Science of Living System

BS20001

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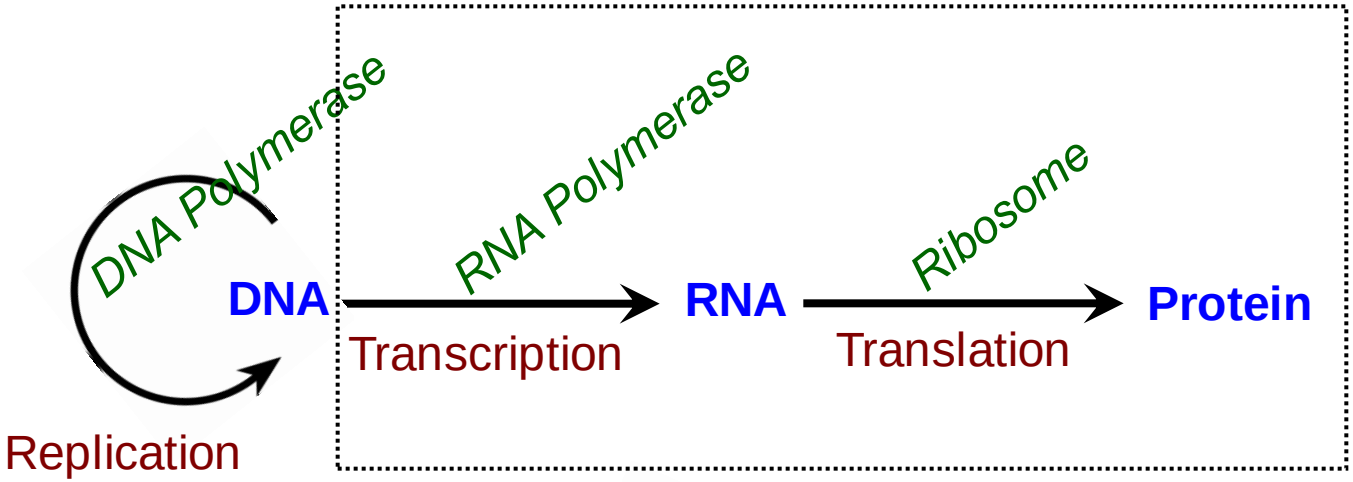
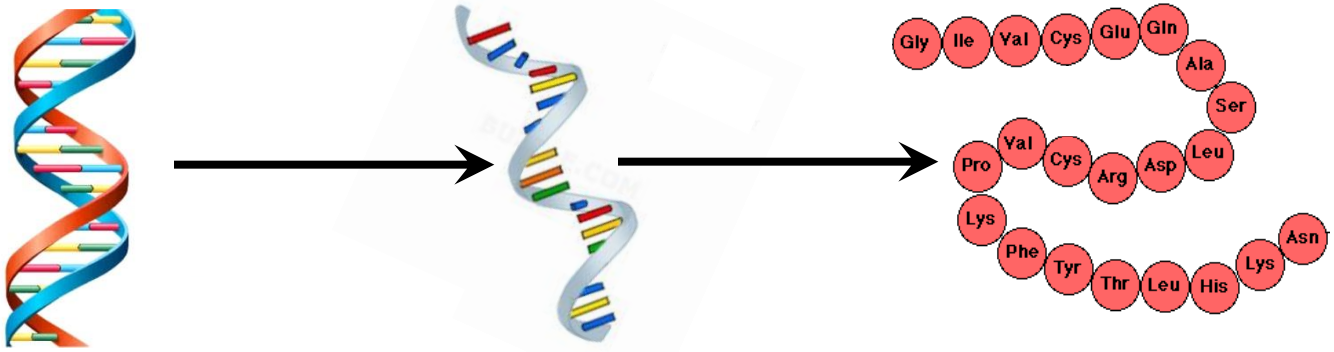
Flow of Genetic Information: The Central Dogma of Molecular Biology





DNA

Polymer of nucleotides

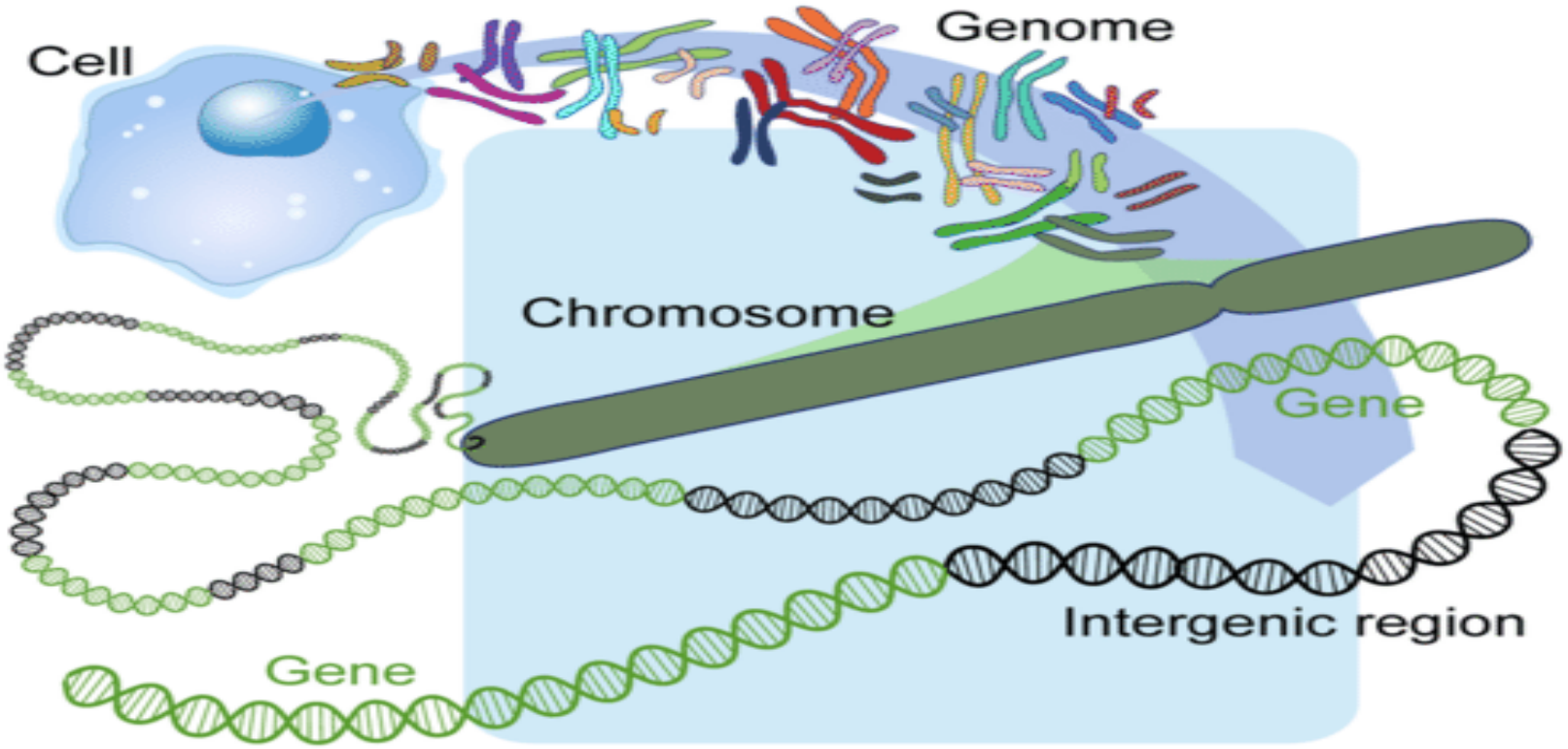
RNA

Polymer of nucleotides

Protein

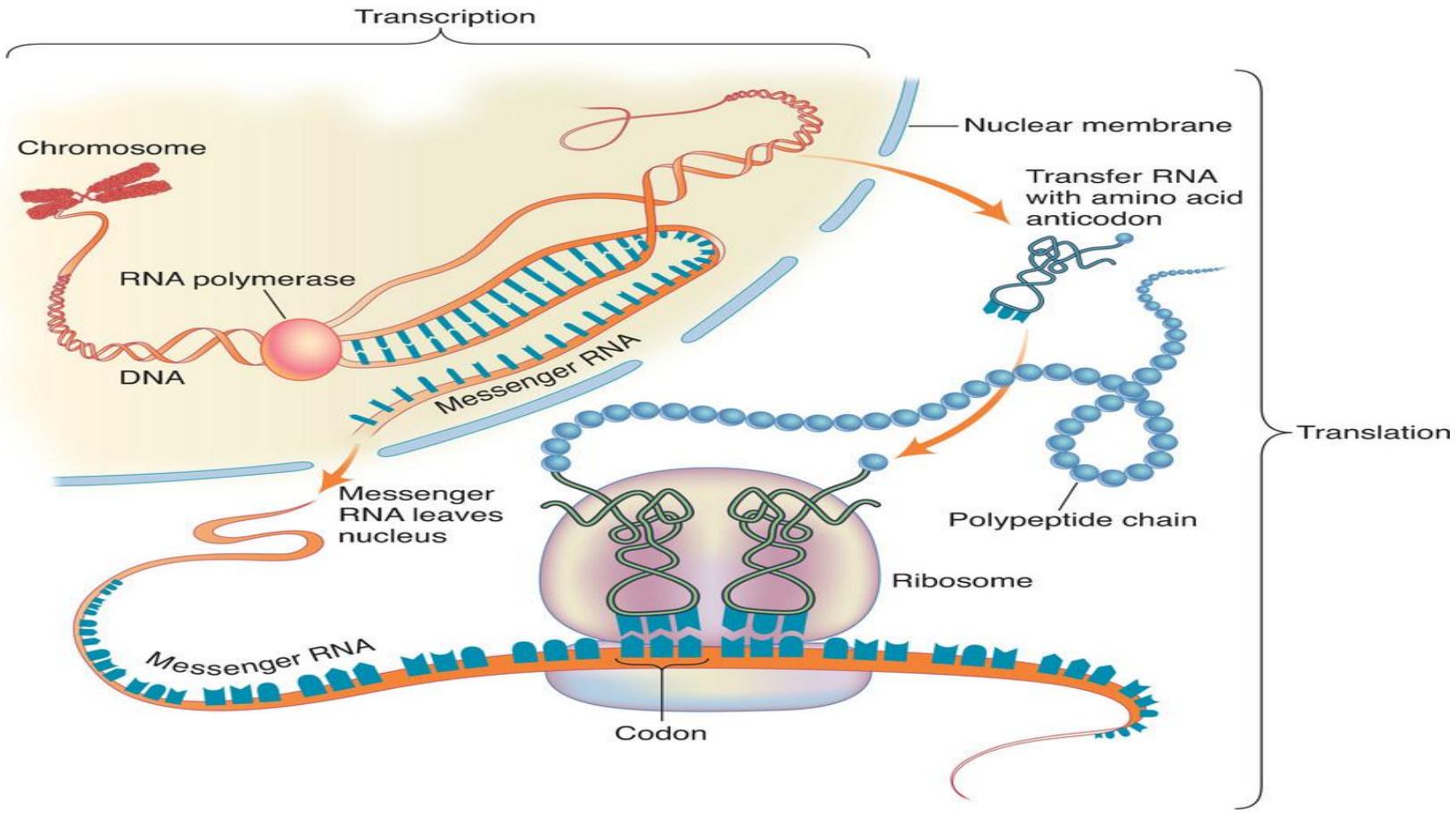
Polymer of amino acids

Genome, Chromosome and Gene



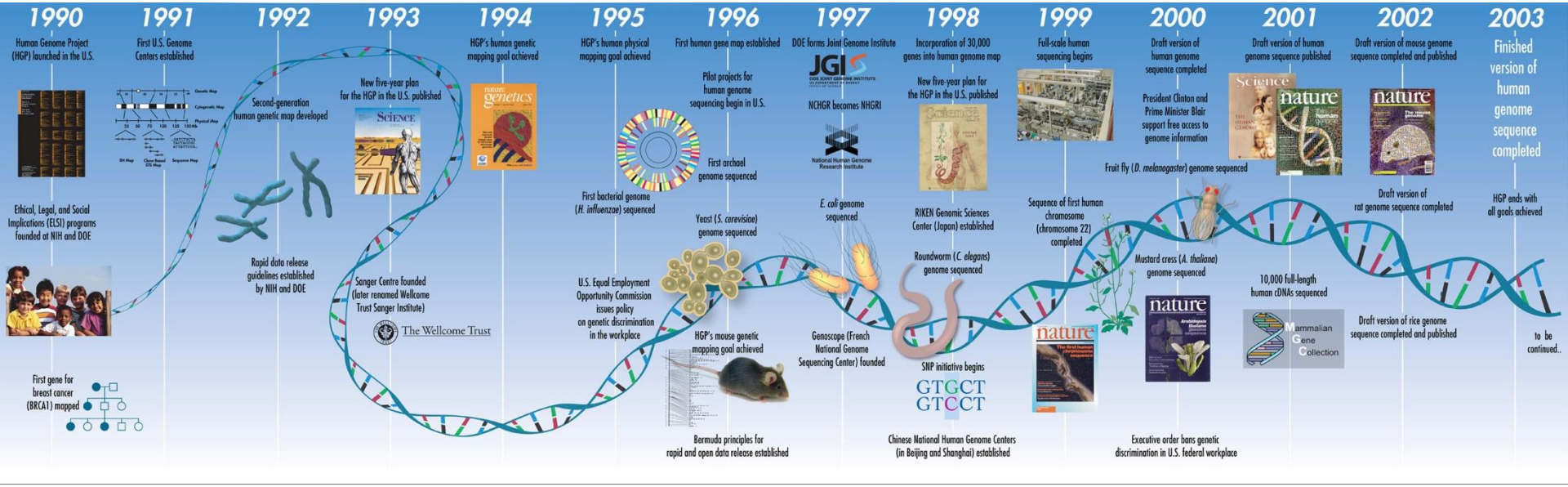
Genome	DNA	Genes	Proteins
• 23 chromosomes • Chromosomes are made of DNA • DNA: A, T, C, and G	• Containing coding regions called genes	• ~20,000 genes in the human genome • Genes code for proteins	• Have biological function in the cell

