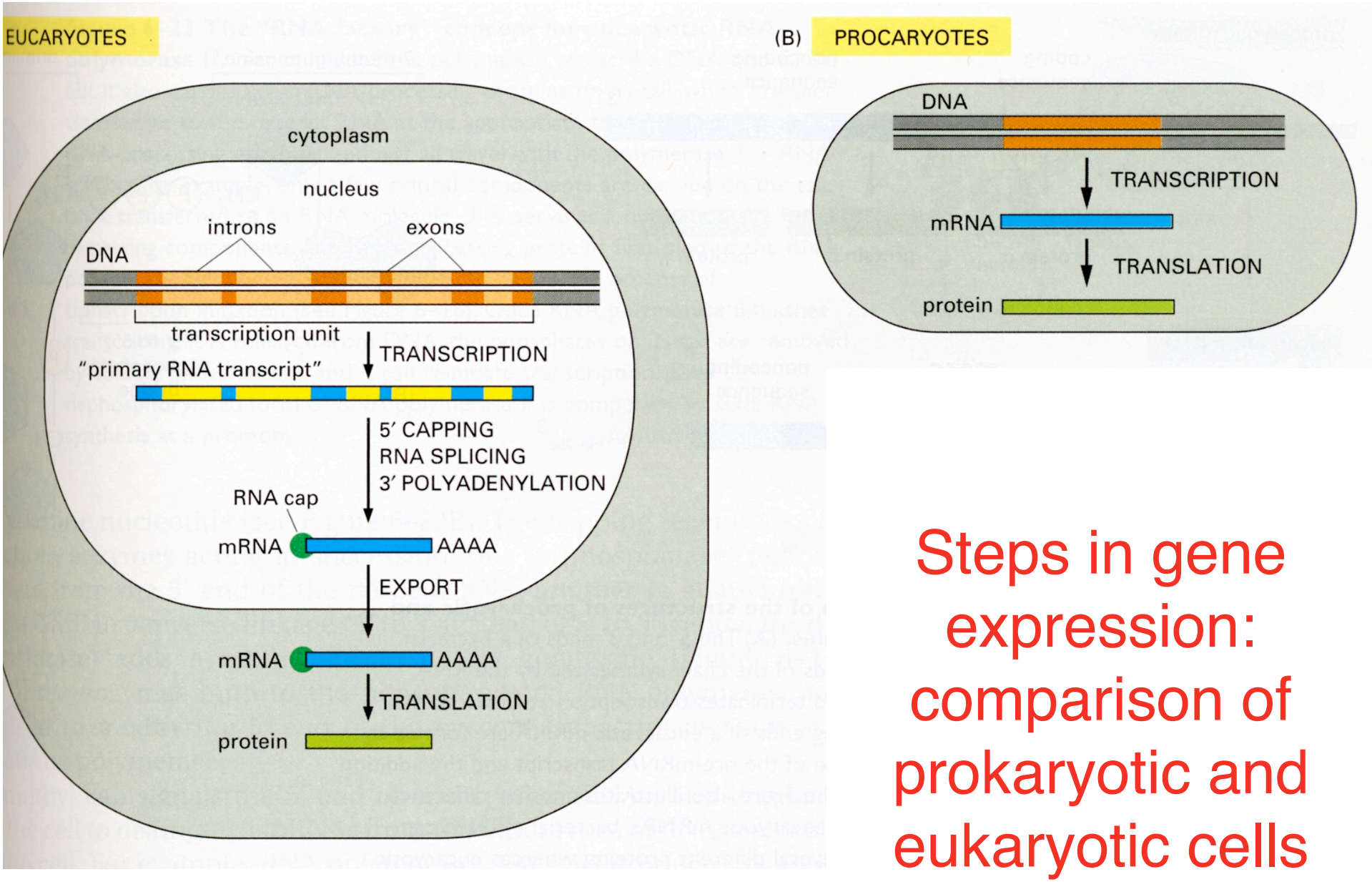
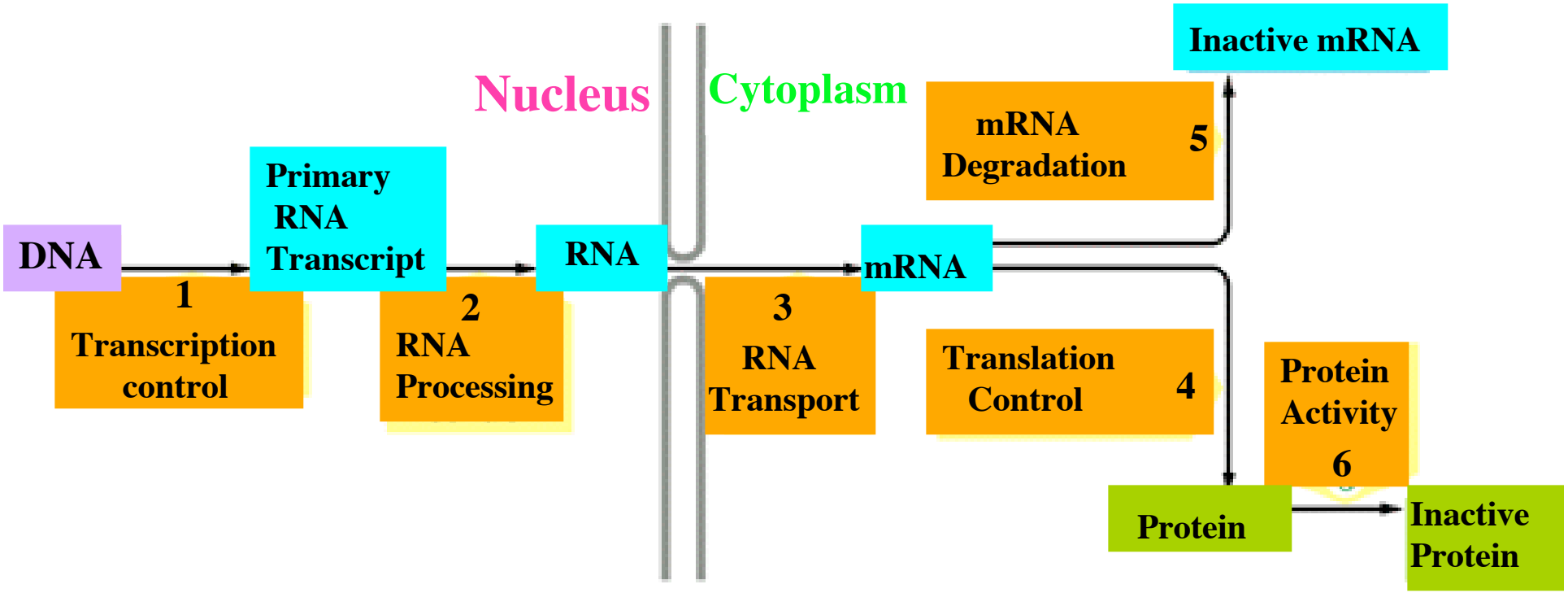
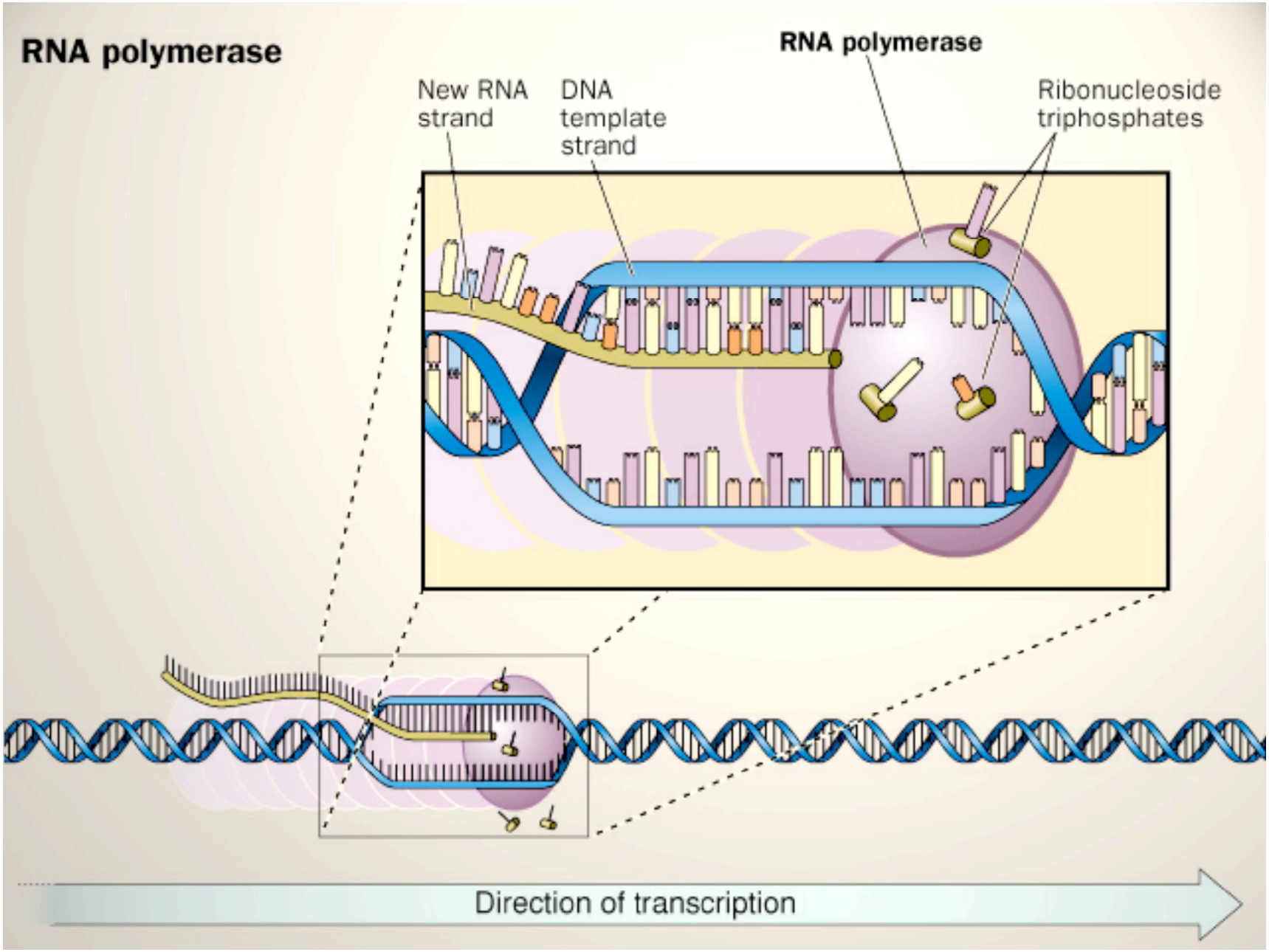


