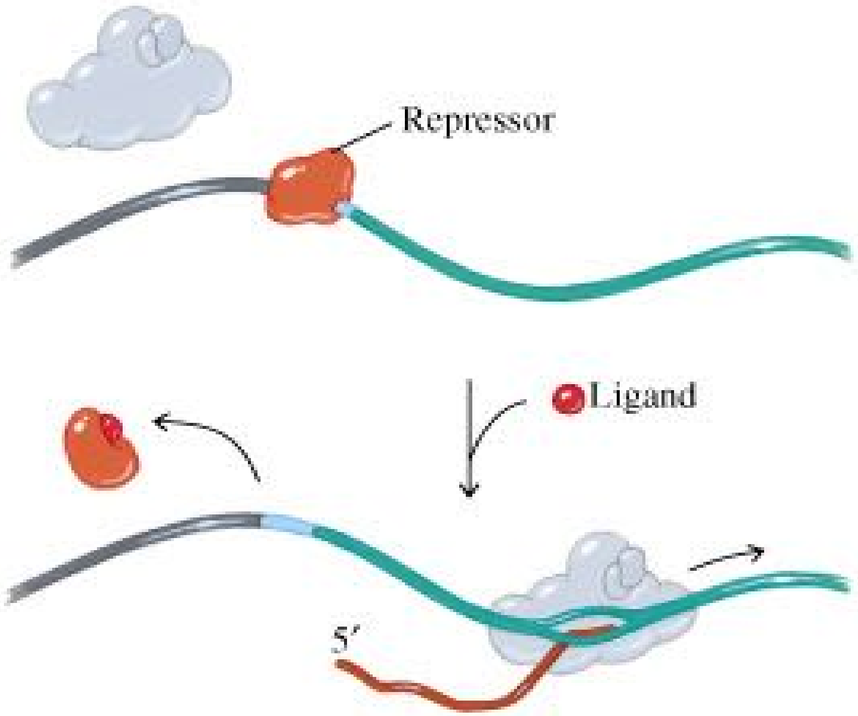
… with some feedback loops

Transcription regulation in bacteria

Activator



Repressor



Regulator concentration -> promoter site occupancy -> transcription level

The basic description of protein binding to DNA

$$AB \underset{k_{on}}{\overset{k_{off}}{\rightleftharpoons}} A+B$$

k_{off} in s^{-1} is the reaction rate constant for dissociation

k_{on} in $M^{-1}\ s^{-1}$ is the reaction rate constant for binding

$$\frac{k_{off}}{k_{on}} = K_d$$

relation to the equilibrium dissociation constant

$$\frac{1}{k_{off}} = \tau$$

life time of the complex

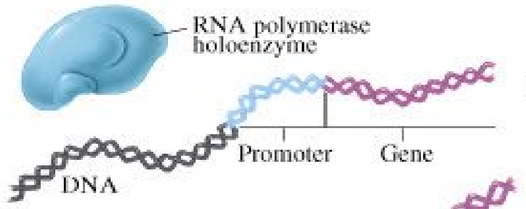
$$\frac{d[AB]}{dt} = k_{on} \cdot [A] \cdot [B] - k_{off} \cdot [AB]$$

rate equation for complex formation,

can be solved but it is already difficult

k_{on} cannot be higher than 10^8 - 10^9 $M^{-1}\ s^{-1}$ for a diffusion controlled reaction

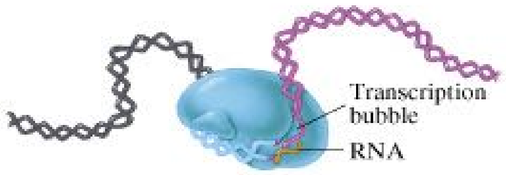
Initiation of transcription in the bacterium E. coli



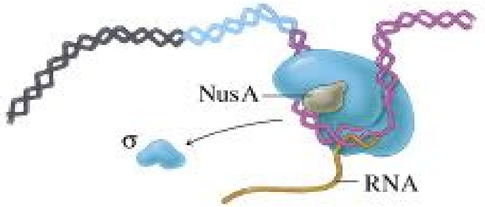
(a) RNA polymerase holoenzyme binds nonspecifically to DNA.

(b) The holoenzyme conducts a one-dimensional search for a promoter.

(c) When a promoter is found, the holoenzyme and the promoter form a closed complex.

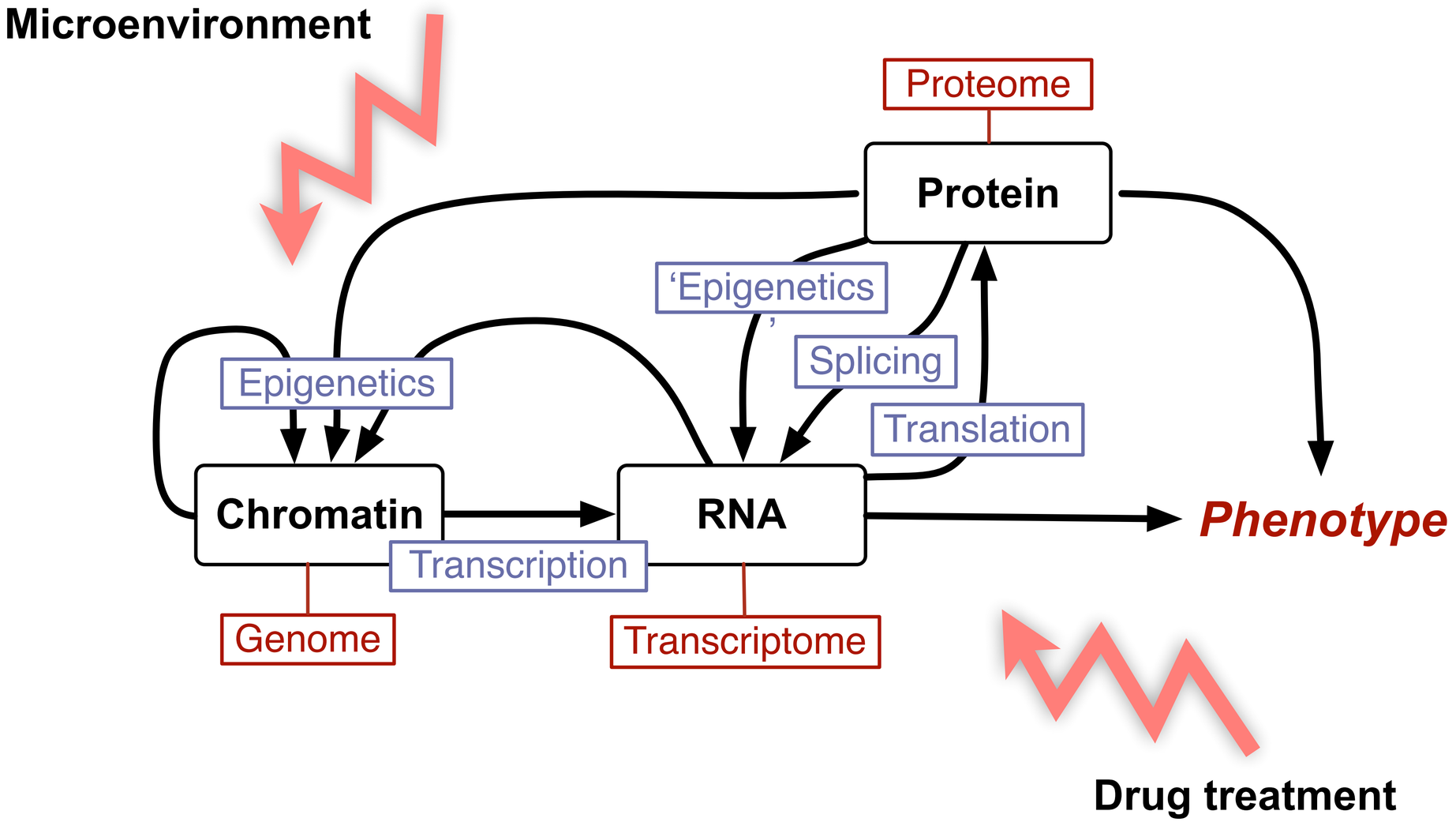


(d) A conformational change from the closed complex to an open complex produces a transcription bubble at the initiation site. A short stretch of RNA is then synthesized.



(e) The σ subunit dissociates from the core enzyme, and RNA polymerase clears the promoter. Accessory proteins, including NusA, bind to the polymerase.

A complex network links DNA to phenotype in eukaryotes

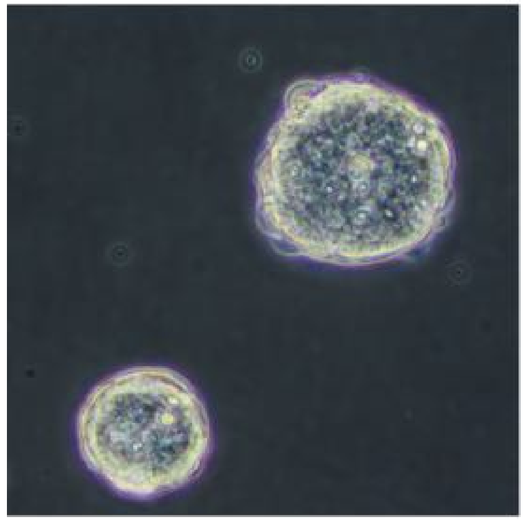


Epigenetic differences determine gene expression programs in higher eukaryotes

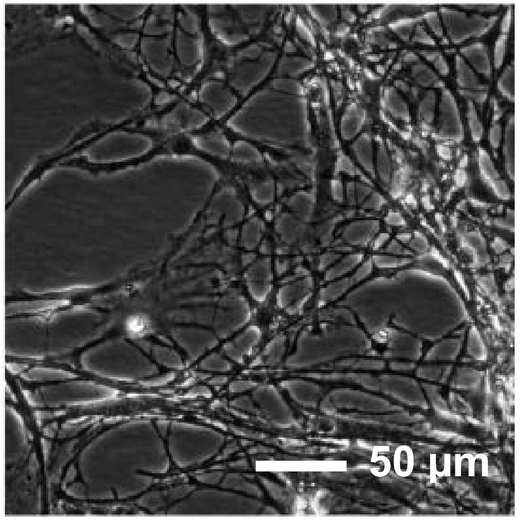
1% genomic differences

Image: James Balog Getty Images

Embryonic stem cells



Neural cells



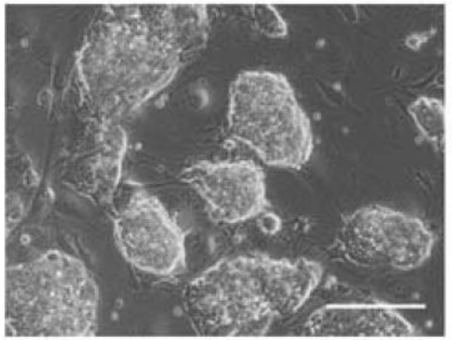
No genomic differences

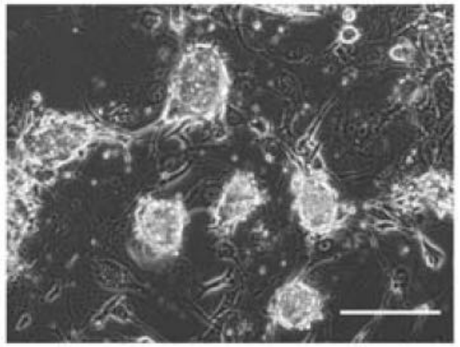
Reversible and heritable (re)programming of cell types

Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors

Kazutoshi Takahashi[1] and Shinya Yamanaka[1,2,*]
[1] Department of Stem Cell Biology, Institute for Frontier Medical Sciences, Kyoto University, Kyoto 606-8507, Japan
[2] CREST, Japan Science and Technology Agency, Kawaguchi 332-0012, Japan
*Contact: yamanaka@frontier.kyoto-u.ac.jp
DOI 10.1016/j.cell.2006.07.024

