In Lesson 1

- Functional genomics is a field of molecular biology based on genome-wide sequencing data.
- Genome-wide sequencing data describe genomic regulatory regions that control gene expression
- Gene expression disregulation may be linked to the disease
- Understanding molecular mechanisms of disease outcome opens the way to discovery drug and identify biomarkers

Genomic regulatory regions are **cell regulatory pattern** that defines cell identity and biological functions. Variants in the genomic regulatory regions may change the molecular mechanisms to control gene expression and may be linked to disease occurance and development.

IDENTIFICATION AND CHARCTERIZATION

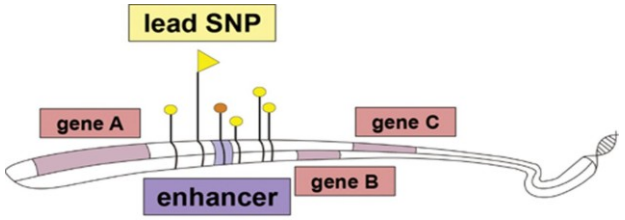
MOLECULAR MECHANISMS

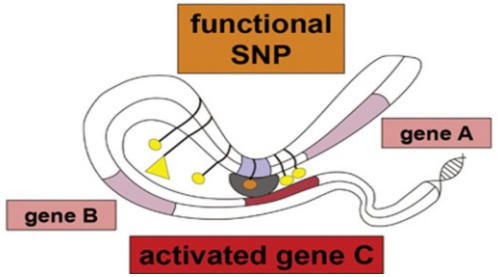
GENOMIC REGULATORY REGIONS

GENE EXPRESSION REGULATION

CELL IDENTITY

BIOLOGICAL FUNCTIONS





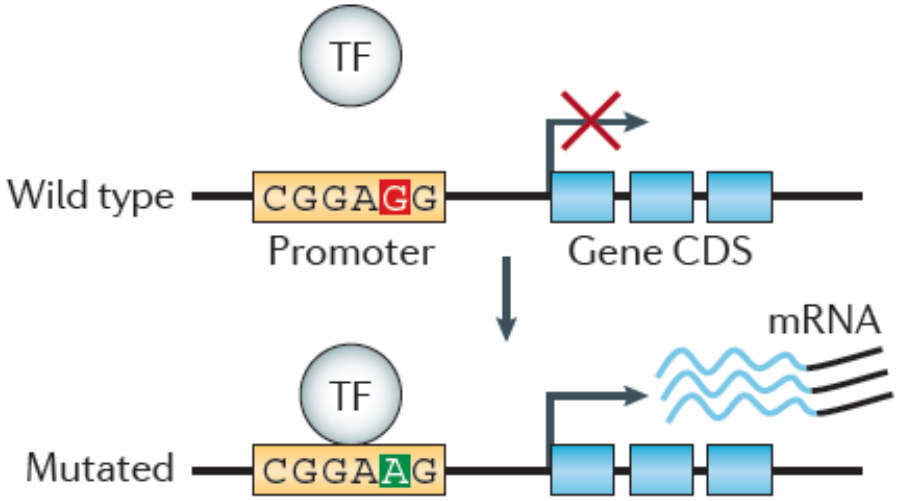
TO UNDERSTAND DISEASES

How SNPs play a FUNCTIONAL role in disease: Impact on transcription

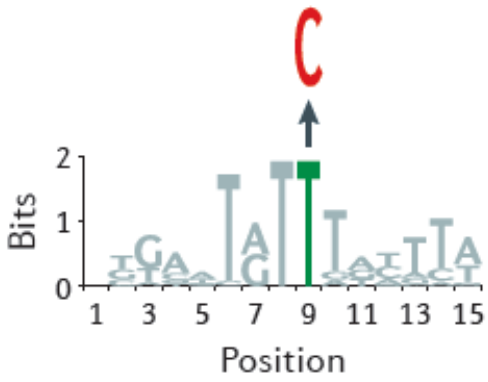
- Changing consensus sequenses for transcription factors binding sites
- Changing interaction between for transcription factors
- Changing epigenetic profiling of specific genomic regions
- Changing long range interaction between two genomic regions

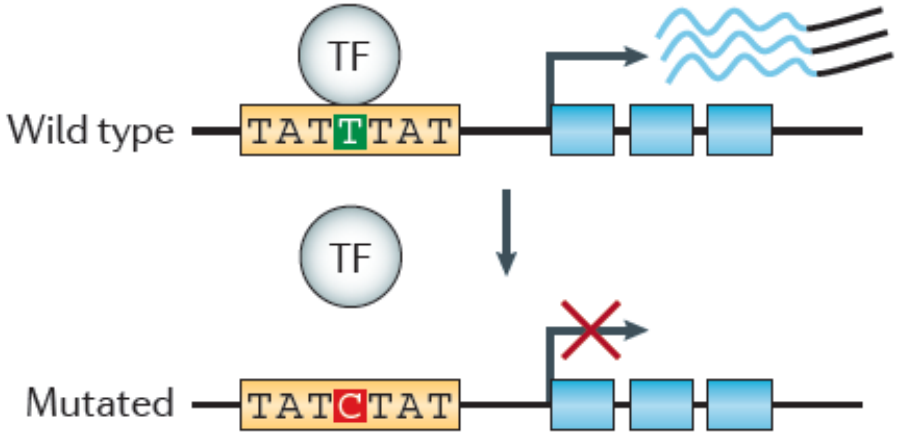
SNPs types functions:

Ba Gain of motif

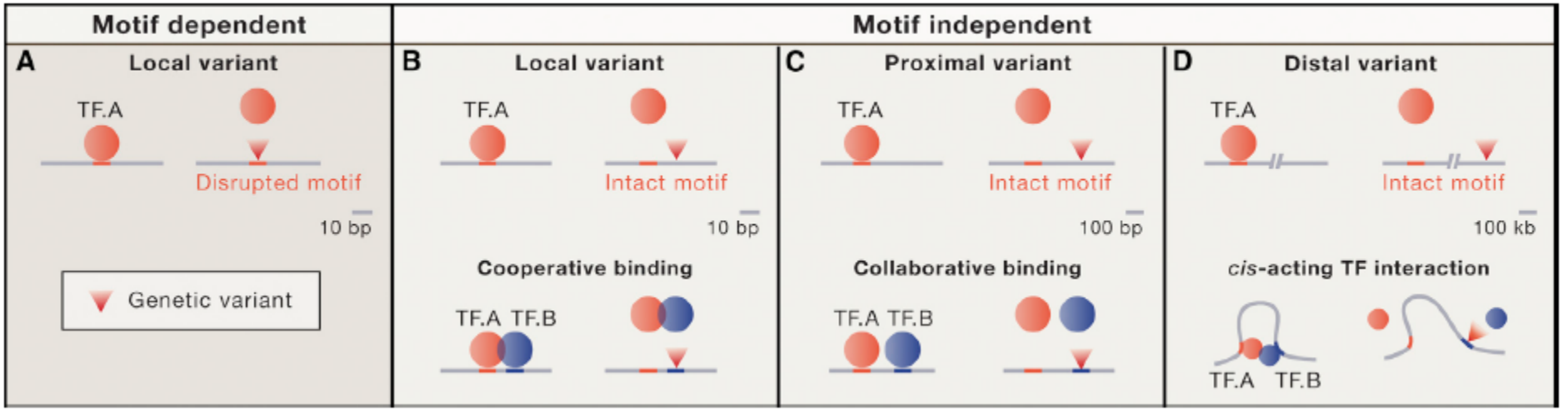


Bb Loss of motif

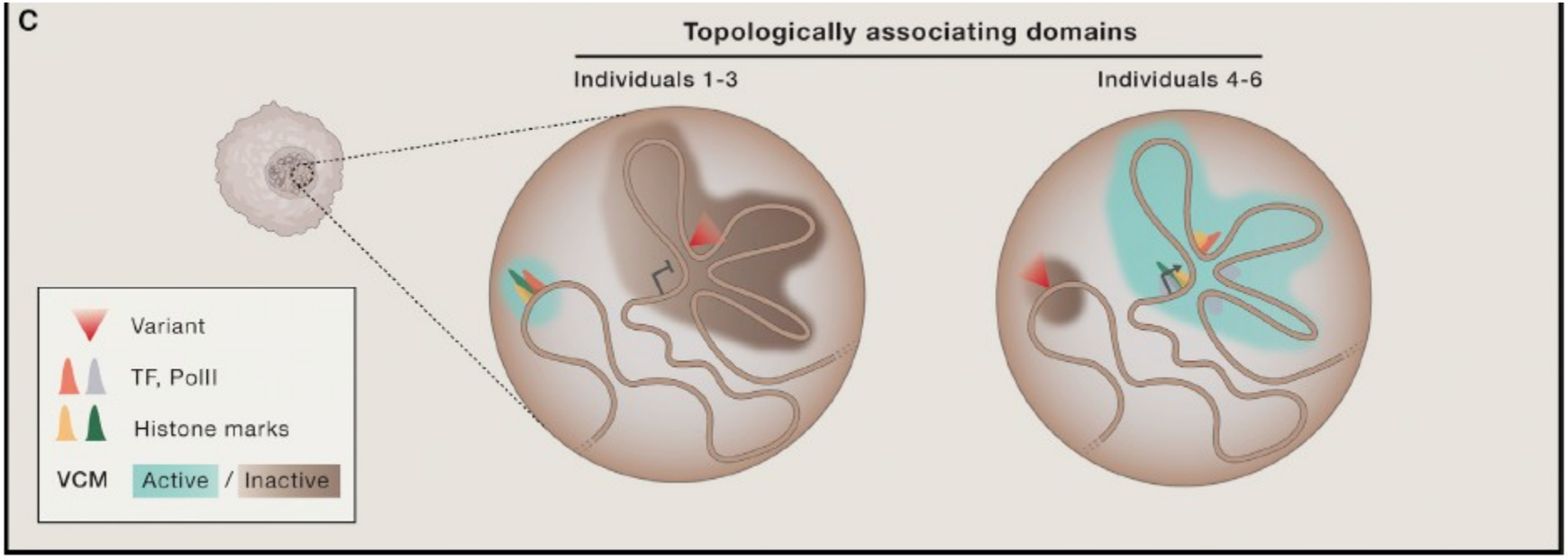




SNPs mechanims for alteration of regulatory transcription factors complexes



SNPs may change long range interactions



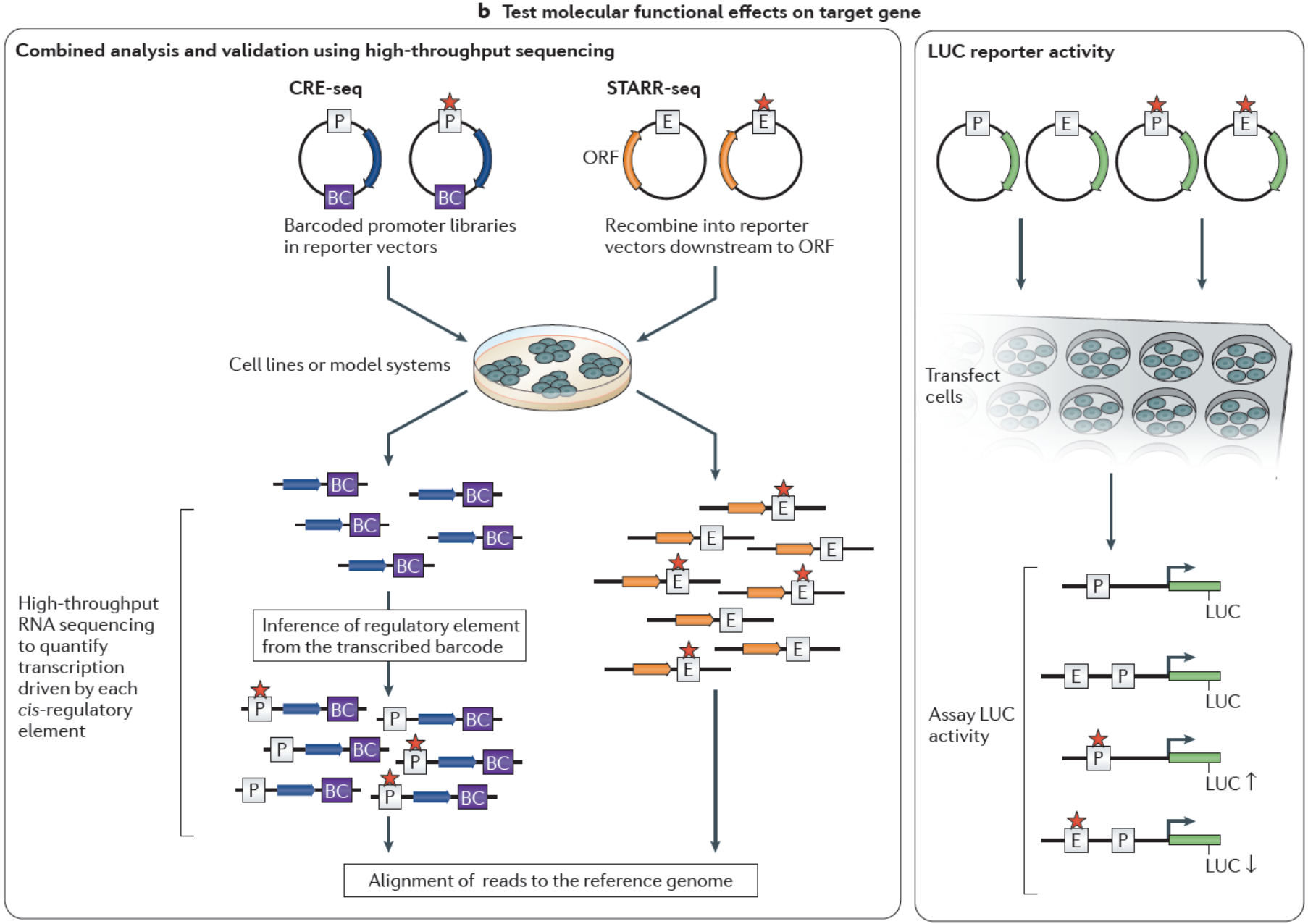
How SNPs play a FUNCTIONAL role in disease:

Alteration of cell identity

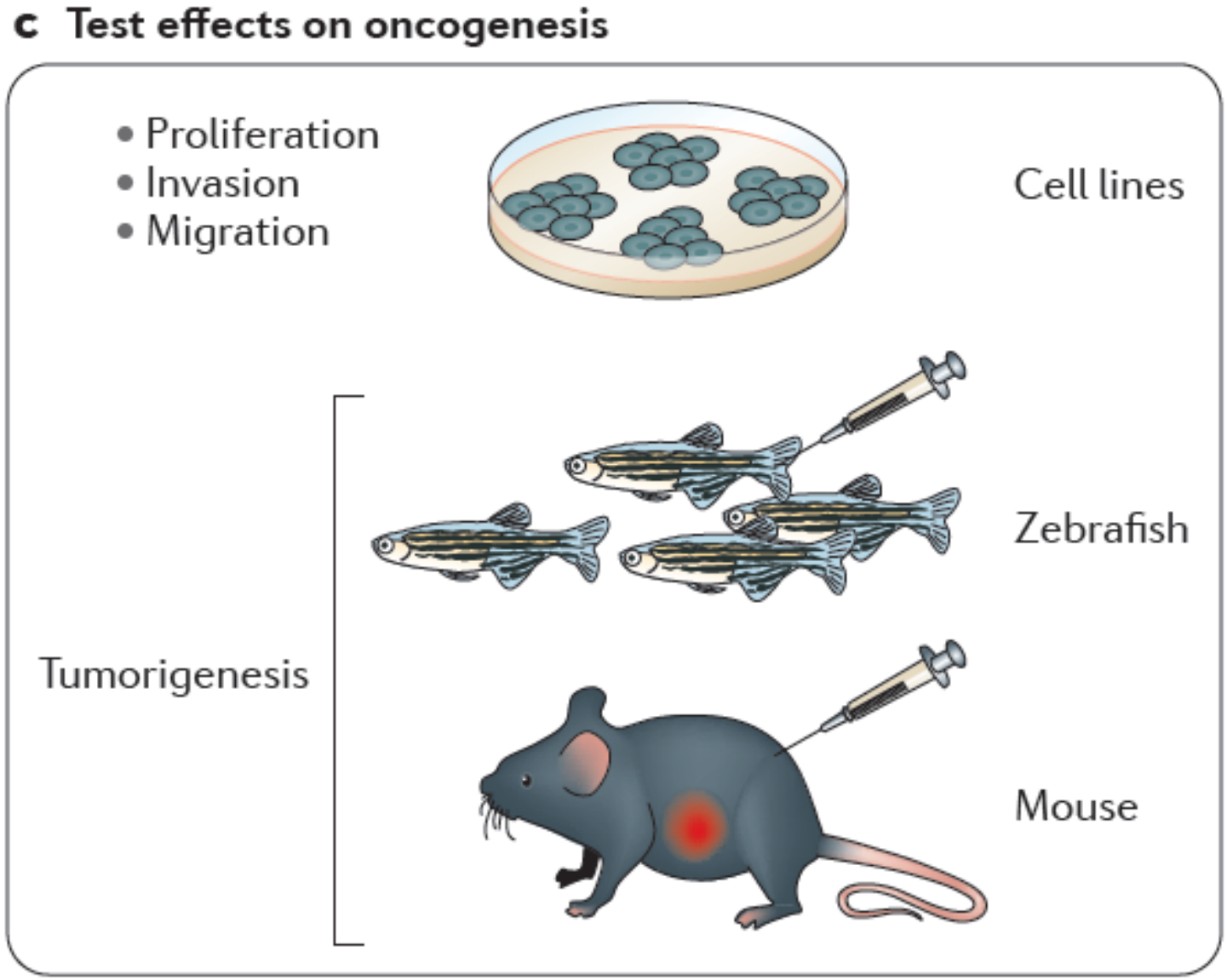
and

biological functions

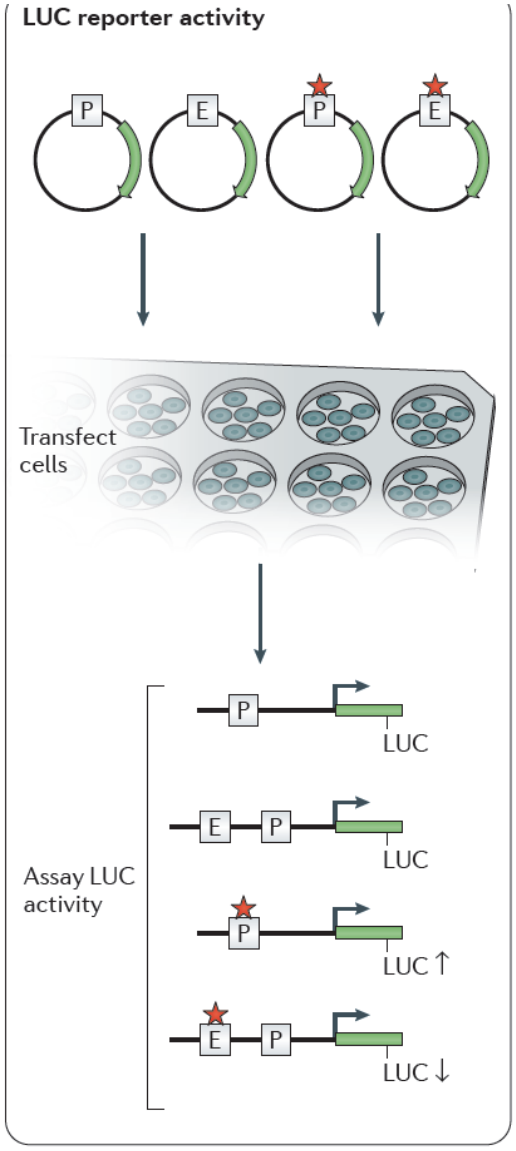
MOLECULAR FUNCTIONAL EFFECTS

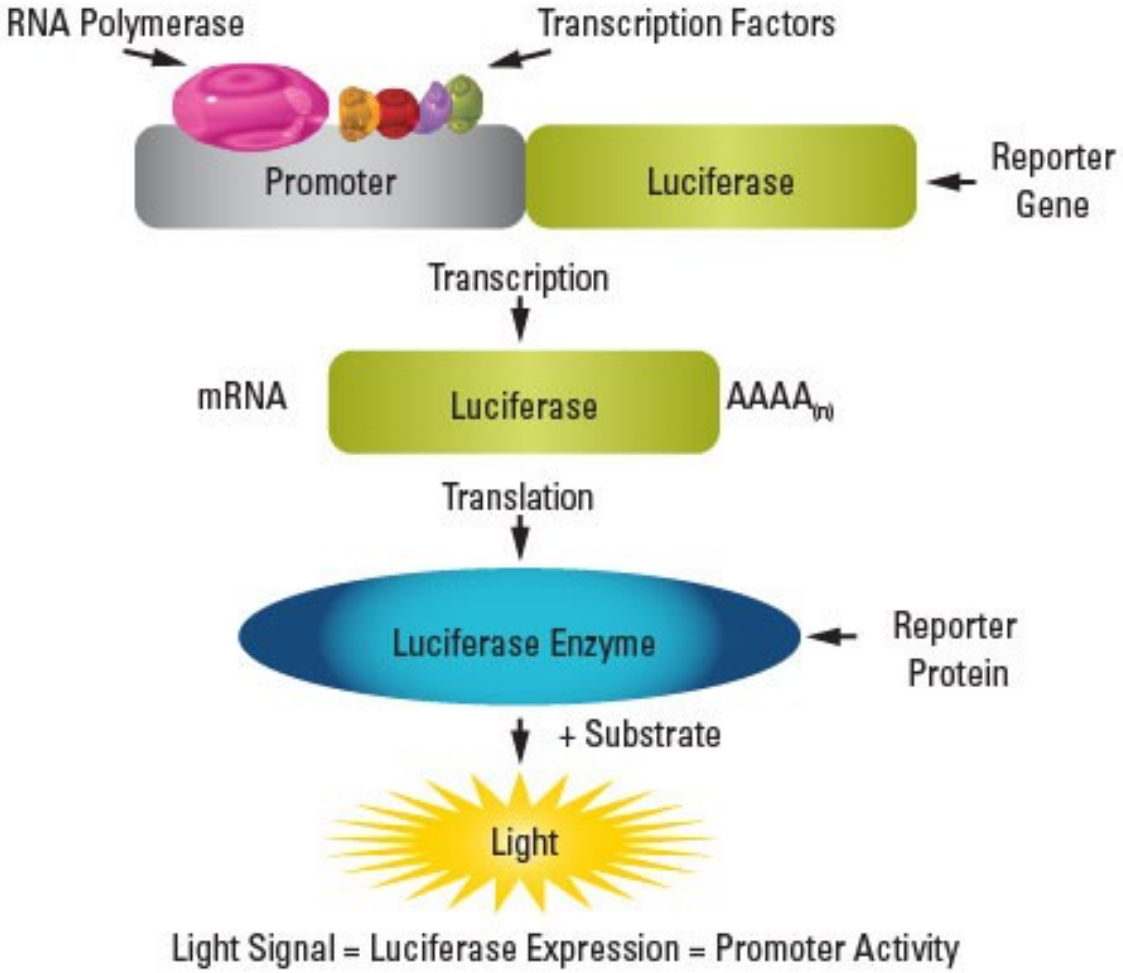


BIOLOGICAL FUNCTION TESTS

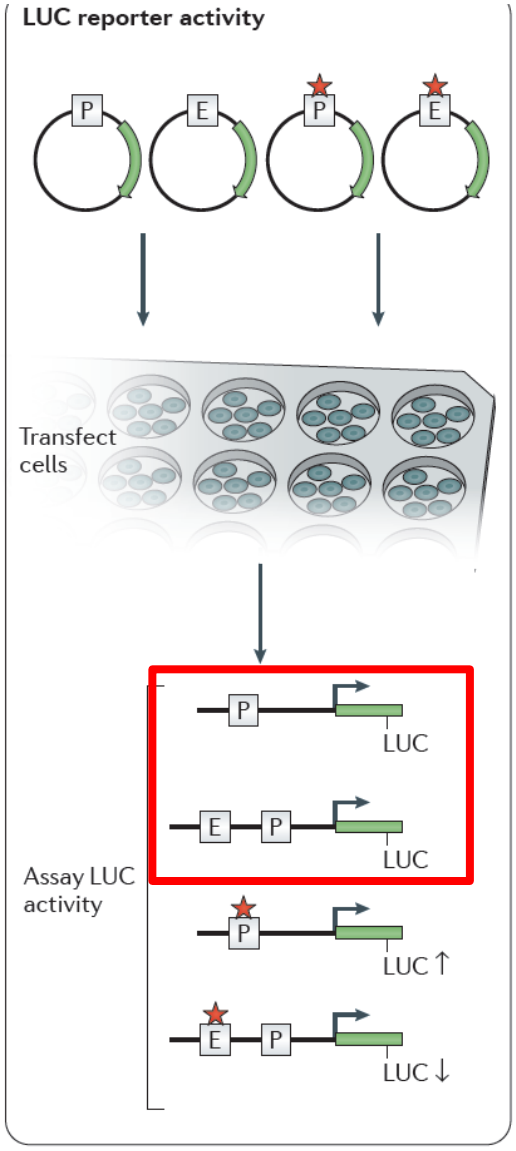


In order to test if SNP has a role in the transcription rate by alteration of TFBS, luciferase assay (REPORTER GENE ASSAY) can be used





In order to test if SNP has a role in the transcription rate by alteration of TFBS, luciferase assay can be used



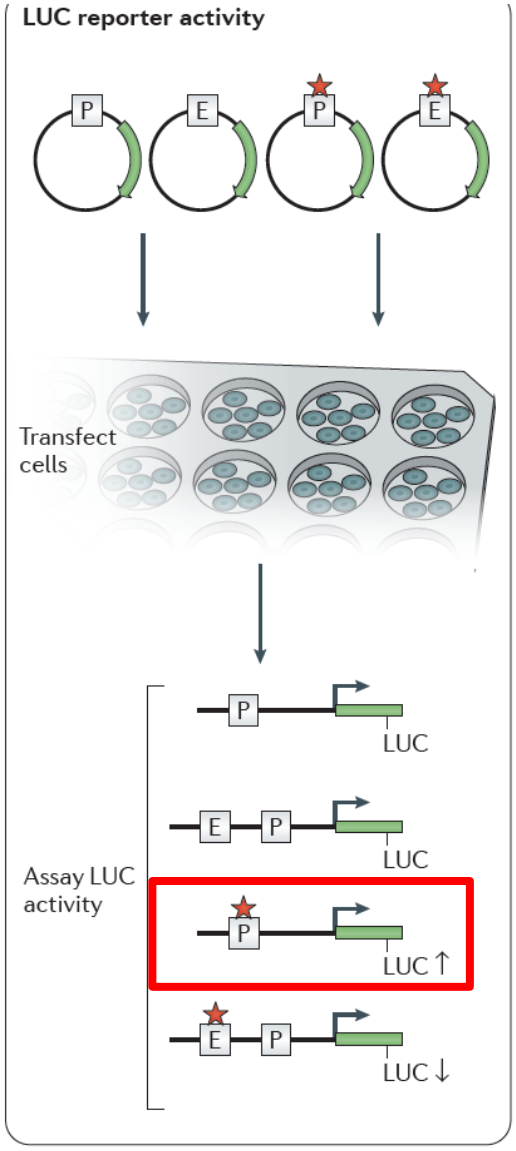
To test enhancer and promoter with SNPs:

Question:
Is the SNP in the promoter or in enhancer able to change trascription activation?

