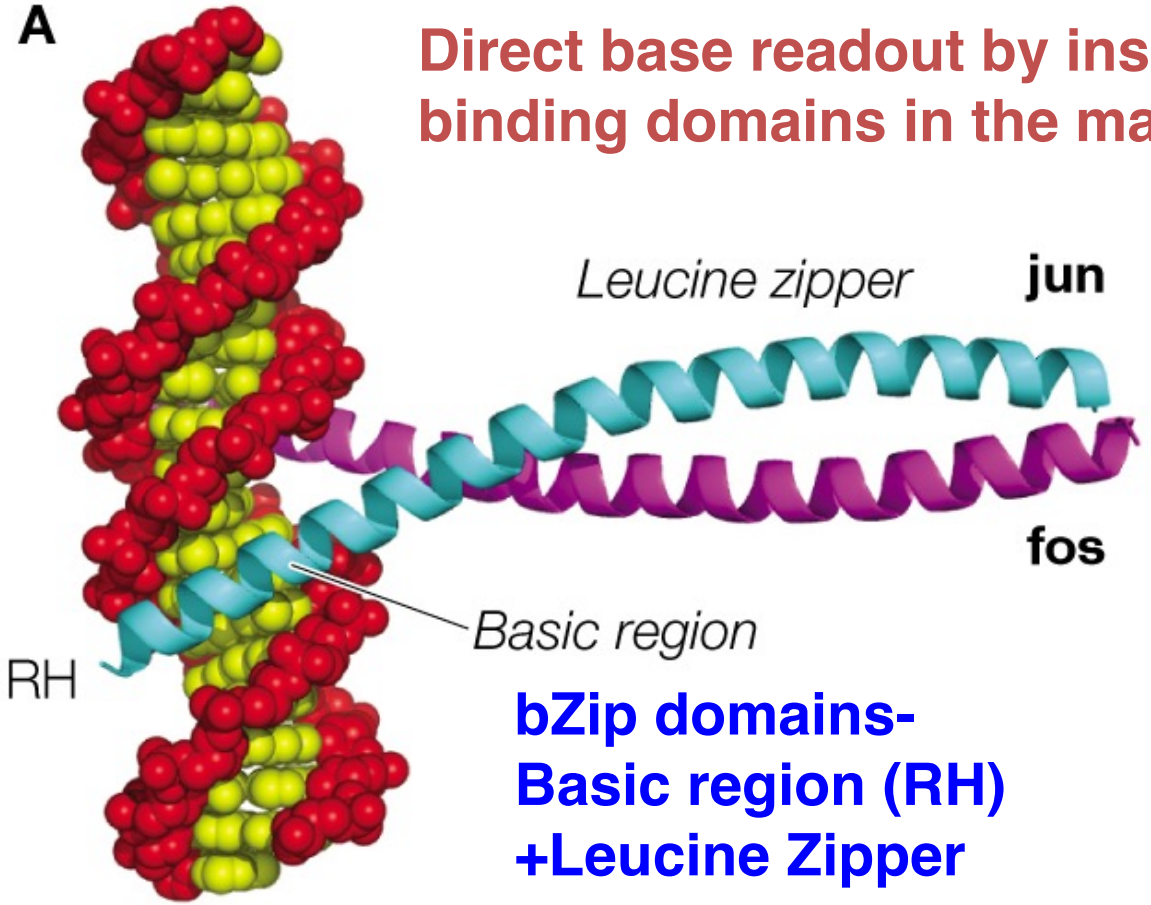
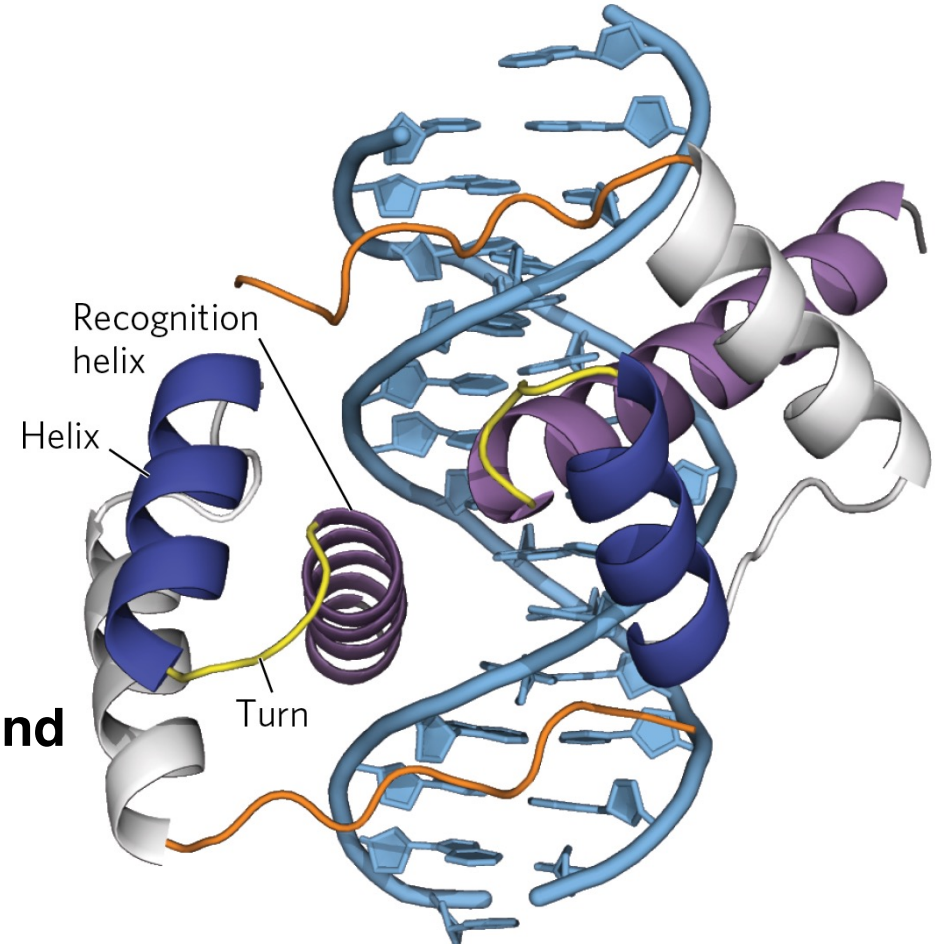
Direct base readout by insertion of alpha-helices of DNA binding domains in the major groove of DNA



bZip domains-
Basic region (RH)
+Leucine Zipper

RH = recognition helix (reads sequence)