Embryonic stem cells

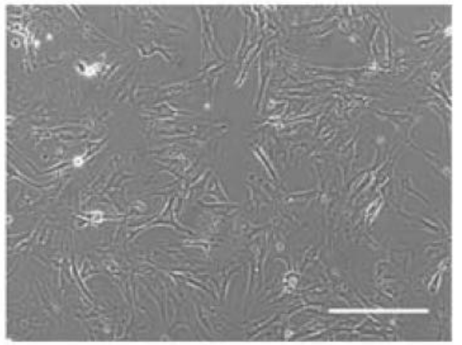




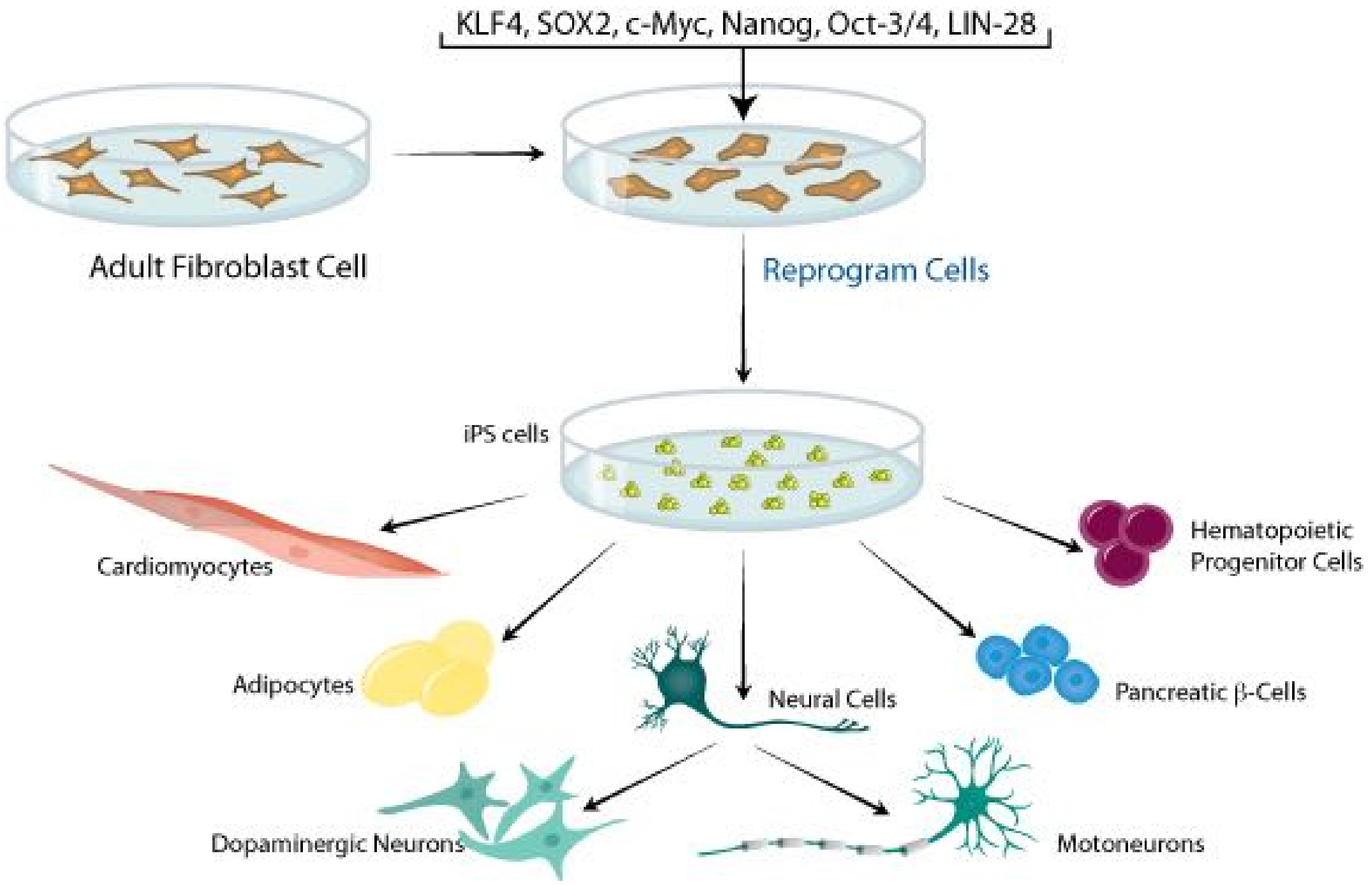
Induced pluripotent cells

Transient expression of
Oct4, Sox2 Klf4, c-Myc,

Fibroblasts

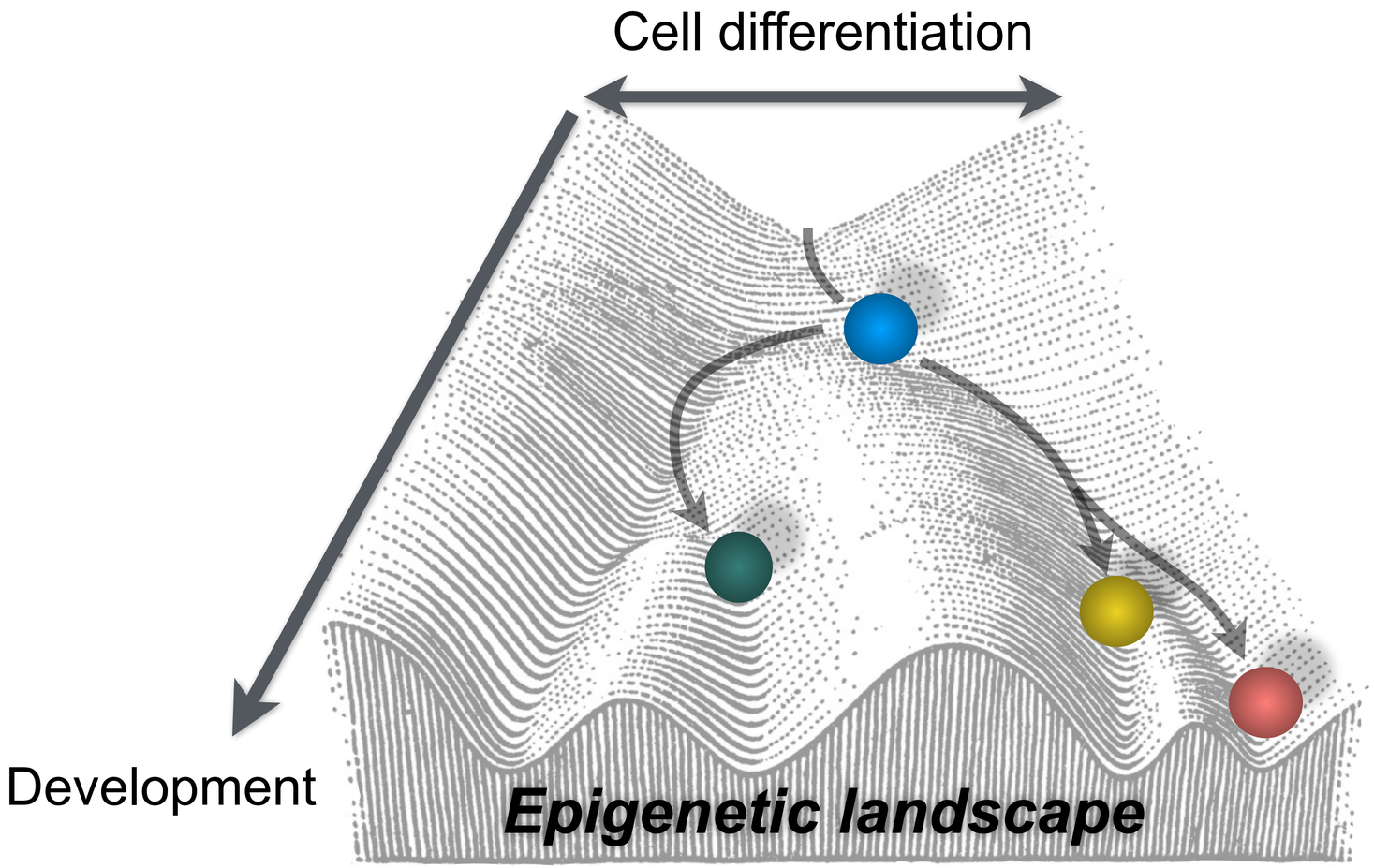


Reprogramming pluripotent stem cells by over-expressing Oct4, Sox2, cMyc and Klf4



Takahashi, K; Yamanaka, S (2006). "Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors". *Cell* **126** (4): 663–76

The “epigenetic landscape” from Conrad Waddington



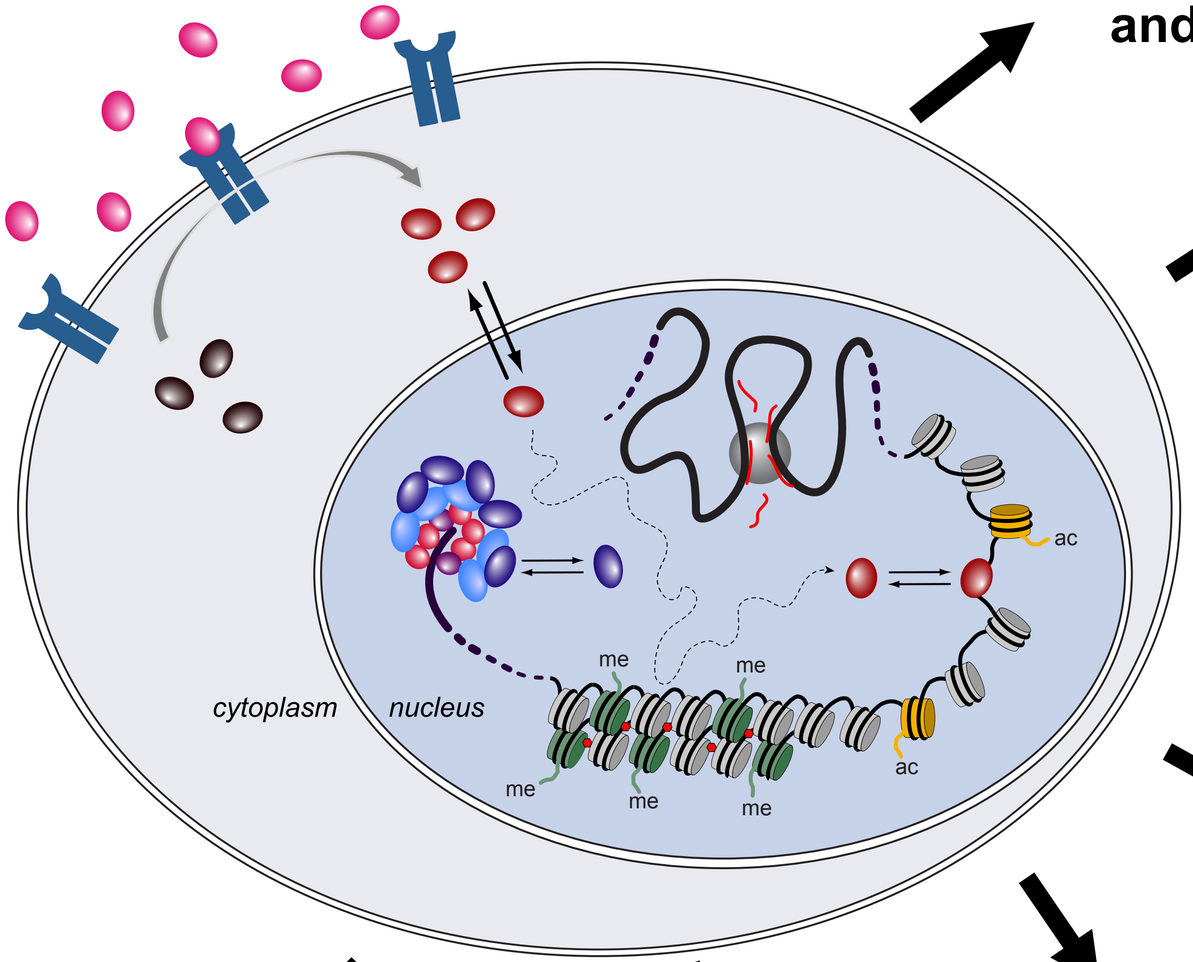
Scheme adapted from Conrad Waddington 1957, The strategy of the genes

A current definition of an epigenetic trait

*An epigenetic trait is **a stably heritable** phenotype resulting from changes in a chromosome without alterations in the DNA sequence.*

Berger et al. 2009, Genes & Development

A cell’s epigenetic landscape reflects…



Gene activity, regulatory mechanisms and deregulation in disease

Cell type/tumor cell of origin

Metabolism (acetyl-CoA, α-ketogluarate, s-adenosyl methionine)

Responsiveness to cell signaling

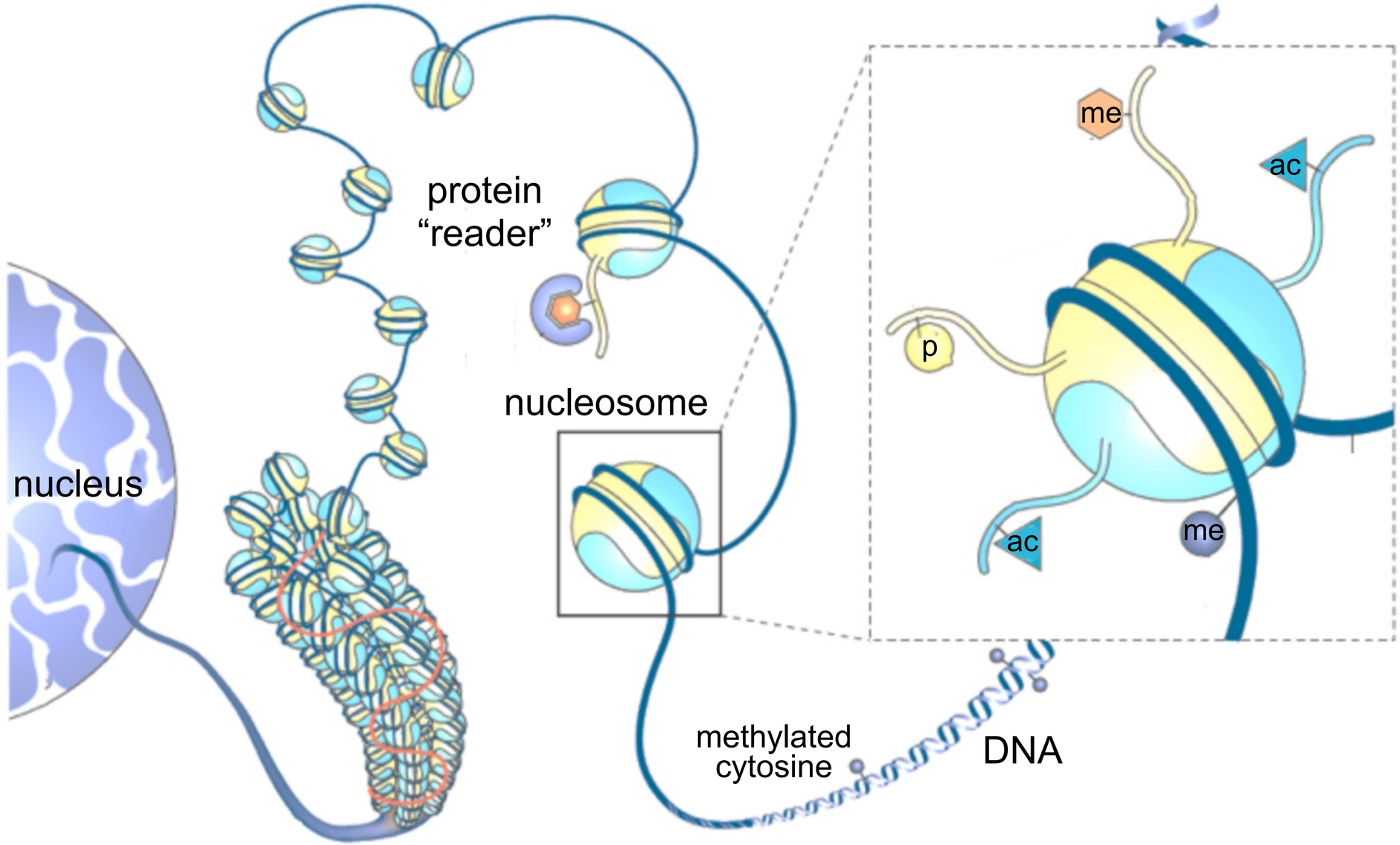
’Memory’ about previous stimulation

Environmental factors (smoking, asbestos, chemicals etc)

