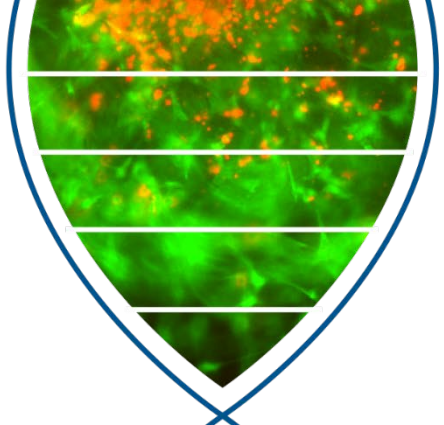
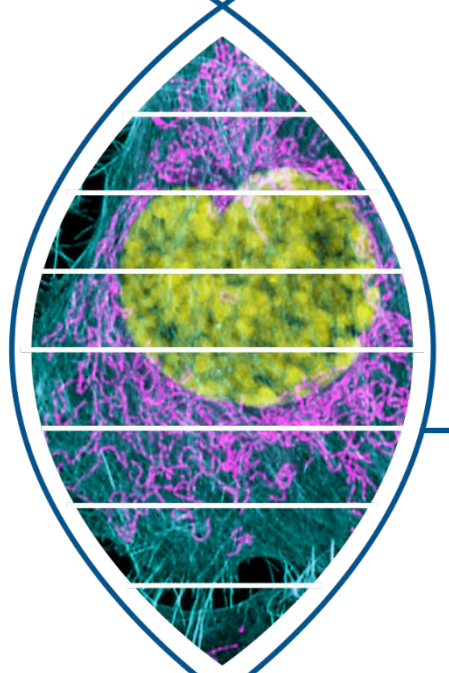
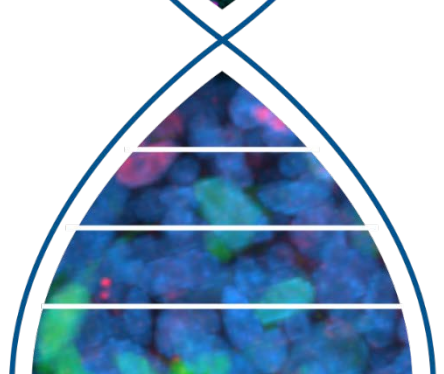




THE WISTAR INSTITUTE

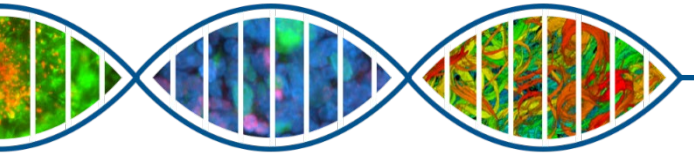
Mechanisms of Transcription Regulation

From RNA Polymerase to the 3D Genome

Alessandro Gardini

BMB 509 – February 2025

NCI Cancer Center
A Cancer Center Designated by the National Cancer Institute



GOALS

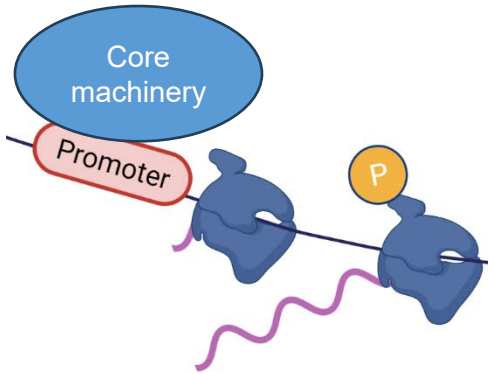
Understanding the different layers of transcriptional regulation in higher eukaryotes

-RNAPII dynamics
-Effect of chromatin environment on transcription
-Proximal vs distal regulation of transcription: enhancers
-The 3D genome: how chromatin conformation and nuclear organization impacts transcription

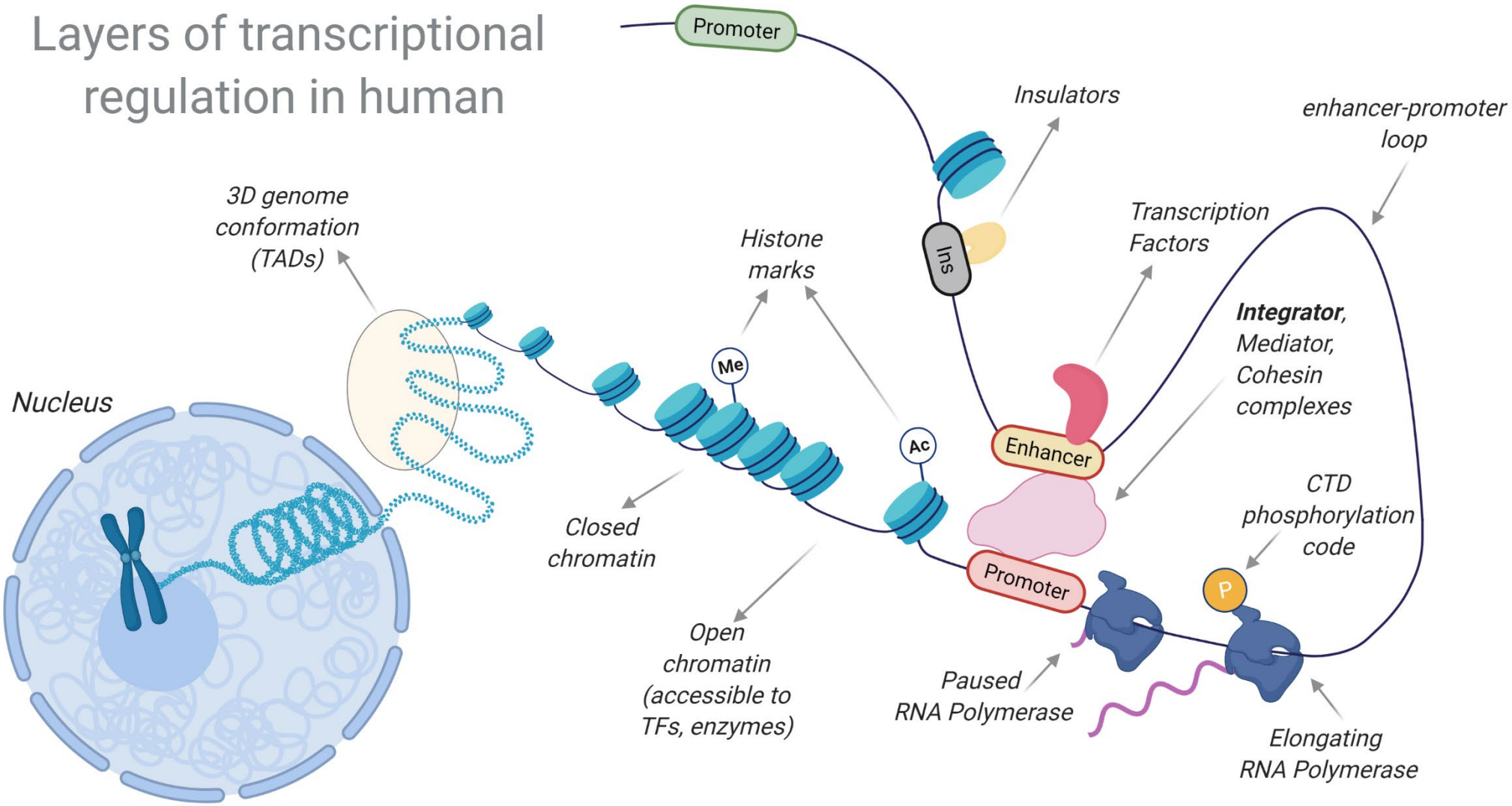
Understanding how the current technology help us *read* the genome

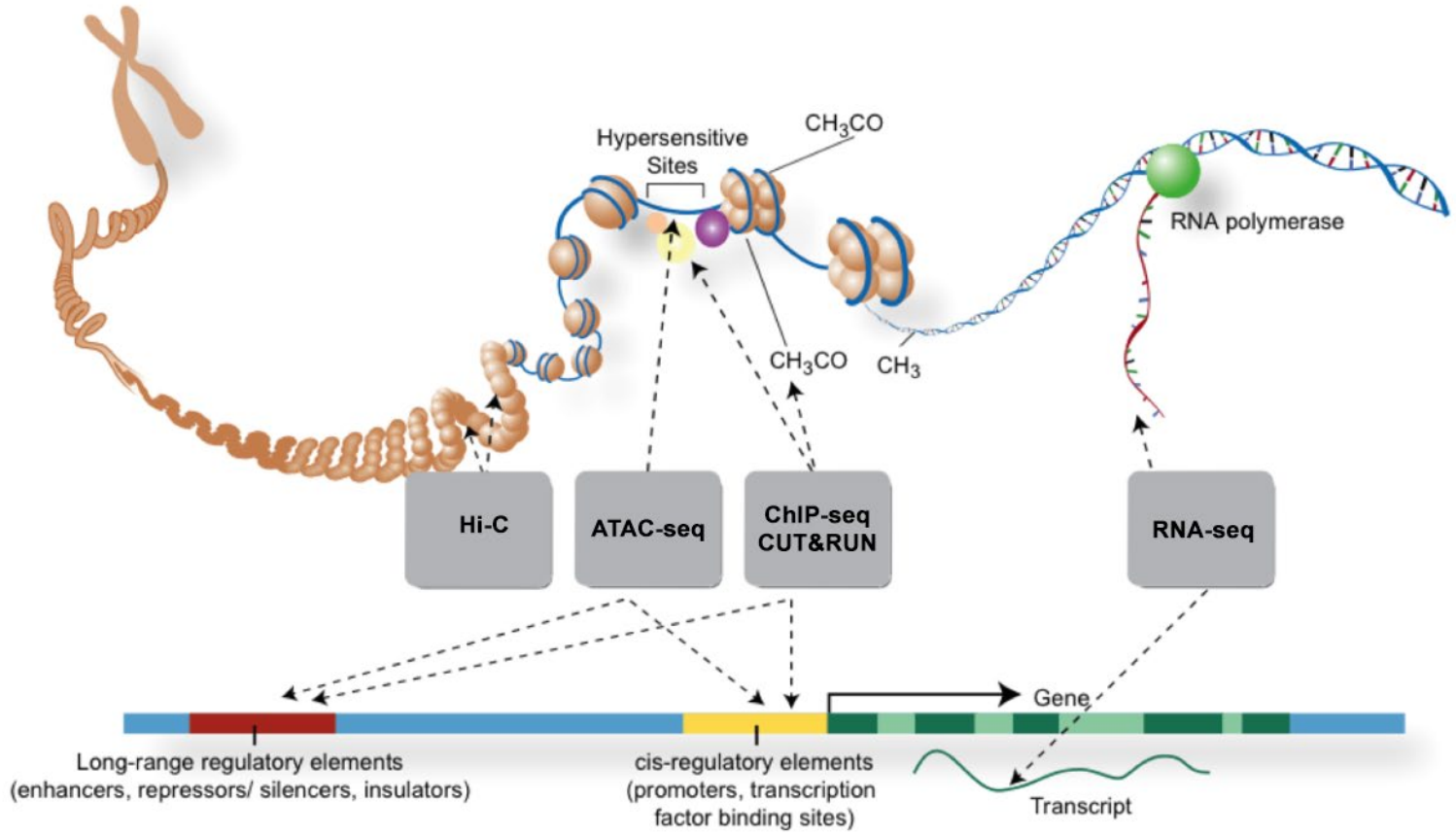
From a promoter-centric world….

