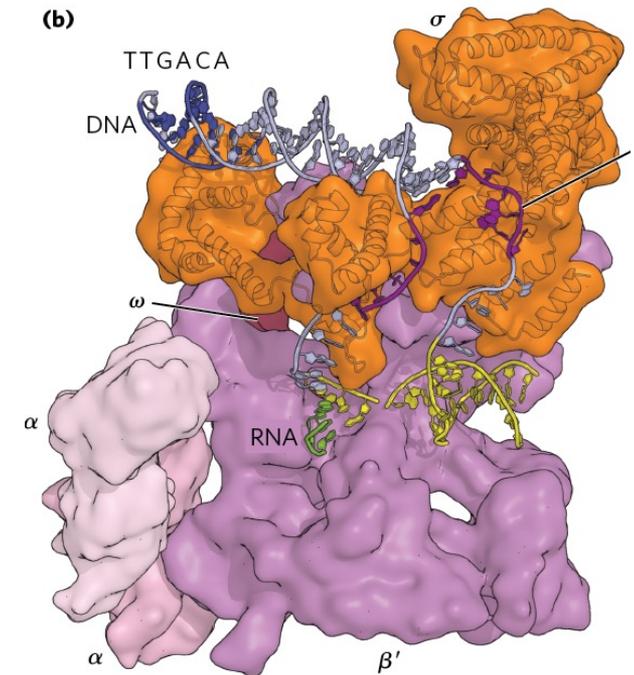# Transcription

Introduction – Definitions

Bacterial Transcription
1) How to detect DNA protein interactions
   in vitro: DNA footprinting
2) Recognition of bacterial promoters by sigma
   factors
3) The prokaryotic transcriptional cycle
4) Transcription regulation in prokaryotes

===

Eukaryotic Transcription
5) RNA polymerases in eukaryotes
6) Assembly of eukaryotic RNA Pol.II on promoters
7) RNA Pol.II Transcriptional activators
8) Transcripion regulation by histone modifications
9) Transcription regulation by pausing

Learning outcomes:
What you need to know/understand after this unit

Understand the mechanisms of recognition of promoters by bacterial RNA polymerase and the basic transcription cycle

Understand the roles of sigma70 in promoter recognition and in the transcription initiation process

Understand the mechanisms of transcription regulation (pos/neg models) and the Lac Operon regulatory model
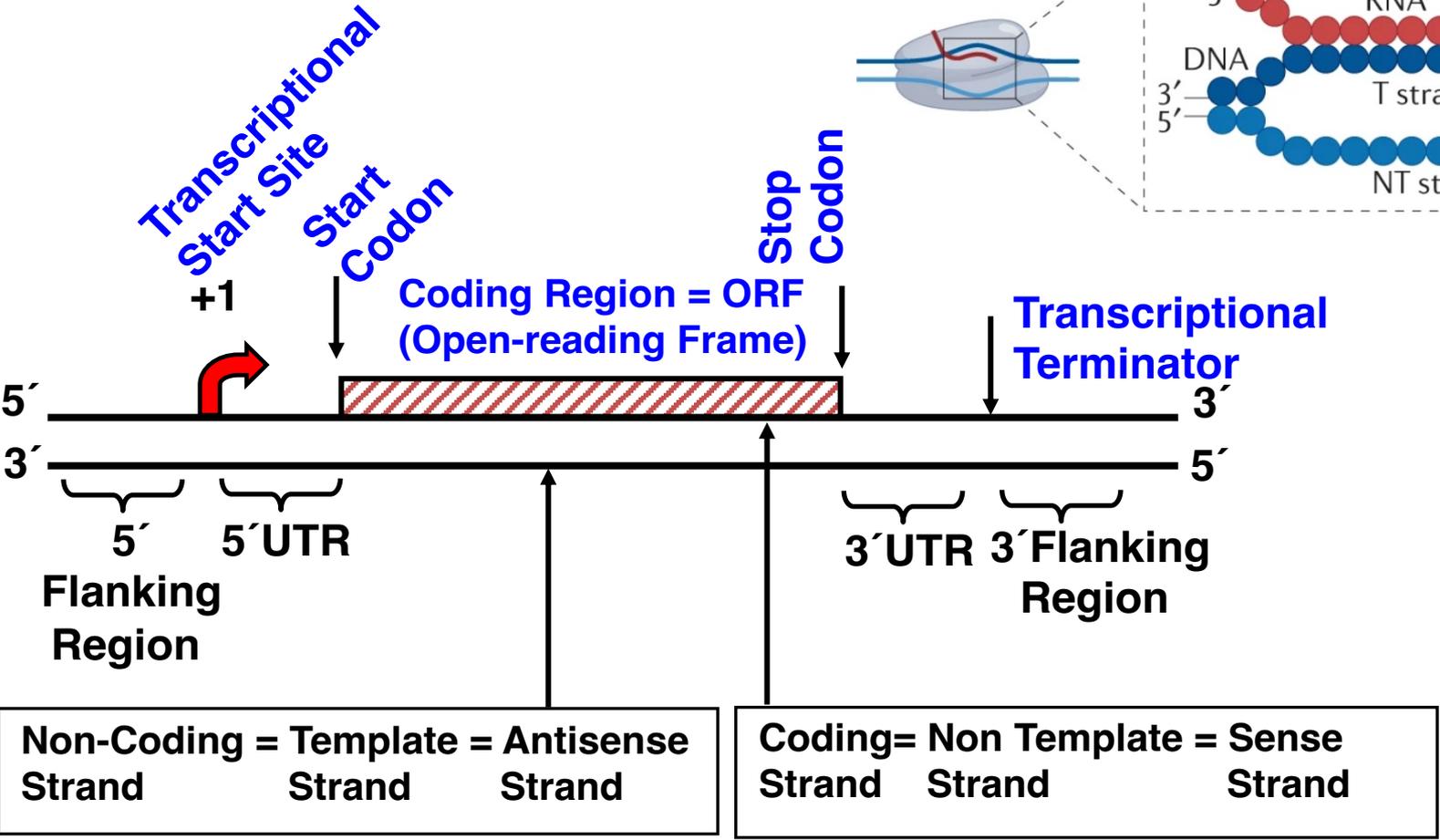
Understand the mechanisms of recognition of promoters by Eukaryotic RNA polymerase II, the basic transcription cycle and role of general Transcription factors

Understand the roles of histones (modifications) in gene regulation

Understand the mechanisms of transcription regulation by activator & Coactivator proteins and by promoter proximal pausing
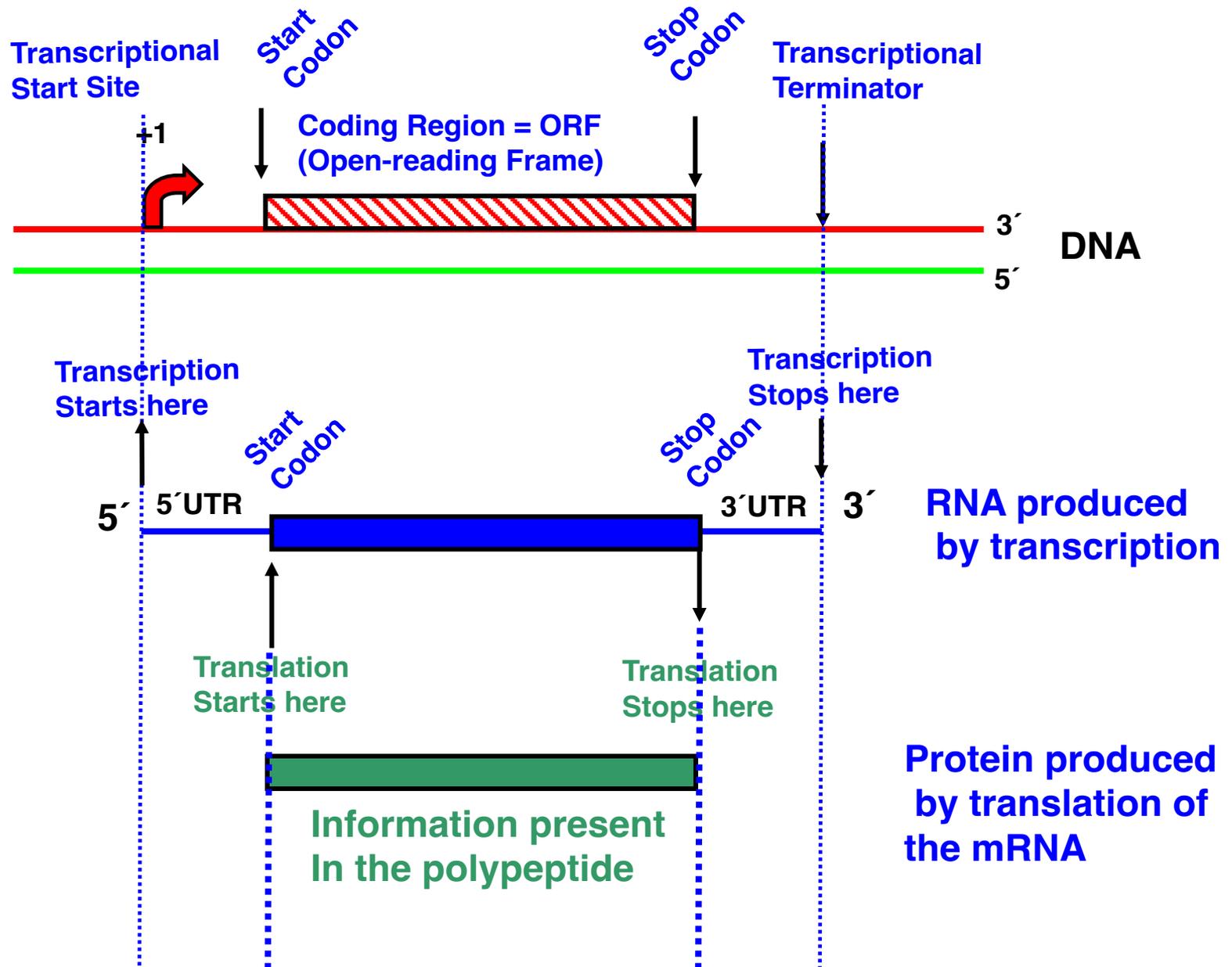
Techniques: understand footprinting and chromatin immunoprecipitation experiments

