Molecular Basis of Inheritance & Bacterial Genetics

What determines an organism's traits?

DNA → **Genes** → Proteins → Traits

OK, What exactly is a gene?

- (Basic Definition) A unit of inheritance that controls a phenotypic character.
- (Better Definition) A nucleotide sequence along a molecule of DNA that codes for a protein.
- **(Best Definition) A region of DNA that can be expressed to produce a final functional product that is either a polypeptide or an RNA molecule.**

DNA..."The Blueprints of Life"

DNA ⟶ **Genes** ⟶ Proteins ⟶ Traits

DNA is the molecule of inheritance!

- This idea was hotly contested through half of the 20th century.
- Finally in 1953 James Watson and Francis Crick not only confirmed that DNA was in fact the molecule of inheritance but they described its structure and hypothesized a replicating mechanism.
- **The story begins in the early 1900's...**

DNA..."The Story"

After the work of Darwin and Mendel the race was on to clarify the vague meaning of "units of heredity"...What exactly were the units of heredity?

- Two leading suspects were nucleic acids and proteins.
- Most thought proteins were more likely suspect.
  - with 20 subunits making up protein and only 4 subunits making up nucleic acids, protein diversity was enormous

DNA…"The Story"

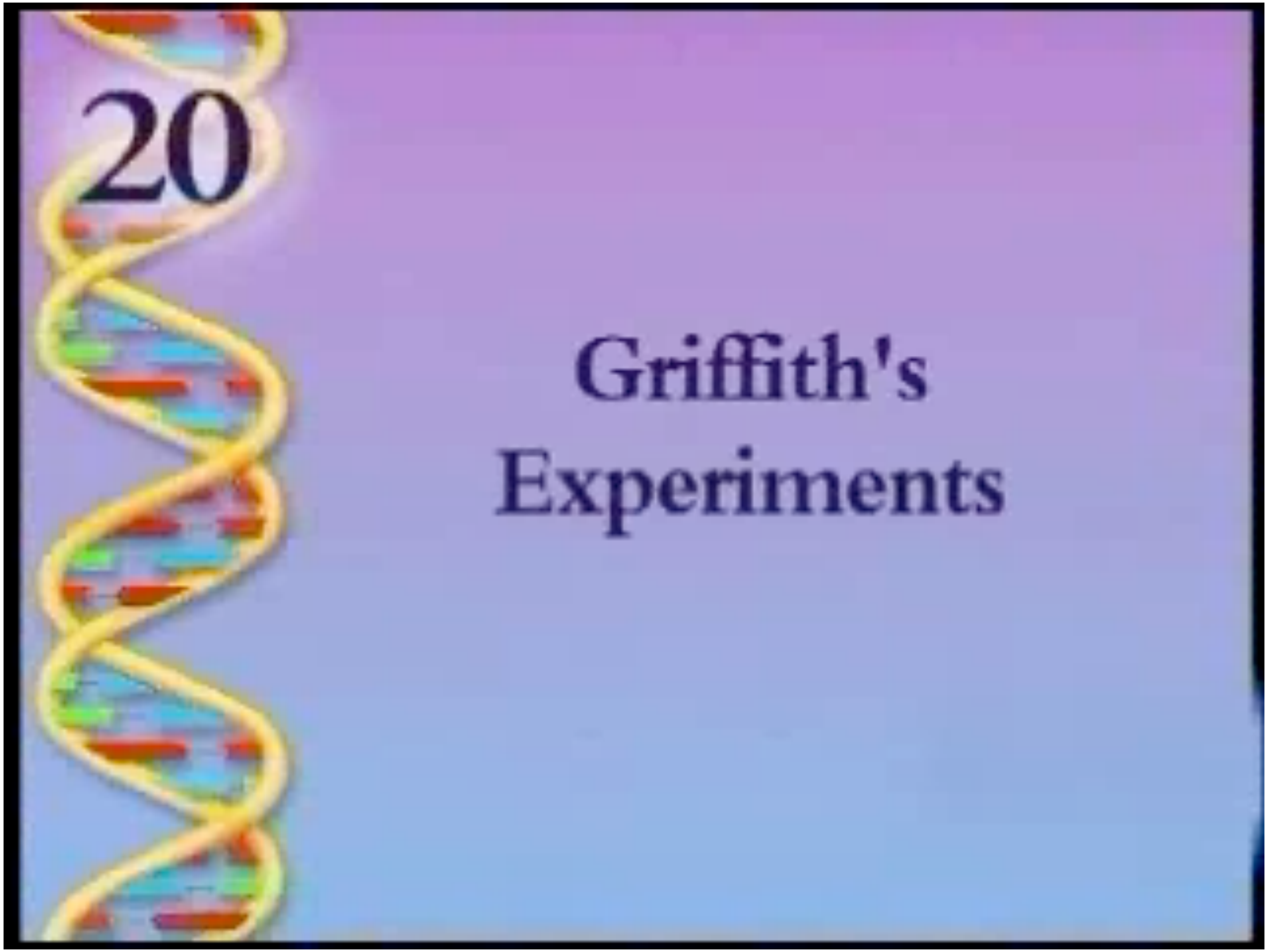
1928 Frederick Griffith

- While trying to develop a vaccine against *Streptoccocus pneumoniae,* he made a rather interesting observation.
- He later explained his observation by a term he coined
    - ***Transformation*** a chemical component moved from one cell to another and changed the phenotype of the recipient cell.
    - Today we know that external DNA was taken up and assimilated by the bacteria whose phenotype was altered

Griffith's Experiment is described on the next slide

DNA..."The Story"

1928 Frederick Griffith

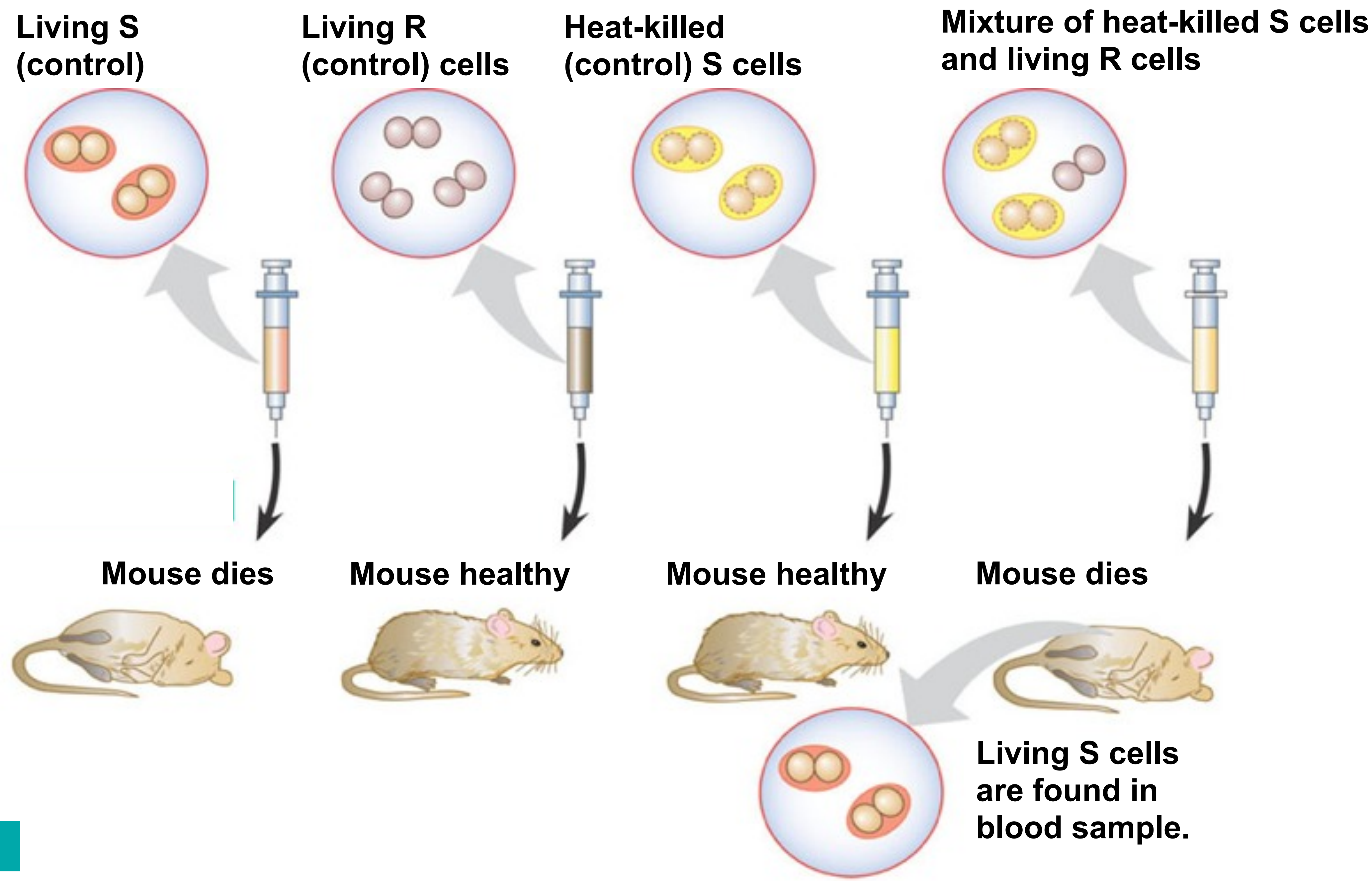


1928 Frederick Griffith

EXPERIMENT

Bacteria of the “S” (smooth) strain of *Streptococcus pneumoniae* are pathogenic because they have a capsule that protects them from an animal’s defense system. Bacteria of the “R” (rough) strain lack a capsule and are nonpathogenic. Frederick Griffith injected mice with the two strains as shown below:

RESULTS



CONCLUSION

Griffith concluded that the living R bacteria had been transformed into pathogenic S bacteria by an unknown, heritable substance from the dead S cells.

DNA..."The Story"

1944 Avery, McCarty & Macleod

- For 14 years Oswald Avery tried to determine the identity of Griffith's "transforming" agent.
- Avery's work centered around purifying molecules from the heat killed bacteria
- Finally in 1944 he and his colleagues identified the agent... DNA!
    - Ironically the results generated interest but many were skeptical and felt that protein was a better suspect.
    - Also many felt even if this were true of bacteria surely "humans" would have a different molecule of inheritance.

DNA..."The Story"

1947-1950 Erwin Chargaff

- A biochemist, Chargaff was analyzing and comparing DNA from different species.
- From his work he two observations emerged which later became known as "Chargaff's Rules"
  - (ironically there was no basis for them at the time)
- **Rule 1: nucleic acid bases vary between species**
  - this was somewhat unexpected
- **Rule 2: within a species the number of A bases are equal to T bases and C bases are equal to G bases**

DNA..."The Story"

Early 20th Century Circumstantial Evidence

- Biologists had noted that the amount of DNA in a cell prior to cell division was "X", prior to mitosis the amount was "2X" and after cell division the amount of DNA returned to "X" amount.
  - It was a fact that biologists could not explain at the time, with their current understandings.
  - Obviously later with new perspectives this makes perfect sense

Makes you wonder... what knowledge today is floating around, going unnoticed, waiting to help us answer one of our many unanswered questions.

DNA..."The Story"

1952 Alfred Hershey & Martha Chase

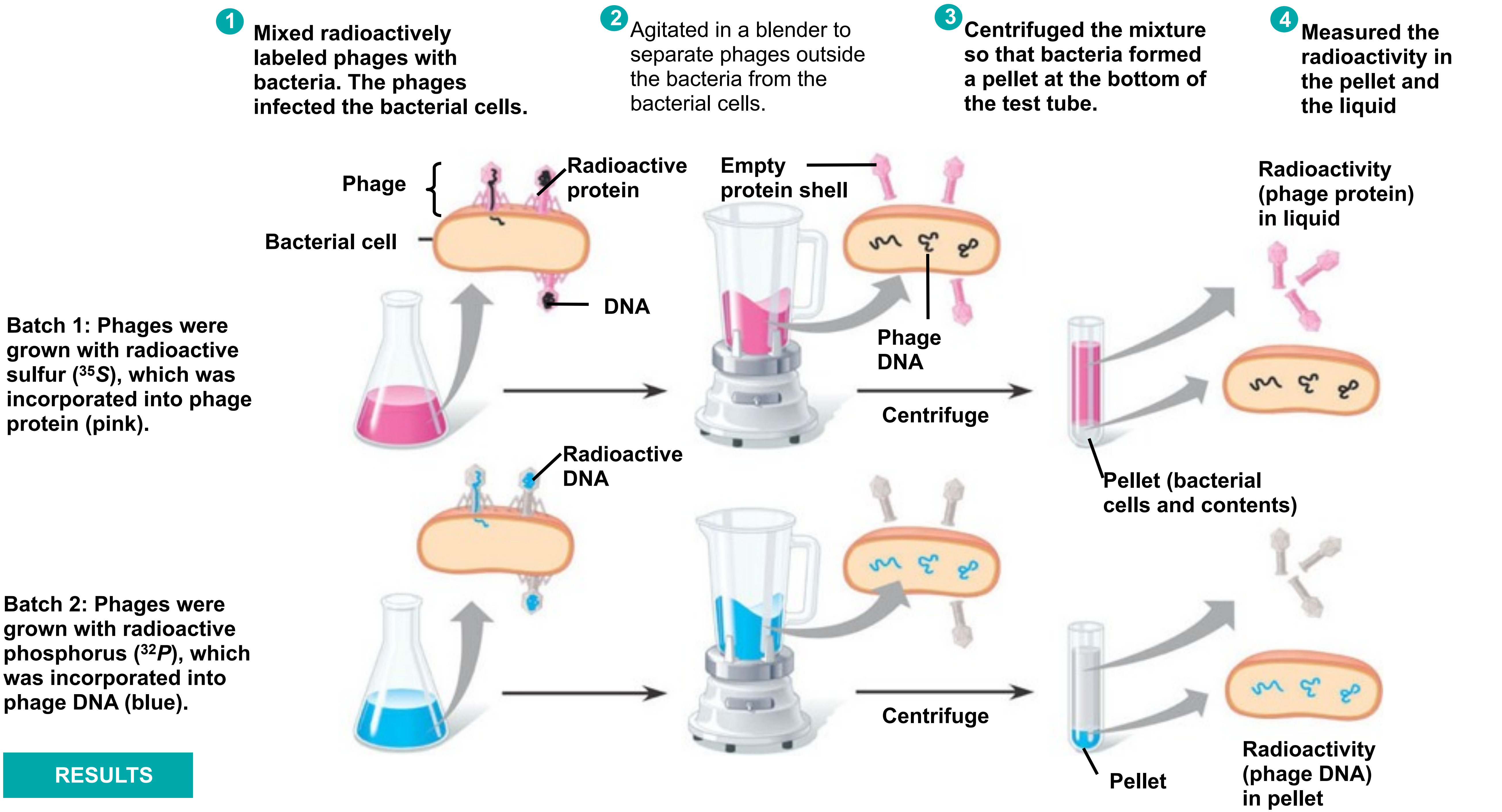
- Hershey and Chase continued the search for the elusive "unit of heredity".
- Hershey and Chase designed simple, elegant and powerful experiment using bacteriophages and radioactive elements.
    - Their experiment definitively showed that DNA was in fact the unit of heredity used by viruses
        - many began to contemplate the idea that DNA may be the unit of heredity for all living organisms
        - The tide was changing!

Hershey and Chase's Experiment is described on the next slide

1952 Alfred Hershey & Martha Chase

EXPERIMENT

In their famous 1952 experiment, Alfred Hershey and Martha Chase used radioactive sulfur and phosphorus to trace the fates of the protein and DNA, respectively, of T2 phages that infected bacterial cells.



RESULTS

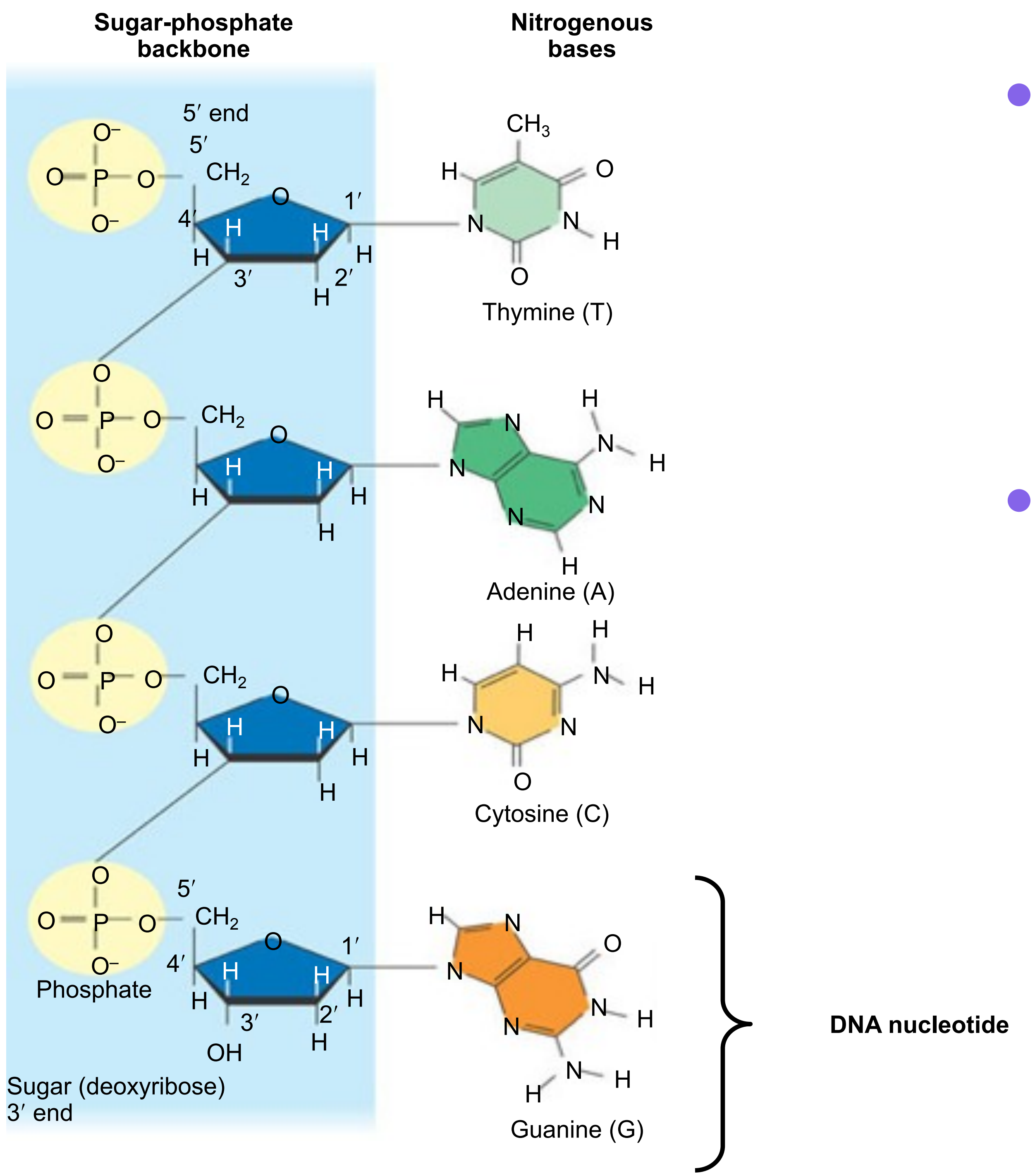
Phage proteins remained outside the bacterial cells during infection, while phage DNA entered the cells. When cultured, bacterial cells with radioactive phage DNA released new phages with some radioactive phosphorus.

CONCLUSION

Hershey and Chase concluded that DNA, not protein, functions as the T2 phage's genetic material.

DNA..."The Story"

1950's Rosalind Franklin, Linus Pauling, Maurice Wilkins



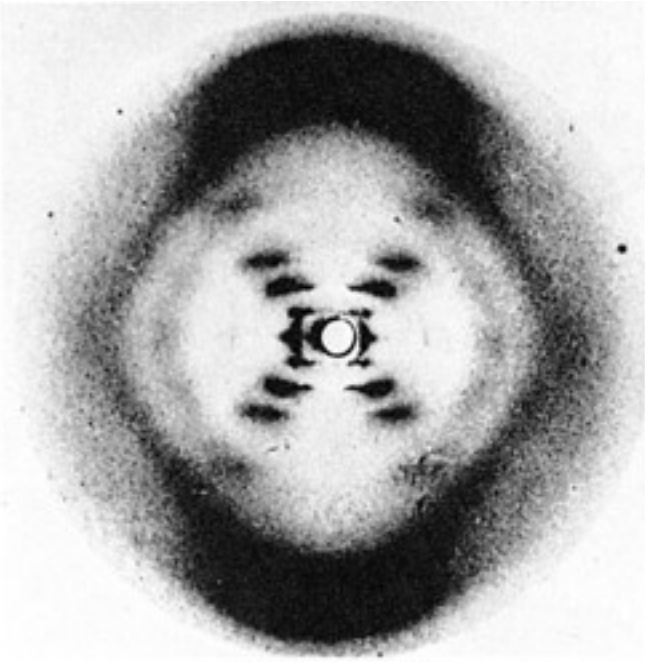
- By now many felt that DNA was the elusive "unit of heredity" and the next step would be to determine its structure.
- Prior to the 1950's chemists already knew that DNA is a polymer of nucleotides, each consisting of three components: a nitrogenous base, a sugar, and a phosphate group.