Steps in gene expression: comparison of prokaryotic and eukaryotic cells



Six steps at which eukaryotic gene expression can be controlled.

In prokaryotic cells, genes do not have introns (no step 2) and transcription and translation are not separated in space and time (no step 3).


RNA polymerase
RNA polymerase
New RNA strand
DNA template strand
Ribonucleoside triphosphates
Direction of transcription

Transcription of DNA into RNA by RNA polymerase.

1. Requires DNA template,
4 ribonucleotide 5′ triphosphates, Mg^{+2}.

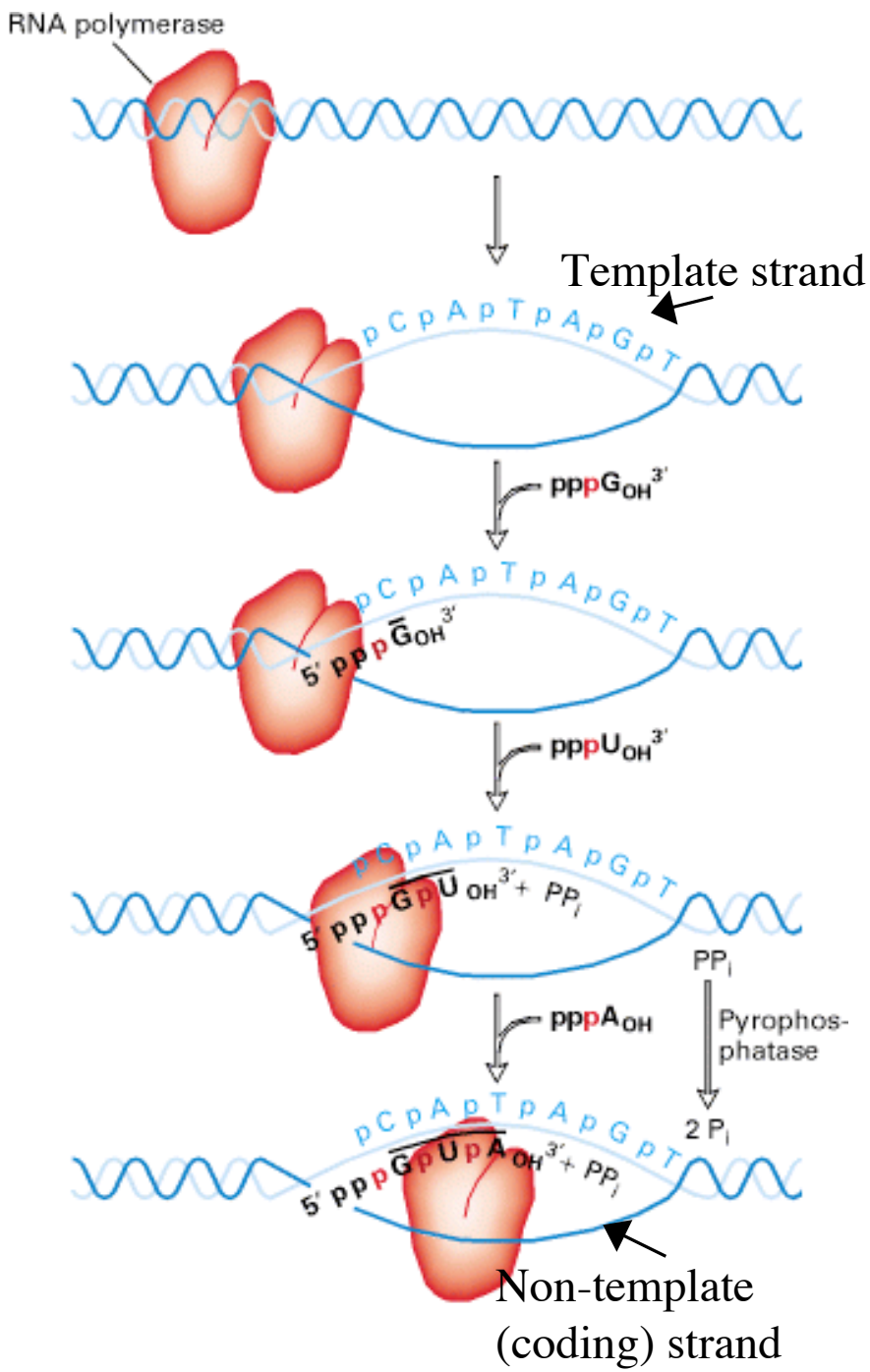
2. De novo synthesis: does not require a primer. Low fidelity compared to DNA polymerase: errors $1/10^4$-10^5. RNA polymerase incorporates ~30 nt/s (much slower than DNA polymerase).