How
Does Transcription Increase or Decrease?

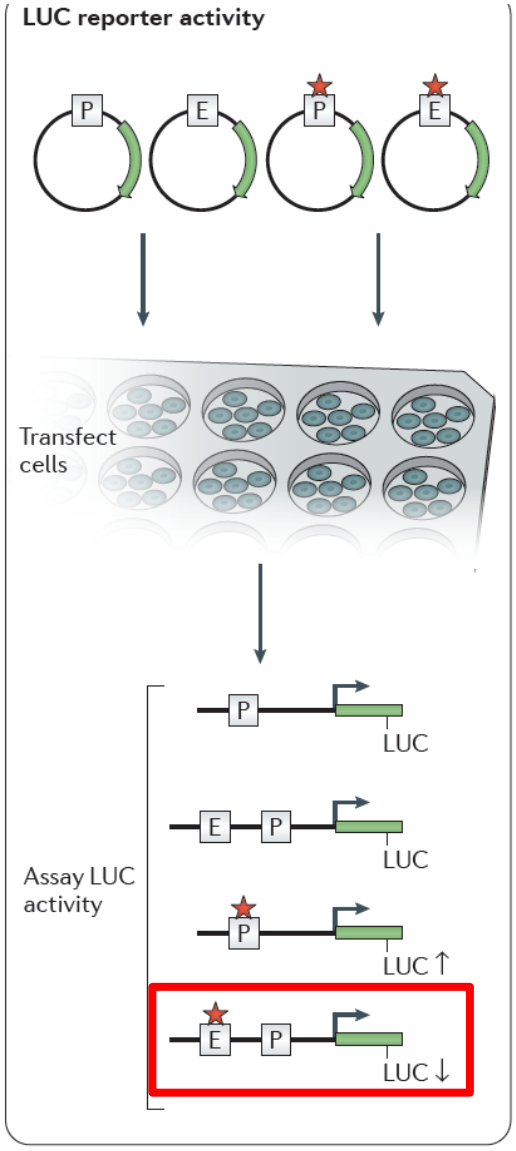
Promoter and Enhancer wild type do not induce transcription

In order to test if SNP has a role in the transcription rate by alteration of TFBS, luciferase assay can be used



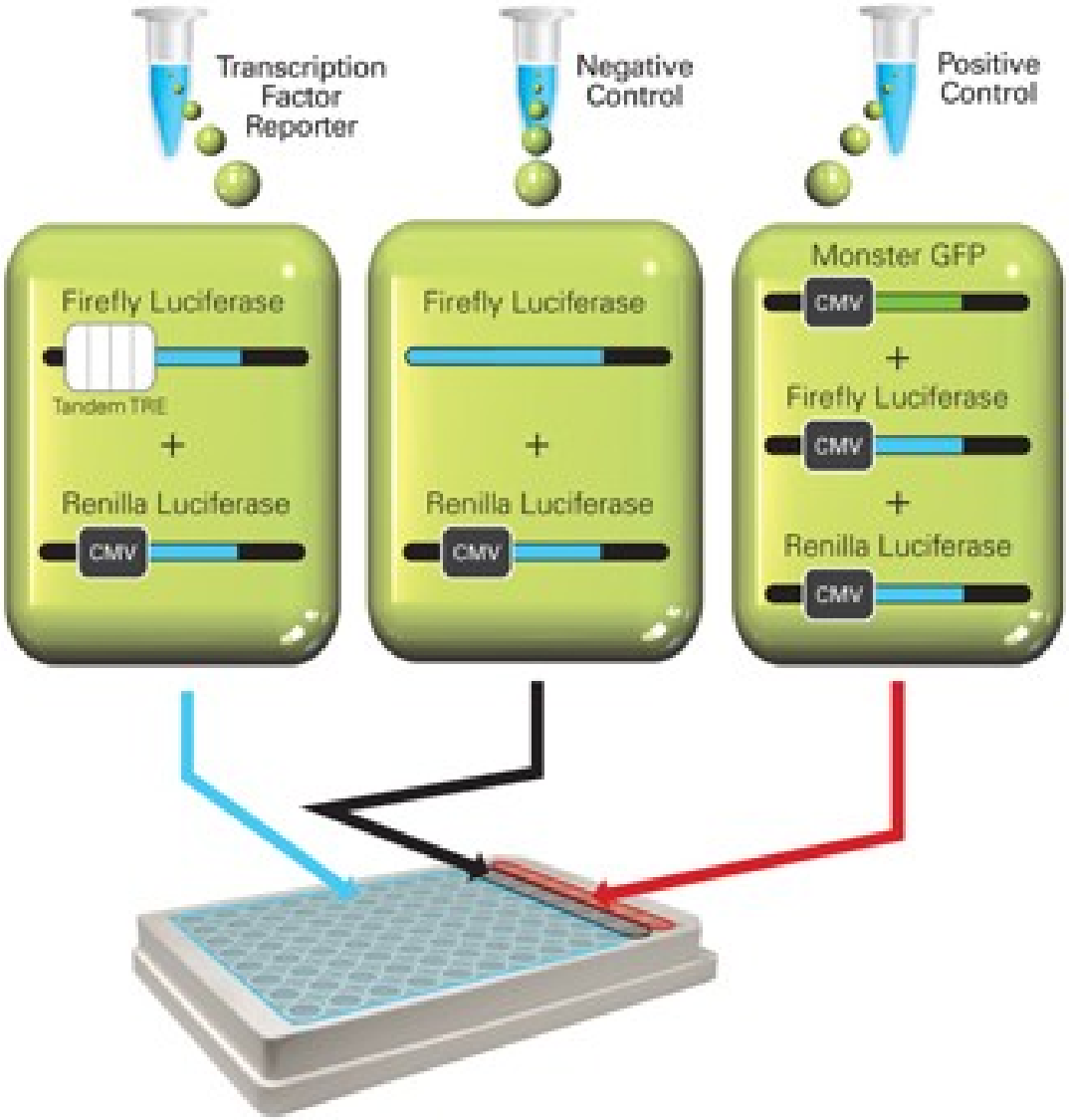
SNP in the Promoter increases trascription

In order to test if SNP has a role in the transcription rate by alteration of TFBS, luciferase assay can be used

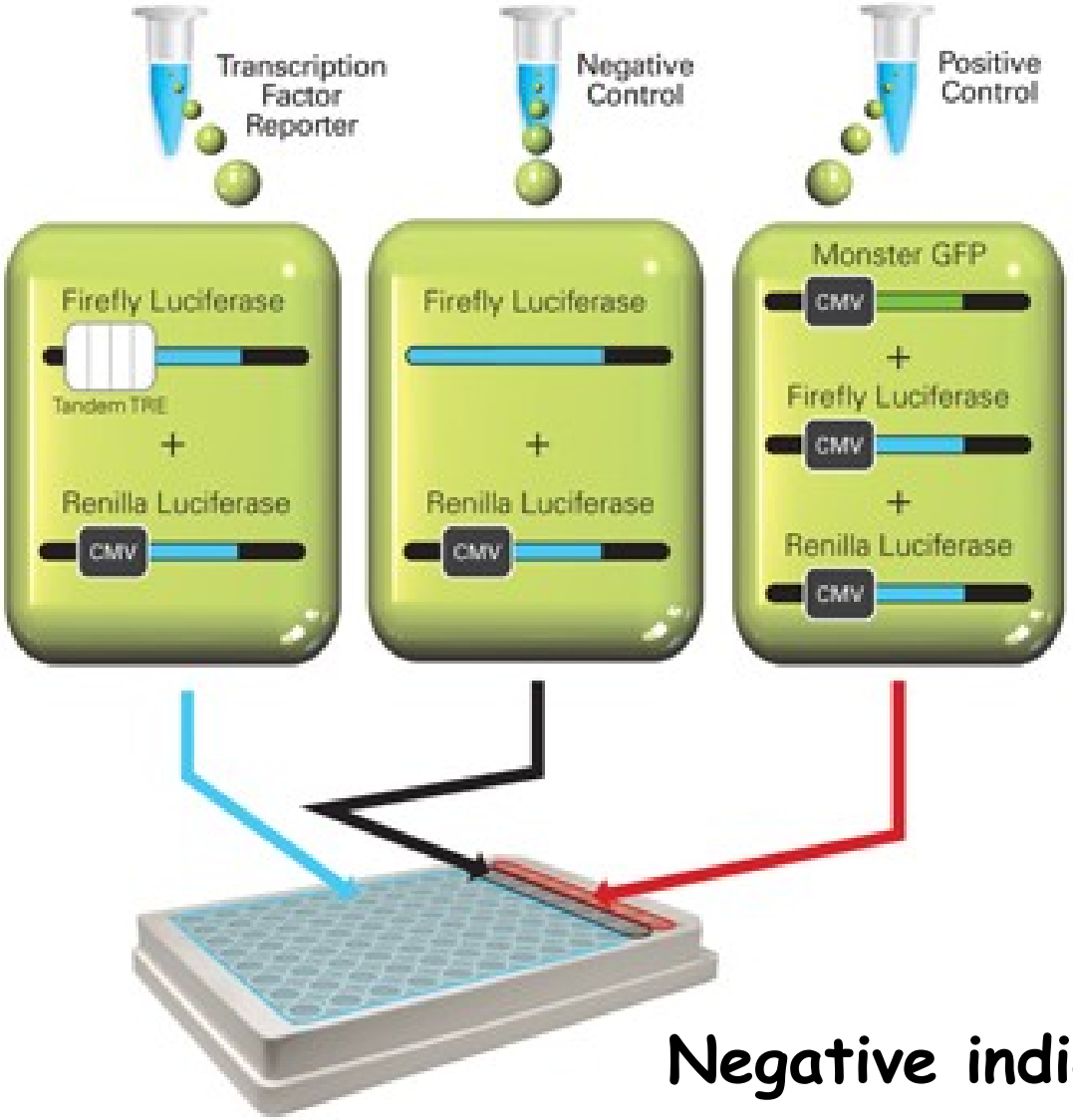


SNP in the Enhancer decrease the trascription

What are positive and negative controls?

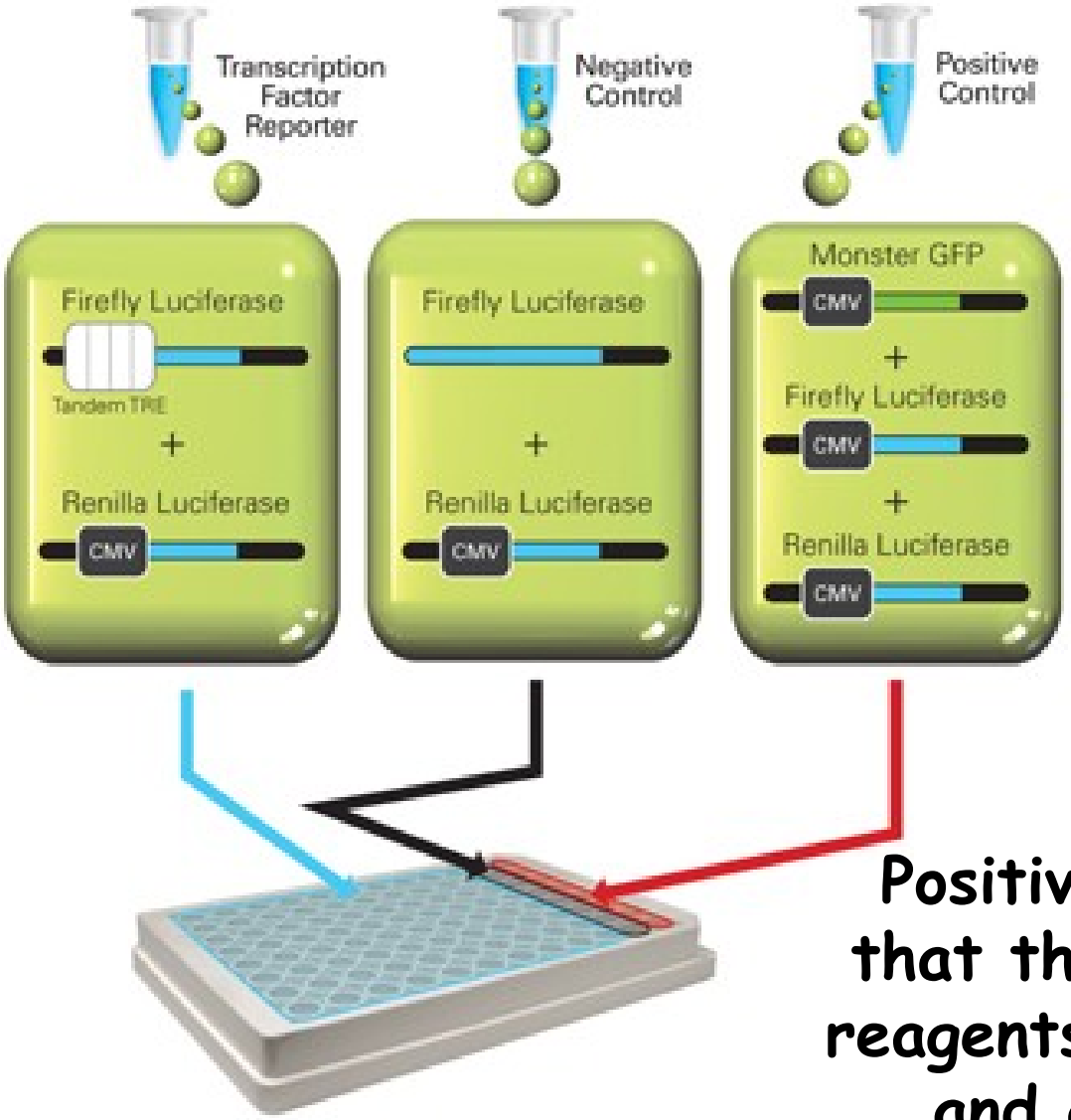


Negative does not have a "regulatory sequence" that you want to test



Negative indicates basal signal

Positive control has a "costitutive active sequence" that induce trascription



Positive control indicates that the assay works well: reagents in the kit are good and are not degrated

In this Lesson

- Enhancer Overview
- Genomic regulatory network to define cell identity
- Genetic variations meaning in cell identity

GENOMIC REGULATORY REGIONS ARE PROMOTER, in proximity of gene target, and ENHANCER, distant from gene target

THE TOPIC IN BRIEF

- Epigenomics is the study of the key functional elements that regulate gene expression in a cell.
- Epigenomes provide information about the patterns in which structures such as methyl groups tag DNA and histones (the proteins around which DNA is packaged to form chromatin), and about interactions between distant sections of chromatin.
- They also contain information about regulatory elements in DNA itself: both those that lie in the promoter region immediately upstream of where a gene's transcription begins, and those in distant enhancer sequences.

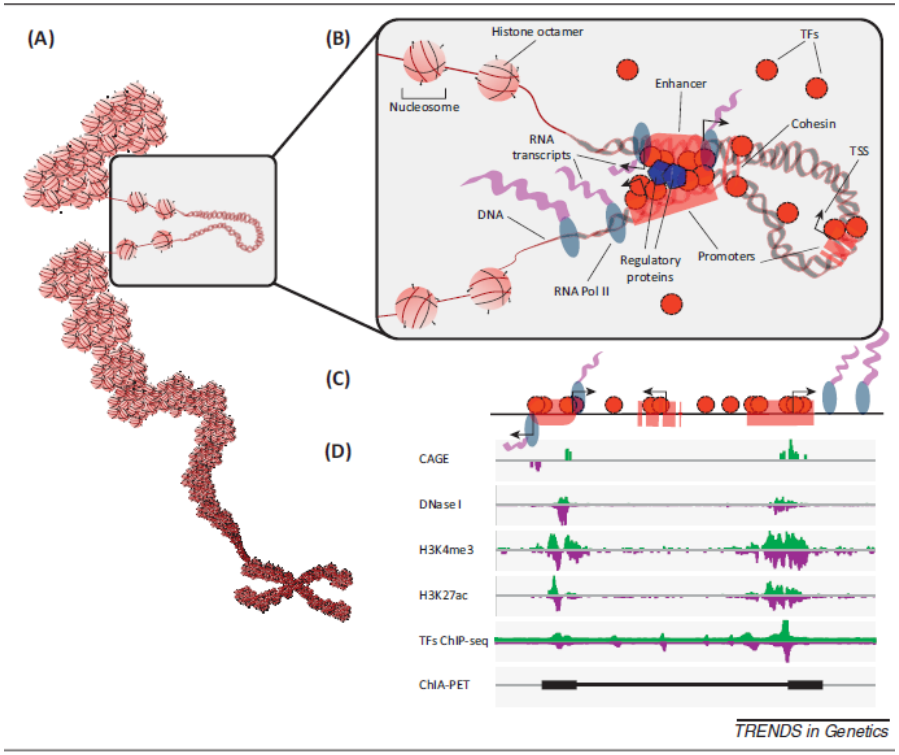
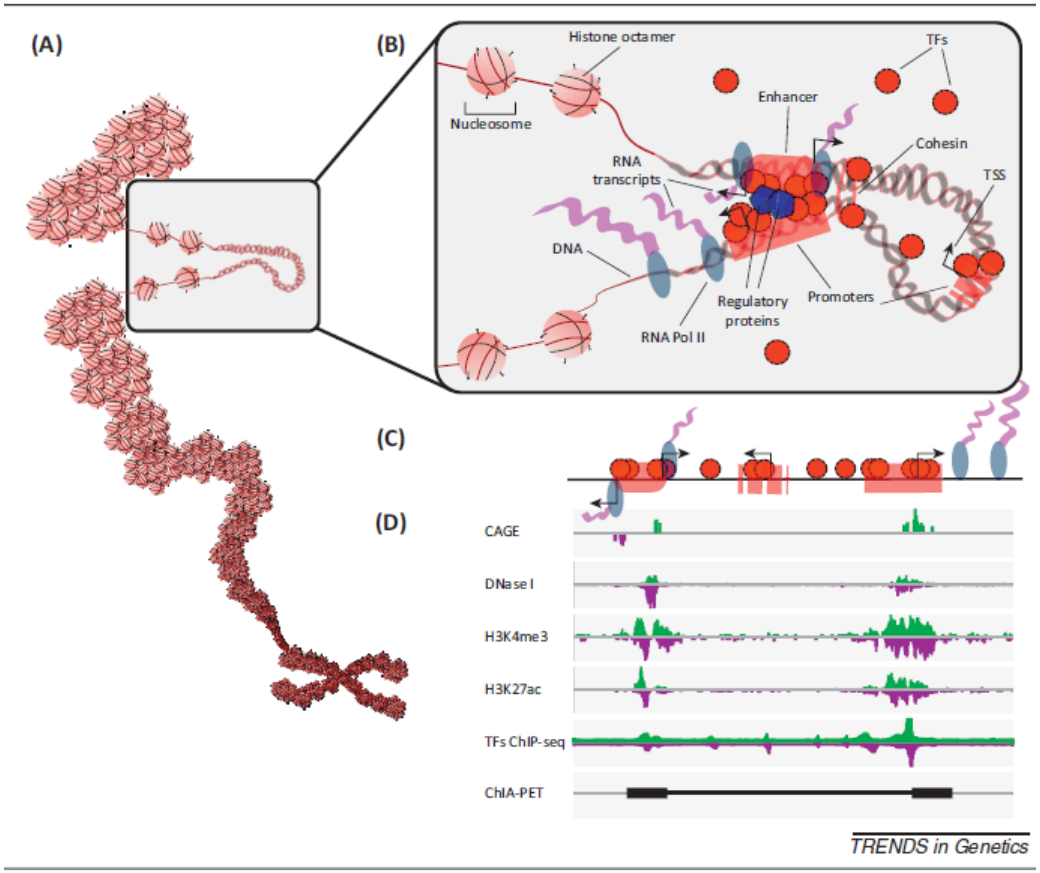


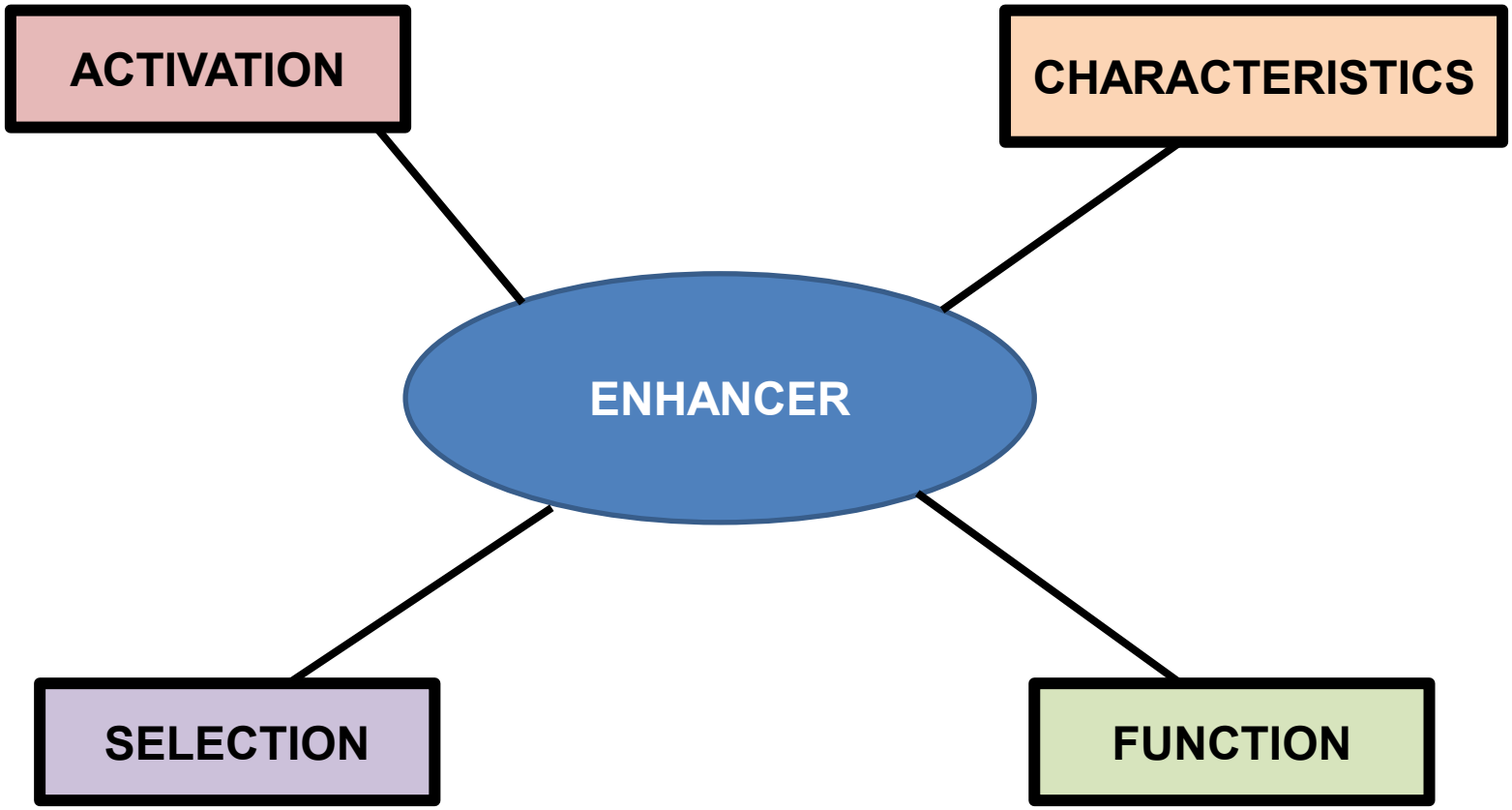
Figure B shows a region of the DNA which is transcribed and in particular it point out the importance of **both trans-activating and cis-activating regulatory elements**. In fact, we can see many **transcripts** and proteins (**Regulatory Factors** and **Transcription factors**), interacting with the DNA and also the formation of a loop with enable two distant regions to be found closer.