DNA…"The Story"

1950's Rosalind Franklin, Linus Pauling, Maurice Wilkins

- Rosalind Franklin wrote that the sugar-phosphate groups made up the backbone on DNA.
- Wilkins and Franklin used X-ray crystallography to determine DNA's 3-D shape but could interpret the images

(a) Rosalind Franklin

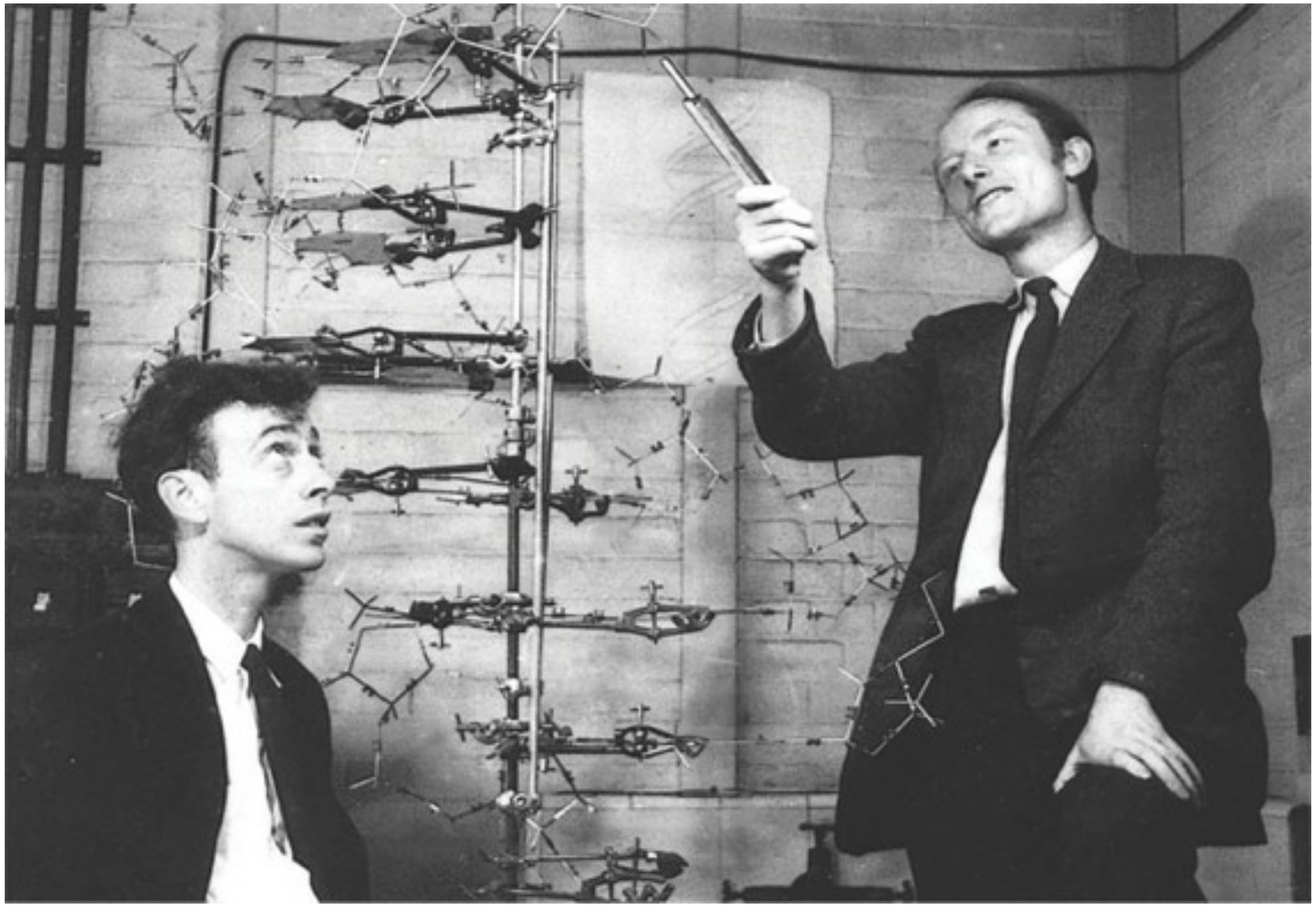


(b) Franklin's X-ray diffraction Photograph of DNA

DNA…"The Conclusion"

1953 James Watson & Francis Crick

- Watson & Crick put all the puzzle pieces together in a 1 page paper that described the structure of DNA.
  - They won Nobel Prize



DNA..."The Conclusion"

The Puzzle Pieces

- Watson recognized the x-ray image from Wilkin's lab as a helix.
- They used what chemists already knew about DNA
- They used Chargaff's observations
- They read Franklin's paper suggesting a sugar-phosphate backbone
    - From these pieces Watson & Crick deduced the following structure of DNA as seen on the next few slides

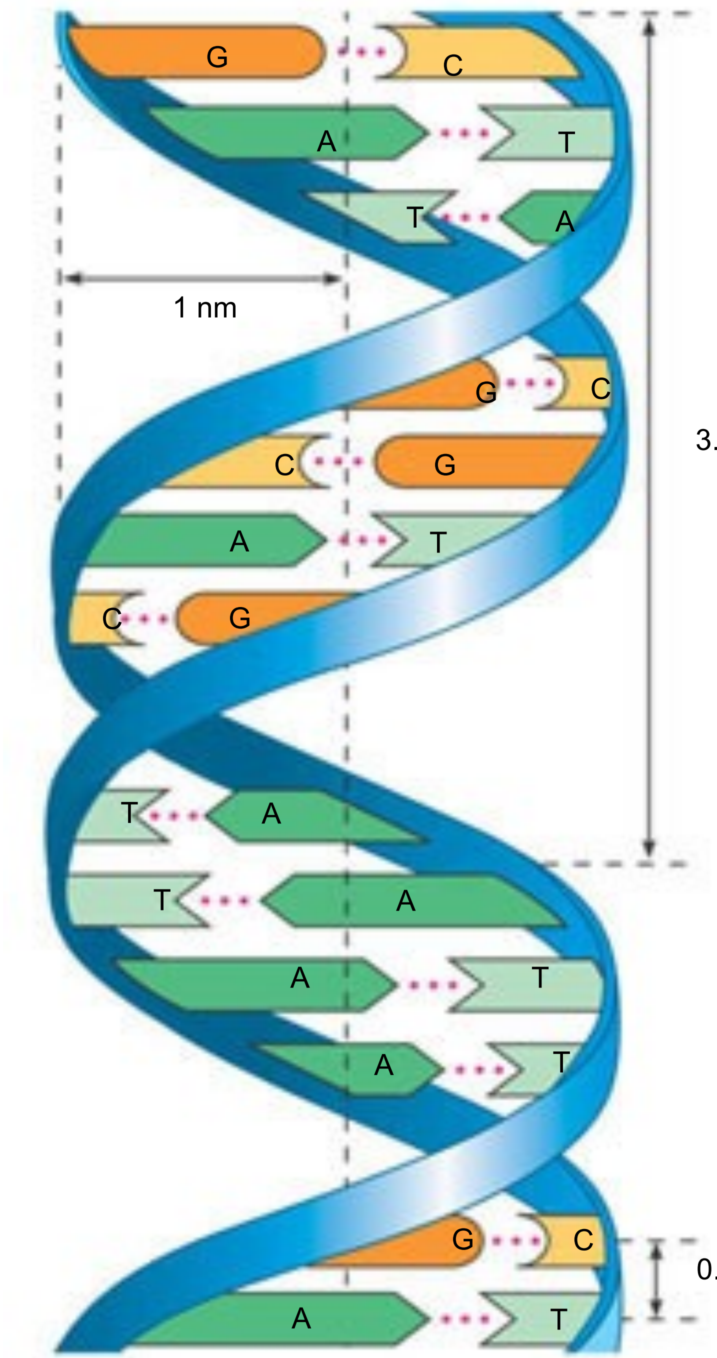
DNA

They knew...

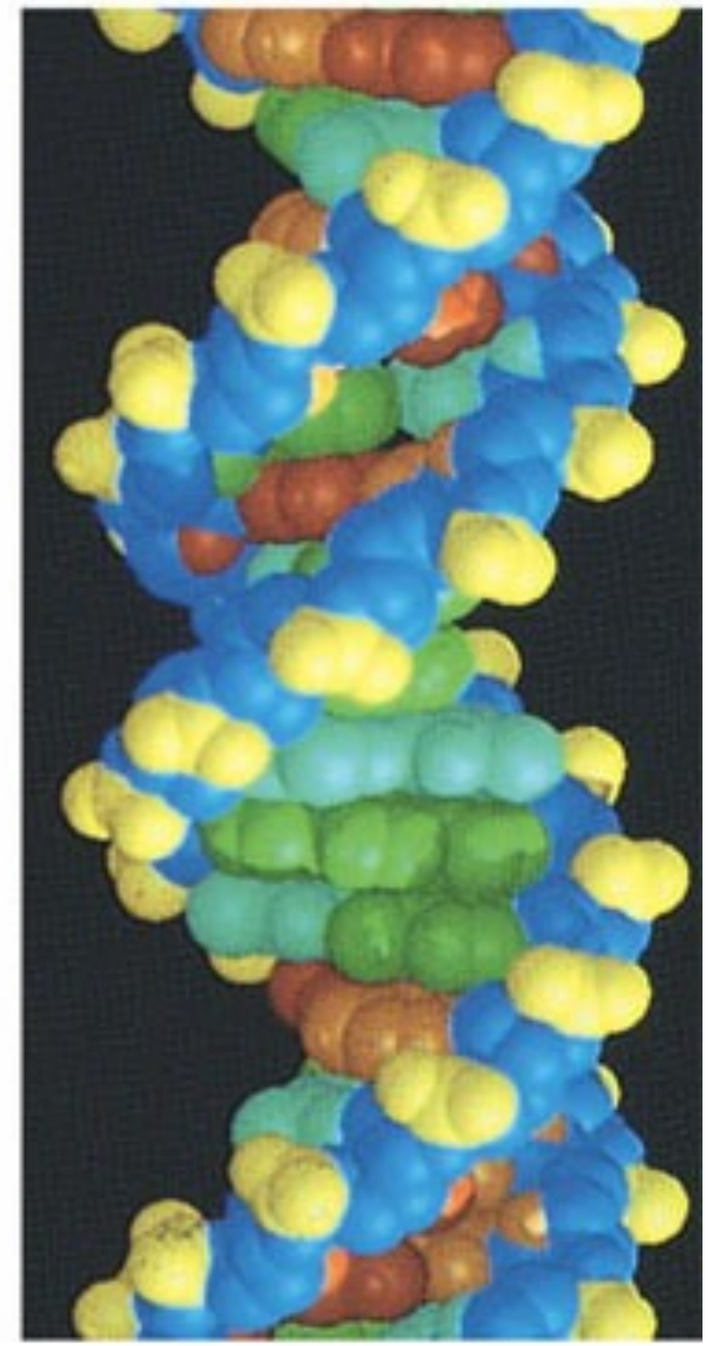
its width

its length

its shape



(a) Key features of DNA structure



(c) Space-filling model

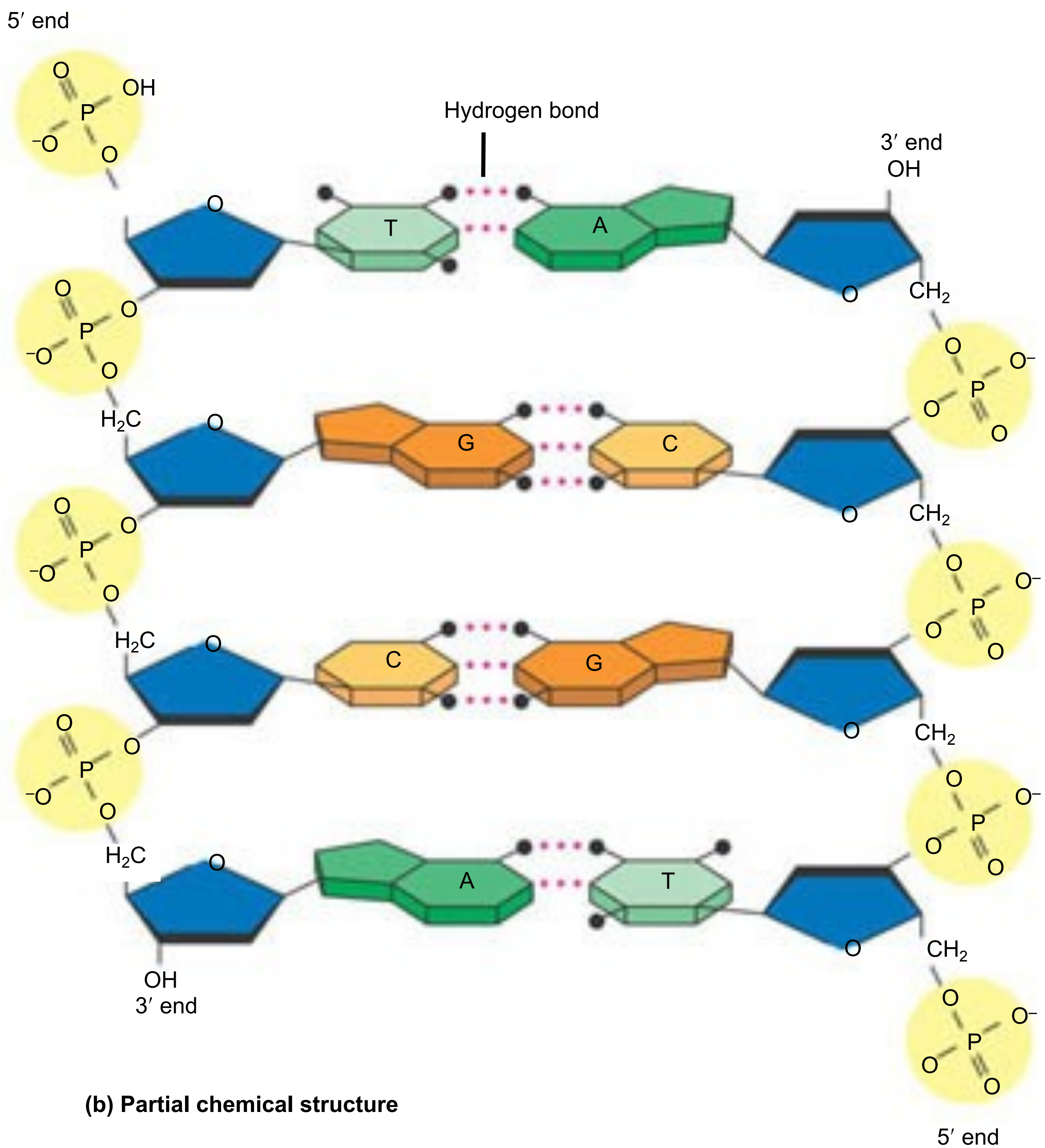
DNA

the structure of the backbone

its anti-parallel nature

in fact they even hypothesized a replicating mechanism

They knew...



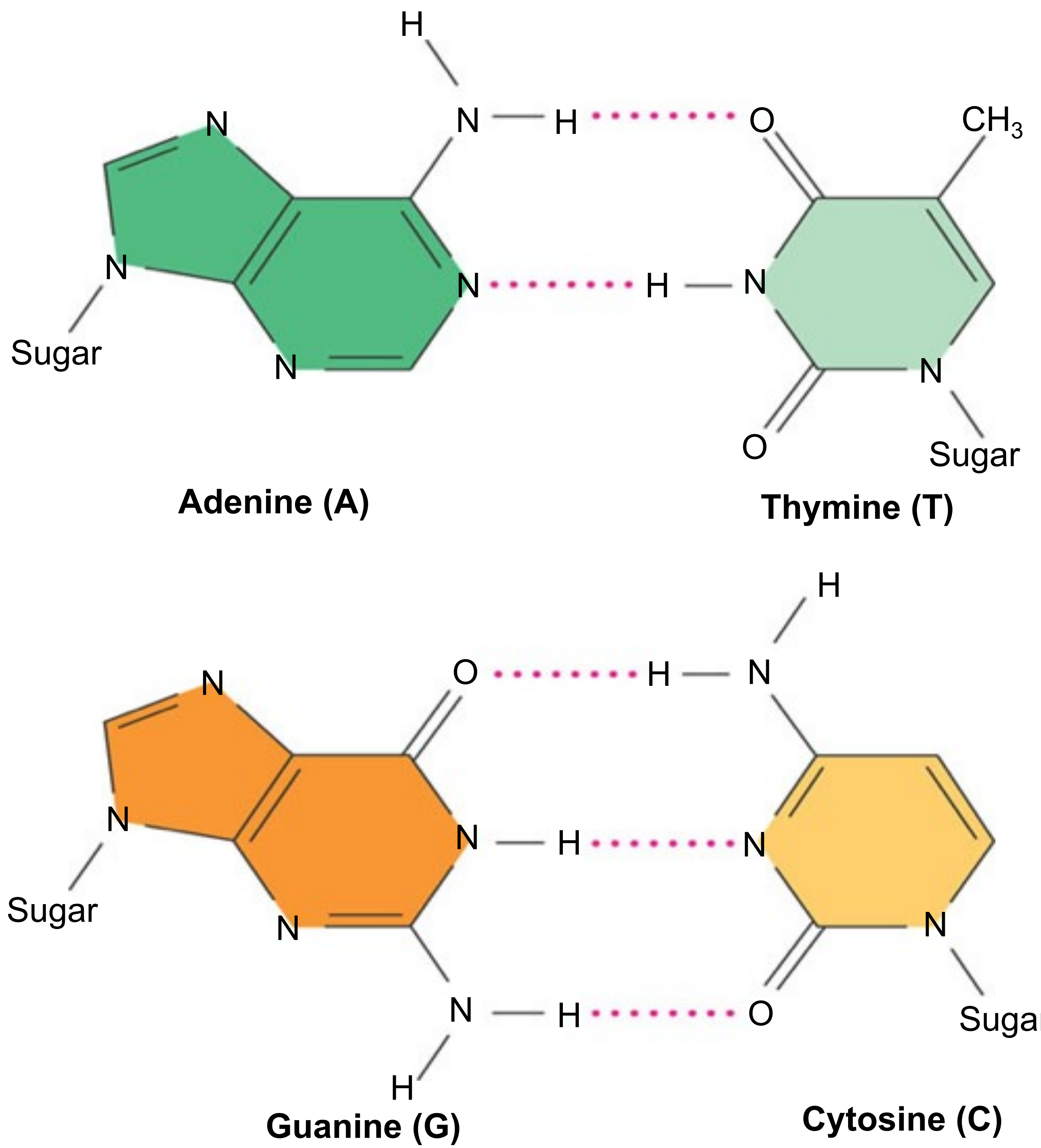
(b) Partial chemical structure

DNA

They knew...

base pairing rules

bonds between the bases



Revisit this idea...

DNA ⟶ **Genes** ⟶ Proteins ⟶ Traits

OK, What exactly is a gene?

- (Basic Definition) A unit of inheritance that controls a phenotypic character.
- (Better Definition) A nucleotide sequence along a molecule of DNA that codes for a protein.
- **(Best Definition) A region of DNA that can be expressed to produce a final functional product that is either a polypeptide or an RNA molecule.**

How do genes produce traits?

Genoytpe ? → **Phenoytpe**

Genes → **Proteins** → Traits

Proteins are the link between genotypes and phenotypes
Proteins are the link between genotypes and phenotypes
Proteins are the link between genotypes and phenotypes
Proteins are the link between genotypes and phenotypes

How do genes produce traits?

Global Flow of Information

DNA → RNA → Protein

- The flow of genetic information involves two processes.
  - Transcription
  - Translation
- Together these two processes represent gene expression.

Gene Expression..."The Story"

Early 1900's Archibald Garrod

- Biochemists knew that cells make and break molecules and that every reaction is catalyzed by a specific enzyme.
- **In 1902, British physician Archibald Garrod hypothesized that "genes" control enzymes which in return control phenotypes.**
    - He was well ahead of his time
- His ideas were sound, he explained how certain diseases might occur, he even referred to these diseases as "inborn errors of metabolism" .
- However he lacked the details: specific reactions, enzymes and even what genes were are largely unknown at this time.

Gene Expression..."The Story"

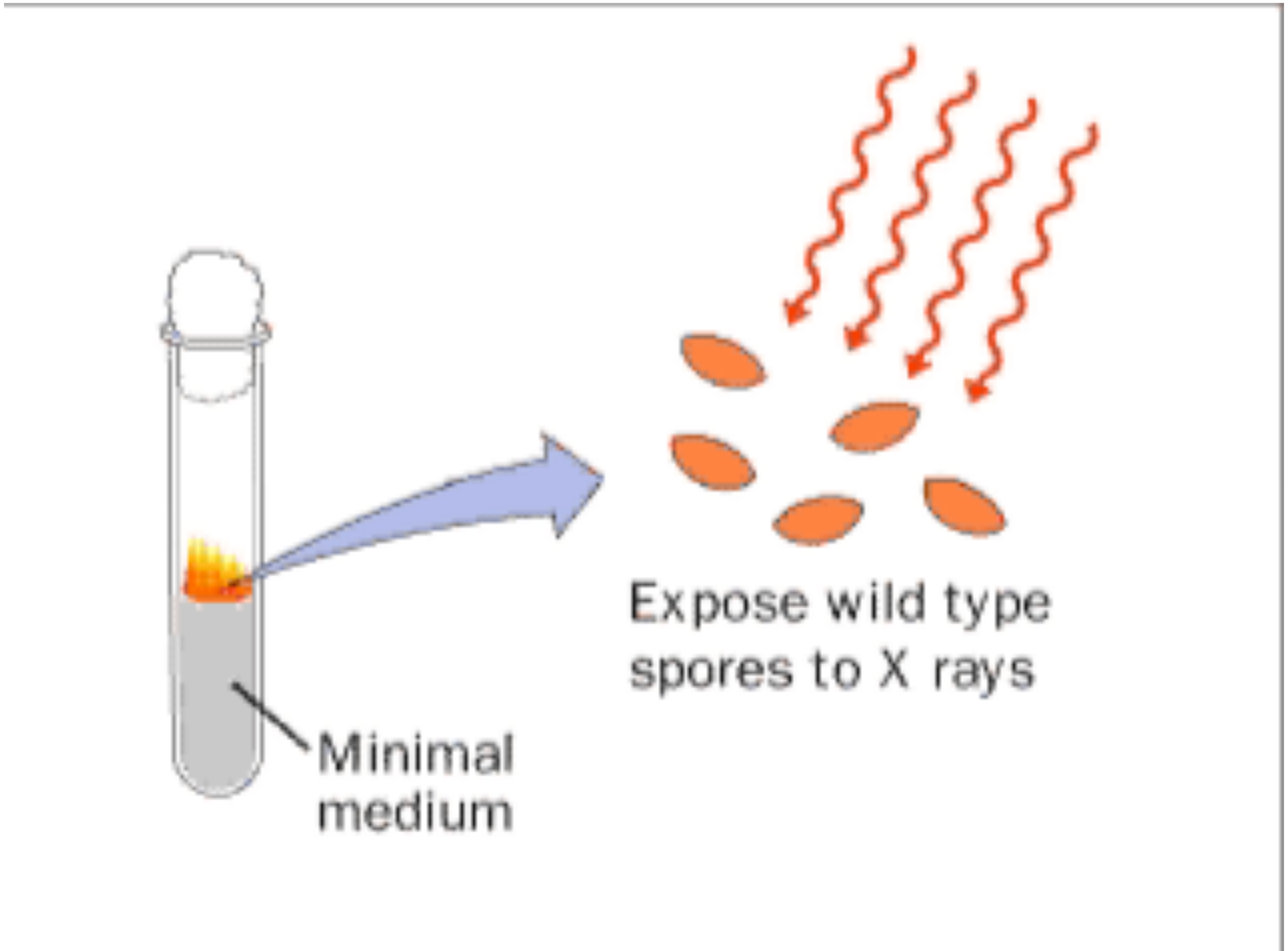
Early 1940's George Beadle & Edward Tatum

- The work of Beadle and Tatum supported the claims made decades earlier by Garrod.
- **Beadle & Tatums's experimental results supported their** ***one gene - one enzyme hypothesis*** **(which states that one dictates the production of a specific enzyme).**
    - Beadle and Tatum shared the 1958 Nobel Prize for their work

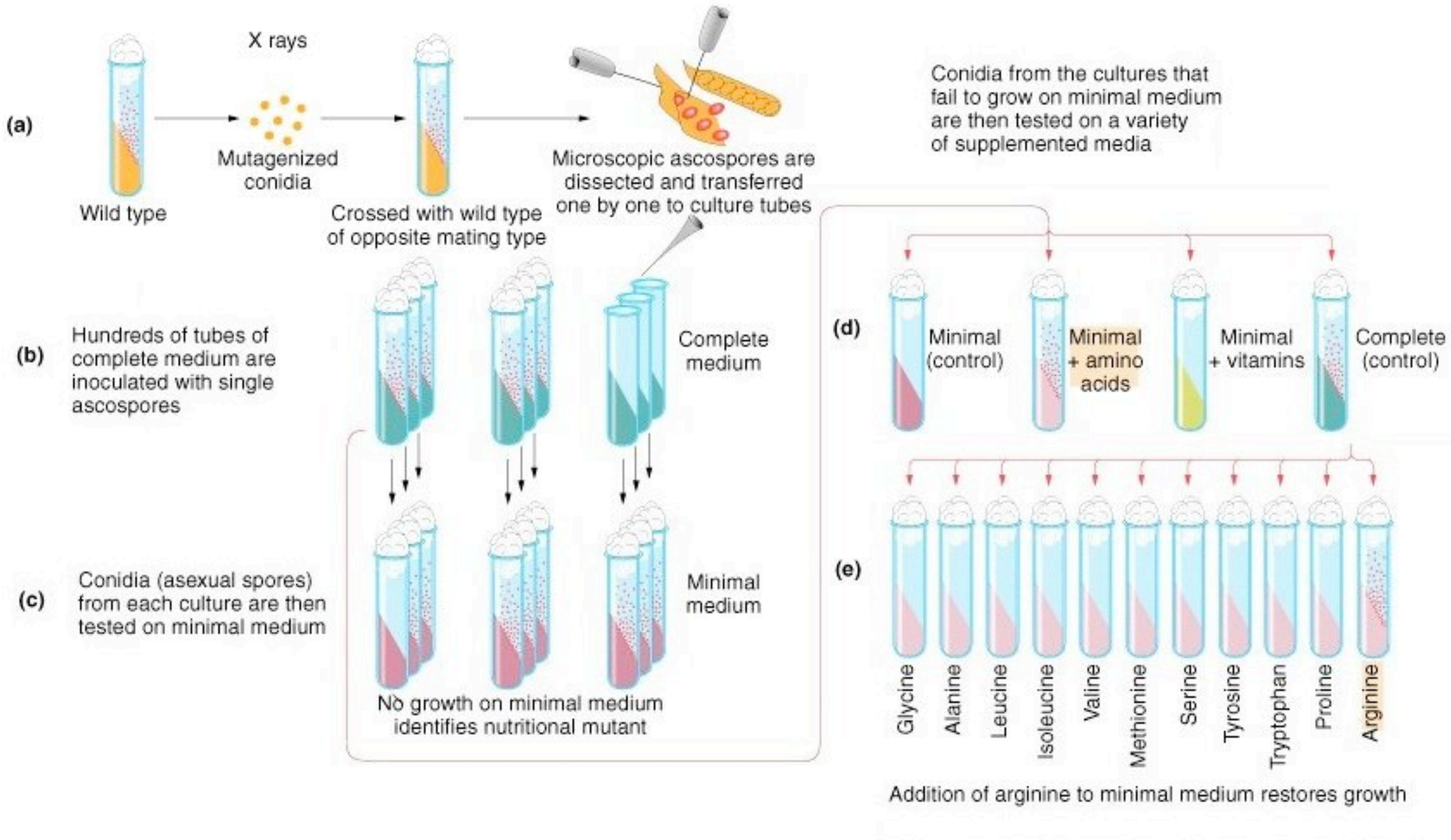
Beadle & Tatum's Experiment is described on the next slide

