

Science of Living System

BS20001

Arindam Mondal

School of Bioscience

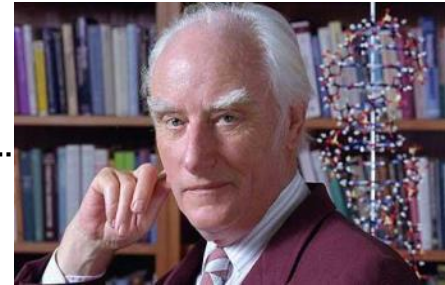
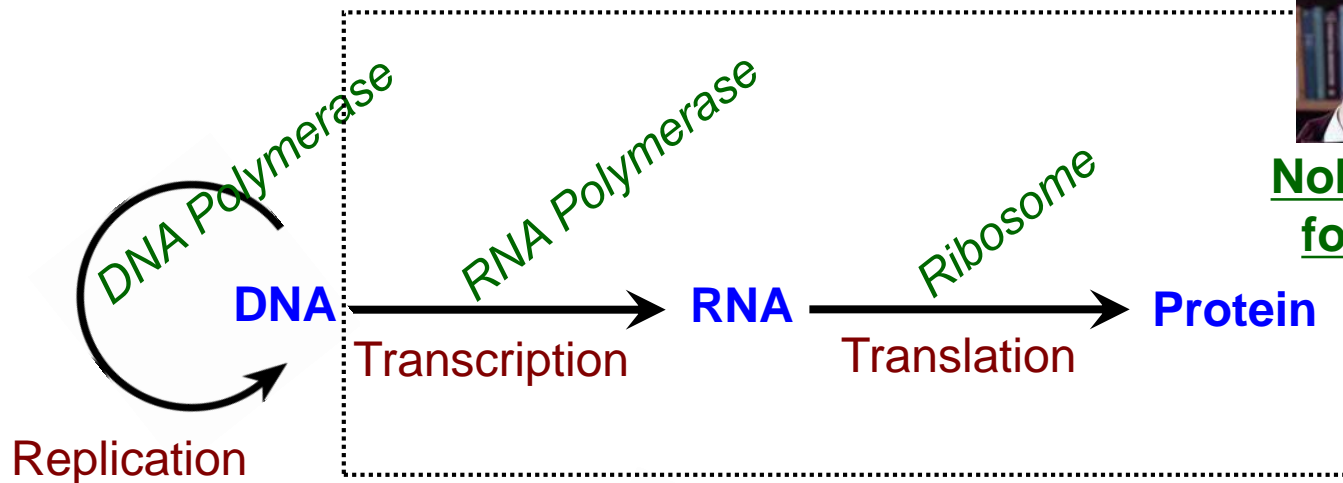
Email: arindam.mondal@iitkgp.ac.in

Tel: 03222-260518

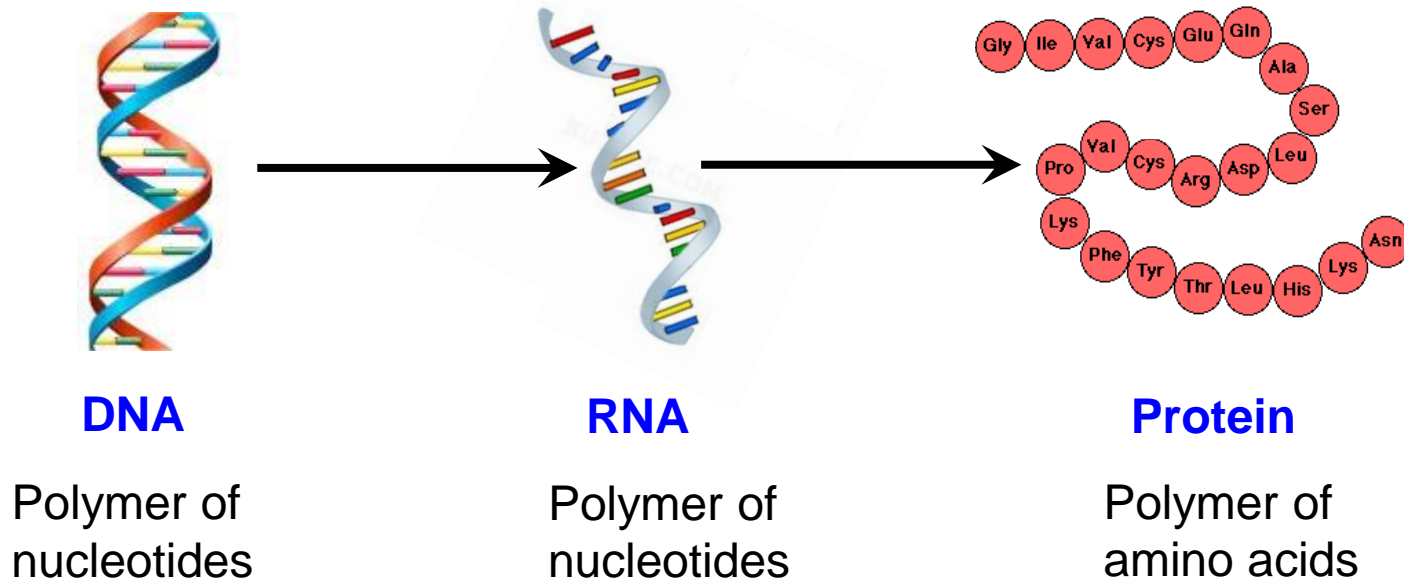
Overview of Transcription and Translation



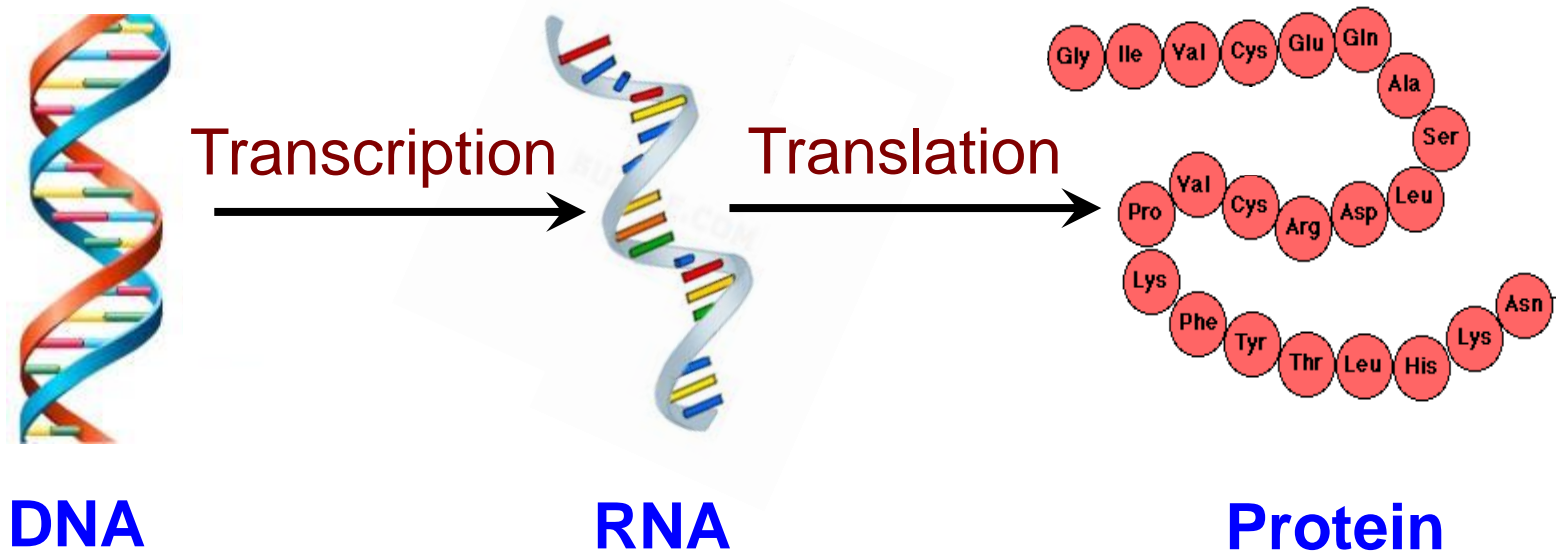
Flow of Genetic Information: The Central Dogma of Molecular Biology



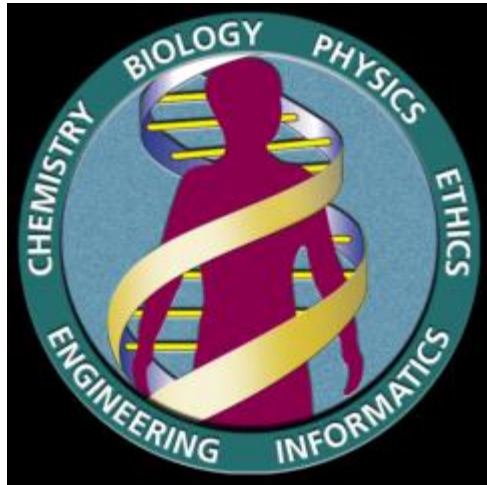
Nobel Prize in 1962
for DNA Structure



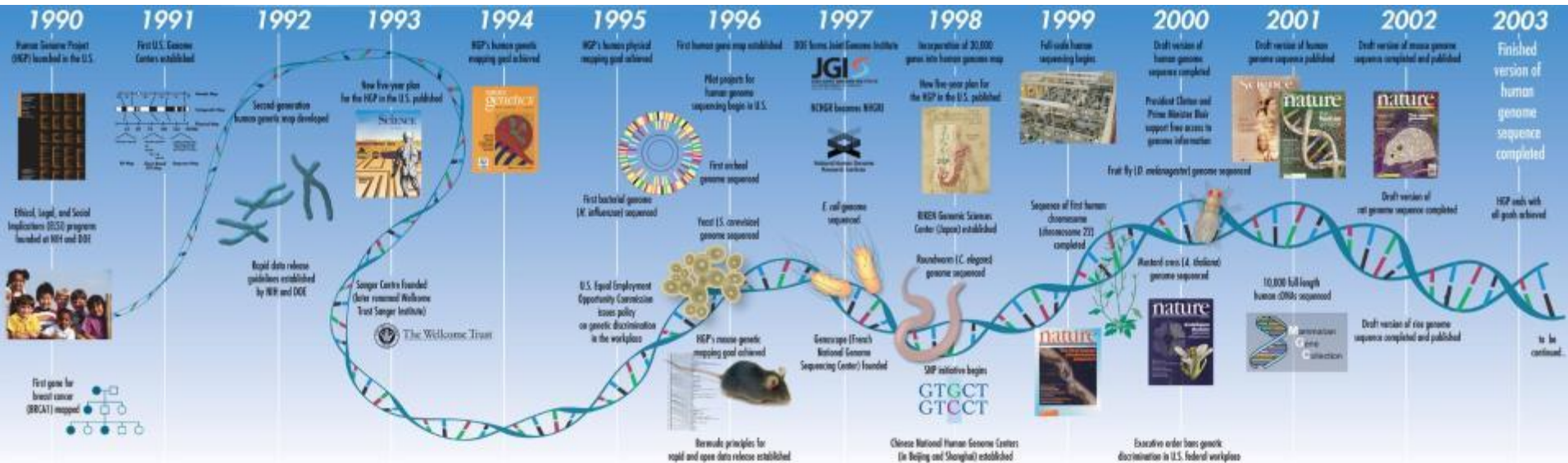
Correlations Between DNA Content and Its Downstream Product




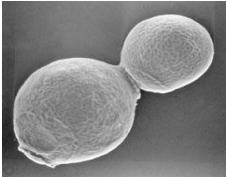



“Human Genome Project” dramatically enhanced our understanding of **Gene Expression**



~21,000 human genes (appeared to be significantly fewer than previous estimates)



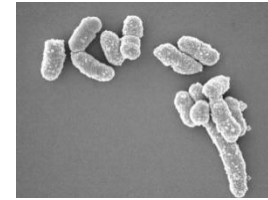
Genome Size, Gene Number, and Complexity of an Organism

	Organism	Genome size (bp)	Protein coding genes
	<i>E. coli</i>	4,600,000	4,250
	<i>S. cerevisiae</i>	12,160,000	5,616
	<i>C. elegans</i>	100,000,000	19,735
	Human	3,200,000,000	21,000
	Marbled lungfish	139,000,000,000	NA

Transcription

Total DNA content vs transcribable content

Genome size (bp)



4,600,000

- ▶ **Protein coding sequences is ~1.5% of total DNA content (human)**

Messenger RNA (mRNA)



3,200,000,000

- ▶ **Besides protein coding region, DNA can be transcribed into:**

Ribosomal RNA (rRNA)
Transfer RNA (tRNA)

- ▶ **Most of the DNA sequences are not transcribed**

What is a machine?

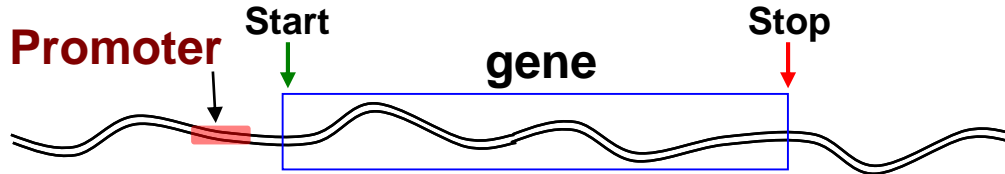
A piece of equipment with several moving parts that uses power to do a particular type of work.