Nomenclature used to describe a gene

Transcriptional Start Site

Start Codon

Stop Codon

+1

Coding Region = ORF (Open-reading Frame)

Transcriptional Terminator

5′

3′

3′

5′

5′ Flanking Region

5′UTR

3′UTR

3′ Flanking Region

Non-Coding = Template = Antisense
Strand   Strand   Strand

Coding= Non Template = Sense
Strand   Strand   Strand

* **Promoter** = The region near the 5′ end of the transcribed region to which the RNA polymerase binds prior to the initiation of transcription

UTR = ·UnTranslated Region

RNA
DNA
T strand
NT strand
i−1 i+1
5′   3′
3′   5′
5′   3′

Transcriptional Start Site

Start Codon

Stop Codon

Transcriptional Terminator

+1

Coding Region = ORF (Open-reading Frame)

3´ DNA

5´

Transcription Starts here

Transcription Stops here

Start Codon

Stop Codon

5´ 5´UTR

3´UTR 3´

RNA produced by transcription

Translation Starts here

Translation Stops here

Information present In the polypeptide

Protein produced by translation of the mRNA

Critically, start codons and stop codons do not regulate where RNA polymerase starts and stops - they only control translation by ribosomes

Example RNA transcript made from dsDNA

(5') C G C T A T A G C G T T T (3')   DNA nontemplate (coding) strand

(3') G C G A T A T C G C A A A (5')   DNA template strand

(5') C G C U A U A G C G U U U (3')   RNA transcript

Non-Coding =	**Template =**	**Antisense**
Strand	**Strand**	**Strand**

Coding=	**Non Template =**	**Sense**
Strand	**Strand**	**Strand**

RNA Polymerase holoenzyme in bacteria: $\alpha_2\beta\beta'$ + one σ factor

$\alpha_2\beta\beta'$ = Core RNA Polymerase

β' = Catalytic Subunit

α, β = Structural Roles/Activation

Without σ factor, $\alpha_2\beta\beta'$ RNA polymerase initiates transcription inefficiently and at random sites

σ factor allows the $\alpha_2\beta\beta'$ RNA polymerase to initiate transcription at gene promoters

Another factor (ω) is also present in the RNA polymerase complex but will not be discussed in this class
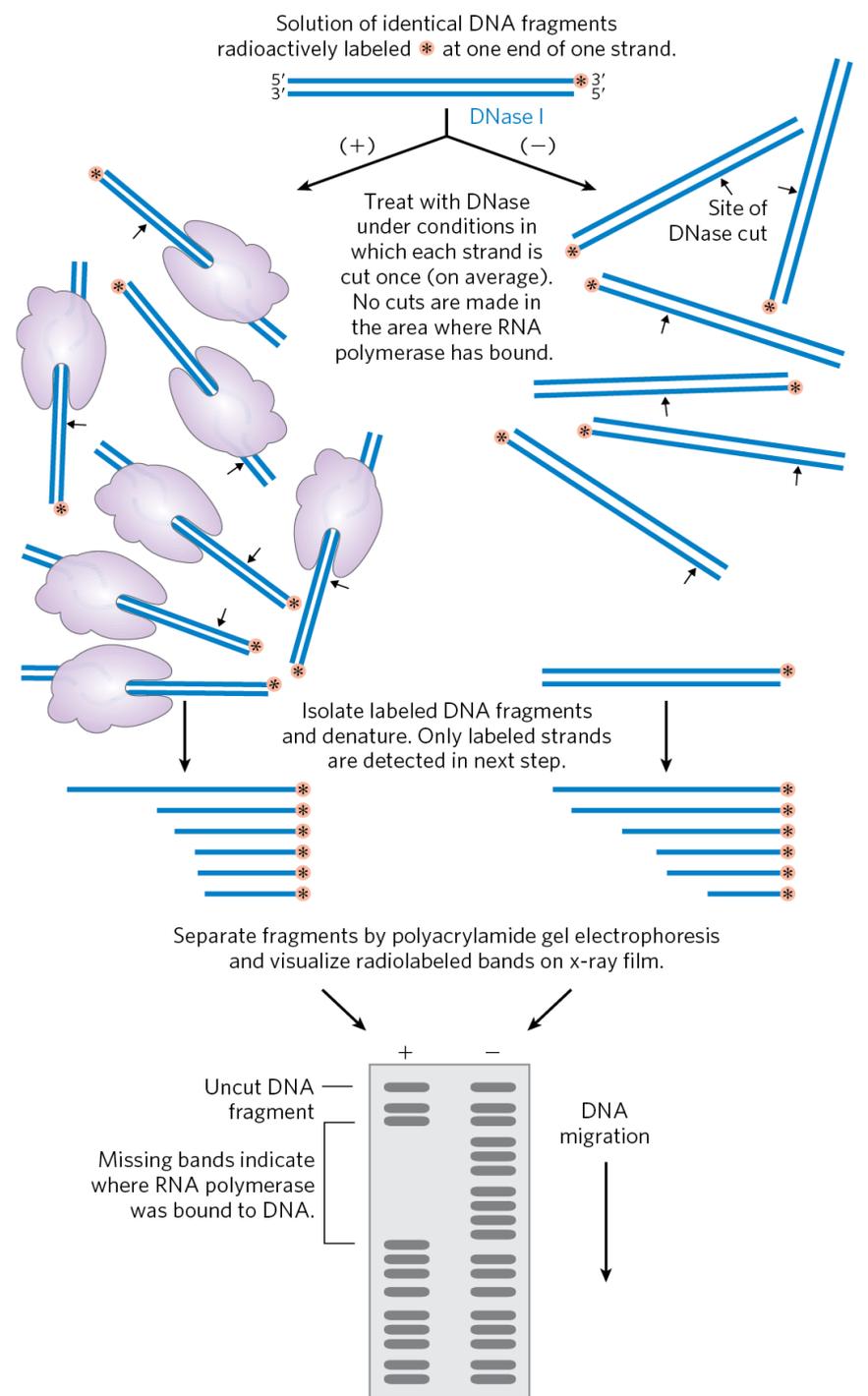

σ

DNA

ω

α

RNA

Catalytic Mg^{++}

α

β'