Gene Expression..."The Story"

Early 1940's George Beadle & Edward Tatum



George Beadle & Edward Tatum Experiment



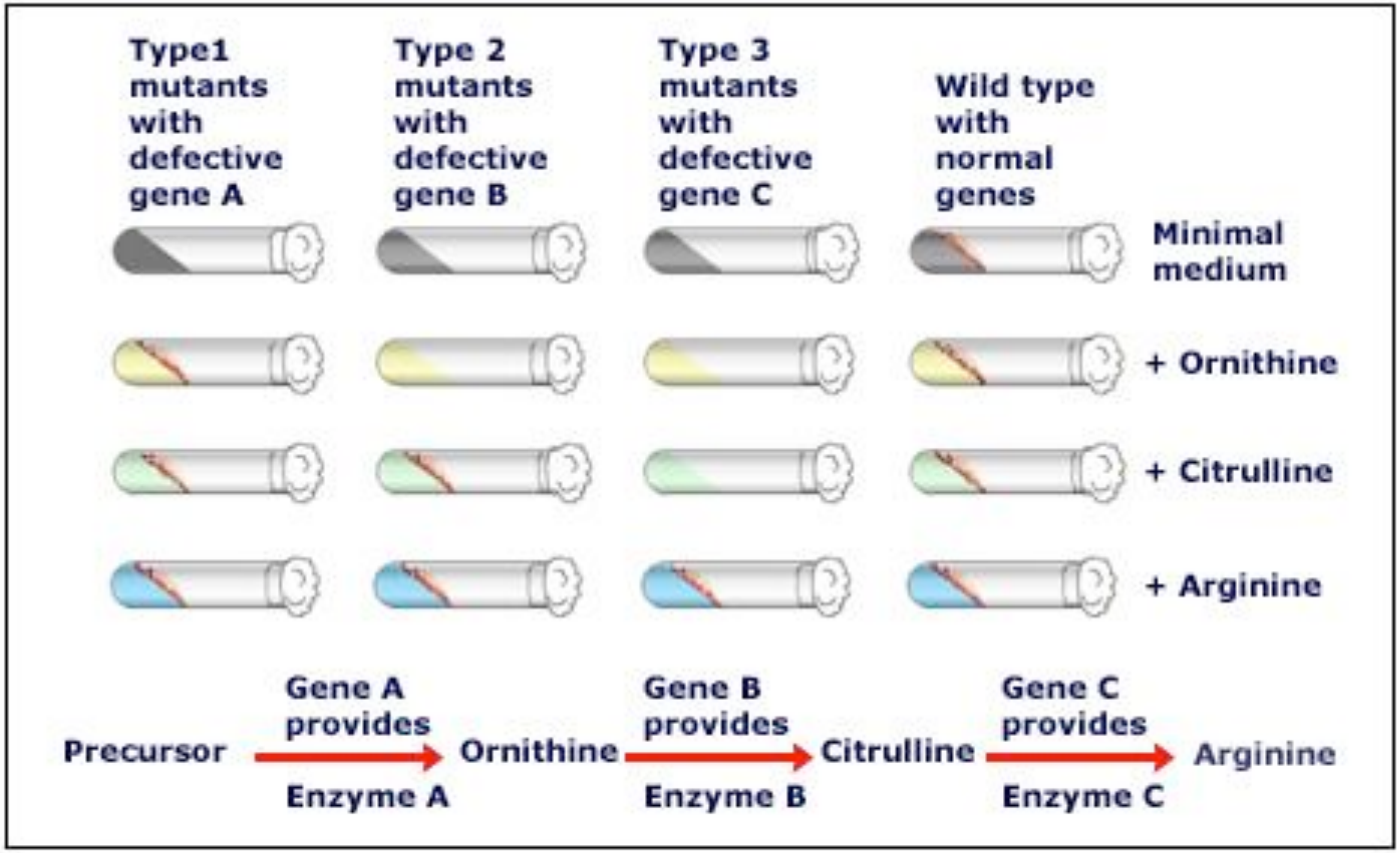
Gene Expression..."The Story"

Early 1940's Adrian Srb & Norman Horowitz

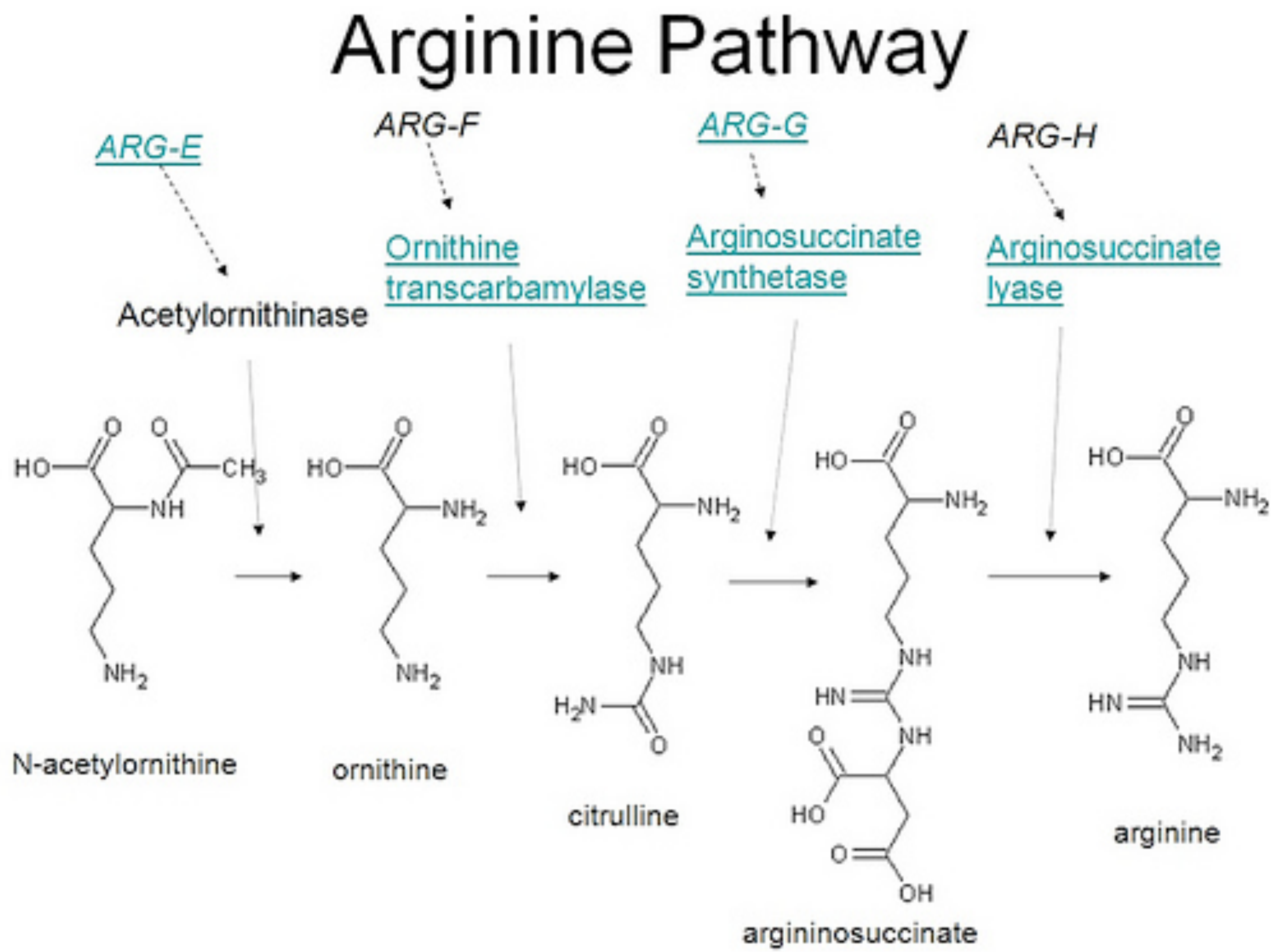
- Colleagues of Beadle and Tatum, Srb and Horowitz used a similar approach to investigate the specific biochemical pathway for *Arginine*.
- **Srb and Horowitz's experimental results provided additional support for the *one gene - one enzyme hypothesis*.**

Srb & Horowitz's Experiment is described on the next slide

Adrian Srb & Norman Horowitz Experiment



Adrian Srb & Norman Horowitz Experiment



Gene Expression..."The Conclusion"

Decades Later

- As researched continued the *one gene - one enzyme hypothesis* was modified as our understanding grew and technologies evolved.
  - First we realize not all proteins are enzymes thus it became
    - *one gene - one protein hypothesis*
  - Later we learn thatmany proteins are constructed from multiple polypeptides thus it becomes
    - *one gene - one polypeptide hypothesis*
  - Today we know that genes also code for RNA molecules...thus

A region of DNA that can be expressed to produce a final functional product that is either a polypeptide or an RNA molecule.

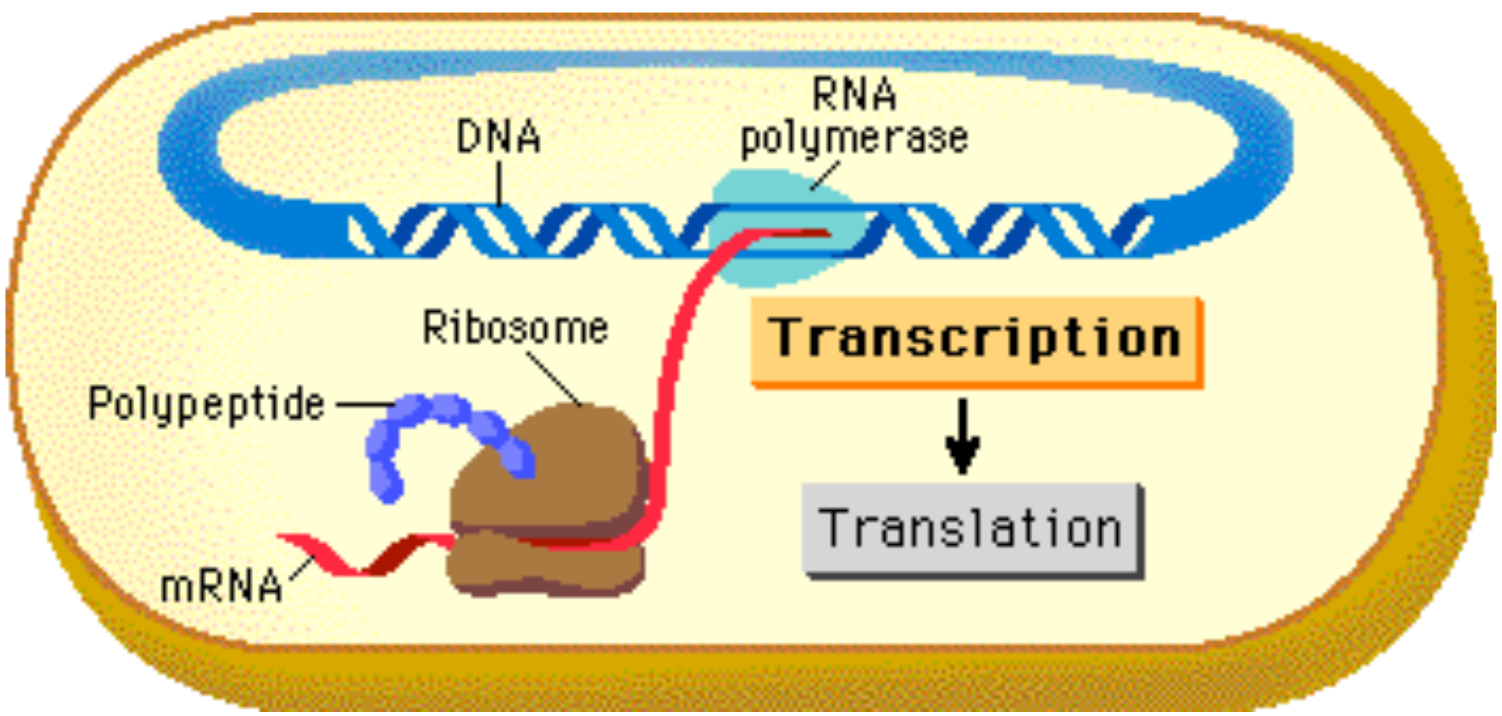
Protein Synthesis (The Basics)

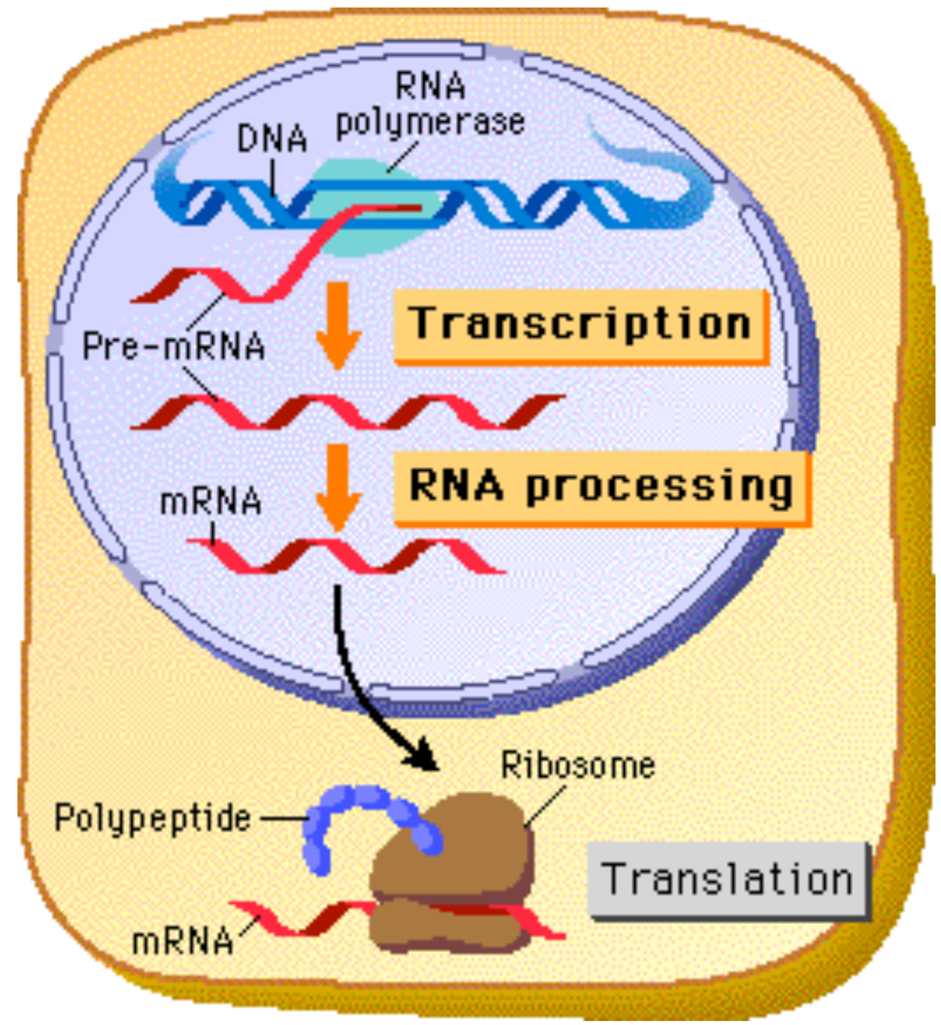
- The flow of genetic information involves two processes.
  - ***Transcription*, the synthesis of RNA using info stored in the DNA**
    - DNA serves as a template for mRNA
    - Their forms differ but their language is the same
  - ***Translation*, is the building of a polypeptide using the info stored in mRNA**
    - The language differs between nucleic acids and proteins
    - The cell must translate a nucleotide sequence into an amino acid sequence of the polypeptide

The Central Dogma

DNA → RNA → Protein

- **Transcription & Translation occurs in every organism.**
  - *The mechanics are the same or very similar in all cells*
  - *However, one very important difference exists between prokaryotes and eukaryotes*





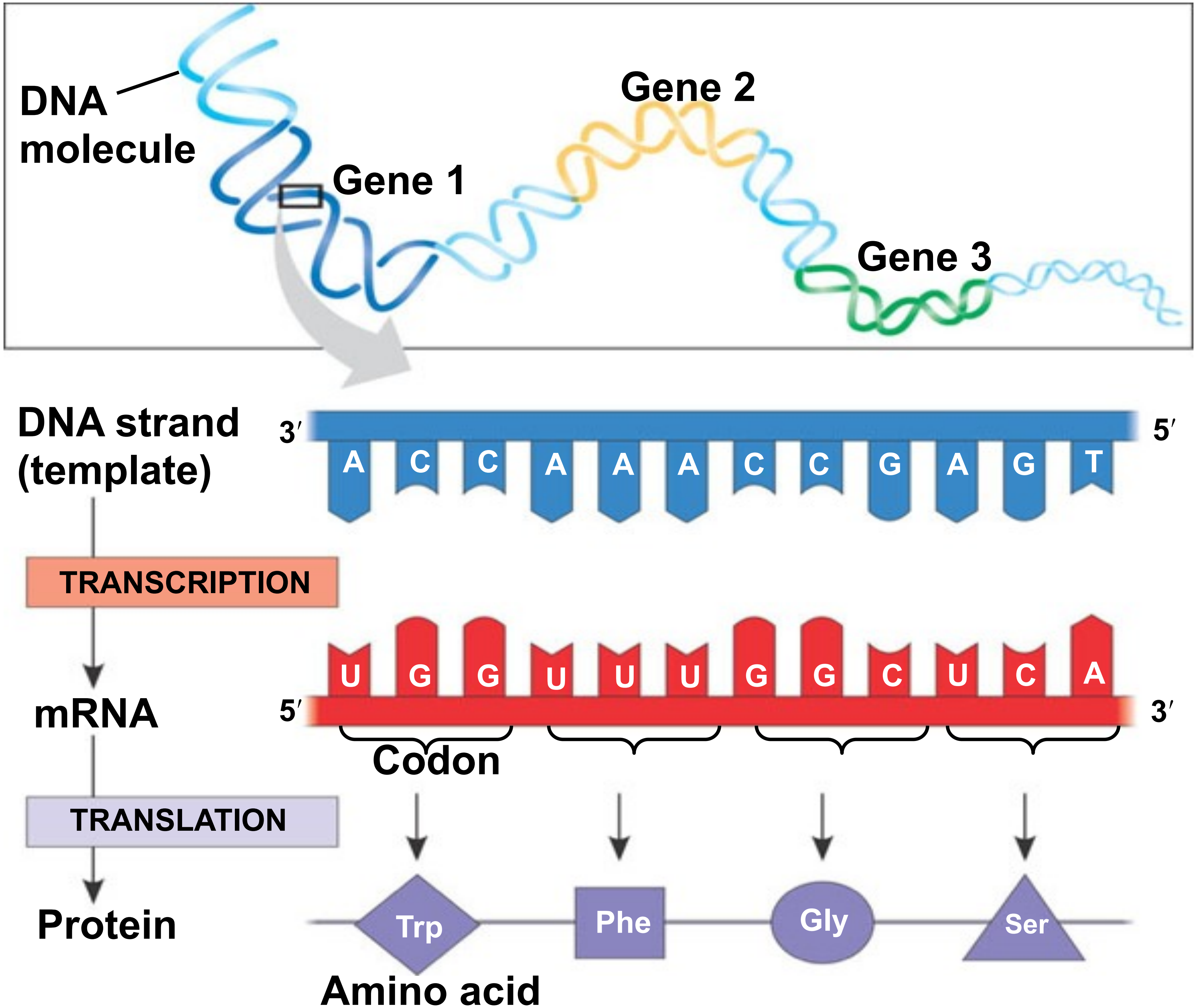
The Genetic Code

- **Once science agreed that DNA was the elusive "unit of inheritance"the next was to "crack the code".**
- **Both nucleic acids and proteins are long polymers made of molecular subunits BUT nucleic acids are built with only 4 nucleotides (subunits) and proteins are built using 20 amino acids (subunits).**
  - **How does a language with 4 characters translate into a language with 20 characters?**

The Genetic Code

- 1 nucleotide could not code for 1 amino acid it would not be enough. $4^1 = 4 < 20$
- 2 nucleotides could not code for 2 amino acids it would not be enough. $4^2 = 16 < 20$
- 3 nucleotides could not code for 3 amino acids it would be more than enough. $4^3 = 64 > 20$
  - We know that the language of life (nucleic acids) is written in a ***triplet code***.
  - ***DNA uses three non-overlapping nucleotides to code for three non-overlapping nucleotides (codons) of mRNA which in turn codes for a single amino acid.***

The Genetic Code



How many nucleotides would it take to build a protein with 250 amino acids?