Helix-turn-Helix
DNA binding domain

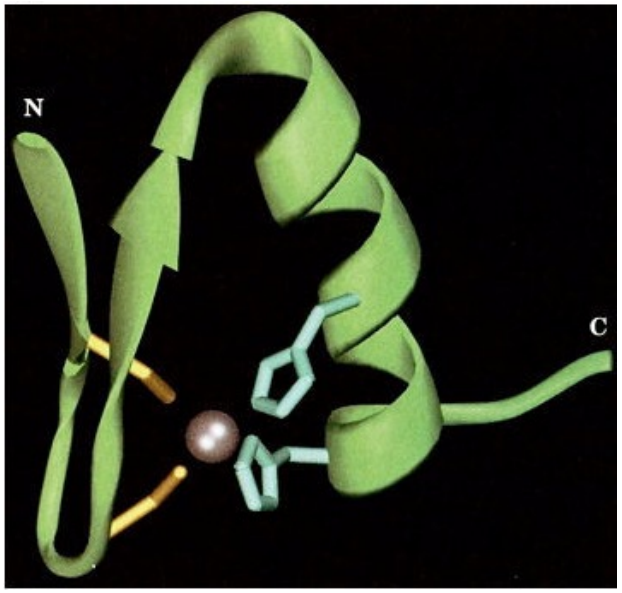


HTH = most frequent DBD in prokaryotes (e.g. Lac Repressor). Also found in eukaryotes. Example of Homeodomain proteins = Transcription Factors that control Development, e.g., Engrailed, Bithorax

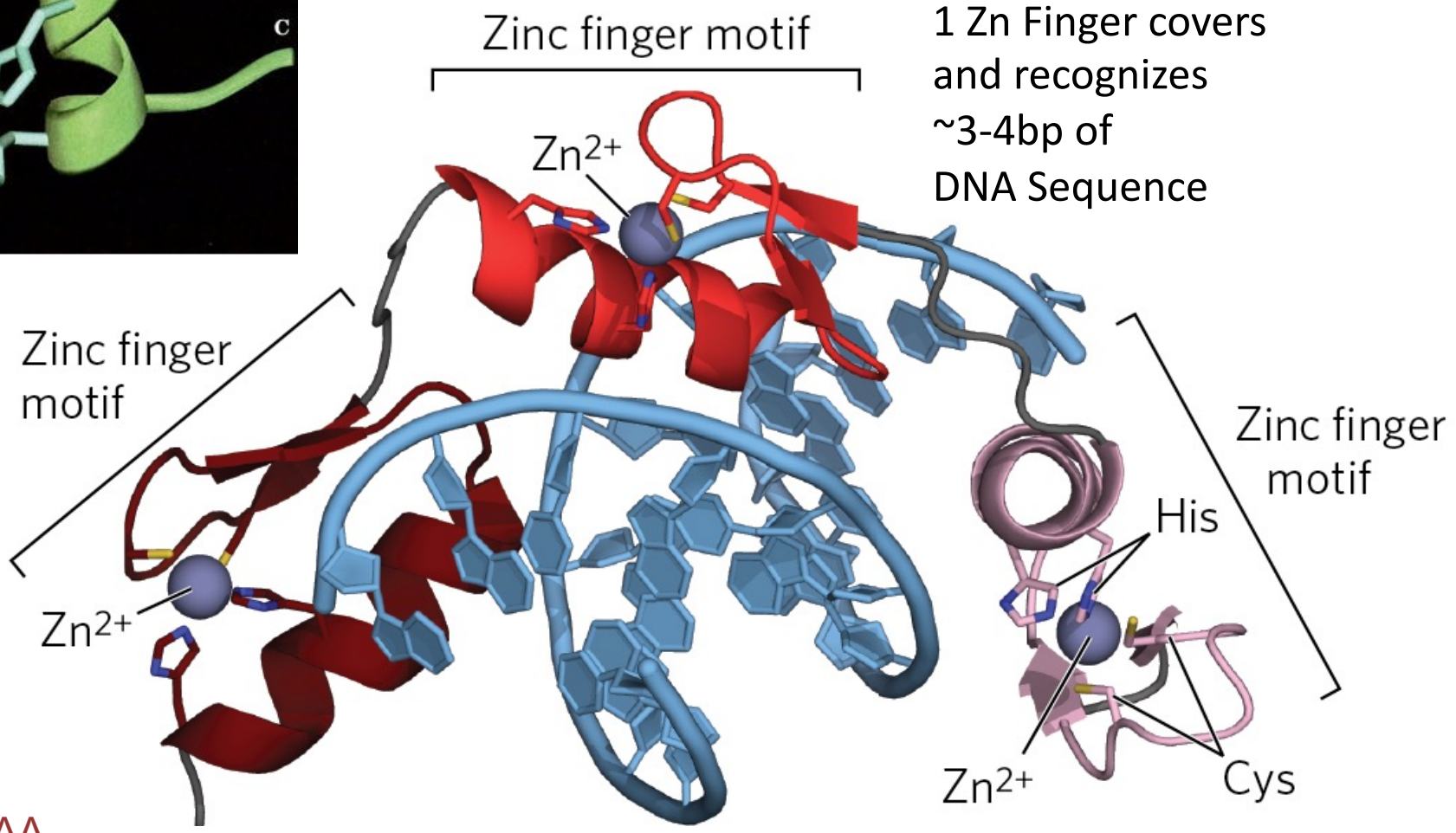
Direct base readout by α-helices of Zinc Fingers DNA binding domains:

1 Zn Finger

3D structure



Presence of several Zn Fingers on the same protein allows recognition of longer/more complex DNA sequences

