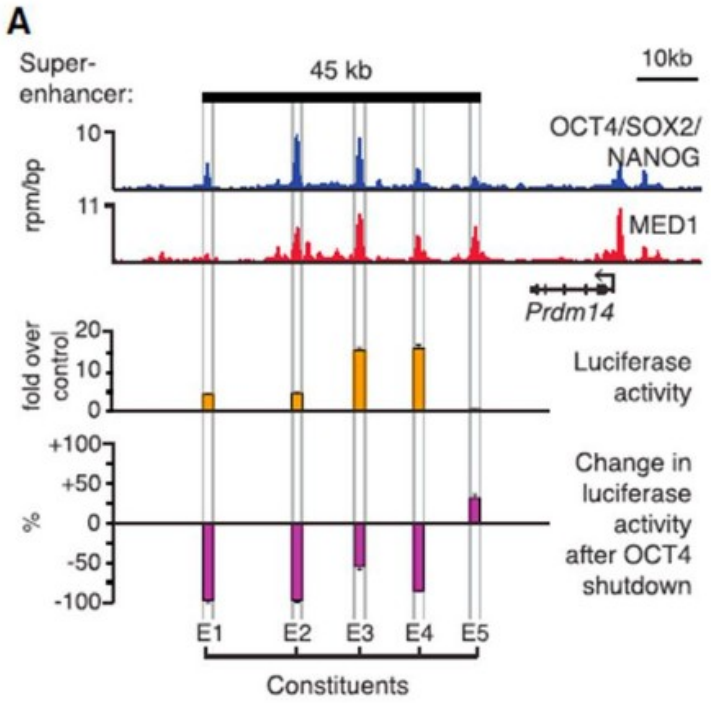
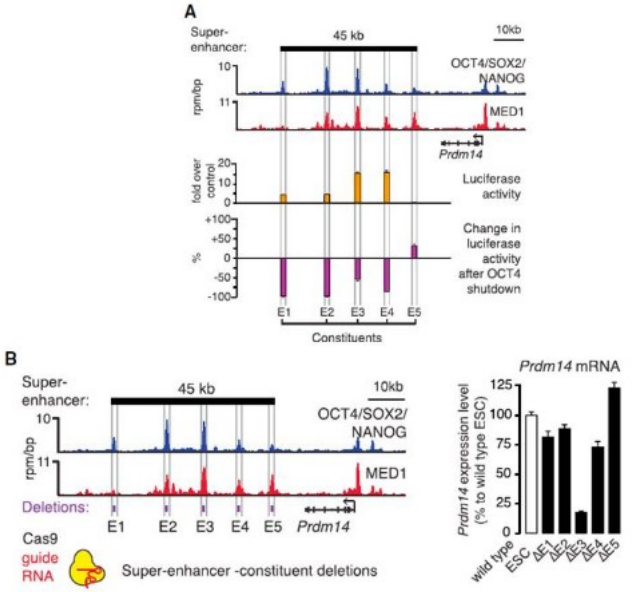


Select one:

- a. OCT4 is bound to costituents enhancers and the OCT4 silencing is used to demonstrate the role of OCT4 in these specific genomic regulatory regions
- b. OCT4 is bound to costituents enhancers and the OCT4 silencing is used to demonstrate the role of OCT4 to induce Prdm14 expression ✗
- c. to demonstrate that OCT4 didn't involve in the activation of transcription
- d. to demonstrate that OCT4 wasn't necessary to induce luciferase expression

What is the meaning to apply two techniques in the SE study?



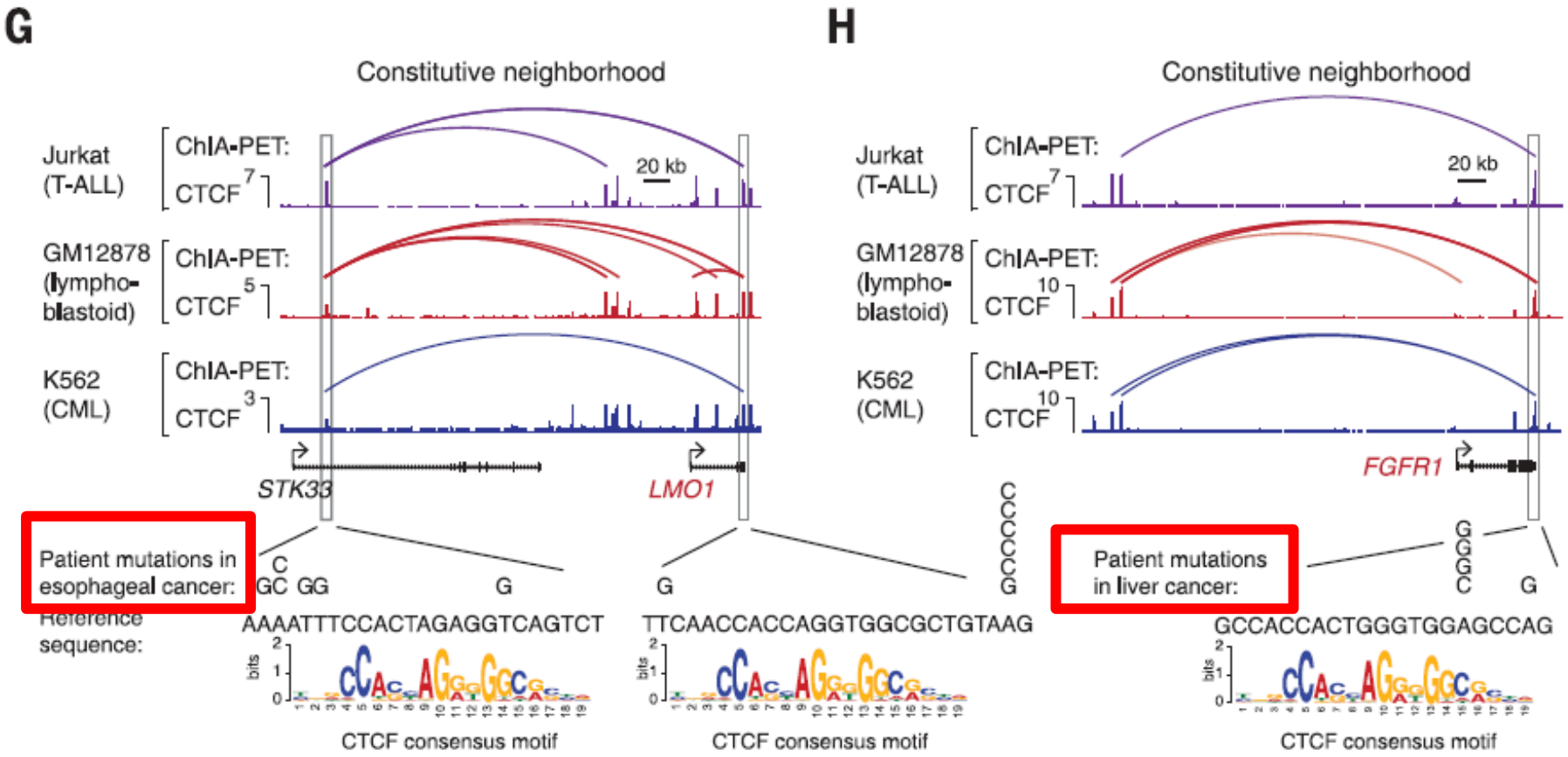
Select one or more:

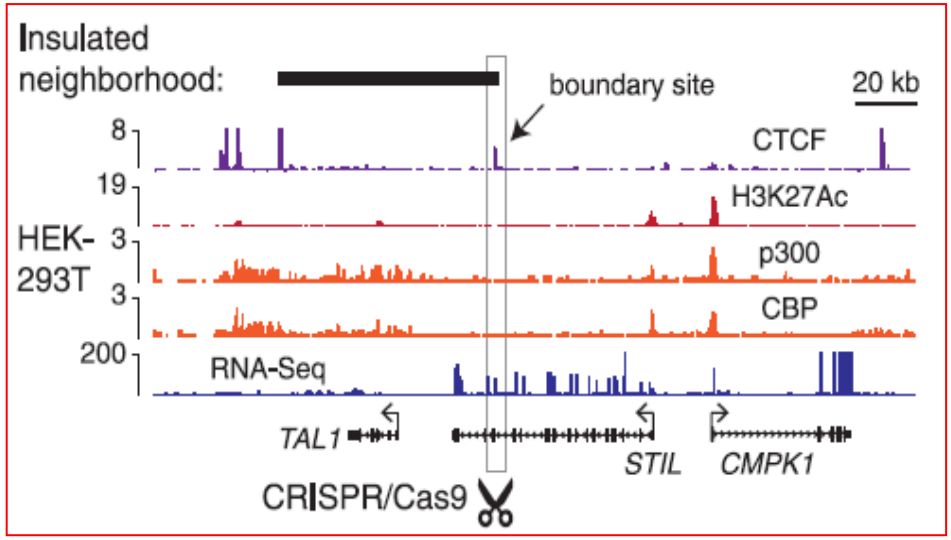
- [ ] a. Luciferase assay is used to test the role of costituent enhancer in the trascription activation and CRISPR-Cas9 assay shows the role of costituent enhancer in the chromatin context.
- [ ] b. E1 is the enhancer that play a crucial role in the regulation of gene expression because it has high level of luciferase activity and low level of Prdm14 expression using CRISPR-Cas9 systems
- [x] c. E3 is the enhancer that play a crucial role in the regulation of gene expression because it has high level of luciferase activity and low level of Prdm14 expression using CRISPR-Cas9 systems ✓
- [ ] d. Luciferase assay is used to test the interaction between TFs and CRISPR-Cas9 assay shows the role of TFs in the genome editing.

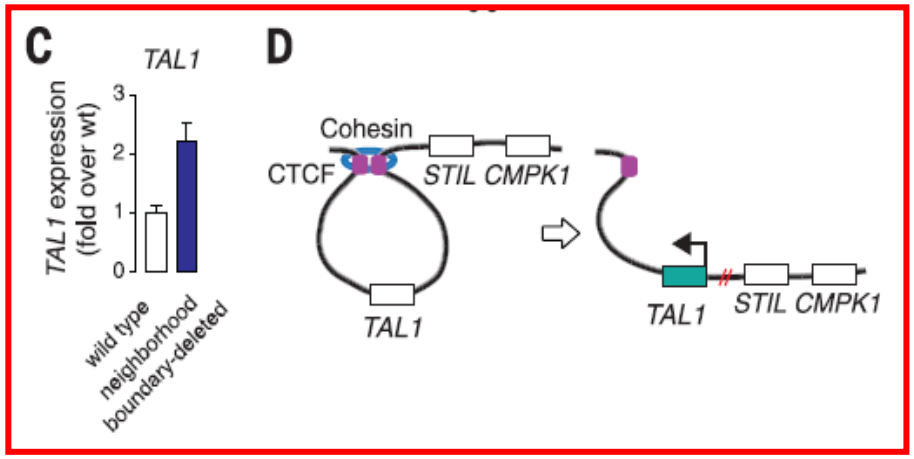
Somatic mutations of neighborhood boundaries

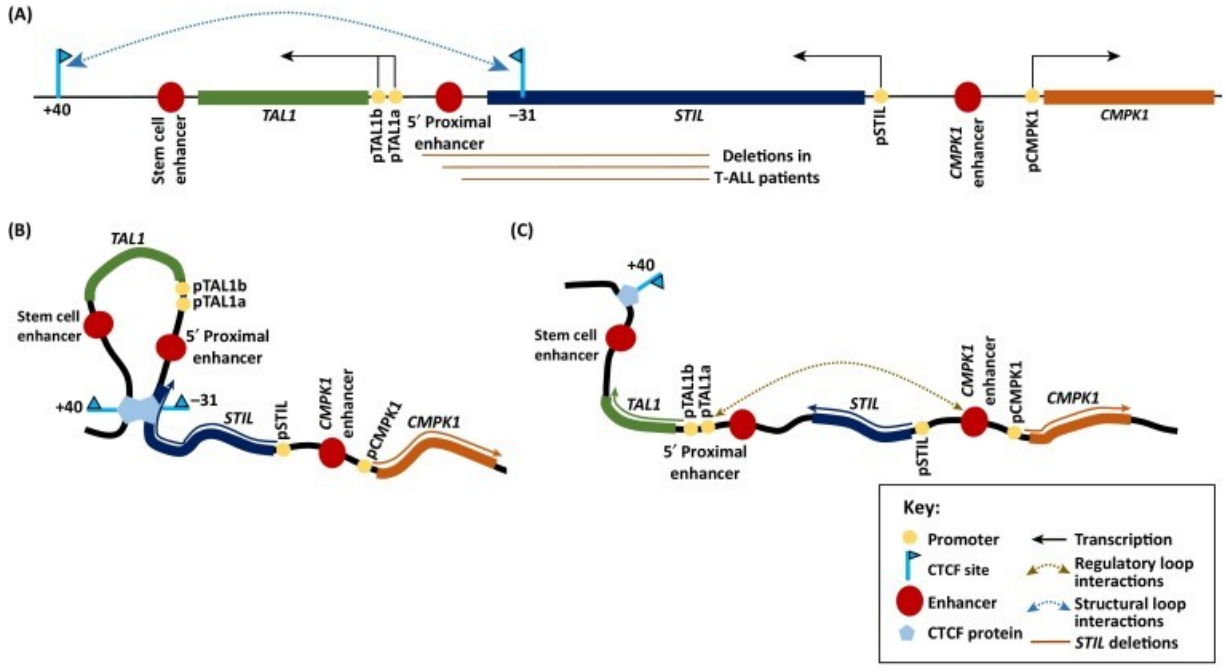
occur in many cancers.

Mutations in the boundery sites
In cancers

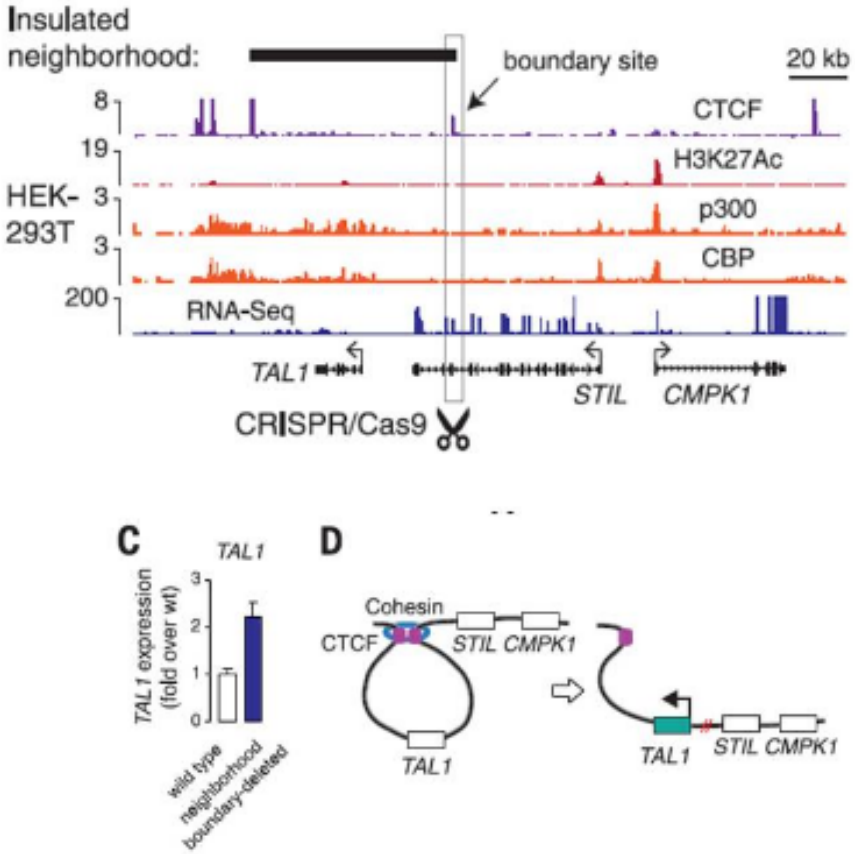




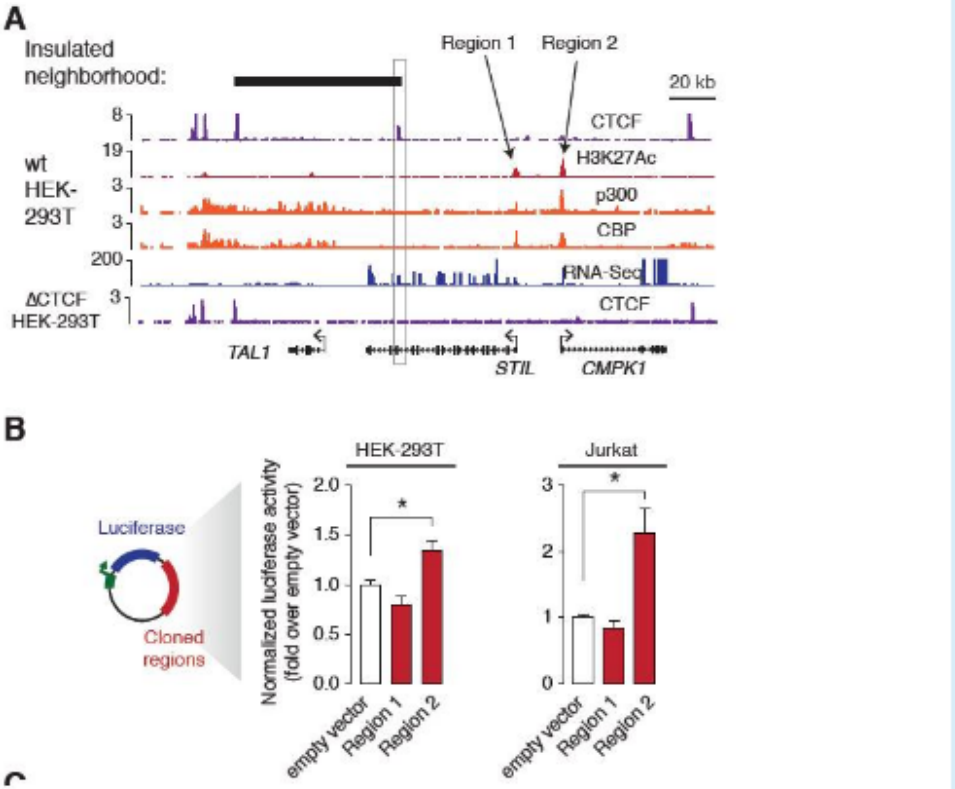




Trends in Molecular Medicine



The CRISPR-Cas9 cleavage of CTCF regions gives an increase in the expression of TAL1 probably under the action of STIL and CMPK1 promoters which will act as Enhancers on TAL1. H3K27Ac is a marker of active enhancer and might be involved in TAL1 expression.

