Regulation via the lac repressor = *negative regulation*

The lac operon also undergoes *positive regulation*.

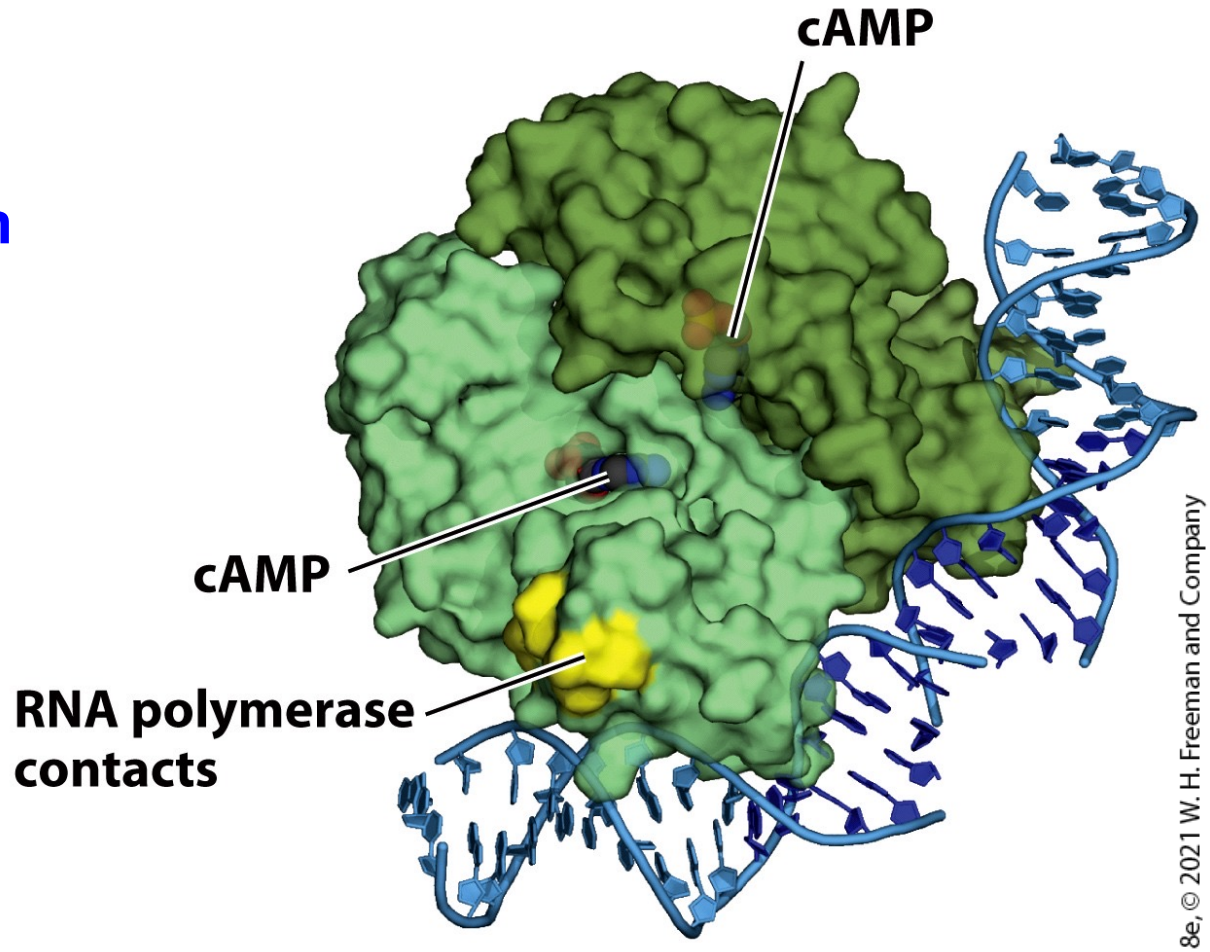
- **Glucose is the preferred energy source in E. coli**
- **If glucose is abundant, we don't want to express the genes that metabolize other sugars**

DNA Binding by CAP a.k.a.CRP
Catabolite Activator Protein
= Cyclic AMP Receptor Protein

High Glucose -> low cAMP
-> No binding of CAP

Low Glucose -> High cAMP
Binding of CAP to Lac promoter

CAP facilitates binding of the RNA polymerase to the Lac Promoter



When lactose is absent → very little transcription

Whether [glucose] is high or low, <u>if lactose is absent</u>:

- repressor stays bound
- no transcription even when

CRP-cAMP binds DNA

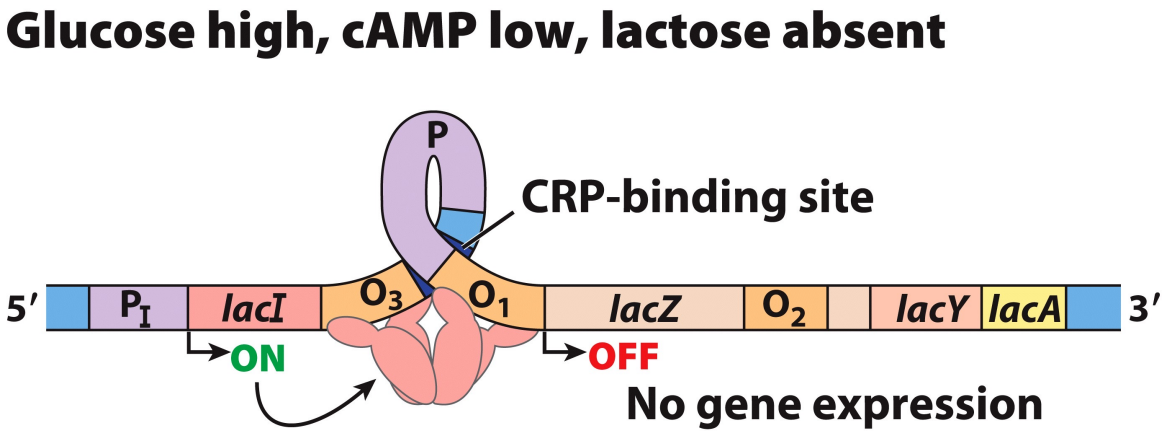


Figure 28-17a
Lehninger Principles of Biochemistry, Sixth Edition

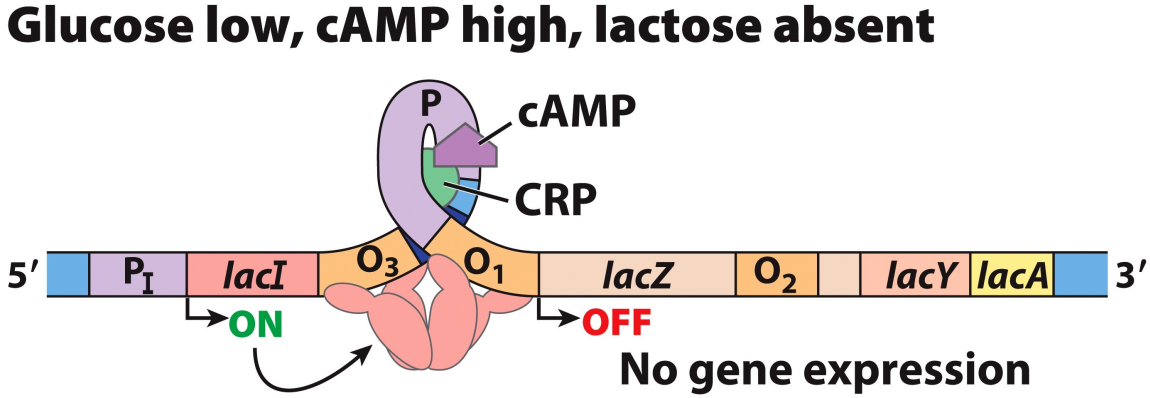


Figure 28-17b
Lehninger Principles of Biochemistry, Sixth Edition

When lactose is present, transcription levels depend on glucose

- **Repressor dissociates, but transcription only stimulated significantly if cAMP rises due to low glucose levels:**