Chronic inflammation

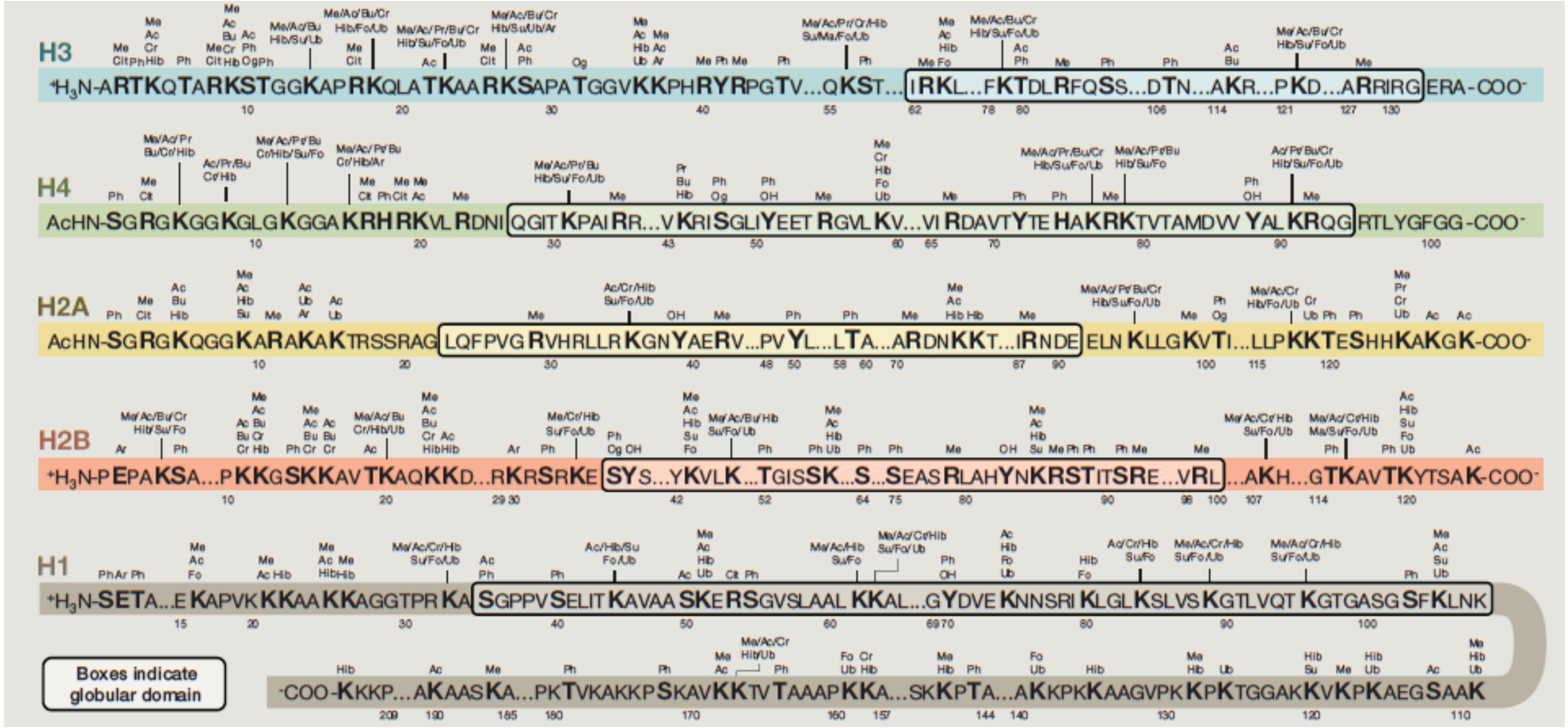
Aging

Epigenetic signals define access to the DNA genome and cell type specific chromatin state patterns



Probst, Nat Rev Mol Cell Biol

The histone code: At least 17 different modifications at 128 core histone sites plus 48 linker histone H1 sites



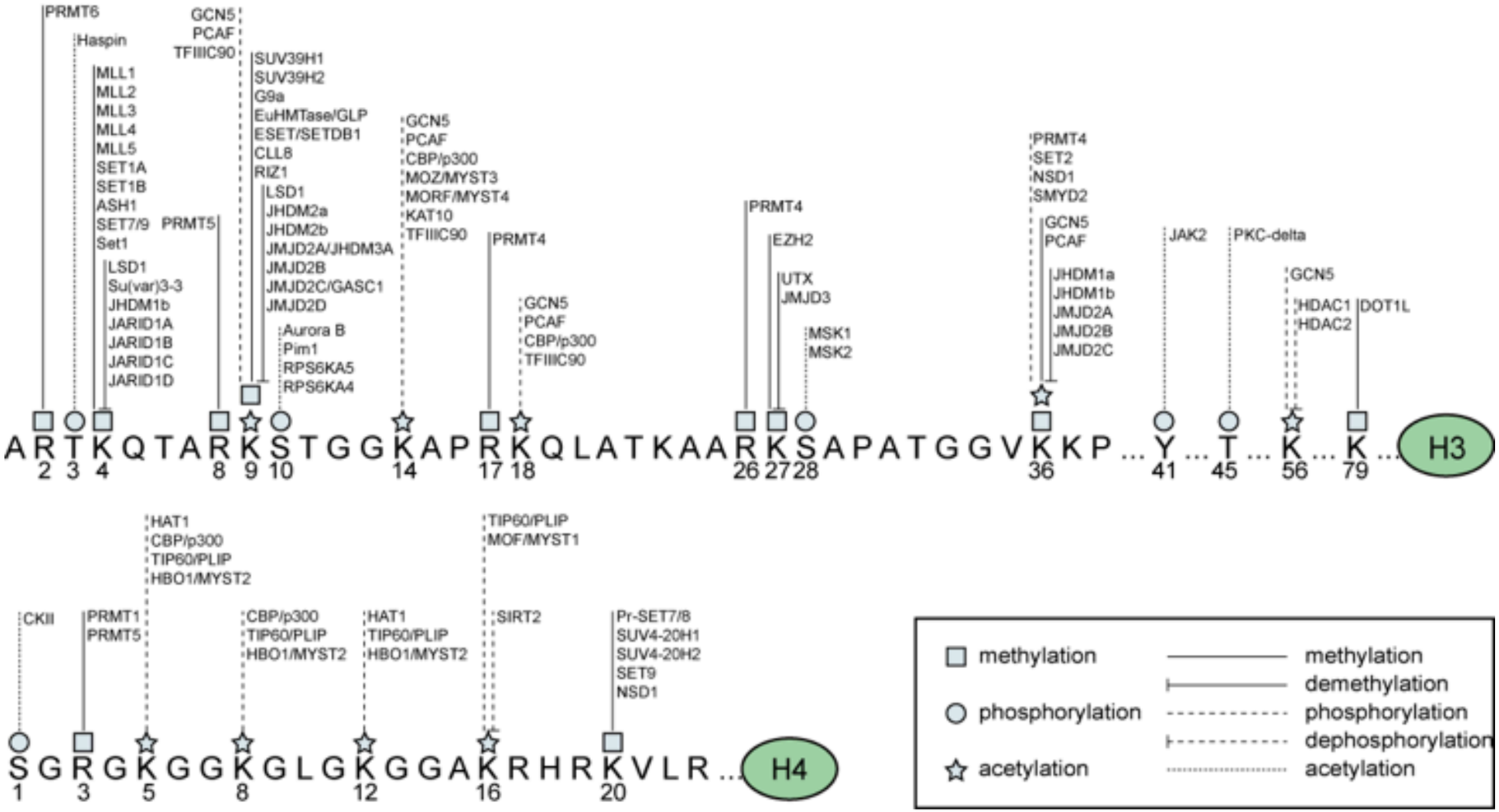
Me1/2/3: Methylation (K, R)
Ac: Acetylation (K, S, T)
Pr: Propionylation (K)
Bu: Butyrylation (K)
Cr: Crotonylation (K)

Cit: Citrullination(R)
Ph: Phosphorylation (S, T, Y, H)
OH: Hydroxylation (Y)
Og: O-GlcNAcylation (S, T)
Ar: ADP ribosylation (K, E)

Hib: 2-Hydroxyisobutyrylation (K)
Ma: Malonylation (K)
Su: Succinylation (K)
Ub: Ubiquitination (K)
Fo: Formylation (K)

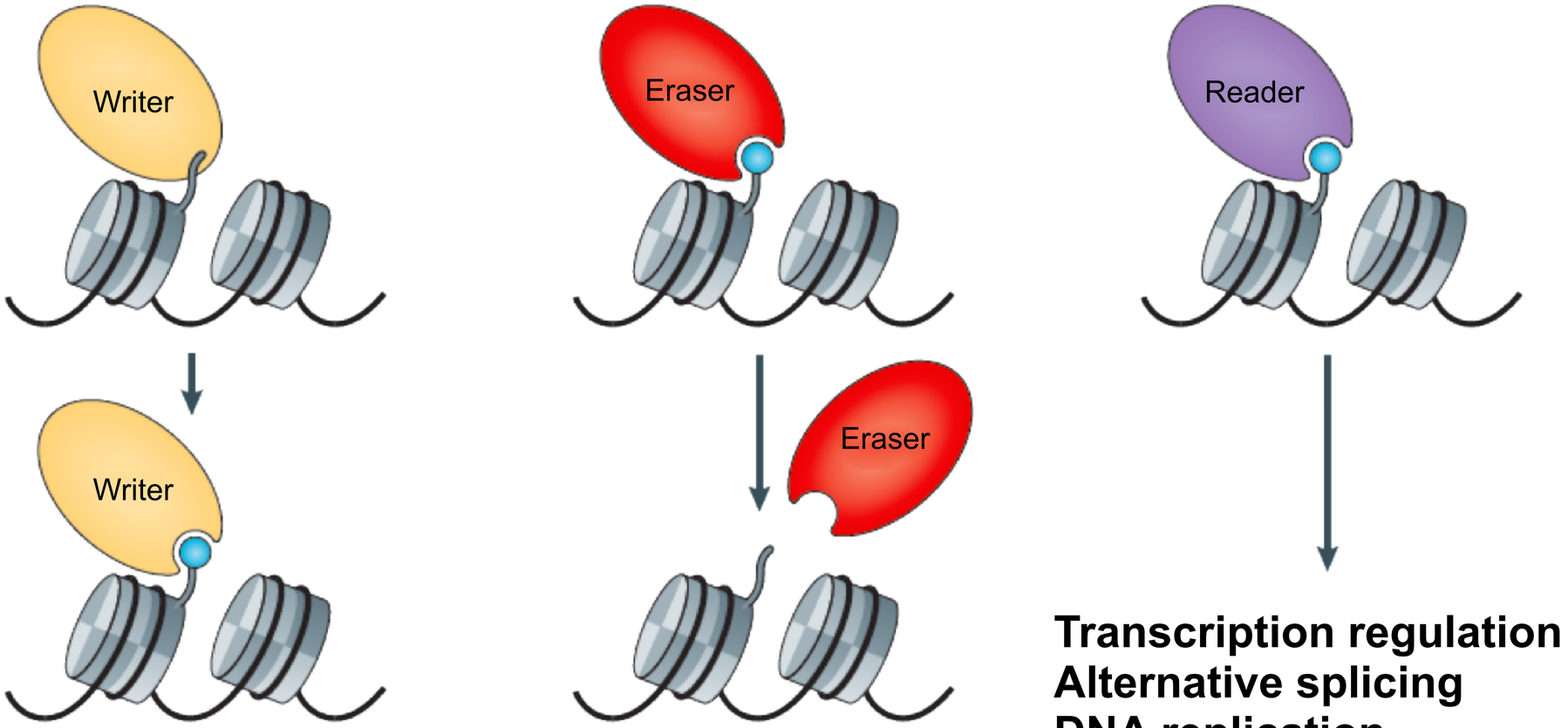
Huang et al. Cell, 2014

A complex network of proteins sets and removes a variety of histone modifications at multiple sites



Kouzarides 2007, Cell

Chromatin modifications are set reversibly and recognized by dedicated readout proteins



Transcription regulation
Alternative splicing
DNA replication
DNA repair
DNA recombination

Cancer genome-sequencing: recurrent mutations in ‘writers’, ‘erasers’ or readers’ of modifications.