…to a context dependent regulation



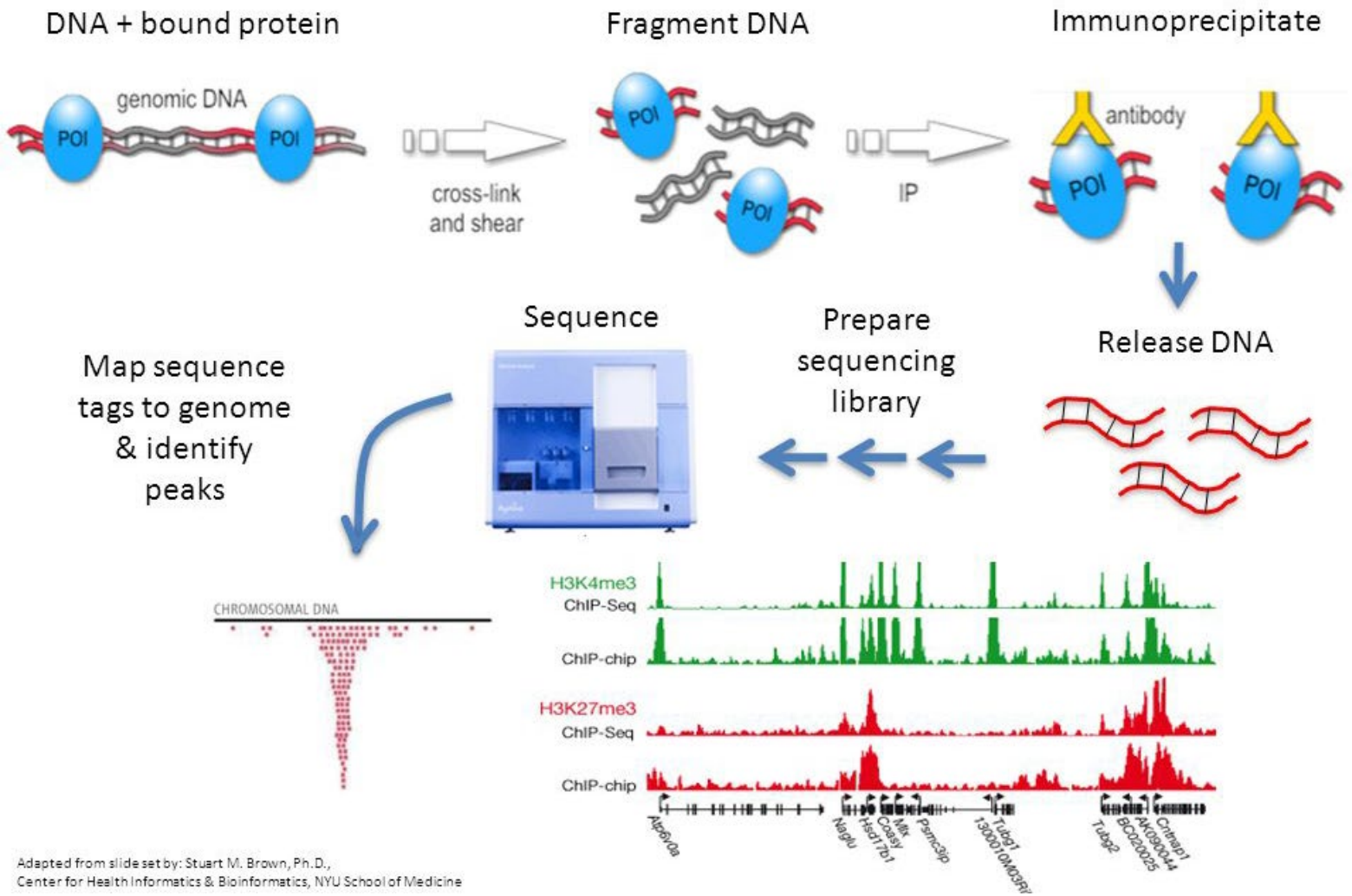
Layers of transcriptional regulation in human





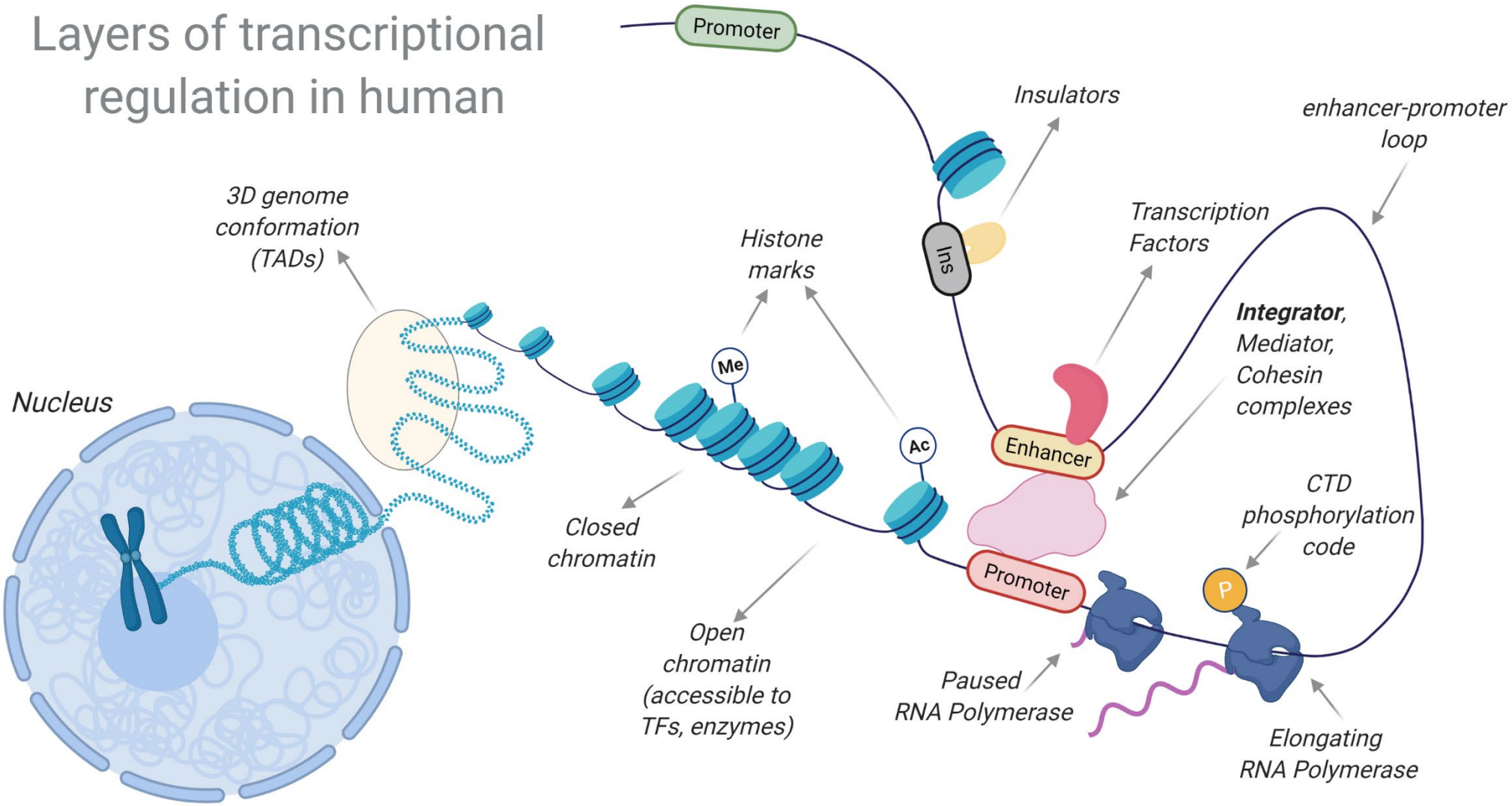
New technological developments accompany the discovery of novel regulatory layers