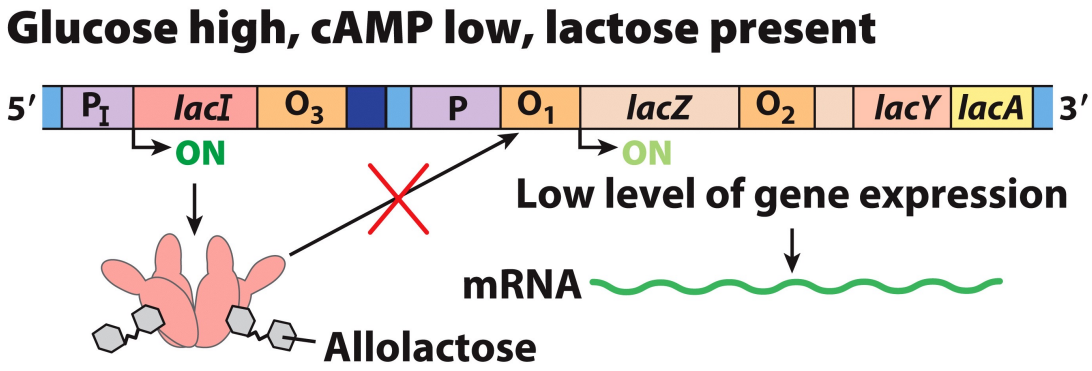


Figure 28-17c
Lehninger Principles of Biochemistry, Sixth Edition

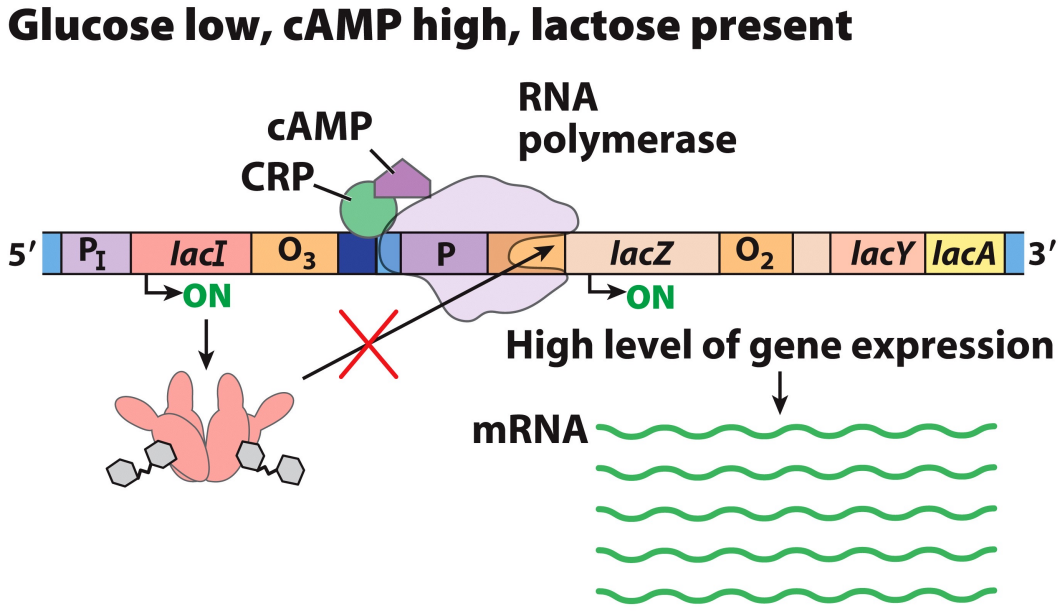
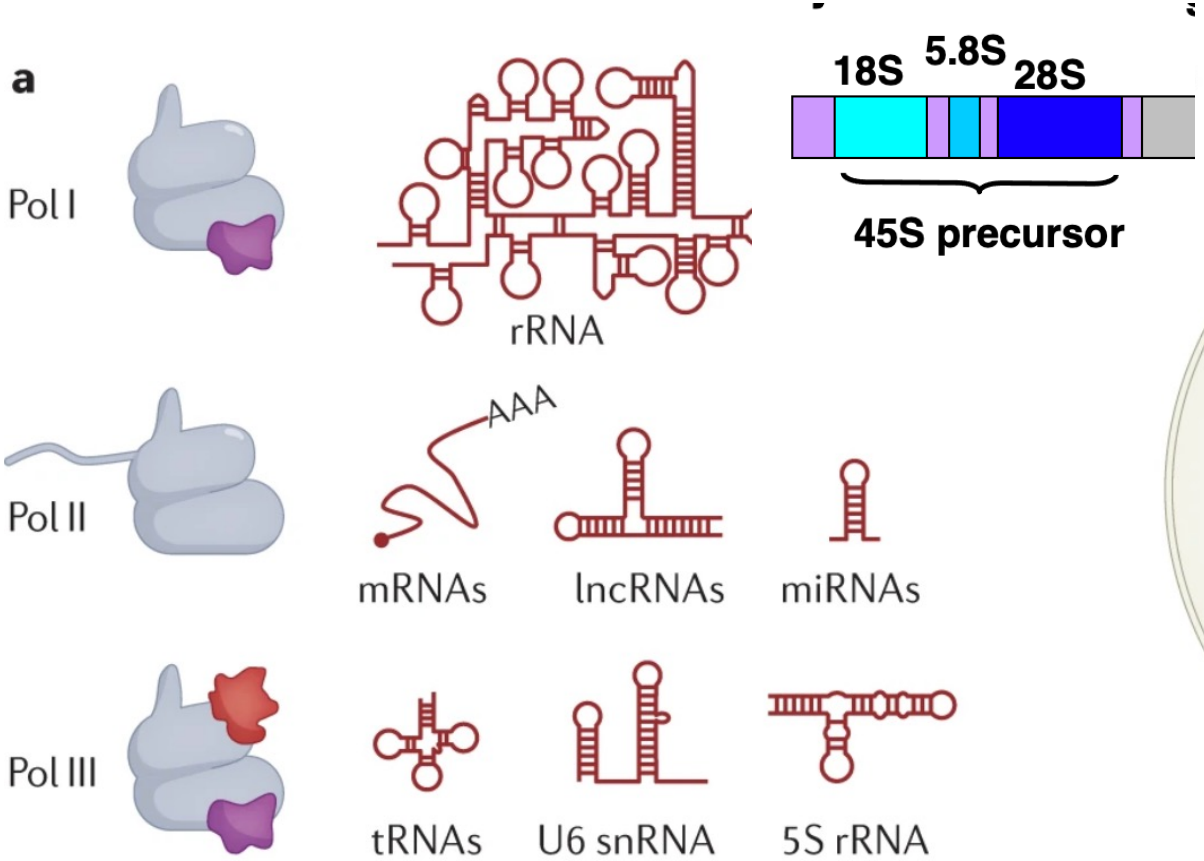
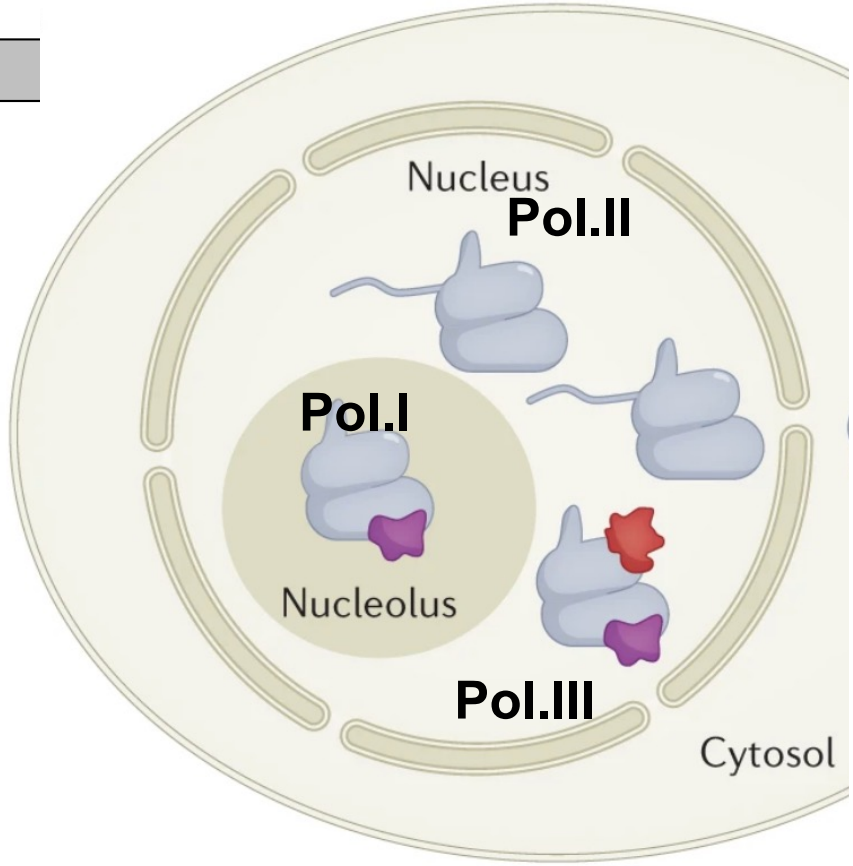


Figure 28-17d
Lehninger Principles of Biochemistry, Sixth Edition

Three RNA polymerases in eukaryotes

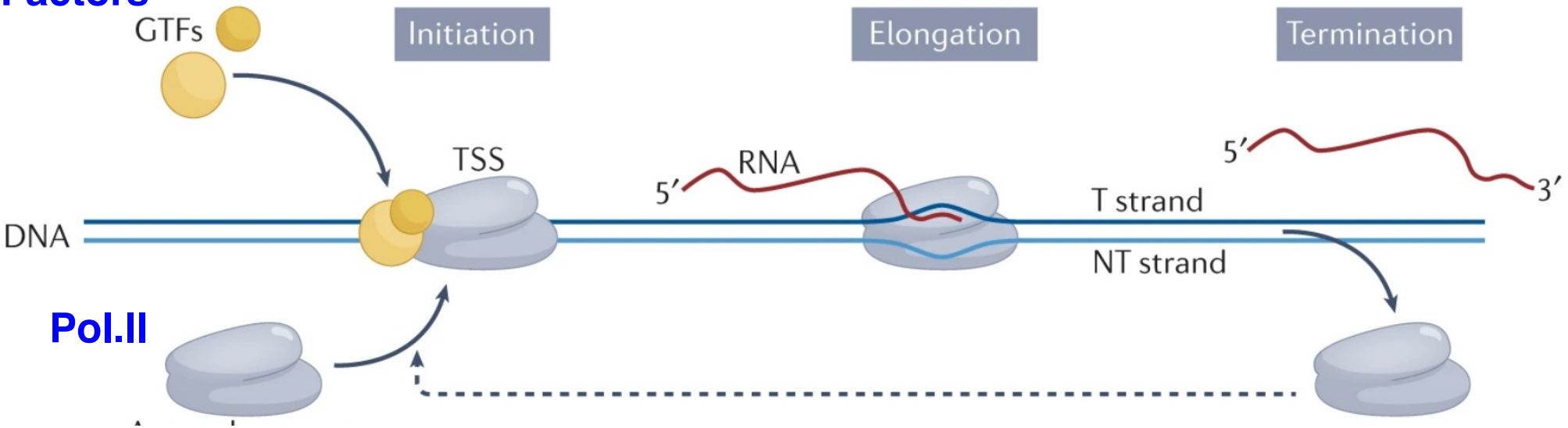




Girbig et al.
Nat. Rev.
Mol. Cell Biol.
2022 |

The basic transcription cycle for RNA Polymerase II

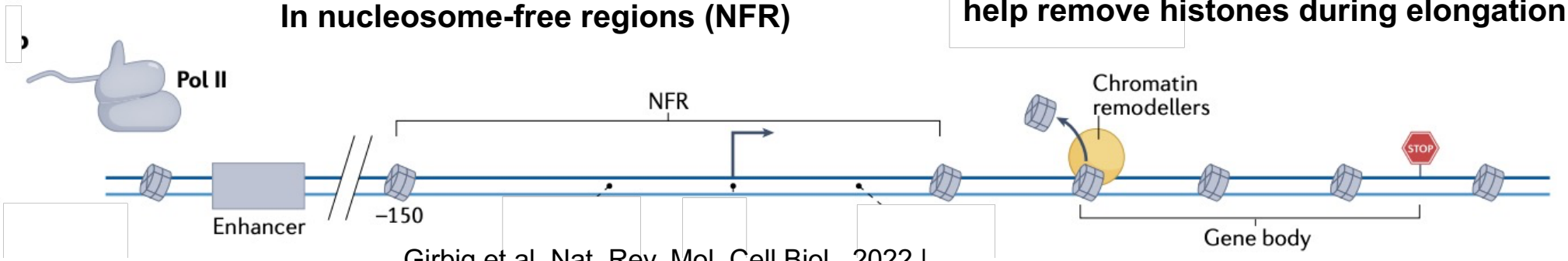
General Transcription Factors



Pol.II

Assemble onto promoters
In nucleosome-free regions (NFR)

