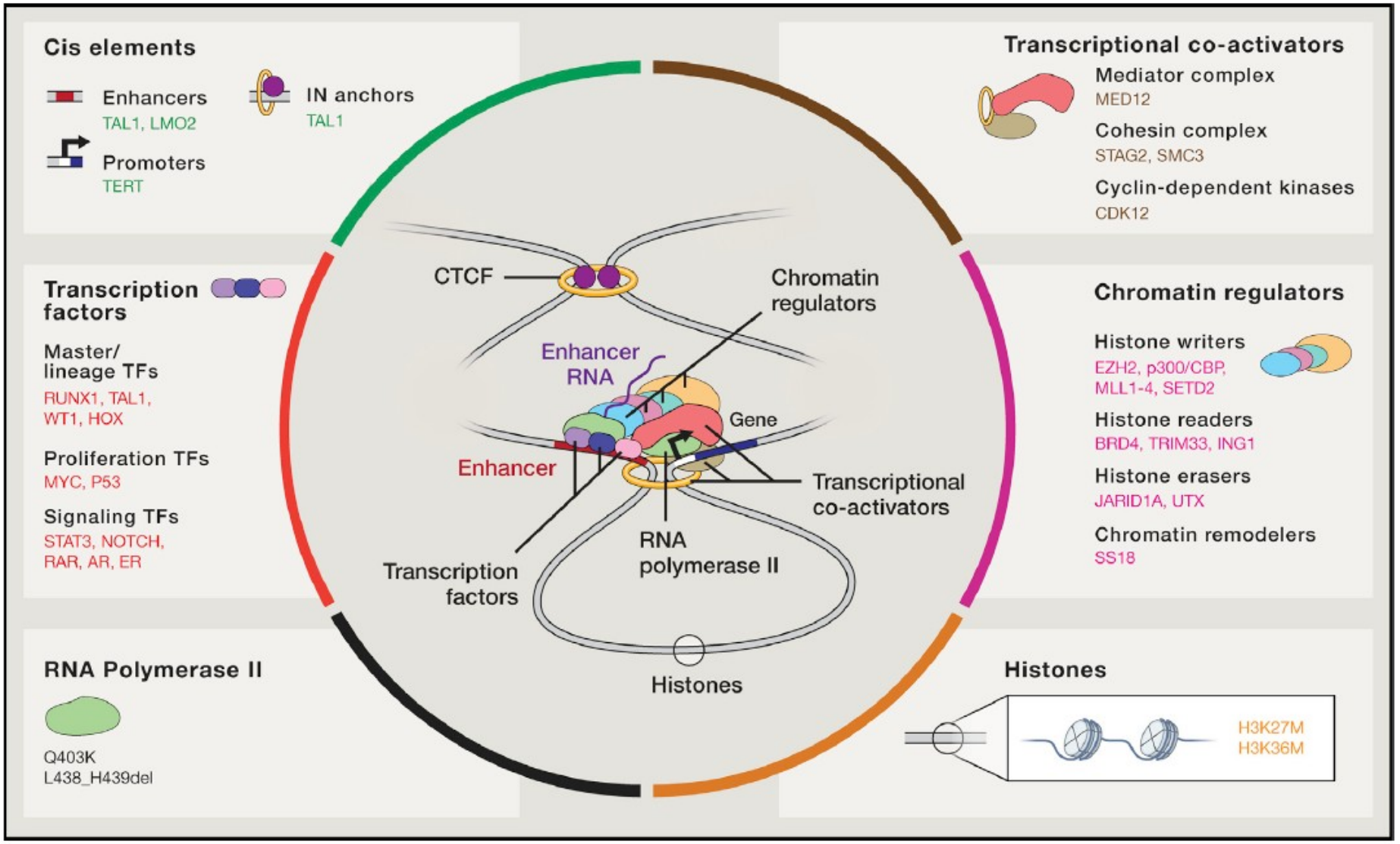
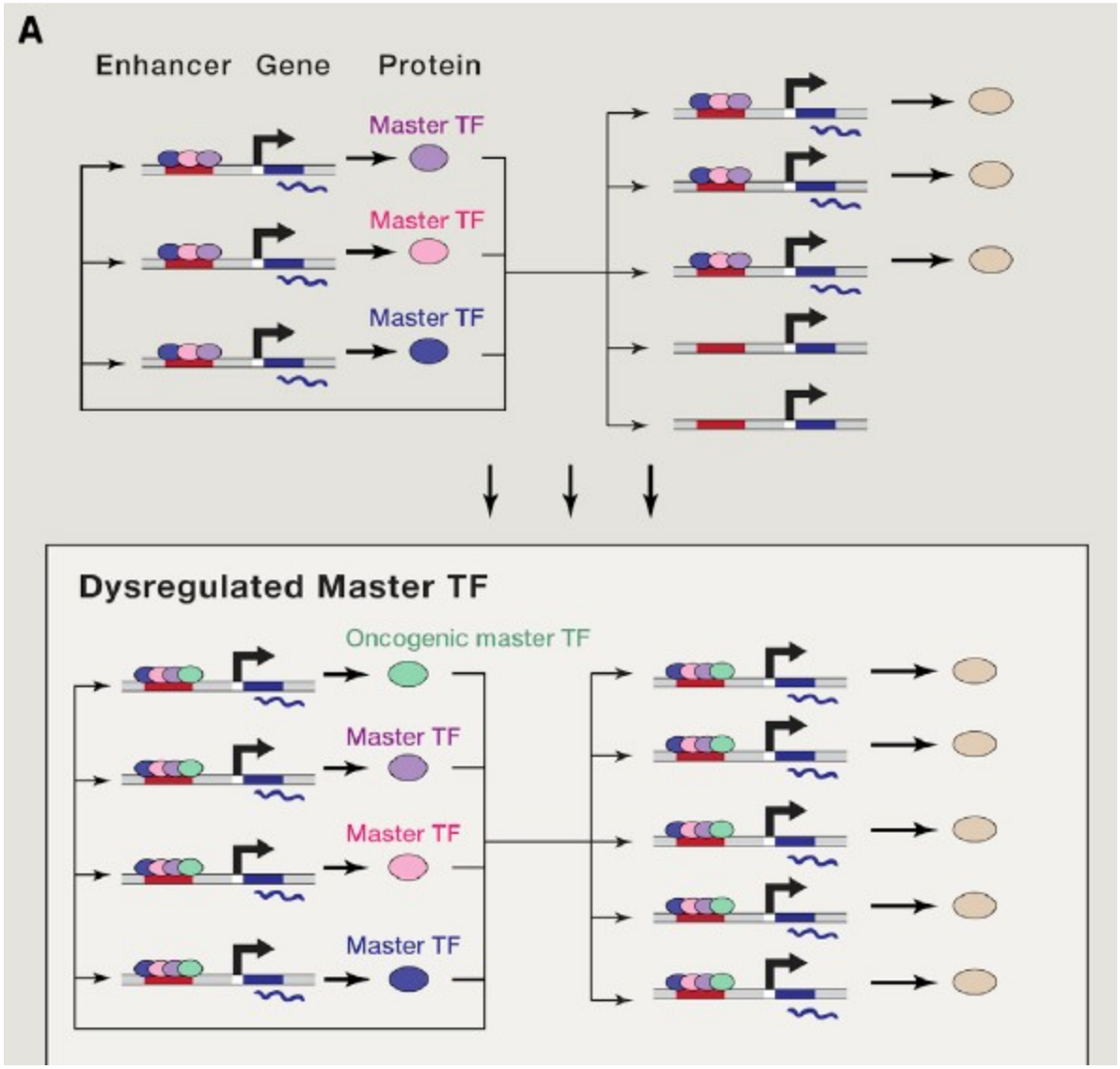
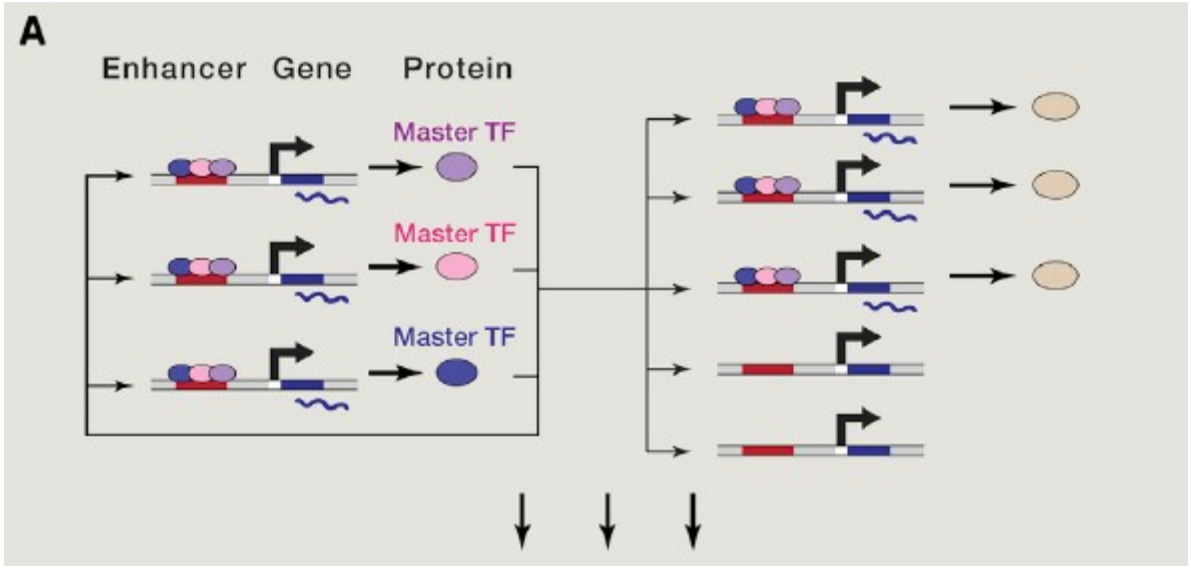
ALTERATIONS OF TRANSCRIPTIONAL REGULATION IN CANCER

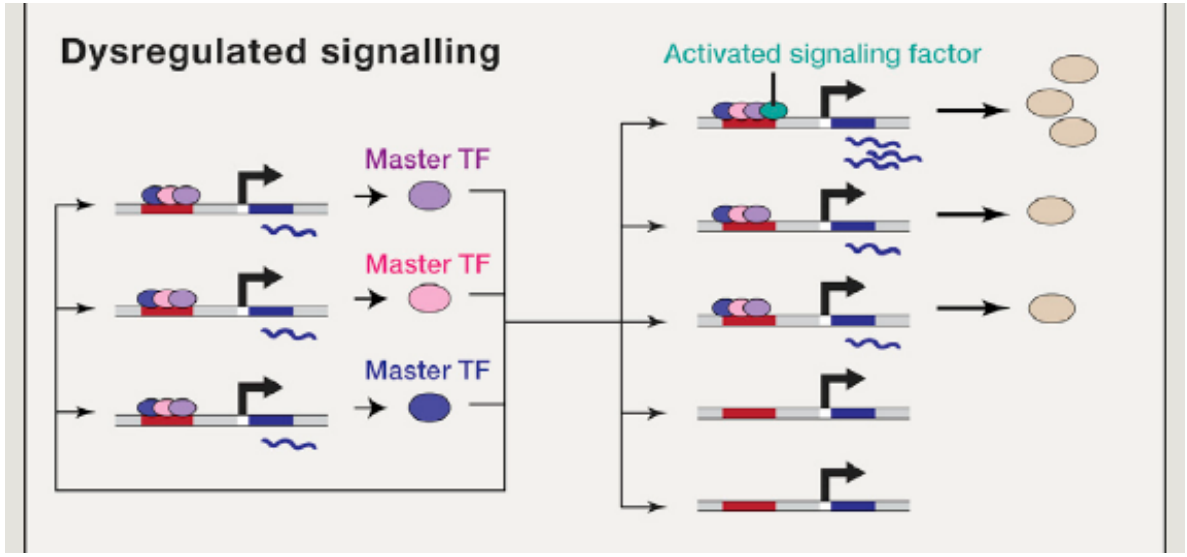

Cis elements
Enhancers
TAL1, LMO2
IN anchors
TAL1
Promoters
TERT
Transcriptional co-activators
Mediator complex
MED12
Cohesin complex
STAG2, SMC3
Cyclin-dependent kinases
CDK12
Transcription factors
Master/ lineage TFs
RUNX1, TAL1, WT1, HOX
Proliferation TFs
MYC, P53
Signaling TFs
STAT3, NOTCH, RAR, AR, ER
CTCF
Chromatin regulators
Enhancer RNA
Gene
Enhancer
Transcriptional co-activators
RNA polymerase II
Transcription factors
Histones
Chromatin regulators
Histone writers
EZH2, p300/CBP, MLL1-4, SETD2
Histone readers
BRD4, TRIM33, ING1
Histone erasers
JARID1A, UTX
Chromatin remodelers
SS18
RNA Polymerase II
Q403K
L438_H439del
Histones
H3K27M
H3K36M