- Cambridge dictionary

Biological machines:

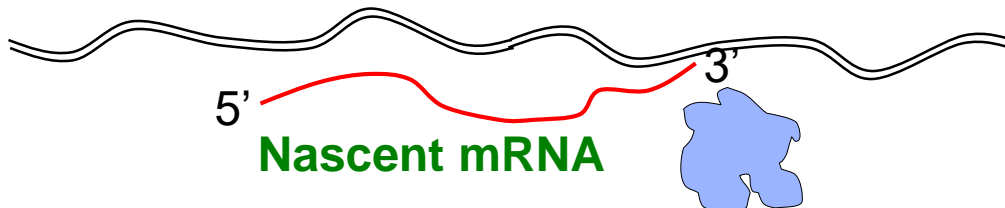
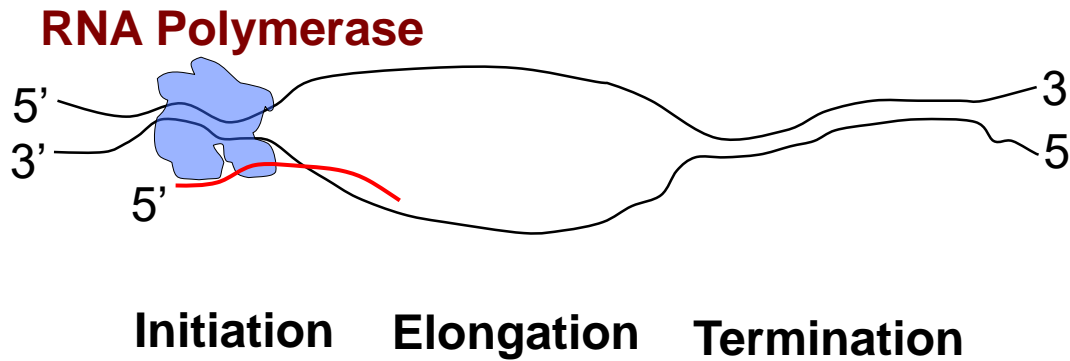
- DNA polymerase
- RNA polymerase
- Ribosome

Transcription: Involved Machineries and Processes



Key points to be discussed

1. Promoter
2. RNA Polymerase
3. RNA synthesis
4. Termination



Promoter for Transcription

Promoter is just like a “pointer” that points to the location of the information (gene) to be copied into mRNA

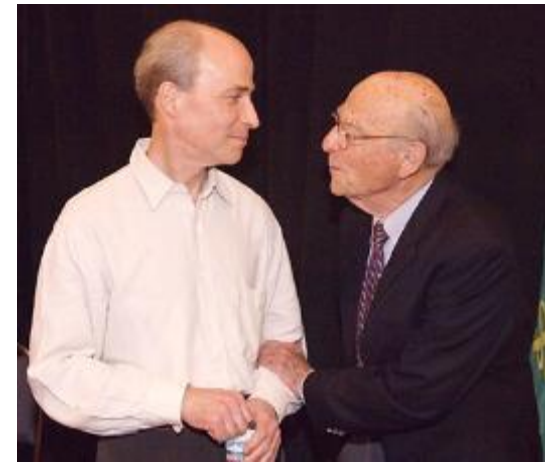
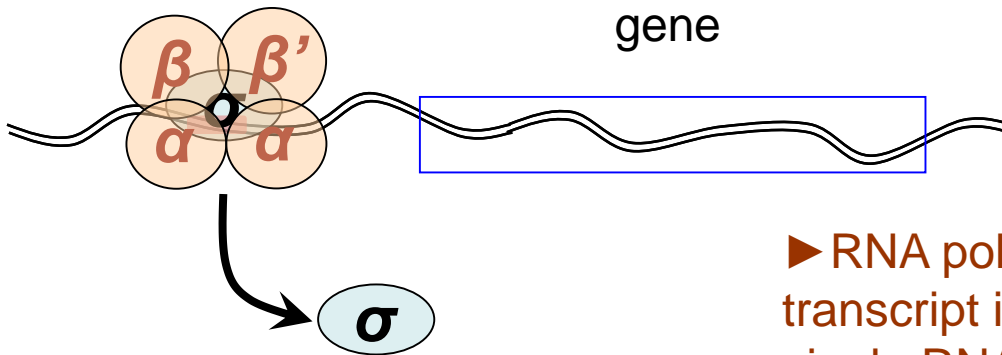


RNA Polymerase

Subunits of RNA Polymerase: α , α , β , β' and σ

Holo-enzyme: α , α , β , β' and σ

Core-enzyme: α , α , β and β'



Roger Kornberg
Nobel Prize in 2006

► RNA polymerase is completely **Processive**: A transcript is synthesized from start to end by a single RNA polymerase molecule.

► RNA polymerase can initiate the synthesis of RNA **de-novo** (No primer required)

RNA Molecules in *E. coli*

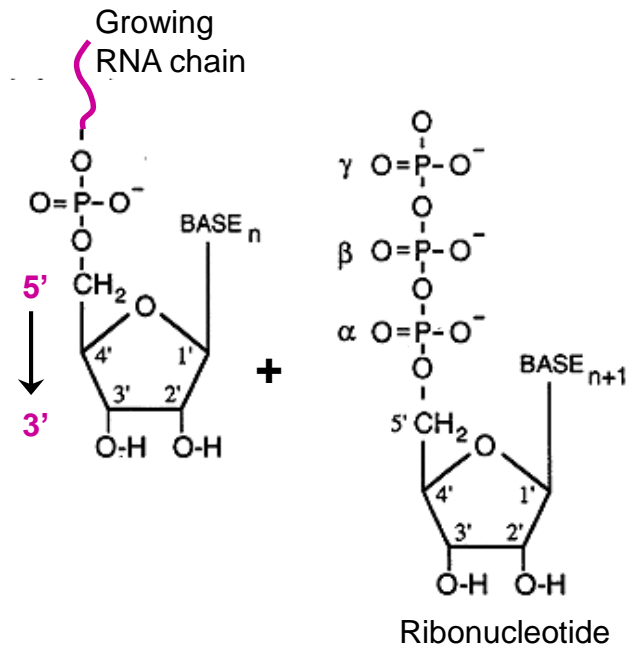
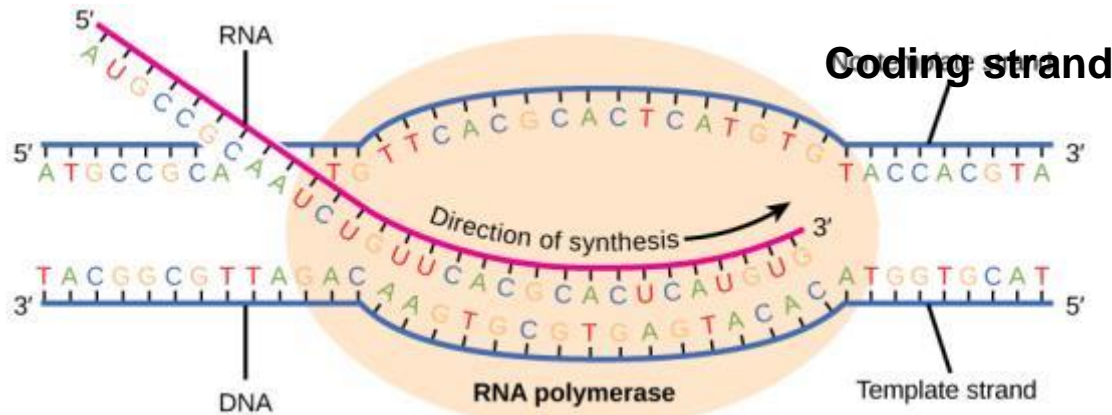
mRNA	5%
tRNA	15%
rRNA	80%

Who transcribes this huge pool of rRNA and tRNA?

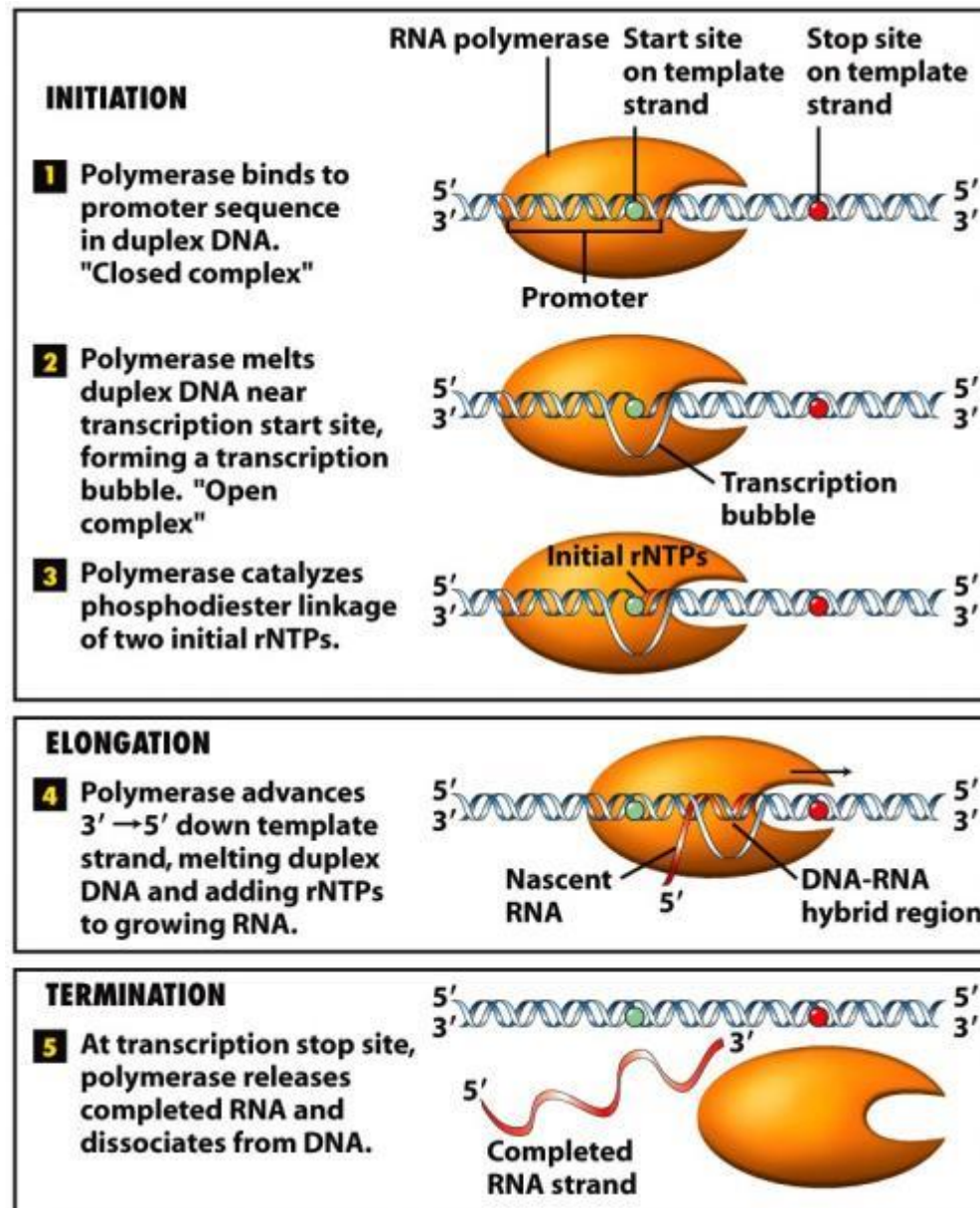
In bacteria same RNA polymerase transcribe all these three types of RNA

In eukaryotes different RNA polymerases are involved in transcription of mRNA, rRNA and tRNA

RNA Synthesis



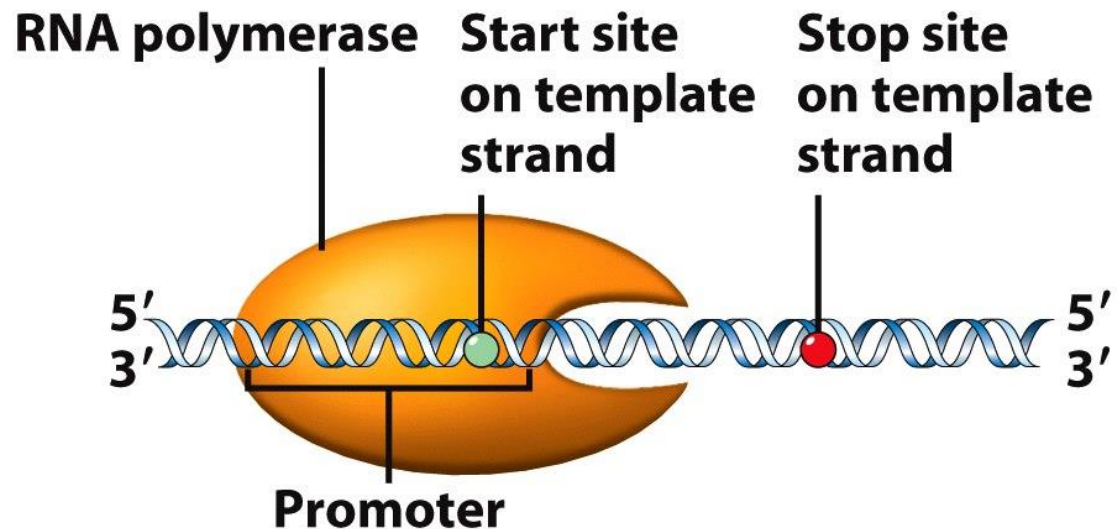
Schematic Representation of Multiple Steps Involved in Transcription



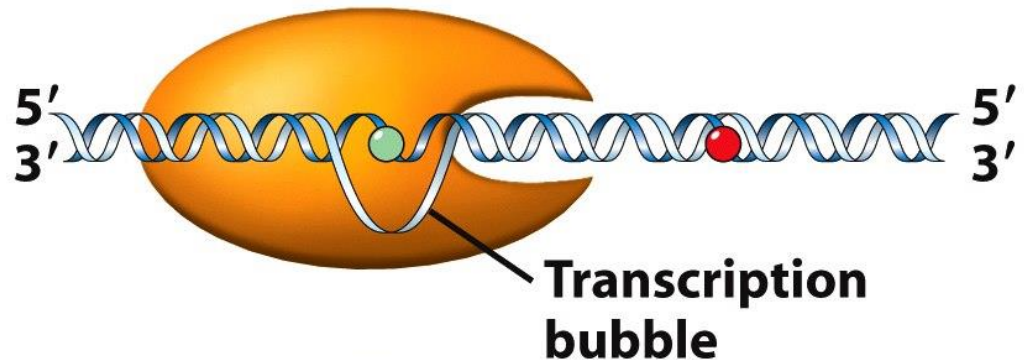
Schematic Representation of Multiple Steps Involved in Transcription

INITIATION

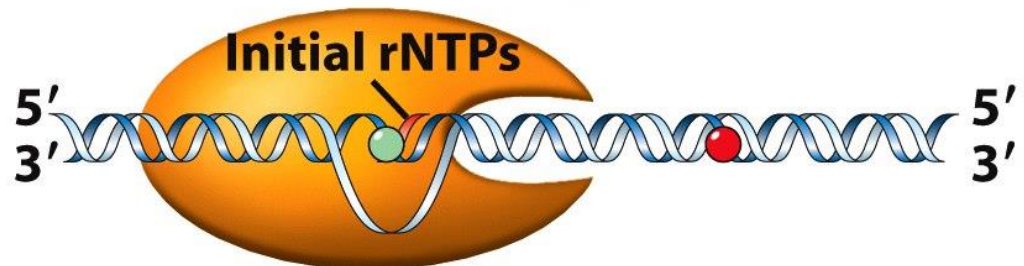
Polymerase binds to promoter sequence in duplex DNA.
"Closed complex"



Polymerase melts duplex DNA near transcription start site, forming a transcription bubble. "Open complex"



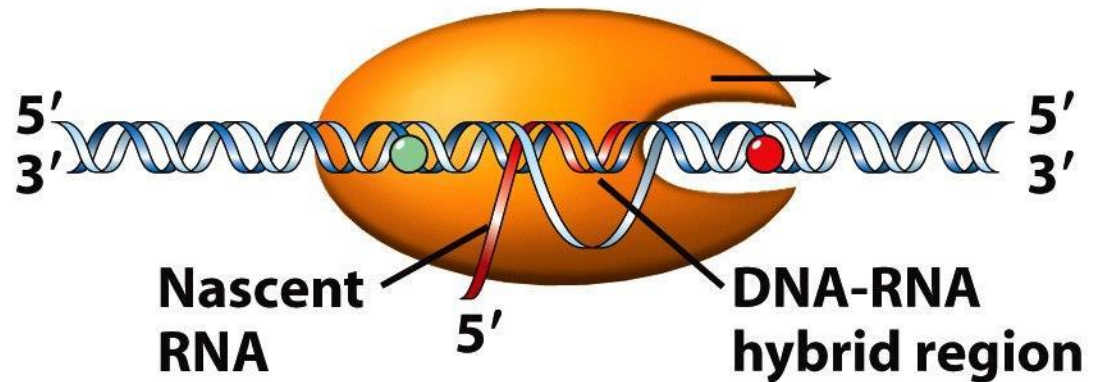
Polymerase catalyzes phosphodiester linkage of two initial rNTPs.



