27th July, 2016

Science of Living System

BS20001 (2-0-0)

Dibyendu Samanta

School of Bio Science

Email: dibyendu.samanta@iitkgp.ac.in

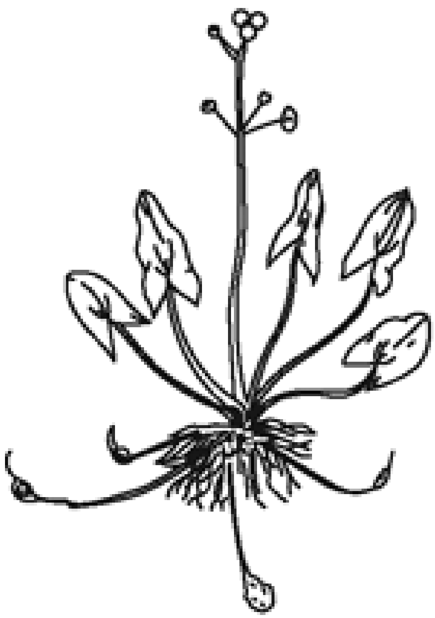
Tel: 03222-260295

Class	Date	Schedule
1	20/7/16	L-1 (Nucleic acids: How is information stored and maintained)
2	27/7/16	L-2 (Transcription & Translation: How stored information get processed)
3	3/8/16	L-3 (Protein structure: Making sense of processed information)
4	10/8/16	L-4 (Enzymes: Working principles & concept of enzyme technology)
5	17/8/16	L-5 (Photosynthesis and Respiration: Making and breaking of sugars)

Overview of Transcription and Translation

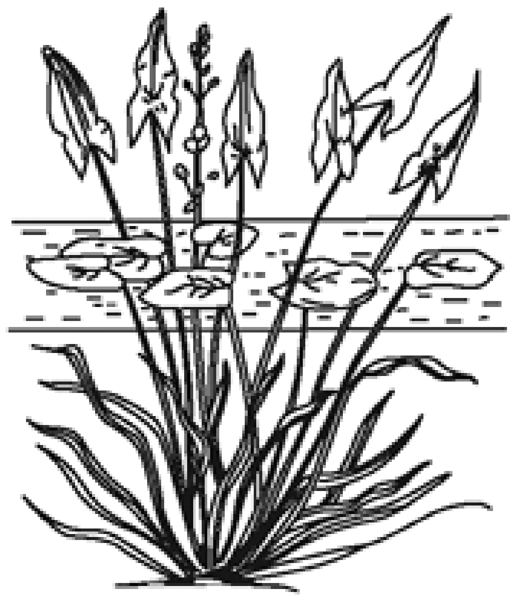
(How stored information get processed)

DNA: Contains the Instruction for Life



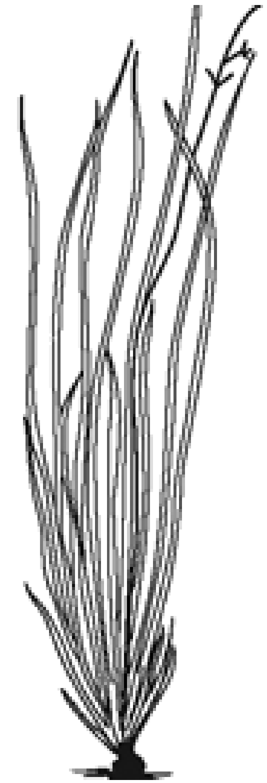
Plant-X

Completely terrestrial



Plant-Y

Partially submerged

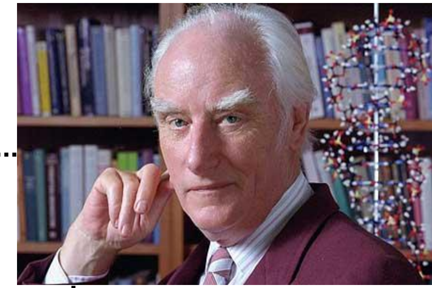


Plant-Z

Completely submerged

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

Flow of Genetic Information: The Central Dogma of Molecular Biology



Nobel Prize in 1962 for DNA Structure

DNA Polymerase

RNA Polymerase

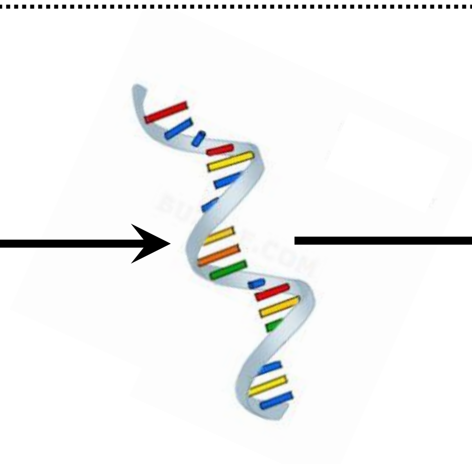
Ribosome

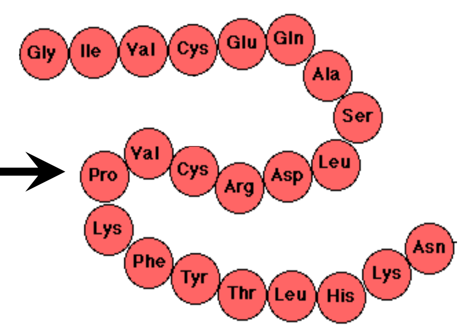
DNA → RNA → Protein

Replication

Transcription

Translation





DNA

Polymer of nucleotides

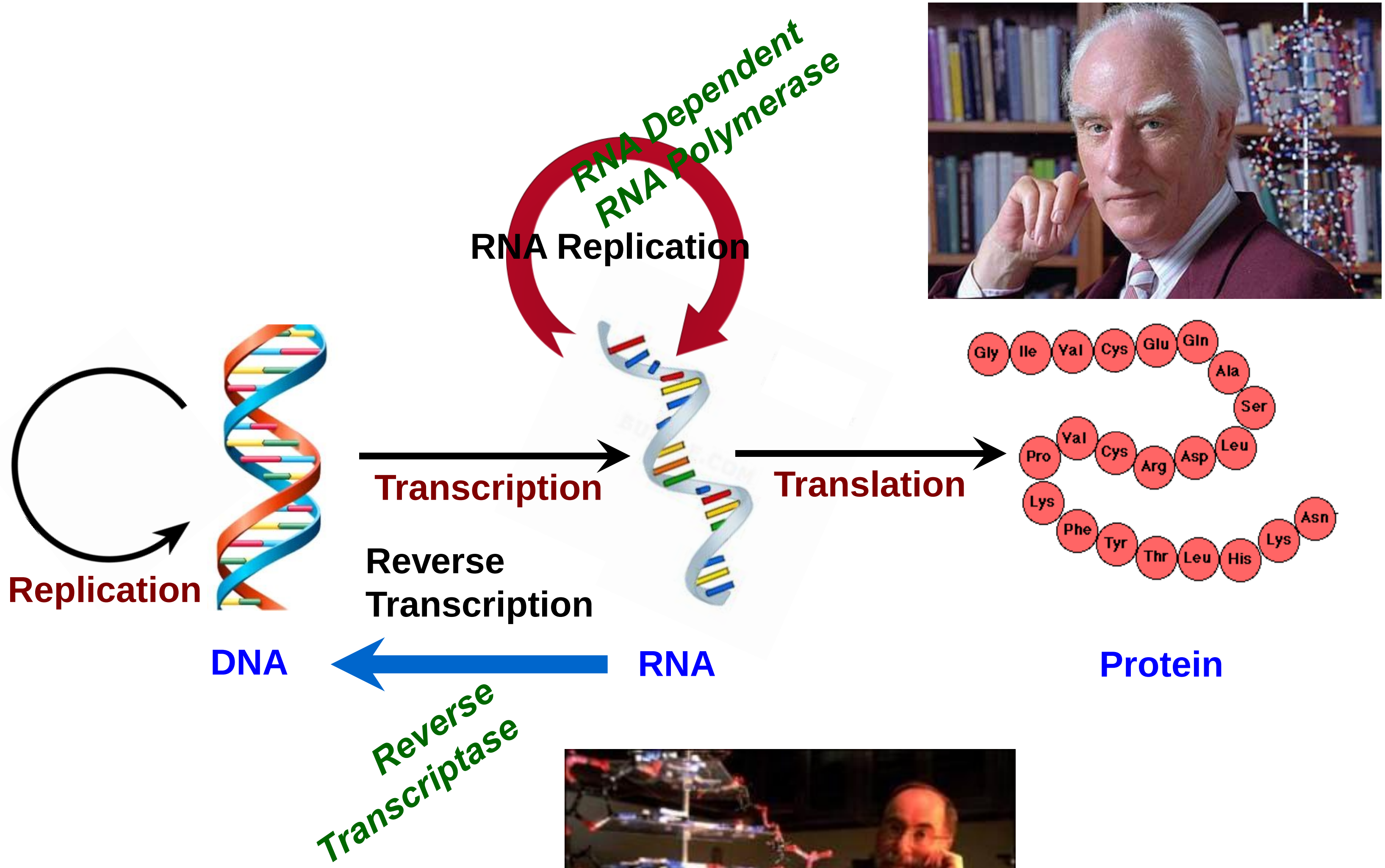
RNA

Polymer of nucleotides

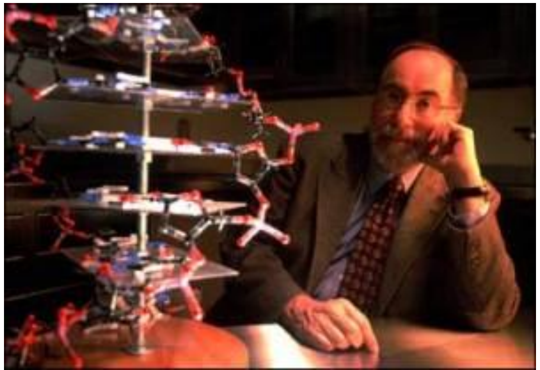
Protein

Polymer of amino acids

Flow of Genetic Information: Updates



David Baltimore
<u>Nobel Prize in 1975</u>

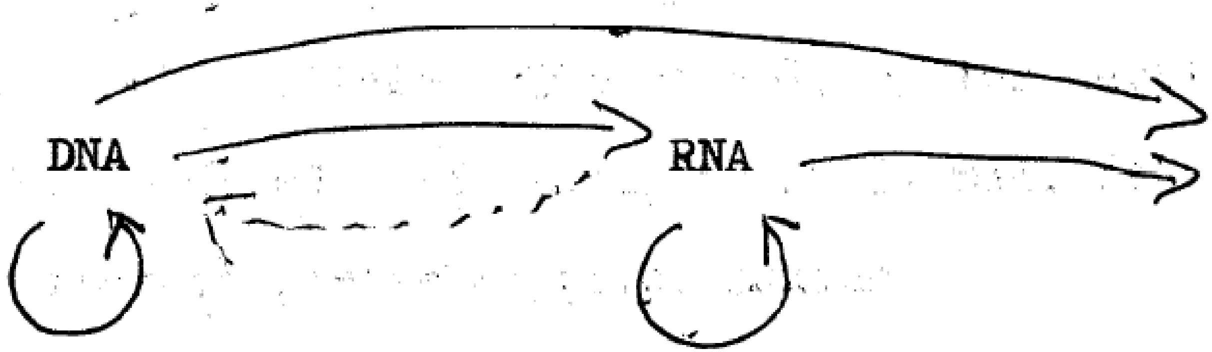


Ideas on Protein Synthesis (Oct. 1956)

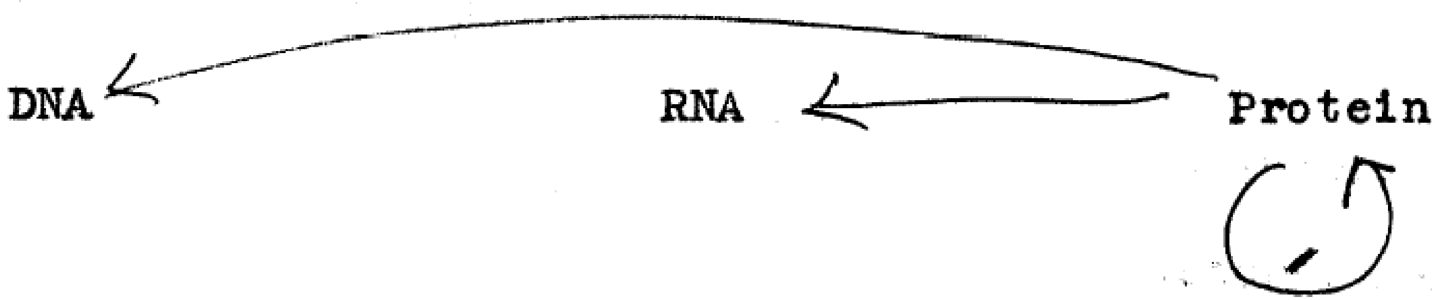
Francis Crick

The Central Dogma: "Once information has got into a protein it can't get out again". Information here means the sequence of the amino acid residues, or other sequences related to it.

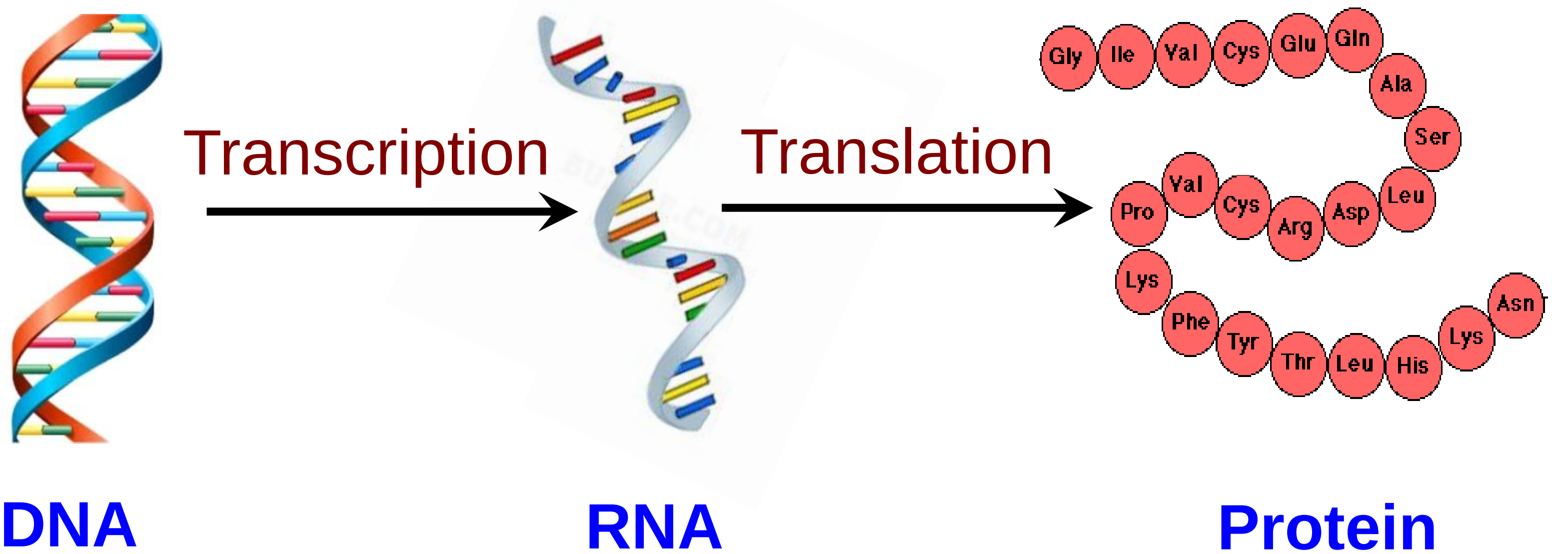
That is, we <u>may</u> be able to have



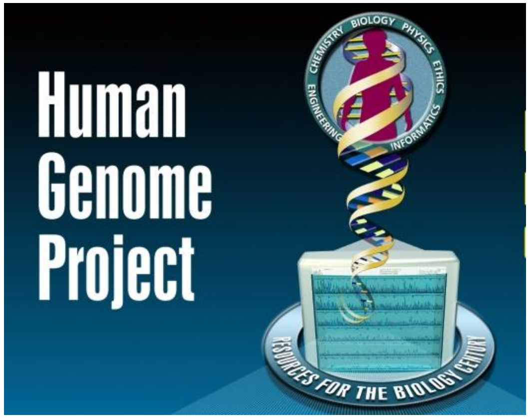
but <u>never</u>



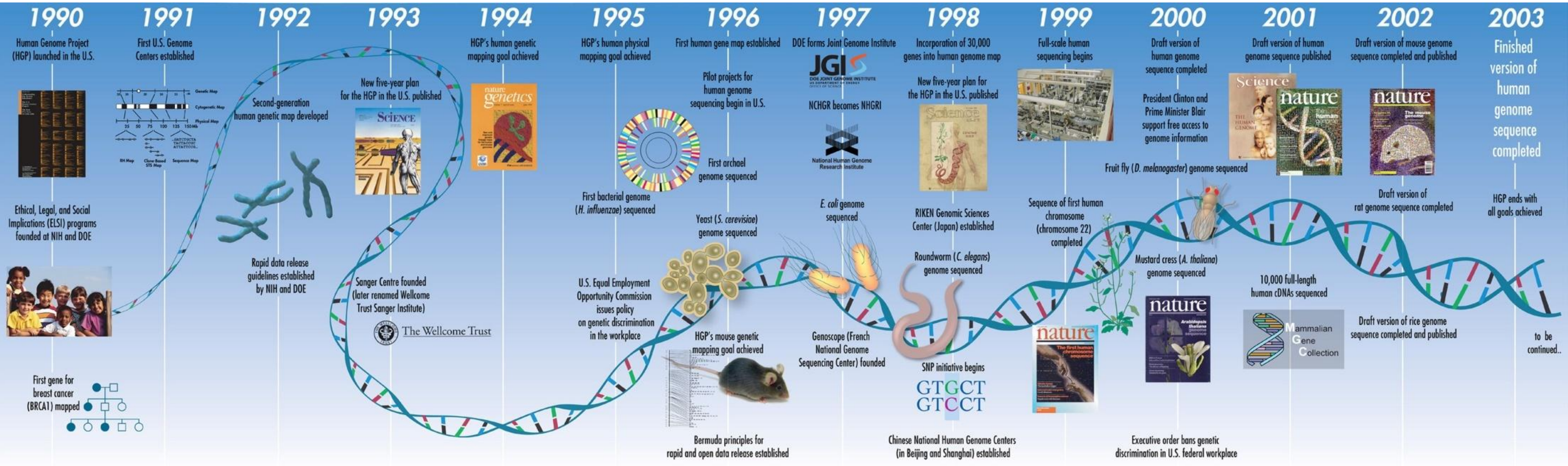
Correlations Between DNA Content and Its Down-stream Product