DYSREGULATION OF MASTER TRANSCRIPTION FACTOR

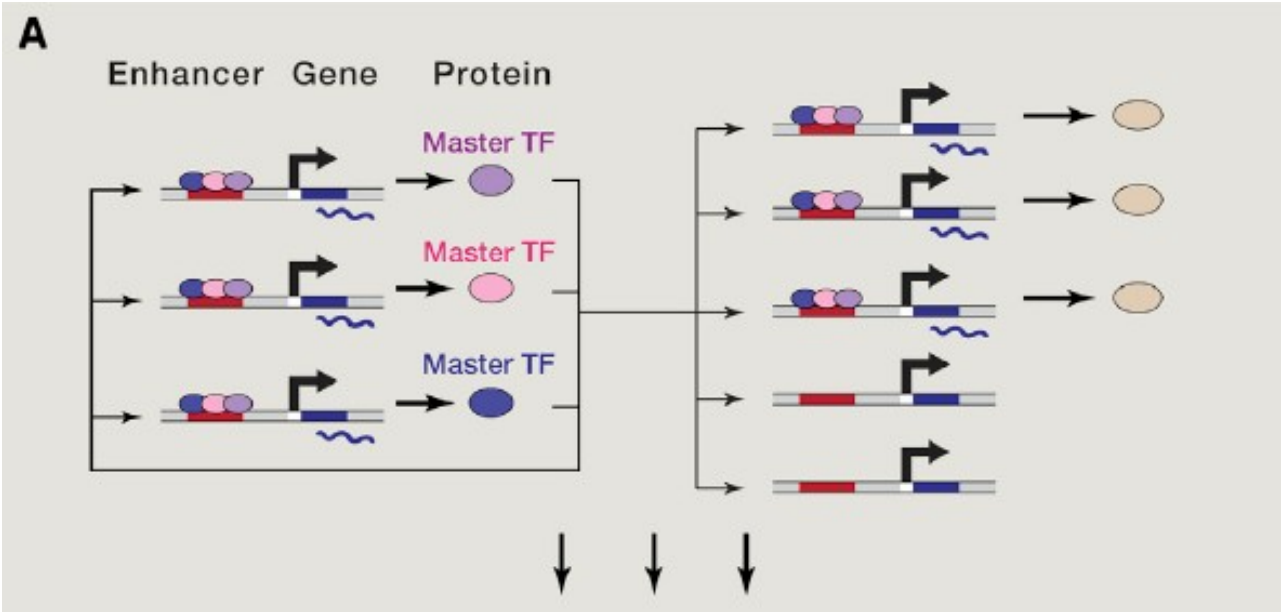


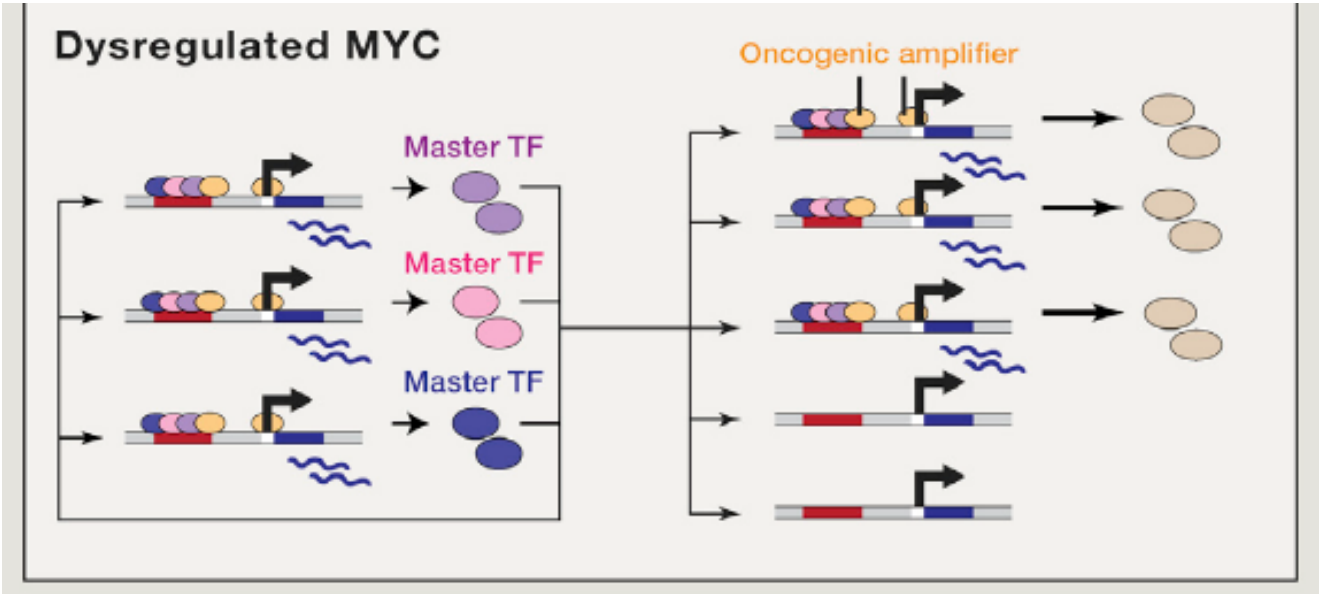
DYSREGULATION OF ACTIVATED SIGNALLING TRANSCRIPTION FACTOR



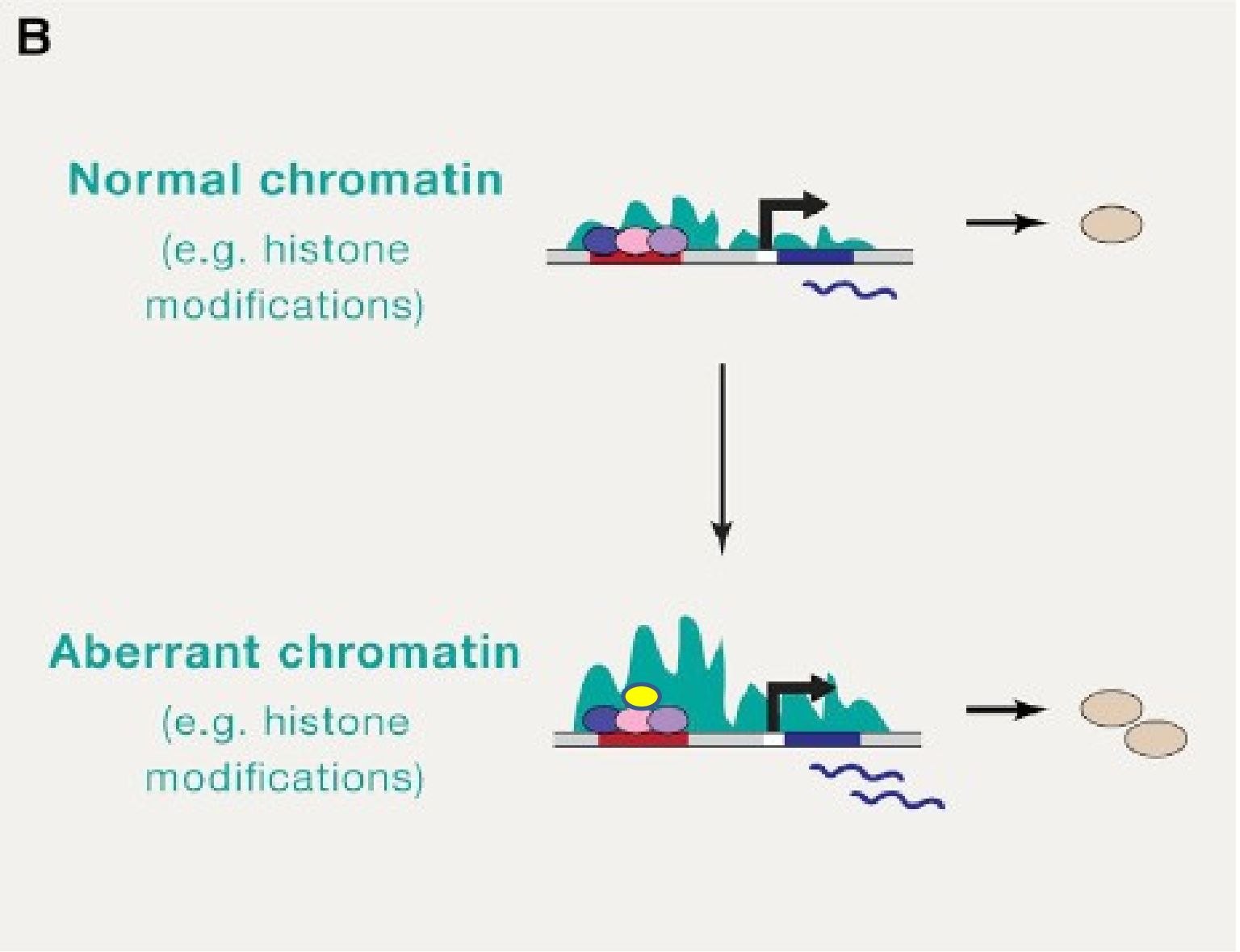


DYSREGULATION OF ONCOGENIC TRANSCRIPTION FACTOR

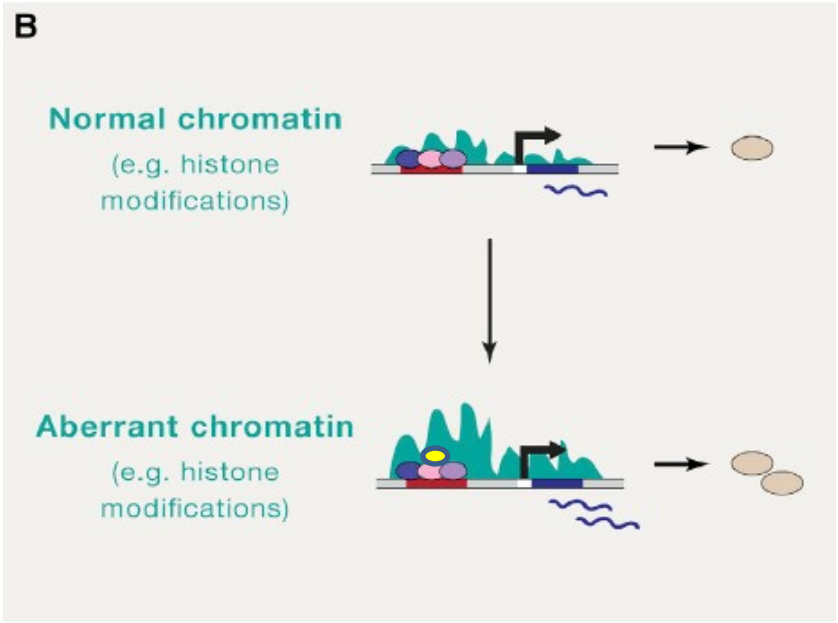




Chromatin remodelling plays a role in disease: transcription activation

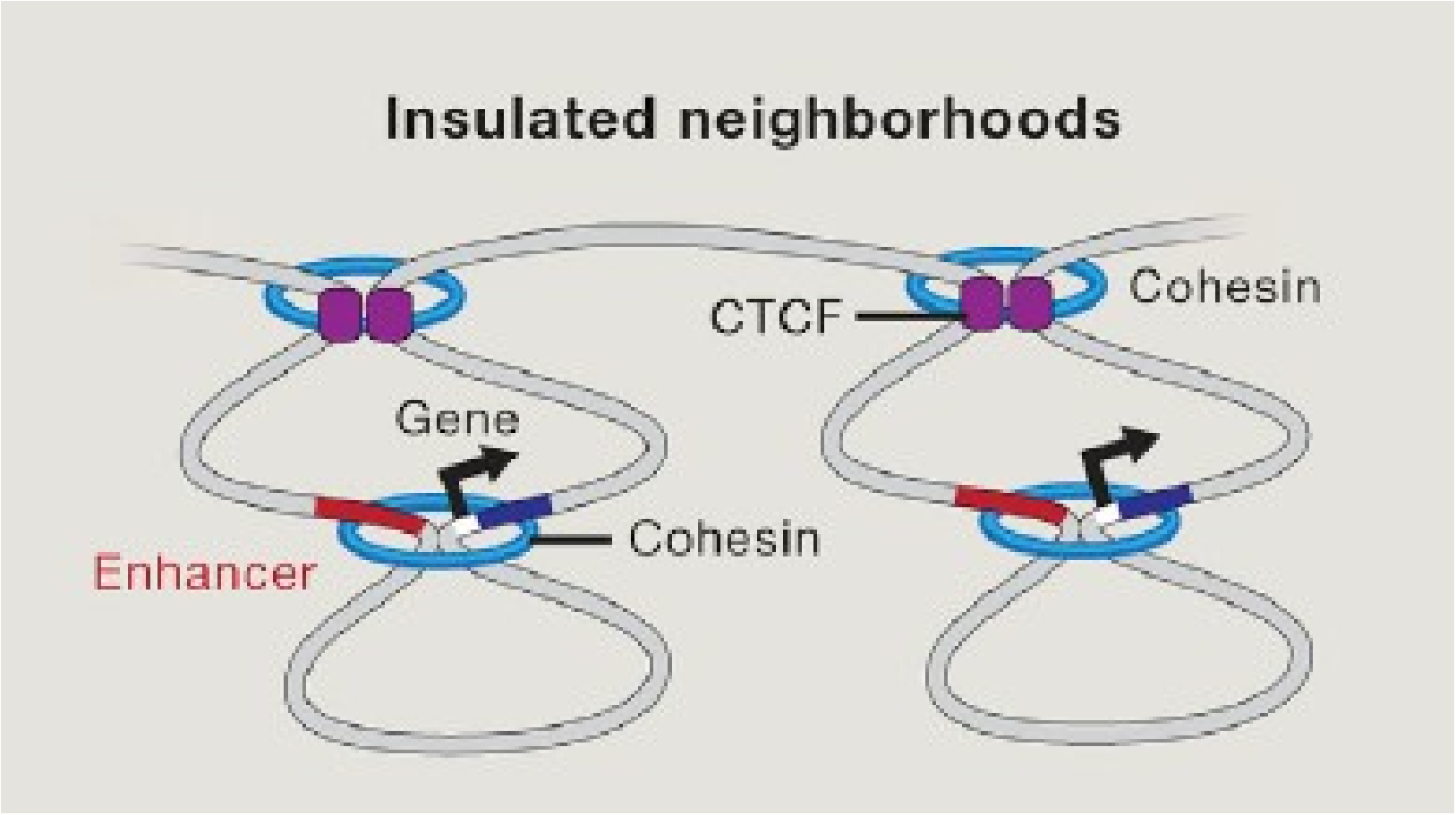


Chromatin remodelling plays a role in disease: transcription activation

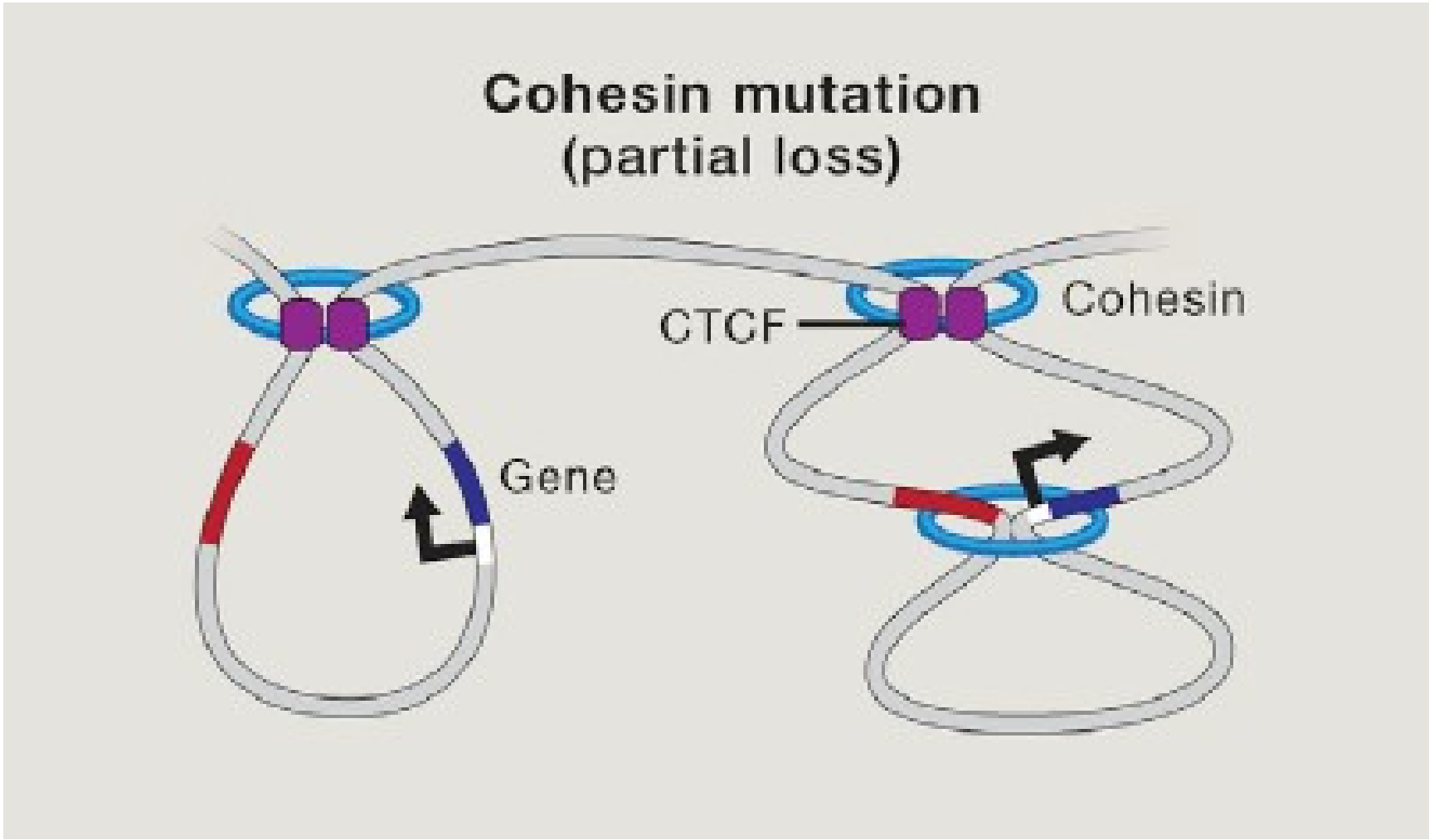


Efficient transcriptional signaling from enhancers to promoters is often **chromatin dependent**, mediated by specialized transcriptional **cofactors** that physically associate with or biochemically modify the genome to reinforce gene activation or repression. **Chromatin regulators function globally**, so their dysregulation can also have profound effects on the gene expression program of cells

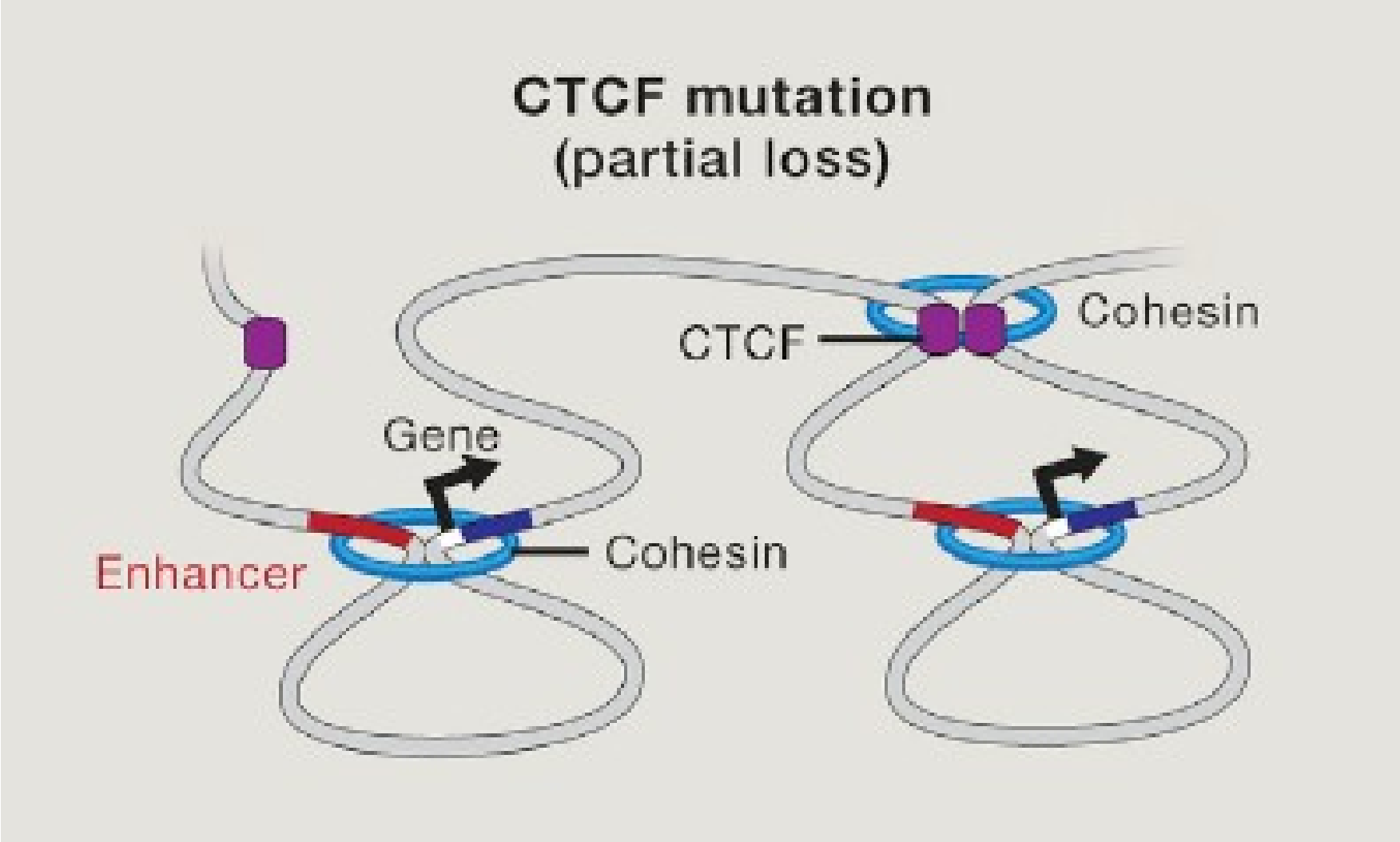
Long range interactions are mediated by CTCF and cohesin and define specific regulatory domain



