Biology from an EE perspective
Lecture 10

Transcription in Prokaryotes

Transcription and post transcription processing in Eukaryotes

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Lecture Overview

- Look at transcription
- An important process in all of life
- One gets some feel of the complex transcription machinery with feedback that exists
- Important for understanding modern literature

Figure credits: Unless mentioned, all jpeg images are from the site of *Molecular Cell Biology* by Lodish et al., published by W H Freeman & Co

RNA needed for

- rRNA – ribosomal RNA
- tRNA – for sequence decoding
- mRNA – template RNA for protein synthesis
- [More recently, other forms of RNA such as microRNA and small interfering RNA (siRNA) are thought to regulate gene expression]

Transcription -- Prokaryotes

- Chromosome structure
  - In cytoplasm a single circular chromosome with ribosomes conglomerated (nucleoid) around it
- RNA polymerase
  - One type
- Post transcription processes
  - Coupled transcription and translation
  - No post transcription modification of RNA

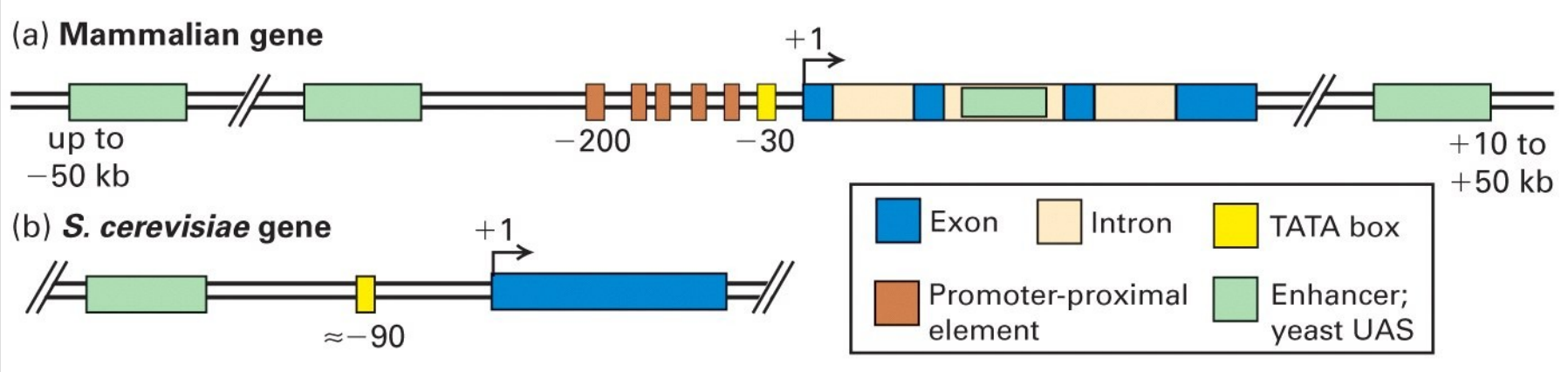
Transcription -- Eukaryotes

- Chomosomes
  - One linear DNA molecule
  - DNA in nucleus wound around histone cores forming chromatin
  - Chromatin exits in extended & condensed form with the help of structural scaffold proteins
- RNA polymerase
  - Three types of RNA polymerase
- Post transcription processing
  - Capped, spliced (because lengthy introns need to be removed and exons ligated) & adenylated

Gene structure

- Prokaryotes
  - Recognition region 50 bp
  - Transcription initiation site
  - 5’ untranslated region
  - Translation initiation
  - Coding region
  - 3’ untranslated region
  - Transcription stop site
- Eukaryotes
  - Recognition region 50kbp
  - Transcription initiation site
  - 5’ UTR untranslated region
  - Translation initiation
  - Splice donor acceptor site
  - Translation stop
  - 3’ UTR
  - Polyadenylation signal
  - Transcription stop site

Partial architecture of a gene



Transcription in prokaryotes -1

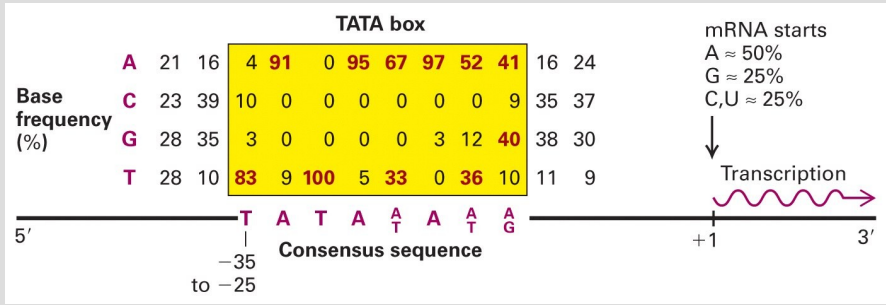
- RNA polymerase + σ factors samples DNA by weakly binding and unbinding from the DNA
- At a site where there is a promoter, binds more strongly
- DNA melted and transcription begun, σ factors released
- About 10-12 nucleotide chain hydrogen bonded to the DNA
- The remaining RNA strand unbonded

Transcription in prokaryotes -1

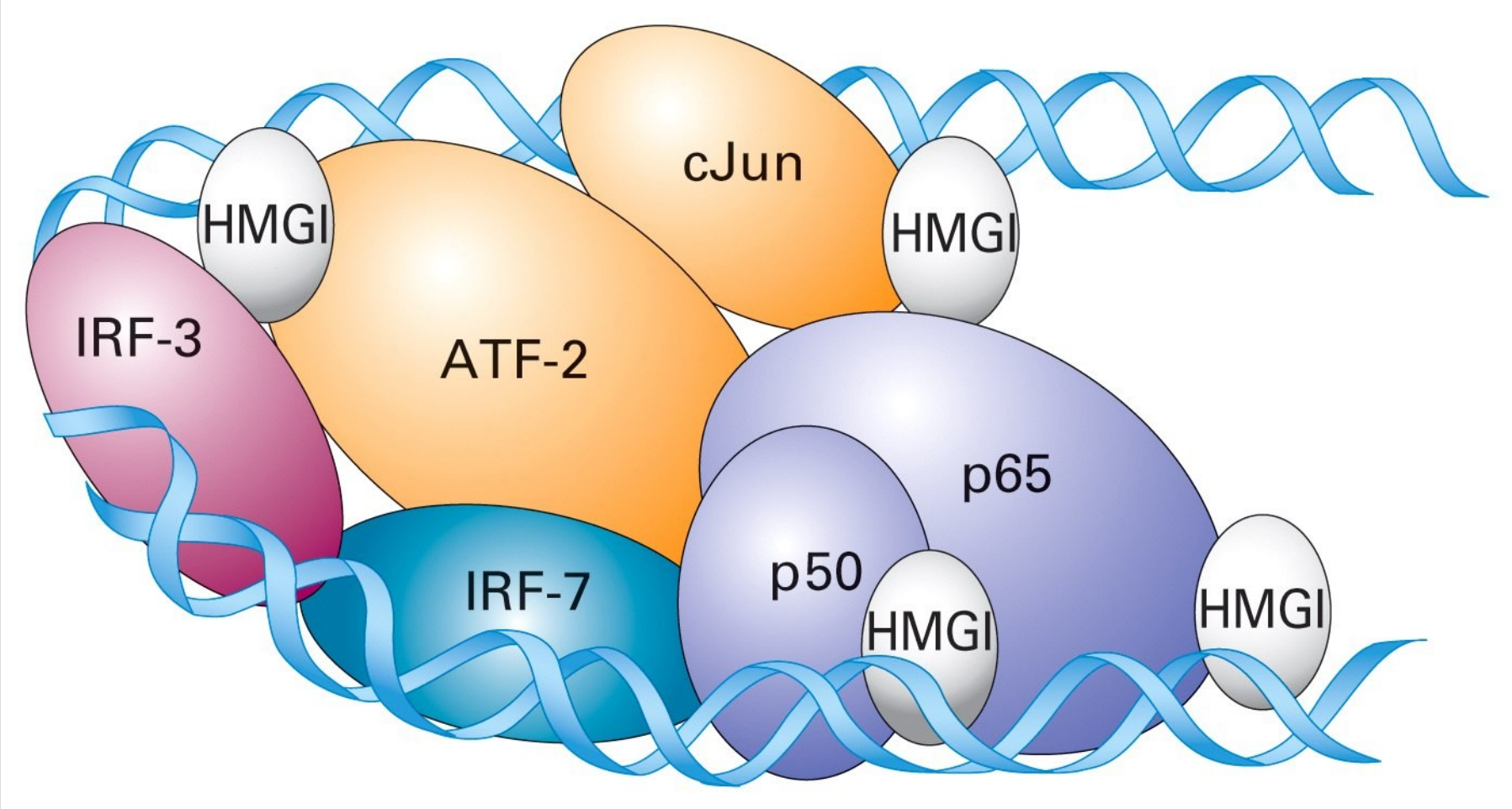
- Translation could begin while transcription still on
- Transcription stops at the stop encoded site
- But for some exceptions, transcription could be with template genes in either strand

Initiation site

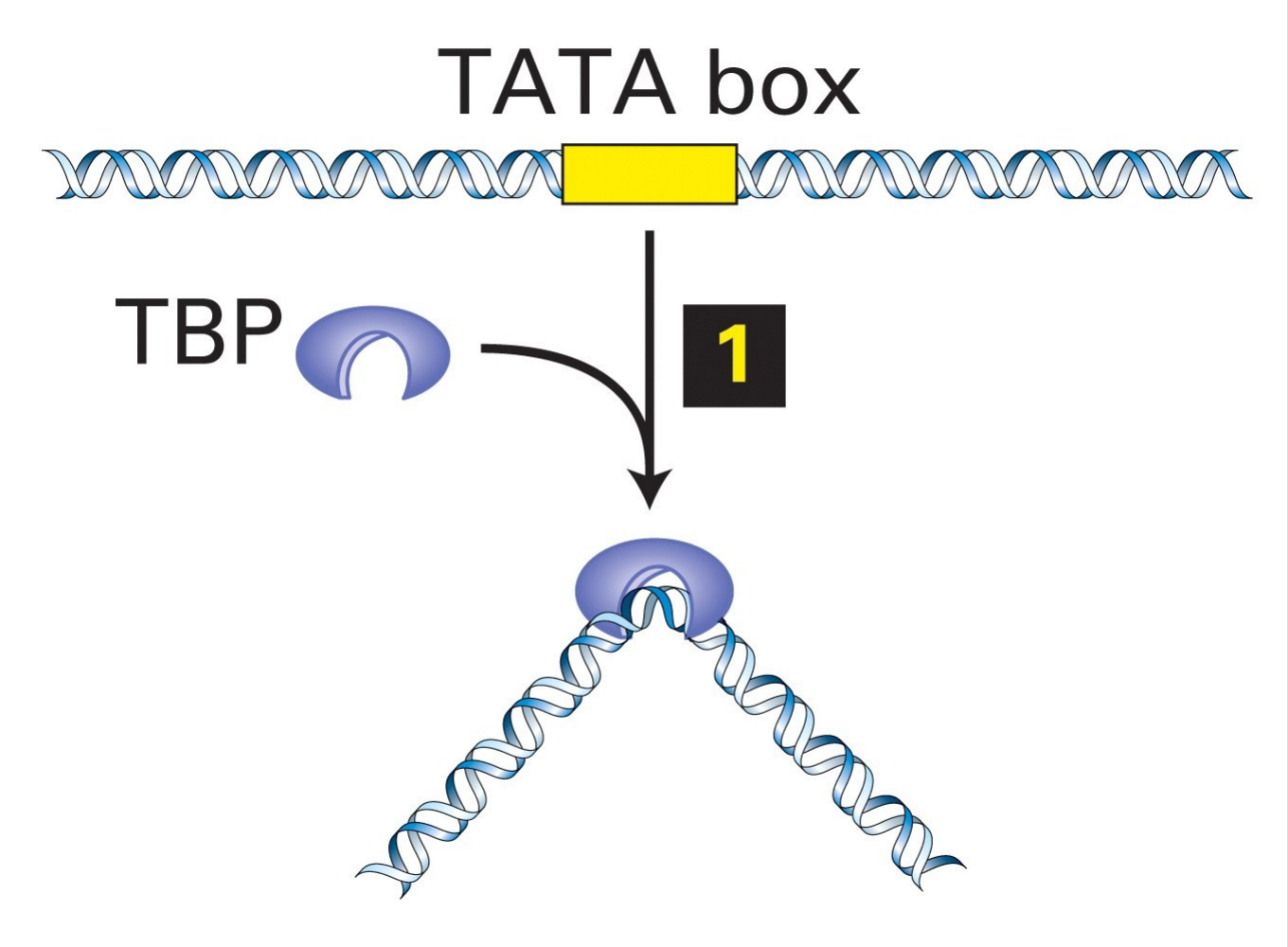
- Pribnow box
- TATTAT sequence
- 5-8 nucleotides
- Start nucleotide +1
- Upstream sites -ve
- Initiation site conserved



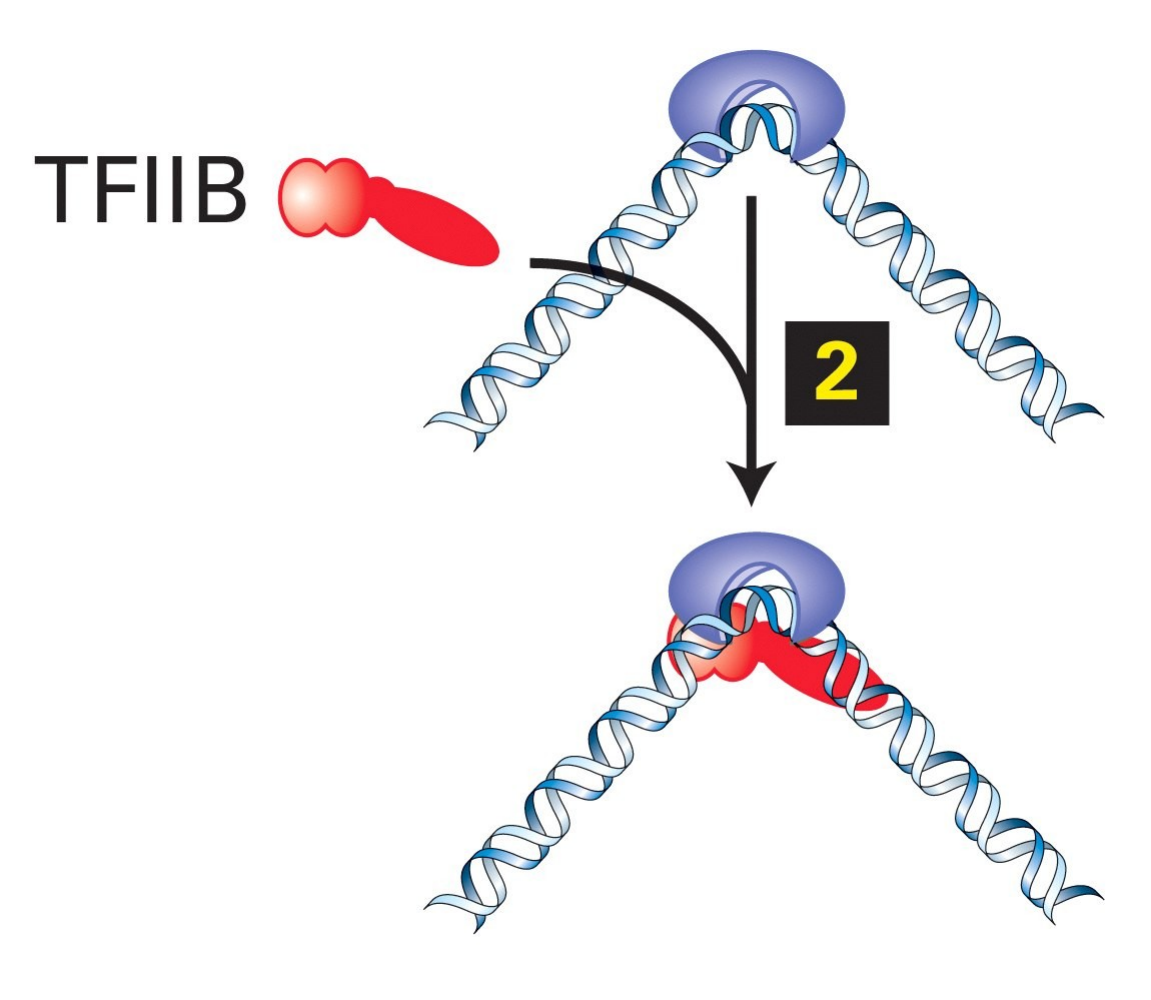
How does the molecular machinery look?