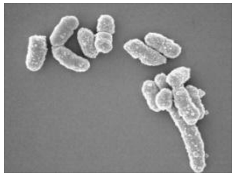
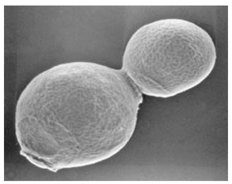
"Human Genome Project" Dramatically Enhanced Our Understanding on Gene Expression



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



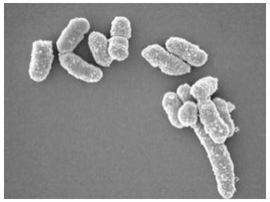
Genome Size, Gene Number, and Complexity of an Organism

	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)
Small nuclear RNA (snRNA)
Regulatory RNA

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

What is a Machine?

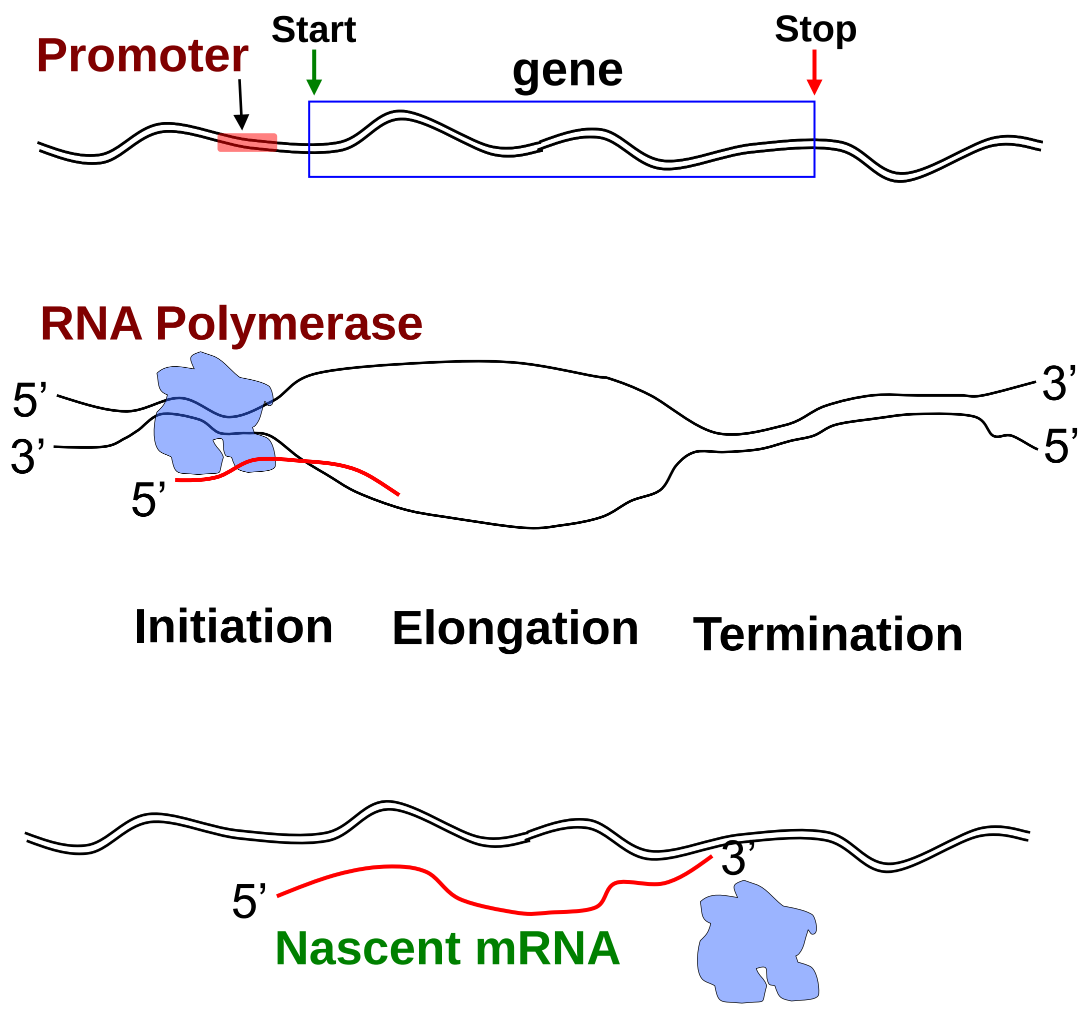
A piece of equipment with several moving parts that uses power to do a particular type of work.

- Cambridge dictionary

Biological machines:

- DNA polymerase
- RNA polymerase
- Ribosome

Transcription: Involved Machineries and Processes

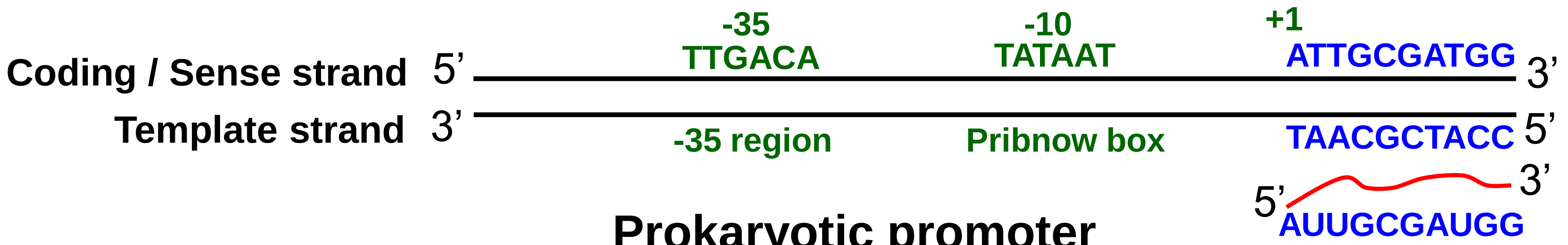


Key points to be discussed

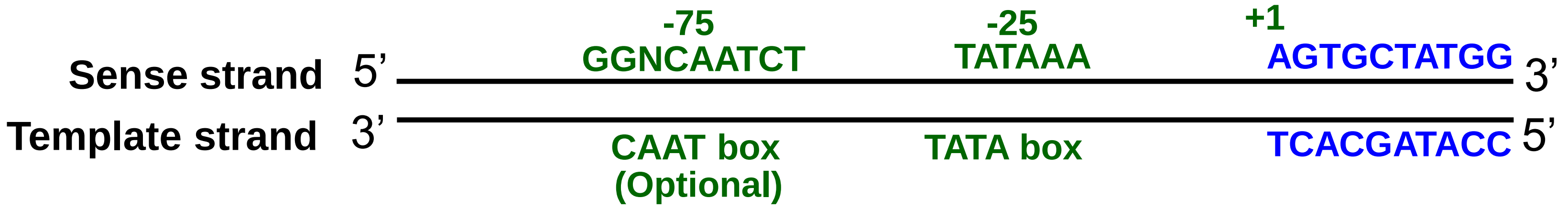
1. **Promoter**
2. **RNA Polymerase**
3. **RNA synthesis**
4. **Termination**

Promoter for Transcription

Promoter is just like a "start button" of an assembly line that starts the conveyor belt moving

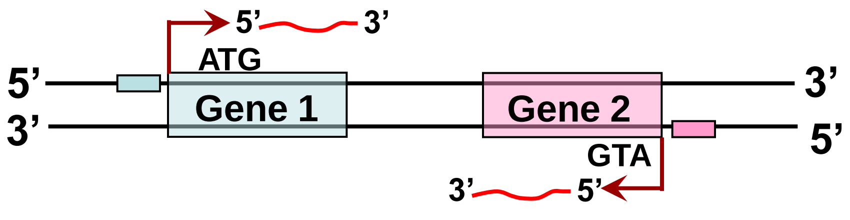


Prokaryotic promoter

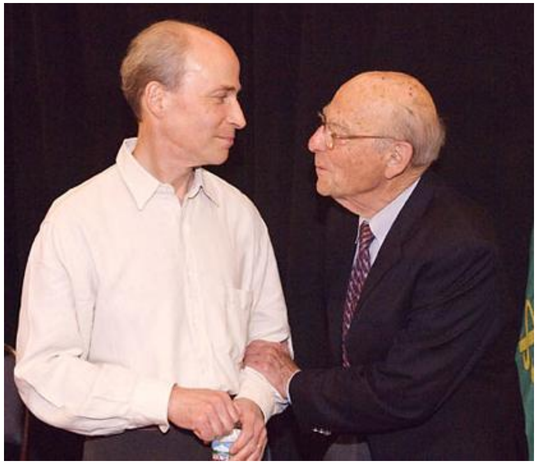


Eukaryotic promoter

►**Transcription can start from both directions**



RNA Polymerase

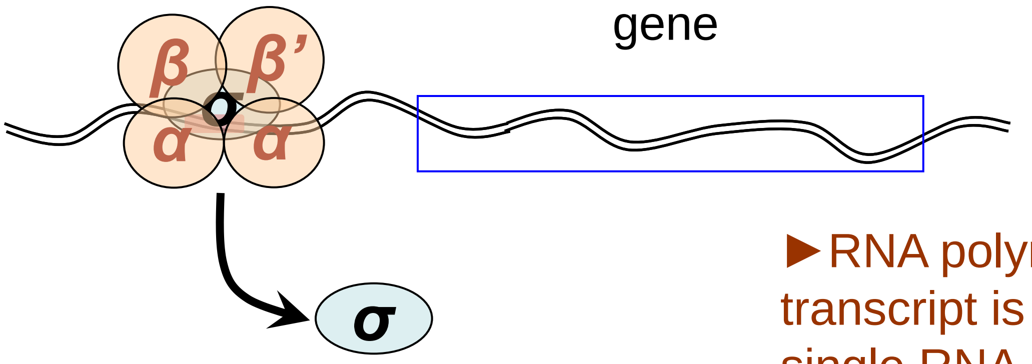


Roger Kornberg
Nobel Prize in 2006

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

Holoenzyme: ***α, α, β, β'*** **and** ***σ***

Coreenzyme: ***α, α, β and β'***



►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

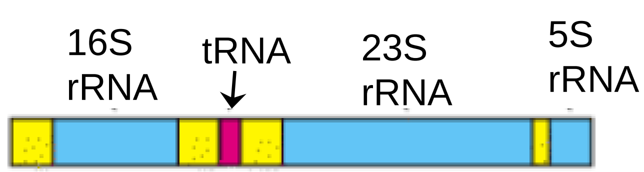
In eukaryotes different RNA polymerases are involved in transcription of mRNA, rRNA and tRNA

RNA Polymerase in Eukaryotes

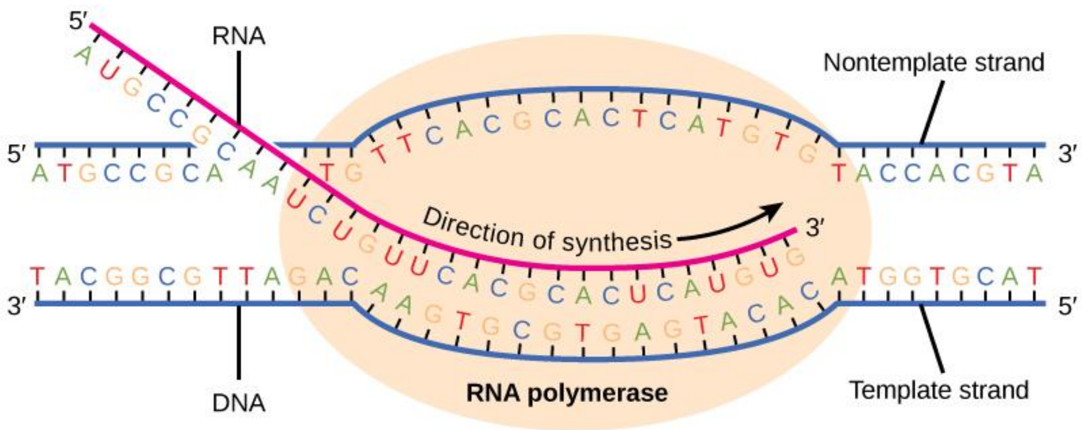
In contrast to bacteria, the nucleus of a eukaryote contains 3 types of RNA polymerase

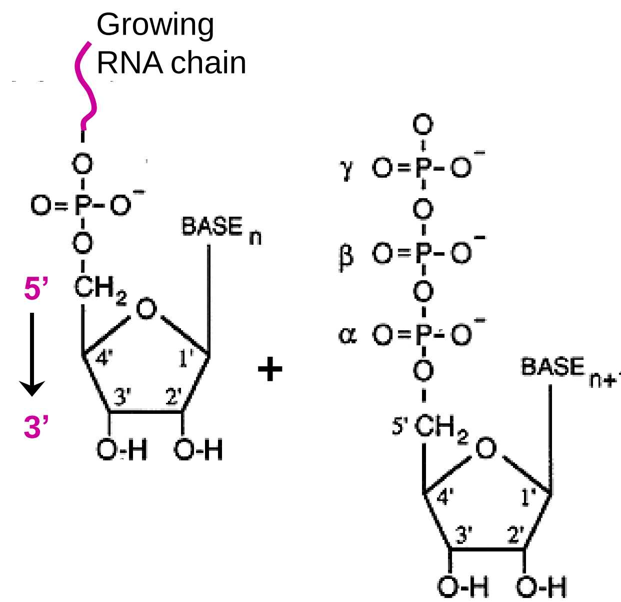
	Template specificity	Location in the nucleus	Susceptibility to inhibitors (α –amanitin)
RNA Pol- I	18S, 5.8S, and 28S r RNA	Nucleoli	Insensitive
RNA Pol- II	mRNA	Nucleoplasm	Highly susceptible
RNA Pol- III	5S rRNA, and tRNA	Nucleoplasm	Susceptible

How rRNA and tRNA are generated in bacteria?