Nelson & Cox, *Lehninger Principles of Biochemistry*, 8e, © 2021 W. H. Freeman and Company
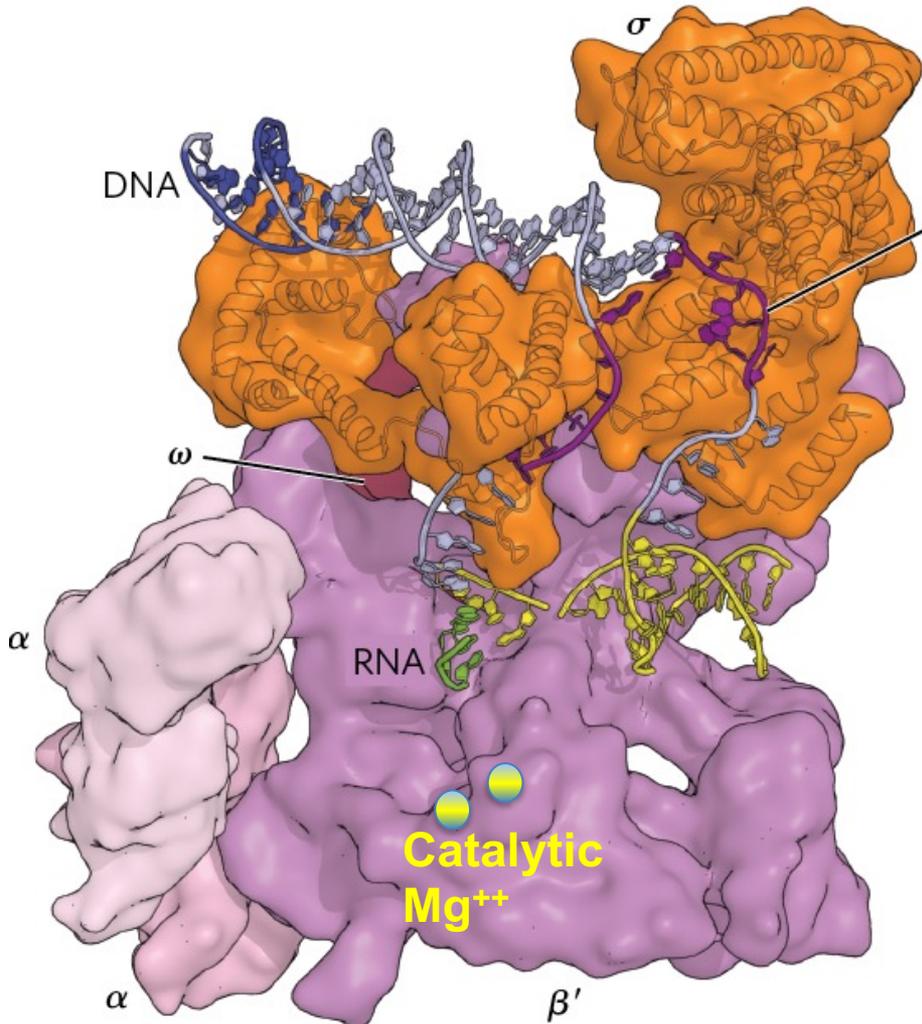
How to detect RNA Polymerase (or any protein) interactions with DNA in vitro (= in test tube) ?
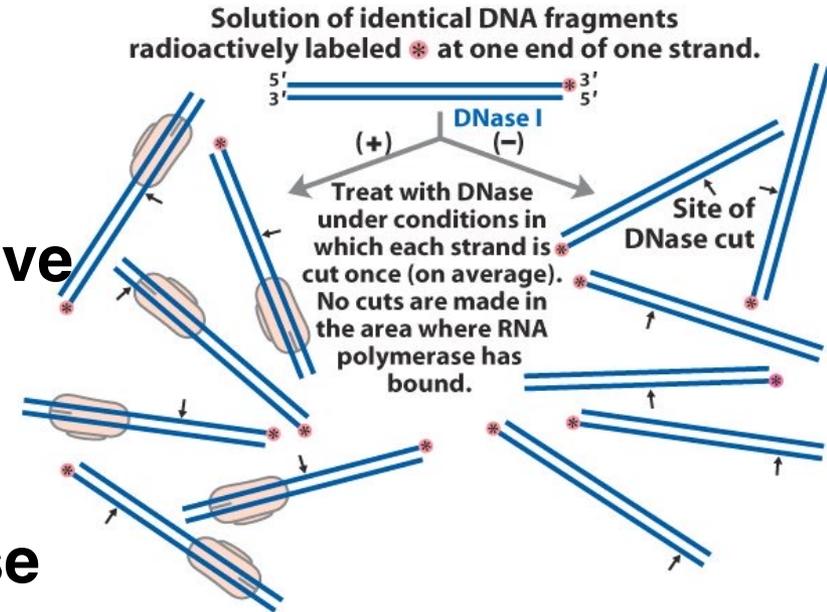
→ DNAse I "footprinting"

• **This experiment requires you purified RNA Polymerase and a DNA containing a promoter sequence**

• **Dnase I cleaves DNA at nucleotides which are not tightly bound and protected by a DNA binding protein**

• **Areas where cleavage bands are absent are called "footprints" and reveal the presence of a tightly bound protein**

Solution of identical DNA fragments radioactively labeled ✳ at one end of one strand.

5′ / 3′ ✳ ✳ 3′ / 5′

DNase I

(+)   (−)

Treat with DNase under conditions in which each strand is cut once (on average). No cuts are made in the area where RNA polymerase has bound.

Site of DNase cut

Isolate labeled DNA fragments and denature. Only labeled strands are detected in next step.

Separate fragments by polyacrylamide gel electrophoresis and visualize radiolabeled bands on x-ray film.

+   −

Uncut DNA fragment

Missing bands indicate where RNA polymerase was bound to DNA.

DNA migration

Why do you need to treat with Dnase I in limited conditions (ie not too much enzyme)?

Solution of identical DNA fragments
radioactively labeled ✳ at one end of one strand.

5'————————3'
3'————————✳5'

DNase I
(+)   (−)

Treat with DNase
under conditions in
which each strand is
cut once (on average).
No cuts are made in
the area where RNA
polymerase has
bound.

Site of
DNase cut

A: Because the enzyme is expensive and funding by NIH is shrinking

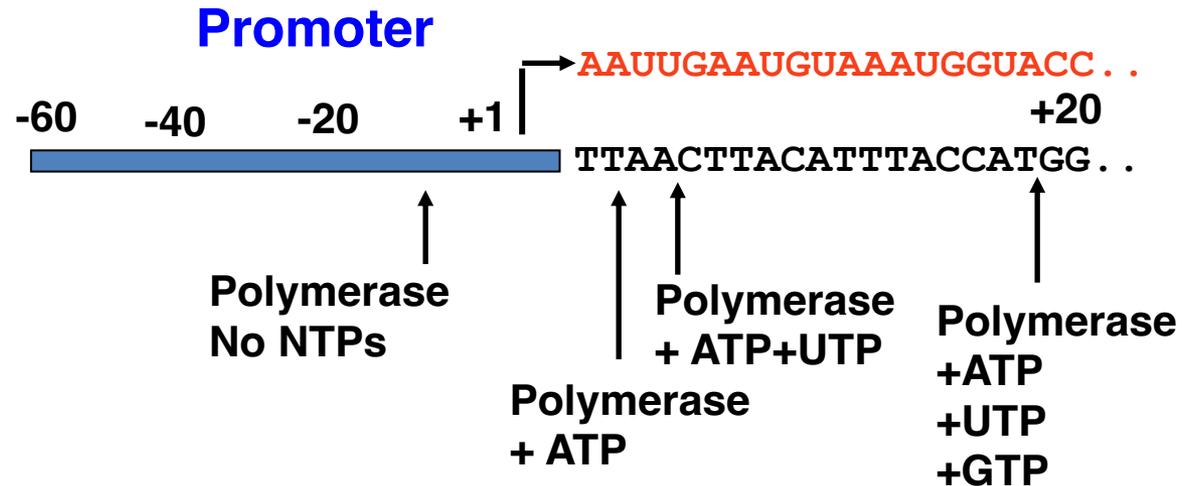B: Adding too much Dnase I will activate its exonuclease activity and will degrade the DNA

C: Adding too much Dnase I would compete for DNA binding and displace the RNA Polymerase from the DNA

D: Adding too much Dnase I would result in multiple cleavage per strand and only small fragments, making it impossible to get a footprint

DNase I Footprinting of RNA polymerase on a bacterial promoter
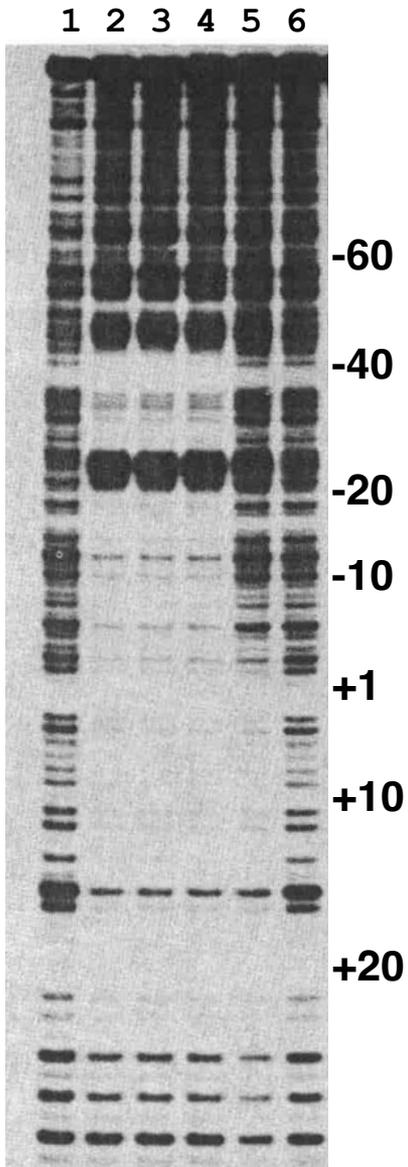
1 = No RNA polymerase/ +DNase I

2 = RNA polymerase/ No NTPs/ +DNase I

3 = RNA polymerase/ +ATP/ +DNase I

4 = RNA polymerase/ +ATP+UTP/ +DNase I

5 = RNA polymerase/ +ATP+UTP+GTP/ +DNase I

6 = RNA polymerase/ +NTPs/ +DNase I

Lane labels: 1 2 3 4 5 6

Positions: -60, -40, -20, -10, +1, +10, +20

Carpoussis & Gralla (1985)

Promoter

AAUUGAAUGUAAAUGGUACC . .