Overview of Transcription and Translation

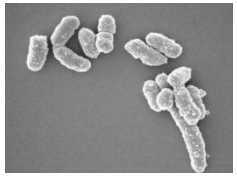
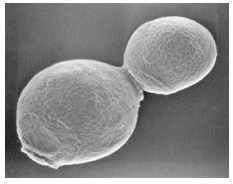


“Human Genome Project” Dramatically Enhanced Our Understanding on Gene Expression

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



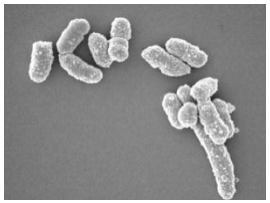
Genome Size, Gene Number, and Complexity of an Organism

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

Transcription

Genome size (bp)

Total DNA content vs transcribable content



4,600,000

3,200,000,000

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

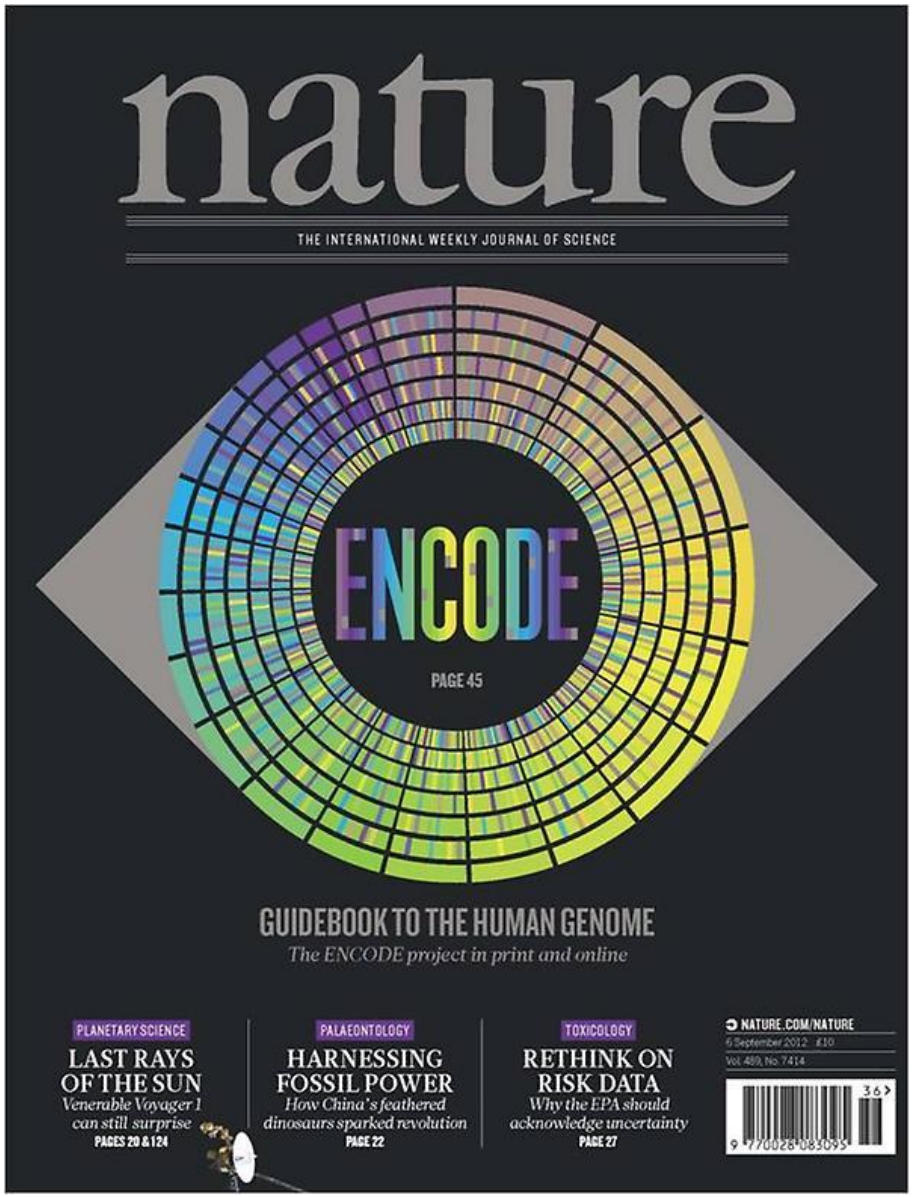
Messenger RNA (mRNA)

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

Ribosomal RNA (rRNA)
Transfer RNA (tRNA)

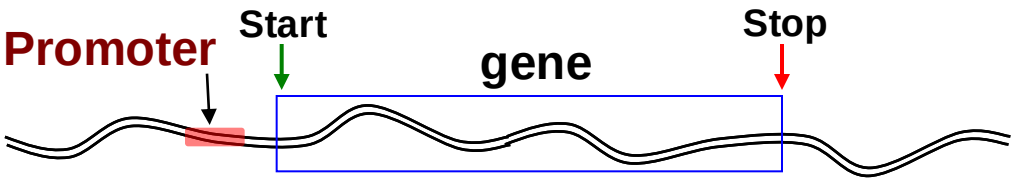
► **Most of the DNA sequences are not transcribed**

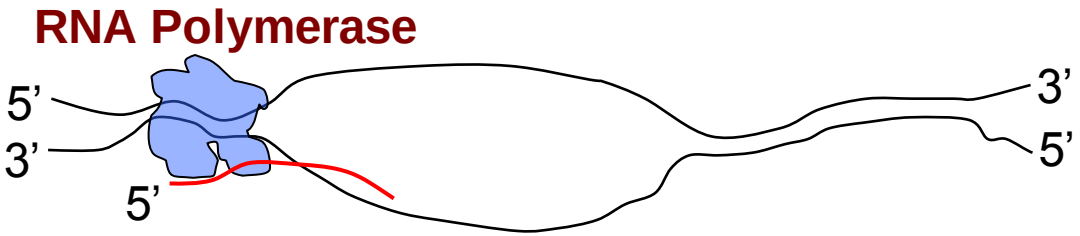
The Encyclopedia of DNA Elements (ENCODE)



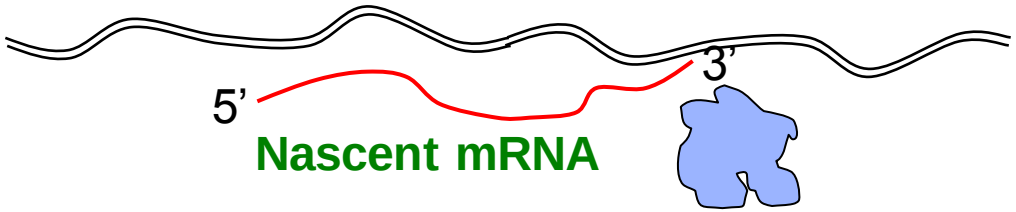
About 80% of the human genome serves some purpose

Transcription: Involved Machineries and Processes





Initiation Elongation Termination



Key points to be discussed

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

Promoter for Transcription

Promoter is a region of a DNA molecule which forms the site at which transcription of a gene starts

Prokaryotic promoter

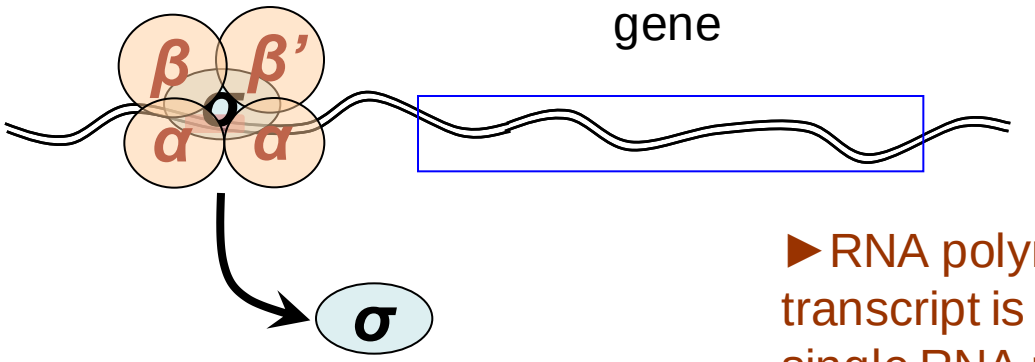
Eukaryotic promoter

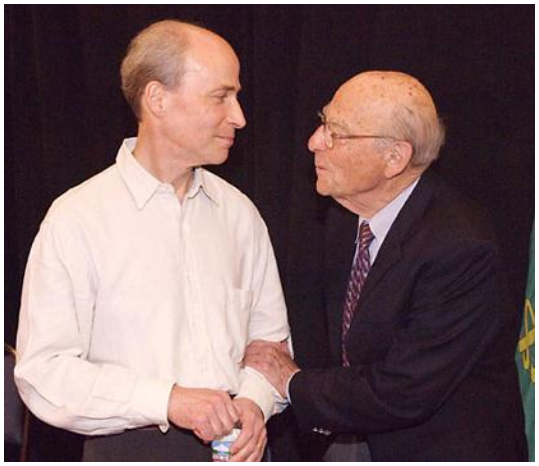
RNA Polymerase

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)

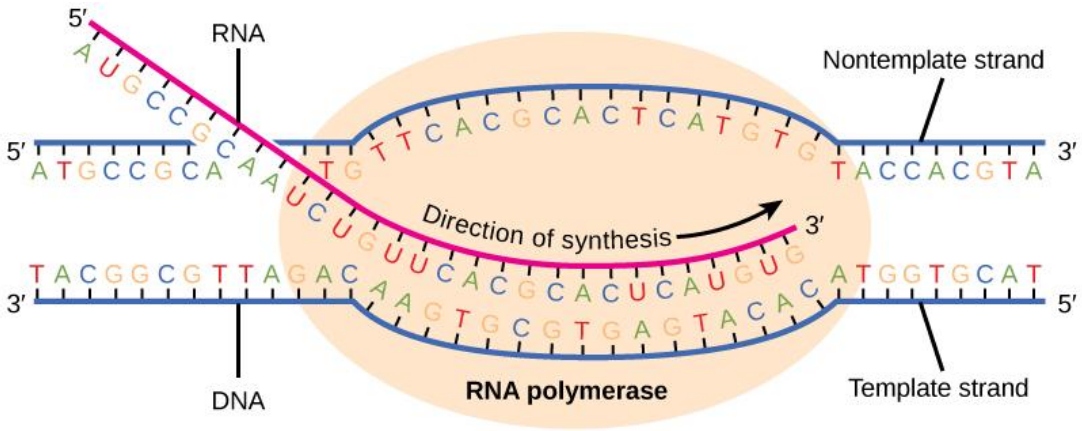
RNA Molecules in *E. coli*	
mRNA	5%
tRNA	15%
rRNA	80%

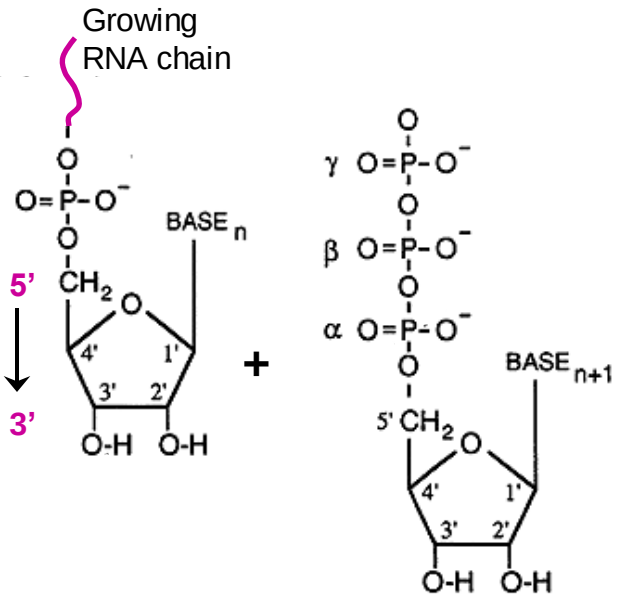
Who transcribes this huge pool of rRNA and tRNA?