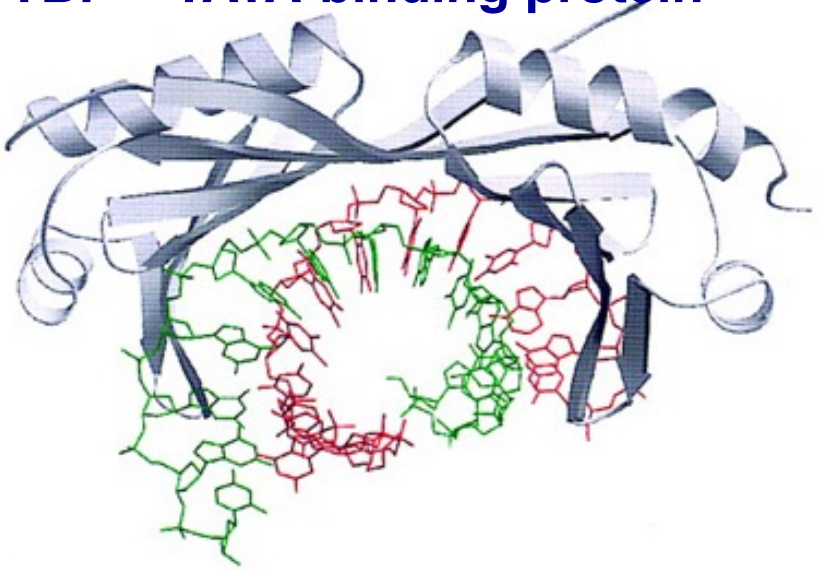
Chromatin Remodeling Enzymes help remove histones during elongation



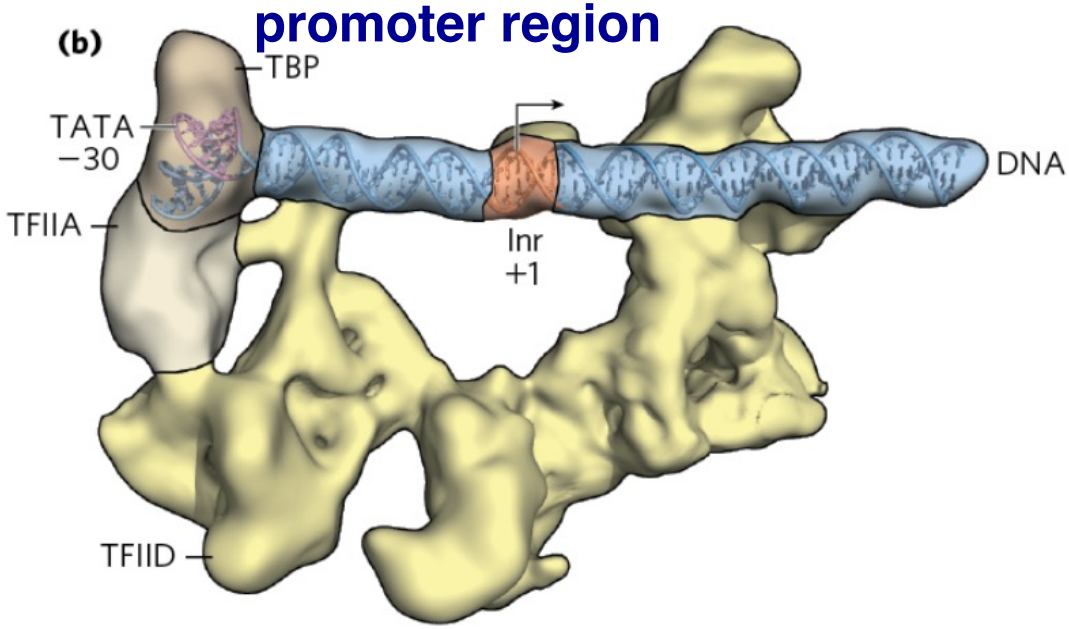
Girbig et al. Nat. Rev. Mol. Cell Biol. 2022 |

General Transcription Factors
TFIID & TBP (TATA-binding) recognize Pol.II Promoters

TBP = TATA-binding protein



Binding of TFIID and TBP to the promoter region



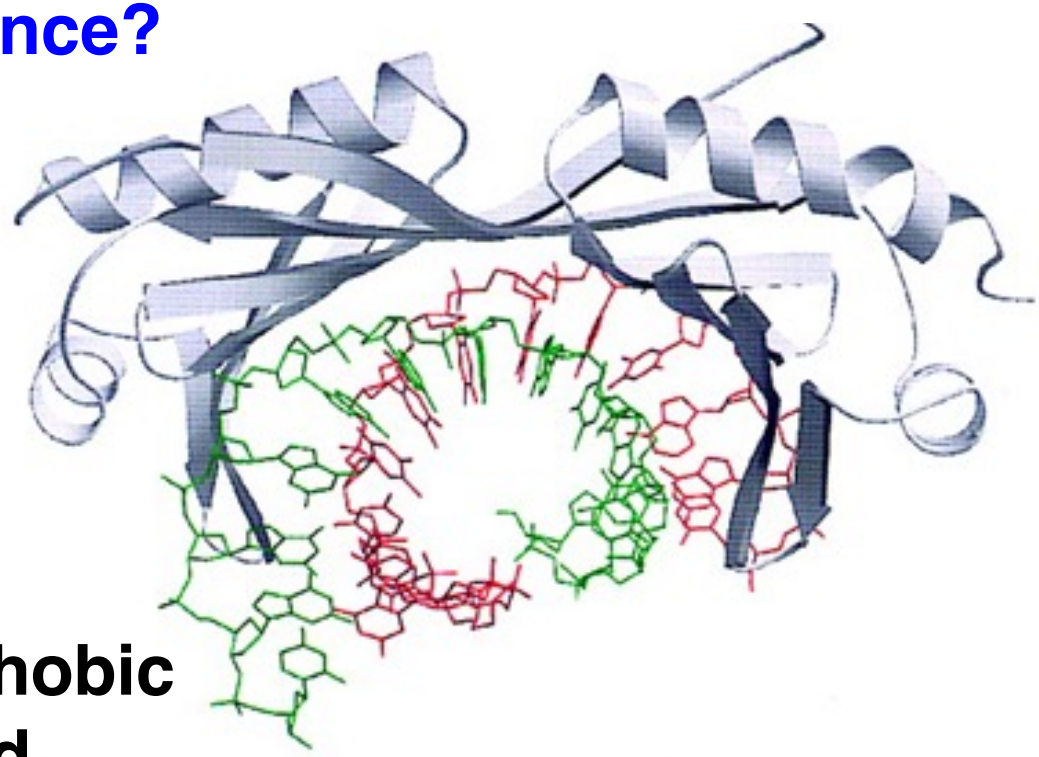
TBP consensus sequence: TATAWAW (W = A or T)

Inr sequence: initiator sequence, where transcription begins

Pymol: TATA_TBPcomplex.pse

https://www.ibiology.org/techniques/transmission-electron-microscopy/

What strategy does TBP use to recognize the TATA box sequence?