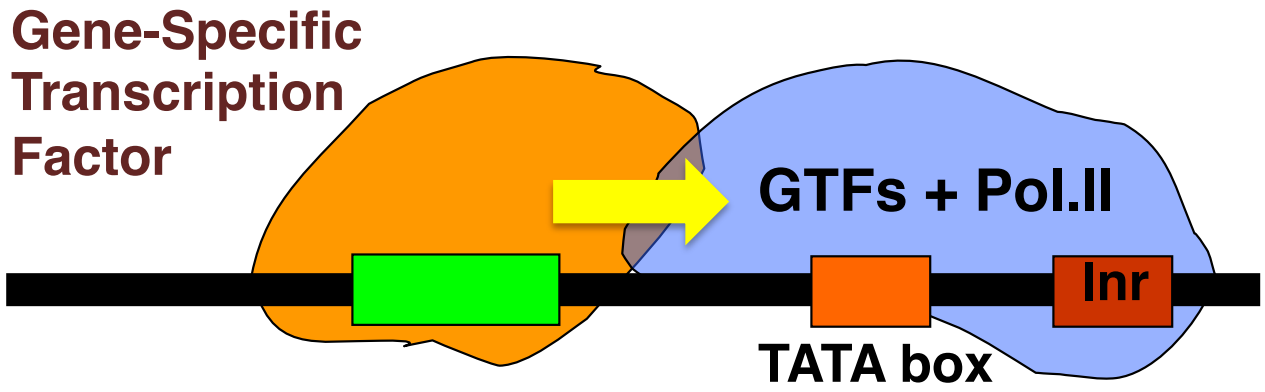


1 Zn Finger covers and recognizes ~3-4bp of DNA Sequence

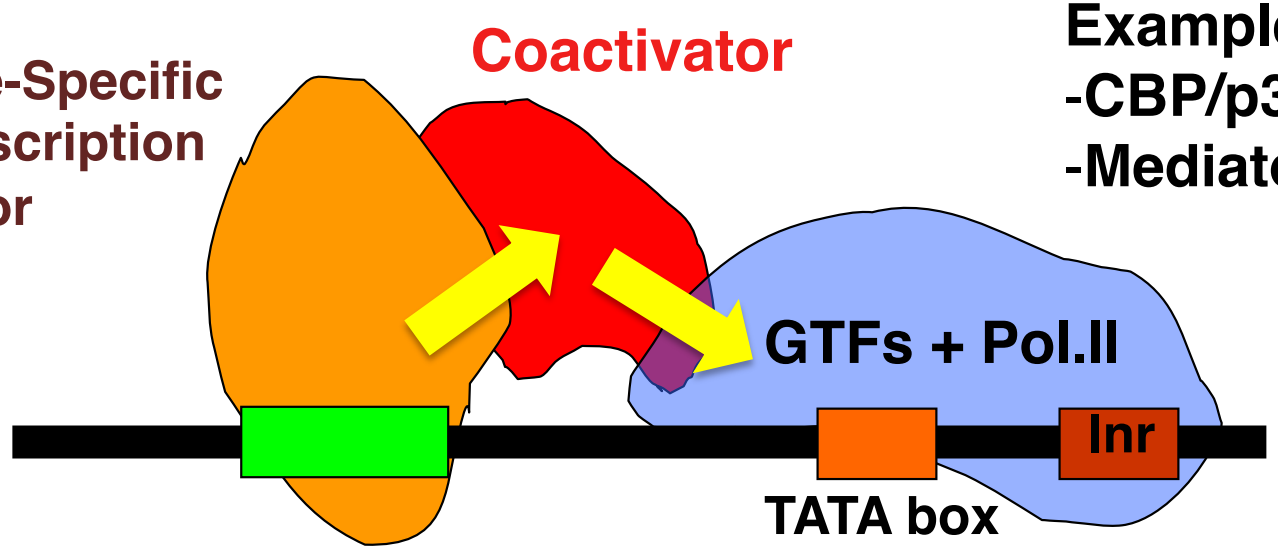
PDB ID = 1ZAA

Direct vs. Indirect Activation by Gene-Specific Transcription Factors

• **Direct Activation**: The Gene-Specific Transcription Factor interacts directly with the GTFs and/or RNA Polymerase II



• **Indirect Activation:** The Gene-Specific Transcription Factor does not interact directly with the GTFs and/or RNA Polymerase II and needs a **Coactivator**



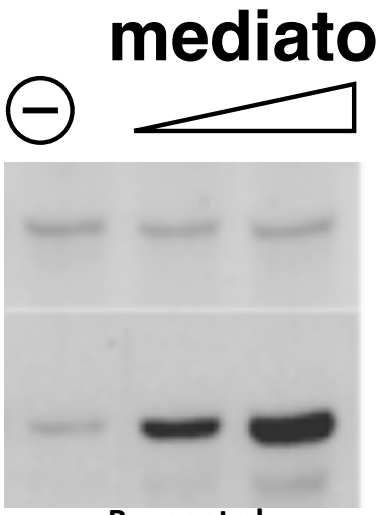
Examples of Coactivators:
-CBP/p300
-Mediator Complex

One Example of Coactivator complex: Mediator

In vitro transcription with two different promoter DNAs:

Promoter 1: ——— TATA – Inr

Promoter 2: – Enhancer – TATA – Inr



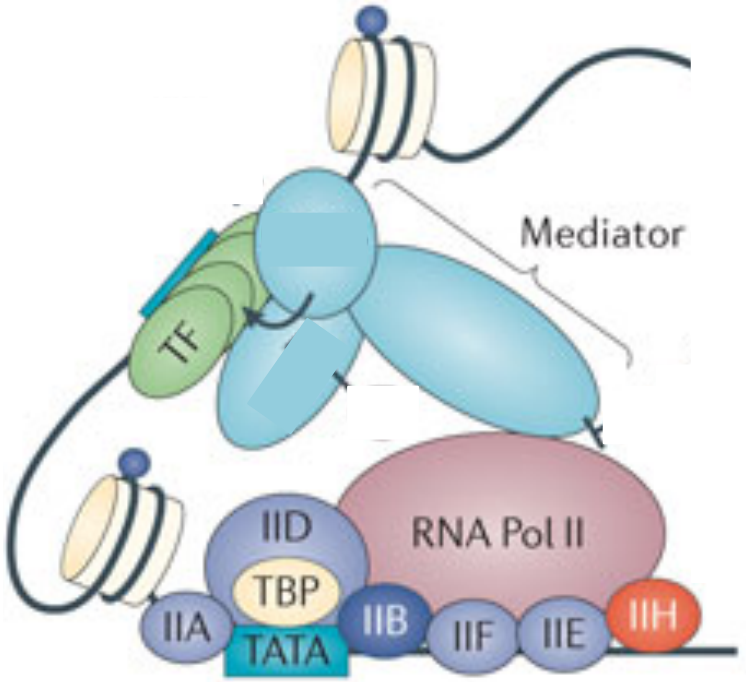
Boyer et al., Nature 1999

The reactions contain:
RNA Pol.II, GTFs, the transcription factor binding to the enhancer sequence
and increasing amounts of mediator complex

Conclusion: The presence of the mediator complex affects RNA produced by "activated" transcription, not by "basal" transcription