750 (at least)

The Genetic Code

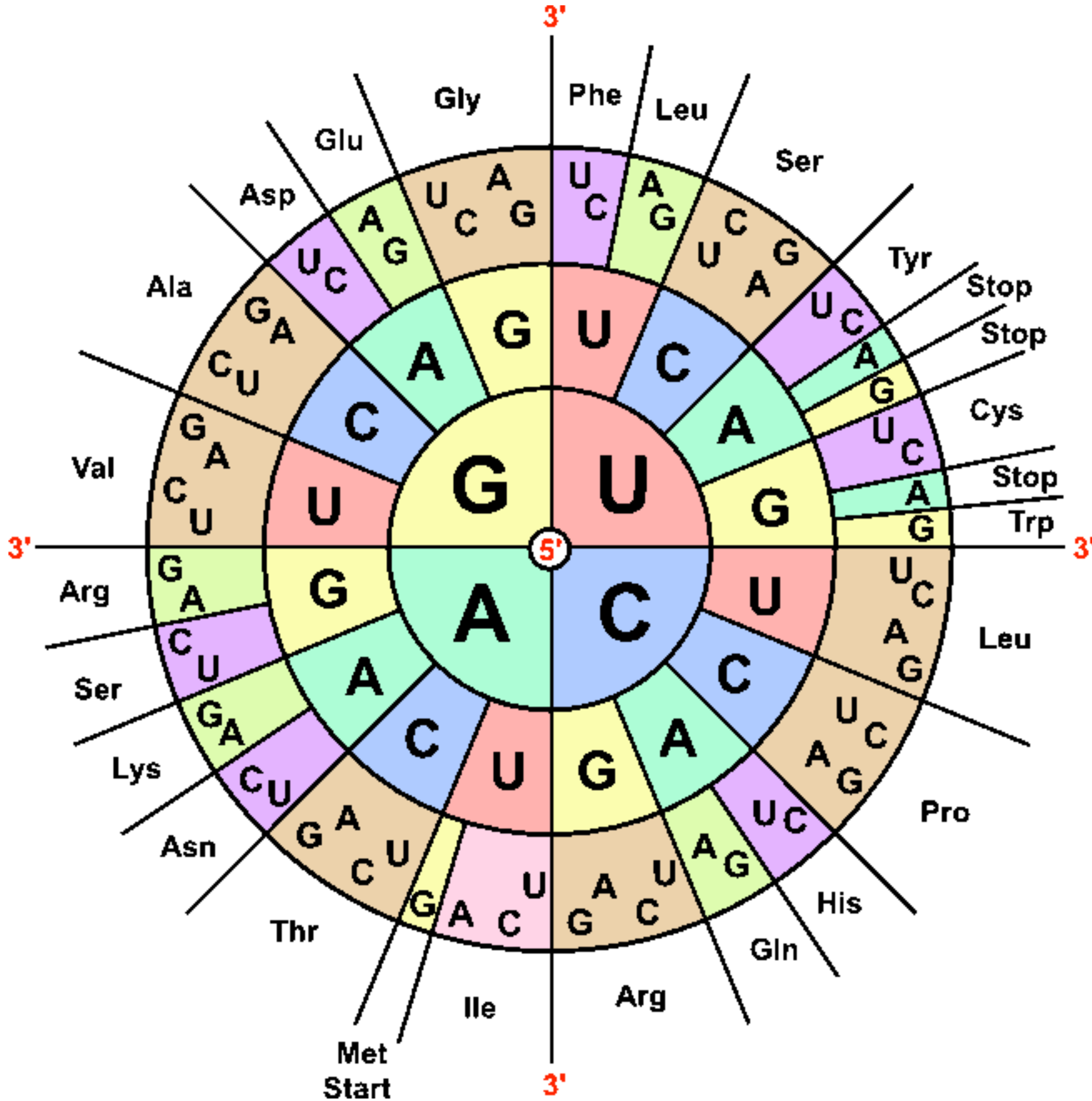
1961 Marshall Nirenberg

- Determined that UUU coded for the amino acid phenylalanine.
    - By the mid 1960's all 64 codons were deciphered.

Second Position

First Position	U		C		A		G		Third Position
	U		C		A		G		
U	UUU	Phe / F	UCU	Ser / S	UAU	Tyr / Y	UGU	Cys / C	U
U	UUC		UCC		UAC		UGC		C
U	UUA	Leu / L	UCA		*UAA*	*STOP*	*UGA*	*STOP*	A
U	UUG		UCG		*UAG*	*STOP*	UGG	Trp / W	G
C	CUU	Leu / L	CCU	Pro / P	CAU	His / H	CGU	Arg / R	U
C	CUC		CCC		CAC		CGC		C
C	CUA		CCA		CAA	Gln / Q	CGA		A
C	CUG		CCG		CAG		CGG		G
A	AUU	Ile / I	ACU	Thr / T	AAU	Asn / N	AGU	Ser / S	U
A	AUC		ACC		AAC		AGC		C
A	AUA		ACA		AAA	Lys / K	AGA	Arg / R	A
A	AUG	Met / M	ACG		AAG		AGG		G
G	GUU	Val / V	GCU	Ala / A	GAU	Asp / D	GGU	Gly / G	U
G	GUC		GCC		GAC		GGC		C
G	GUA		GCA		GAA	Glu / E	GGA		A
G	GUG		GCG		GAG		GGG		G

Another Amino Acid Look Up Table

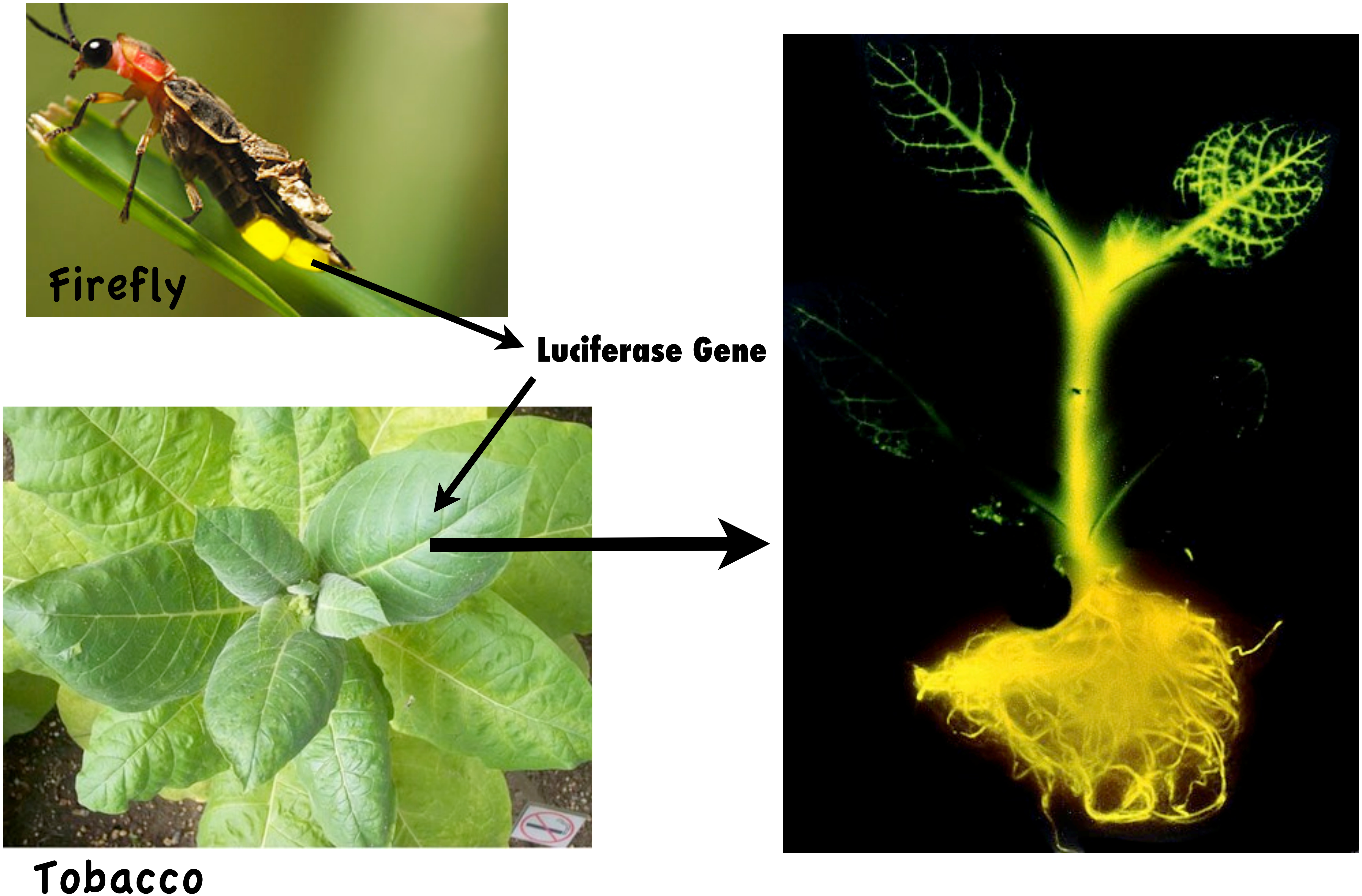


The Genetic Code

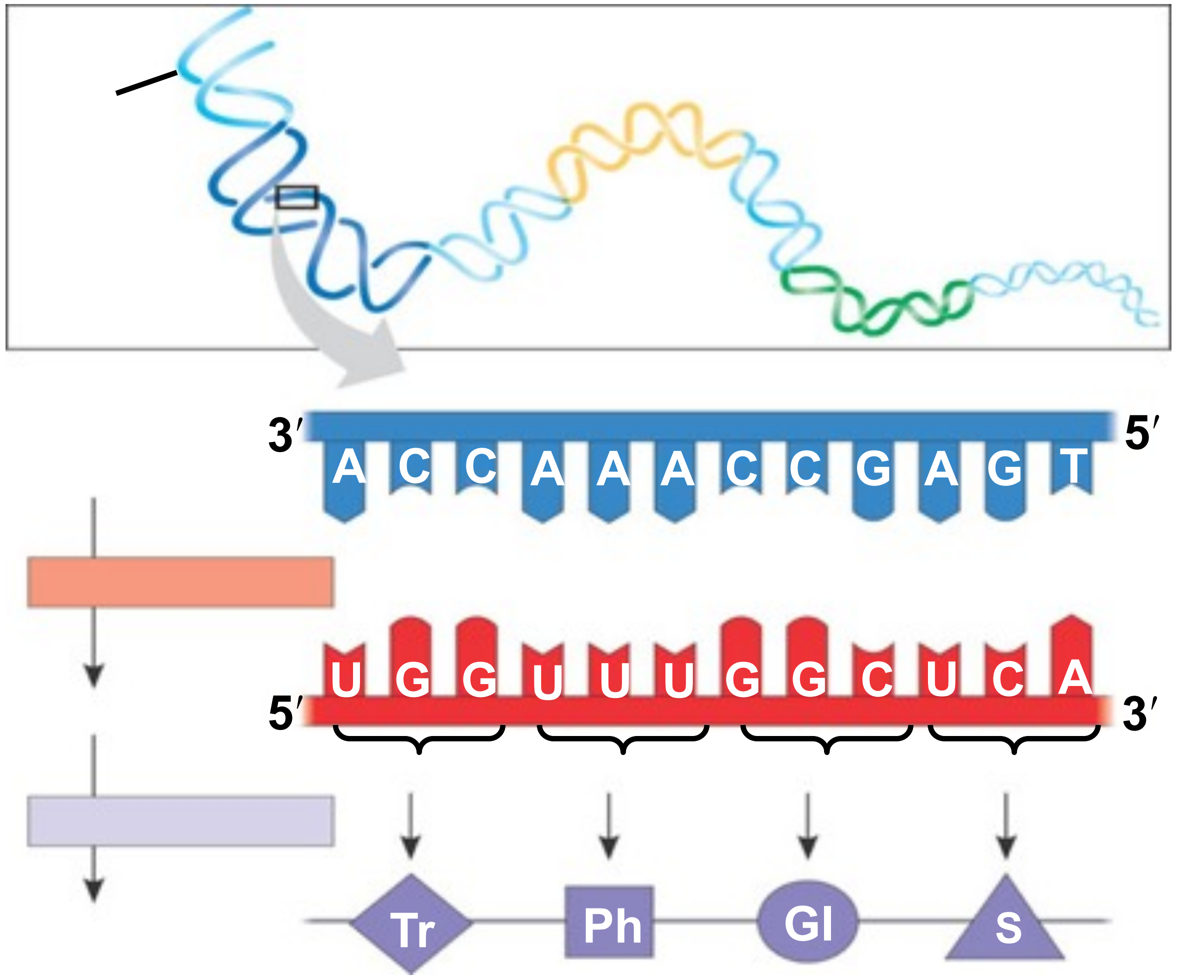
- The genetic code has some noteworthy characteristics.
  - **Redundancy**
    - AGU = serine, AGC = serine, multiple codons exist for the same amino acid
  - **No Ambiguity**
    - AGU = serine, any codon always codes for the same amino acid, it never changes
  - **Universal* (nearly)**
    - This code is identical from bacteria to blue whales!

*A shared genetic code supports the idea common ancestry among all living organisms

Universal Genetic Code



A Short Side Trip...

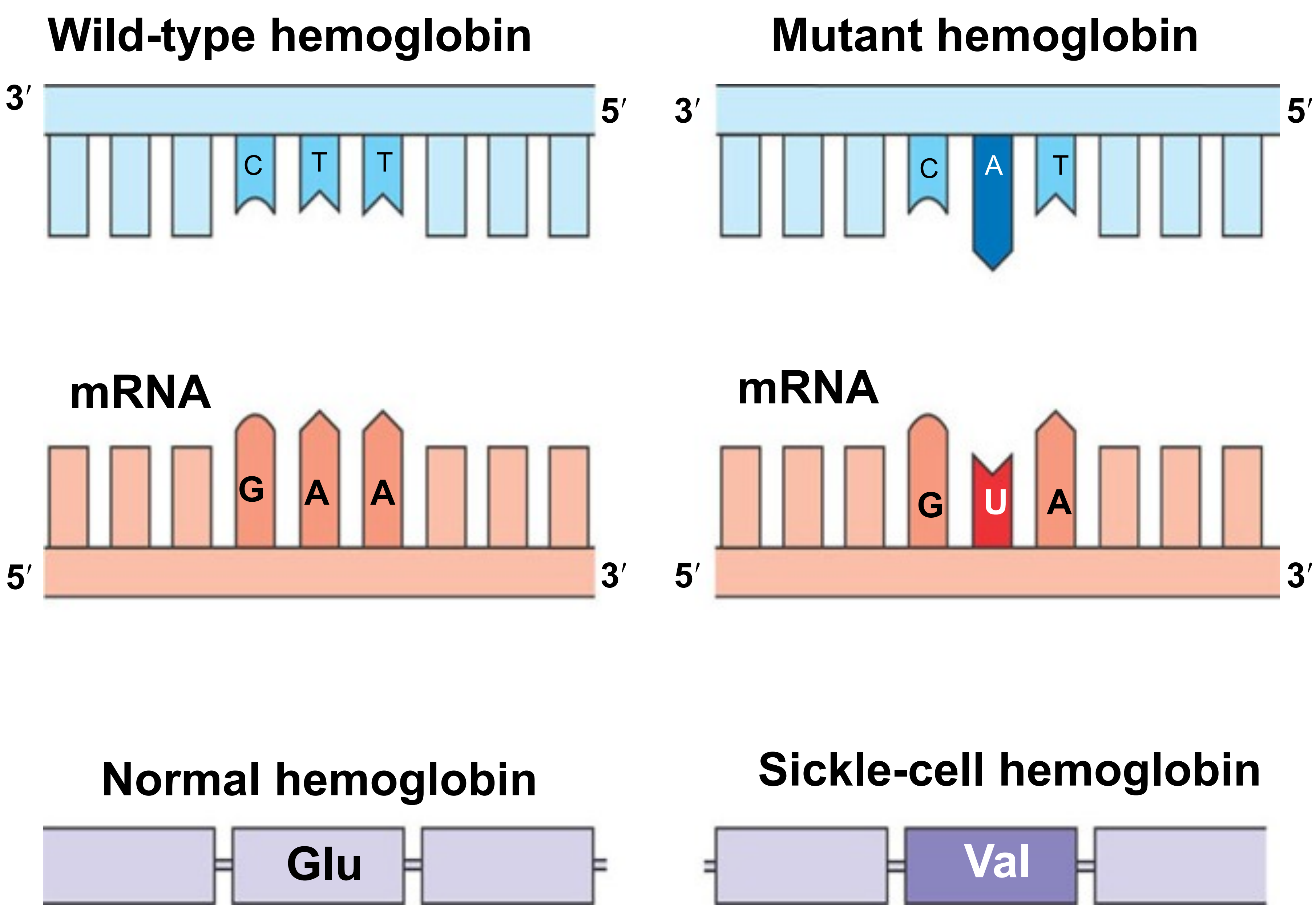


Quick Reminder...

What happens when one or more of these nucleotides changes?

Point Mutations

Changes in one base pair of a gene.

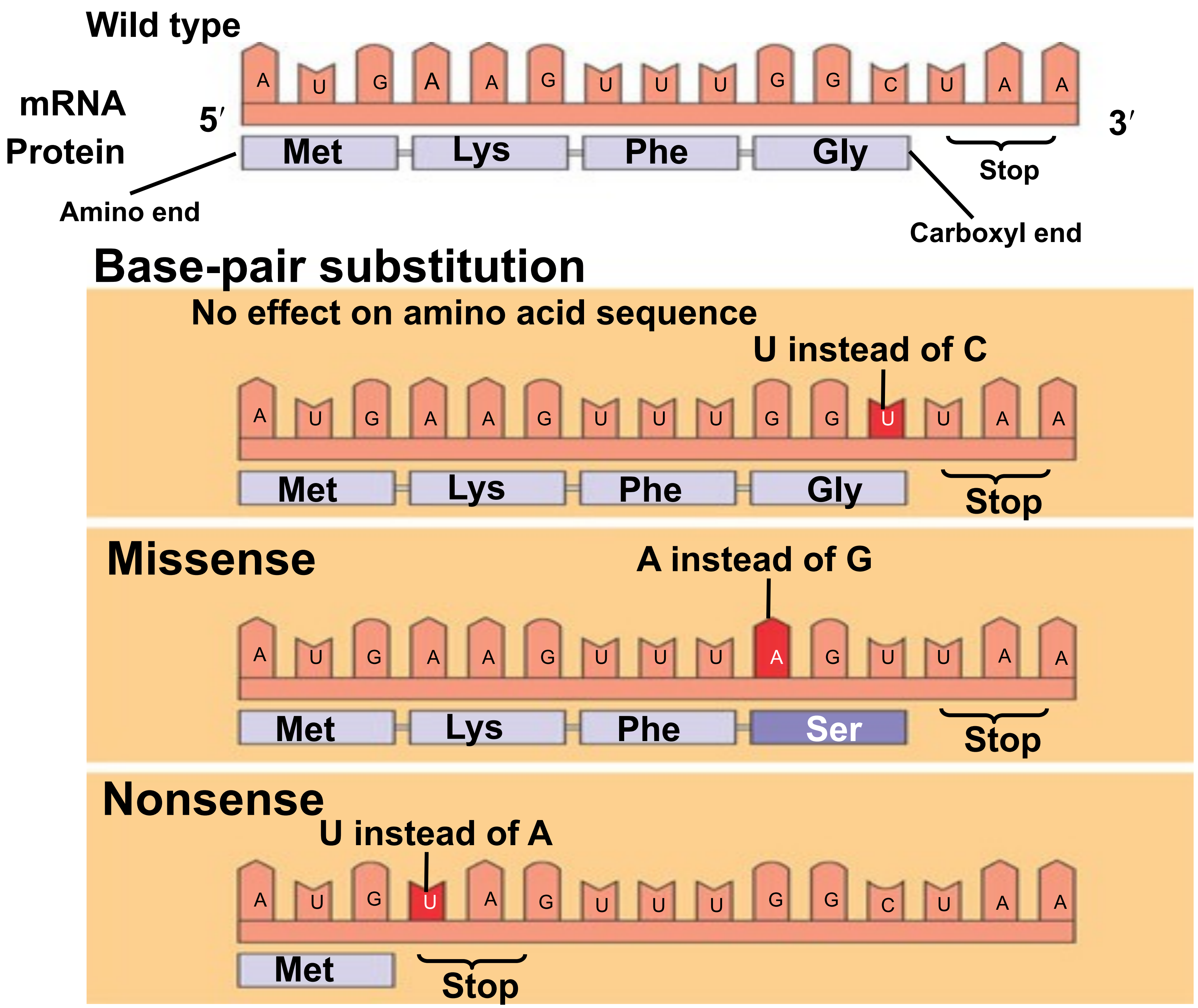


In the DNA, the mutant template strand has an A where the wild-type template has a T.

The mutant mRNA has a U instead of an A in one codon.

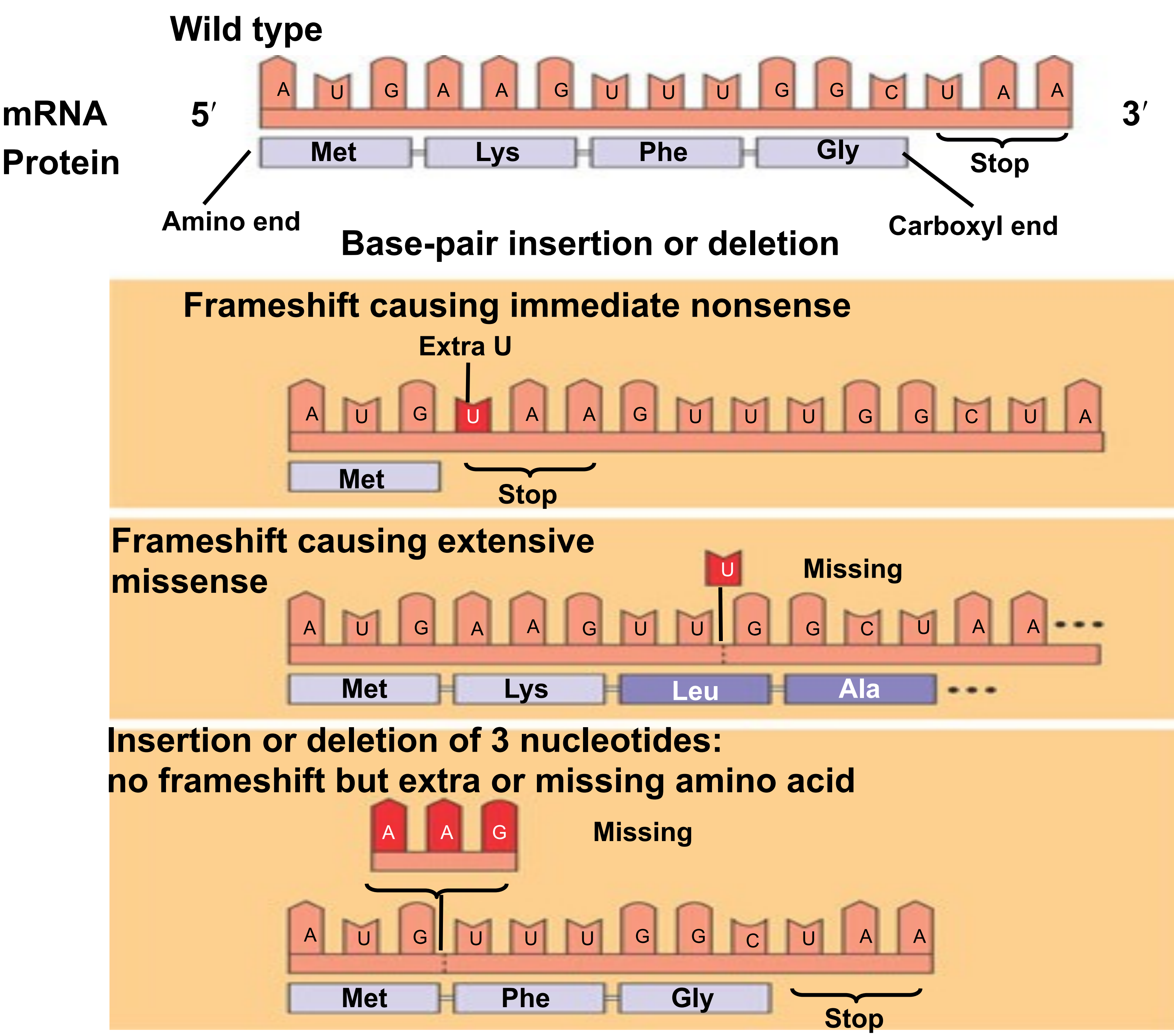
The mutant (sickle-cell) hemoglobin has a valine (Val) instead of a glutamic acid (Glu).