Schematic Representation of Multiple Steps Involved in Transcription

ELONGATION

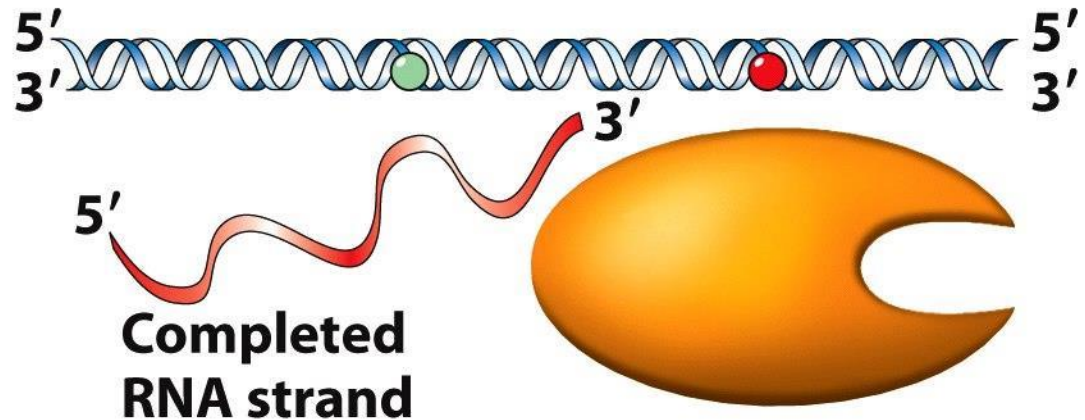
Polymerase advances 3' → 5' down template strand, melting duplex DNA and adding rNTPs to growing RNA.



Schematic Representation of Multiple Steps Involved in Transcription

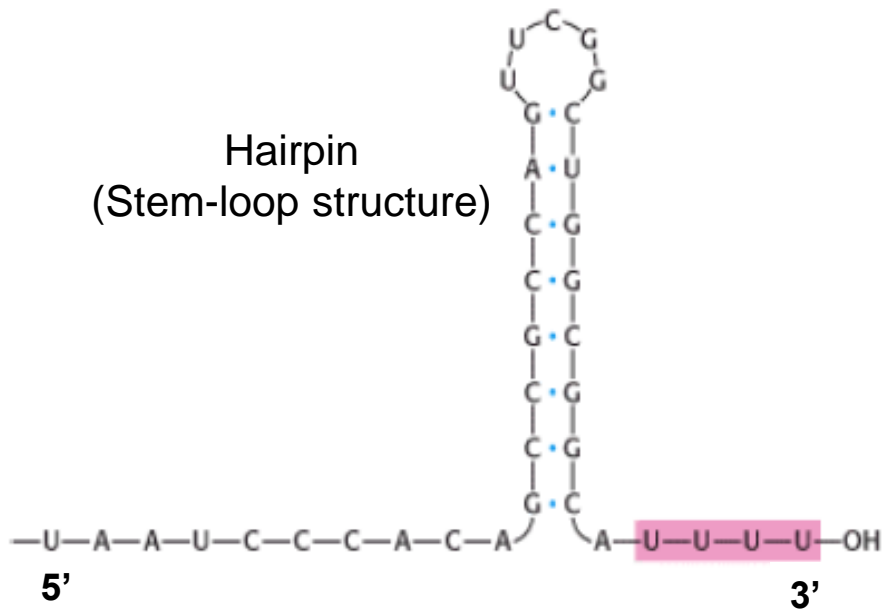
TERMINATION

At transcription stop site, polymerase releases completed RNA and dissociates from DNA.

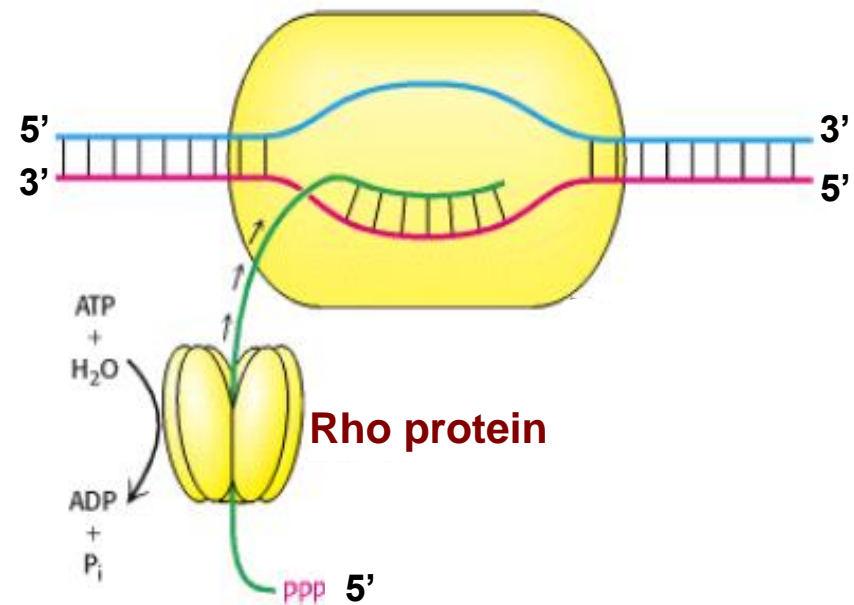


Termination of Transcription in Prokaryotes

An RNA hairpin followed by several uracil residues terminates transcription

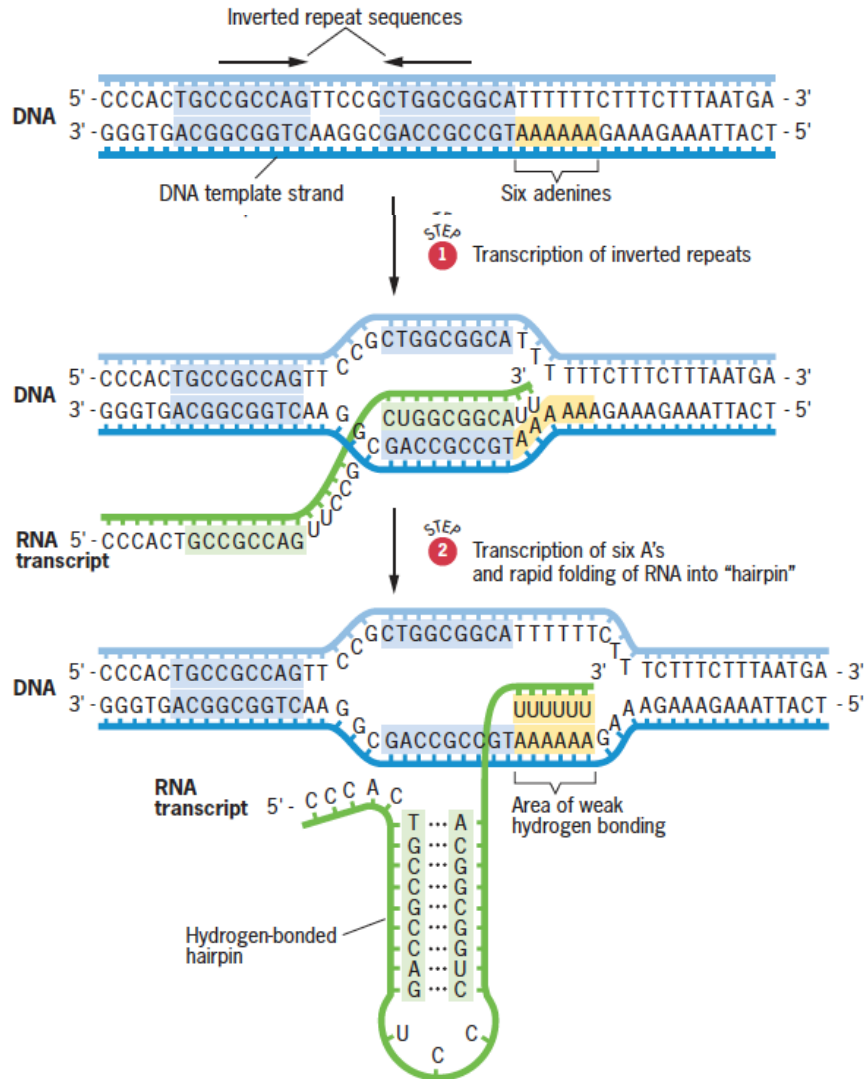


Rho binds the nascent RNA chain and pulls it away from RNA polymerase and the DNA template.



Termination of Transcription in Prokaryotes

Factor independent

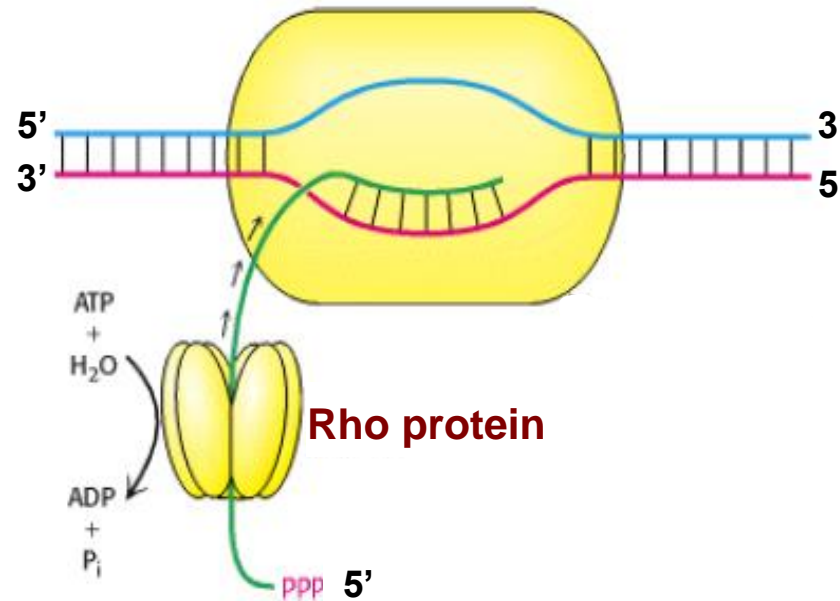


An RNA hairpin followed by several uracil residues terminates transcription

Termination of Transcription in Prokaryotes

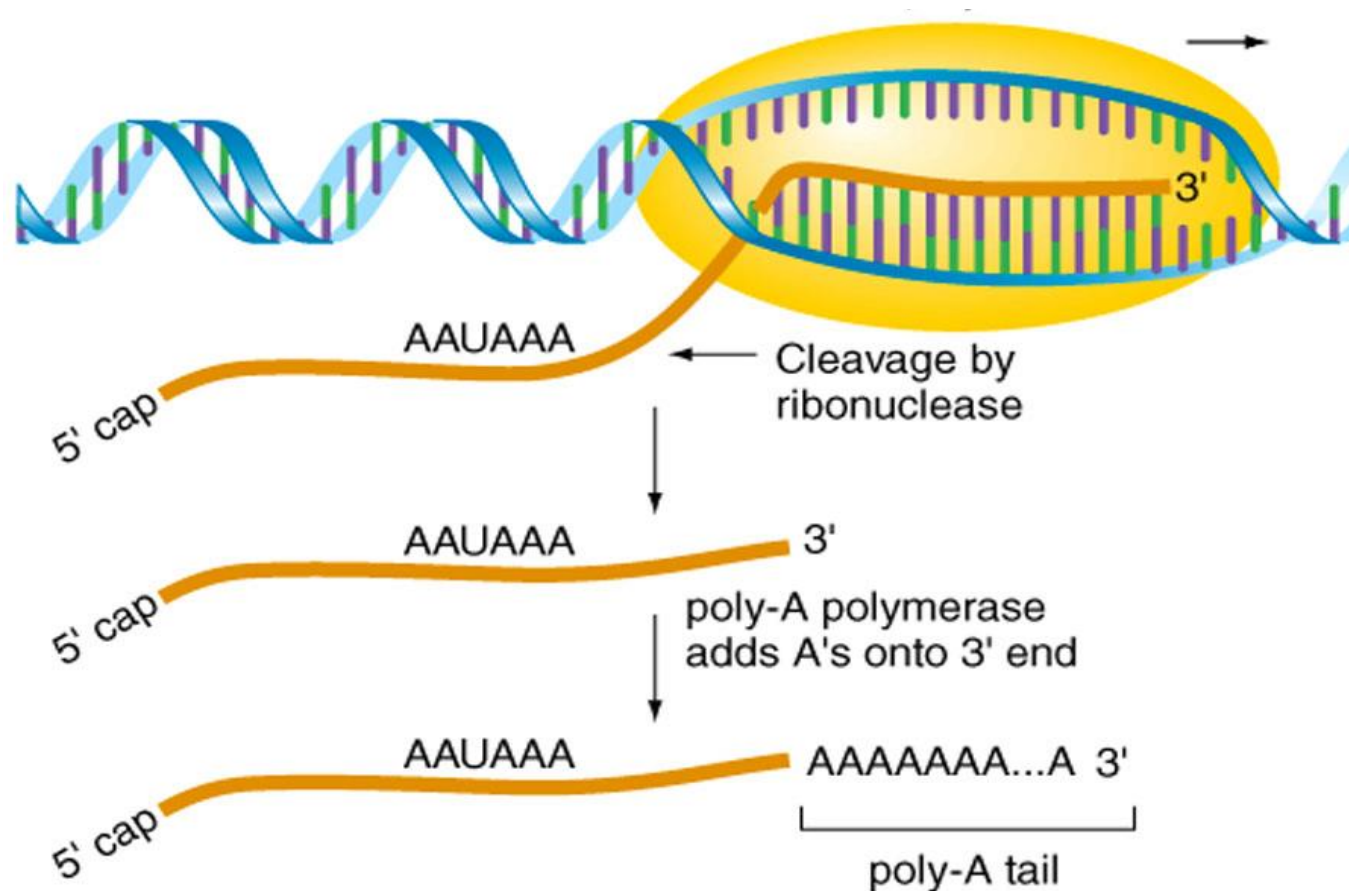
Factor dependent

Rho binds the nascent RNA chain and pulls it away from RNA polymerase and the DNA template.



