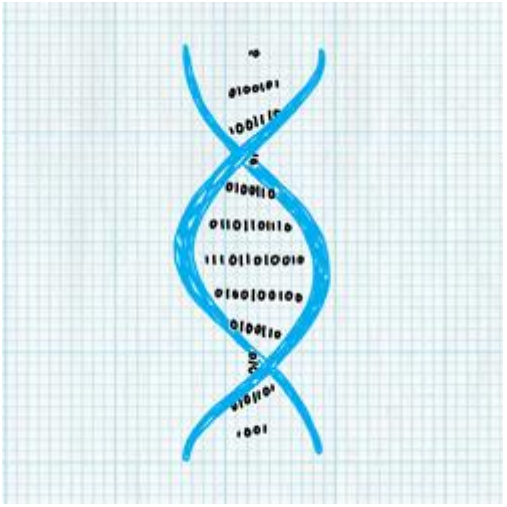
February, 2022

Science of Living System

BS20001 (2-0-0)



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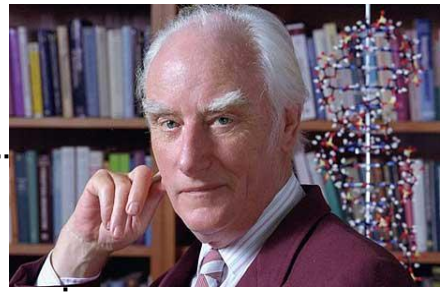
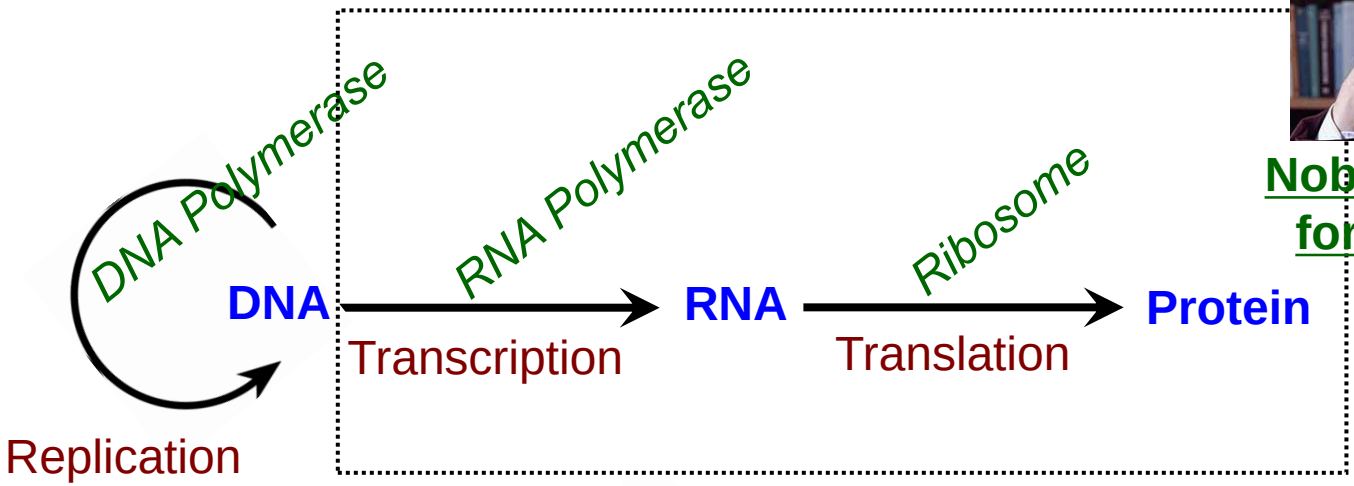
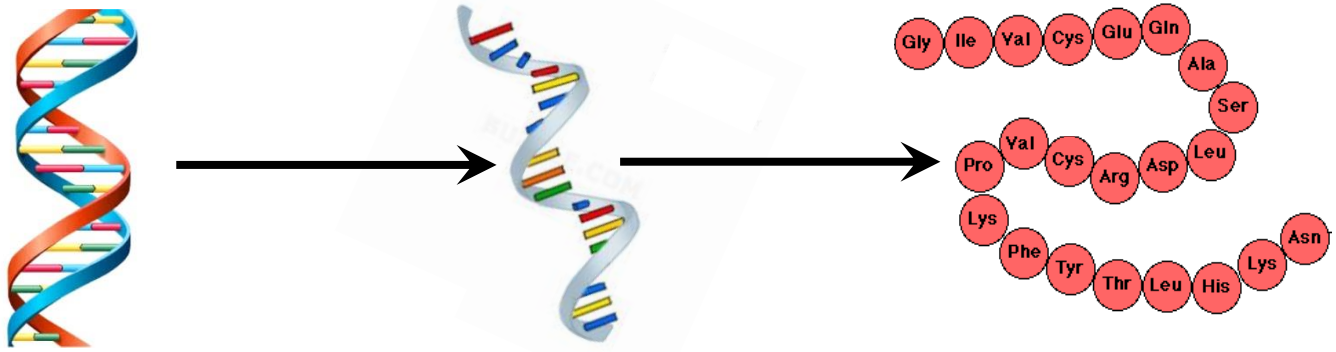
Overview of Transcription and Translation

Flow of Genetic Information: The Central Dogma of Molecular Biology



Nobel Prize in 1962 for DNA Structure





DNA

Polymer of nucleotides

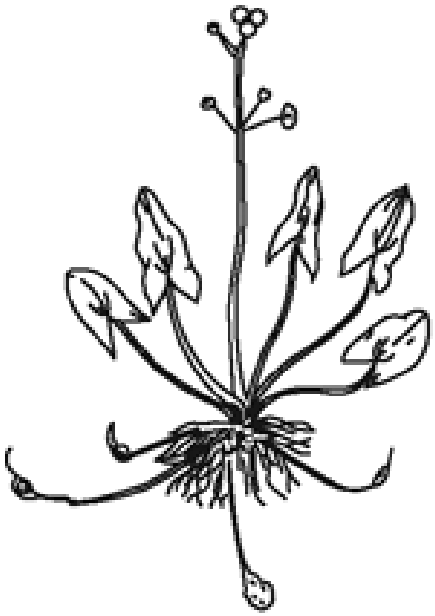
RNA

Polymer of nucleotides

Protein

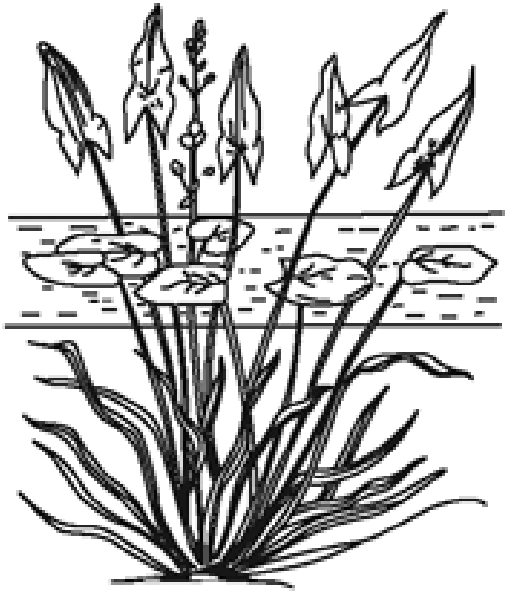
Polymer of amino acids

DNA: Contains the Instruction for Life



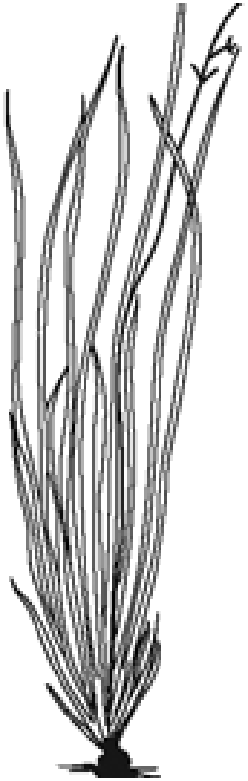
Plant-X

Completely terrestrial



Plant-Y

Partially submerged

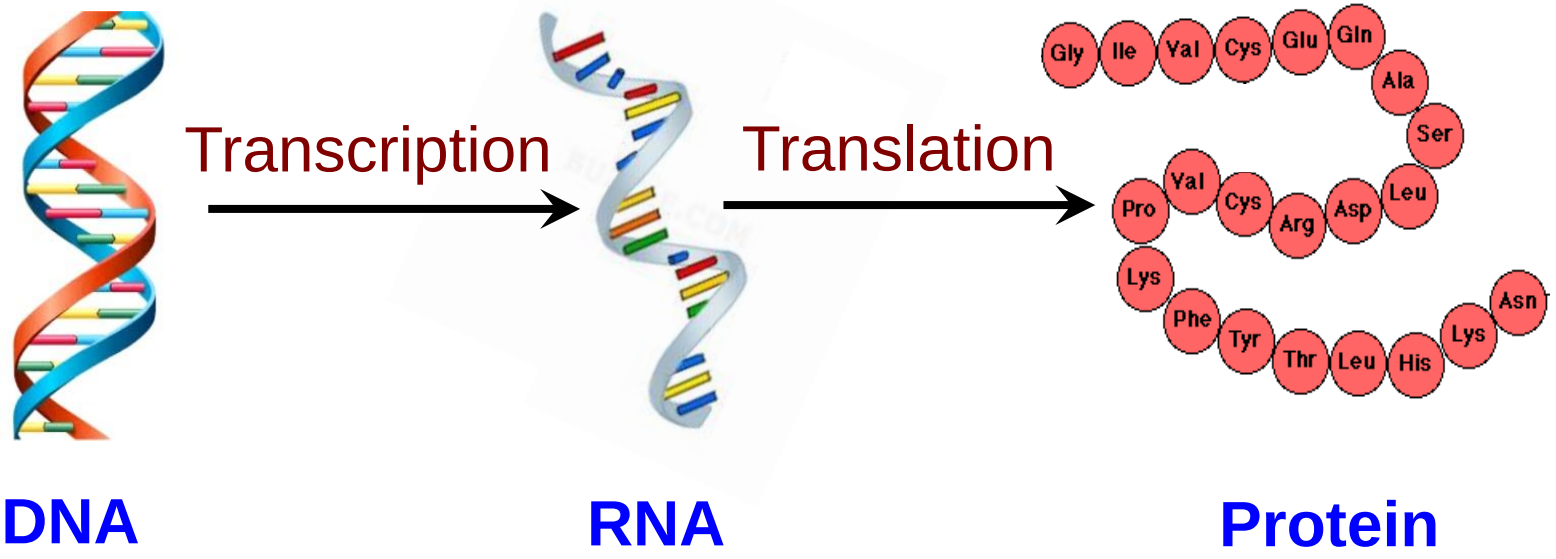


Plant-Z

Completely submerged

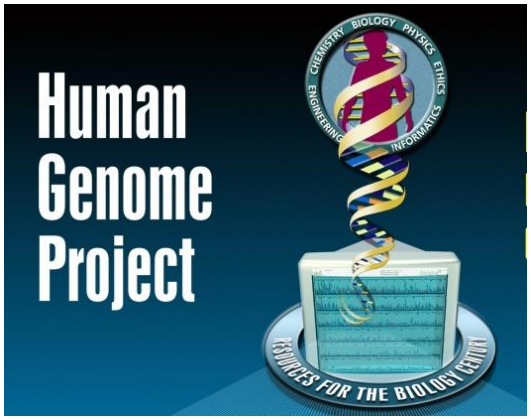
The phenotype (visible configuration) of the marsh plant *Sagittaria sagittifolia* depends on its environment