3. Activity highly regulated *in vivo*: at initiation, elongation and termination.

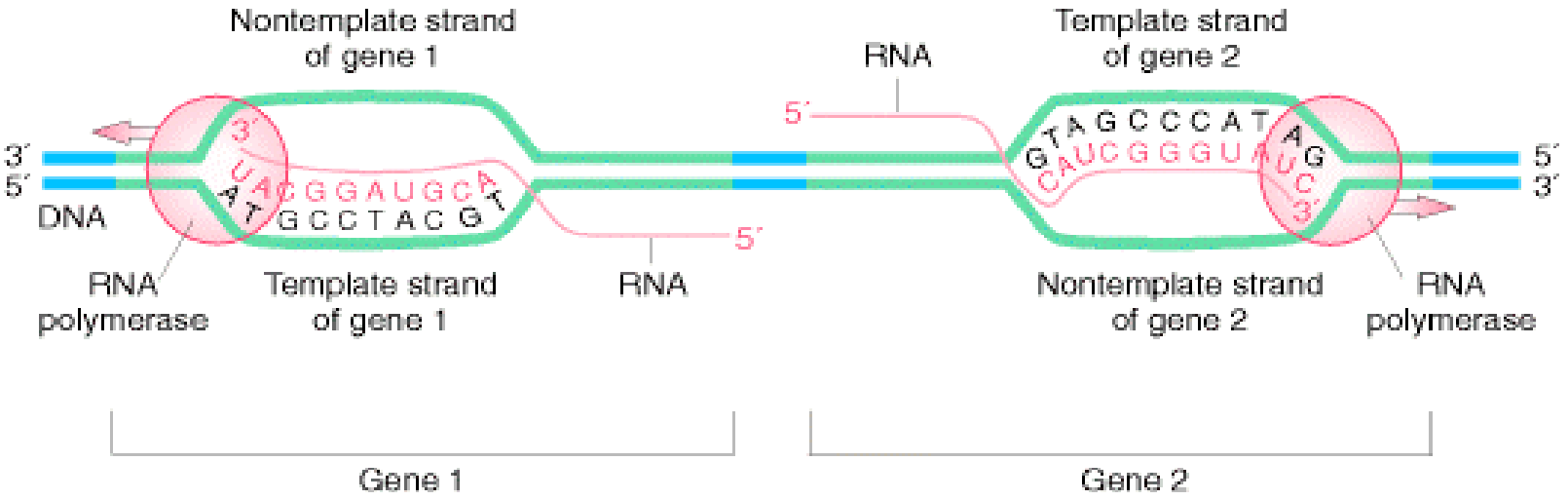
4. The nucleotide at the 5′ end of an RNA strand retains all three of its phosphate groups; all subsequent nucleotides release pyrophosphate (PPi) when added to the chain and retain only their □ phosphate (red).

5. The released PPi is subsequently hydrolyzed by pyrophosphatase to Pi, driving the equilibrium of the overall reaction toward chain elongation.

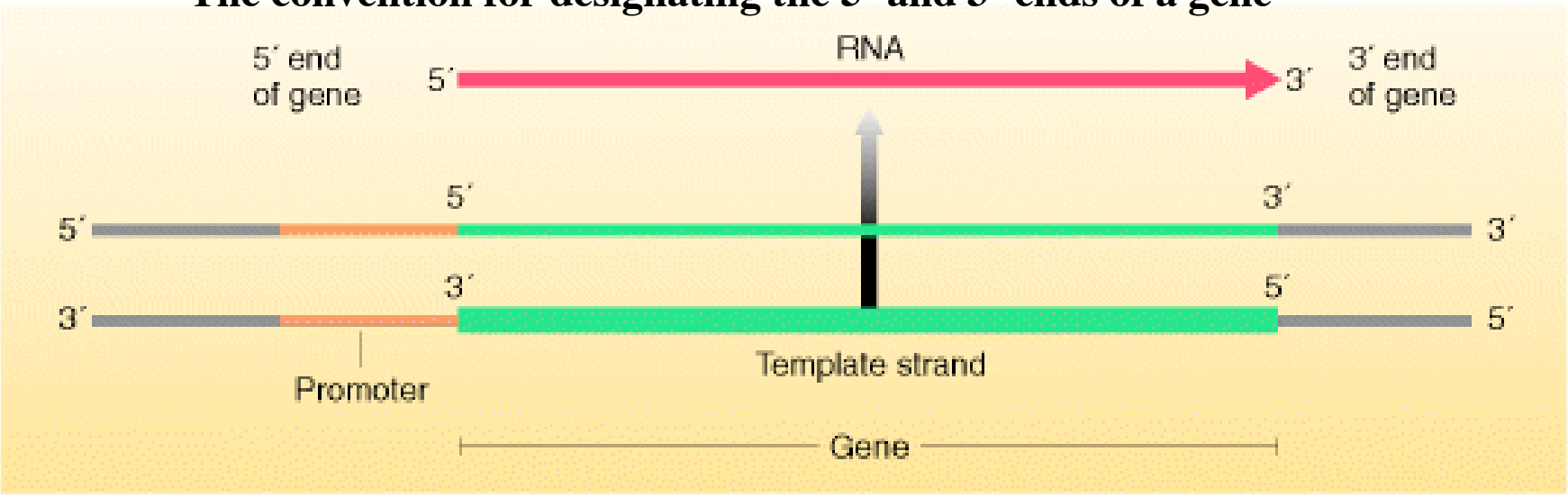
6. In most cases, only one DNA strand is transcribed into RNA.



Transcription of two genes on different template strands. Note that growth of the transcript always occurs in the 5'-to-3' direction.



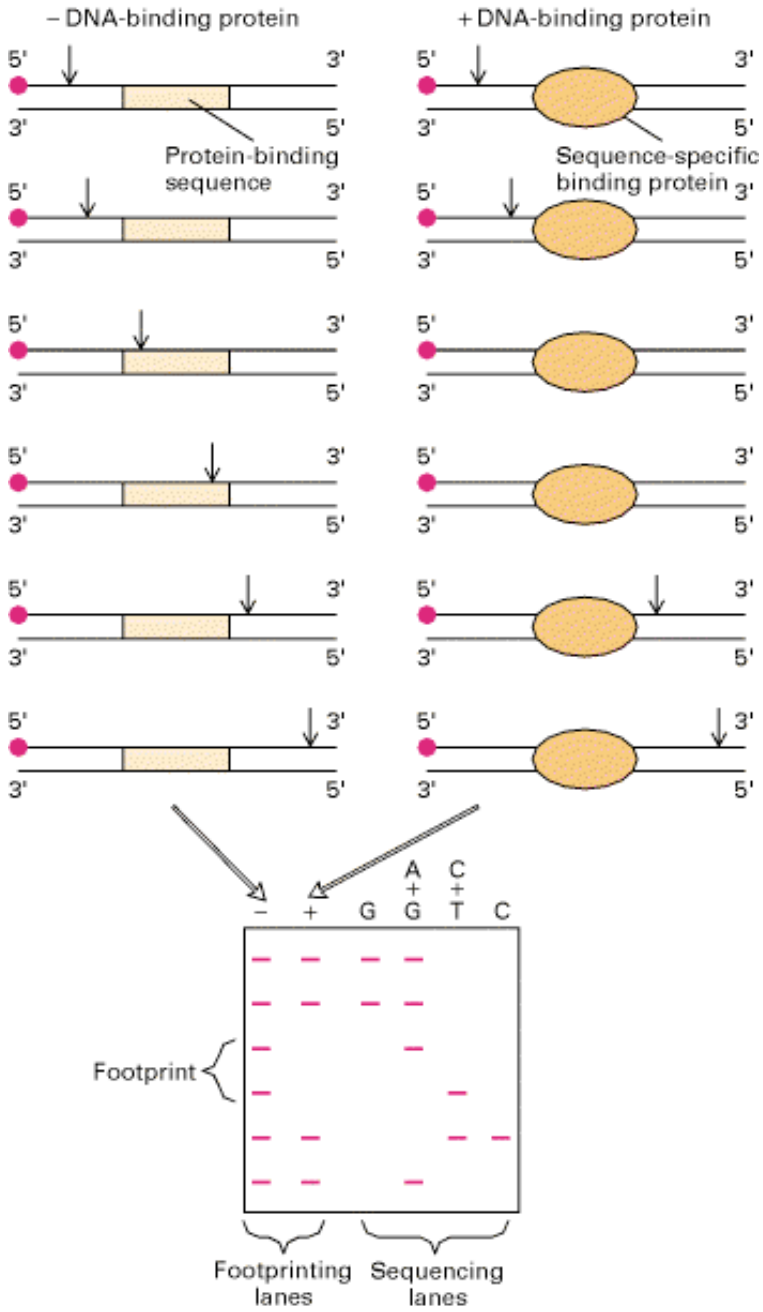
The convention for designating the 5' and 3' ends of a gene



Biochemical studies of bacterial RNA polymerase

1. DNA binding assays- DNaseI footprinting
   Gel shift

2. Role of individual subunits- dissociation of holoenzyme
   by column chromatography



DNase I footprinting, a common technique for identifying protein-binding sites in DNA.

1. A DNA fragment is labeled at one end with ^{32}P (red dot).

2. Portions of the sample then are digested with DNase I in the presence and absence of a protein that binds to a specific sequence in the fragment.