Eukaryotic Transcripts Need to be Processed

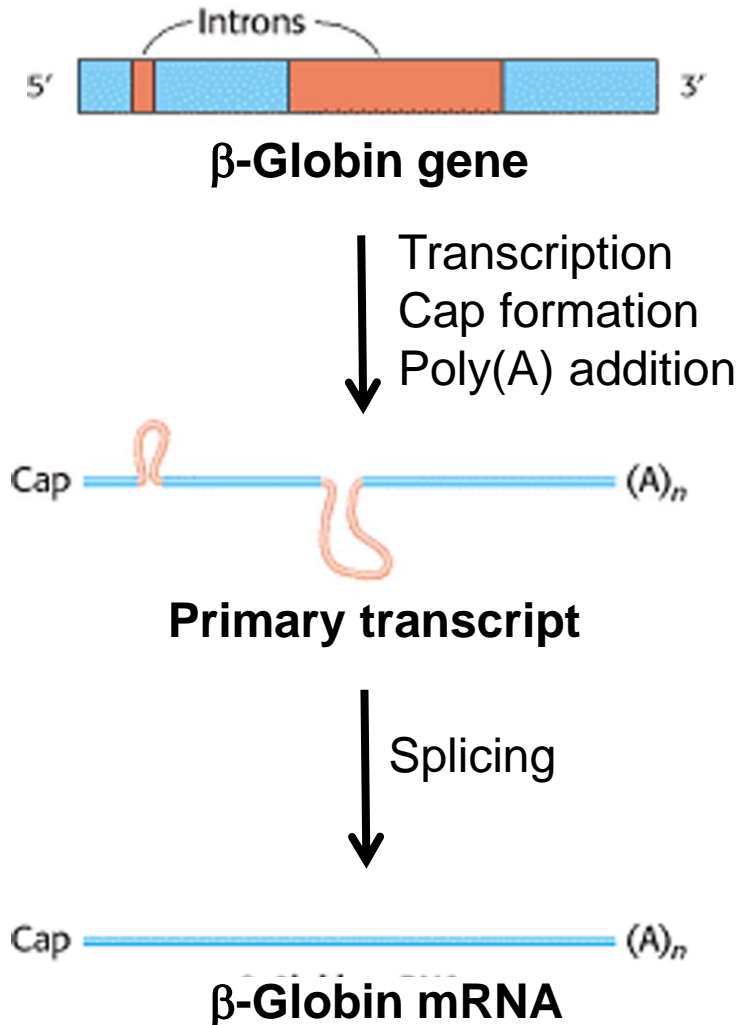
- Ends of a nascent mRNA acquire a 5' cap and a 3' poly A tail



- Increase stability of mRNA
- More effective template for translation

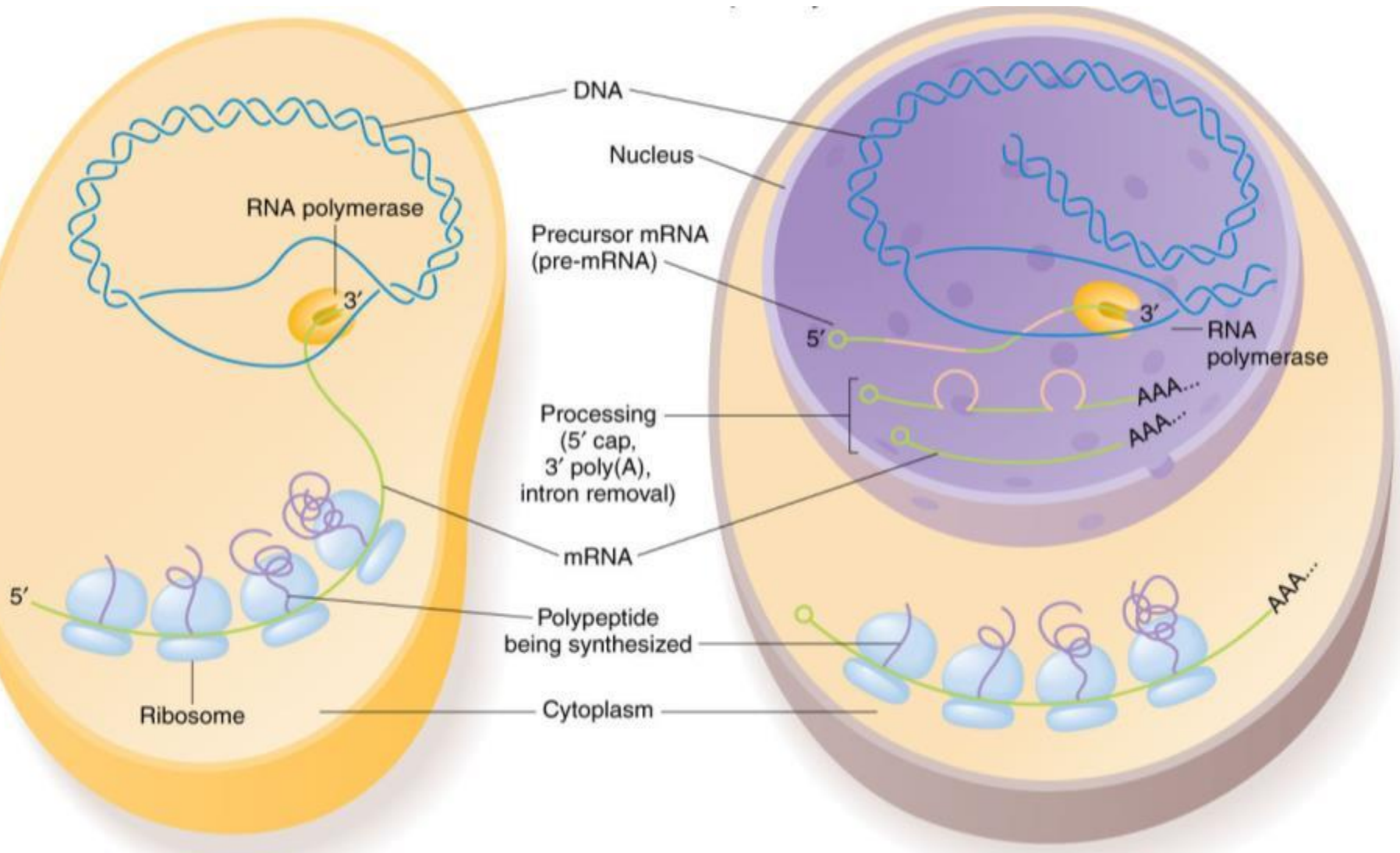
Eukaryotic Transcripts Need to be Processed

► **Splicing** (mediated by specialized enzymatic machineries consisting of snRNAs and proteins) removes introns from nascent mRNA



Thomas Cech
Nobel prize in 1989

Transcription: At a Glance

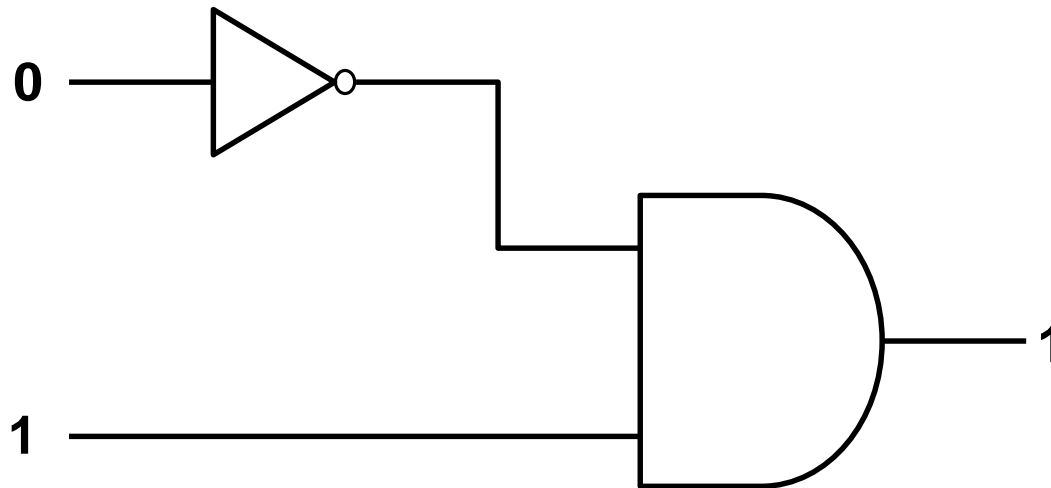


Bacteria

Eukaryote

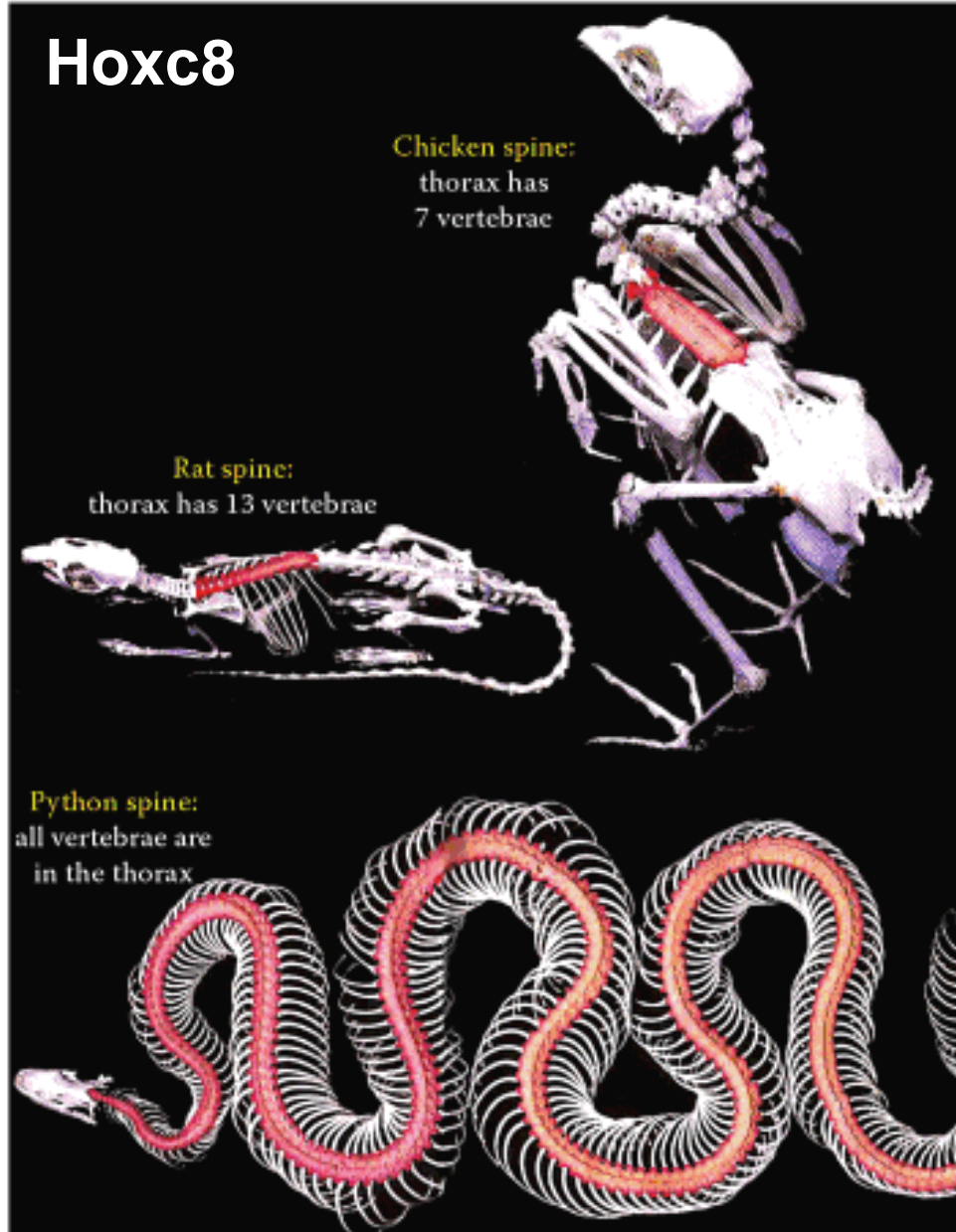
Regulation of Gene Expression

(Biological circuits)



DNA: Contains the Instruction for Life

Hoxc8



Regulation of Gene Expression

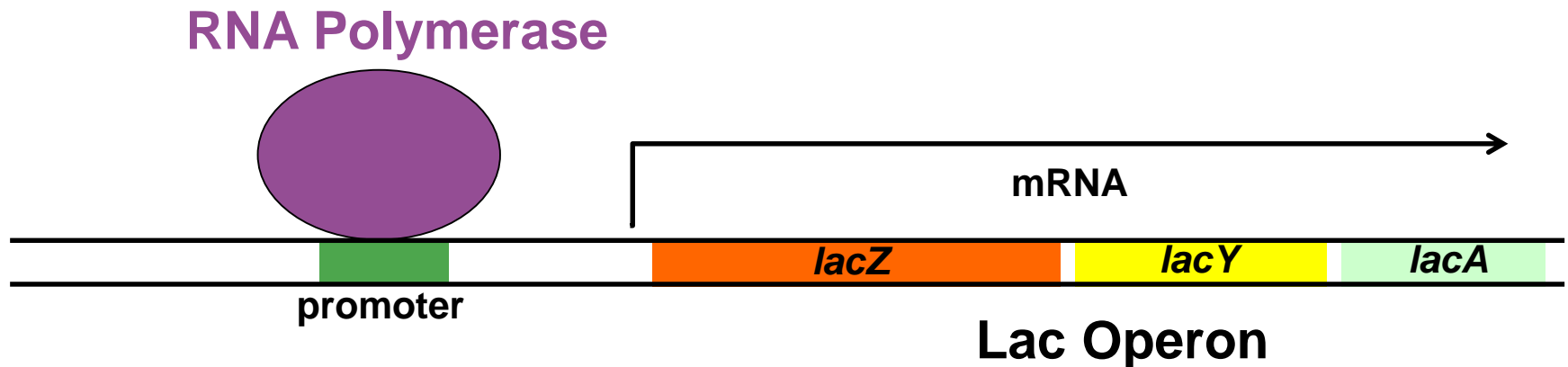
Each cell contains all the genetic material for growth and development

Some of these genes are expressed all the time

Other genes are not expressed all the time. They are switched on and off at need

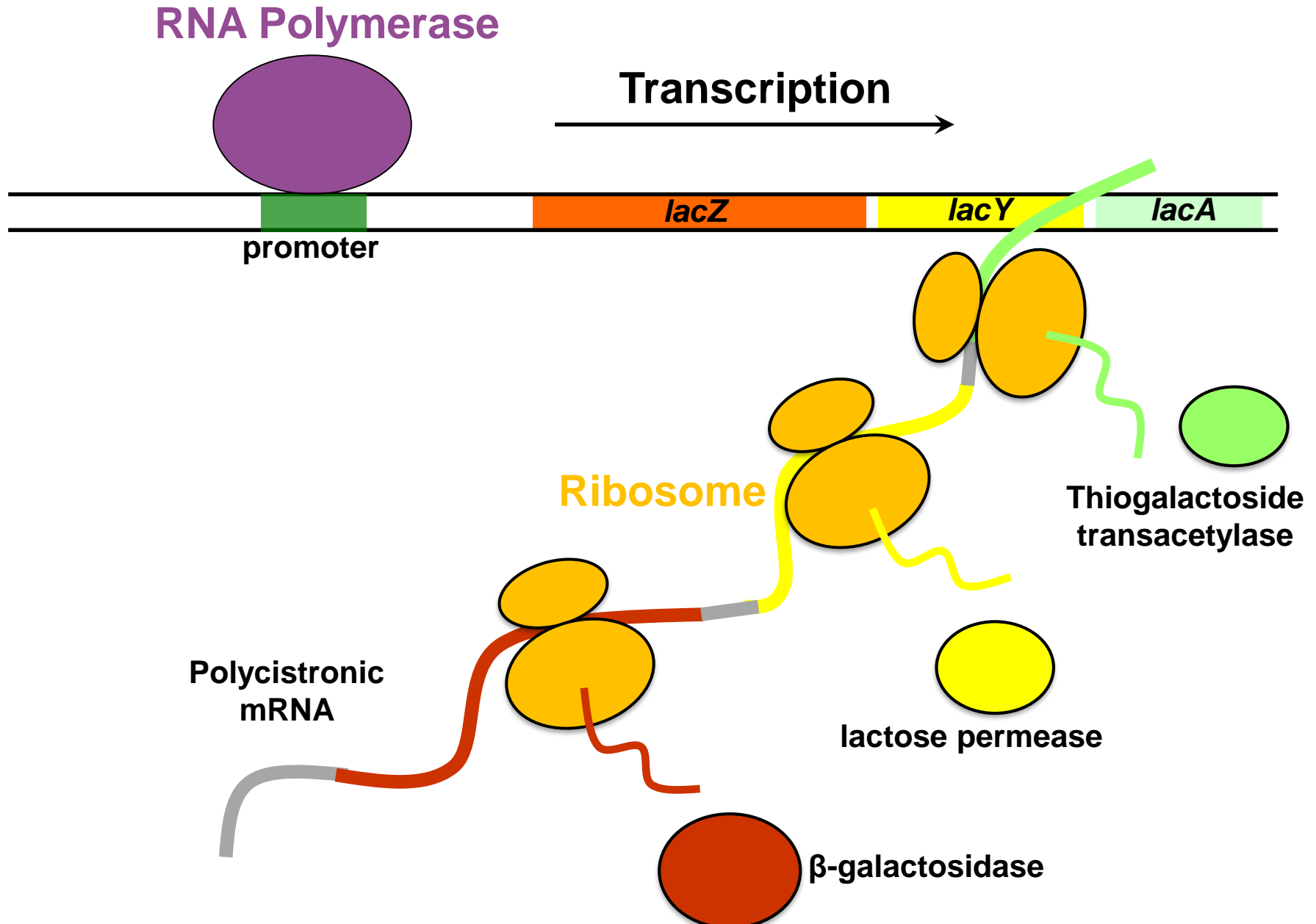
Lac Operon: A Classic Example of Bacterial Gene Expression Control

Operon: Cluster of genes, related by function, regulated by a single promoter and transcribed into one mRNA (polycistronic).