Plass et al 2013, Nat Rev Mol Cell Biol

The epigenetic landscape is very dynamic and determined by > 600 proteins

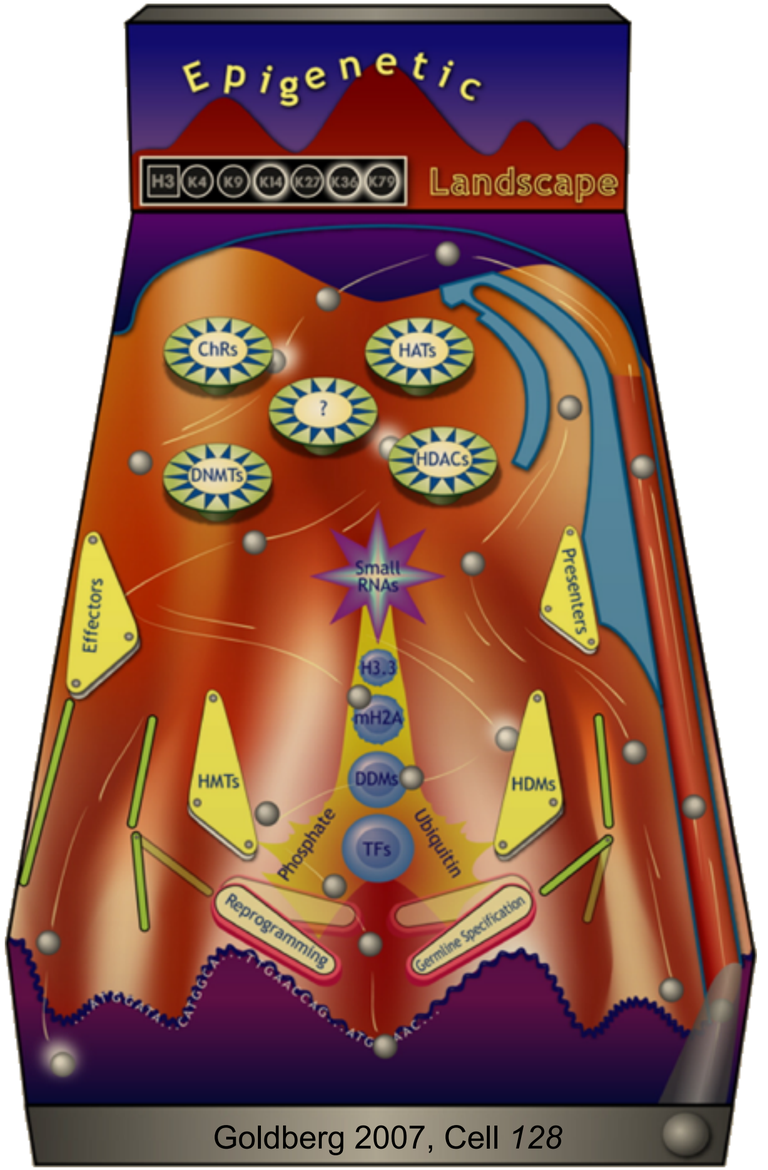
DNA methylases (DNMT1/3a/3b)
Removal via hydroxylation (TET1/2/3)
$t_{1/2}$ = 10 min to days

Histone lysine methylase (KMT)
Histone lysine demethylase (KDM)
$t_{1/2}$ = hours to days

Histone acetylases (HAT)
Histone deacetylases (HDAC)
$t_{1/2}$ = 2-40 min (80%)

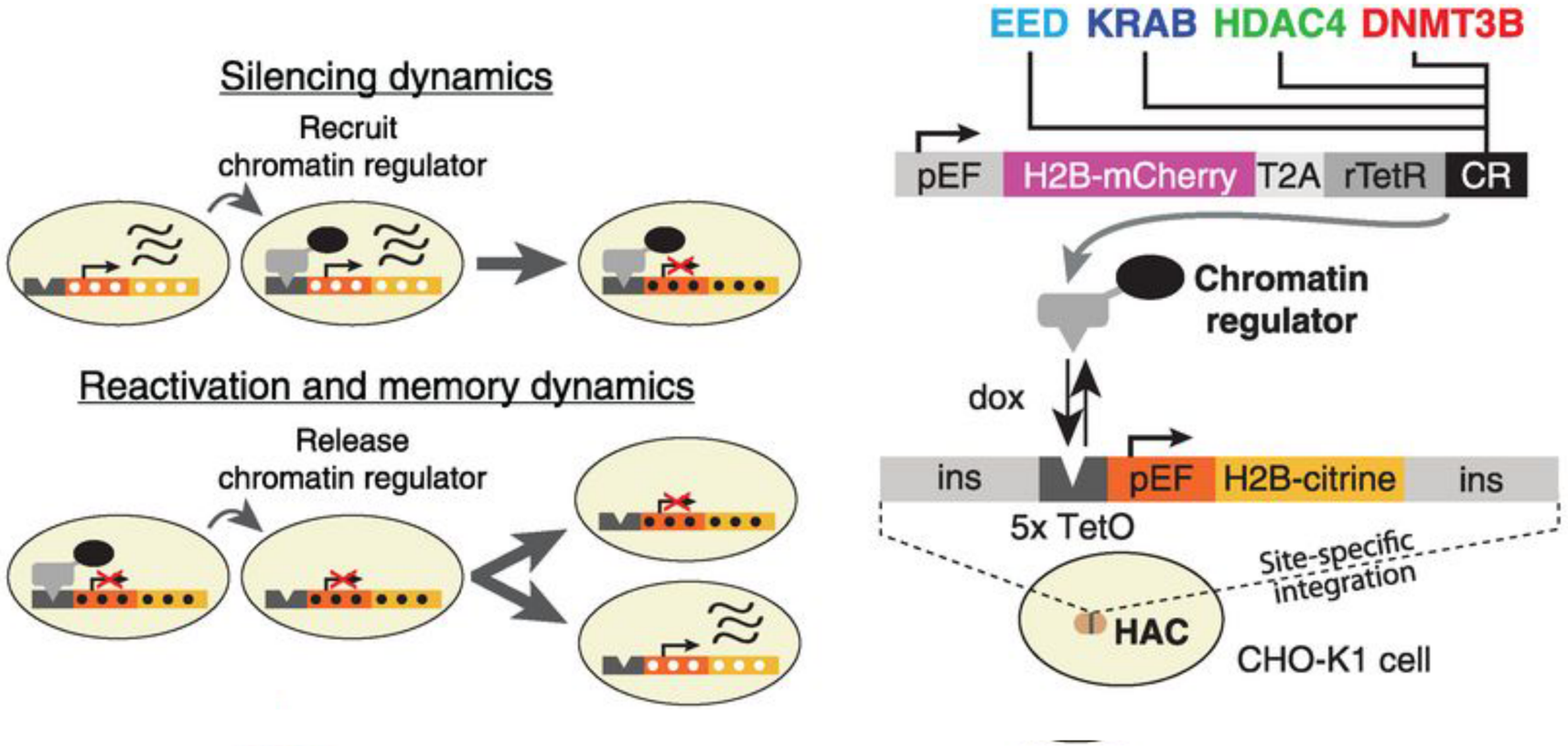
Reader proteins (MBD, bromo, chromo, tudor, PHD domains)
$t_{1/2}$ = 1-10 sec

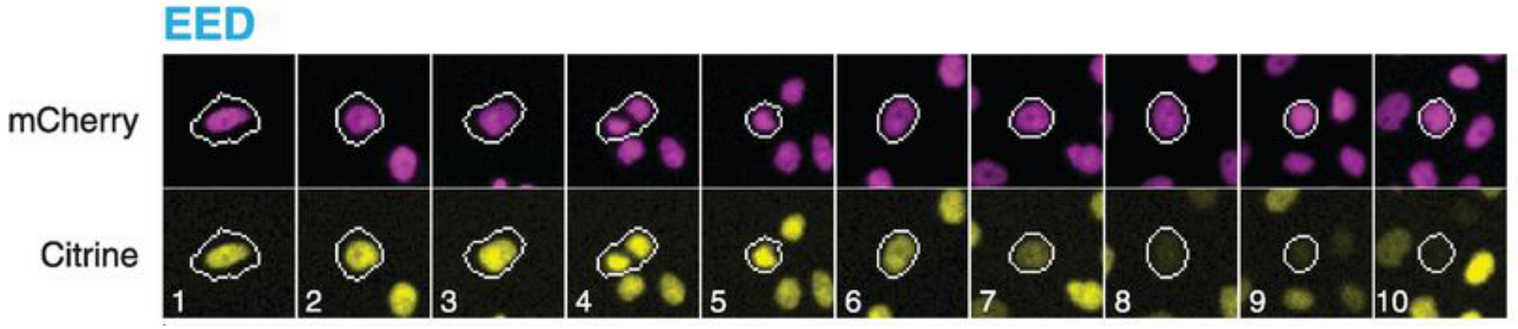
Chromatin remodeler (SNF2 family)
~30 sec (nucleosome translocation)



Goldberg 2007, Cell *128*

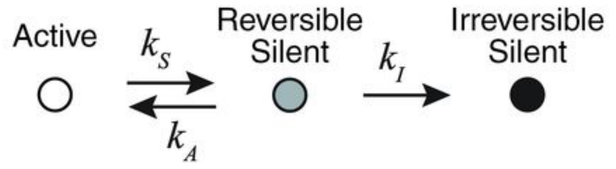
Tracing epigenetic memory in living single cells

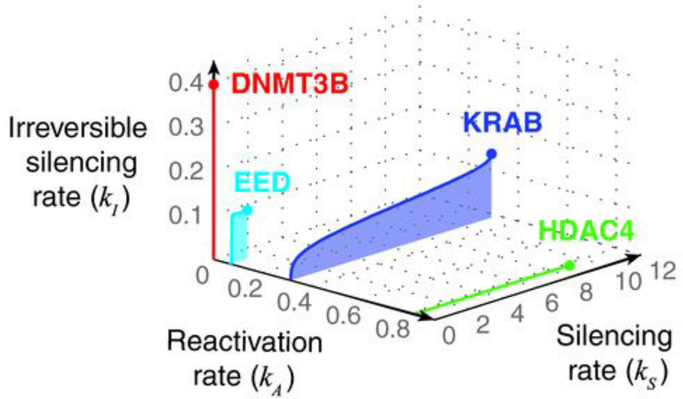


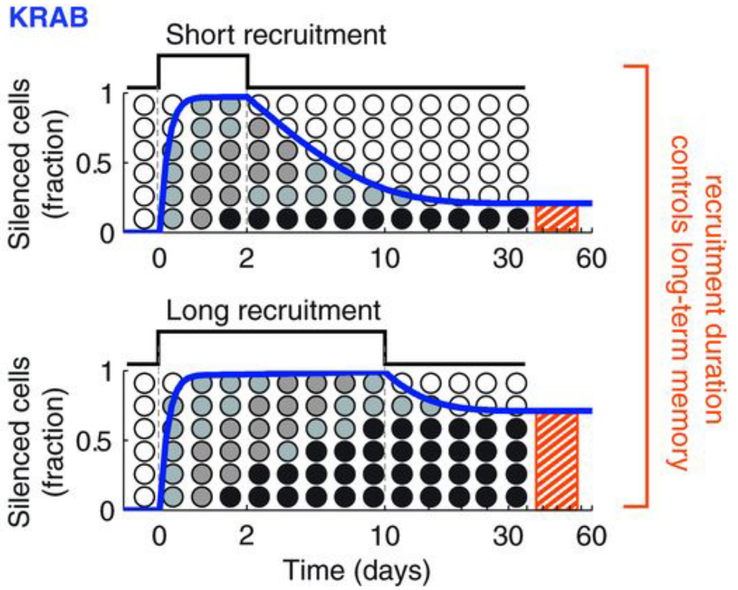


Bintu et al. Science 2016;351:720-724

Epigenetic memory is different for different modifications







H3K27me3

EED
Slow-silencing, partial committer

H3K9me3

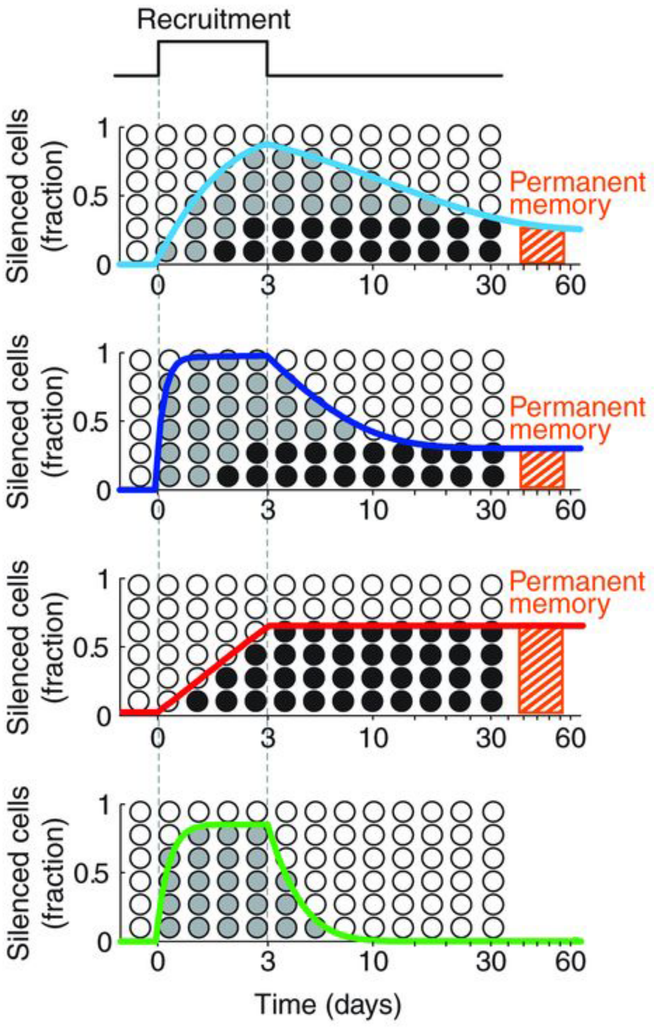
KRAB
Fast-silencing, partial committer

DNA methylation

DNMT3B
Slow, complete committer

Histone deacetylation

HDAC4
Fast reverter



Bintu et al. Science 2016;351:720-724

Maintenance methylation by DNMT1 can make *de novo* methylated sites self-sustained