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

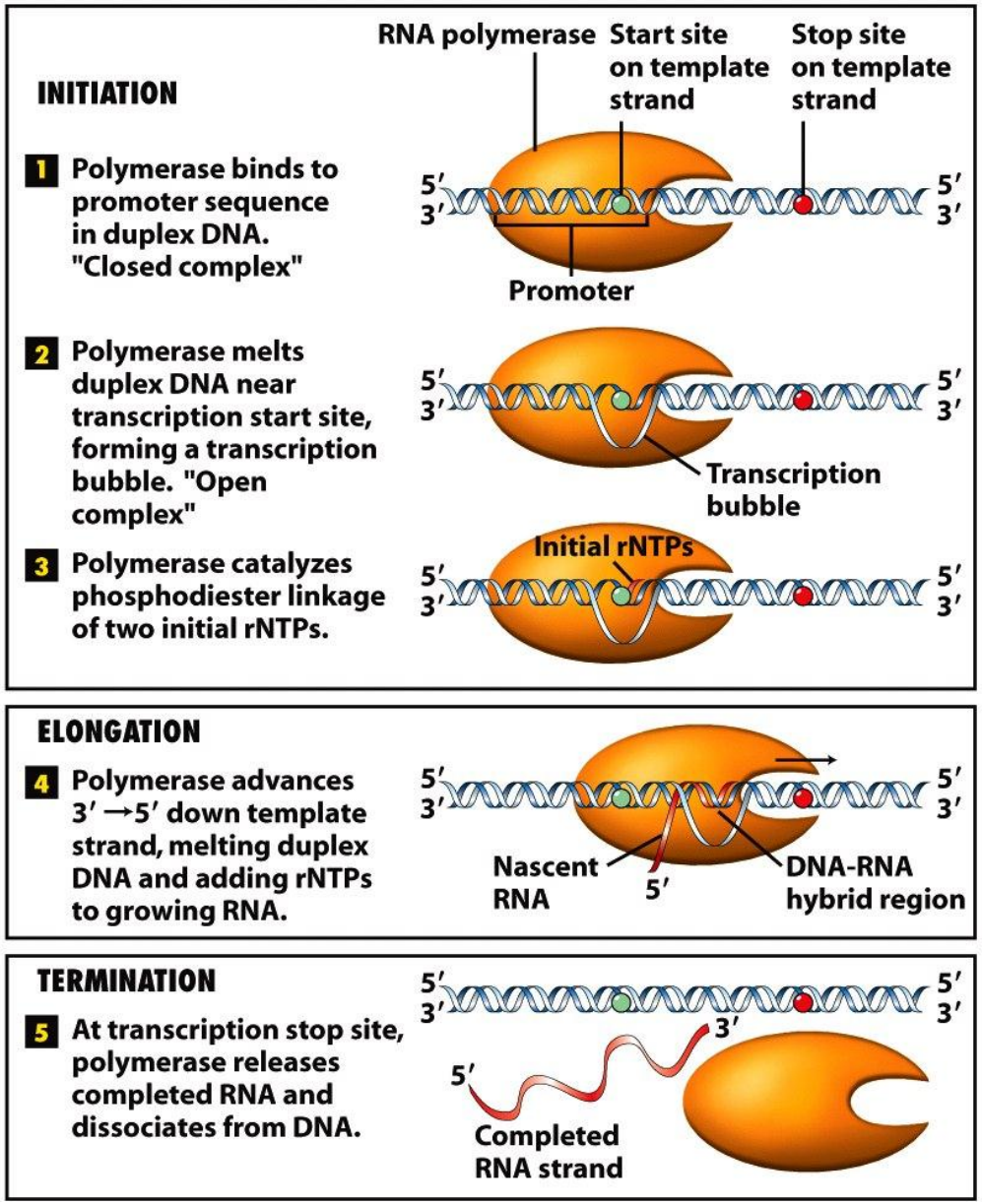
In eukaryotes different RNA polymerases are involved in transcription of mRNA, rRNA and tRNA

RNA Synthesis





Schematic Representation of Multiple Steps Involved in Transcription



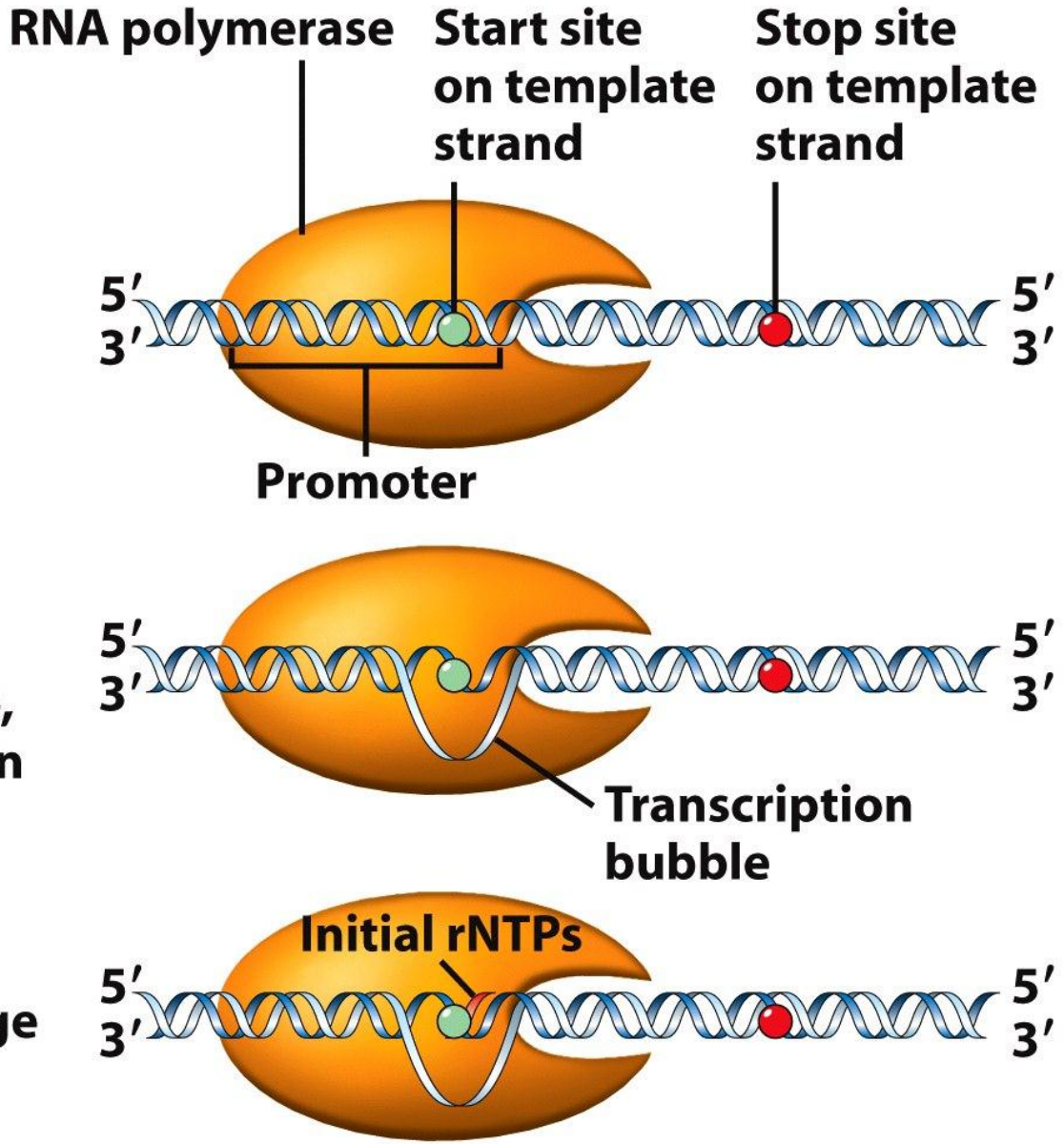
Schematic Representation of Multiple Steps Involved in Transcription

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"

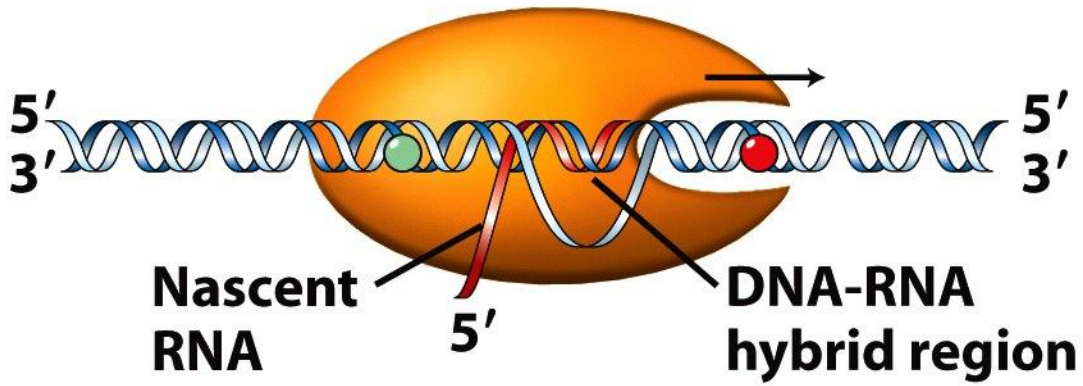
Polymerase catalyzes phosphodiester linkage of two initial rNTPs.



Schematic Representation of Multiple Steps Involved in Transcription

ELONGATION

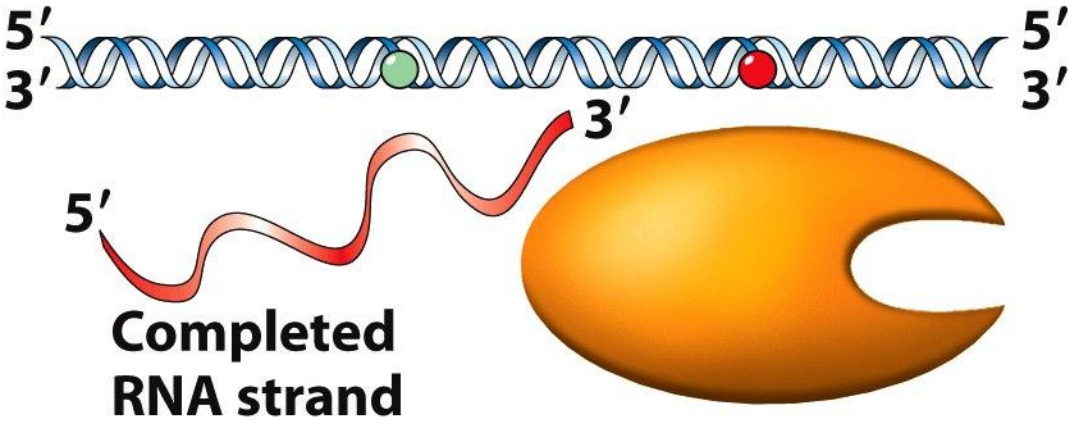
Polymerase advances 3′ →5′ down template strand, melting duplex DNA and adding rNTPs to growing RNA.



Schematic Representation of Multiple Steps Involved in Transcription

TERMINATION

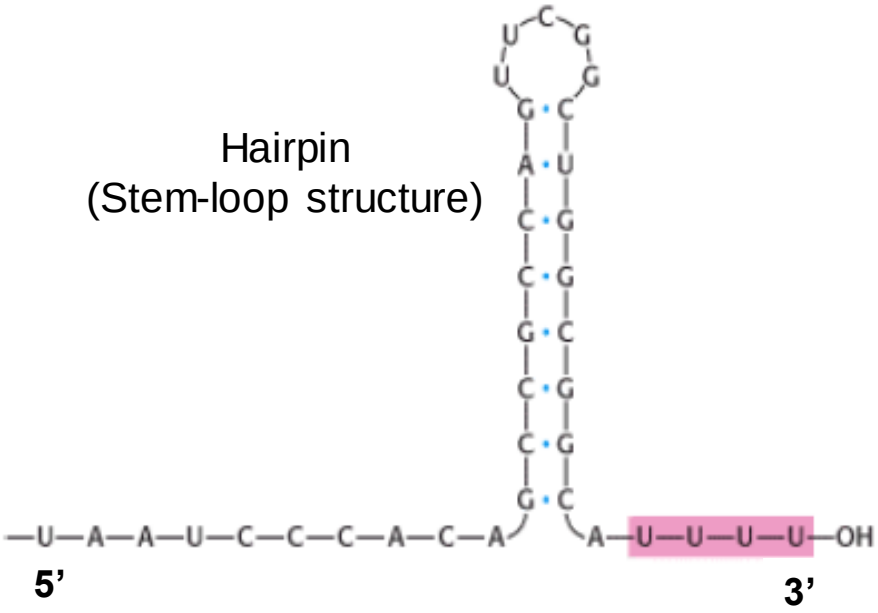
At transcription stop site, polymerase releases completed RNA and dissociates from DNA.