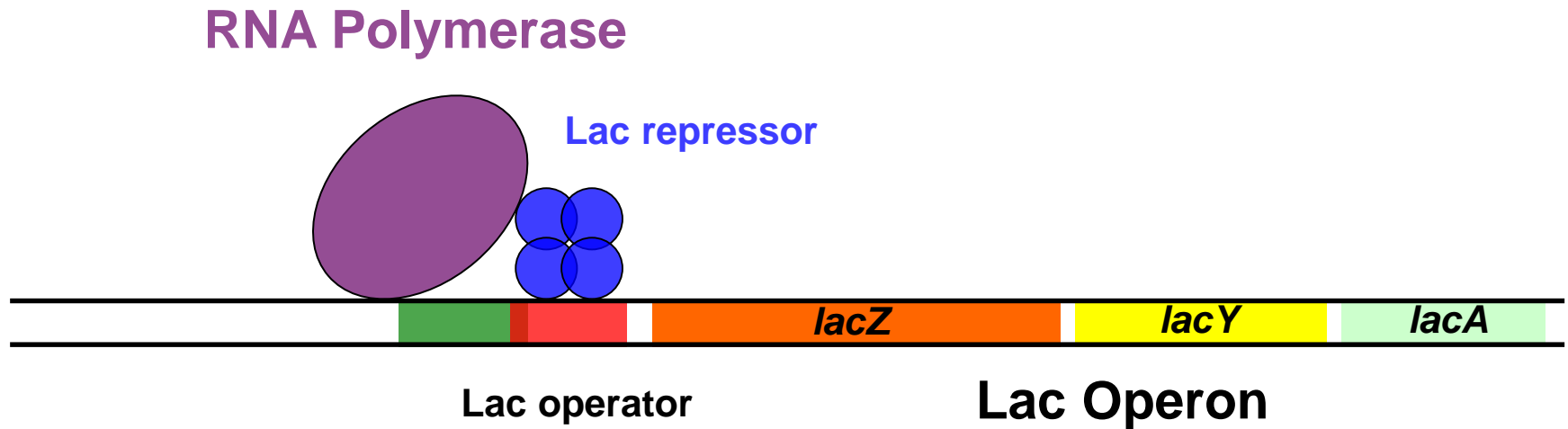


<i>lacZ</i>	β -galactosidase	Breaks lactose into galactose and glucose.
<i>lacY</i>	lactose permease	Imports lactose into the bacterial cell.
<i>lacA</i>	thiogalactoside transacetylase	Cell detoxification.

Functional Outcome of Lac Operon

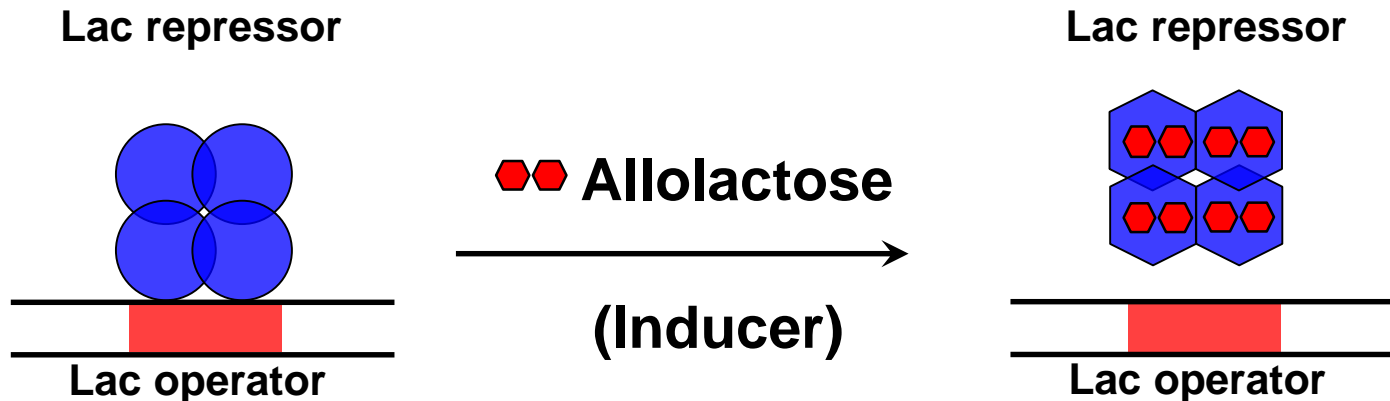
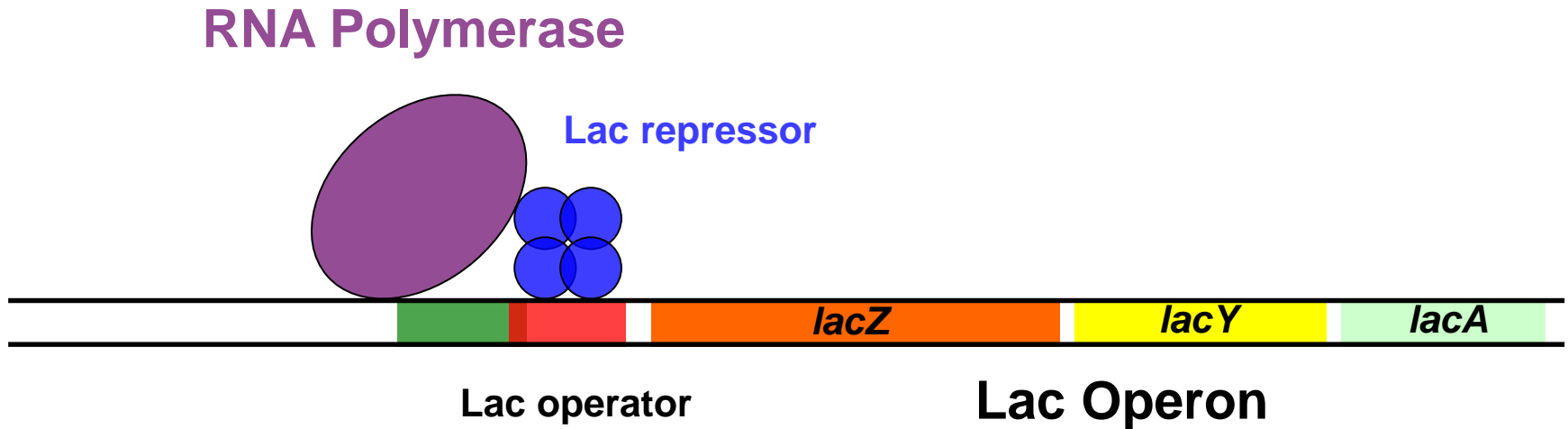


Lac repressor is a negative regulator of the Lac operon



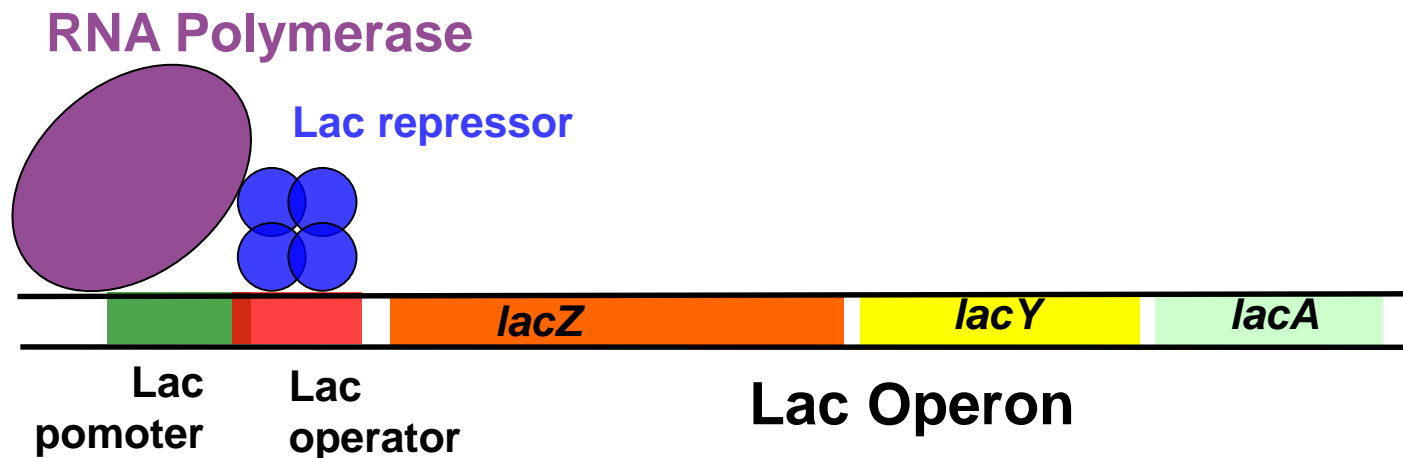
<i>lacZ</i>	β -galactosidase	Breaks lactose into galactose and glucose.
<i>lacY</i>	lactose permease	Imports lactose into the bacterial cell.
<i>lacA</i>	thiogalactoside transacetylase	Cell detoxification.