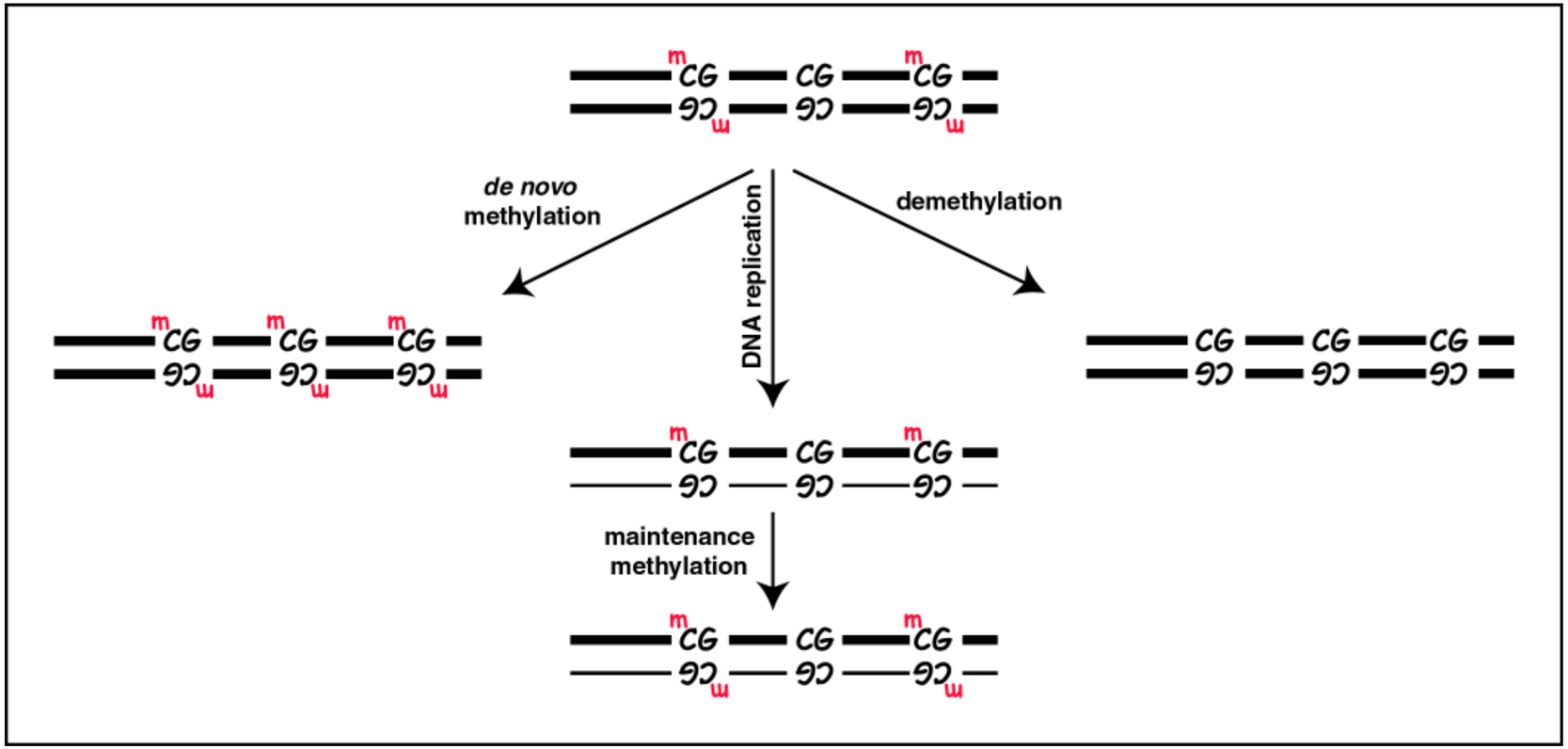
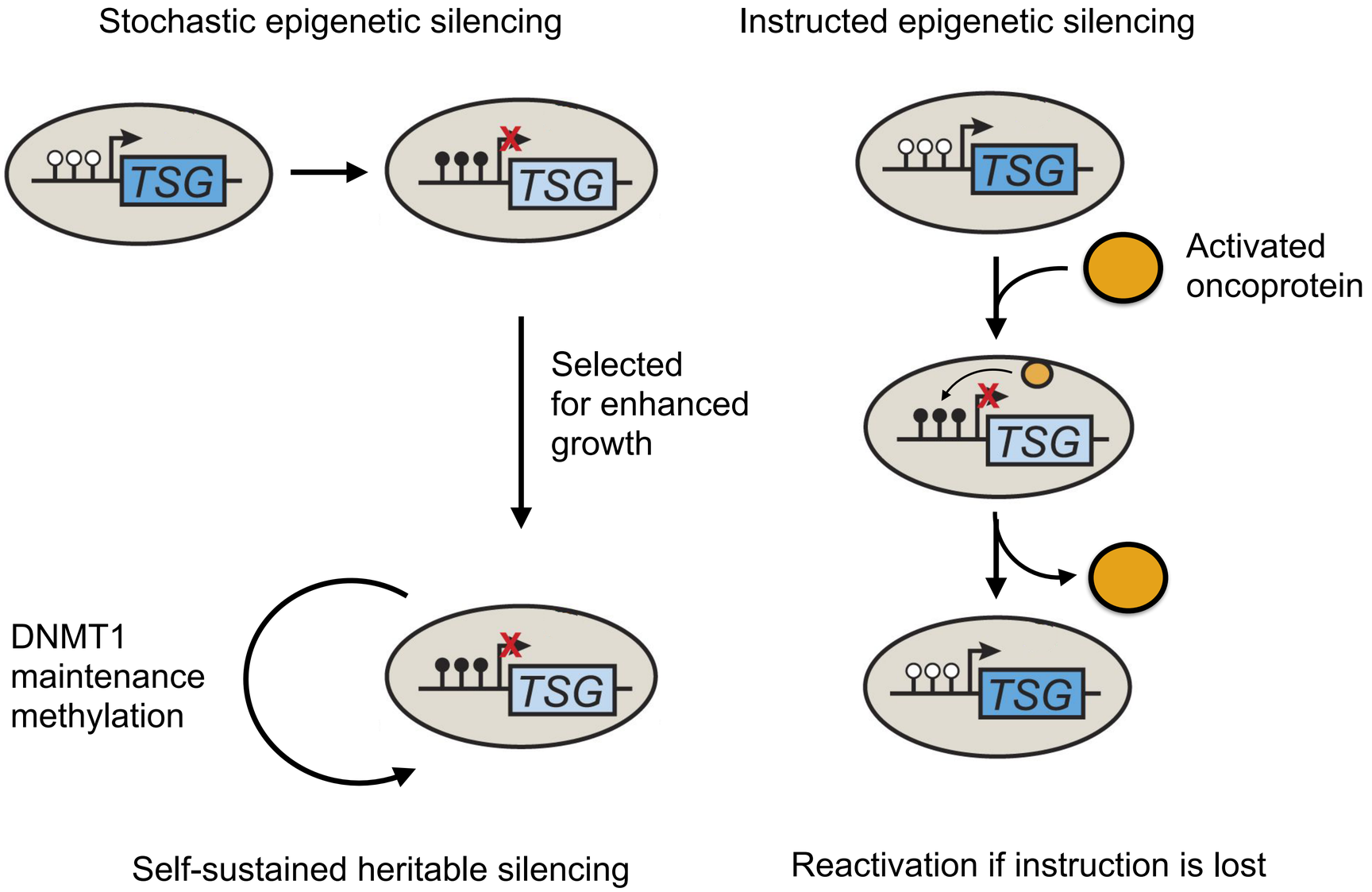


Figure adapted from Easwaran, 2003

Self-sustained versus instructed DNA methylation



Struhl 2014, eLife

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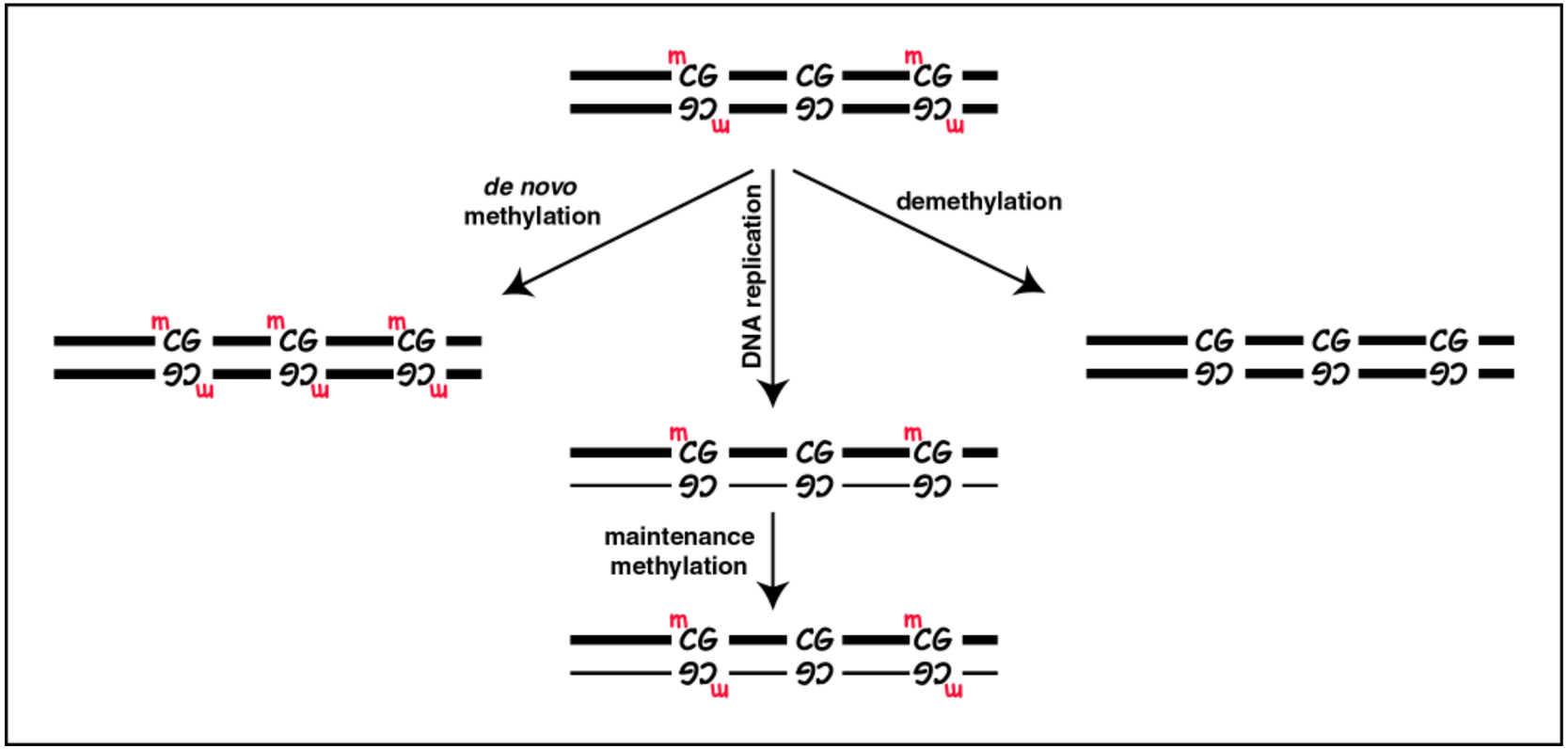

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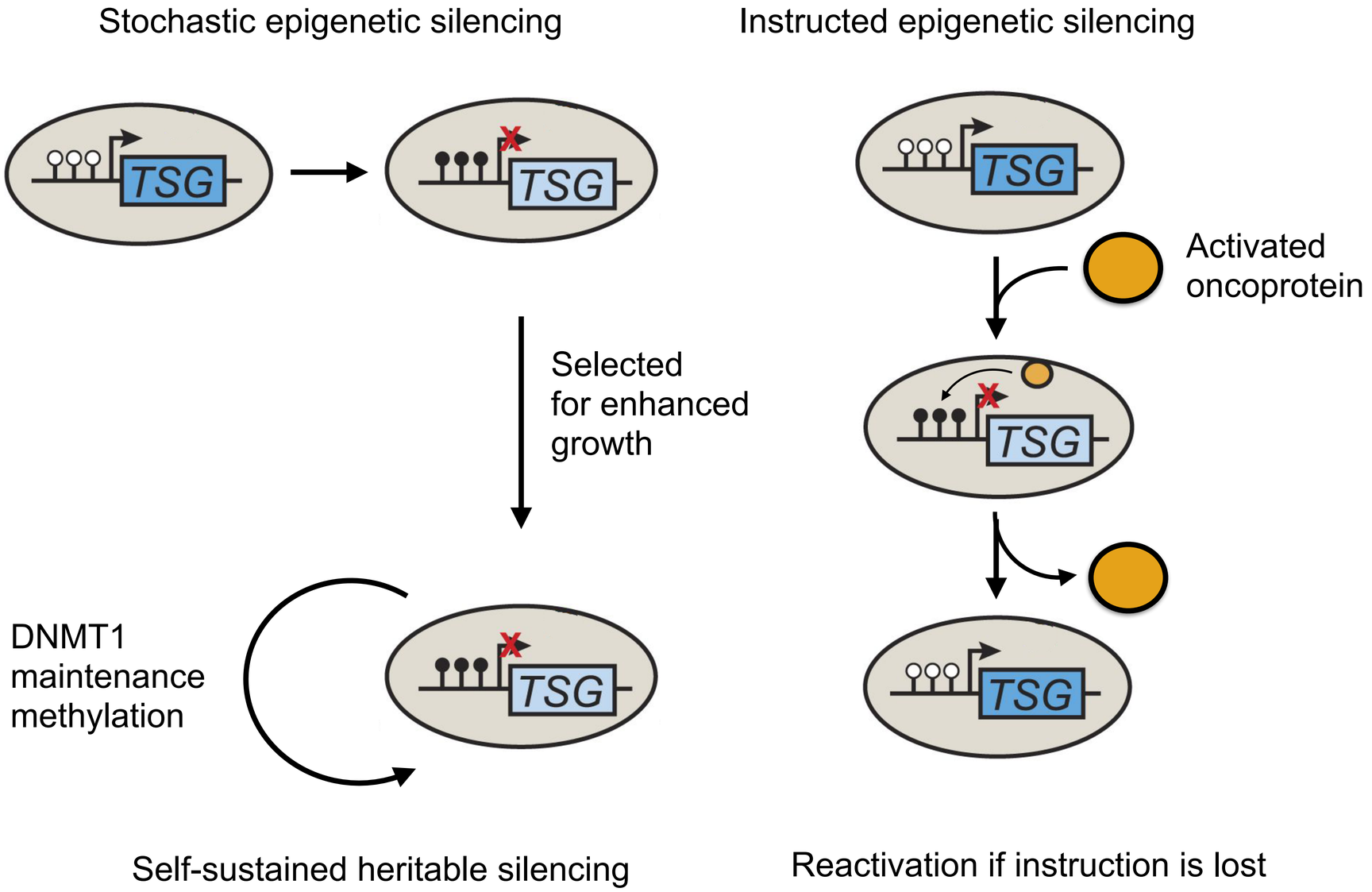
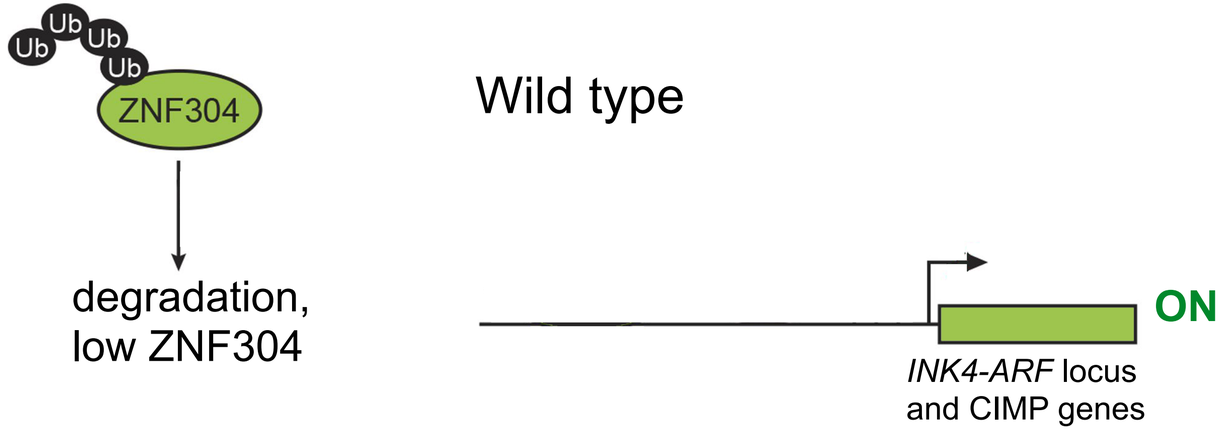
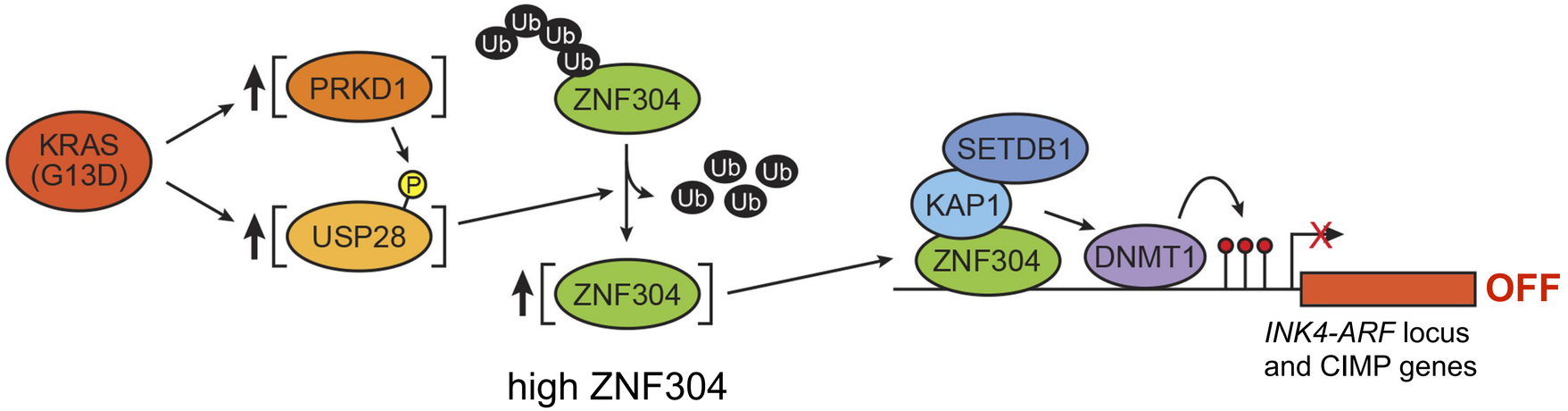