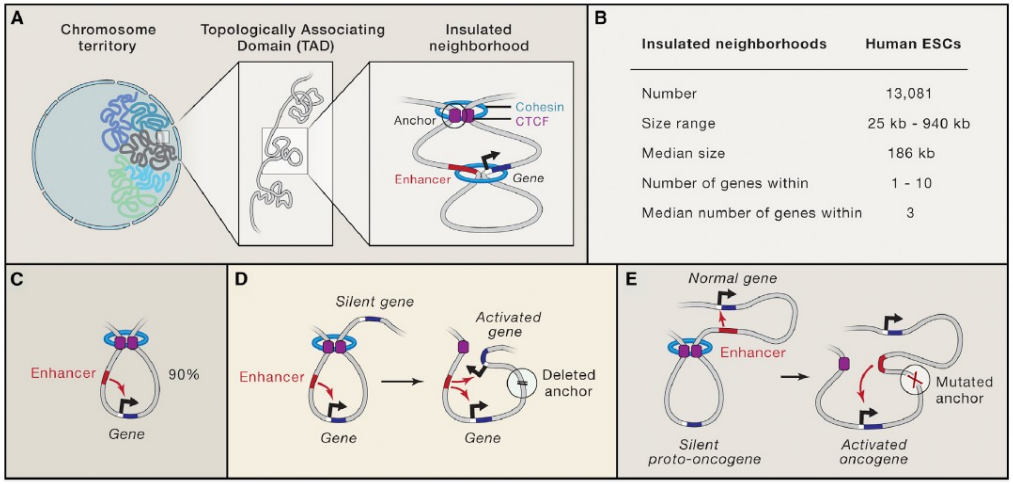


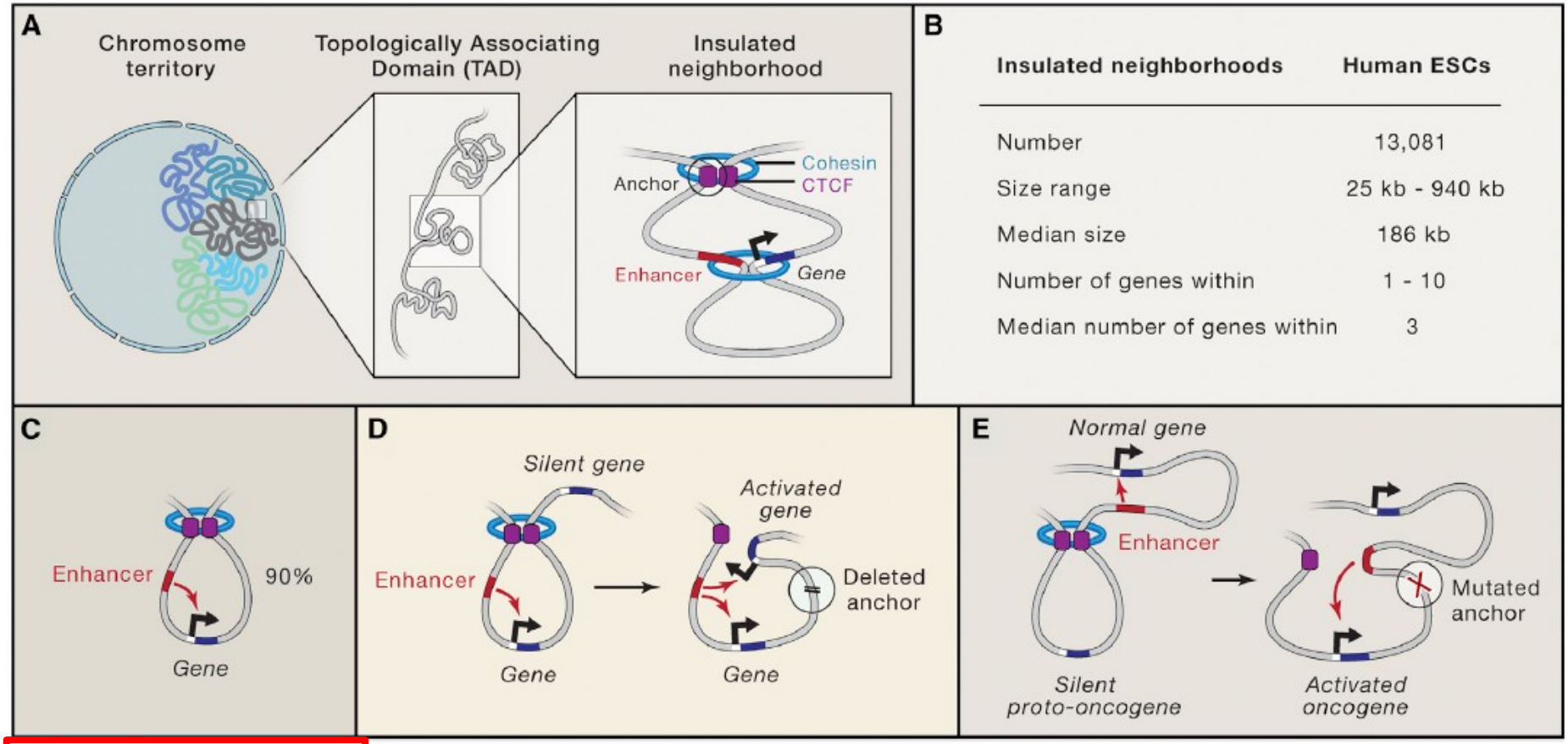
They test these two regions to see if they are responsabile of the attivation of TAl1. These regions are attive as is possibile to see from Chip seq analysis for H3k27ac, p300 and CBP and to verify if they are responsible for the proto oncogene activation they clone these regions in a vector carrying the luciferase reporter.From these analysis they see that only the region 2 leads to an increase in luciferase expression indicating that this region is the responsible for tal1 expression.

Insulated Neighborhoods: Structural and Functional Units of Mammalian Gene Control

Denes Hnisz,[1,3,*] Daniel S. Day,[1,3,*] and Richard A. Young[1,2,*]
[1]Whitehead Institute for Biomedical Research, 9 Cambridge Center, Cambridge, MA 02142, USA
[2]Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139, USA
[3]Co-first author
*Correspondence: hnisz@wi.mit.edu (D.H.), dsday@wi.mit.edu (D.S.D.), young@wi.mit.edu (R.A.Y.)
http://dx.doi.org/10.1016/j.cell.2016.10.024

Understanding how transcriptional enhancers control over 20,000 protein-coding genes to maintain cell-type-specific gene expression programs in all human cells is a fundamental challenge in regulatory biology. Recent studies suggest that gene regulatory elements and their target genes generally occur within insulated neighborhoods, which are chromosomal loop structures formed by the interaction of two DNA sites bound by the CTCF protein and occupied by the cohesin complex. Here, we review evidence that insulated neighborhoods provide for specific enhancer-gene interactions, are essential for both normal gene activation and repression, form a chromosome scaffold that is largely preserved throughout development, and are perturbed by genetic and epigenetic factors in disease. Insulated neighborhoods are a powerful paradigm for gene control that provides new insights into development and disease.





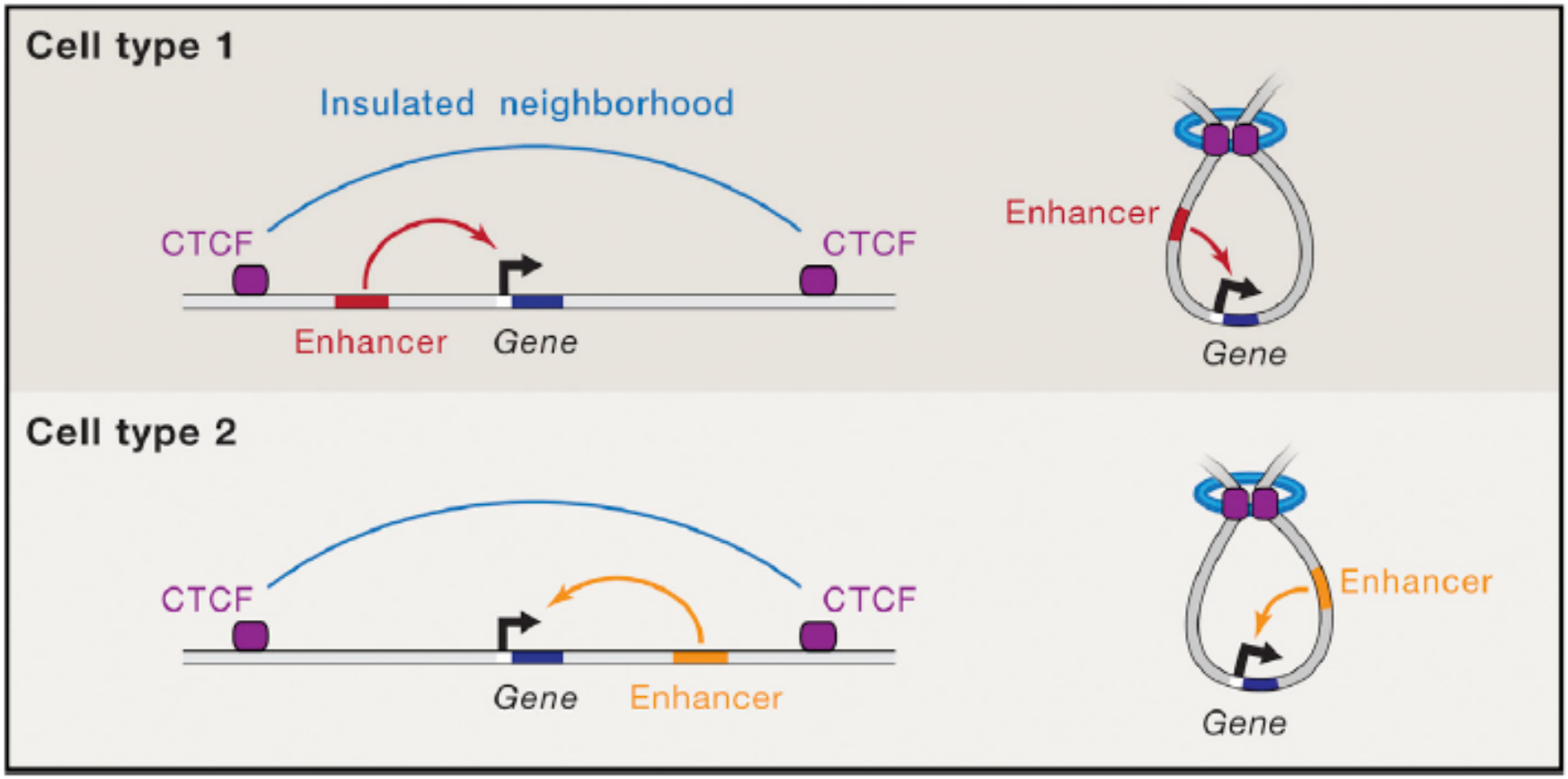
90% of enhancer-gene interactions occur **within insulated neighborhoods** in human ESCs

deletion of insulated neighborhood anchors leads to gene misregulation

mutations of insulated neighborhood anchors in tumor cells lead to oncogene activation

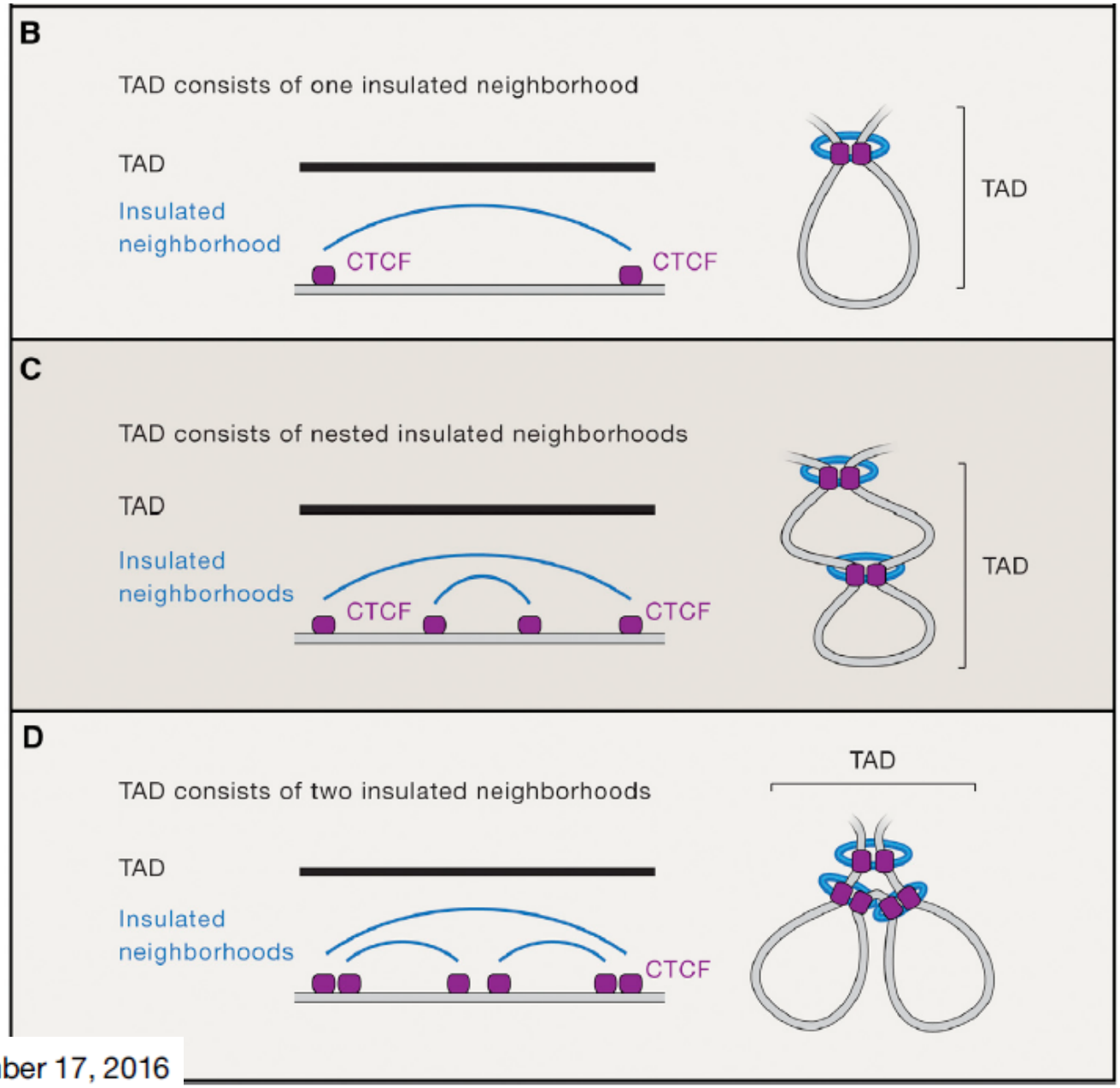
Cell *167*, November 17, 2016

Cell-type specific enhancers –gene interactions occur within the boundaries



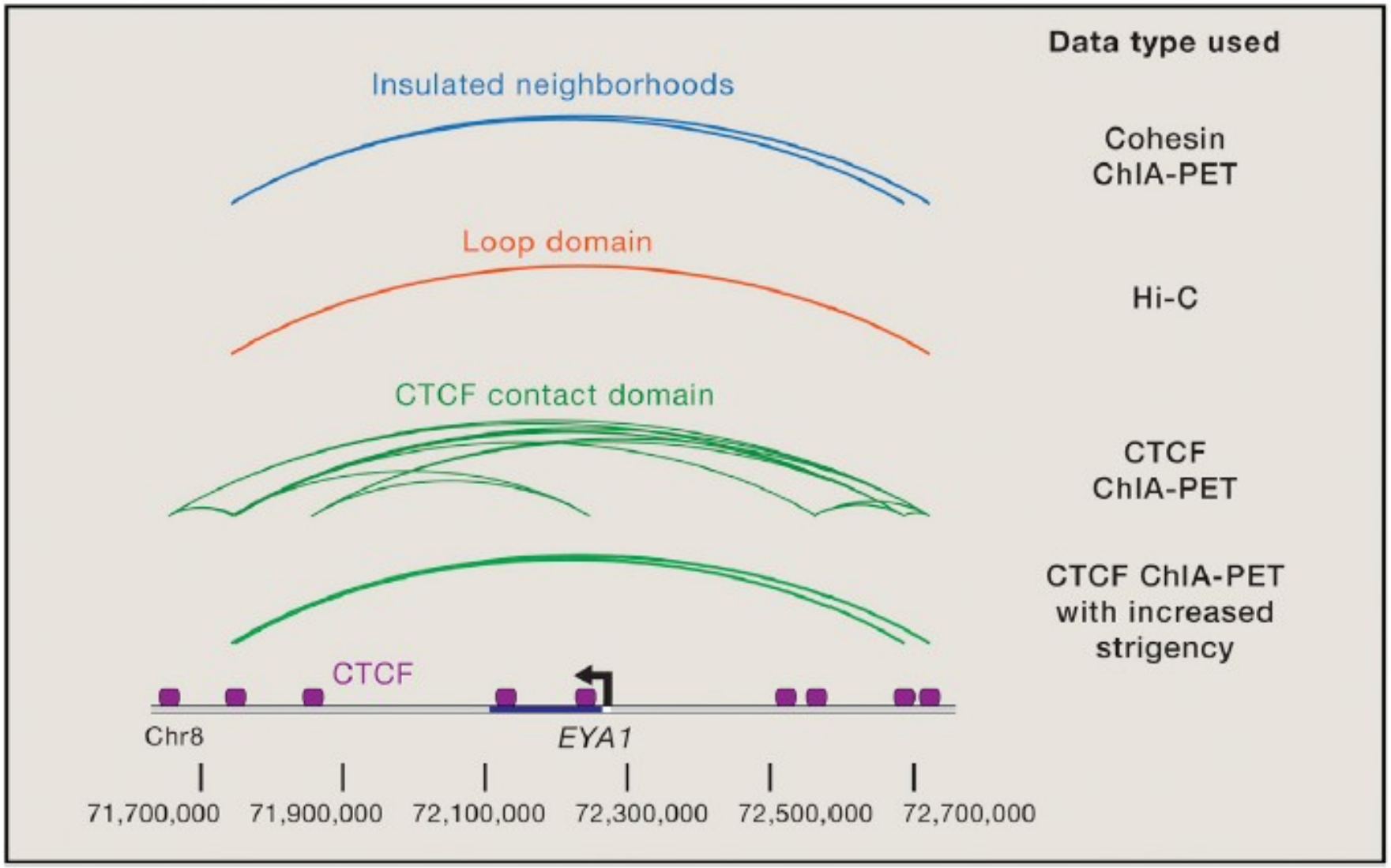
Cell *167*, November 17, 2016

Insulated neighborhoods are a major structuring component of TADs.