3. A low concentration of DNase I is used so that on average each DNA molecule is cleaved just once (vertical arrows).

4. The two samples of DNA then are separated from protein, denatured to separate the strands, and electrophoresed. The resulting gel is analyzed by autoradiography, which detects only labeled strands and reveals fragments extending from the labeled end to the site of cleavage by DNase I.

DNaseI footprint of RNAP on the *lac* promoter

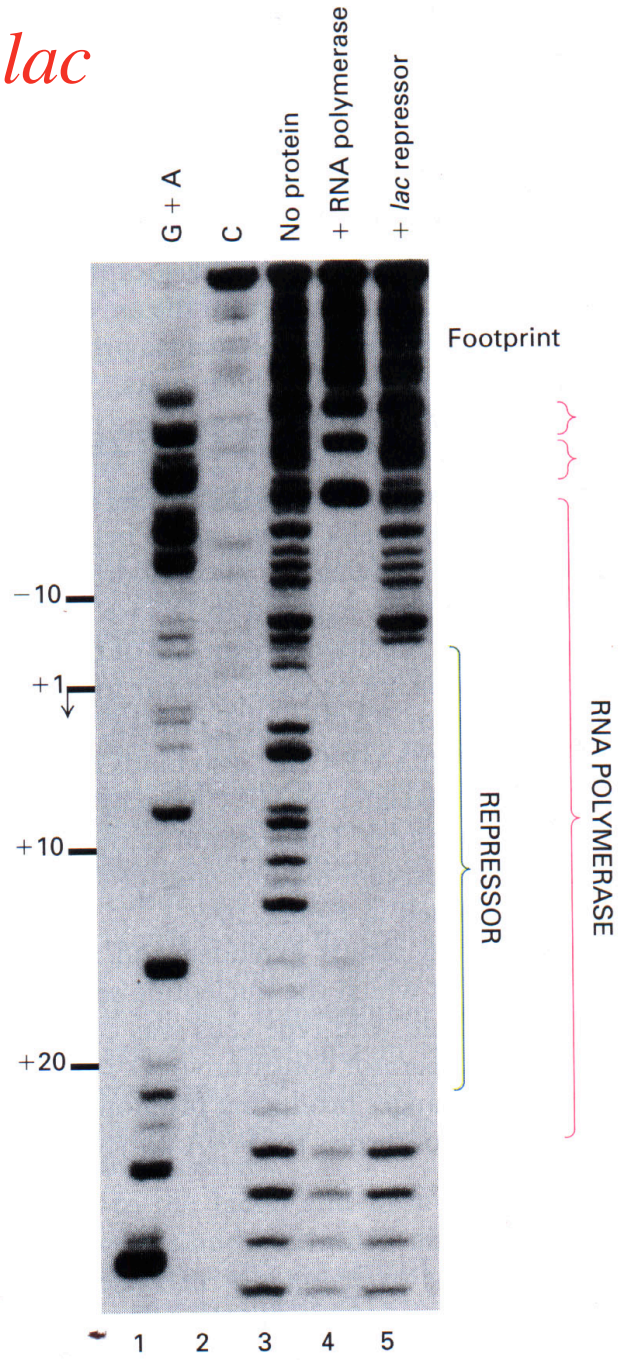
Lanes 1 and 2 are DNA sequencing reactions for orientation

Lane 3 is the no protein control

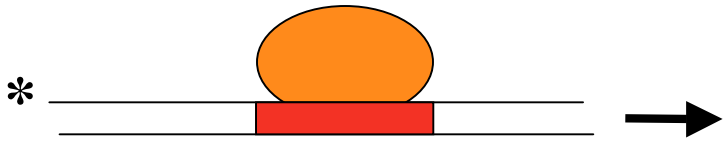
Lane 4 contained RNAP

Footprint:
Advantage- can assay short-lived interactions; proteins have very characteristic binding patterns

Disadvantage- requires nearly stoichiometric binding

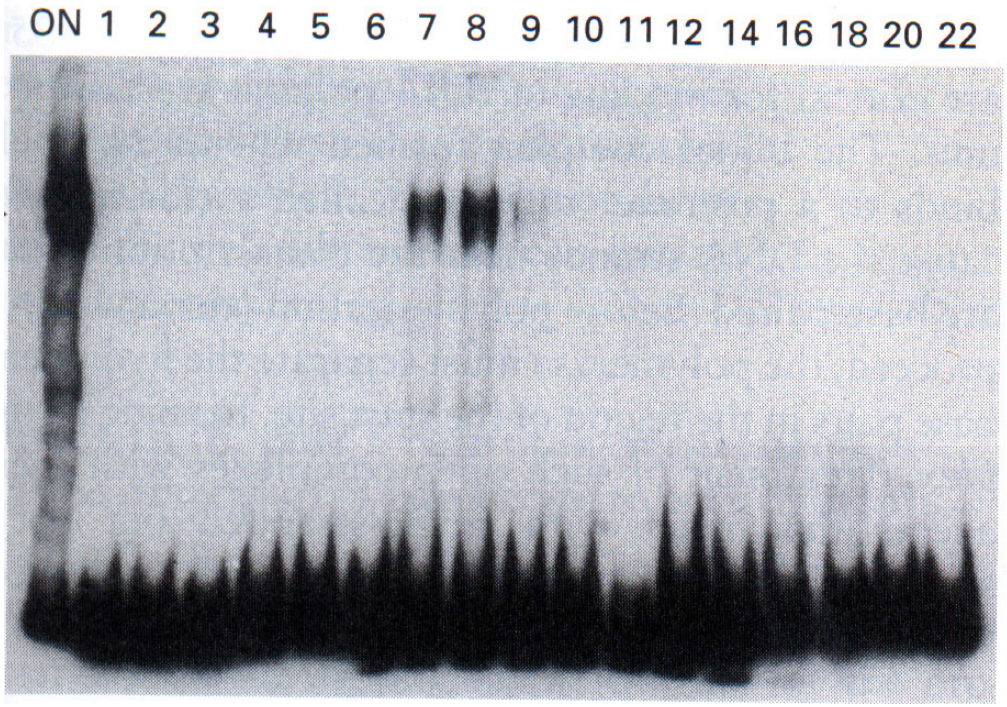


"Gel shift": electrophoretic mobility shift assay ("EMSA") for DNA-binding proteins