Termination of Transcription in Prokaryotes

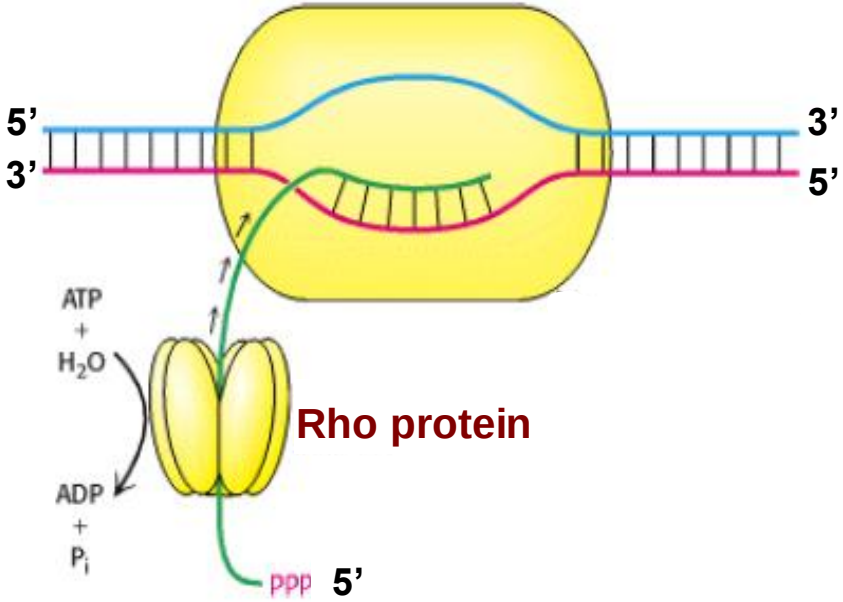
Factor independent

An RNA hairpin followed by several uracil residues terminates transcription



Factor dependent

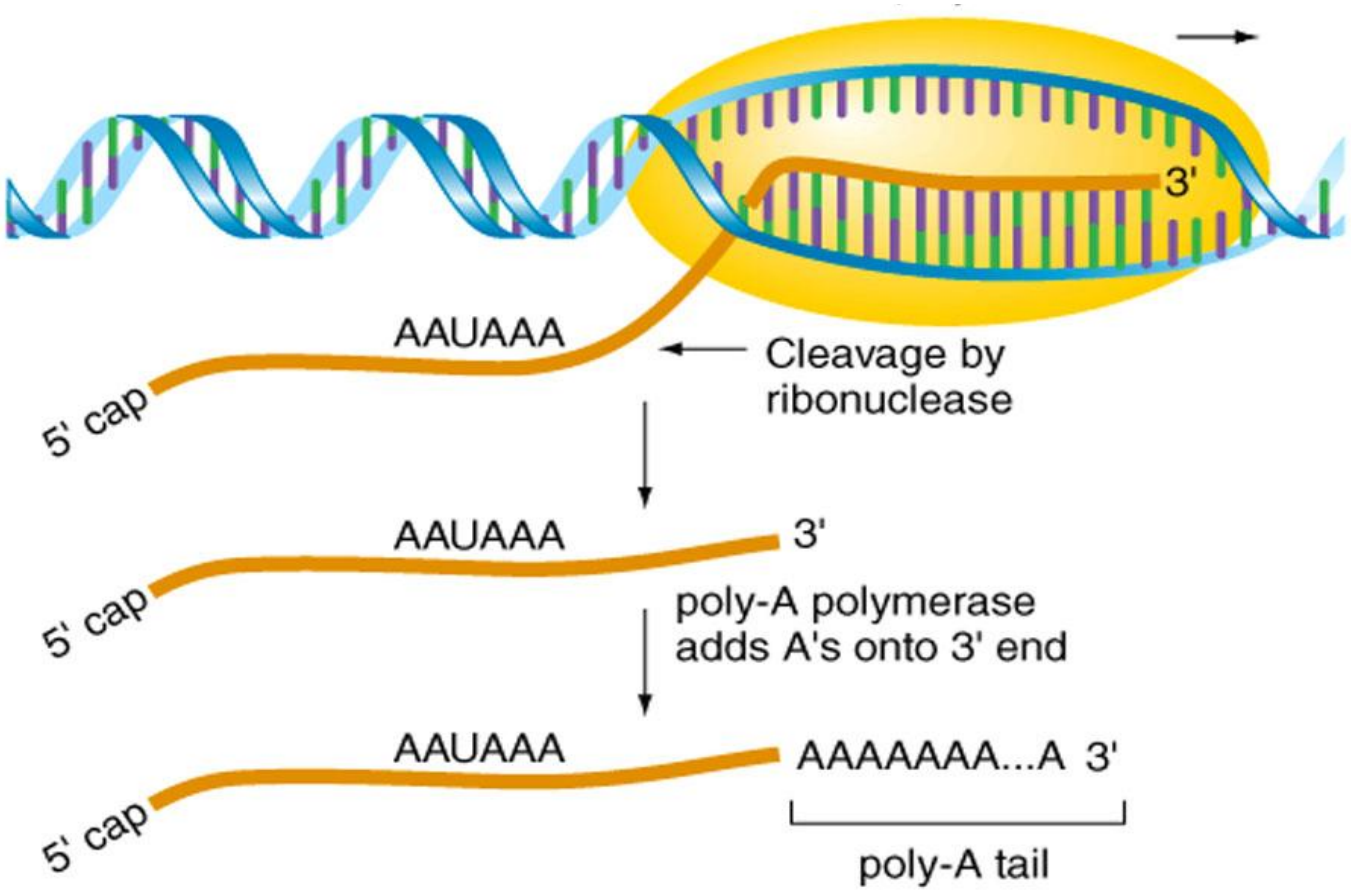
Rho binds the nascent RNA chain and pulls it away from RNA polymerase and the DNA template.



Stryer, Biochemistry, 4th edition

Eukaryotic Transcripts Need to be Processed

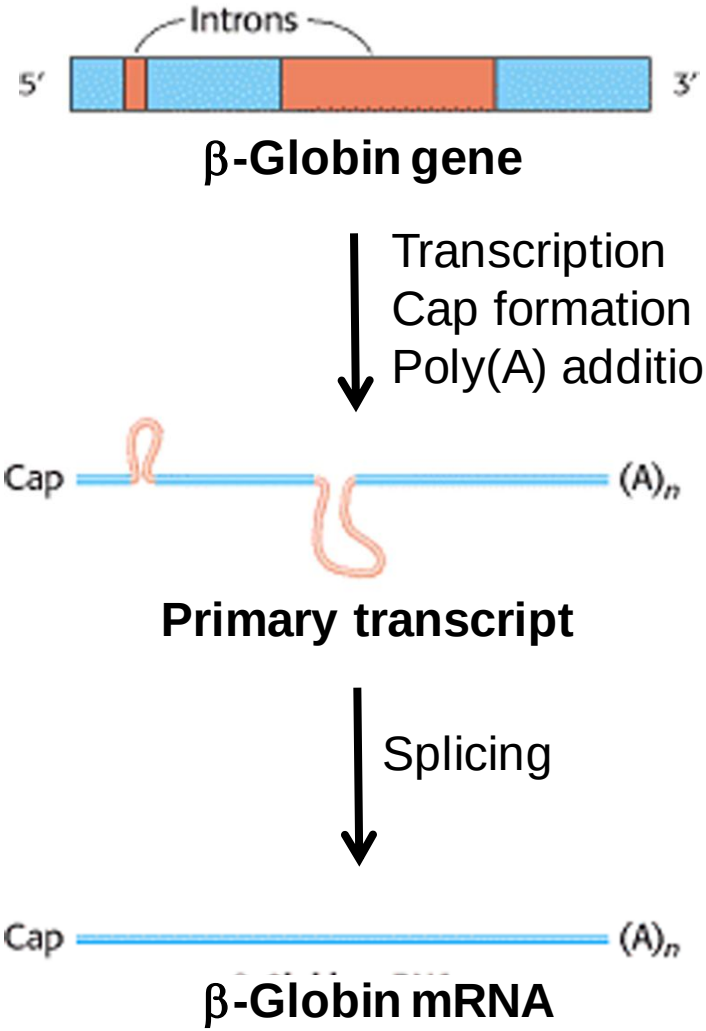
► Ends of a nascent mRNA acquire a 5' cap and a 3' poly A tail



- Increase stability of mRNA
- More effective template for translation

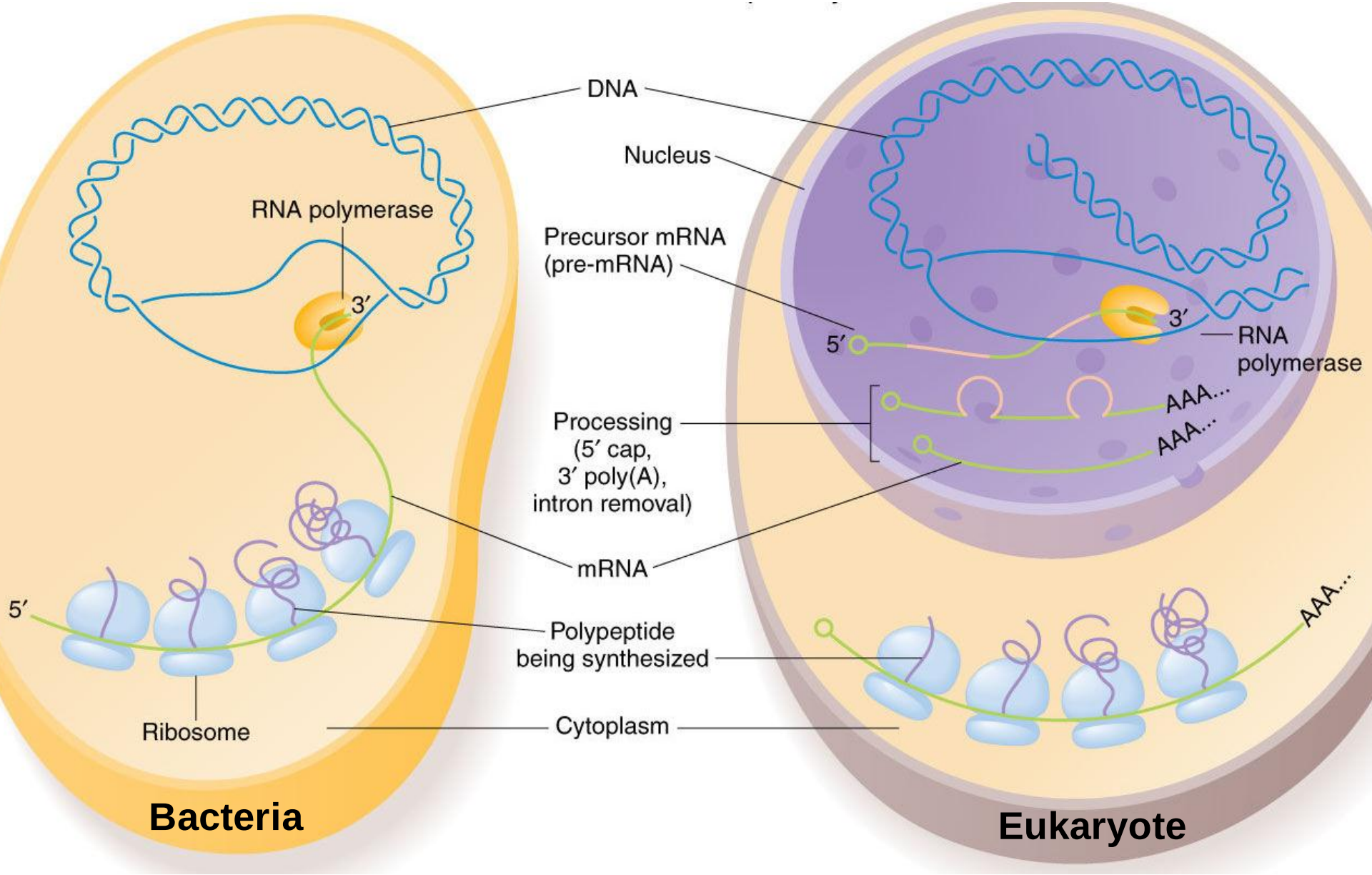
Eukaryotic Transcripts Need to be Processed

