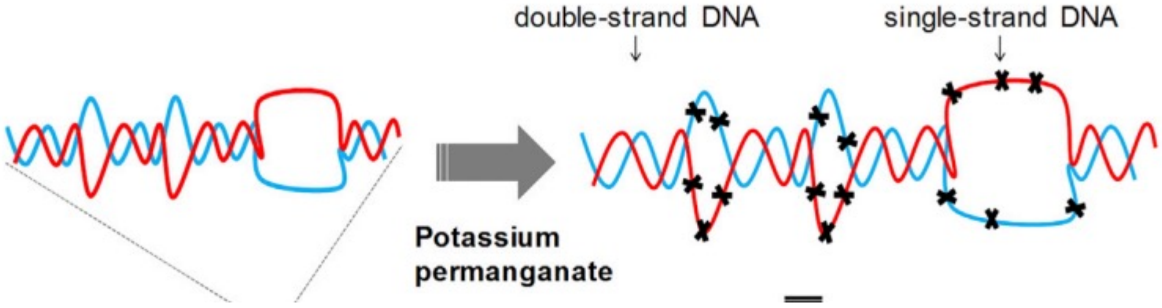
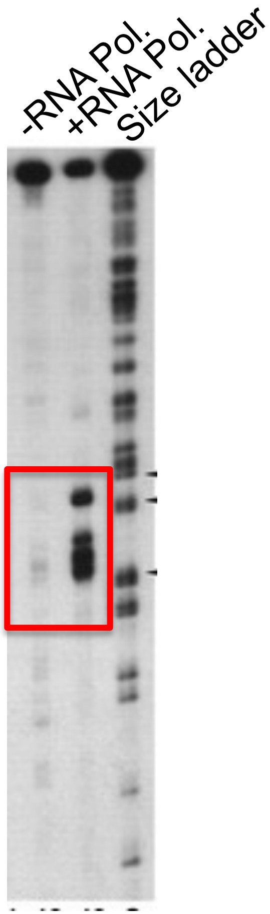
Permanganate (KMnO4) footprinting + primer extension: Detects single stranded regions



Kouzine et al., 2017, Cell Systems



DNA region unwound by the addition of RNA Pol.

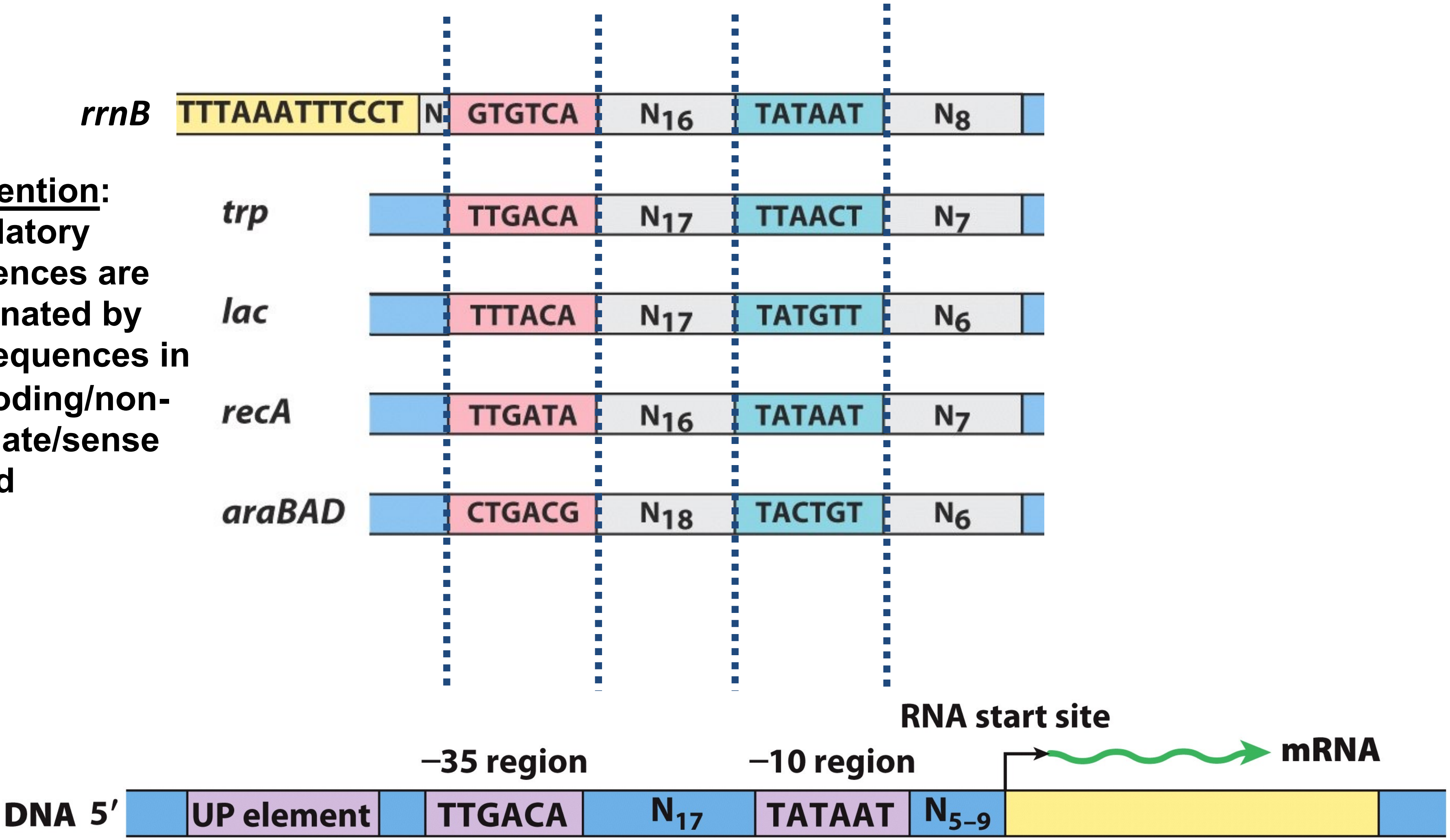
<u>Step 1</u>: Permanganate chemically modifies DNA in single stranded regions

<u>Step 2</u>: Primer extension — polymerase will stop at chemically modified positions

<u>Step 3</u>: Read out on a gel

Alignments of the sequences at the site of contact between the RNA polymerase and the promoter reveals conserved promoter DNA sequences

<u>Convention</u>: Regulatory sequences are designated by the sequences in the coding/non-template/sense strand


rrnB TTTAAATTTCCT N GTGTCA N_{16} TATAAT N_8

trp TTGACA N_{17} TTAACT N_7

lac TTTACA N_{17} TATGTT N_6

recA TTGATA N_{16} TATAAT N_7

araBAD CTGACG N_{18} TACTGT N_6

RNA start site

−35 region

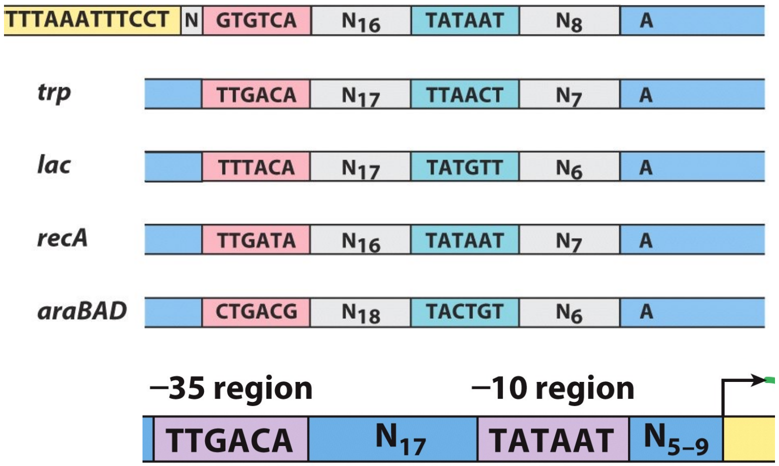
−10 region

mRNA

DNA 5′ UP element TTGACA N_{17} TATAAT N_{5-9}

Figure 28-2
Lehninger Principles of Biochemistry, Sixth Edition

These promoter sequences don’t match the consensus sequence completely. Why?



A: Perfect match would result in too strong binding by RNA polymerase making it difficult to regulate its assembly and thus transcription.