-60   -40   -20   +1   +20

TTAACTTACATTTACCATGG . .

Polymerase
No NTPs

Polymerase
+ ATP

Polymerase
+ ATP+UTP

Polymerase
+ATP
+UTP
+GTP

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

double-strand DNA   single-strand DNA

Potassium permanganate
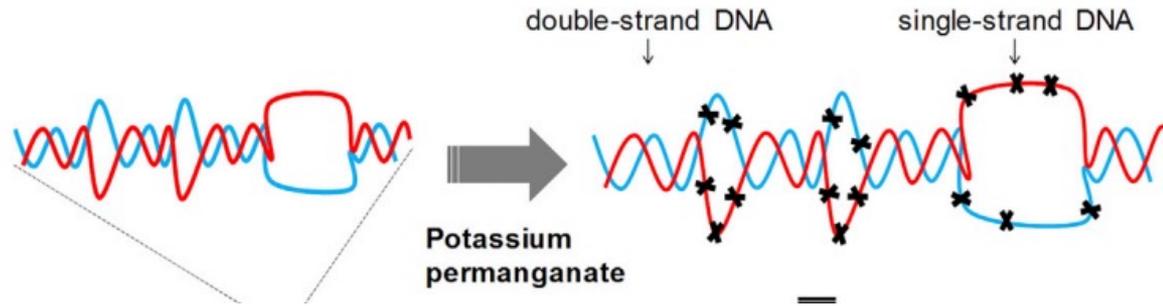
Kouzine et al., 2017, Cell Systems
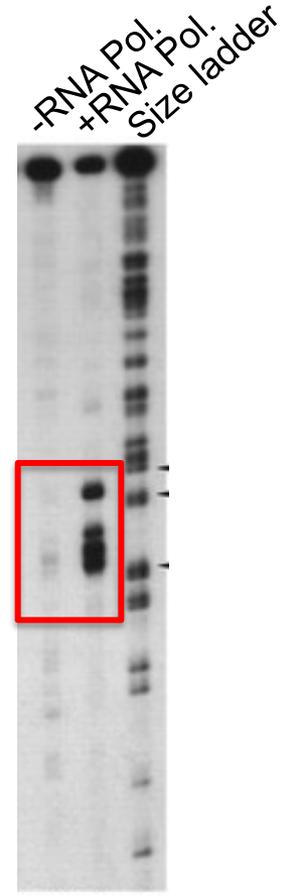
-RNA Pol.
+RNA Pol.
Size ladder

DNA region unwound by the addition of RNA Pol.

Step 1: Permanganate chemically modifies DNA in single stranded regions
Step 2: Primer extension — polymerase will stop at chemically modified positions
Step 3: 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
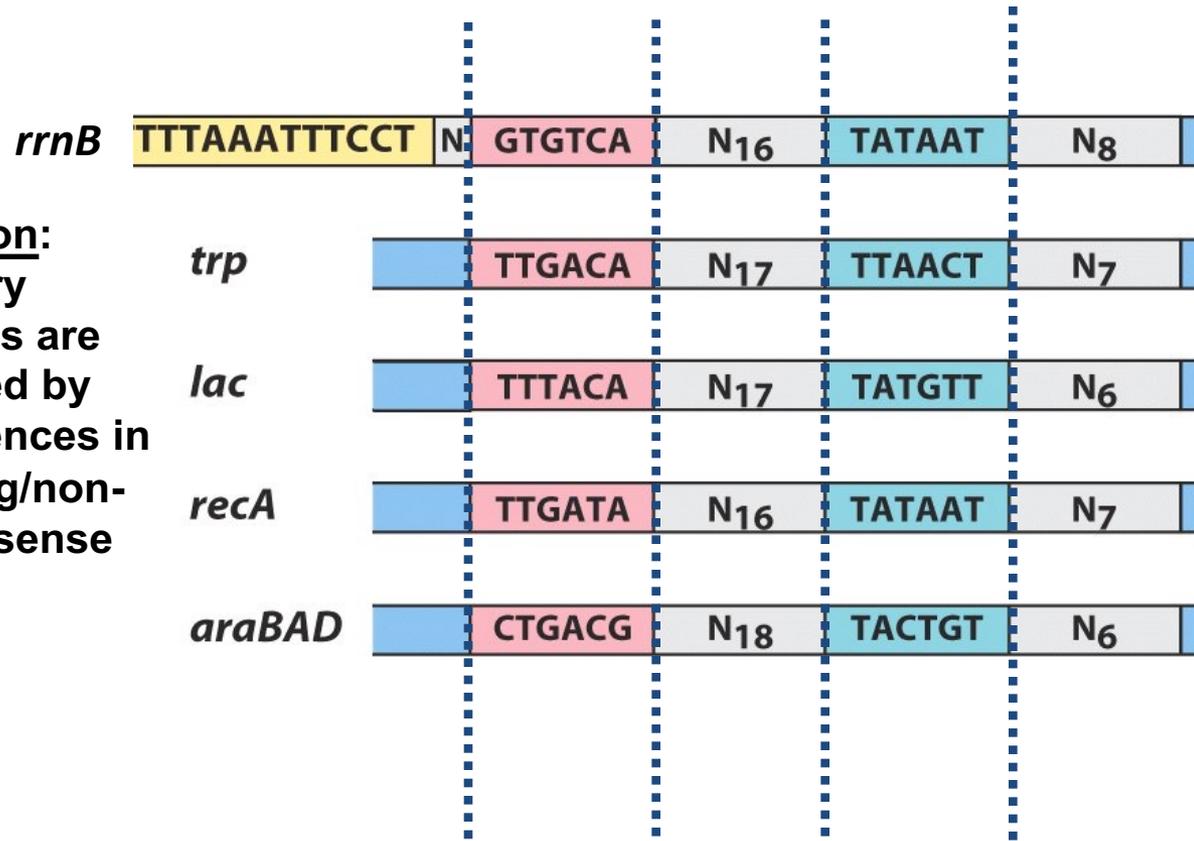
Convention:
Regulatory sequences are designated by the sequences in the coding/non-template/sense strand

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

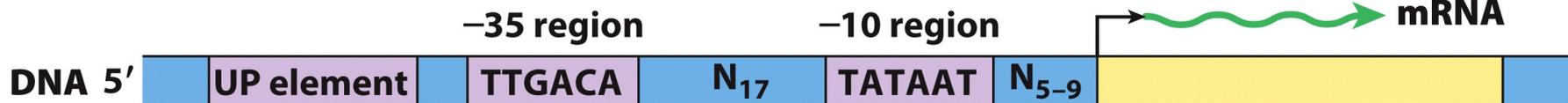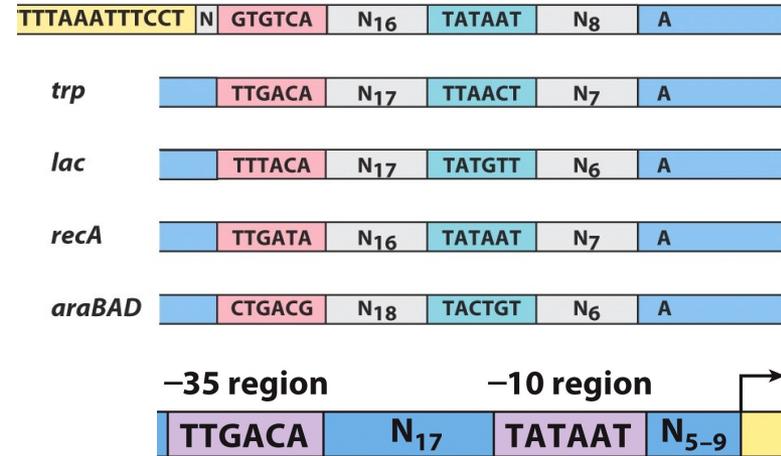DNA 5′ | UP element | TTGACA | N_{17} | TATAAT | N_{5-9} |

Figure 28-2
Lehninger Principles of Biochemistry, Sixth Edition
© 2013 W. H. Freeman and Company