Point Mutations- Base Pair Substitutions



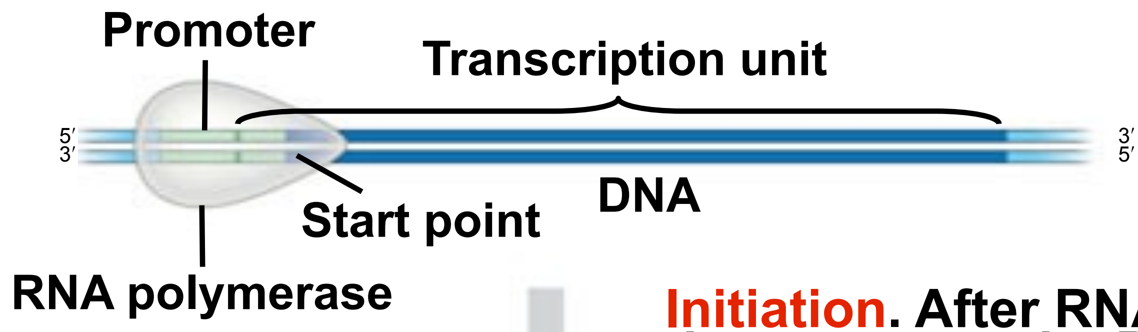
Replacement of one nucleotide and its partner with another pair of nucleotides, can result in missense or nonsense mutations

Point Mutations-
Base Insertions & Deletions

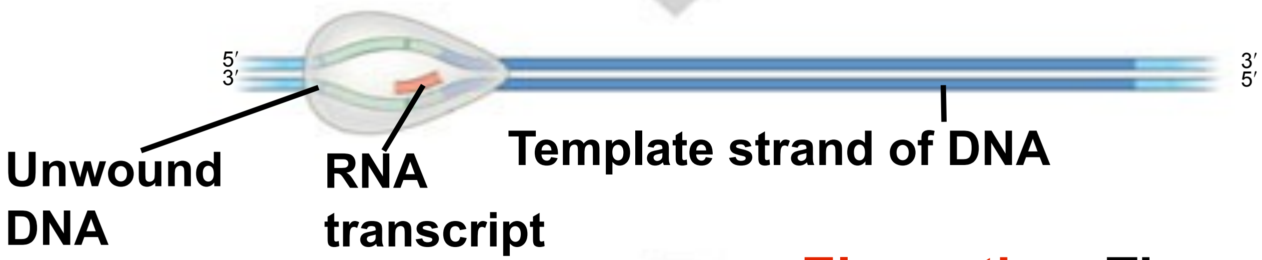


Additions and losses of nucleotide pairs in a gene, can cause frameshifts

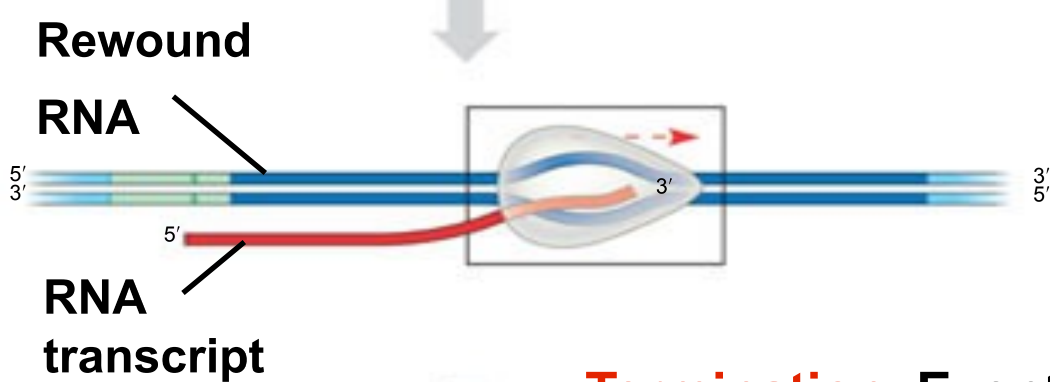
Prokaryotic Transcription



Initiation. After RNA polymerase binds to the promoter, the DNA strands unwind, and the polymerase initiates RNA synthesis at the start point on the template strand.



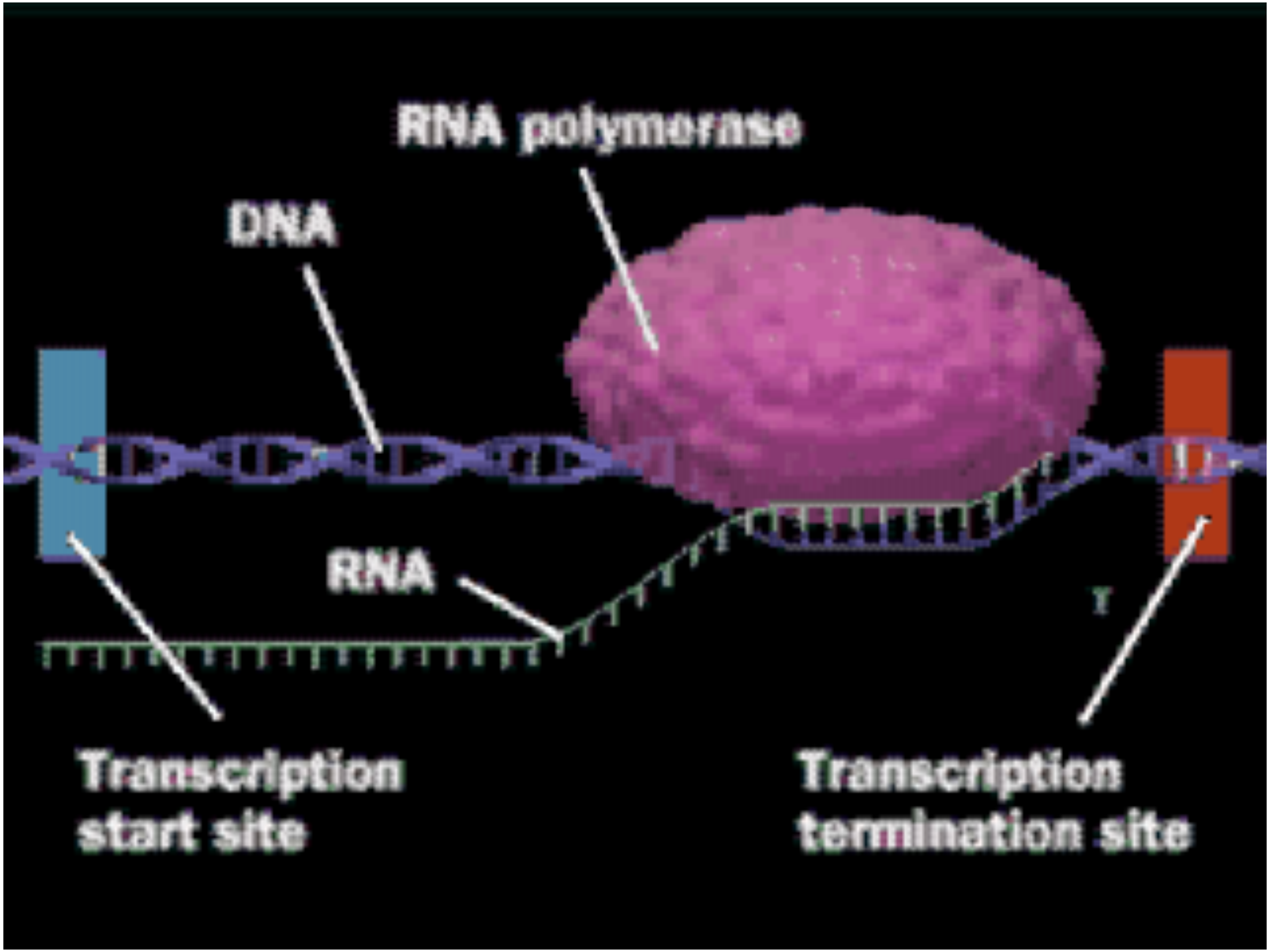
Elongation. The polymerase moves downstream, unwinding the DNA and elongating the RNA transcript 5′ → 3′. In the wake of transcription, the DNA strands re-form a double helix



Termination. Eventually, the RNA transcript is released, and the polymerase detaches from the DNA.

As soon as one RNA polymerase moves off of promoter another one can bind and so forth like a convoy of trucks

Transcription



Prokaryotic Transcription

Elongation

RNA polymerase

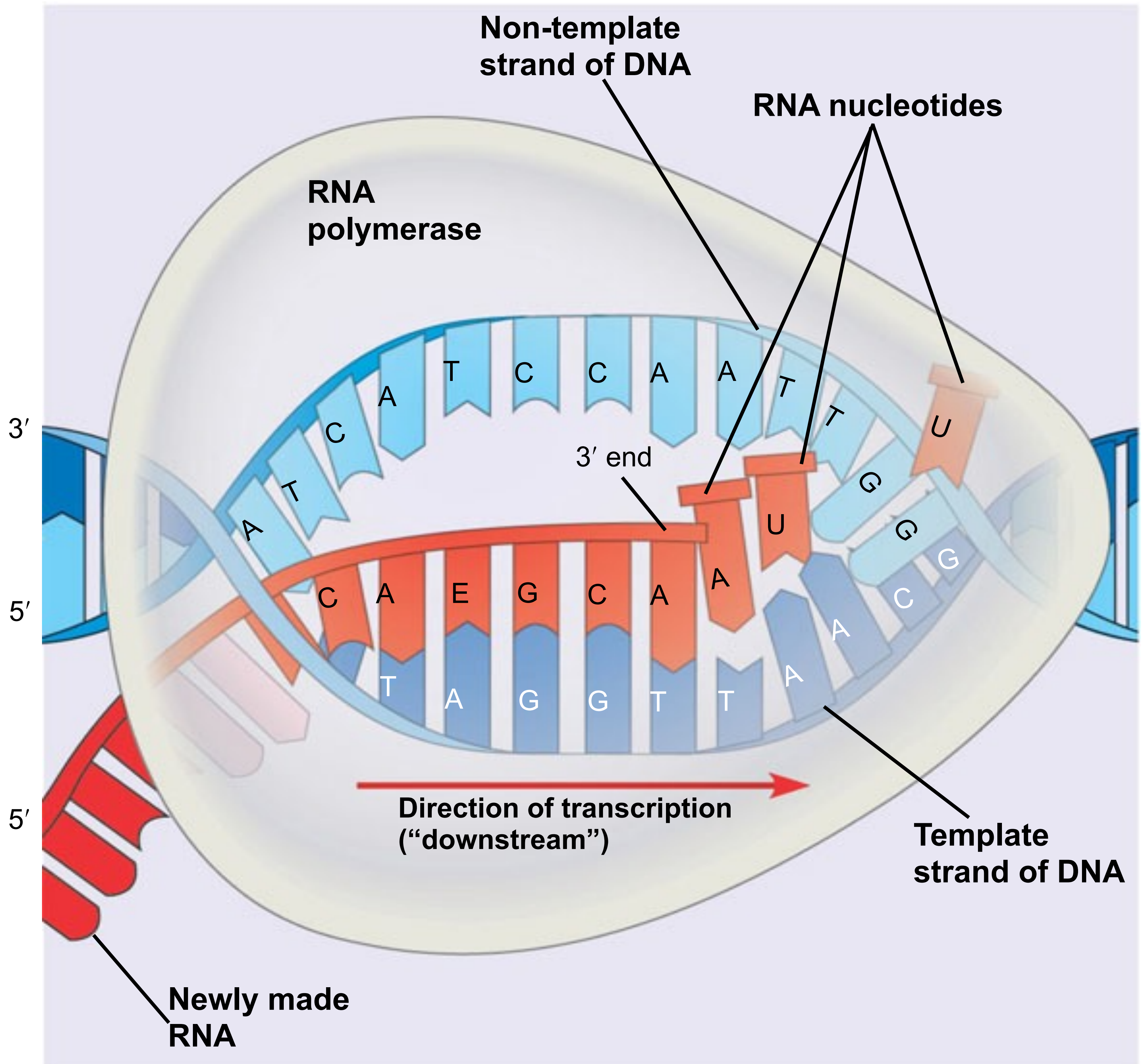
-uncoils DNA

-splits DNA

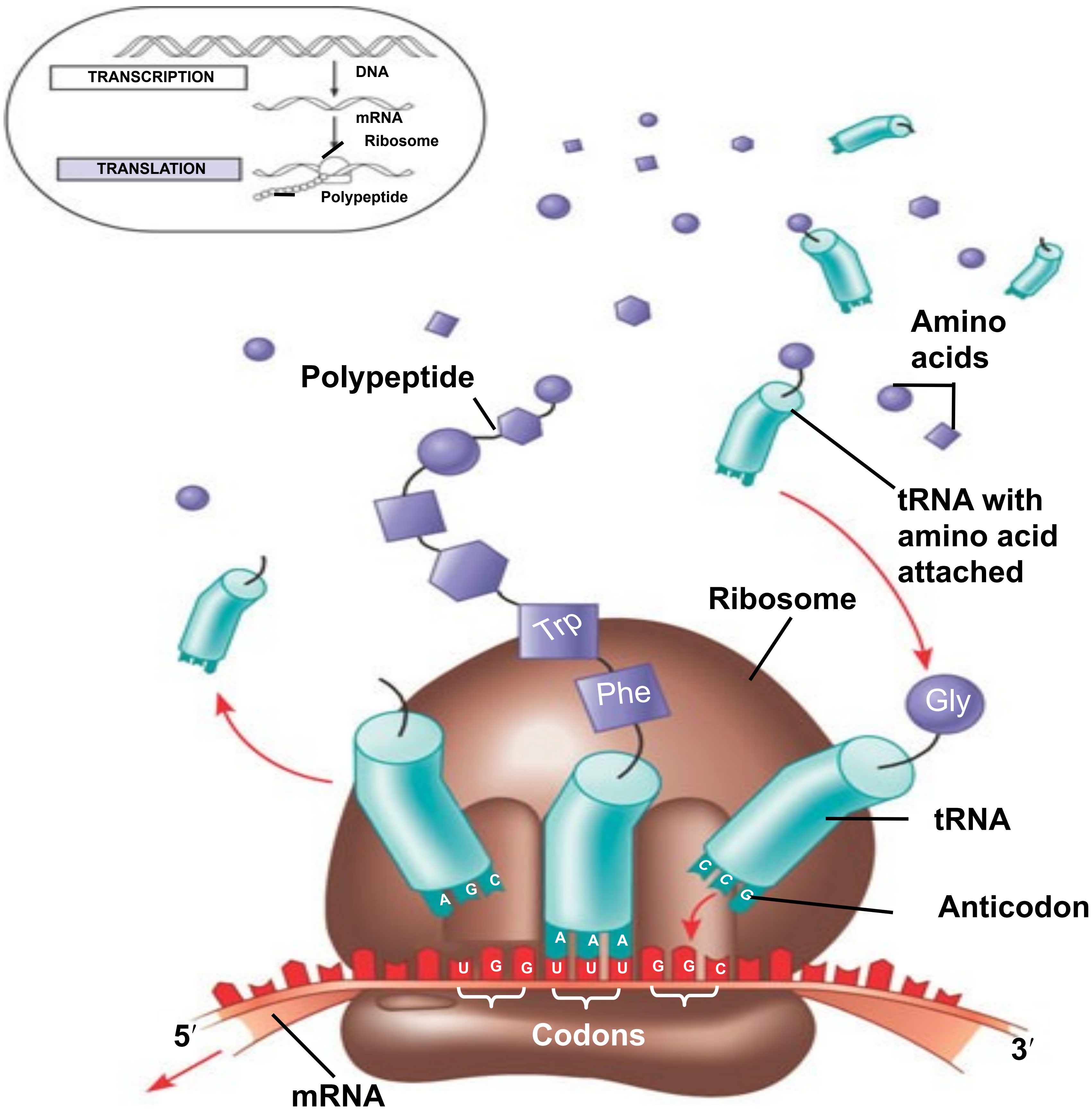
-holds DNA open

-adds RNA nucleotides

proceeds at a rate of approx. 40 nucleotides per second



Prokaryotic Translation



the cytosol is stocked with free floating amino acids

tRNA's are also floating freely

every specific amino acid is carried by a tRNA carrying specific anticodon

Prokaryotic Translation

- Translation involves 3 steps, also named...
  - **Initiation**
  - **Elongation**
  - **Termination**
- Translation involves a number of different "characters"...
  - **tRNA**
  - **ribosomes (small & large subunits)**
  - **mRNA**
  - **amino acids**

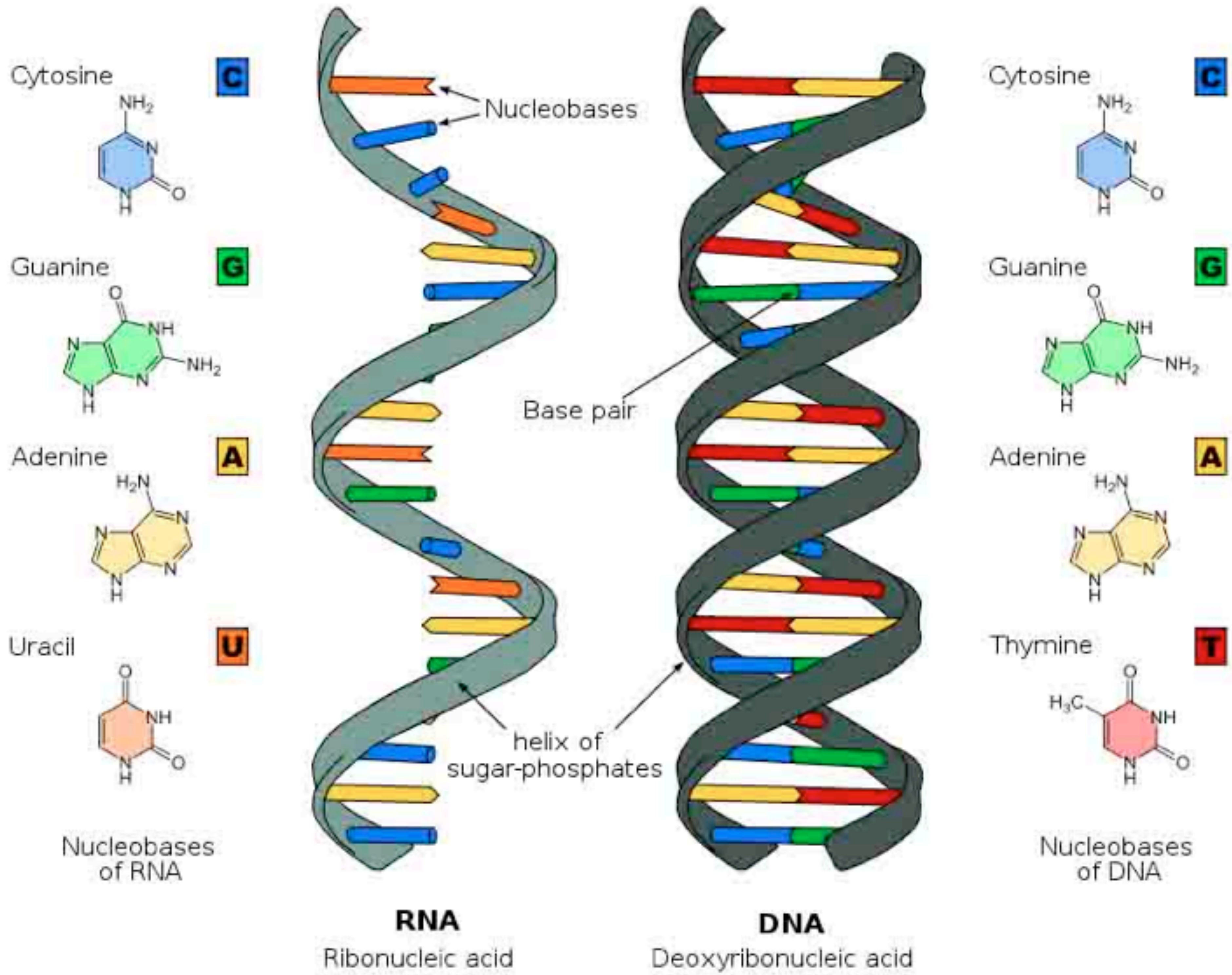
mRNA

"Our Cast of Characters"

single stranded

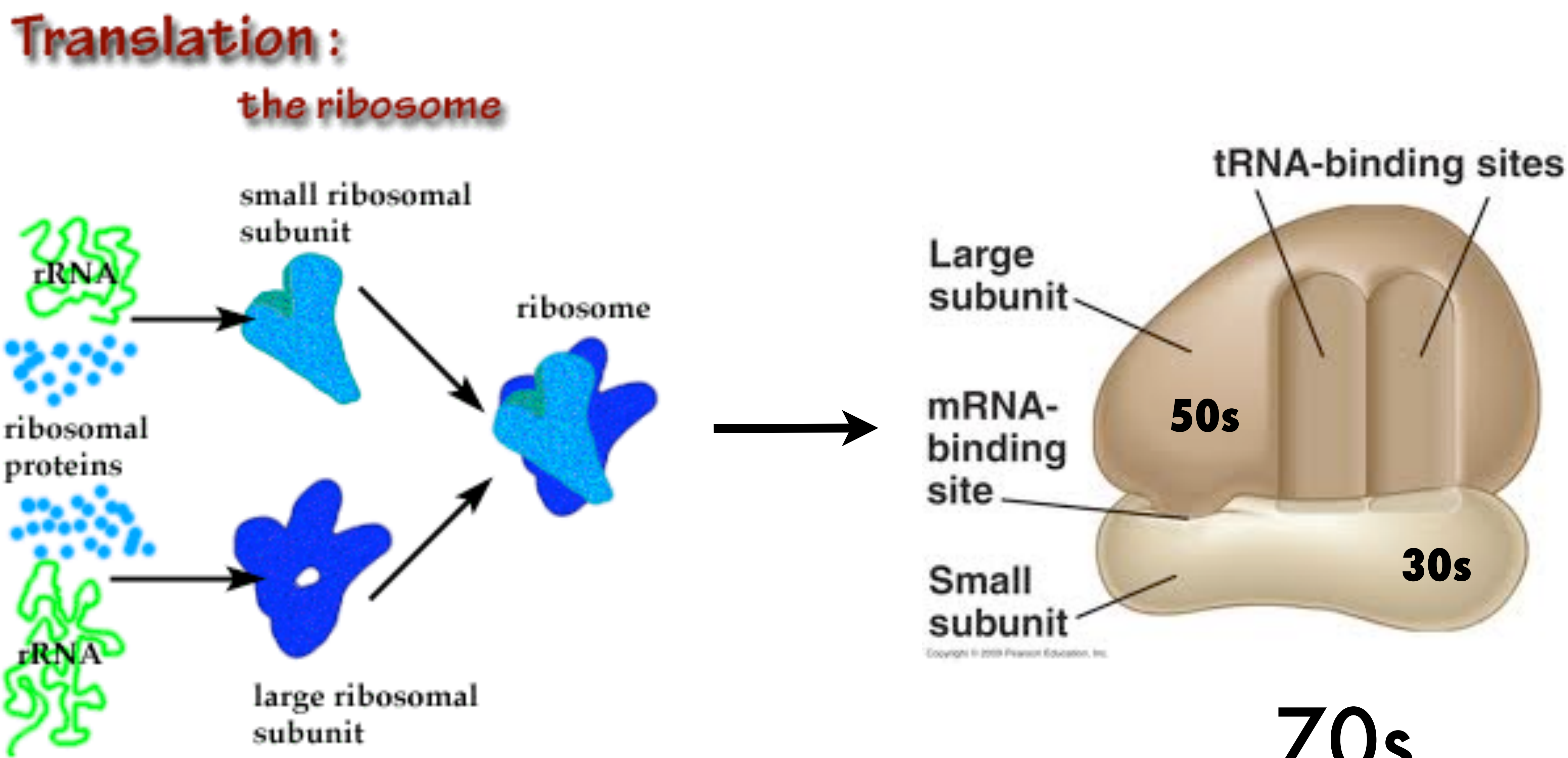
Uracil

ribose sugar in the backbone



Ribosomes

"Our Cast of Characters"