RNA Synthesis

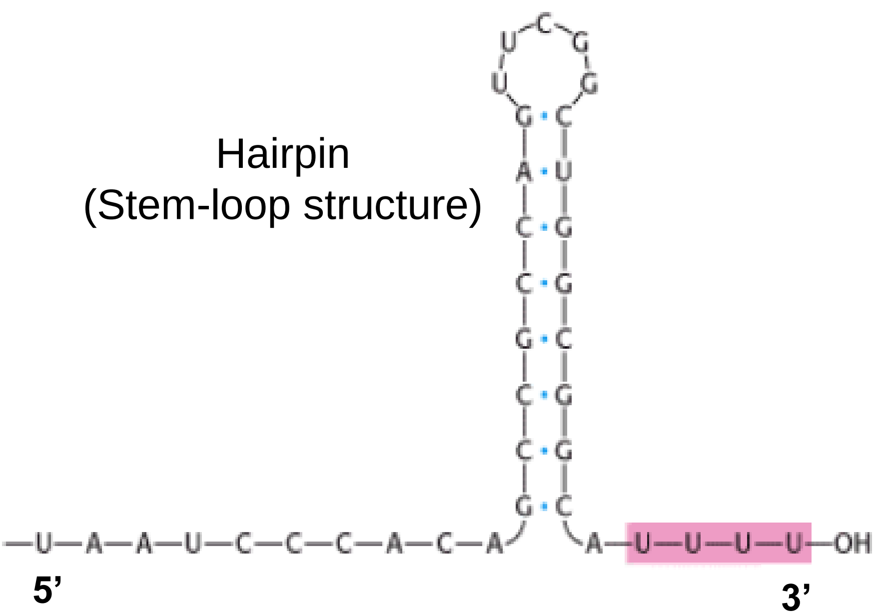




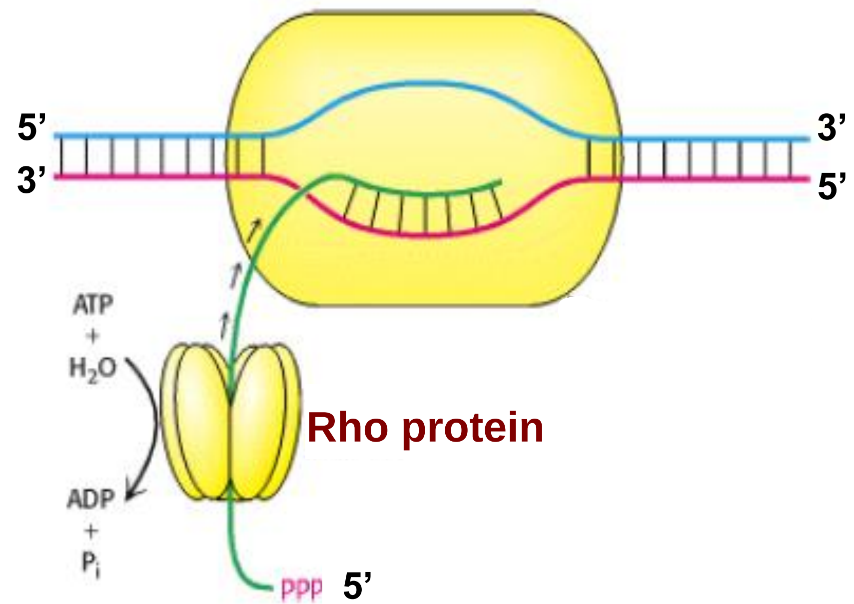
Ribonucleotide

Termination of Transcription in Prokaryotes

An RNA hairpin followed by several uracil residues terminates transcription



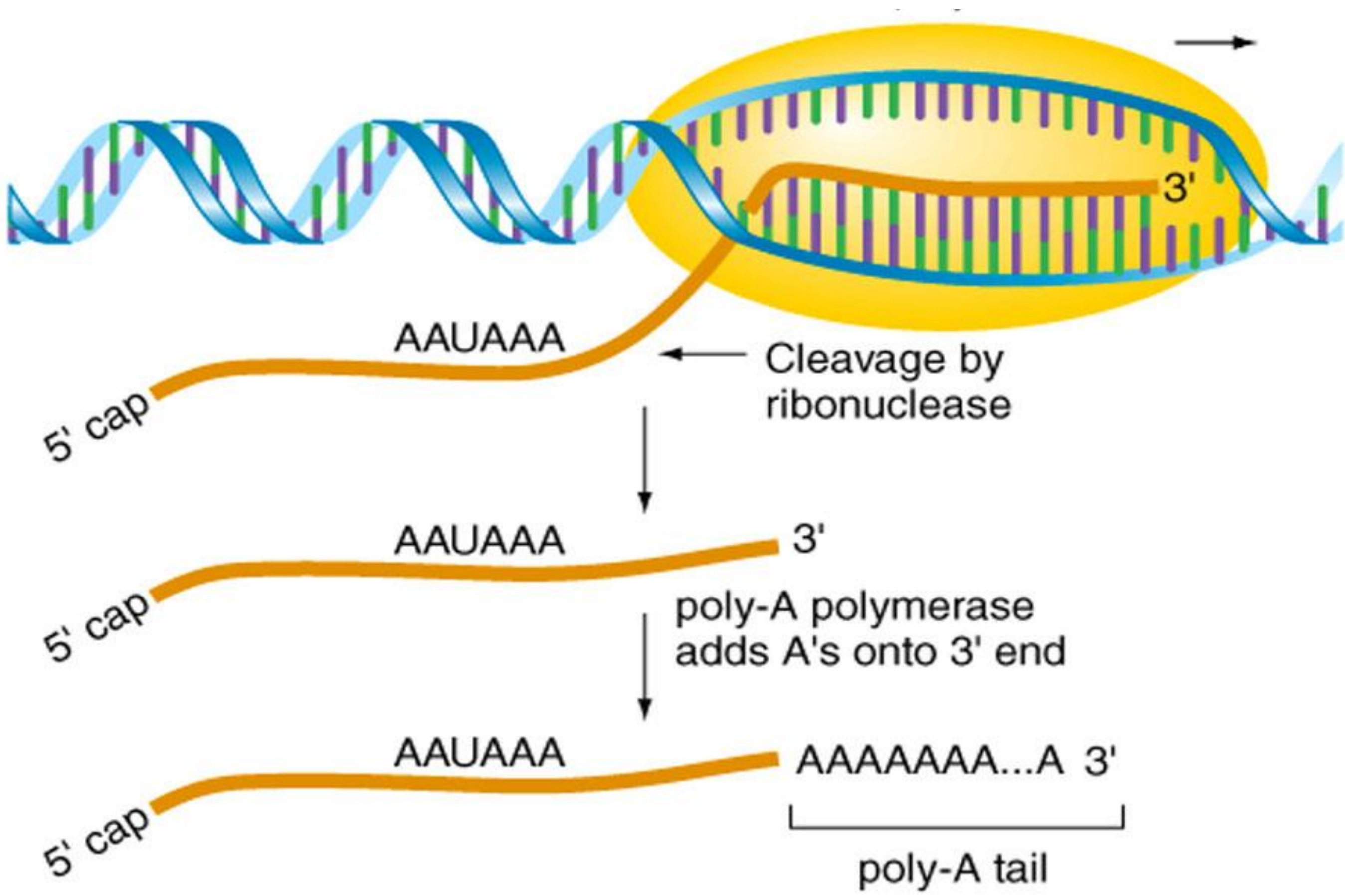
Rho binds the nascent RNA chain and pulls it away from RNA polymerase and the DNA template.



Stryer, Biochemistry, 4th edition

Eukaryotic Transcripts Need to be Processed

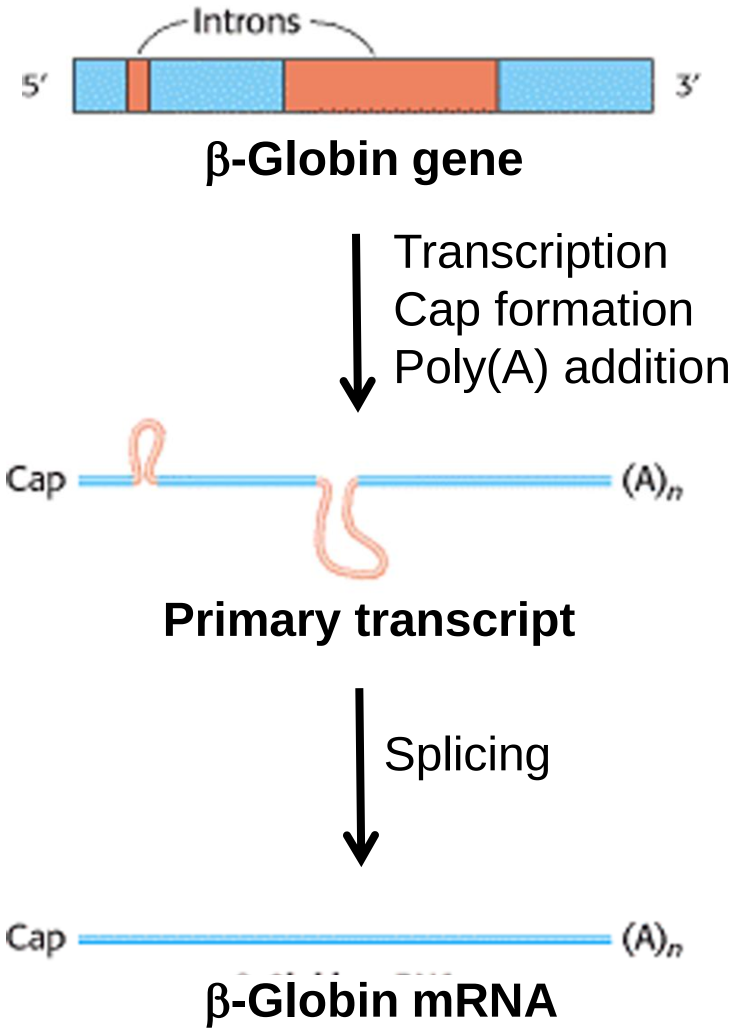
► **Ends of a nascent mRNA acquire a 5’ cap and a 3’ poly A tail**



- **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**

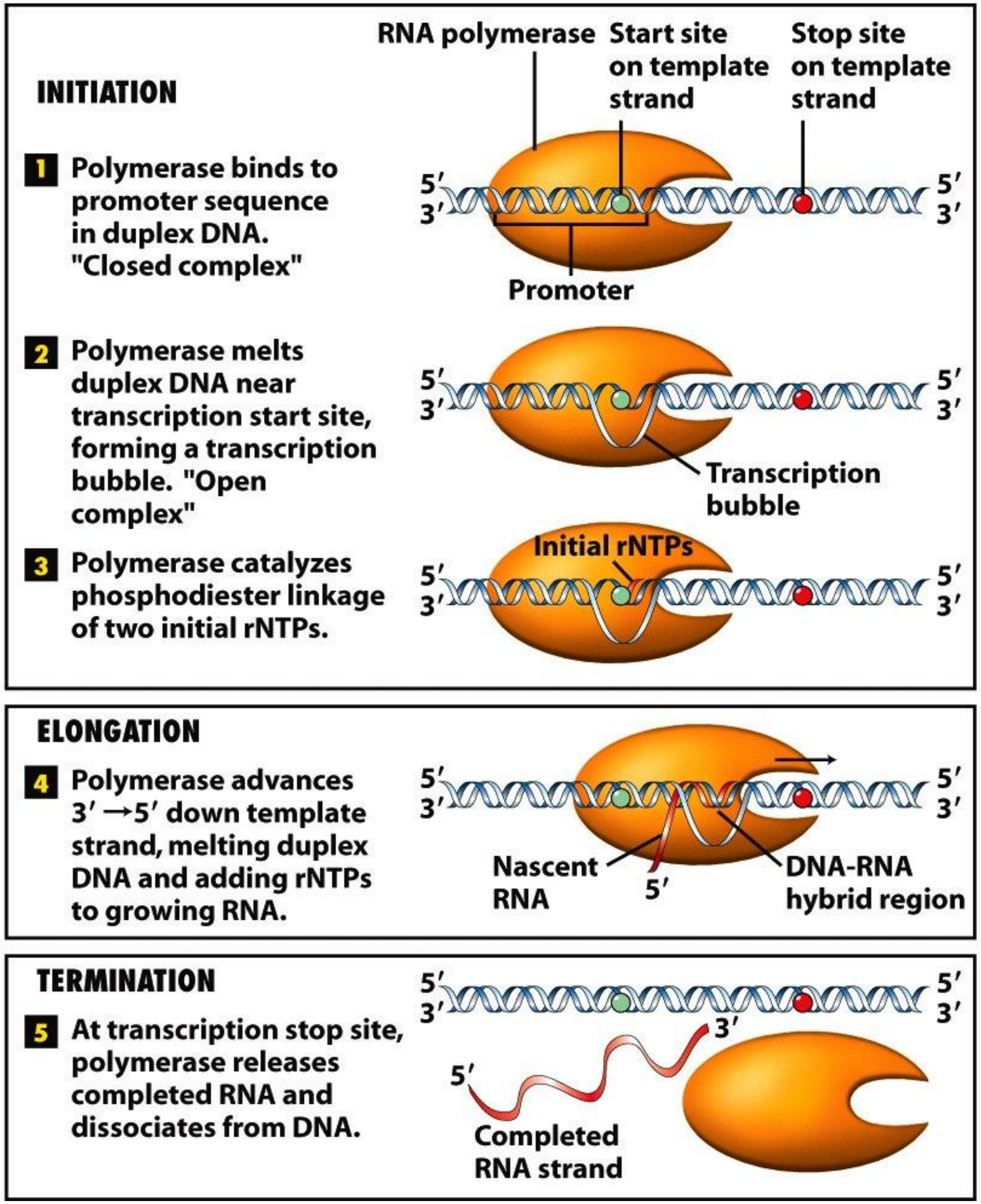


Thomas Cech
Nobel prize in 1989

****Splicing generates more variation**

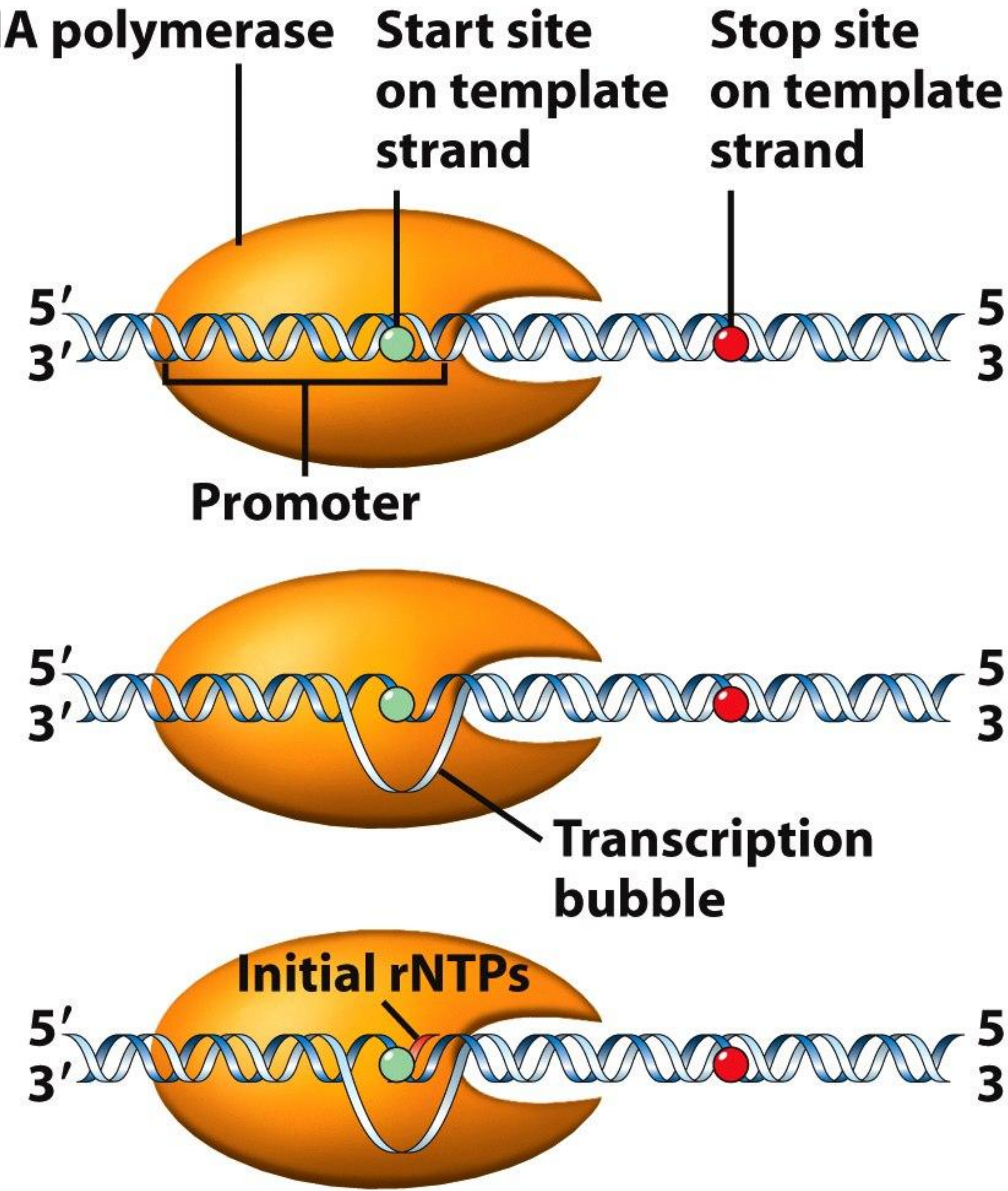
Membrane bound vs soluble form of a protein

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"

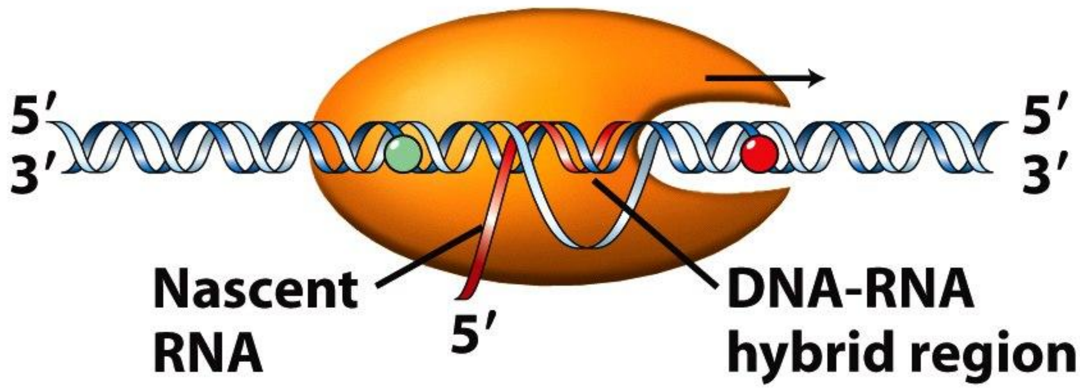
Polymerase melts duplex DNA near transcription start site, forming a transcription bubble. "Open complex"

Polymerase catalyzes phosphodiester linkage of two initial rNTPs.