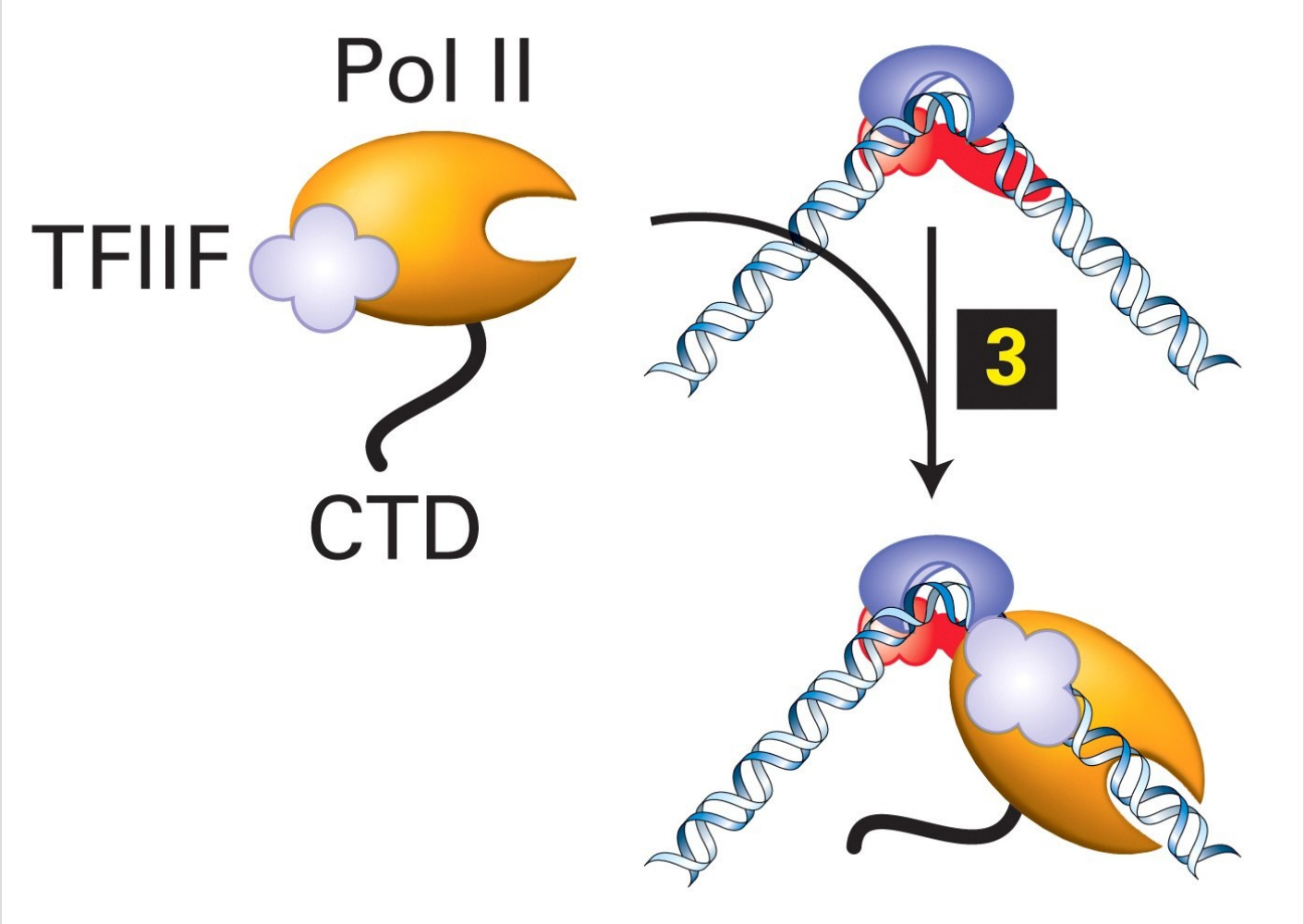
Transcription process -1



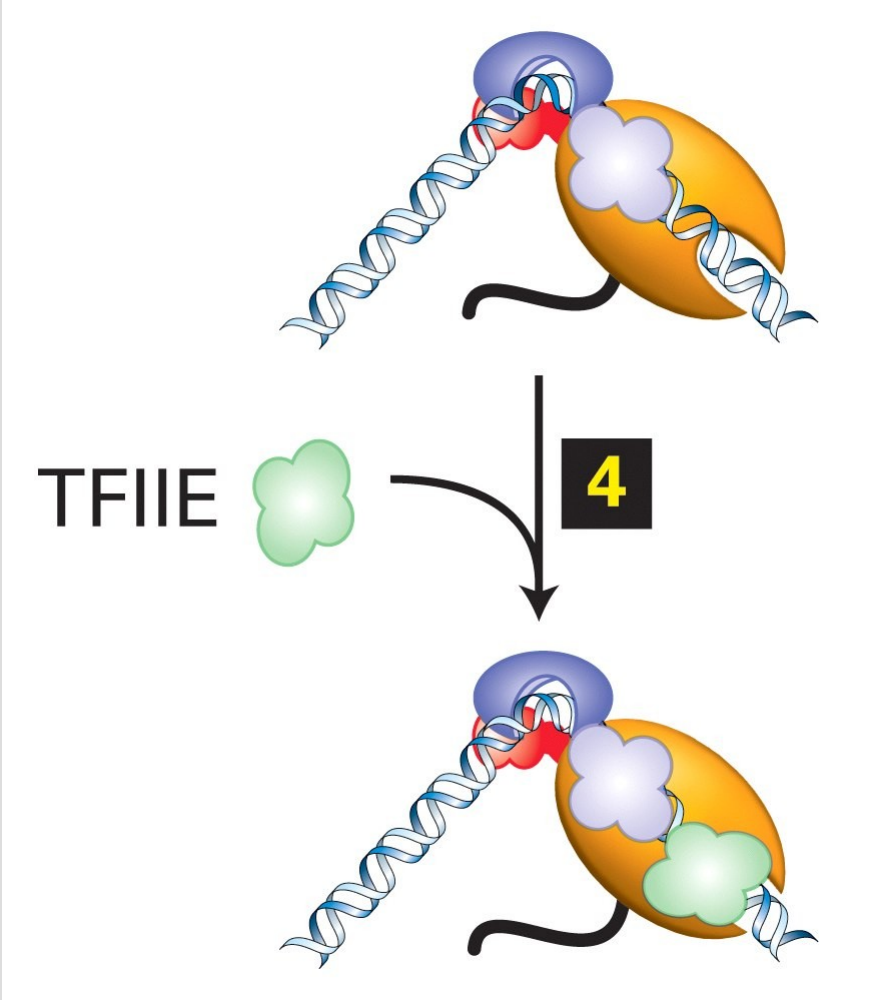
Transcription process -2



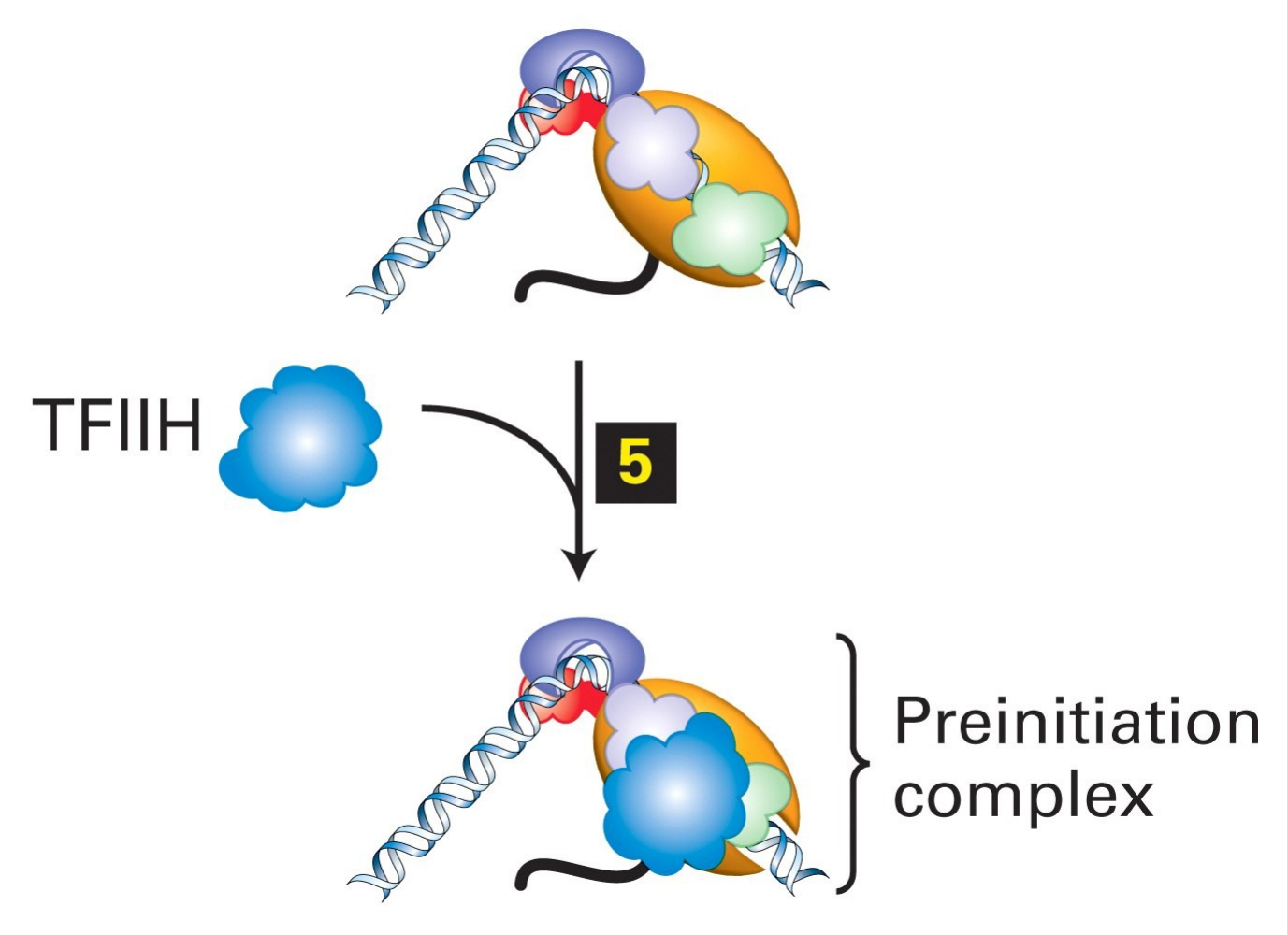
Transcription process -3



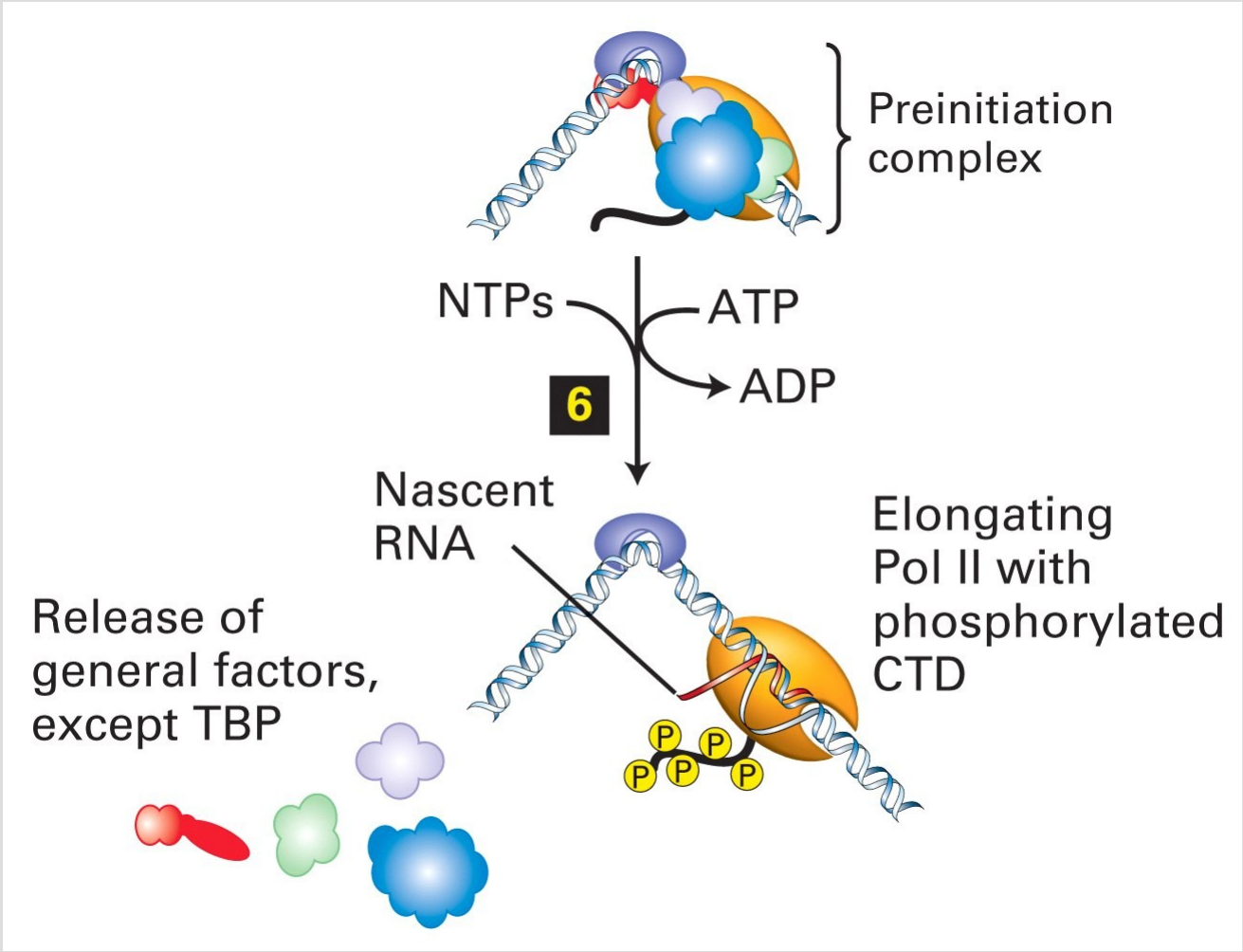
Transcription process -4



Transcription process -5



Transcription process -6



Transcription control

- Structural genes: transcribed all the time at a slow rate
  - For membrane or other proteins and RNA needed for homeostatis
  - RNA degraded with time
- Regulatory genes: transcribed in response to an event/stimulus
  - In response to a stimuli for a required protein