Assessing chromatin binding by ChIP-seq



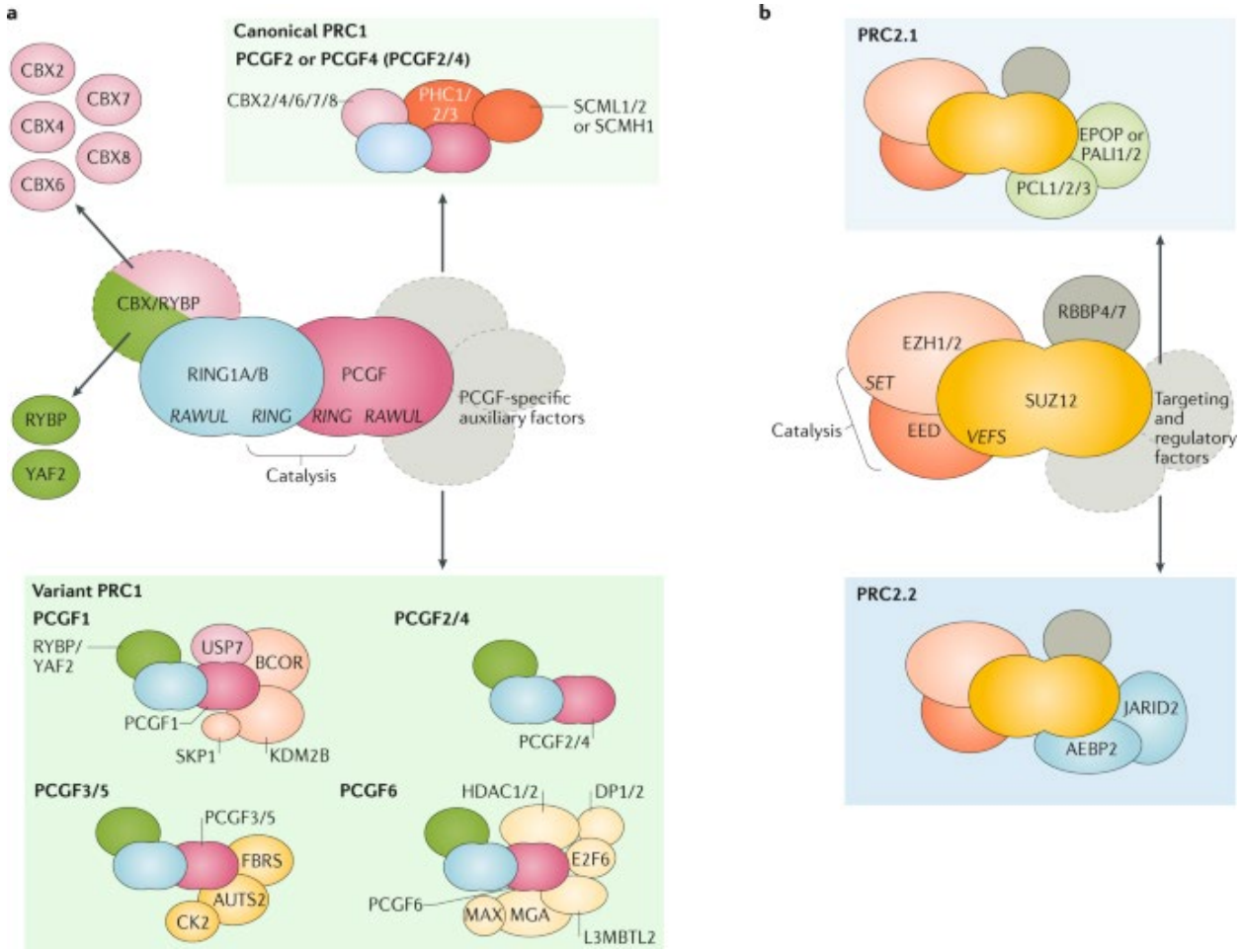
Adapted from slide set by: Stuart M. Brown, Ph.D.,
Center for Health Informatics & Bioinformatics, NYU School of Medicine

Layers of transcriptional regulation in human



How do we block transcription and direct cell fate?

The Polycomb family of epigenetic regulators



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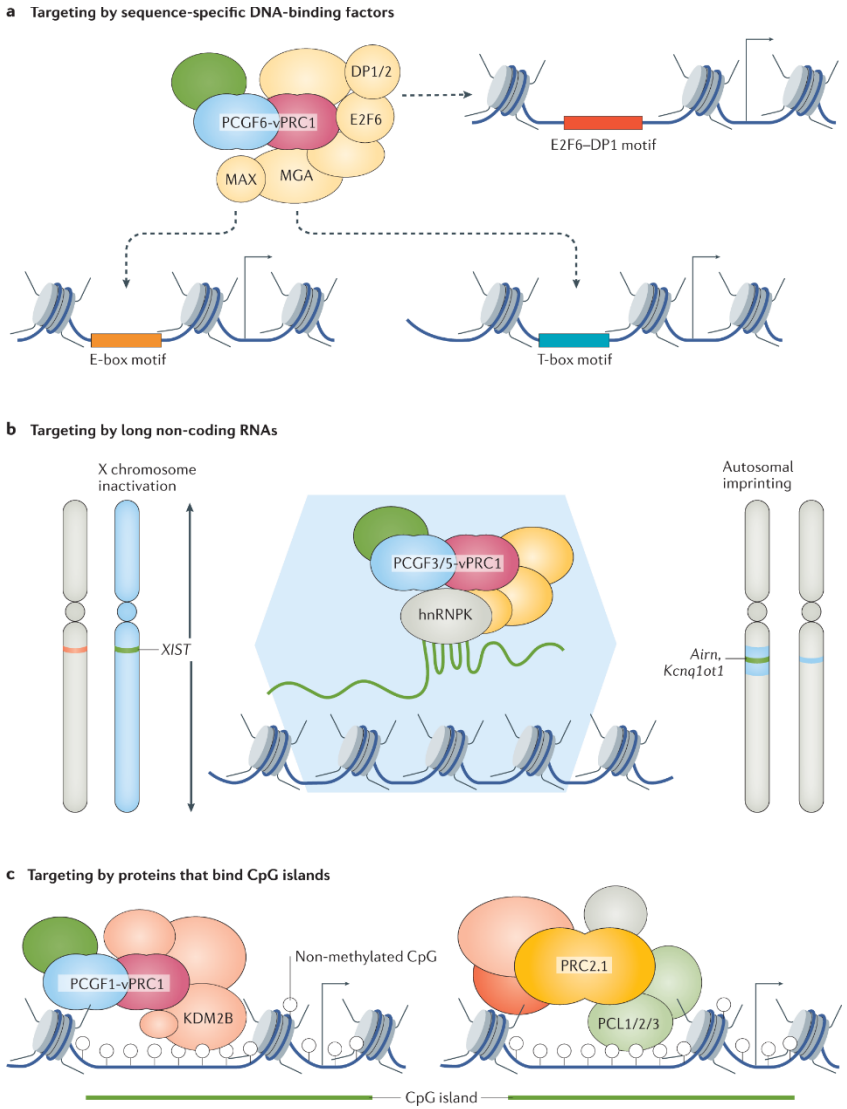
Phase I: targeting



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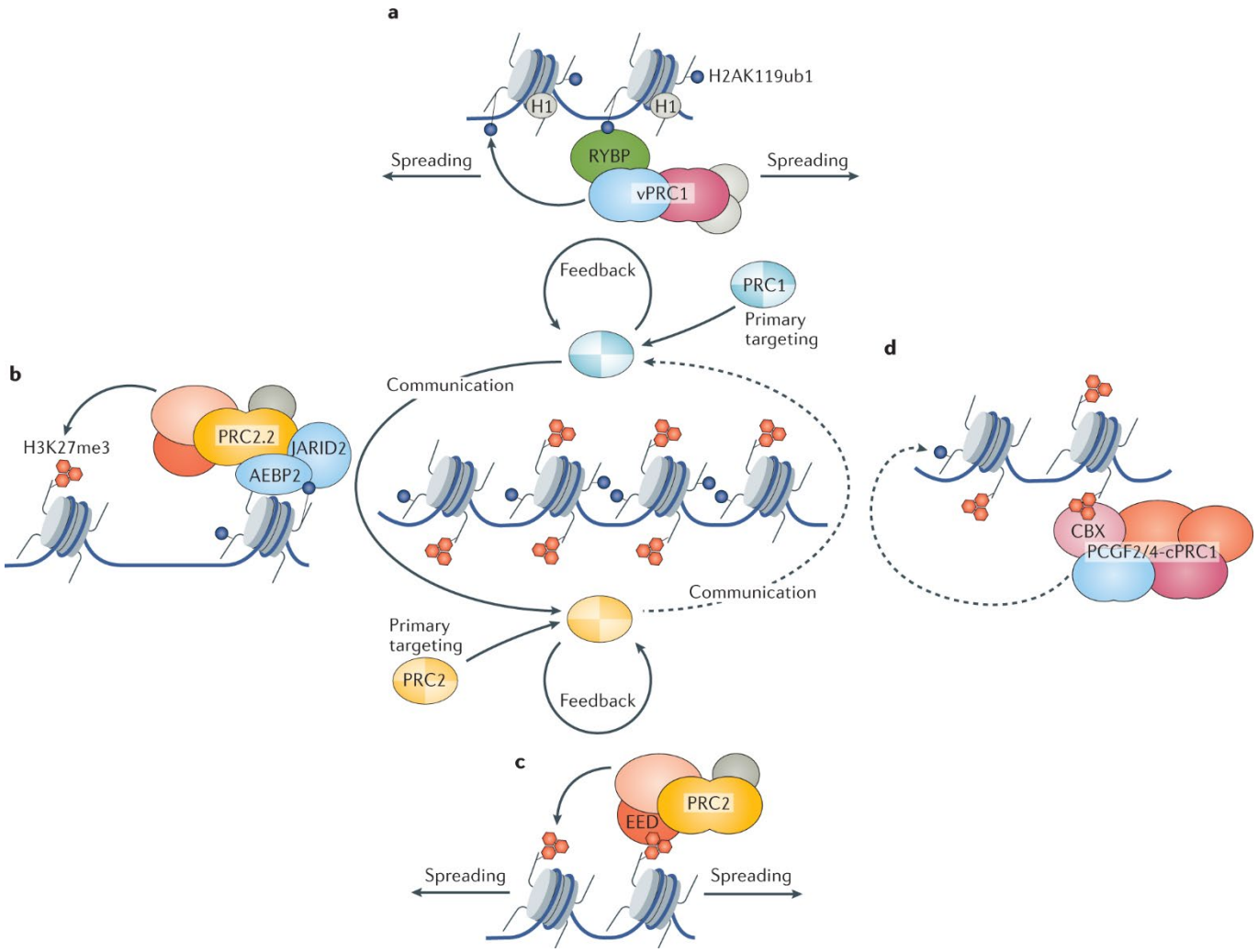
Phase II: histone marks deposition and spreading