Schematic Representation of Multiple Steps Involved in Transcription

ELONGATION

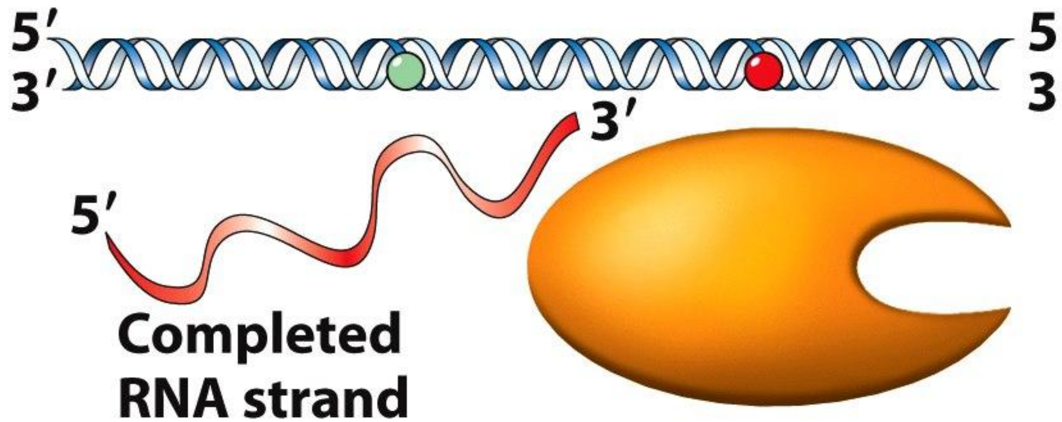
Polymerase advances 3′ → 5′ down template strand, melting duplex DNA and adding rNTPs to growing RNA.



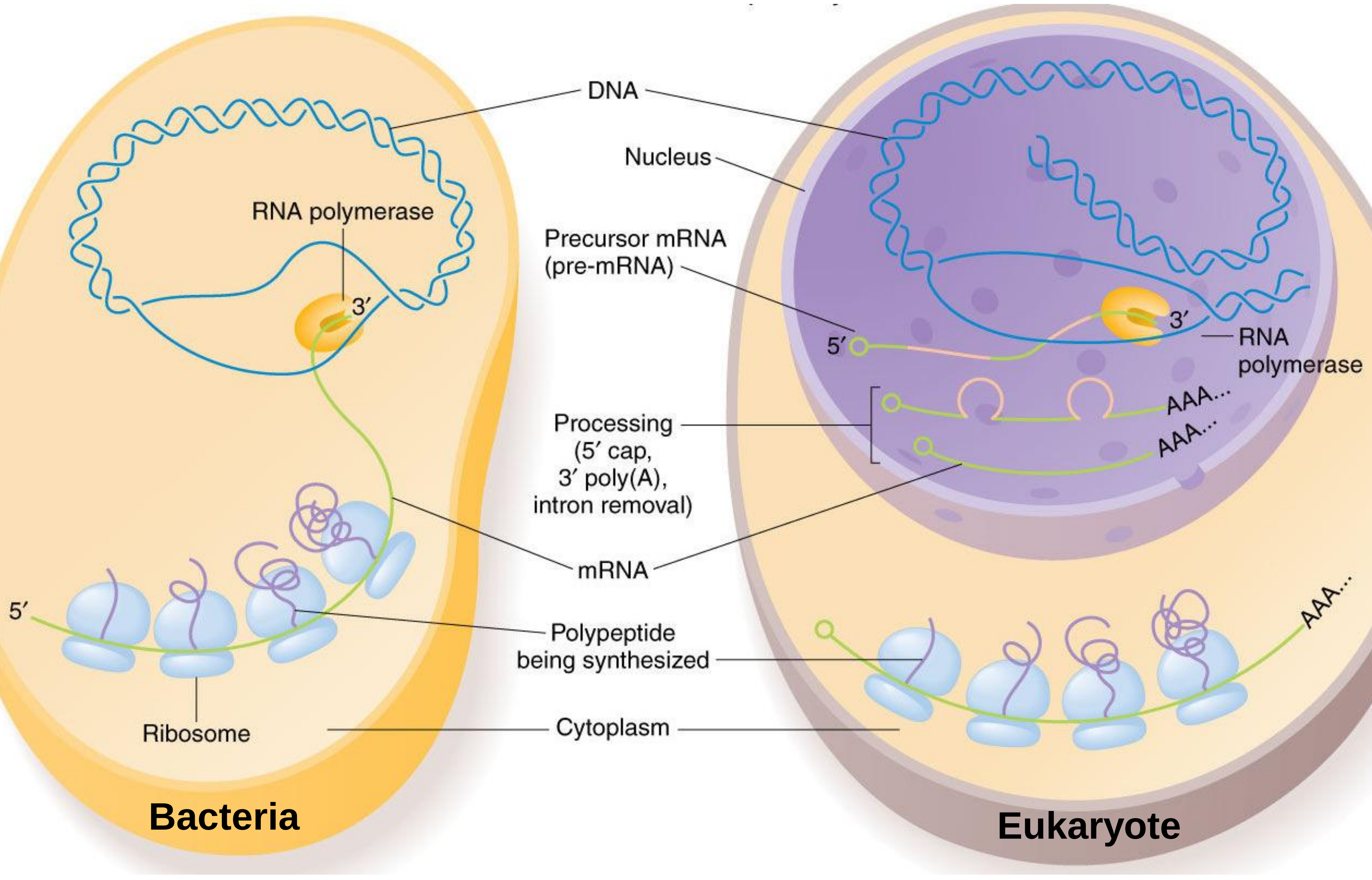
Schematic Representation of Multiple Steps Involved in Transcription

TERMINATION

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



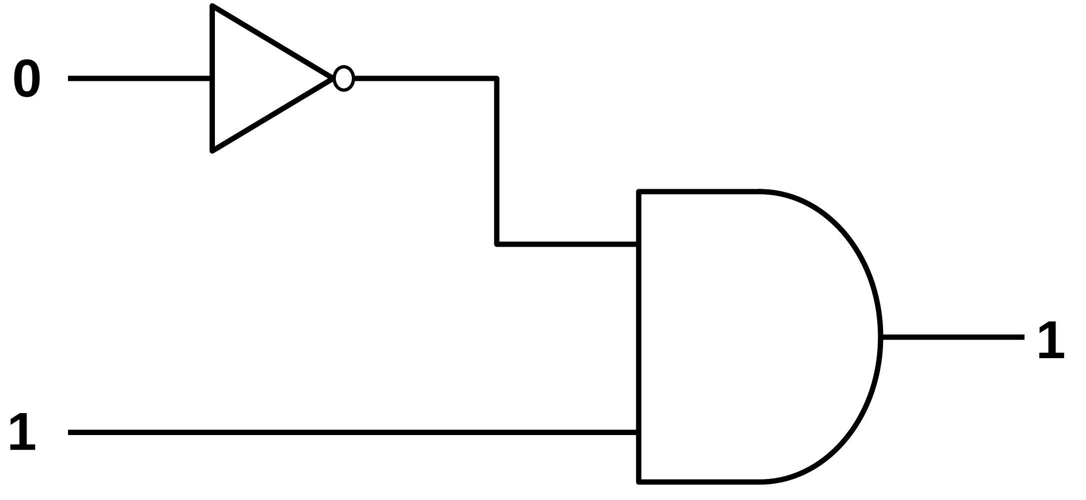
Transcription: At a Glance



Bacteria

Eukaryote

Regulation of Gene Expression
(Biological circuits)



Regulation of Gene Expression

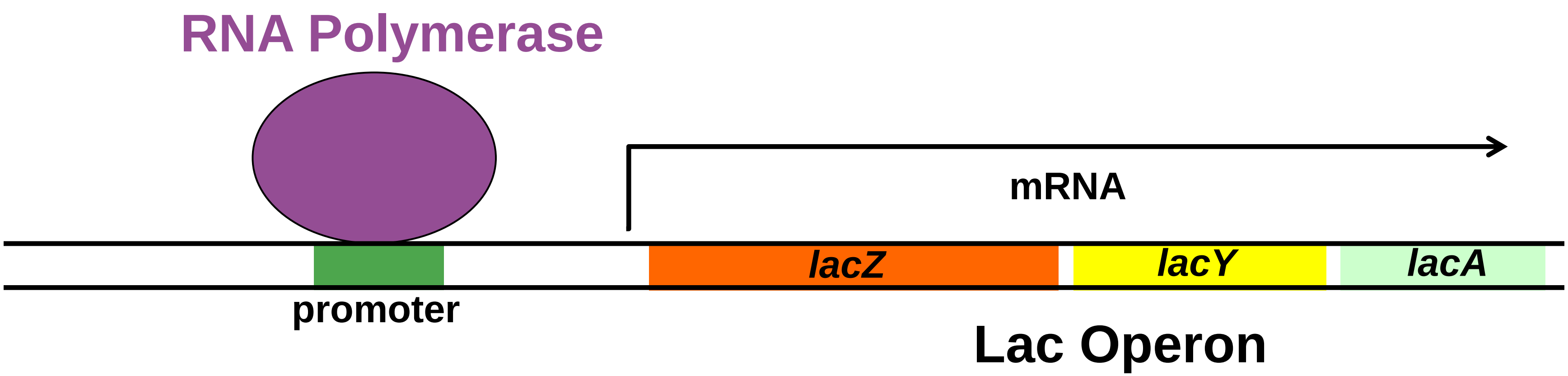
Each cell contains all the genetic material for growth and development

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

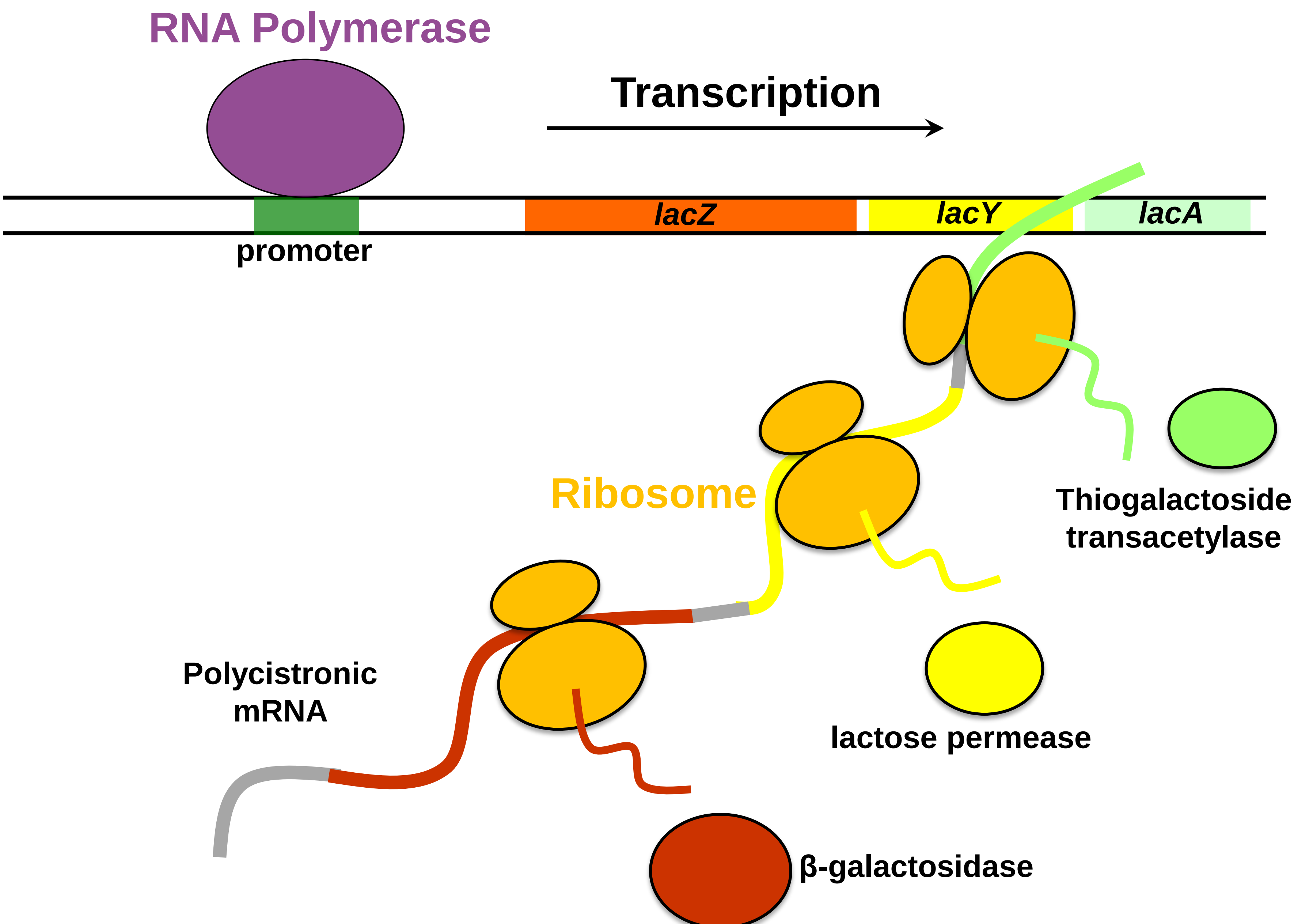
Lac Operon: A Classic Example of Bacterial Gene Expression Control

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

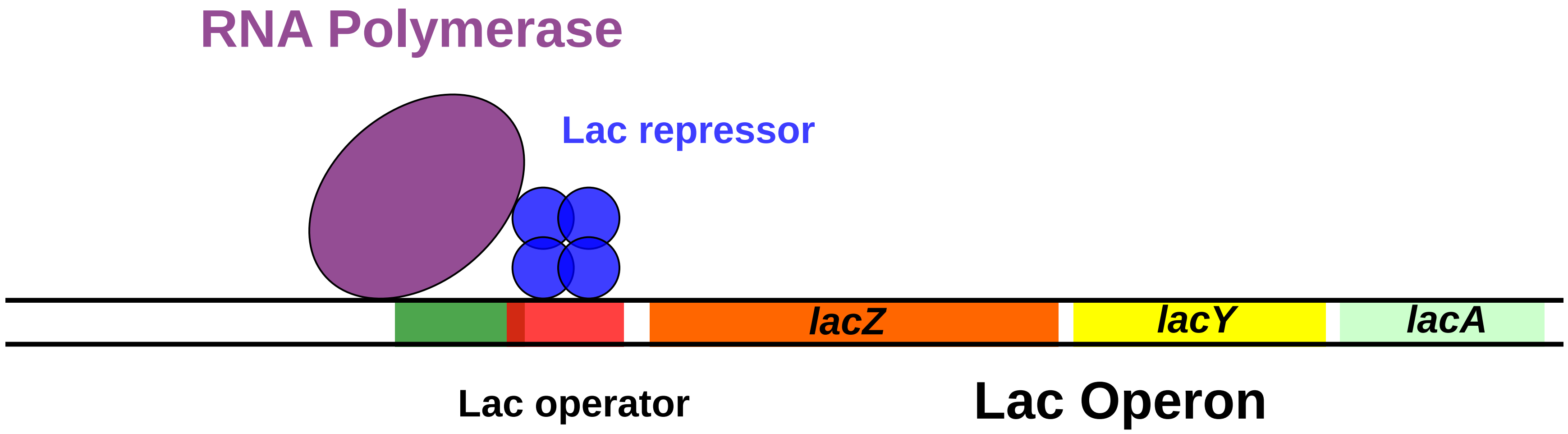


lacZ	β-galactosidase	Breaks lactose into galactose and glucose.
lacY	lactose permease	Imports lactose into the bacterial cell.
lacA	thiogalactoside transacetylase	Cell detoxification.