Regulatory genes

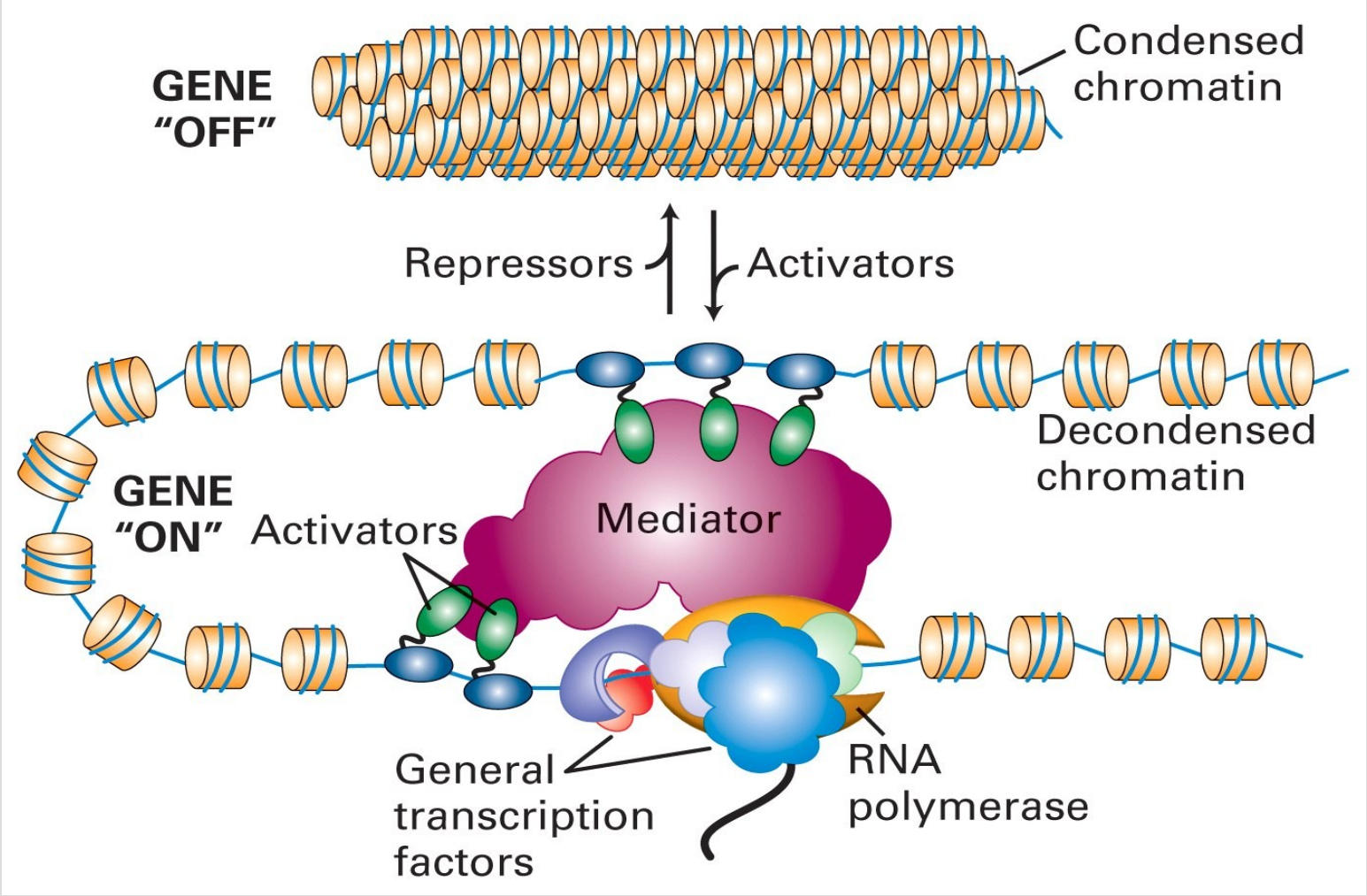
- Encode regulatory proteins
  - Positive and negative control
    - Repressors: Activity reduced by binding to operator site (close to promoter)
    - Co-repressors: Binds with repressor and inhibits transcription
    - Effectors: Induces decreased binding affinity
    - Activators bind near promoter site and increase affinity of RNA polymerase binding to DNA
  - Sometimes both
  - Regulatory proteins are dimers – have alpha helices that fit into major grove of DNA

Termination of transcription

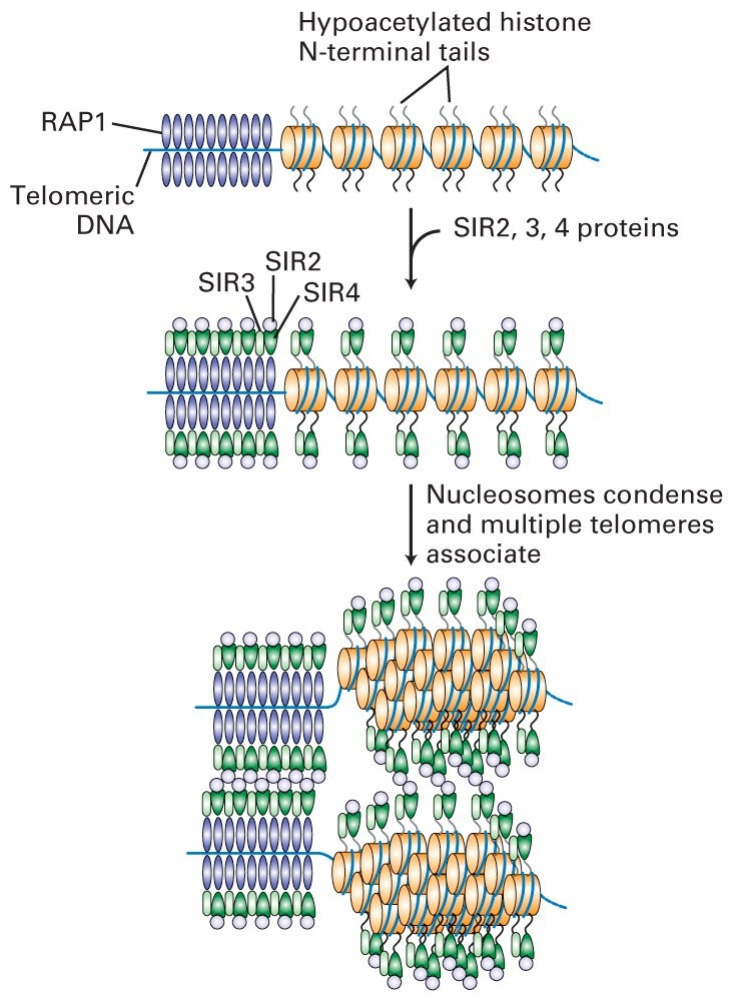
- Rho dependent
- Rho independent

Transcription in Eukaryotes -1

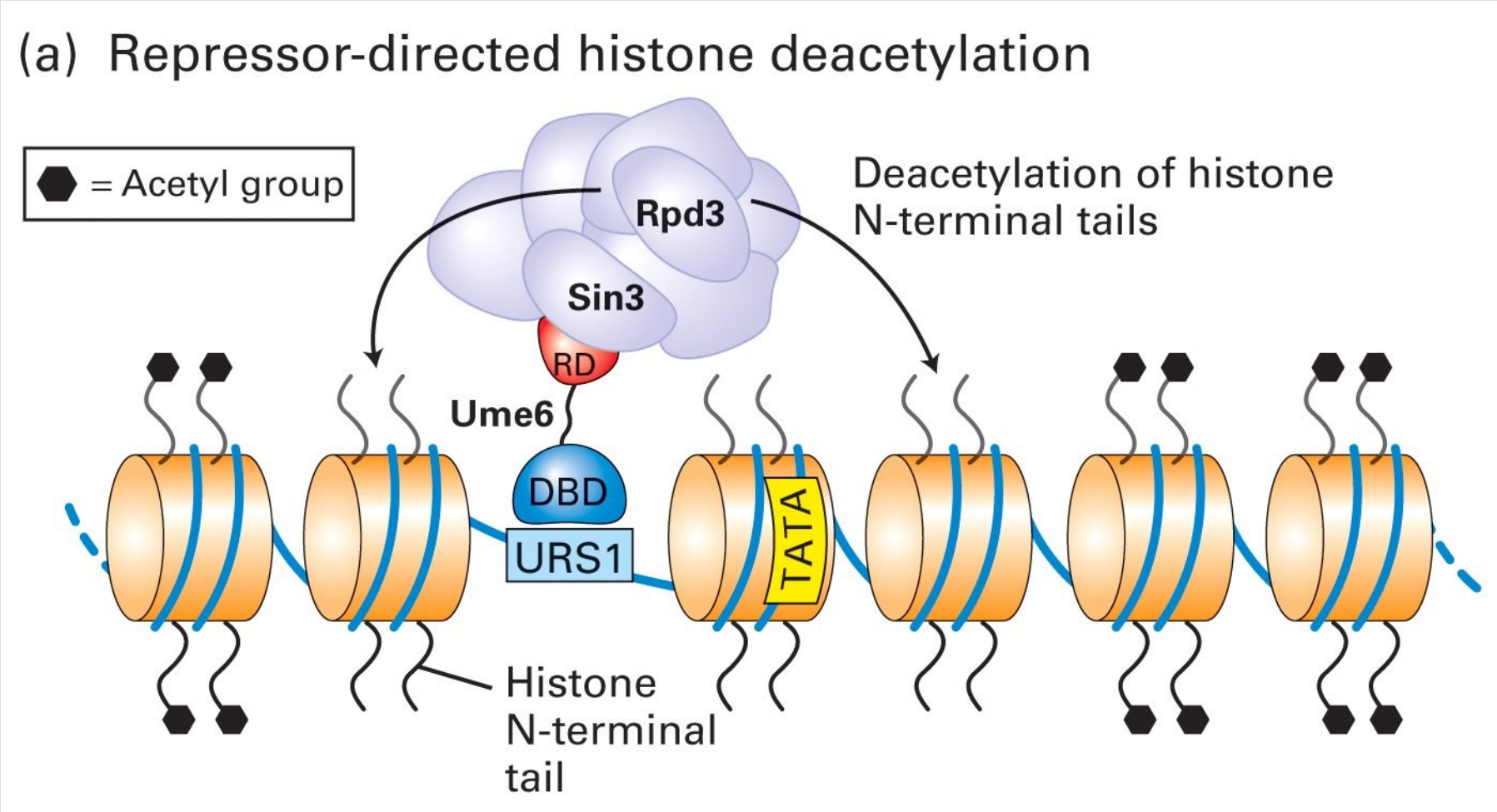
Gene needs to be on for transcription



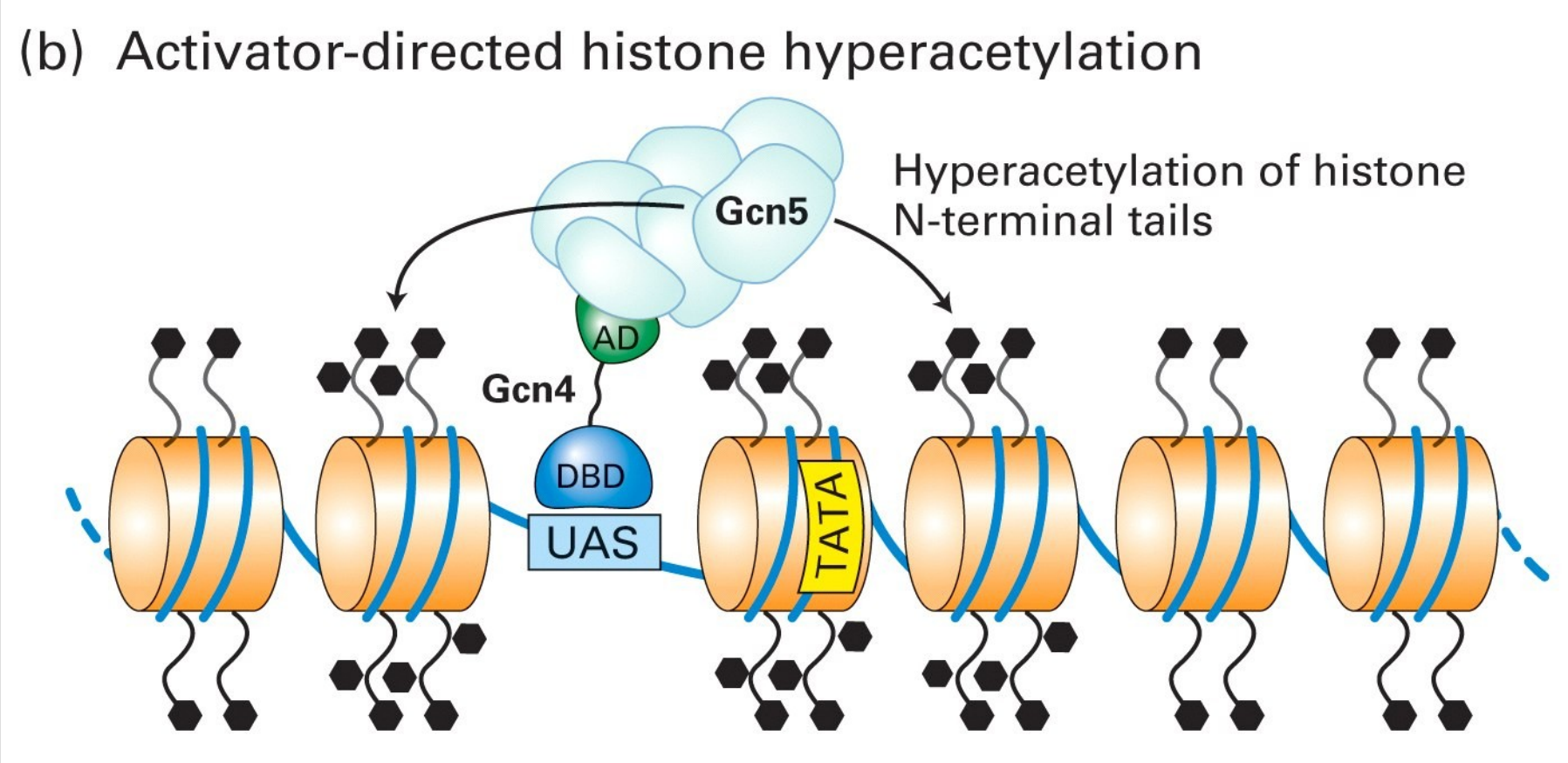
Transcription in Eukaryotes -2



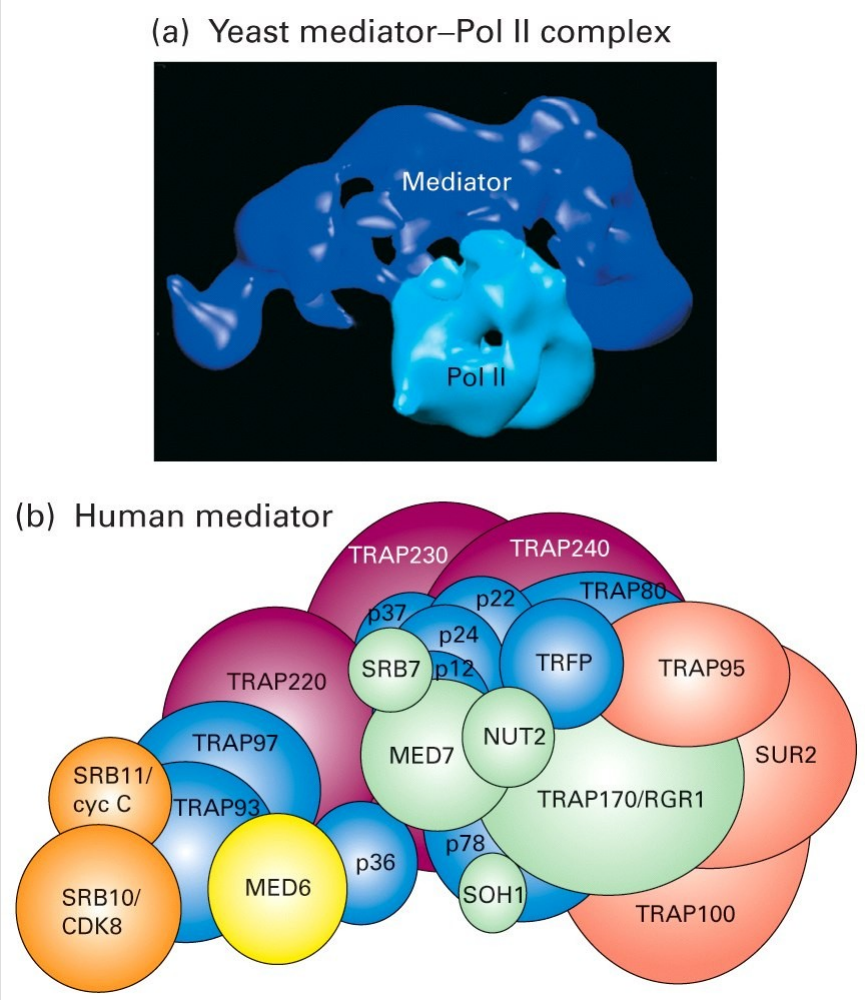
Histone deacetylation leads to more positive charge on histones & DNA wraps more tightly blocking access of transcription factors