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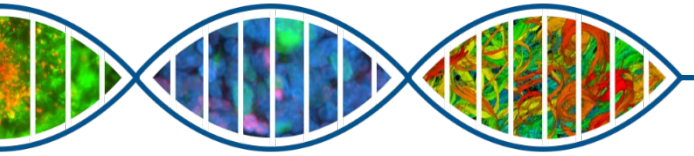
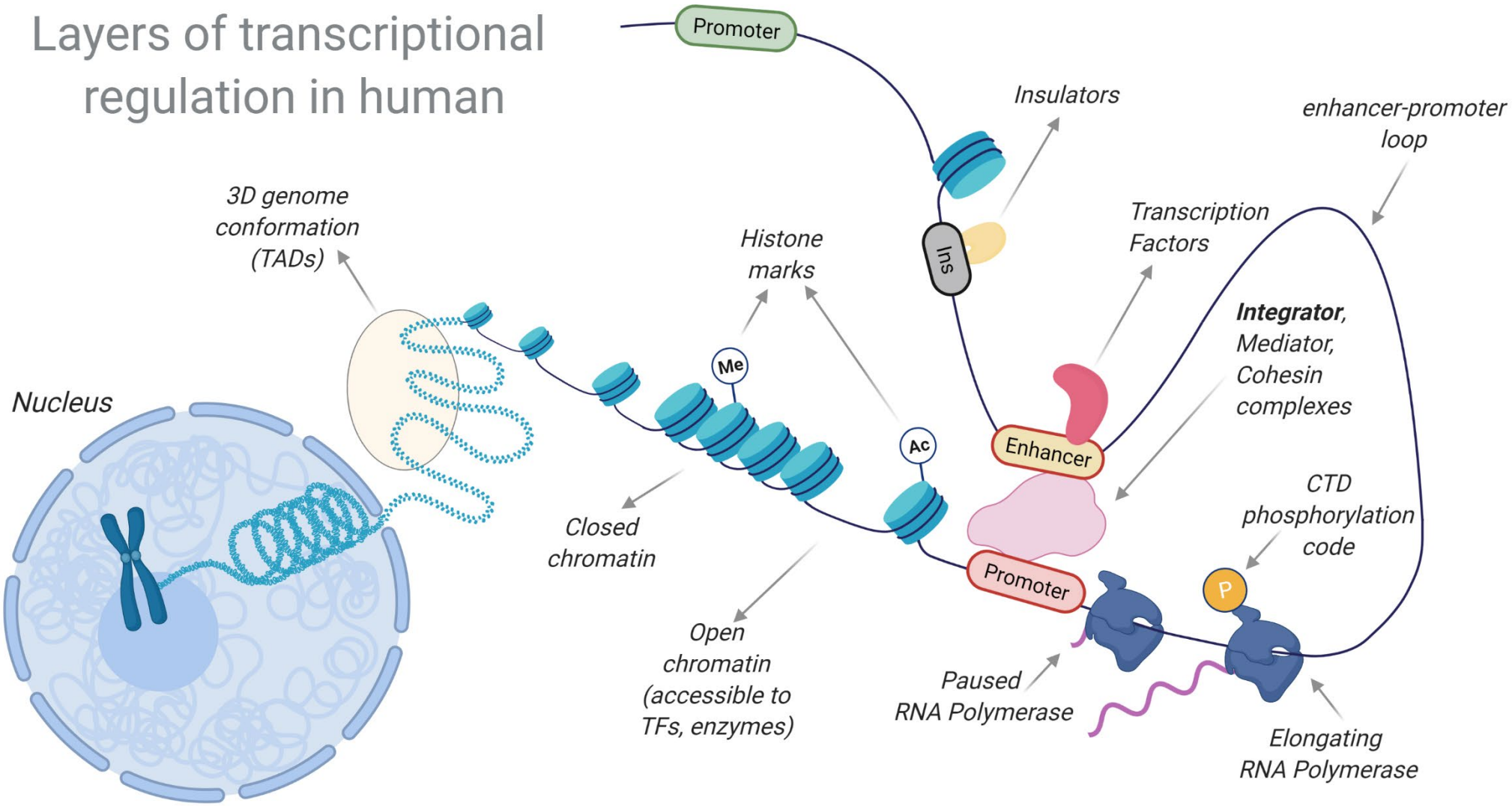


Regulating transcription from a distance

Layers of transcriptional regulation in human



Enhancers are a fixture of gene regulation in higher eukaryotes

nature genetics

Distinct and predictive chromatin signatures of transcriptional promoters and enhancers in the human genome

Nathaniel D Heintzman[1,2], Rhona K Stuart[1], Gary Hon[1,3], Yutao Fu[4], Christina W Ching[1], R David Hawkins[1], Leah O Barrera[1,3], Sara Van Calcar[1], Chunxu Qu[1], Keith A Ching[1], Wei Wang[5], Zhiping Weng[4,6], Roland D Green[7], Gregory E Crawford[8] & Bing Ren[1,9]

nature

Histone modifications at human enhancers reflect global cell-type-specific gene expression

Nathaniel D. Heintzman[1,2]*, Gary C. Hon[1,3]*, R. David Hawkins[1]*, Pouya Kheradpour[5], Alexander Stark[5,6], Lindsey F. Harp[1], Zhen Ye[1], Leonard K. Lee[1], Rhona K. Stuart[1], Christina W. Ching[1], Keith A. Ching[1], Jessica E. Antosiewicz-Bourget[7], Hui Liu[8], Xinmin Zhang[8], Roland D. Green[8], Victor V. Lobanenkov[9], Ron Stewart[7], James A. Thomson[7,10], Gregory E. Crawford[11], Manolis Kellis[5,6] & Bing Ren[1,4]

LETTER

doi:10.1038/nature09692

A unique chromatin signature uncovers early developmental enhancers in humans

Alvaro Rada-Iglesias[1], Ruchi Bajpai[1], Tomek Swigut[1], Samantha A. Brugmann[1], Ryan A. Flynn[1] & Joanna Wysocka[1,2]

Cell

Long Noncoding RNAs with Enhancer-like Function in Human Cells

Ulf Andersson Ørom,[1] Thomas Derrien,[2] Malte Beringer,[1] Kiranmai Gumireddy,[1] Alessandro Gardini,[1] Giovanni Bussotti,[2] Fan Lai,[1] Matthias Zytnicki,[2] Cedric Notredame,[2] Qihong Huang,[1] Roderic Guigo,[2] and Ramin Shiekhattar[1,2,3,*]
[1]The Wistar Institute, 3601 Spruce Street, Philadelphia, PA 19104, USA
[2]Centre for Genomic Regulation (CRG), UPF, Barcelona, Spain
[3]Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain
*Correspondence: shiekhattar@wistar.org
DOI 10.1016/j.cell.2010.09.001

nature

Widespread transcription at neuronal activity-regulated enhancers

Tae-Kyung Kim[1]*†, Martin Hemberg[2]*, Jesse M. Gray[1]*, Allen M. Costa[1], Daniel M. Bear[1], Jing Wu[3], David A. Harmin[1,4], Mike Laptewicz[1], Kellie Barbara-Haley[5], Scott Kuersten[6], Eirene Markenscoff-Papadimitriou[1]†, Dietmar Kuhl[7], Haruhiko Bito[8], Paul F. Worley[3], Gabriel Kreiman[2] & Michael E. Greenberg[1]

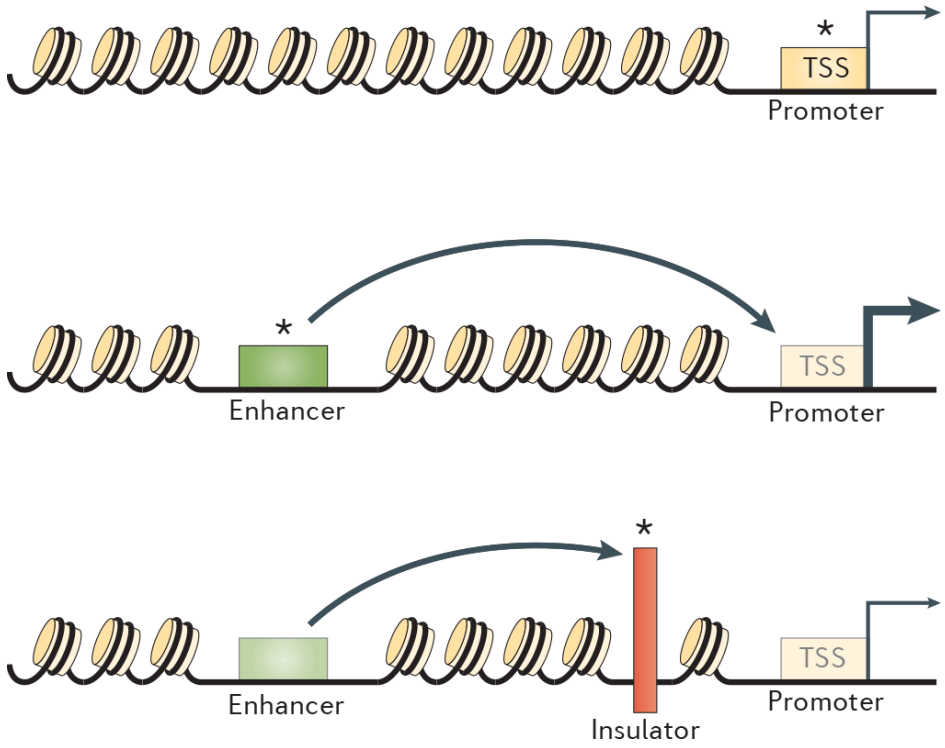
LETTER

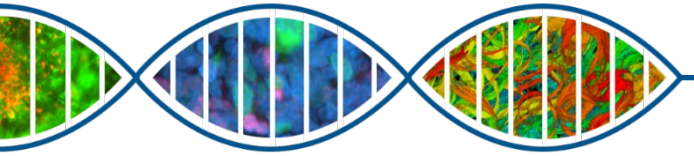
doi:10.1038/nature12209

Rev-Erbs repress macrophage gene expression by inhibiting enhancer-directed transcription

Michael T. Y. Lam[1], Han Cho[2], Hanna P. Lesch[1], David Gosselin[1], Sven Heinz[1], Yumiko Tanaka-Oishi[1], Christopher Benner[1], Minna U. Kaikkonen[1], Aneeza S. Kim[3], Mika Kosaka[1], Cindy Y. Lee[1], Andy Watt[3], Tamar R. Grossman[3], Michael G. Rosenfeld[4,5], Ronald M. Evans[2,5] & Christopher K. Glass[1,4]

What are enhancers?