Correlations Between DNA Content and Its Down-stream Product

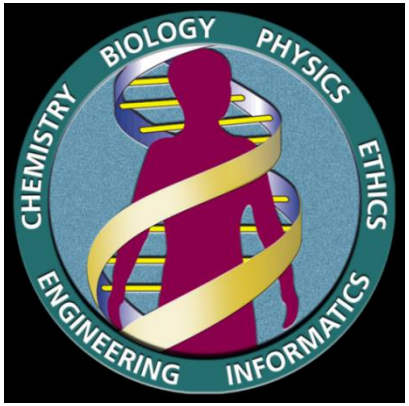


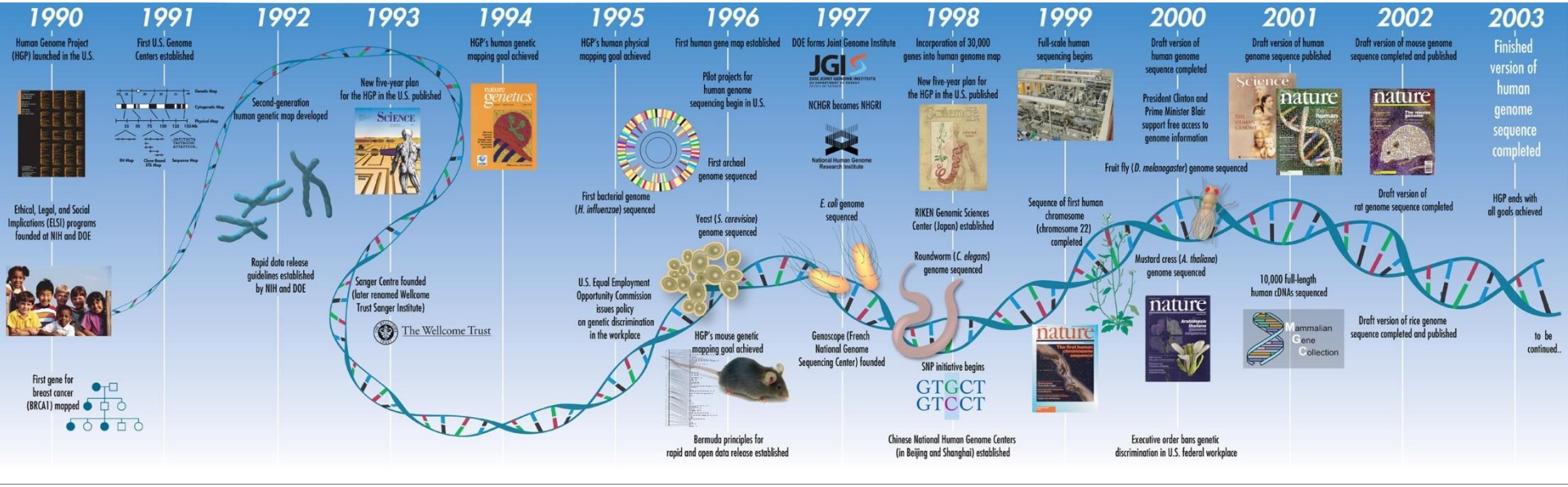
DNA **RNA** **Protein**

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

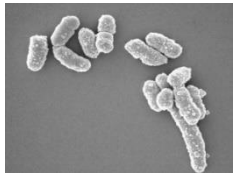
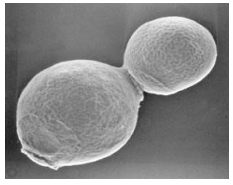


~21,000 human genes (appeared to be significantly fewer than previous estimates)





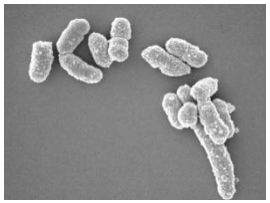
Genome Size, Gene Number, and Complexity of an Organism

	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	19,042
	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**

What is a Machine?

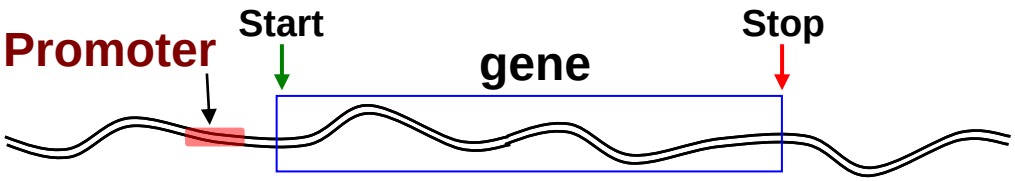
A piece of equipment with several moving parts that uses power to do a particular type of work.

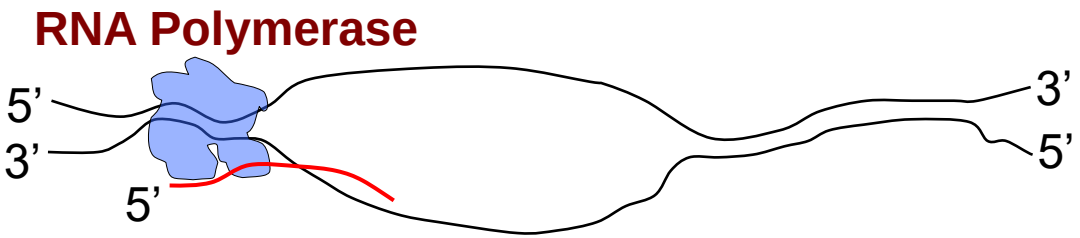
- Cambridge dictionary

Biological machines:

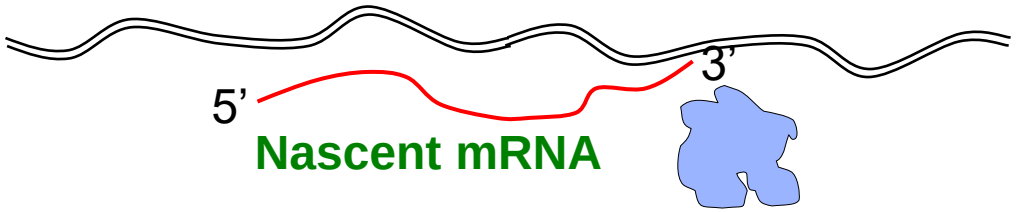
- DNA polymerase
- RNA polymerase
- Ribosome

Transcription: Involved Machineries and Processes





Initiation Elongation Termination



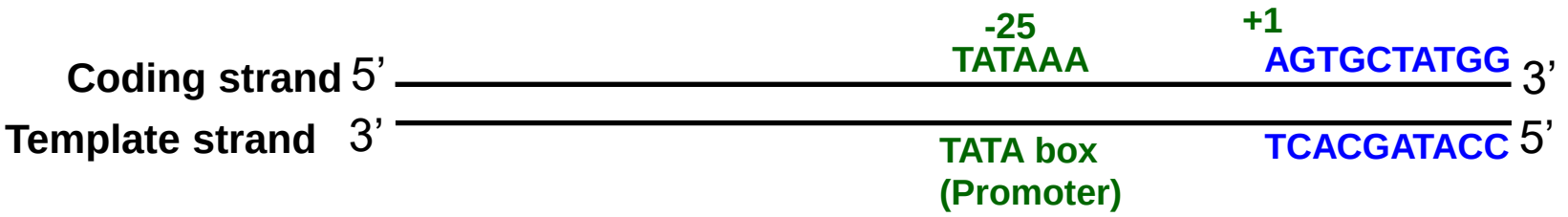
Key points to be discussed

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

Promoter for Transcription

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

Prokaryotic promoter



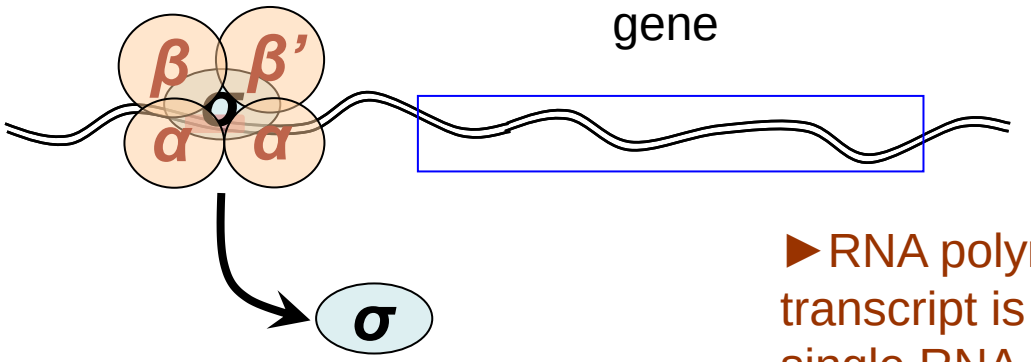
Eukaryotic promoter

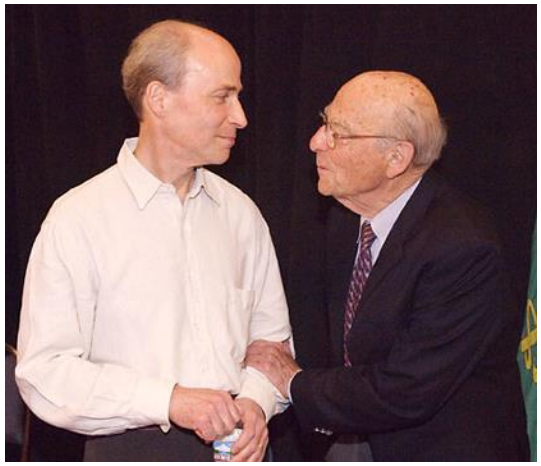
RNA Polymerase

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

Holoenzyme: ***α, α, β, β' and σ***

Coreenzyme: ***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)