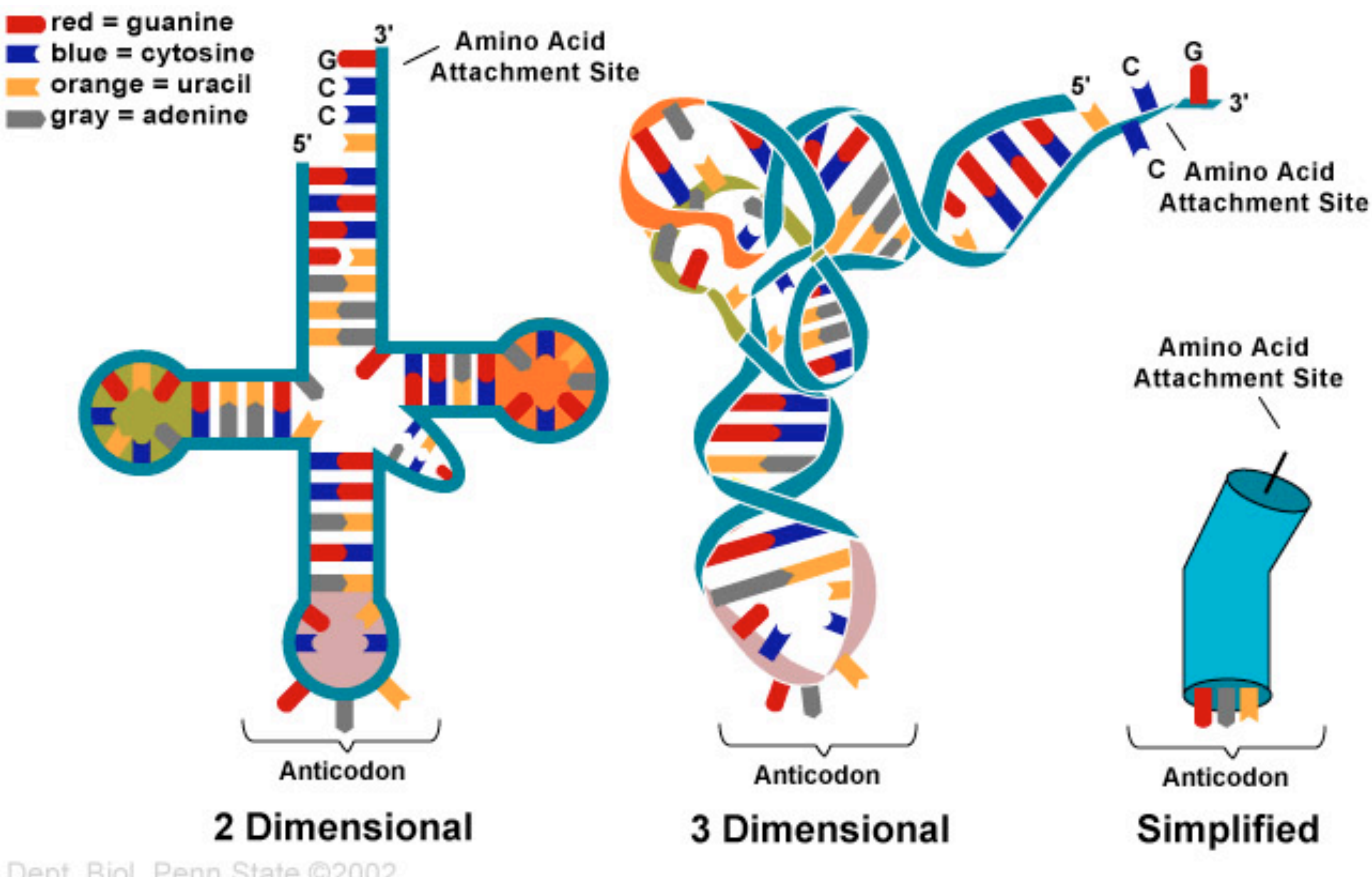


70s

rRNA is the most abundant type of cellular RNA

tRNA

"Our Cast of Characters"

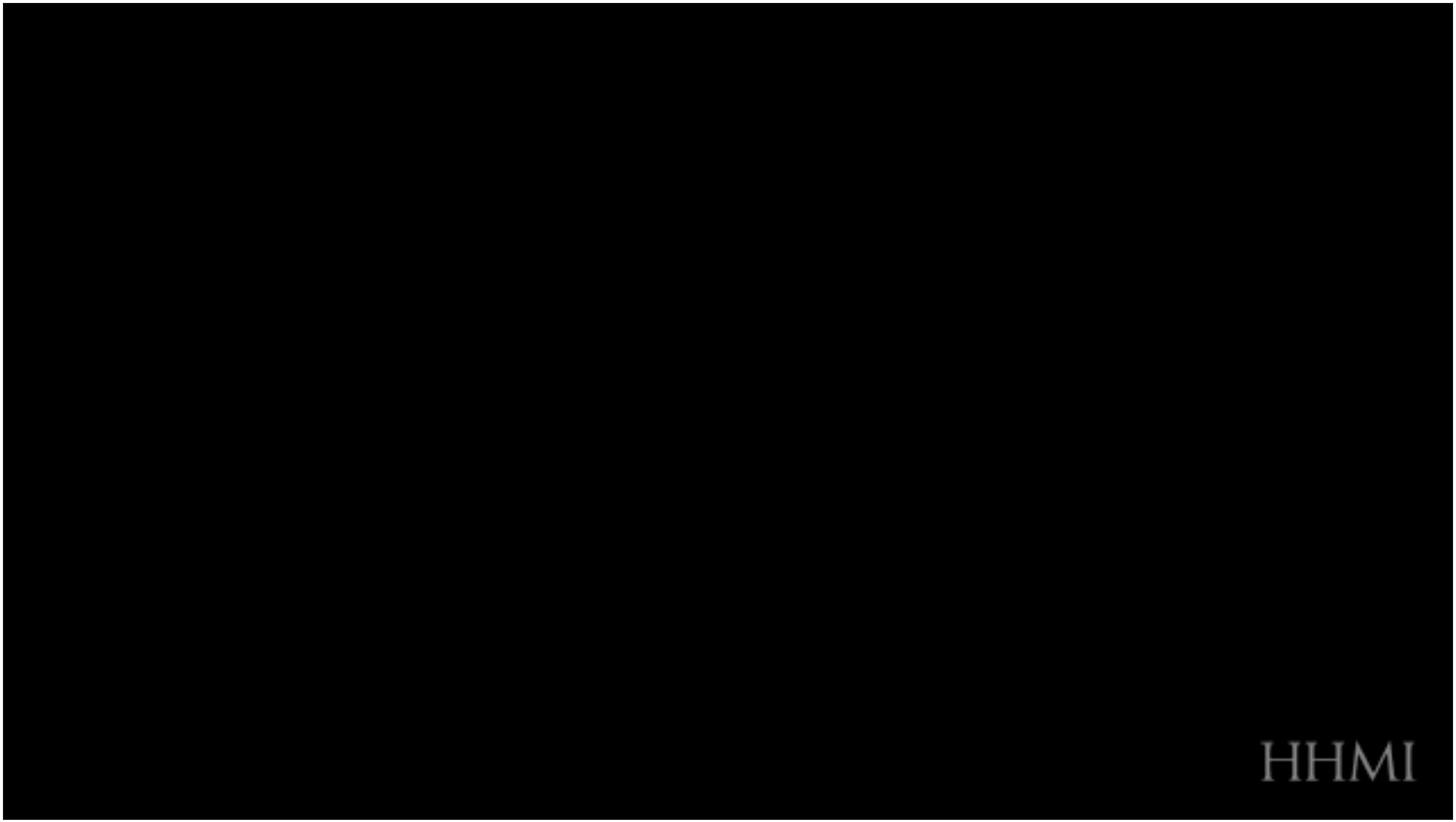


~80 nucleotides

"L" shaped

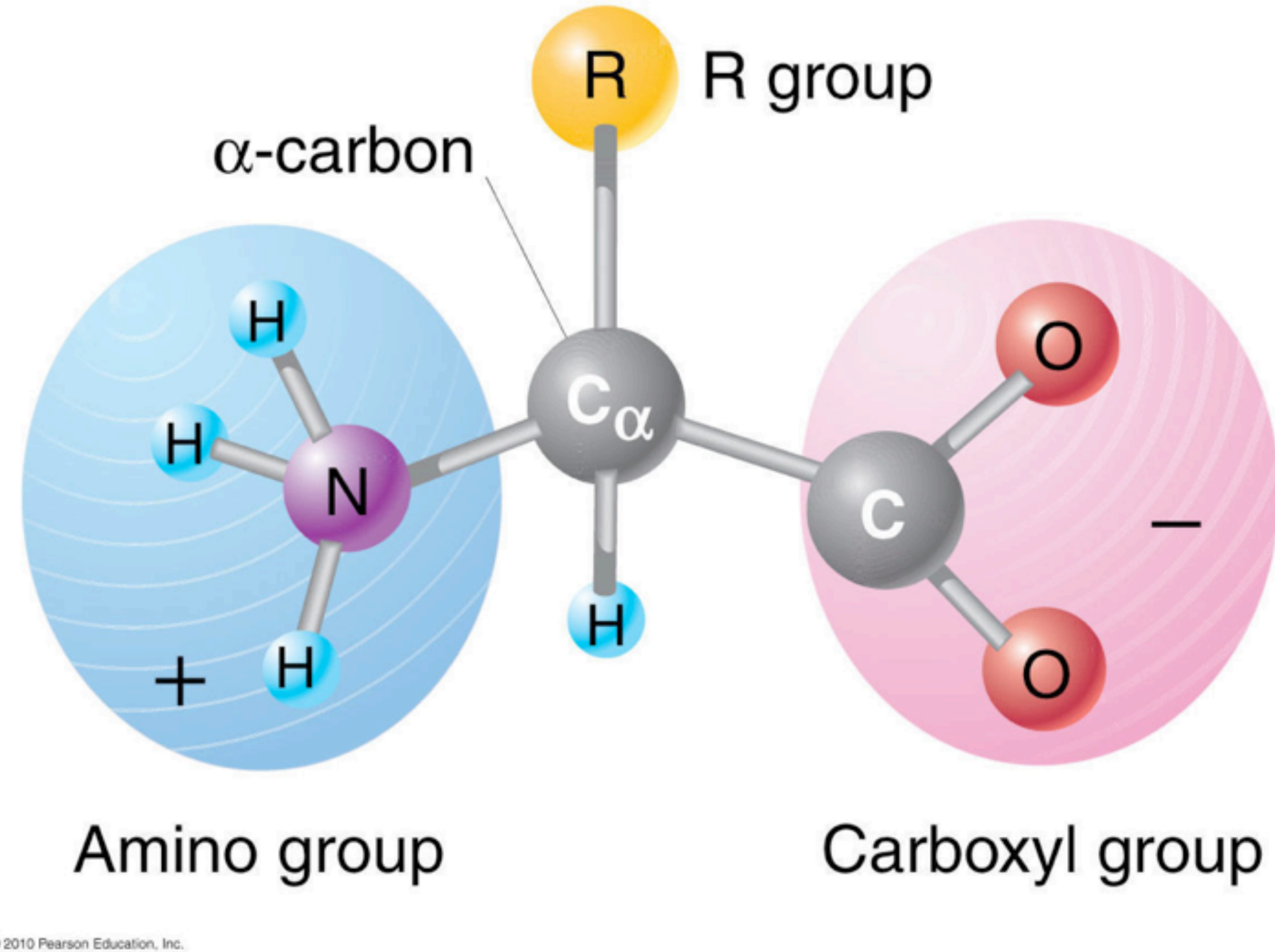
tRNA

"Our Cast of Characters"



Amino Acids

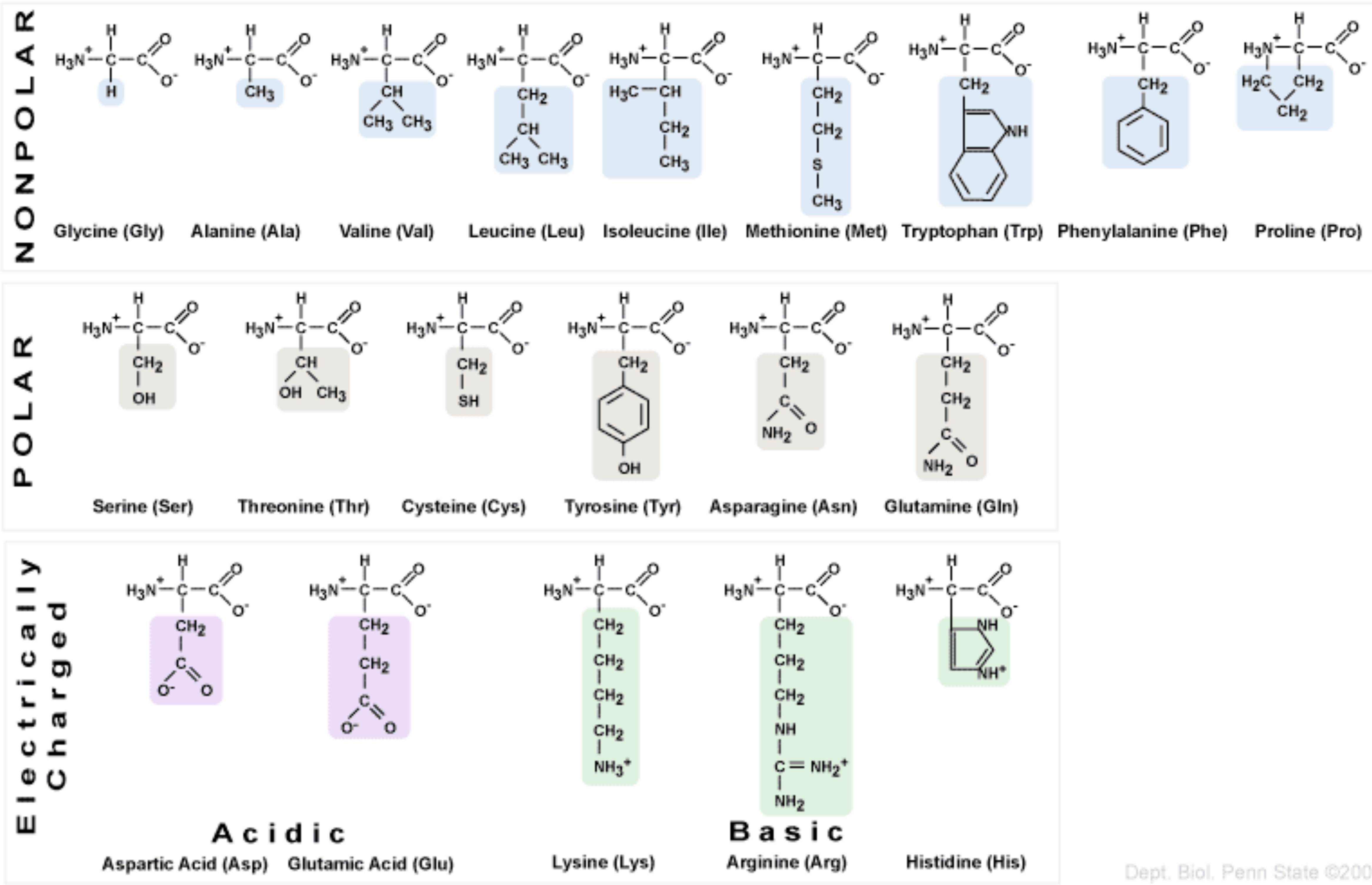
"Our Cast of Characters"



General Amino Acid Structure in Solution

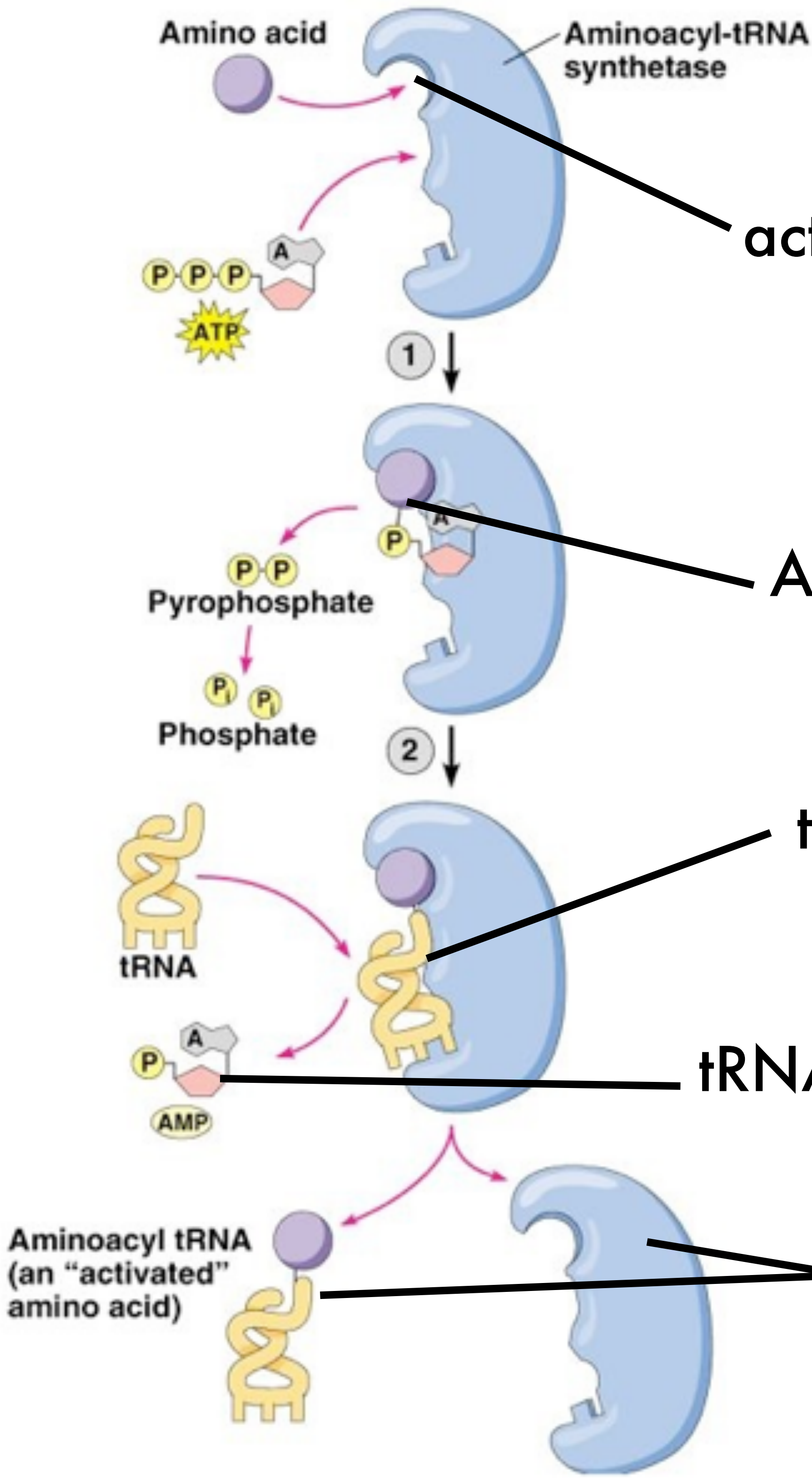
Amino Acids

"Our Cast of Characters"



tRNA

“The Processes”



20 different enzymes one for each amino acid

active site binds amino acid and ATP

ATP loses two phosphate groups

tRNA binds to active site

tRNA displaces AMP

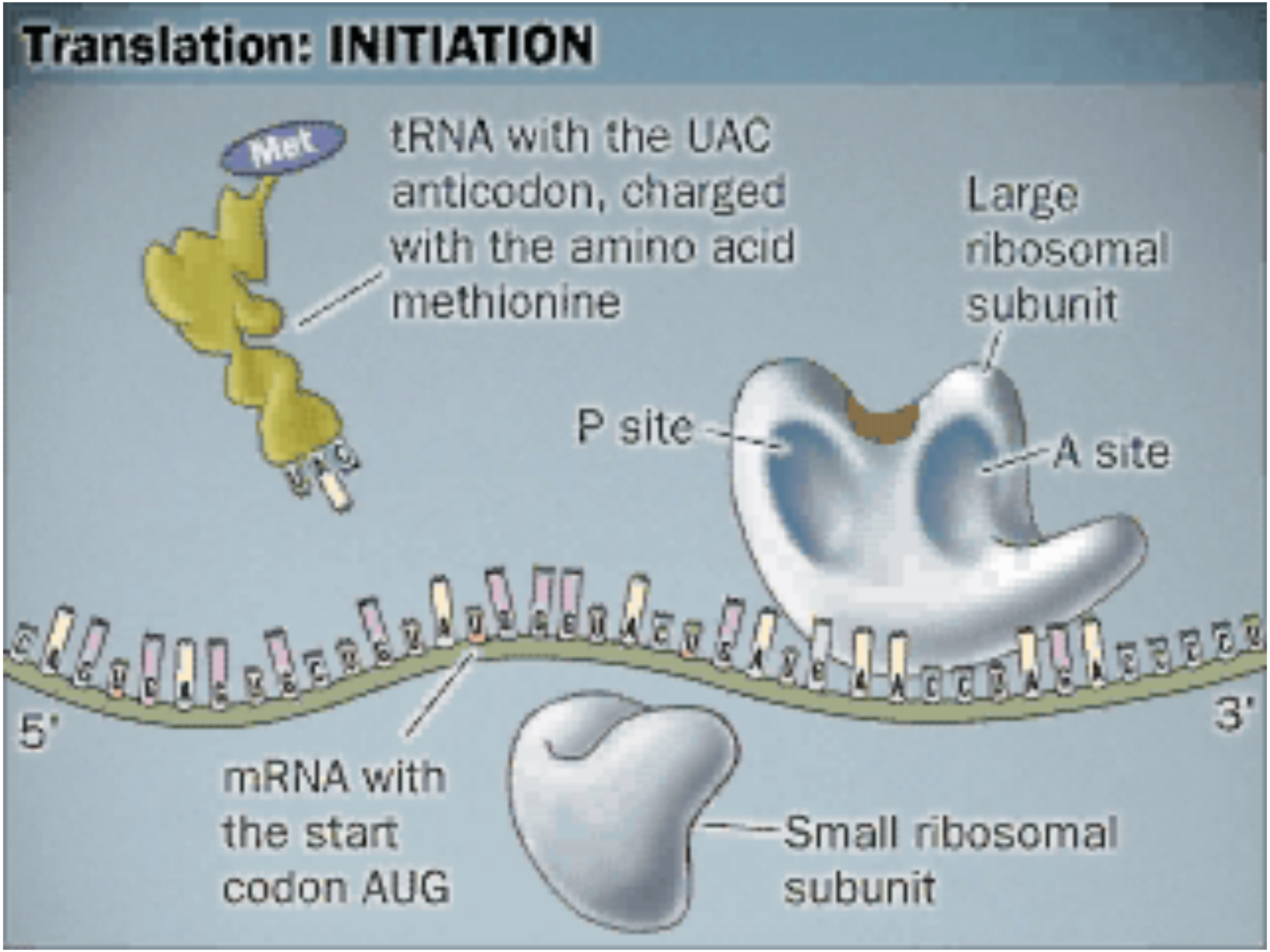
enzyme releases loaded tRNA

Only 45 different tRNA’s (not 61) some tRNA’s bind more than one amino acid.