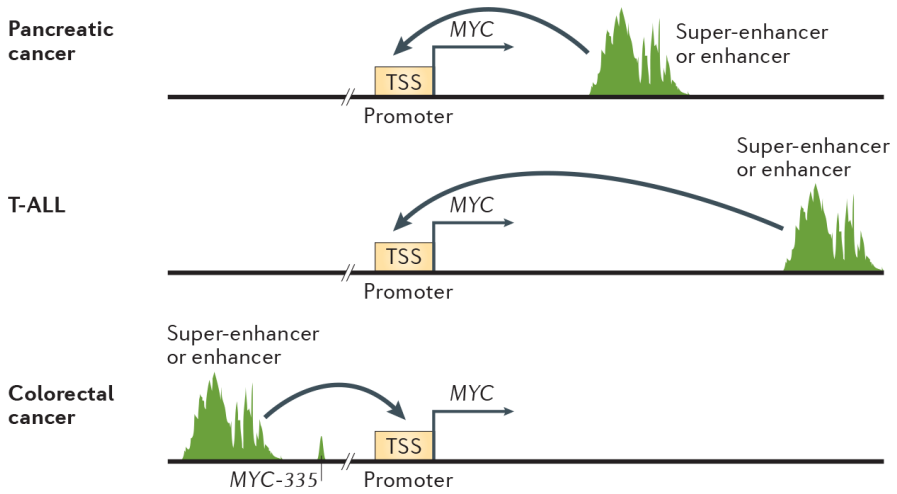


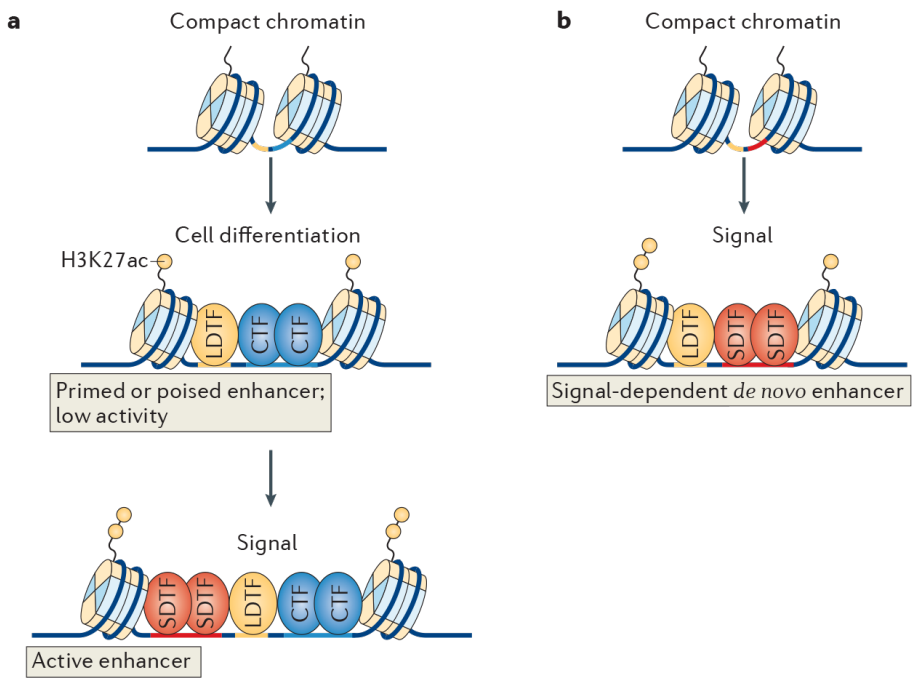


How many enhancers?

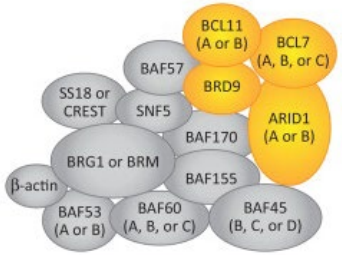
How do we define them?

Are enhancers pathologically relevant?



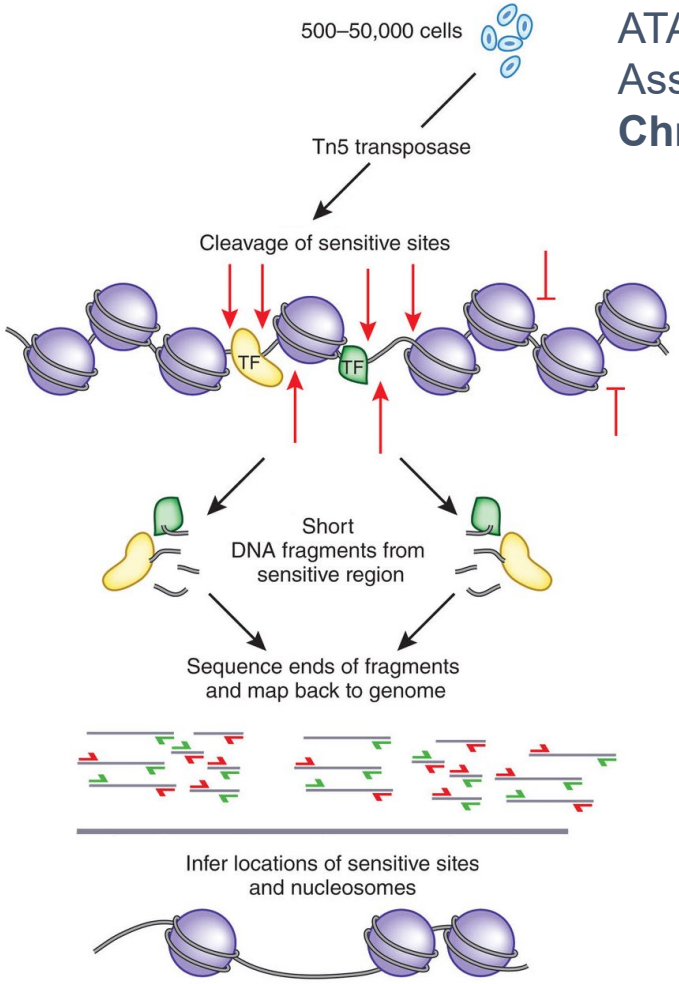


A group of enzymes called **chromatin remodelers** regulate nucleosome positioning at enhancers and promoters

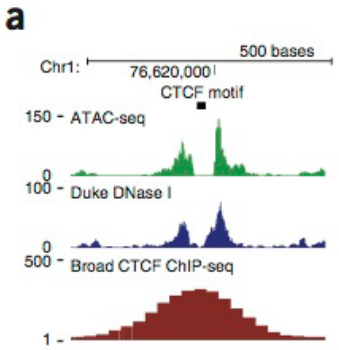


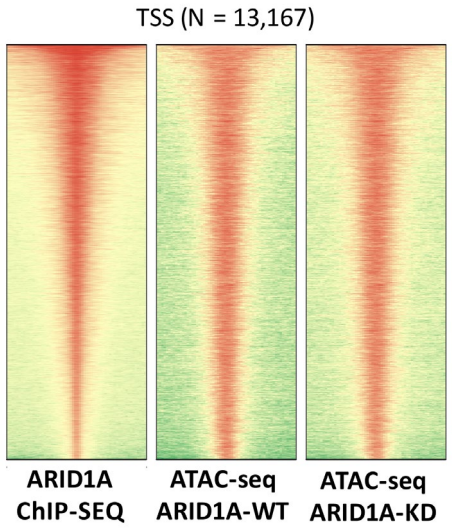
The SWI/SNF (BAF) complex

Nature Reviews Mol Cell Biol, Mar 2015



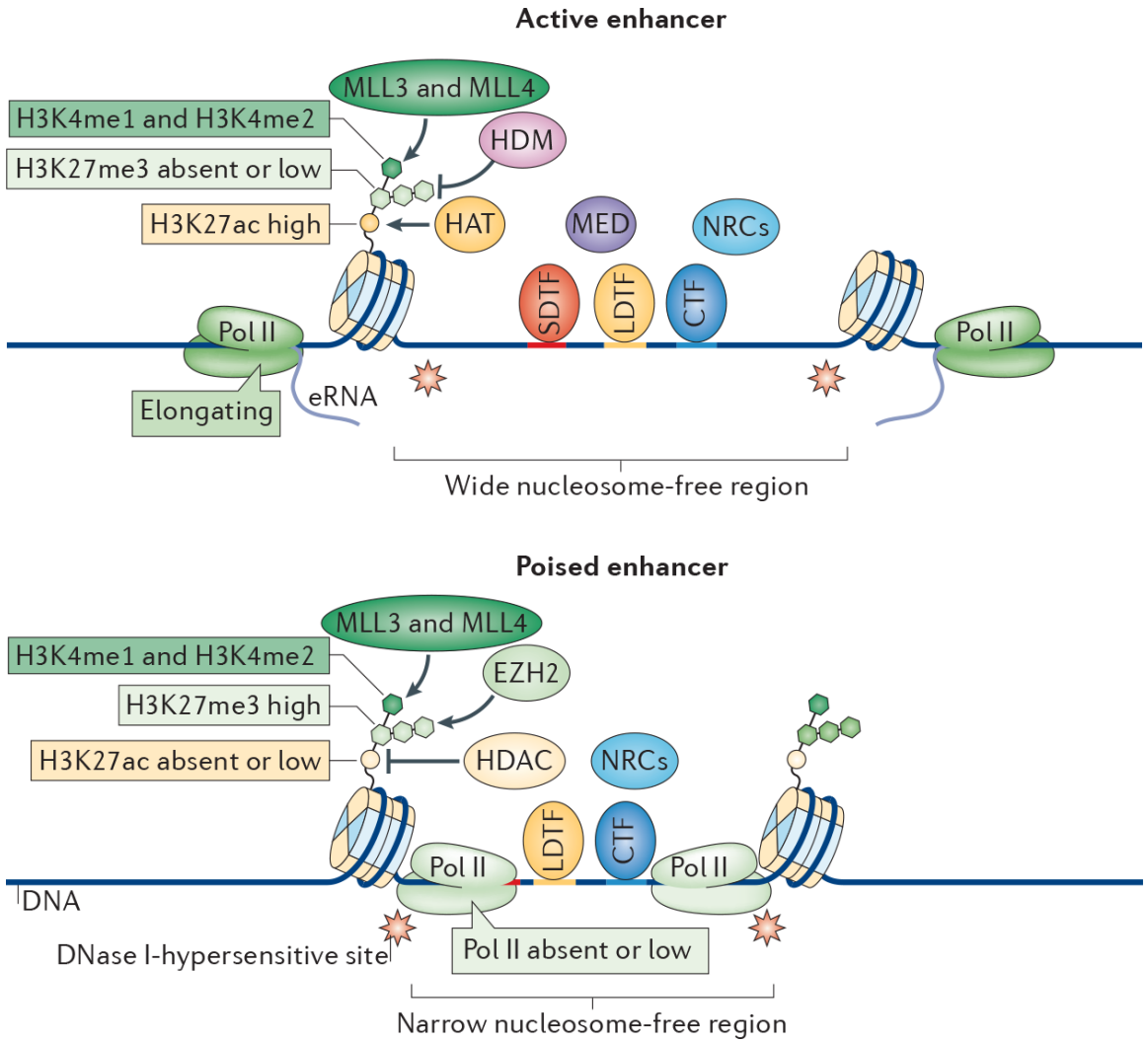
ATAC-seq
Assay for **Transposase-Accessible Chromatin** using sequencing