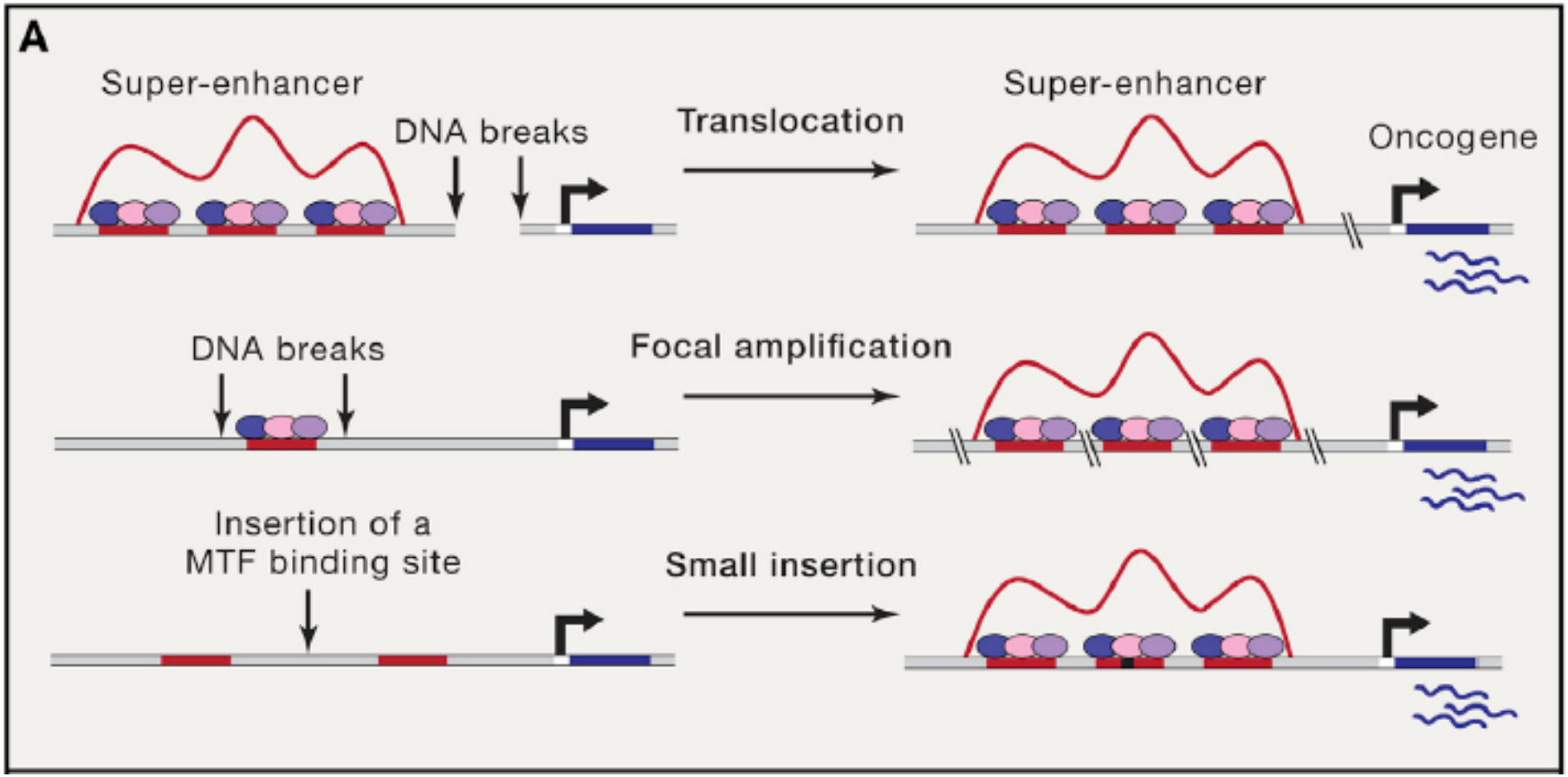
Cohesin mutation may disrupt long range interactions between enhancer-promoter



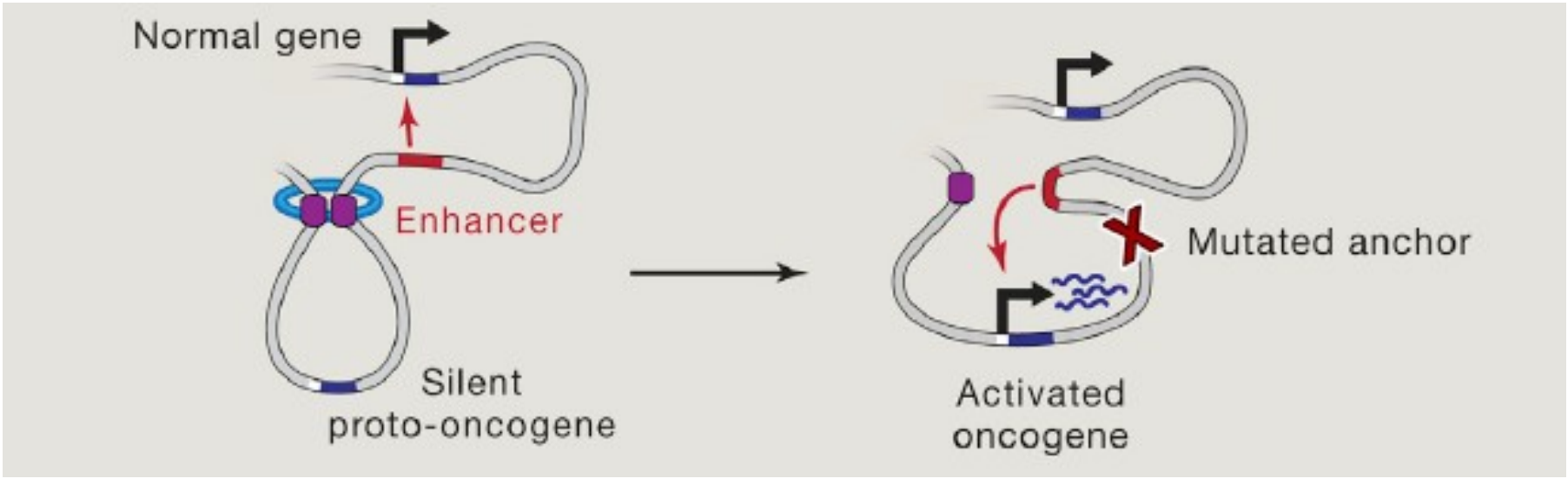
CTCF mutation may favorite long range interactions between enhancer-promoter



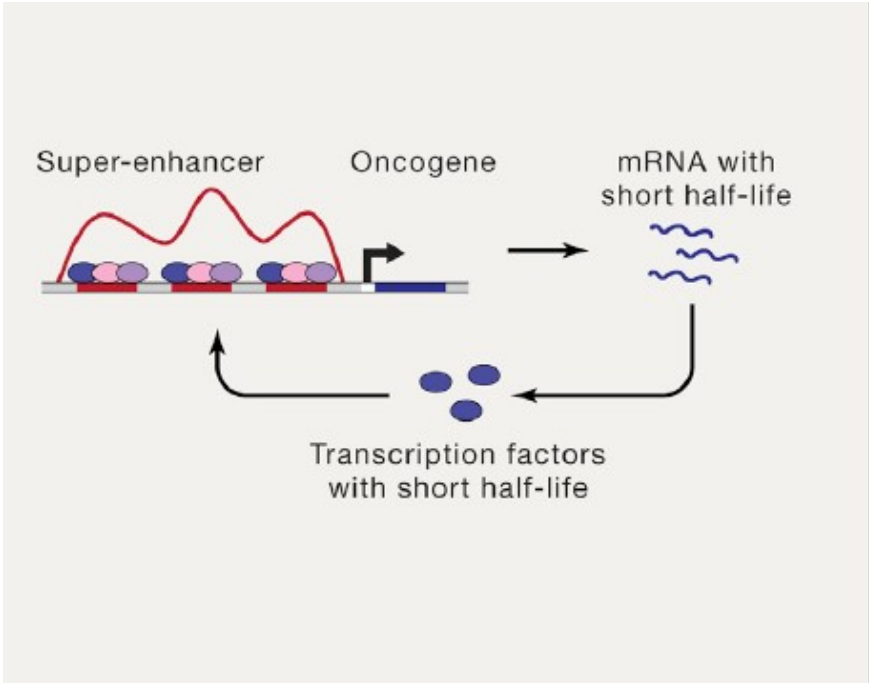
Mechanisms that lead SE formation

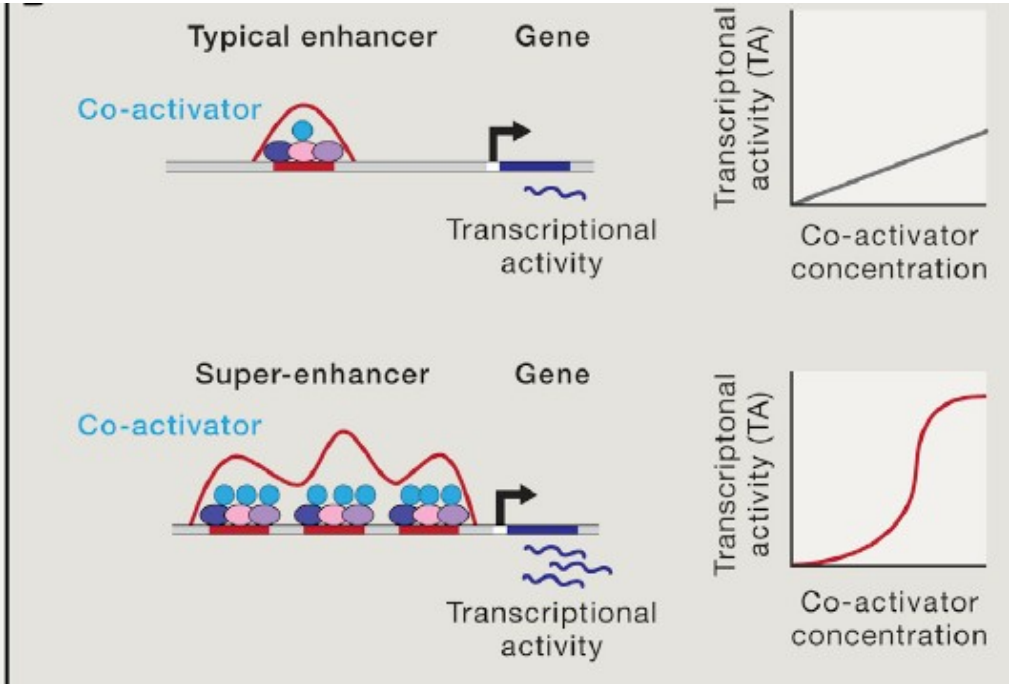


Activation of silent proto-oncogenes by somatic mutations that disrupt insulated neighborhood anchor sites



Molecular mechanisms that may be used for drug discovery

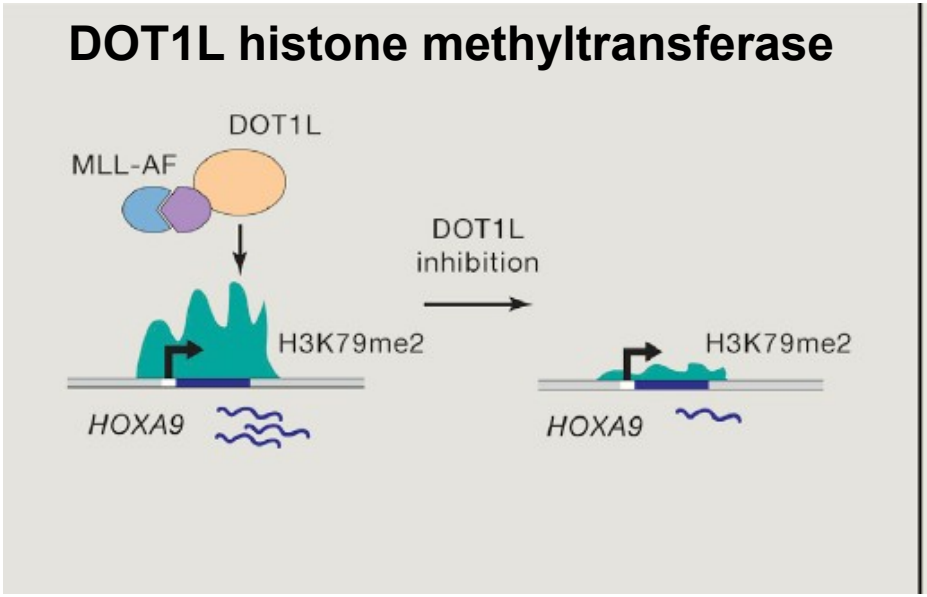


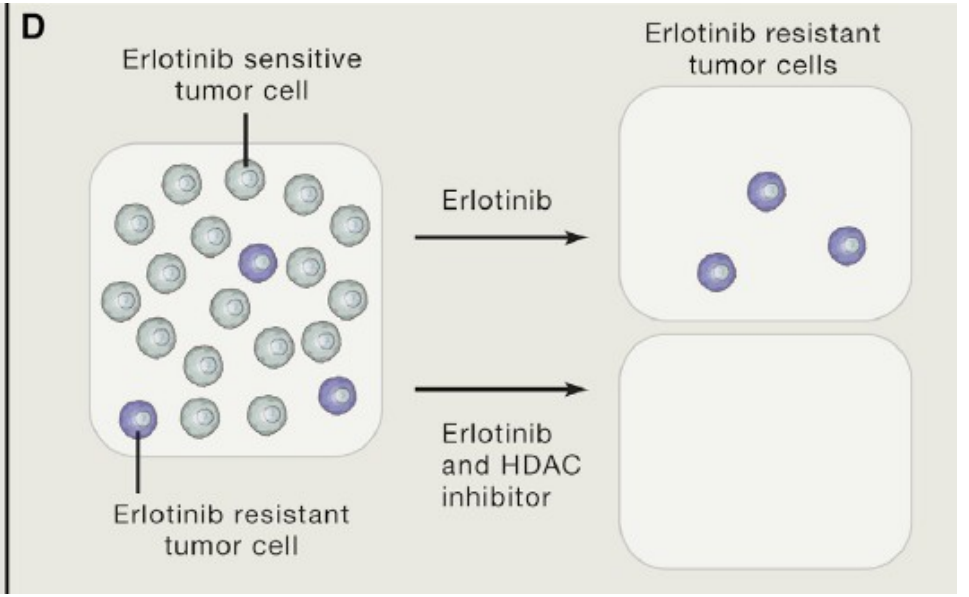


Increased the turnover of TFs

Cooperative Cofactors function about TFs

The drug-tolerant tumor cells can, in turn, be ablated with histone deacetylase inhibitors, establishing a paradigm of **combination therapy** using inhibitors of chromatin regulators against drug resistance