Protein-DNA complex

Free DNA probe

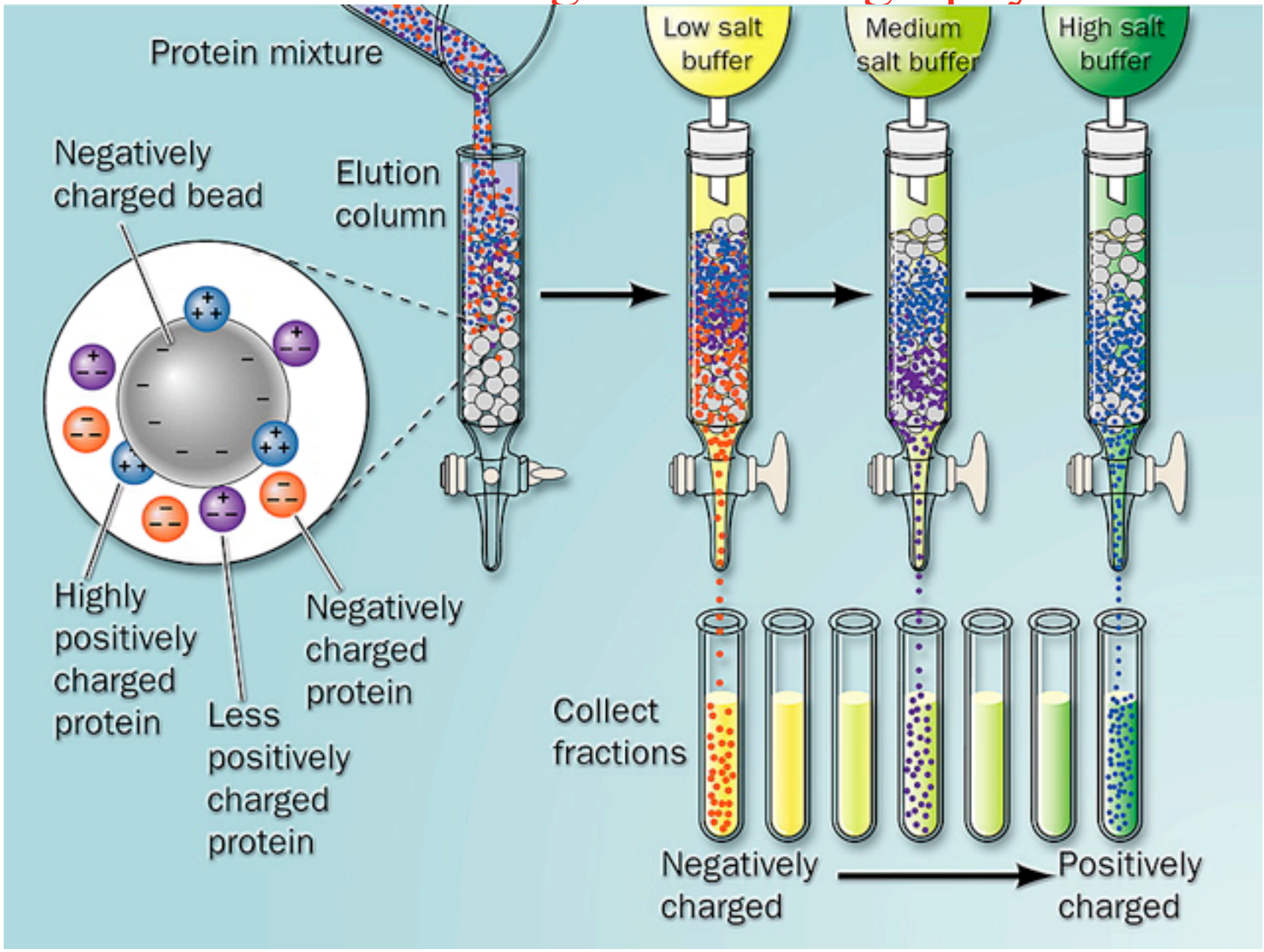


1. Prepare labeled DNA probe
2. Bind protein
3. Native gel electrophoresis

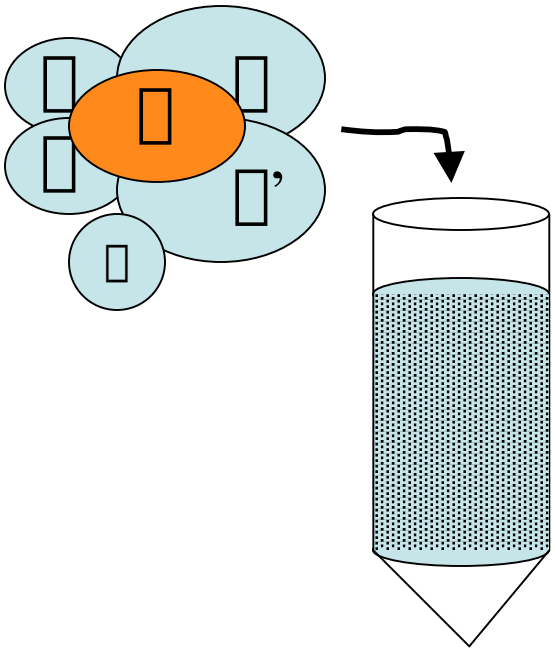
Advantage: sensitive

Disadvantage: requires stable complex; little "structural" information about which protein is binding