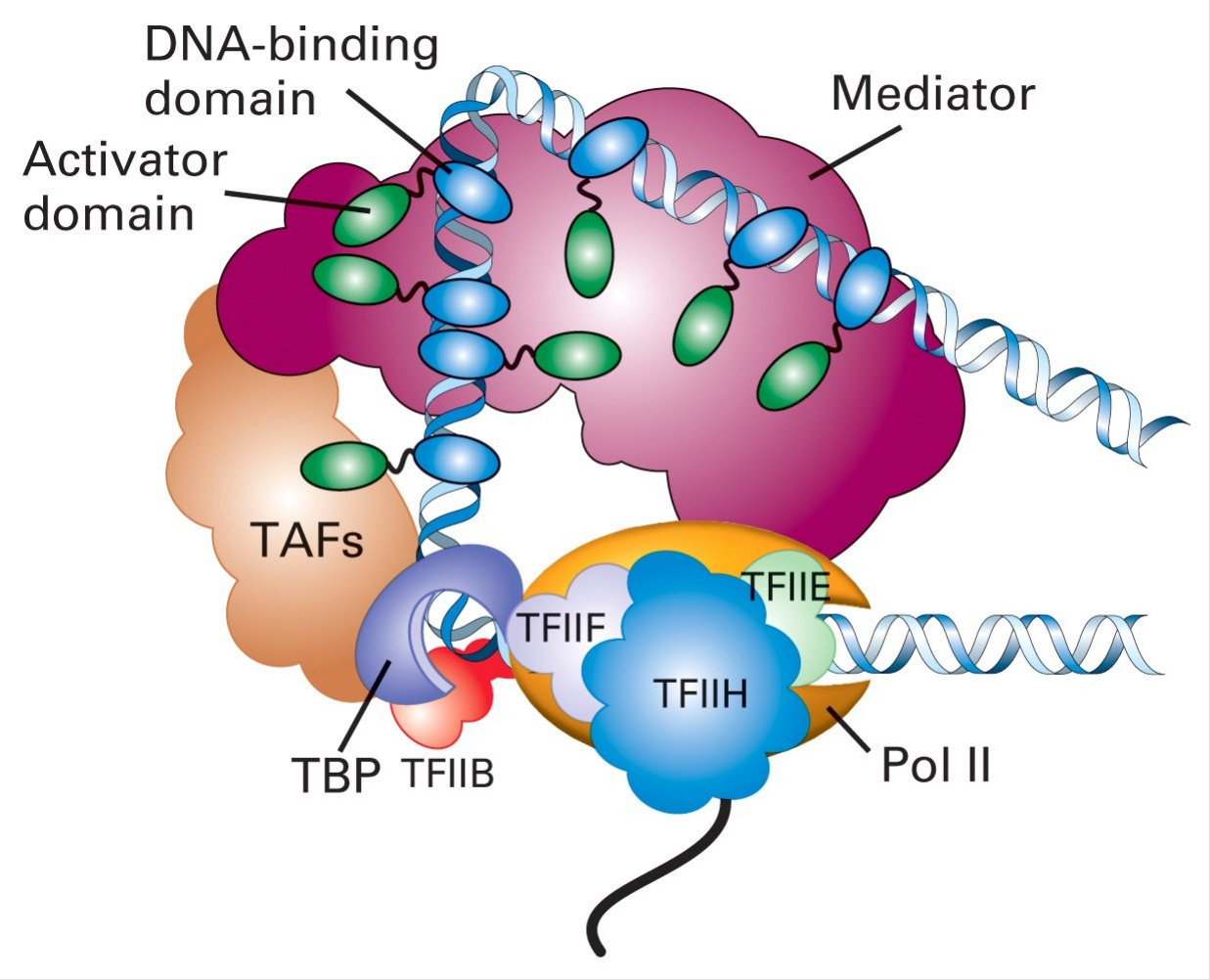
Activators increase acetylation & enable access of promotors



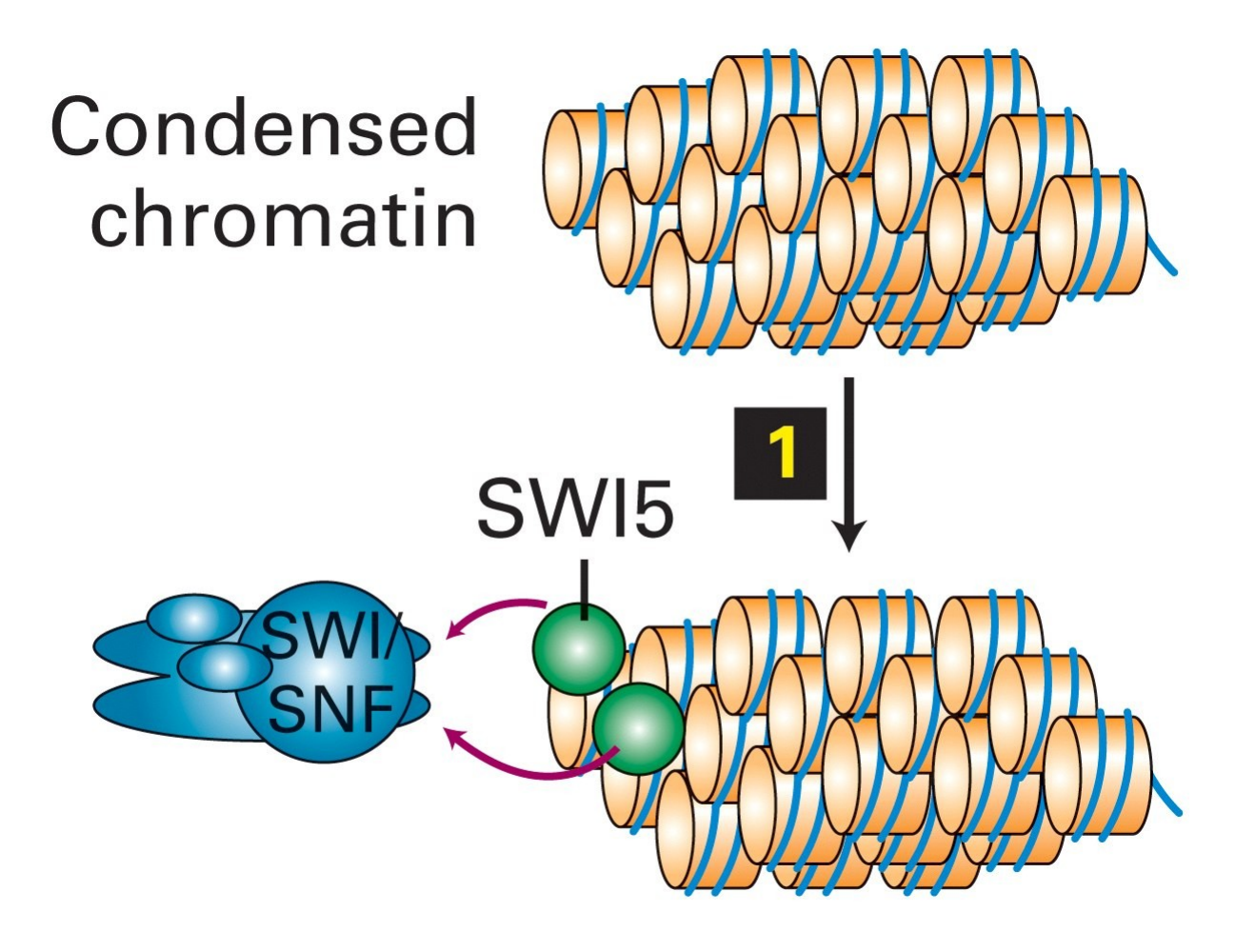
Mediators --


(a) Yeast mediator–Pol II complex
Mediator
Pol II
(b) Human mediator
TRAP230
TRAP240
TRAP80
p22
p37
p24
SRB7
p12
TRFP
TRAP95
TRAP220
TRAP97
NUT2
MED7
SUR2
SRB11/
cyc C
TRAP93
TRAP170/RGR1
p78
p36
MED6
SRB10/
CDK8
SOH1
TRAP100

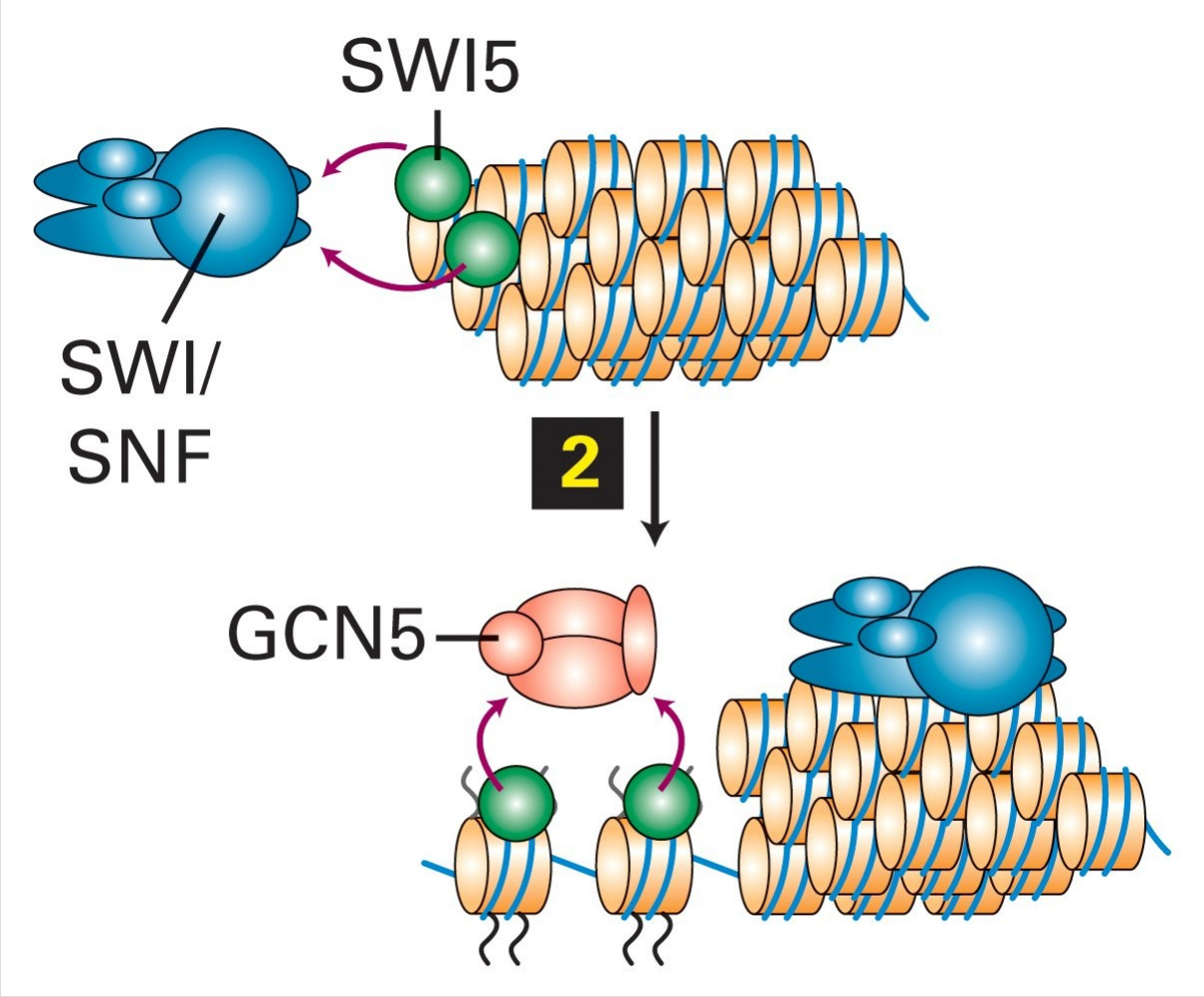
RNA polymerase complex



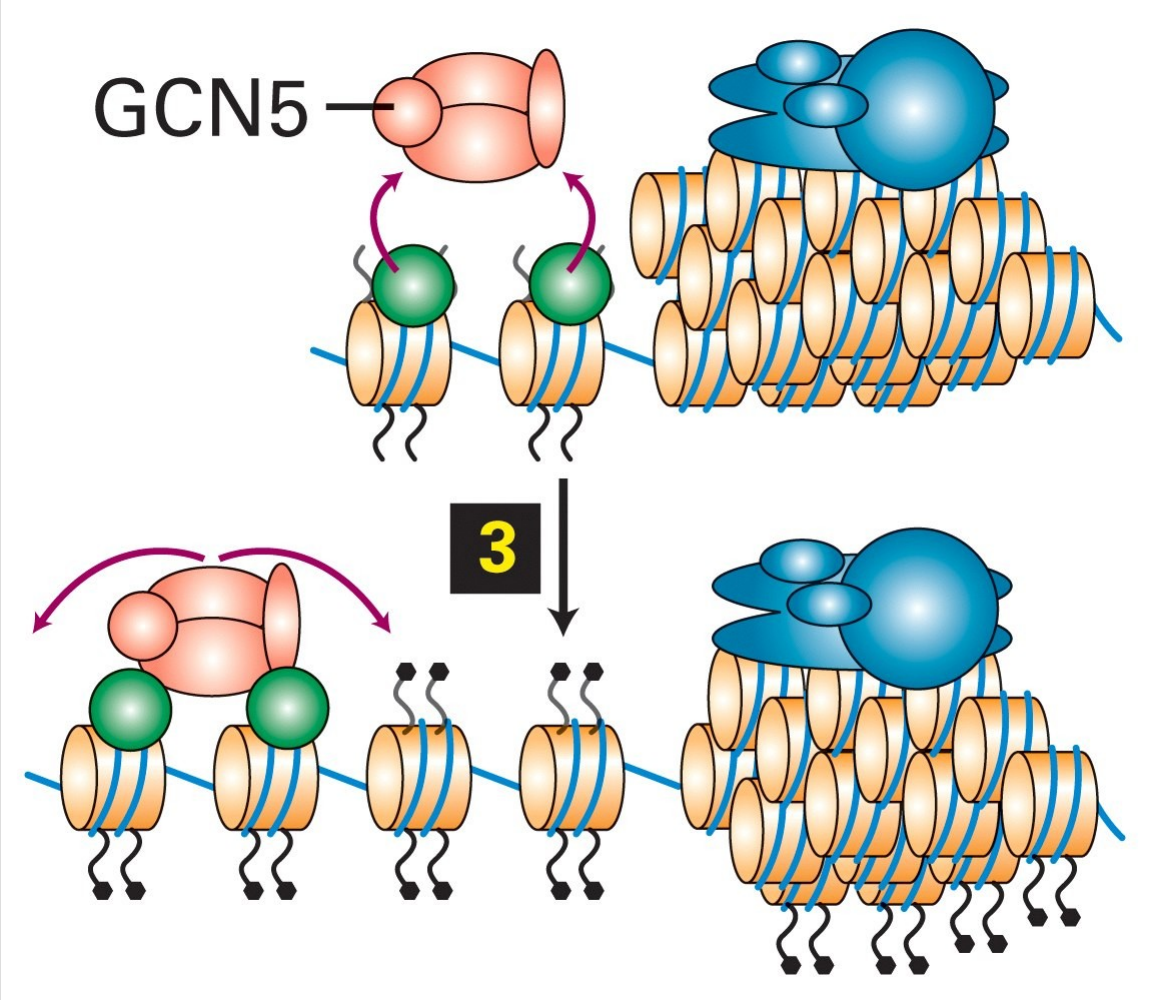
Opening condensed chromatin – SWI5 activates transcription of genes