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

TTTAAATTTCCT	N	GTGTCA	N_{16}	TATAAT	N_8	A

trp		TTGACA	N_{17}	TTAACT	N_7	A

| lac | | TTTACA | N_{17} | TATGTT | N_6 | A |

| recA | | TTGATA | N_{16} | TATAAT | N_7 | A |

| araBAD | | CTGACG | N_{18} | TACTGT | N_6 | A |

−35 region   −10 region

TTGACA	N_{17}	TATAAT	N_{5-9}	

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

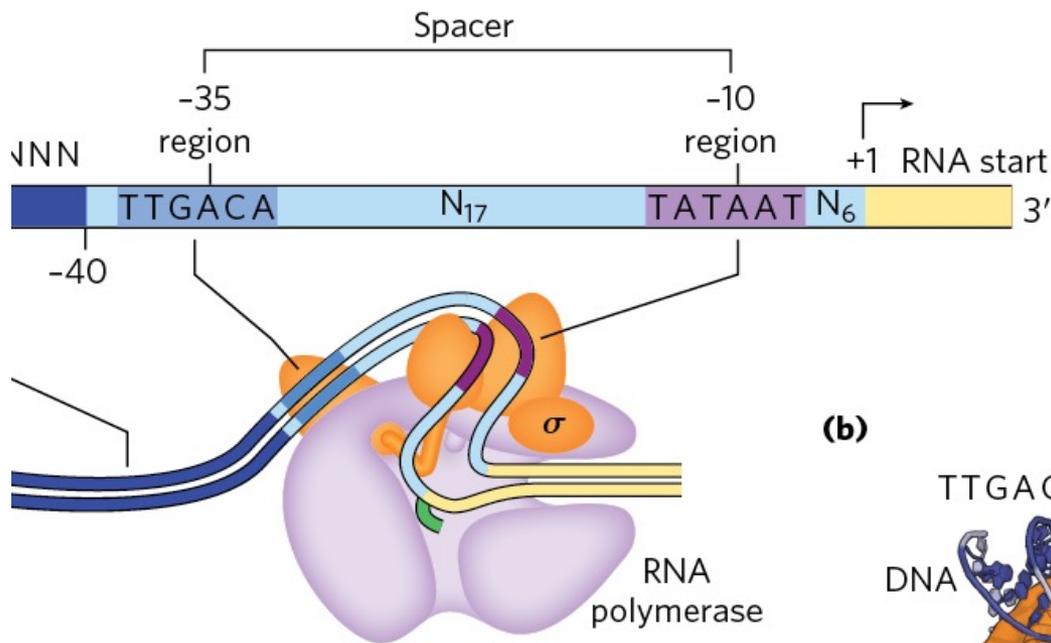# Different σ factors -> Different classes of genes are activated

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

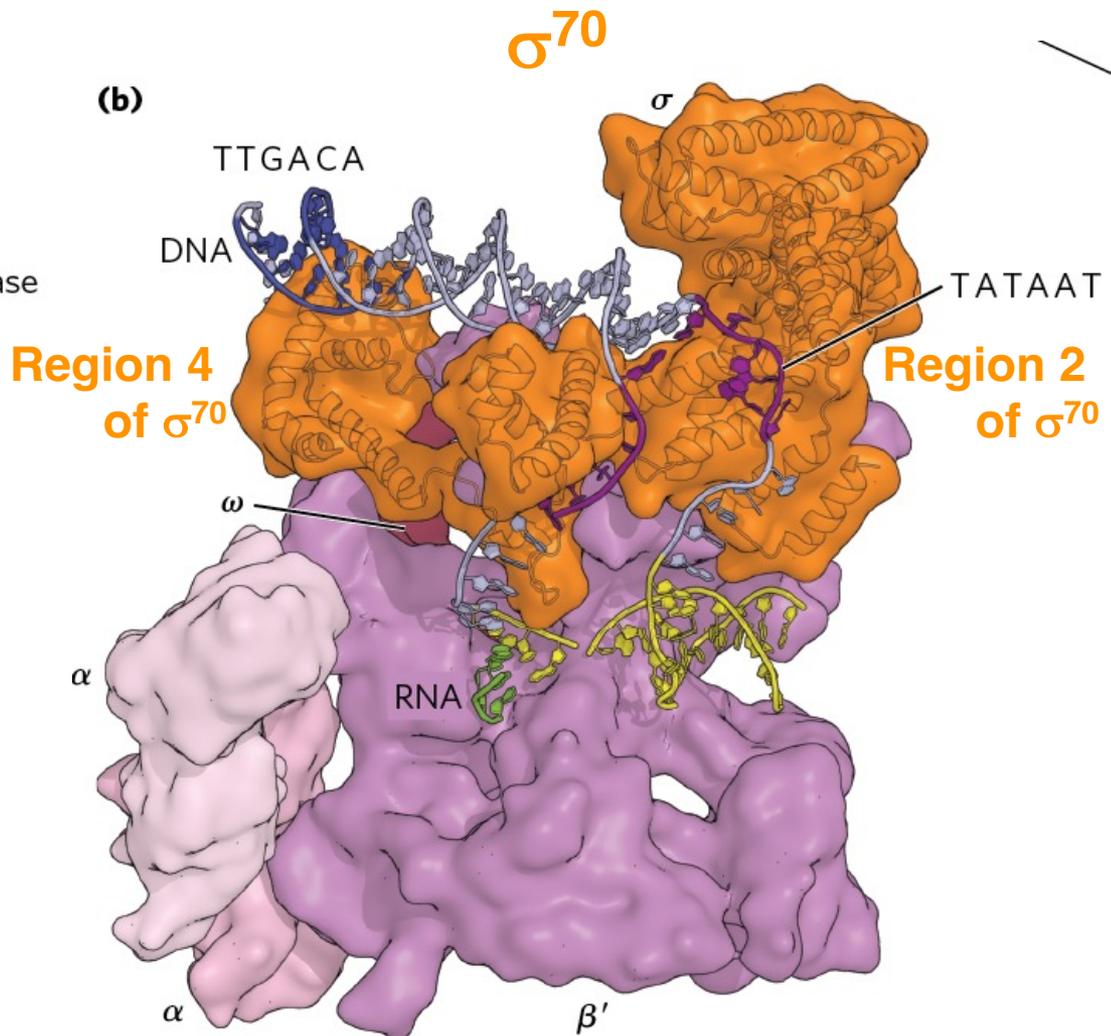| σ subunit	K_d (nM)	Molecules/cell*	Holoenzyme ratio (%)*	Function
σ^{70}	0.26	700	78	Housekeeping
σ^{54}	0.30	110	8	Modulation of cellular nitrogen levels
σ^{38}	4.26	<1	0	Stationary phase genes
σ^{32}	1.24	<10	0	Heat shock genes
σ^{28}	0.74	370	14	Flagella and chemotaxis genes
σ^{24}	2.43	<10	0	Extracytoplasmic 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
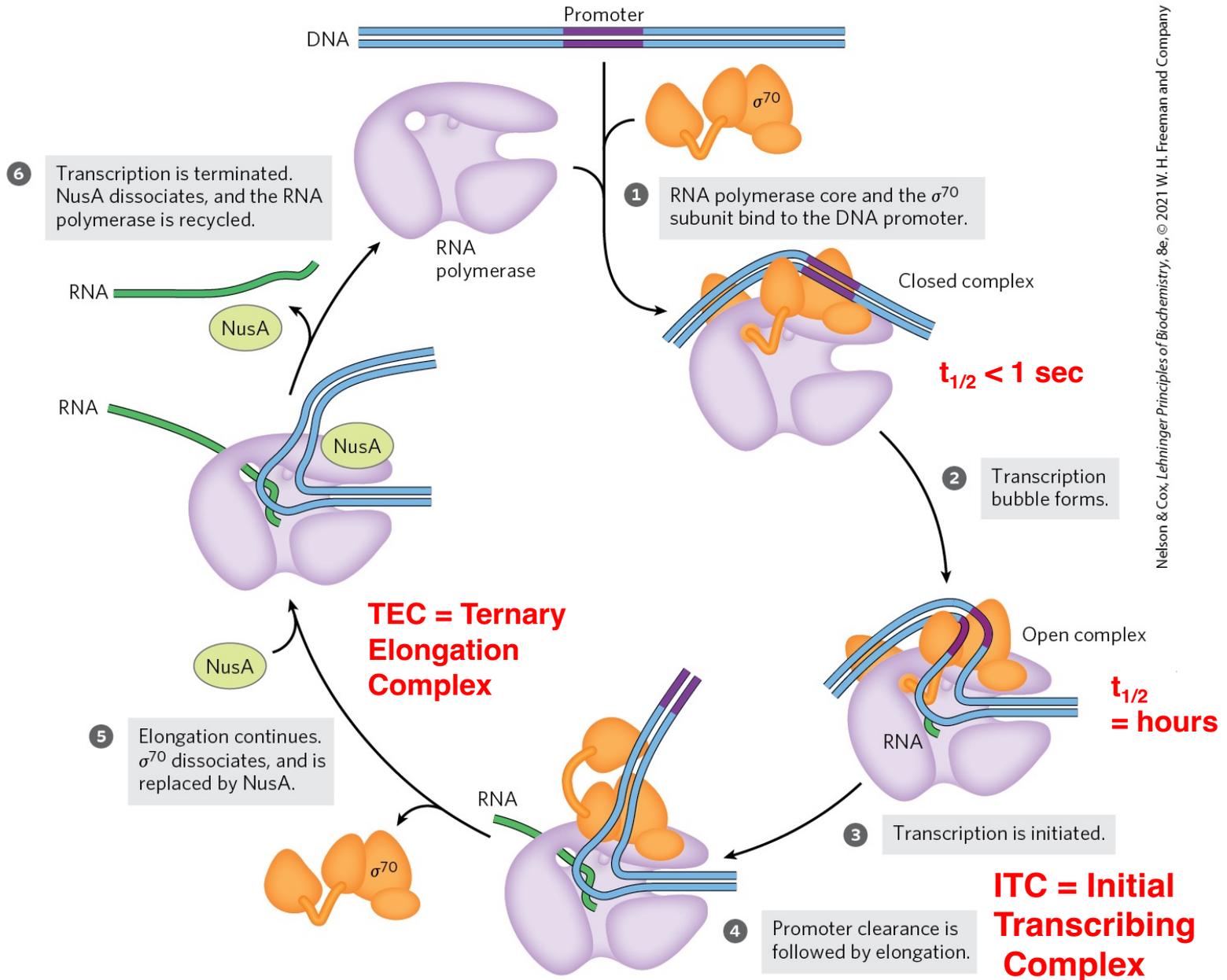(no need to memorize these sequences):