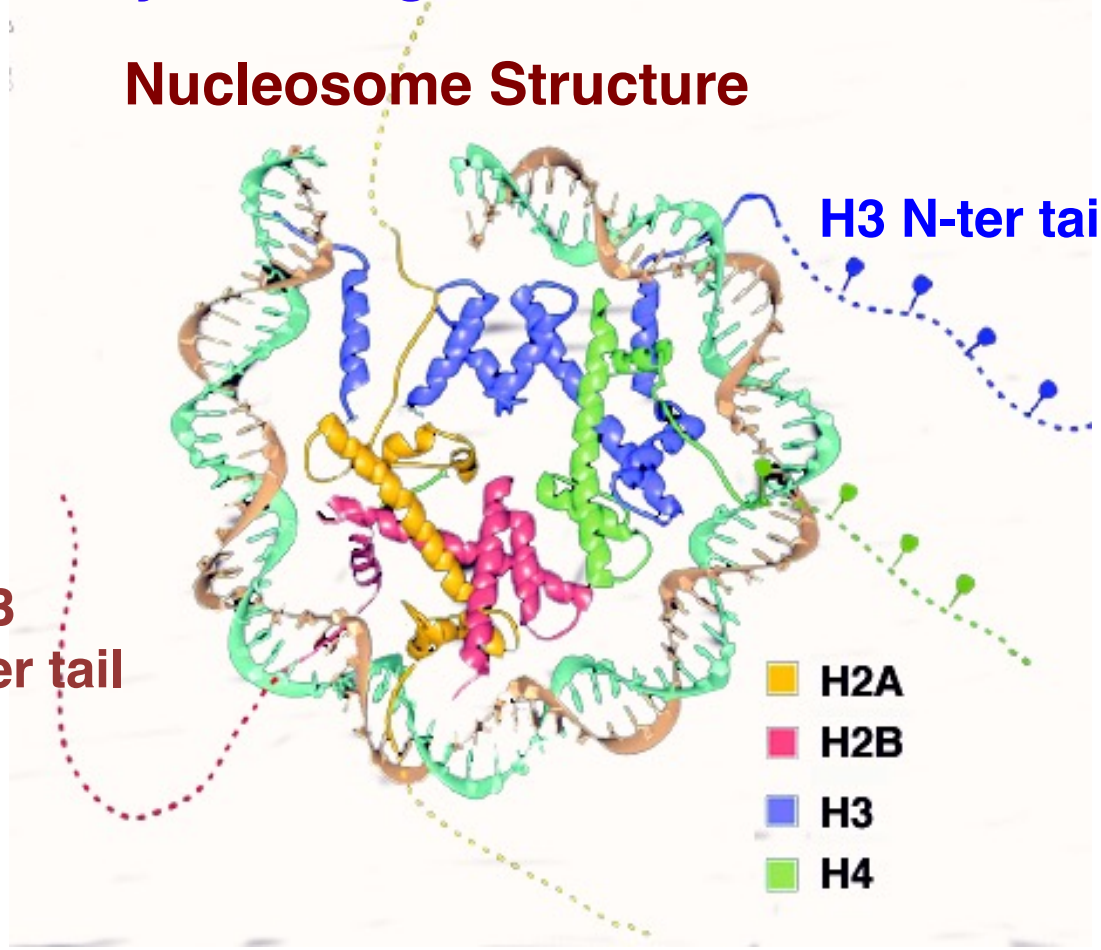
- The Mediator complex stimulates transcription of genes containing enhancer sequences.

- The action of the Mediator complex is dependent on the presence of transcription factors (TF) binding to the activator sequences.



Chromatin in Eukaryotic Cells
How can the transcription machinery access genes in this context ??

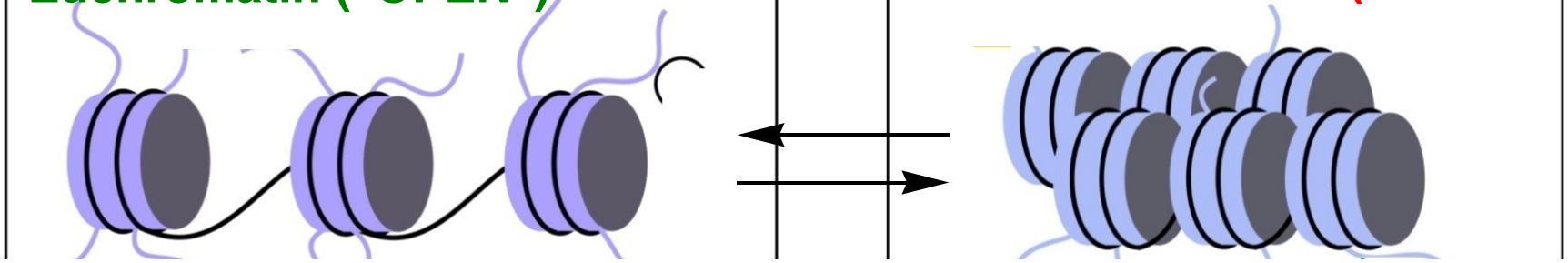
Covalent modifications of amino acids located on the the N-terminal tails of histones by:
- **acetylation**
- **deacetylation**
- **methylation**
- **ubiquitination**



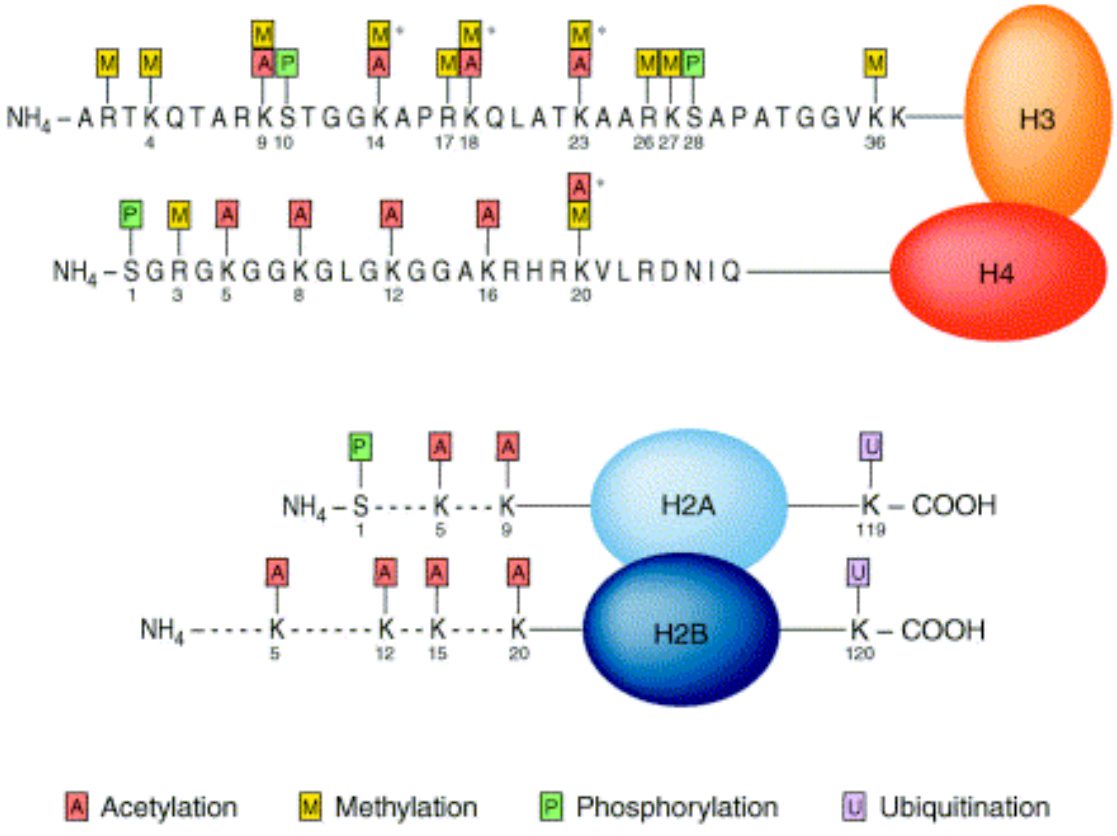
These covalent modifications influence the equilibrium between:

Transcriptionally Active State = Euchromatin ("OPEN")

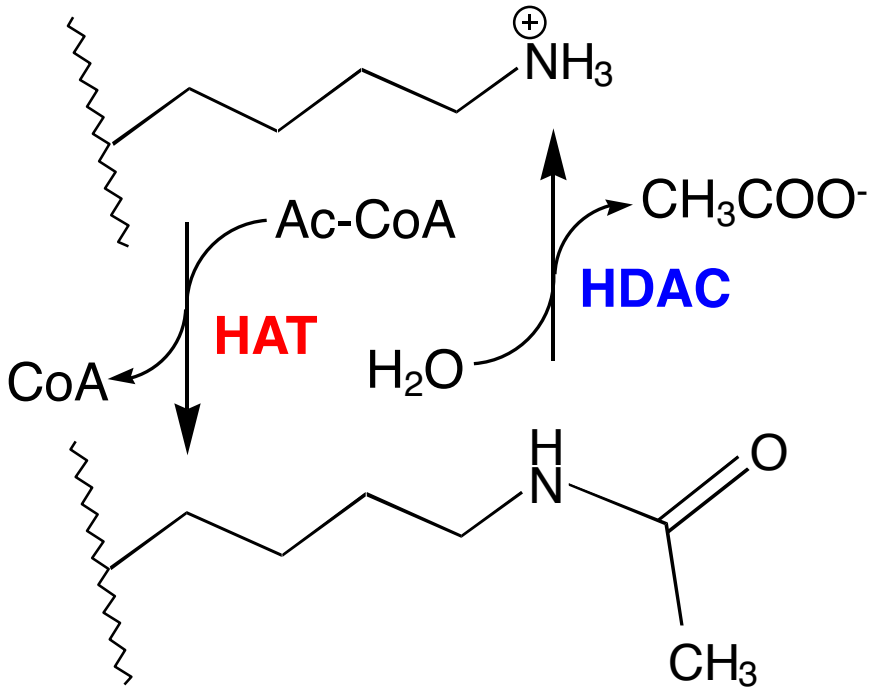
Transcriptionally Inactive State = Heterochromatin ("CLOSED")



Post-translational modifications of histones modulate chromatin accessibility and transcription



Lys side chain in a histone tail



HAT = histone acetyl transferase
HDAC = histone deacetylase

- **Acetylation of Lysines**
- **Methylation of Lysines/Arginines**
- **Phosphorylation of Serines/Arginines**
- **Ubiquitination of Lysines**

Covalent modifications are transmitted to daughter cells during DNA replication (see histone chaperones) –> epigenetic marks

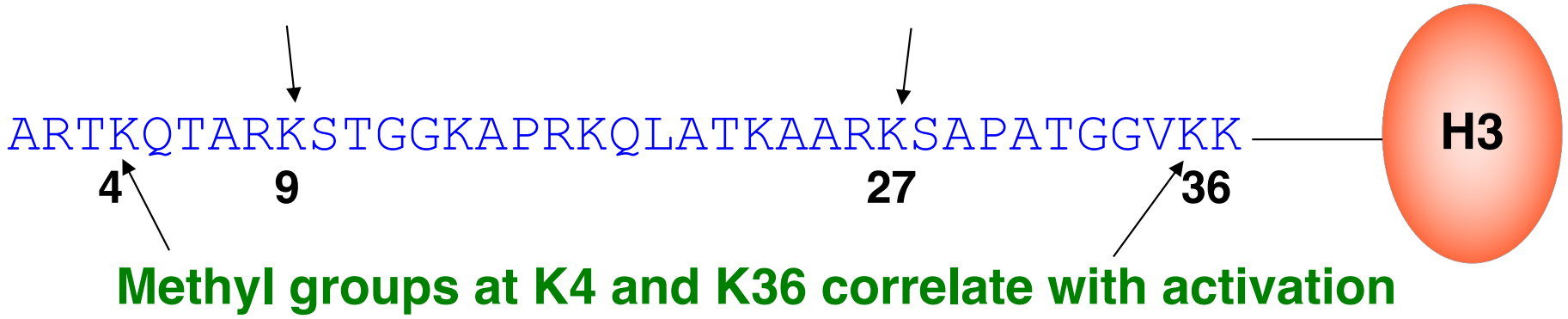
Current Opinion in Plant Biology 2002

Histone post-translational modifications and transcriptional activity

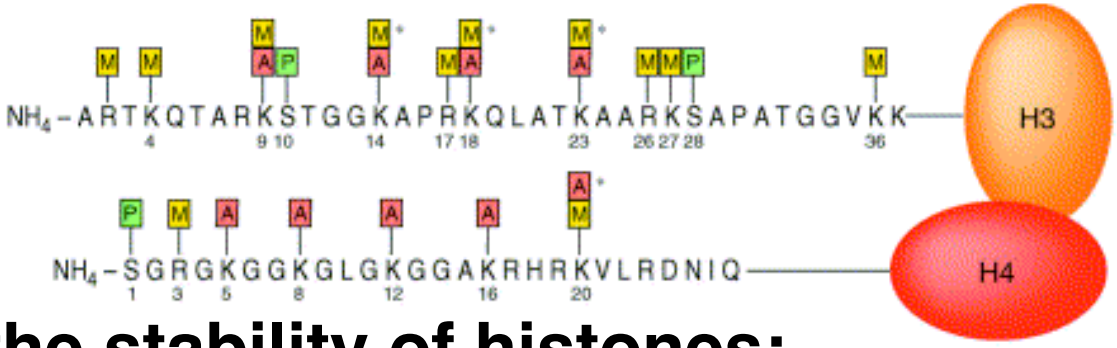
Lysine acetylation and methylation are of primary importance:

- **Lysine acetylation generally leads to activation (some exceptions)**
- **Lysine methylation can lead to activation or repression depending on the site**

Example of modifications at specific residues for histone H3:



How do these covalent modifications of histones influence transcriptional activity?