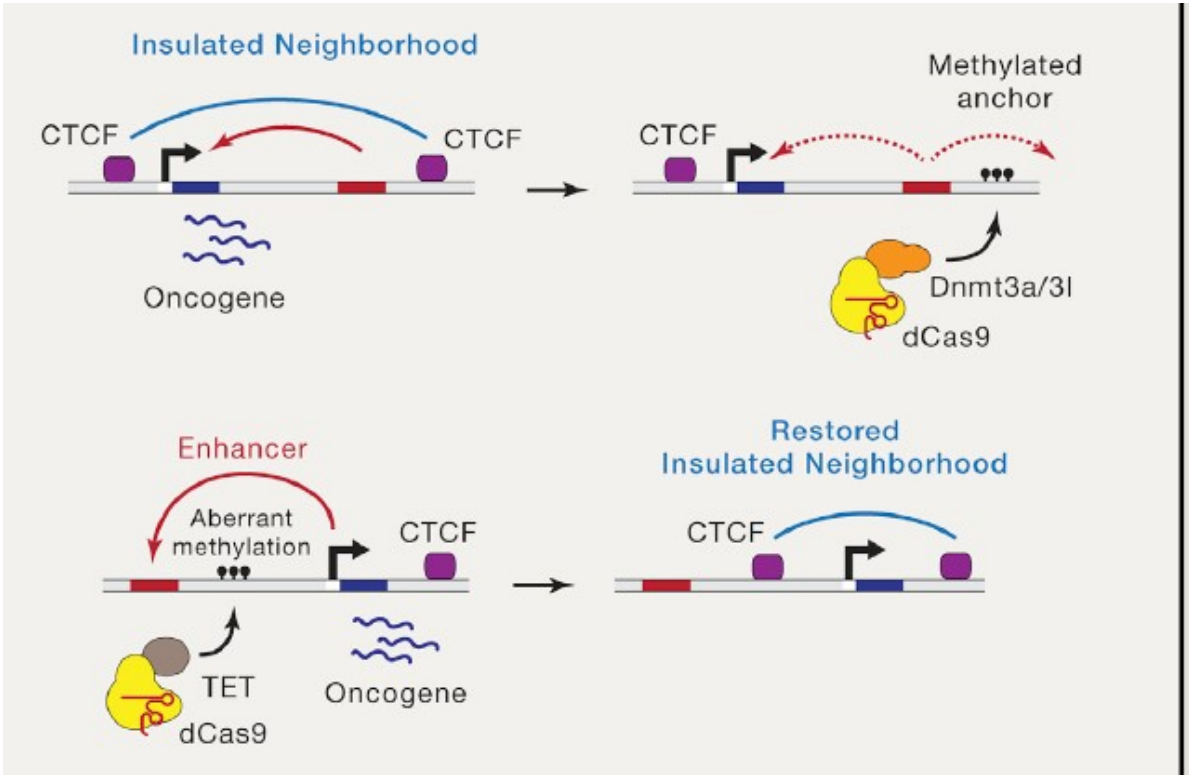


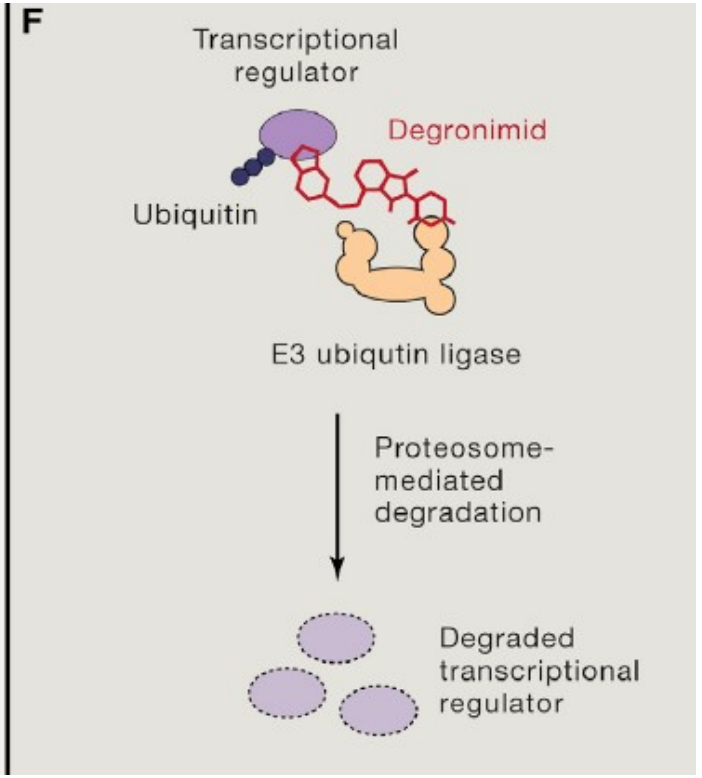


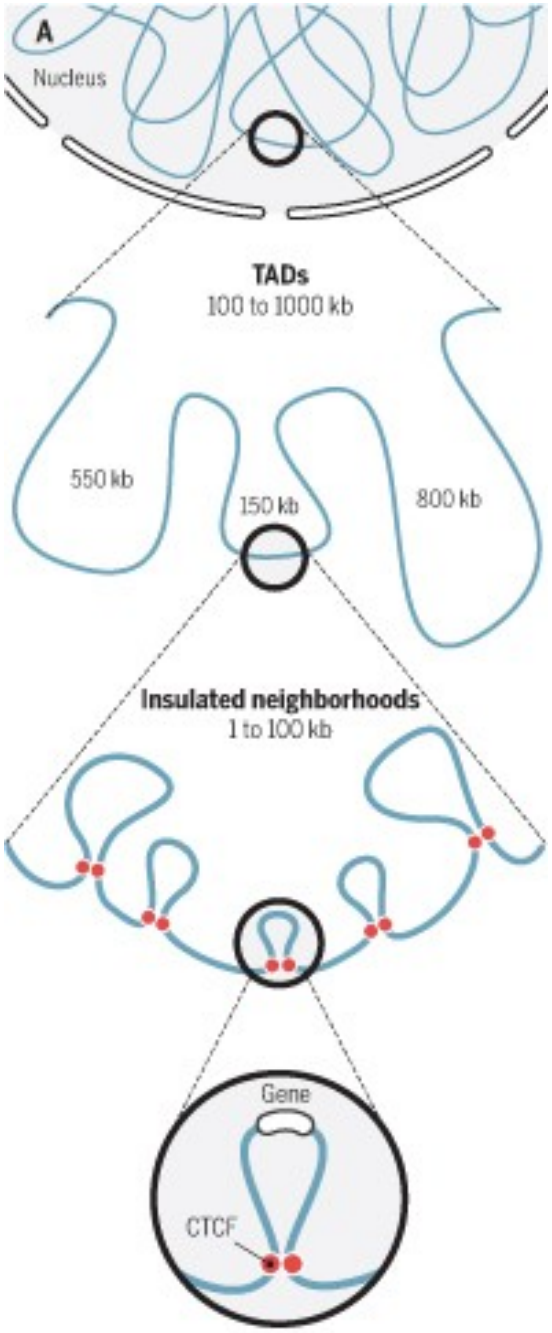
DOT1L histone methyltransferase

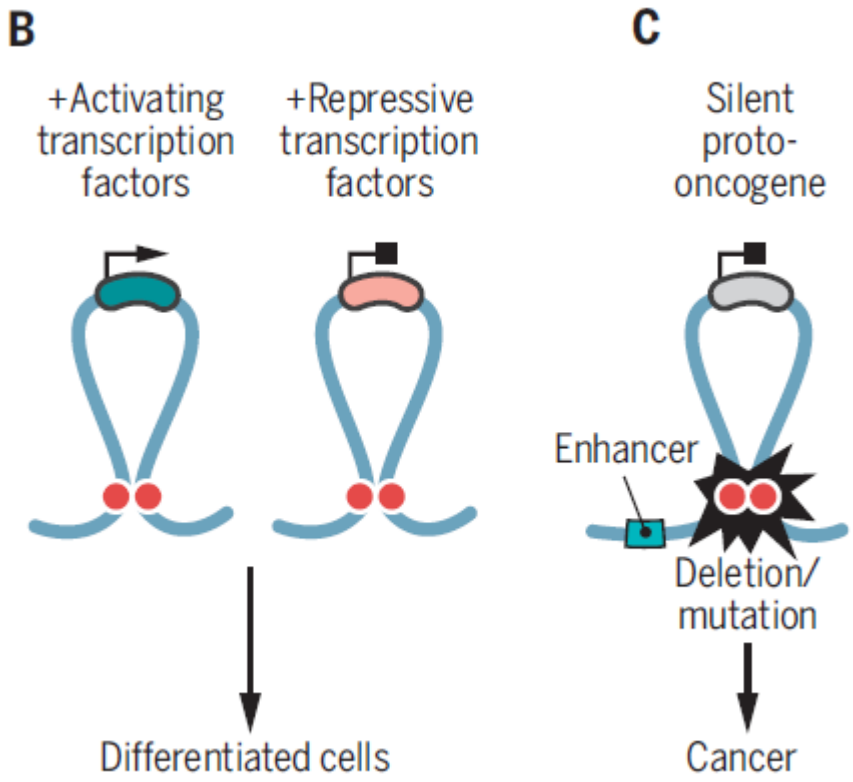
Somatic Mutations and aberrant DNA methylation drive oncogenesis

- Genome editing may represent a promise technology to reverse disease mechanisms
  - Activation of degradation pathway of TFs





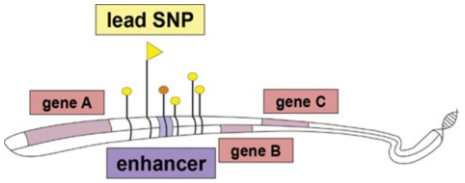

A
Nucleus
TADs
100 to 1000 kb
550 kb
150 kb
800 kb
Insulated neighborhoods
1 to 100 kb
Gene
CTCF


B
+Activating transcription factors
+Repressive transcription factors
Differentiated cells
C
Silent proto-oncogene
Enhancer
Deletion/ mutation
Cancer

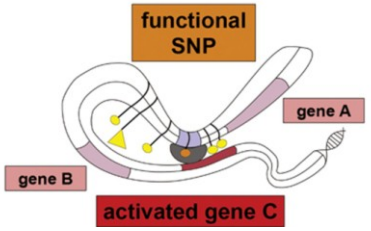
IDENTIFICATION AND CHARCTERIZATION

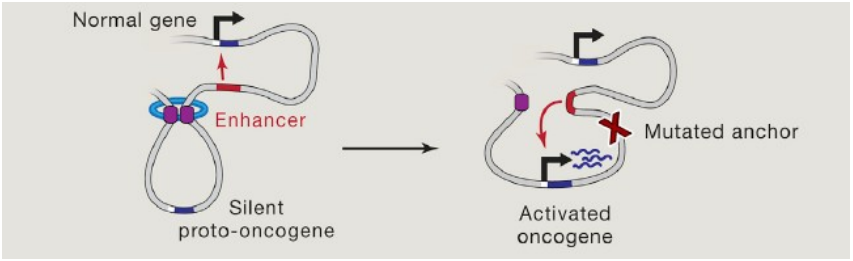
MOLECULAR MECHANISMS

GENOMIC REGULATORY REGIONS



GENE EXPRESSION REGULATION





CELL IDENTITY

BIOLOGICAL FUNCTIONS

TO UNDERSTAND DISEASES

In summary:

-Oncogenesis is based on growth tumors and one molecular mechanism is the transcription activation

- Aberrant transcription activation depends on:

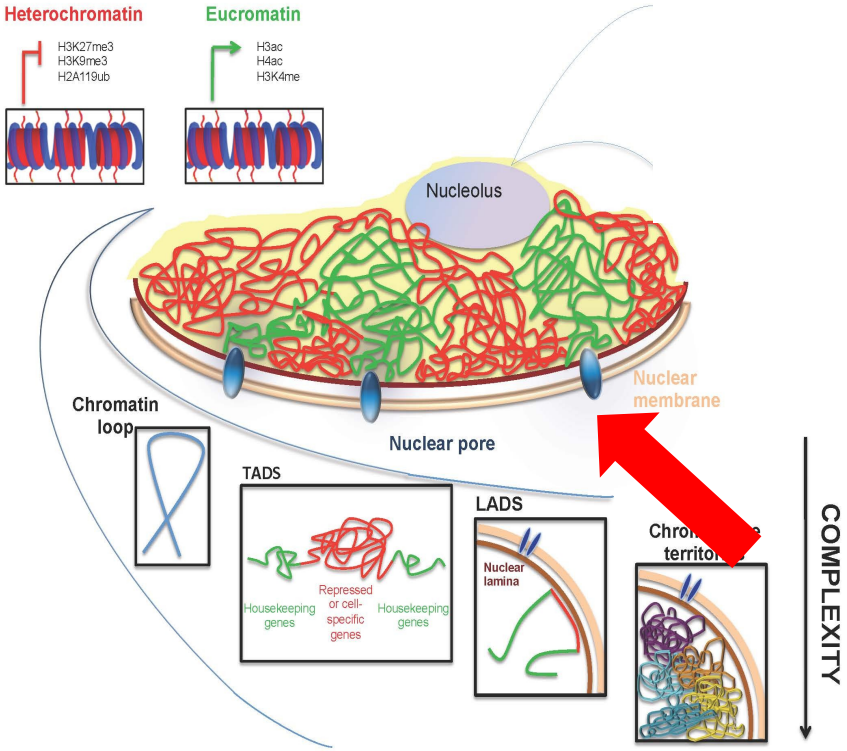
- super-enhancers formation
- transcription factors and cofactors dyregulation
- Long range interactions dynamic

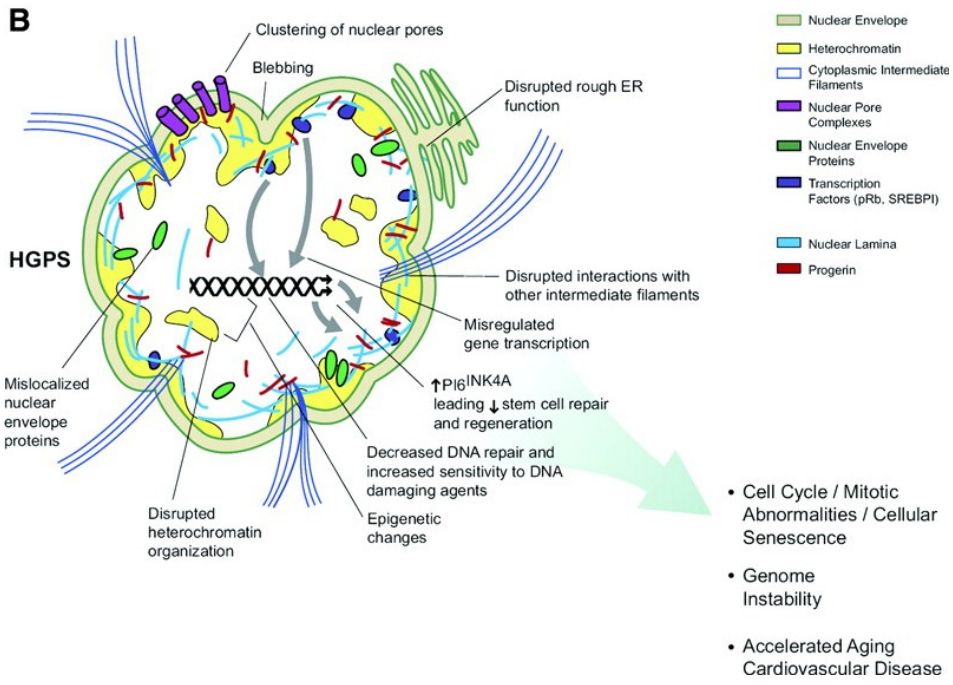
In summary II:

Dysregulated transcriptional programs may be target for drug discovery:

- Increased turnover of oncogenes
- Interfering with cooperation between TFs and cofactors
- Targeting chromatin remodeling enzymes
- Genome editing of specific regulatory regions
- Activation of proteasome degradation machinery

SINGLE NUCLEOTIDE VARIATIONS ASSOCIATED WITH PROTEIN IMPORTANT IN CHROMATIN ORGANIZATION, LAMININ, INDUCES LAMINOPATHIES





Lamina-Associated Domains: Links with Chromosome Architecture, Heterochromatin, and Gene Repression

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http://dx.doi.org/10.1016/j.cell.2017.04.022

In metazoan cell nuclei, hundreds of large chromatin domains are in close contact with the nuclear lamina. Such lamina-associated domains (LADs) are thought to help organize chromosomes inside the nucleus and have been associated with gene repression. Here, we discuss the properties of LADs, the molecular mechanisms that determine their association with the nuclear lamina, their dynamic links with other nuclear compartments, and their proposed roles in gene regulation.

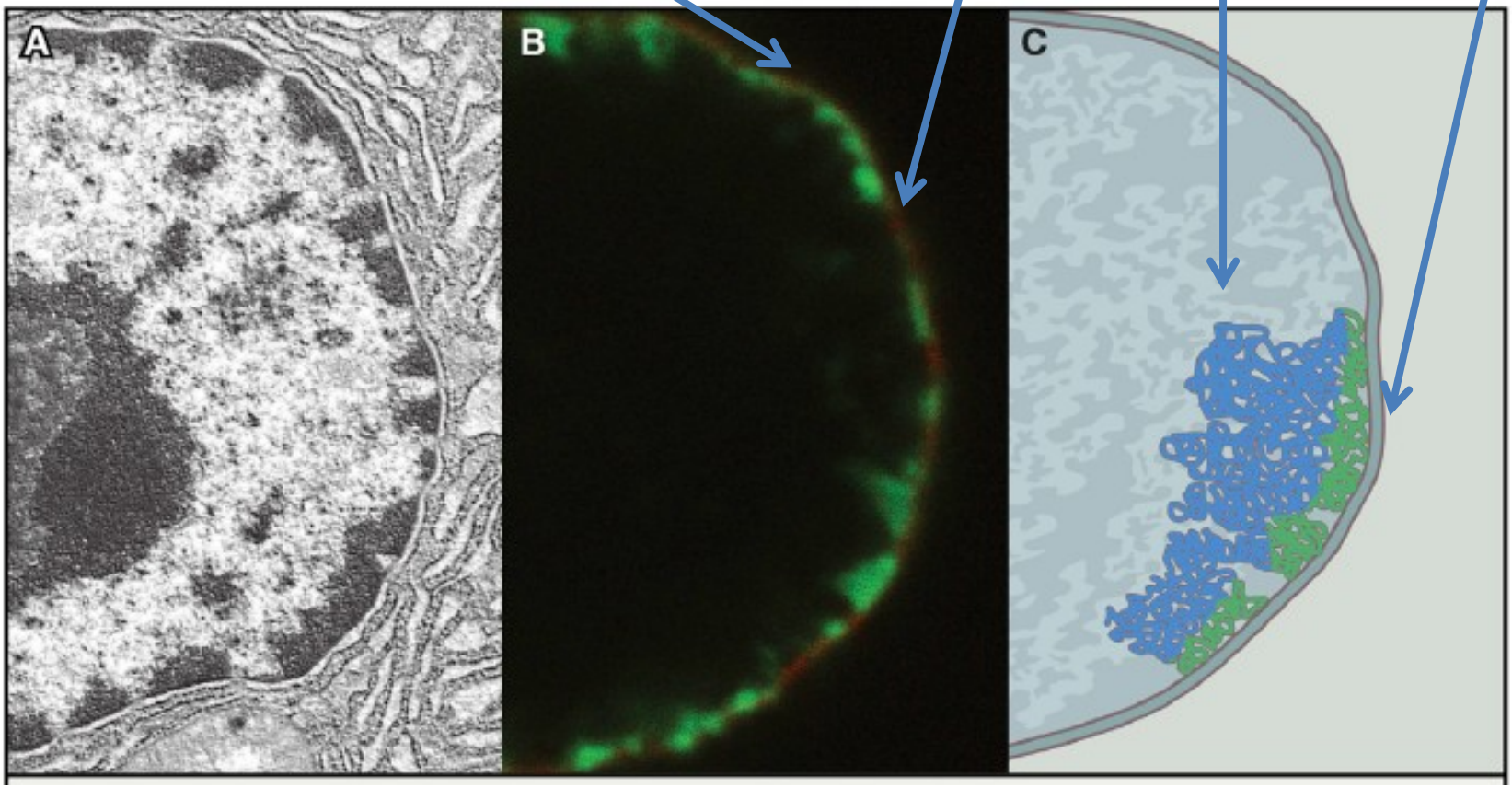
NUCLEAL LAMINA- ASSOCIATED HETEROCHROMATIN

GFP-tagged m6A-tracer protein that binds to adenine-methylated DNA (green)

Dam-Lamin B1 (red)

Chromosome (red)

LAD- Lamina associated domain (green)