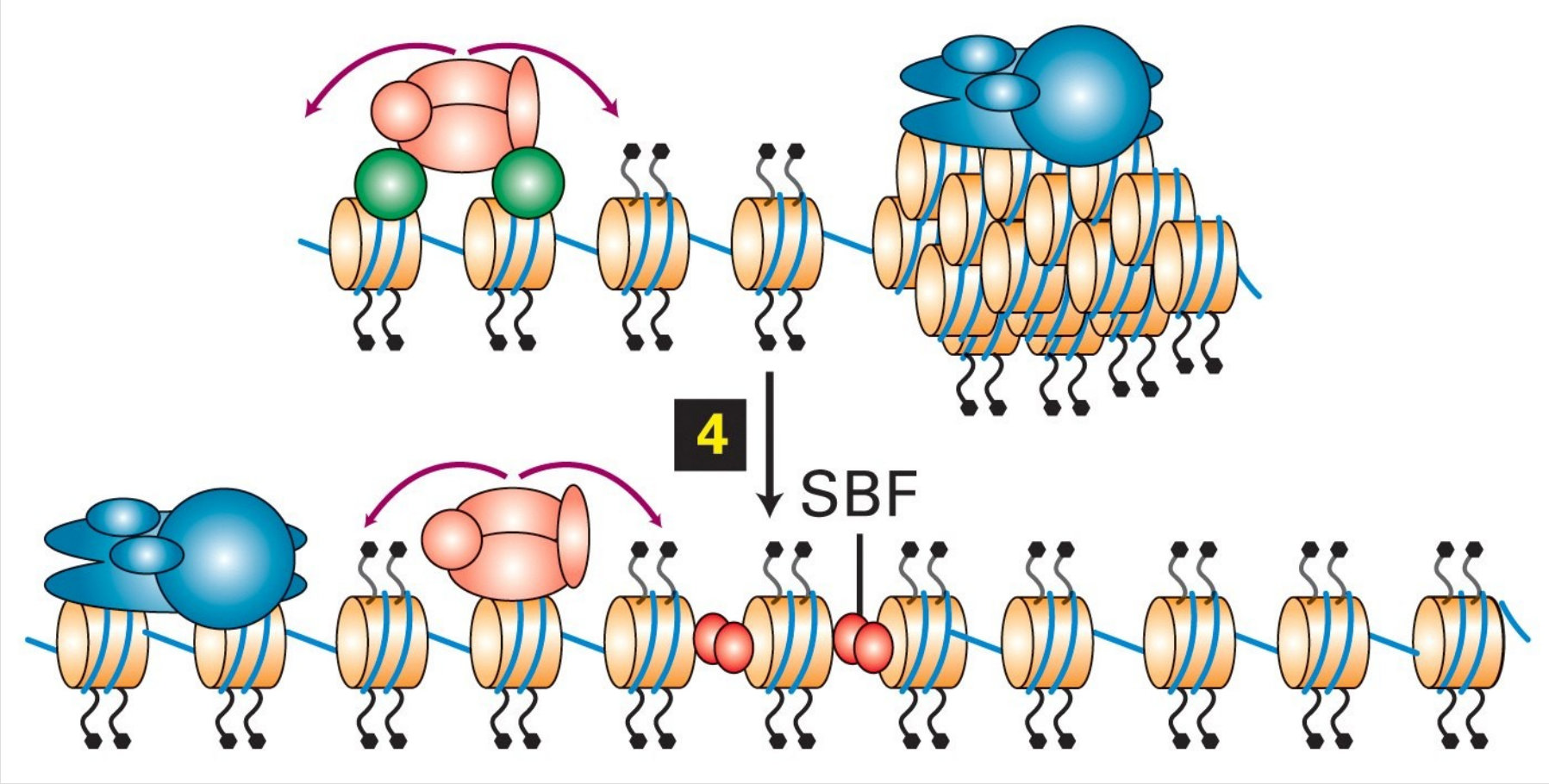
GCN5 acetylates histones -1



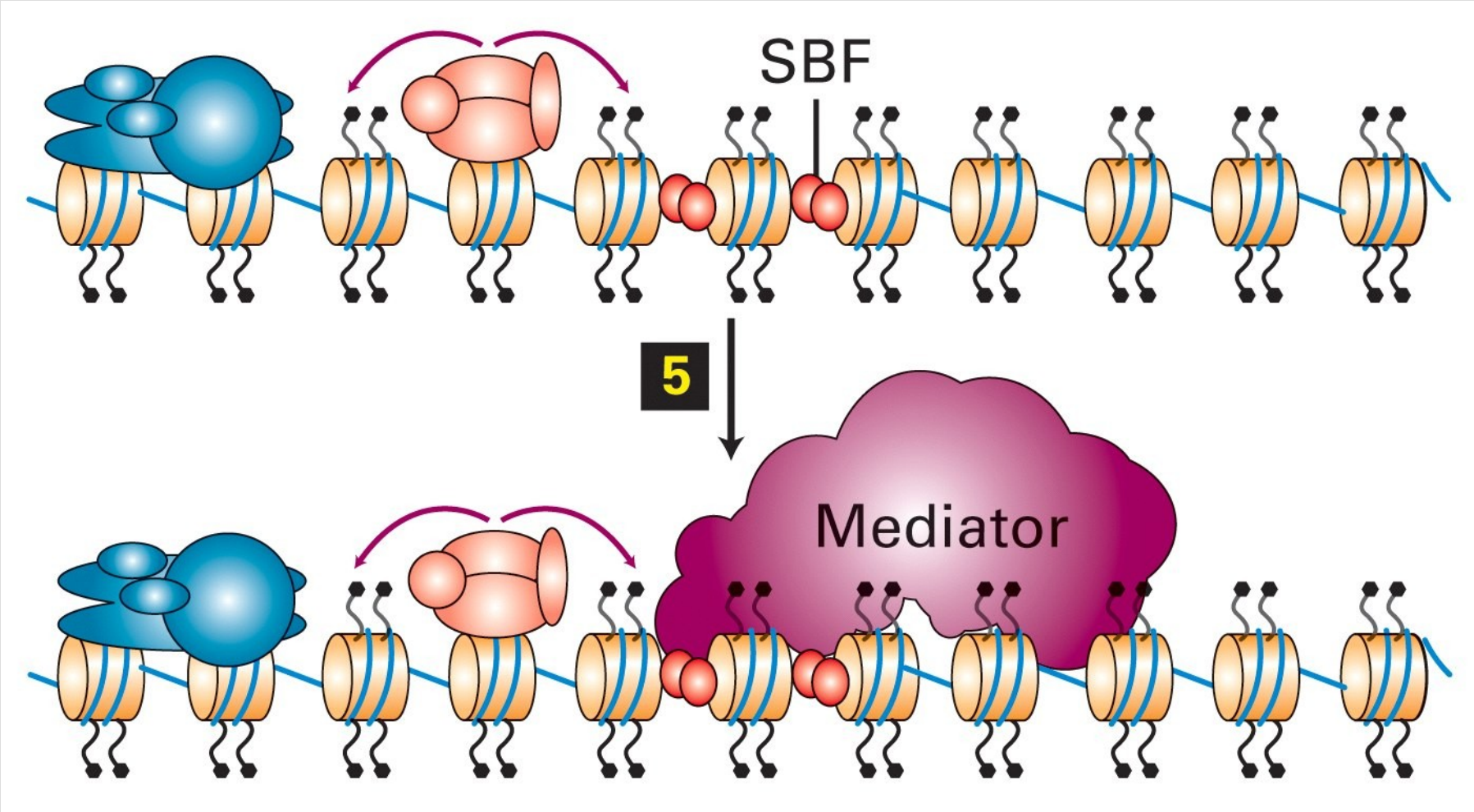
GCN5 acetylates histones -2



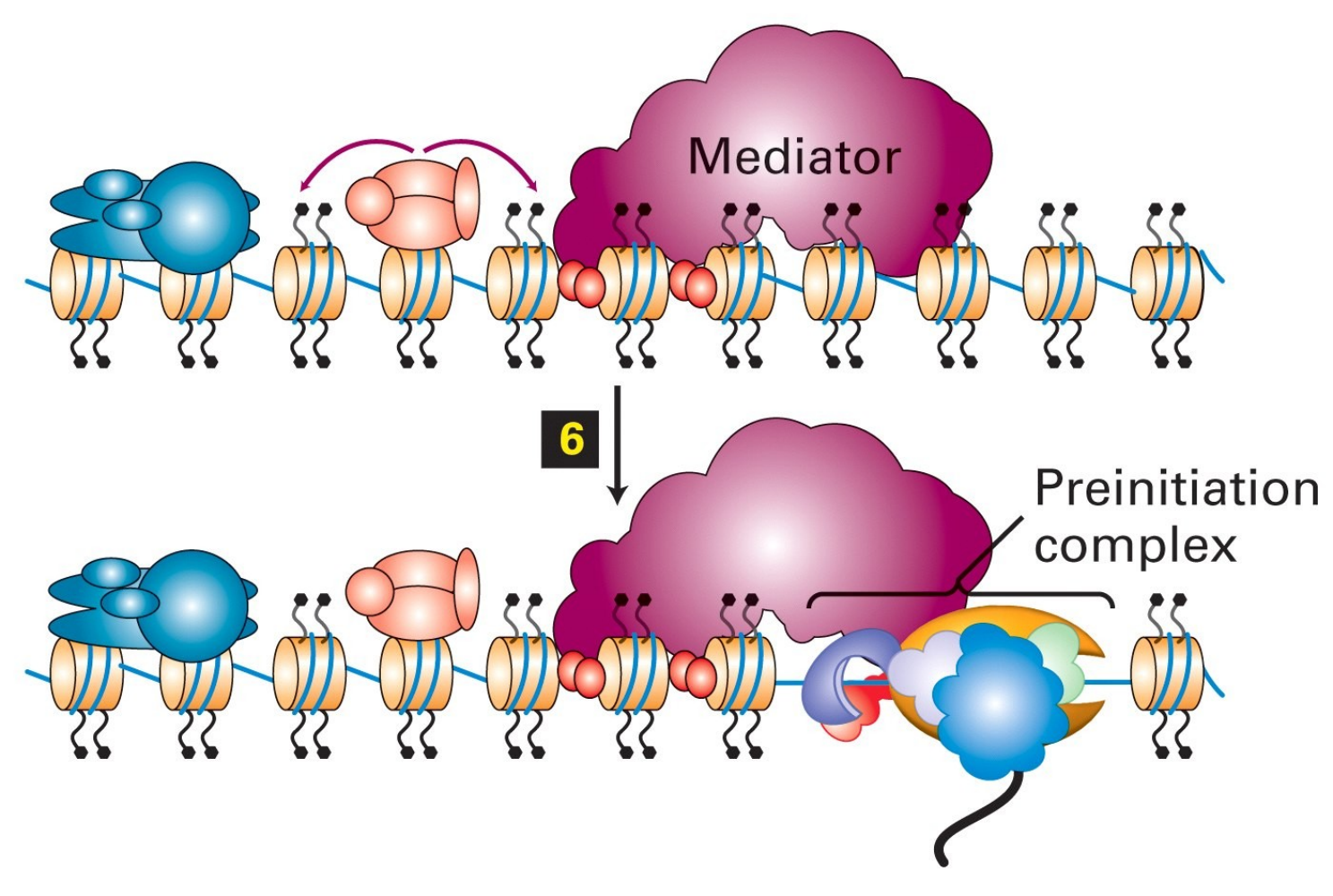
SBF a transcription factor attaches



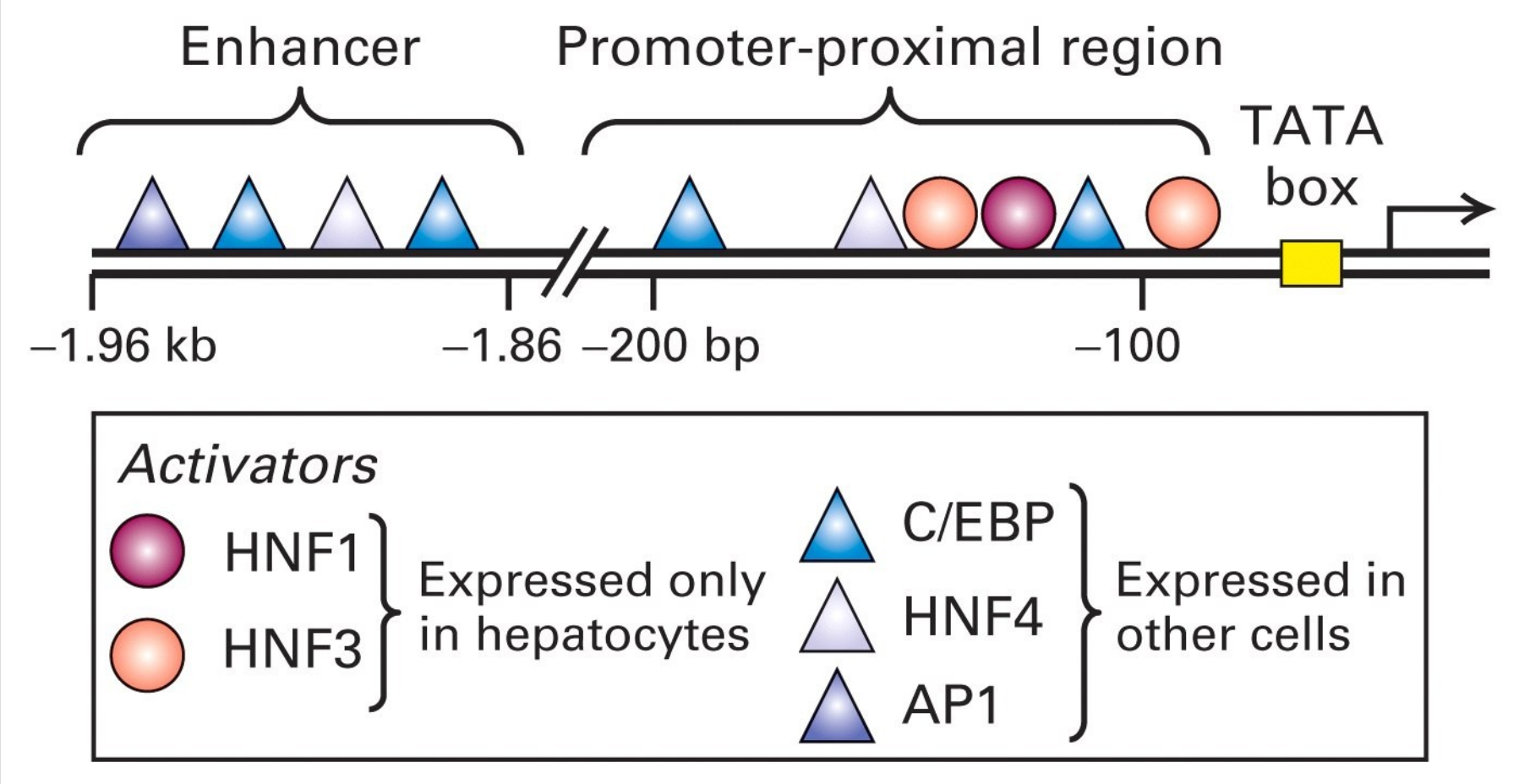
Mediator attaches



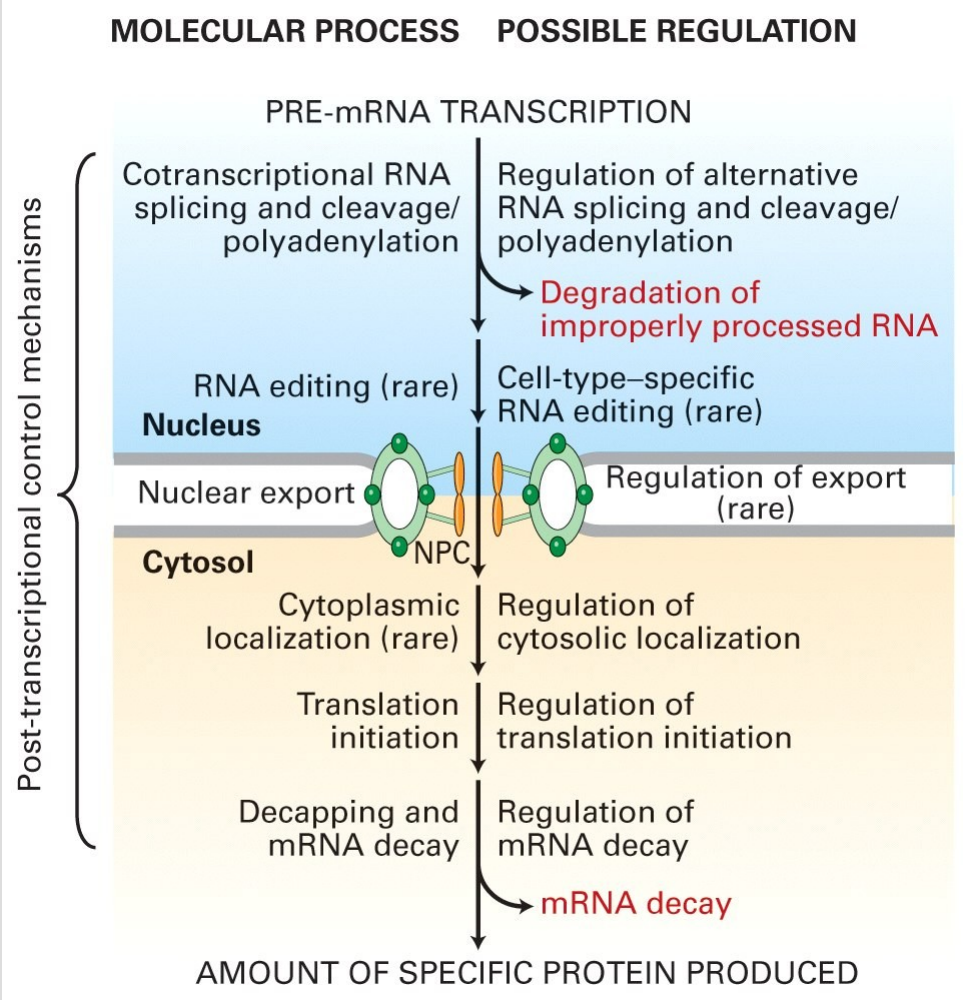
Preinitiation complex forms & RNA polymerization begins