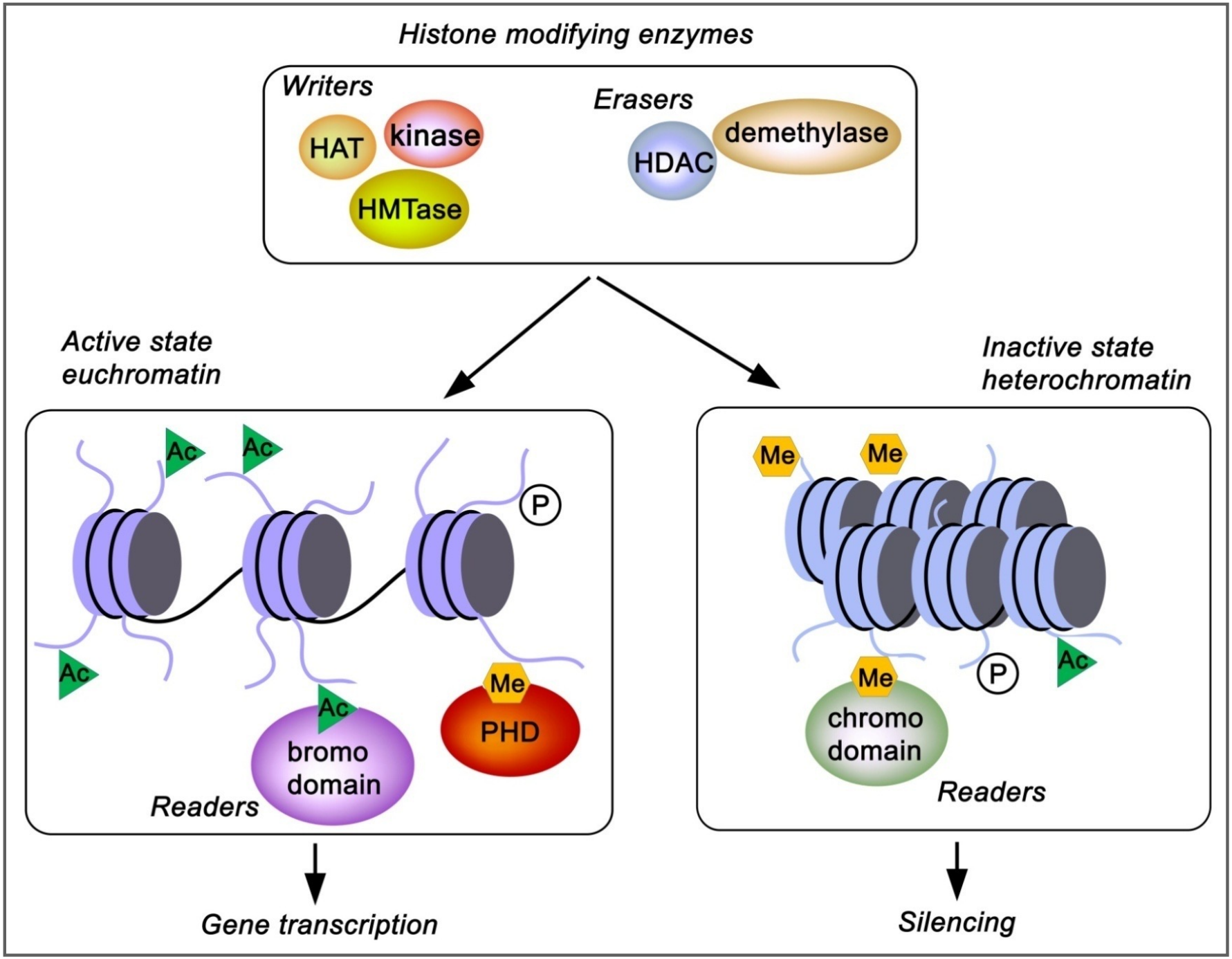
A: Modifications change the stability of histones: ubiquitination triggers degradation and thus limit nucleosome formation

B: Modification of the N-ter tails of Histones changes the ability of Histones to form nucleosomes and therefore affect compaction and accessibility to RNA polymerase II

C: Modifications are recognized by specific proteins which remodel the chromatin and make it more/less accessible to the transcriptional machinery

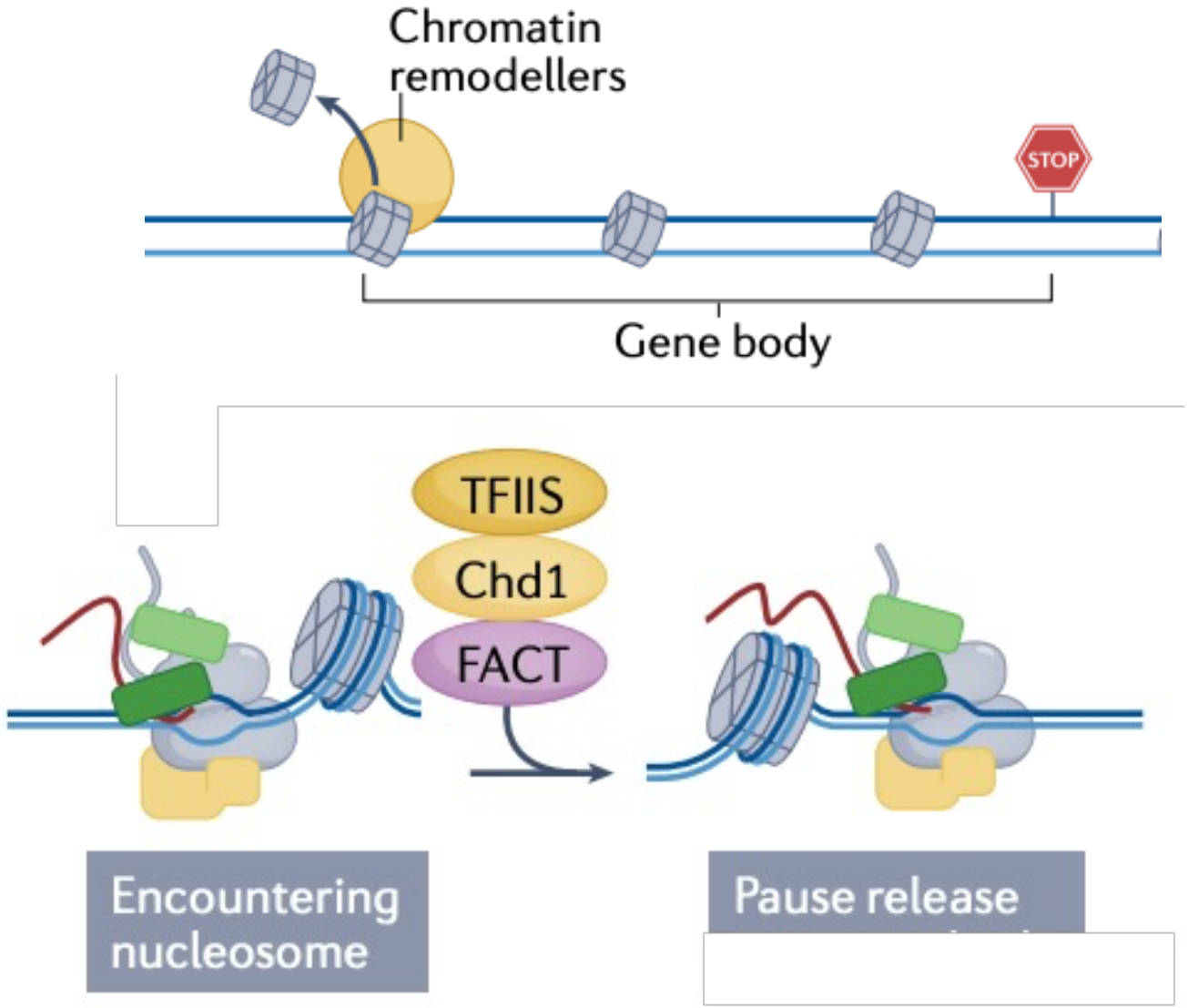
D: Since modifications affect mostly K/R residues (+), they change the net charge of histones and thus DNA binding