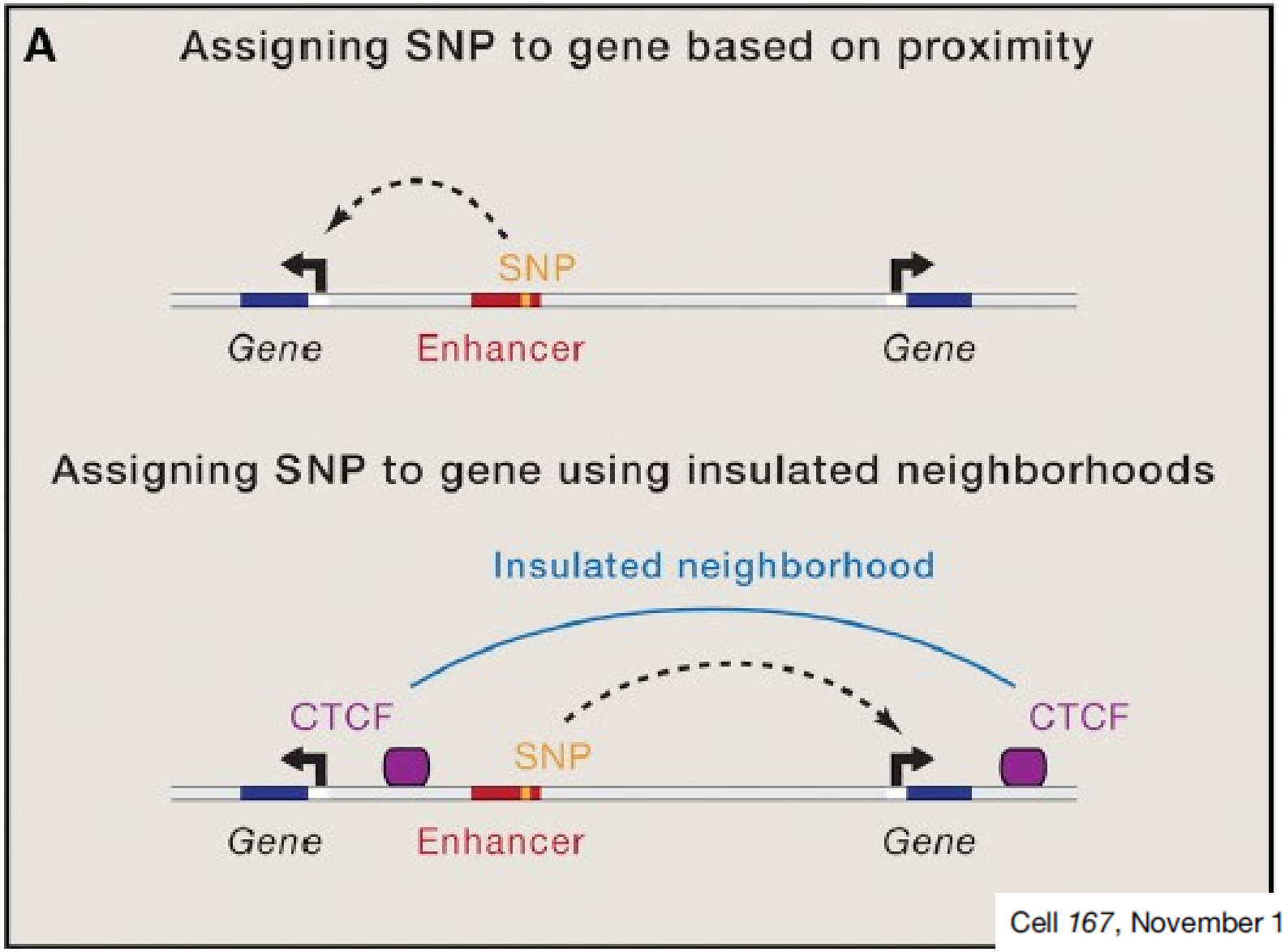
Cell *167*, November 17, 2016

Comparation between several techniques for identification of long range interaction



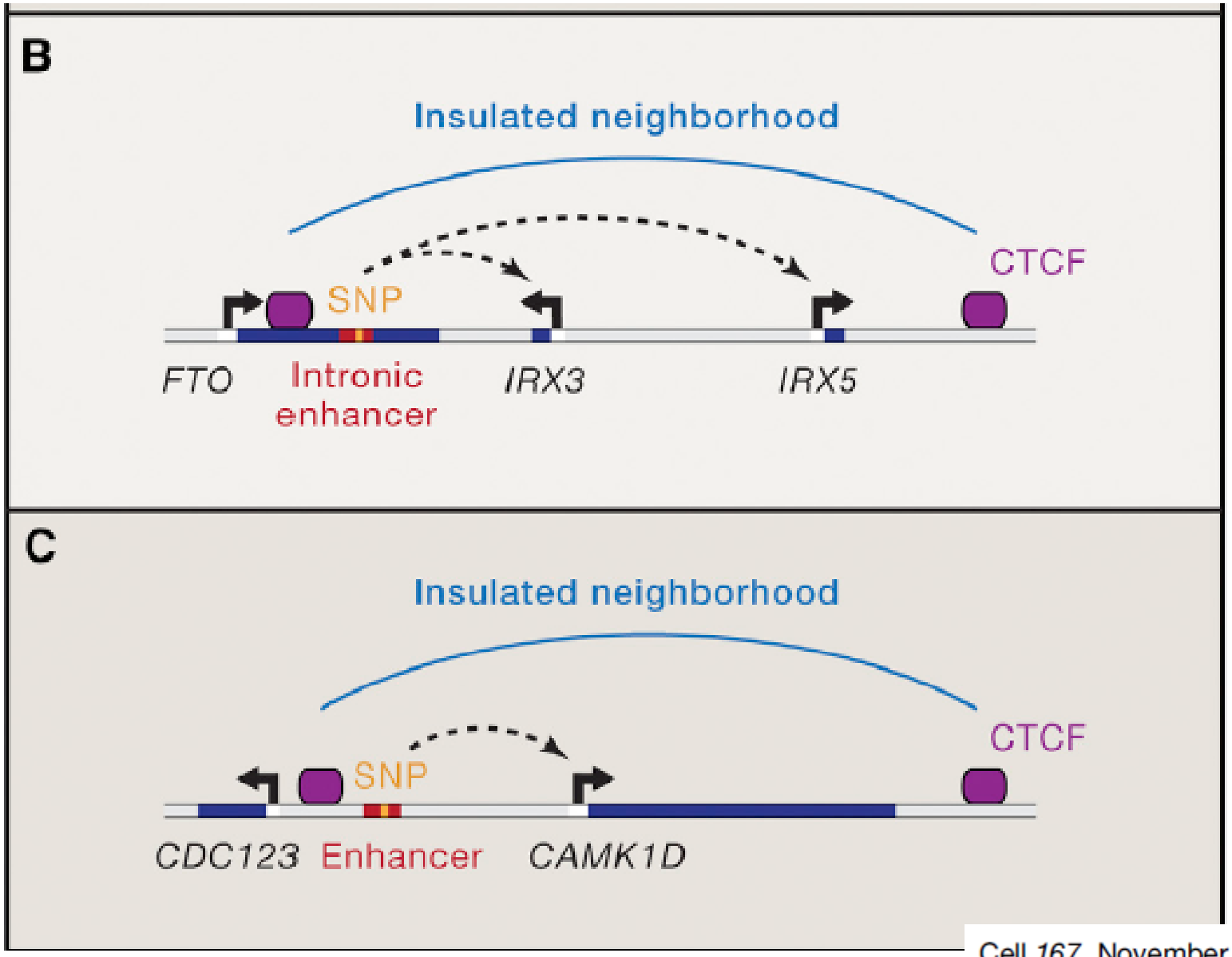
Cell *167*, November 17, 2016

How SNPs affect long range interactions of the chromatin



Cell *167*, November 17, 2016

How SNPs affect long range interactions of the chromatin



Cell *167*, November 17, 2016

Insulated neighborhoods:

are structural and functional units of gene control

are used during development to control the diverse cell identities that contribute to complex animals

form the mechanistic basis of higher-order chromosome structures, such as topologically associating domains (TADs)

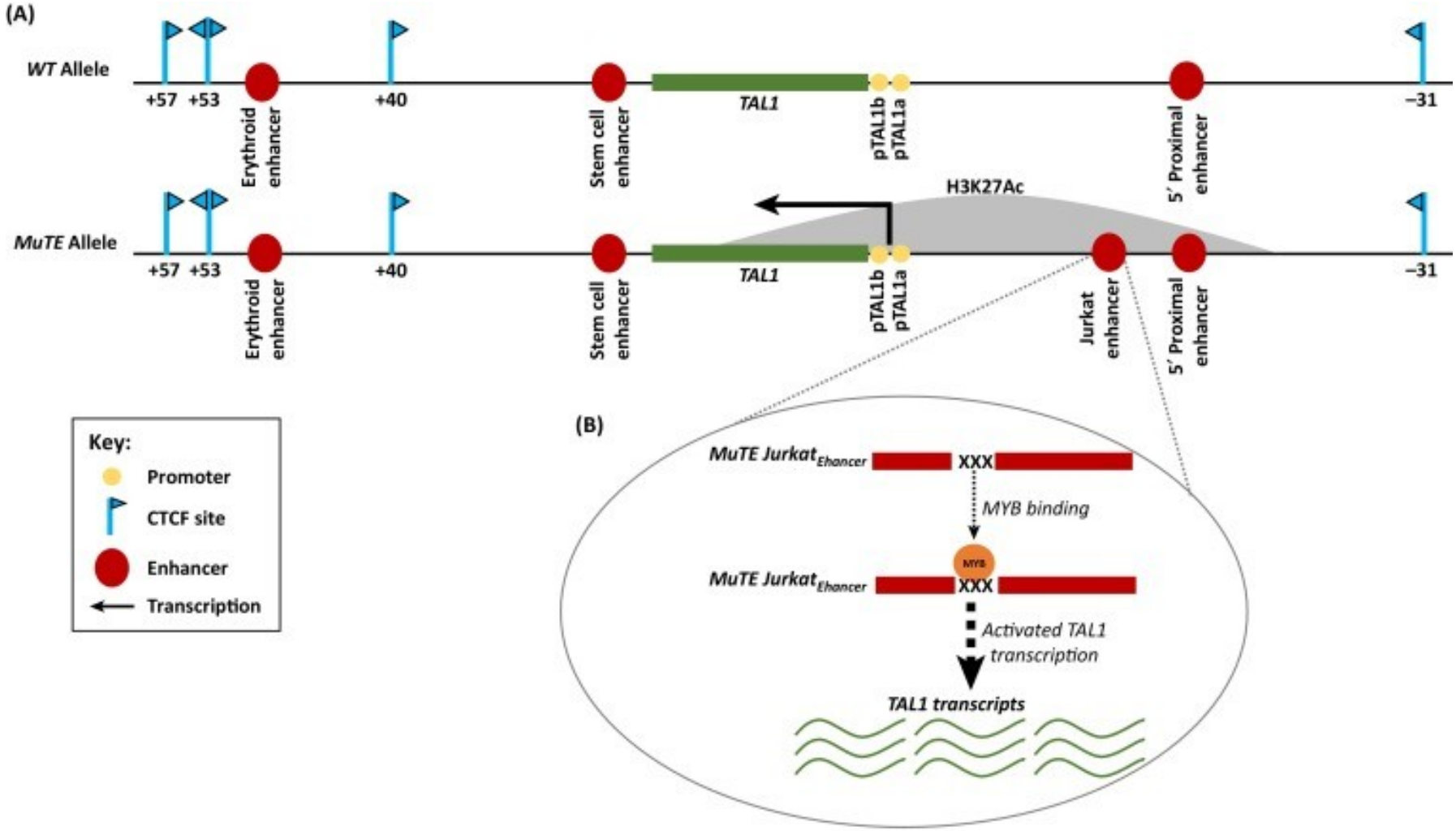
genetic and epigenetic perturbations of neighborhood boundaries contribute to disease.

LESSON 5

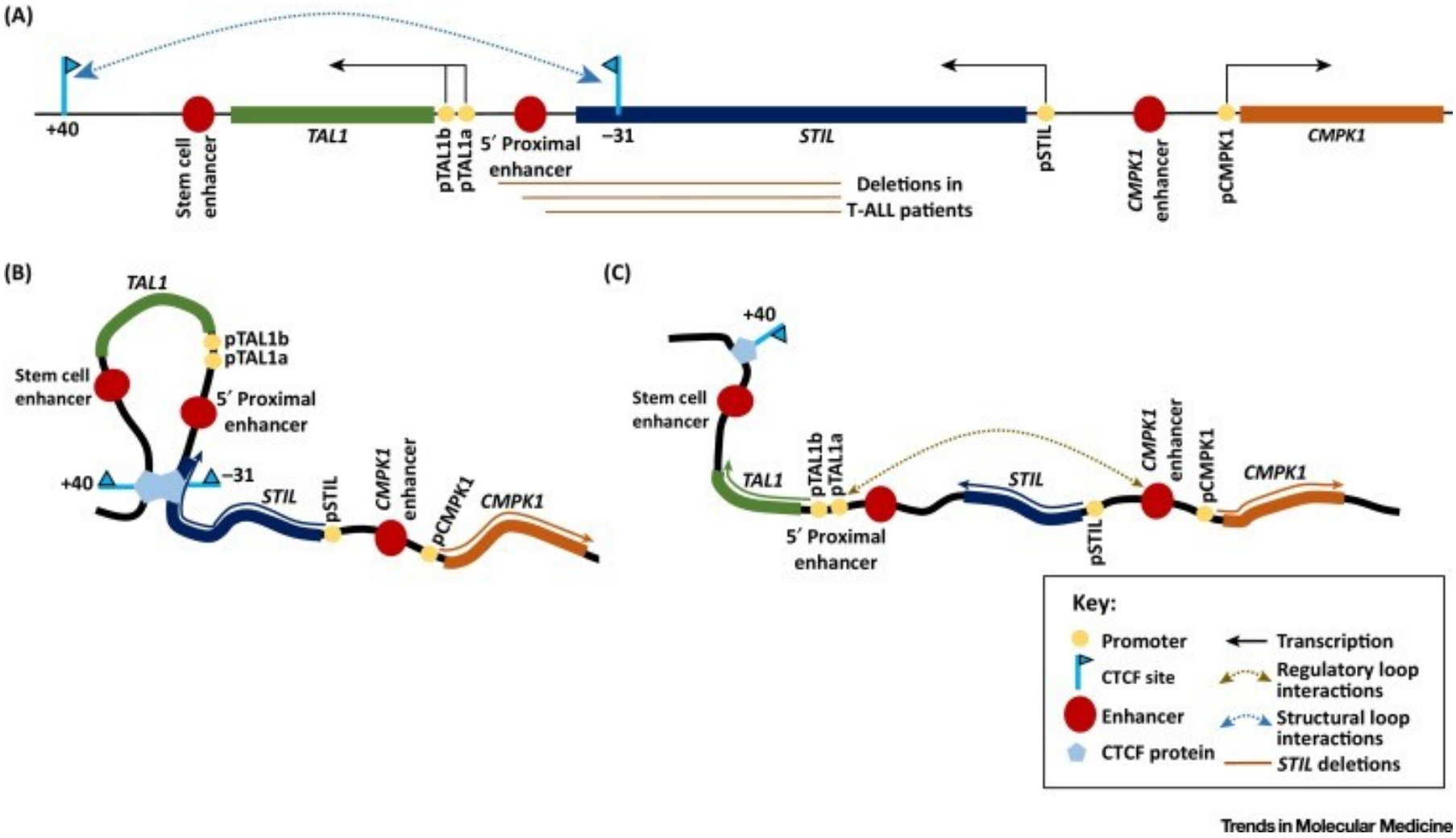
- **An Acquired Super-Enhancer Activates Monoallelic *TAL1* Transcription in T-ALL (T cell acute lymphoblastic leukemia) Cells**

- **Disruption of the TAL1 Insulated Neighborhood Border Activates TAL1 Transcription in T-ALL Cells.**

An Acquired Super-Enhancer Activates Monoallelic *TAL1* Transcription in T-ALL (T cell acute lymphoblastic leukemia) Cells



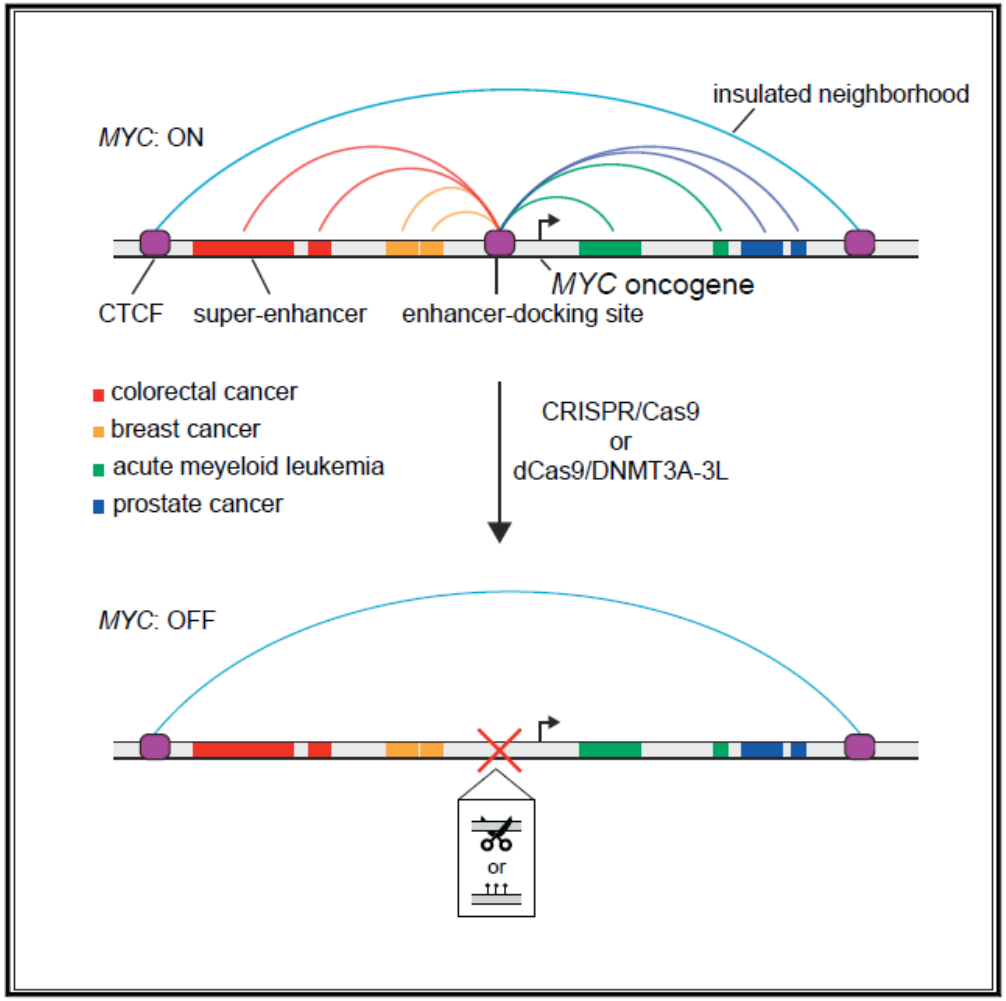
Disruption of the TAL1 Insulated Neighborhood Border Activates TAL1 Transcription in T-ALL Cells.