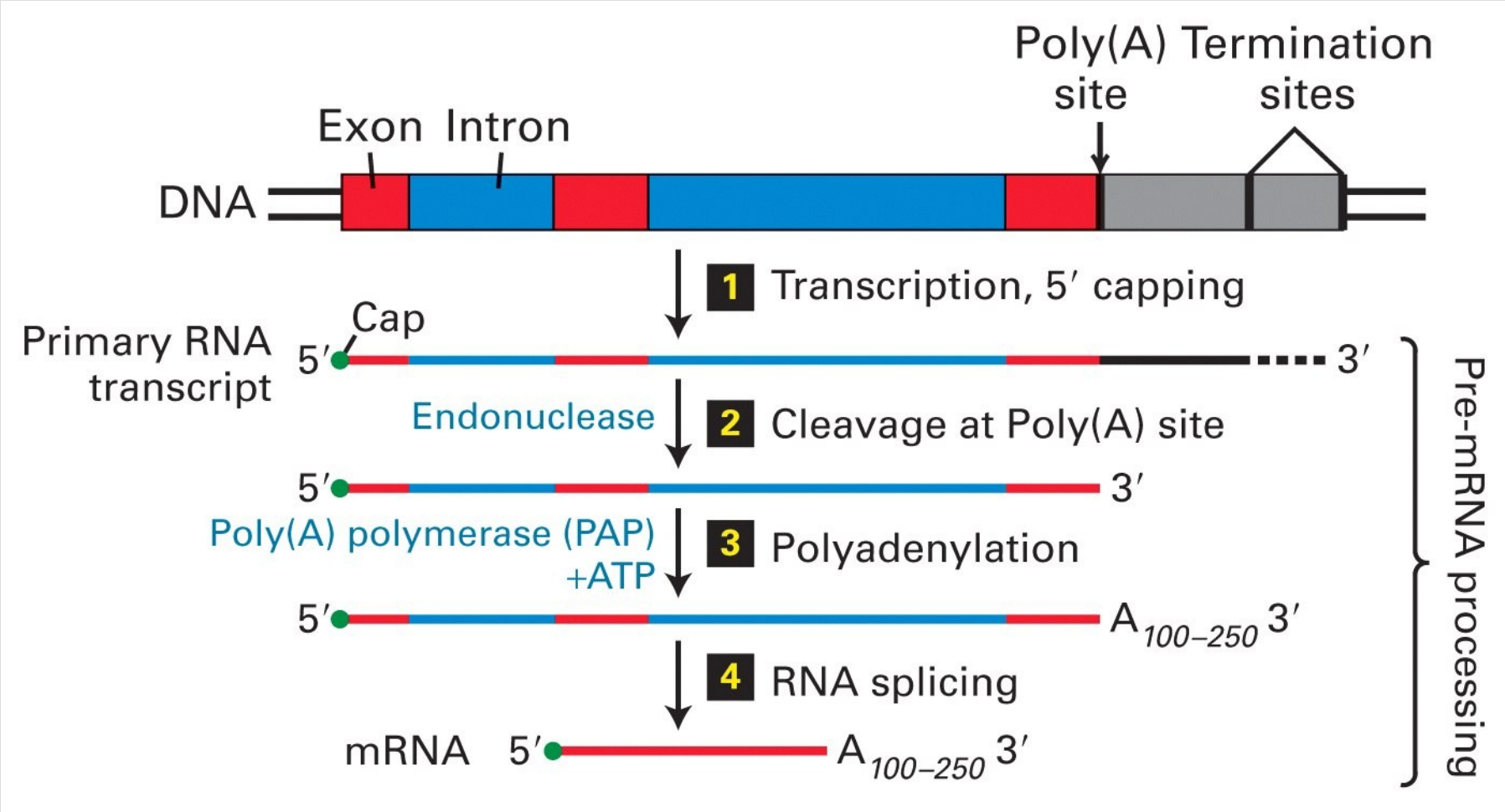
How things line up



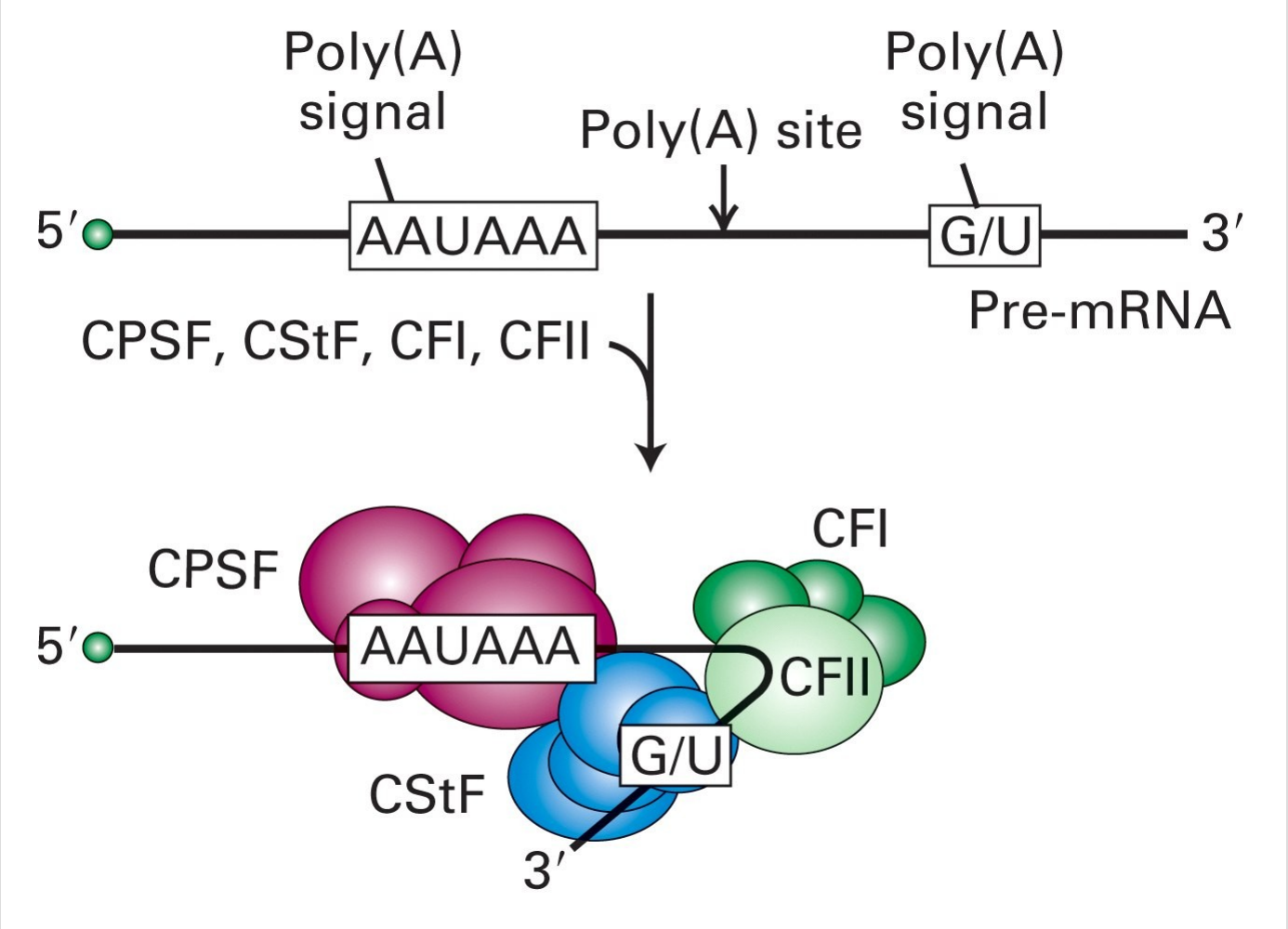
Post transcriptional processes



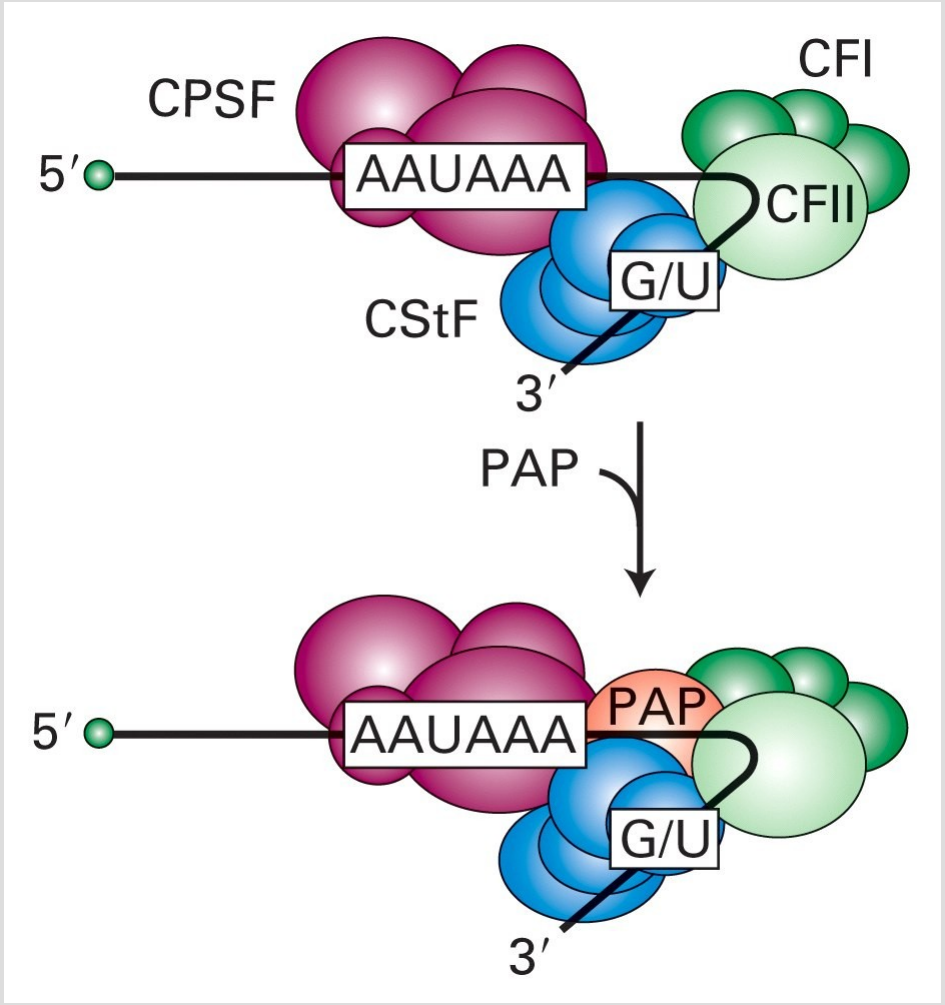
Cis splicing



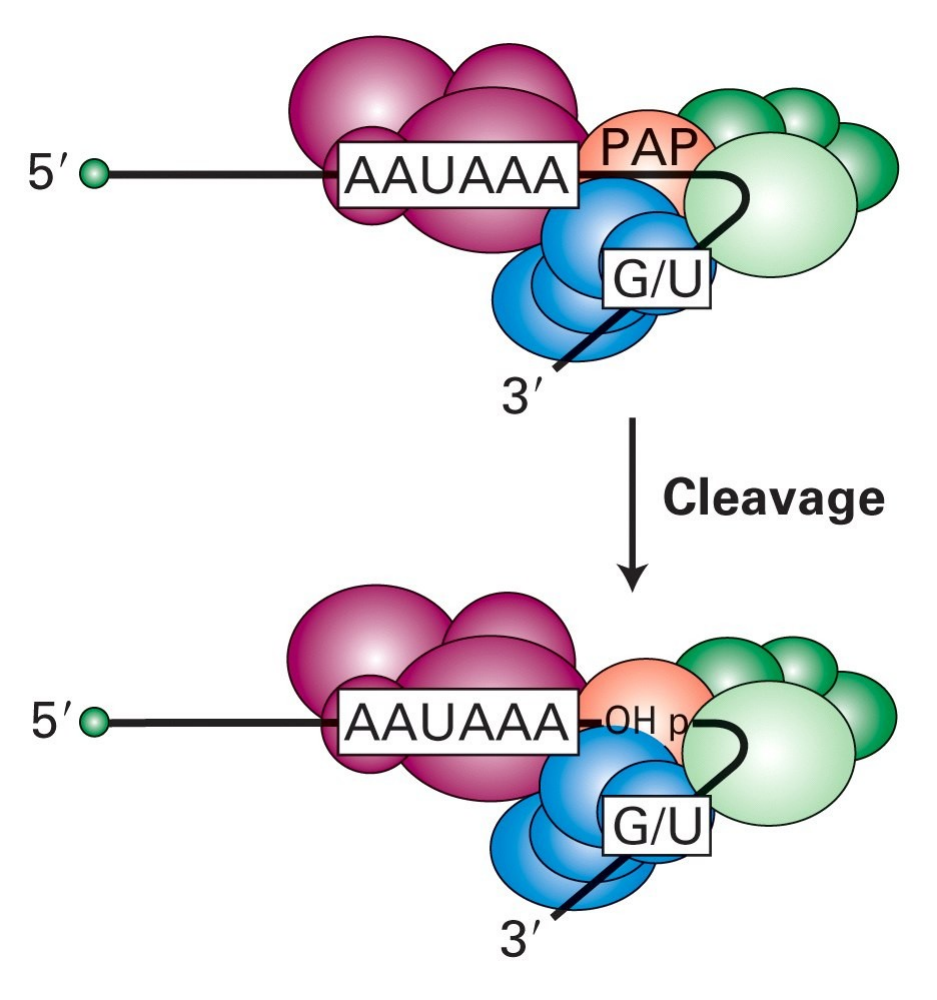
Cleaving at poly-A site



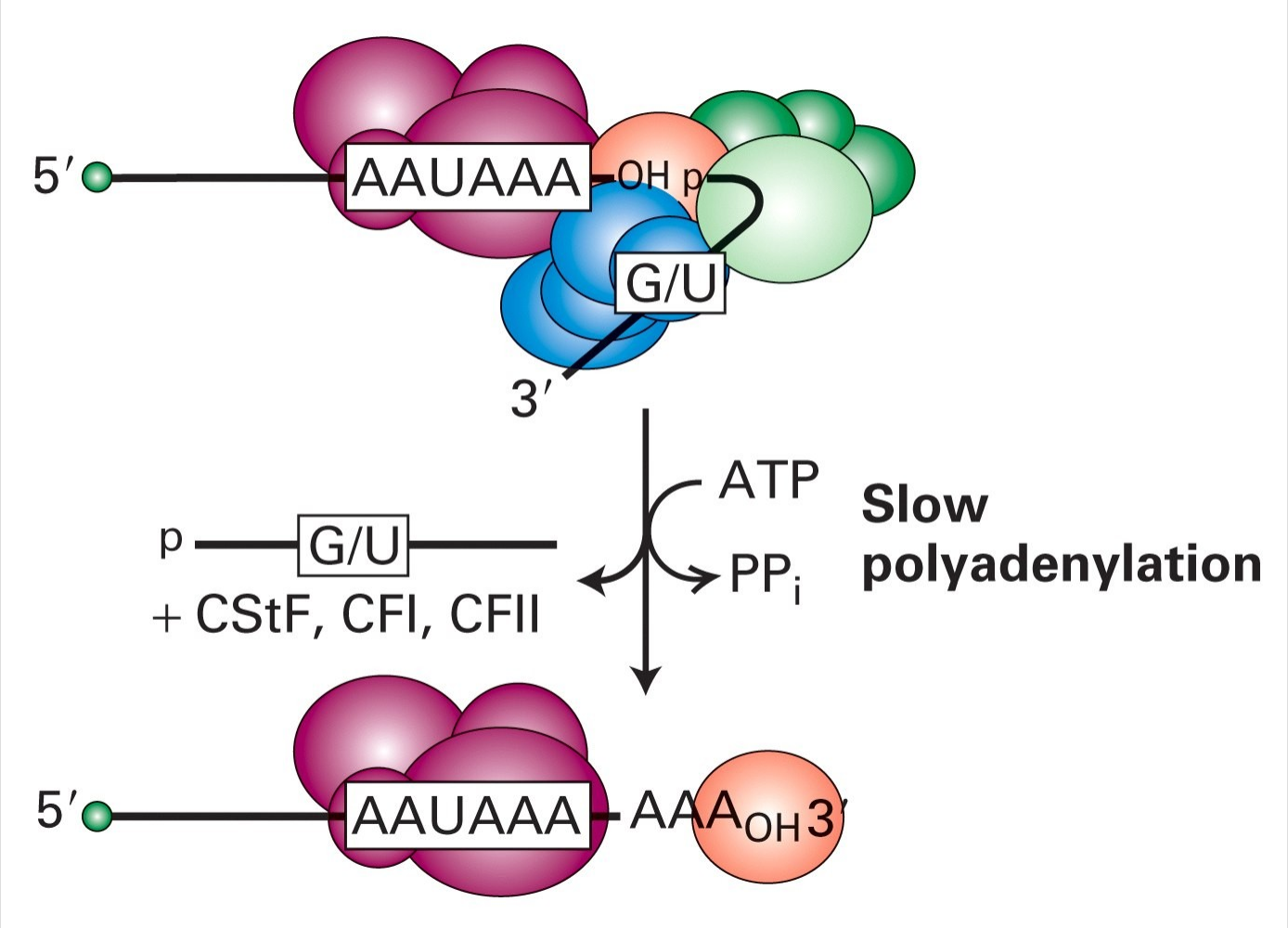
Cleaving -1



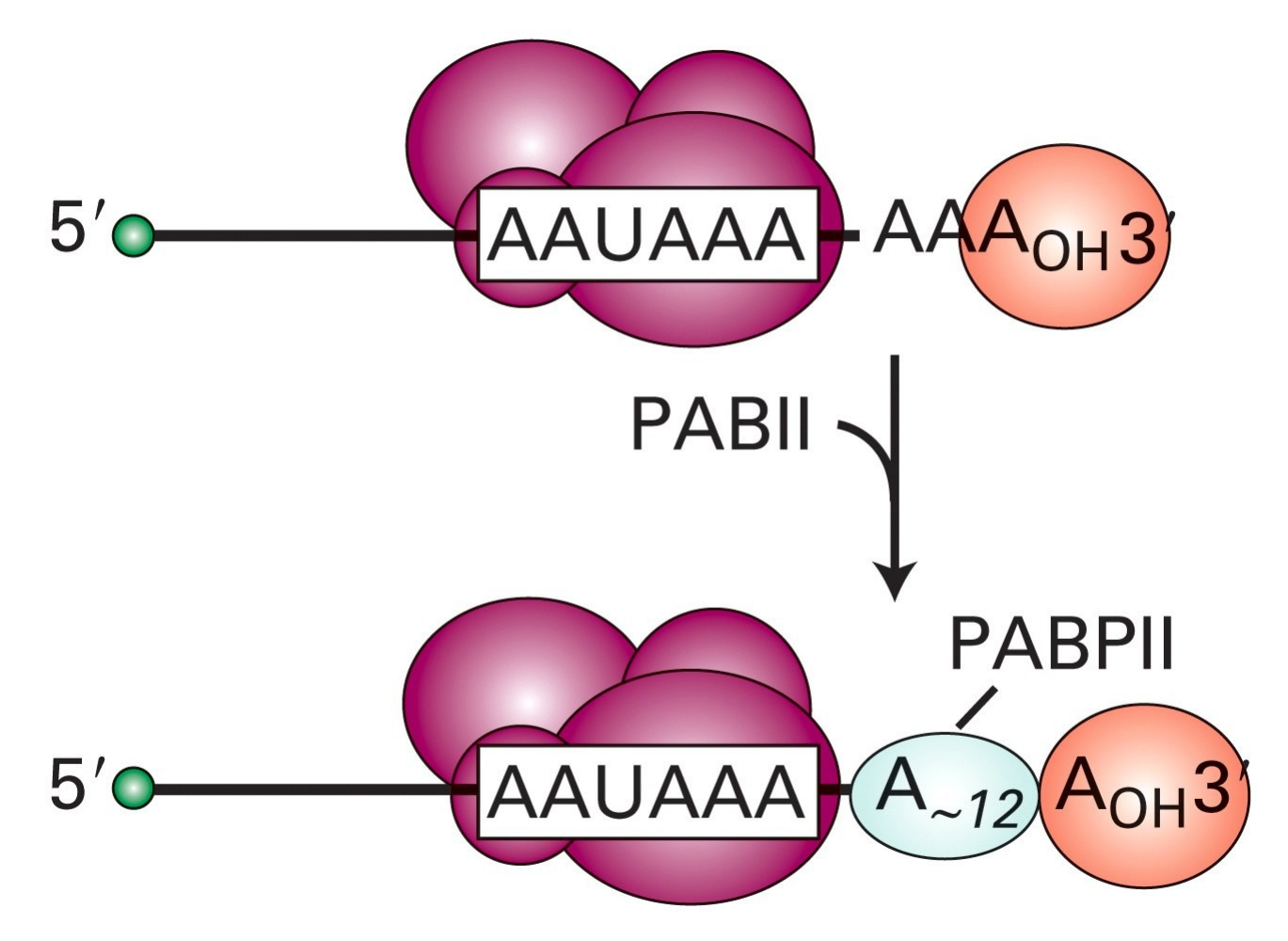
Cleaving -2



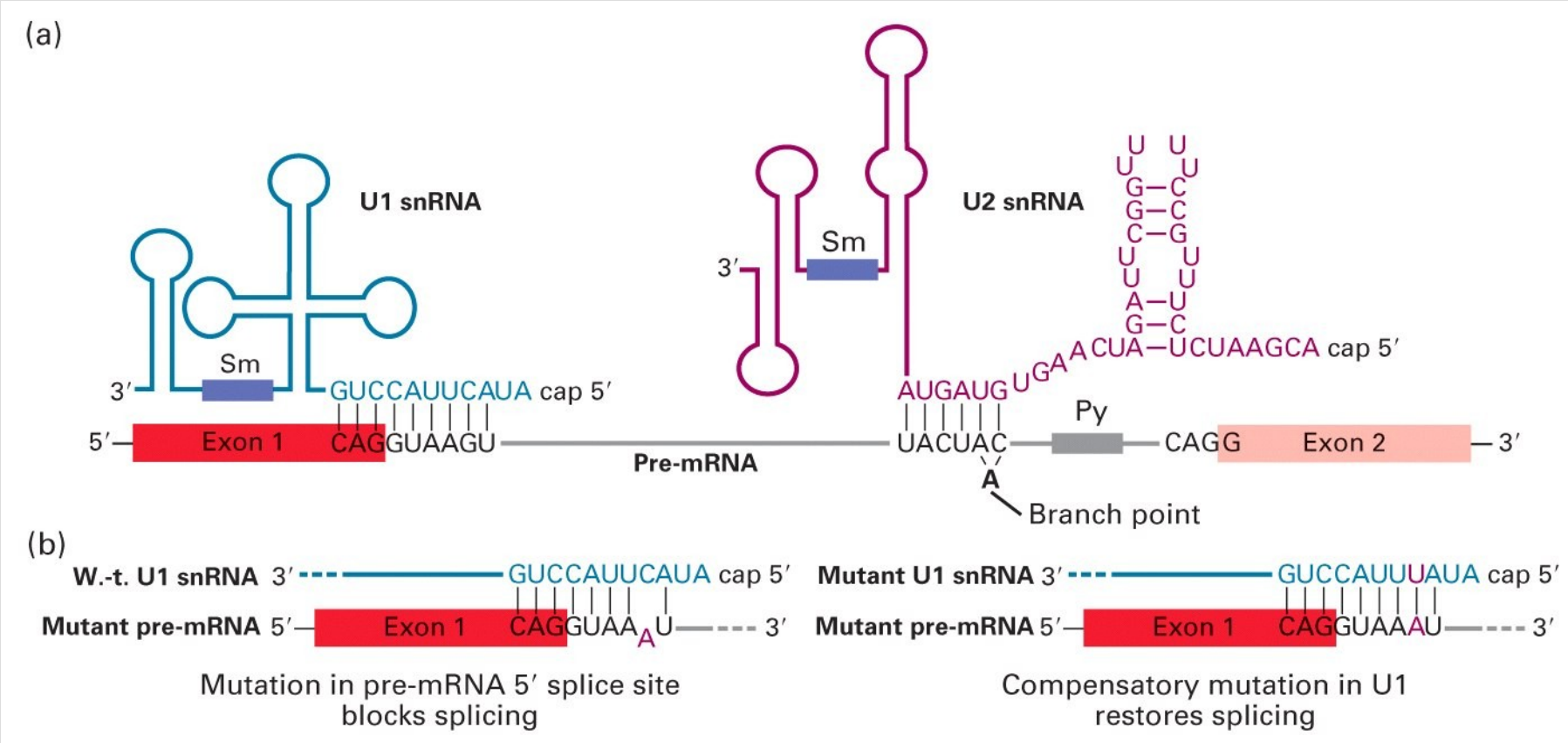
Polyadenylation -1



Polyadenylation -2

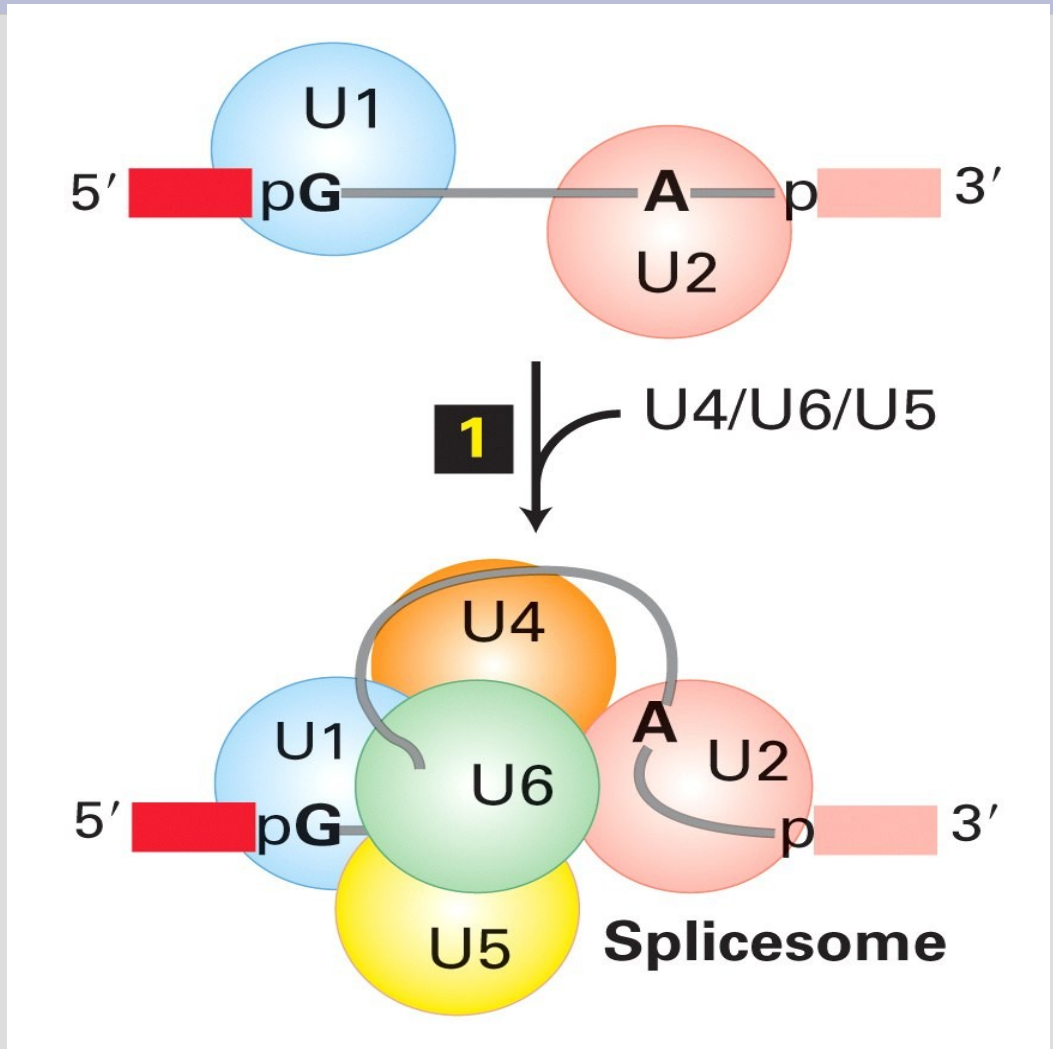