Lactose (Allolactose) Can Displace Lac Repressor From the Operator Site

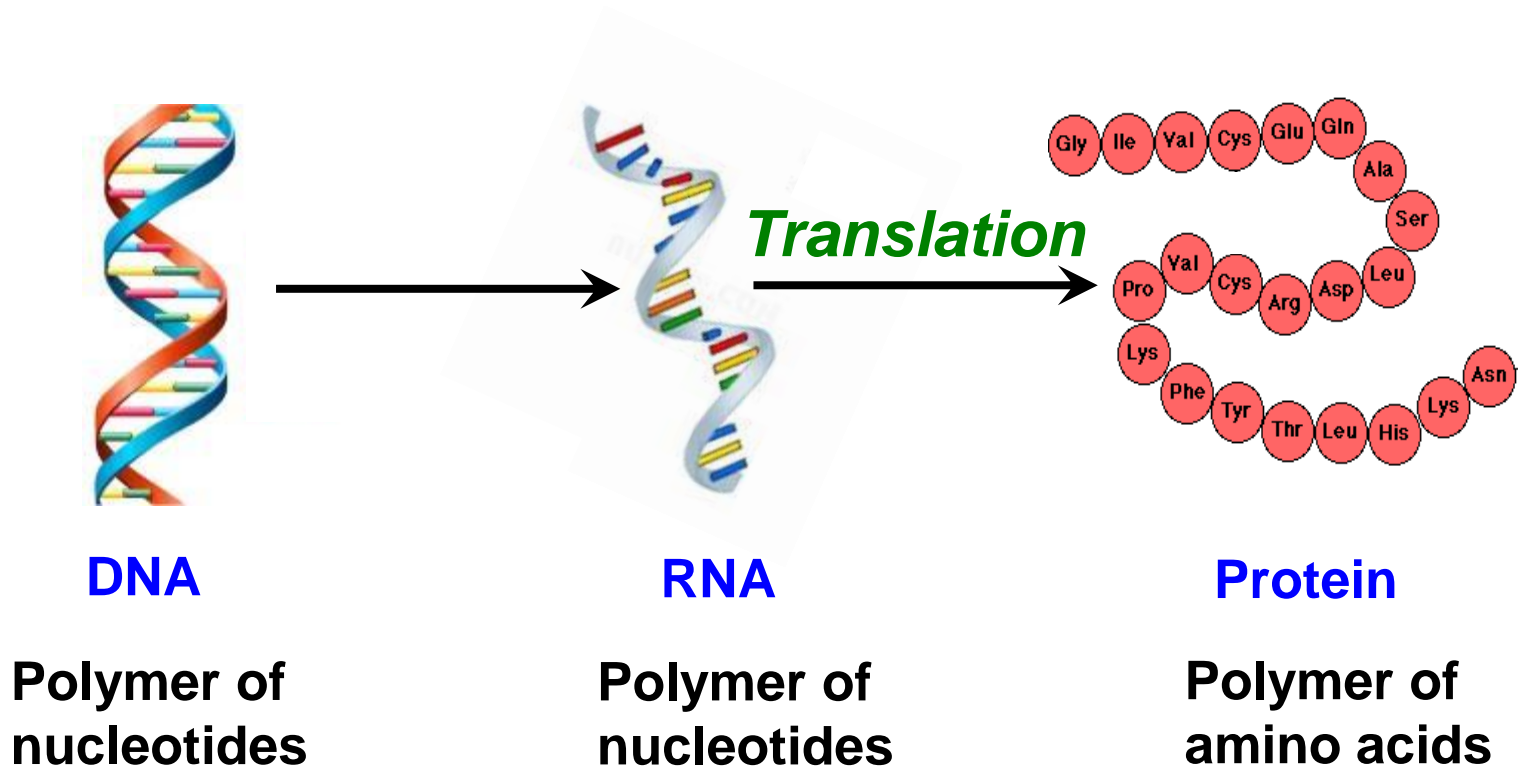


Four Possible Situations

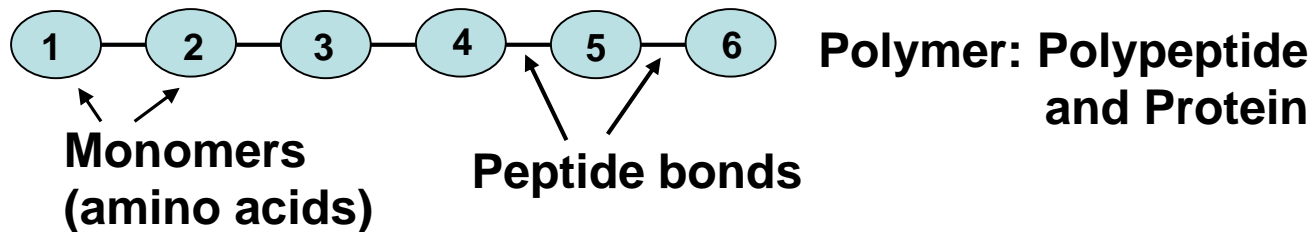
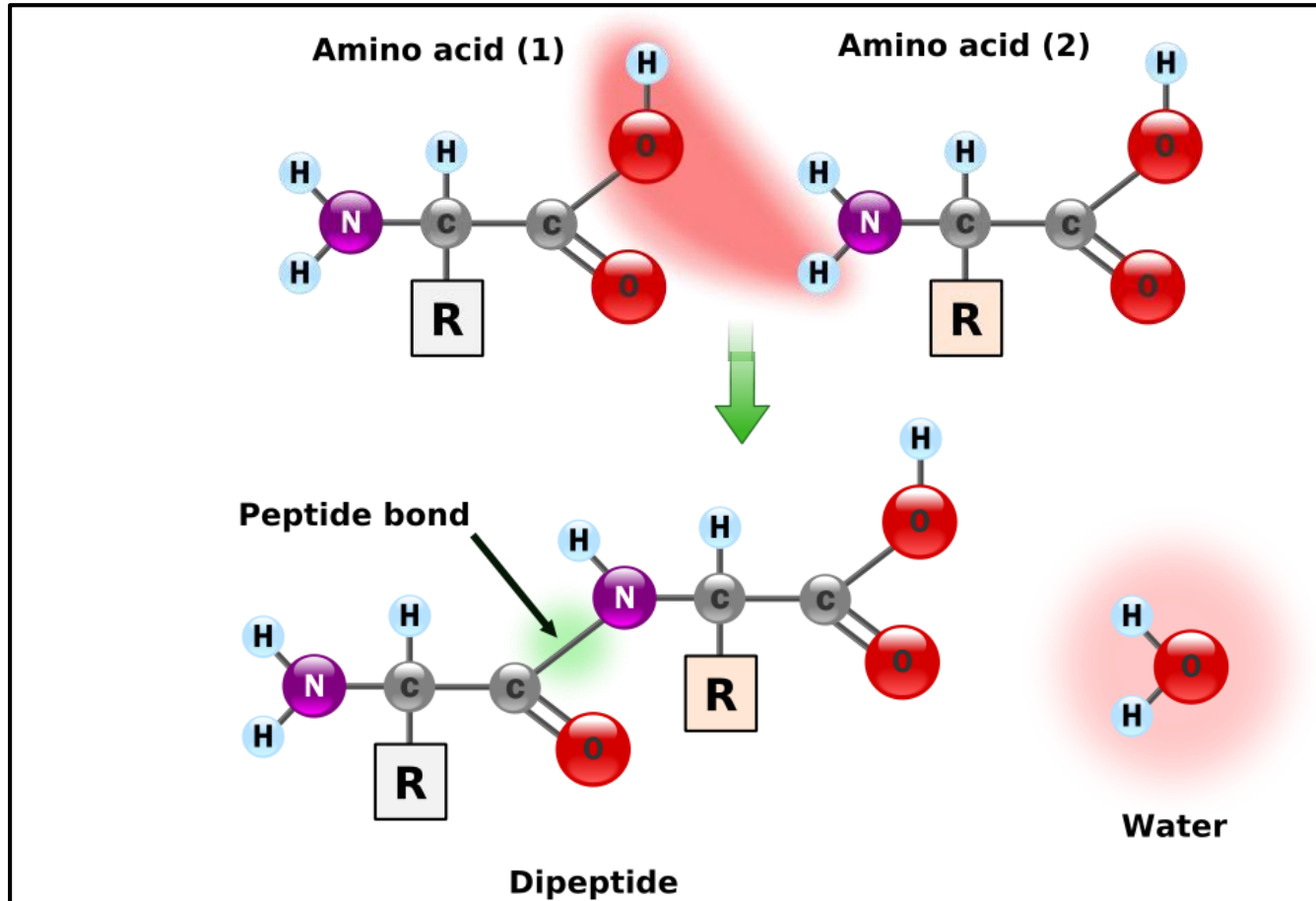
Glucose	Lactose	Lac repressor bound	Lac operon
+	-	YES	OFF (0)
+	+	YES	OFF (0)
-	+	NO	ON (1)
-	-	YES	OFF (0)



Translation



How Amino Acids are Linked Together



Genetic code

Genetic code is the relation between the sequence of bases in DNA (or its RNA transcripts) and the sequence of amino acids in proteins

A codon is a set of 3 nucleotides that specifies a particular amino acid

Why three nucleotides?

64 Codons present. Three of them (UAA, UAG, UGA) can't code any amino acids, called STOP codons

AUG serves as the “initiator” or “start codon, which starts the synthesis of a protein

We have 61 codons that code for amino acids, and we have 20 amino acids. So, more than one codon may specify one particular amino acid



Khorana, Nirenberg, Holley
Nobel Prize in 1968

Genetic code

		Second Letter					
		U	C	A	G		
1st letter	U	UUU Phe UUC UUA Leu UUG	UCU UCC Ser UCA UCG	UAU Tyr UAC UAA Stop UAG Stop	UGU Cys UGC UGA Stop UGG Trp	U C A G	3rd letter
	C	CUU CUC Leu CUA CUG	CCU CCC Pro CCA CCG	CAU His CAC CAA Gln CAG	CGU CGC Arg CGA CGG	U C A G	
	A	AUU AUC Ile AUA AUG Met	ACU ACC Thr ACA ACG	AAU Asn AAC AAA Lys AAG	AGU Ser AGC AGA Arg AGG	U C A G	
	G	GUU GUC Val GUA GUG	GCU GCC Ala GCA GCG	GAU Asp GAC GAA Glu GAG	GGU GGC Gly GGA GGG	U C A G	

Don't have to memorize!



Translation

Venki Ramakrishnan, Thomas A. Steitz & Ada Yonath,
Nobel Prize 2009

Template for protein synthesis

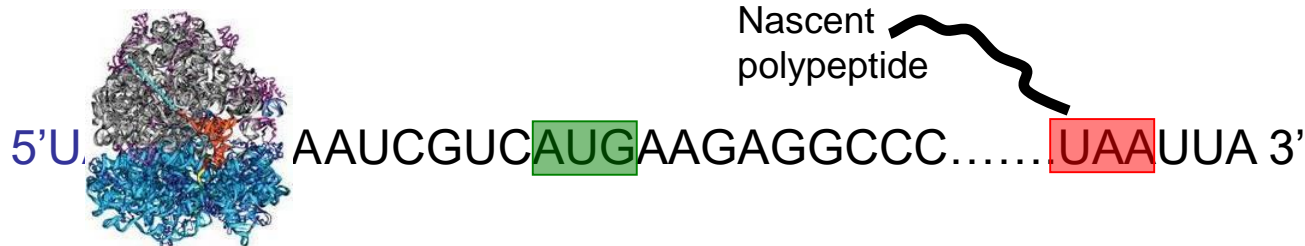
5' ————— 3' mRNA

5'UAAGGAGAAUCGUCAUGAAGAGGCC...UAAUUA 3'
(RBS)

Start
codon

Stop
codon

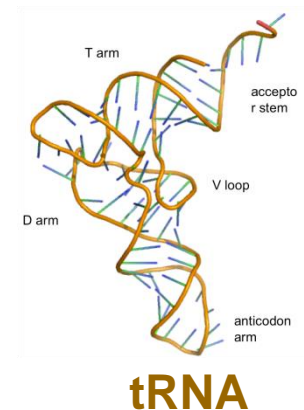
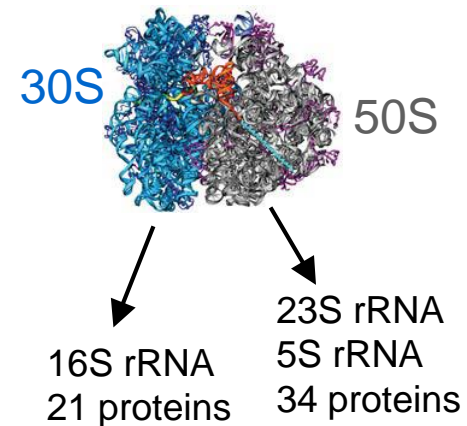
Met—Lys—Arg—Pro.....
Polypeptide



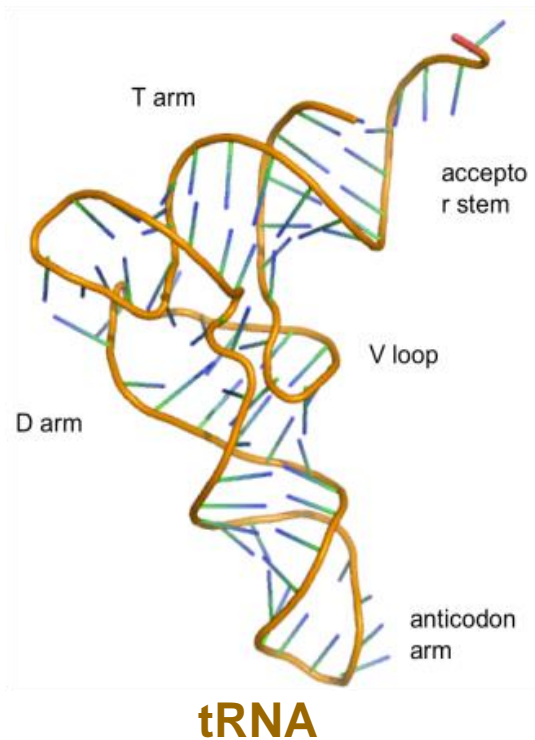
► In Eukaryotes, 5' 7mG cap is recognized by ribosome

Protein synthesizing machinery

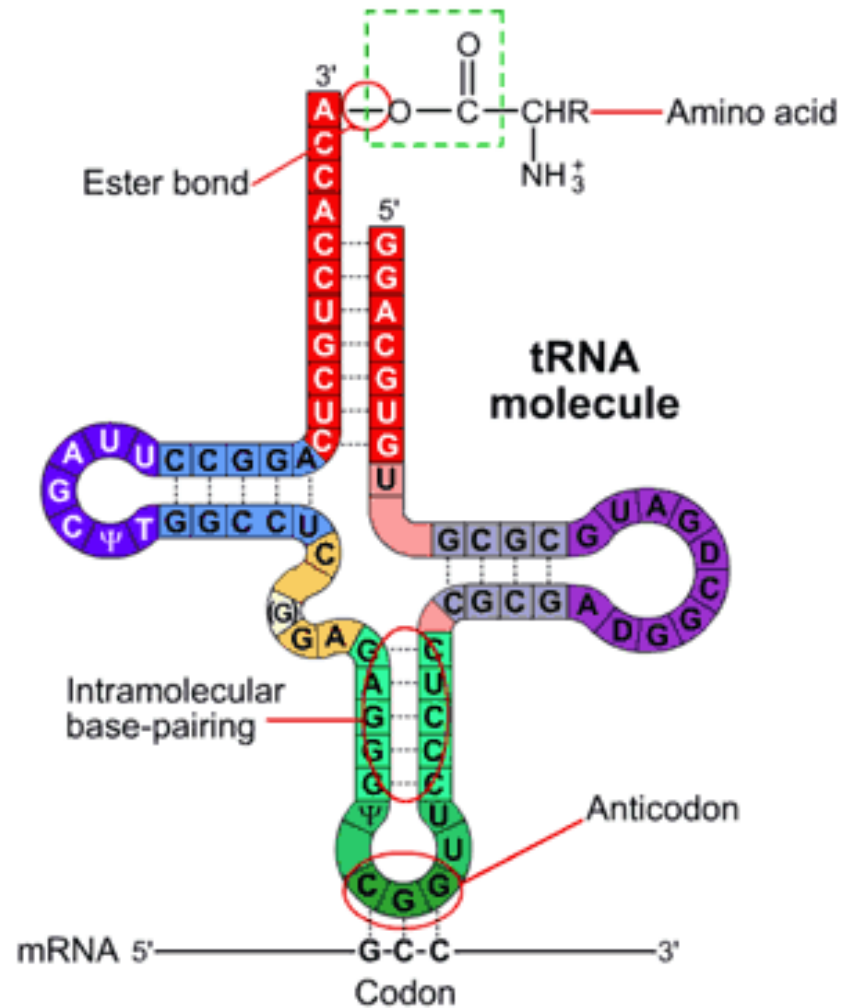
Ribosome: (rRNA + proteins)



Transfer RNA: the adapter molecule

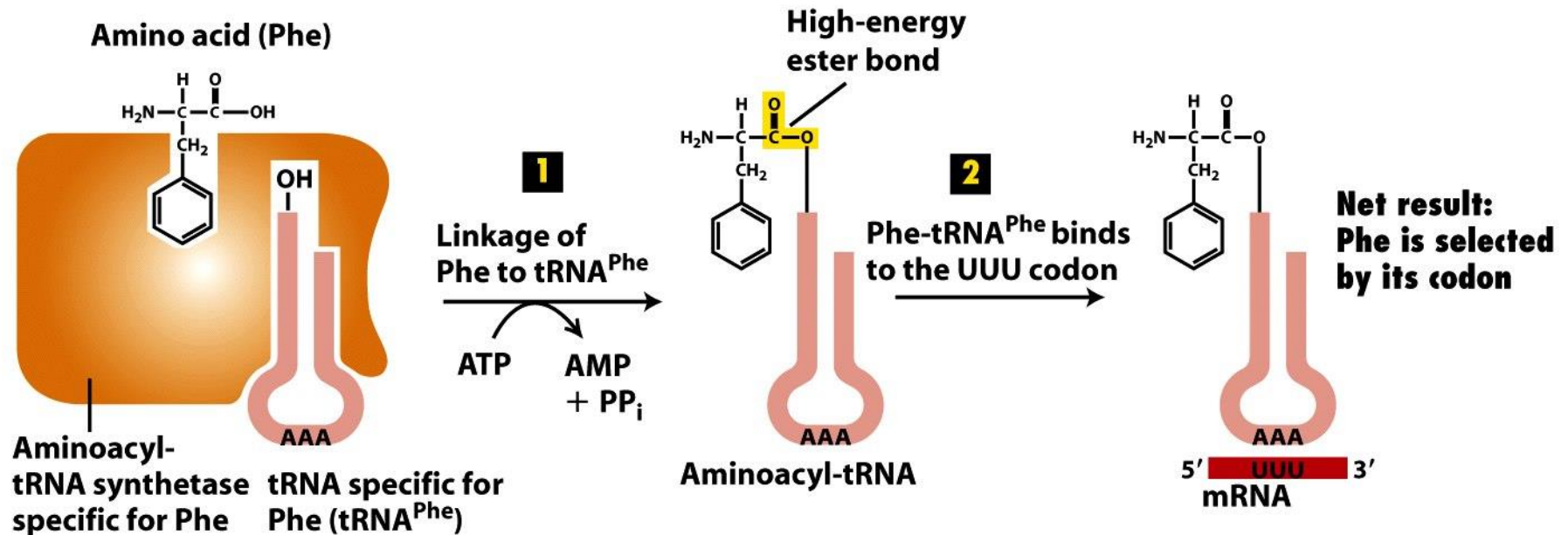


3D structure

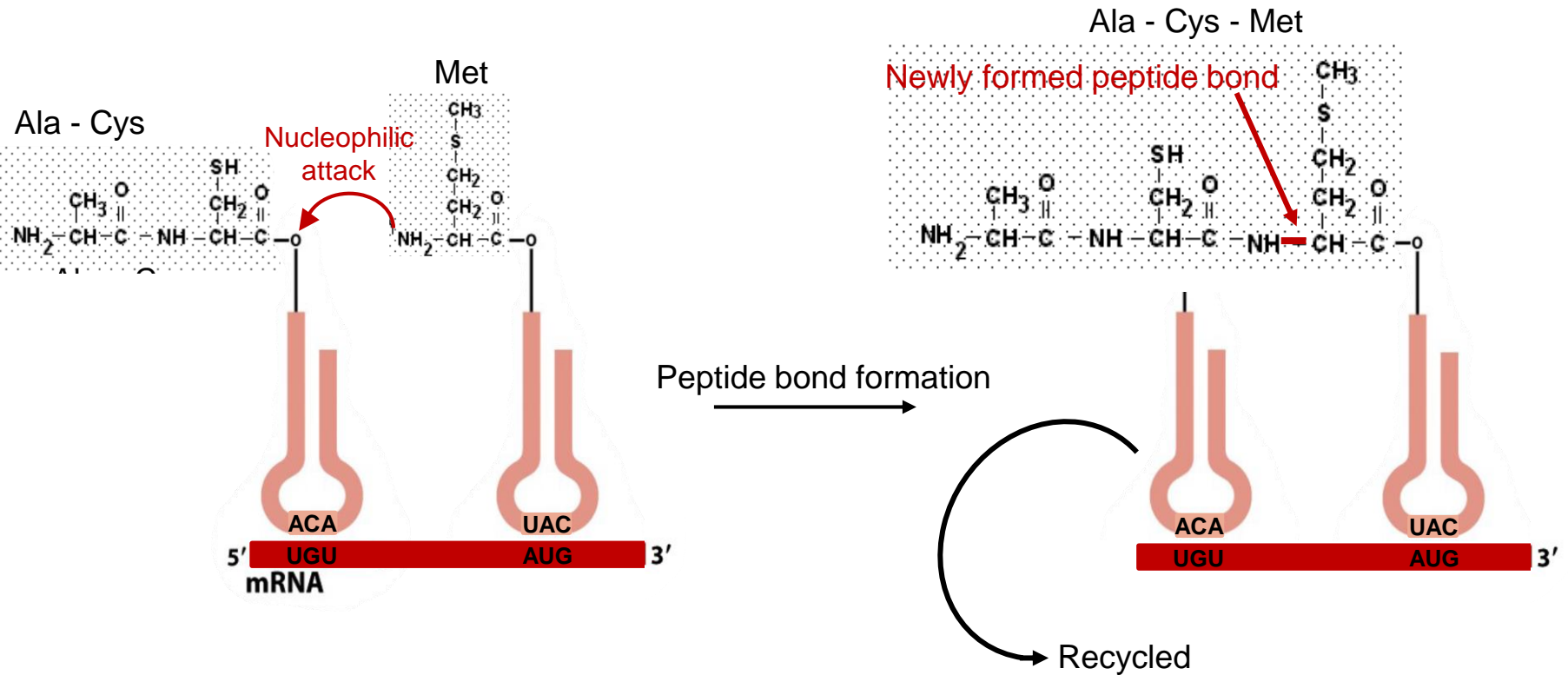


2D structure

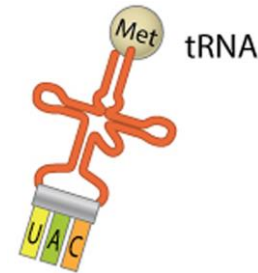
How Correct Amino Acids are Selected During Protein Synthesis