σ^{70}:   TTGACA   TATAAT
        −35   −10

σ^{32}:   CNCTTGAA   CCCCATNT
        −35   −10

σ^{54}:   CTGGNA   TTGCA
        −24   −12

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

Nelson & Cox, *Lehninger Principles of Biochemistry*, 8e, © 2021 W. H. Freeman and Company

The Transcription Cycle

Promoter

DNA

σ^{70}

6 Transcription is terminated. NusA dissociates, and the RNA polymerase is recycled.

RNA polymerase

RNA

NusA

1 RNA polymerase core and the σ^{70} subunit bind to the DNA promoter.

Closed complex

t$_{1/2}$ < 1 sec

RNA

NusA

2 Transcription bubble forms.

TEC = Ternary Elongation Complex

NusA

Open complex

t$_{1/2}$ = hours

RNA

5 Elongation continues. σ^{70} dissociates, and is replaced by NusA.

RNA

σ^{70}

RNA

3 Transcription is initiated.

4 Promoter clearance is followed by elongation.

ITC = Initial Transcribing Complex

The Transcription Cycle

RNA Polymerase functions like a helicase to open the 2 strands of the DNA at promoters:
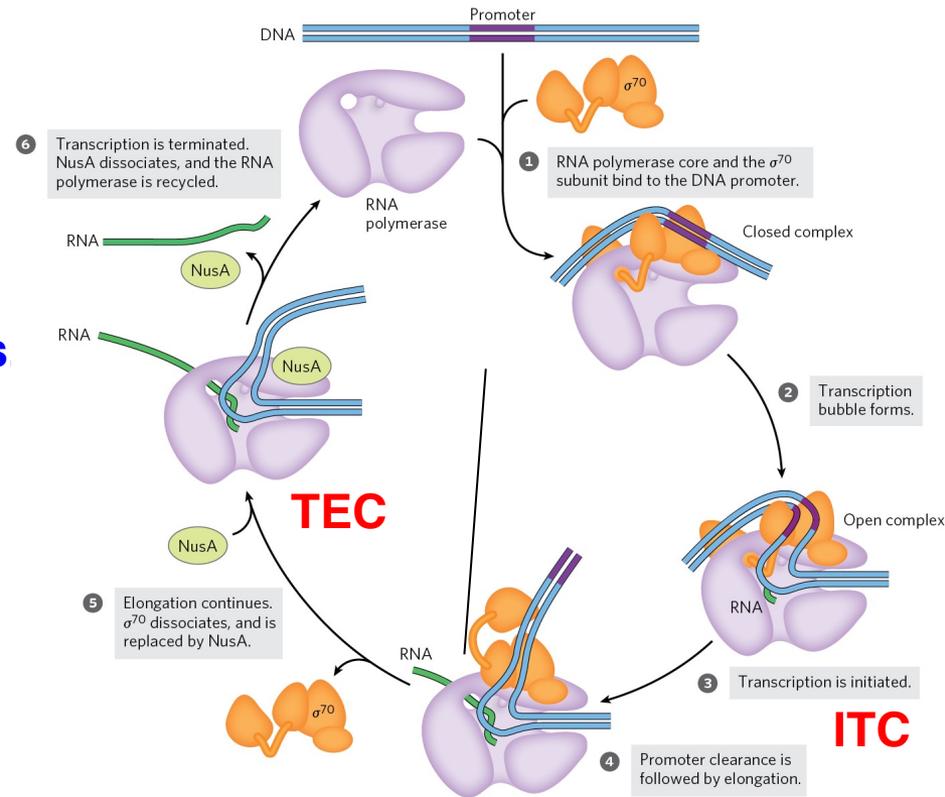
σ^{70} holoenzyme does not require ATP for open complex formation

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
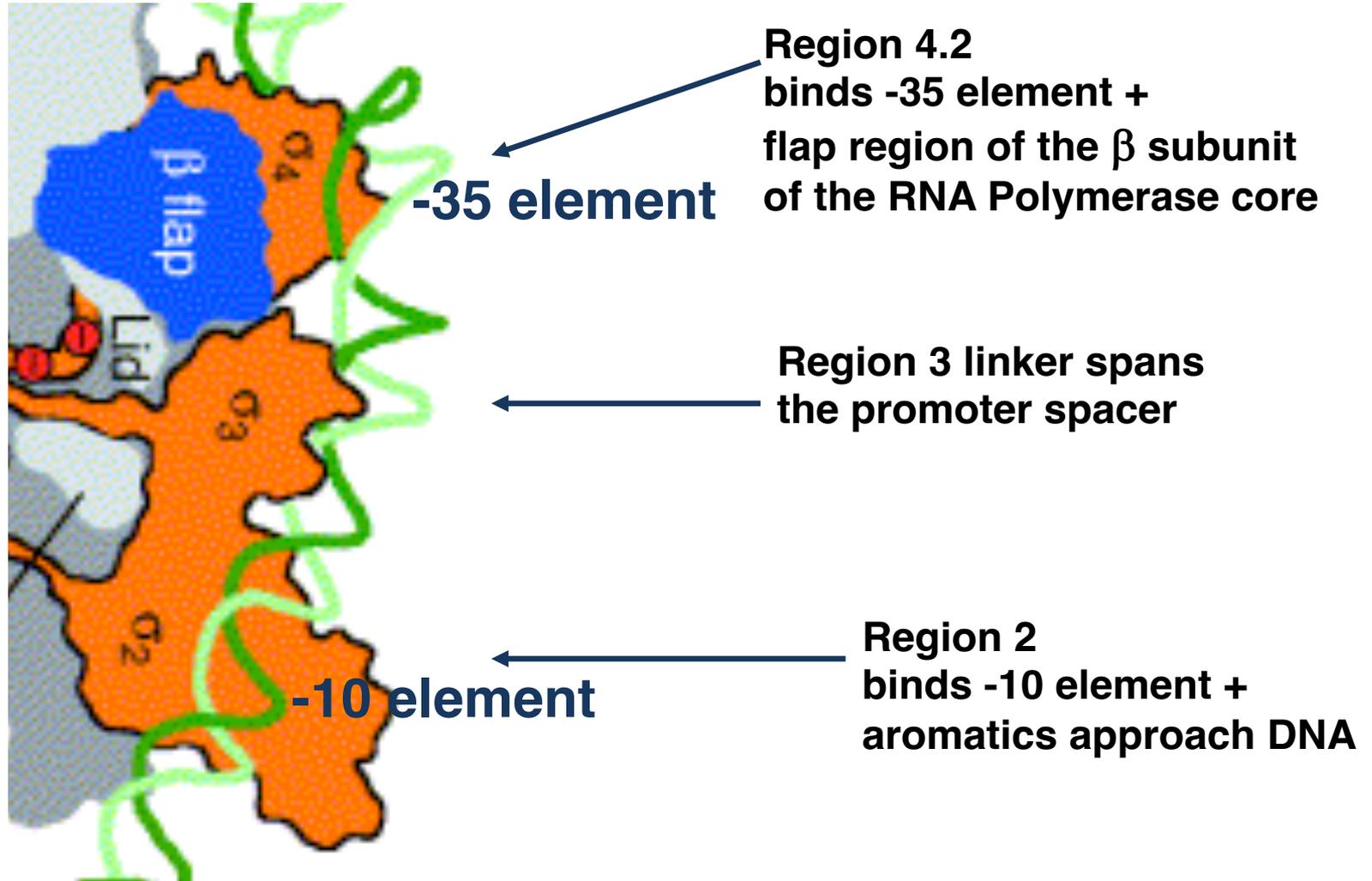- 100s of cycles of abortive initiation sometimes precede the next step.