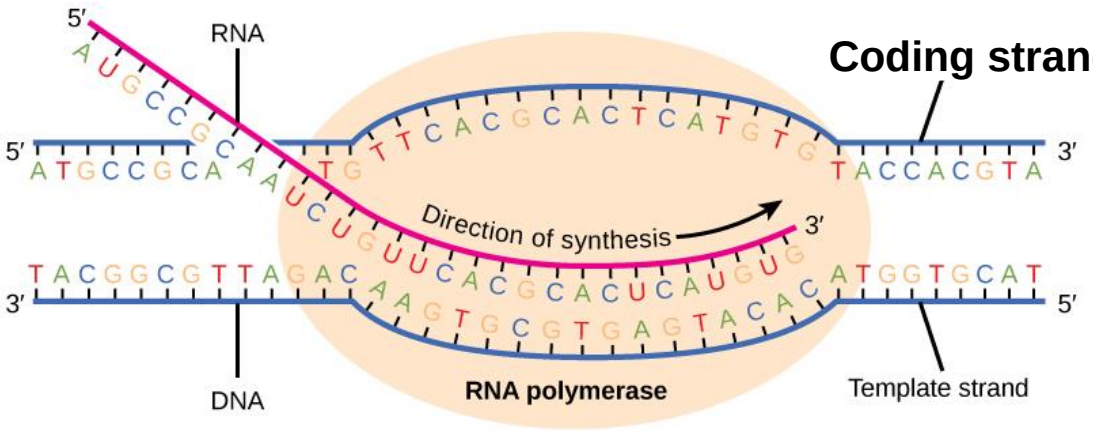
RNA Molecules in *E. coli*	
mRNA	5%
tRNA	15%
rRNA	80%

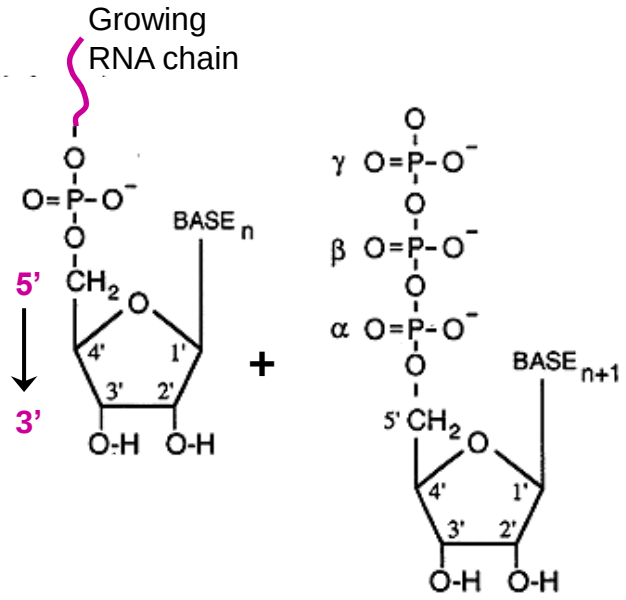
Who transcribes this huge pool of rRNA and tRNA?

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

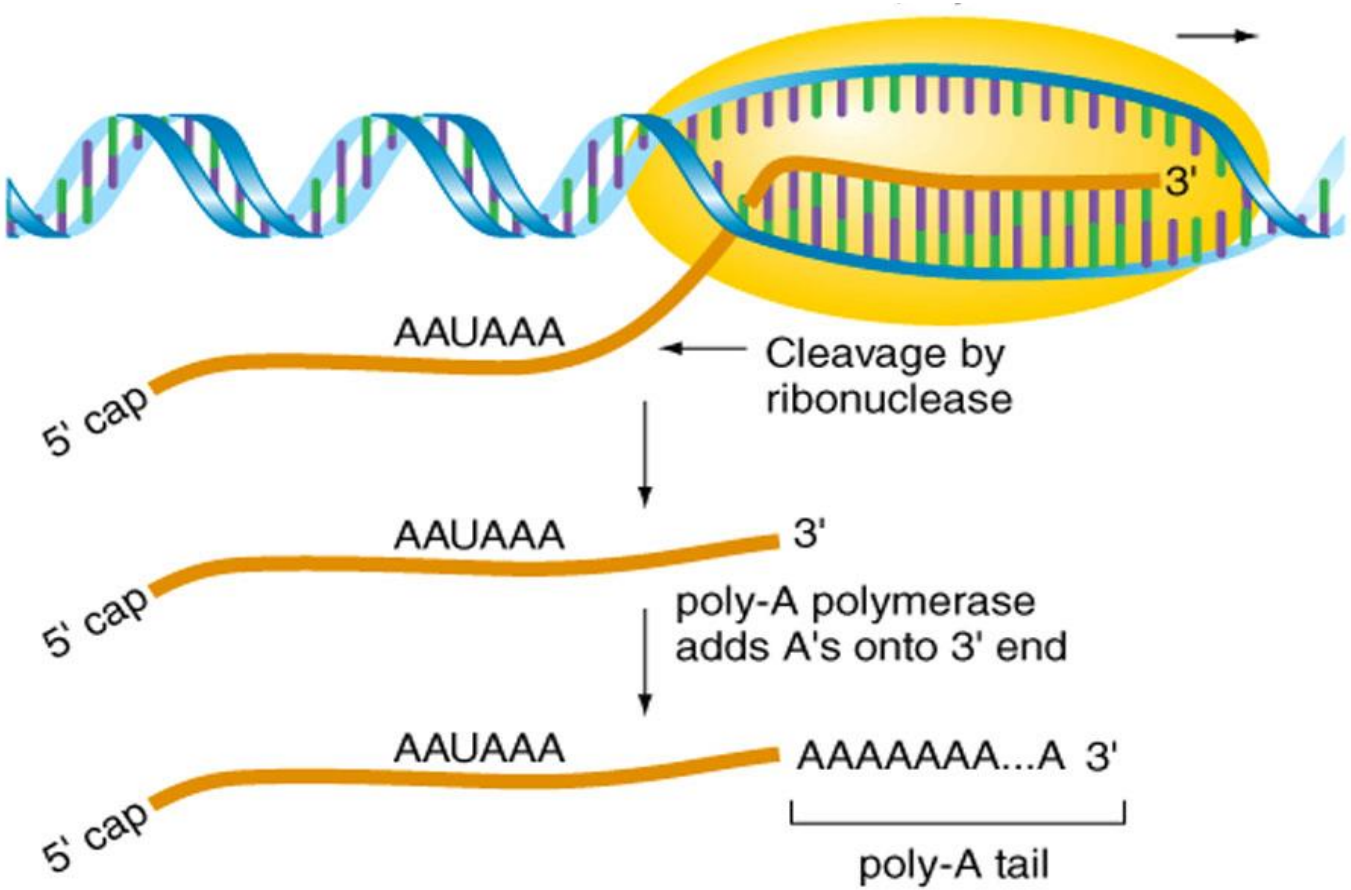




Ribonucleotide

Eukaryotic Transcripts Need to be Processed

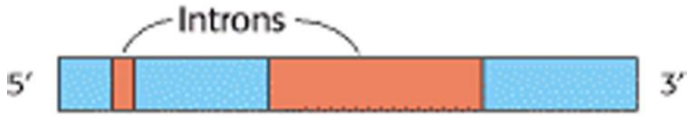
►**Ends of a nascent mRNA acquire a 5’ cap and a 3’ poly A tail**



- **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



β-Globin gene

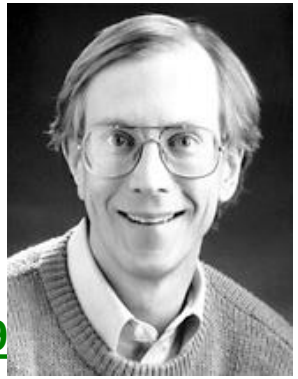
Transcription
Cap formation
Poly(A) addition