Histone modifications modulate transcriptional status by recruiting "readers" which modify chromatin state/accessibility



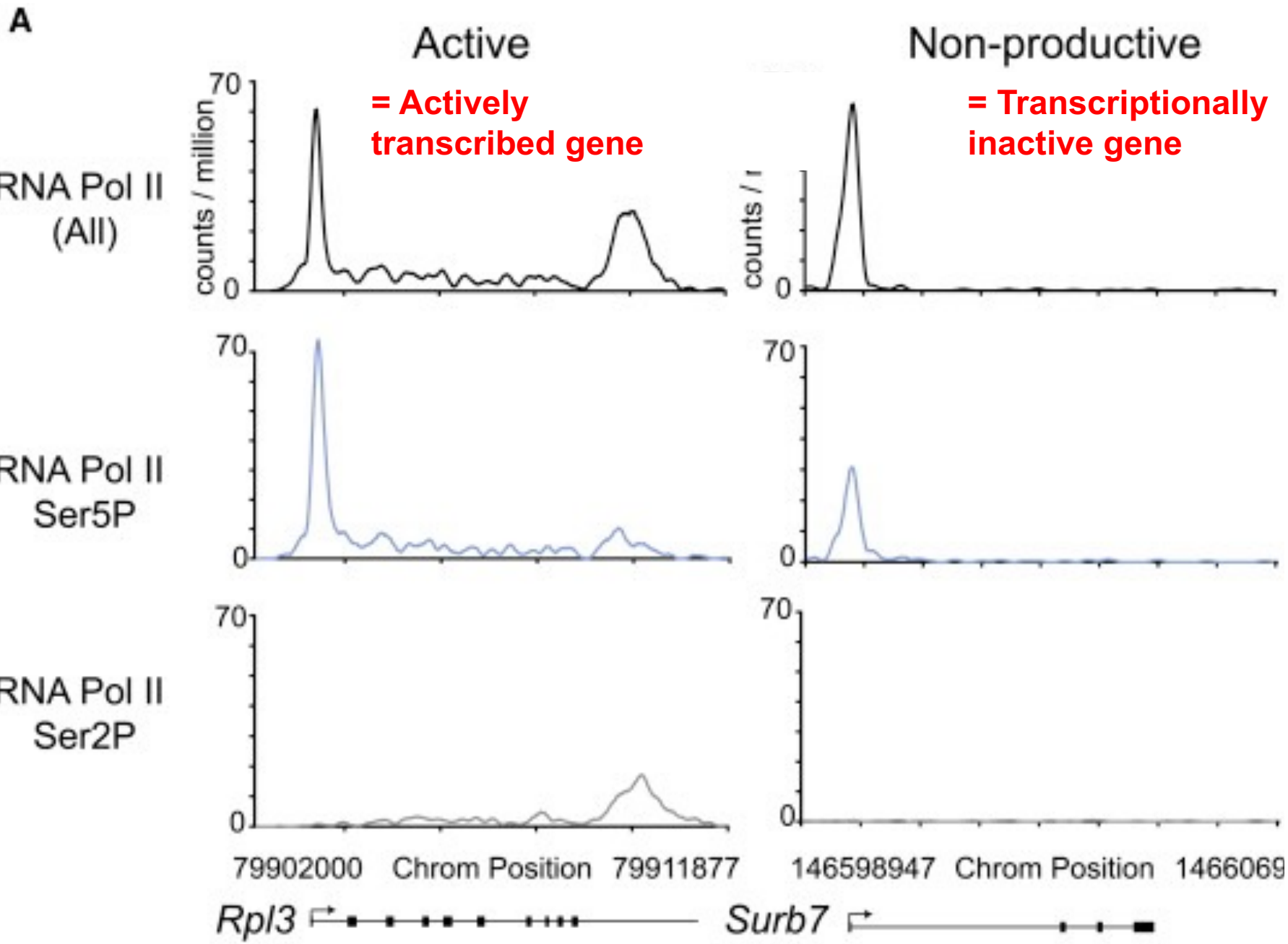
Transcription through Gene Body requires remodeling of the chromatin by specific enzymes (Chromatin Remodelers) which:

- **allow RNA Pol.II to transcribe through the gene body**
- **reconstitute the nucleosome after transcription by RNA Pol.II**



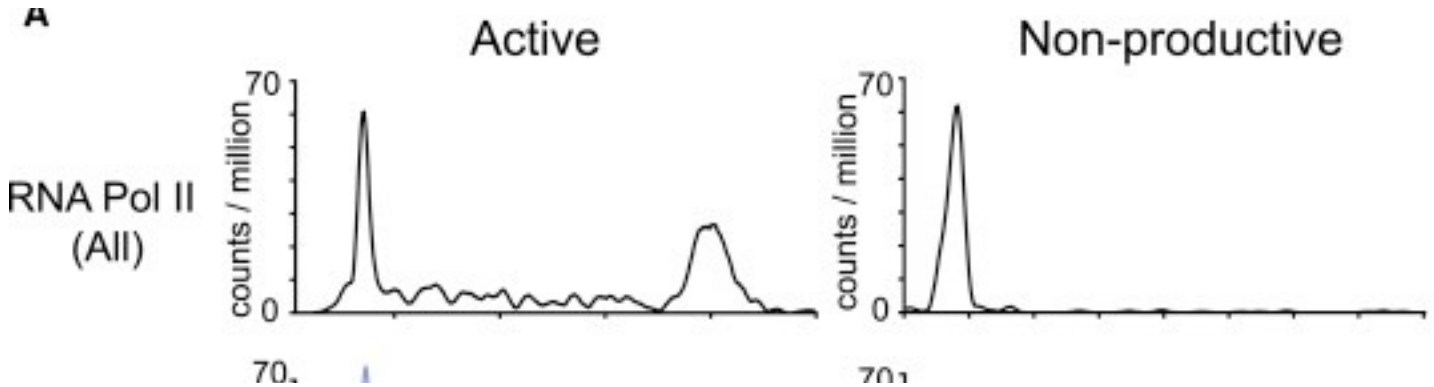
Girbig et al. Nat. Rev. Mol. Cell Biol. 2022 |

Transcriptional Control by regulation of RNA Pol. II elongation: promoter proximal pausiing