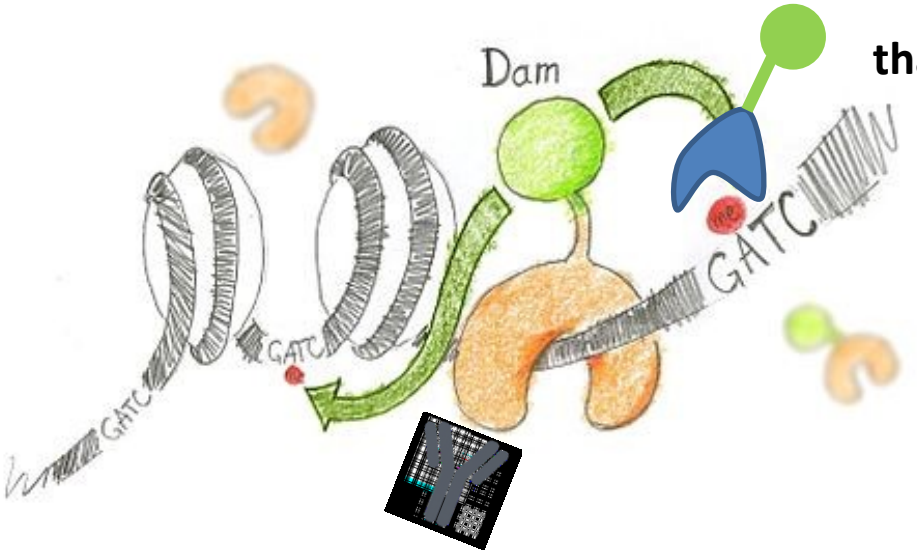


Electron microscopy

Confocal microscopy

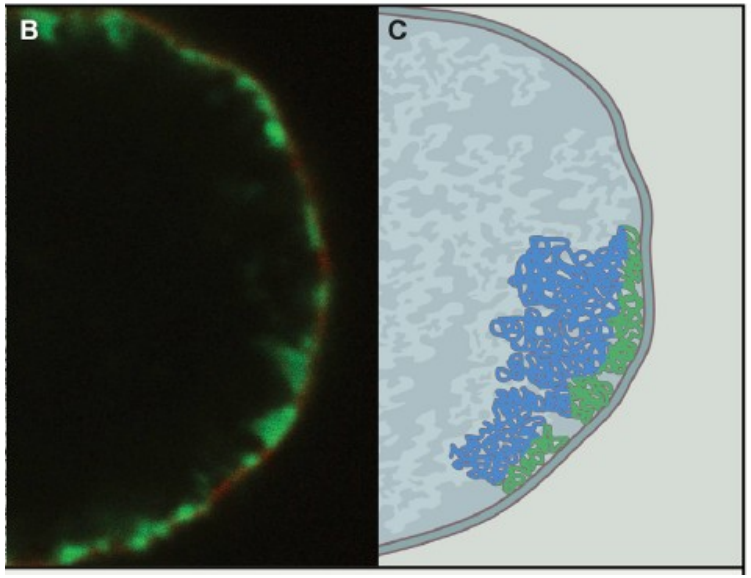
DamID (DNA adenine methyltransferase identification)

DamID identifies binding sites by expressing the proposed DNA-binding protein as a fusion protein with DNA methyltransferase. Binding of the protein of interest to DNA localizes the methyltransferase in the region of the binding site

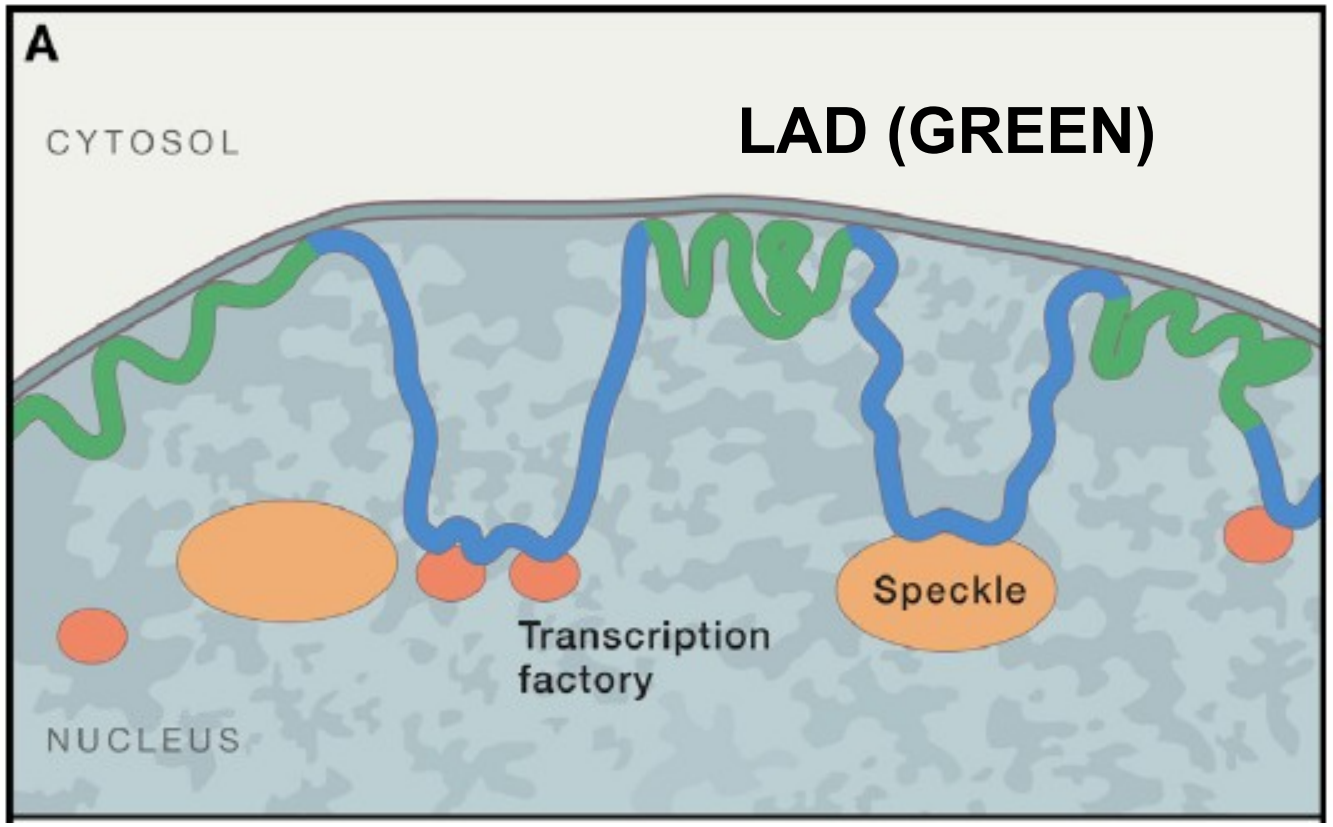


GFP-tagged m6A -tracer protein that binds to adenine-methylated DNA (green)

Antibodies against laminB1 red



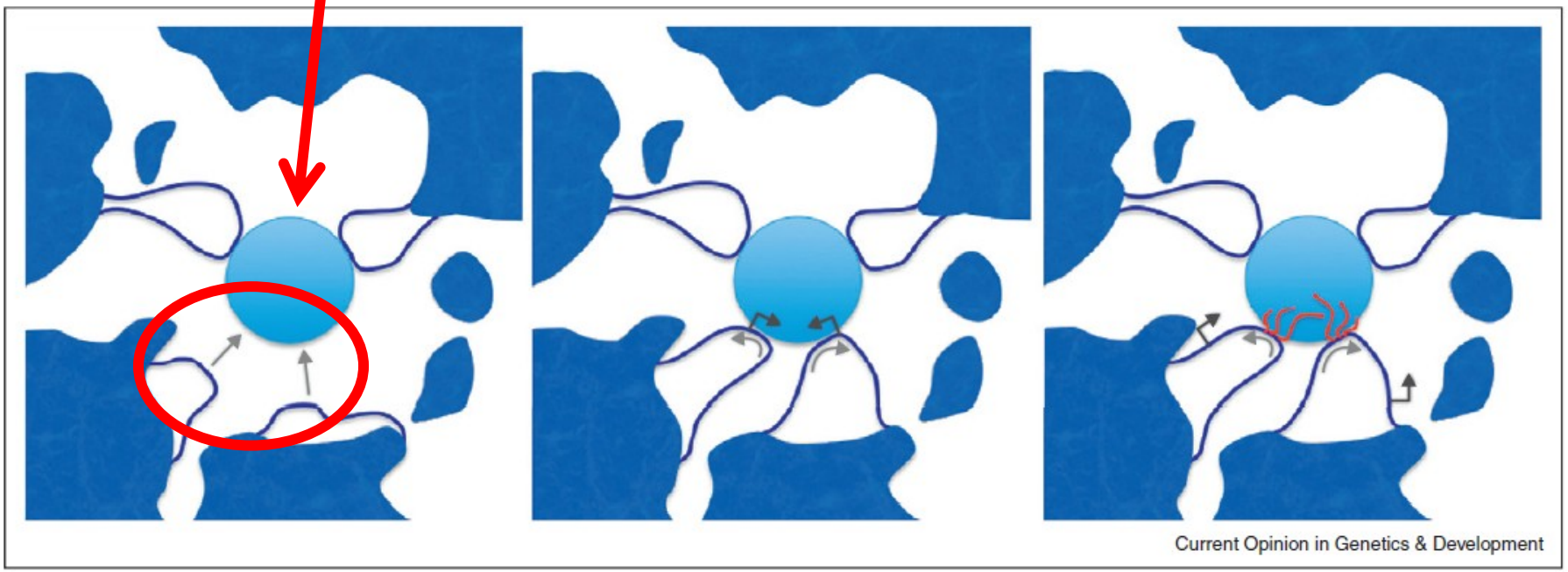
Transcription factories are discrete subnuclear foci composed of **active, phosphorylated RNAPII** and other transcriptional accessory and regulatory factors (RED)



SPLINCING FACTOR SPECKLE

Dynamic juxtapositioning of transcription units at transcription factories

Transcription factory



Active genes recruit to transcription factory

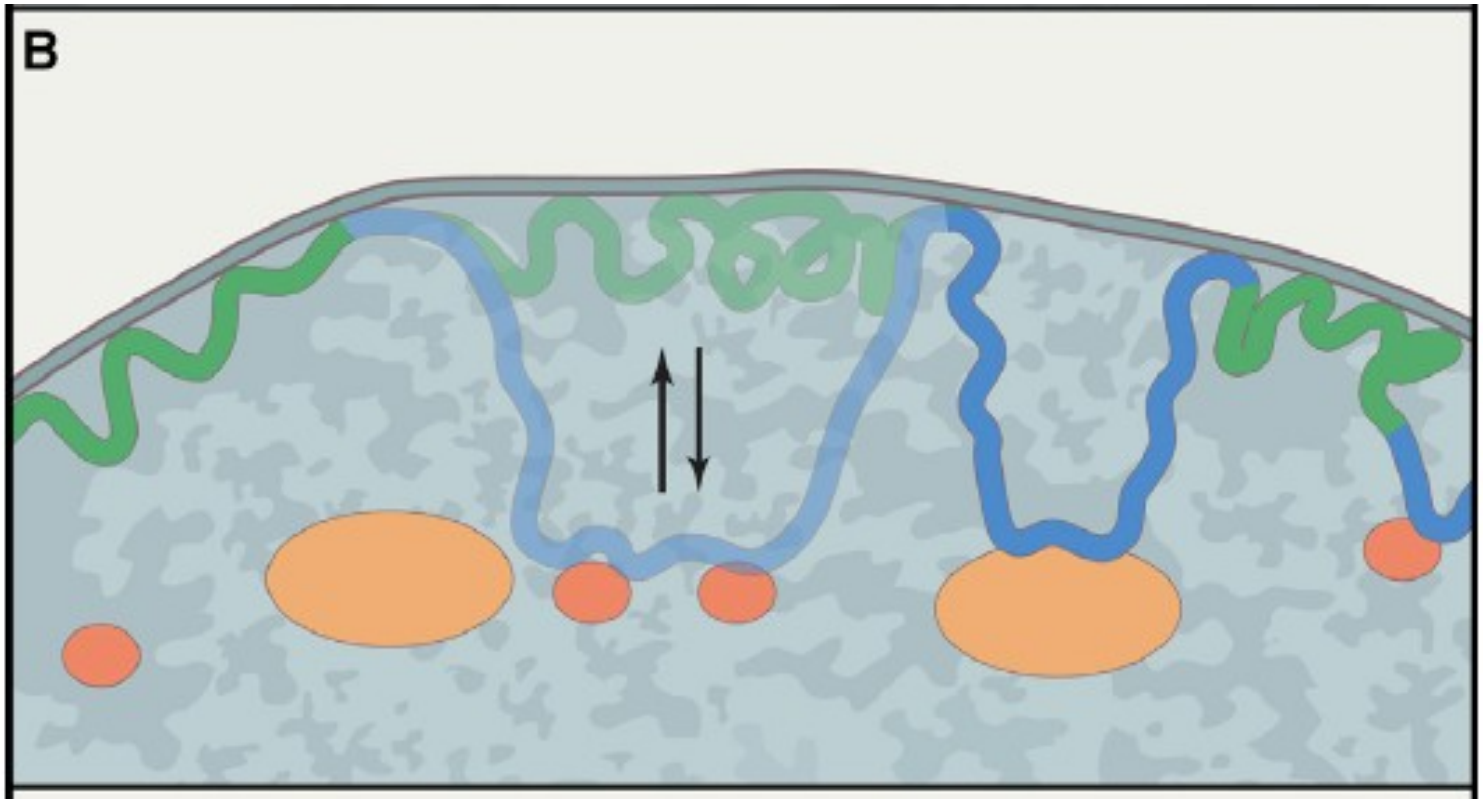
Active genes associates to transcription factory: RNA pol II complex formation

Transcription activation with RNA nascent (red). Induction of genes in proximity activation

Edelman et al., 2012

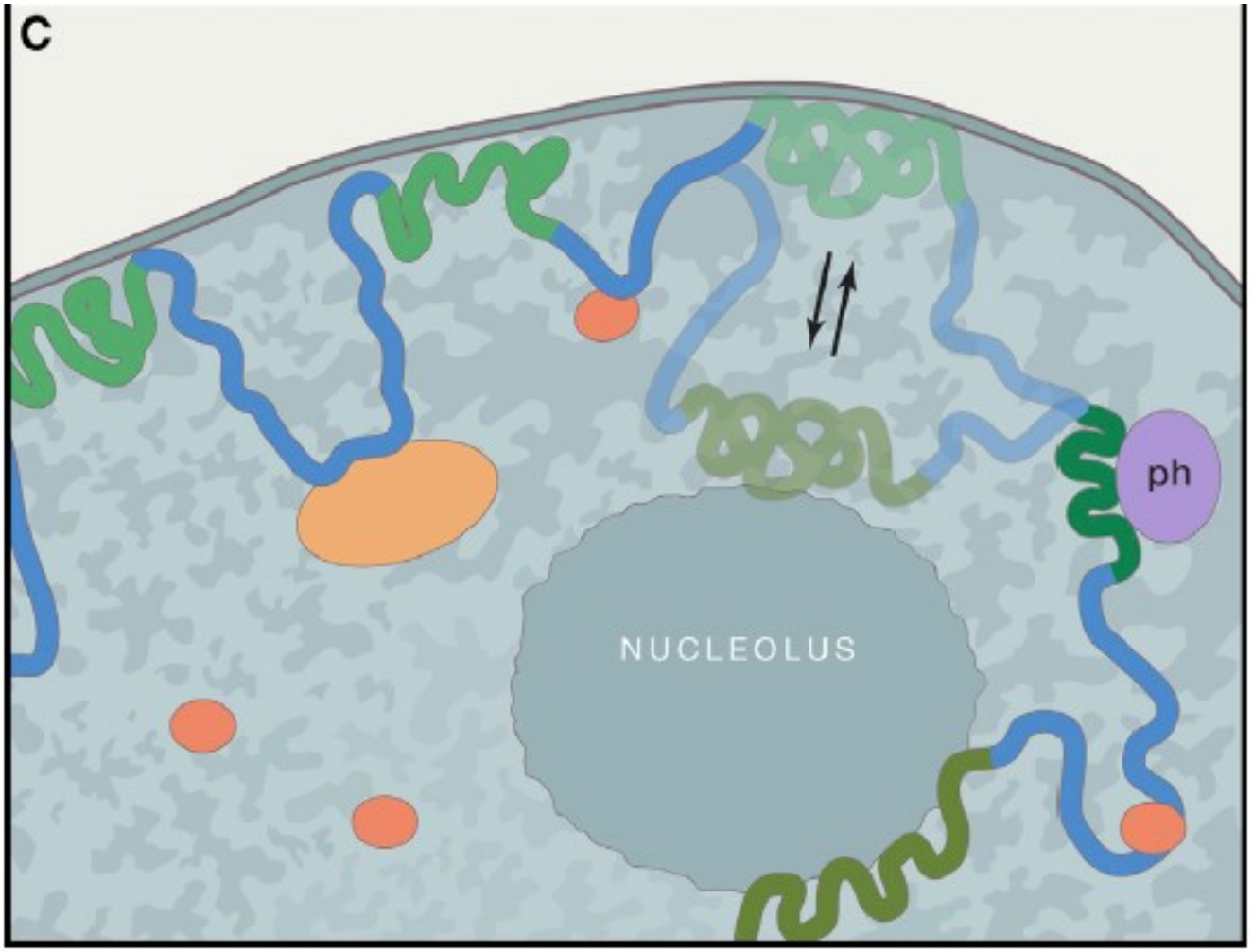
DYNAMIC TRANSCRIPTIONAL ACTIVATION

Some LADs (semi-transparent green) contact the NL erratically (i.e., in a subset of cells) and may **become transcriptionally active** when associated with a permissive compartment (semi-transparent blue).