B: These regions are also used to bind other proteins than the RNA Polymerase (activator, repressor) and their sequence must be adapted to bind both proteins

C: Because you don’t need a perfect match for the RNA polymerase to bind the promoter

D: Because of natural polymorphisms in DNA sequences

Different σ factors -> Different classes of genes are activated

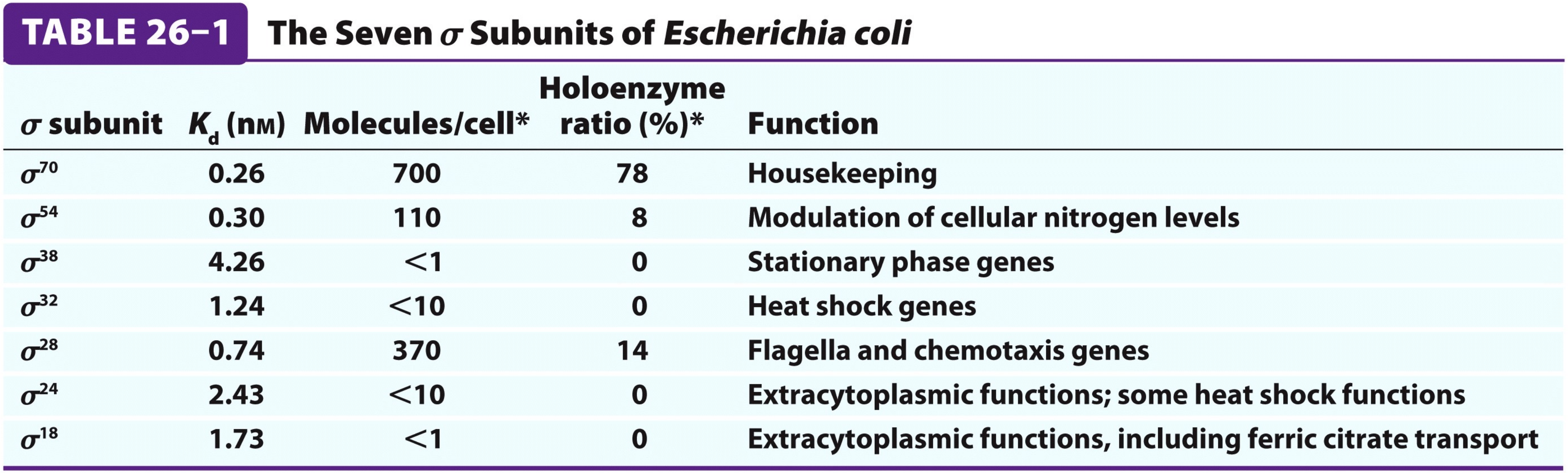


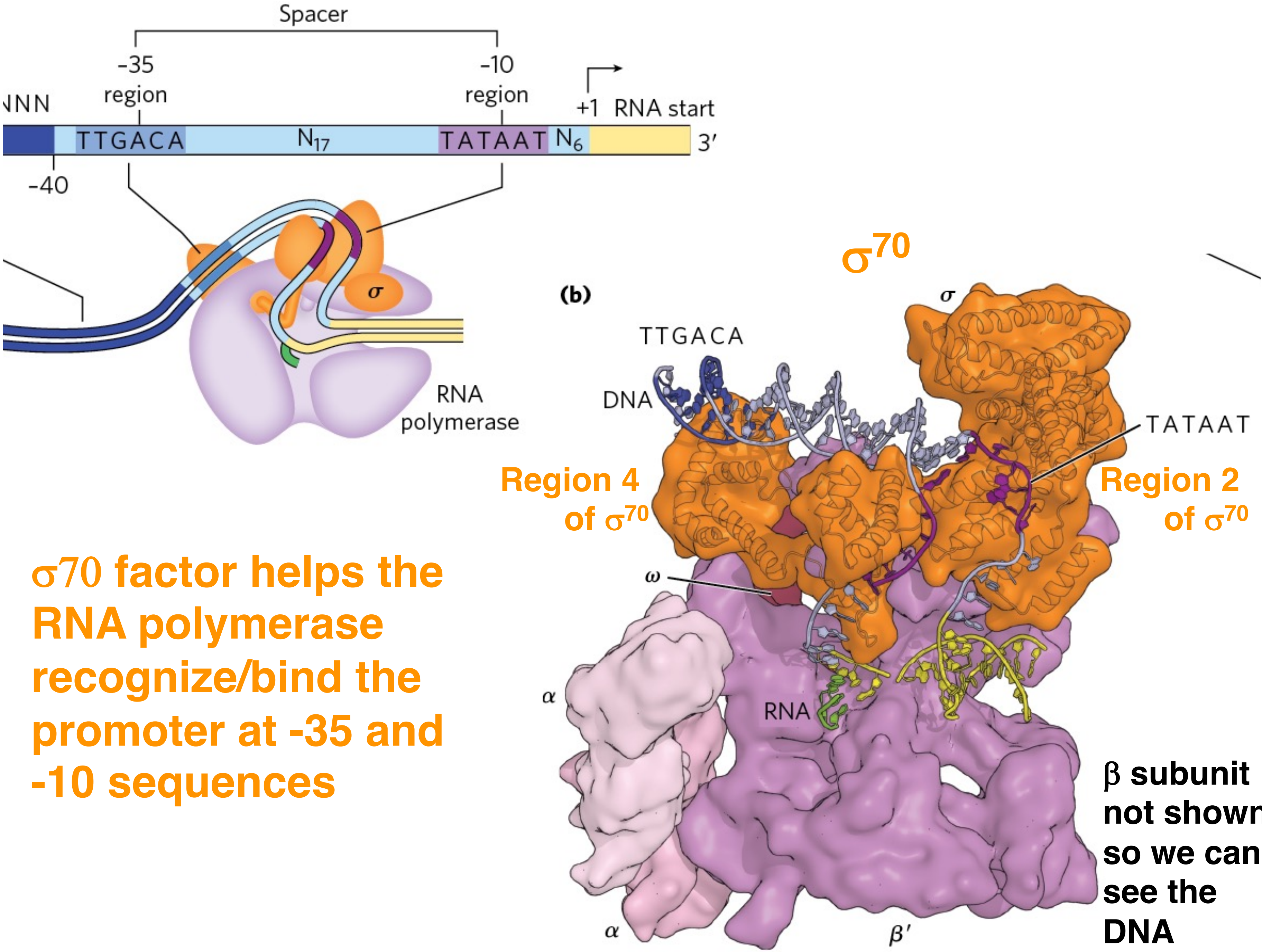
TABLE 26–1 The Seven σ Subunits of *Escherichia coli*

σ subunit	K_d (nM)	Molecules/cell*	Holoenzyme ratio (%)*	Function
σ^{70}	0.26	700	78	Housekeeping
σ^{54}	0.30	110	8	Modulation of cellular nitrogen levels
σ^{38}	4.26	<1	0	Stationary phase genes
σ^{32}	1.24	<10	0	Heat shock genes
σ^{28}	0.74	370	14	Flagella and chemotaxis genes
σ^{24}	2.43	<10	0	Extracytoplasmic functions; some heat shock functions
σ^{18}	1.73	<1	0	Extracytoplasmic functions, including ferric citrate transport

Number of σ factors vary depending on bacteria: 7 in *E.coli*, 1 in *Mycoplasma genitalium*, 63 in *Streptomyces coelicolor*

Different σ factors recognize different promoter consensus sequences (no need to memorize these sequences):

σ^{70}:	TTGACA -35	TATAAT -10
σ^{32}:	CNCTTGAA -35	CCCCATNT -10
σ^{54}:	CTGGNA -24	TTGCA -12



σ^{70}

Region 4 of σ^{70}

Region 2 of σ^{70}

σ70 factor helps the RNA polymerase recognize/bind the promoter at -35 and -10 sequences

β subunit not shown so we can see the DNA

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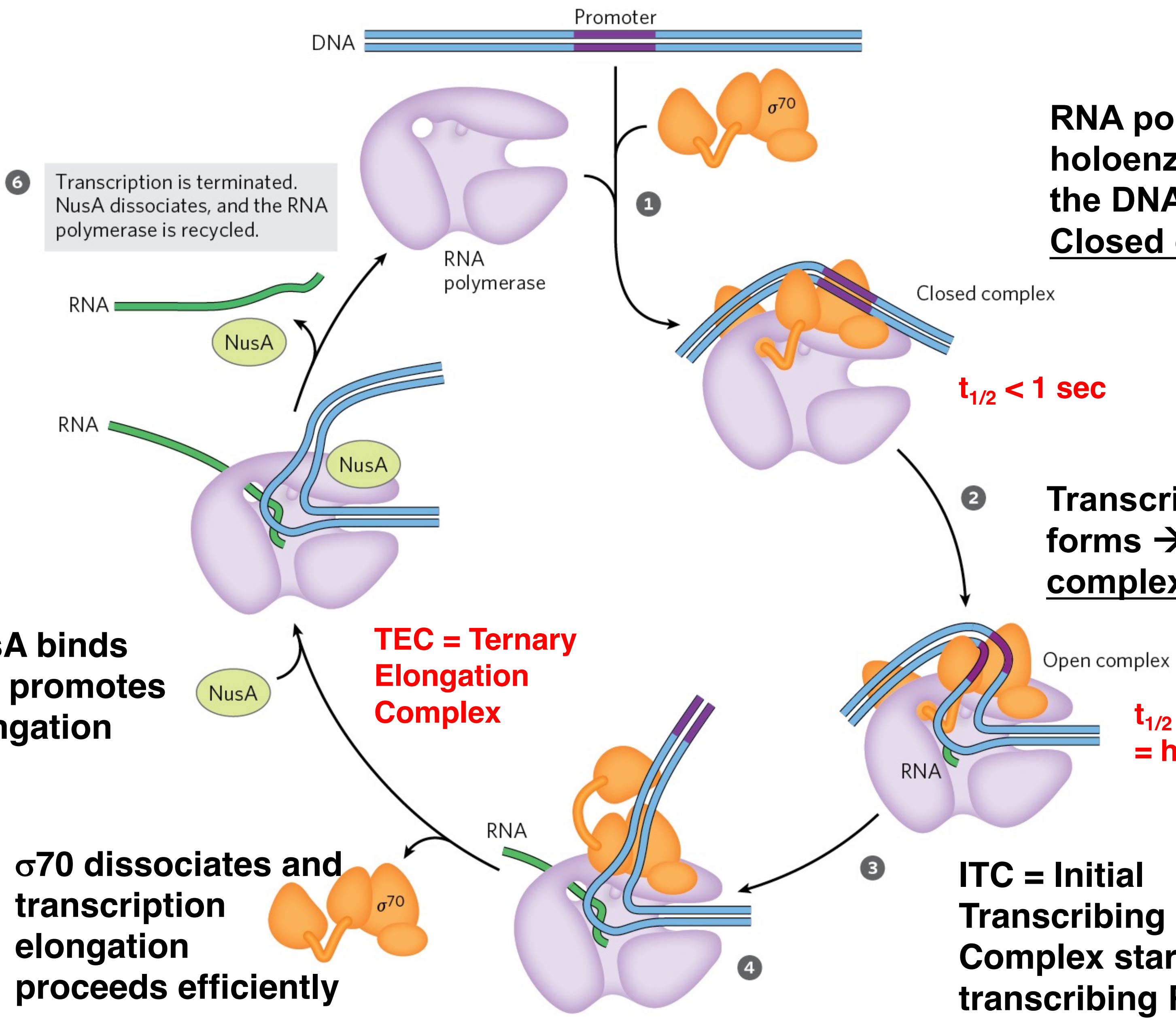
The Transcription Cycle