Functional Outcome of Lac Operon

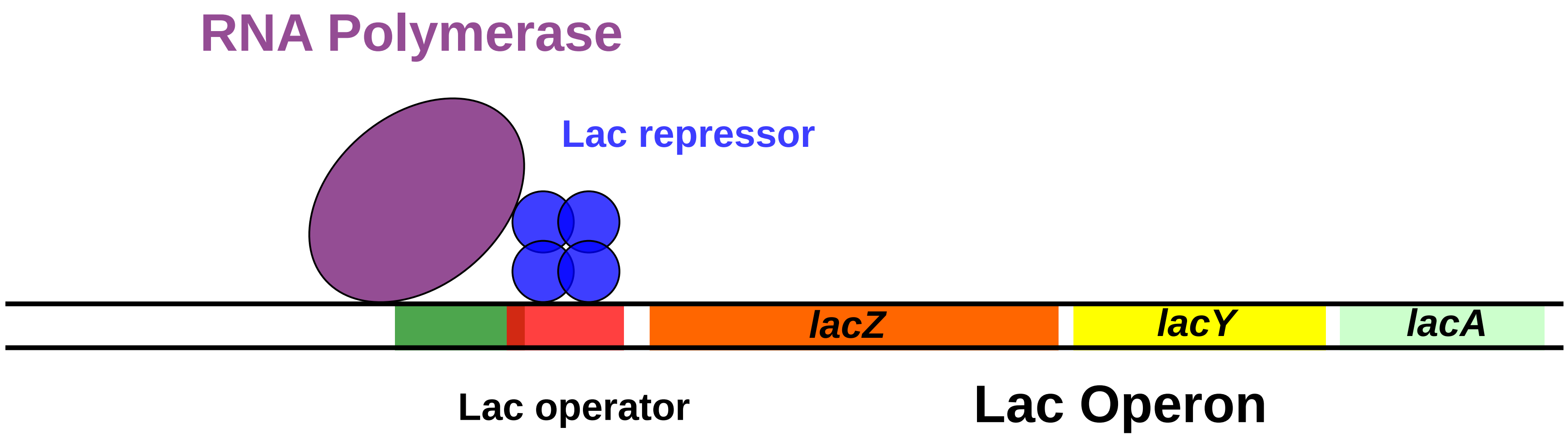


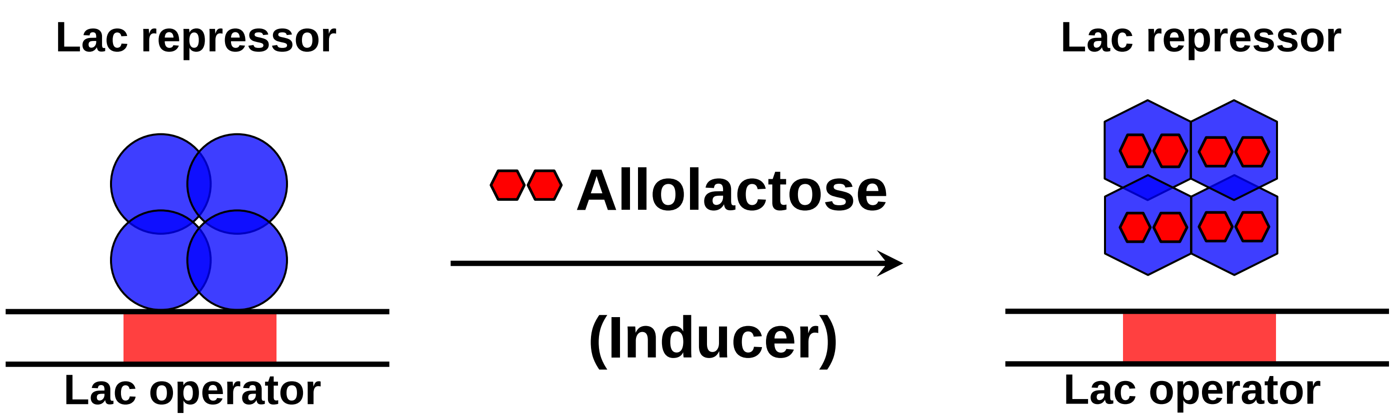
Lac repressor is a negative regulator of the Lac operon



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

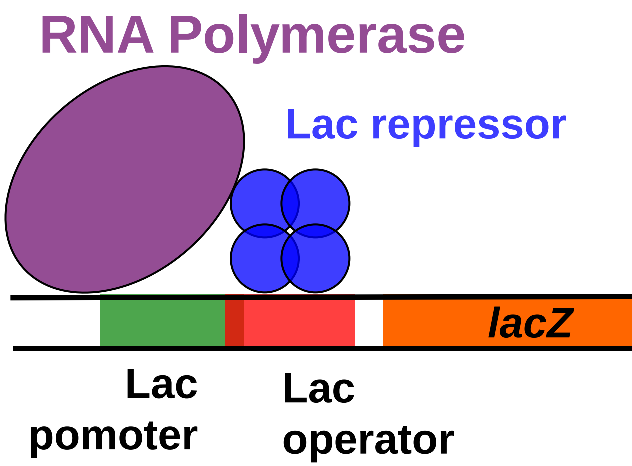
Lactose (Allolactose) Can Displace Lac Repressor From the Operator Site

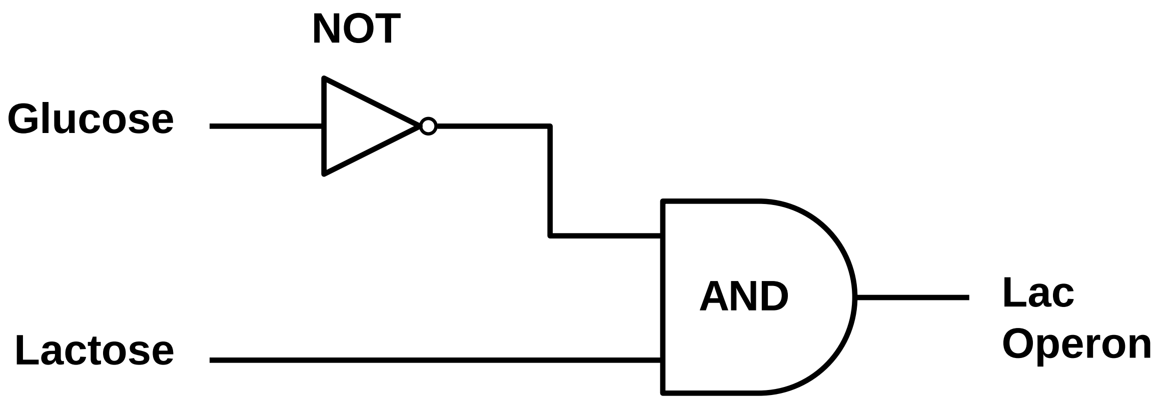




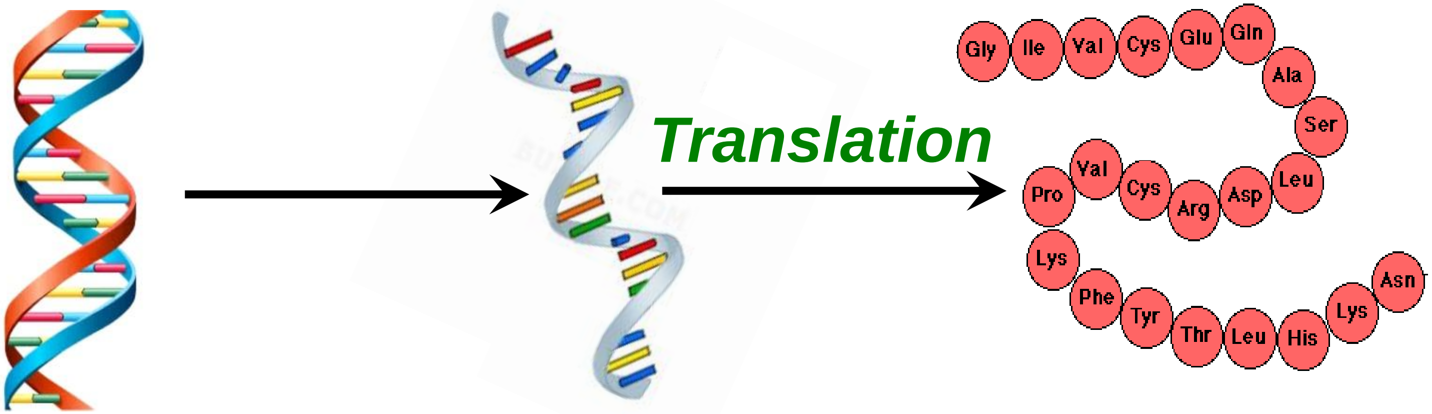
Four Possible Situations

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

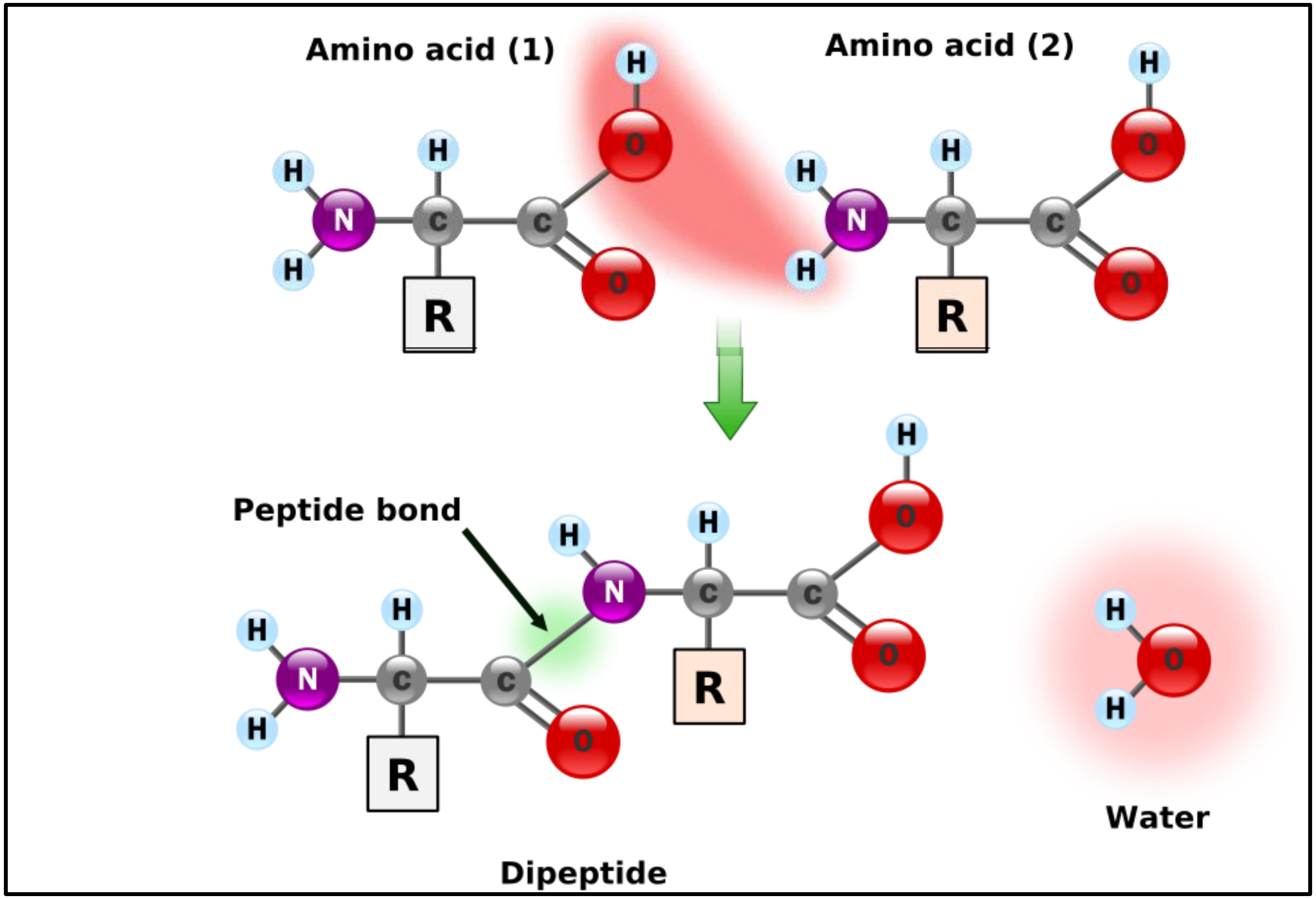
RNA

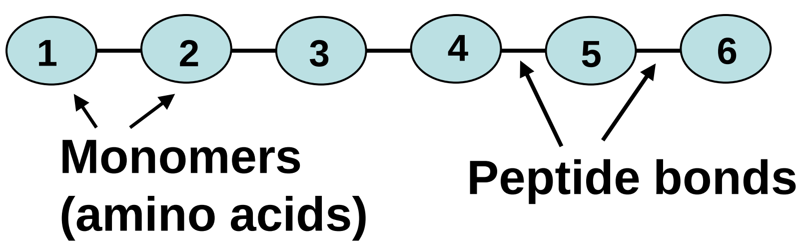
Polymer of nucleotides

Protein

Polymer of amino acids

How Amino Acids are Linked Together





Polymer: Polypeptide and Protein

Translation

Template for protein synthesis

5' ——— 3' mRNA

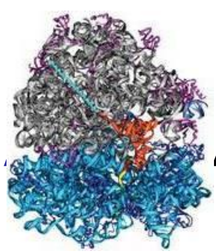
5'UAAGGAGAAUCGUCAUGAAGAGGCCC…….UAAUUA 3'
(RBS)

Start codon

Stop codon

Met—Lys—Arg—Pro……
Polypeptide

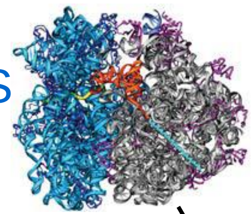
Nascent polypeptide

5'U  AAUCGUCAUGAAGAGGCCC…….UAAUUA 3'

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

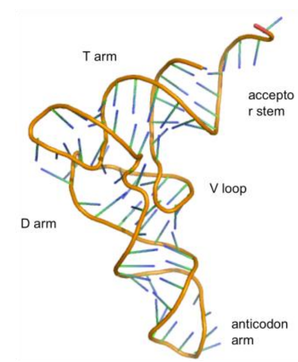
Protein synthesizing machinery

Ribosome: (rRNA + proteins)

30S  50S

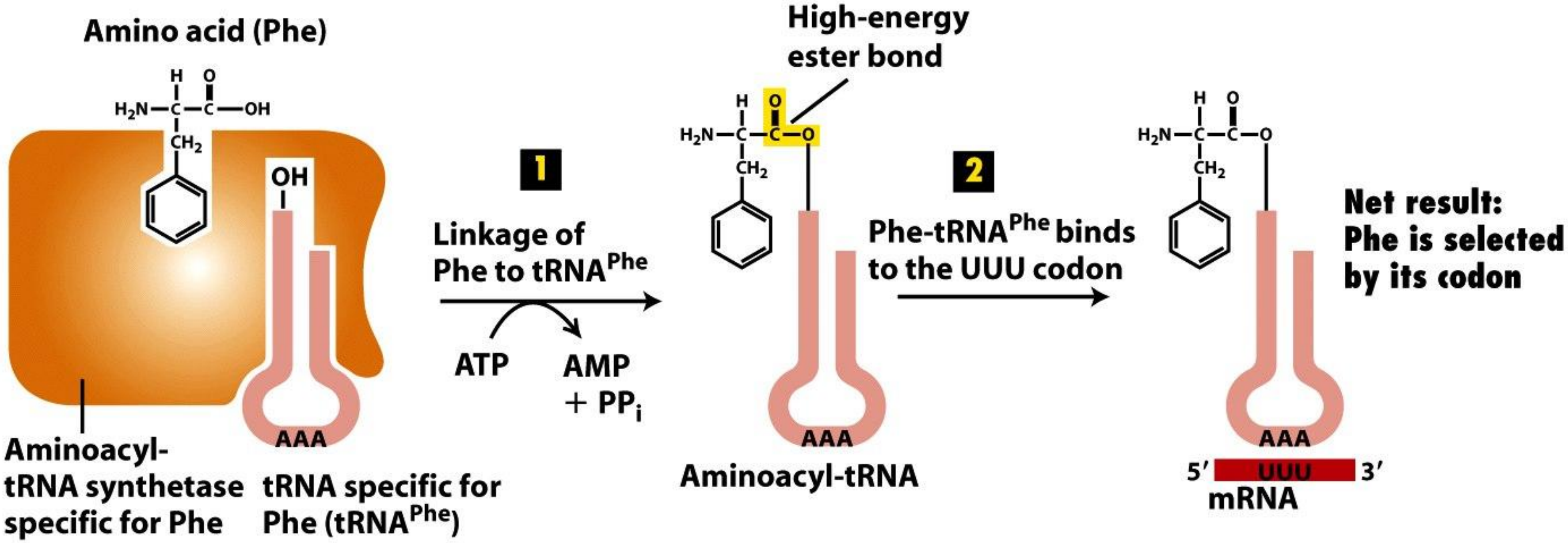
16S rRNA
21 proteins

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 codon may specify more than one amino acids