Primary transcript

Splicing

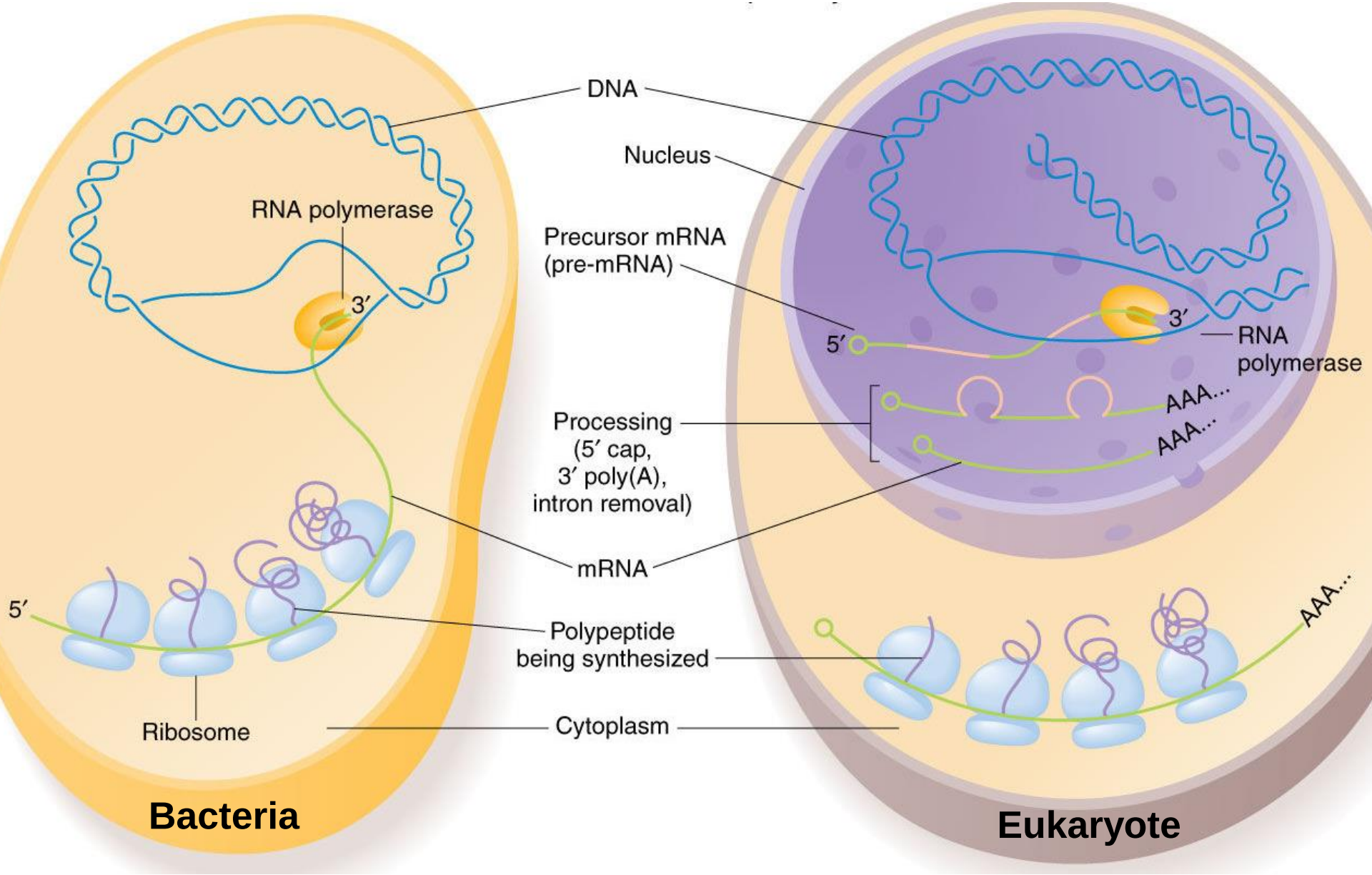
β-Globin mRNA

Thomas Cech
Nobel prize in 1989

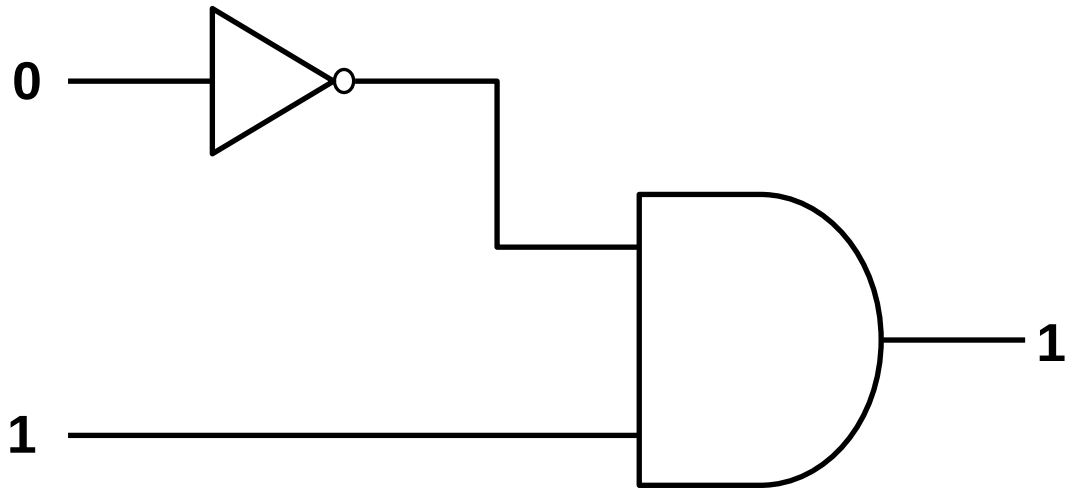


****Splicing generates more variation**

Transcription: At a Glance



Regulation of Gene Expression
(Biological circuits)



Regulation of Gene Expression

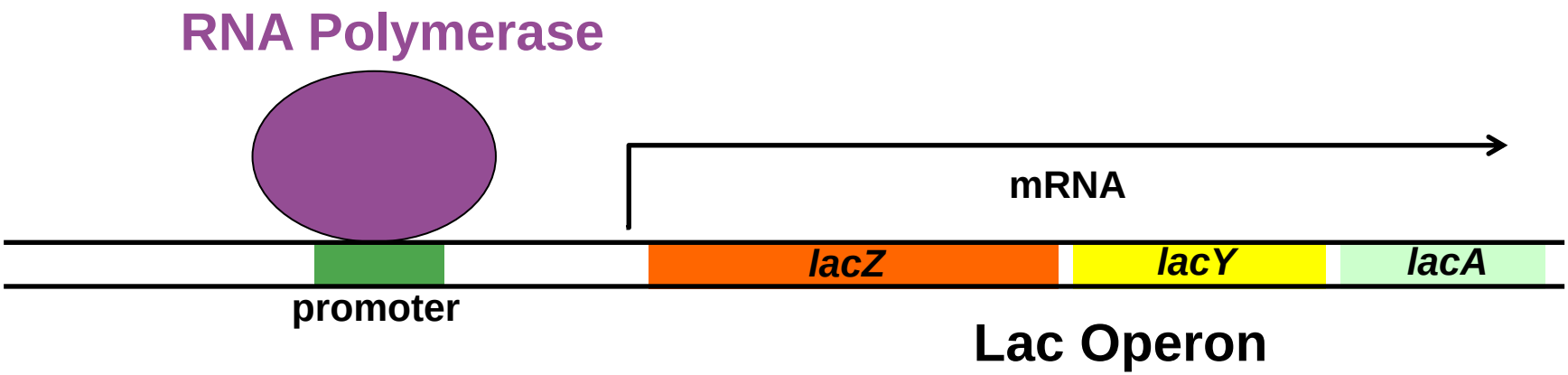
Each cell contains all the genetic material for growth and development

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

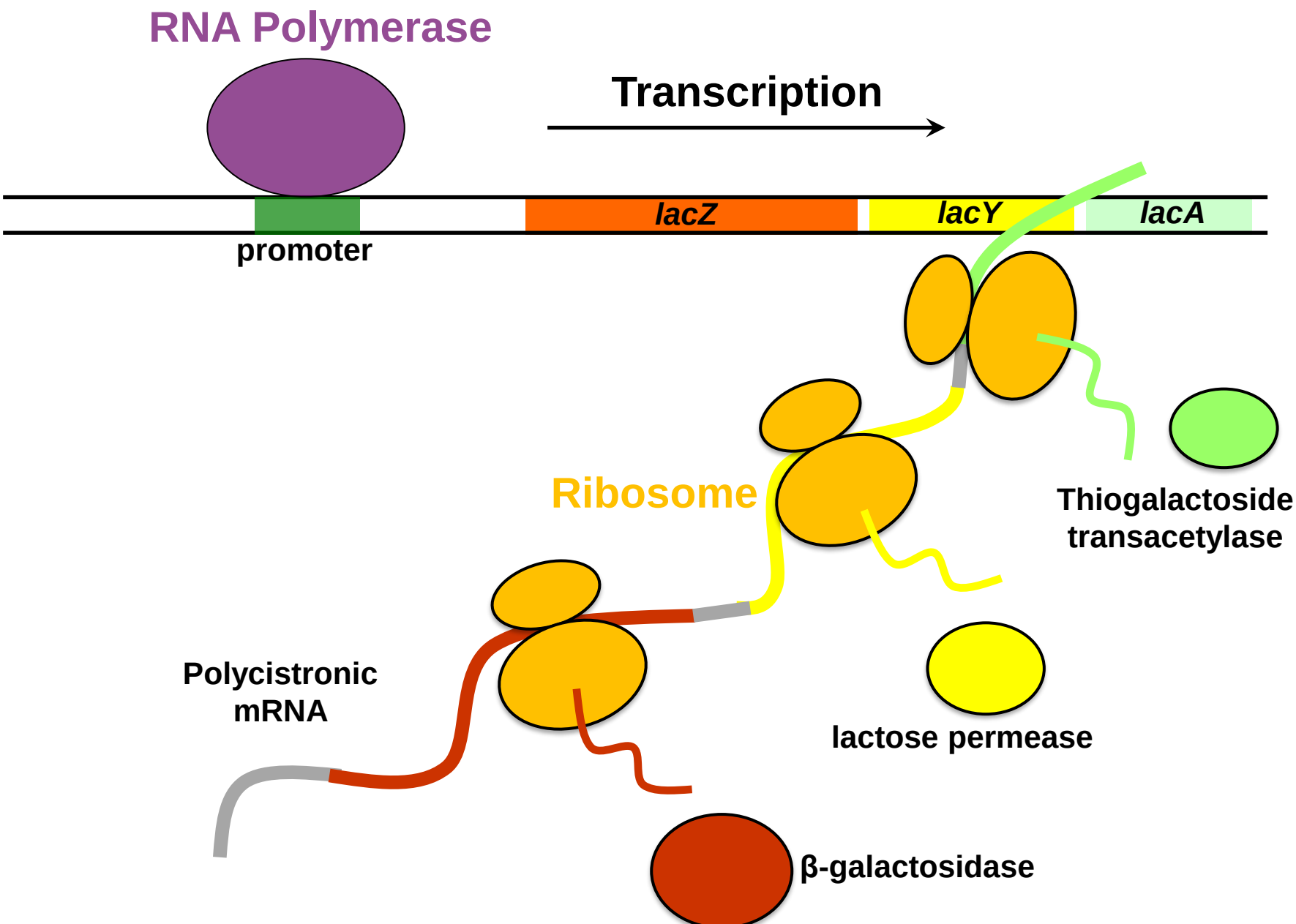
Lac Operon: A Classic Example of Bacterial Gene Expression Control

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

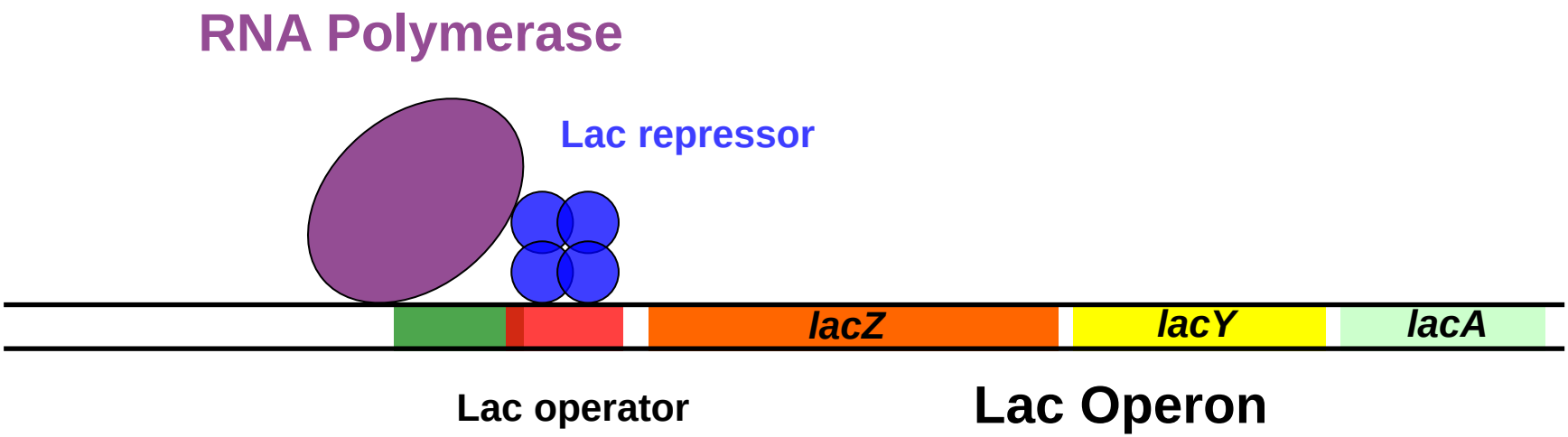


lacZ	β-galactosidase	Breaks lactose into galactose and glucose.
lacY	lactose permease	Imports lactose into the bacterial cell.
lacA	thiogalactoside transacetylase	Cell detoxification.