RNA polymerase holoenzyme binds the DNA promoter → <u>Closed complex</u>

$t_{1/2} < 1$ sec

Transcription bubble forms → <u>Open complex</u>

$t_{1/2}$ = hours

ITC = Initial Transcribing Complex starts transcribing RNA

σ70 dissociates and transcription elongation proceeds efficiently

TEC = Ternary Elongation Complex

NusA binds and promotes elongation

The Transcription Cycle

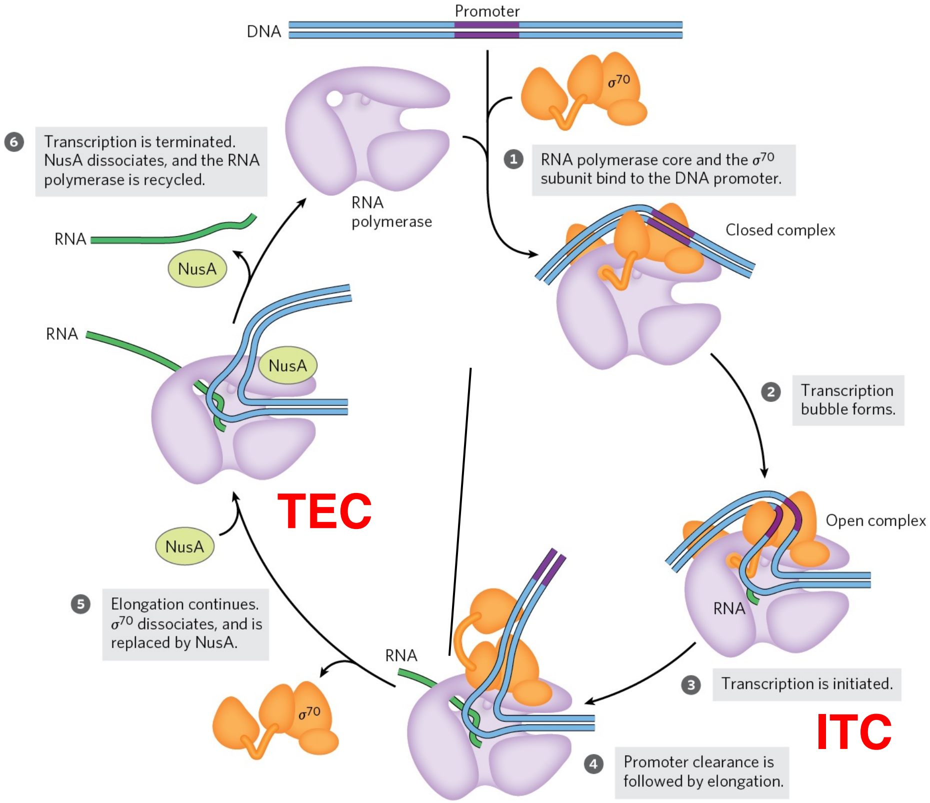
RNA Polymerase functions like a helicase to open the 2 strands of the DNA at promoters:

σ^{70} holoenzyme does not require ATP for open complex formation

(other σ factors can work differently, eg σ^{54} requires ATP for open complex formation)

- **In ITC, association of the transcript is weak resulting in release of short RNAs.**
- **100s of cycles of abortive initiation sometimes precede the next step.**

Ejection of σ increases the stability of the complex. The enzyme is now highly processive. Switch into TEC = Ternary Elongation Complex



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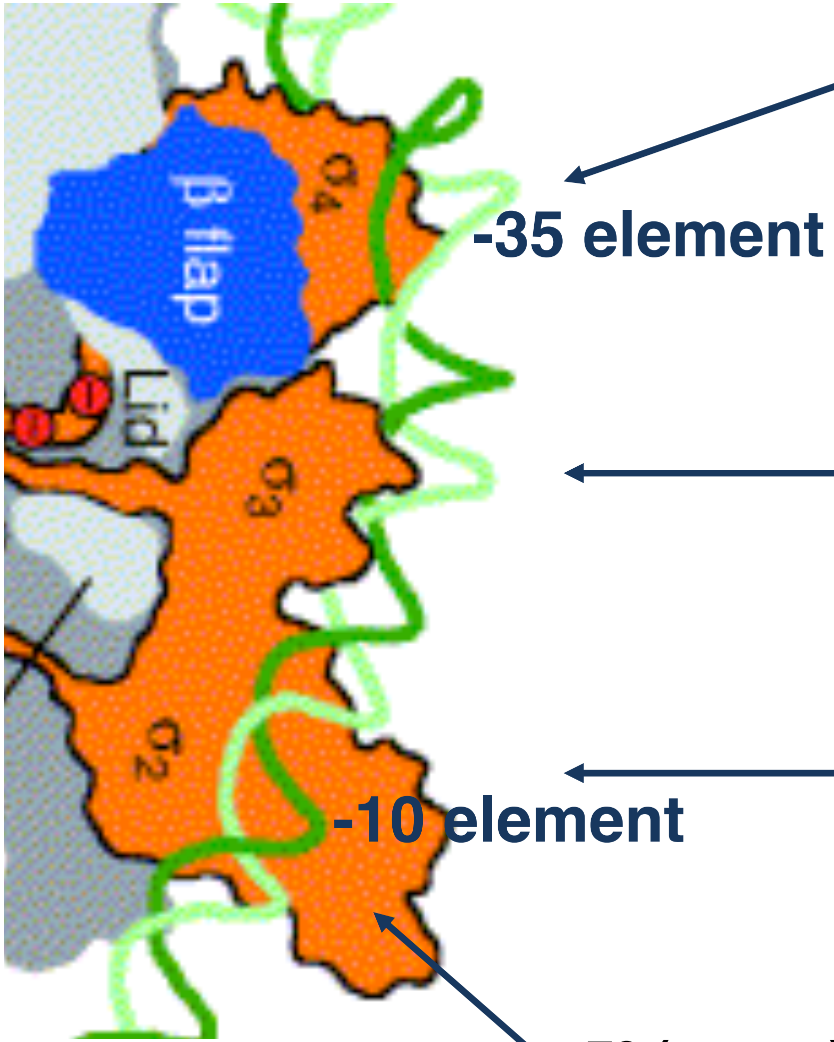
How does promoter recognition and the transition from the Closed -> Open Complex occur?

σ70 contains multiple DNA recognition regions:



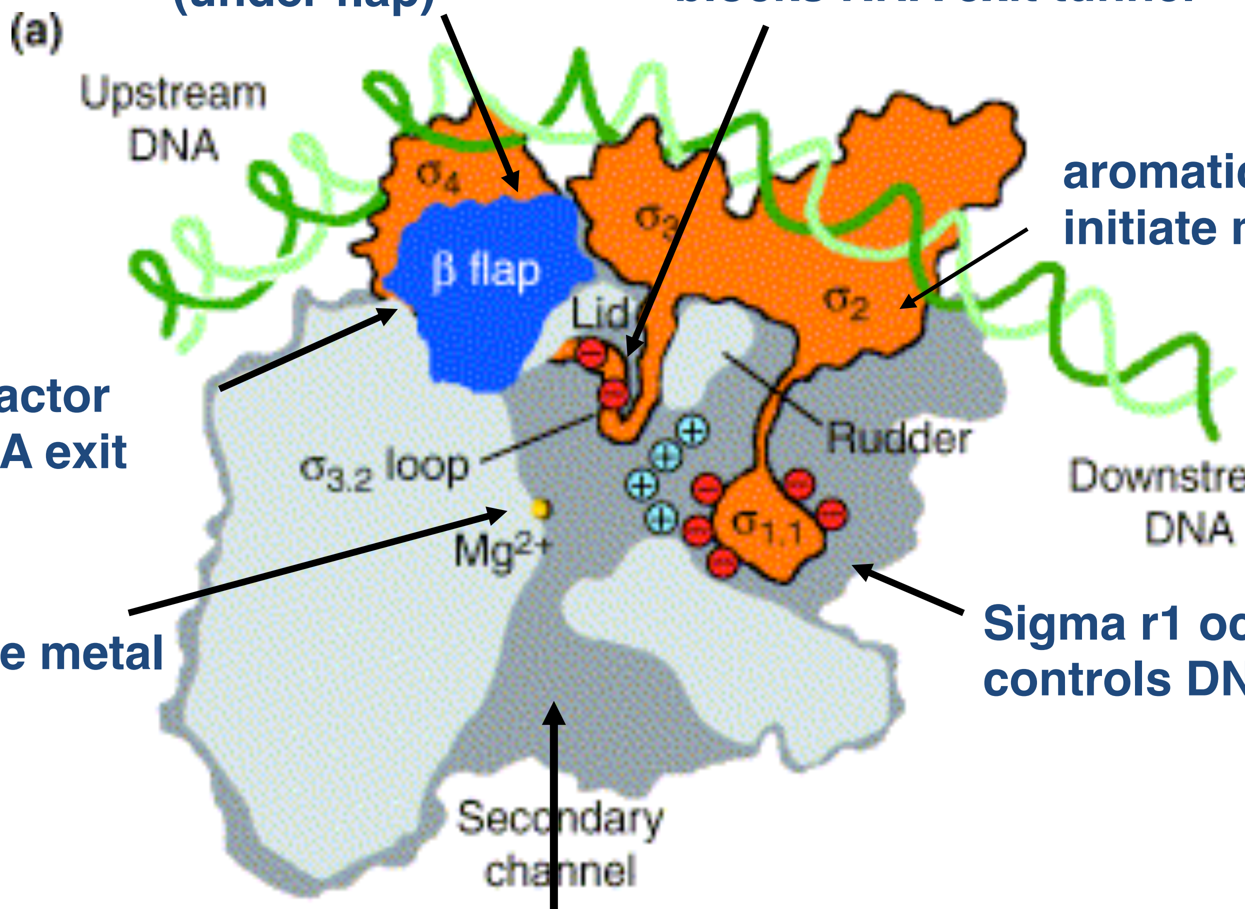
Region 4.2
binds -35 element +
flap region of the β subunit
of the RNA Polymerase core

Region 3 linker spans
the promoter spacer

Region 2
binds -10 element +
aromatics approach DNA

σ70 (orange)

Features of bacterial RNAP that influence transcription initiation and escape



RNA exit channel (under flap)

σ factor occlusion blocks RNA exit tunnel

aromatic residues initiate melting

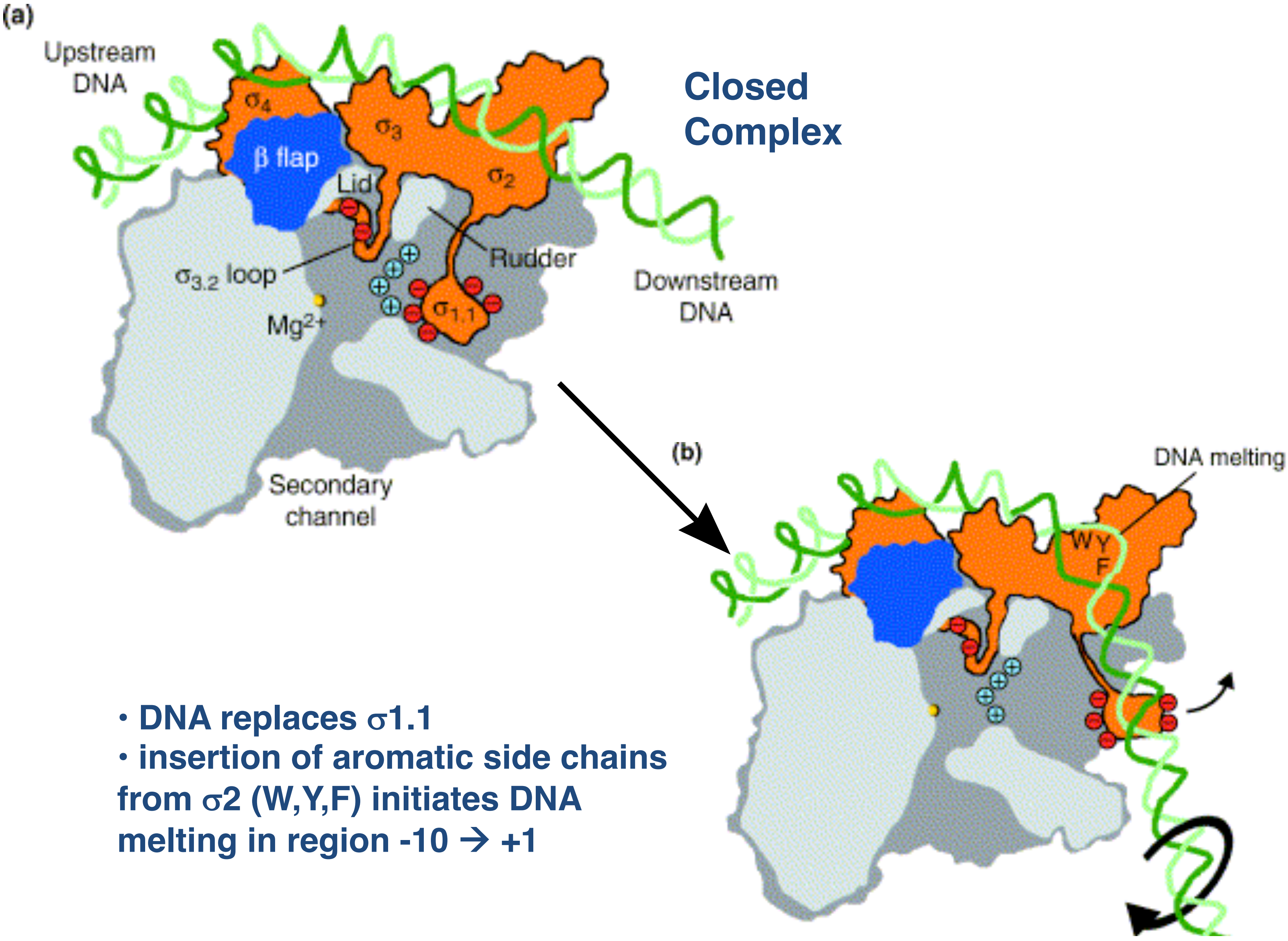
flap & σ factor cover RNA exit channel

Active site metal

Sigma r1 occlusion controls DNA melting

Site of entry of riboNTPs

Model for initiation (steps 1 & 2)