How the chromatin is organized near the lamina-associated domain

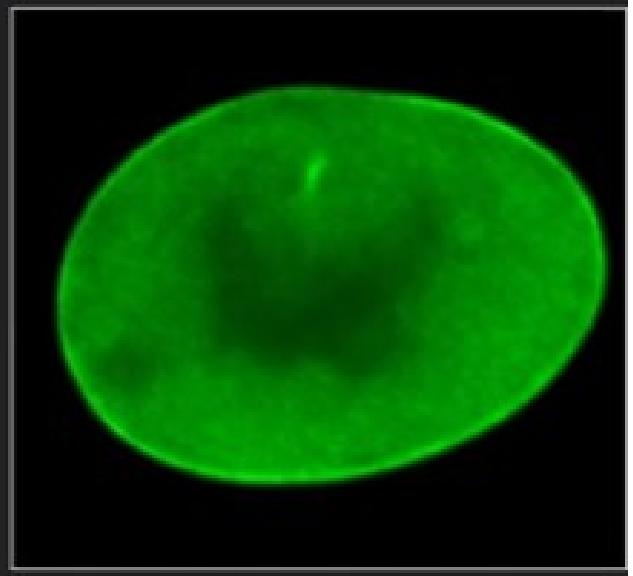
Some LADs are apparently **stochastically** distributed between the NL, nucleoli, and pericentromeric heterochromatin (ph), which are all repressive environments.



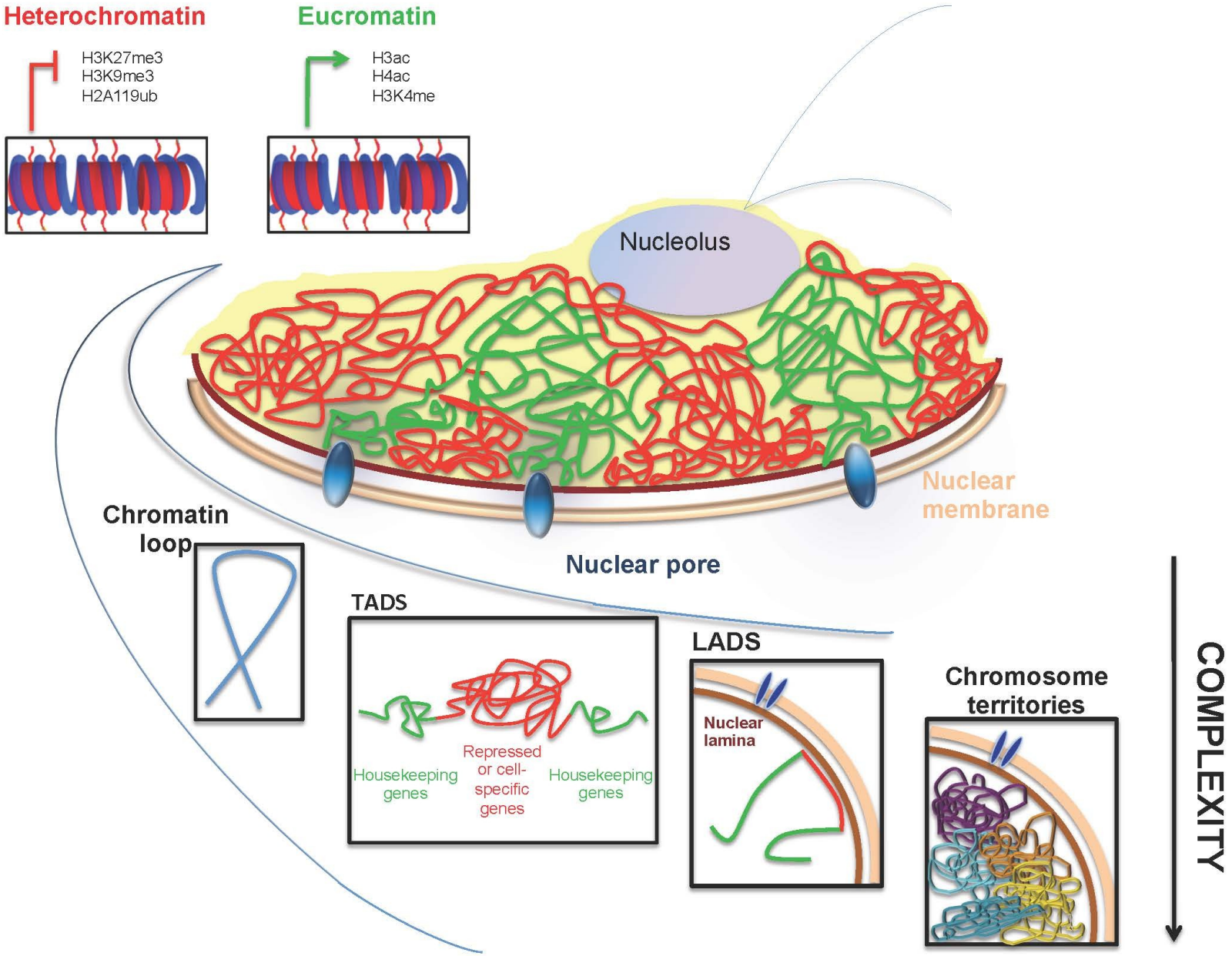
The Nuclear Lamins

Structural Properties and Functions

Major structural proteins of the lamina
Located throughout the nucleoplasm
Determinants of nuclear size and shape
Nuclear envelope assembly/ disassembly
Mitotic spindle assembly
DNA synthesis (chain elongation phase)
DNA damage repair
Transcription (RNA Pol II)
Cell proliferation and senescence
Structural support for nuclear memb.
Support and positioning of nuclear pores
Chromatin anchorage and organization

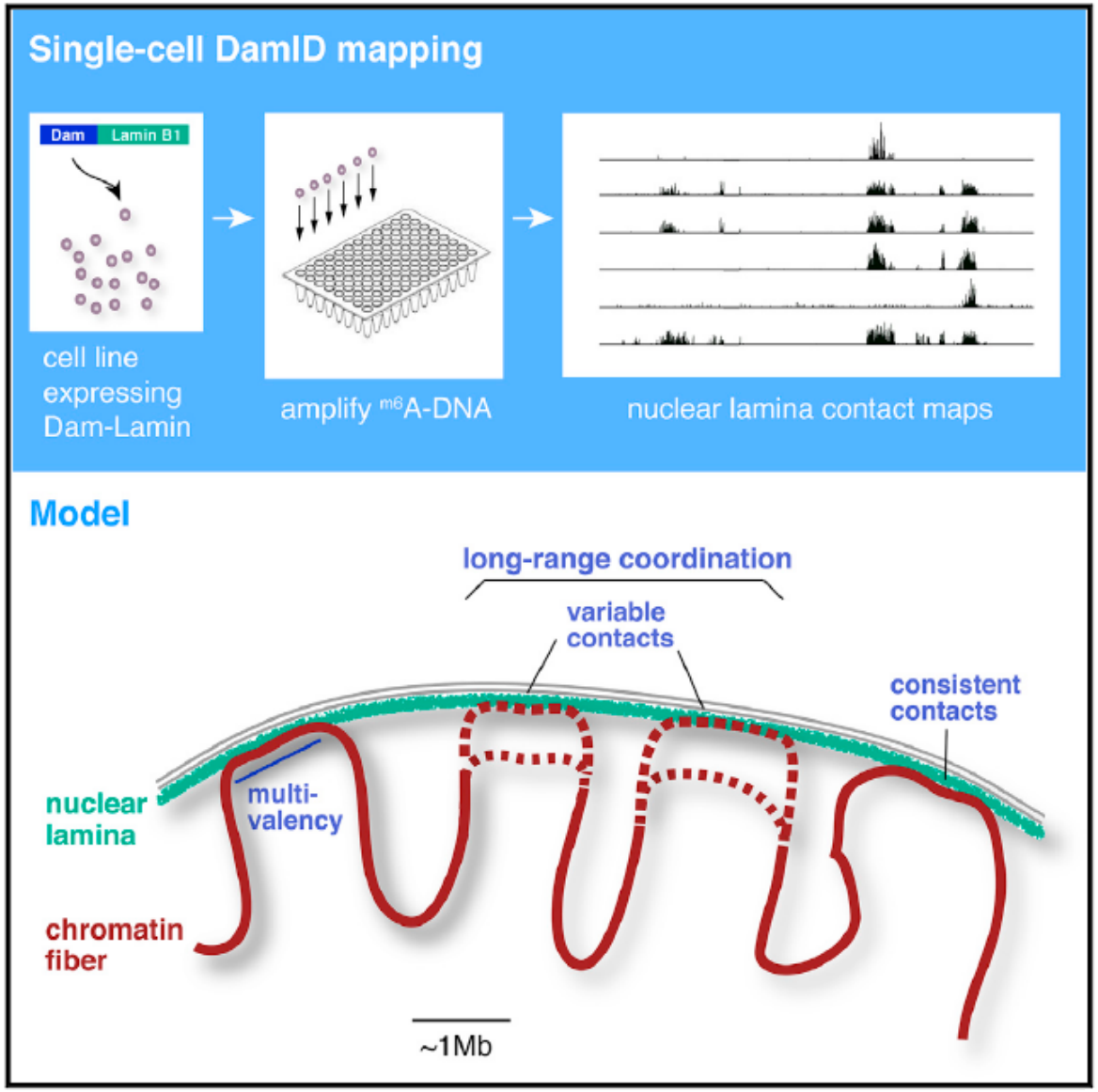


OVERVIEW OF CHROMATIN ARCHITECTURE: RELATIONSHIP BETWEEN TAD AND LAD



DamID : DNA adenine methyltransferase identification

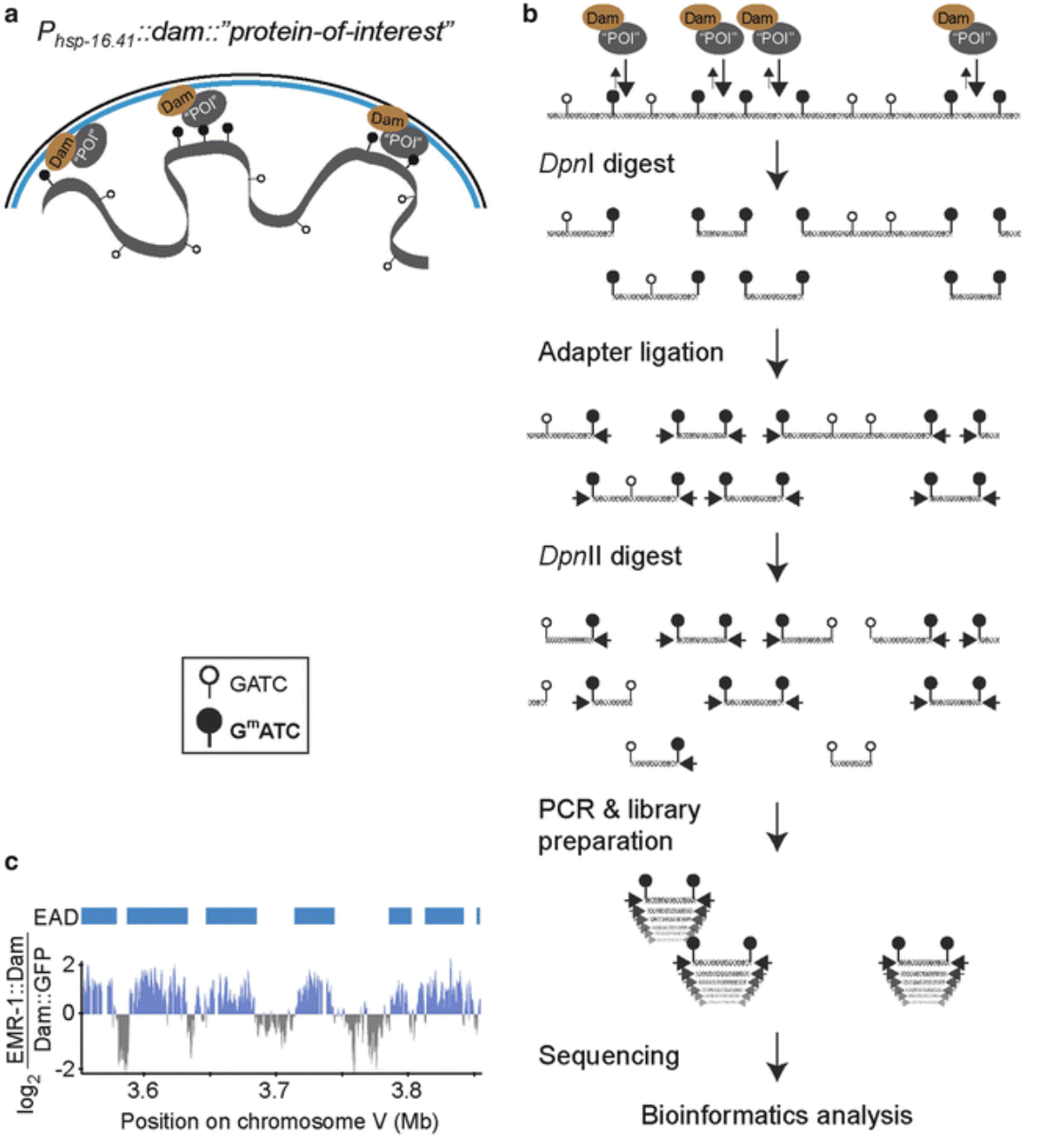
Graphical Abstract



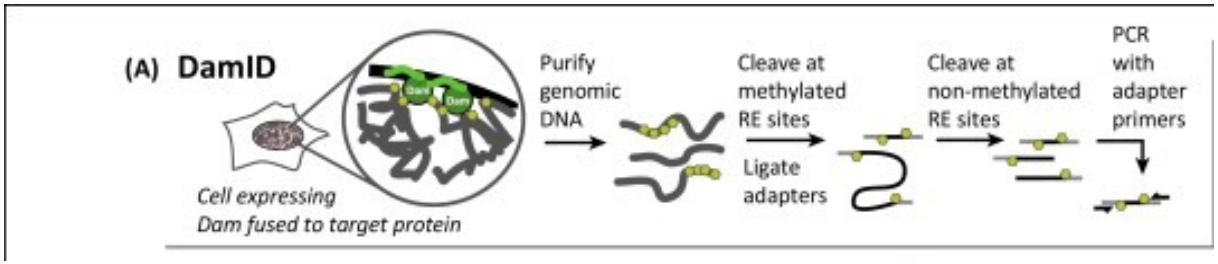
A modified DamID method enables the mapping of genome-wide nuclear lamina interactions in single human cells, providing insight into the cell-to-cell variation in the interphase chromosome architecture and suggesting extensive intra-chromosomal coordination of nuclear lamina contacts.

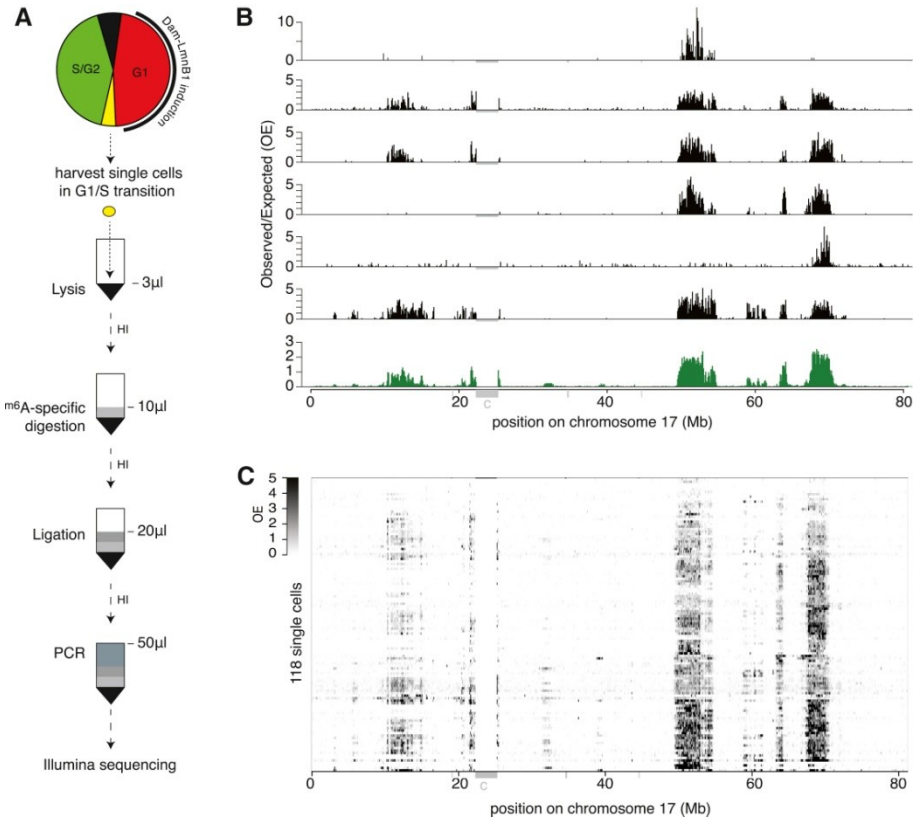
Kind et al., 2015, Cell *163*, 134–147

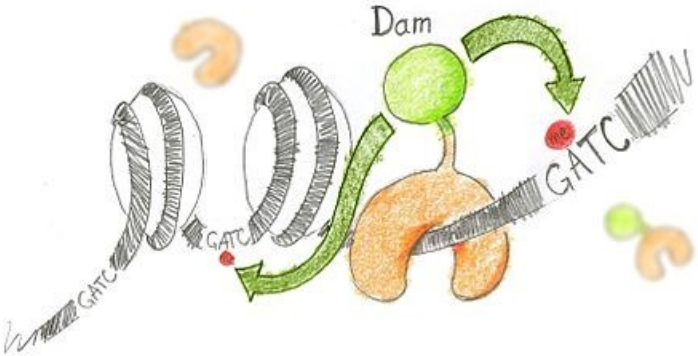
DamID : DNA adenine methyltransferase identification