How enhancers work

2) The chromatin landscape



Chromatin architecture of enhancers and promoters is very similar but not identical (i.e. higher K27ac, higher H3k4me1, lower H3K4me3)

Important for genome-wide annotation of enhancers

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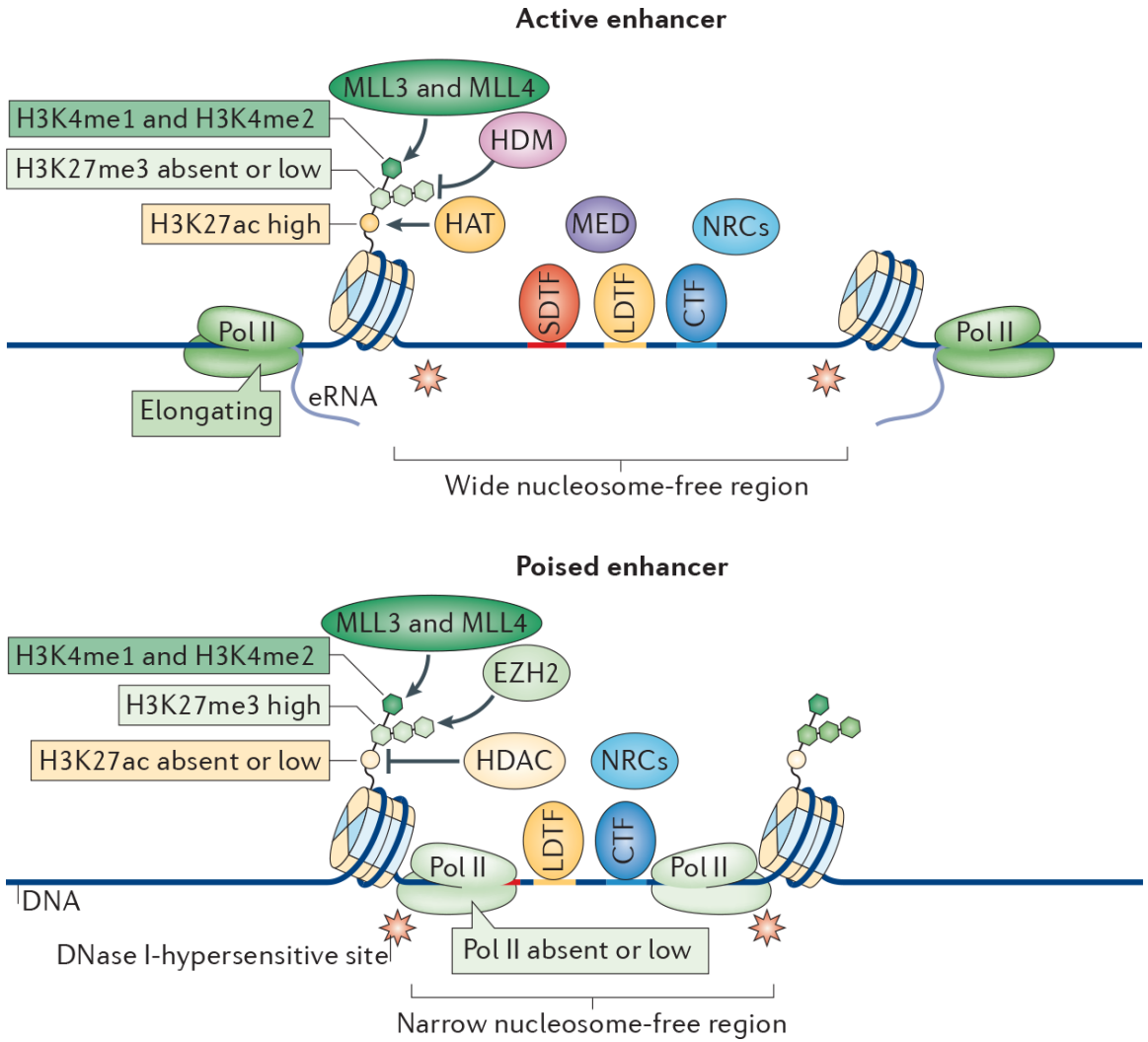
A Cancer Center Designated by the National Cancer Institute

Nature Reviews Mol Cell Biol, Mar 2015



An active chromatin status does not necessarily imply activation

NCI Cancer Center
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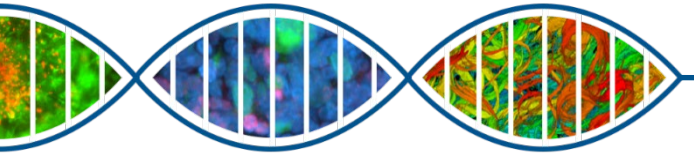
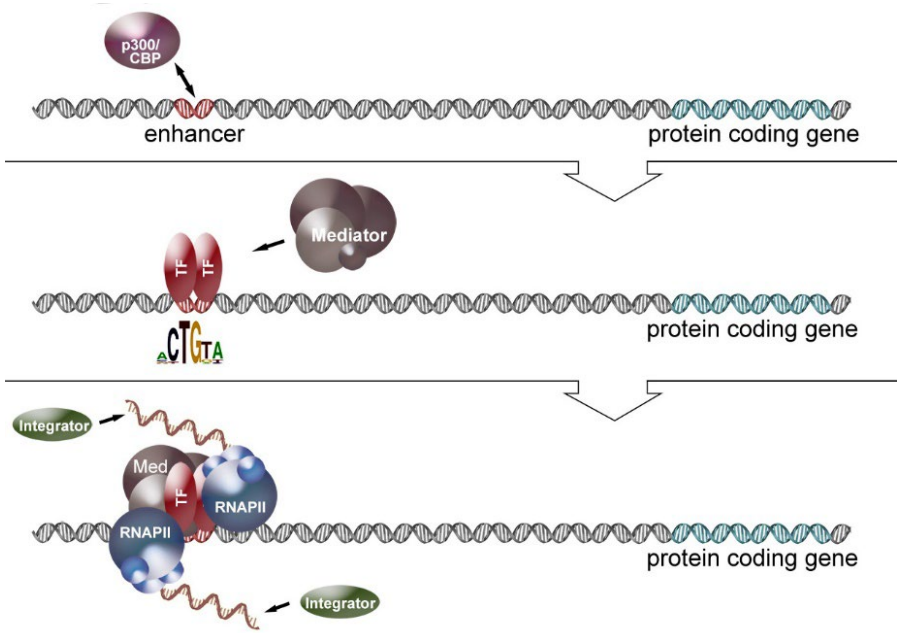
Nature Reviews Mol Cell Biol, Mar 2015

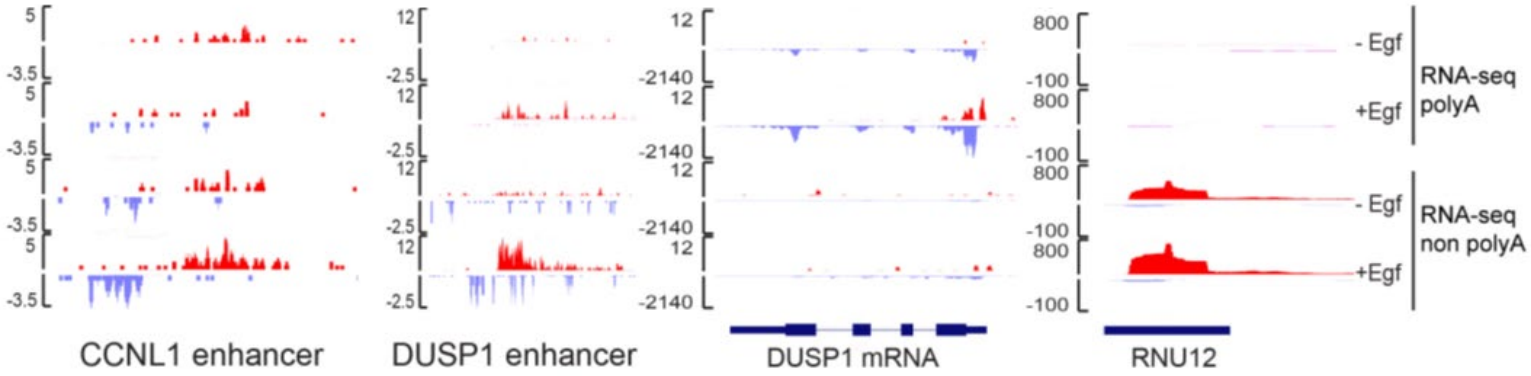


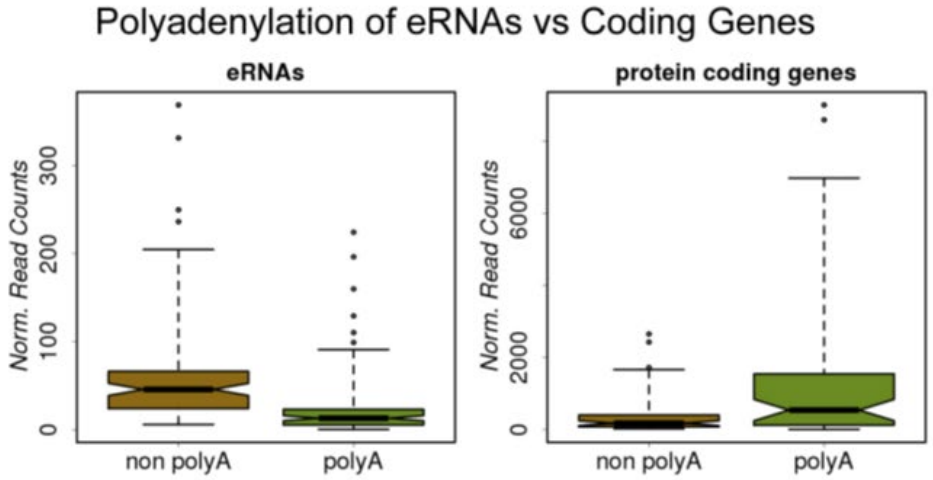
Noncoding RNAs and enhancer activity

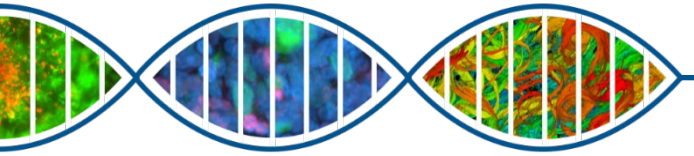


Enhancers-associated noncoding RNAs (eRNAs)