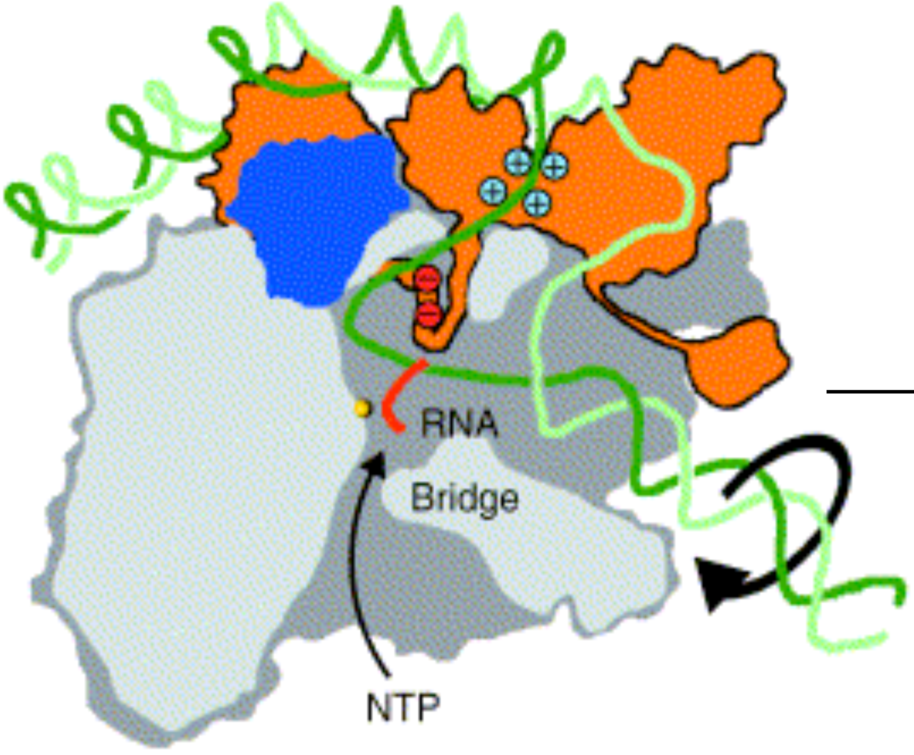


Closed Complex

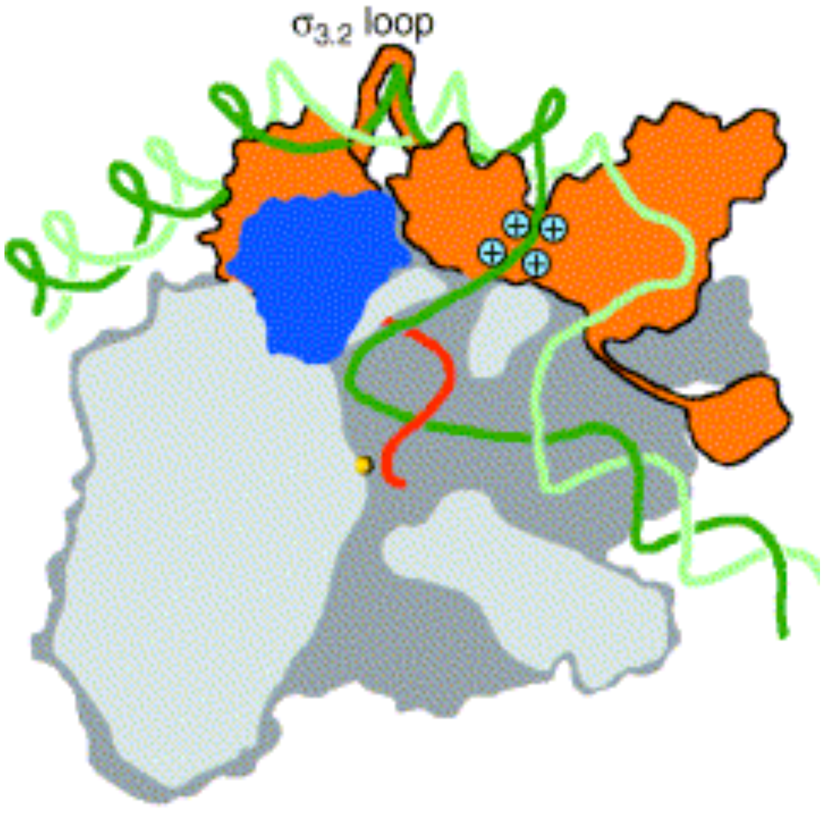
- **DNA replaces σ1.1**
- **insertion of aromatic side chains from σ2 (W,Y,F) initiates DNA melting in region -10 → +1**

The polymerase initiates RNA synthesis However, the exit tunnel is blocked by the σ3.2 loop, forcing abortive initiation and resulting in release of short RNAs and reinitiation

The RNA (red) manages to displace the σ3.2 loop, resulting in the switch to productive elongation



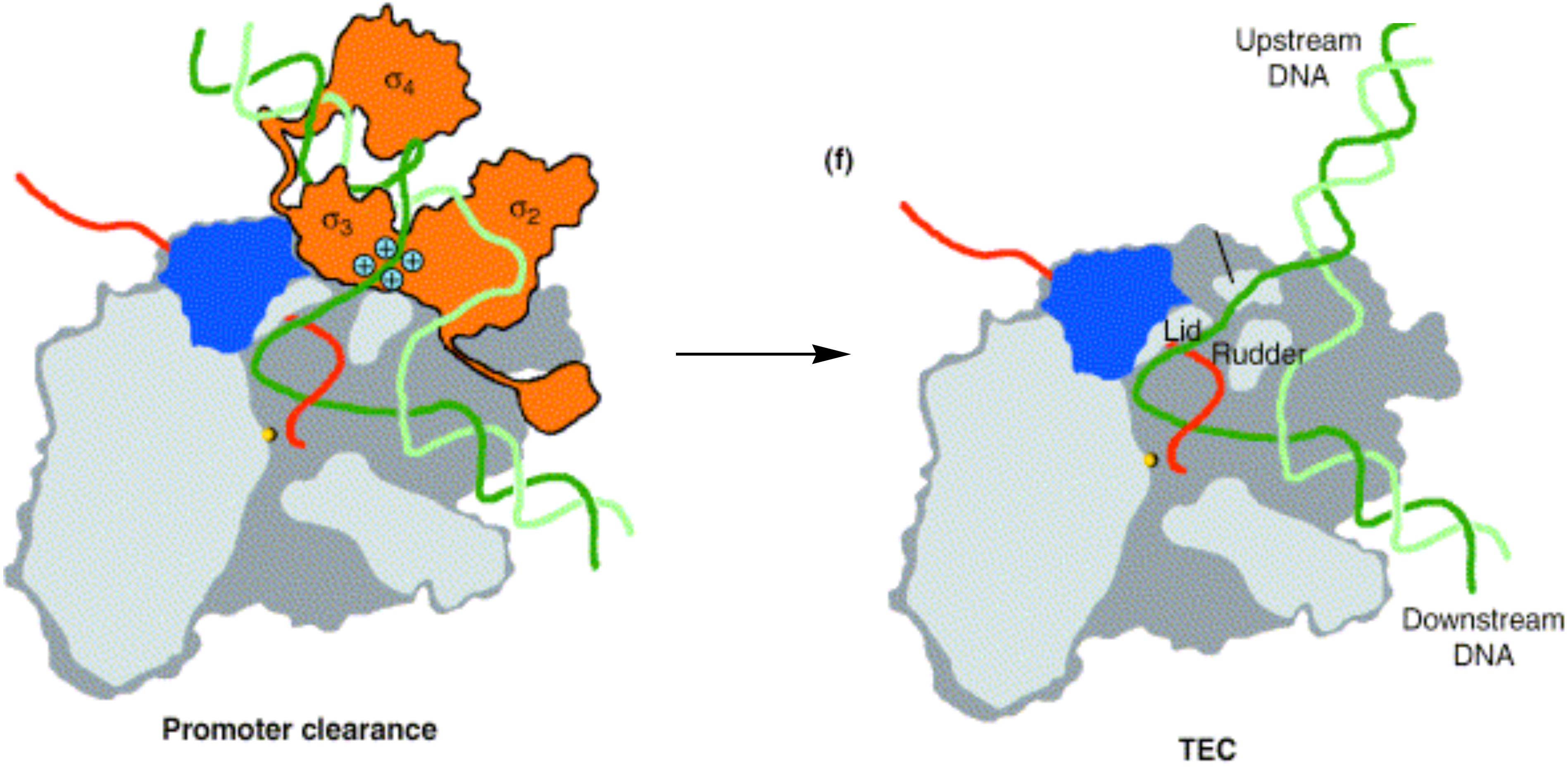
RP$_o$ and abortive initiation



End of abortive initiation

The flap opens to allow RNA to exit. This initiates σ release and escape from the promoter

Stable Ternary Elongation Complex (TEC)