This flexibility, called “wobble” occurs at the third position of the codon/anticodon

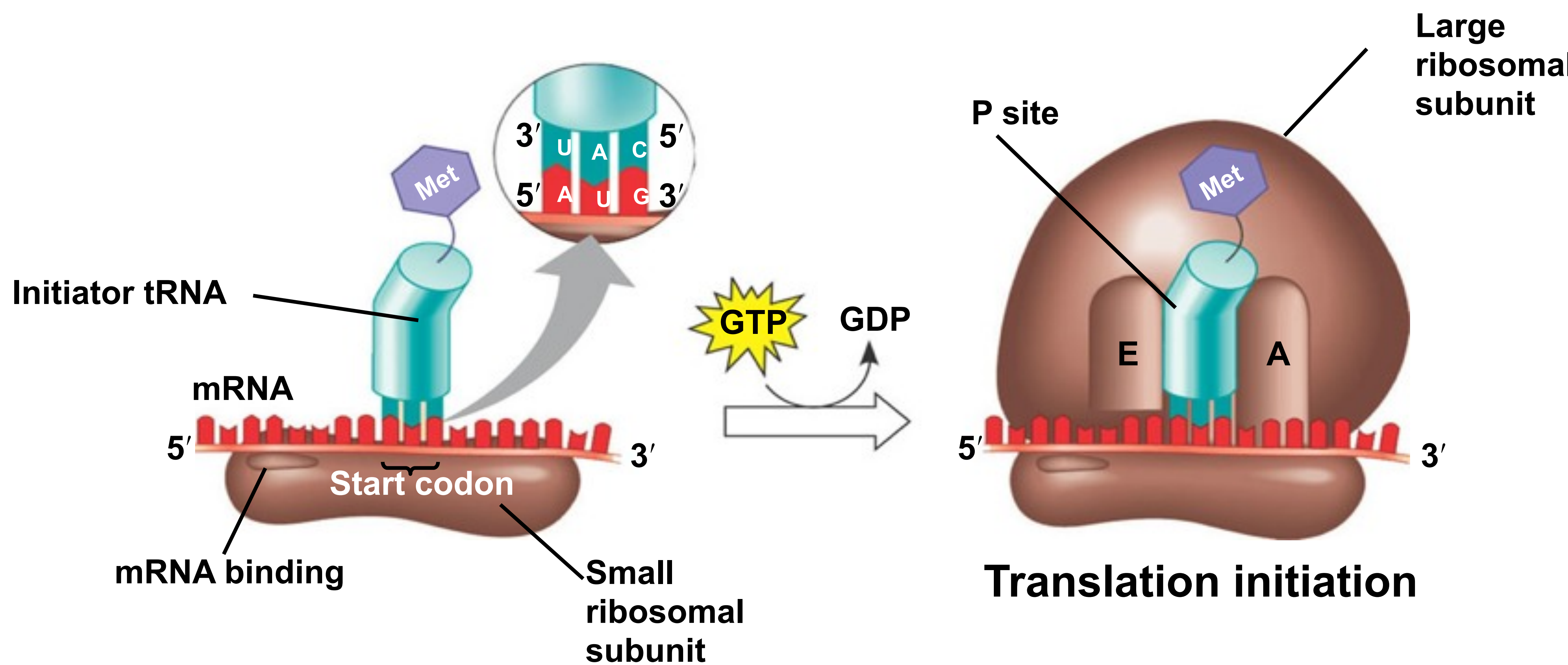
Ex. anticodon 3′-UCU-5′ can bind to either 3′-AGA-5′ or 3′-AGG-5′ both code for arginine

Translation



Translation- Initiation

"The Processes"



1.

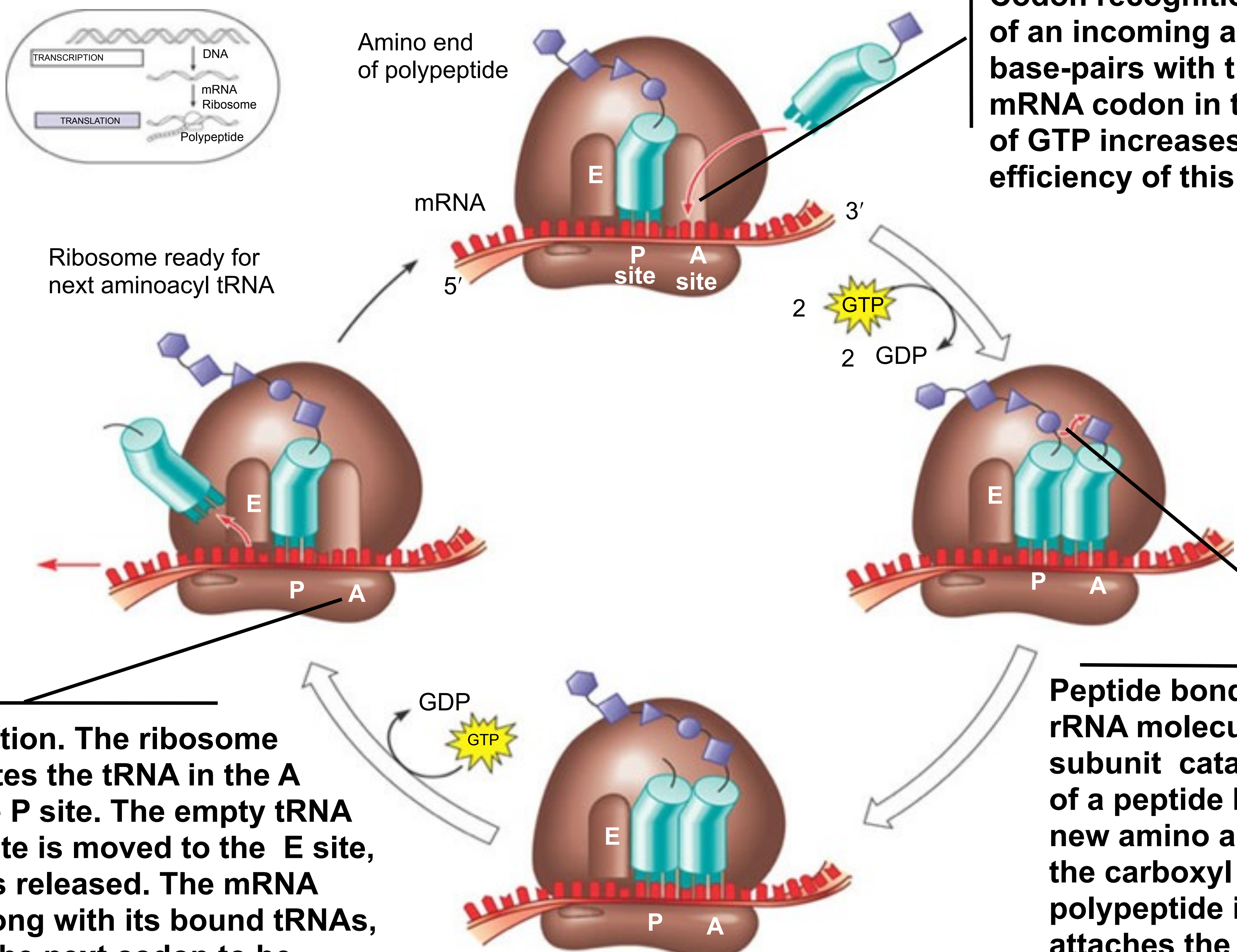
A small ribosomal subunit binds to a molecule of mRNA. In a prokaryotic cell, the mRNA binding site on this subunit recognizes a specific nucleotide sequence on the mRNA just upstream of the start codon. An initiator tRNA, with the anticodon UAC, base-pairs with the start codon, AUG. This tRNA carries the amino acid methionine (Met).

2.

The arrival of a large ribosomal subunit completes the initiation complex. Proteins called initiation factors (not shown) are required to bring all the translation components together. GTP provides the energy for the assembly. The initiator tRNA is in the P site; the A site is available to the tRNA bearing the next amino acid.

Translation- Elongation

"The Processes"



1.

Codon recognition. The anticodon of an incoming aminoacyl tRNA base-pairs with the complementary mRNA codon in the A site. Hydrolysis of GTP increases the accuracy and efficiency of this step.

2.

Peptide bond formation. An rRNA molecule of the large subunit catalyzes the formation of a peptide bond between the new amino acid in the A site and the carboxyl end of the growing polypeptide in the P site. This step attaches the polypeptide to the tRNA in the A site.

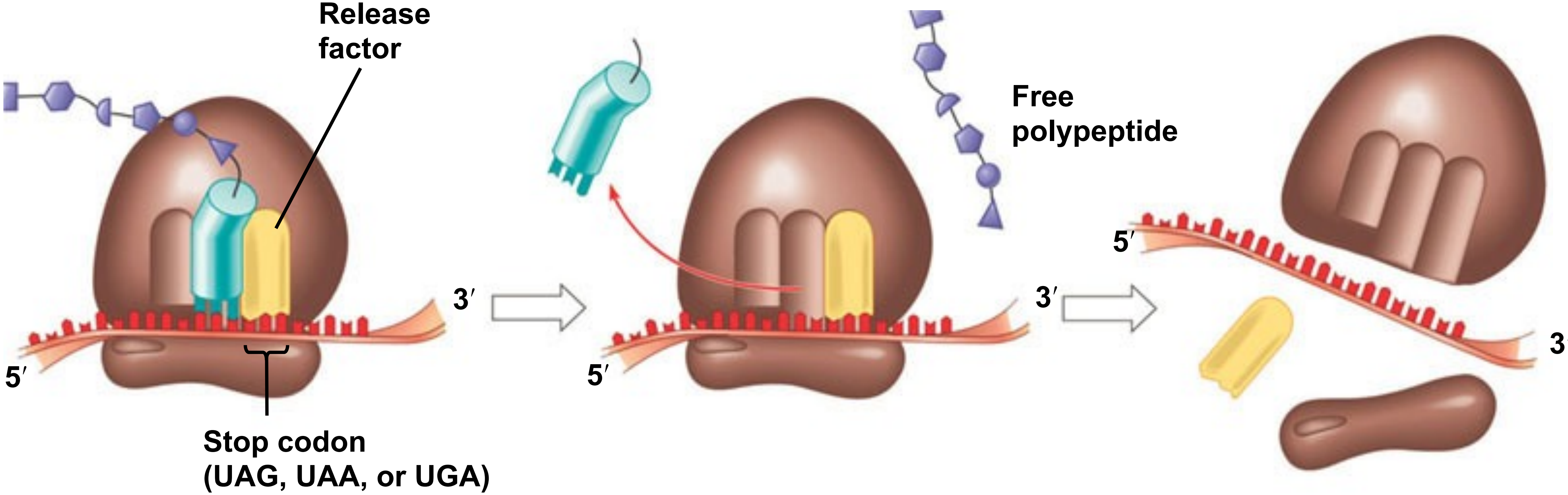
3.

Translocation. The ribosome translocates the tRNA in the A site to the P site. The empty tRNA in the P site is moved to the E site, where it is released. The mRNA moves along with its bound tRNAs, bringing the next codon to be translated into the A site.

Translation- Termination

"The Processes"

Keep in mind this polypeptide now must fold into a 3-D molecule before it becomes functional



When a ribosome reaches a stop codon on mRNA, the A site of the ribosome accepts a protein called a release factor instead of tRNA.

1.

The release factor hydrolyzes the bond between the tRNA in the P site and the last amino acid of the polypeptide chain. The polypeptide is thus freed from the ribosome.

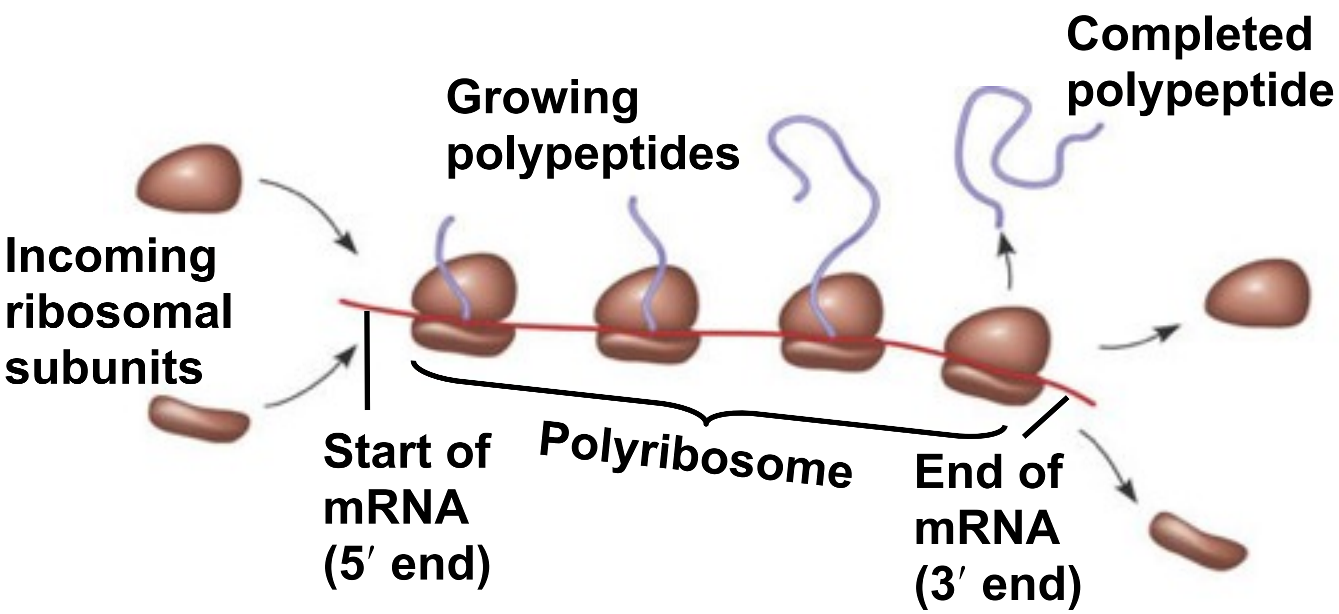
2.

The two ribosomal subunits and the other components of the assembly dissociate. This also requires energy- 2GTP molecules.

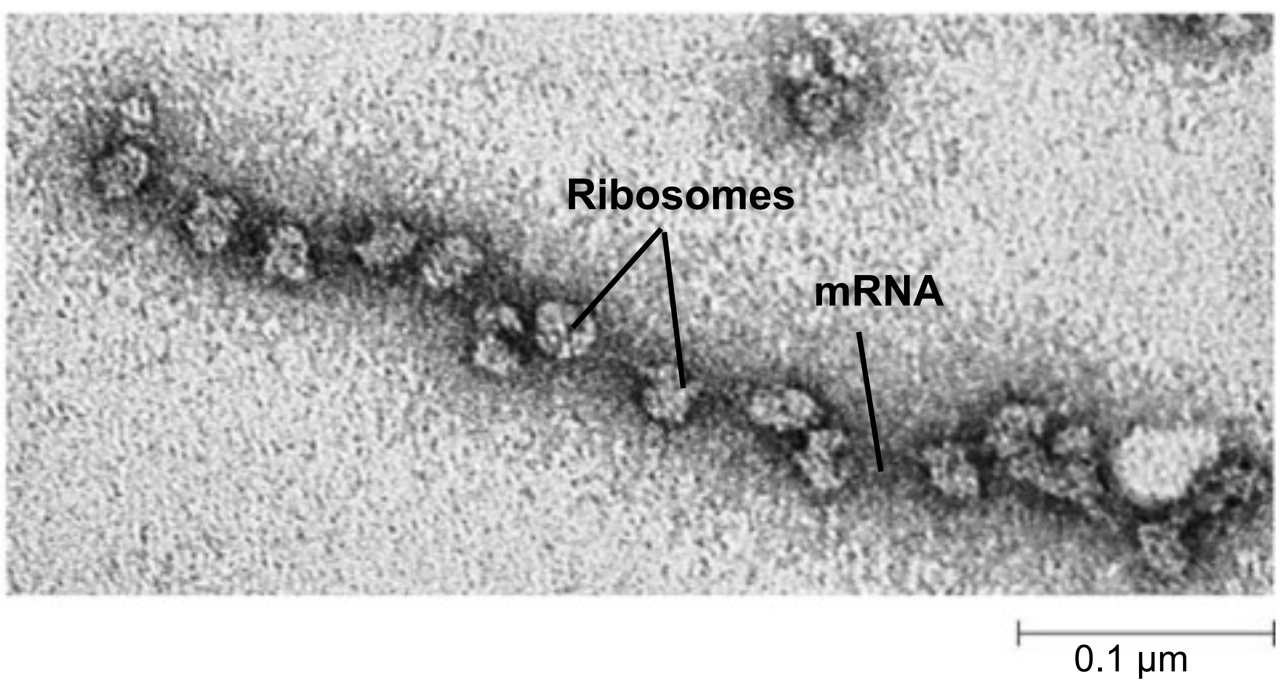
3.

Translation- Side Note

"The Processes"



(a) An mRNA molecule is generally translated simultaneously by several ribosomes in clusters called polyribosomes.



(b) This micrograph shows a large polyribosome in a prokaryotic

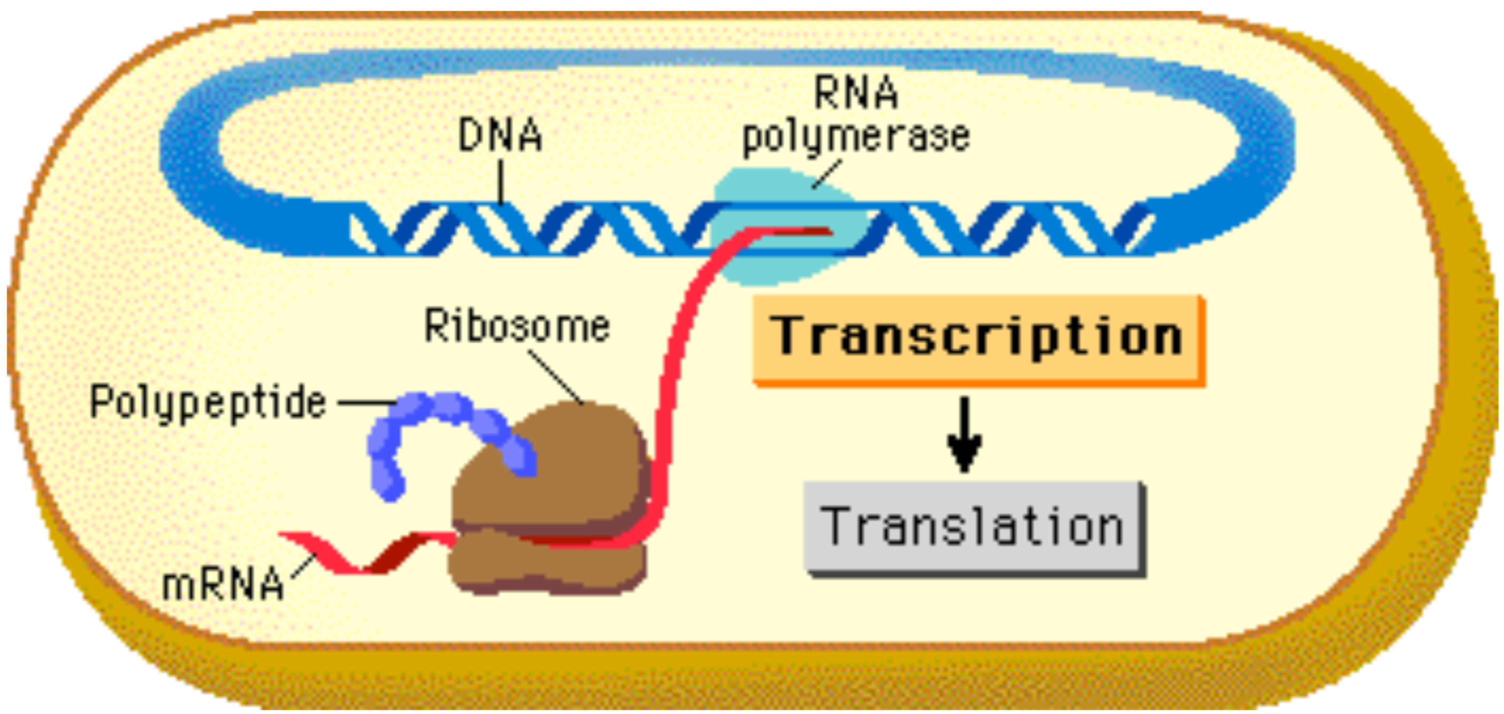
Recall- As soon as one RNA polymerase moves off of promoter another one can bind and so forth like a convoy of trucks

New- As soon as one ribosome moves off of start codon another one can bind and so forth like a convoy of trucks

Both help to increase the number of polypeptides a bacteria can make per unit time!

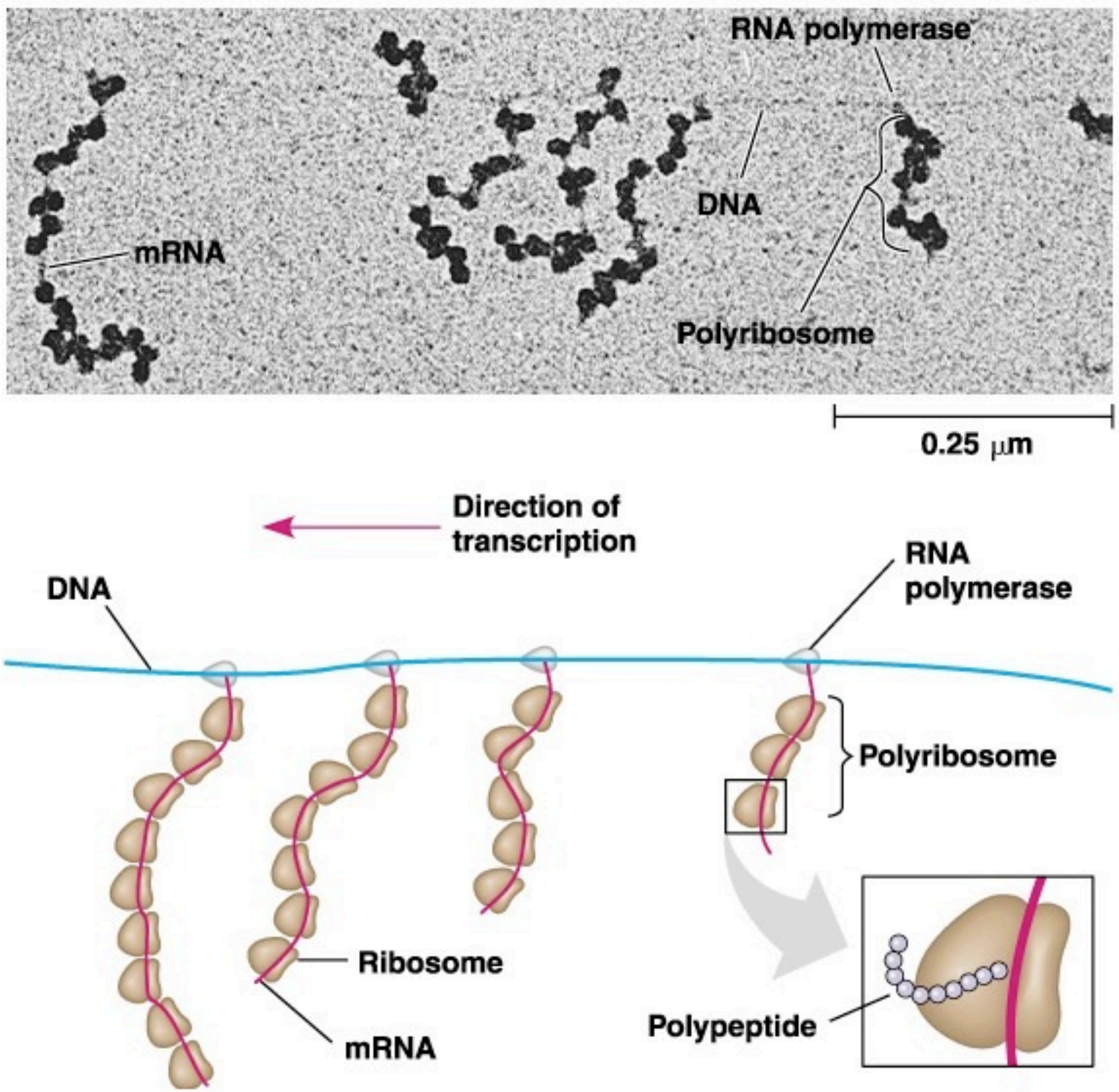
A Final Reminder

- **Transcription & Translation occurs in every organism.**
  - ***The mechanics are the same or very similar in all cells***
  - ***However, one very important difference exists between prokaryotes and eukaryotes***