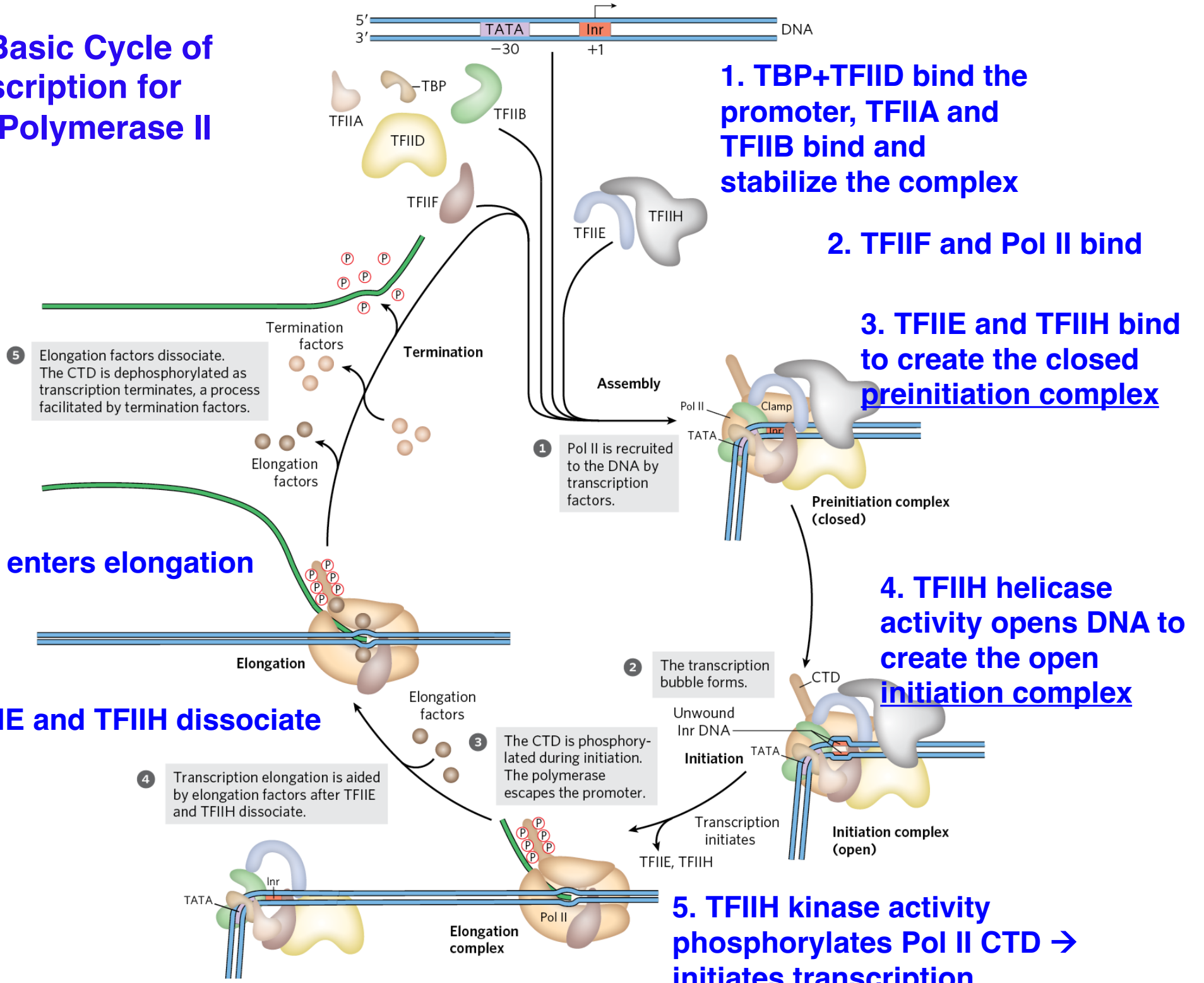
A: It inserts side chains in the major groove of the TATA sequence

B: It builds a cavity of hydrophobic amino acids that can only bind Ts and As

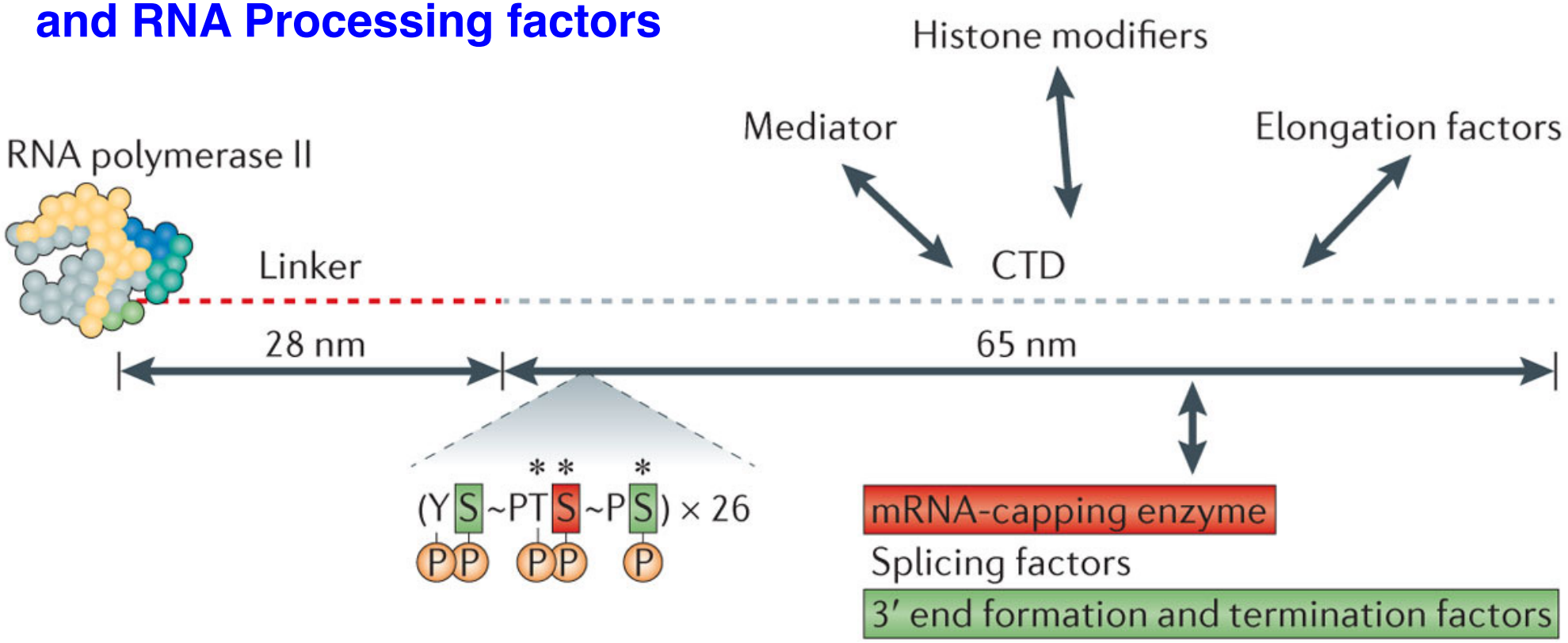
C: It recognizes the curvature of the DNA that is created by the high roll of T/A steps in DNA sequence

D: It makes sequence-specific contacts with the minor groove side

The Basic Cycle of Transcription for RNA Polymerase II



Covalent modifications of the C-Terminal Domain (CTD) of the largest subunit of RNA Polymerase II (Rpb1) and interaction of the CTD with transcription and RNA Processing factors



P = phosphorylation (P) sites

Nature Reviews | Genetics

Eukaryote-specific features of transcriptional control

- Gene-specific transcription factors
(as opposed to sigma factors)

- Coactivators of transcription
these do not bind DNA but help enhance transcription

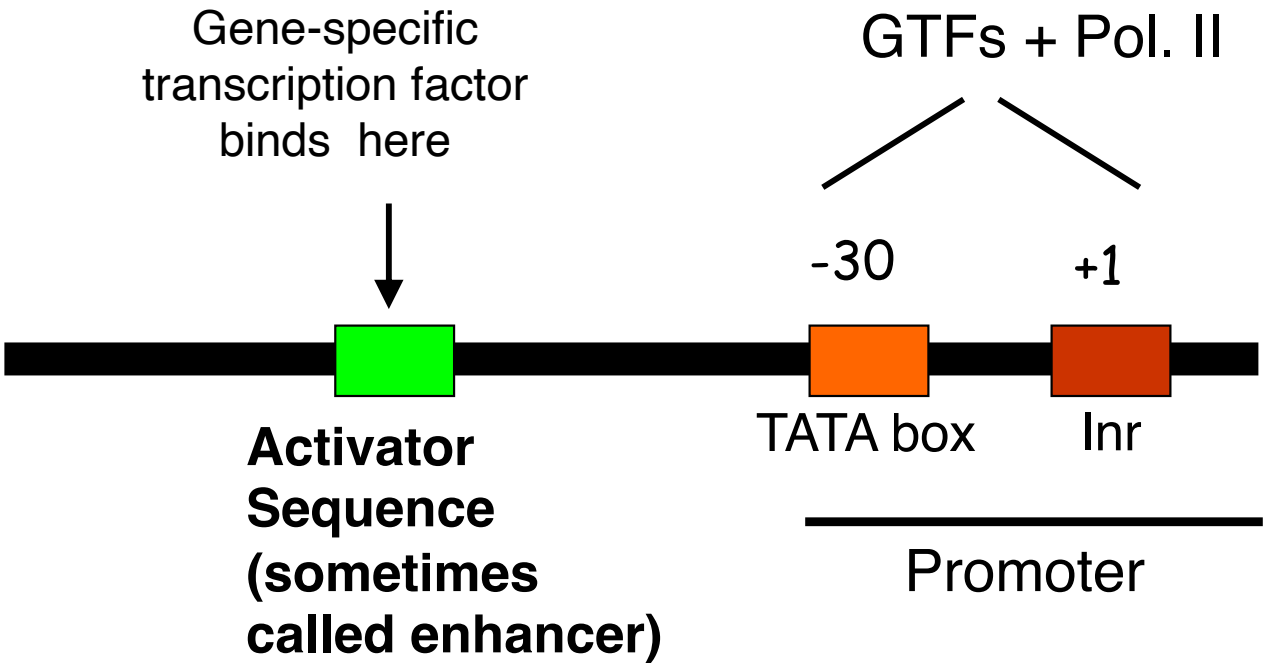
- Transcriptional control and chromatin modifications
("epigenetics")

(control of transcription by controlling RNA Pol.II binding)

- Pausing of the RNA polymerase near promoters
("promoter-proximal pausing")

(control of transcription by controlling RNA Pol.II elongation)

Transcriptional Activation in Eukaryotes: Genes-specific transcription factors

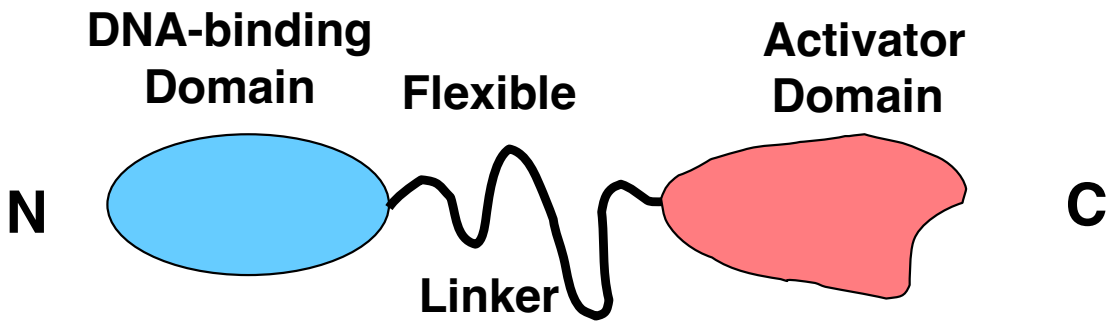


Why Are activator sequences necessary ?