Trends in Molecular Medicine

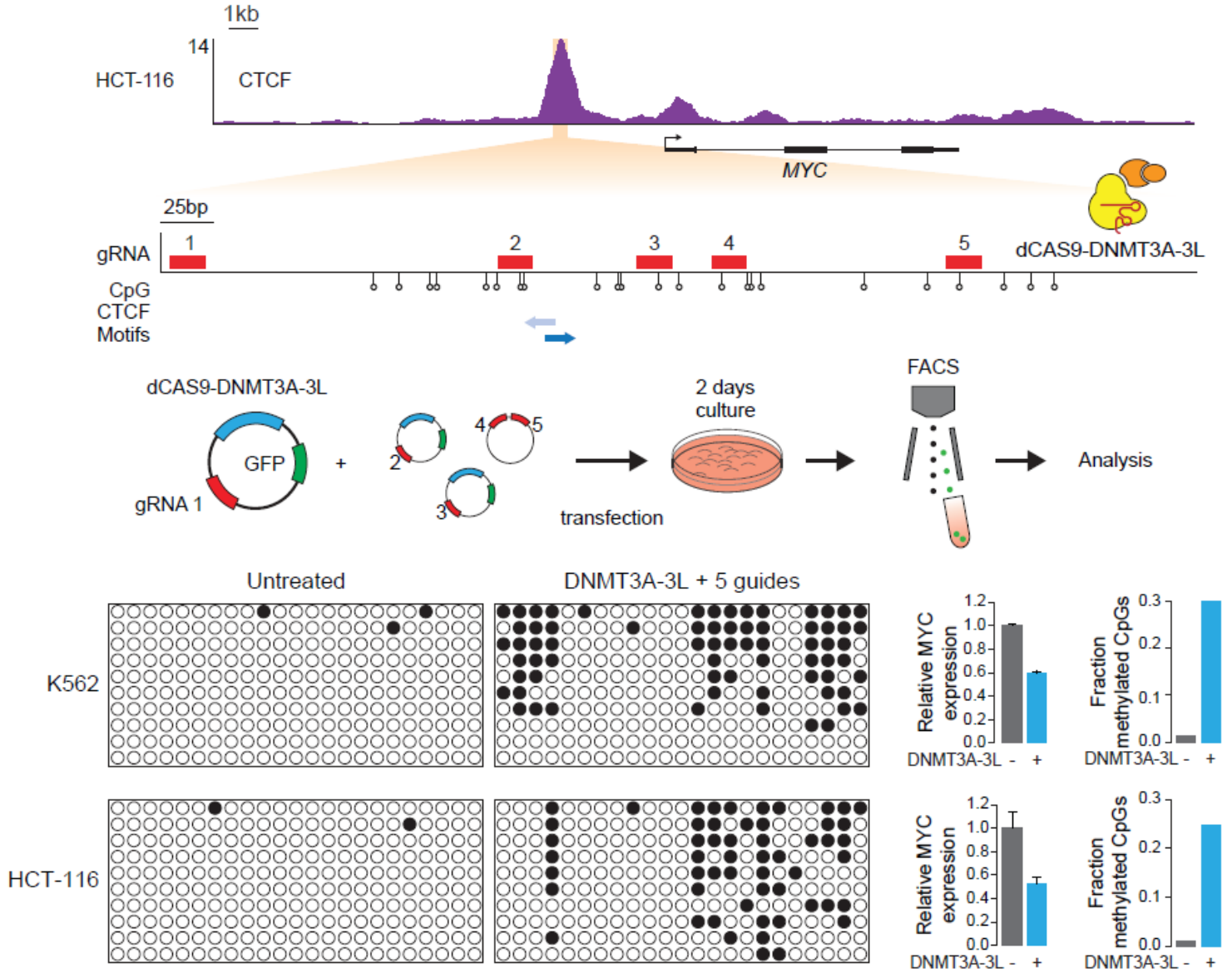
Cell Reports

Transcriptional Dysregulation of *MYC* Reveals Common Enhancer-Docking Mechanism

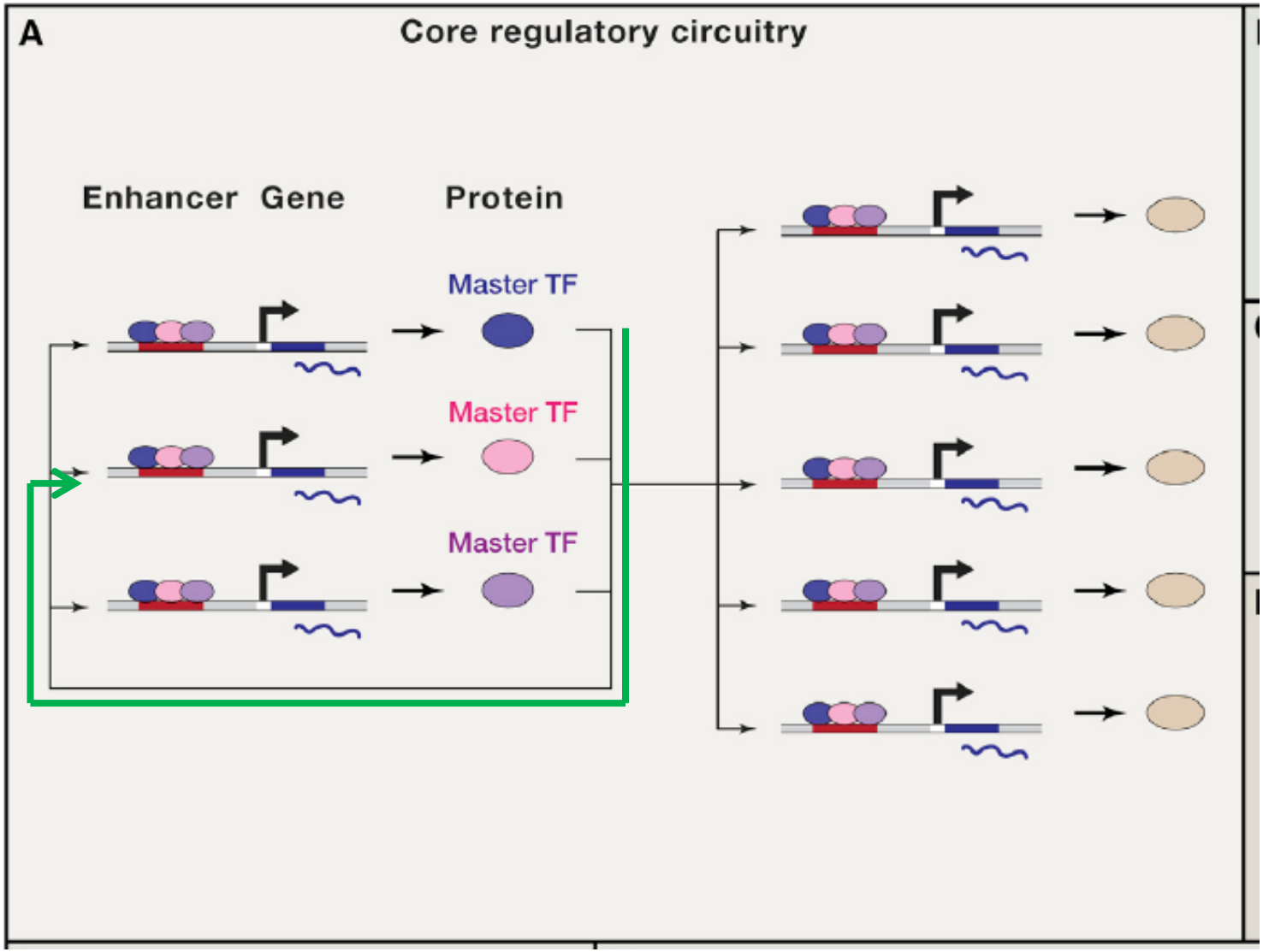


Cell Reports 23, 349–360, April 10, 2018

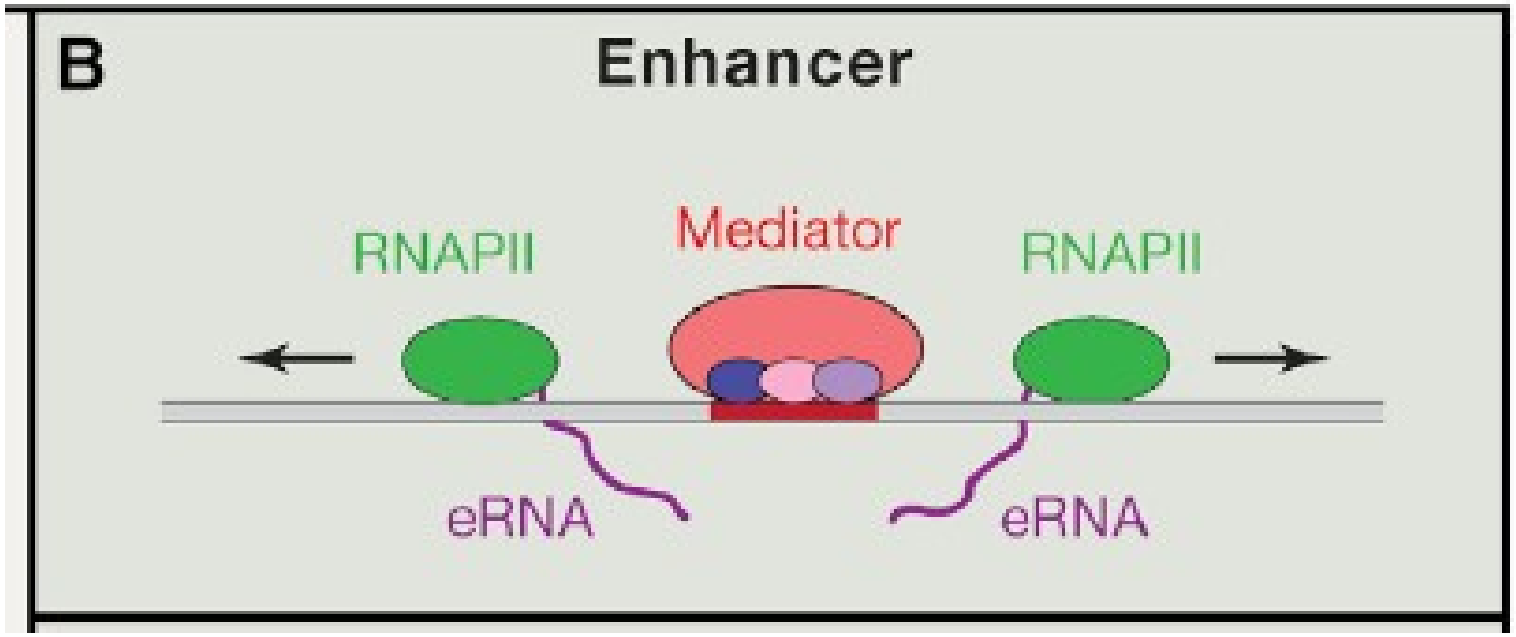
dCas9-Mediated Methylation of the CTCF Loop-Anchor Site Reduces MYC Expression in Tumor Cells



In normal differented cells,
lineage determining TFs cooperate to regulate gene expression
and autoregulate own genes forming CORE REGULATORY CIRCUITRY

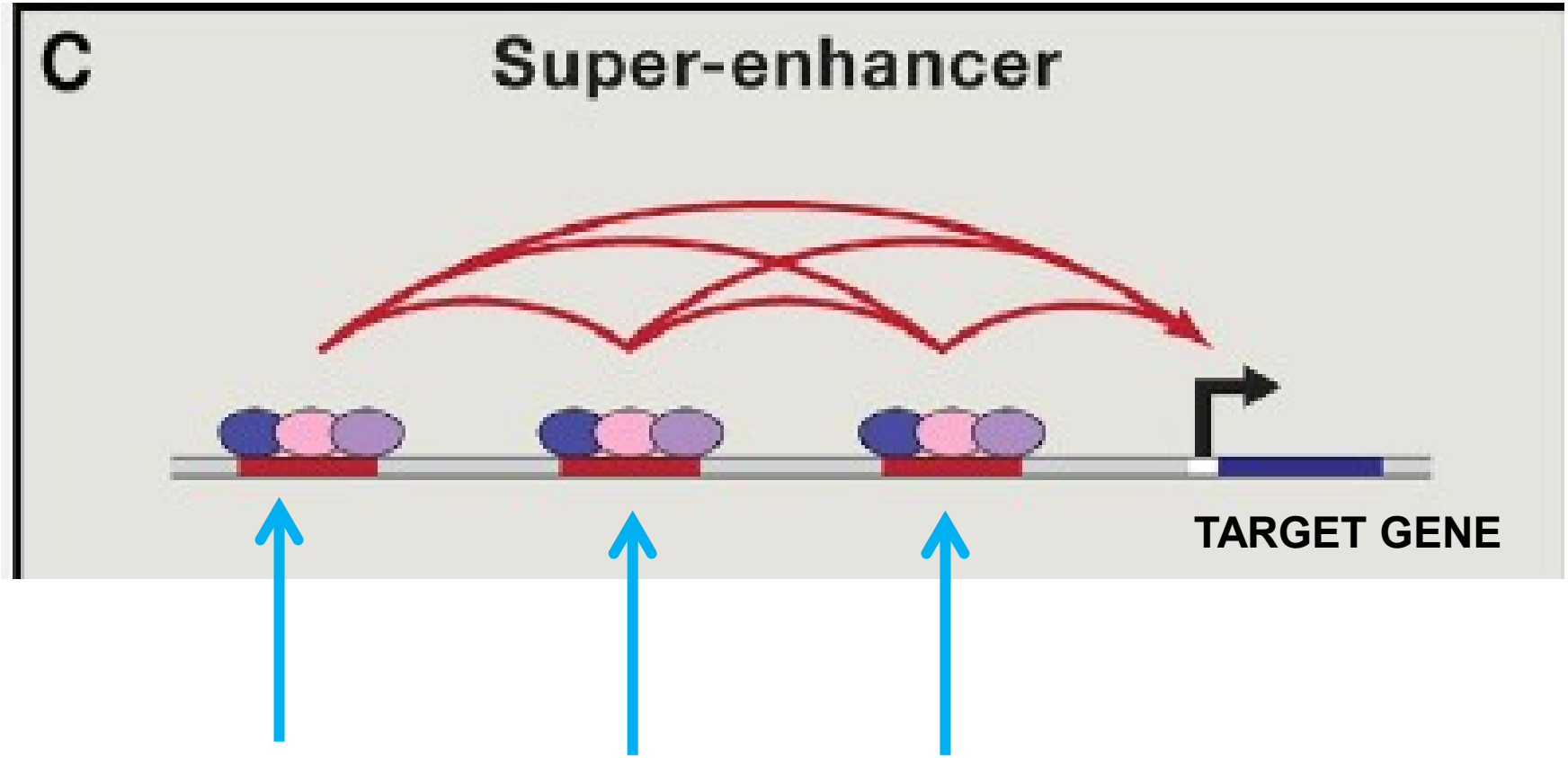


The master TFs bind cooperatively to enhancer DNA elements and **recruit coactivators and the transcription apparatus**.



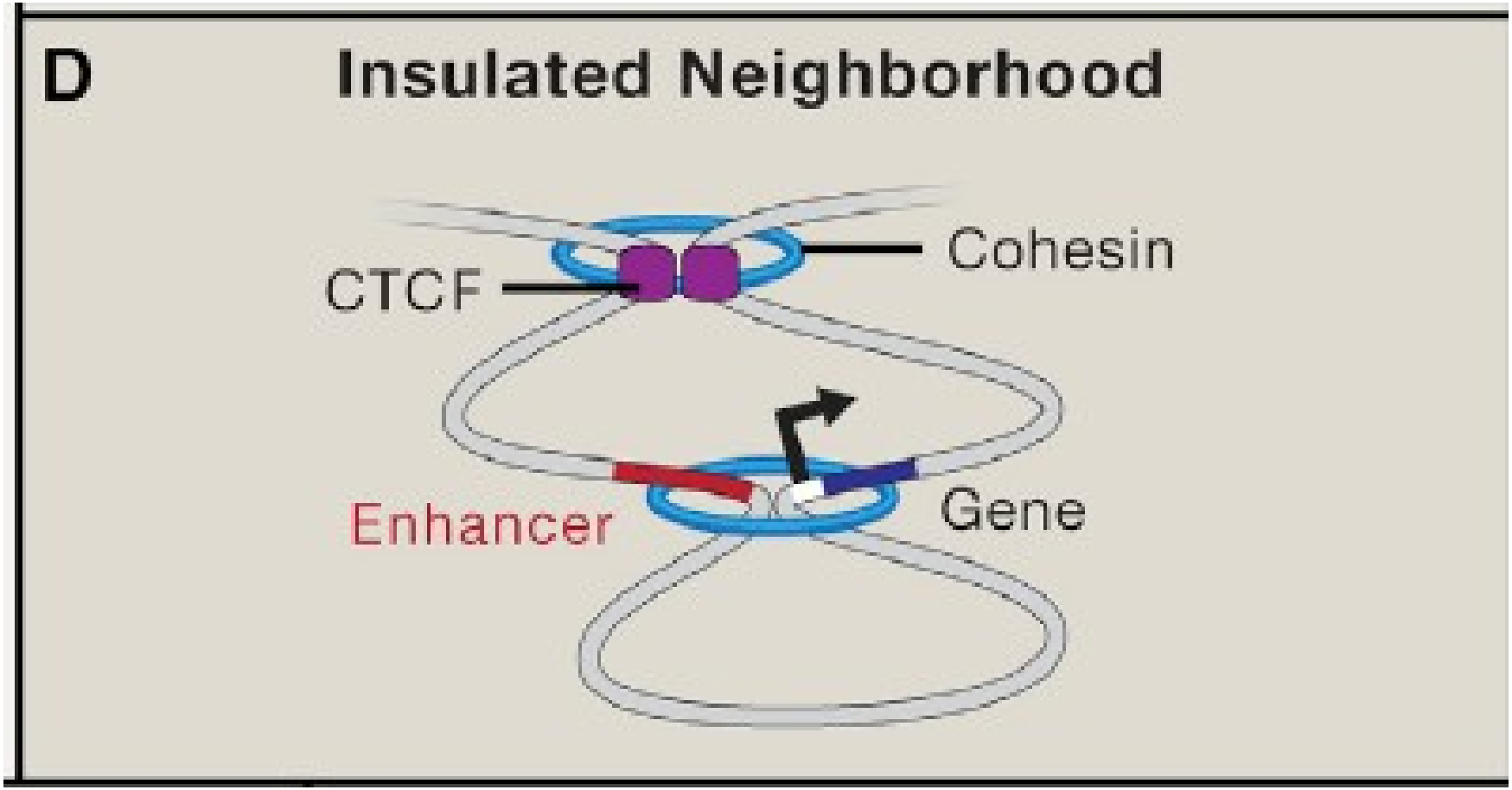
These **TFs can activate transcription from the enhancer elements** themselves, producing **enhancer RNAs (eRNAs)** that bind certain TFs and cofactors and contribute to enhancer maintenance and dynamics

The **constituent enhancers of SEs** physically associate with one another and can function as **independent** or **interdependent components** of these large transcription-regulating complexes to drive high-level expression of their associated genes.