Peptidyl transferase reaction



Translation: Involved Machineries and Processes

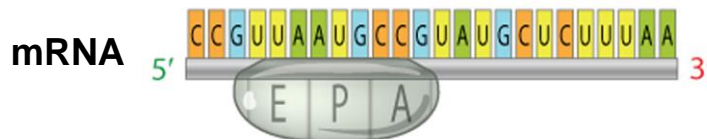


mRNA

Ribosome

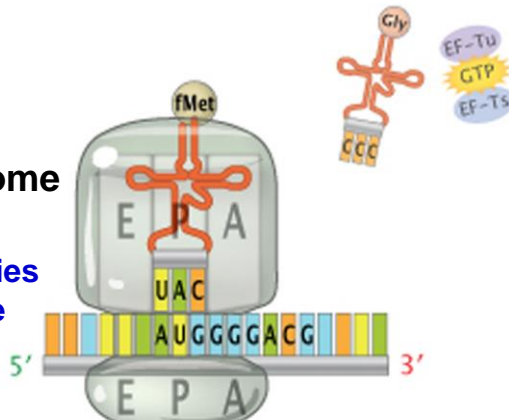
Amino acid

tRNA

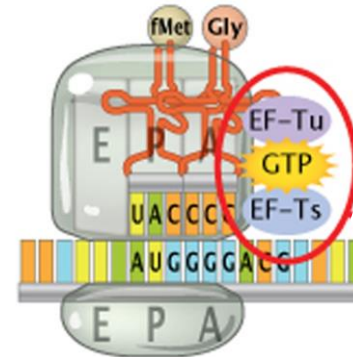


50S ribosome

tRNA^{fMet} occupies the P site of the ribosome

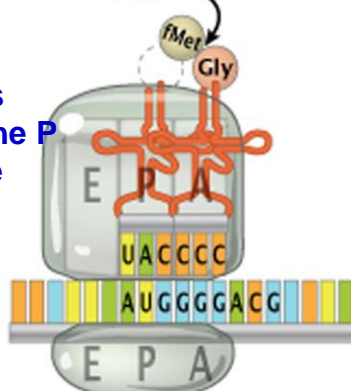


Next charged tRNA and associated translation factors enter the A site

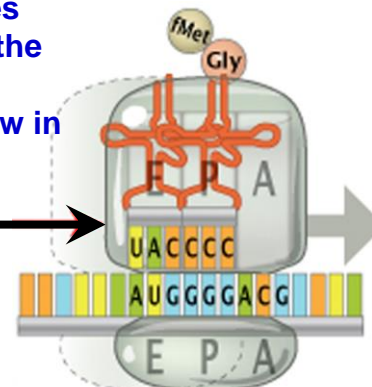


Peptide bond

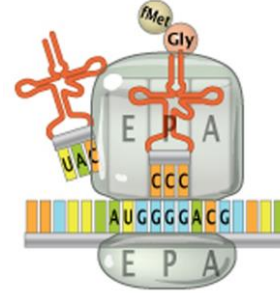
Peptide bond forms between the aa in the P and A sites, and the tRNA in the P site releases its aa



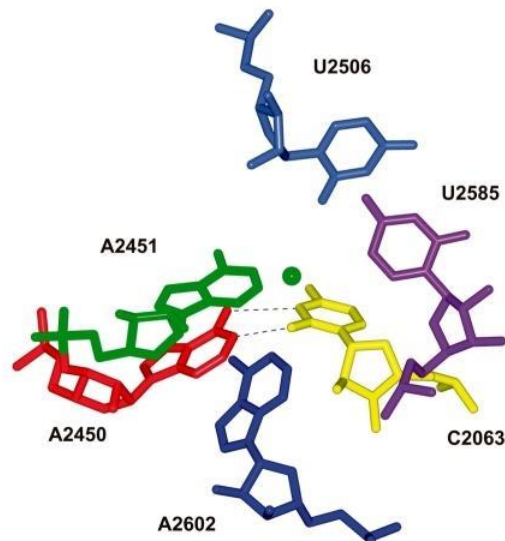
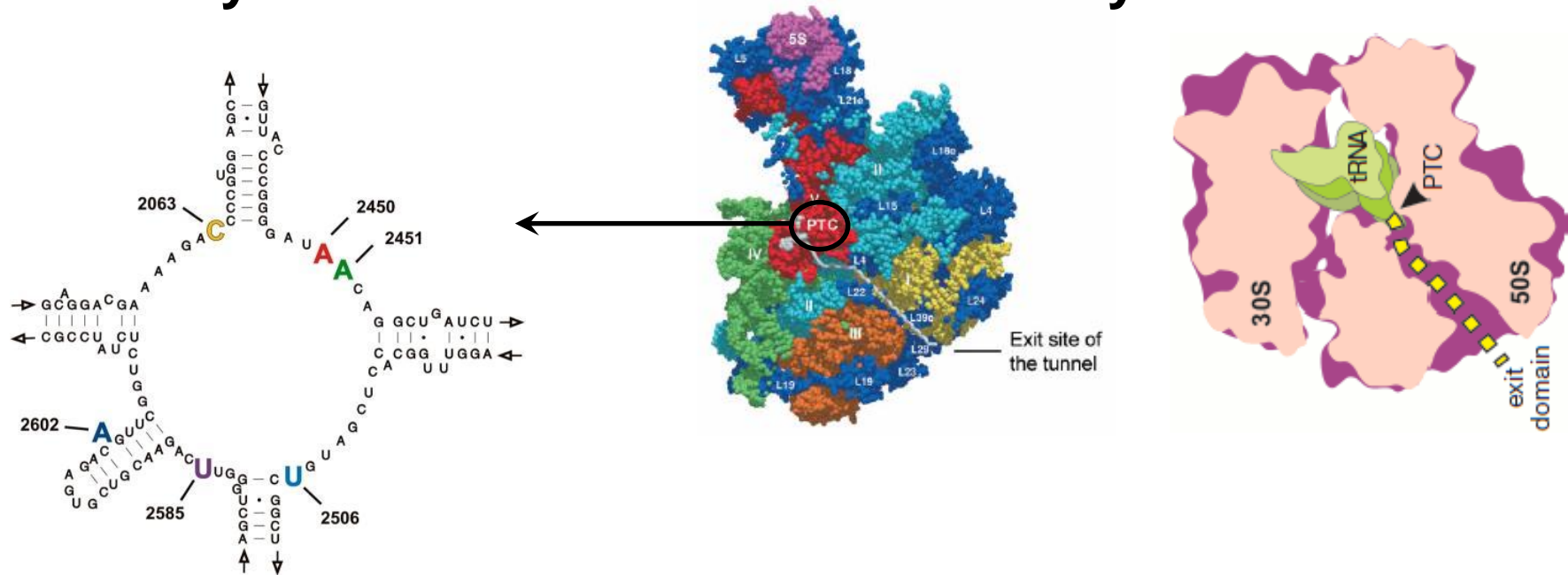
The ribosome moves down the mRNA to the next codon and the uncharged tRNA now in the E site



E site tRNA releases and A site is now ready to receive another tRNA

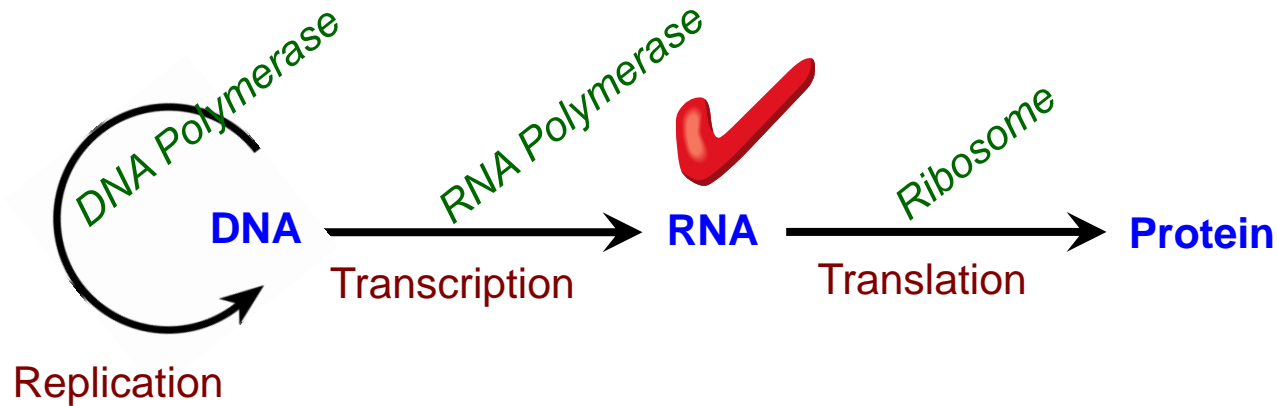


What Happens Inside the Ribosome? Chemical and Physical Consideration of Protein Synthesis