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

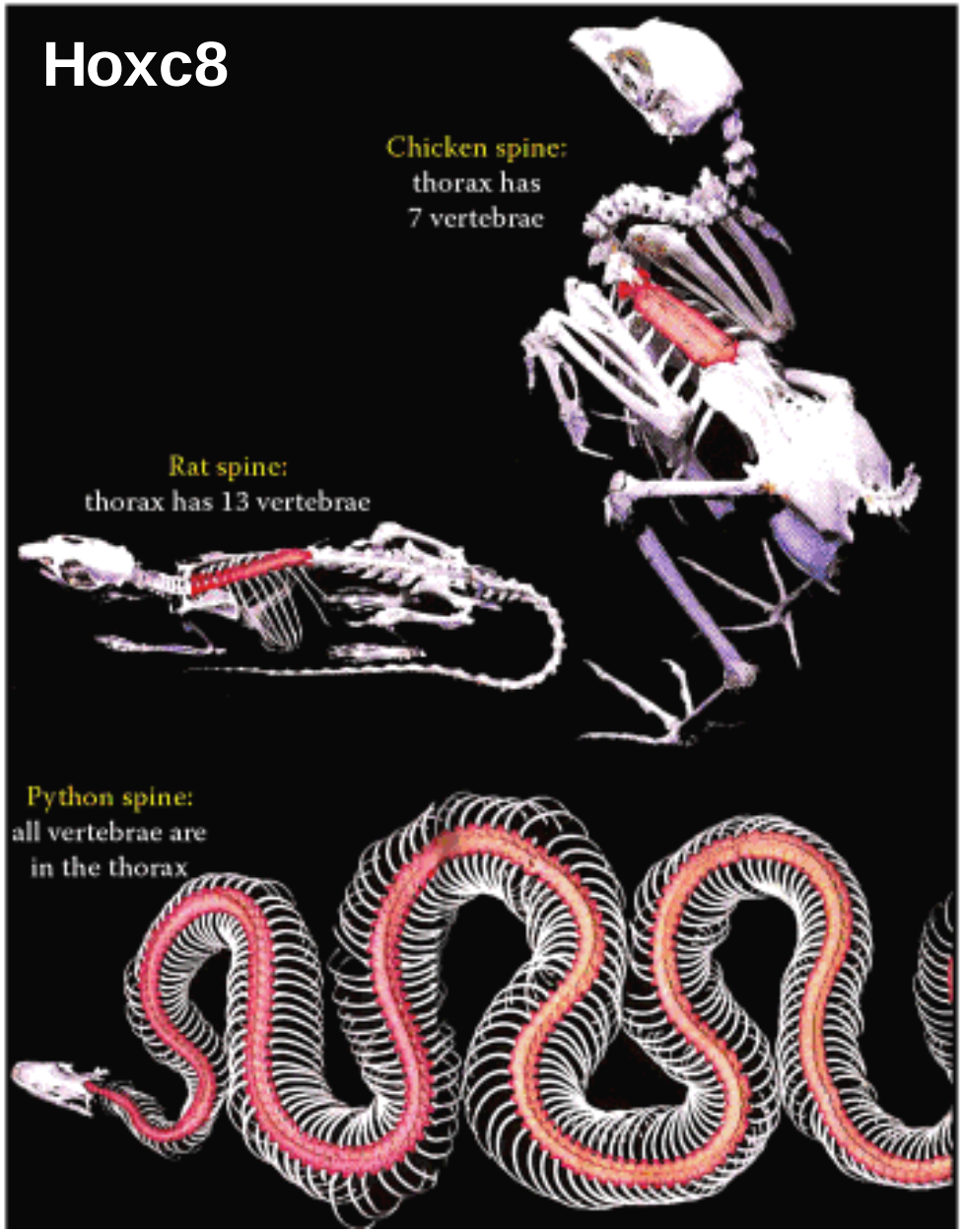


Thomas Cech
Nobel prize in 1989

Transcription: At a Glance



DNA: Contains the Instruction for Life



Regulation of Gene Expression

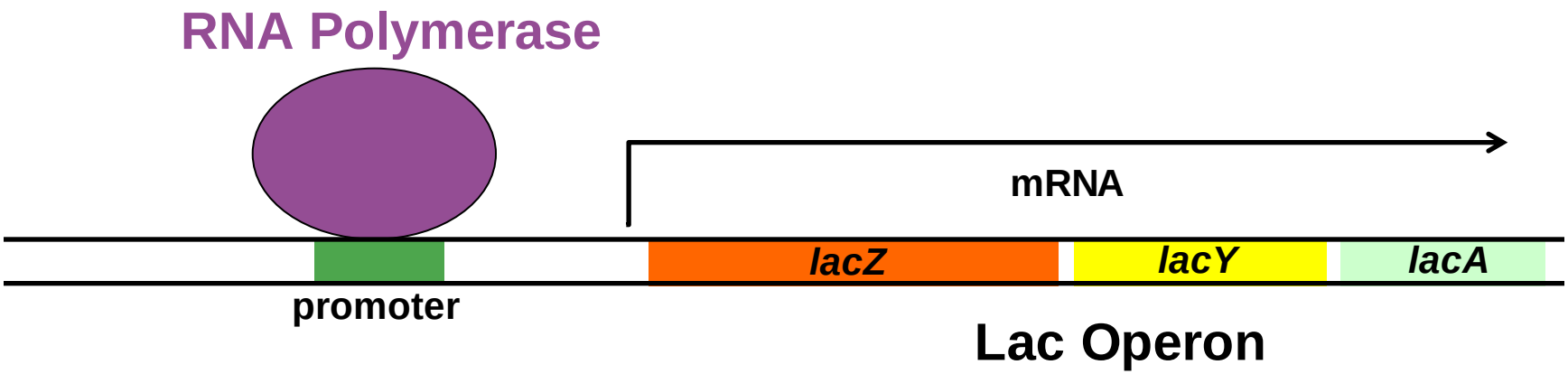
Each cell contains all the genetic material for growth and development

Some of these genes are expressed all the time

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

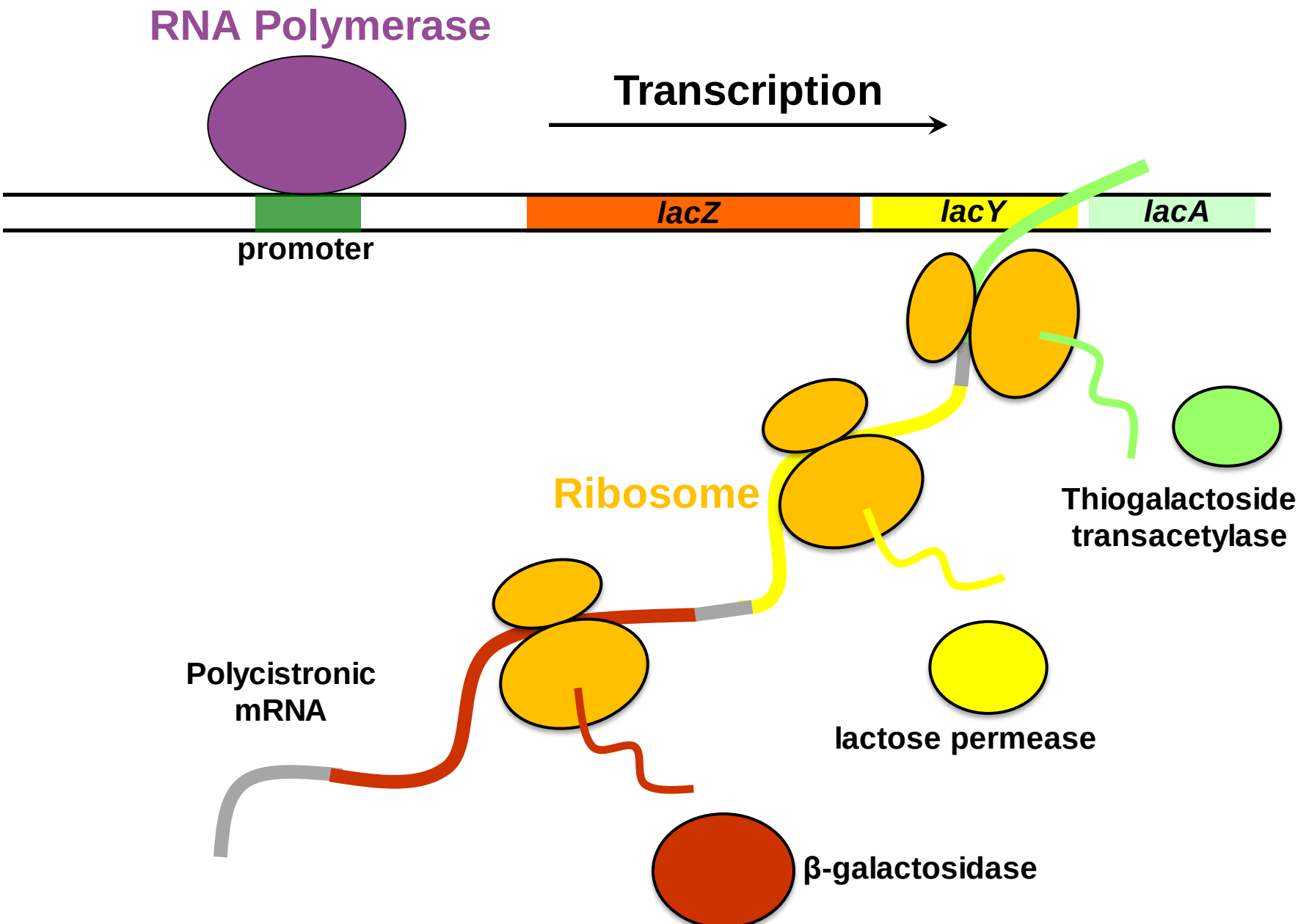
Lac Operon: A Classic Example of Bacterial Gene Expression Control

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

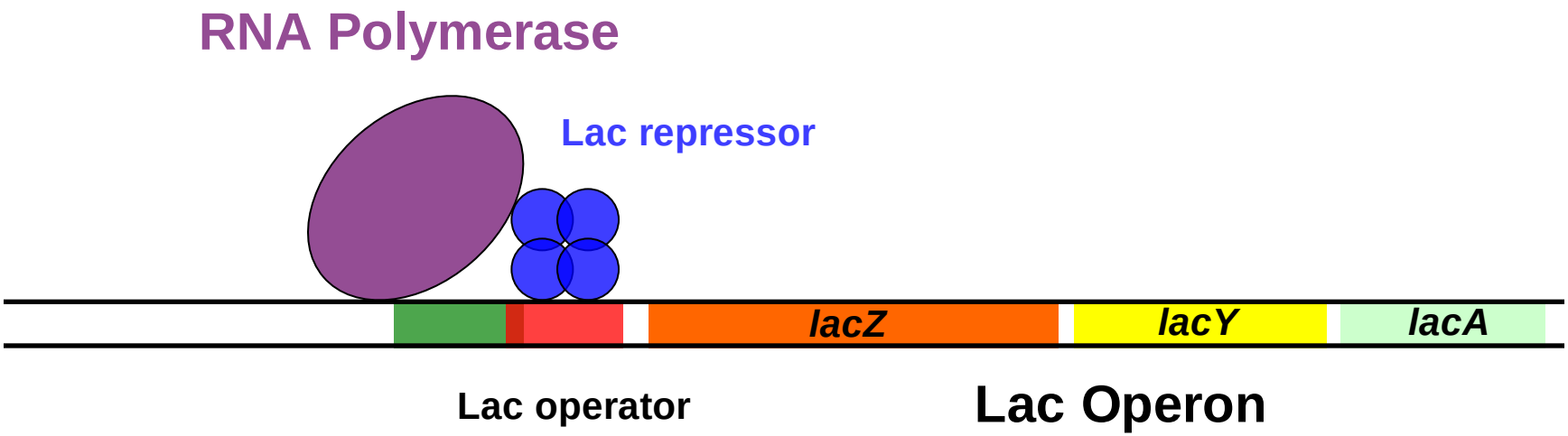


lacZ	β-galactosidase	Breaks lactose into galactose and glucose.
lacY	lactose permease	Imports lactose into the bacterial cell.
lacA	thiogalactoside transacetylase	Cell detoxification.

Functional Outcome of Lac Operon

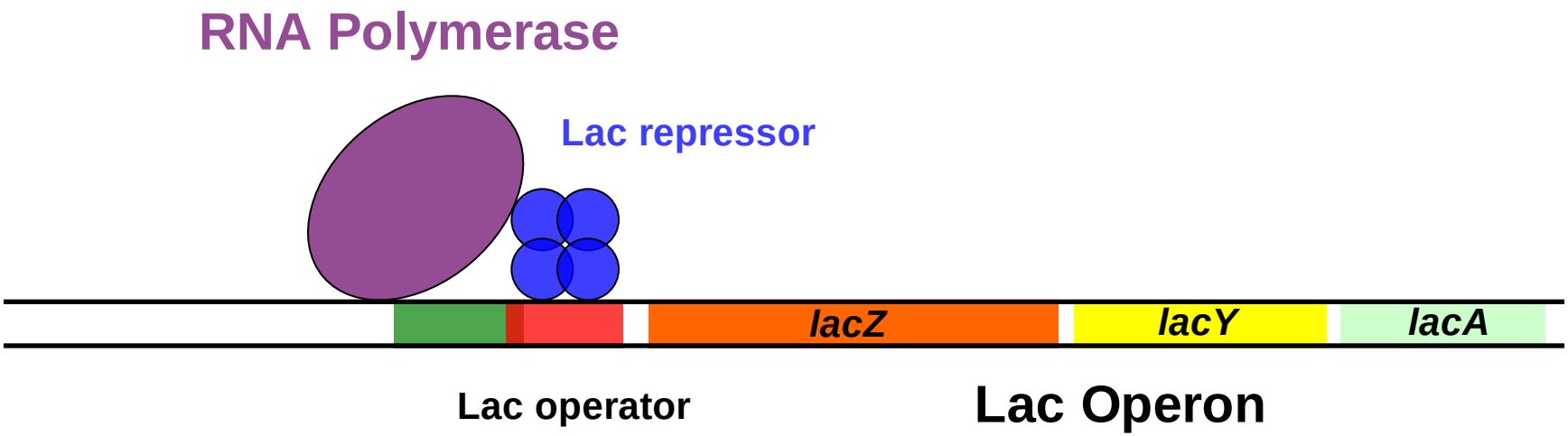


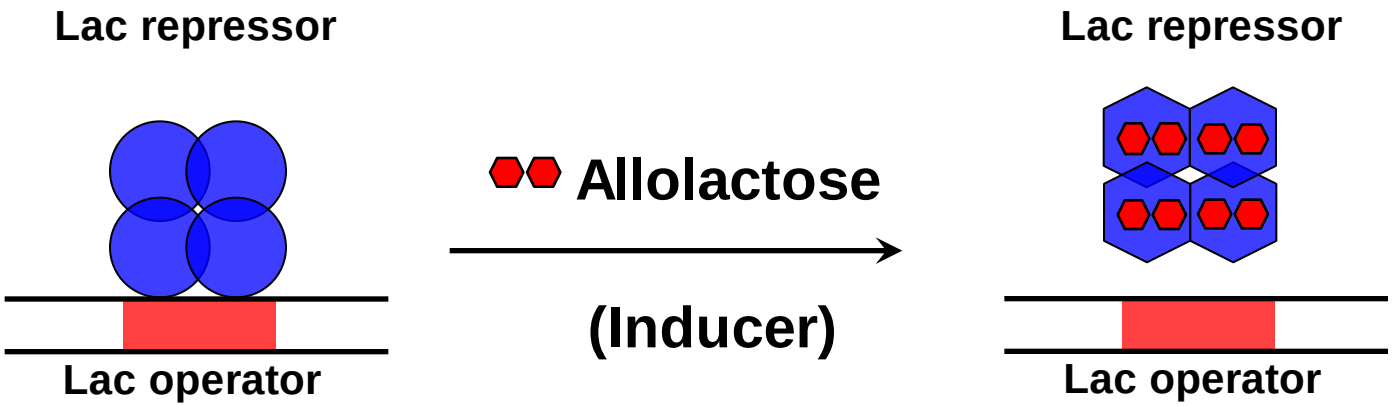
Lac repressor is a negative regulator of the Lac operon