Splicing

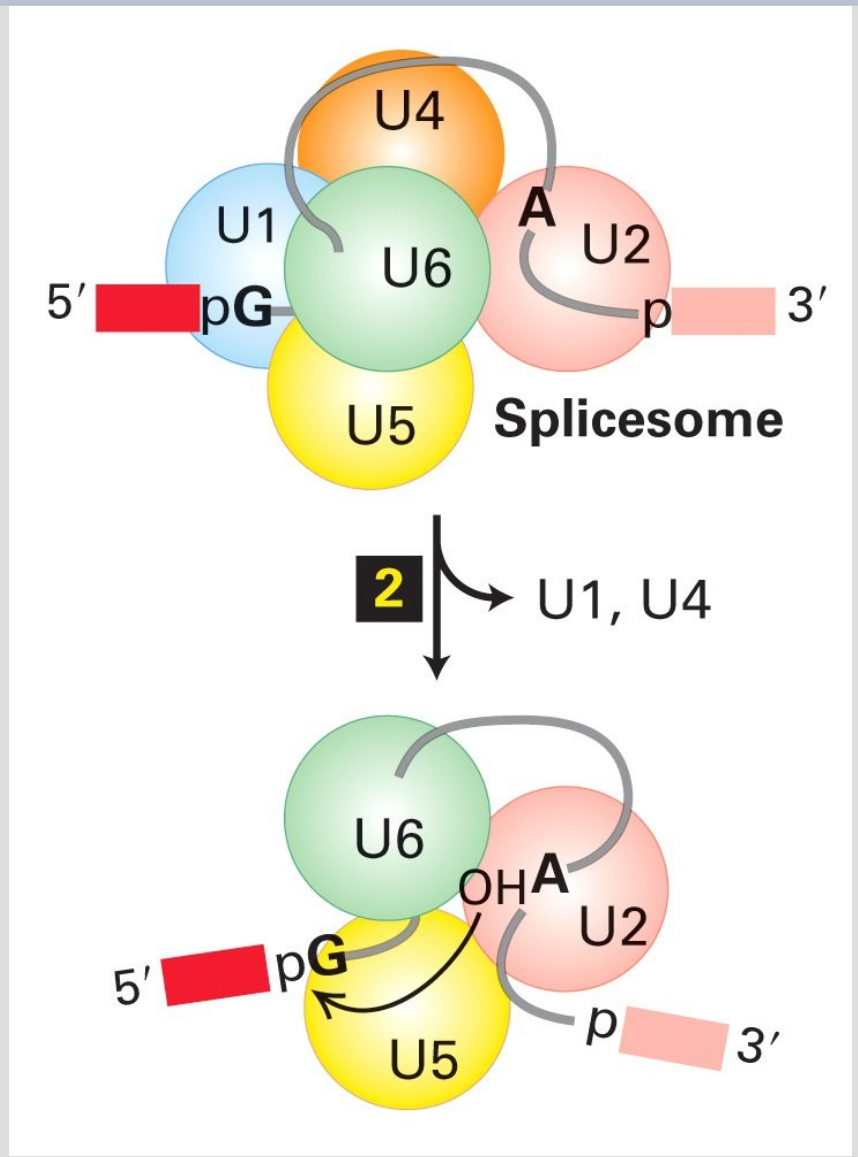

(a)
U1 snRNA
Sm
3′
GUCCAUUCAUA cap 5′
5′
Exon 1
CAGGUAAGU
Pre-mRNA
U2 snRNA
Sm
3′
AUGAUG
UGA
ACUA—UCUAAGCA cap 5′
Py
UACUAC
CAGG
Exon 2
3′
A
Branch point
(b)
W.-t. U1 snRNA 3′
GUCCAUUCAUA cap 5′
Mutant pre-mRNA 5′
Exon 1
CAGGUAAAU
3′
Mutation in pre-mRNA 5′ splice site blocks splicing
Mutant U1 snRNA 3′
GUCCAUUUAUA cap 5′
Mutant pre-mRNA 5′
Exon 1
CAGGUAAAU
3′
Compensatory mutation in U1 restores splicing