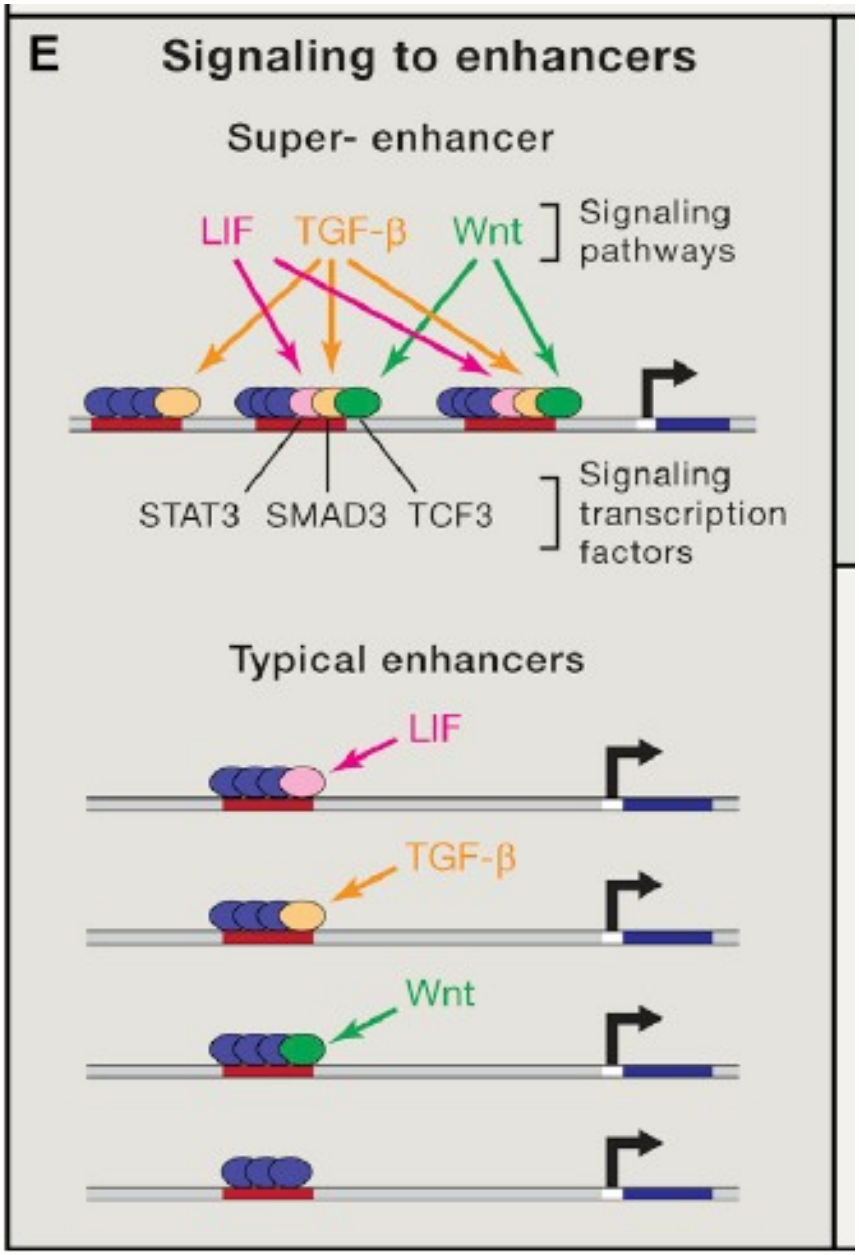
Constituent enhancers of SUPER-ENHANCERS

Insulated neighborhoods are **chromosomal loop structures formed by the interaction of two DNA sites bound by the CTCF protein and occupied by the cohesin complex**

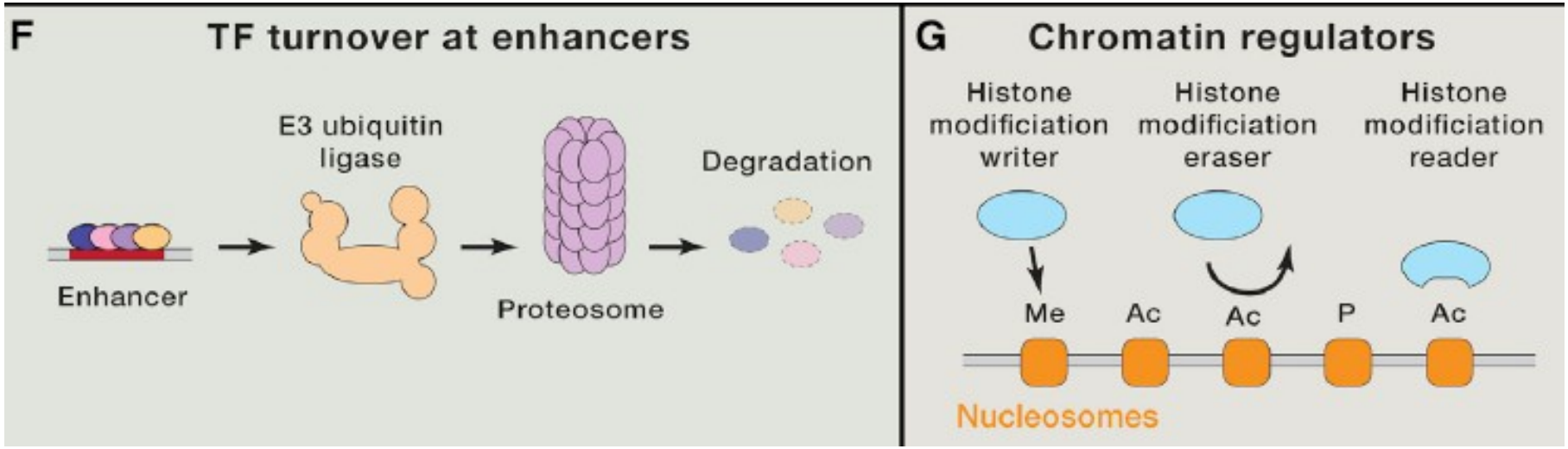


These chromosomal neighborhoods engender specific enhancer-gene interactions and are essential for **normal gene activation and repression**

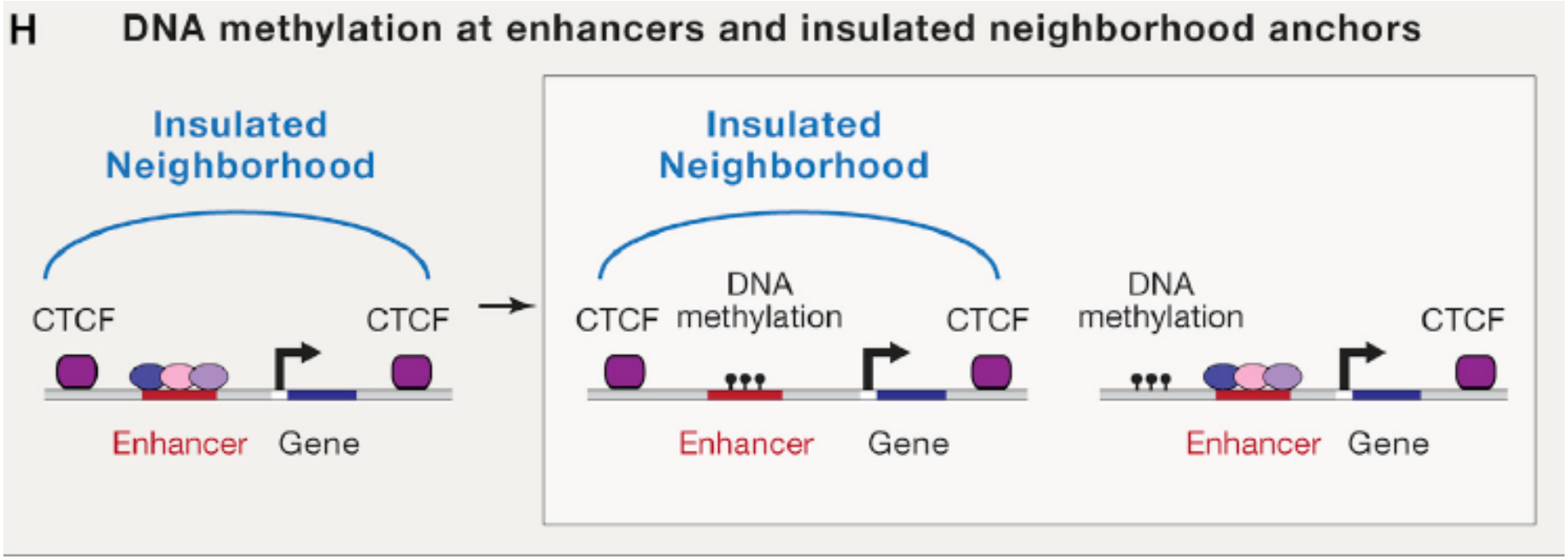
Signals derived from cell environment activate TFs bound at SE for gene expression regulation.



Chromatin dynamics

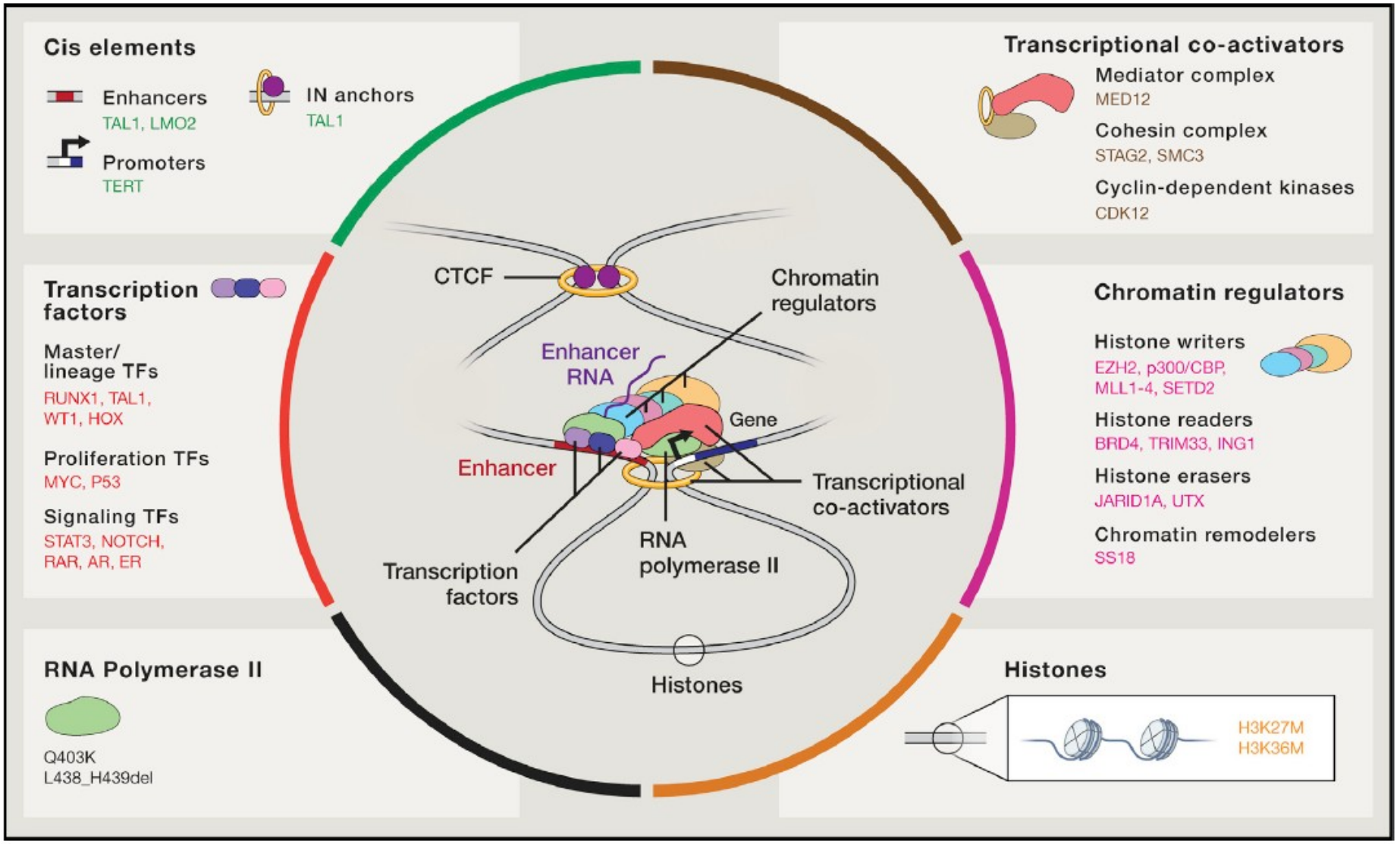


Role of DNA methylation in the gene regulation

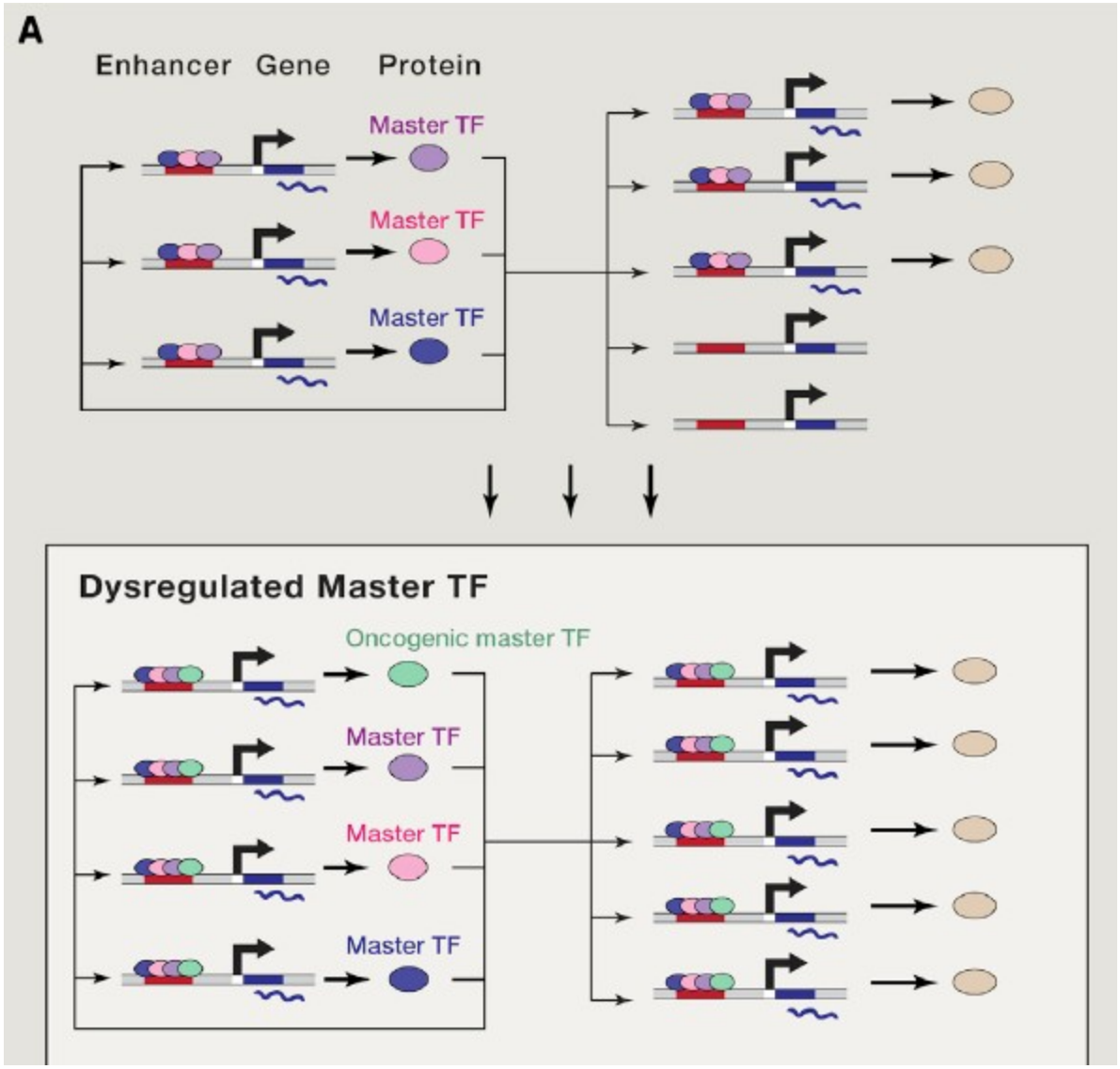


START the TRAINING TEST

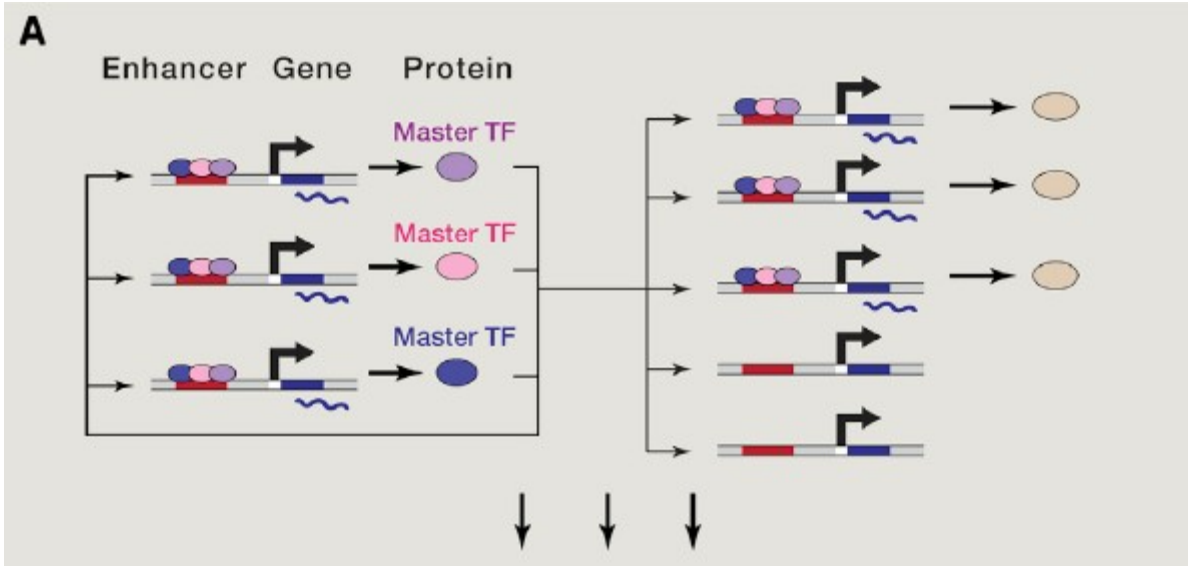
ALTERATIONS OF TRANSCRIPTIONAL REGULATION IN CANCER