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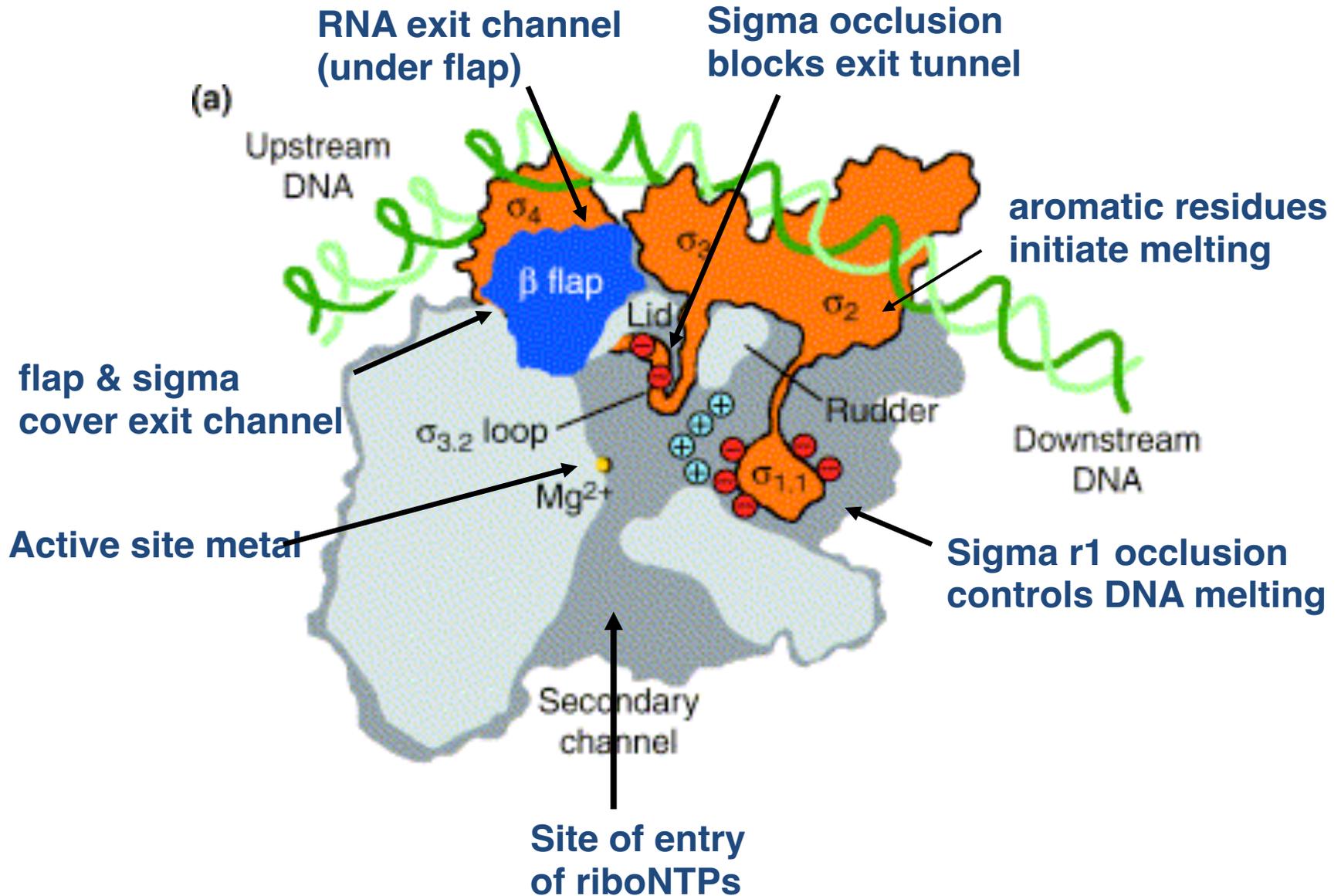

DNA

Promoter

6 Transcription is terminated. NusA dissociates, and the RNA polymerase is recycled.

RNA polymerase

NusA

RNA

NusA

TEC

NusA

5 Elongation continues. σ^{70} dissociates, and is replaced by NusA.

RNA

σ^{70}

1 RNA polymerase core and the σ^{70} subunit bind to the DNA promoter.

Closed complex

2 Transcription bubble forms.

Open complex

RNA

3 Transcription is initiated.

ITC

4 Promoter clearance is followed by elongation.

How does promoter recognition and the transition Closed -> Open Complex occur ?
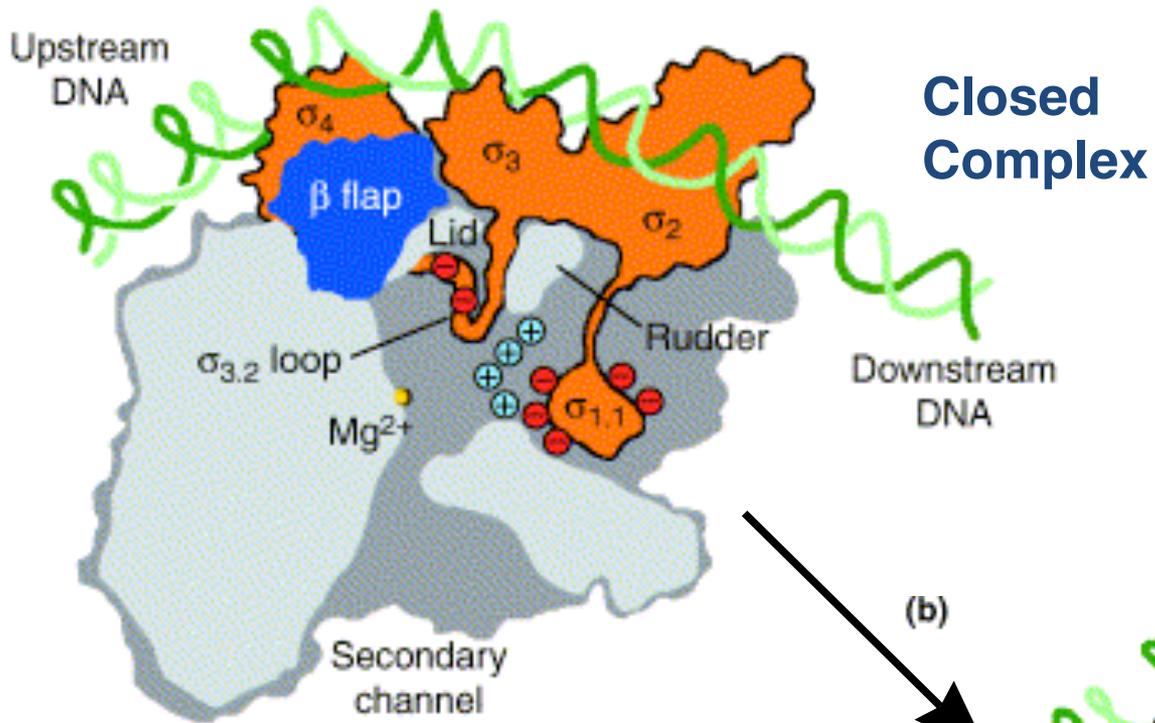
Sigma70 contains 2 DNA recognition regions:

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

Features of bacterial RNAP that influence transcription initiation and escape

(a)

RNA exit channel (under flap)

Sigma occlusion blocks exit tunnel

aromatic residues initiate melting

flap & sigma cover exit channel

Upstream DNA

σ4

β flap

Lid

σ3

σ2

Rudder

Downstream DNA

Active site metal

σ3.2 loop

Mg2+

σ1.1

Sigma r1 occlusion controls DNA melting

Secondary channel

Site of entry of riboNTPs

Model for initiation (steps 1 & 2)

(a)

Upstream DNA

σ4

β flap

Lid

σ3.2 loop

Mg²⁺

σ3

σ2

Rudder

σ1.1

Downstream DNA

Secondary channel

Closed Complex

(b)