1) Assembly of GTFs and Pol. II is inefficient. The binding of gene-specific TFs facilitate assembly of GTFs and Pol. II (default state of eukaryotic genes is "off")

2) Transcription is cell- or time-specific The presence of a combination of gene specific activators in a particular cell type at a particular stage of differentiation ensures the transcription of the proper set of genes.

Modular Structure of Gene-Specific Transcription Factors



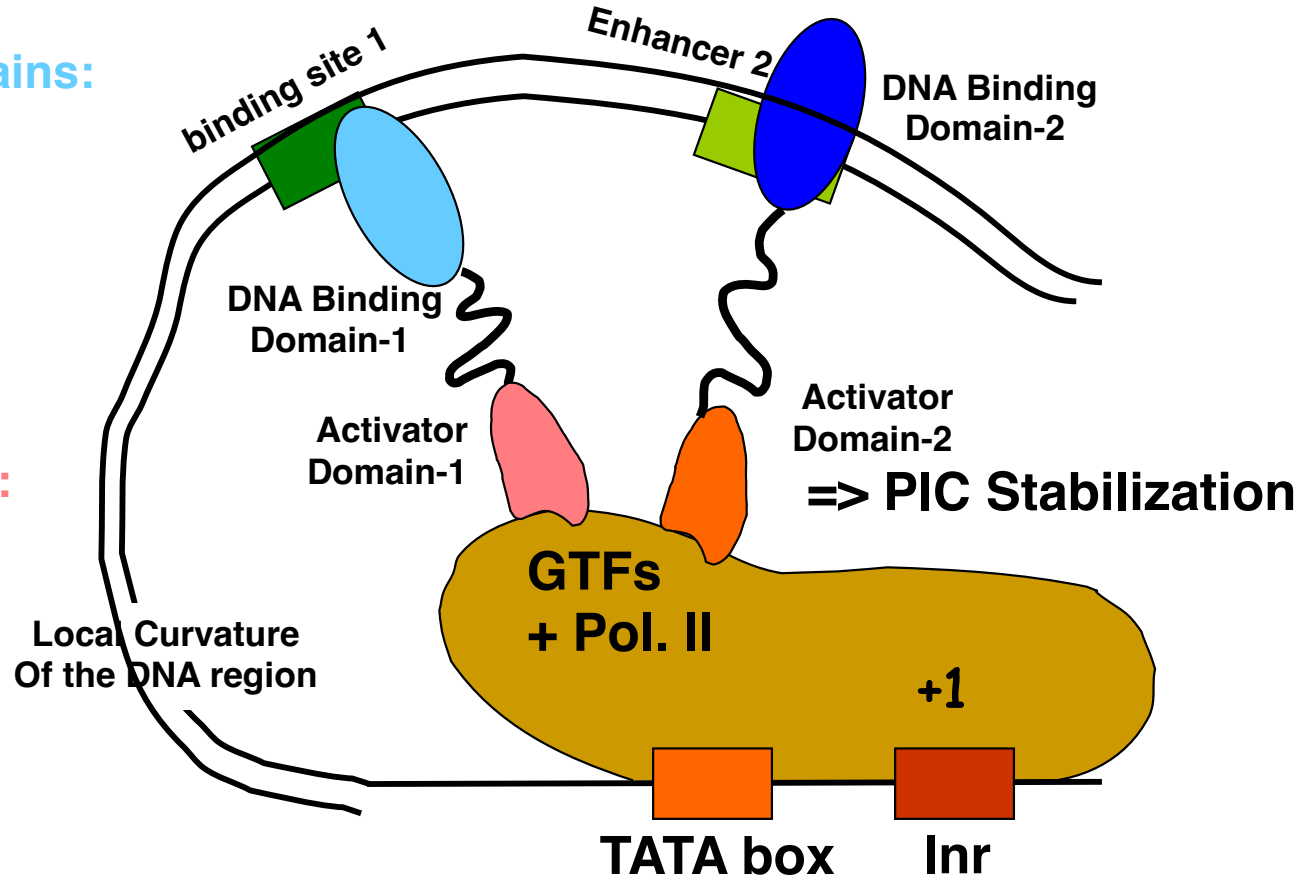
How do long distance (enhancer-promoter) relationships work ?

Examples of DNA-binding Domains:

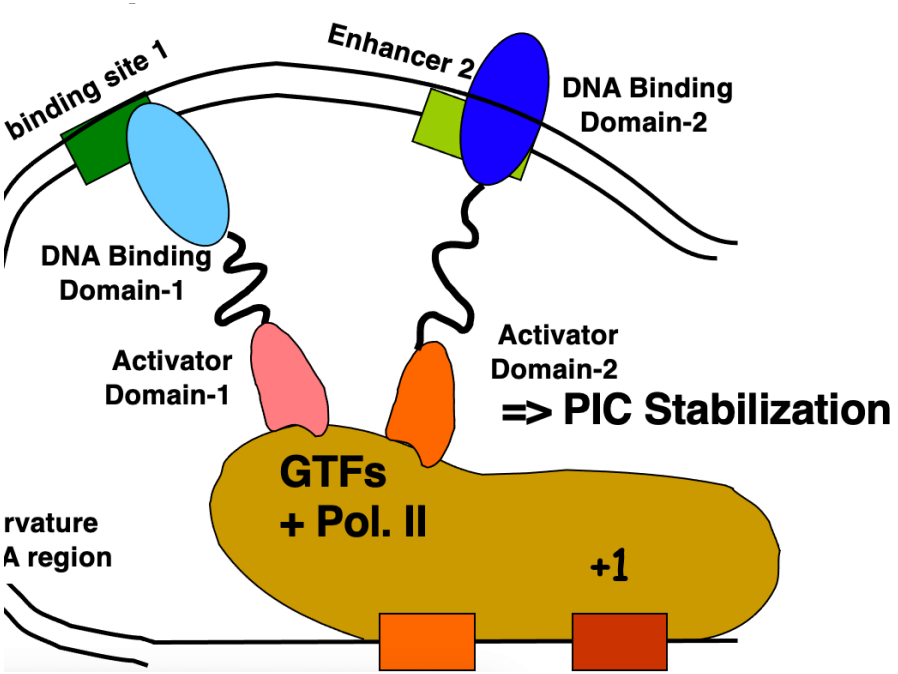
- Helix Turn Helix
- Zinc Finger
- leucine Zippers/bZip

Examples of Activator Domains:

- Acidic (e.g. p53)
- Glutamine-rich
- Proline-rich



Eukaryotic transcription initiation is almost always dependent on the action of multiple activator proteins. Why might this be an advantageous way for eukaryotes to regulate transcription?



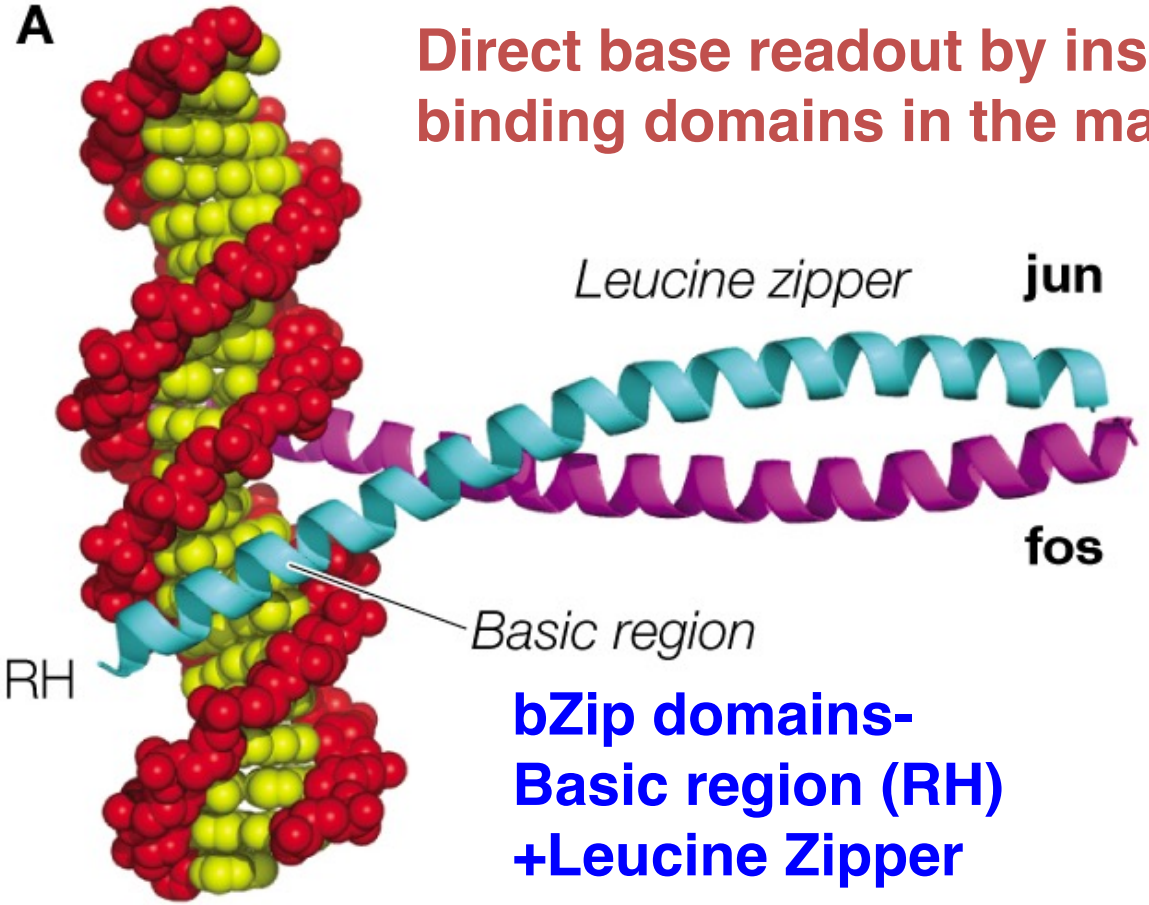
A: It enables combinatorial control (a limited number of activator proteins can combinatorially regulate many genes).