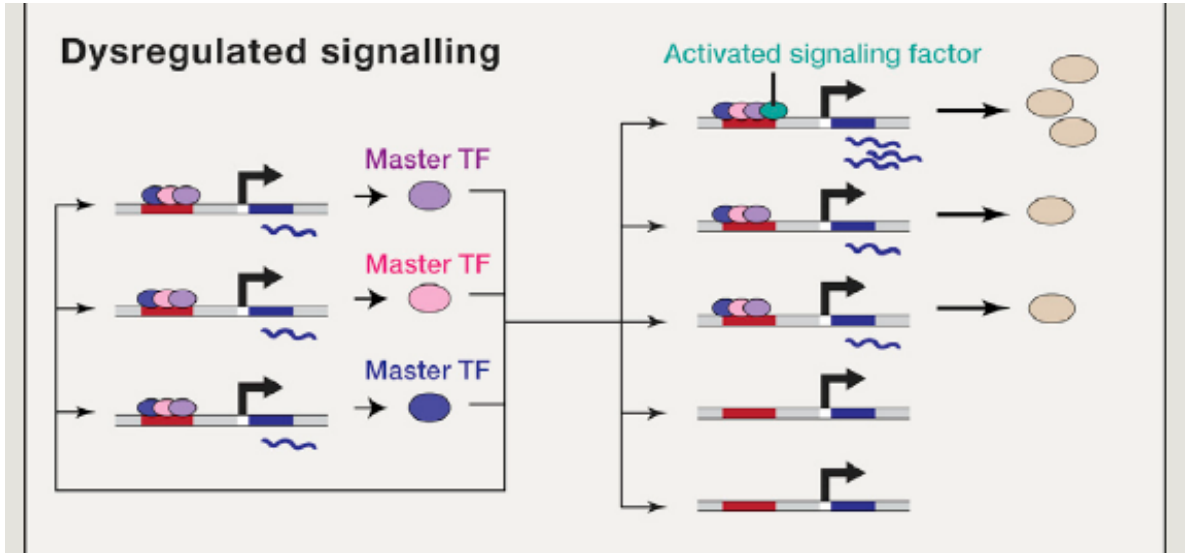

Cis elements
Enhancers
TAL1, LMO2
IN anchors
TAL1
Promoters
TERT
Transcriptional co-activators
Mediator complex
MED12
Cohesin complex
STAG2, SMC3
Cyclin-dependent kinases
CDK12
Transcription factors
Master/ lineage TFs
RUNX1, TAL1, WT1, HOX
Proliferation TFs
MYC, P53
Signaling TFs
STAT3, NOTCH, RAR, 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

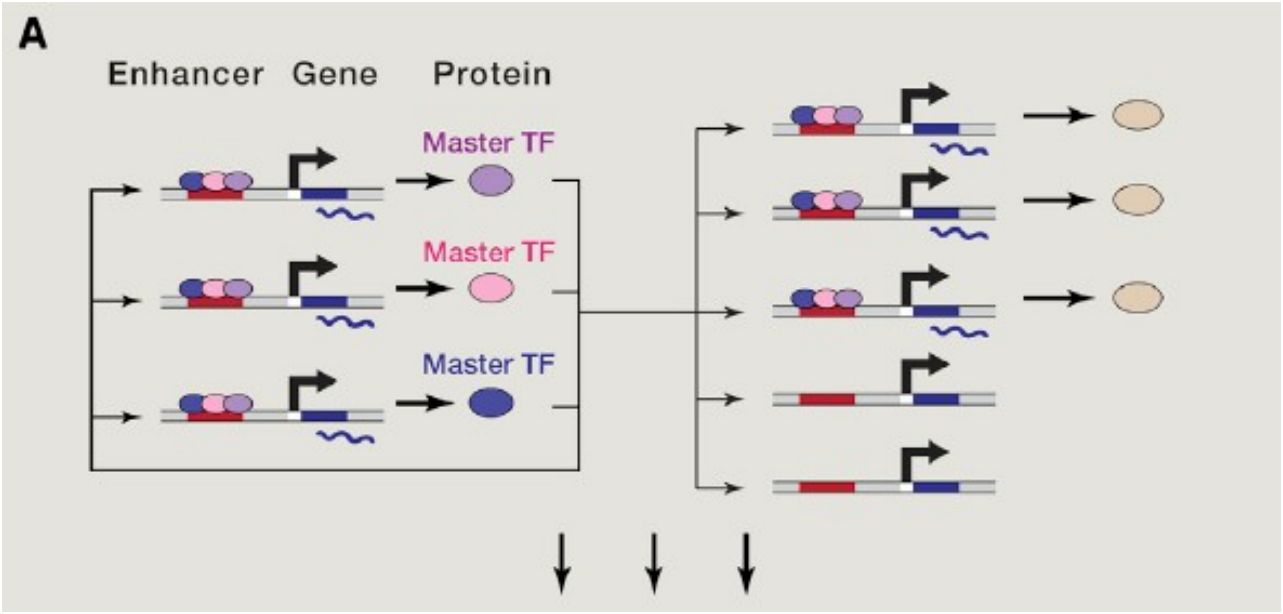


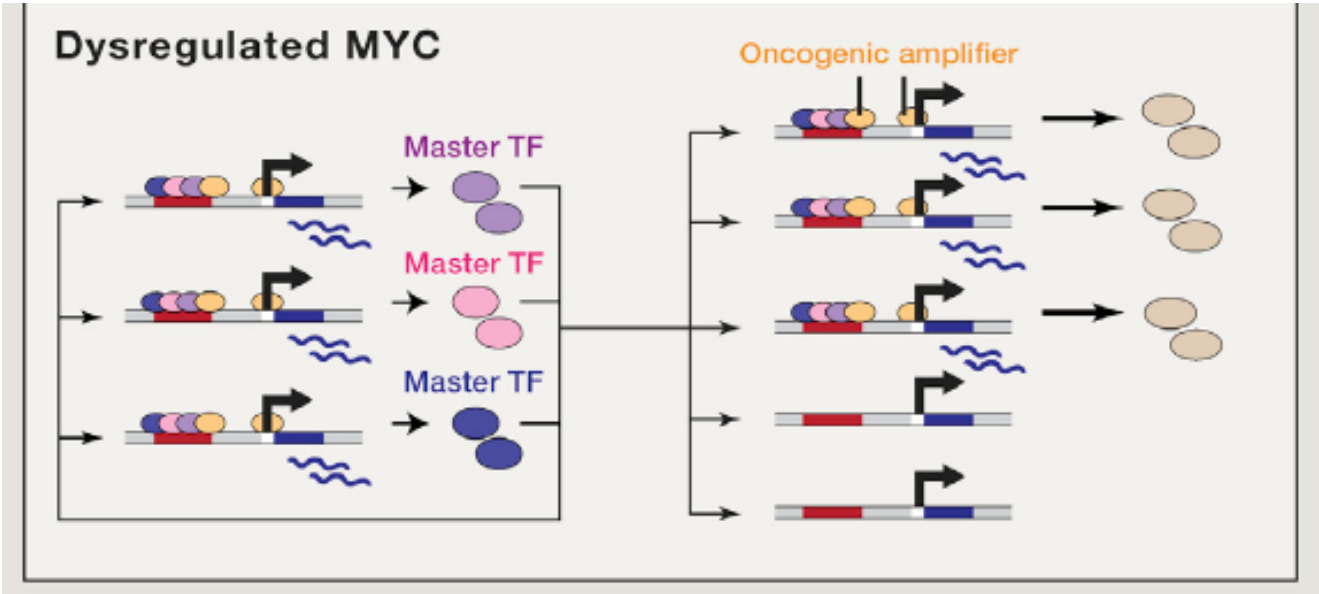
DYSREGULATION OF ACTIVATED SIGNALLING TRANSCRIPTION FACTOR



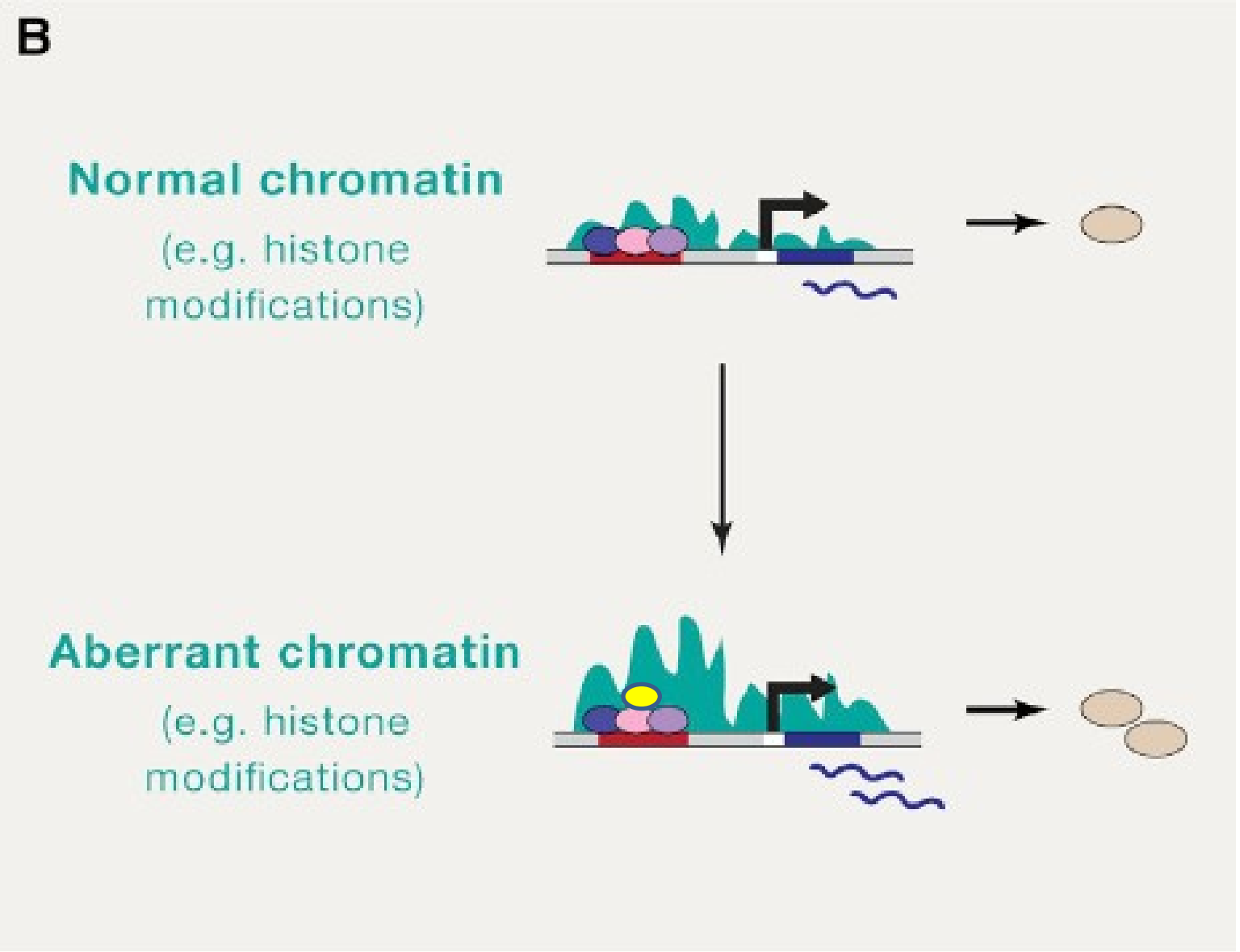


DYSREGULATION OF ONCOGENIC TRANSCRIPTION FACTOR

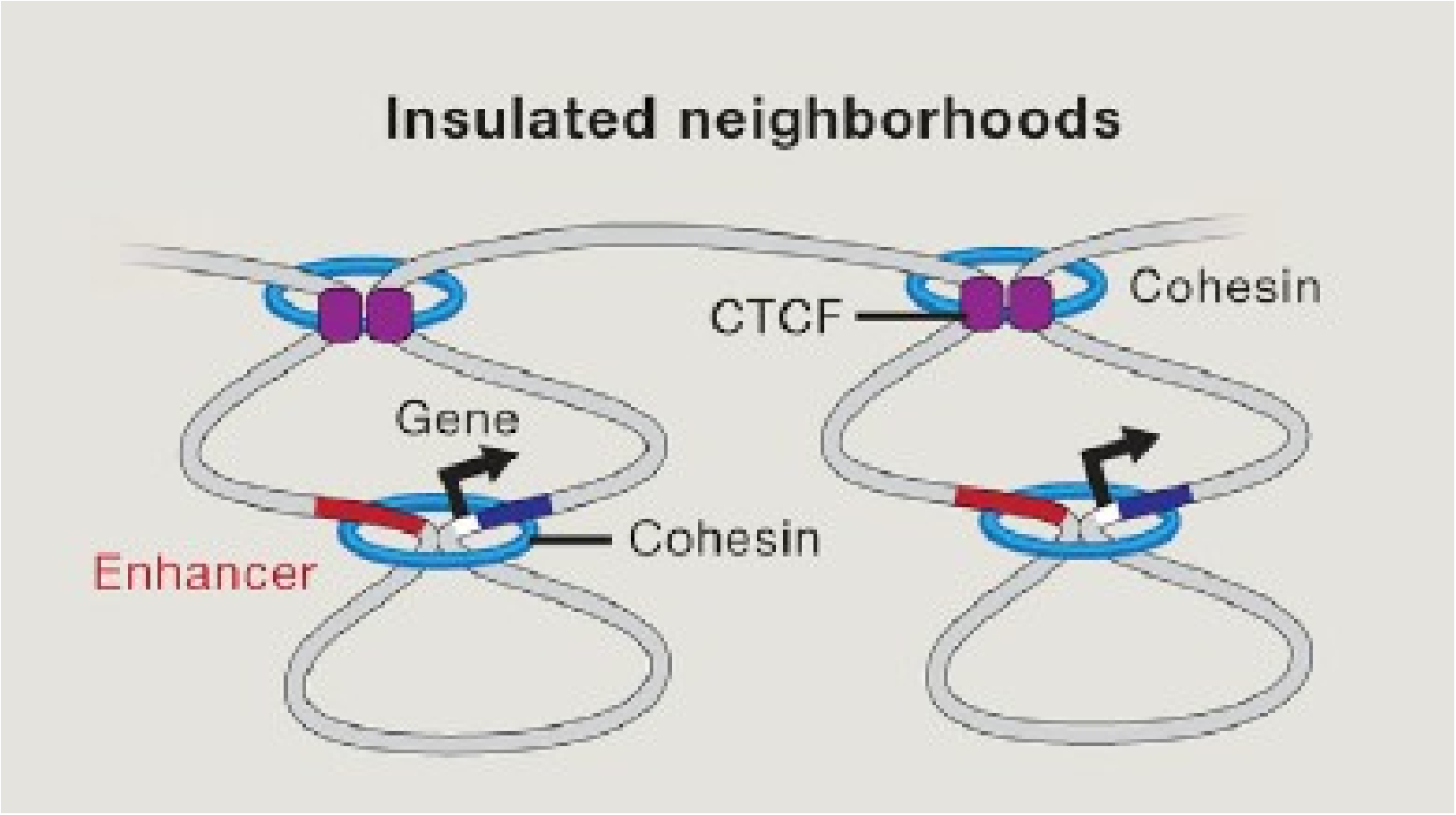




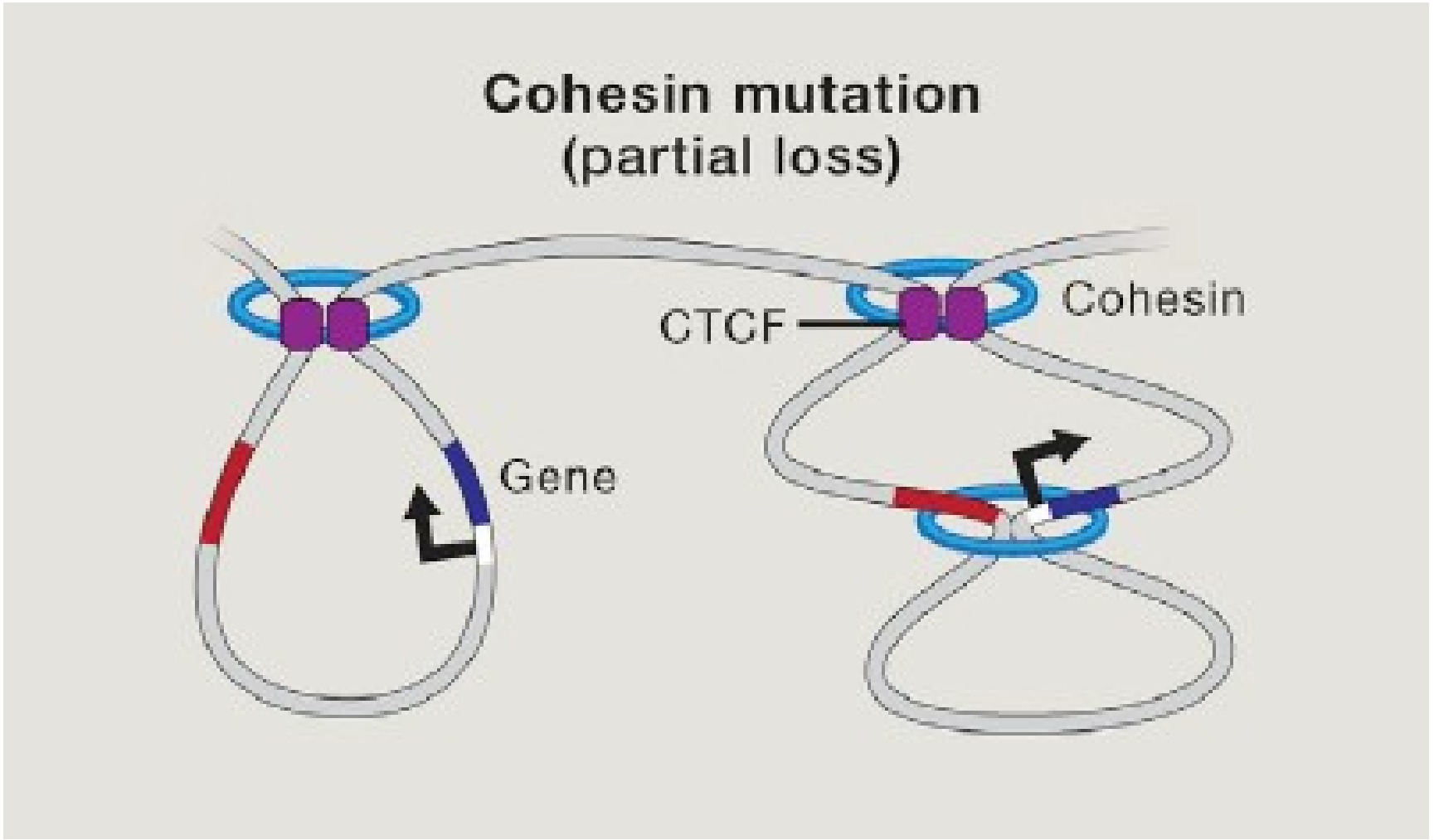
Chromatin remodelling plays a role in disease: transcription activation