Struhl 2014, eLife

Silencing of tumor suppressors in the *INK4-ARF* locus for colorectal cancers is instructed and not self-sustained

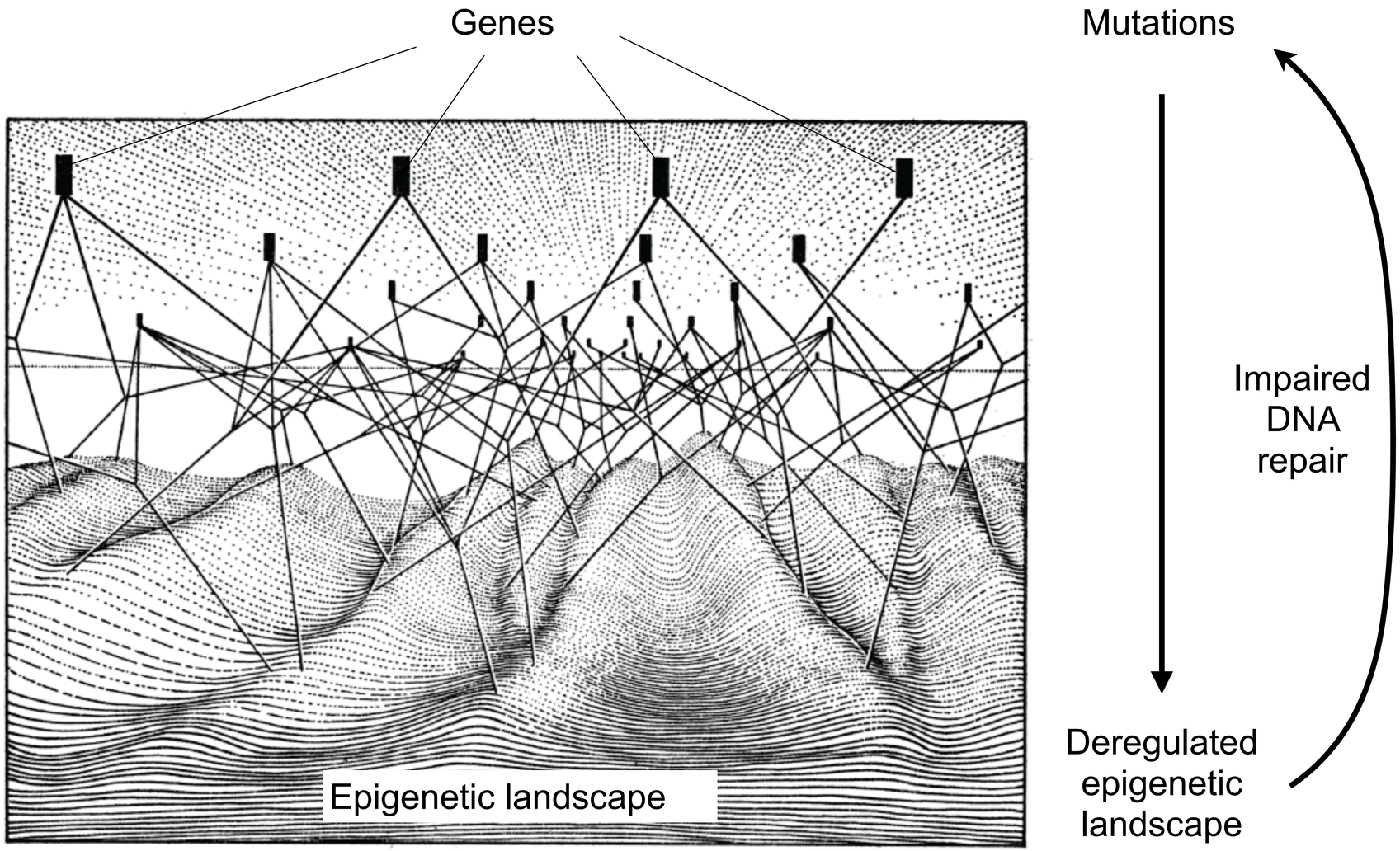


KRAS mutation with CpG island dependent methylator phenotype (CIMP)



Serra et al 2014, eLife; Wajapeyee et al 2013, Genes Dev

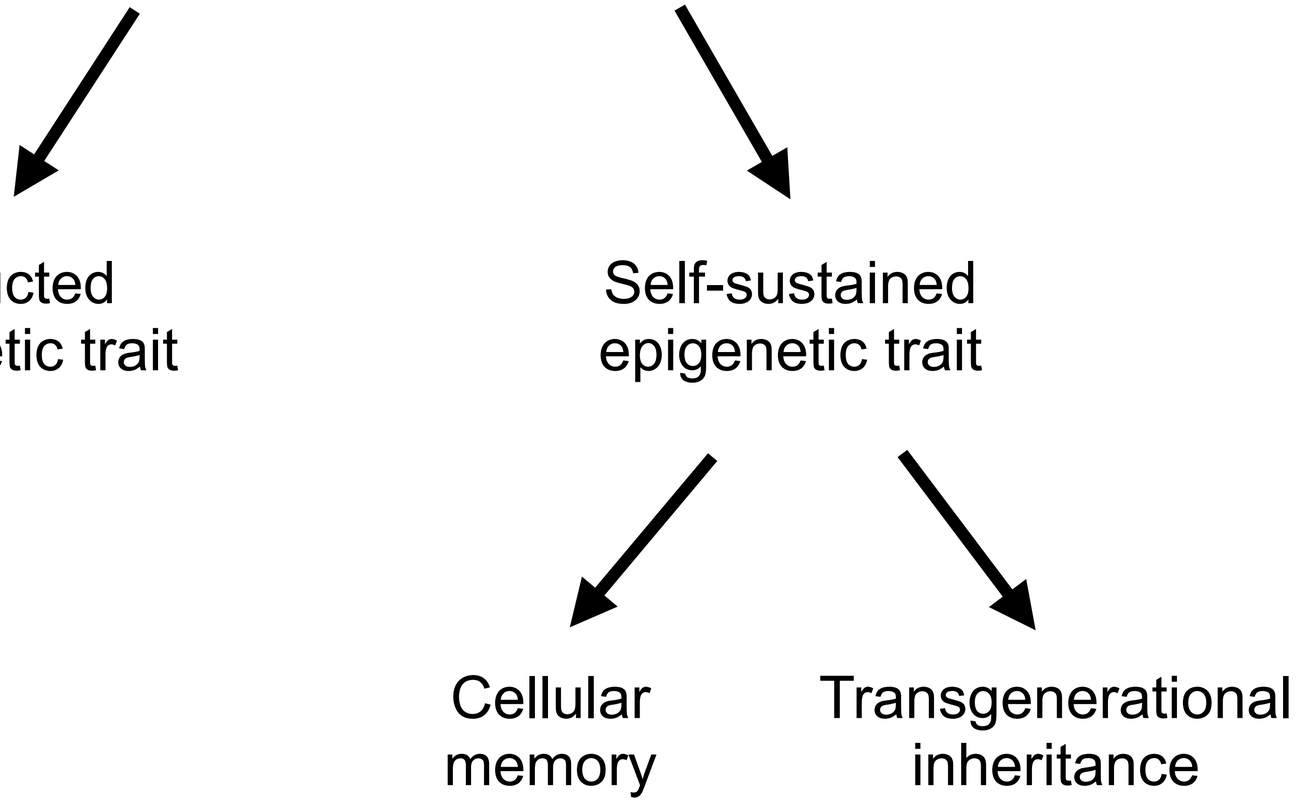
Waddington: “The modeling of the epigenetic landscape… is anchored to the genes”



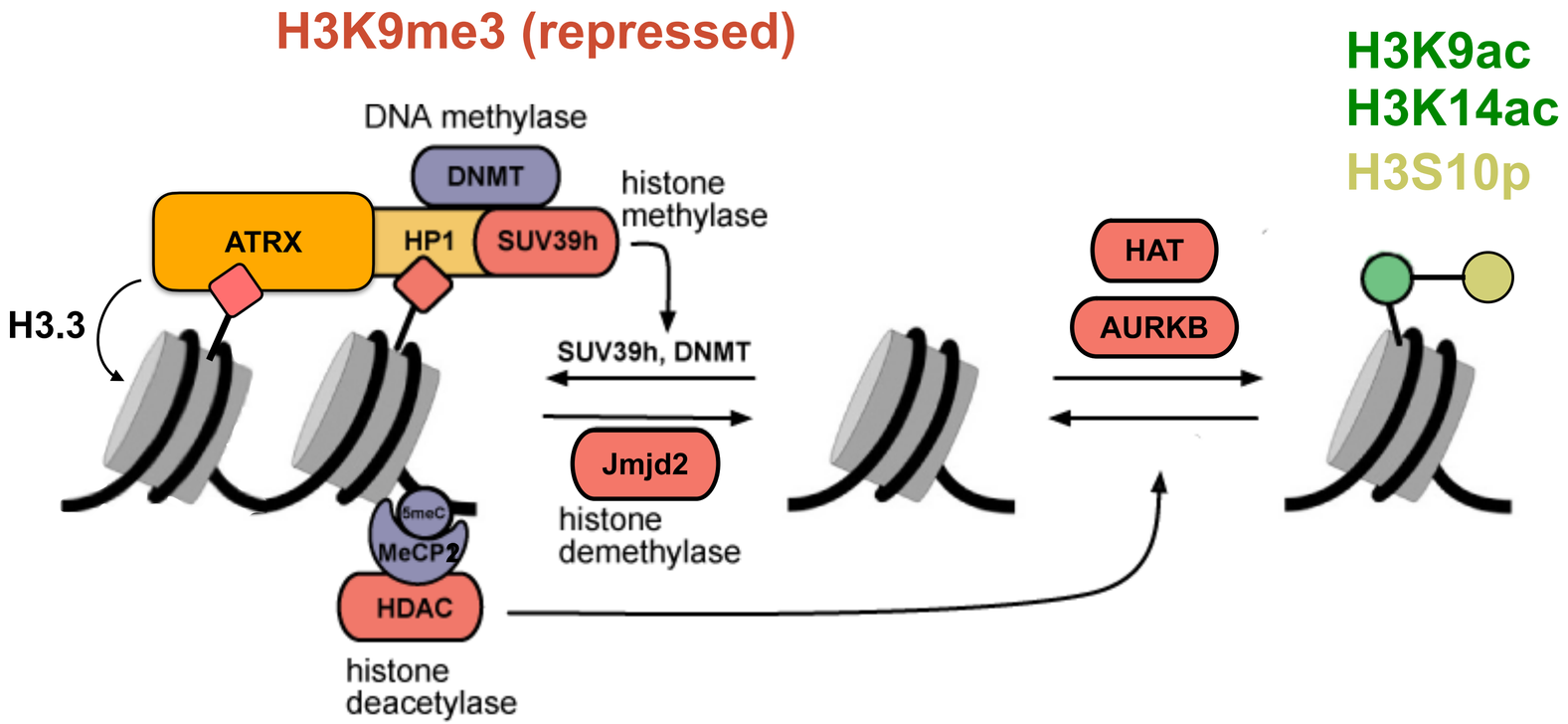
The strategy of the genes, 1957 (image rotated by 180º)

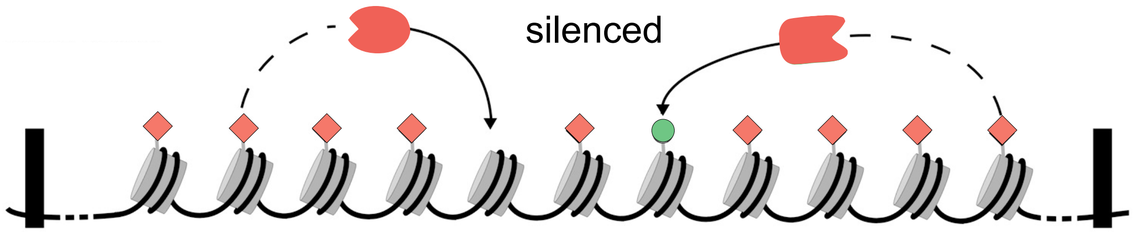
A broader definition of epigenetics

An epigenetic trait is a phenotype that is dependent on the chromatin state without alterations of the DNA sequence.