Functional Outcome of Lac Operon

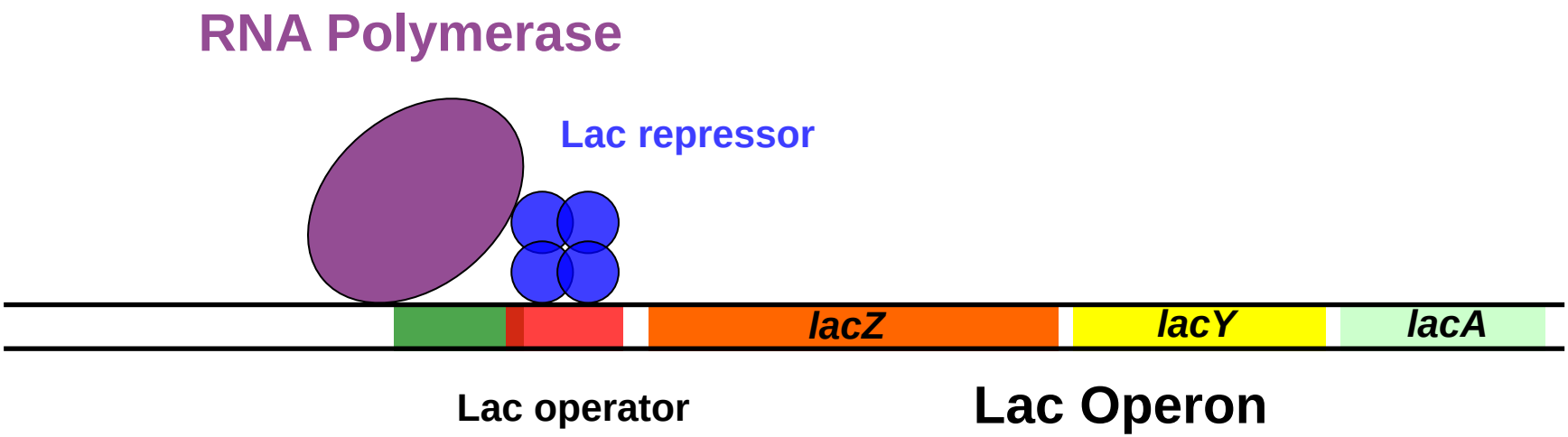


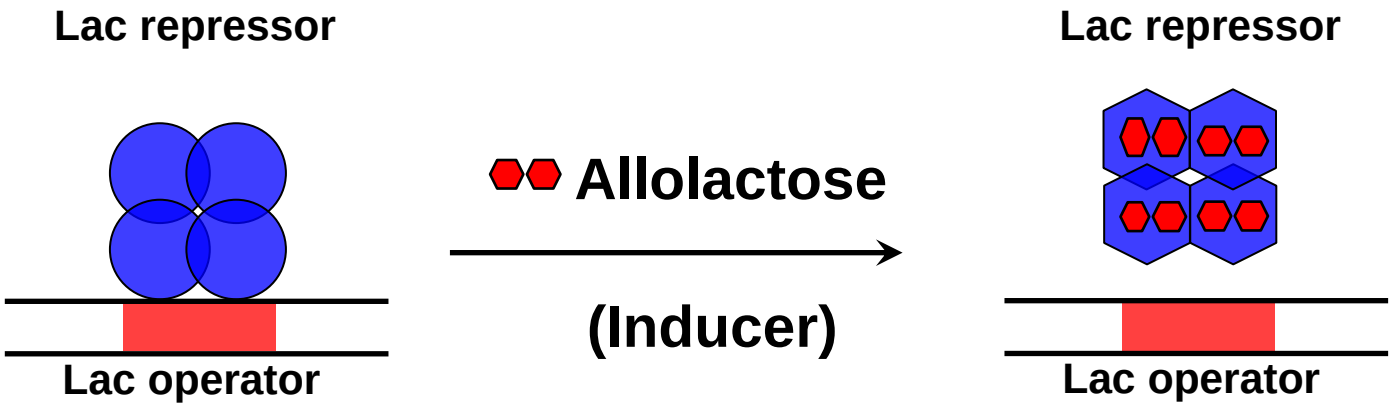
Lac repressor is a negative regulator of the Lac operon



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

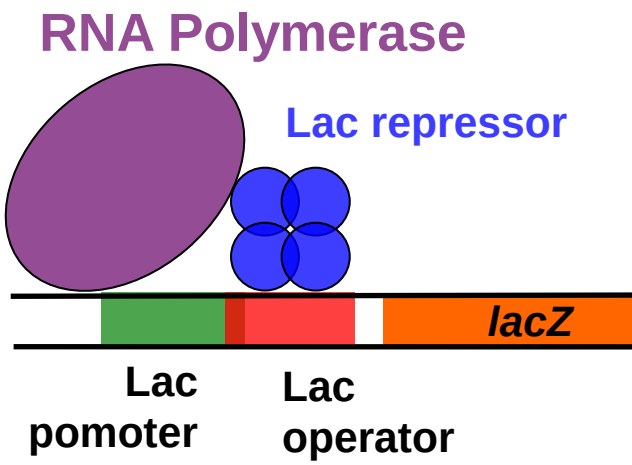
Lactose (Allolactose) Can Displace Lac Repressor From the Operator Site