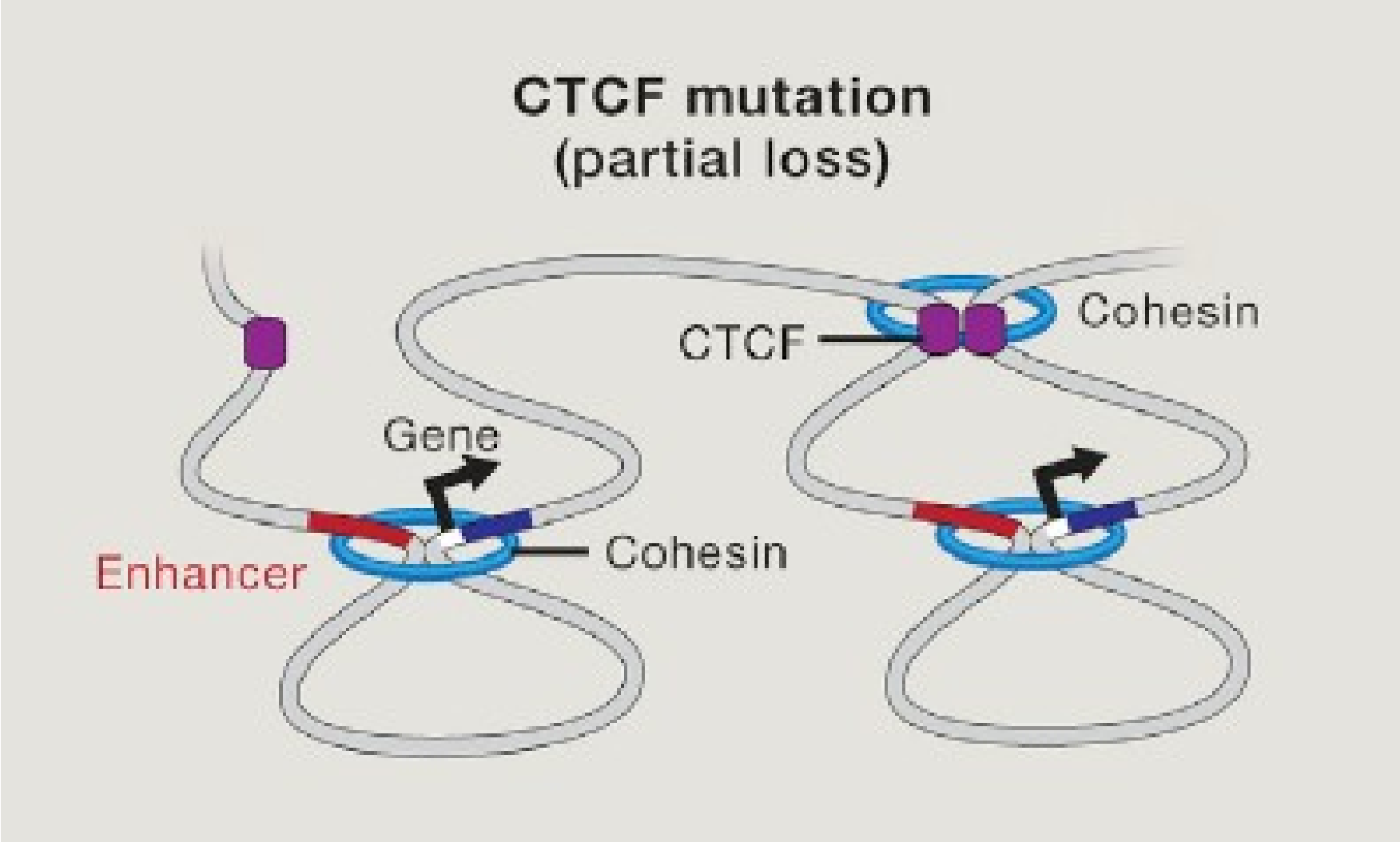
Long range interactions are mediated by CTCF and cohesin and define specific regulatory domain



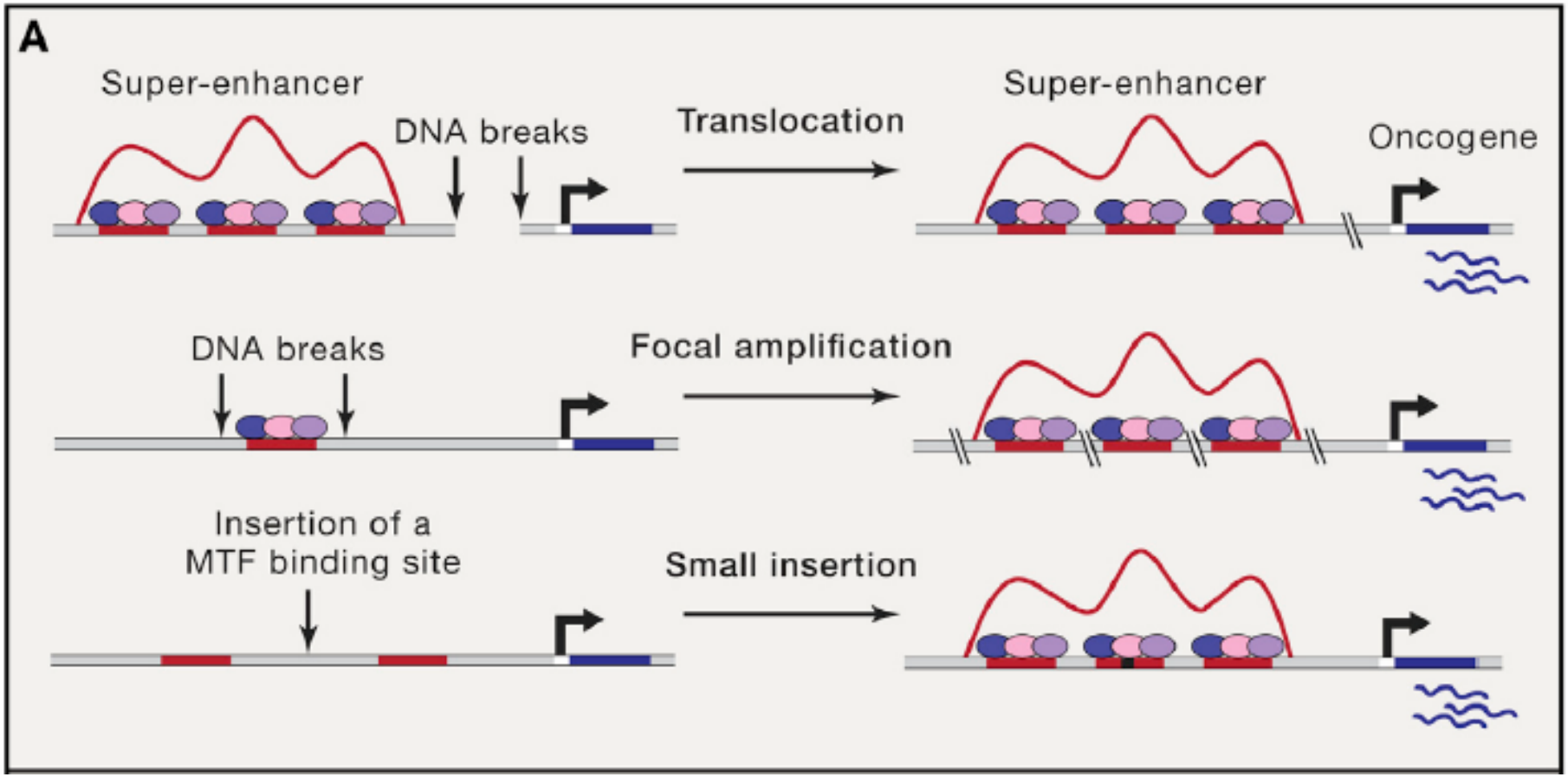
Cohesin mutation may disrupt long range interactions between enhancer-promoter



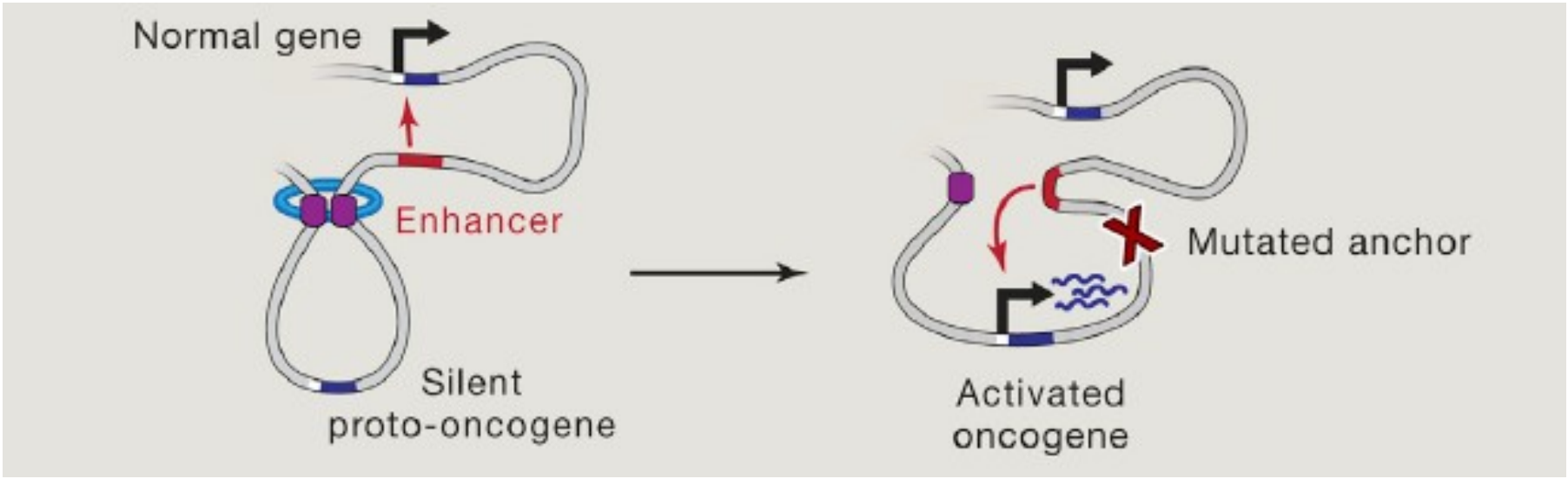
CTCF mutation may favorite long range interactions between enhancer-promoter



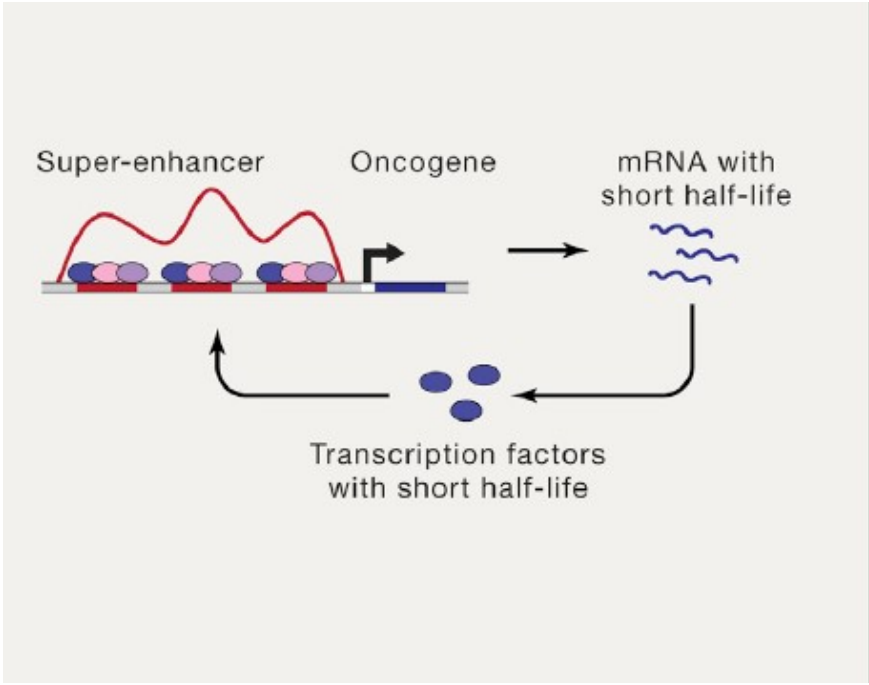
Mechanisms that lead SE formation

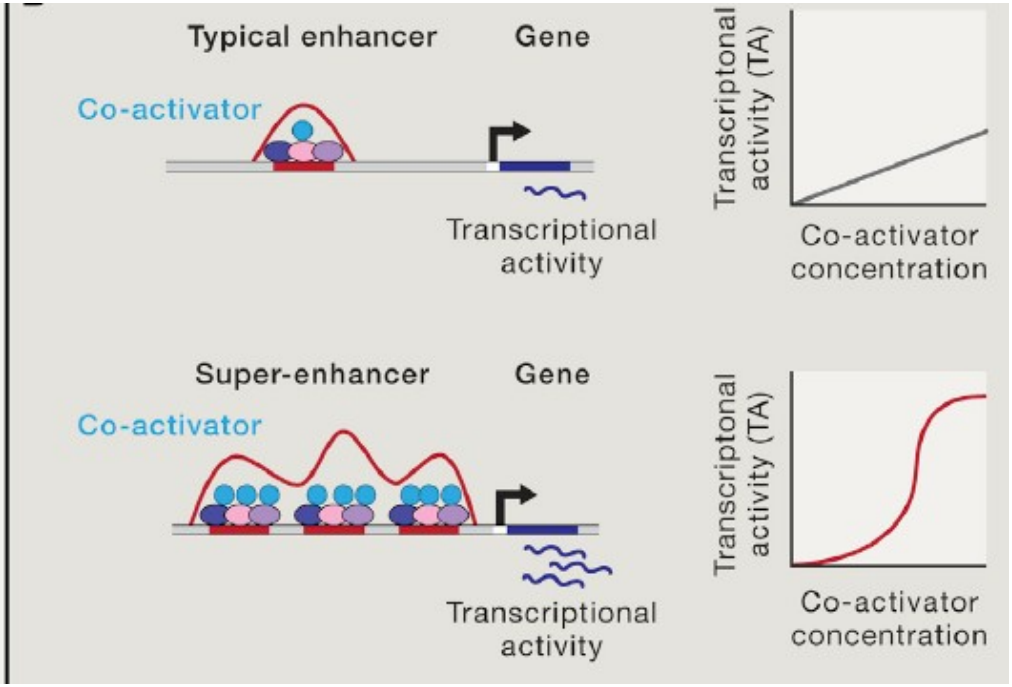


Activation of silent proto-oncogenes by somatic mutations that disrupt insulated neighborhood anchor sites