DamID : DNA adenine methyltransferase identification

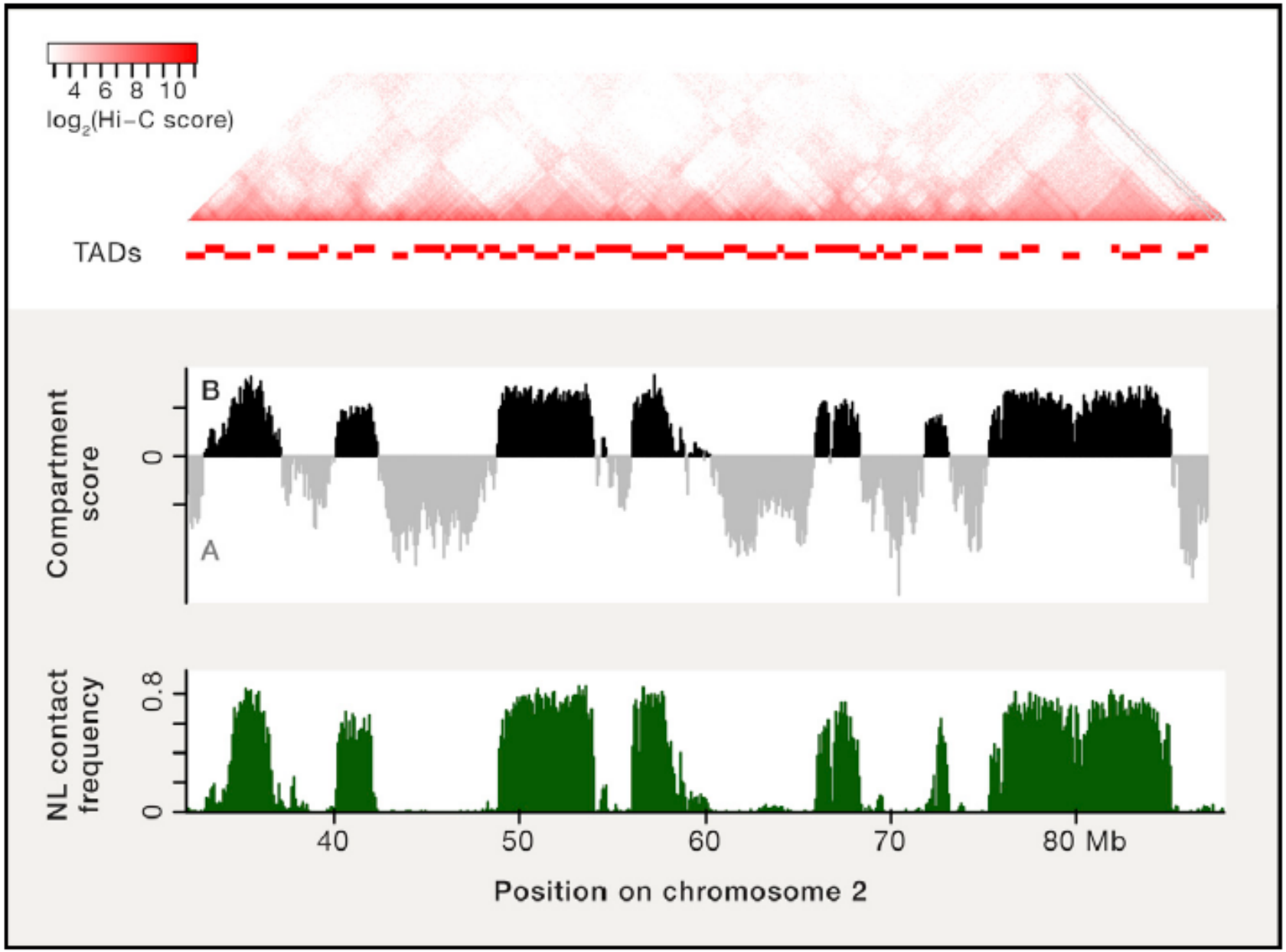




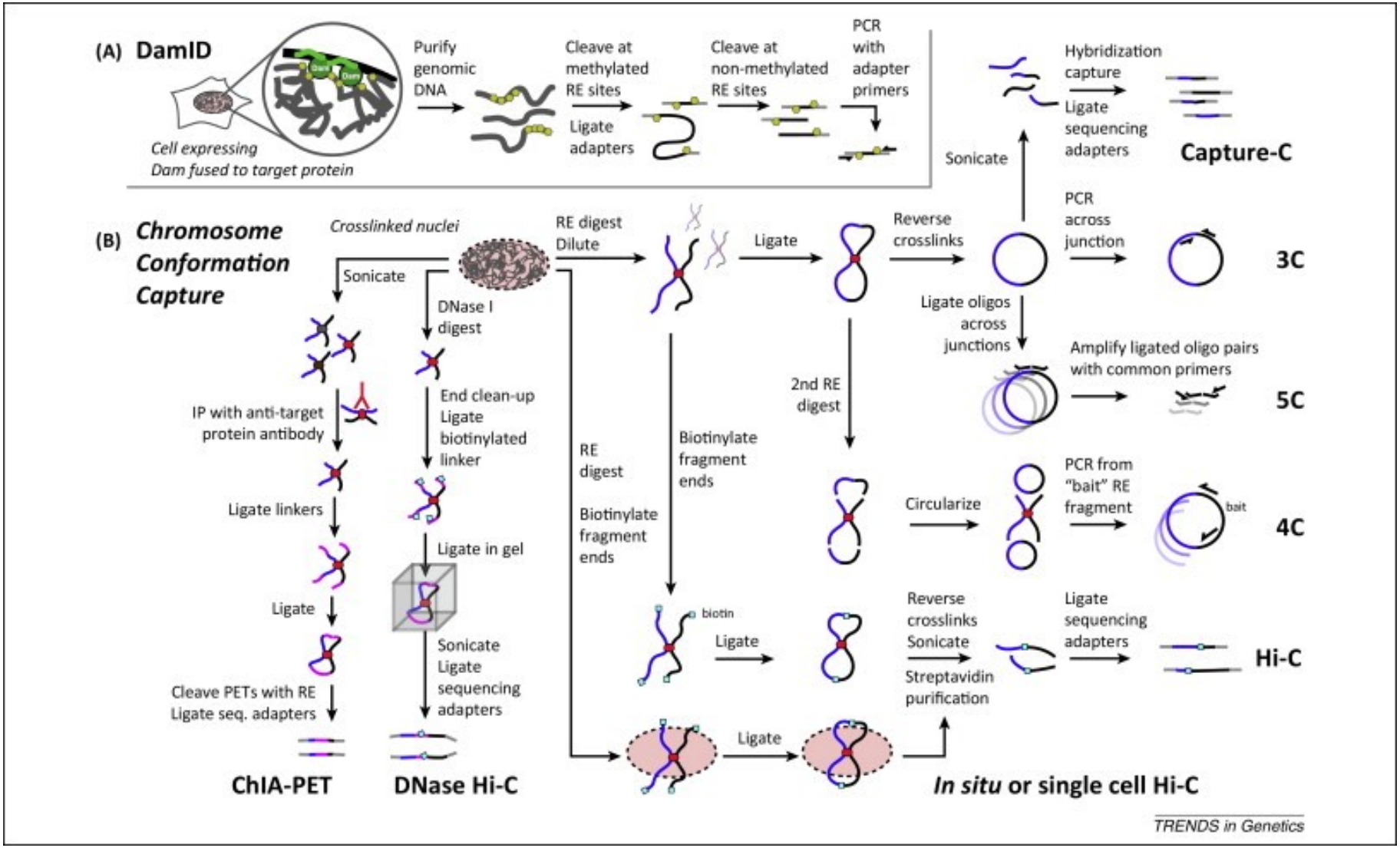


DamID is a molecular biology protocol used to map the binding sites of DNA- and chromatin-binding proteins in eukaryotes. DamID identifies binding sites by expressing the proposed DNA-binding protein as a fusion protein with DNA methyltransferase. Binding of the protein of interest to DNA localizes the methyltransferase in the region of the binding site.

Model derived from comparation of signals between Hi-C and DamID assays



CHROMATIN ORGANIZATION IN THE NUCLEUS USING CHROMATIN LOOPING TECHNIQUES



Hutchinson-Gilford progeria syndrome (HGPS)
PROGERIA

is caused by a **point mutation in the LMNA gene** that activates a cryptic donor splice site and yields a truncated form of prelamin A called progerin

LAMINA ALTERATIONS INDUCE DISEASE

Progeria, or Hutchinson–Gilford progeria syndrome (HGPS), is a rare, fatal genetic disease characterized by an **appearance of accelerated aging in children**.

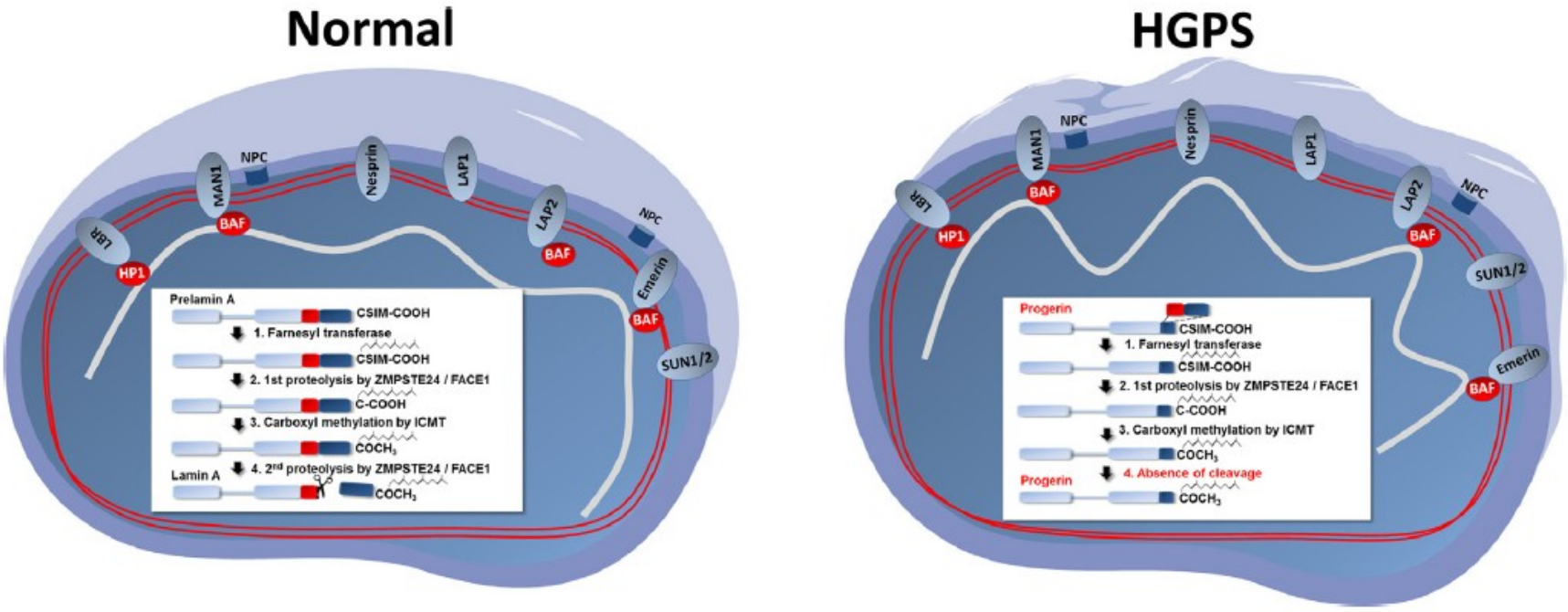
This syndrome is typically caused by mutations **in codon 1824, cryptic splincing site (p.G608G, no change aminoacid)** of the LMNA, leading to the production of a mutated form of lamin A precursor called **progerin**.

Progerin accumulates in cells causing progressive molecular defects, including nuclear shape abnormalities, chromatin disorganization, damage to DNA and delays in cell proliferation.

LAMINA ALTERATIONS INDUCE DISEASE

Progeria, or Hutchinson–Gilford progeria syndrome (HGPS)

Codon 608 (p.G608G) of the LMNA: mutated form of lamin A precursor called **progerin**.