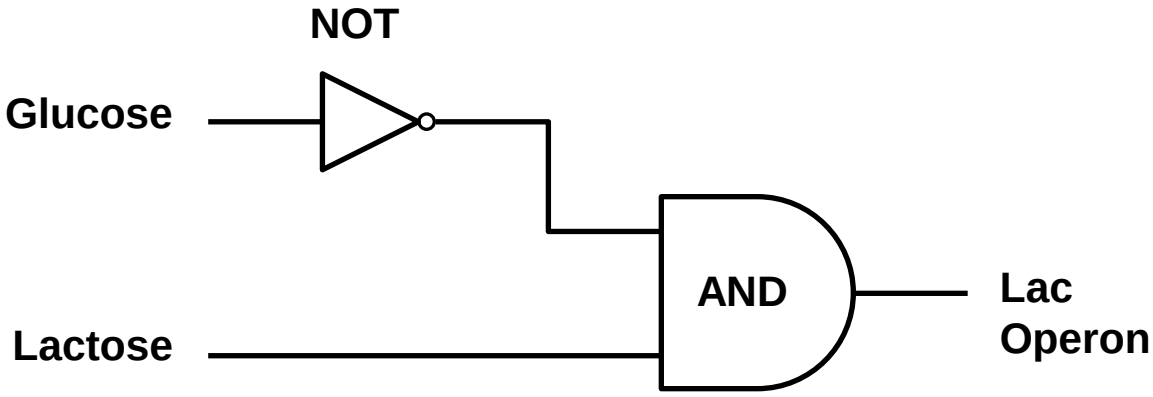




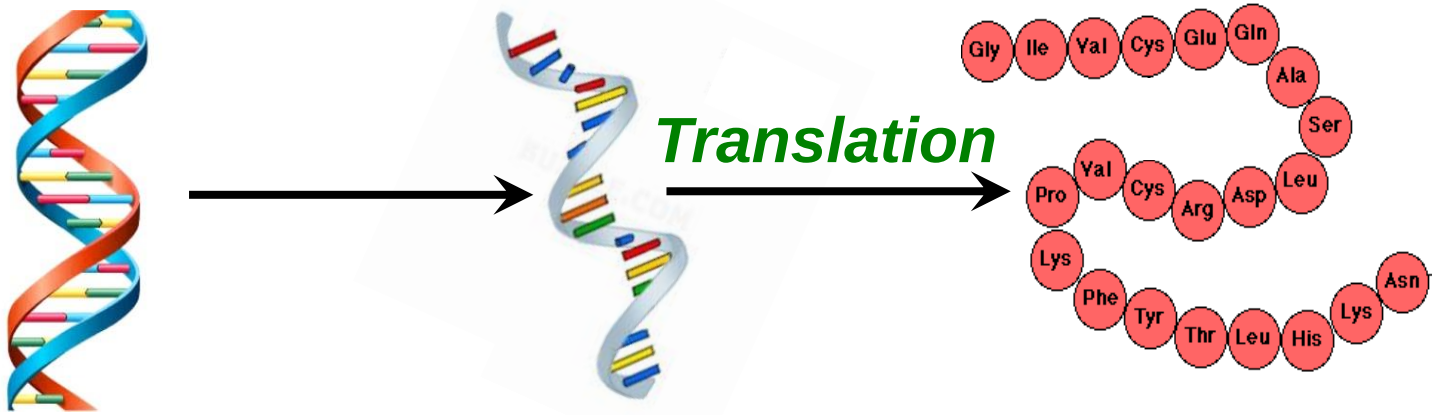
Four Possible Situations

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

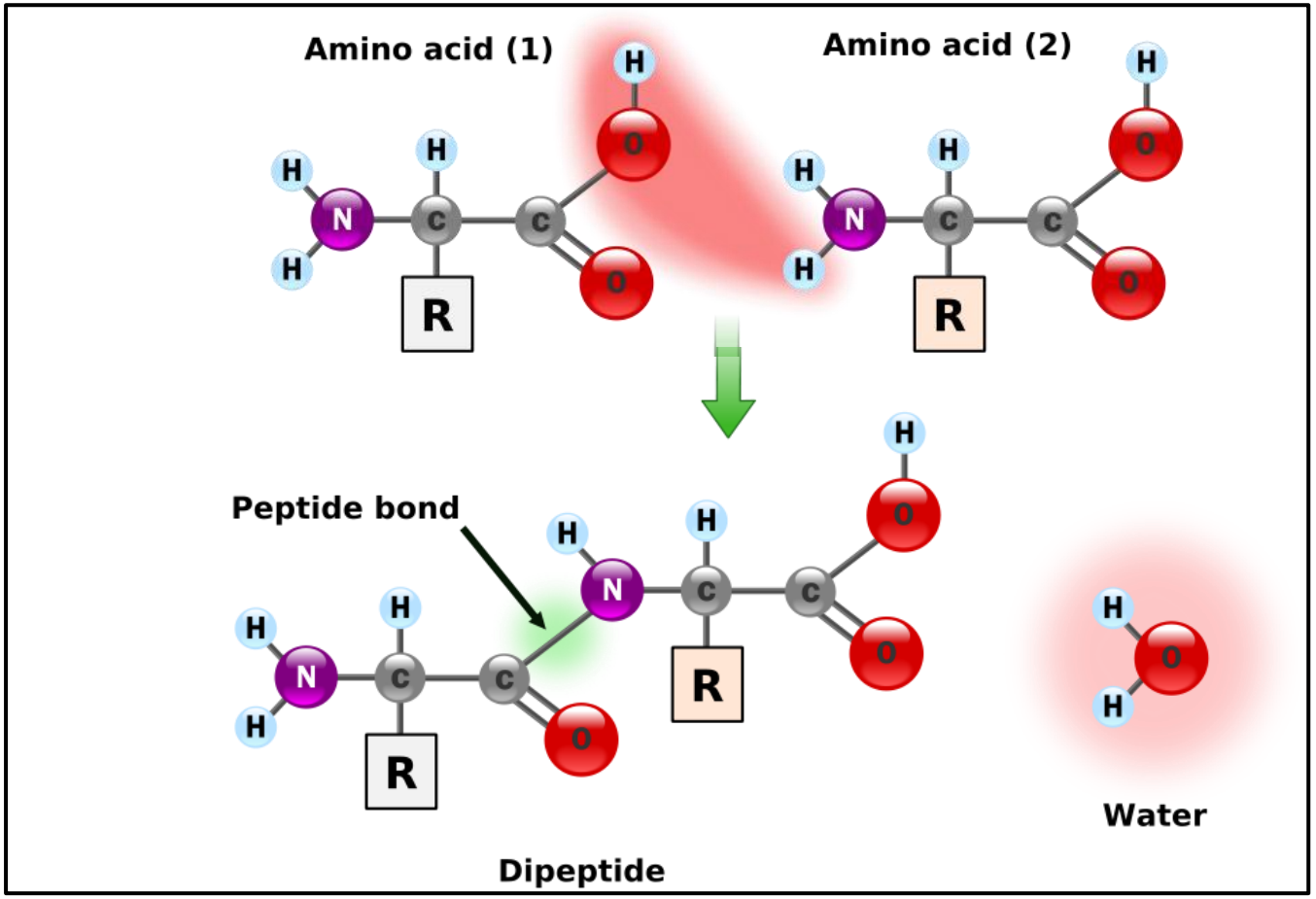
RNA

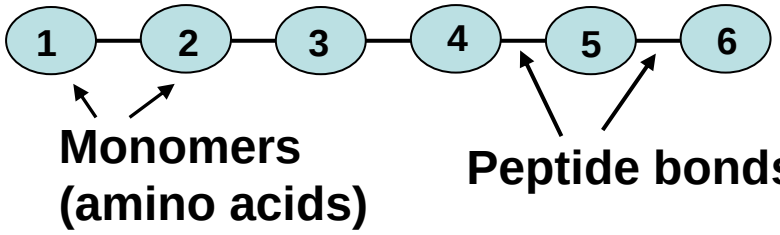
Polymer of nucleotides

Protein

Polymer of amino acids

How Amino Acids are Linked Together





Polymer: Polypeptide and Protein

Translation

Template for protein synthesis

5’ ——————————— 3’ mRNA

5’UAAGGAGAAUCGUCAUGAAGAGGCCC……UAAUUA 3’

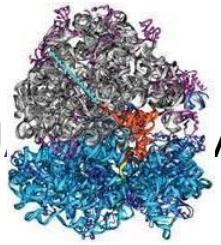
(RBS)

Start codon

Stop codon

Met–Lys–Arg–Pro……

Polypeptide



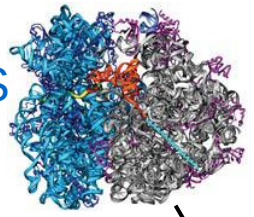
Nascent polypeptide

5’U AAUCGUCAUGAAGAGGCCC……UAAUUA 3’

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

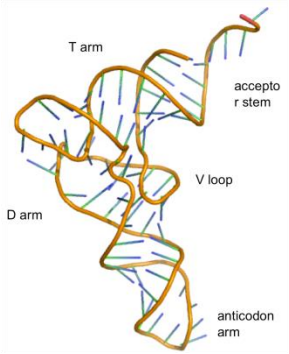
Protein synthesizing machinery

Ribosome: (rRNA + proteins)



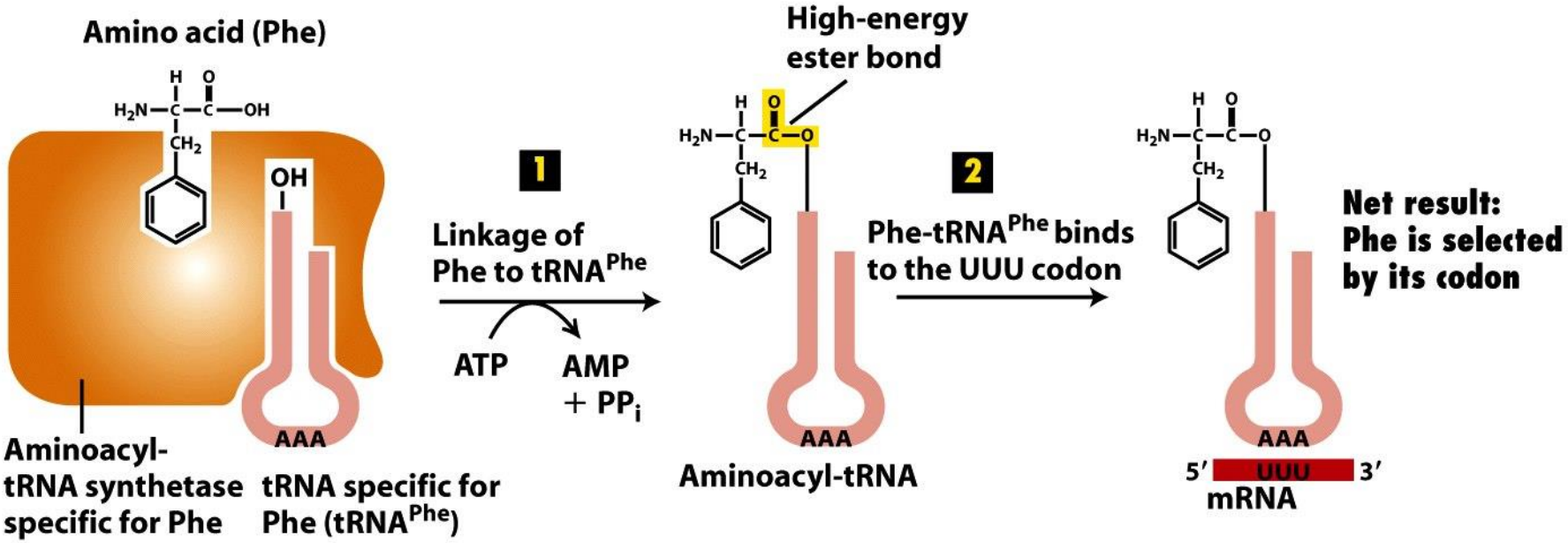
16S rRNA
21 proteins

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, multiple codons may specify a single amino acid

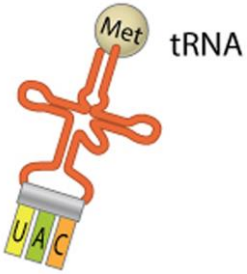
Khorana, Nirenberg, Holley

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 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
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

Translation: Involved Machineries and Processes

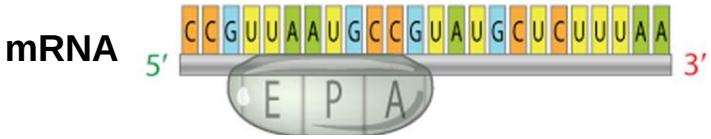


mRNA

Ribosome

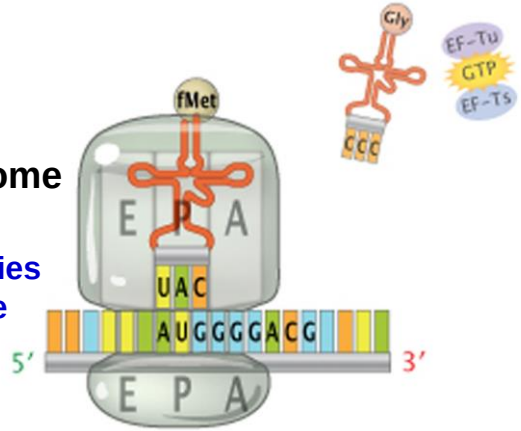
Amino acid

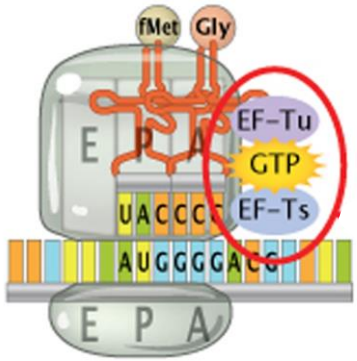
tRNA



50S ribosome

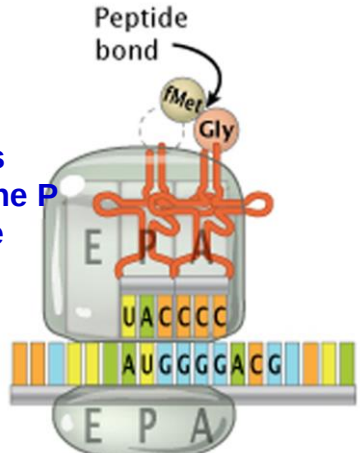
tRNA^{fMet} occupies the P site of the ribosome



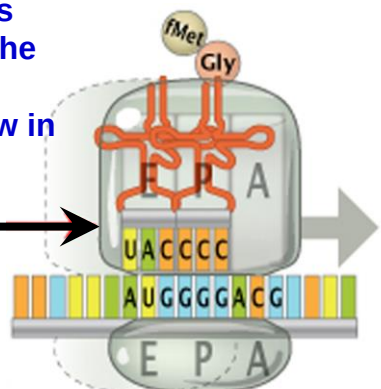


Next charged tRNA and associated translation factors enter the A site

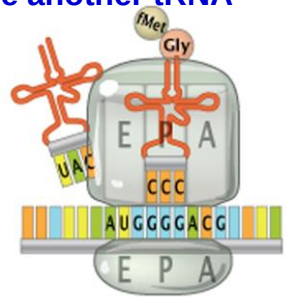
Peptide bond forms between the aa in the P and A sites, and the tRNA in the P site releases its aa