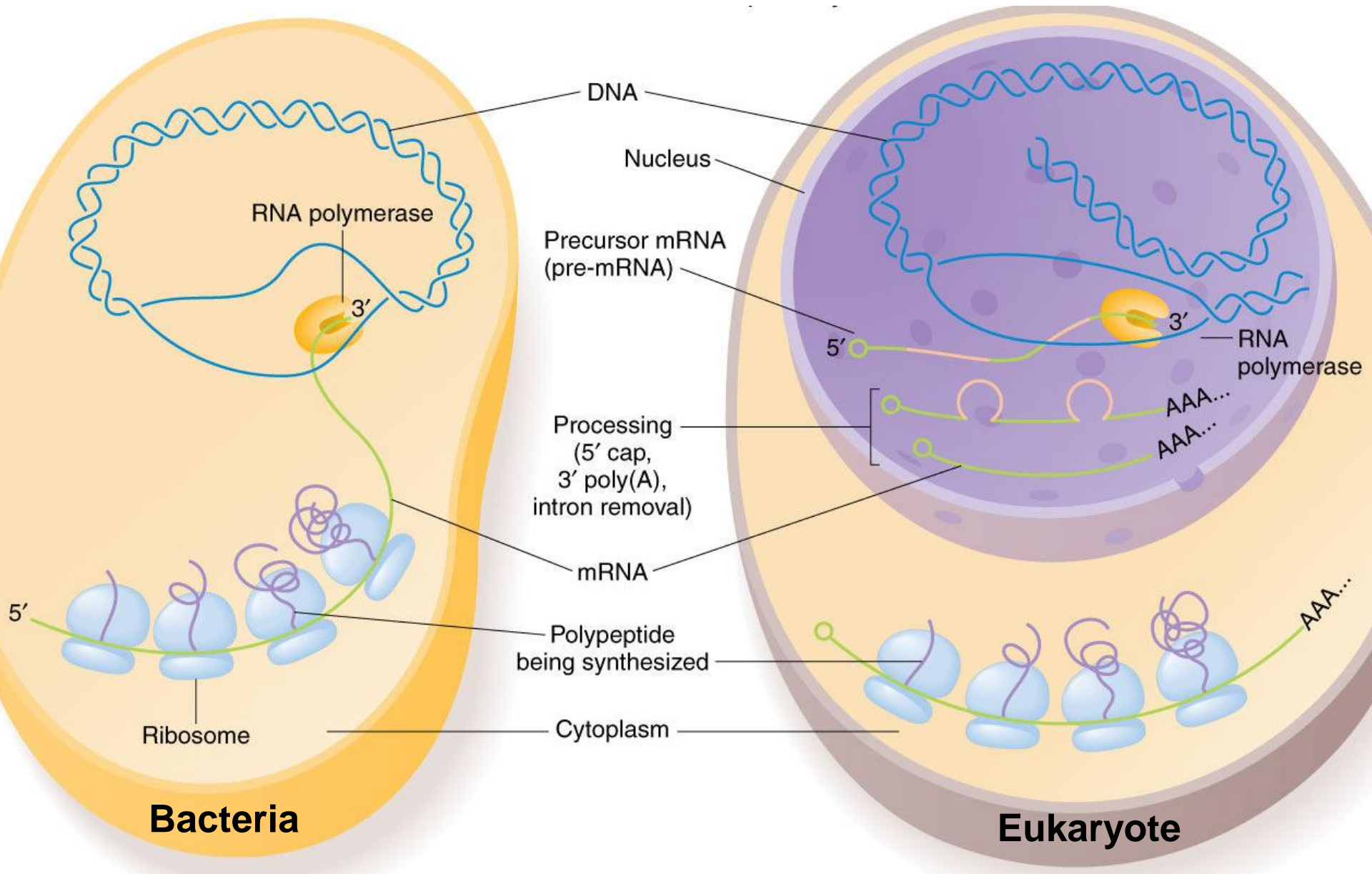
Peptidyl transferase center

Which Came First? **Nucleic acids or Proteins**

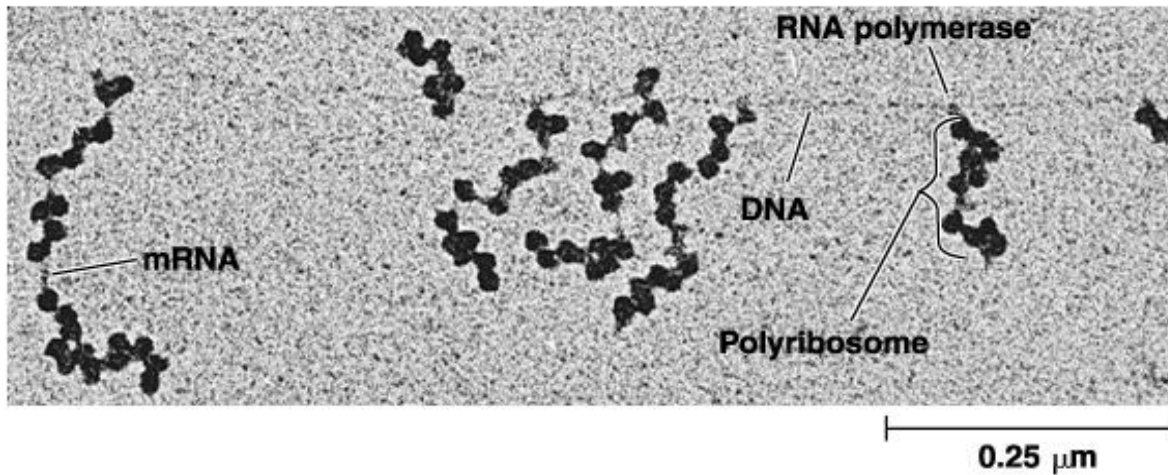


► **RNA has enzymatic activity**

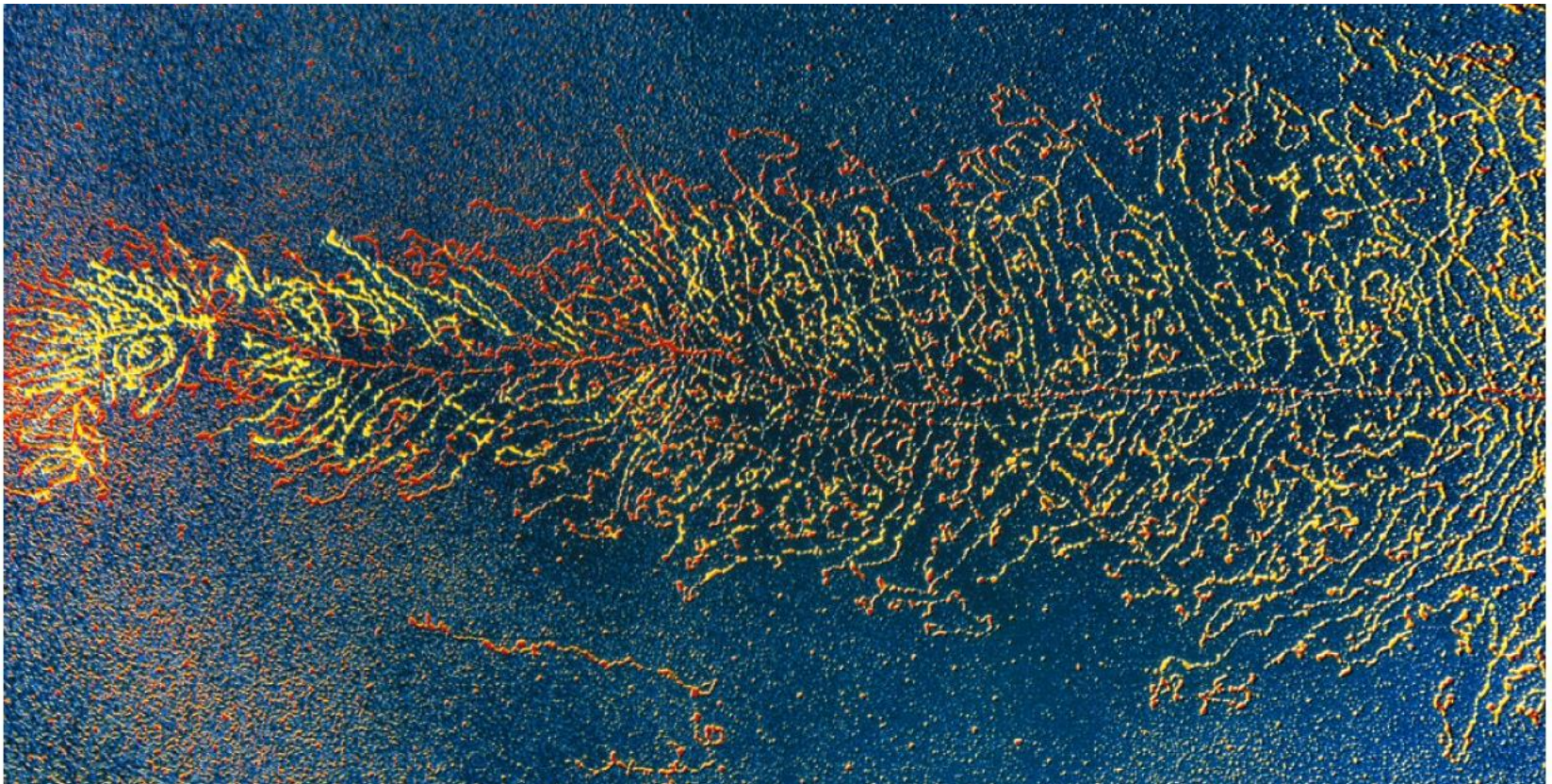
Transcription & Translation is coupled for prokaryotes



Time, Space and Correlation between Transcription and Translation



Overview of Transcription and Translation



Translation Machineries: Attractive Targets For Therapeutics

Tetracycline

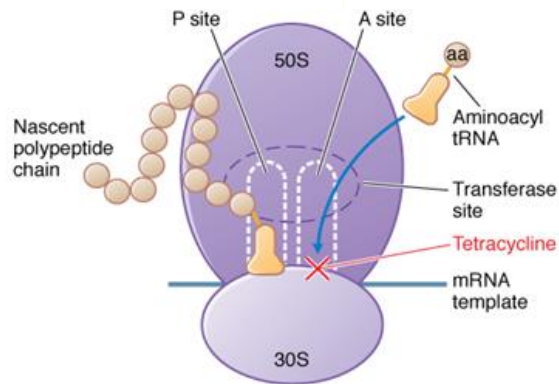
250 mg



500 mg

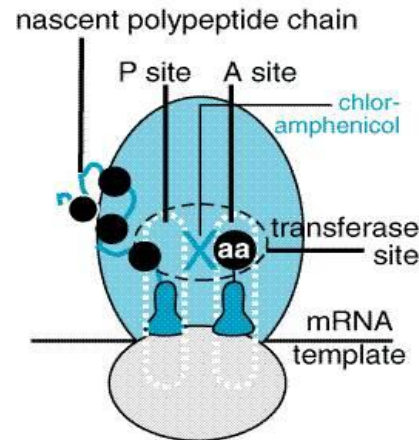


Binds to the 30S ribosome, and blocks binding of aminoacyl-tRNA to the A-site



Chloramphenicol

Blocks the peptidyl transferase reaction on 50S ribosomes



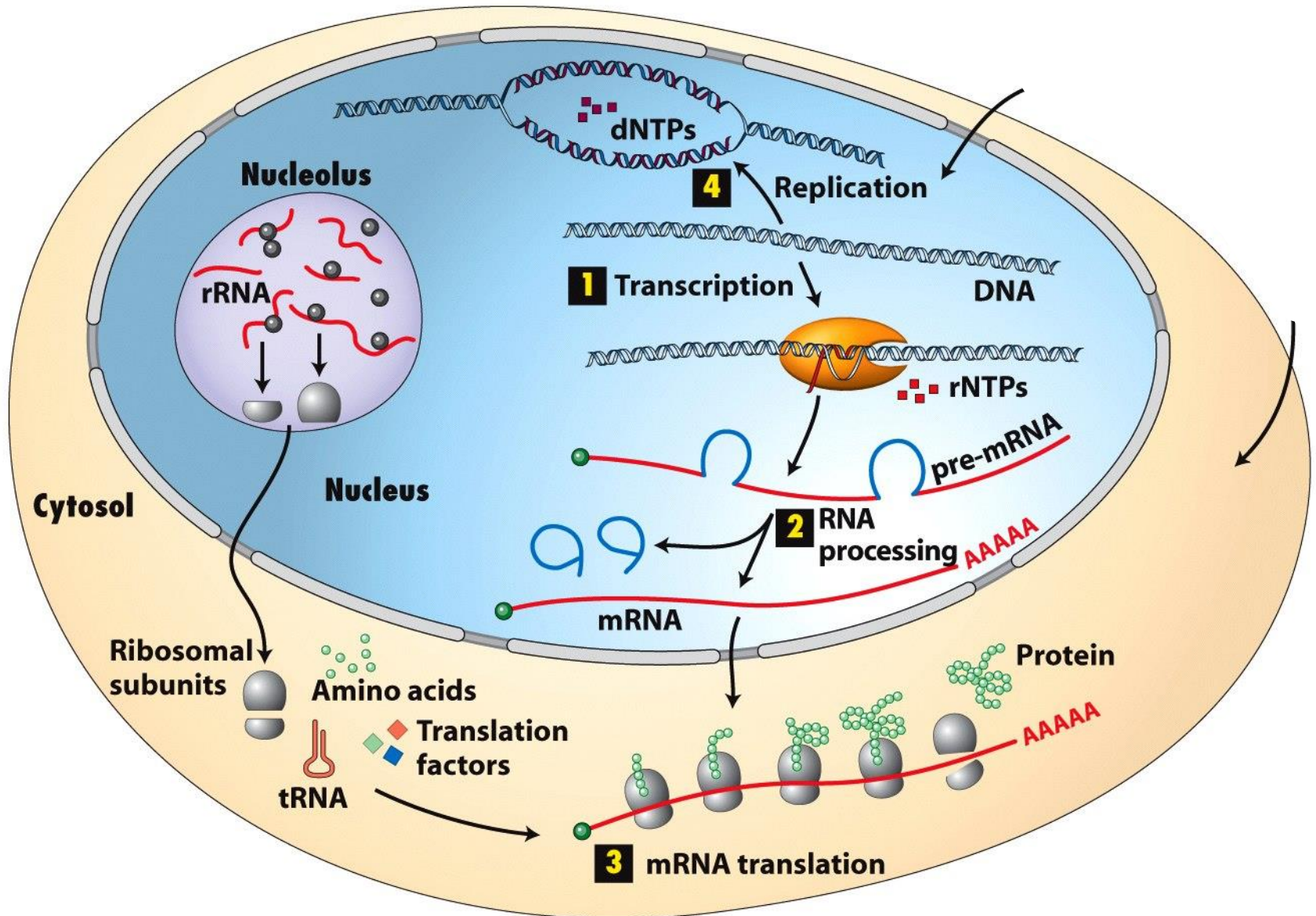
Streptomycin

Binds to the 30S ribosome, prevents the transition from initiation to chain-elongation

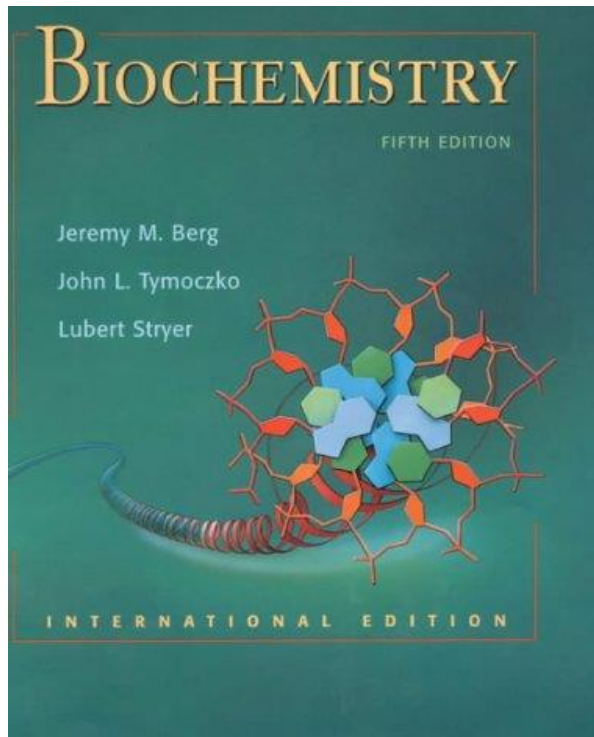
Erythromycin

Binds to the 50S ribosome, and blocks the translocation

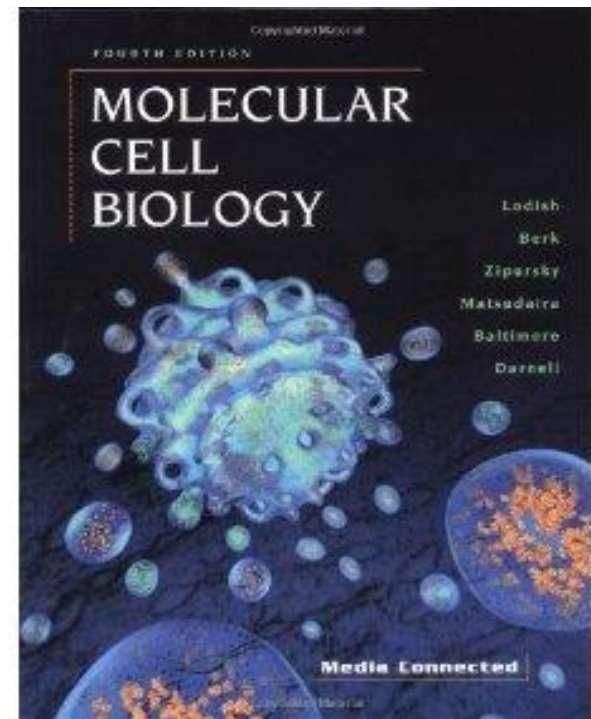
Different genetic events: At a Glance



Suggested Textbook...



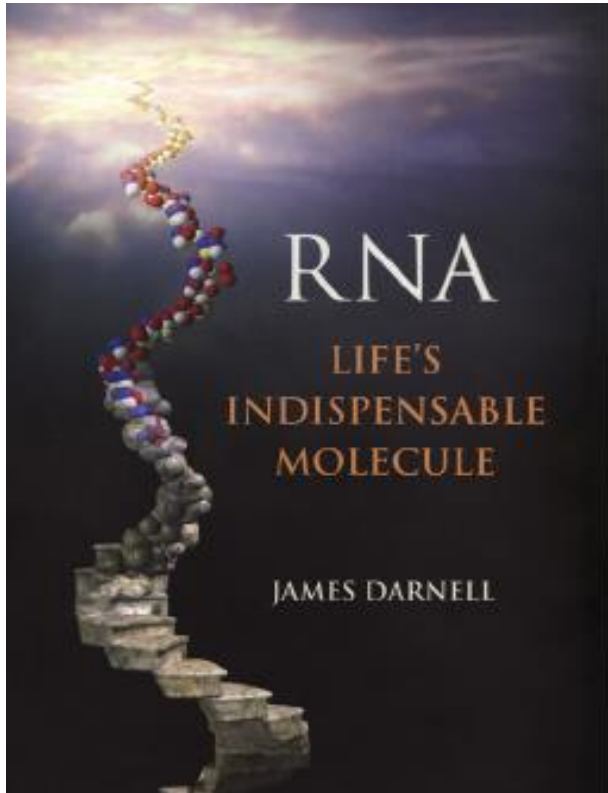
Stryer...



Baltimore, Lodish..

Extra Resources

Further Reading...



James Darnell

Videos...

mRNA synthesis (Transcription)

http://highered.mheducation.com/sites/0072507470/student_view0/chapter3/animation_mrna_synthesis_transcription_quiz_2.html

Protein synthesis (Translation)

<https://www.youtube.com/watch?v=lkq9AcBcohA>

Overview

<https://www.youtube.com/watch?v=gG7uCskUOrA>