Rahl et al. Cell 2010

What is the conclusion from this part of the experiment



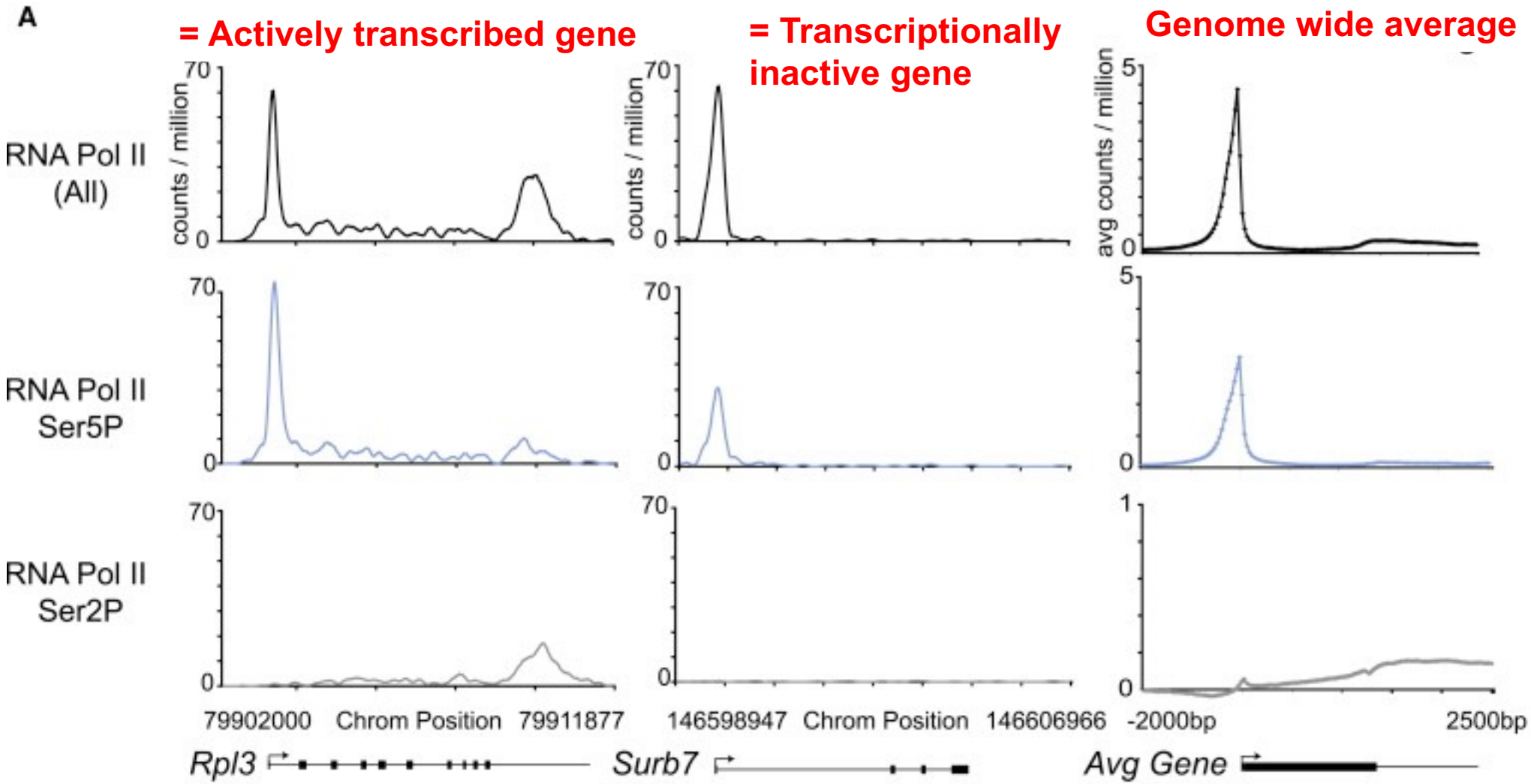
A: There is a lot of Pol.II phosphorylated at Ser5 near promoters

B: There is a lot of Pol.II bound to promoters, regardless of whether or not the genes are transcribed actively

C: There is more RNA corresponding to the 5´ region of genes regardless of whether or not the genes are transcribed actively

D:Pol.II only reaches the 3´-end of genes when genes are transcribed actively

Transcriptional Control by regulation of RNA Pol. II elongation

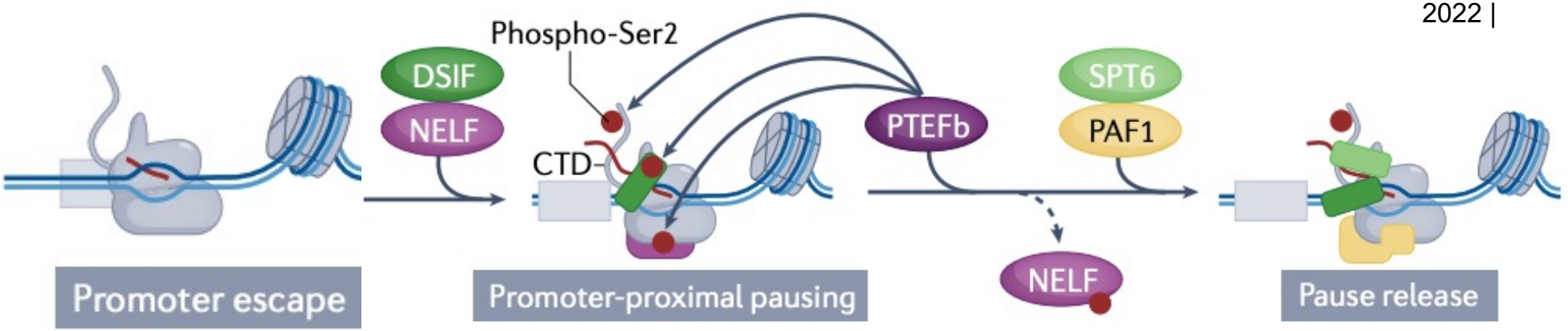


Chromatin immunoprecipitation of RNA pol.II across genomes reveals the enrichment of the polymerase nearby the transcription start site ("poised")-- whether the gene is actively transcribed or not -- These polymerases are phosphorylated at Ser5 on the CTD

Rahl et al. Cell 2010

Promoter proximal pausing is controlled by the pausing factor NELF and helps control gene activation by rapid switch from paused state to productive elongation

Girbig et al. Nat. Rev. Mol. Cell Biol. 2022 |



► **Pausing factors DSIF/NELF are detected with Pol II in promoter-proximal regions**

► **P-TEFb stimulates Pol II pause release at most active genes by:**

- **Phosphorylating S2 of the CTD repeats**
- **Phosphorylating DSIF and NELF which results in NELF release**
- **Promoting binding of the PAF1 elongation complex**