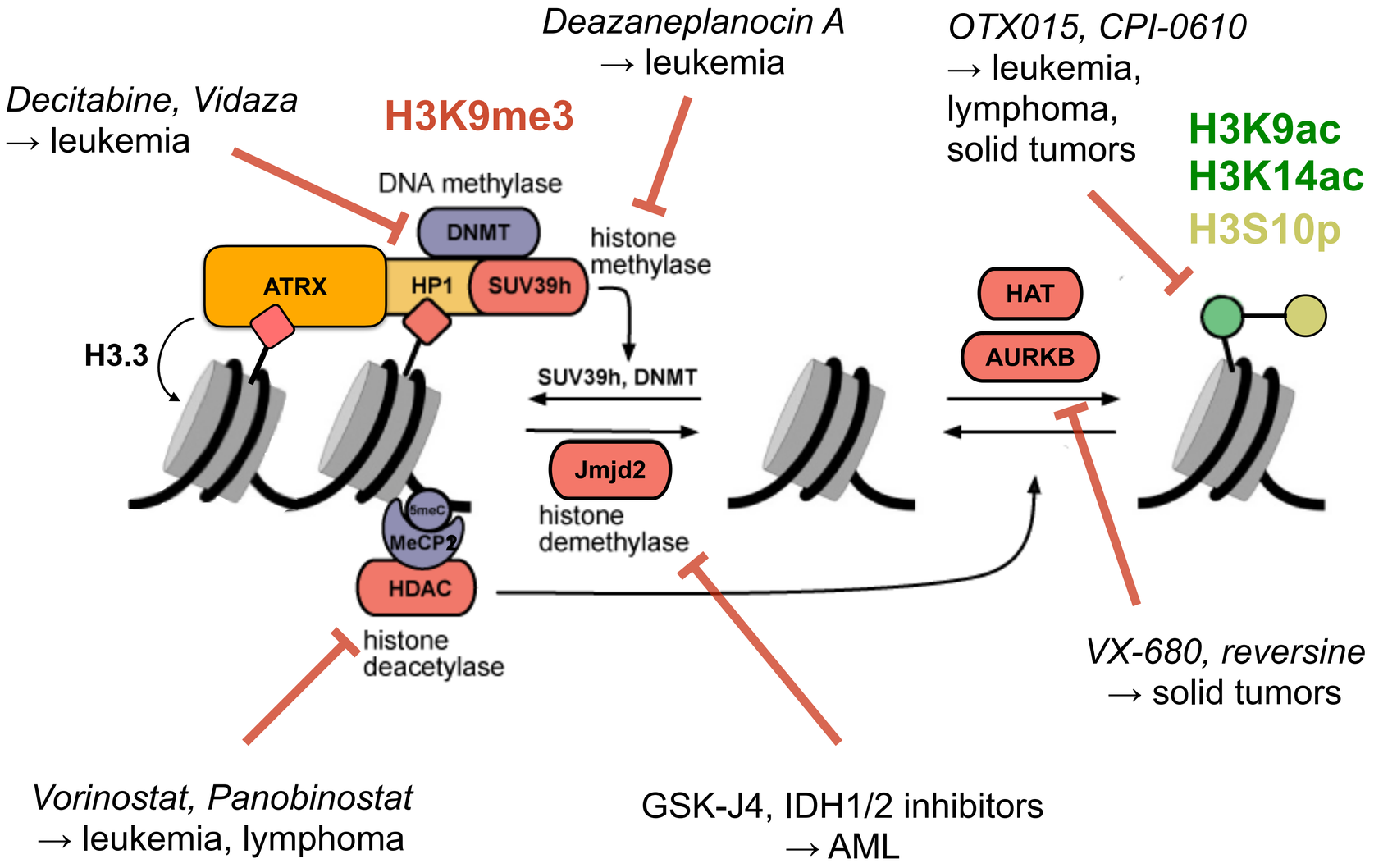
Silencing transcription of (peri)centromeric and telomeric repeats





Müller-Ott et al 2014, *Mol Sys* Biol; Mallm & Rippe 2015, *Cell* Rep; Rippe & Luke 2015, *Nat Struct Mol Biol*

‘Epigenetic drugs’ that target chromatin networks



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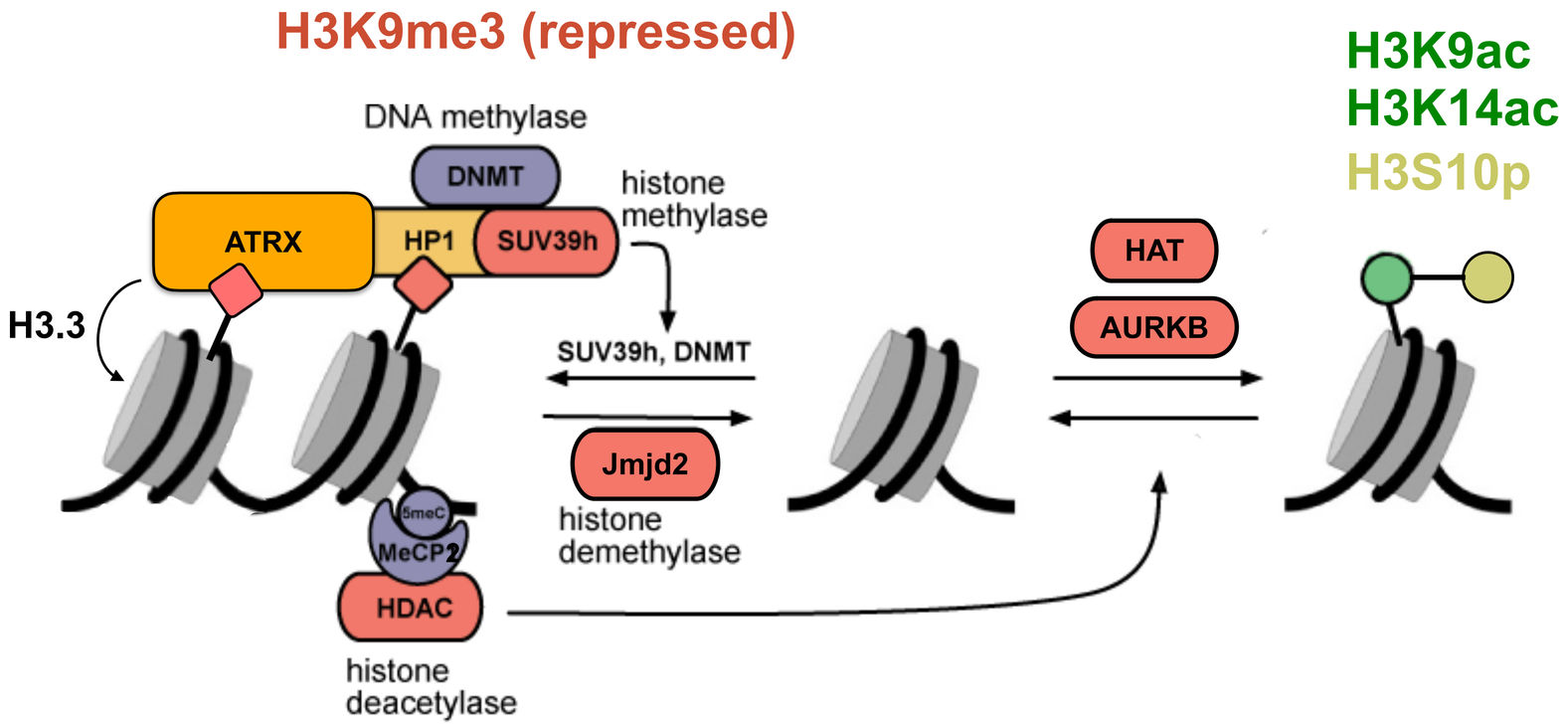
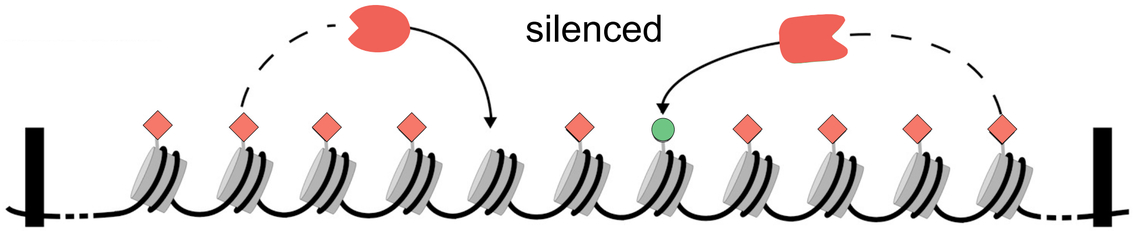
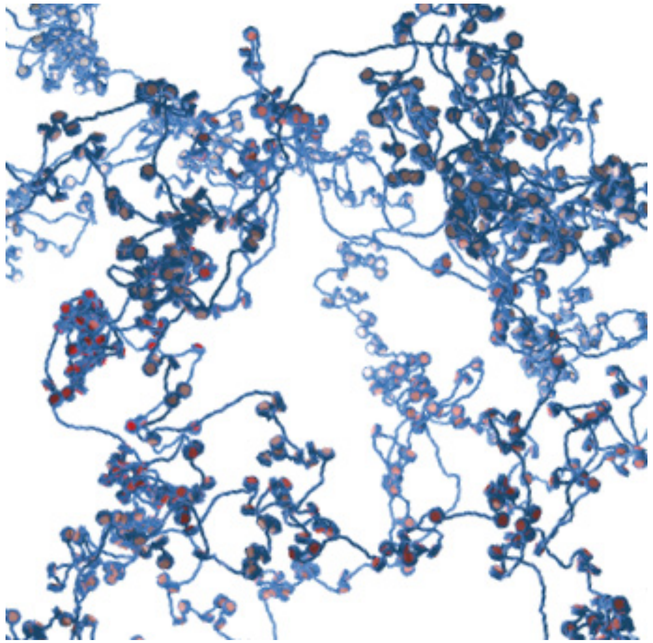




The domino cascade model for spreading histone modifications along the DNA

Spreading in three dimensions?



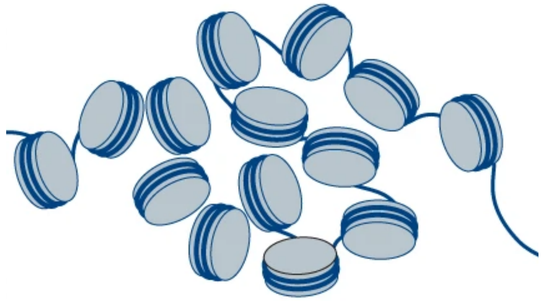
in 1D: ~25 nm
(extended chain)

in 3D: ~28 nm
(“sea of nucleosomes”)

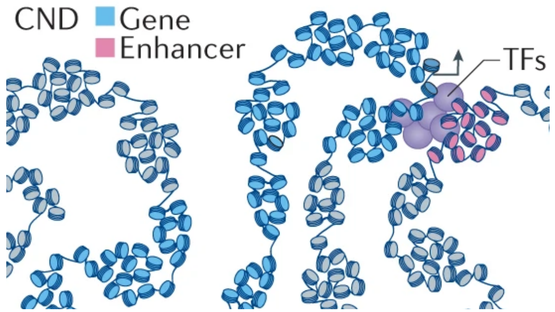
3D constructions with dominos are very fragile…

from Annodomino2007 on Youtube

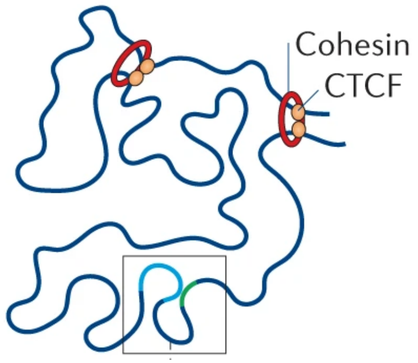
Chromatin organization provides an additional regulatory layer in eukaryotes