Molecular mechanisms that may be used for drug discovery

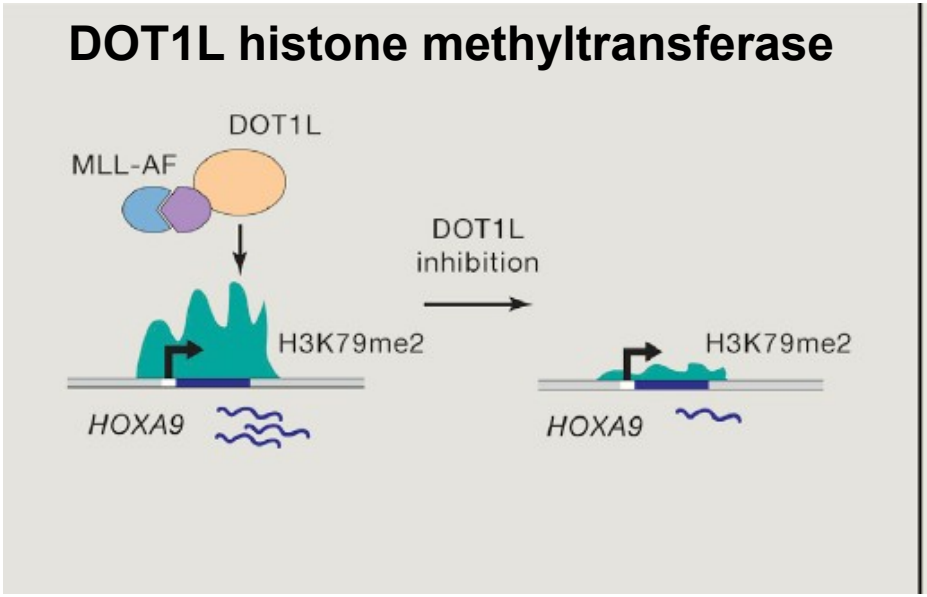


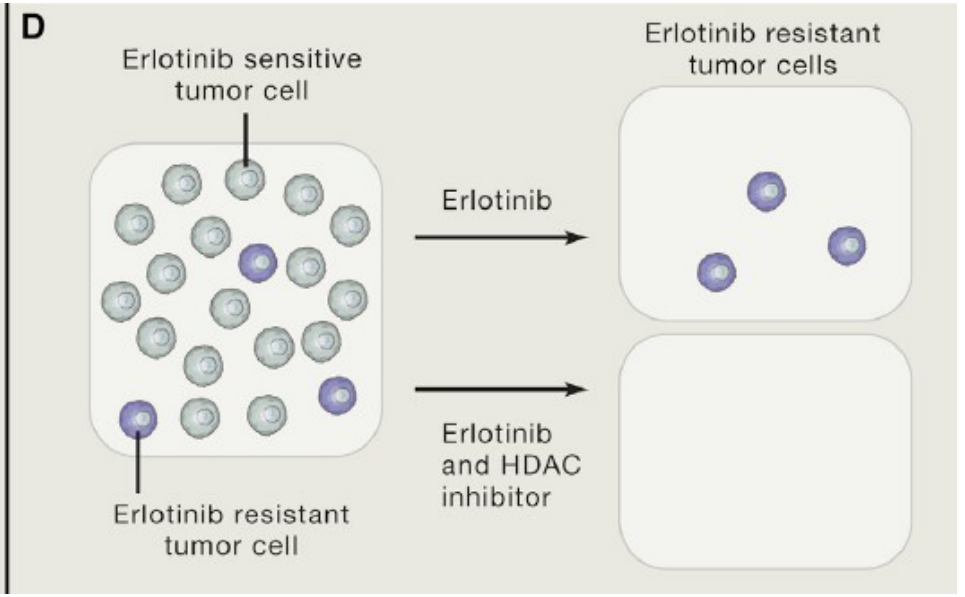


Increased the turnover of TFs

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

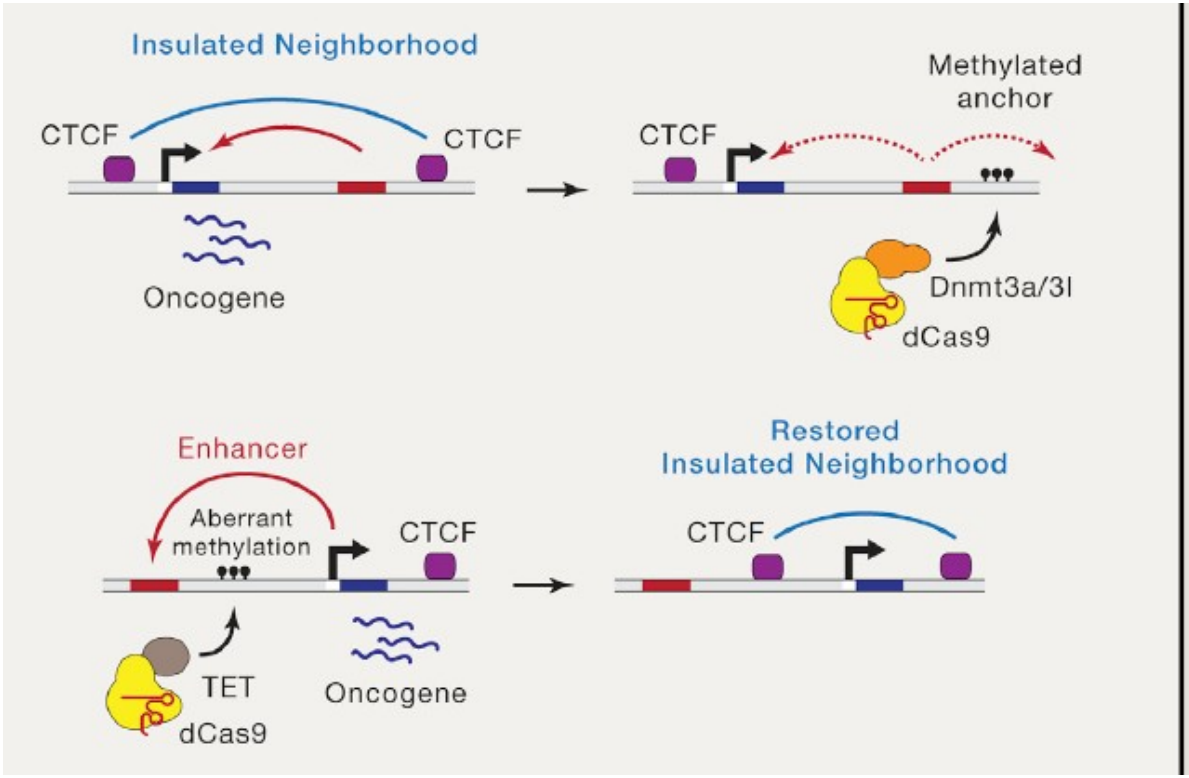


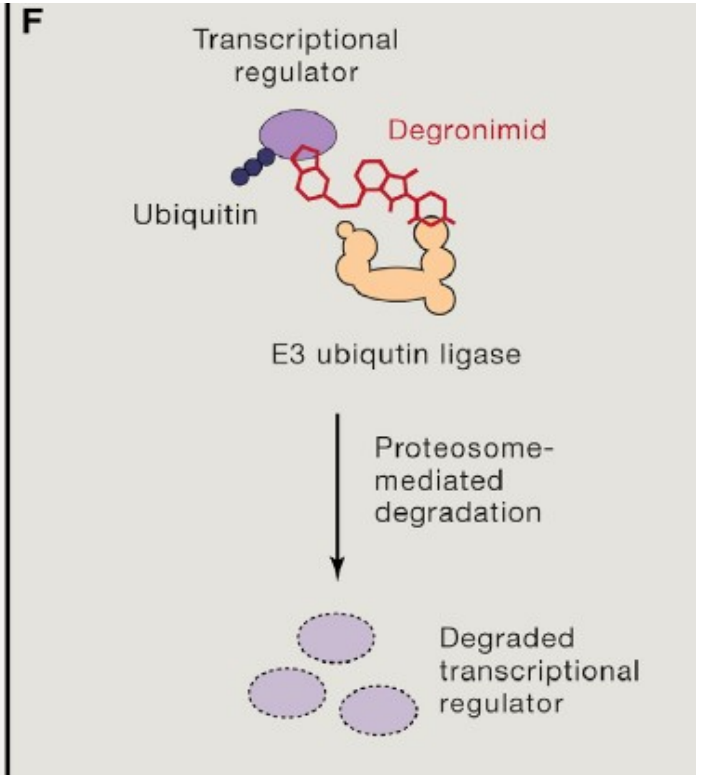


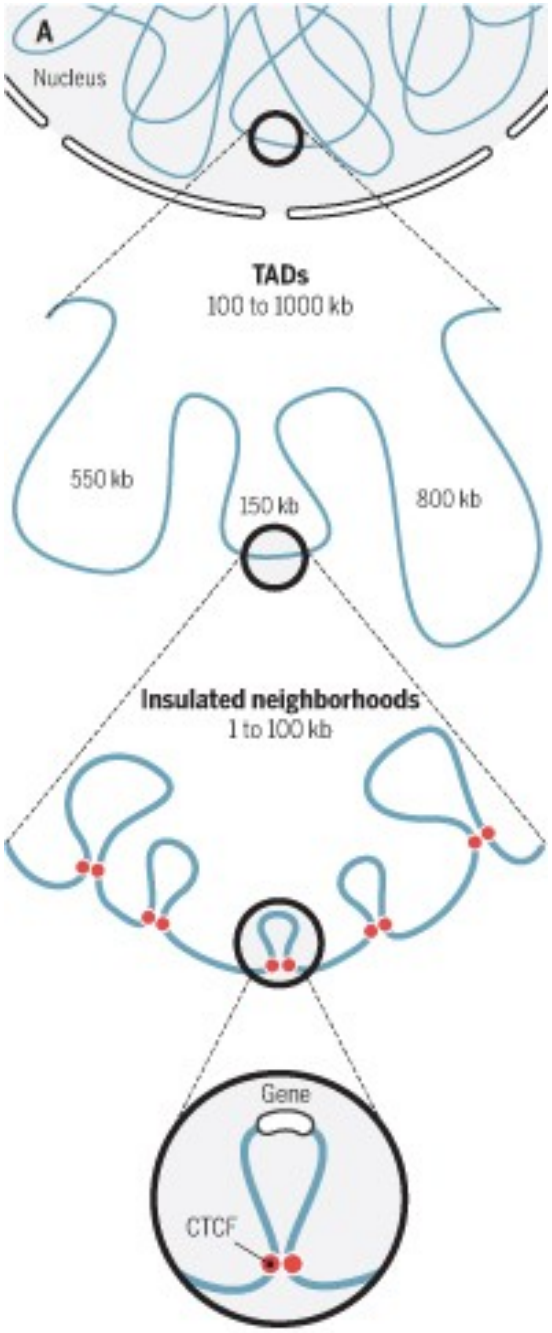
DOT1L histone methyltransferase

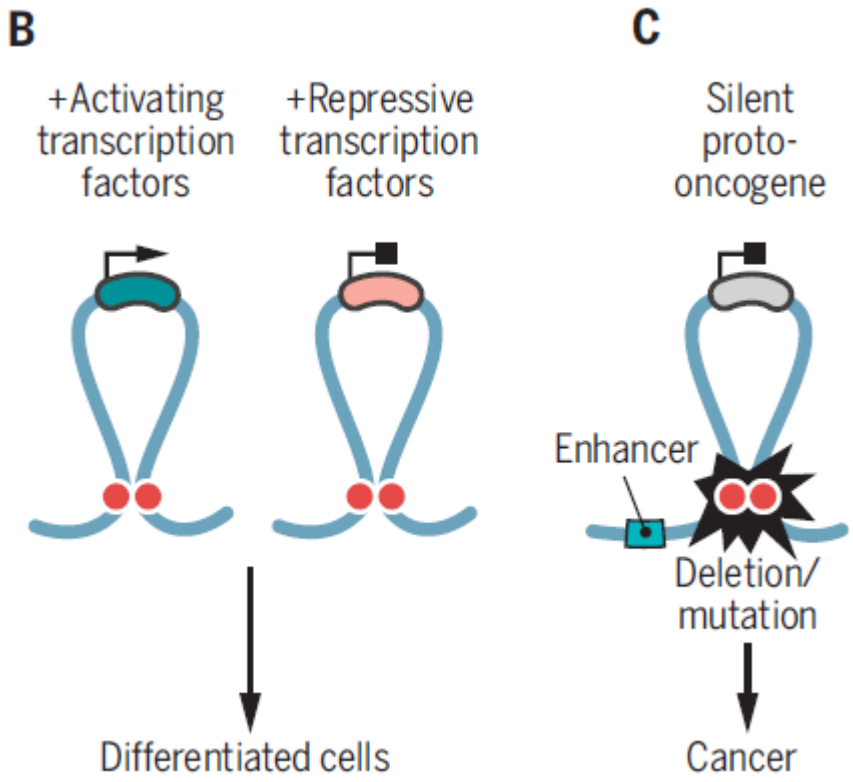
Somatic Mutations and aberrant DNA methylation drive oncogenesis

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





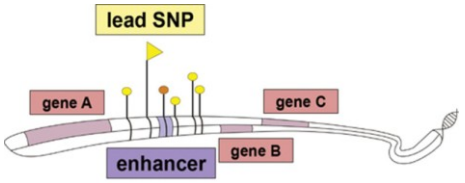

A
Nucleus
TADs
100 to 1000 kb
550 kb
150 kb
800 kb
Insulated neighborhoods
1 to 100 kb
Gene
CTCF


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

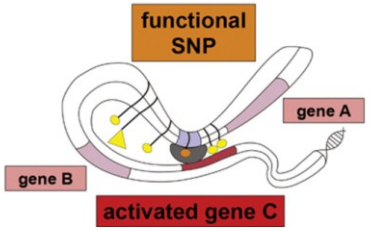
IDENTIFICATION AND CHARCTERIZATION

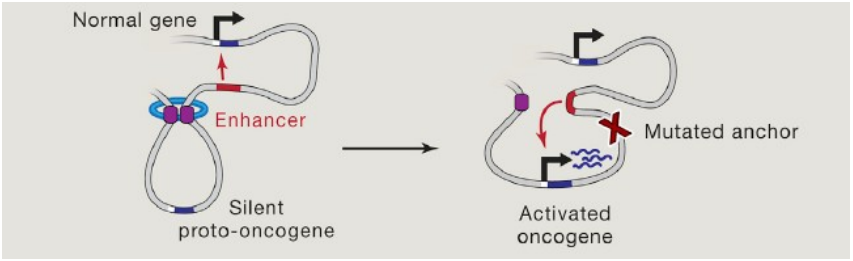
MOLECULAR MECHANISMS

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

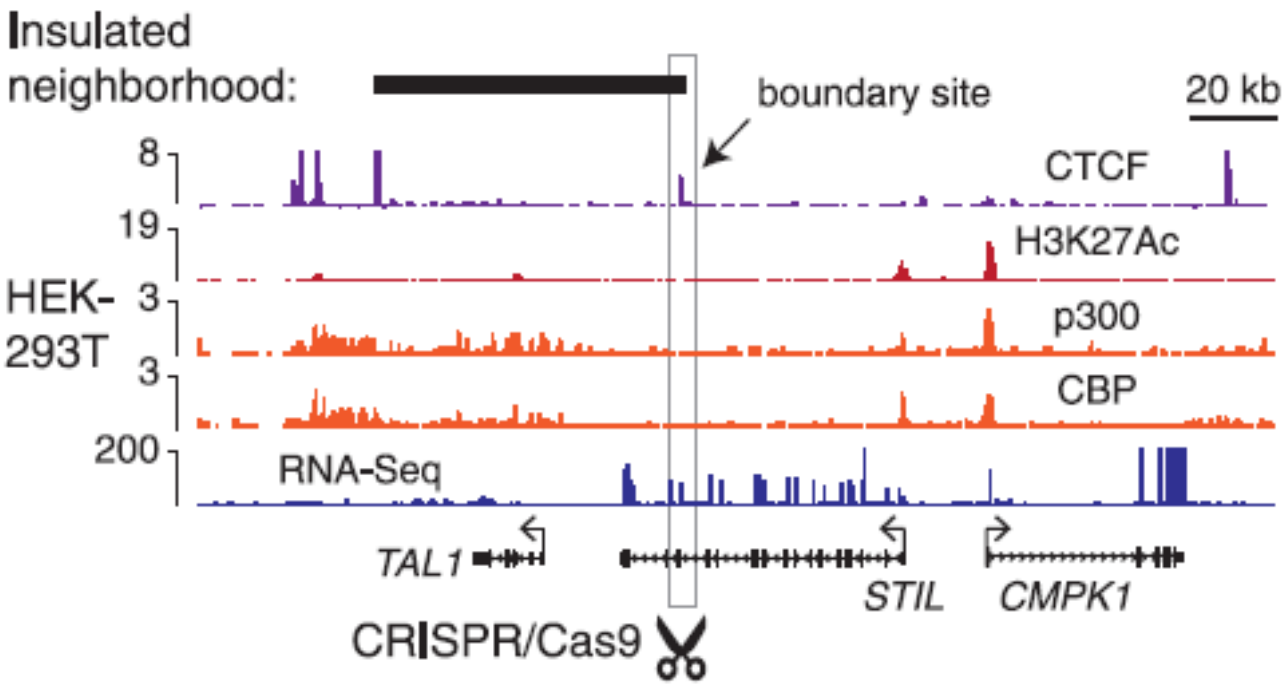
- Aberrant transcription activation depends on:

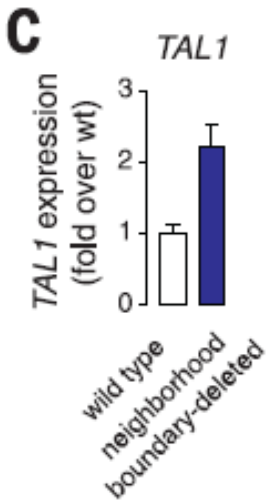
- super-enhancers formation
- transcription factors and cofactors
- Long range interactions dynamic

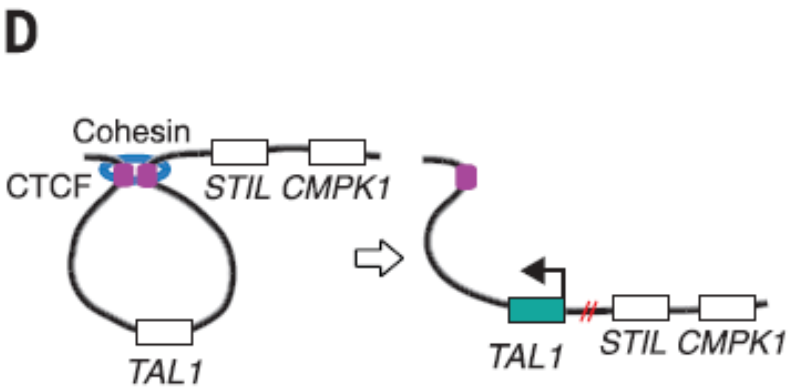
In summary II:

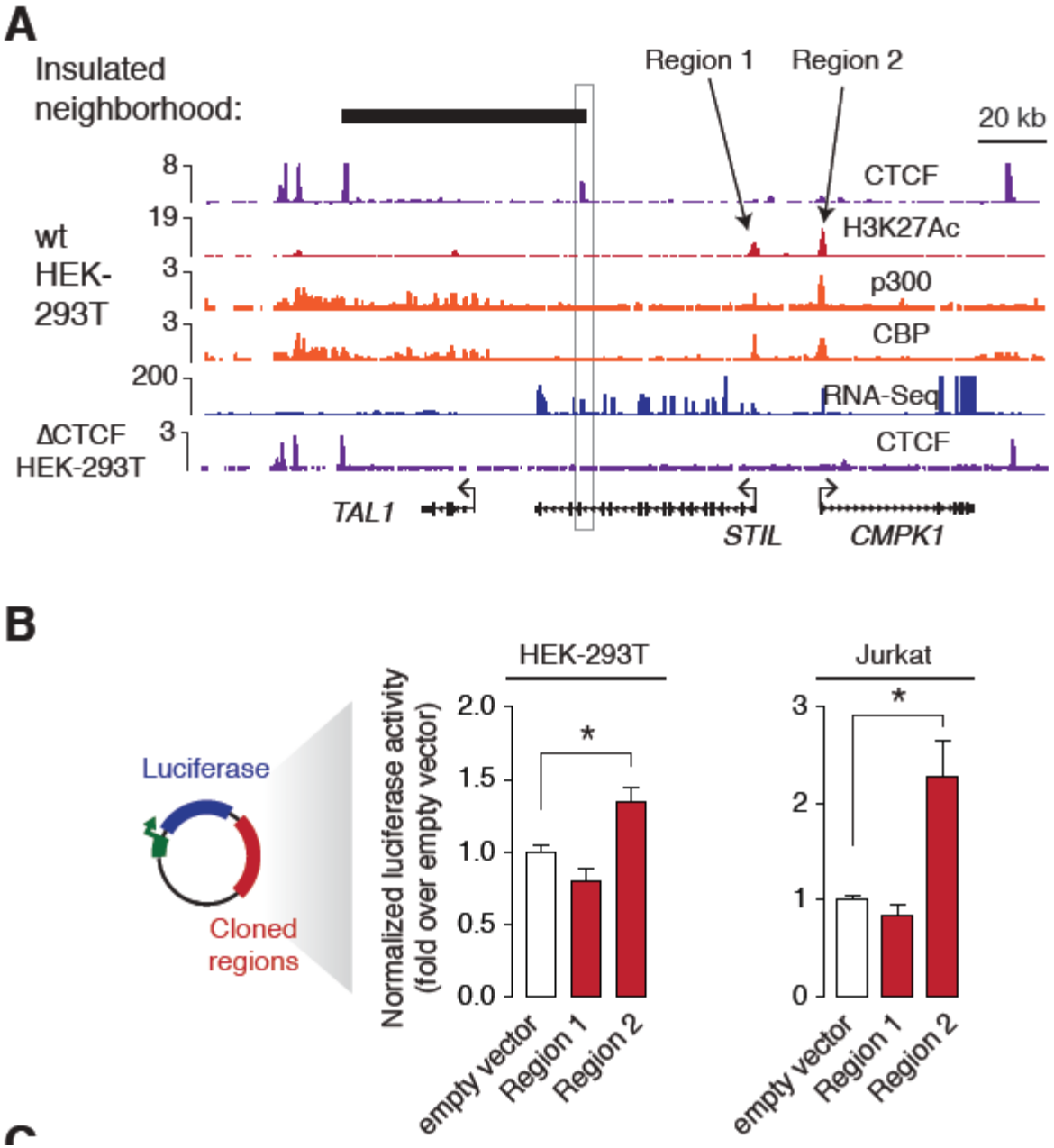
Dysregulated transcriptional programs may be target for drug discovery:

- 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


Insulated
neighborhood:
boundary site
20 kb
HEK-
293T
8
19
3
3
200
CTCF
H3K27Ac
p300
CBP
RNA-Seq
TAL1
STIL
CMPK1
CRISPR/Cas9


C
TAL1
TAL1 expression
(fold over wt)
3
2
1
0
wild type
neighborhood
boundary-deleted


D
Cohesin
CTCF
STIL CMPK1
TAL1
TAL1
STIL CMPK1


A
Insulated neighborhood:
Region 1
Region 2
20 kb
wt HEK-293T
CTCF
H3K27Ac
p300
CBP
RNA-Seq
ΔCTCF HEK-293T
CTCF
TAL1
STIL
CMPK1
B
Luciferase
Cloned regions
HEK-293T
Jurkat
Normalized luciferase activity (fold over empty vector)
empty vector
Region 1
Region 2