The selection and function of cell type-specific enhancers

Sven Heinz[1], Casey E. Romanoski[2], Christopher Benner[1] and Christopher K. Glass[2,3]

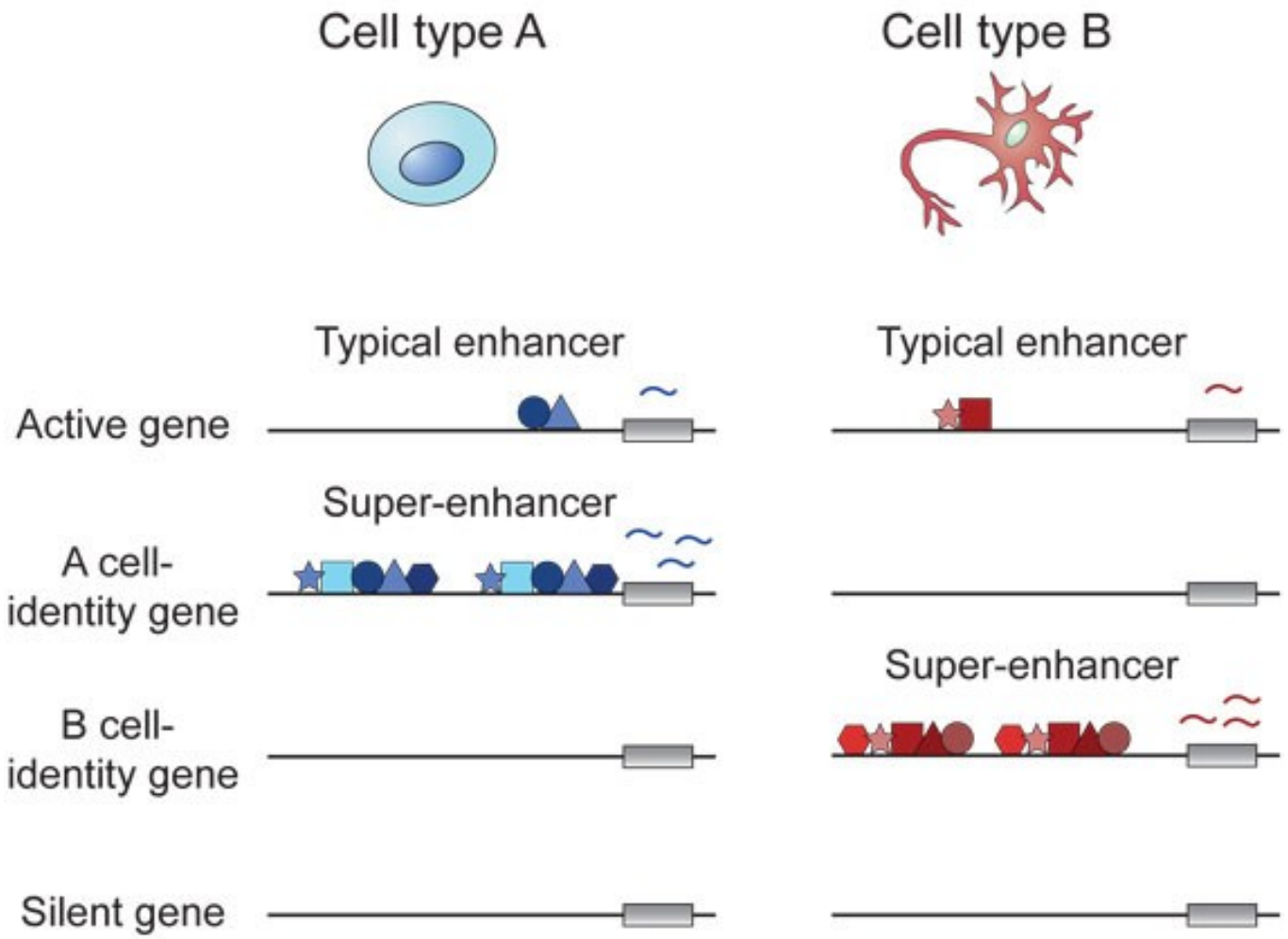
Abstract | The human body contains several hundred cell types, all of which share the same genome. In metazoans, much of the regulatory code that drives cell type-specific gene expression is located in distal elements called enhancers. Although mammalian genomes contain millions of potential enhancers, only a small subset of them is active in a given cell type. Cell type-specific enhancer selection involves the binding of lineage-determining transcription factors that prime enhancers. Signal-dependent transcription factors bind to primed enhancers, which enables these broadly expressed factors to regulate gene expression in a cell type-specific manner. The expression of genes that specify cell type identity and function is associated with densely spaced clusters of active enhancers known as super-enhancers. The functions of enhancers and super-enhancers are influenced by, and affect, higher-order genomic organization.


ACTIVATION
CHARACTERISTICS
ENHANCER
SELECTION
FUNCTION

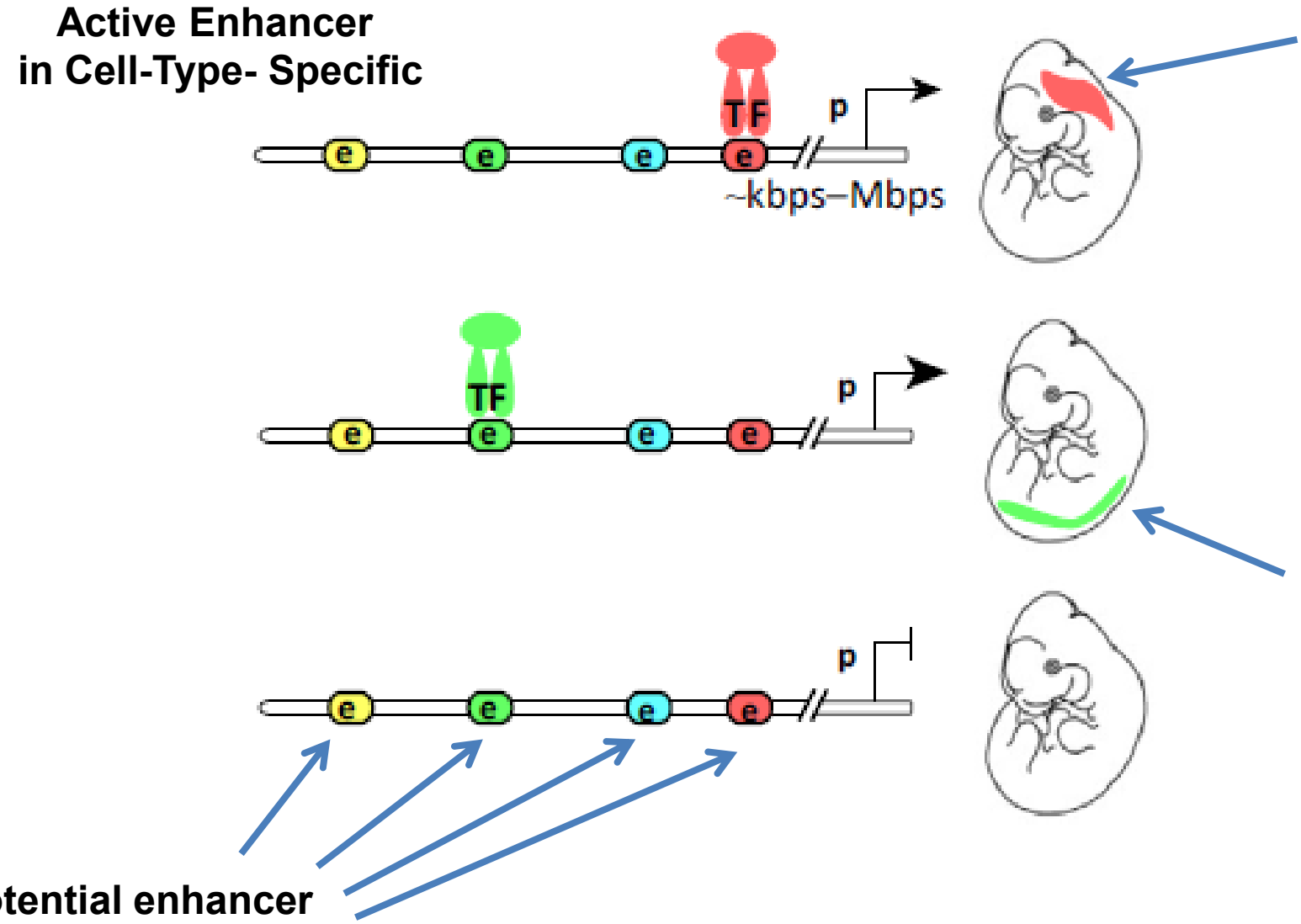
Enhancer Characteristitcs

- Enhancers are cis-regulatory elements in proximity of genes
- Each cell has a set of enhancers
- Enhancers have motifs for sequence-specific transcription factors
- Enhancers are marked with epigenetic modifications
- Enhancers are in different states of activation

MUCH OF THE REGULATORY CODE THAT DRIVES CELL-TYPE-SPECIFIC GENE EXPRESSION IS LOCATED IN DISTAL ELEMENTS CALLED ENHANCERS



CELL TYPE USE A SMALL SUBSET OF MILLIONS OF POTENTIAL ENHANCERS

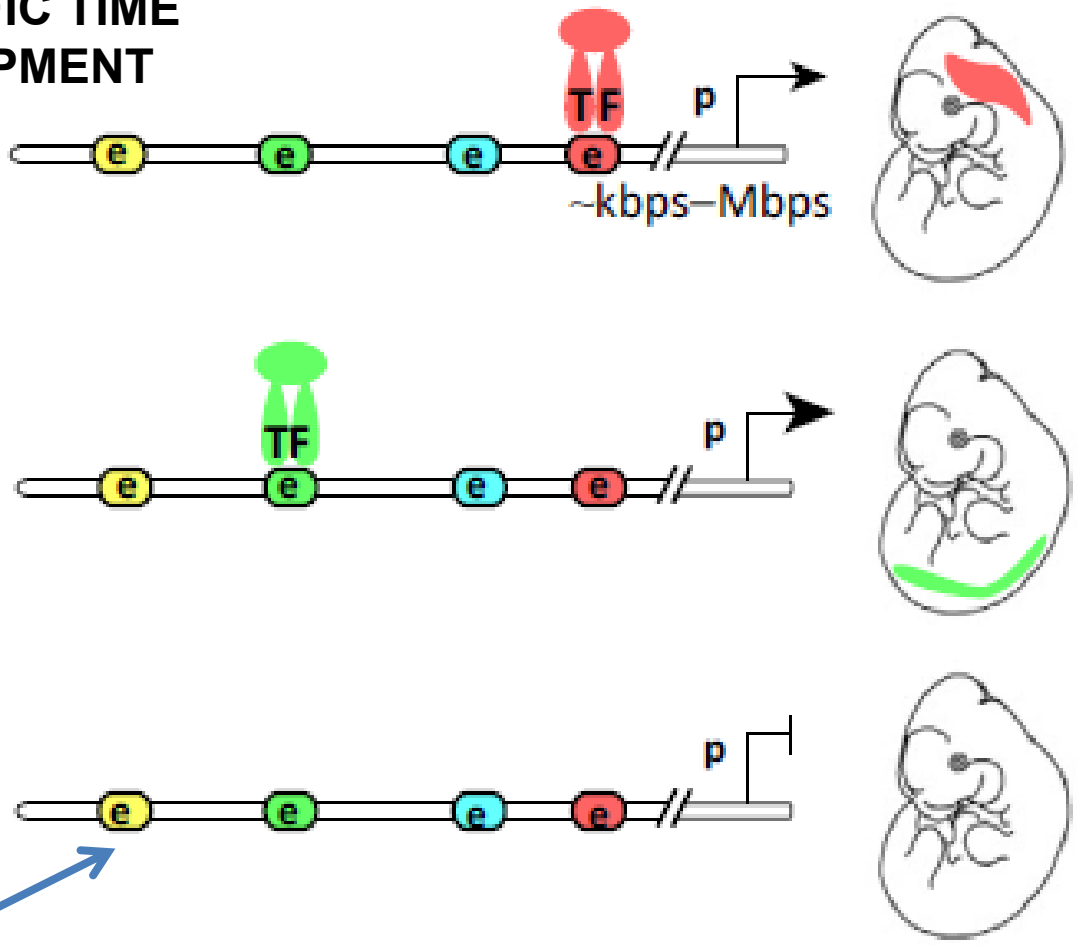


Enhancers in tissue/cell-specific gene expression

Trends in Biochemical Sciences April 2014, Vol. 39, No. 4

CELL TYPE USE A SMALL SUBSET OF MILLIONS OF POTENTIAL ENHANCERS

Active Enhancer during SPECIFIC TIME OF DEVELOPMENT



Enhancers in tissue/cell-specific gene expression

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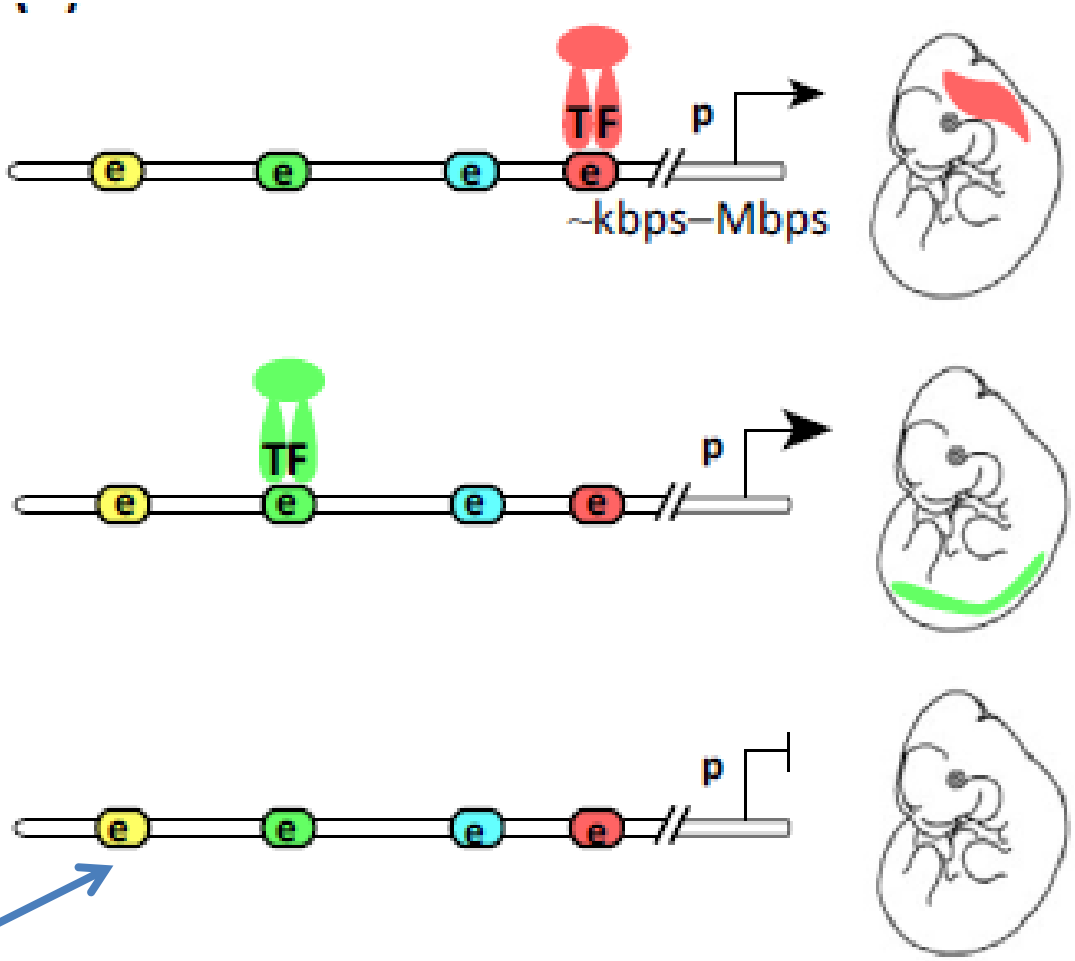
LINEAGE-DETERMINING TRANSCRIPTION FACTORS BIND AT CELL-TYPE SPECIFIC ENHANCERS



TF RED

TF GREEN

Potential enhancers

Enhancers in tissue/cell-specific gene expression

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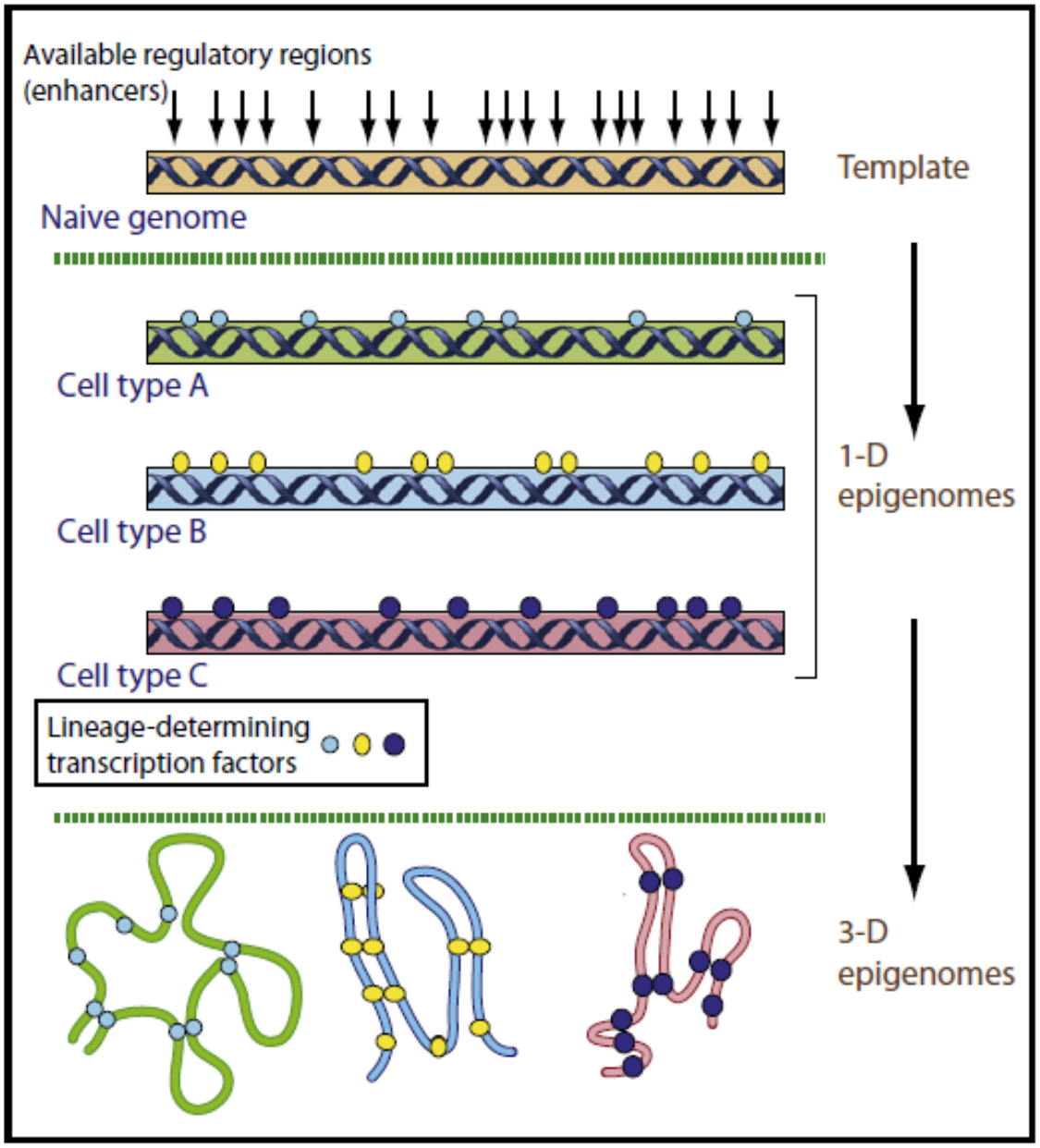
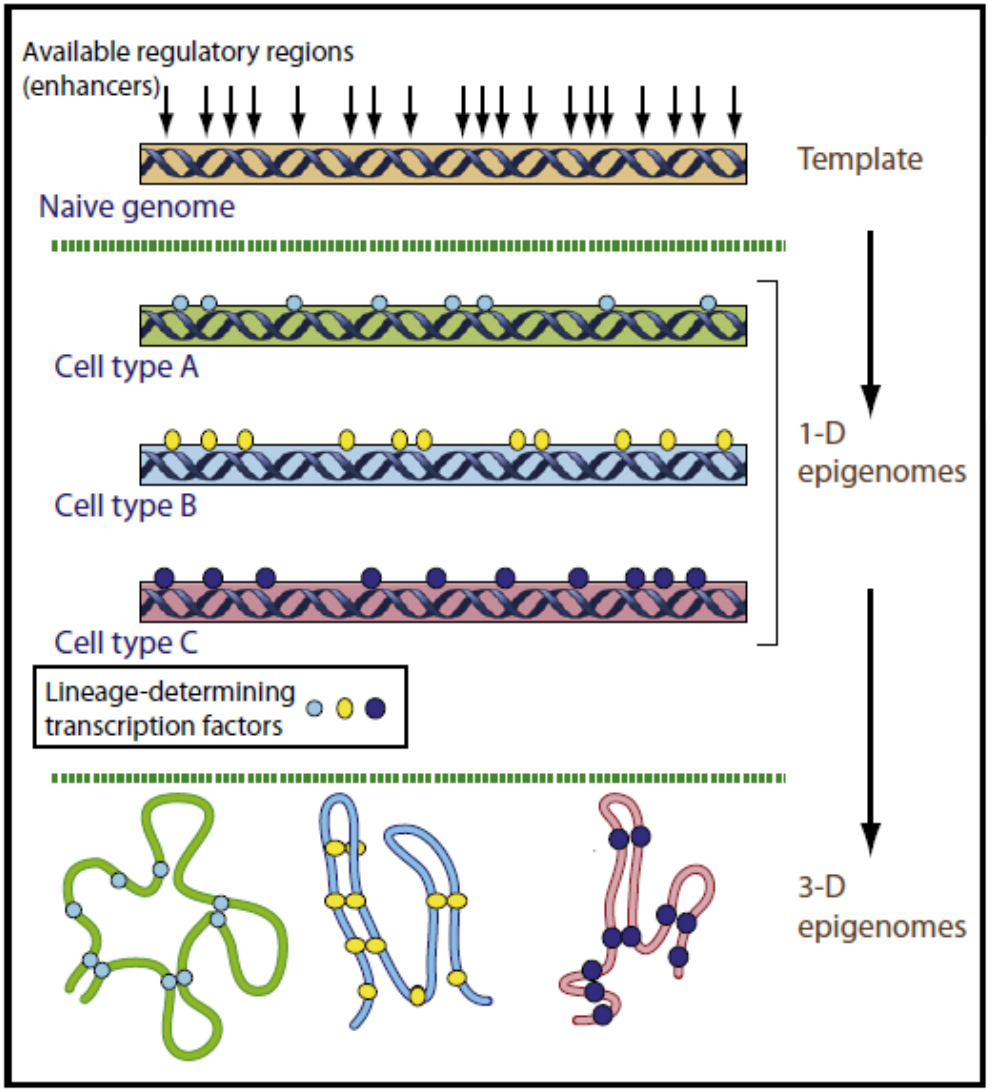
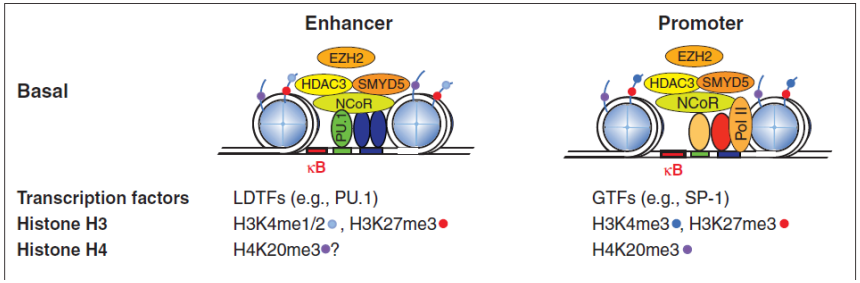
EACH CELL HAS ACTIVE ENHANCERS