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

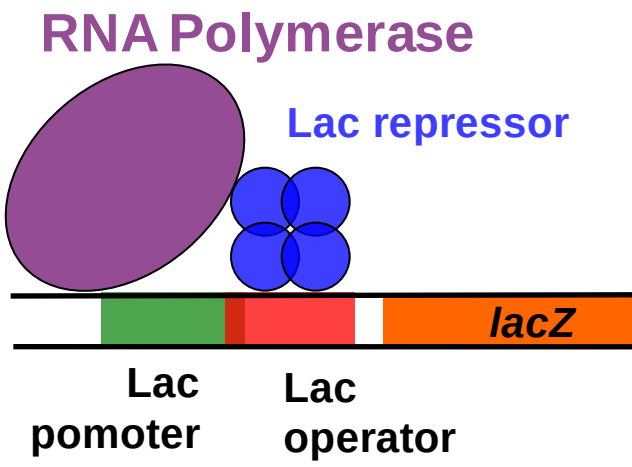
Lactose (Allolactose) Can Displace Lac Repressor From the Operator Site



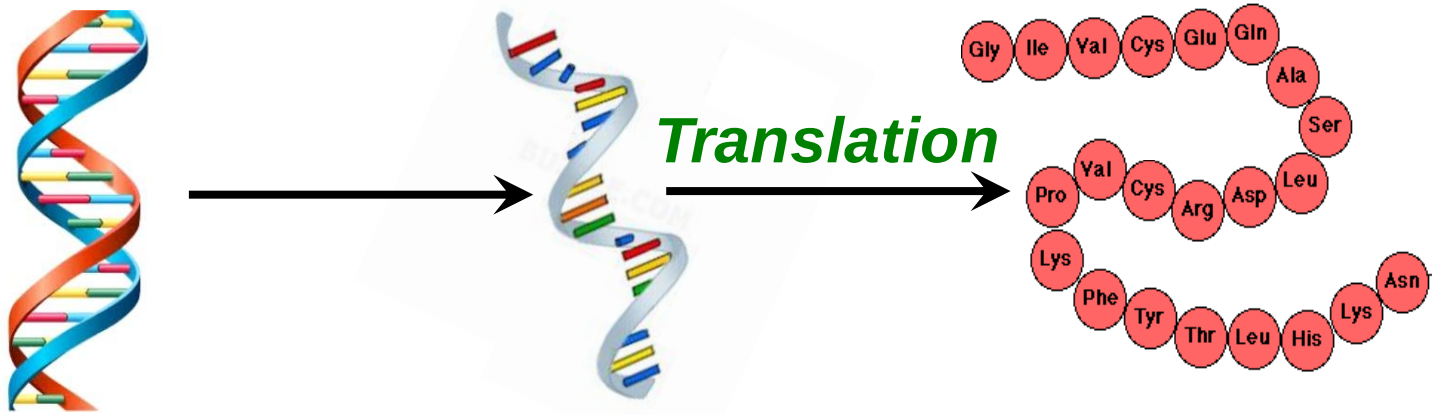


Four Possible Situations

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



Translation



DNA

Polymer of nucleotides

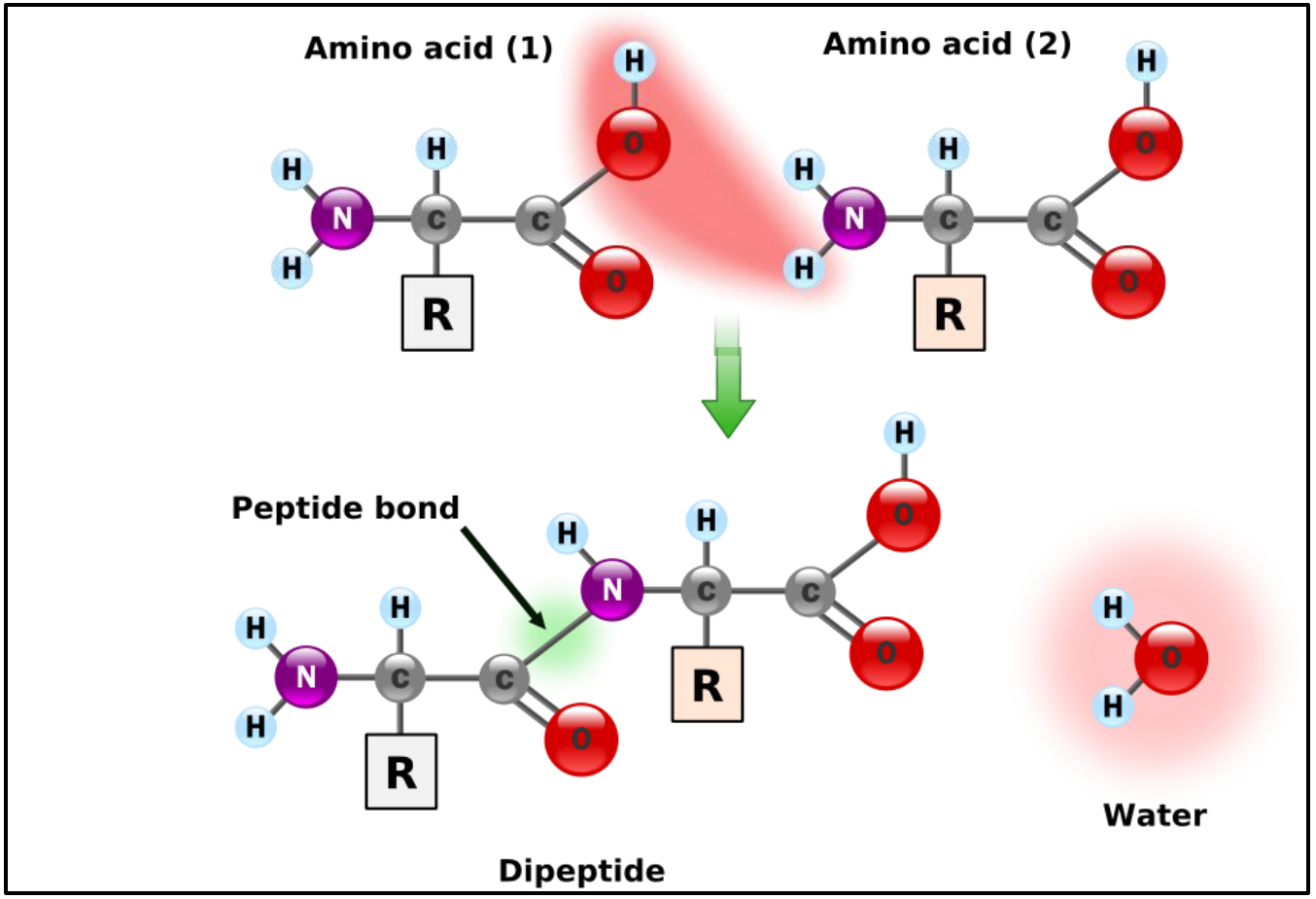
RNA

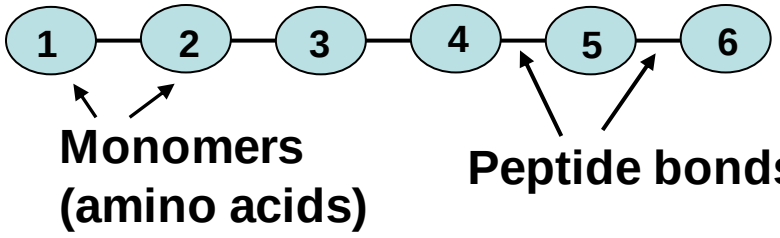
Polymer of nucleotides

Protein

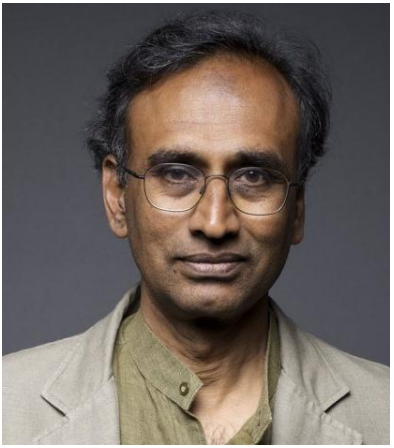
Polymer of amino acids

How Amino Acids are Linked Together





Polymer: Polypeptide and Protein



Translation

Venki Ramakrishnan
Nobel Prize 2009

Template for protein synthesis

5’ ——————————— 3’ mRNA

5’UAAGGAGA AUCGUCAUGAAGAGGCCC…….UAAUUA 3’

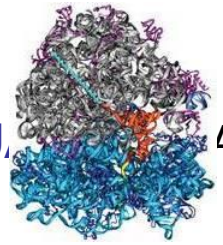
(RBS)

Start codon

Stop codon

Met–Lys–Arg–Pro……

Polypeptide



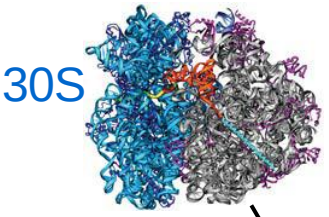
Nascent polypeptide

5’U A AUCGUCAUGAAGAGGCCC…….UAAUUA 3’

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

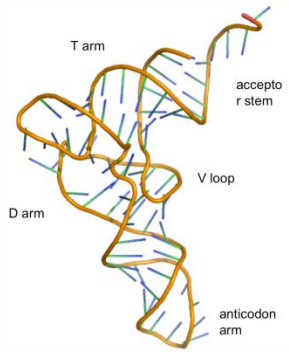
Protein synthesizing machinery

Ribosome: (rRNA + proteins)



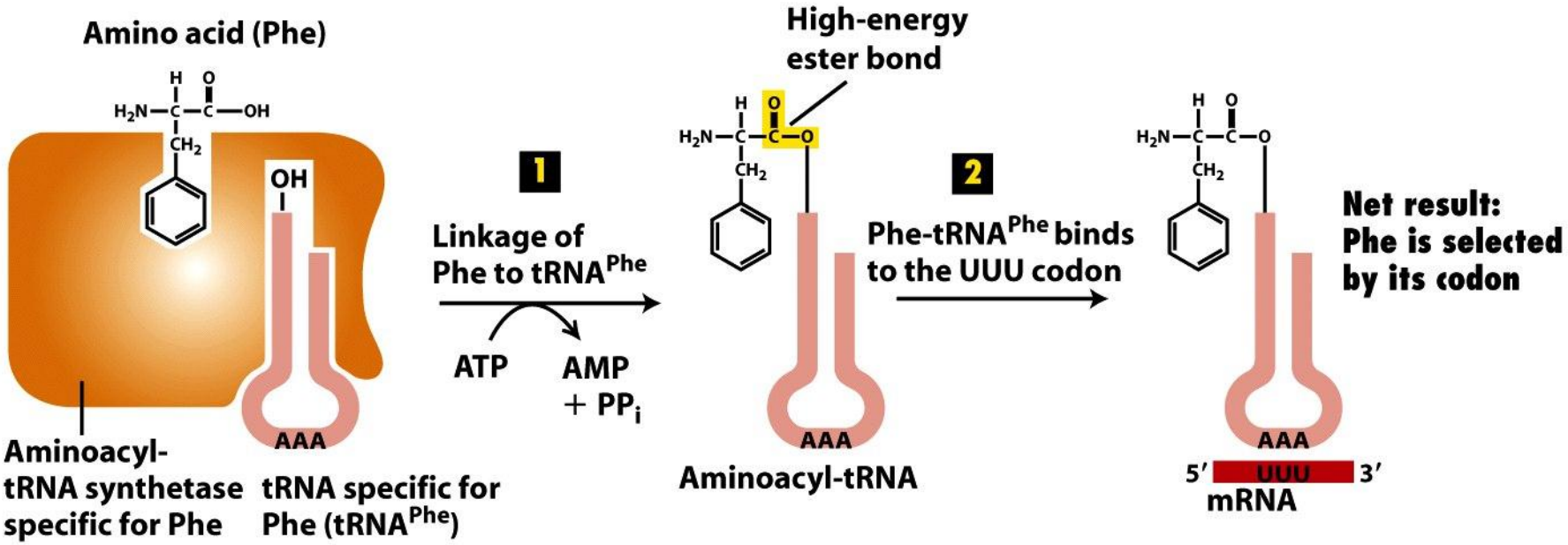
16S rRNA
21 proteins

23S rRNA
5S rRNA
34 proteins



tRNA

How Correct Amino Acids are Selected During Protein Synthesis



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, one amino acid may be specified by more than one codon