Khorana, Nirenberg, Holley

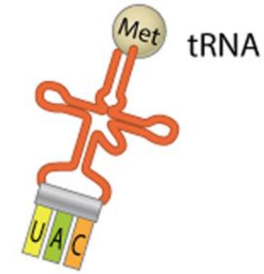
Nobel Prize in 1968

Genetic code

Second Letter

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

Translation: Involved Machineries and Processes



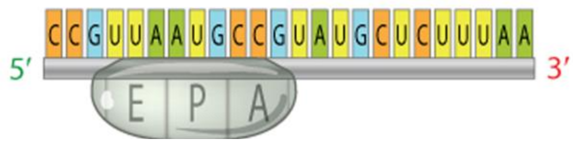
mRNA

Ribosome

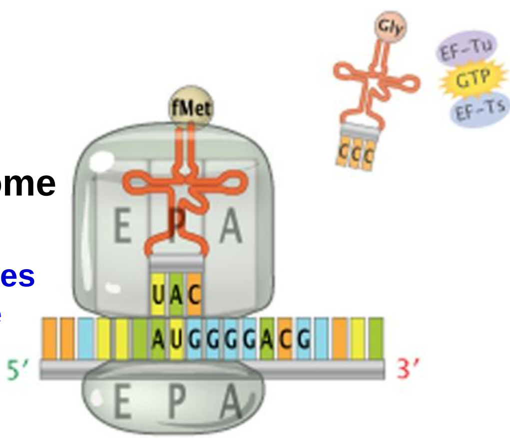
Amino acid

tRNA

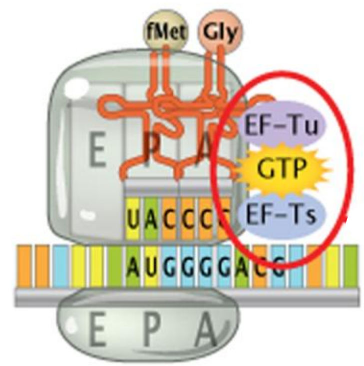
mRNA



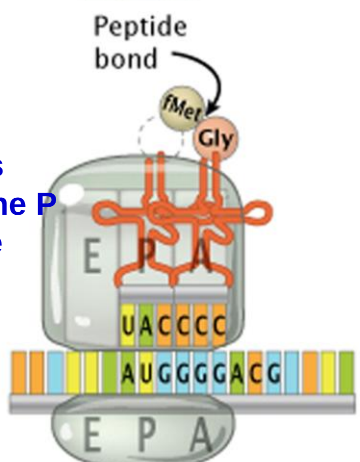
50S ribosome



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

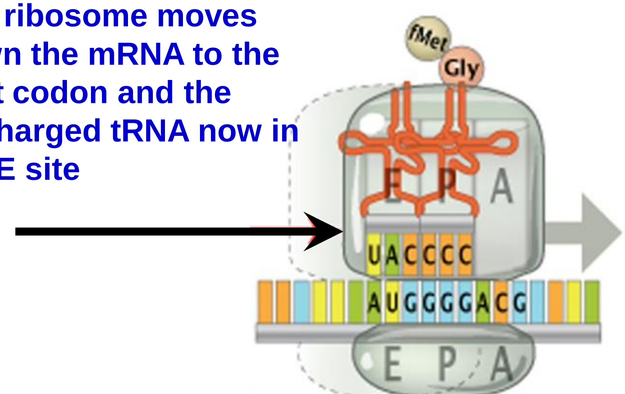


Next charged tRNA and associated translation factors enter the A site

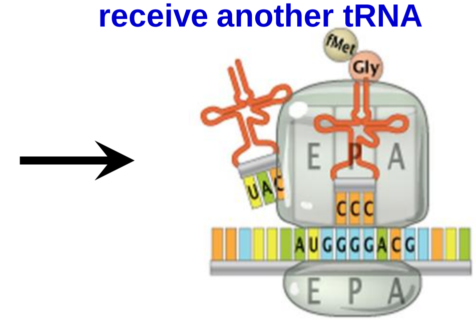


Peptide bond forms between the aa in the P and A sites, and the tRNA in the P site releases its aa