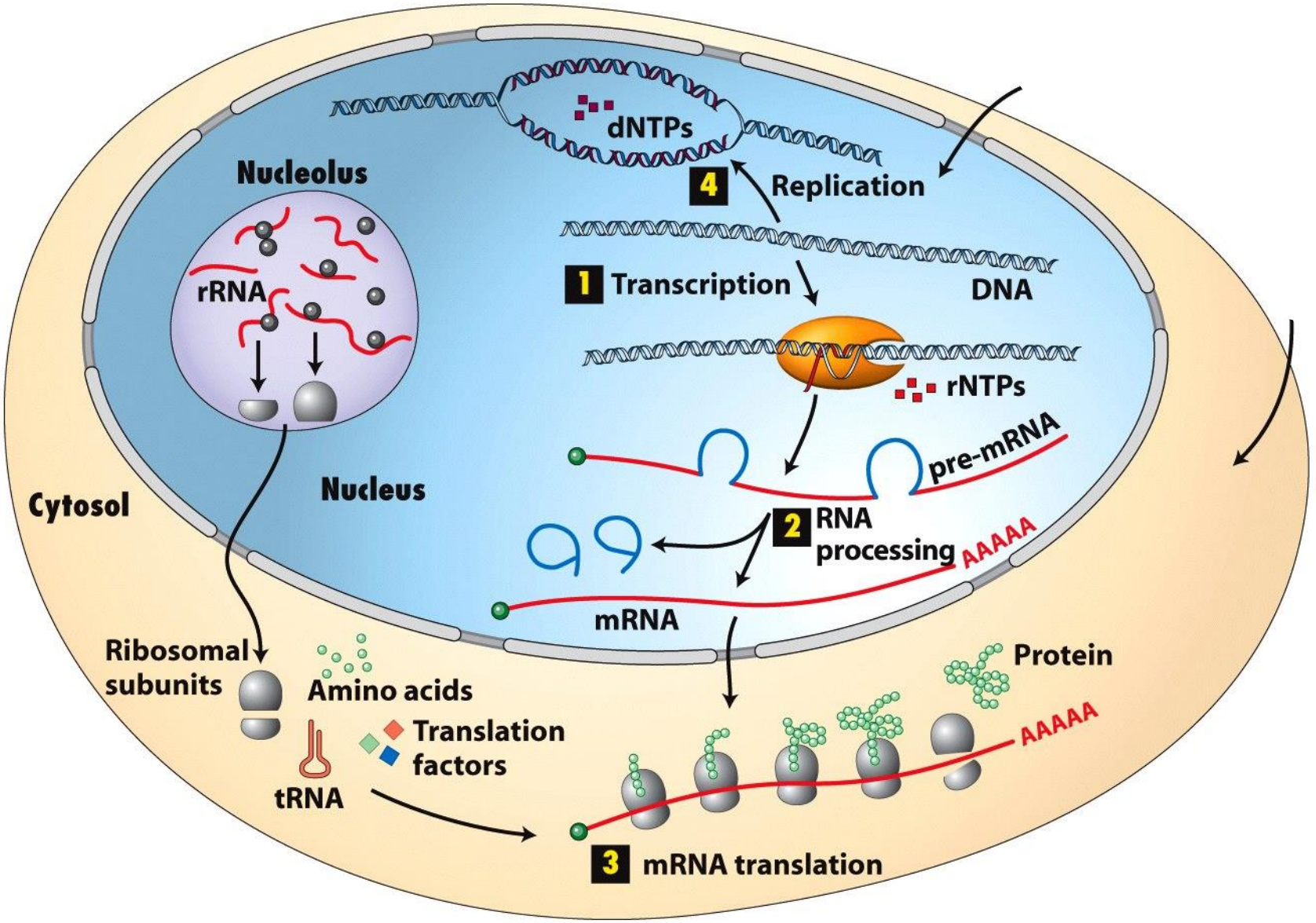
The ribosome moves down the mRNA to the next codon and the uncharged tRNA now in the E site



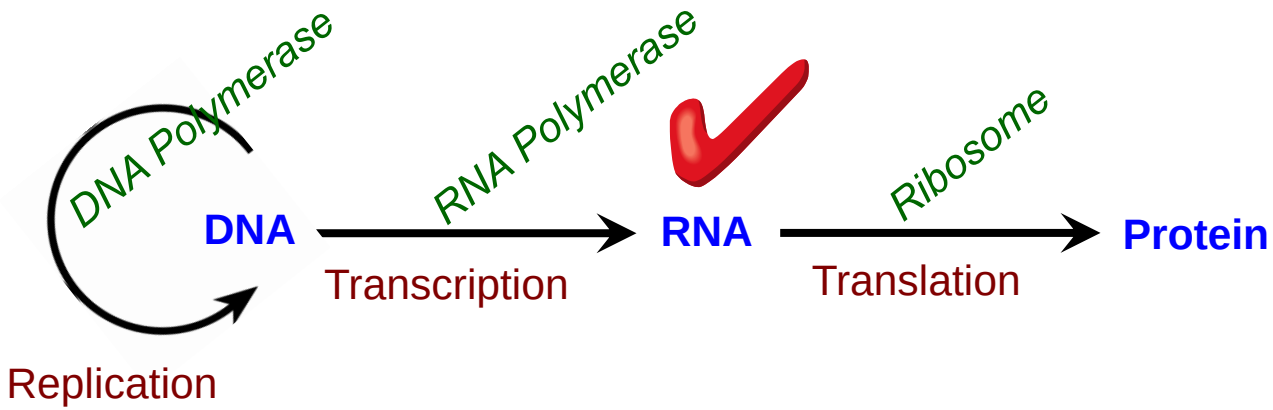
E site tRNA releases and A site is now ready to receive another tRNA

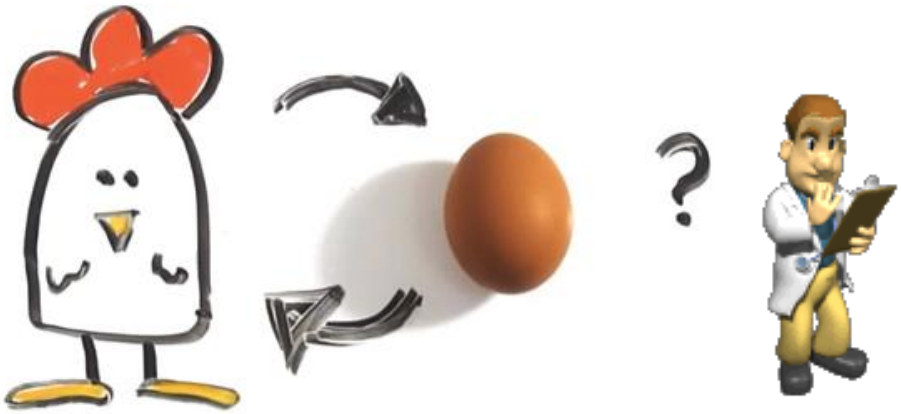


Translation: At a Glance



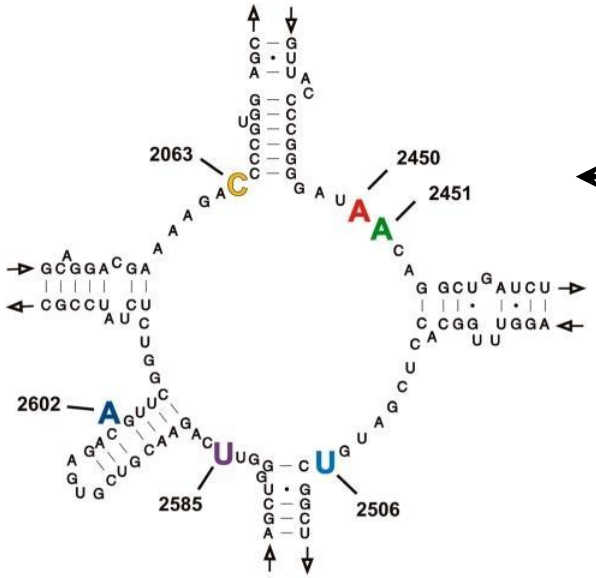
Which Came First? Nucleic acids or Proteins