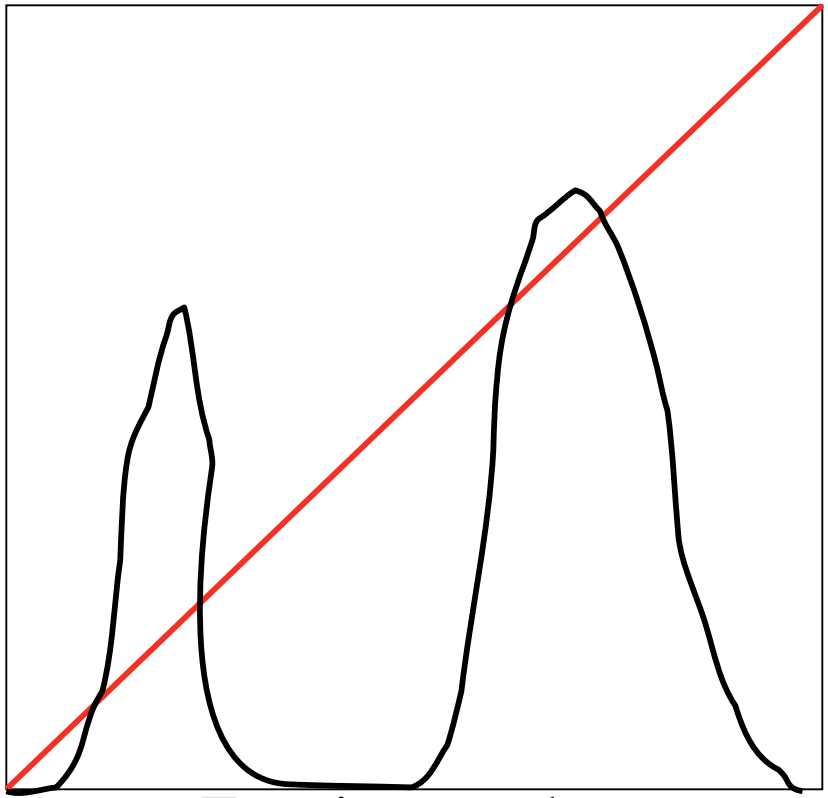
Ion-exchange chromatography

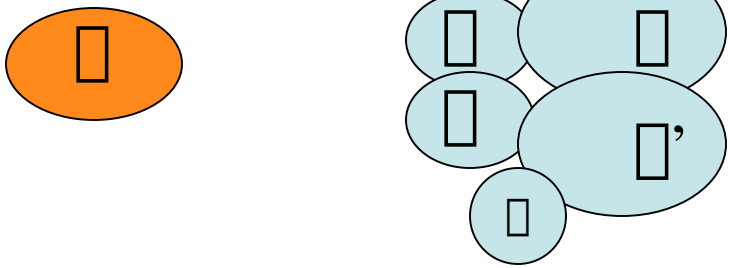


Dissociation of RNAP and purification of
by ion-exchange chromatography

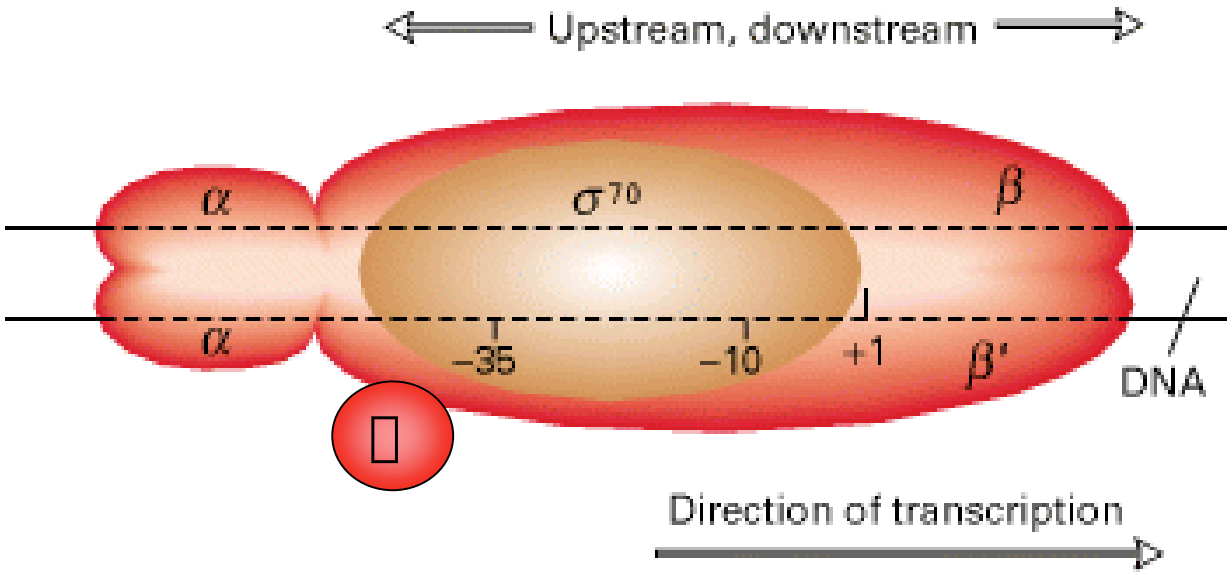


Carboxymethyl- ($-CO_2^{-2}$) or
phospho- ($-PO_3^{-2}$) cellulose



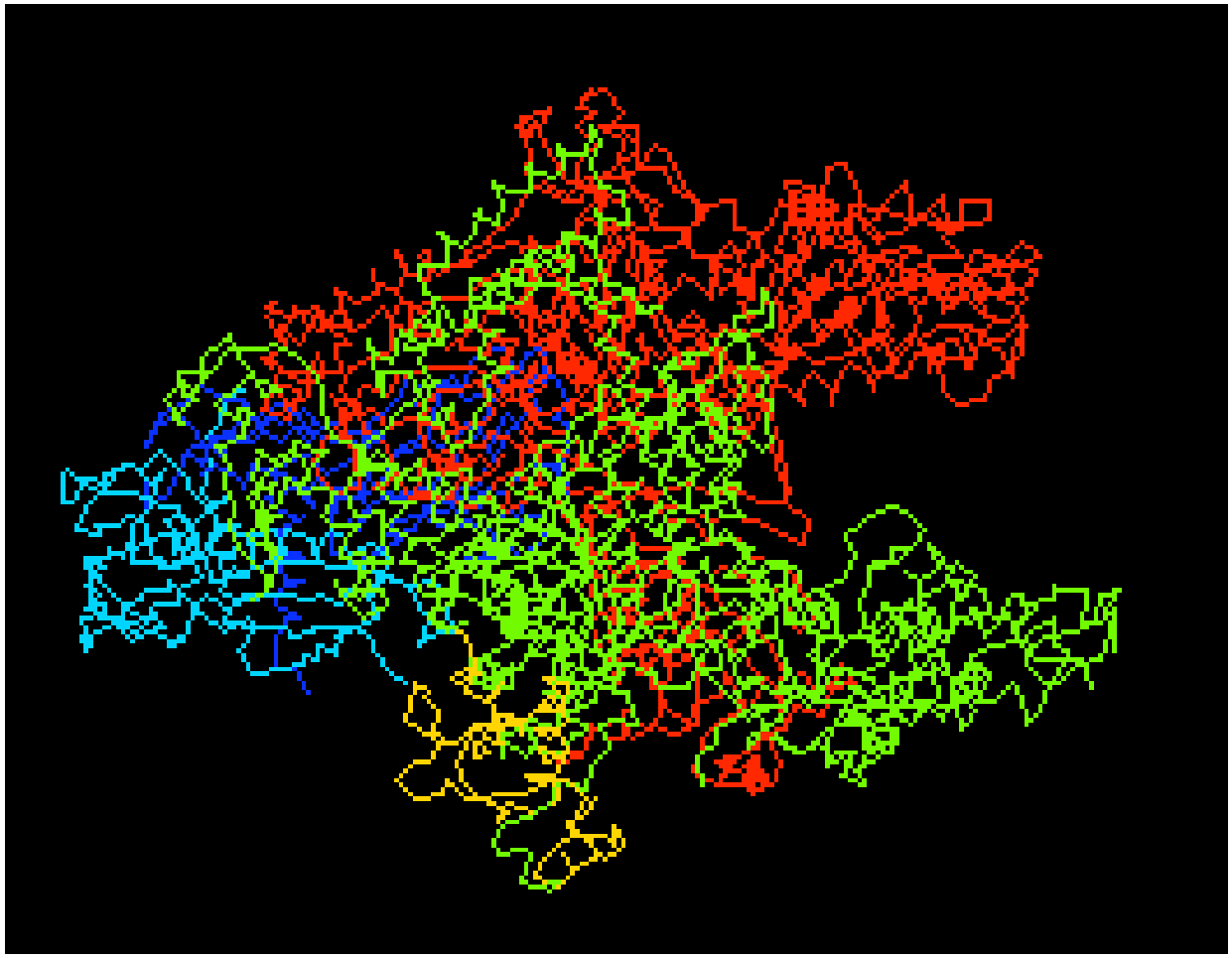


E. coli RNA polymerase holoenzyme bound to DNA.



Subunit	Stoichiometry in holoenzyme	Role
	2	Binds regulatory sequences/proteins
	1	Polymerase; Binds DNA template
’	1	Polymerase; Binds DNA template
	1	Promoter recognition
	1	RNAP assembly

Thermus aquaticus core RNA polymerase



Blue
Red
’ Green
Orange

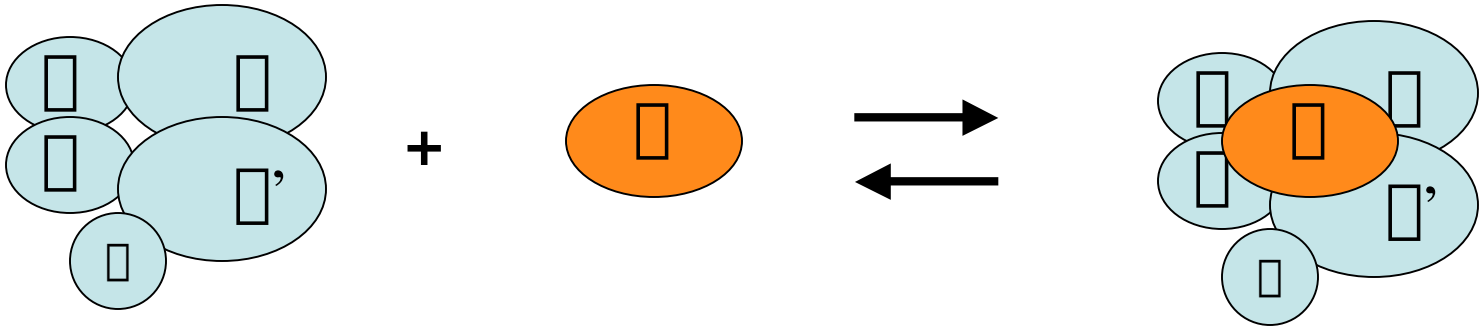
PDB (Protein Data Bank)
http://www.rcsb.org/pdb/
File 1HQM
Minakhin, L. et al. *Proc.Nat.Acad.Sci.USA* 98 *pp.* 892 (2001)

A single RNA polymerase makes multiple types of RNAs in prokaryotes

TABLE 5.2 **RNA molecules in *E. coli***

Type	Relative amount (%)	Sedimentation coefficient (S)	Mass (kd)	Number of nucleotides
Ribosomal RNA (rRNA)	80	23	1.2×10^3	3700
		16	0.55×10^3	1700
		5	3.6×10^1	120
Transfer RNA (tRNA)	15	4	2.5×10^1	75
Messenger RNA (mRNA)	5		Heterogeneous	

The dissociable sigma subunit gives promoter specificity to prokaryotic RNA polymerase (RNAP)