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

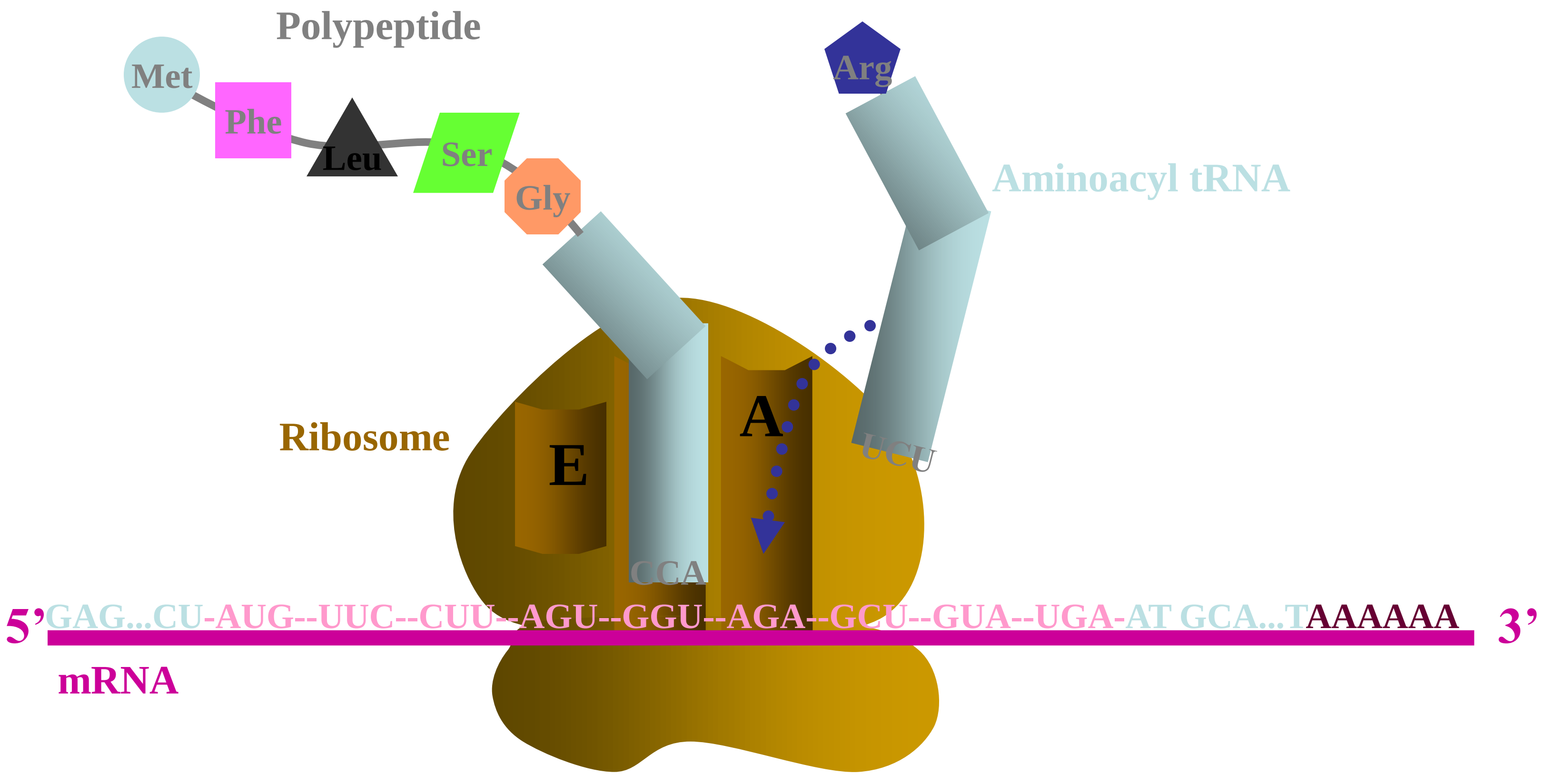


E site tRNA releases and A site is now ready to receive another tRNA

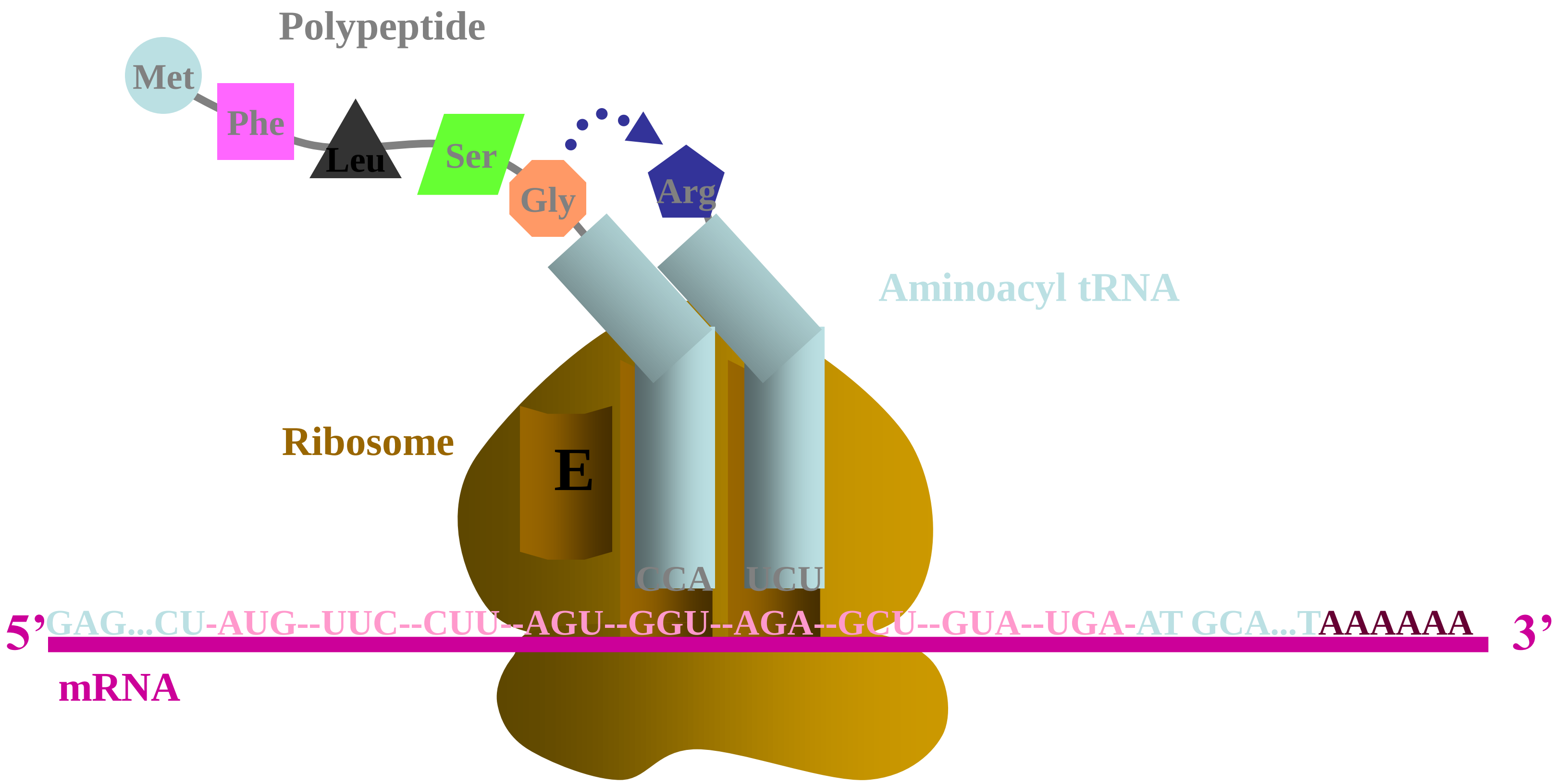


Translation - Initiation

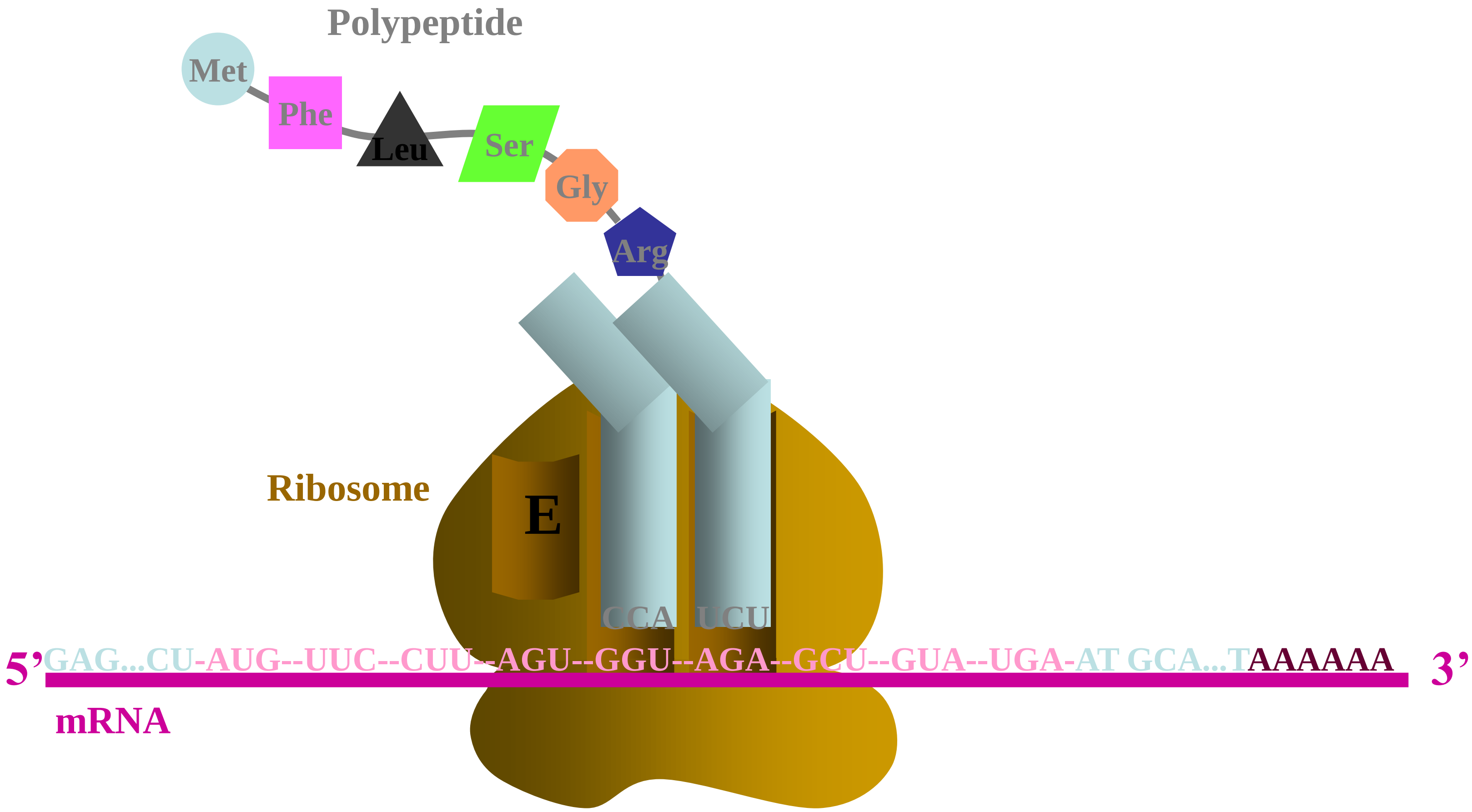
Translation - Elongation



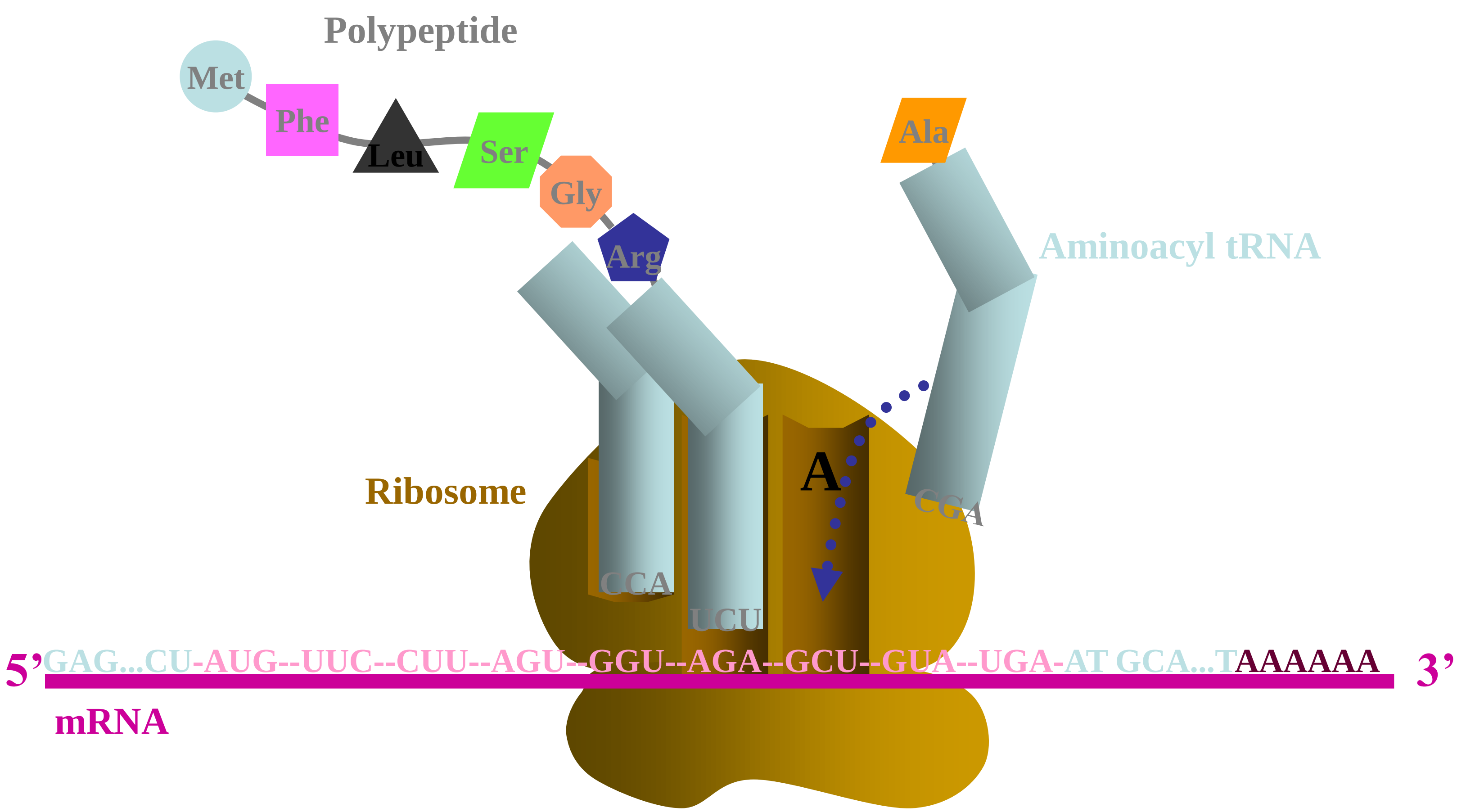
Translation - Elongation



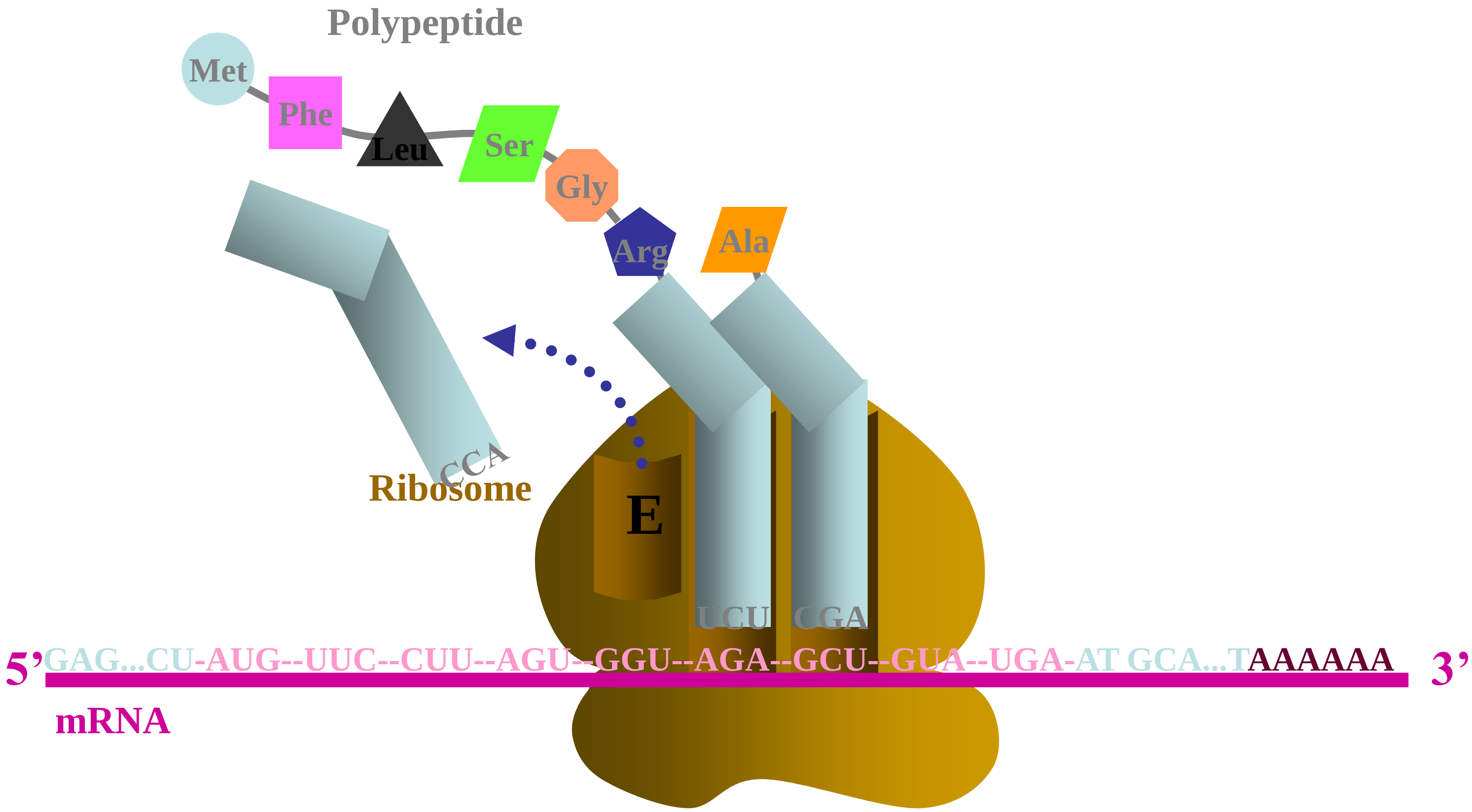
Translation - Elongation



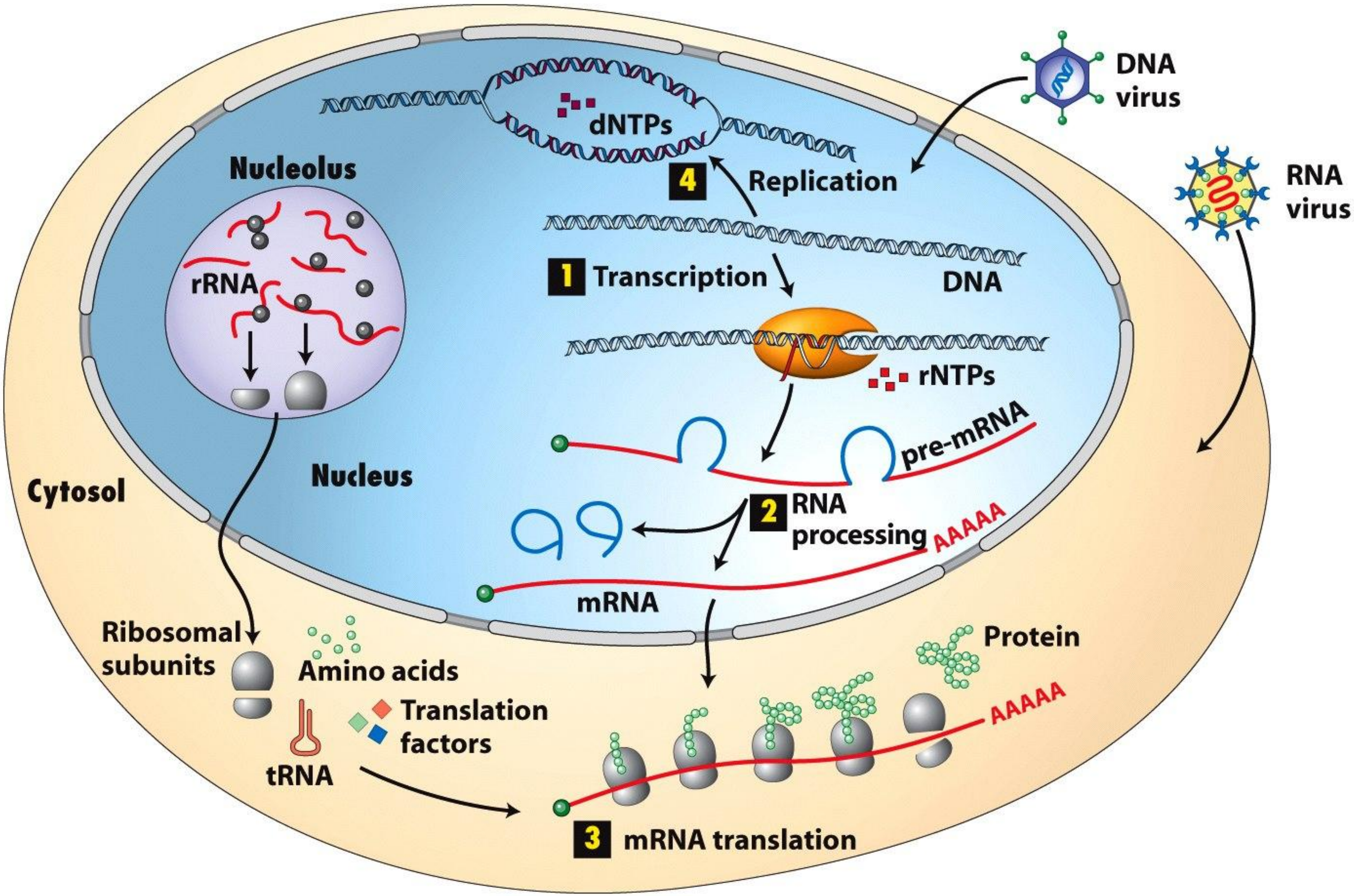
Translation - Elongation



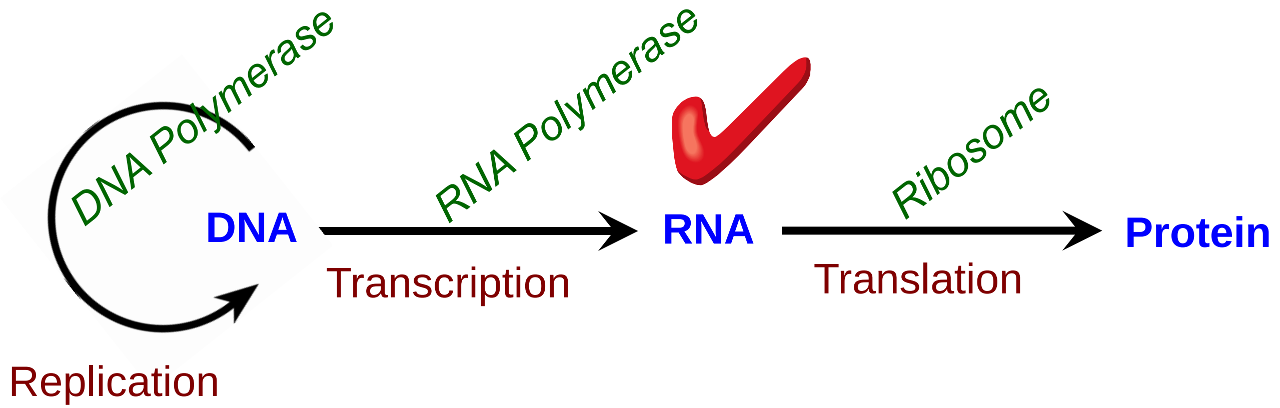
Translation - Elongation

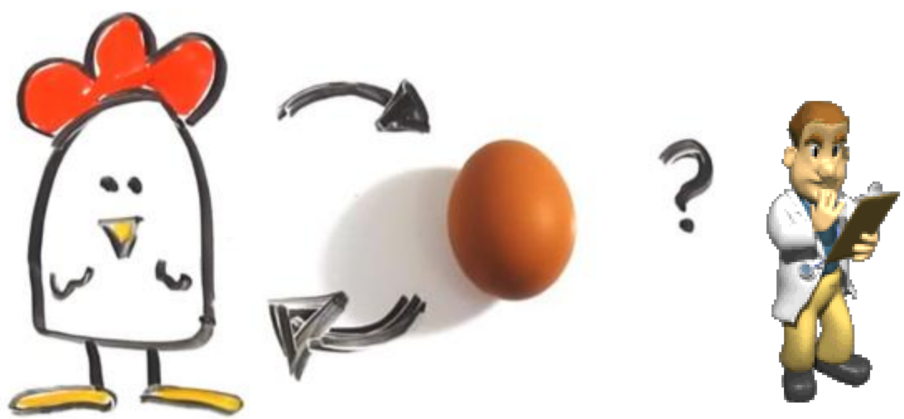


Translation: At a Glance



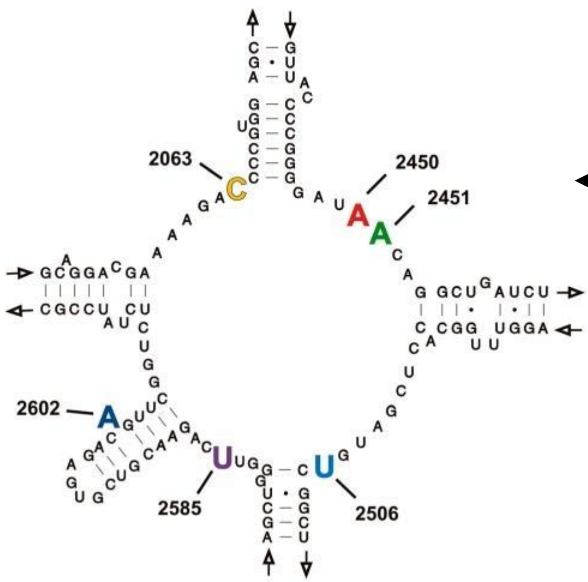
Which Came First? Nucleic acids or Proteins