B: It decreases the chance that nonspecific activator binding will accidentally activate transcription.

C: Multiple activators can improve the efficiency of DNA unwinding.

D: Multiple activators can create high specificity and tunability.

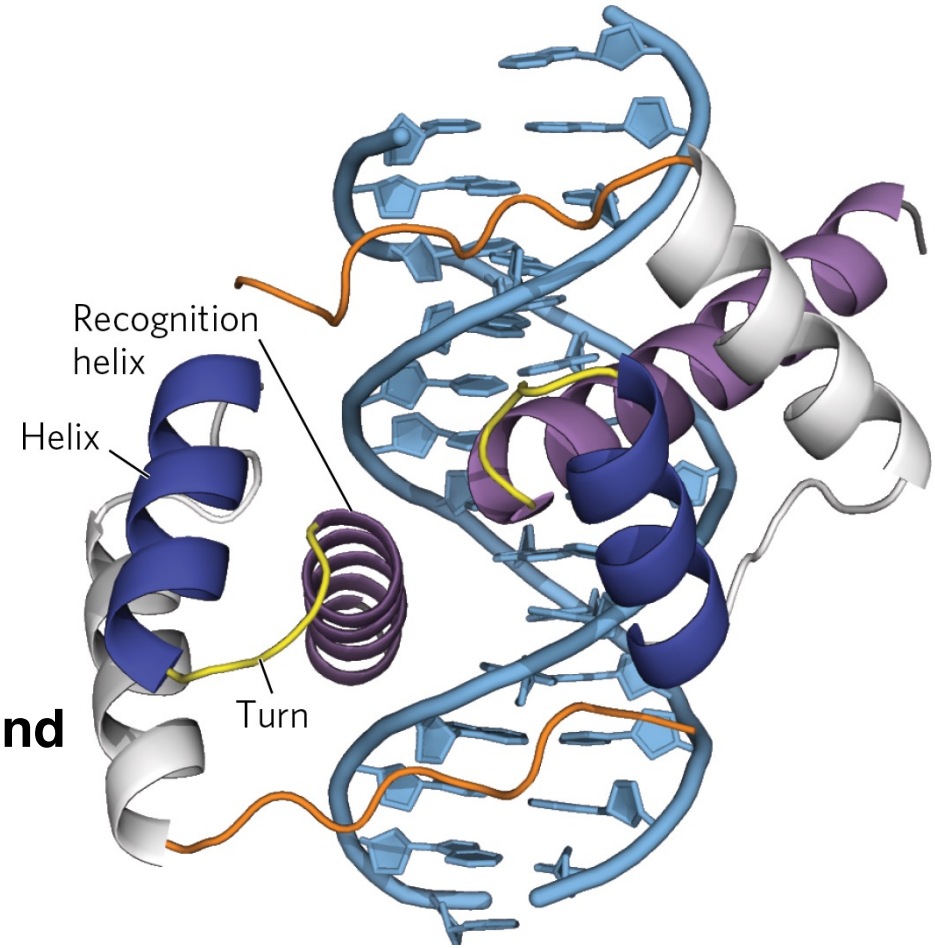
Direct base readout by insertion of alpha-helices of DNA binding domains in the major groove of DNA



bZip domains-
Basic region (RH)
+Leucine Zipper

RH = recognition helix (reads sequence)

Helix-turn-Helix
DNA binding domain

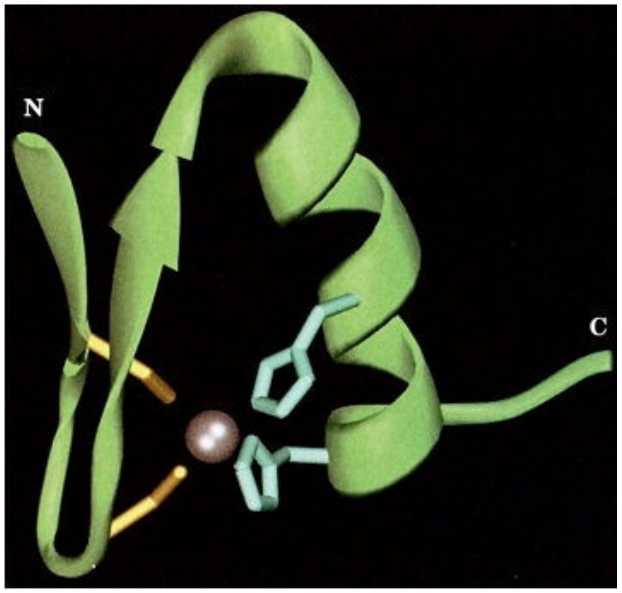


HTH = most frequent DBD in prokaryotes (e.g. Lac Repressor). Also found in eukaryotes. Example of Homeodomain proteins = Transcription Factors that control Development, e.g., Engrailed, Bithorax

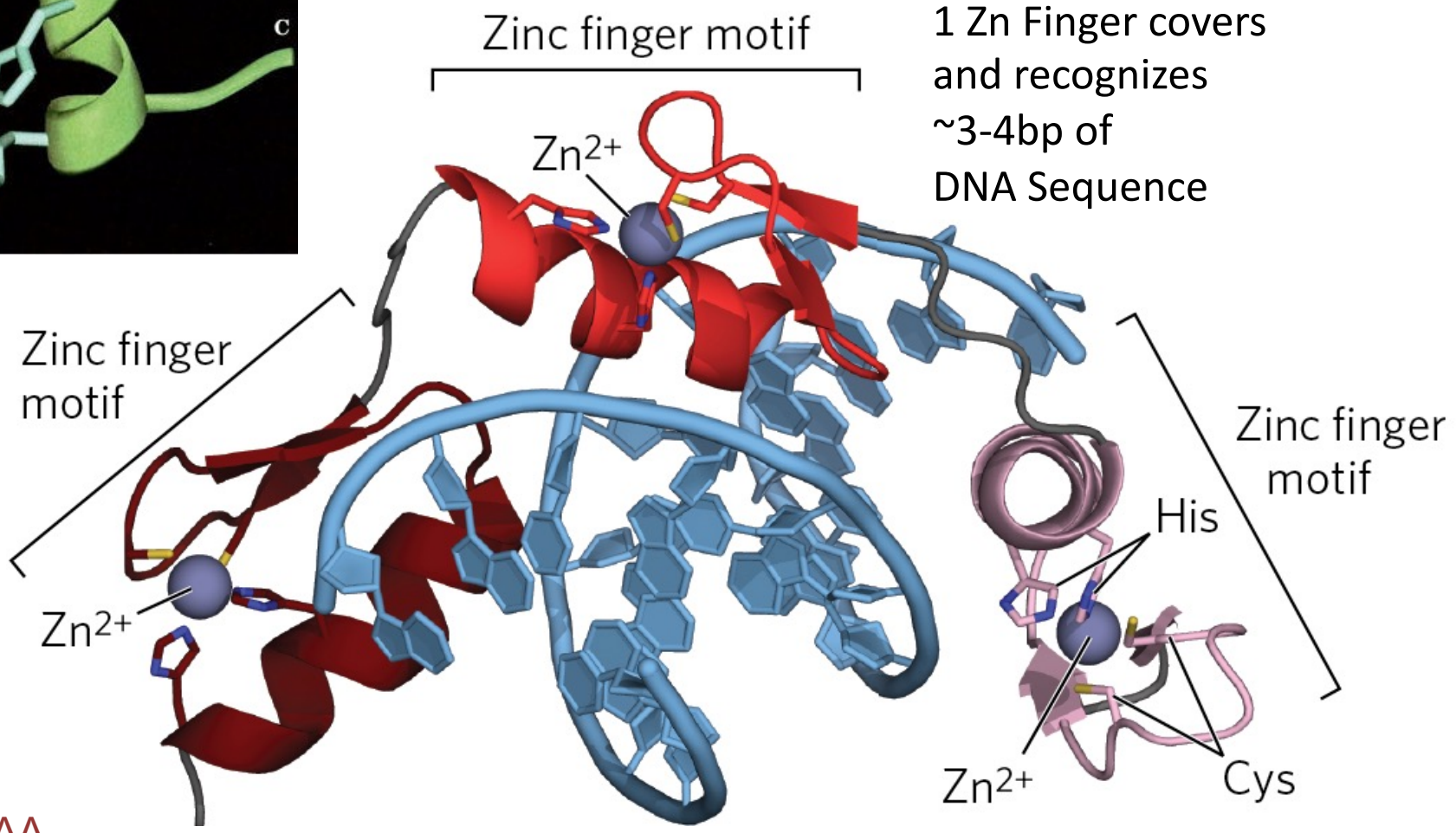
Direct base readout by α-helices of Zinc Fingers DNA binding domains:

1 Zn Finger

3D structure



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

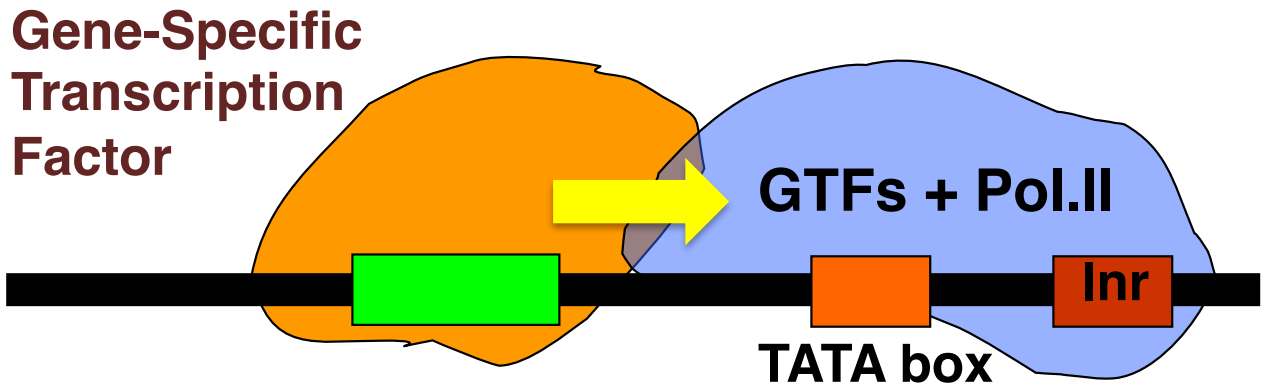


1 Zn Finger covers and recognizes ~3-4bp of DNA Sequence

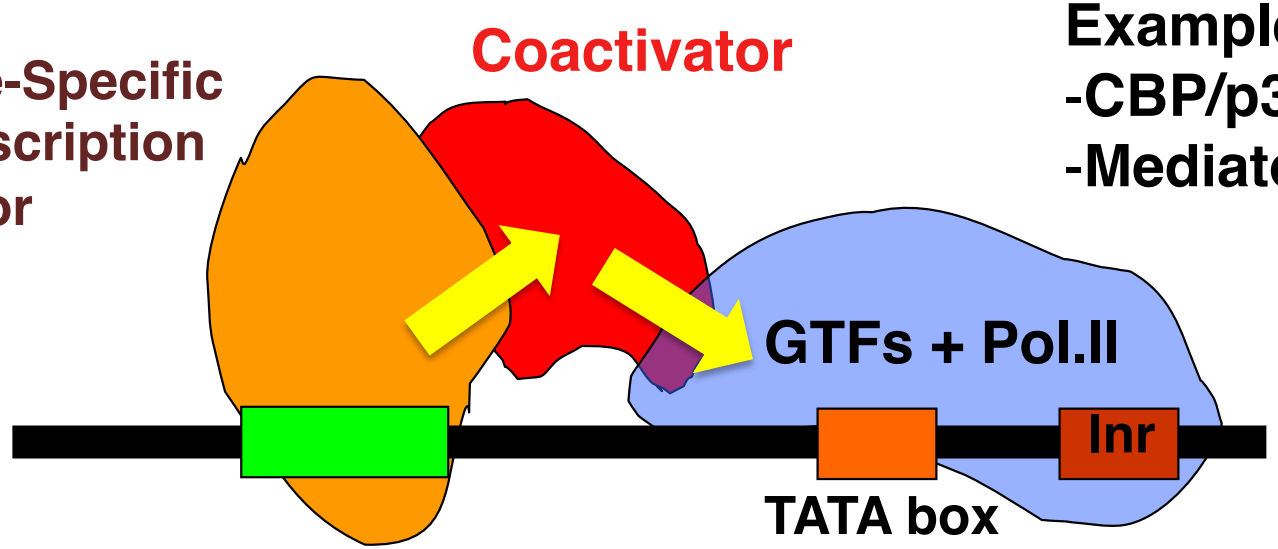
PDB ID = 1ZAA

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**



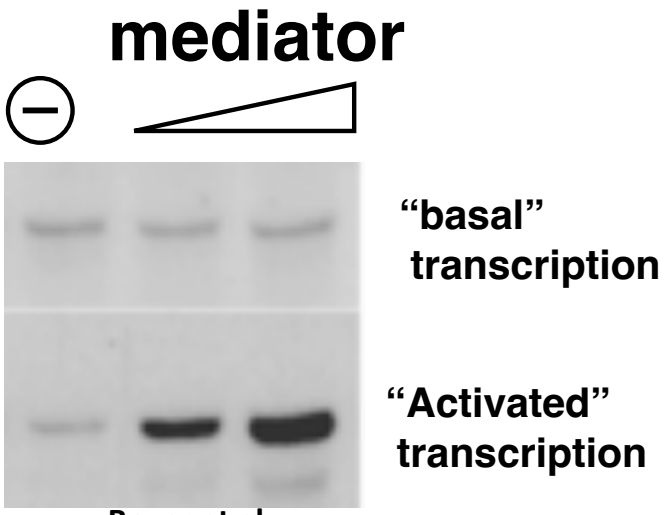
Examples of Coactivators:
-CBP/p300
-Mediator Complex

One Example of Coactivator complex: Mediator

In vitro transcription with two different promoter DNAs:

Promoter 1: ——— TATA – Inr

Promoter 2: – Enhancer – TATA – Inr



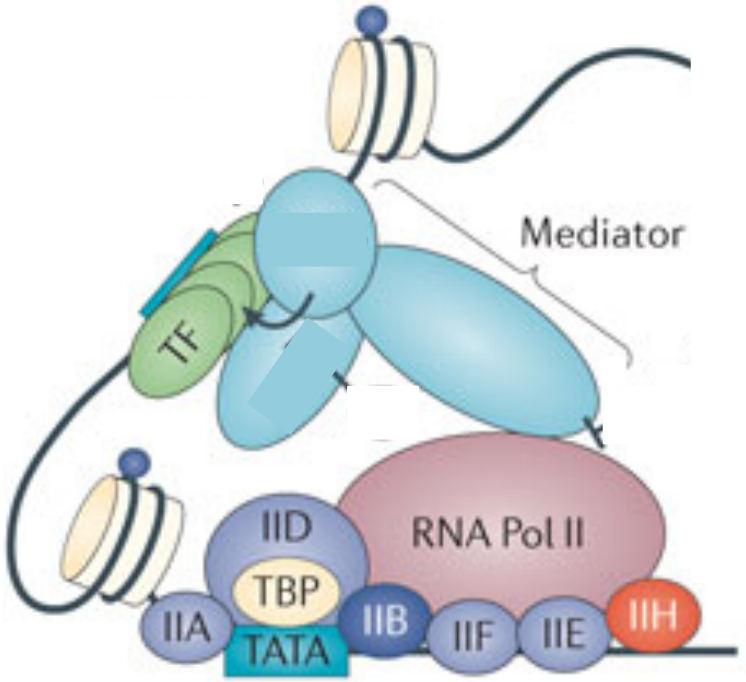
Boyer et al., Nature 1999

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

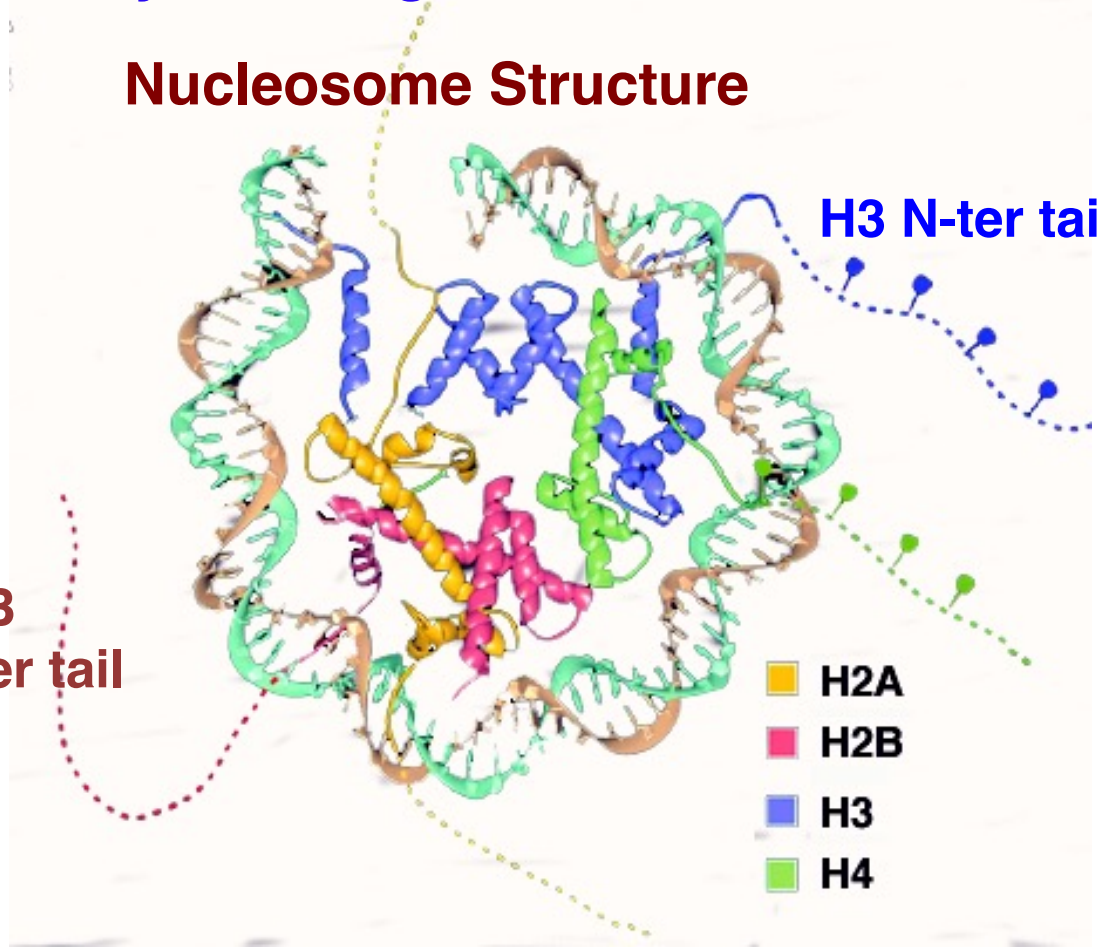
- The Mediator complex stimulates transcription of genes containing enhancer sequences.