eRNAs are non polyadenylated transcripts





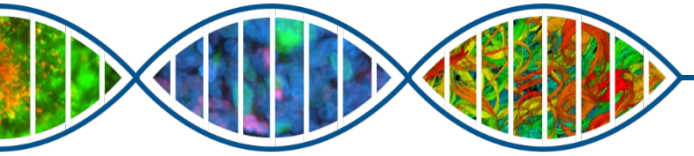


eRNAs appear necessary for enhancer function

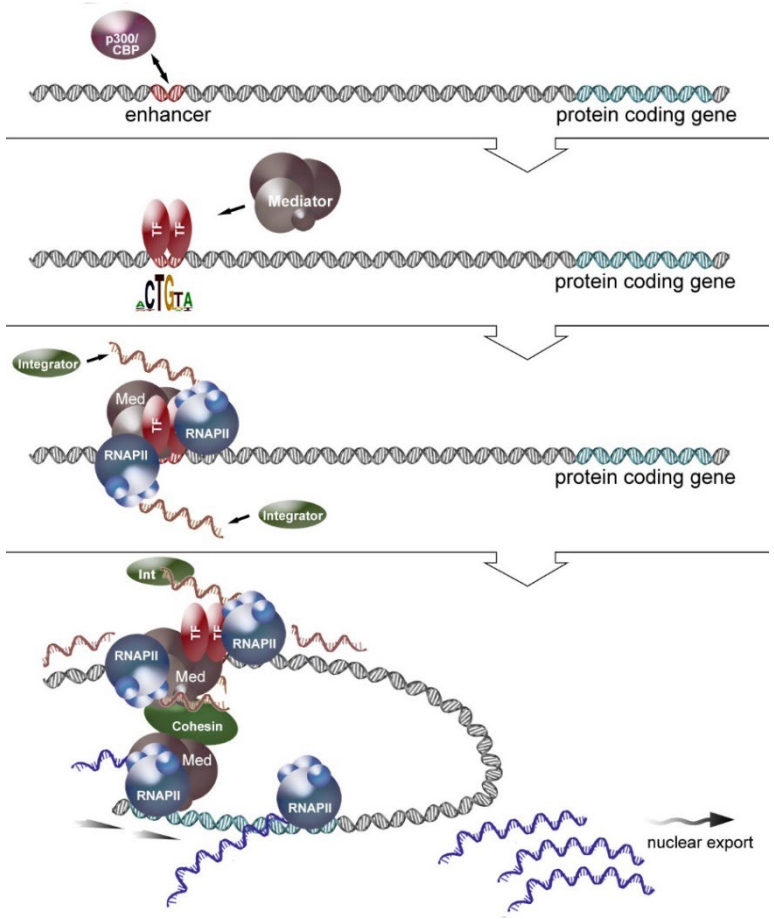
(several depletion studies show eRNA requirement for activation in many cell types)

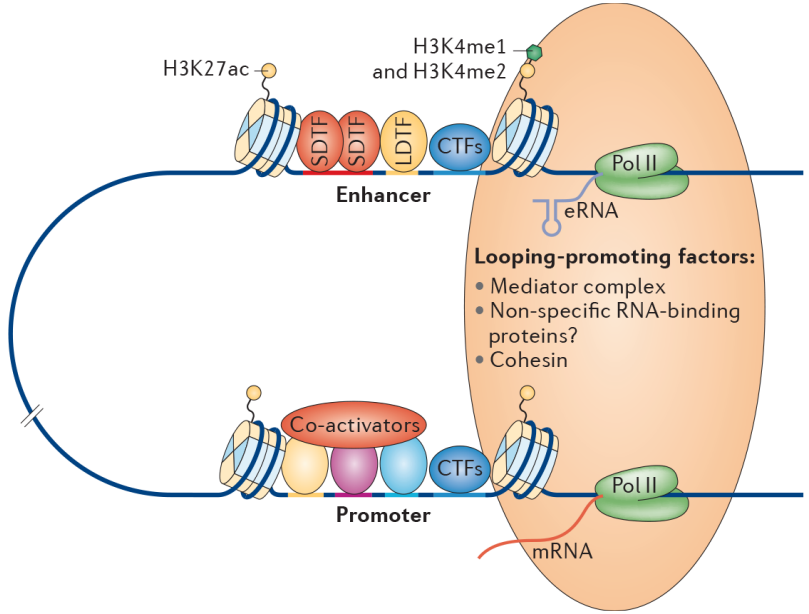
but HOW?

Very much debated (regulating pausing, increasing local acetylation, etc.)



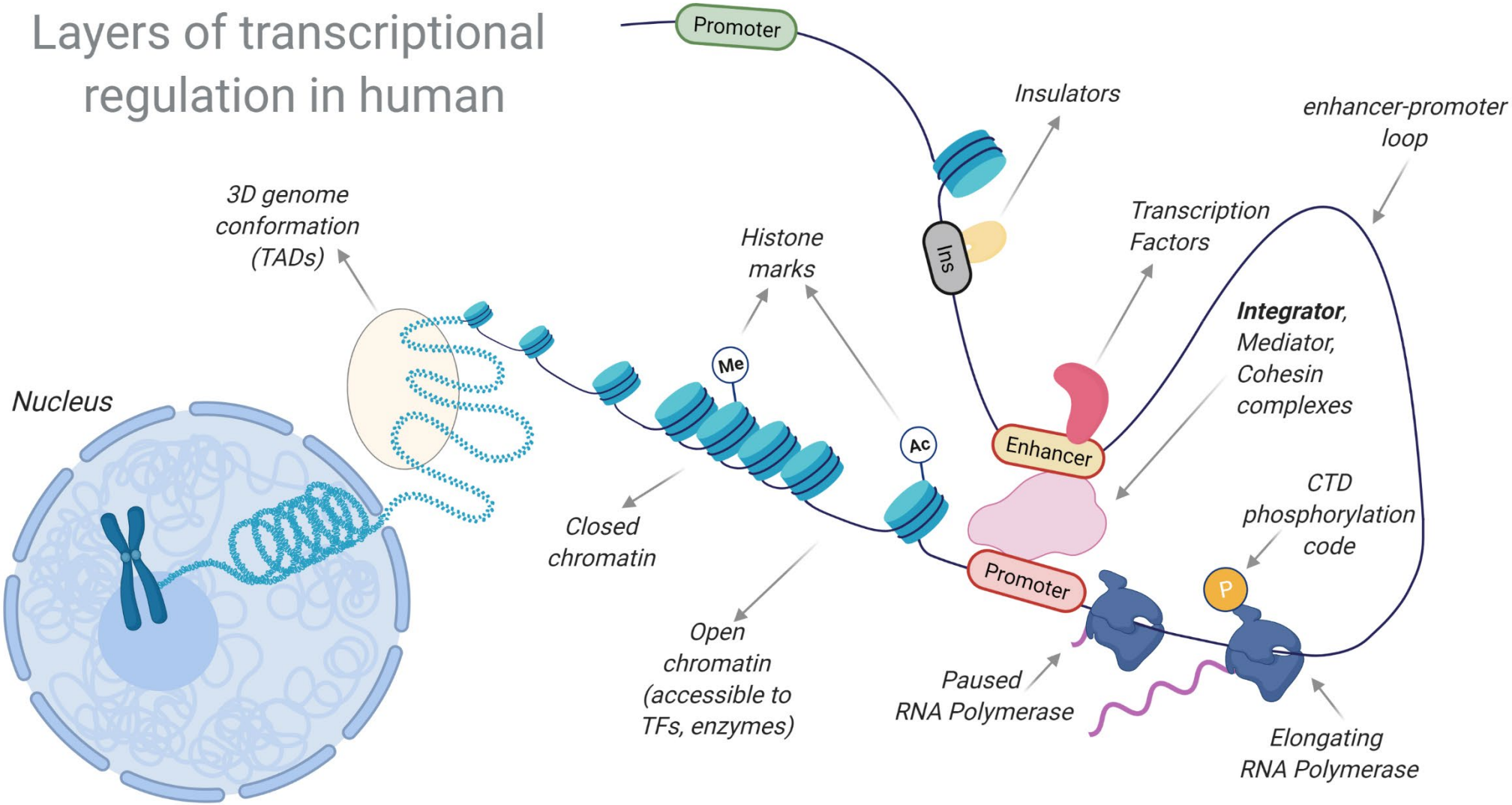
Role of chromatin conformation at enhancers