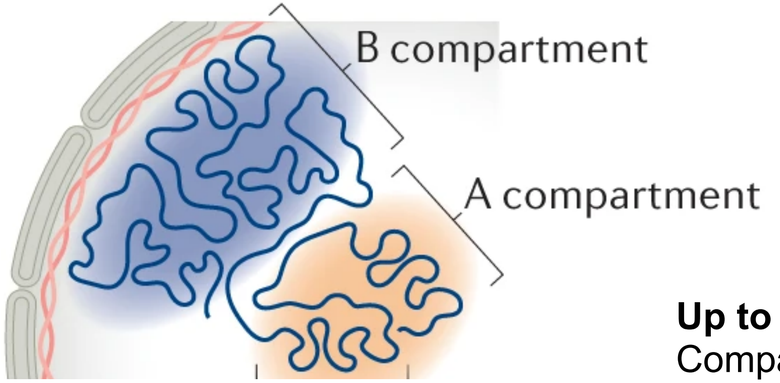
1-2 kb: Nucleosome clutches



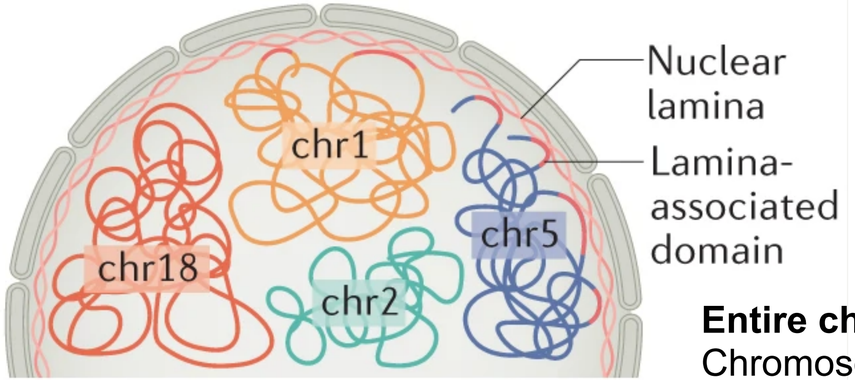
10-100 kb: Chromatin domains and functional loops (E-P contacts)



100 kb to a few Mb: Chromatin loops and topologically associating domains (TADs)



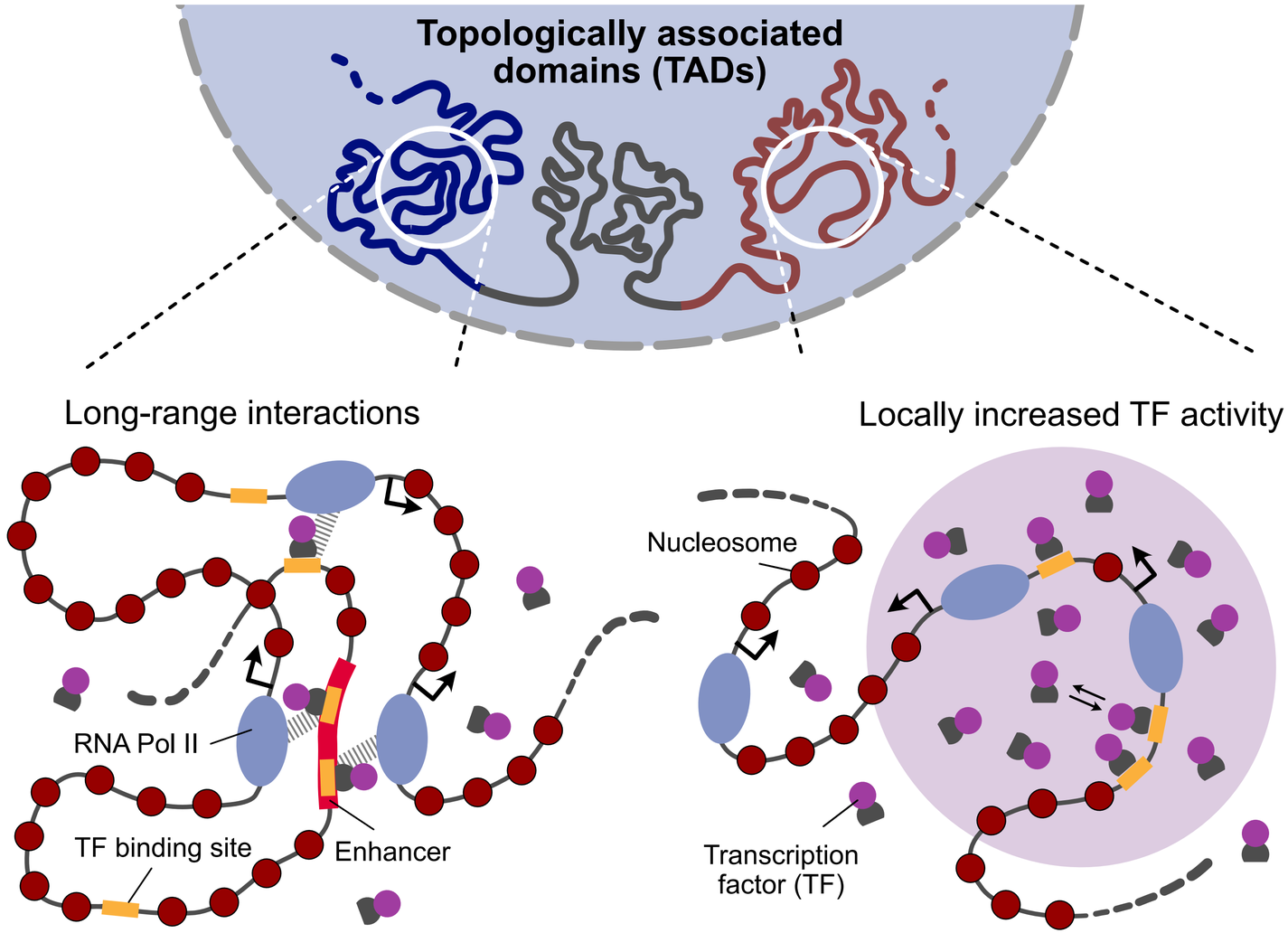
Up to 100s of Mb: Compartments and hubs



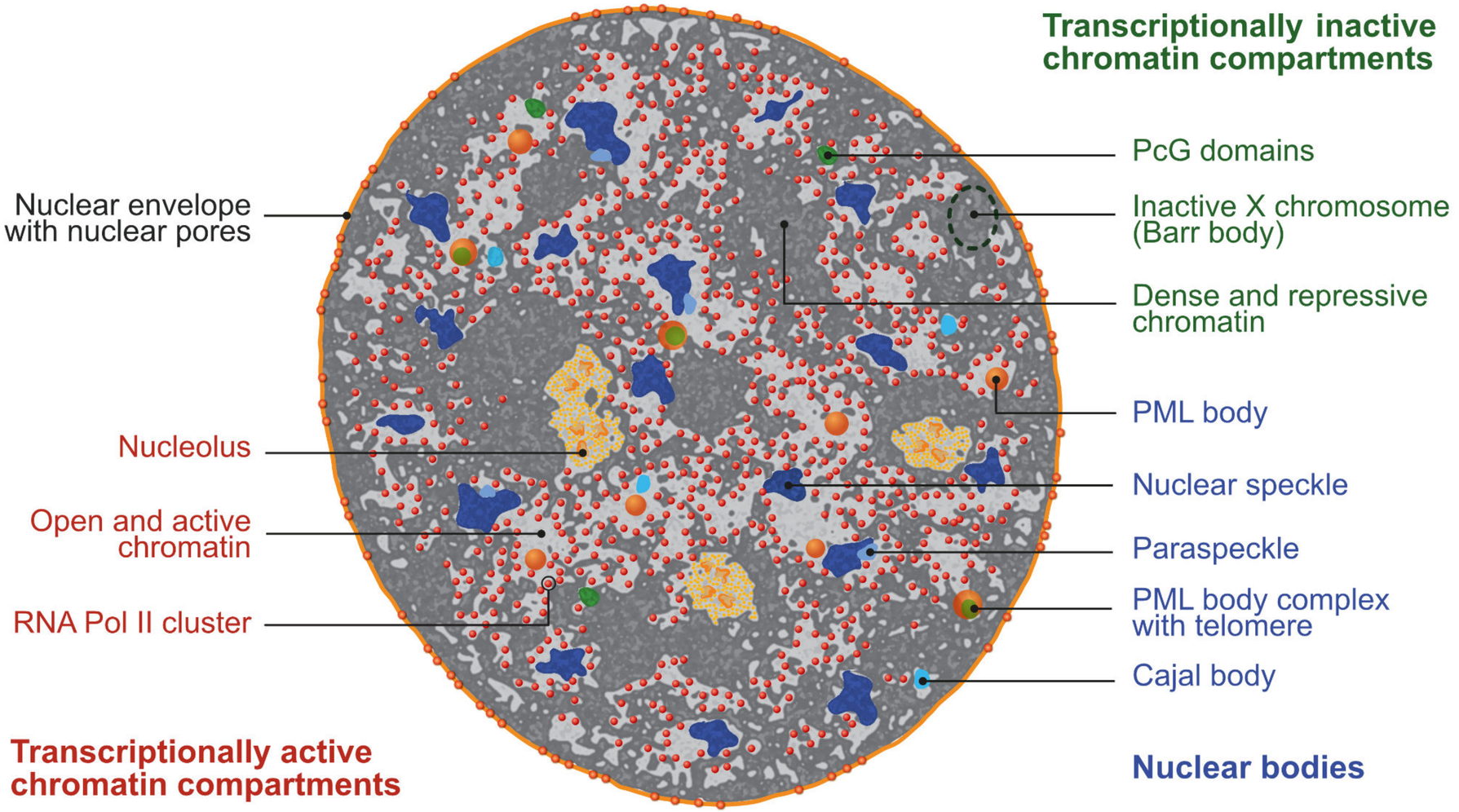
Entire chromosomes: Chromosome territories

Adapted from Fitz-James & Cavalli 2022 *Nat Rev Genet*

Genome organization can regulate gene expression



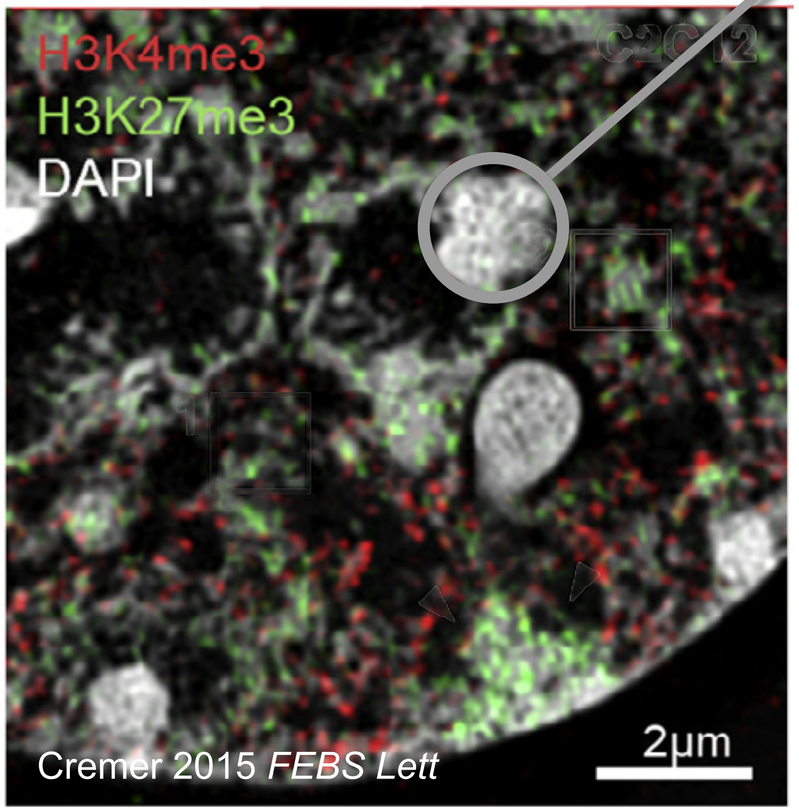
The mammalian nucleus organizes genome functions in subcompartments

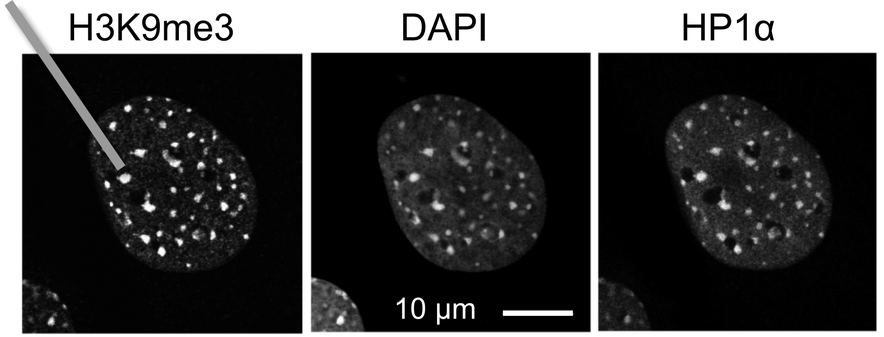


Caudron-Herger & Rippe 2012 *Curr Opin Genet Dev*

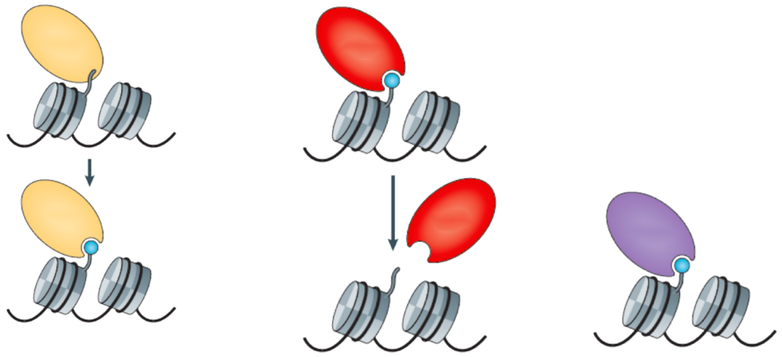
Mouse pericentric heterochromatin - a model system for a large silenced chromatin domain

Pericentric heterochromatin ("chromocenters")





H3K9me3 modification



Writer
SUV39H1/H2

Eraser
JMJD2

Readers
HP1α/β/γ
SUV39H
ATRX

Mouse chromocenters
- Nucleosomes: 230 µM
- HP1α/β total: 10 µM dimer