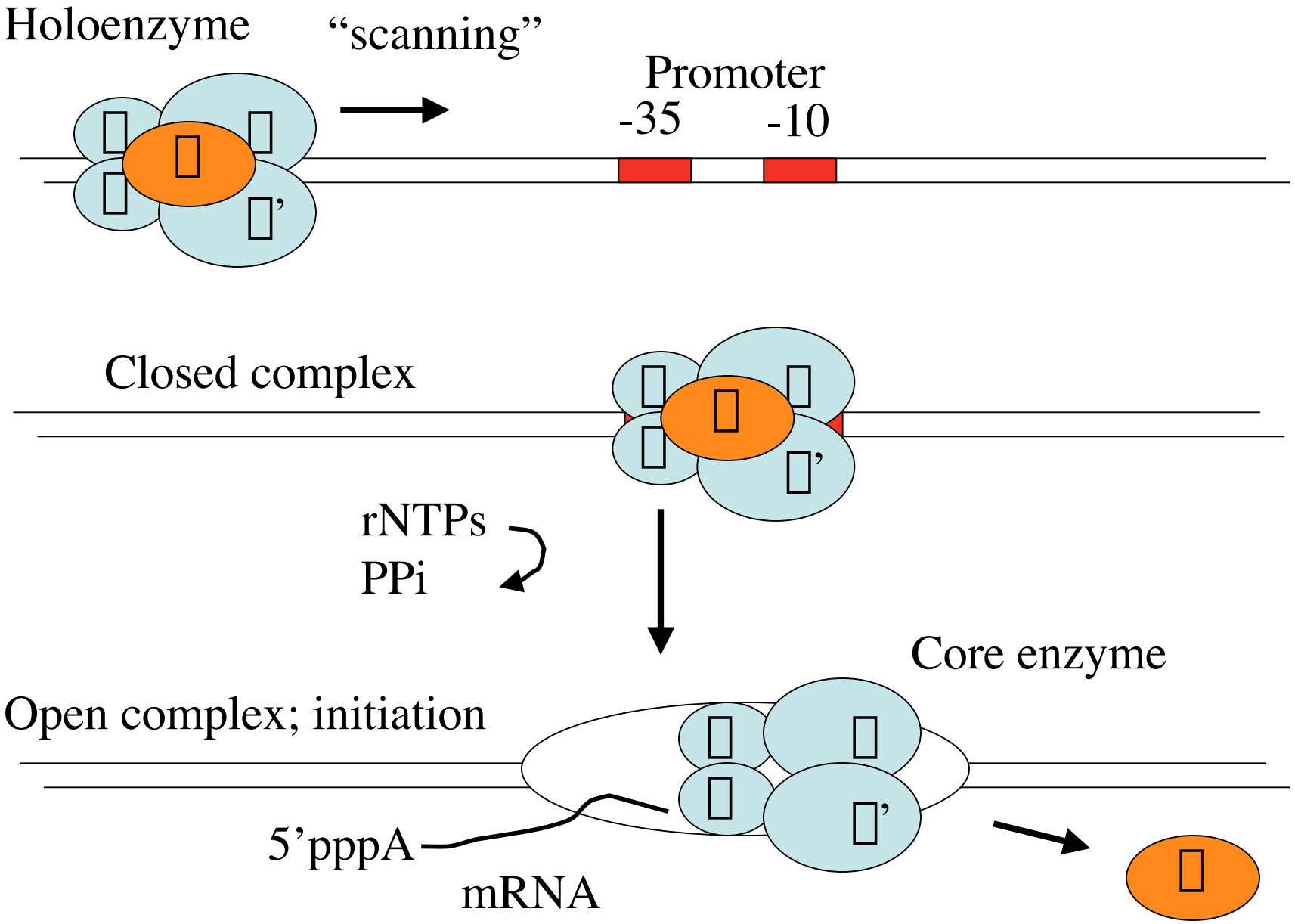
Core enzyme

Holoenzyme

No specific promoter binding;
tight non-specific DNA binding
(K_d ~5 x 10^{-12} M)

Specific promoter binding; weak
non-specific DNA binding (K_d ~10^{-7} M)

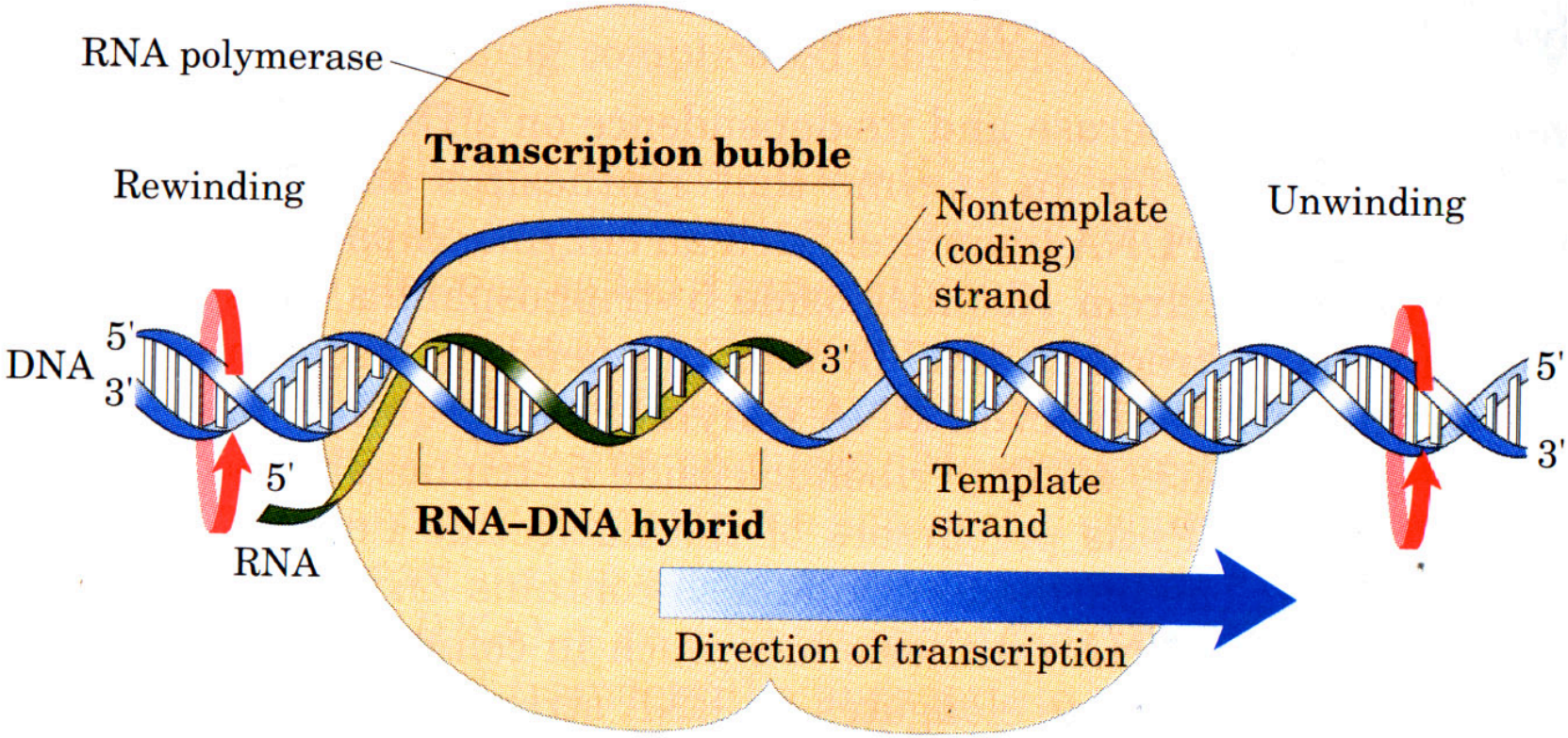
Transcription initiation by prokaryotic RNA polymerase