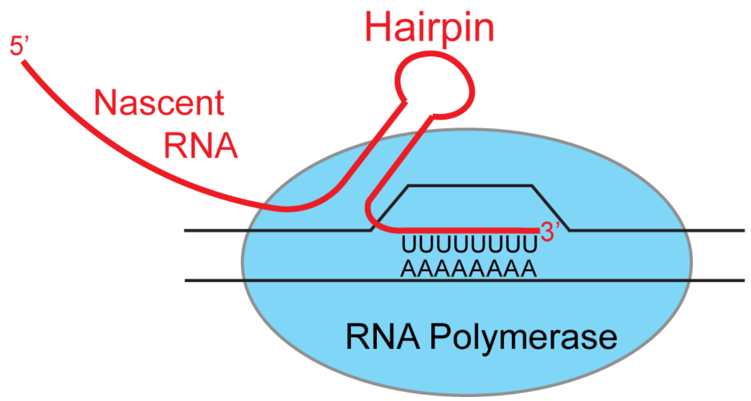


Promoter clearance

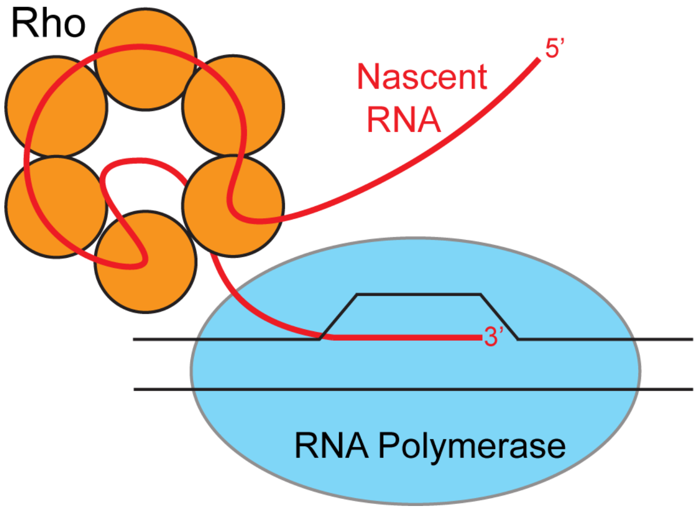
TEC

The transcription cycle: 2 possible termination mechanisms

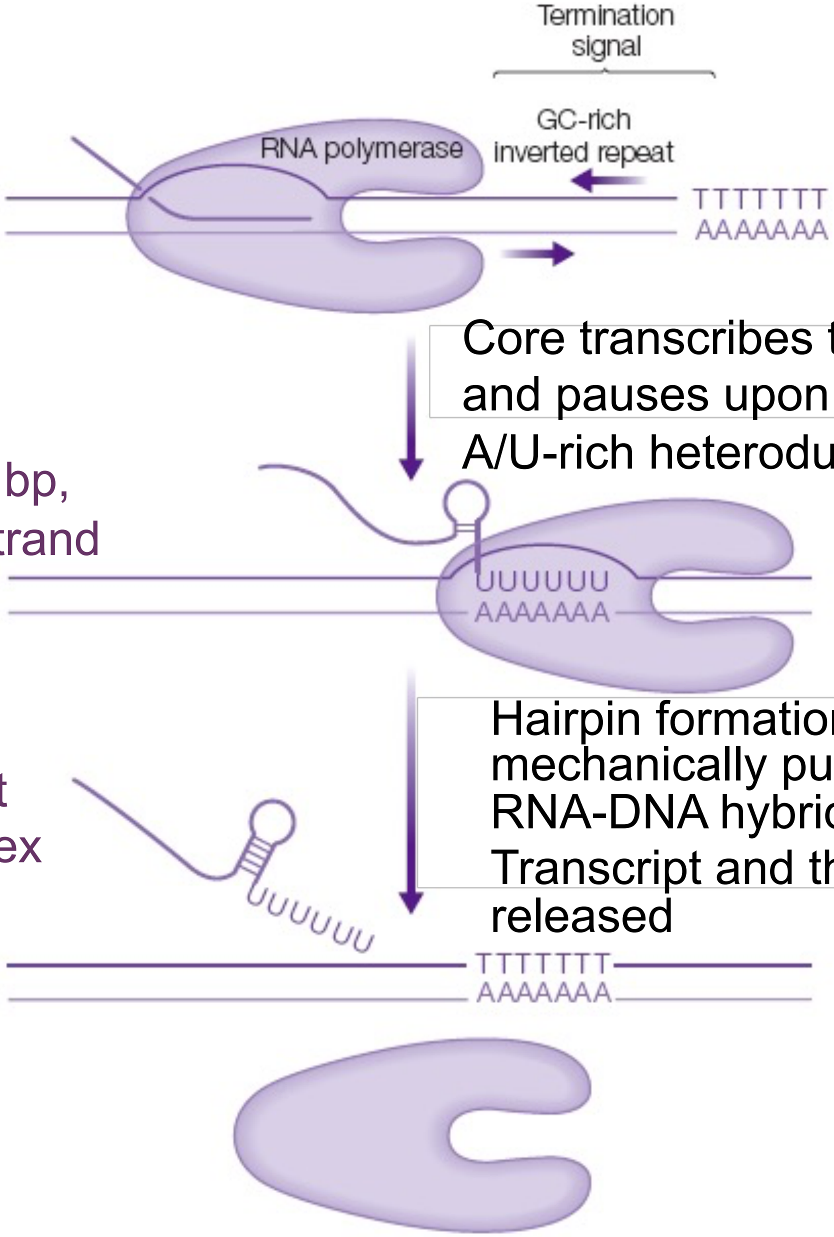
A Intrinsic Termination



B Rho-dependent termination



Intrinsic termination (ρ independent)



Termination sequence characterized by

a) G-C rich palindrome, followed by

b) 4-10 consecutive A-T bp, with As on template strand

Core transcribes through palindrome and pauses upon formation of A/U-rich heteroduplex

Dissociation of transcript aided by low heteroduplex stability of dA•rU region

Hairpin formation helps to mechanically pull the RNA out of the RNA-DNA hybrid.

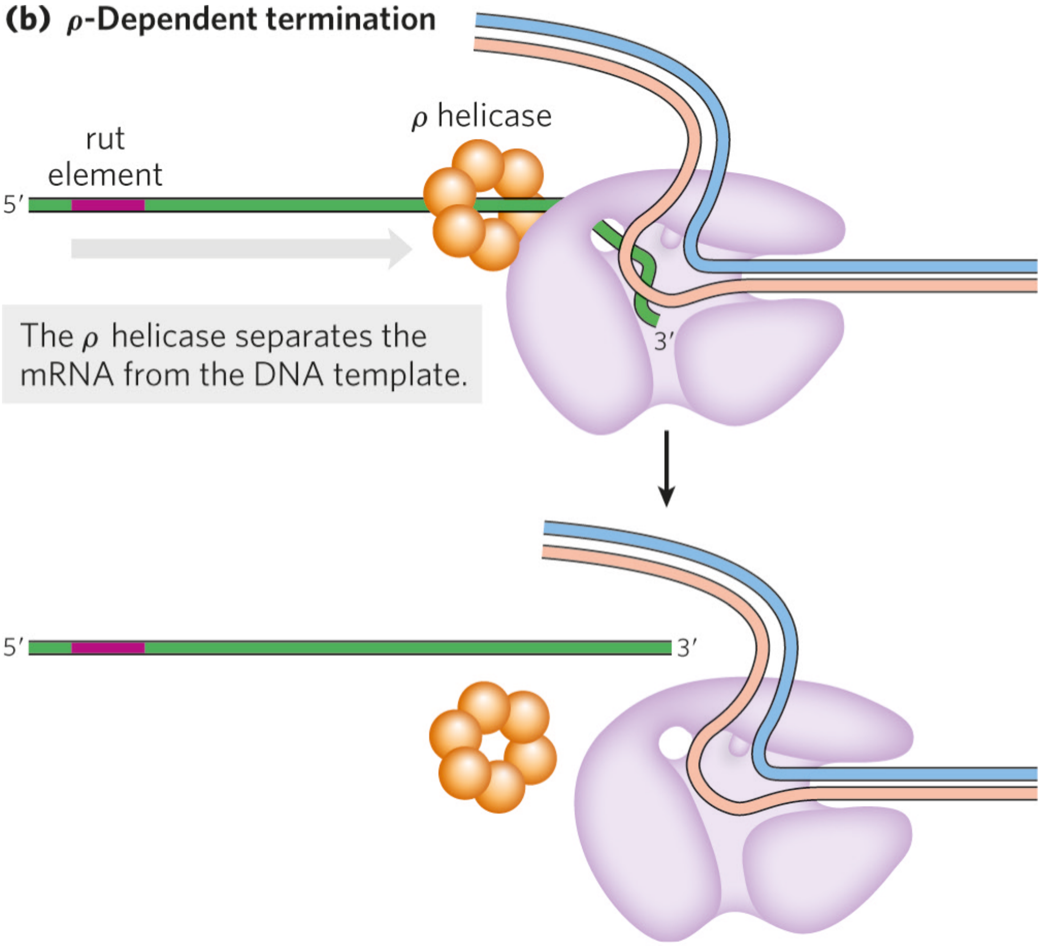
Transcript and then polymerase are released

Rho-dependent transcription termination

ρ (rho) = protein factor that has an ATP-dependent RNA-DNA **helicase** activity.

ρ-dependent terminators = class of terminators that rely on the protein rho

- Have a CA-rich sequence called a *rut* (*r*ho *ut*ilization) element
- Rho promotes release of the RNA



The ρ helicase separates the mRNA from the DNA template.

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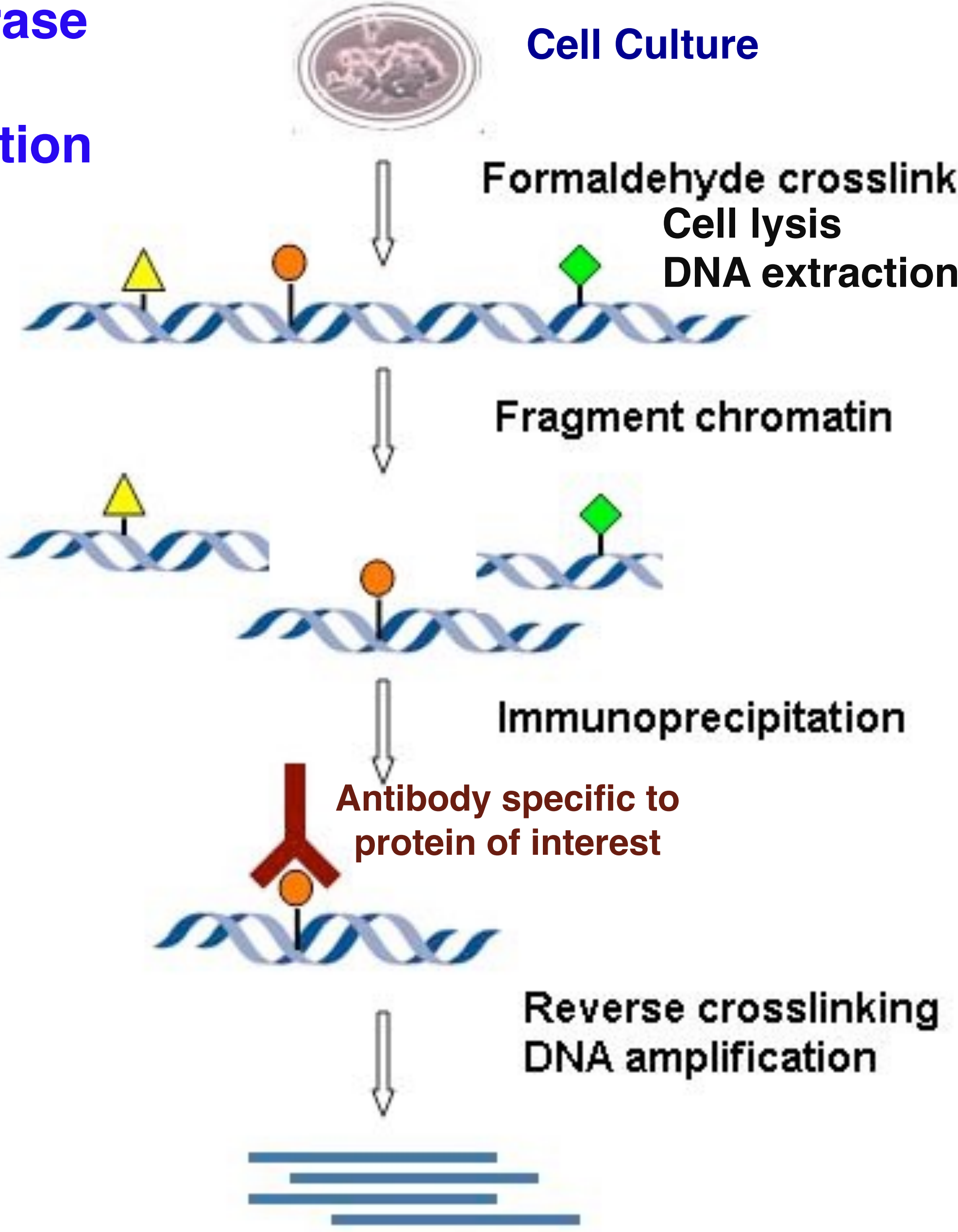
Identifying the sites of RNA polymerase binding to genes <u>in vivo</u> (=in cells) using "chromatin" immunoprecipitation = ChIP (technique developed in eukaryotes but adapted to bacterial cells)

- Crosslink proteins to DNA in cells
- Lyse cells
- Fragment DNA into small fragments
- immunoprecipitate proteins

of choice bound to DNA.