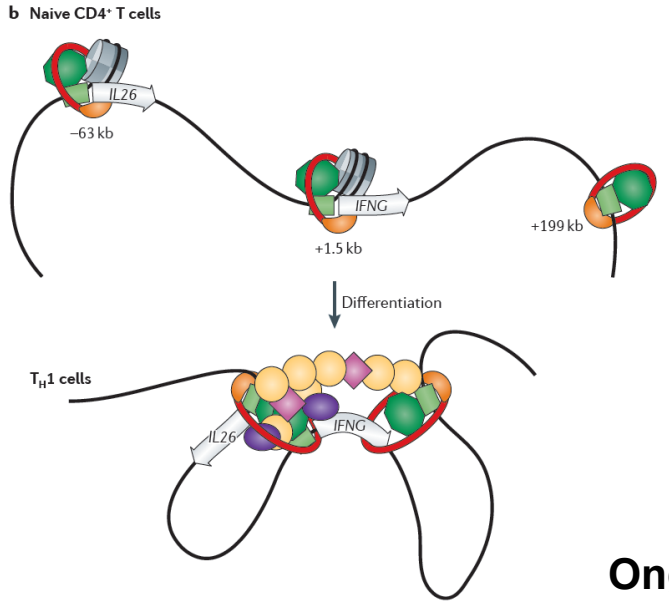


Maintaining Cell Identity through Global Control of Genomic Organization

Gioacchino Natoli[1,*]





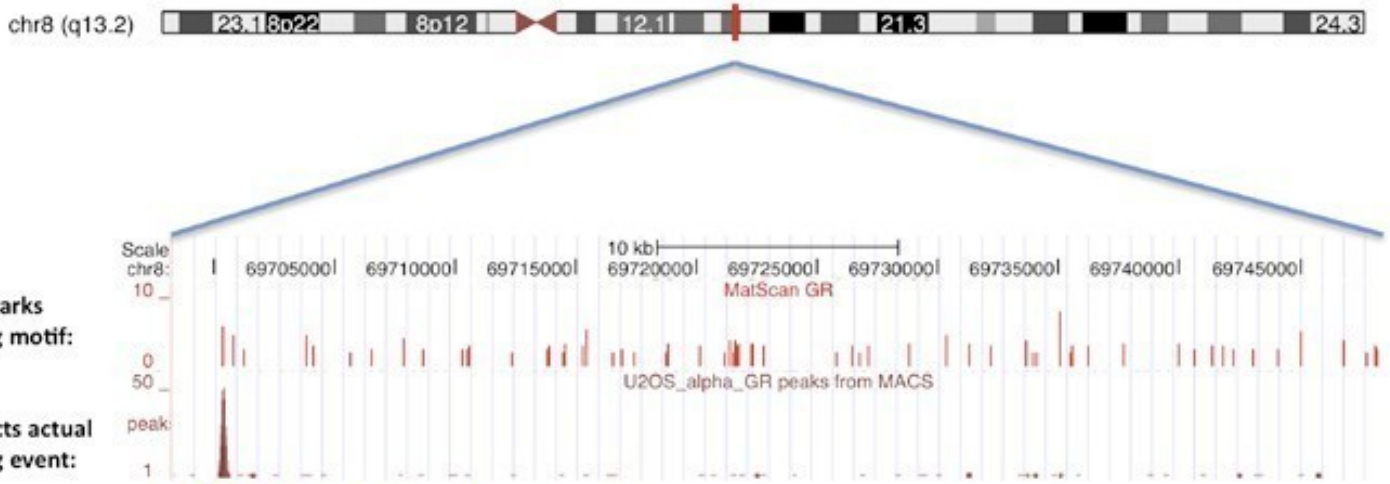


Ong 2011

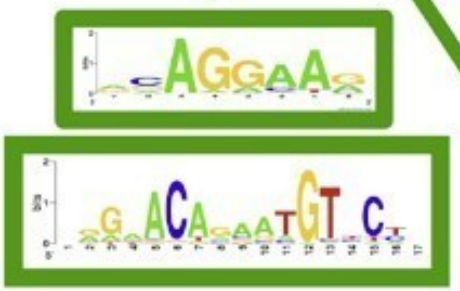
Immunity 33, July 23, 2010

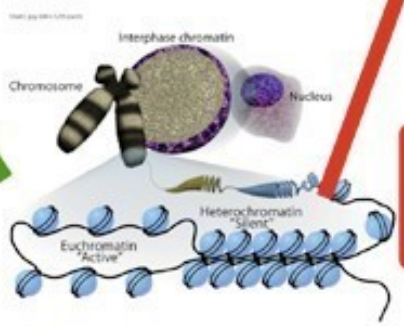
TRANSCRIPTION FACTOR BINDS SPECIFIC CONSENSUS SEQUENCE IN ACTIVE ENHANCER

GR binding site motif found in approx. every 1000bp in genome



Genetic and epigenetic inputs directing binding





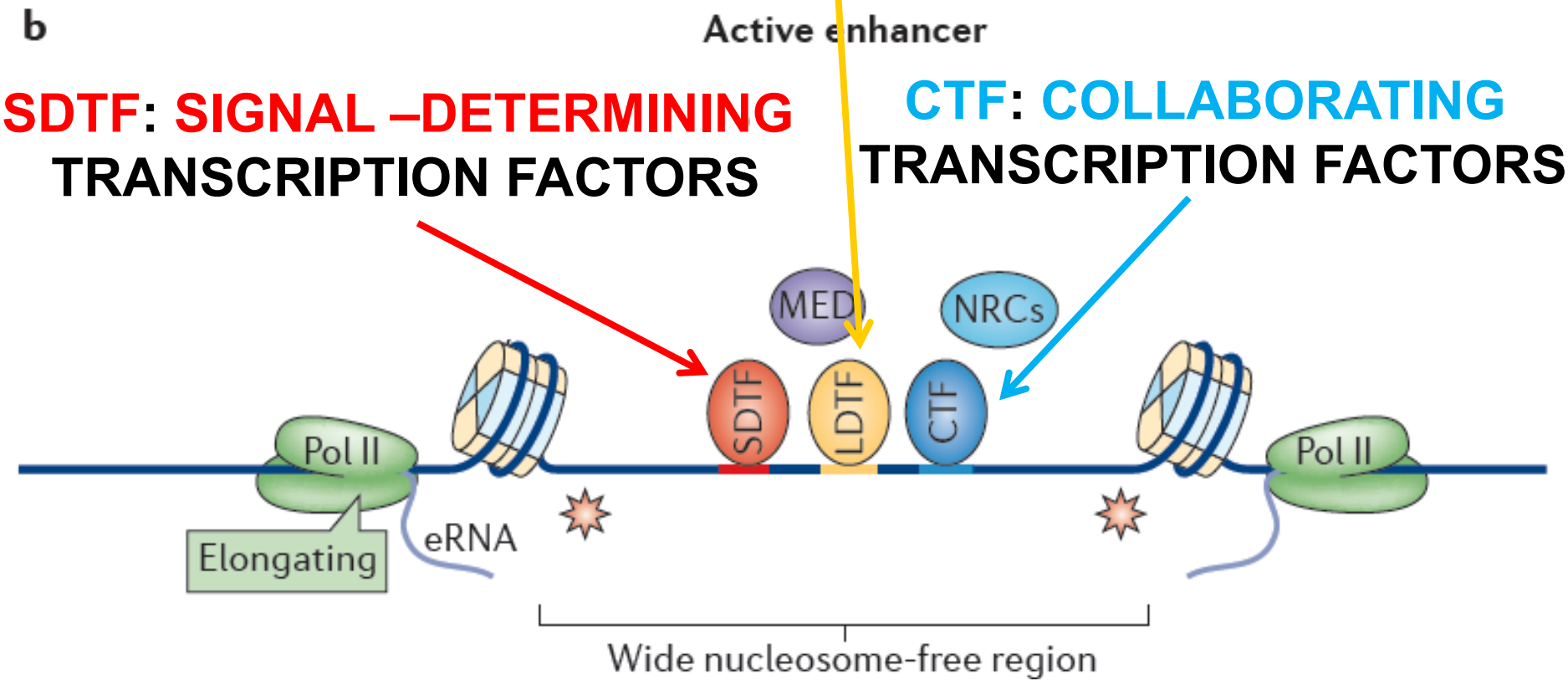
Genetic and epigenetic inputs that restrict binding

TRANSCRIPTION FACTORS THAT BIND ENHANCERS

LDTF: LINEAGE –DETERMINING TRANSCRIPTION FACTORS

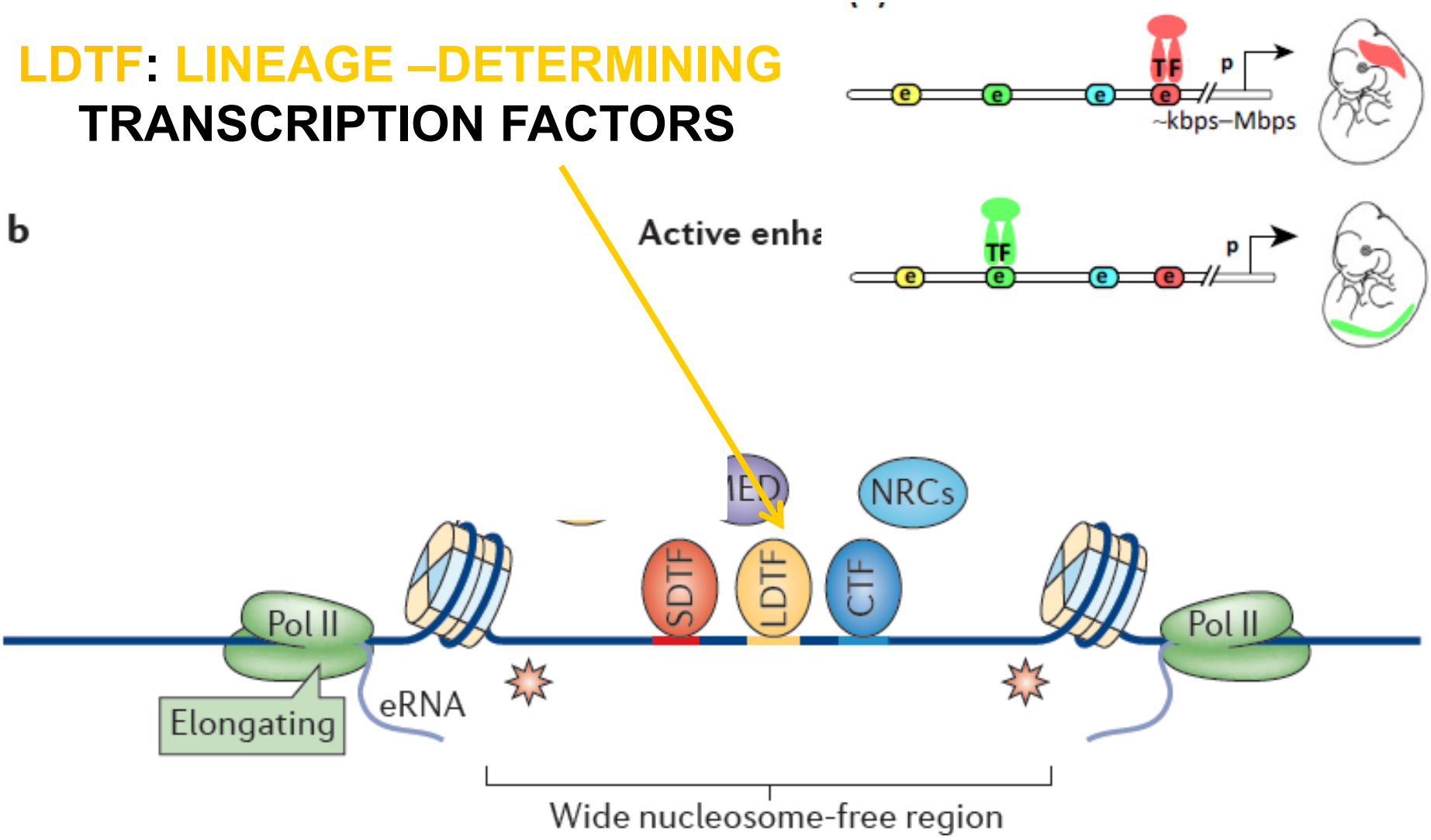
SDTF: SIGNAL –DETERMINING TRANSCRIPTION FACTORS

CTF: COLLABORATING TRANSCRIPTION FACTORS



TRANSCRIPTION FACTORS THAT BIND ENHANCERS

LDTF: LINEAGE –DETERMINING TRANSCRIPTION FACTORS

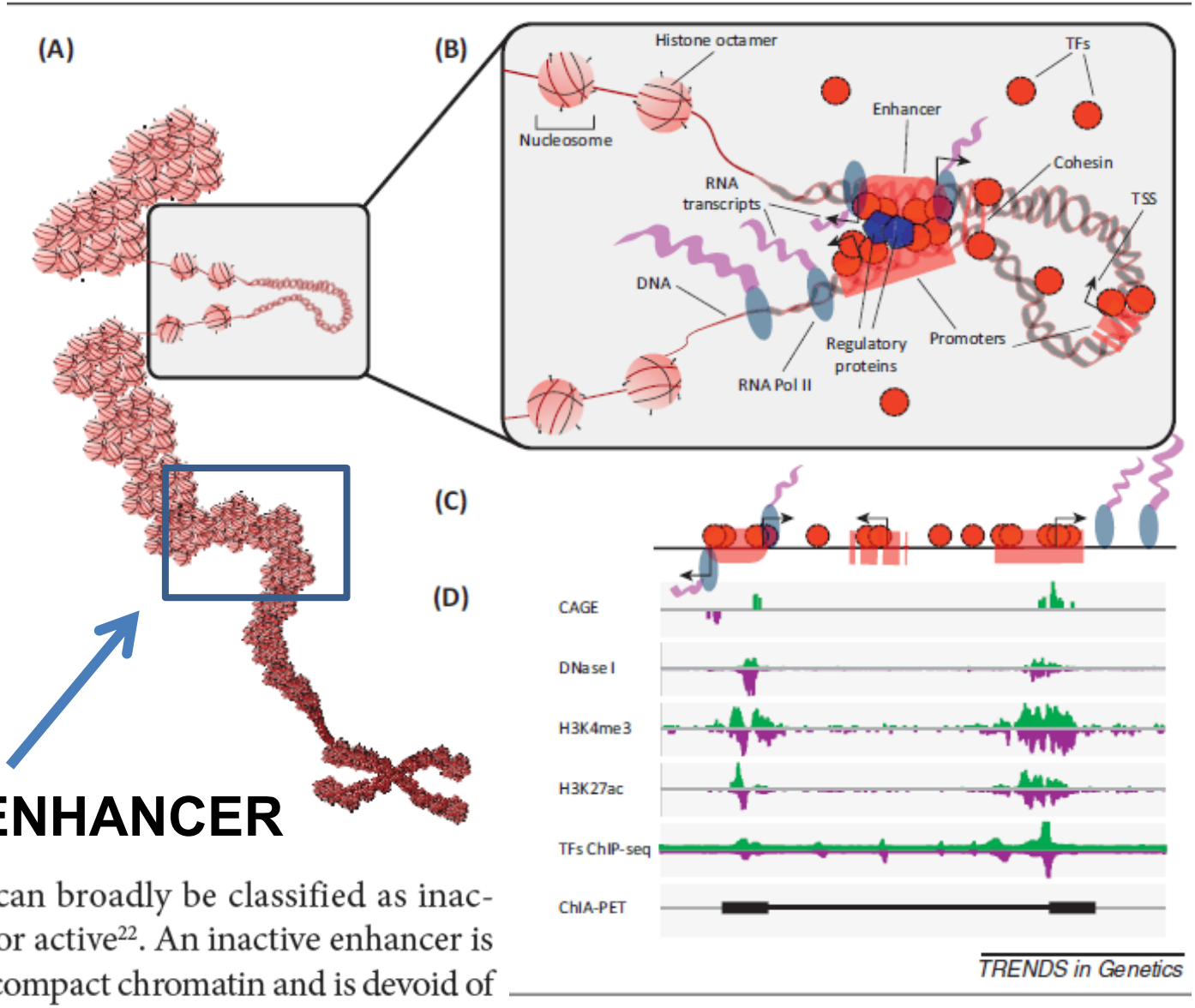


Enhancer Activation

- Transcription Factors bind specific genomic regions and allow access to other proteins remodelling chromatin

- Differentiation states and external stimuli induce enhancers activation

ACTIVE ENHANCER



INACTIVE ENHANCER

Enhancer states can broadly be classified as inactive, primed, poised or active[22]. An inactive enhancer is essentially buried in compact chromatin and is devoid of transcription factor binding and histone modifications.

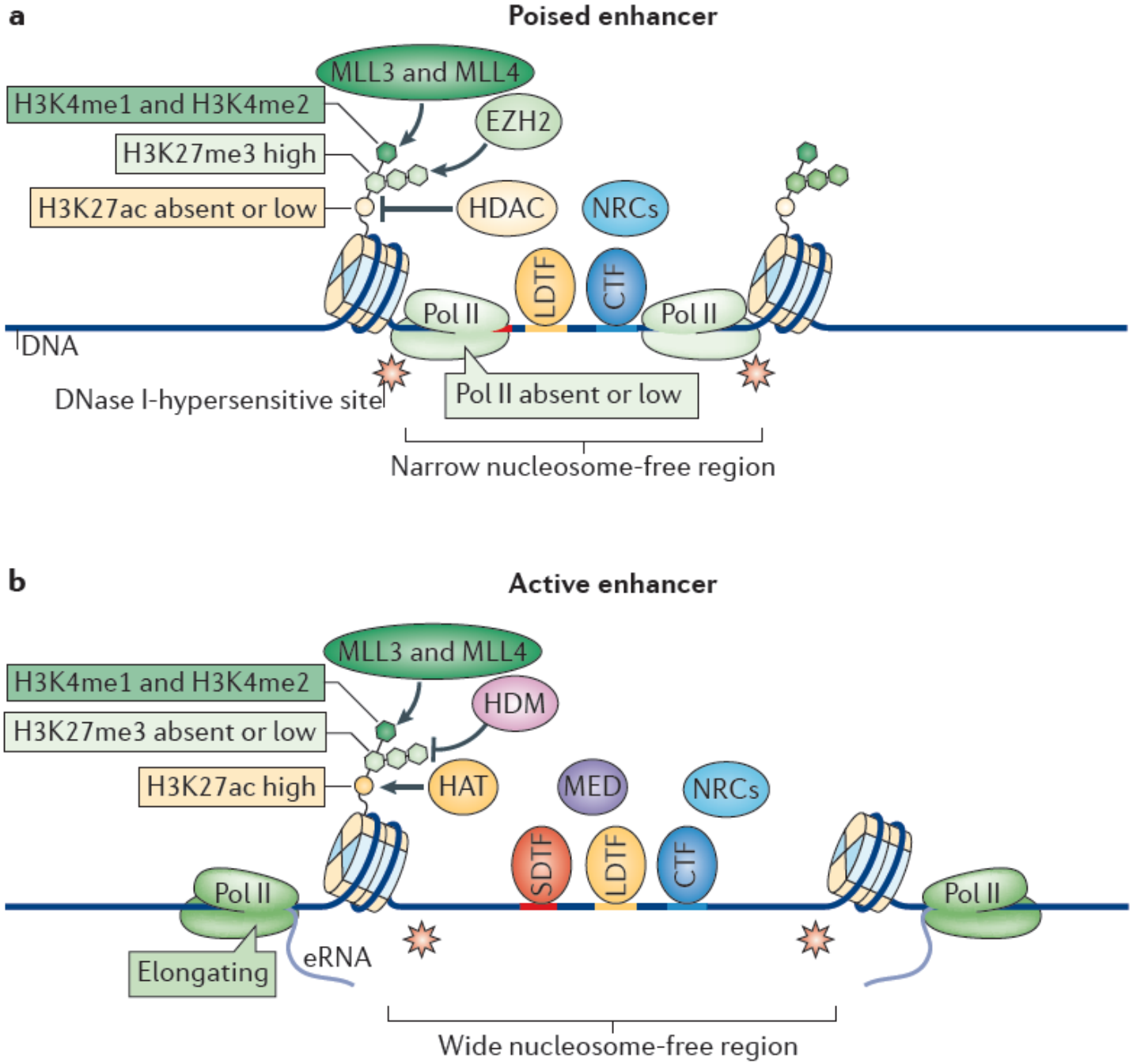
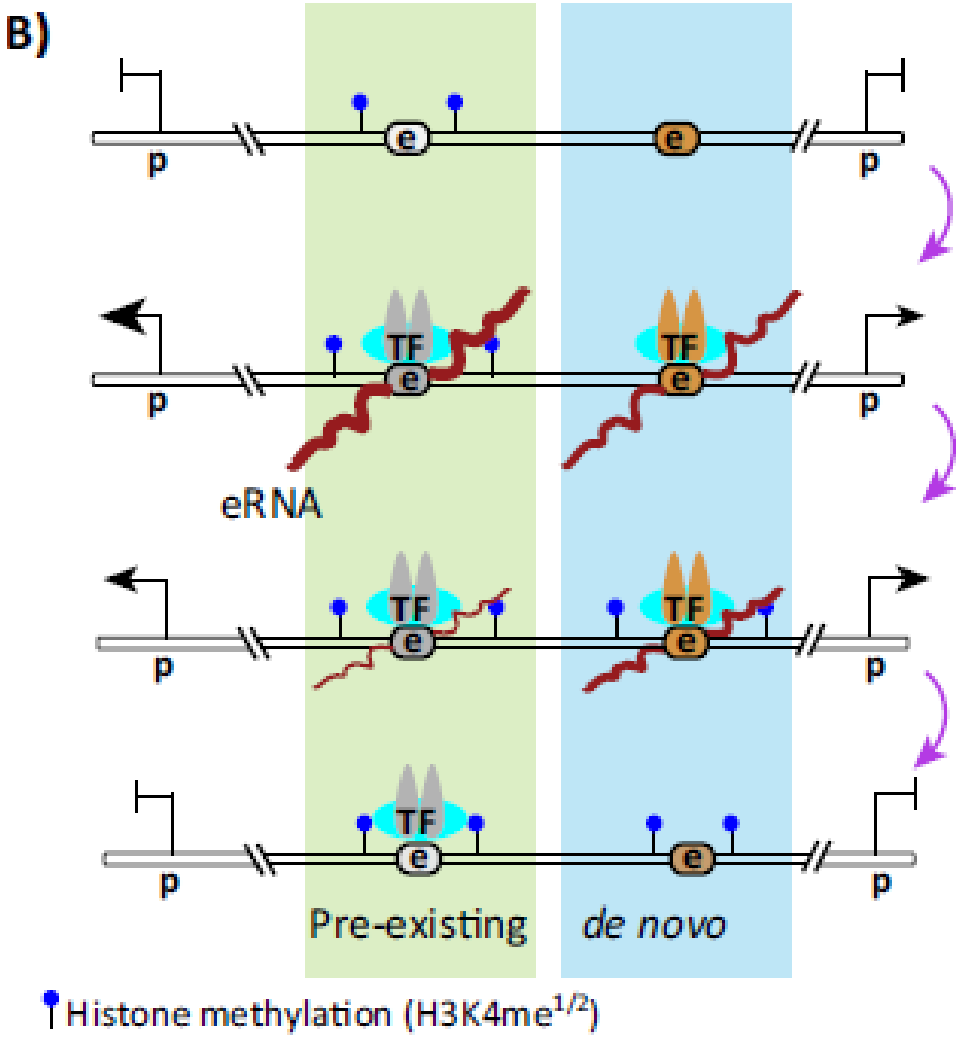