DNA melting

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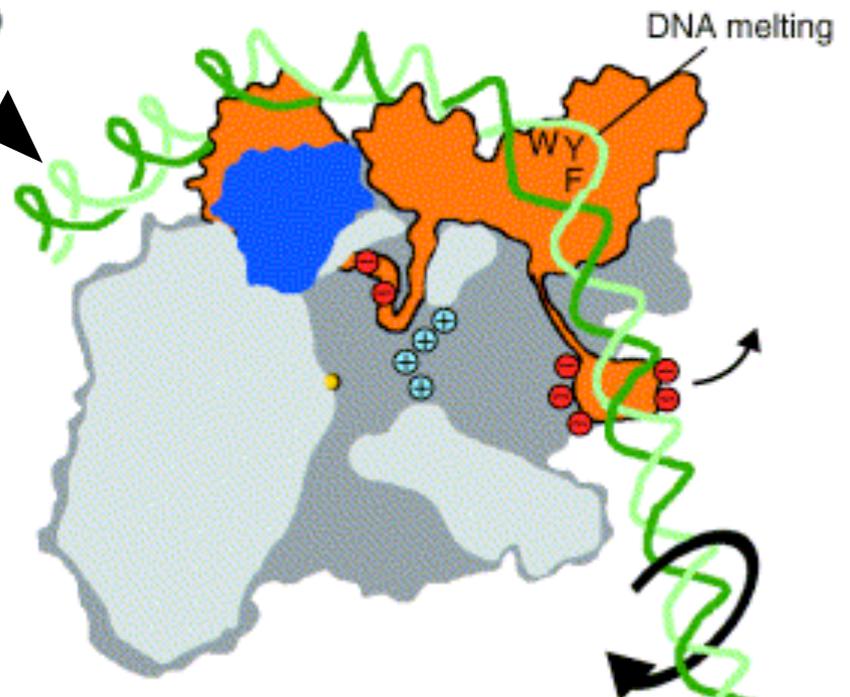
• **DNA replaces σ1.1**
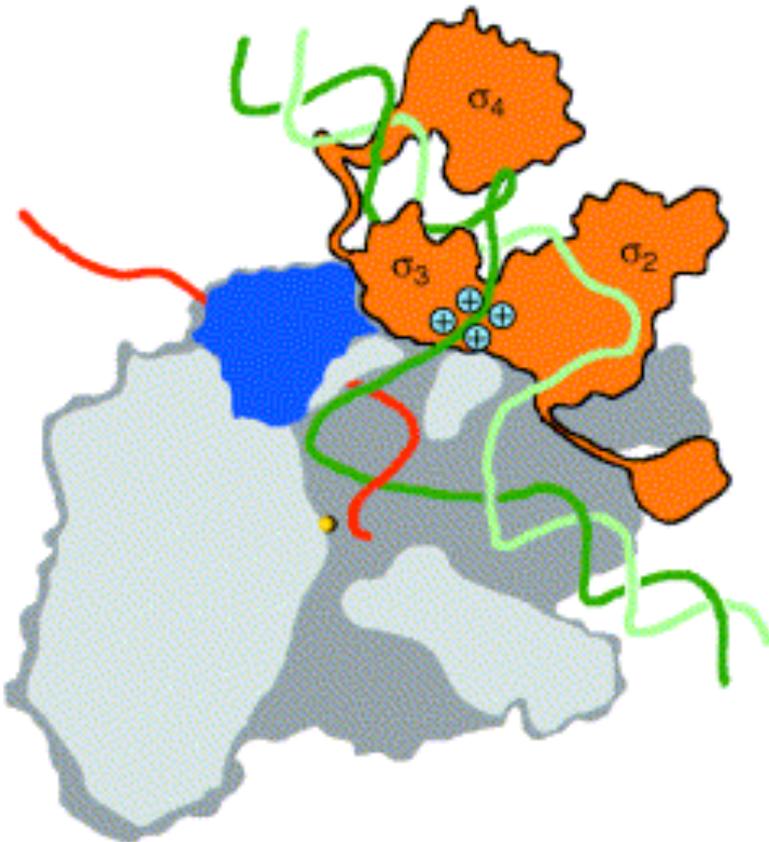• **insertion of aromatic side chains from σ2 (W,Y,F) initiates DNA melting in region -10-+1**

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

RNA

Bridge

NTP

RP$_o$ and abortive initiation

σ$_{3.2}$ loop

End of abortive initiation
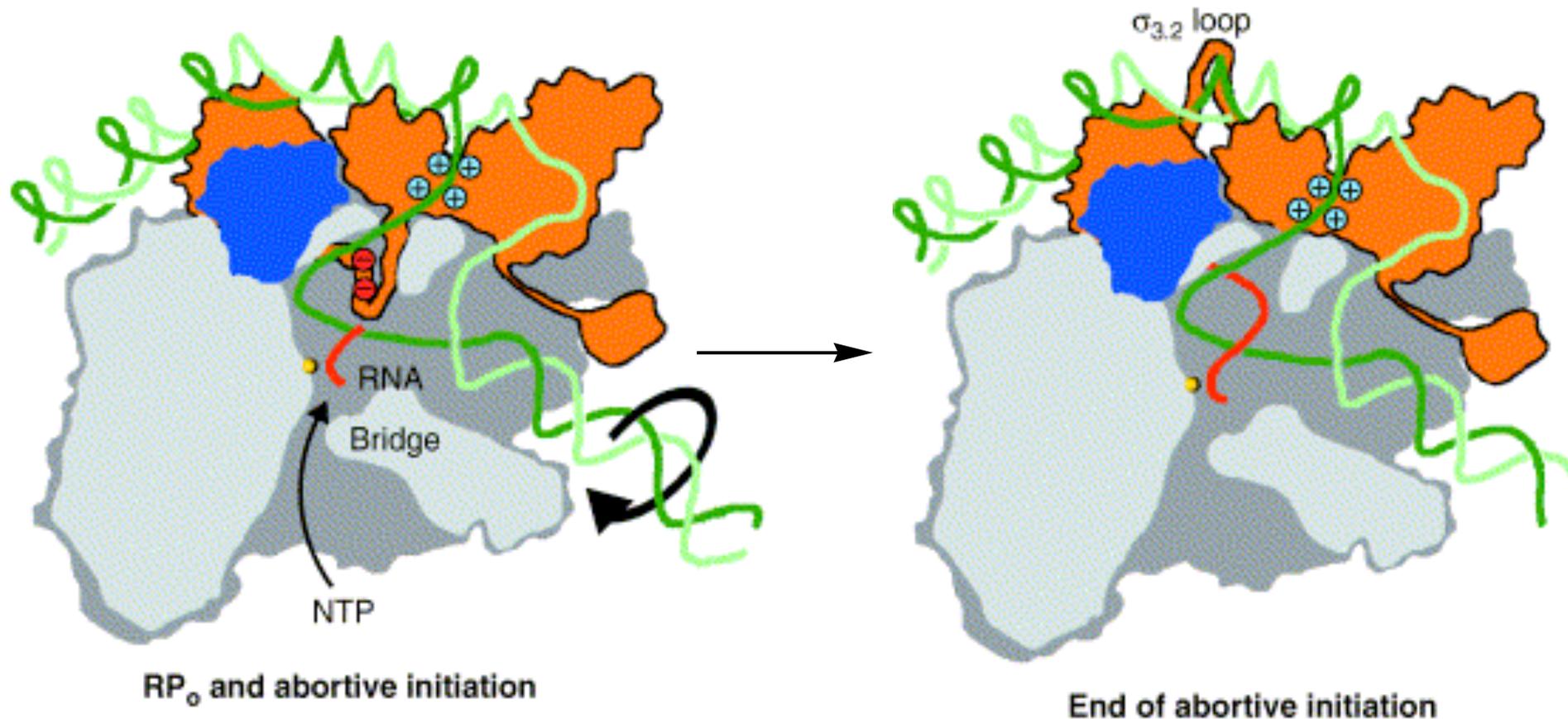
The flap opens to allow RNA to exit. This initiates sigma release and escape from the promoter
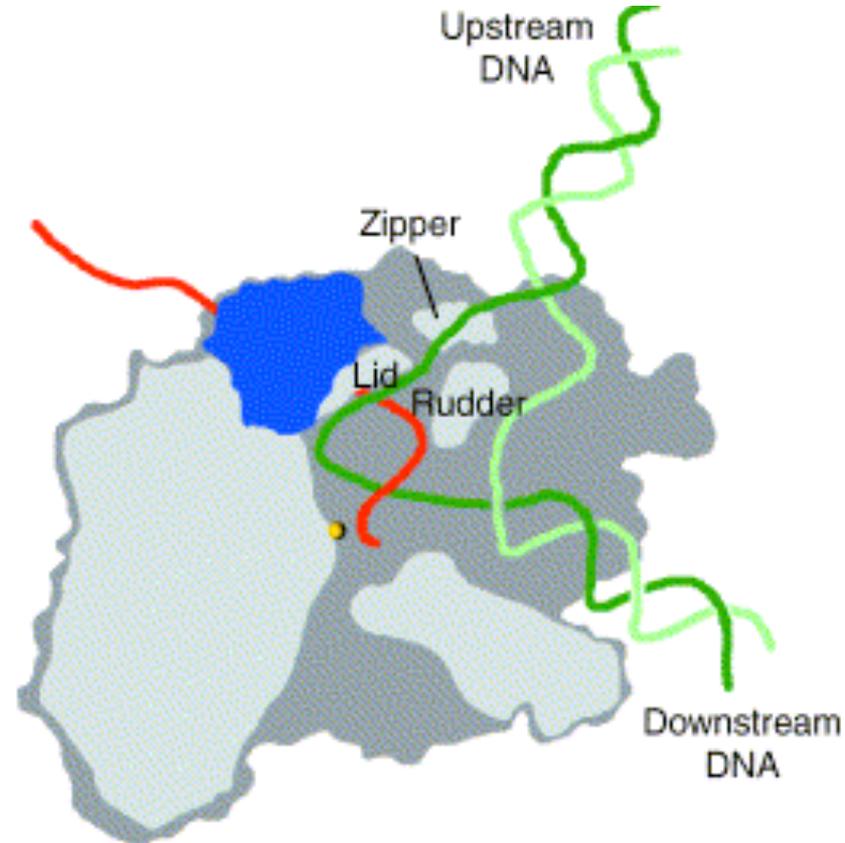
Stable Ternary Elongation Complex (TEC)

Promoter clearance

TEC