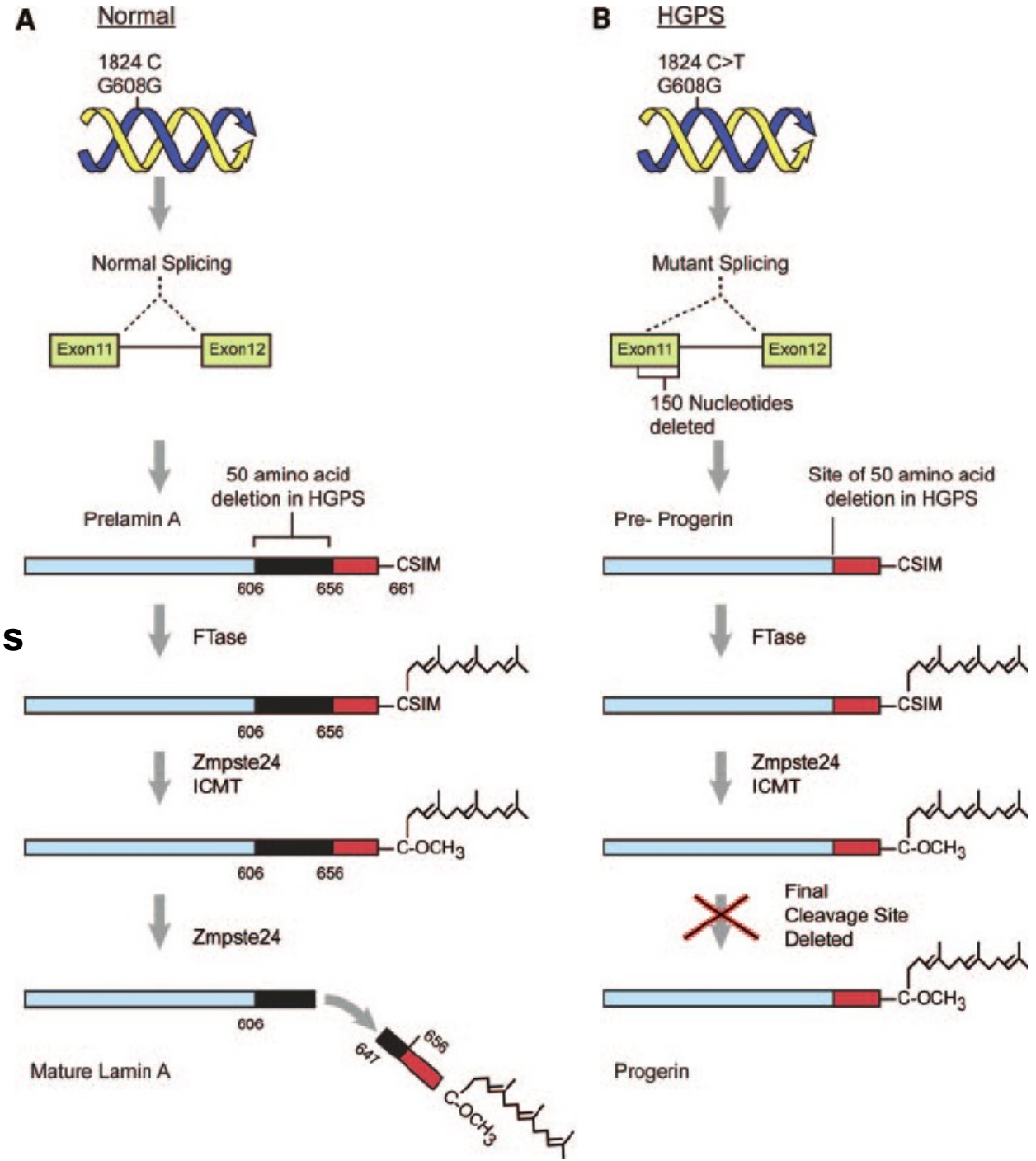


PRELAMININ A

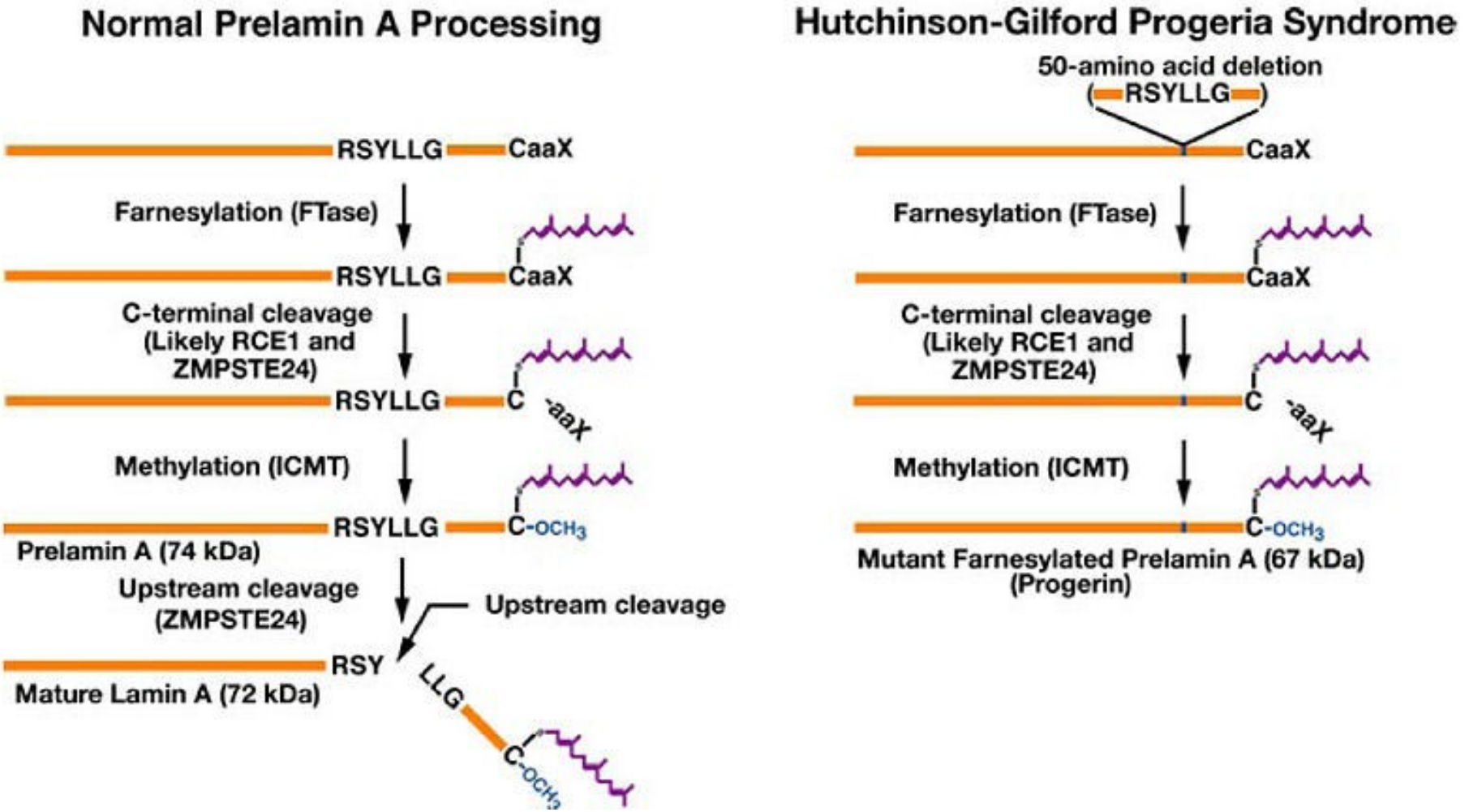
farnesylated C-terminus

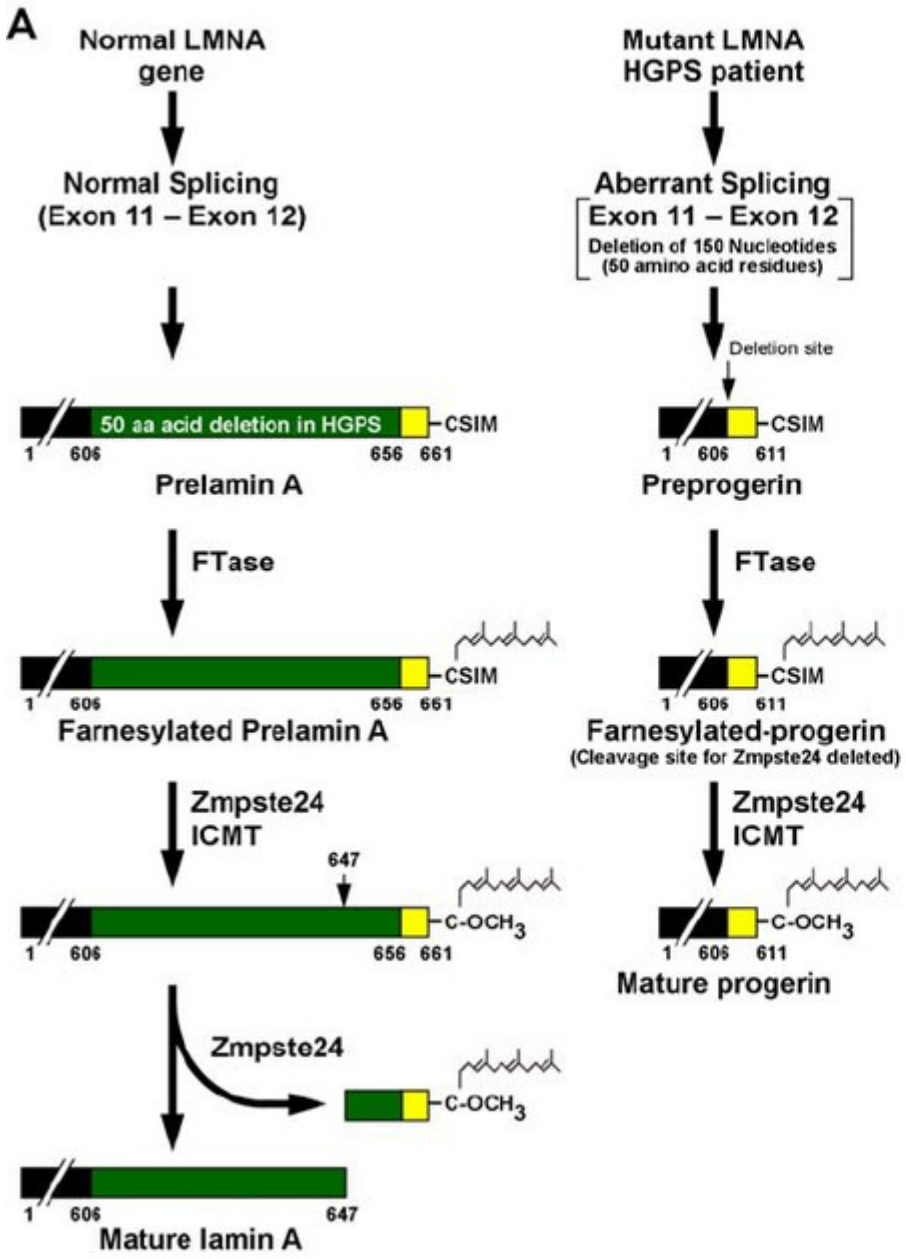
ZMPSTE24 enzyme cleave the farnesylated C-terminus

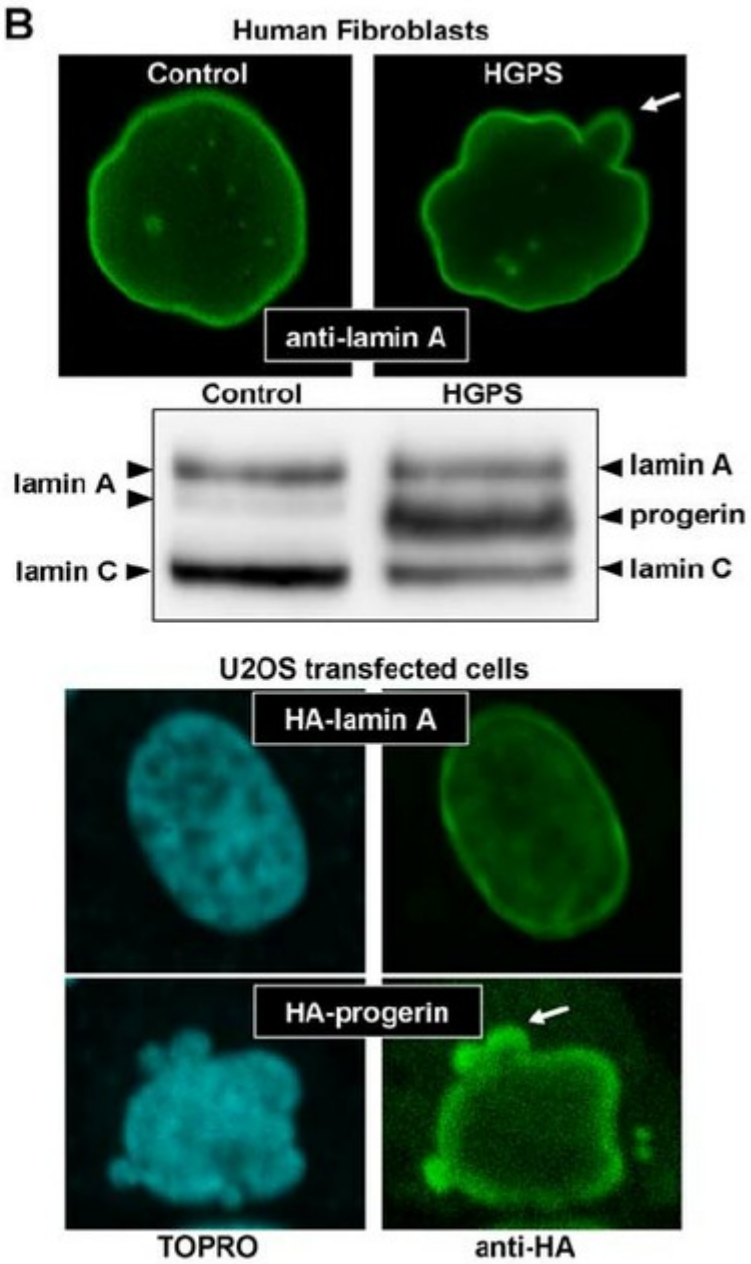
LAMININ A



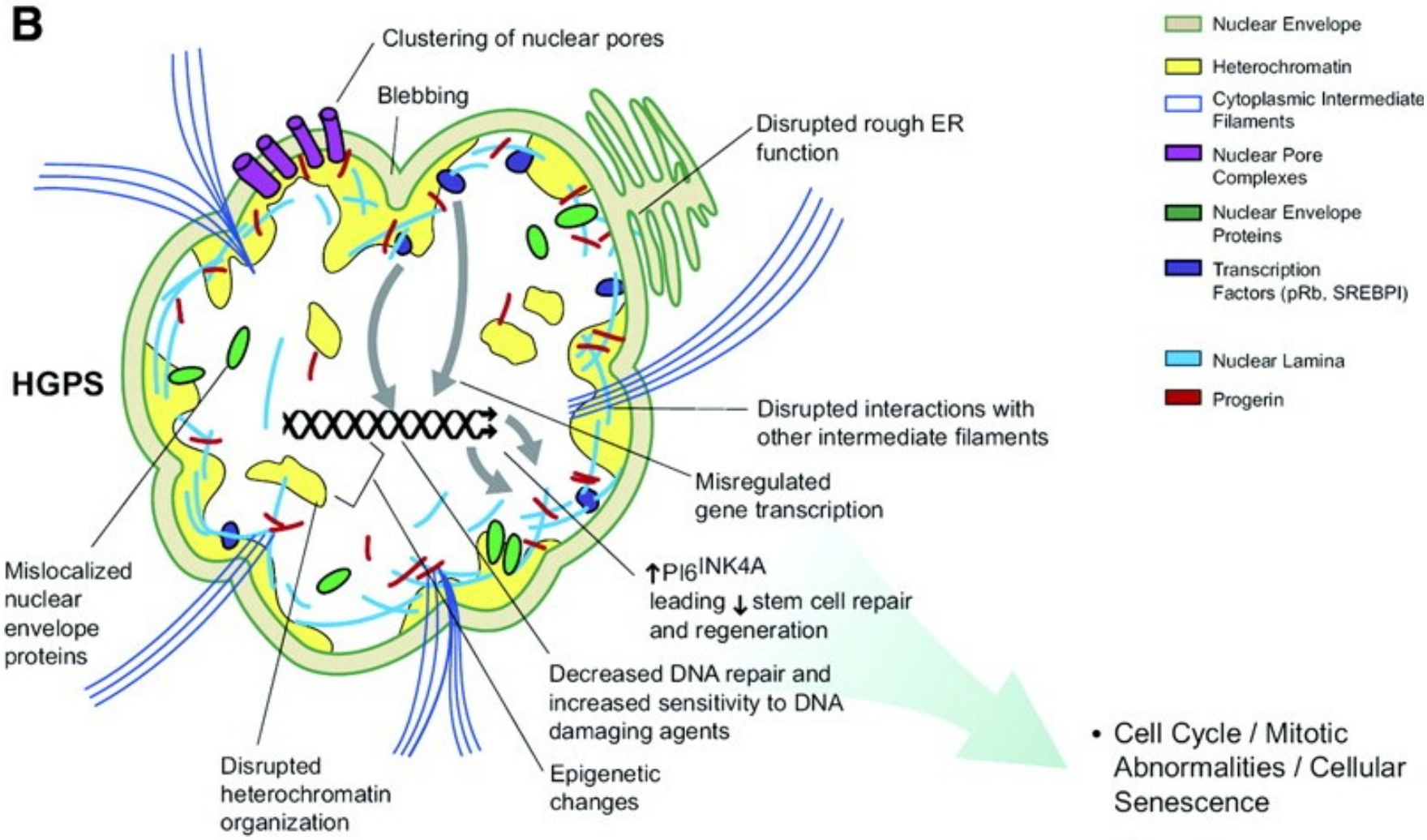
Loss of splicing site induce a deletion of aminoacid sequence that is recognized by ZMPSTE24 enzyme




A
Normal LMNA gene
Normal Splicing (Exon 11 – Exon 12)
50 aa acid deletion in HGPS
CSIM
1 606 656 661
Prelamin A
FTase
Farnesylated Prelamin A
Zmpste24 ICMT
647
C-OCH3
Zmpste24
Mature lamin A
Mutant LMNA HGPS patient
Aberrant Splicing
Exon 11 – Exon 12
Deletion of 150 Nucleotides (50 amino acid residues)
Deletion site
1 606 611
Preprogerin
Farnesylated-progerin
(Cleavage site for Zmpste24 deleted)
Mature progerin

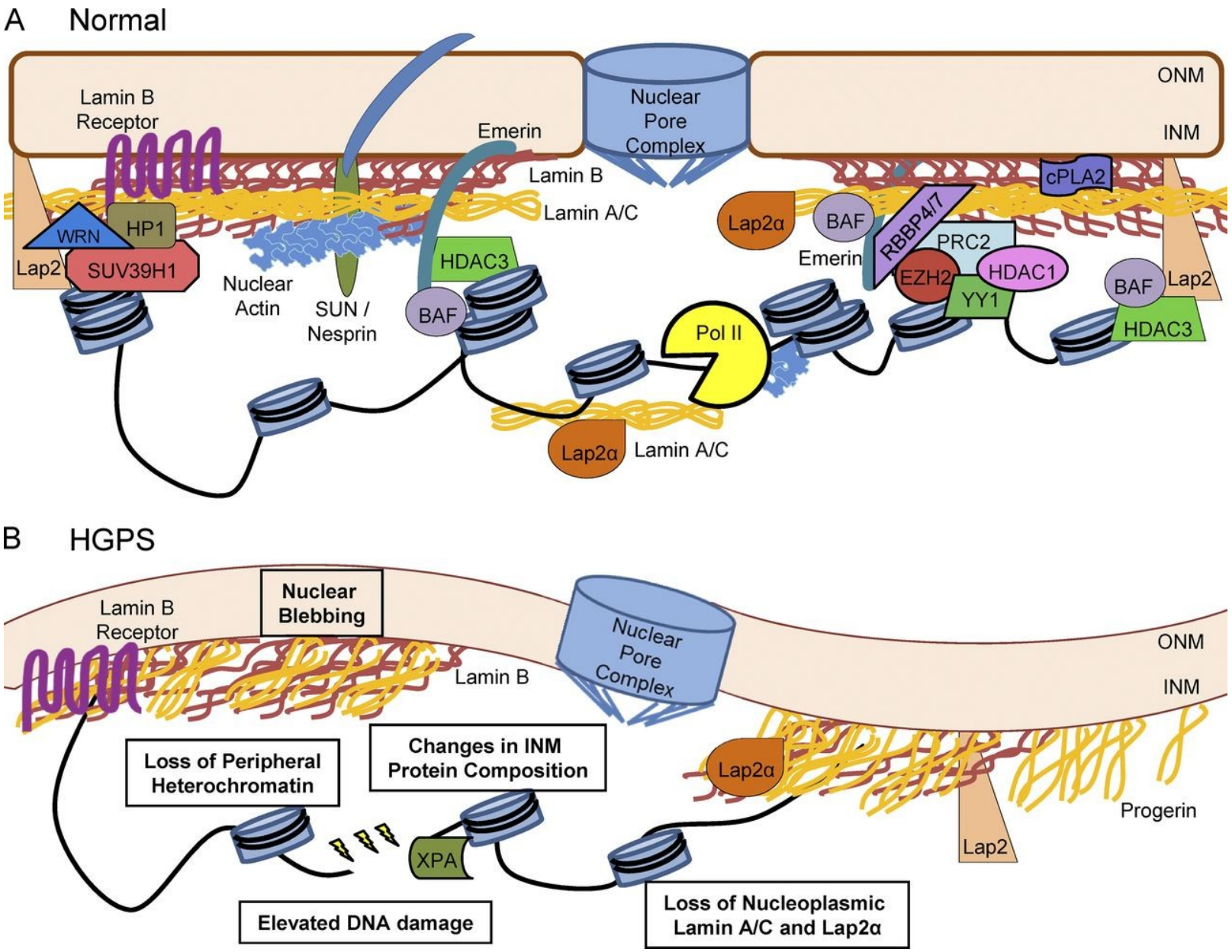

B
Human Fibroblasts
Control
HGPS
anti-lamin A
Control
HGPS
lamin A
lamin C
lamin A
progerin
lamin C
U2OS transfected cells
HA-lamin A
HA-progerin
TOPRO
anti-HA

PROGERIA EFFECTS ON THE BIOLOGICAL FUNCTIONS



- Cell Cycle / Mitotic Abnormalities / Cellular Senescence
- Genome Instability
- Accelerated Aging Cardiovascular Disease

Loss of protein complexs organization in HGPS



In summary

In HGPS:

- the mutation leads to alternative splicing in exon 11 and to the loss of 50 amino acids in prelamin A
- ZMPSTE24 enzyme not cleave the farnesylated C-terminus of this protein.
- This mutant protein, called progerin, remains permanently farnesylated
- Alteration of lamin A processing induce nuclear shape and protein complexes dysorganization.