Khorana, Nirenberg, Holley

Nobel Prize in 1968

Genetic code

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

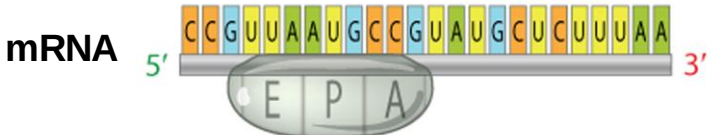
Translation: Involved Machineries and Processes

mRNA

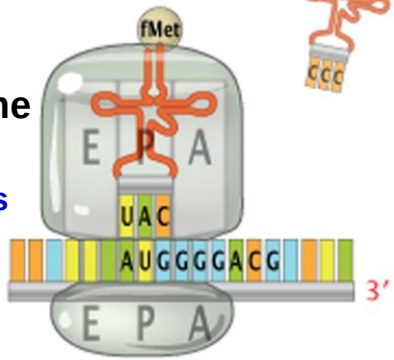
Ribosome

Amino acid

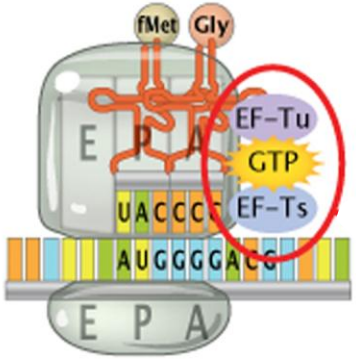
tRNA



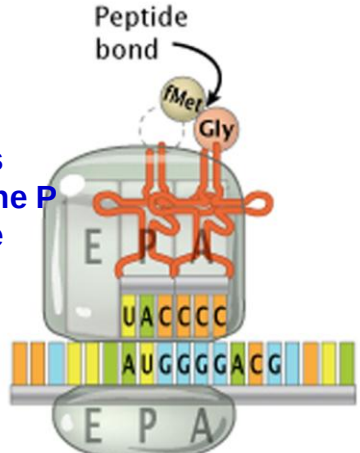
50S ribosome



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

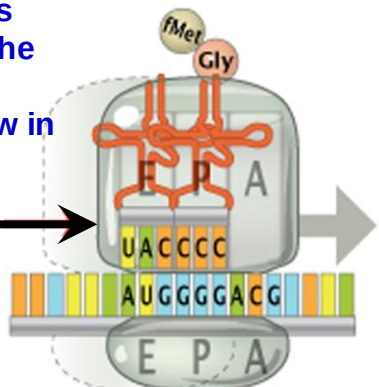


Next charged tRNA and associated translation factors enter the A site

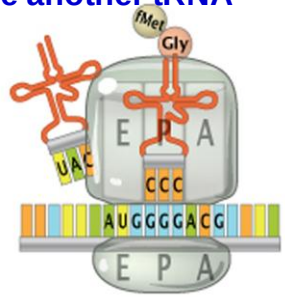


Peptide bond forms between the aa in the P and A sites, and the tRNA in the P site releases its aa

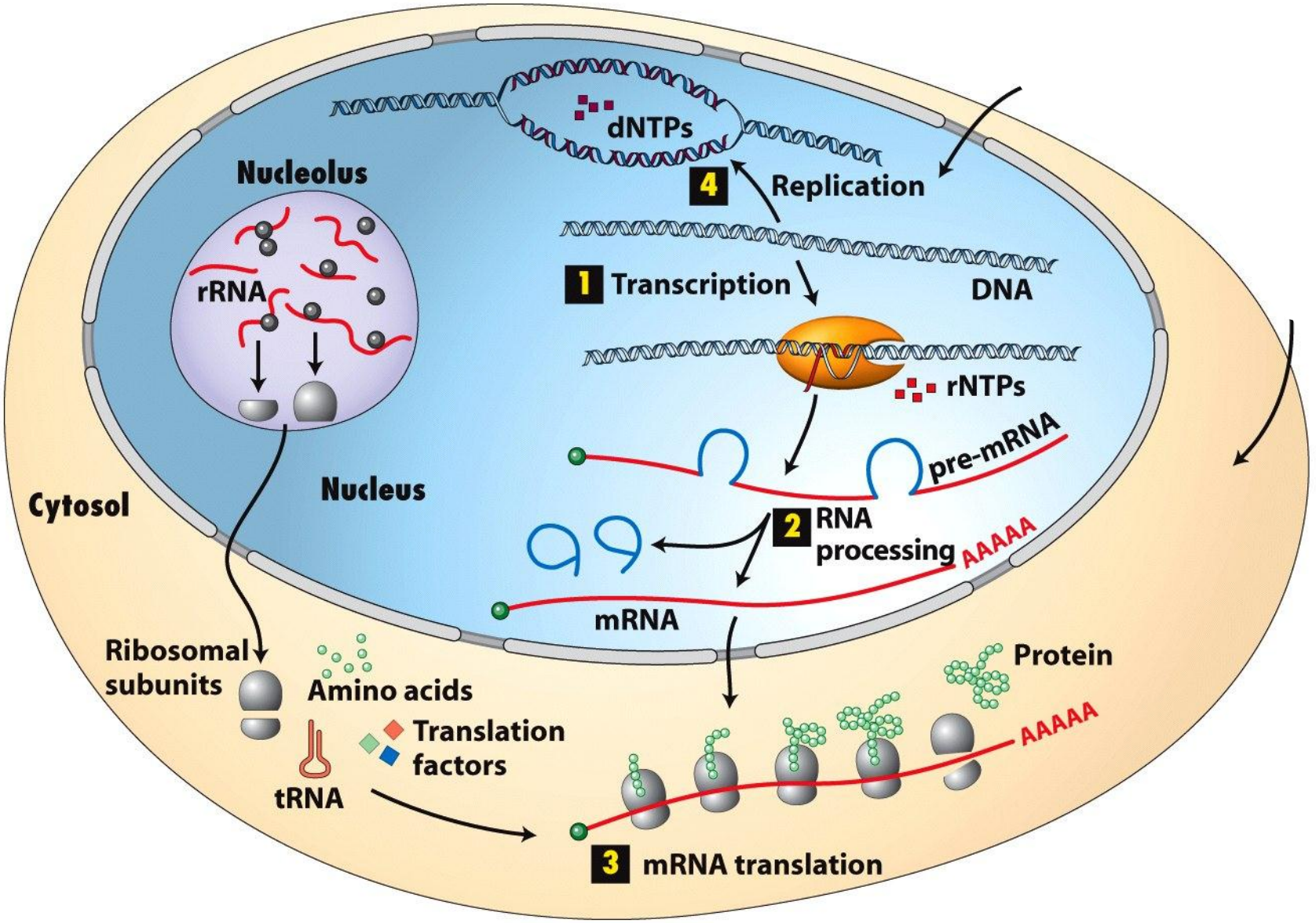
The ribosome moves down the mRNA to the next codon and the uncharged tRNA now in the E site



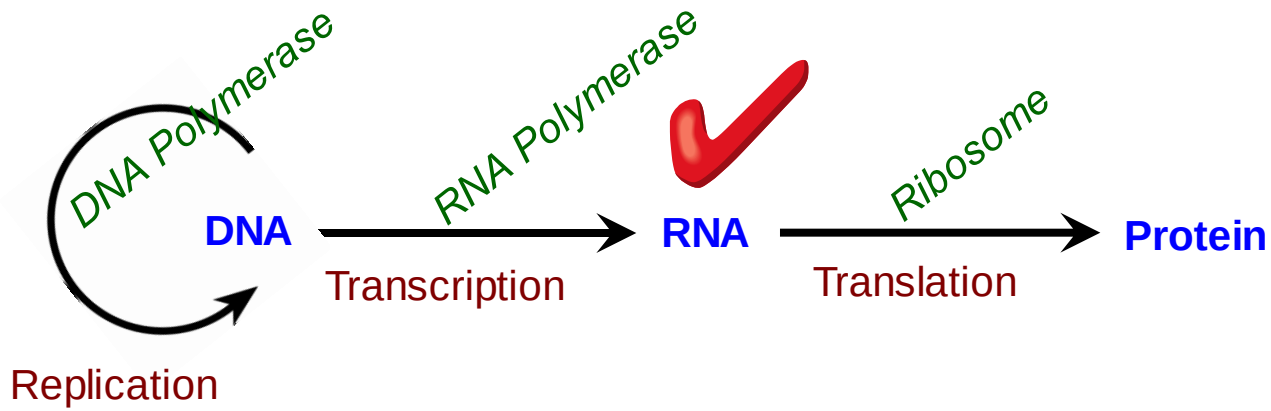
E site tRNA releases and A site is now ready to receive another tRNA

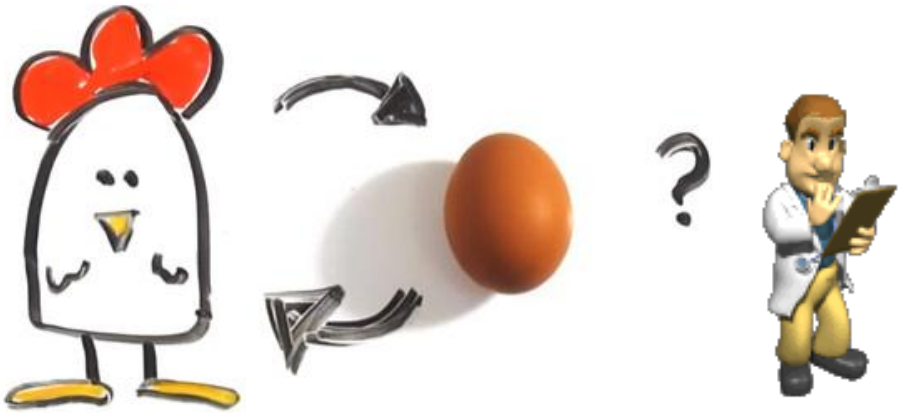


Nucleic Acid to Protein: At a Glance



Which Came First? Nucleic acids or Proteins



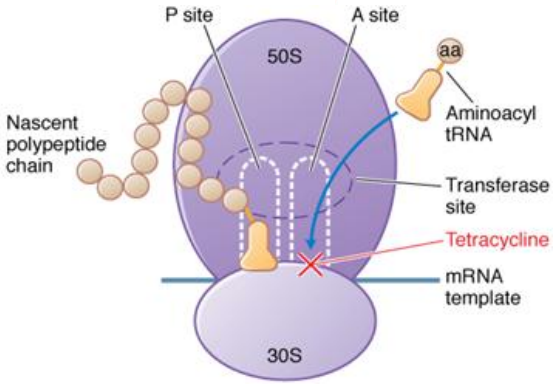


► **RNA has enzymatic activity**

Translation Machineries: Attractive Targets For Therapeutics

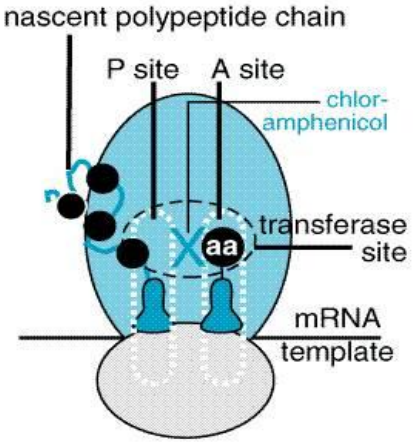
Tetracycline

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



Chloramphenicol

Blocks the peptidyl transferase reaction on 50S ribosomes

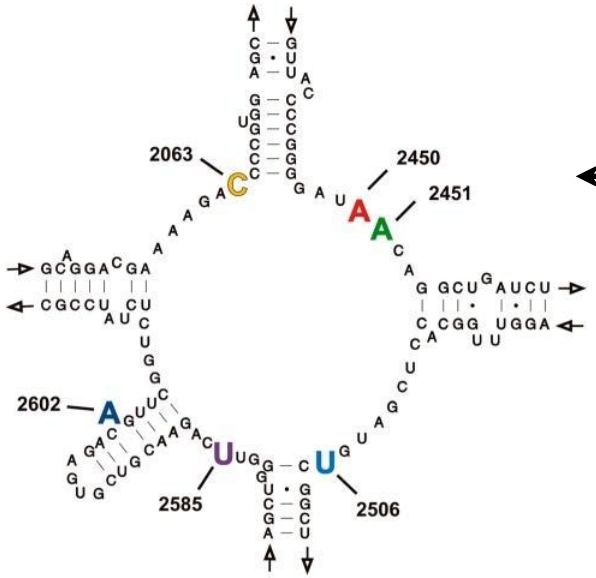


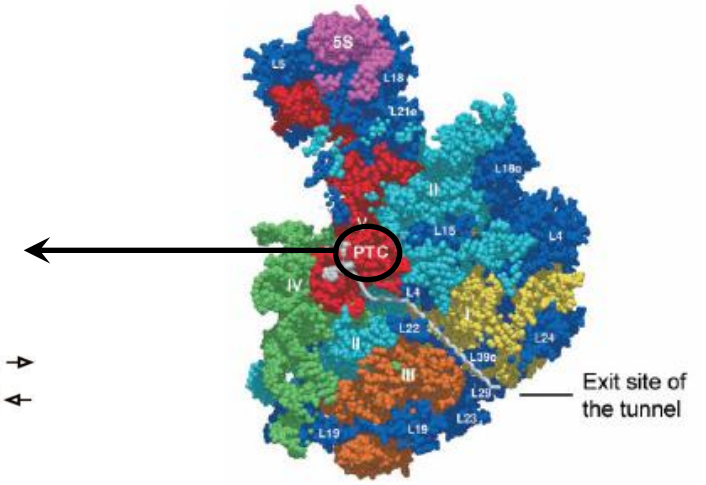
Streptomycin Binds to the 30S ribosome, prevents the transition from initiation to chain-elongation

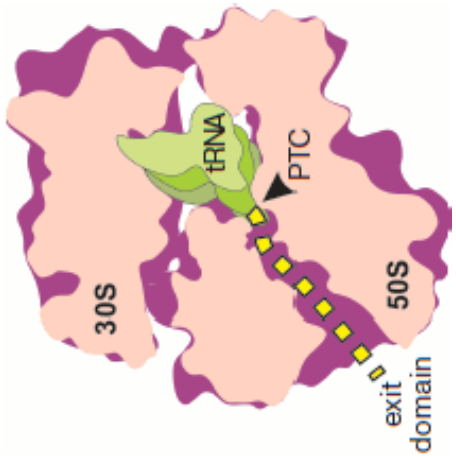
Erythromycin Binds to the 50S ribosome, and blocks the translocation

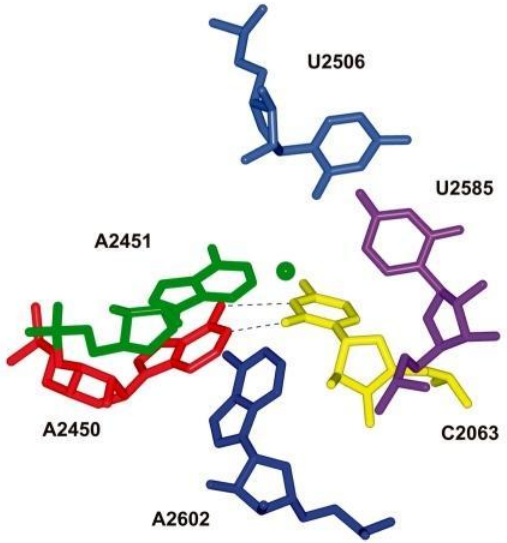
What Happens Inside the Ribosome?