- Recover bound DNA, amplify and

identify the DNA bound by specific proteins using sequencing technology



Cell Culture

Formaldehyde crosslink
Cell lysis
DNA extraction

Fragment chromatin

Immunoprecipitation

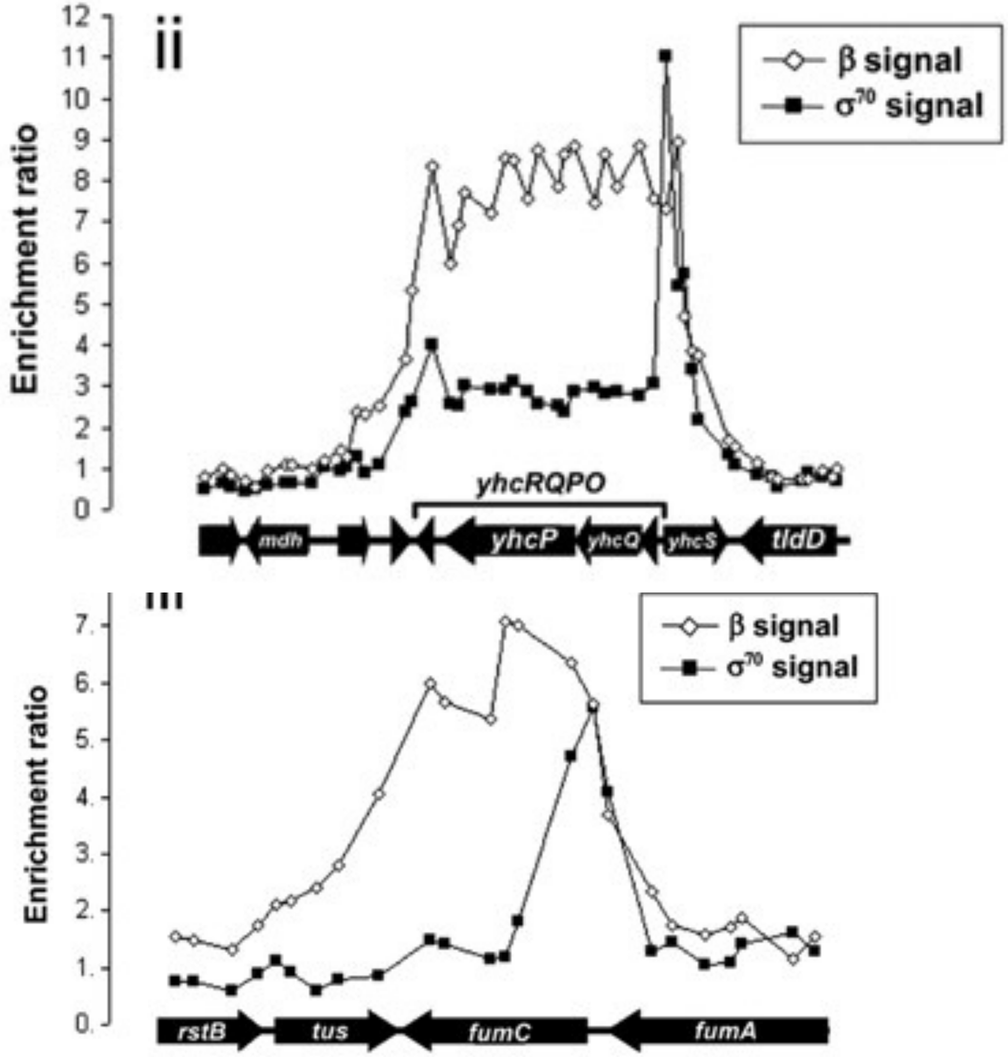
Antibody specific to protein of interest

Reverse crosslinking
DNA amplification

-> amplify and identify the DNA bound by specific proteins by sequencing

Using ChIP to localize σ70 and β subunits on genes in the bacterial genome

Y-axis = Enrichment-> shows how much the protein analyzed by ChIP crosslinks to the DNA in the region studied

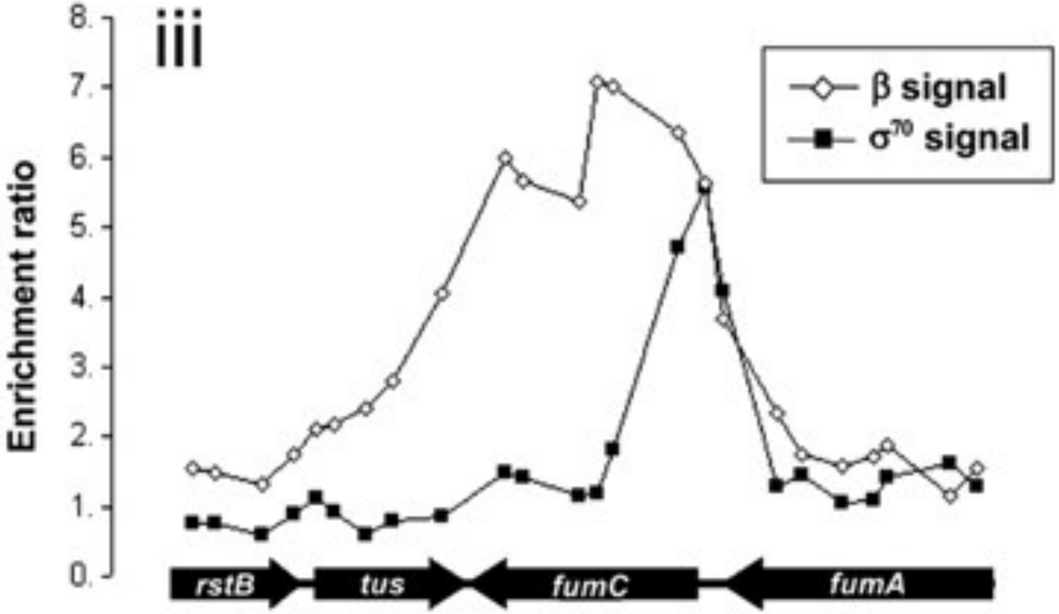


The β subunit associates with highly expressed genes/ operons
(broad distribution of β along the operon)

the sigma factor mostly associates with promoters and does not travel with the core RNA polymerase
(sharp peak of enrichment of σ70 in the promoter)

Grainger et. al Proc Natl Acad Sci U S A. 2005 Dec 6;102(49):17693-8

What ChIP signal would be expected for the two alpha subunits of RNAP?



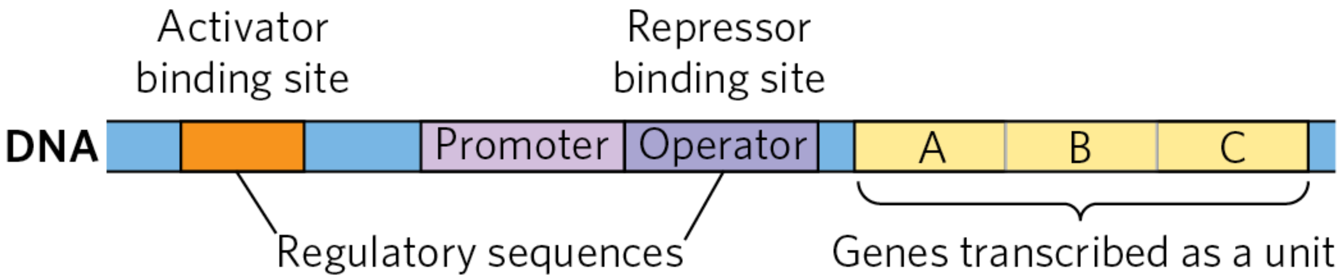
A: Same as sigma70 (peaks at promoter)

B: Same as beta subunit = promoter+transcribed region

C: No way to know as there are two alpha subunits on RNAP and you cannot differentiate the signal for each

D: There would be a higher enrichment in the transcribed region than in the promoter since alpha subunits join the RNA polymerase after transcription has begun