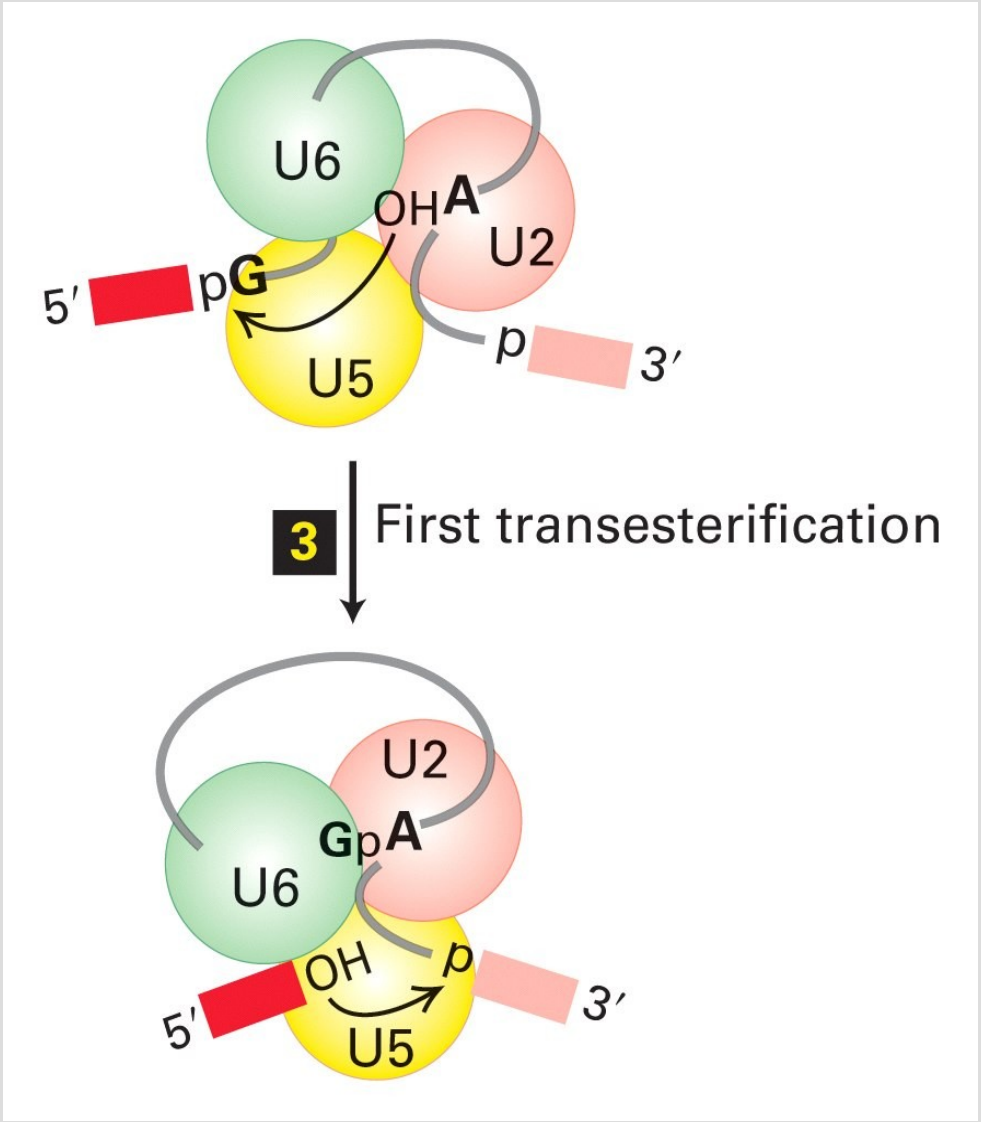
Spliceosome action -1

cis splicing, i.e. intron excised exons spliced from the same strand

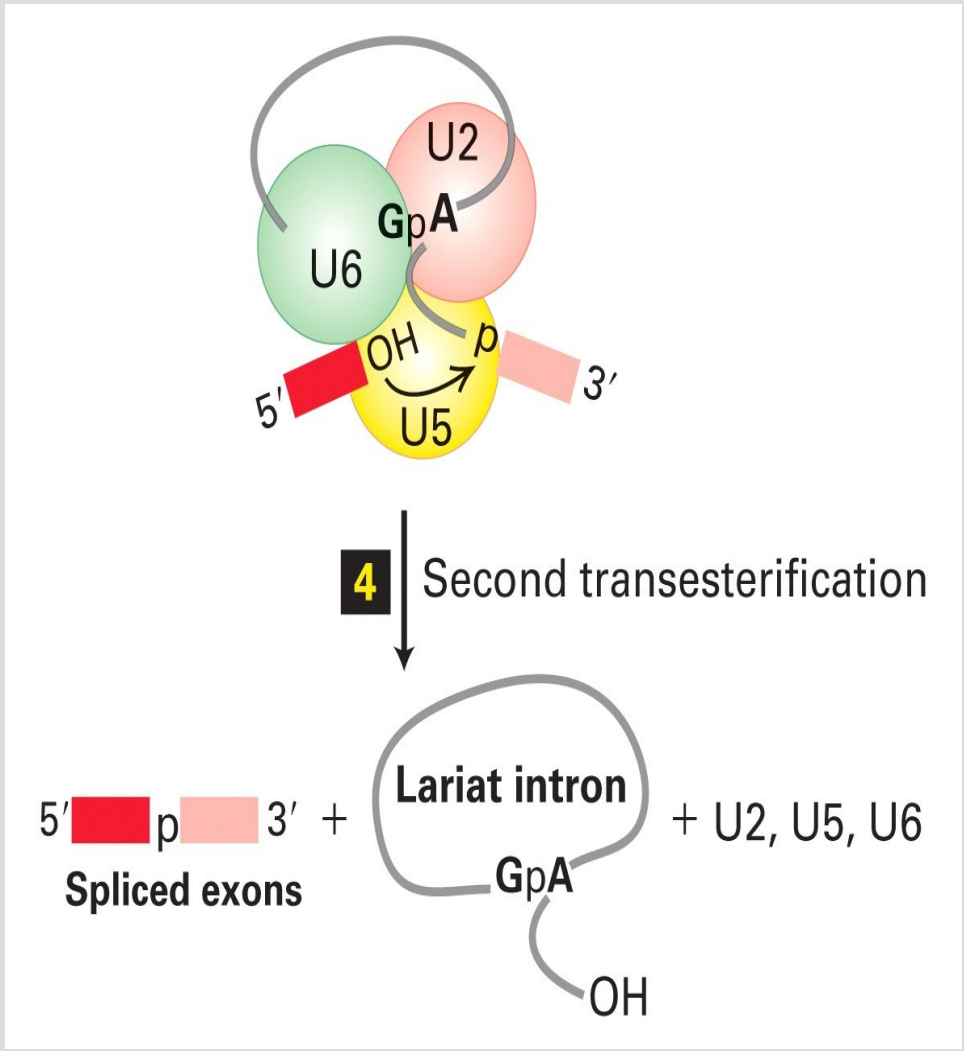


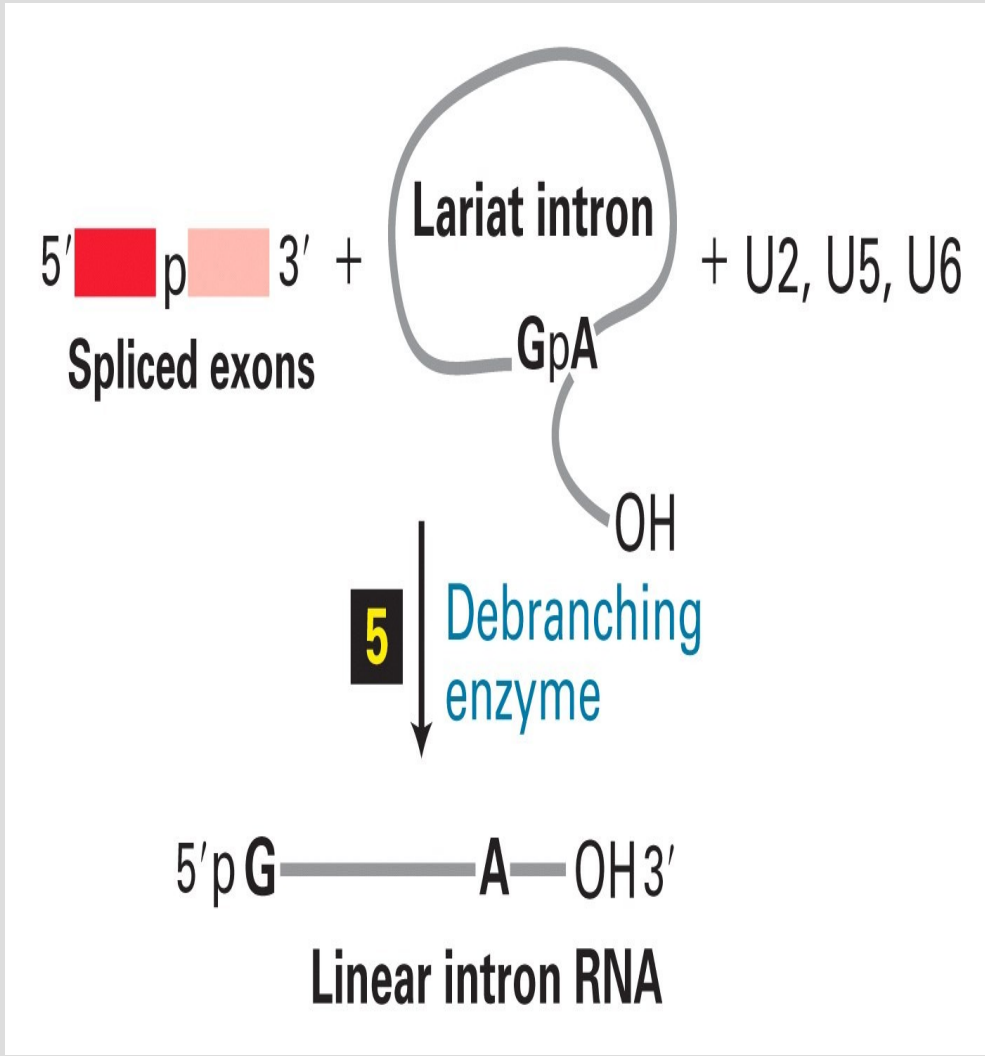
Spliceosome action -2



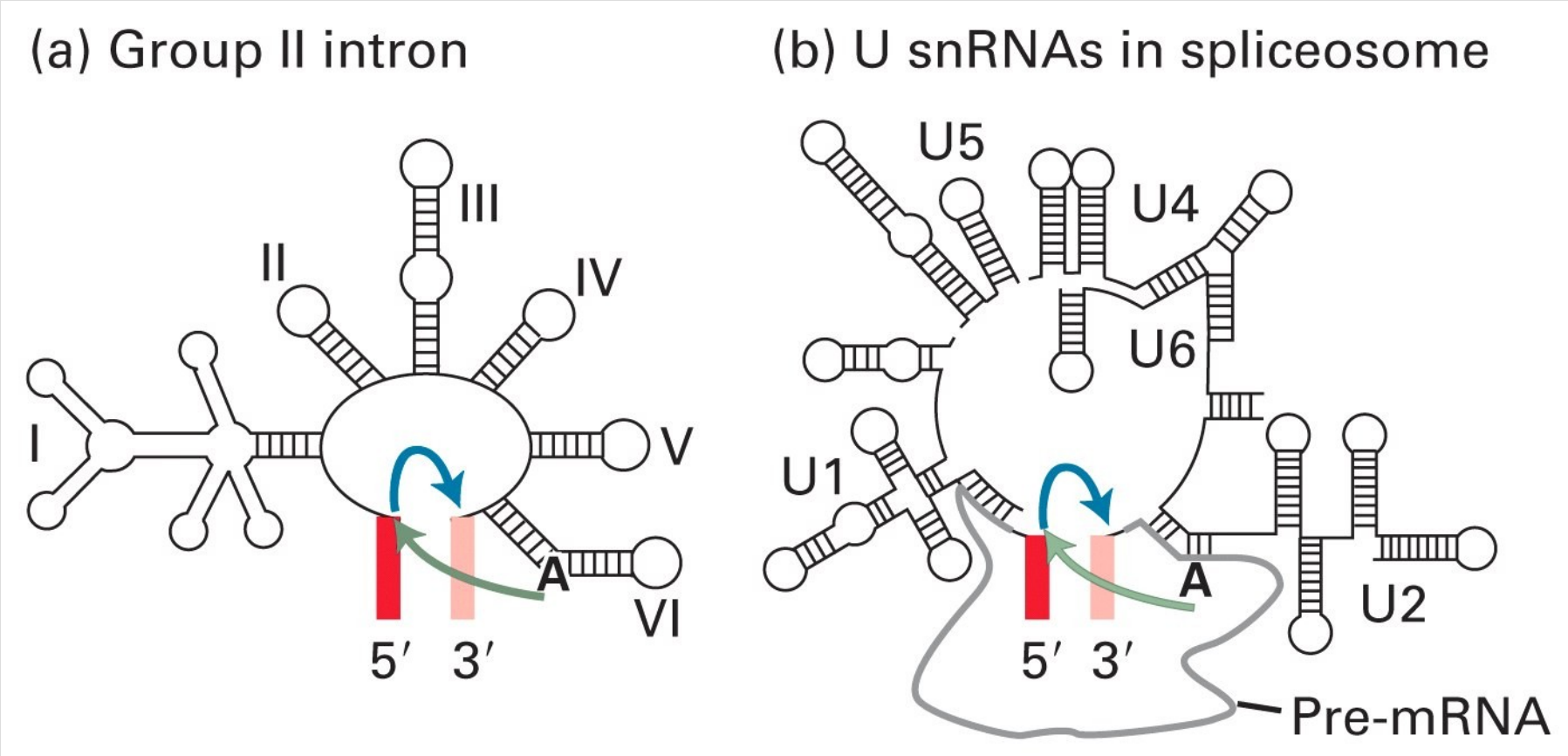


Spliceosome action -3





Sketch of core spliceosome structure



Summary

- In prokaryotes transcription and translation could occur concurrently
- In eukaryotes, transcription and translation in two different locations with an intervening process that excises introns and spices exons – this gives additional possibilities for protein synthesis (alternative splicing)
- Also regulation of expression more complex for exons, because gene might be in an inactive condensed state