- 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 ??

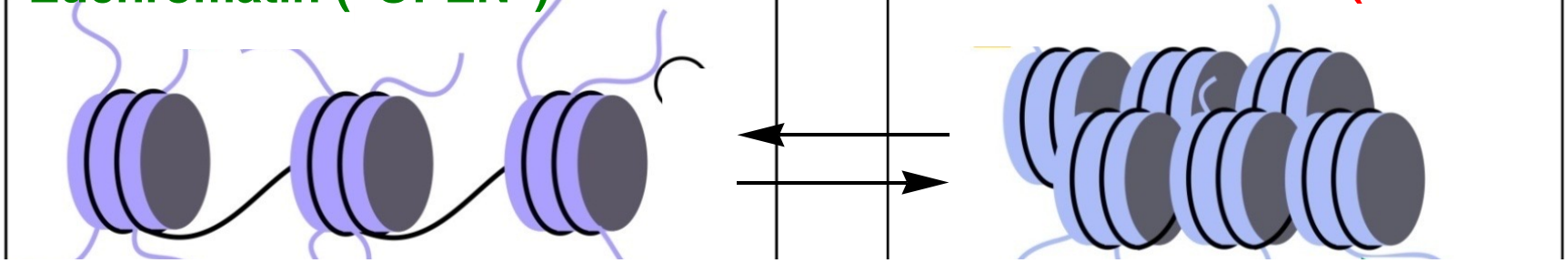
Covalent modifications of amino acids located on the the N-terminal tails of histones by:
- **acetylation**
- **deacetylation**
- **methylation**
- **ubiquitination**



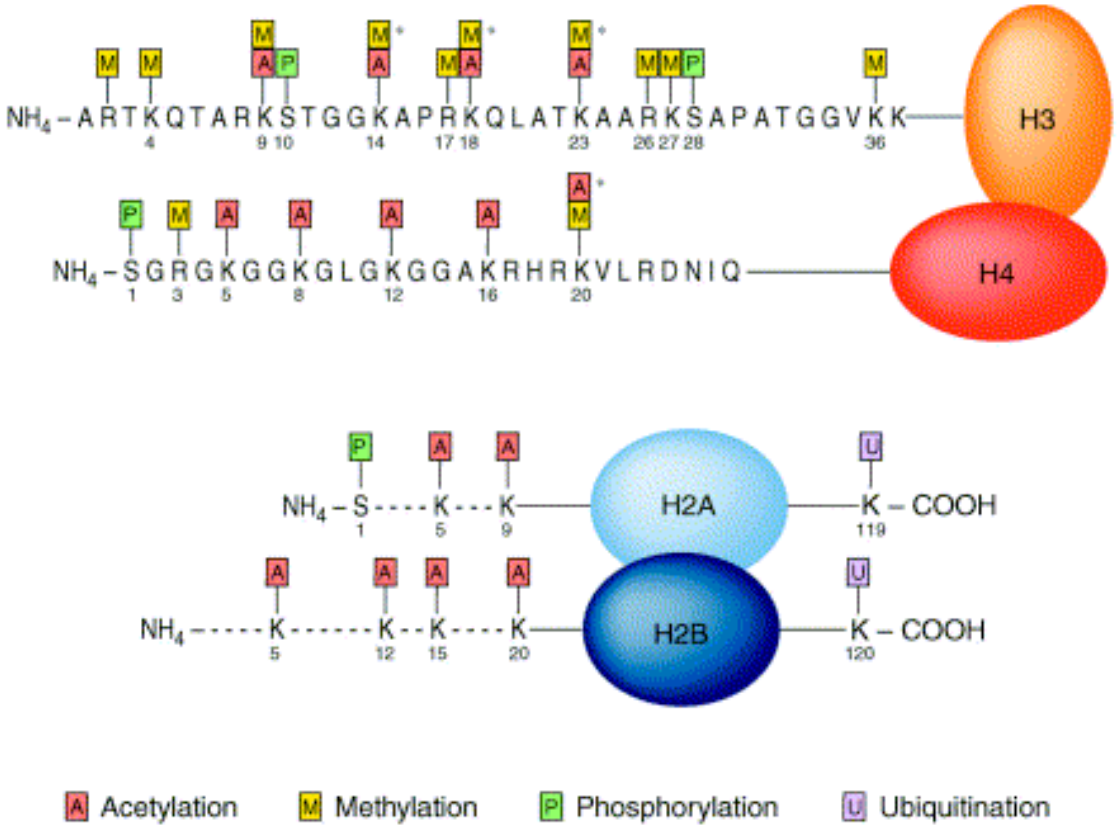
These covalent modifications influence the equilibrium between:

Transcriptionally Active State = Euchromatin ("OPEN")

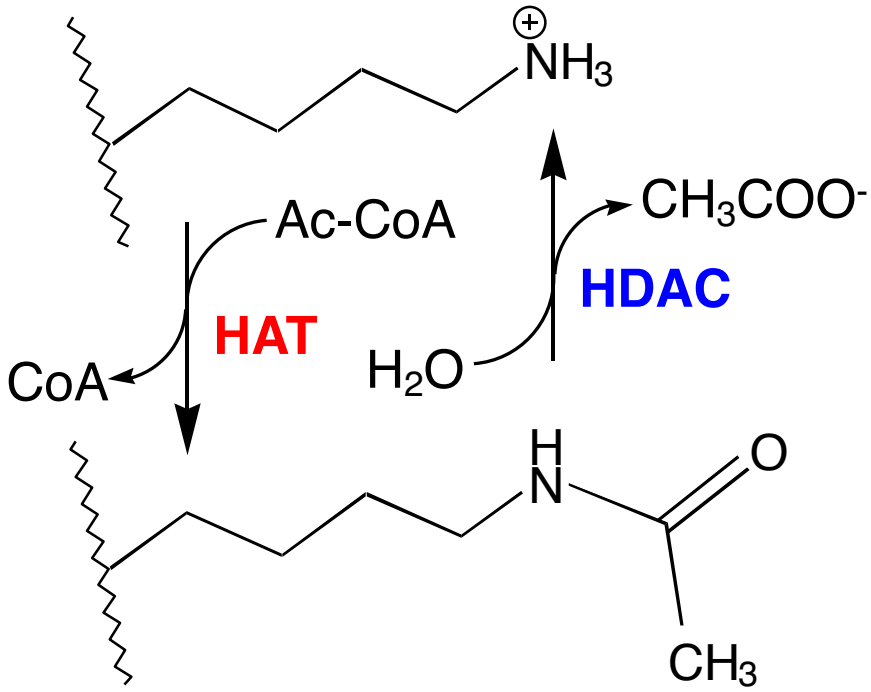
Transcriptionally Inactive State = Heterochromatin ("CLOSED")



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

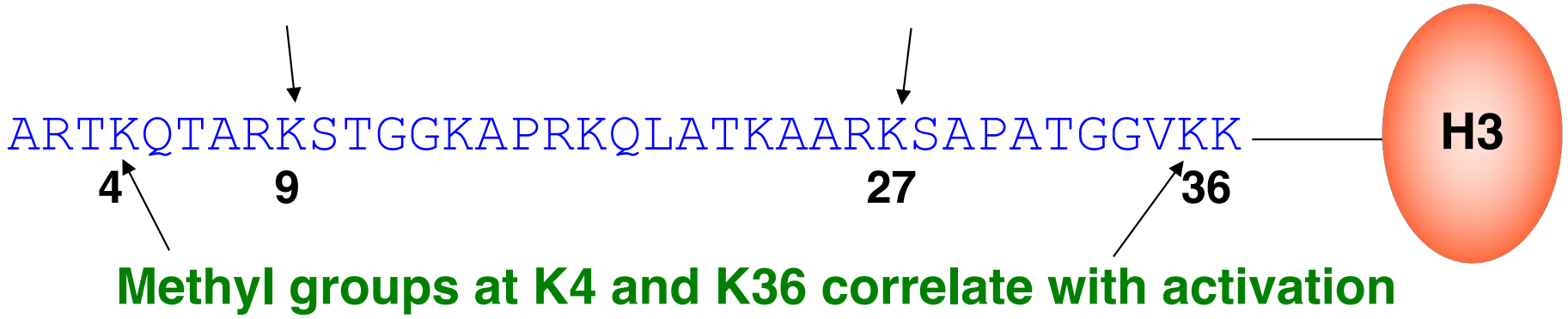
Current Opinion in Plant Biology 2002

Histone post-translational modifications and transcriptional activity

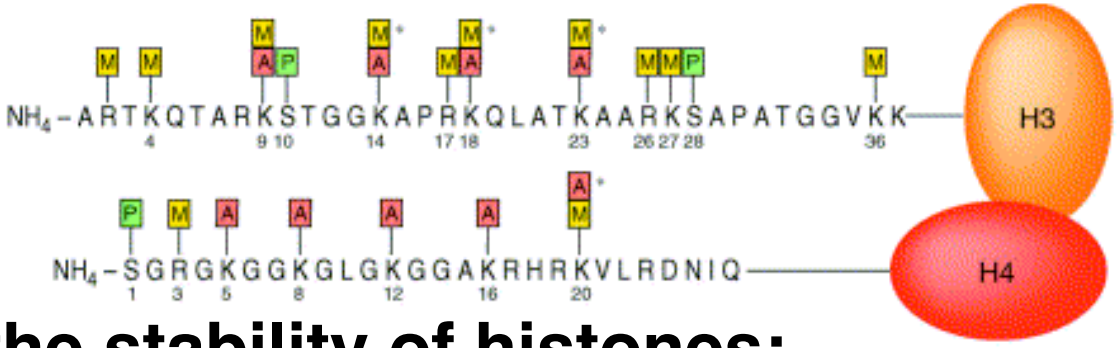
Lysine acetylation and methylation are of primary importance:

- **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