A representative bacterial operon



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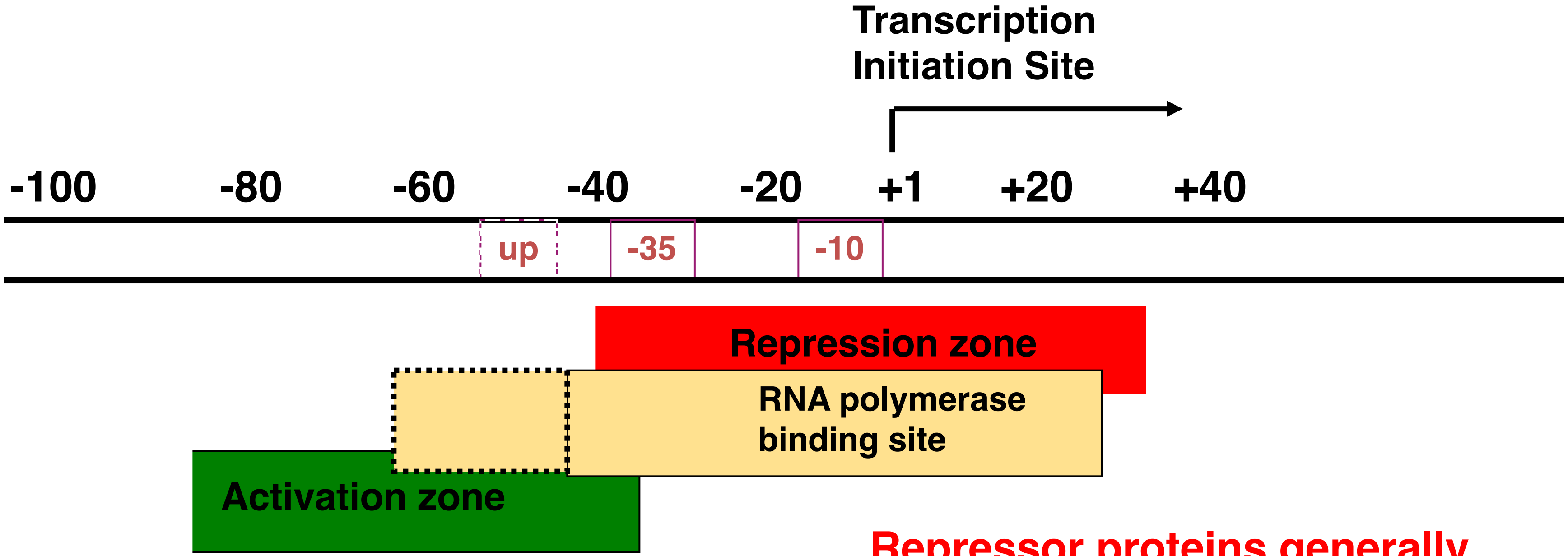
Polycistronic mRNA: multiple genes on a single transcript
Monocistronic mRNA: single gene on a transcript
Operator: DNA sequence that a repressor binds to
Operon: gene cluster + promoter + regulatory sequences

Genes within an operon are usually related (e.g. multiple subunits of a larger complex)

Transcription Regulation by Activator and Repressor Proteins: General Principles



Activators proteins generally bind upstream the promoter and interact with RNA Polymerase to help load it onto the Promoter

Repressor proteins generally block RNA polymerase binding by competing for binding with sequences near the promoter

Negative and positive regulation

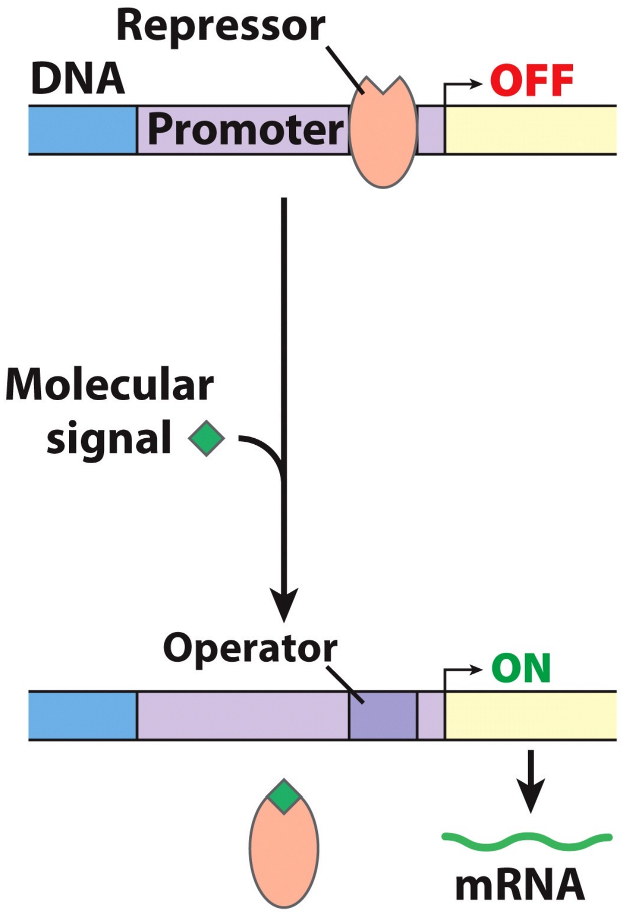
Negative regulation: regulation via a repressor (a protein that blocks transcription)

Positive regulation: regulation via an activator (a protein that facilitates/enhances transcription)

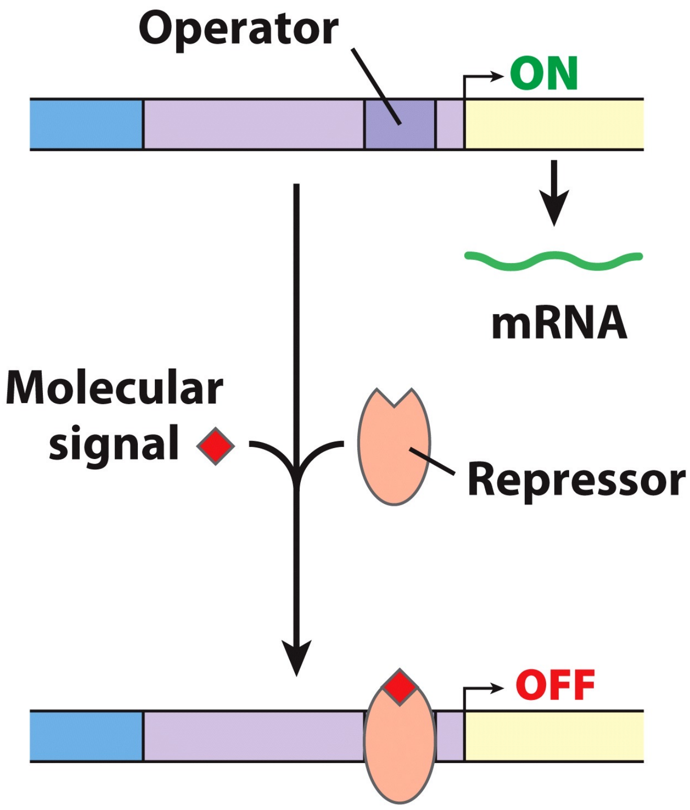
Transcriptional Regulatory Models:

Negative Regulation

Negative regulation
Molecular signal causes dissociation of repressor from DNA, inducing transcription.



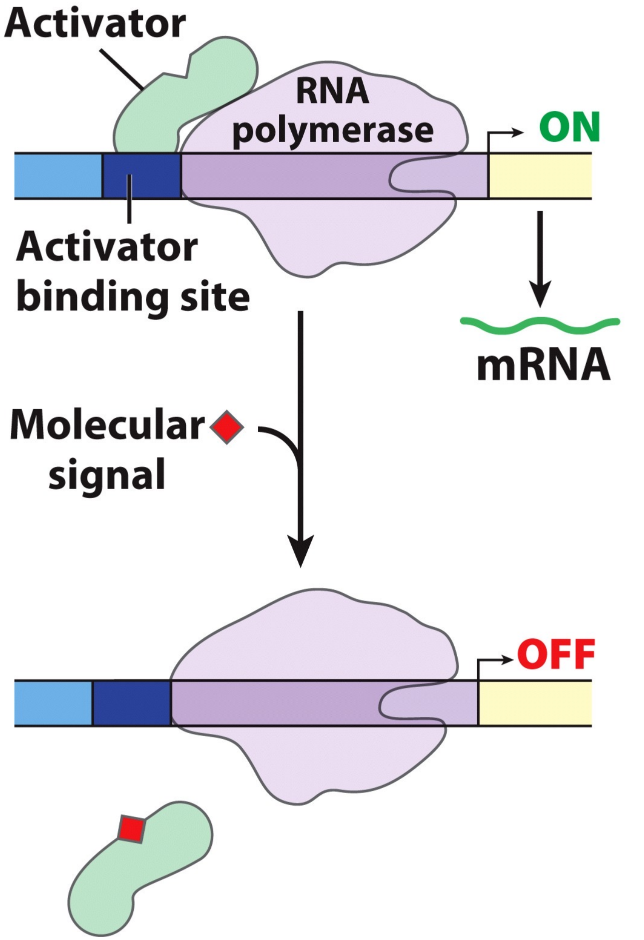
Negative regulation
Molecular signal causes binding of repressor to DNA, inhibiting transcription.



Transcriptional Regulatory Models:

Positive Regulation

Positive regulation
Molecular signal causes dissociation of activator from DNA, inhibiting transcription.



Positive regulation
Molecular signal causes binding of activator to DNA, inducing transcription.