Figure 1 | **The anatomies of poised and active enhancers.** The characteristic features

LDTF: LINEAGE –DETERMINING TRANSCRIPTION FACTORS



Enhancers in stimulus-induced gene activation

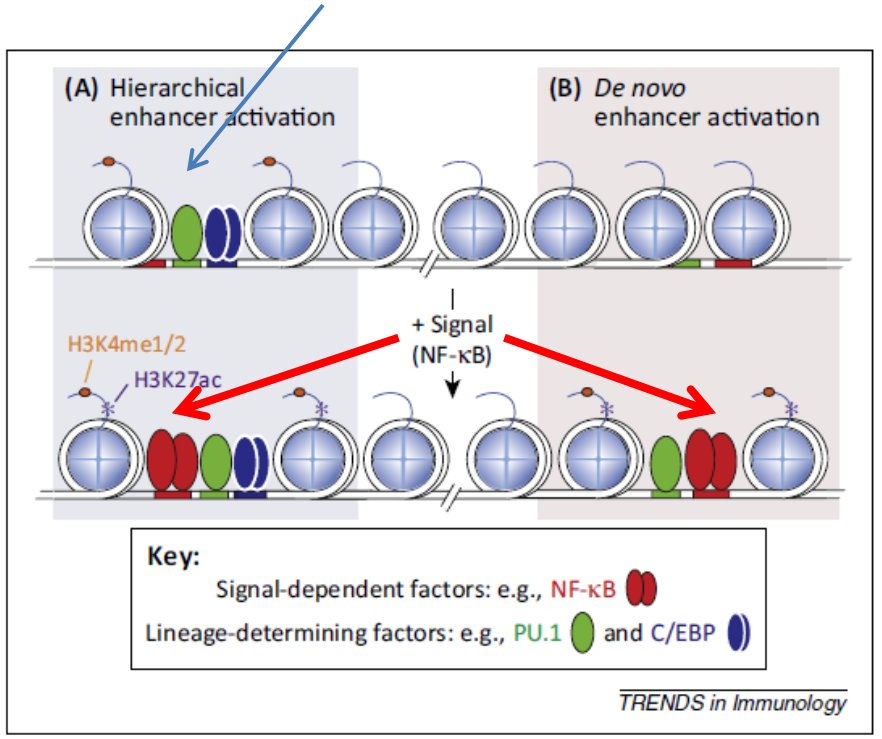
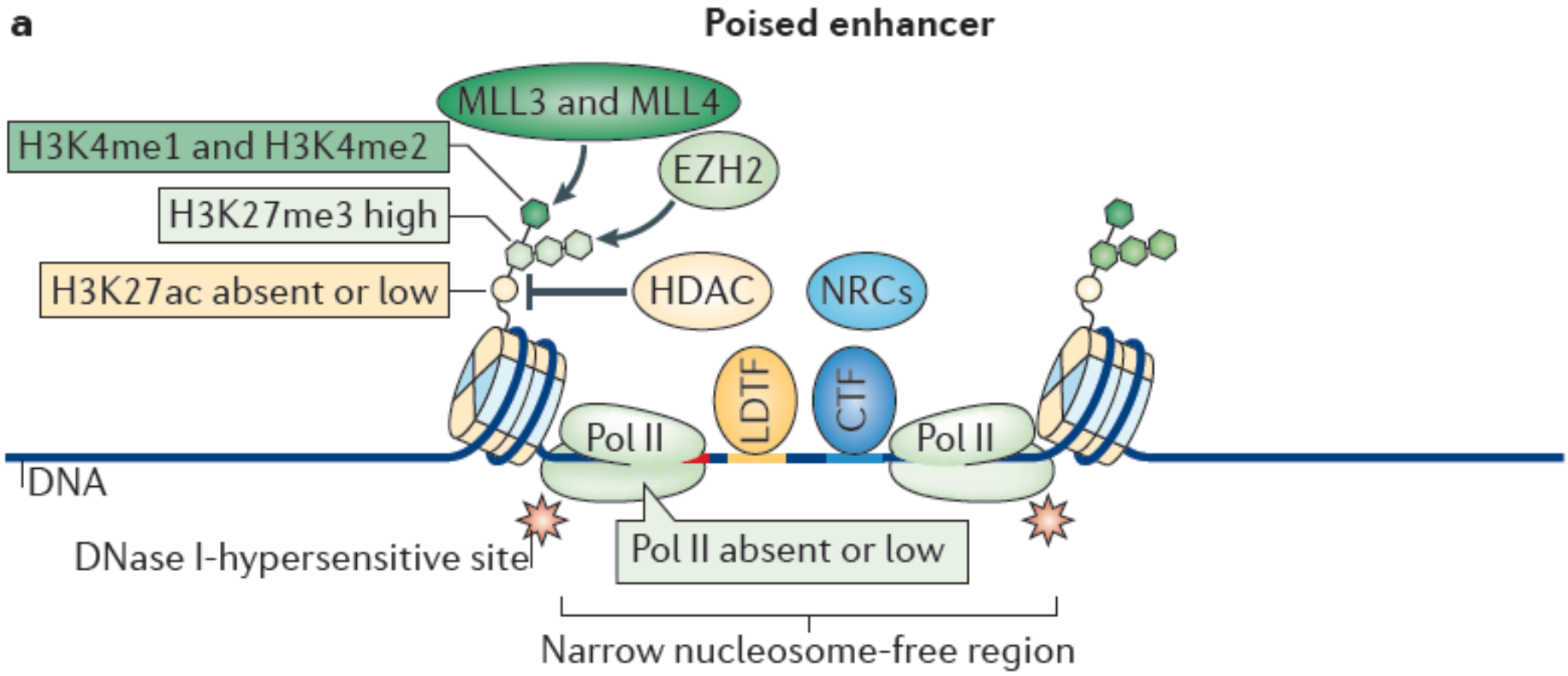


Figure 3. Chromatin transitions to active enhancers involve interactions between cell lineage-determining transcription factors and signal-dependent factors. **(A)** Enhancers primed by lineage-determining factors frequently require signal-dependent transcription factor binding to gain H3K27ac and become active. **(B)** Active enhancers can also be selected by interactions between signal-dependent factors and lineage-determining factors. Abbreviaions: C/EBP, CCAAT/enhancer binding protein; NF-κB, nuclear factor-κB; PU.1, transcription factor originally named spleen focus forming virus (SFFV) proviral integration oncogene.

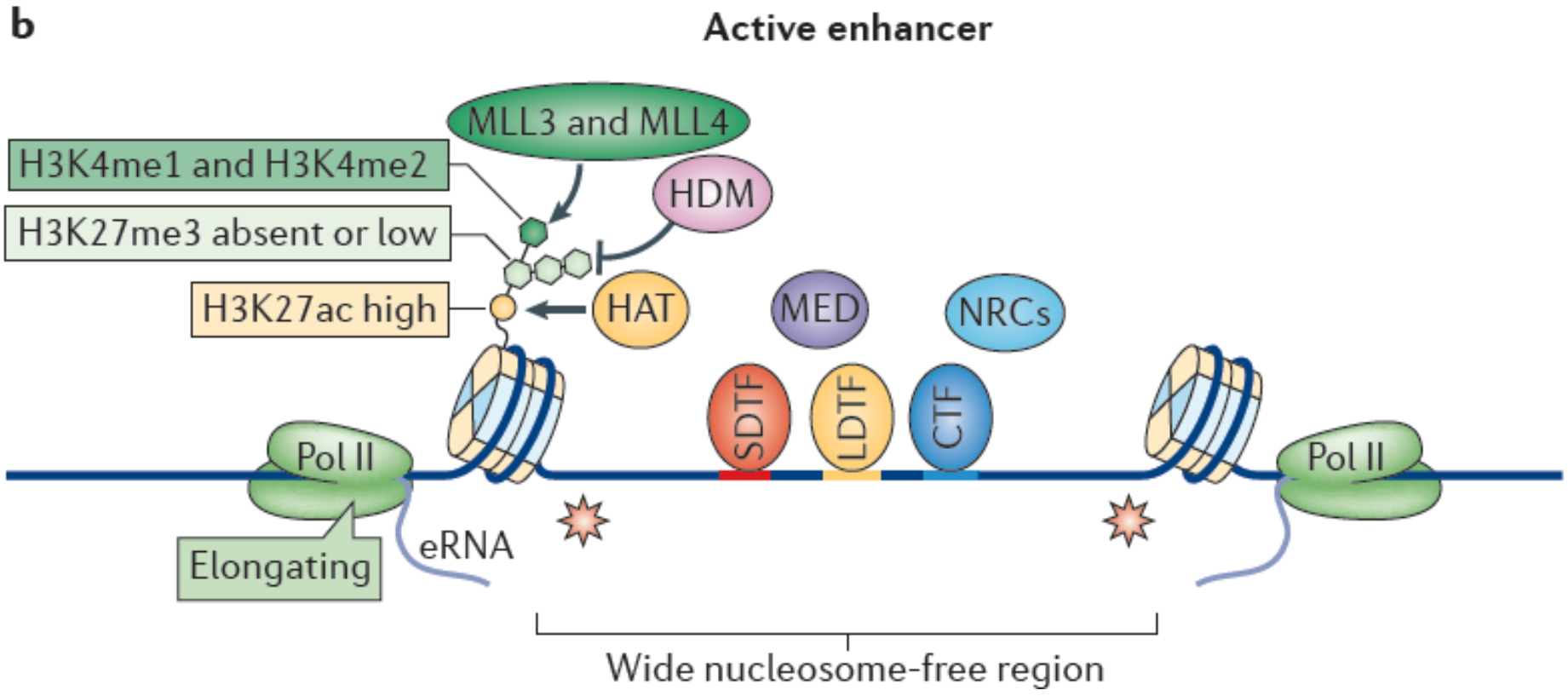
Trends in Immunology September 2015, Vol. 36, No. 9

H3K4me1, H3K4me2, lack histone acetylation and Pol II, high H3K27me3

mark POISED ENHANCERS

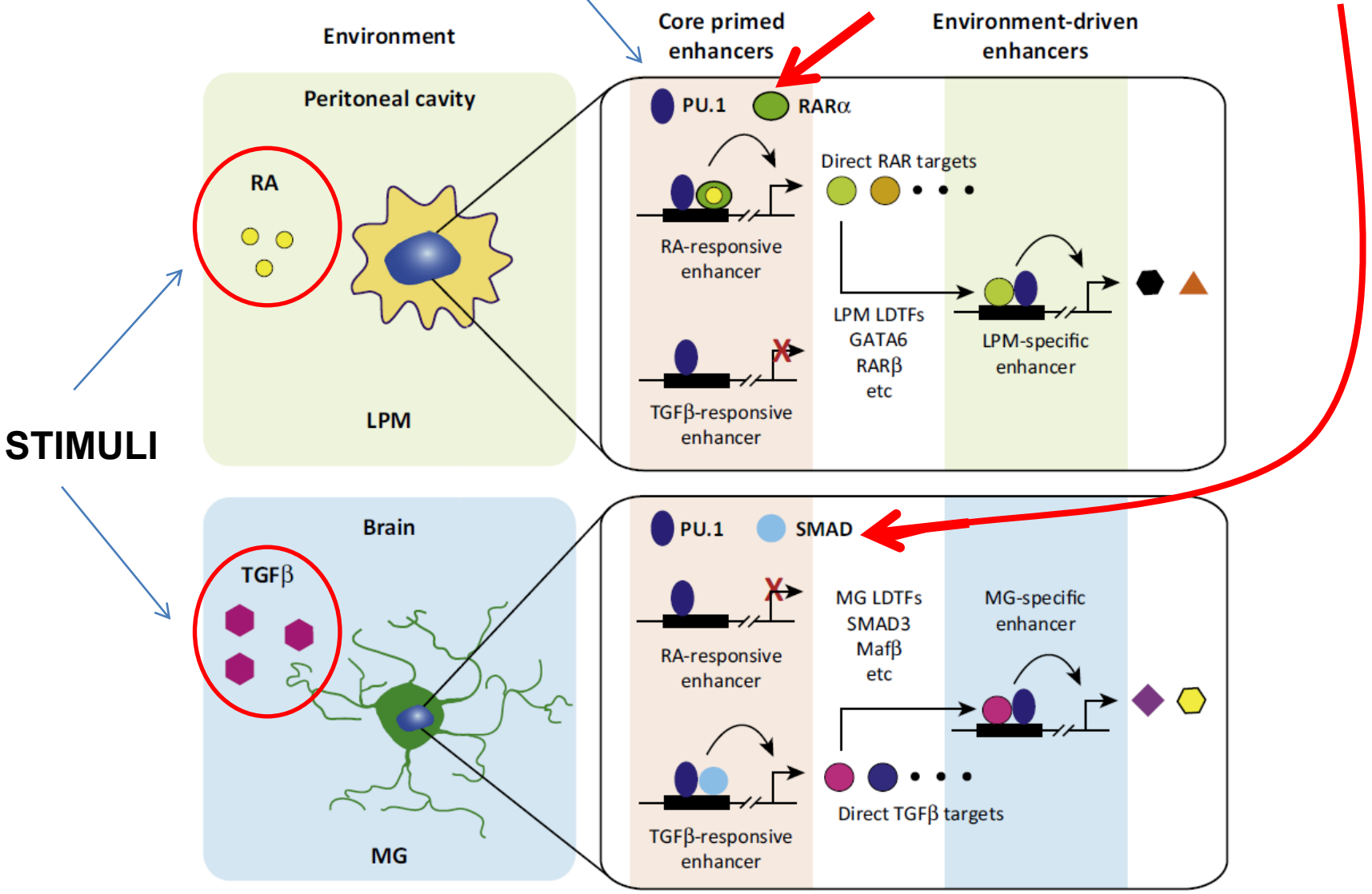


H3K4me1, H3K4me2, high H3K27Ac, lack H3K27me3, presence of Pol II and RNA transcript mark ACTIVE ENHANCERS



LDTF: LINEAGE –DETERMINING TRANSCRIPTION FACTORS

SDTF: SIGNAL –DETERMINING TRANSCRIPTION FACTORS



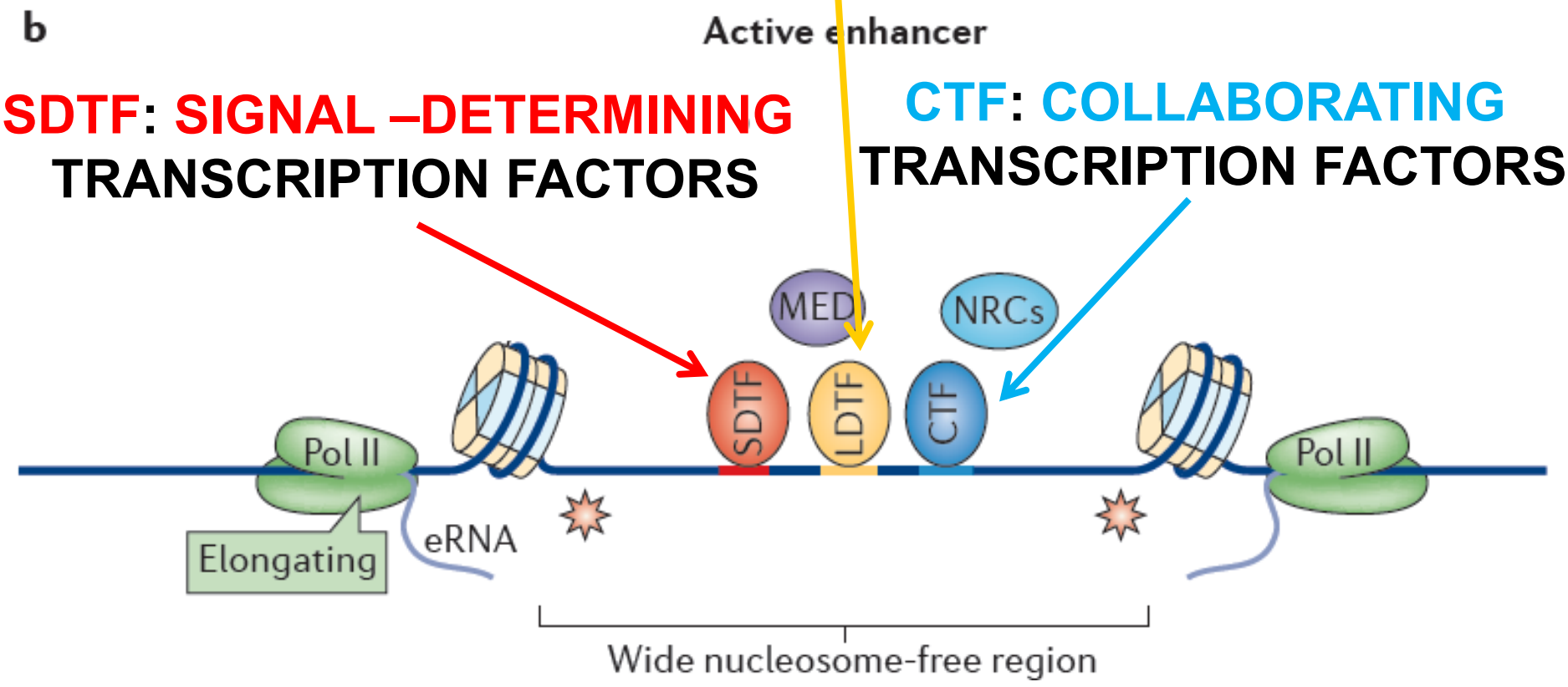
Romanoski et al., 2015

Trends in Immunology September 2015, Vol. 36, No. 9

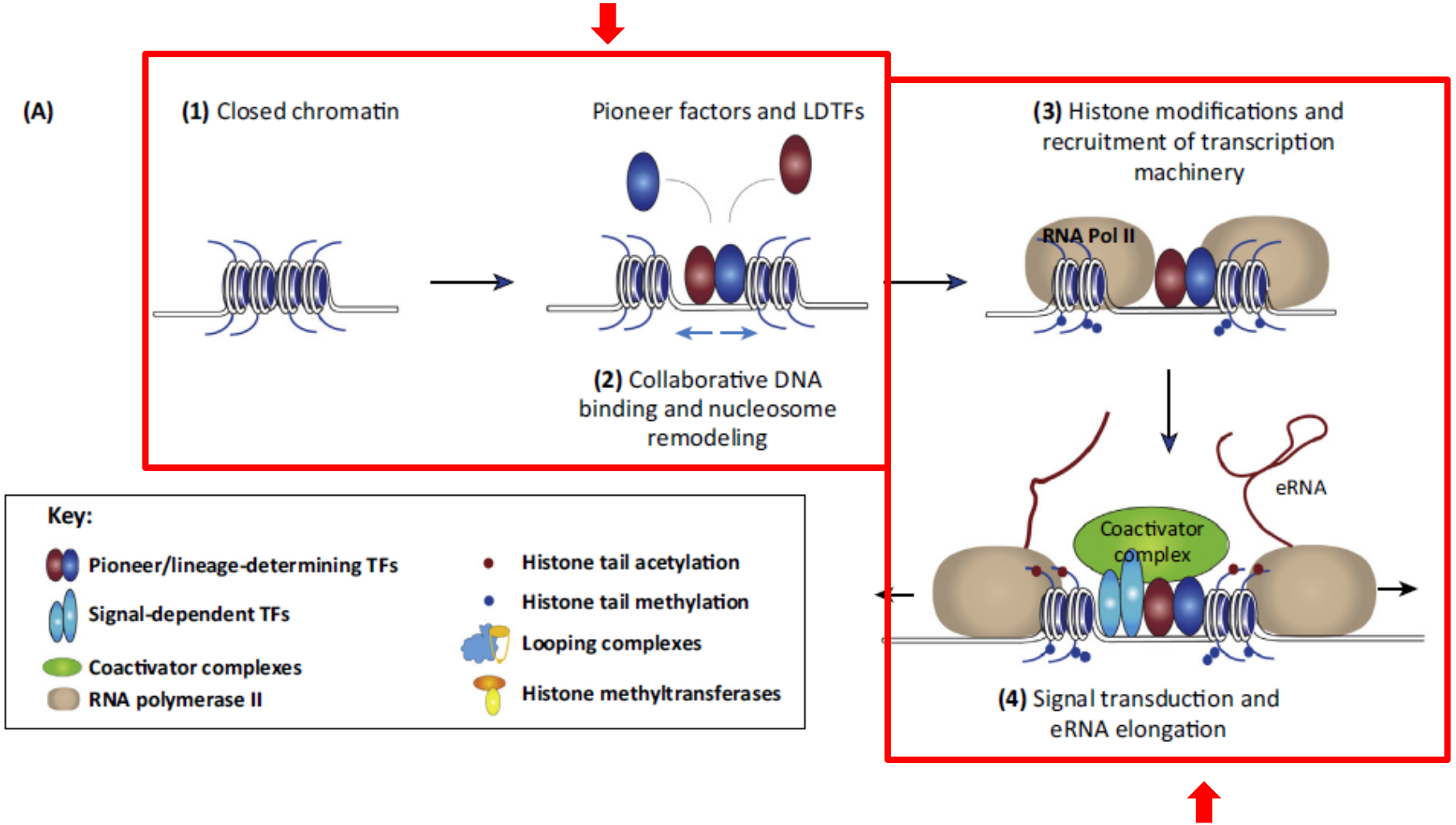
Enhancer Selection

- The role of lineage-determining transcription factors.
- The role of signal-dependent transcription factors.