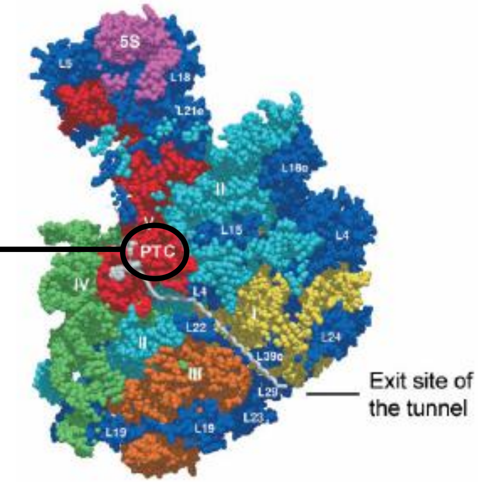


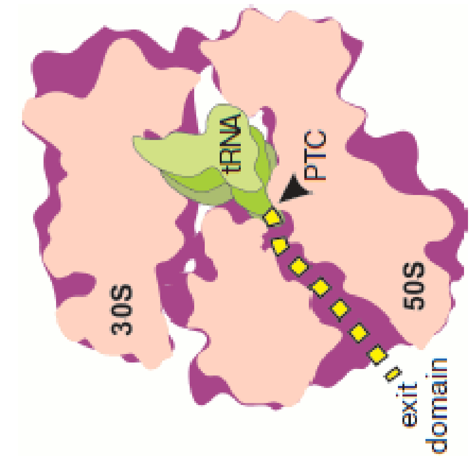


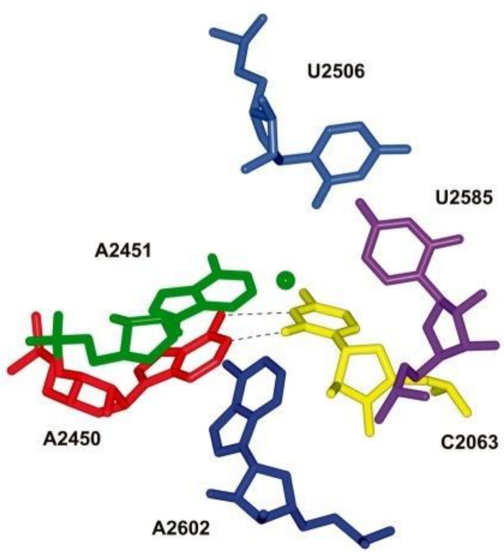
► **RNA has enzymatic activity**

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



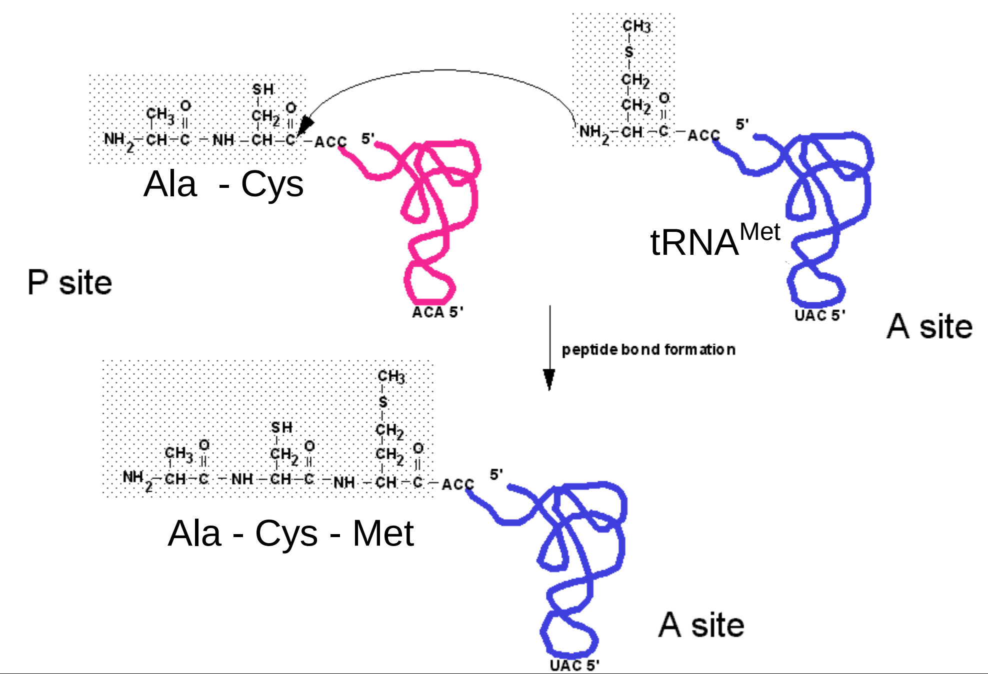




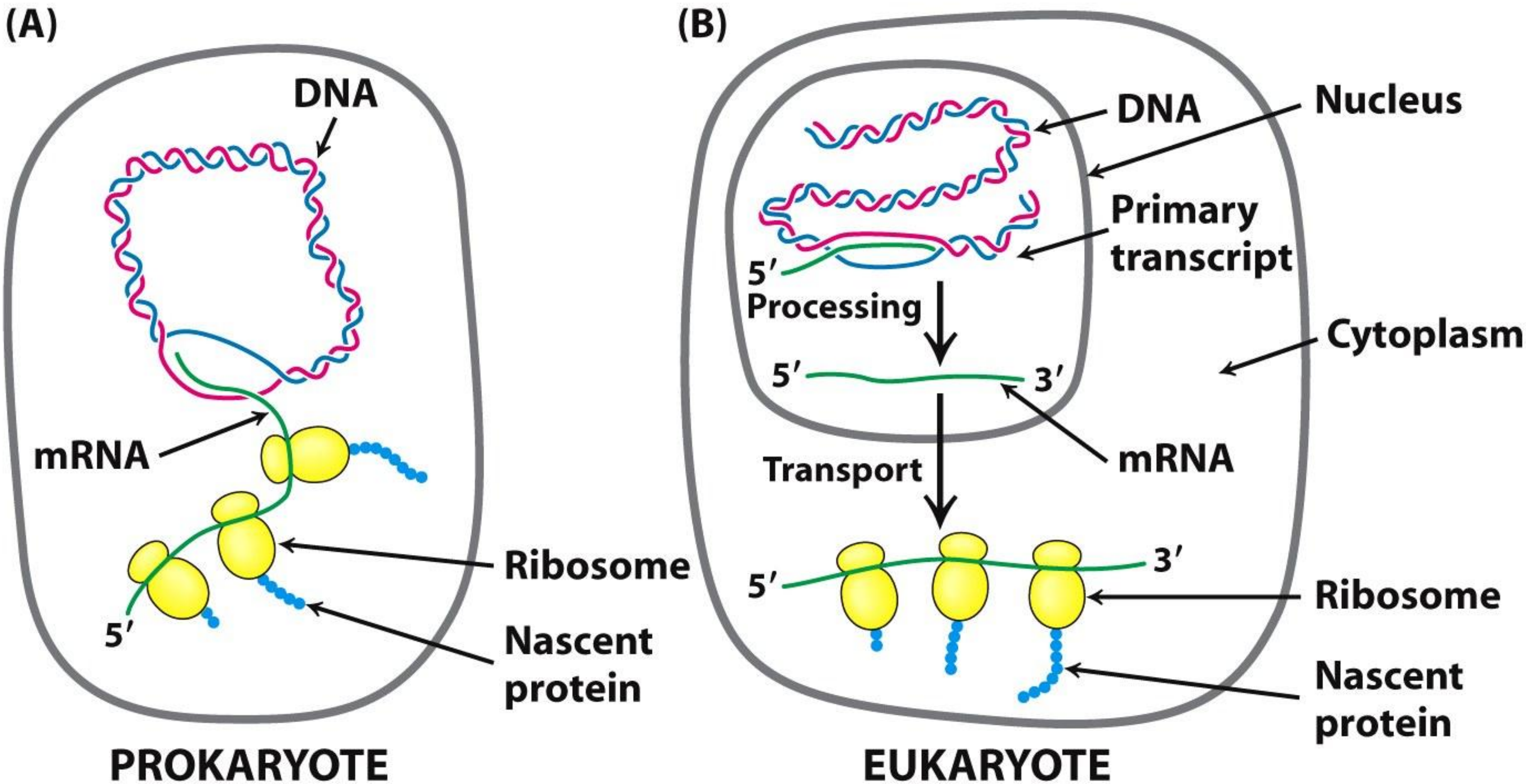


Peptidyl transferase center

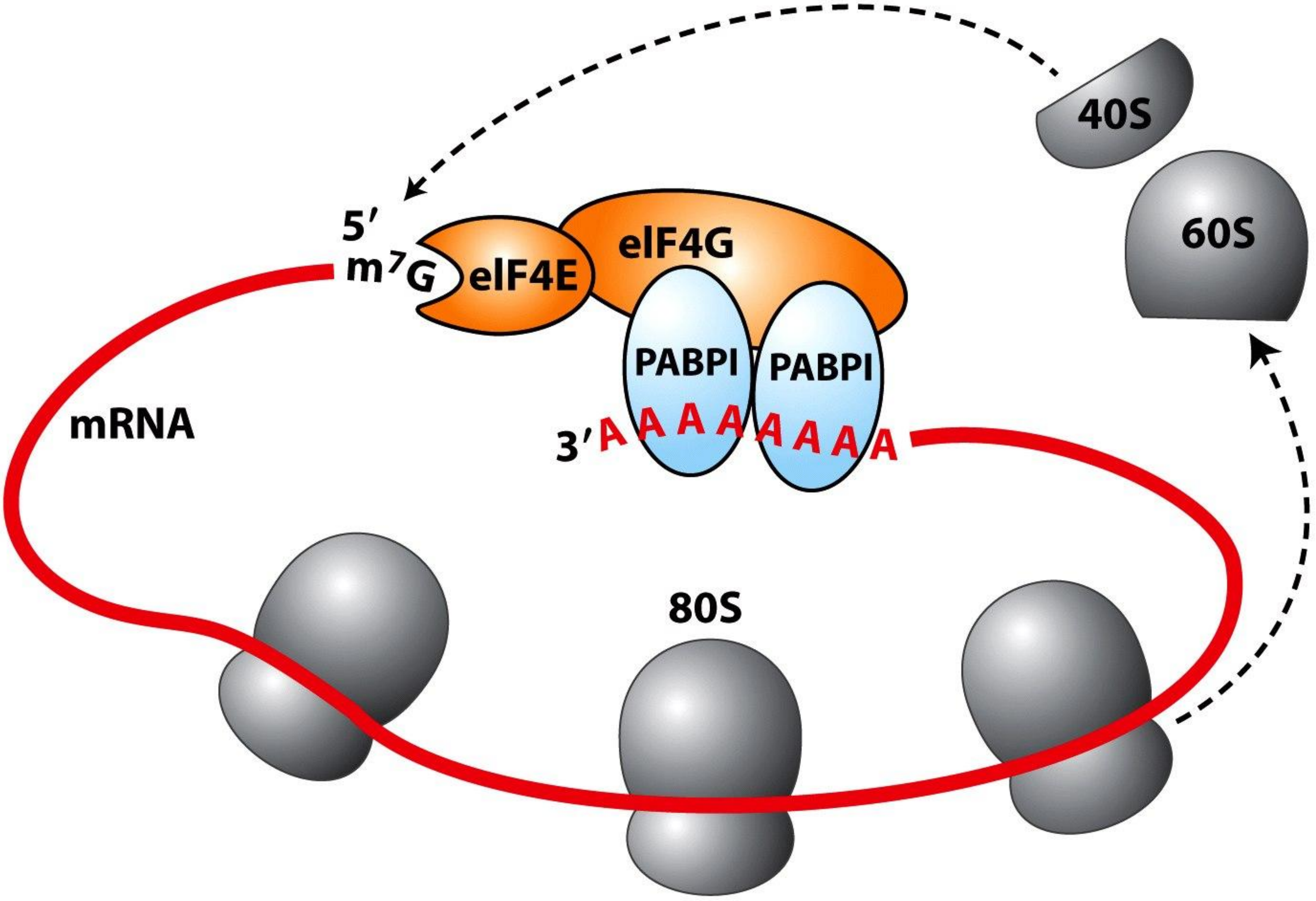
Peptidyl transferase reaction



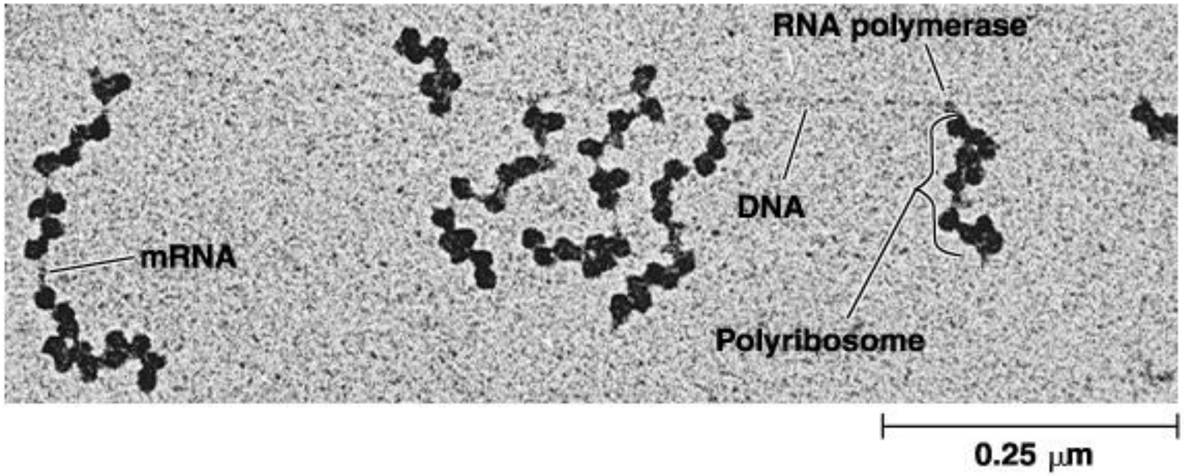
Time, Space and Correlation between Transcription and Translation



Time, Space and Correlation between Transcription and Translation



Time, Space and Correlation between Transcription and Translation

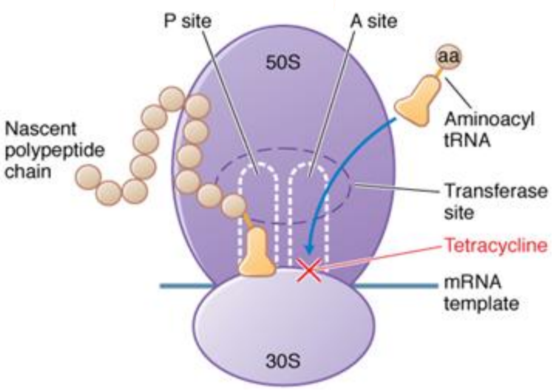


Jonathan Warner

Translation Machineries: Attractive Targets For Therapeutics

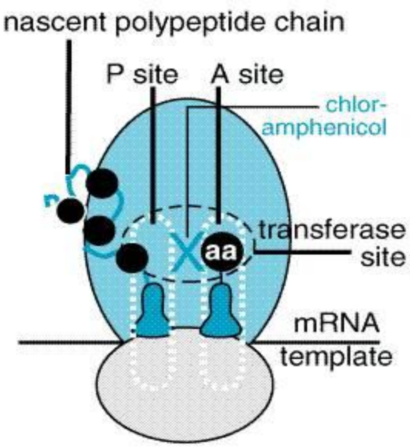
Tetracycline

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



Chloramphenicol

Blocks the peptidyl transferase reaction on 50S ribosomes

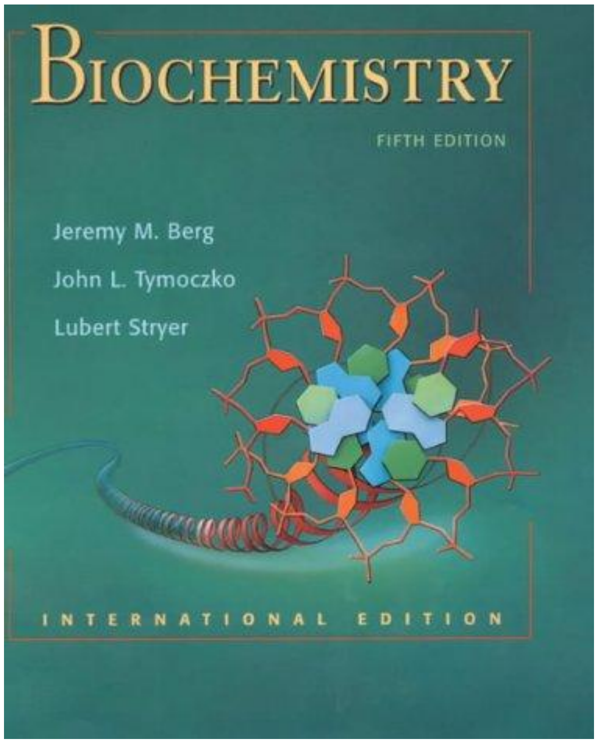


Streptomycin Binds to the 30S ribosome, prevents the transition from initiation to chain-elongation

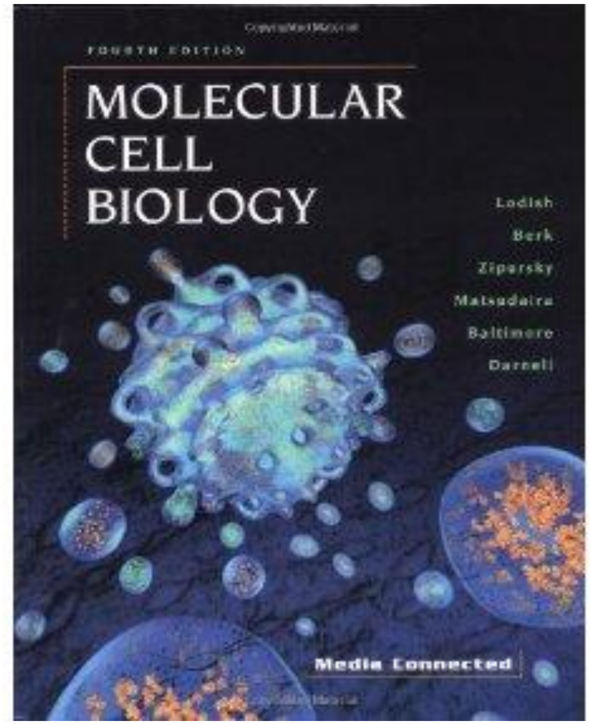
Erythromycin Binds to the 50S ribosome, and blocks the translocation

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

Suggested Textbook…



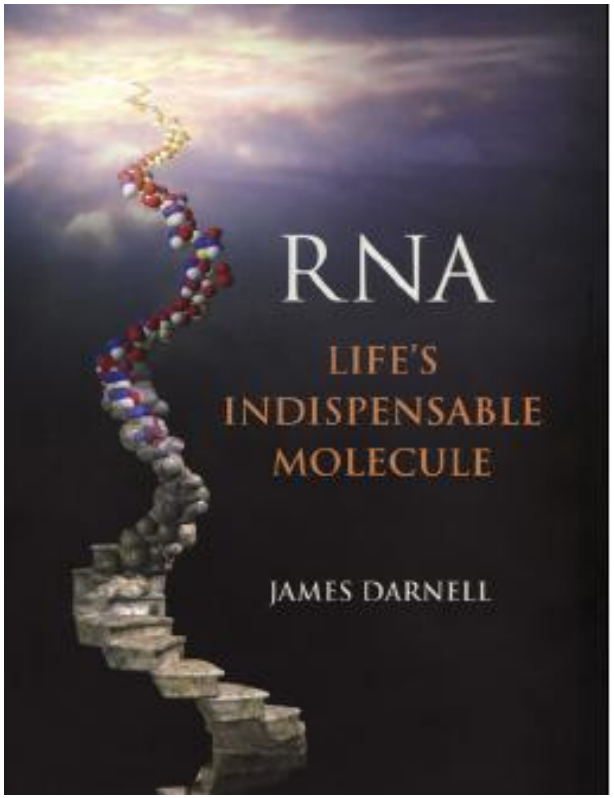
Stryer…



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