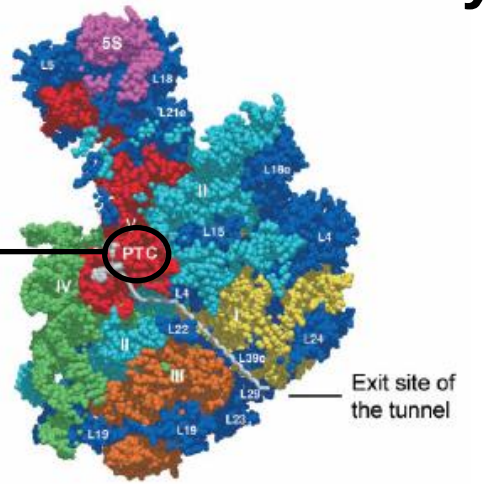


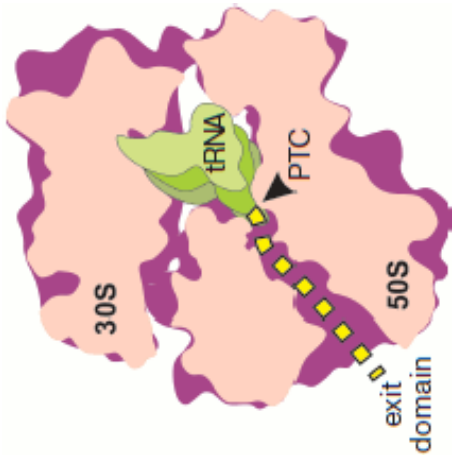


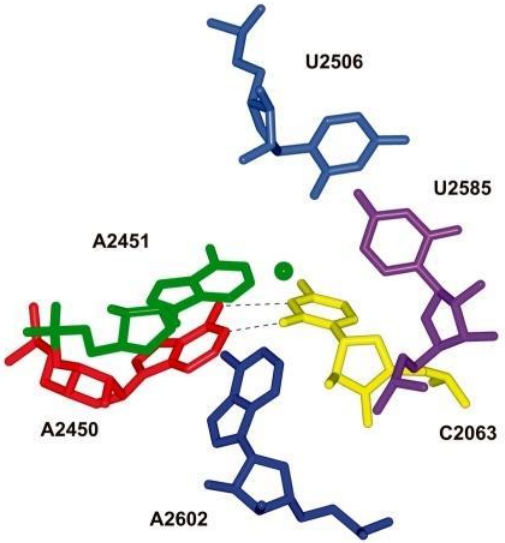
►**RNA has enzymatic activity**

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



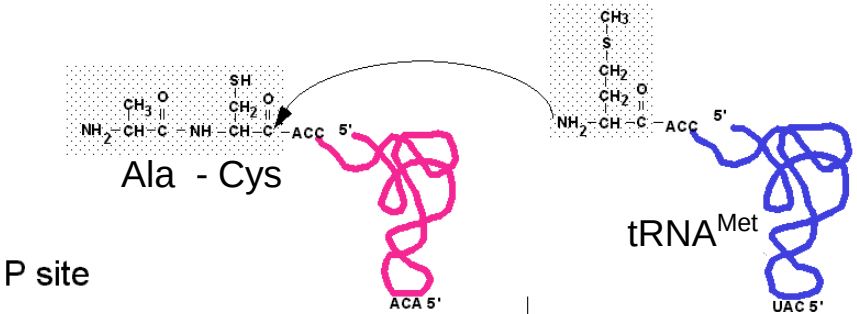




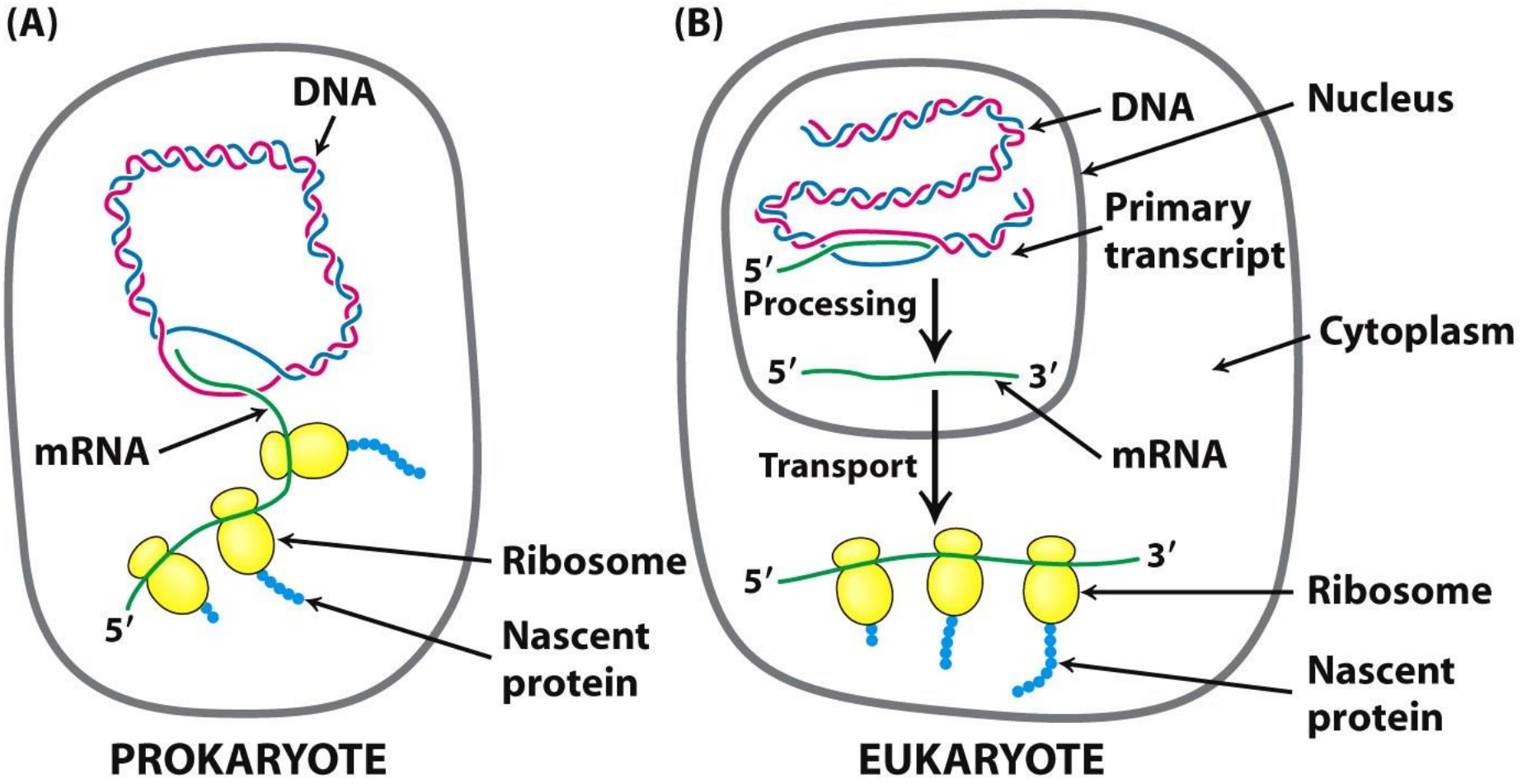


Peptidyl transferase center

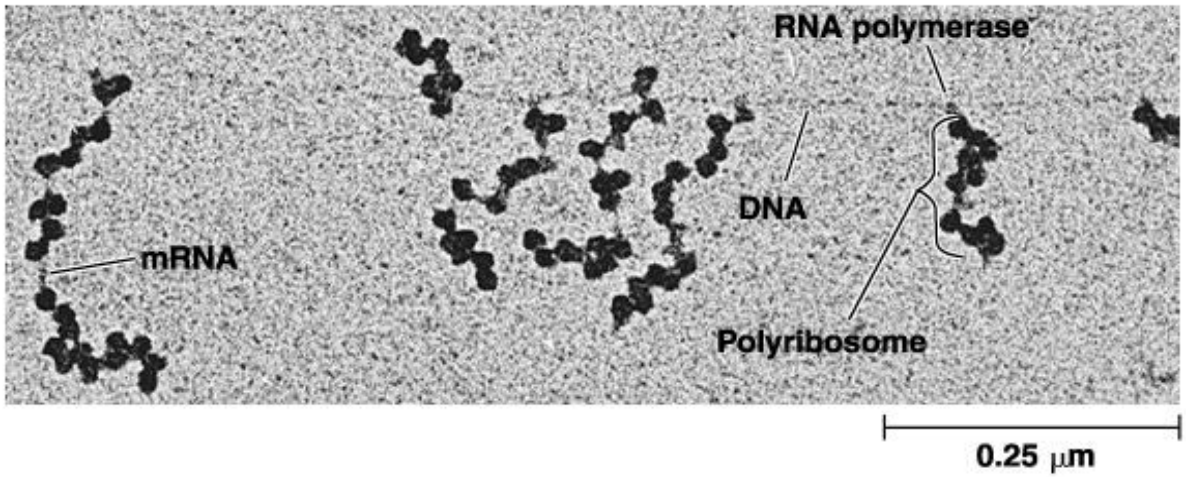
Peptidyl transferase reaction



Time, Space and Correlation between Transcription and Translation



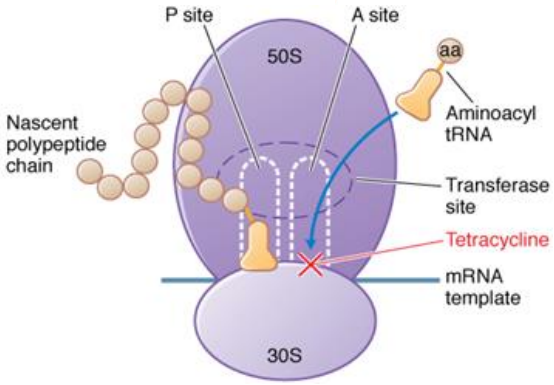
Time, Space and Correlation between Transcription and Translation



Translation Machineries: Attractive Targets For Therapeutics

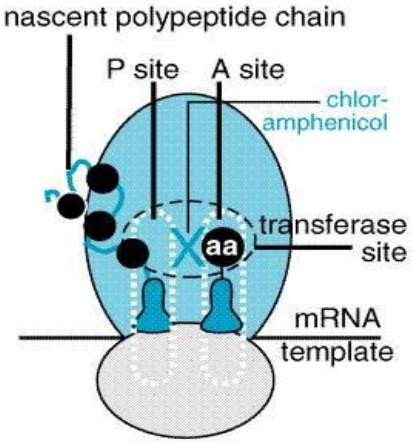
Tetracycline

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



Chloramphenicol

Blocks the peptidyl transferase reaction on 50S ribosomes

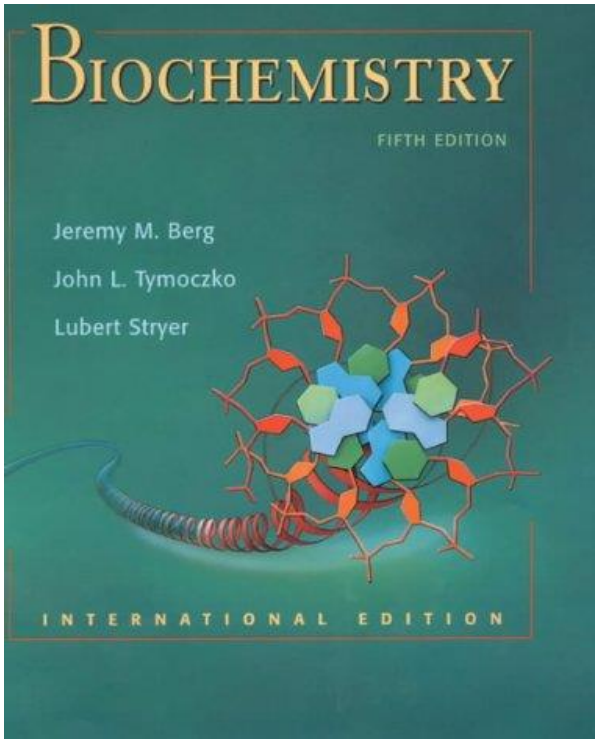


Streptomycin Binds to the 30S ribosome, prevents the transition from initiation to chain-elongation

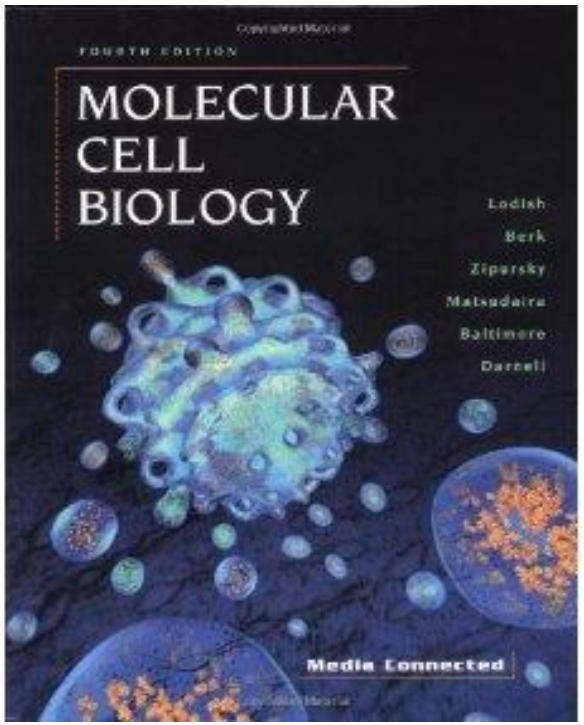
Erythromycin Binds to the 50S ribosome, and blocks the translocation

Why ribosome is an attractive target for the development of antibiotics?

Suggested Textbook…



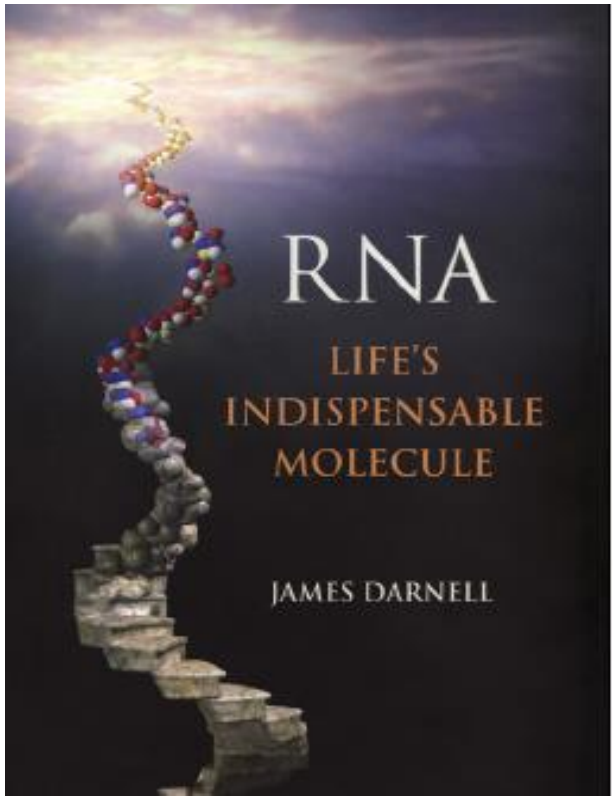
Stryer…



Baltimore, Lodish..

Extra Resources

Further Reading...



James Darnell

Videos…

mRNA synthesis (Transcription)
https://www.youtube.com/watch?v=_C9Un4dlpR4

Protein synthesis (Translation)
https://www.youtube.com/watch?v=Ikq9AcBcohA

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