LDTF: LINEAGE –DETERMINING TRANSCRIPTION FACTORS



Pioneer Factors and Lineage-determining Transcription Factors leads to nucleosome remodeling and increased chromatin accessibility



Pioneer Factors and Lineage-determining Transcription Factors leads to histone modifications and basal transcription machinery

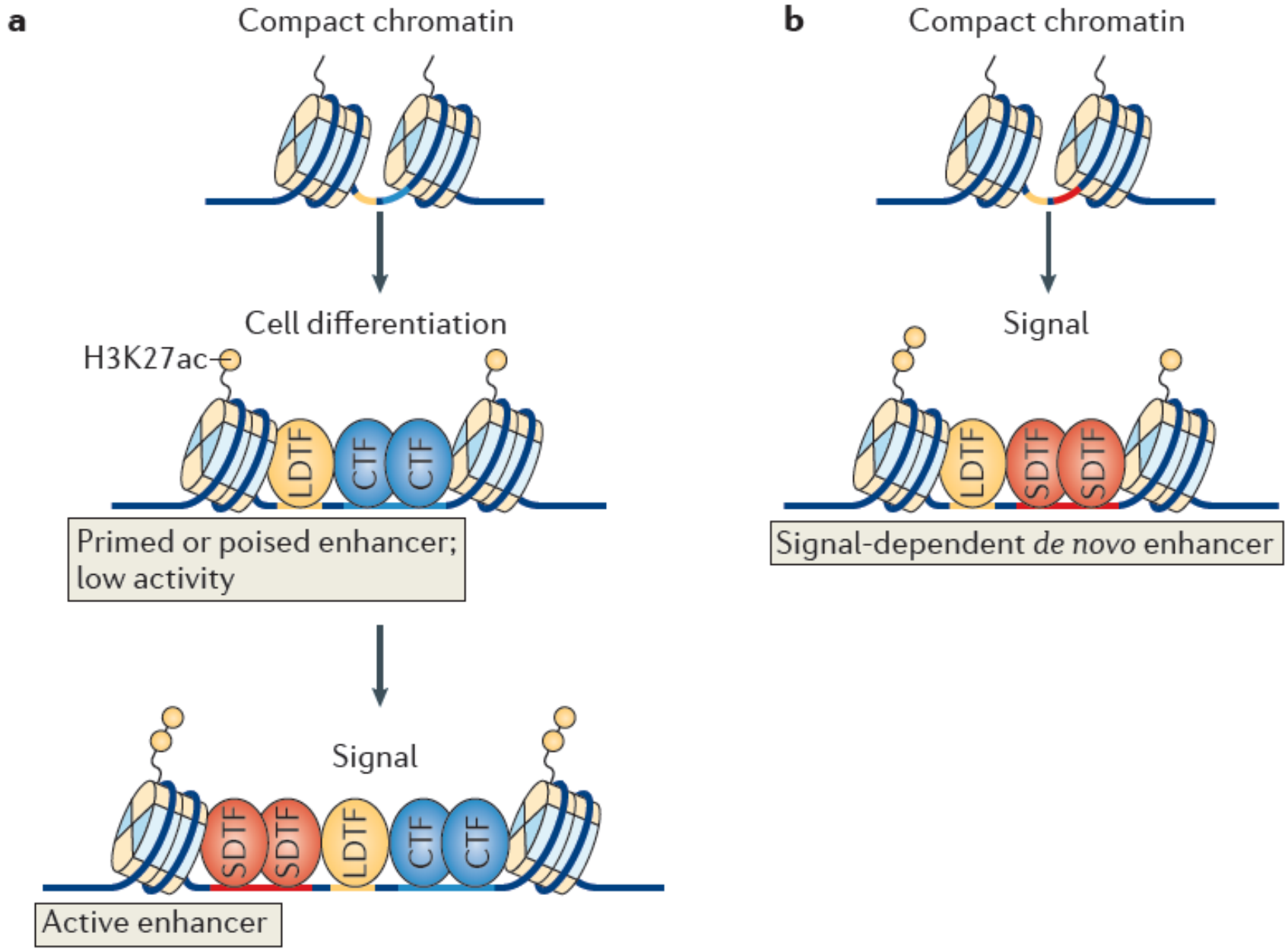
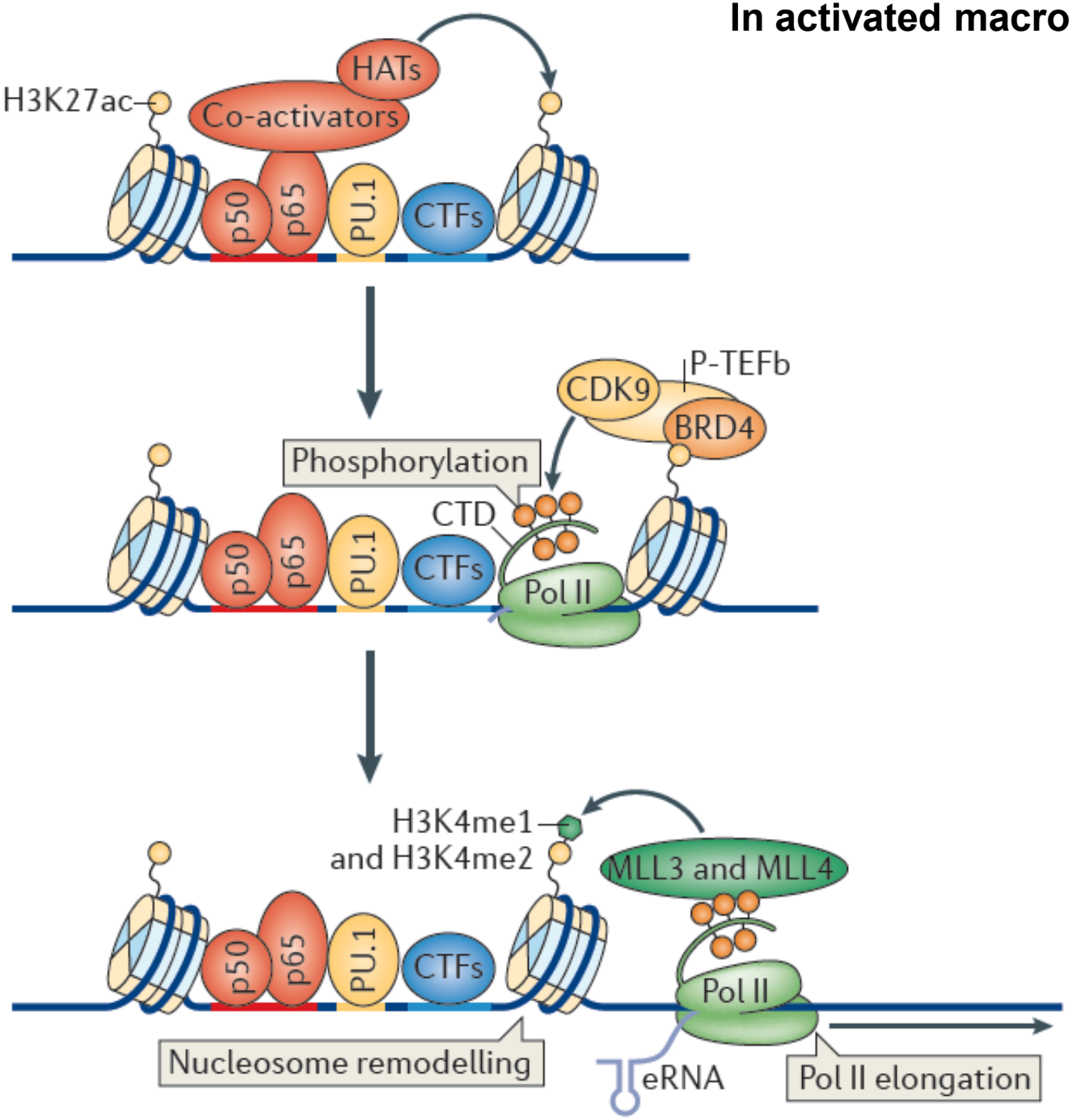


Figure 3 | **Cell type-specific enhancer selection and activation.** **a** | Collaborative

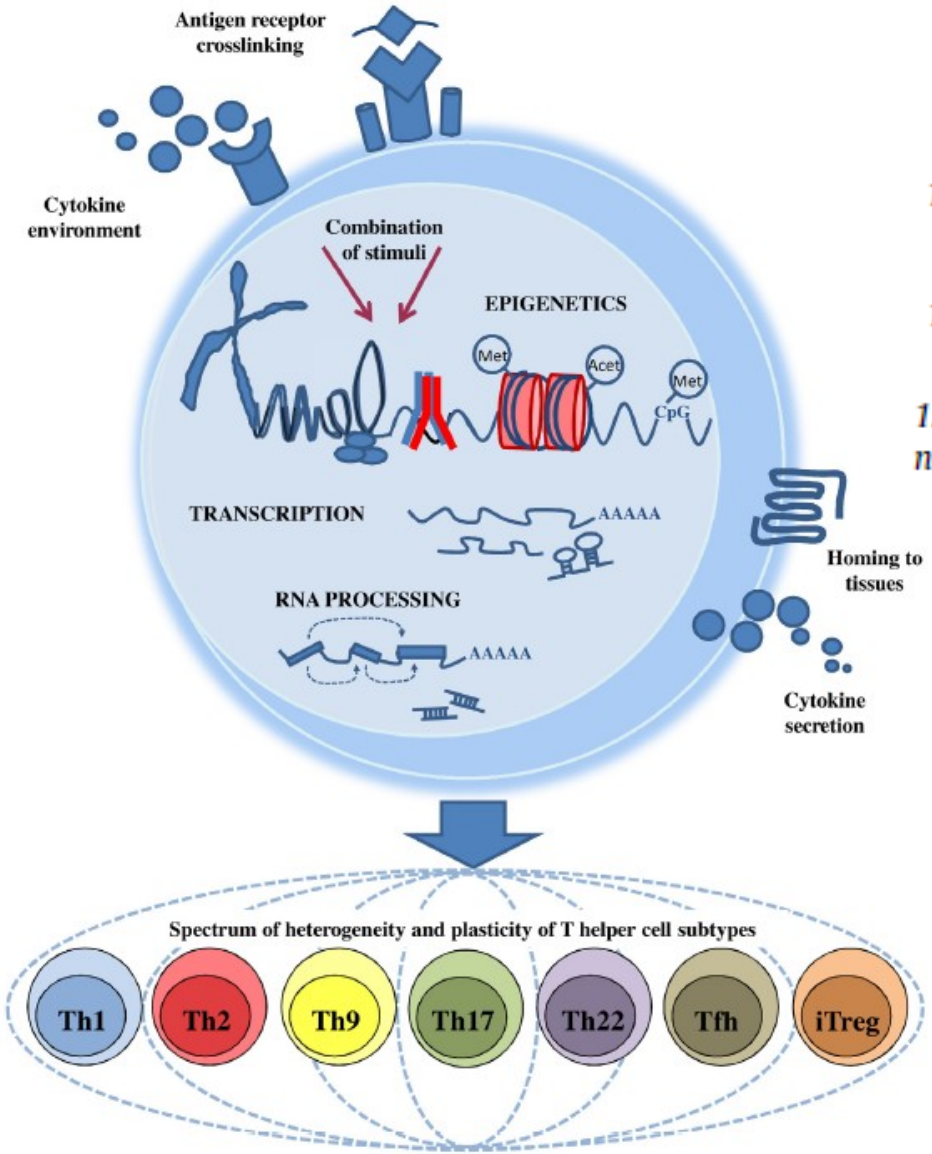
In activated macrophages

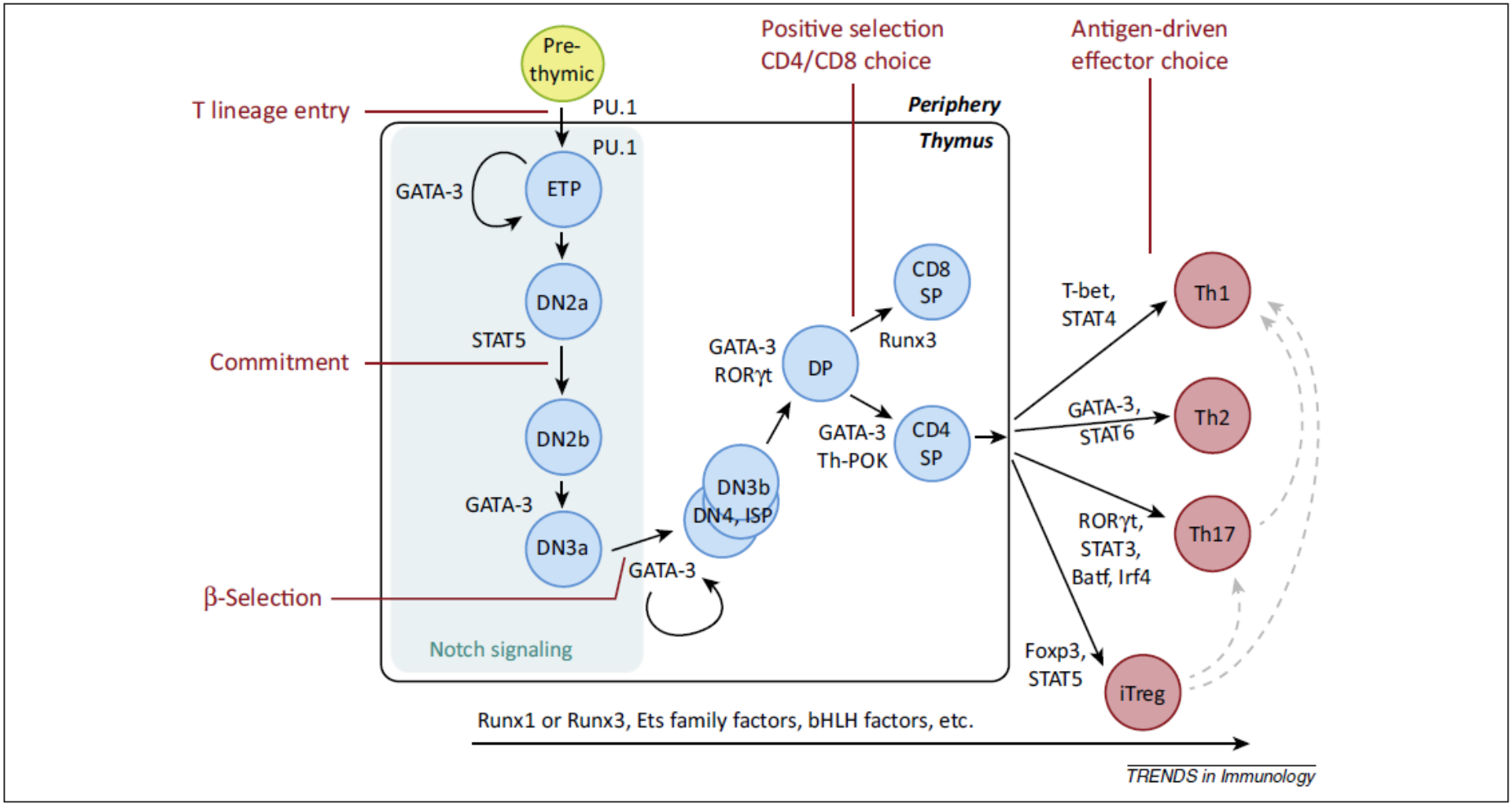


Review

Early T helper cell programming of gene expression in human

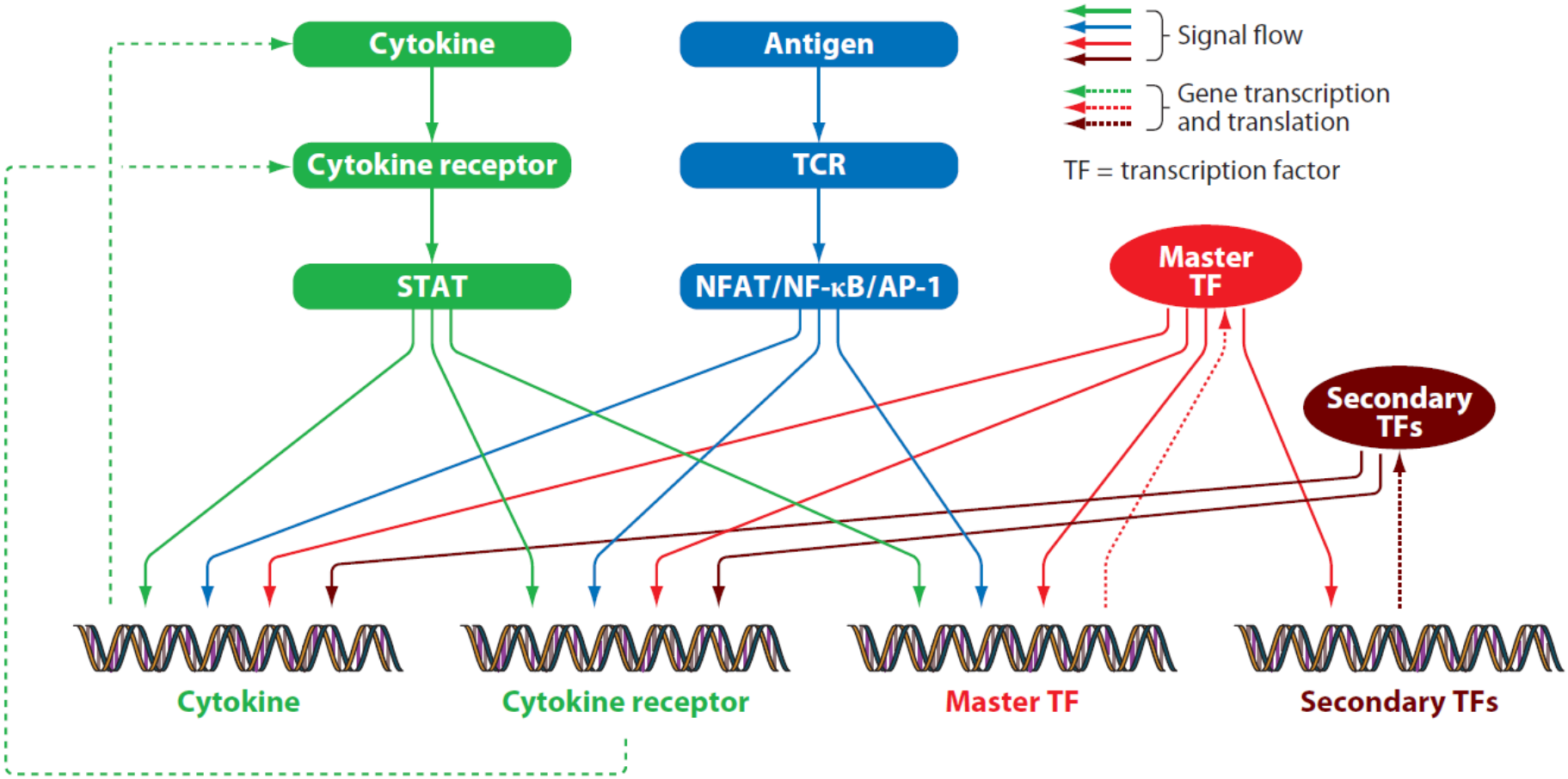
Soile Tuomela, Riitta Lahesmaa*




Pre-thymic
T lineage entry
PU.1
Positive selection
CD4/CD8 choice
Antigen-driven
effector choice
Periphery
Thymus
PU.1
GATA-3
ETP
DN2a
STAT5
Commitment
DN2b
GATA-3
DN3a
β-Selection
GATA-3
DN3b
DN4, ISP
GATA-3
RORγt
DP
CD8
SP
Runx3
GATA-3
Th-POK
CD4
SP
T-bet,
STAT4
Th1
GATA-3,
STAT6
Th2
RORγt,
STAT3,
Batf, Irf4
Th17
Foxp3,
STAT5
iTreg
Notch signaling
Runx1 or Runx3, Ets family factors, bHLH factors, etc.
TRENDS in Immunology

Differentiation of Effector CD4 T Cell Populations*

Jinfang Zhu, Hidehiro Yamane, and William E. Paul



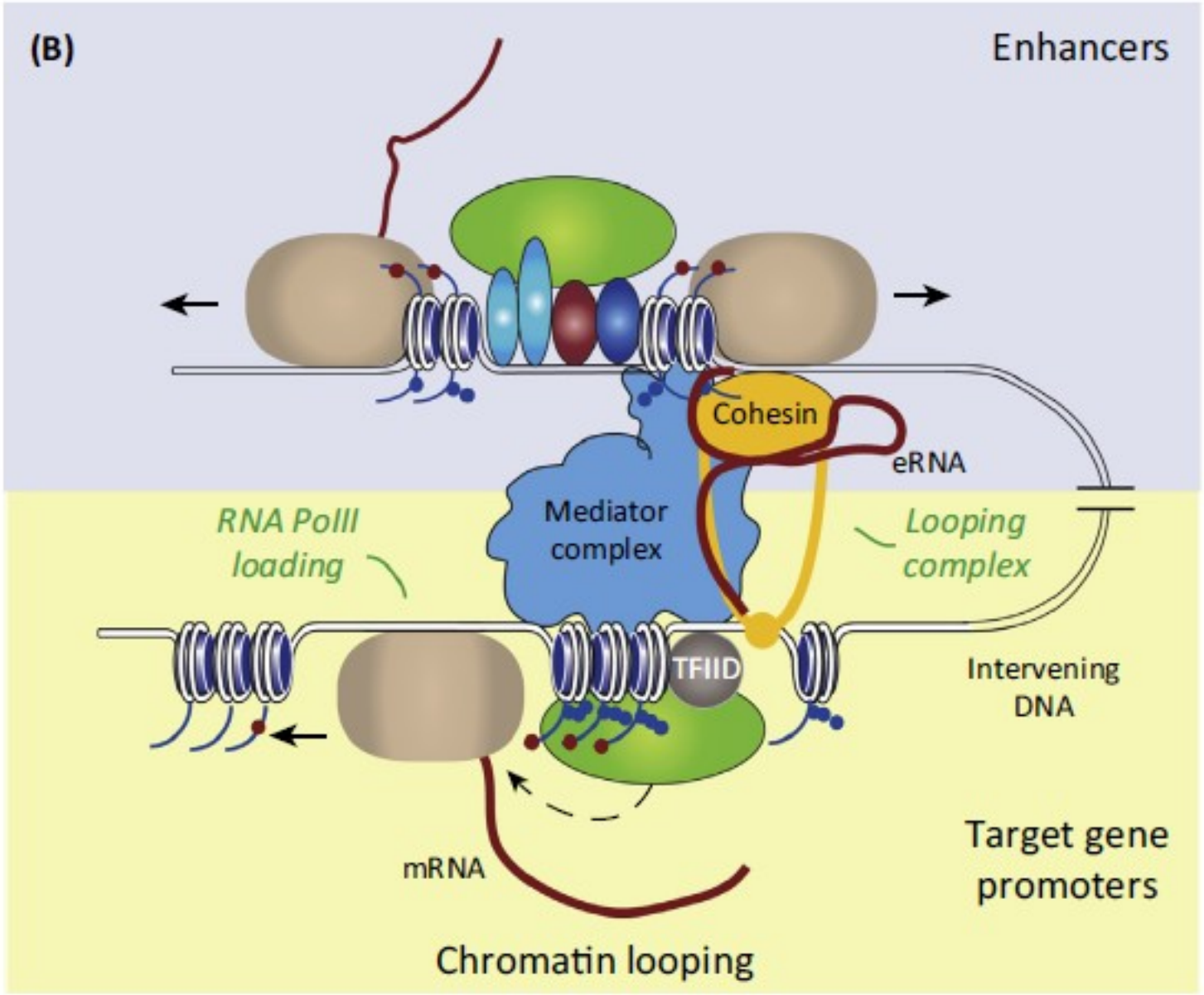
Annu. Rev. Immunol. 2010. 28:445–89

Enhancer RNAs and regulated transcriptional programs

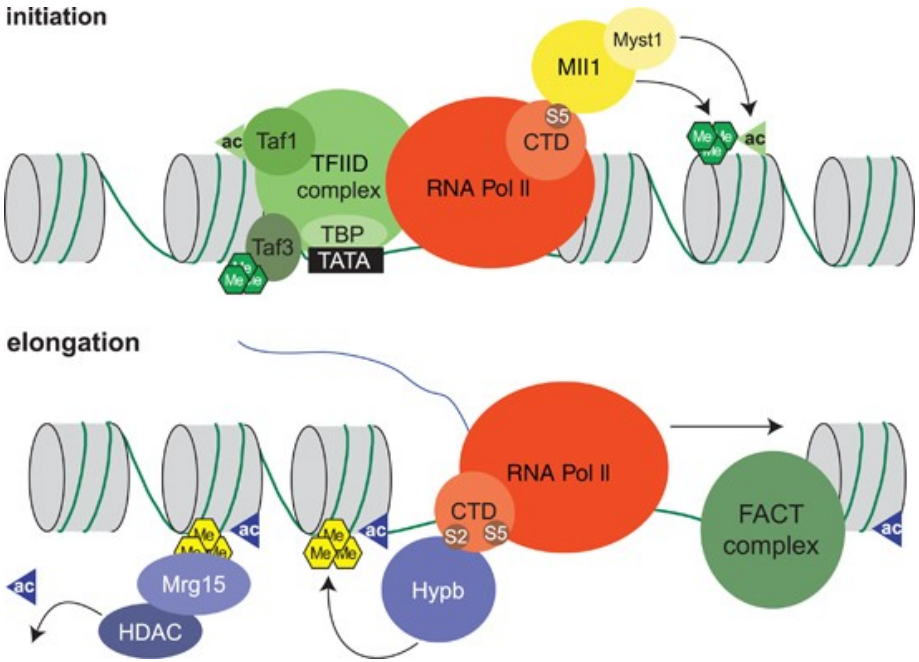
Michael T.Y. Lam[1], Wenbo Li[2], Michael G. Rosenfeld[2], and Christopher K. Glass[1,2]