Chemical and Physical Consideration of Protein Synthesis



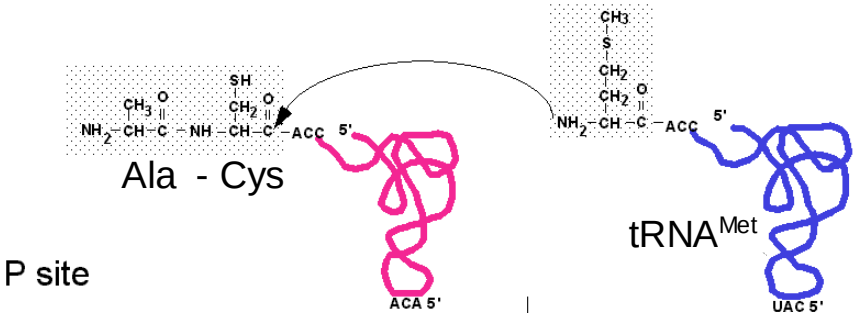


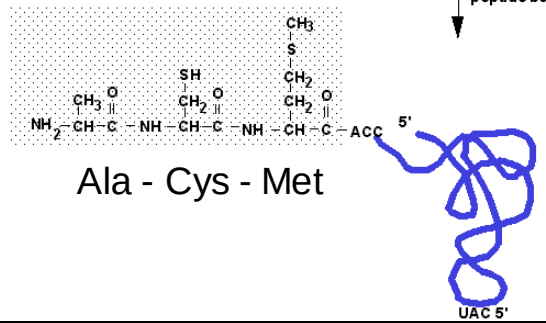




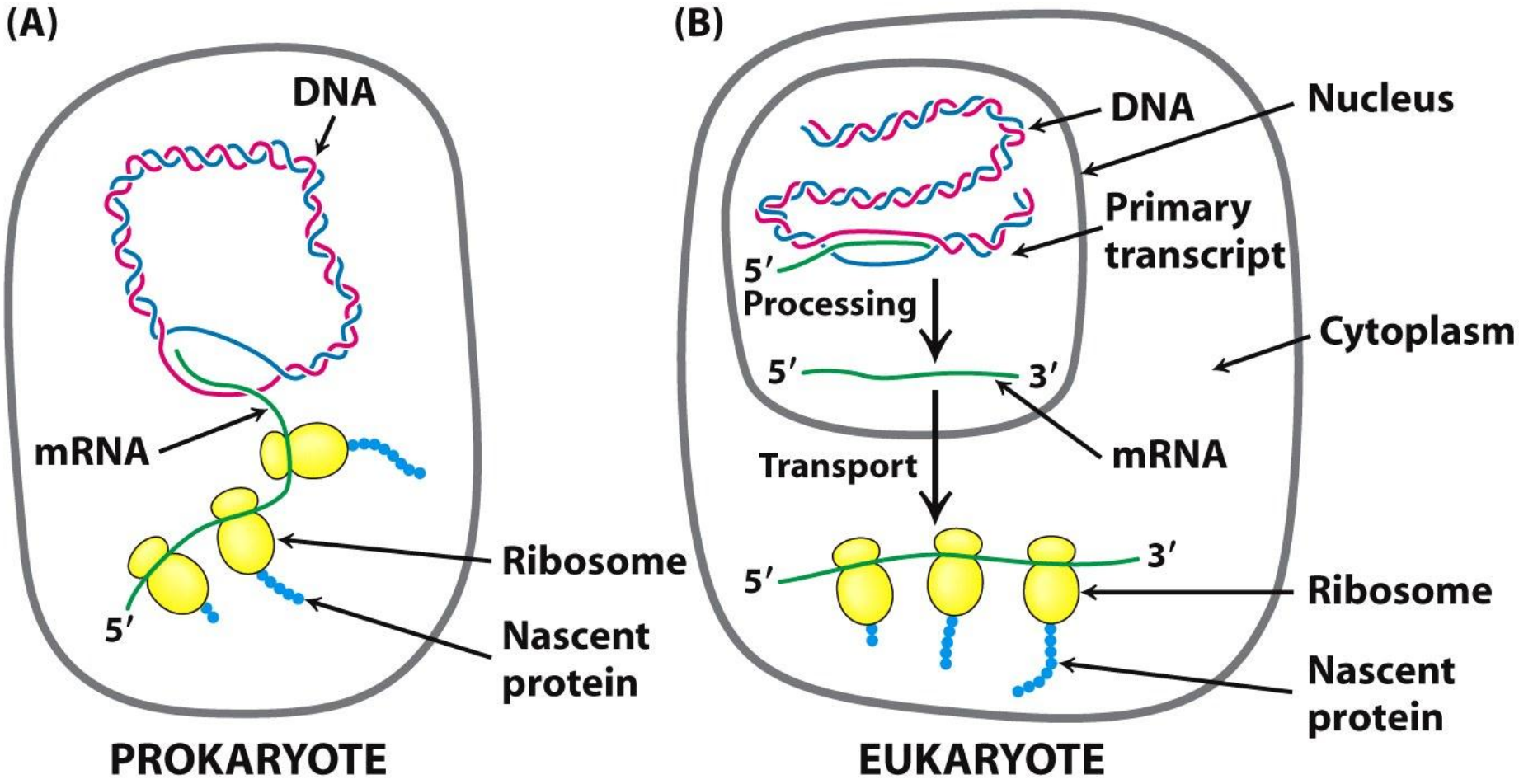
Peptidyl transferase center

Peptidyl transferase reaction

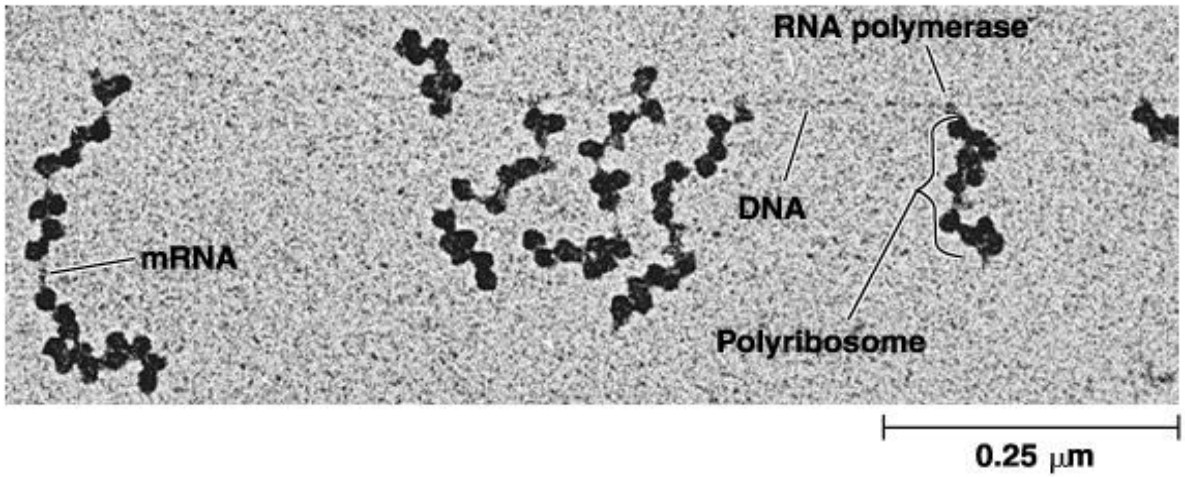




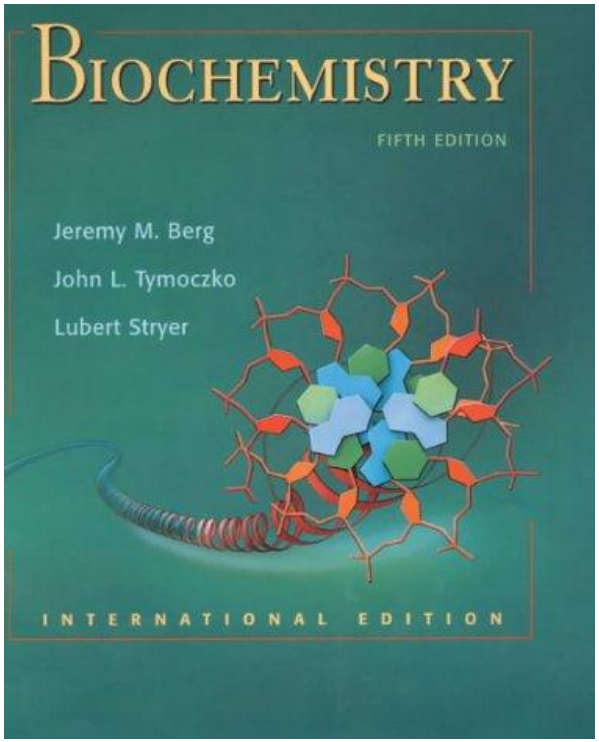
Time, Space and Correlation between Transcription and Translation



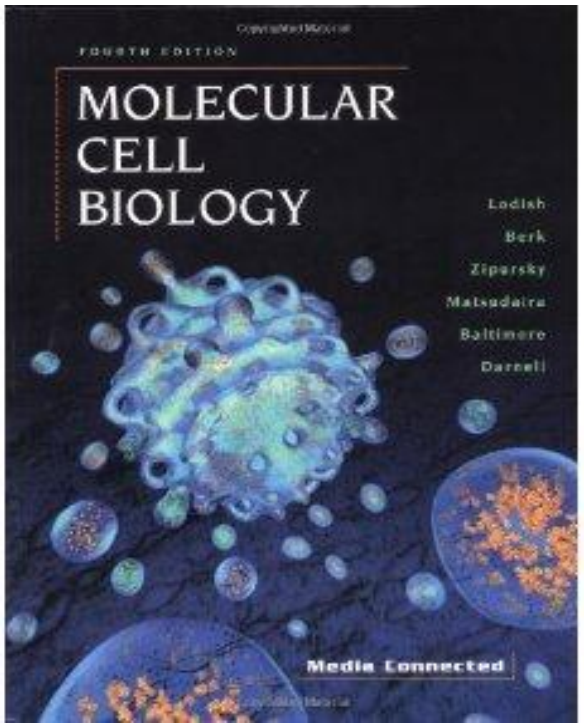
Time, Space and Correlation between Transcription and Translation



Suggested Textbook…



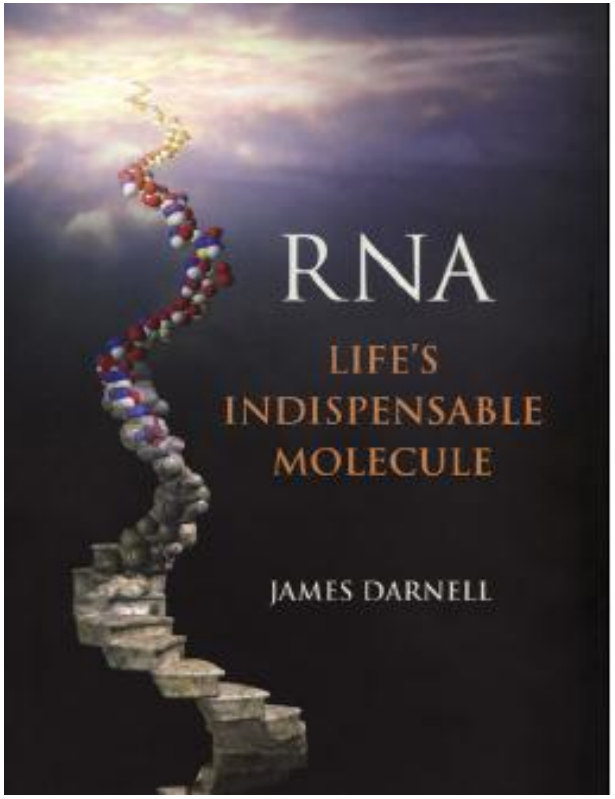
Stryer…



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=Ikq9AcBcohA

Overview

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