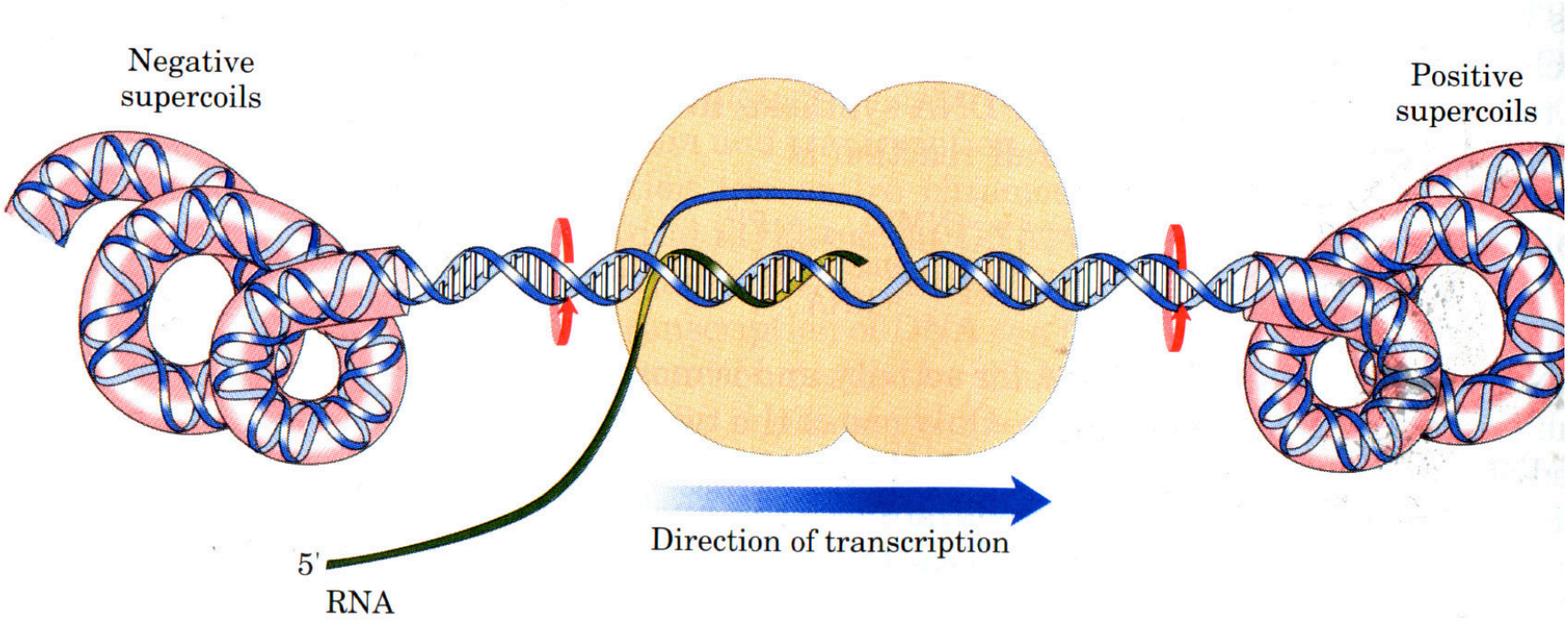
Sigma Factors of *E. coli*

Sigma Factor	Promoters Recognized	Promoter Consensus	
		-35 Region	**-10 Region**
σ^{70}	Most genes	TTGACAT	TATAAT
σ^{32}	Genes induced by heat shock	TCTCNCCCTTGAA	CCCCATNTA
σ^{28}	Genes for motility and chemotaxis	CTAAA	CCGATAT
σ^{38}	Genes for stationary phase and stress response	?	?
		-24 Region	**-12 Region**
σ^{54}	Genes for nitrogen metabolism and other functions	CTGGNA	TTGCA

Transcriptional elongation



Supercoiling of DNA during transcription causes a requirement for topoisomerases



Rho-independent prokaryotic transcription termination

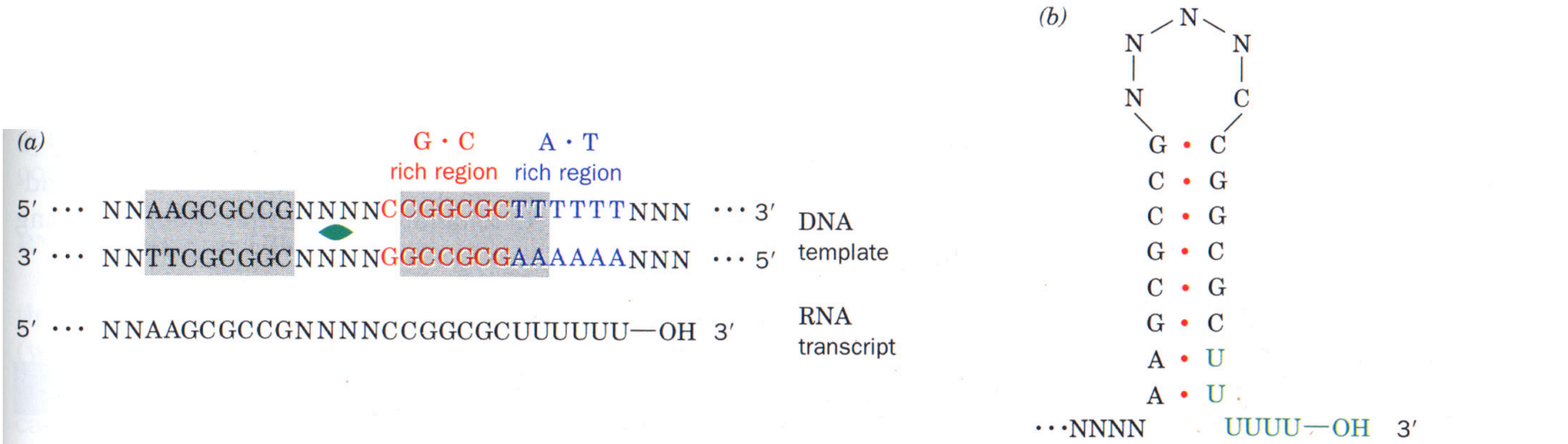
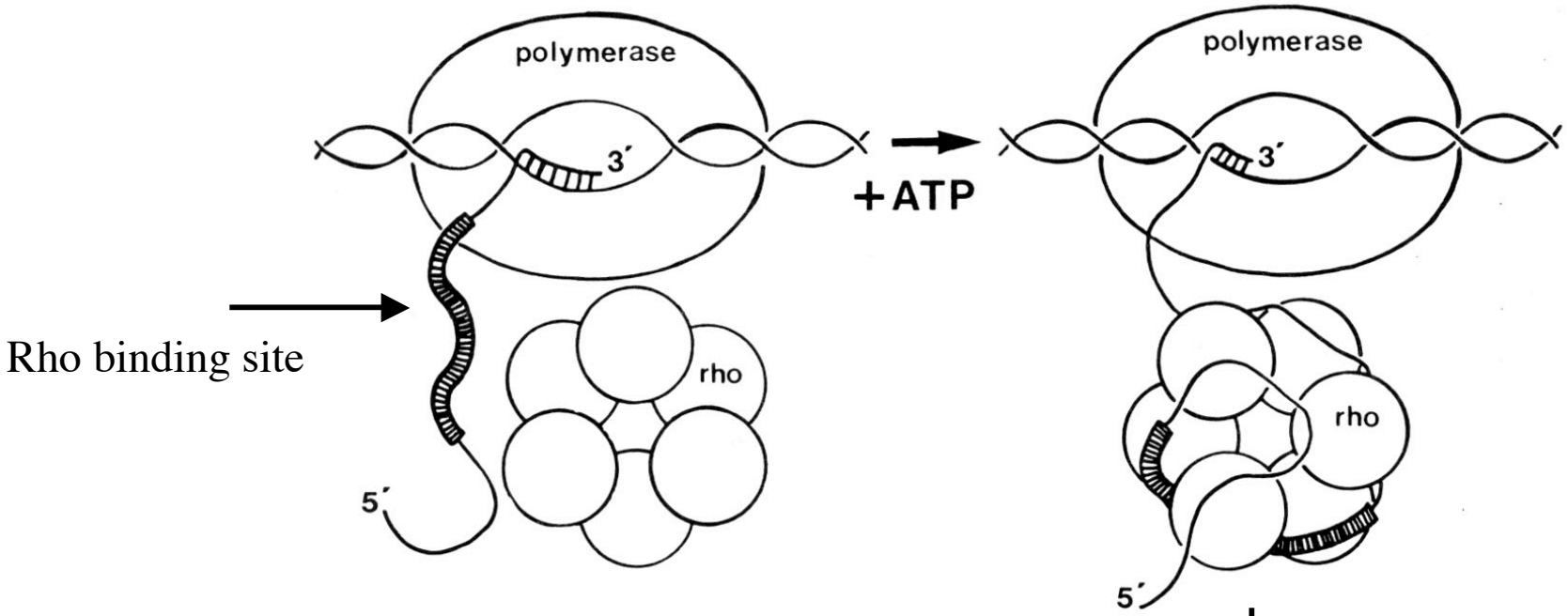


Figure 25-10. **A hypothetical strong (efficient) *E. coli* terminator.** The base sequence was deduced from the sequences of several transcripts. (*a*) The DNA sequence together with its corresponding RNA. The A · T-rich and G · C-rich sequences are shown in blue and red, respectively. The twofold symmetry axis (*green symbol*) relates the flanking shaded segments that form an inverted repeat. (*b*) The RNA hairpin structure and poly(U) tail that trigger transcription termination. [After Pribnow, D., *in* Goldberger, R.F. (Ed.), *Biological Regulation and Development,* Vol. 1, p. 253, Plenum Press (1979).]

Rho-dependent transcription termination



Rho: forms RNA-dependent hexameric ATPase,
translocates along RNA 5'-to-3',
promoting dissociation from polymerase

termination

Platt, *Ann. Rev. Biochem.* 55: 339 (1986)