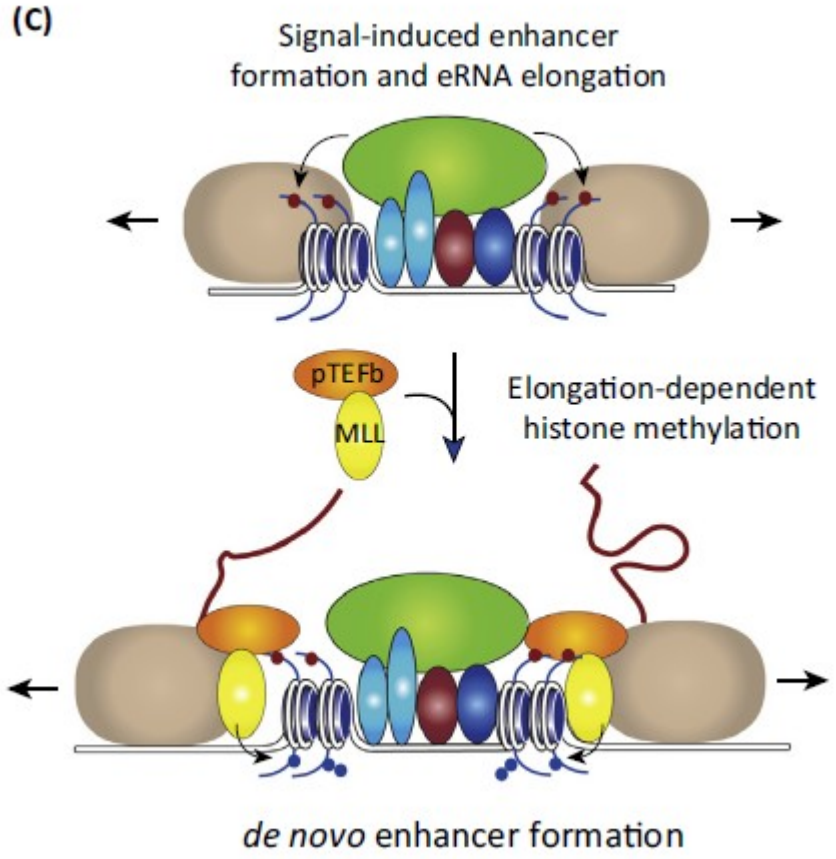
Trends in Biochemical Sciences April 2014, Vol. 39, No. 4

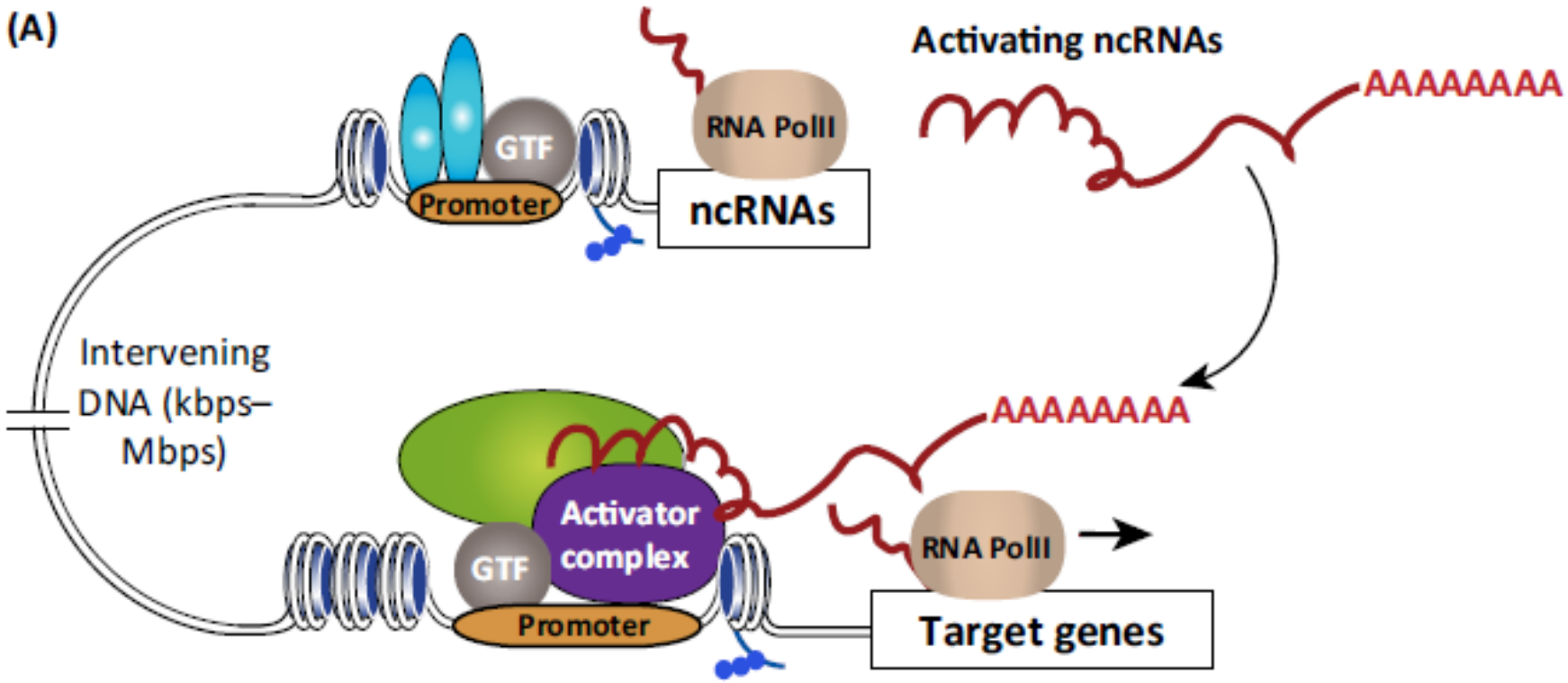
eRNA mediates the long range interactions



Molecular mechanisms that underline enhancer activation

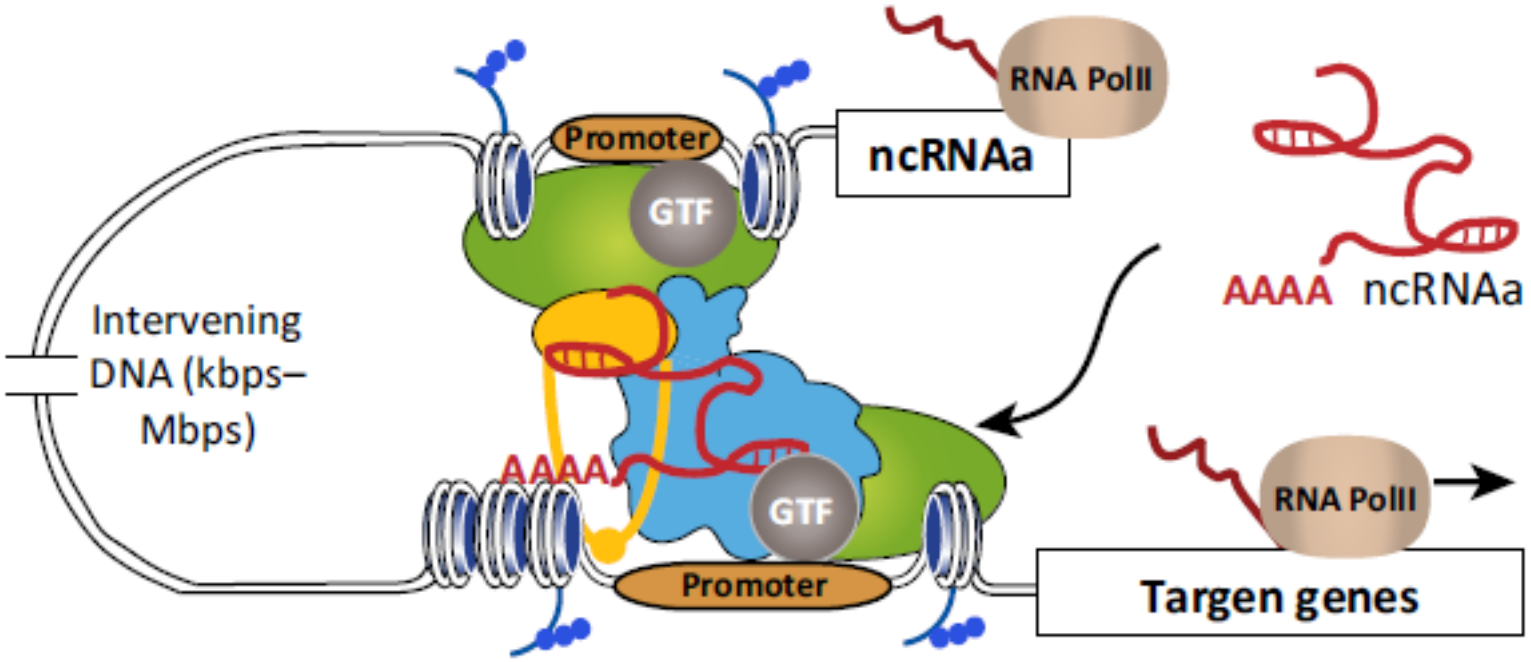




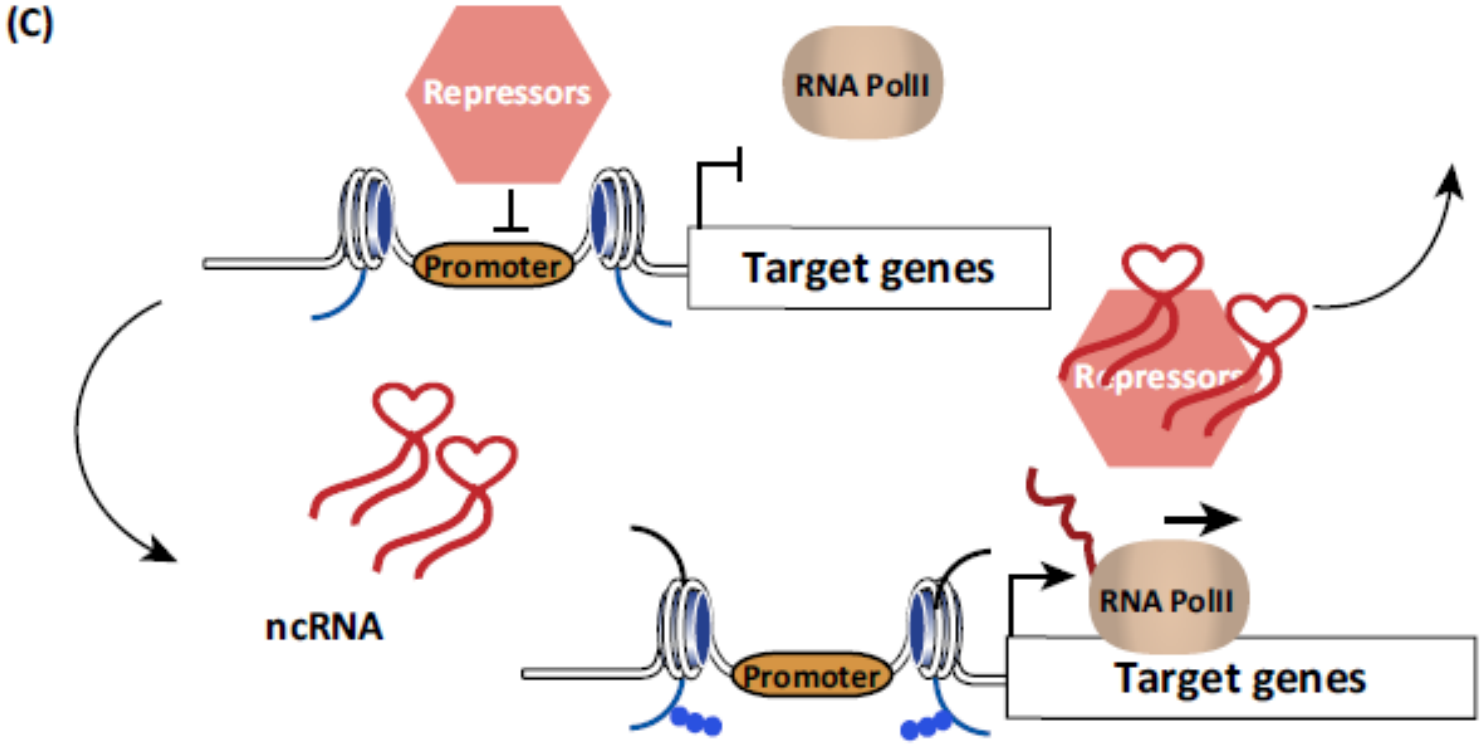


Model 1: ncRNAs collaborate with transcriptional activators

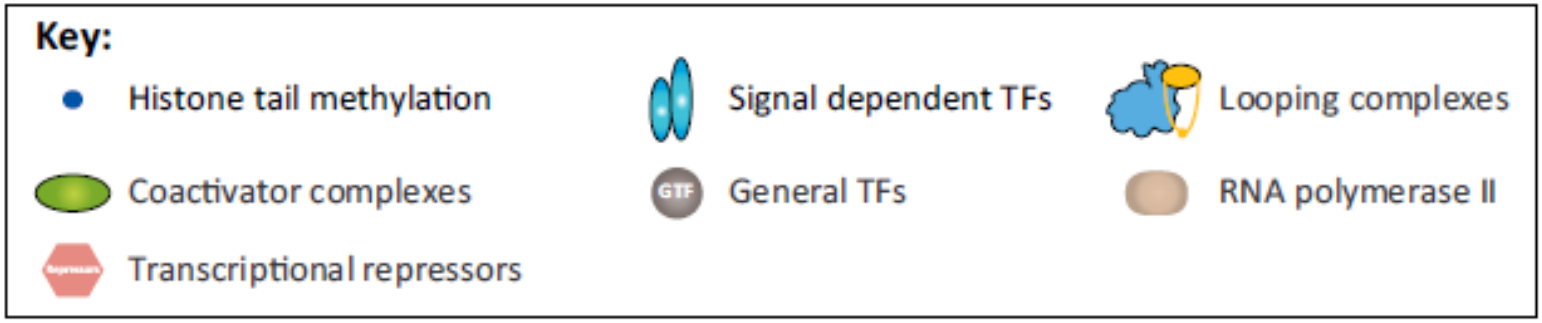
(B)



Model 2: ncRNAs modulate chromatin loops



Model 3: ncRNAs evict transcriptional repressors

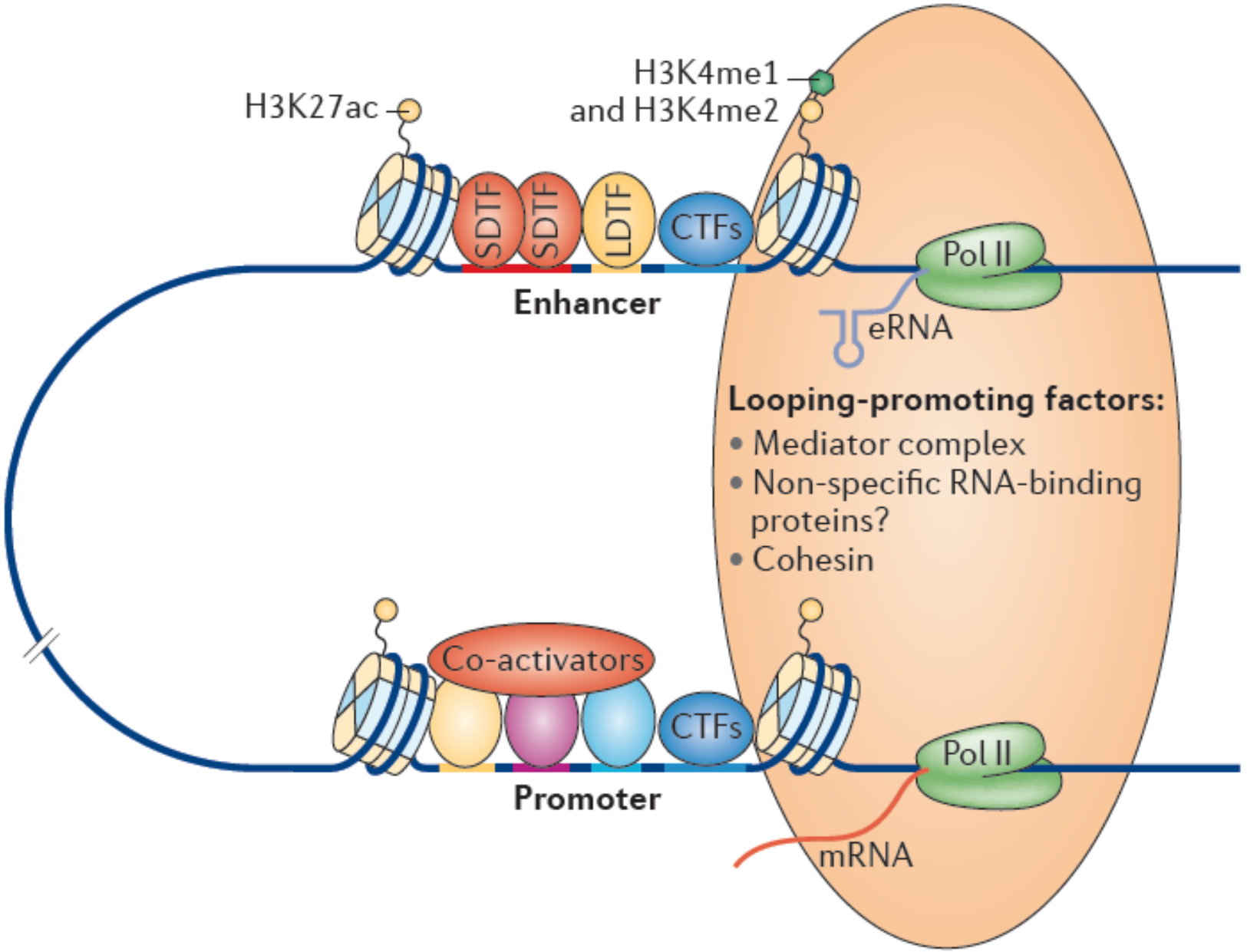


Enhancer Function

- Chromatin looping

- Super-enhancers, cluster of enhancers, key player in the cell identity and differentiation

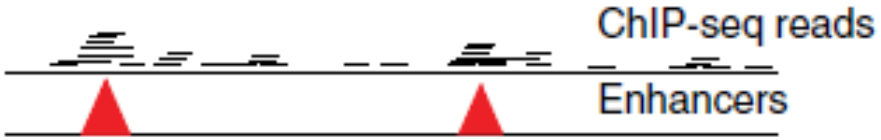
CHROMATIN LOOPING



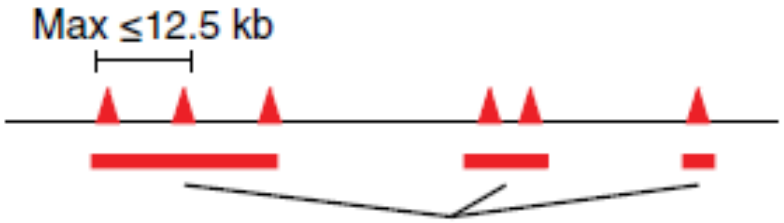
Super-enhancers.

a

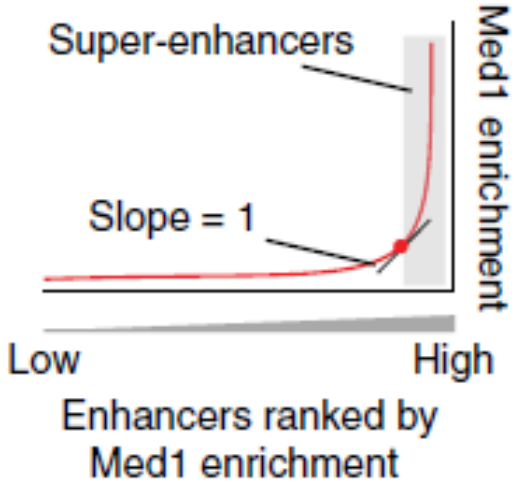
Step 1. Identification of enhancer locations



Step 2. Clustering of enhancers



Step 3. Identify super-enhancers



b

Factor used for step 1	Factor used for step 3	Reference
Oct4 + Sox2 + Nanog, Pu.1	Med1	Whyte *et al.*
MyoD, T-bet, C/EBPα	MyoD, T-bet, C/EBPα	Whyte *et al.*
H3K27ac	H3K27ac	Hnisz *et al.*
Med1	Med1	Loven *et al.*

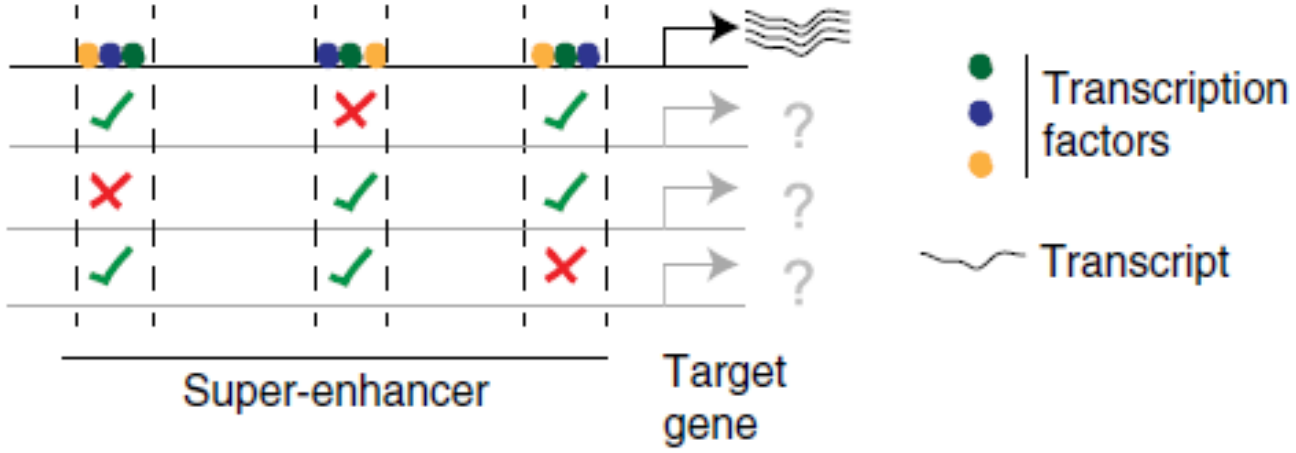


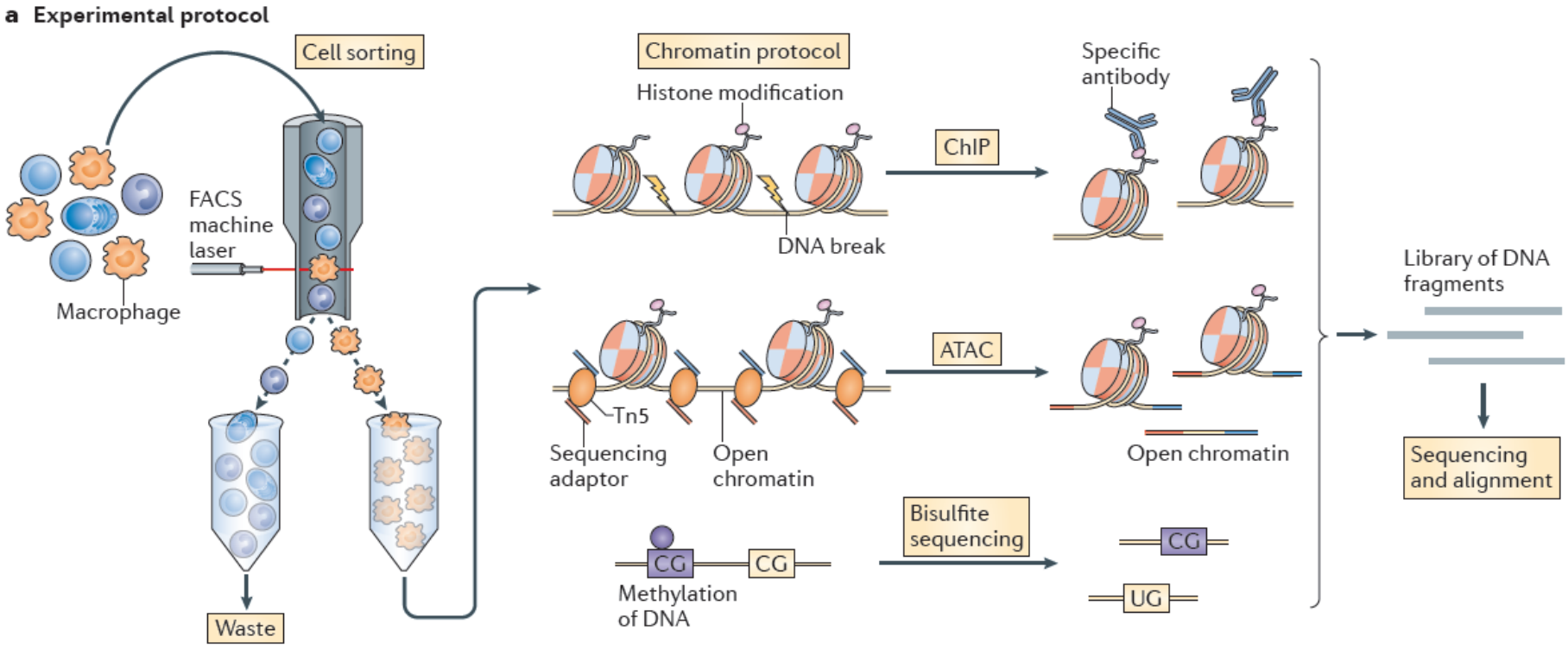
Figure 2 Schematic of an experimental approach to characterizing super-enhancers. Use of genome editing tools, such as the CRISPR-Cas9 system, provides a methodology to create a minimal targeted deletion to test the activity of specific putative enhancers within super-enhancer loci by assessing the consequences of genetic deletions on gene activity.