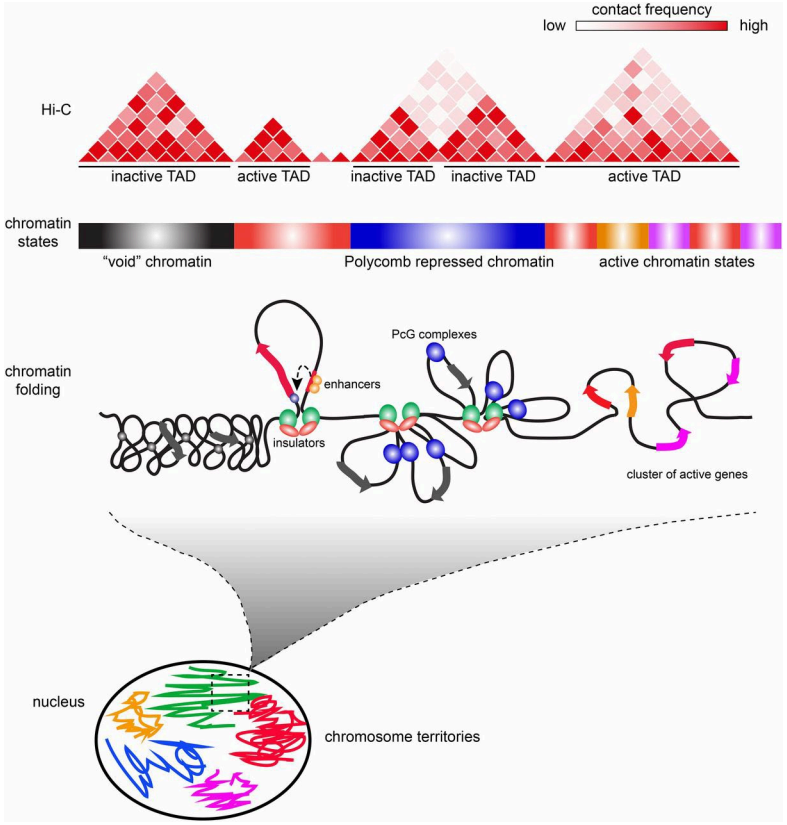


Chromatin looping is an essential step of enhancer-mediated gene activation

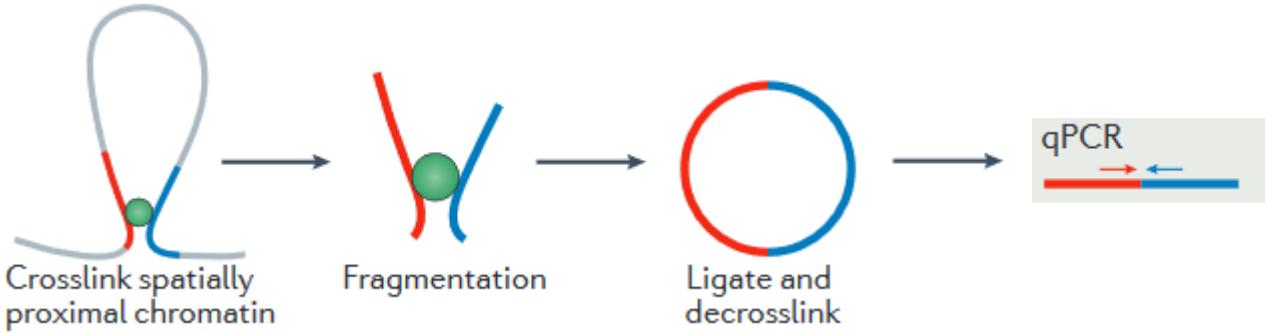
Layers of transcriptional regulation in human



How do we assay the 3D genome structure?

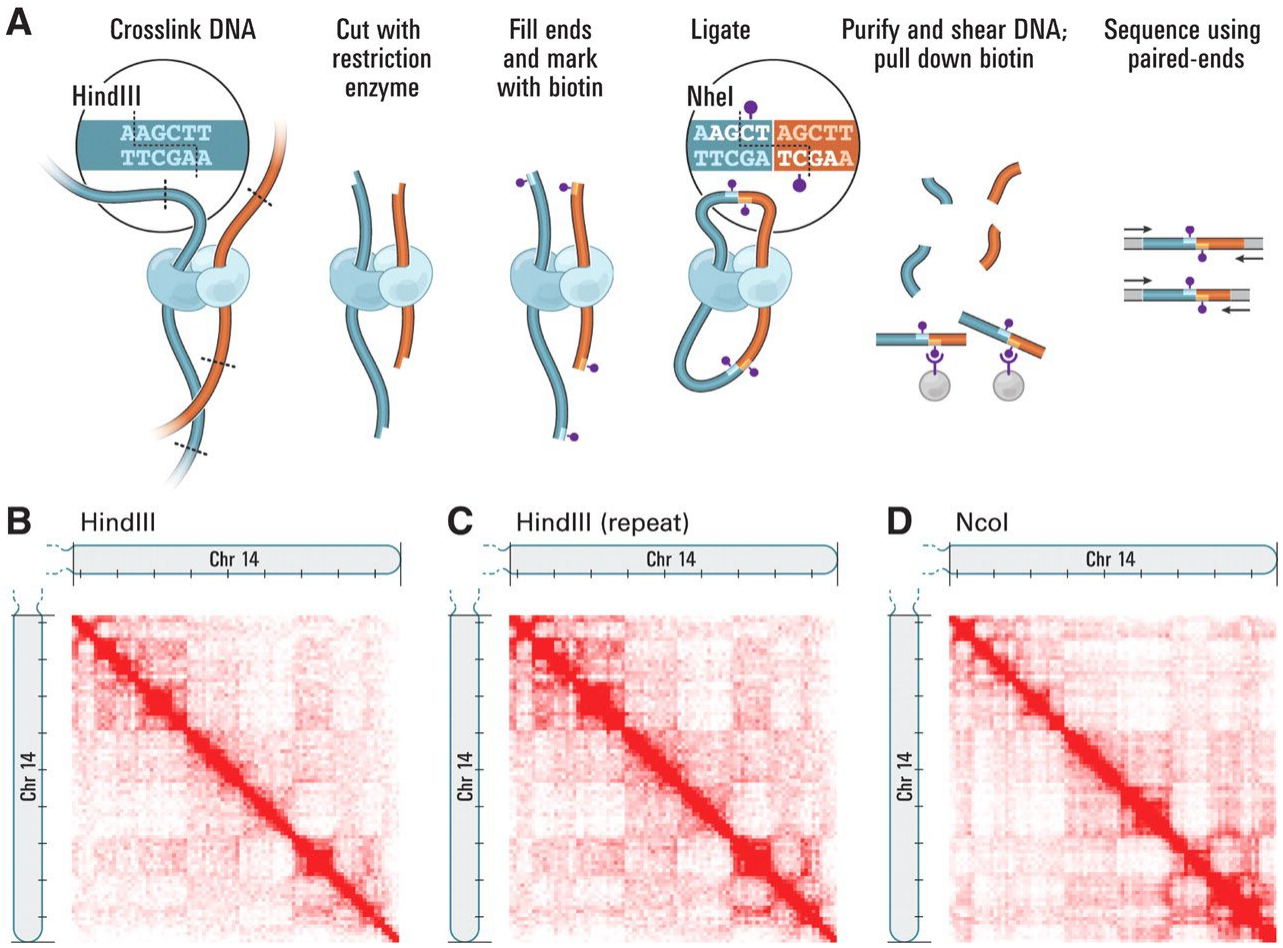


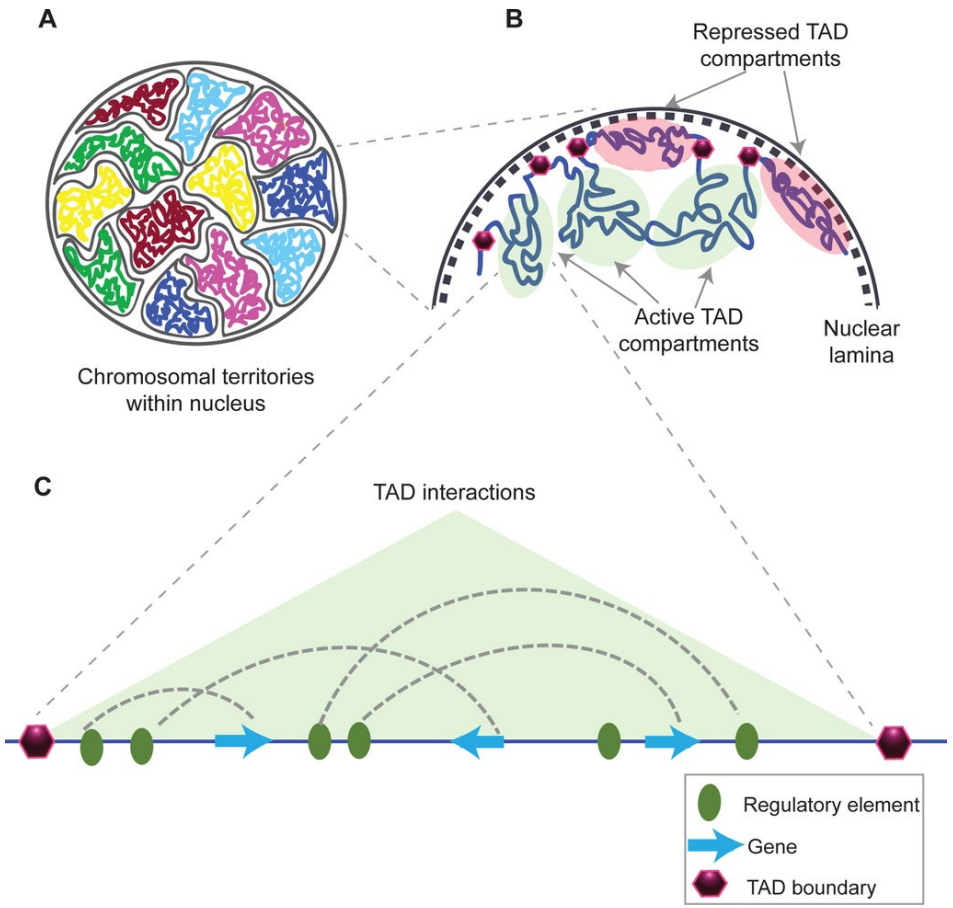
Chromosome Conformation Capture 3C



Modified from Krijger, de Laat, *Nat Rev Mol Cell Biol*. 2016

Scaling up: Hi-C maps of chromosome interactions




A
B
Repressed TAD compartments
Chromosomal territories within nucleus
Active TAD compartments
Nuclear lamina
C
TAD interactions
Regulatory element
Gene
TAD boundary

Is the 3D chromatin structure pathologically relevant?