Making the case for chromatin profiling: a new tool to investigate the immune-regulatory landscape

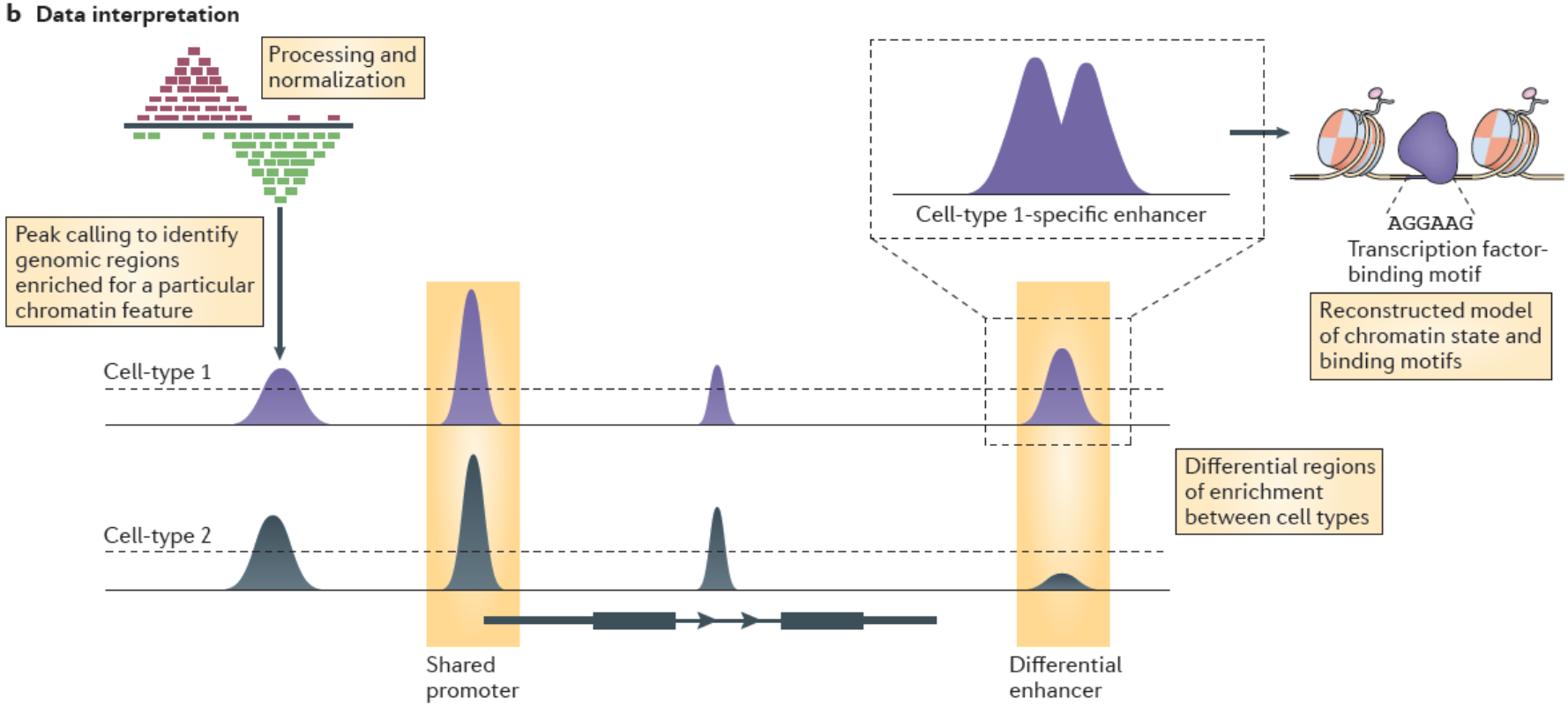
Deborah R. Winter, Steffen Jung and Ido Amit

Abstract | Recent technological advances have enabled researchers to accurately and efficiently assay the chromatin dynamics of scarce cell populations. In this Opinion article, we advocate the application of these technologies to central questions in immunology. Unlike changes to other molecular structures in the cell, chromatin features can reveal the past (developmental history), present (current activity) and future (potential response to challenges) of a given immune cell type; chromatin profiling is therefore an important new tool for studying the immune-regulatory networks of health and disease.

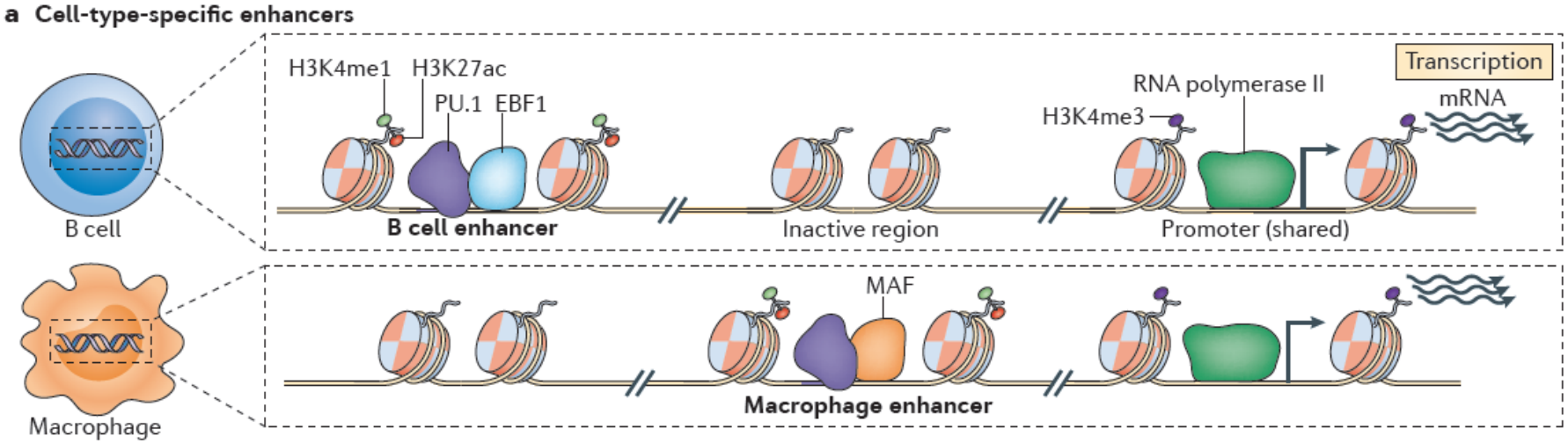
Methods for identification of genomic regulatory regions



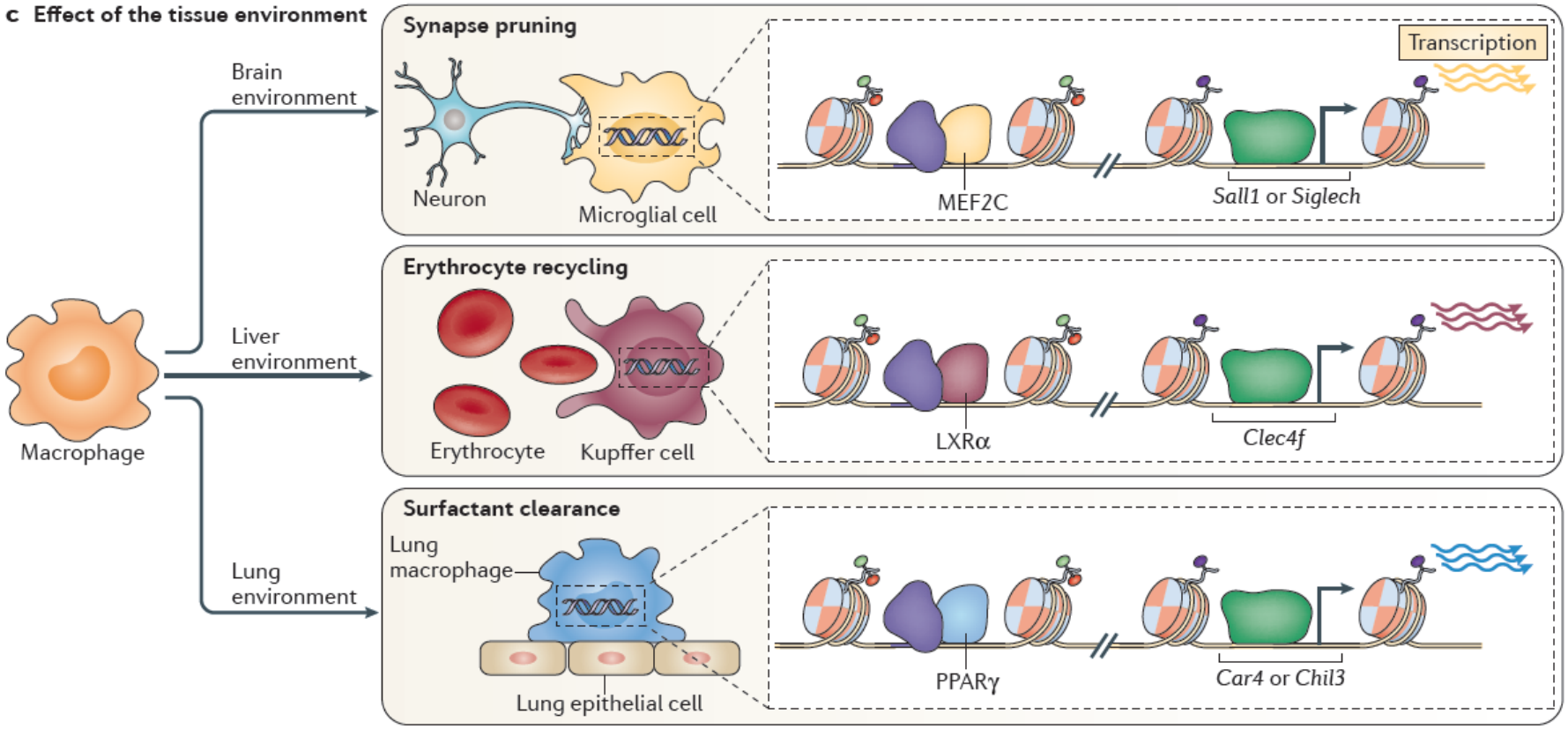
From reads to DNA elements function



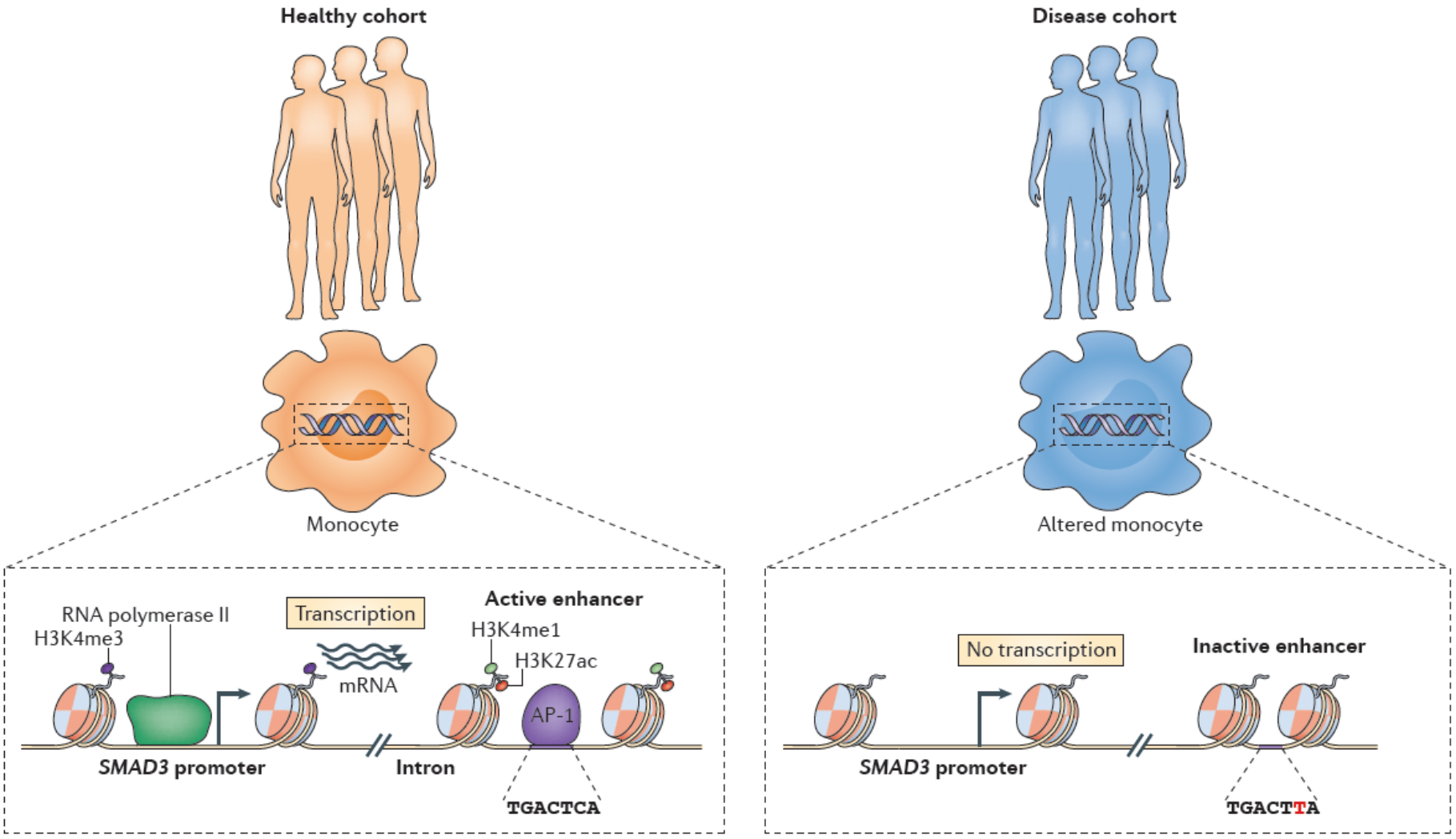
Cell-type-specific enhancers to regulate same genes



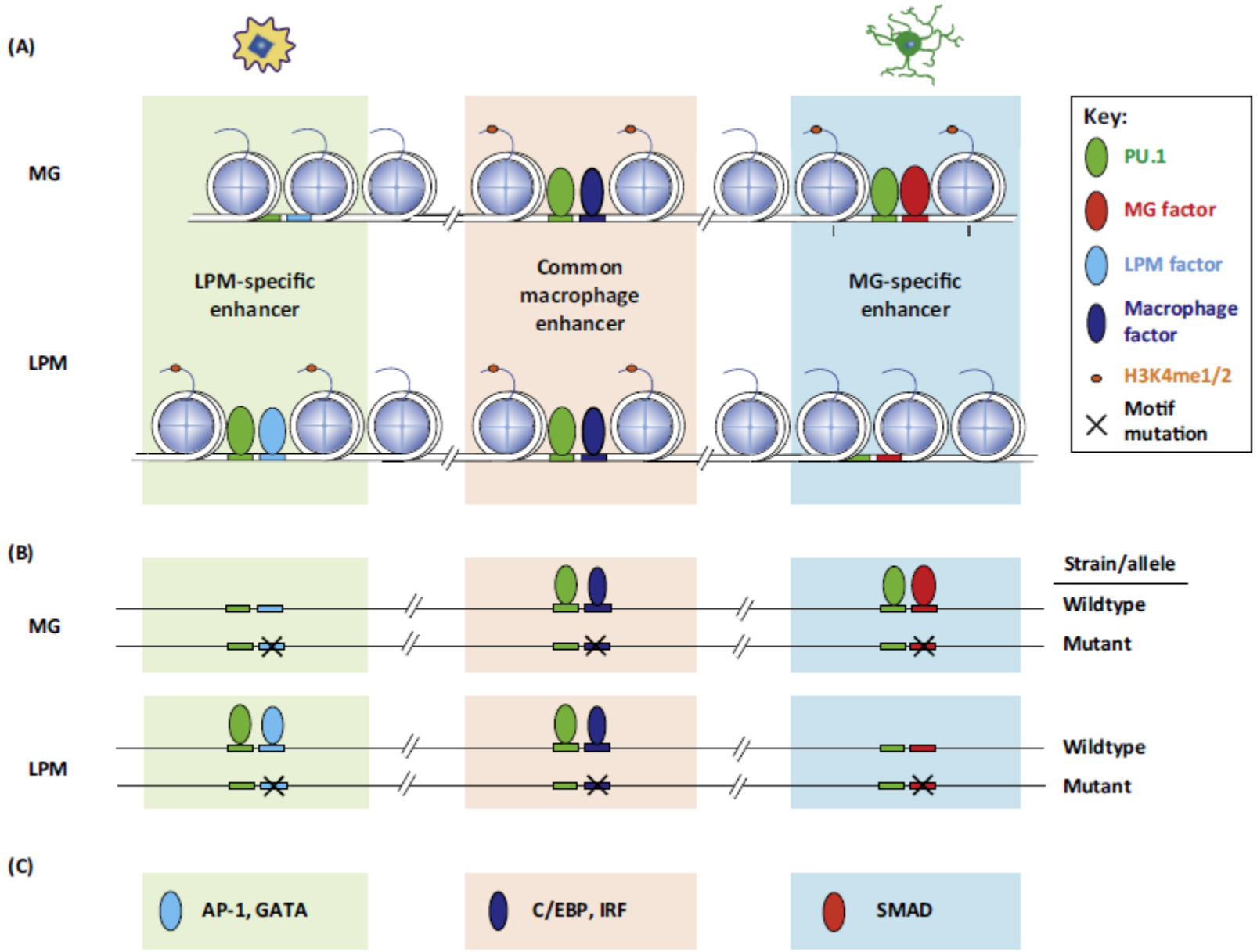
Effect of the tissue environment



Association of human chromatin data and susceptibility to immune disease



NATURAL GENETIC VARIATION IS ASSOCIATED WITH TF BINDING



Romanoski et al., 2015

TRENDS in Immunology

ENHANCER

ACTIVATION

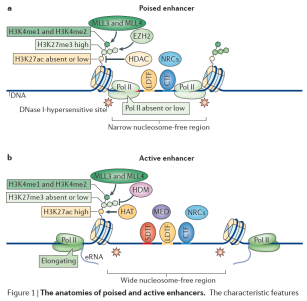
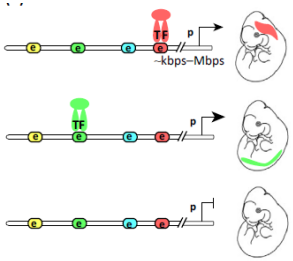


Figure 1 | **The anatomies of poised and active enhancers.** The characteristic features

CHARACTERISTICS



Enhancers in tissue/cell-specific gene expression

SELECTION

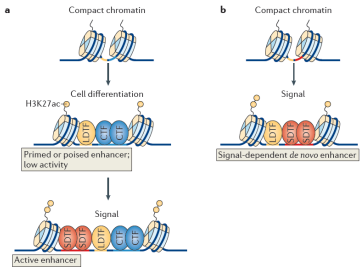
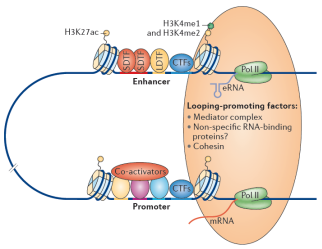


Figure 3 | **Cell type-specific enhancer selection and activation.** **a** | Collaborative

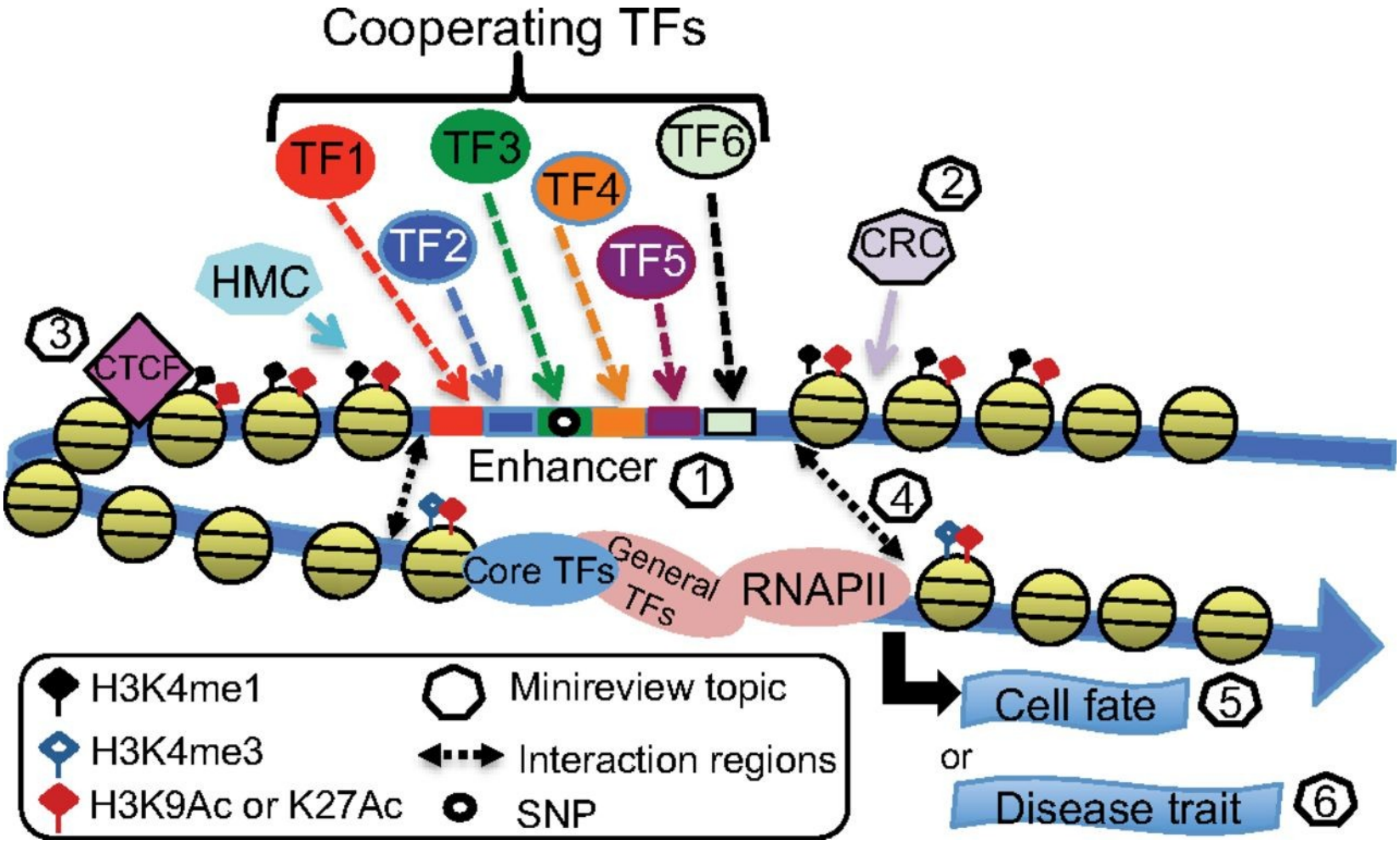
FUNCTION



SNPs in the genomic regulatory regions may affect:

- **Enhancer Activation: loss of TFs interaction or TFs recruitment.**
- **Enhancer Selection: loss or association of LTDF**
- **Alteration of timing or specific tissues activation**
- **Long range interaction between genomic regulatory regions**

Genome-wide characterizations of regulatory regions.



Peggy J. Farnham J. Biol. Chem. 2012;287:30885-30887