Prokaryotic Transcription and Translation are not separated by time and space

Prokaryotic Protein Synthesis



Prokaryotic Transcription and Translation occur simultaneously

Regulating Gene Expression

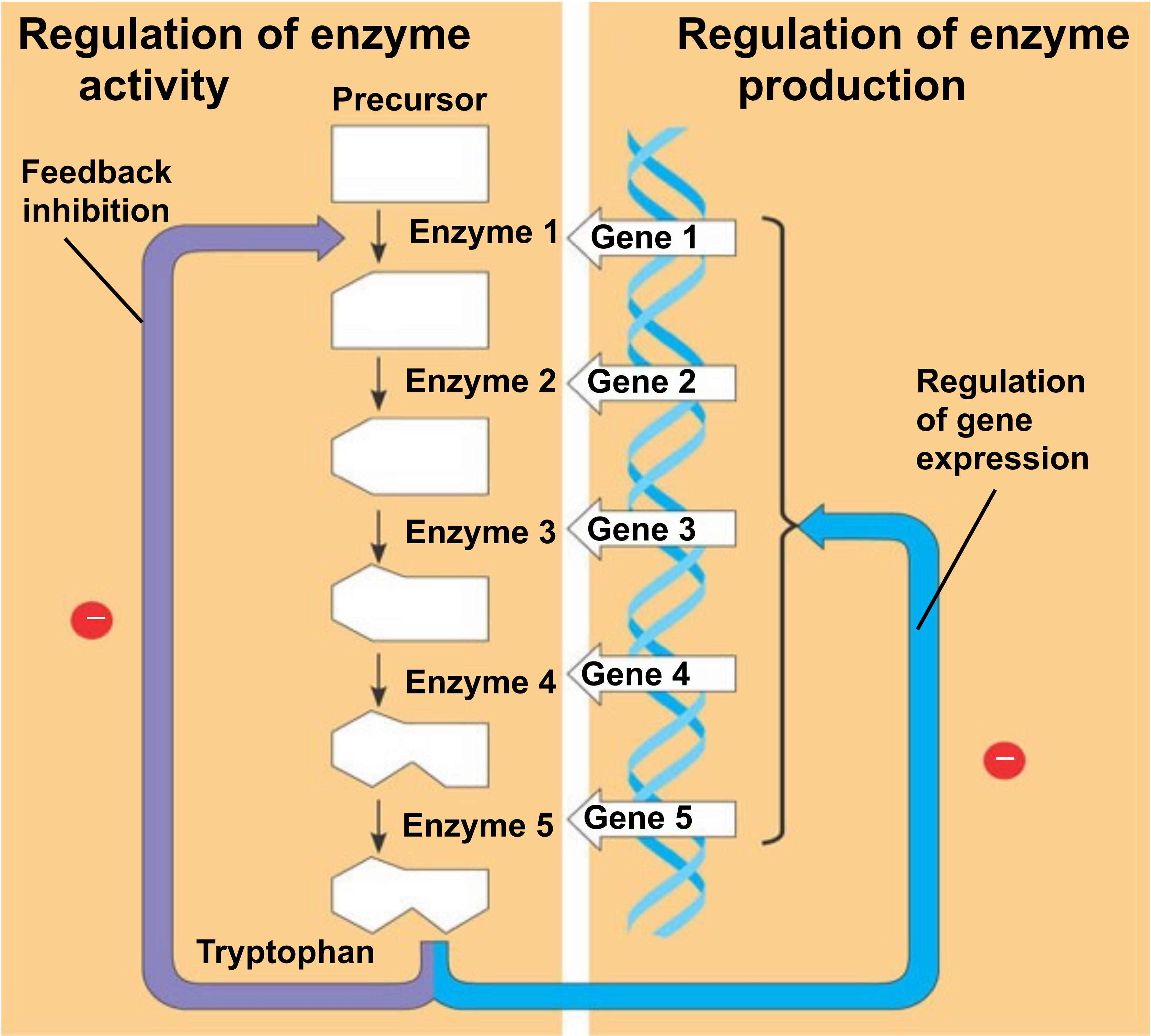
- A cell's genome consists of all its genes.
- NOT ALL genes need to be expressed at ALL times.
- ONLY certain genes are expressed at certain times.
- Bacteria turn on and turn off genes in response to the environmental conditions.
- As environmental conditions change so to does gene expression.

Regulating Gene Expression

- A bacteria that can turn its genes on and off in response to environmental changes **will save both resources and energy over time**.
- Natural selection has favored these bacteria over those which have less control.
  - *Consider E-coli that live in a human colon, if human meal includes a particular nutrient then they need not produce it (save energy) BUT if human meal does not include a particular nutrient then they need produce it.*
  - This fundamentally requires that the E-coli turn on/off certain genes depending on the presence/absence of a particular nutrient.

How are bacterial genes controlled?

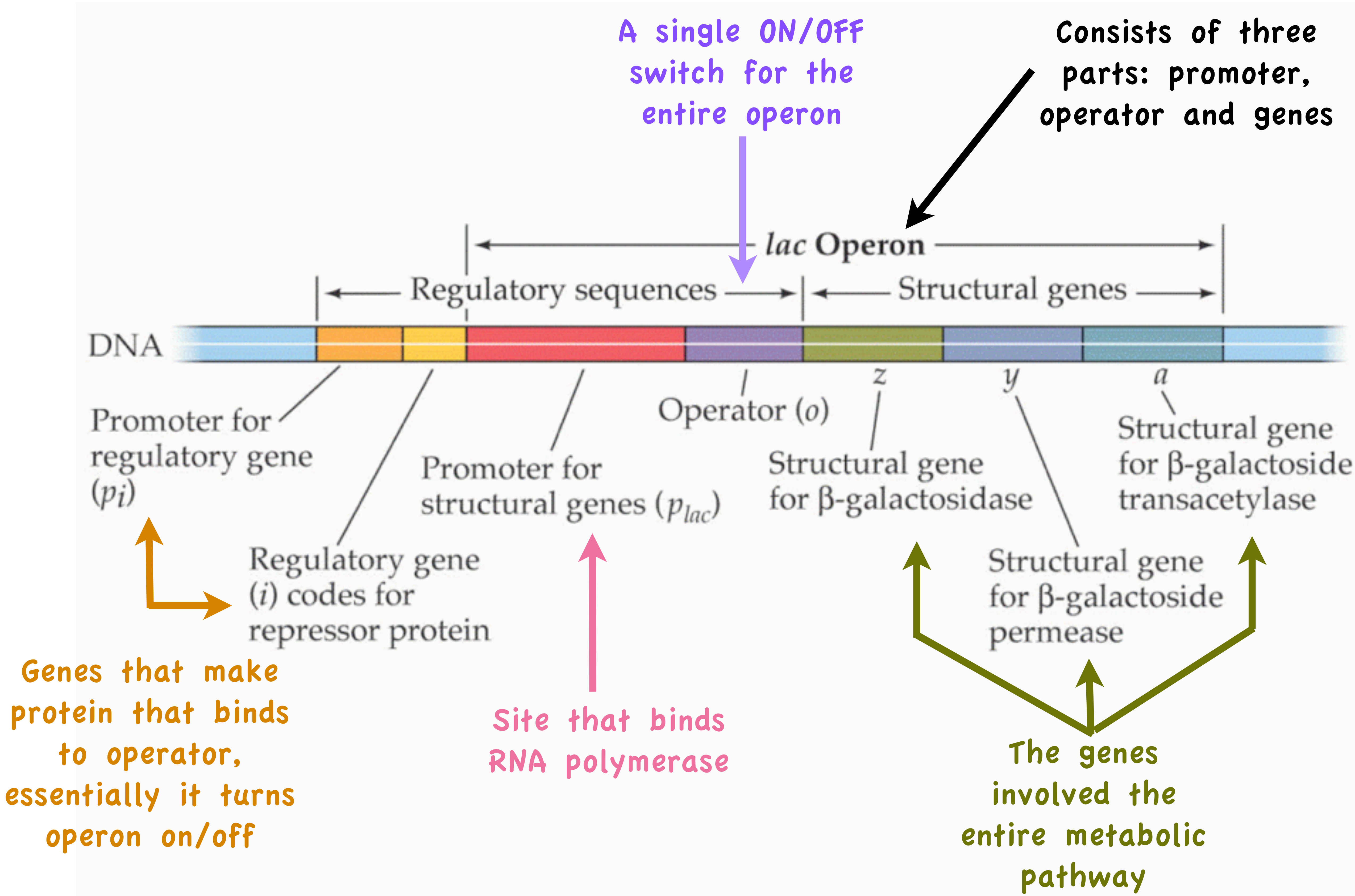
"order from the bakery case"



"place a custom order"

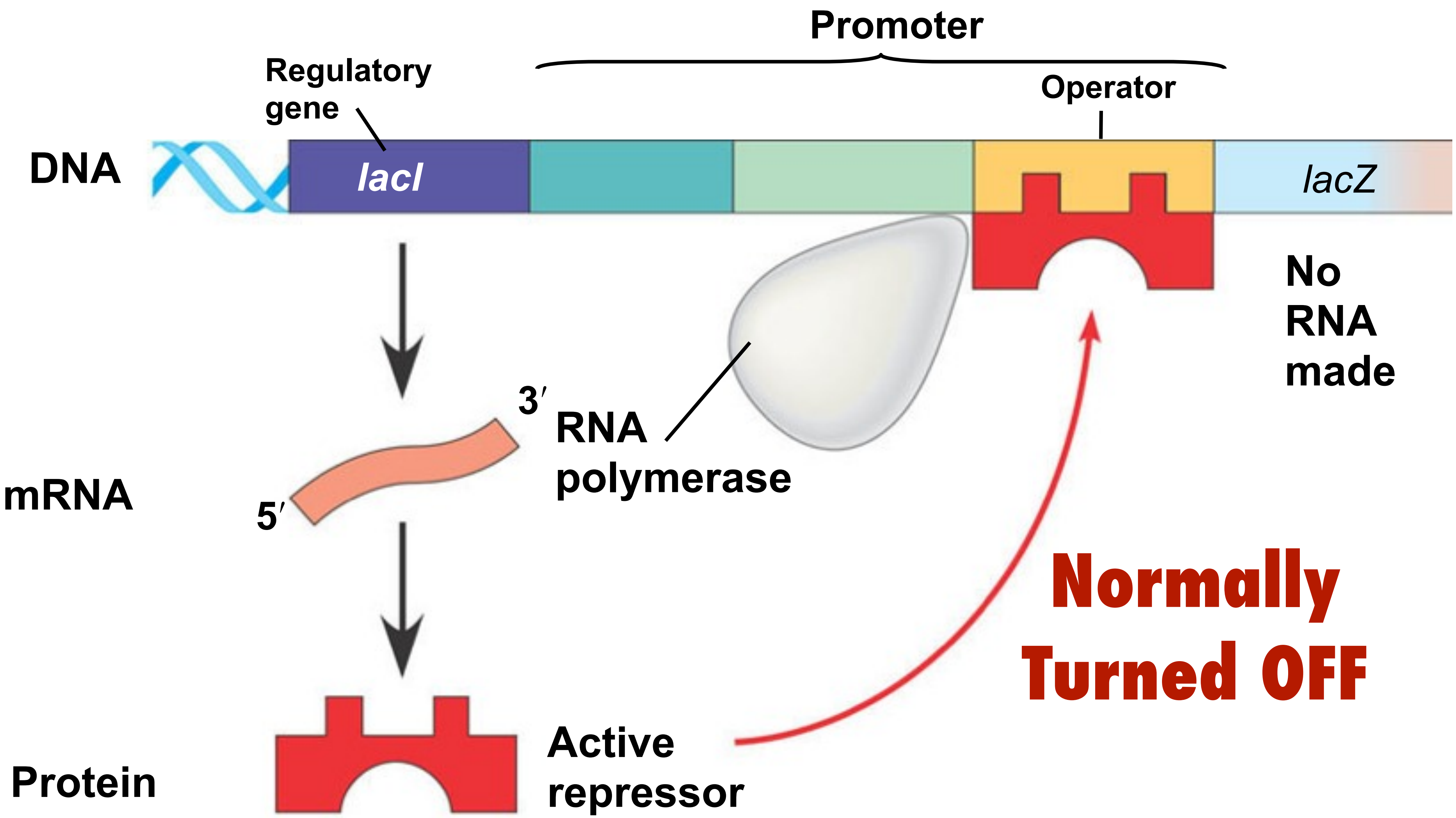
"two general ways of eliciting a cellular response"

Bacterial Operon Concept



Inducible Operons "turn-on-able"

Negative Gene Regulation

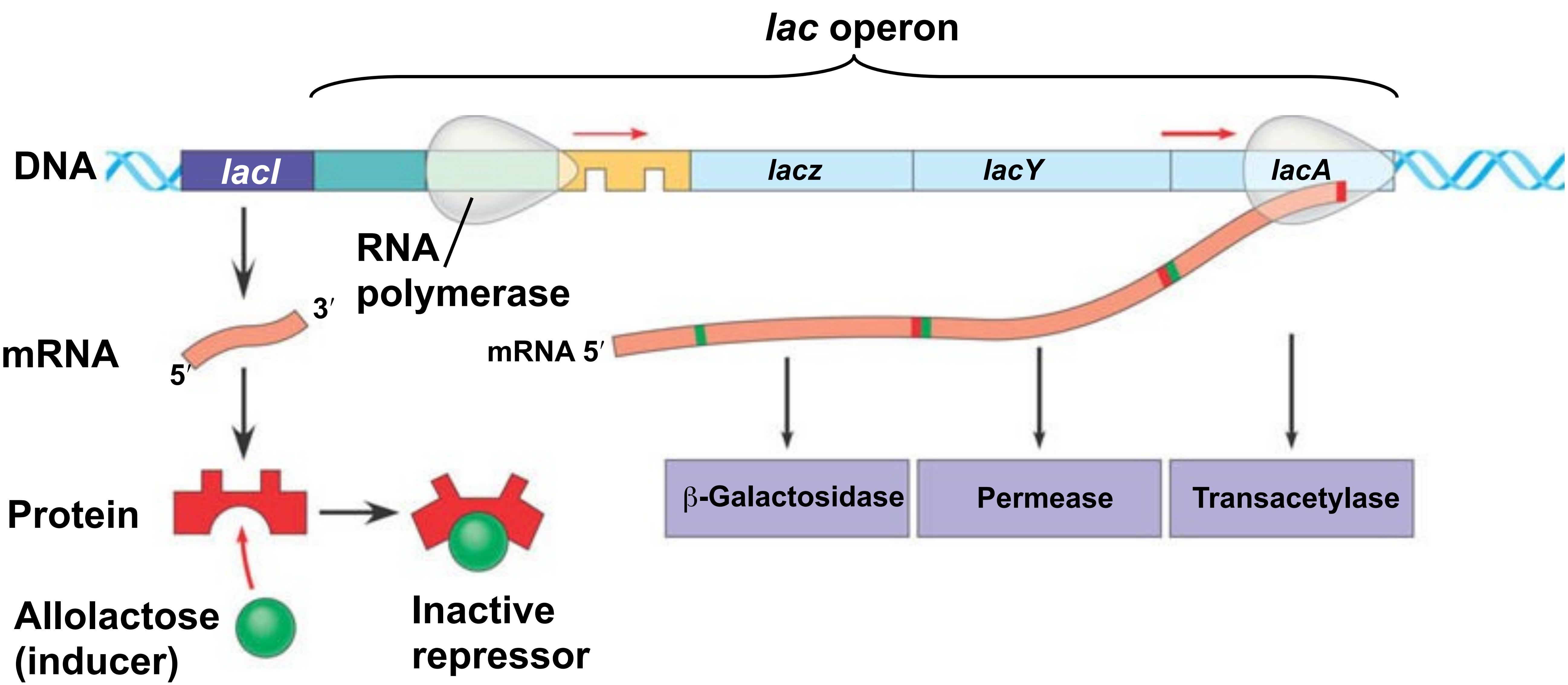


Lactose absent, repressor active, operon off. The *lac* repressor is innately active, and in the absence of lactose it switches off the operon by binding to the operator.

Inducible Operons "turn-on-able"

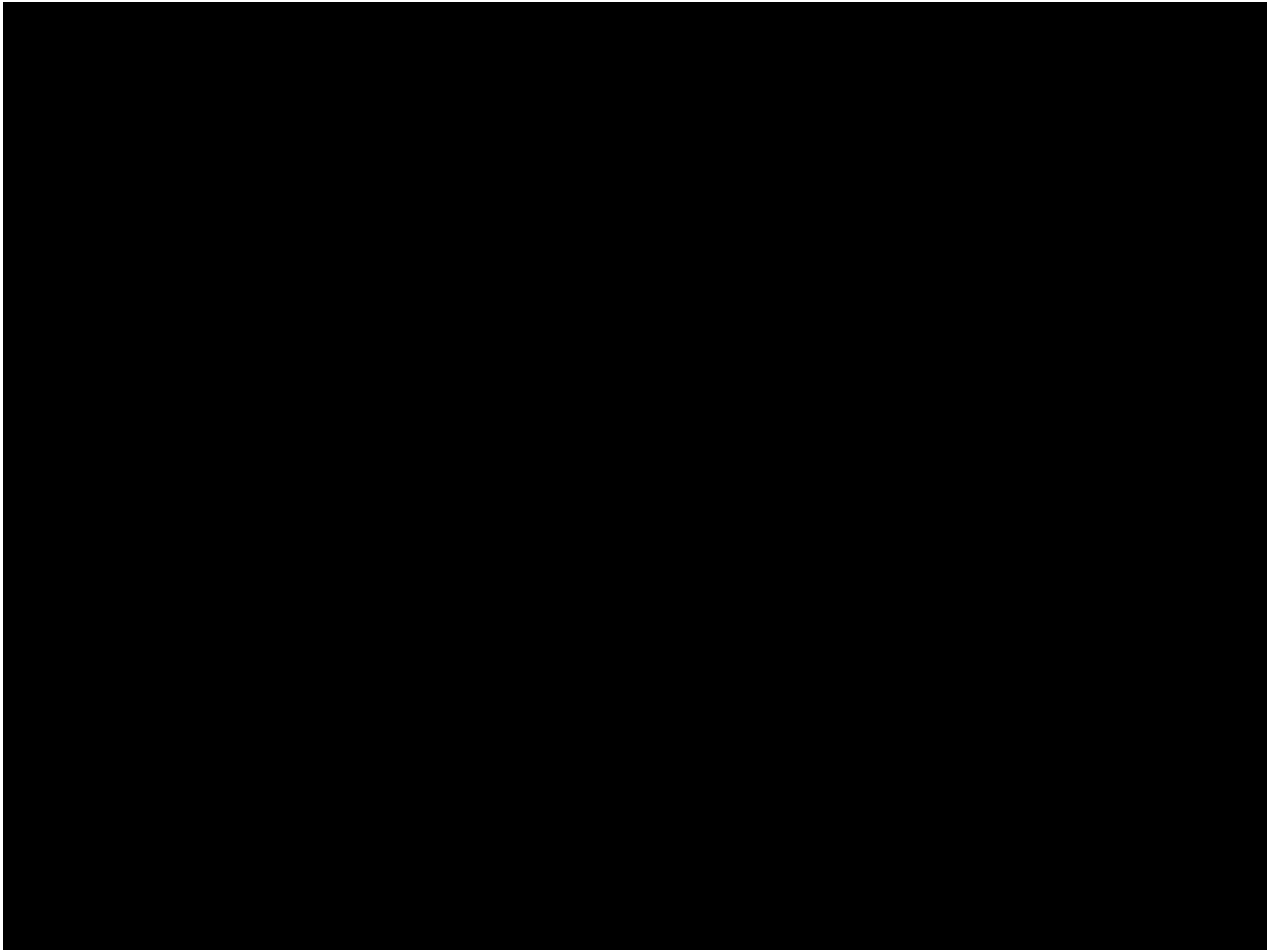
Negative Gene Regulation

Normally Turned OFF



Lactose present, repressor inactive, operon on. Allolactose, an isomer of lactose, derepresses the operon by inactivating the repressor. In this way, the enzymes for lactose utilization are induced.

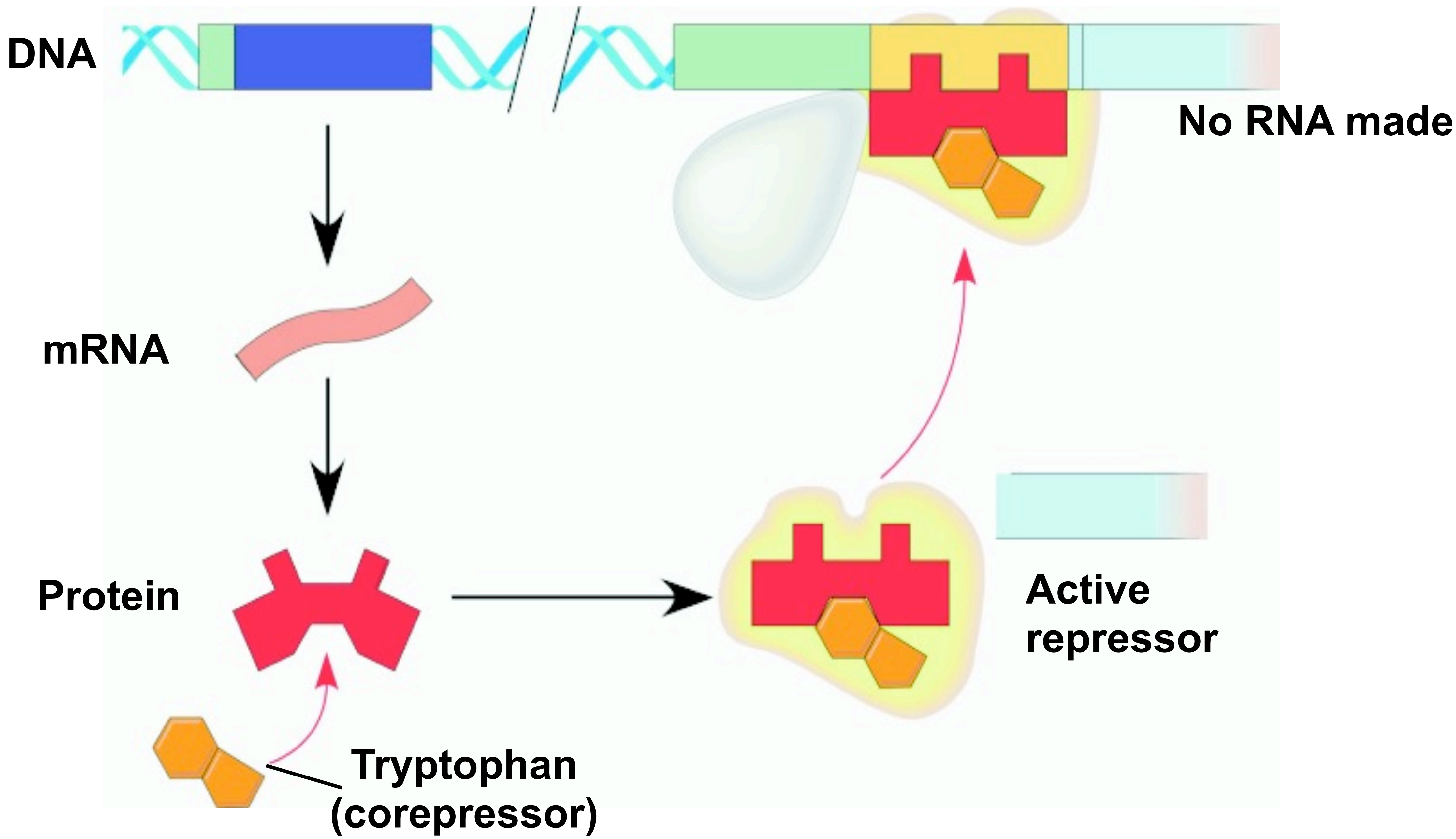
lac Operons "turn-on-able"



Repressible Operons "turn-off-able"

Negative Gene Regulation

Normally Turned ON

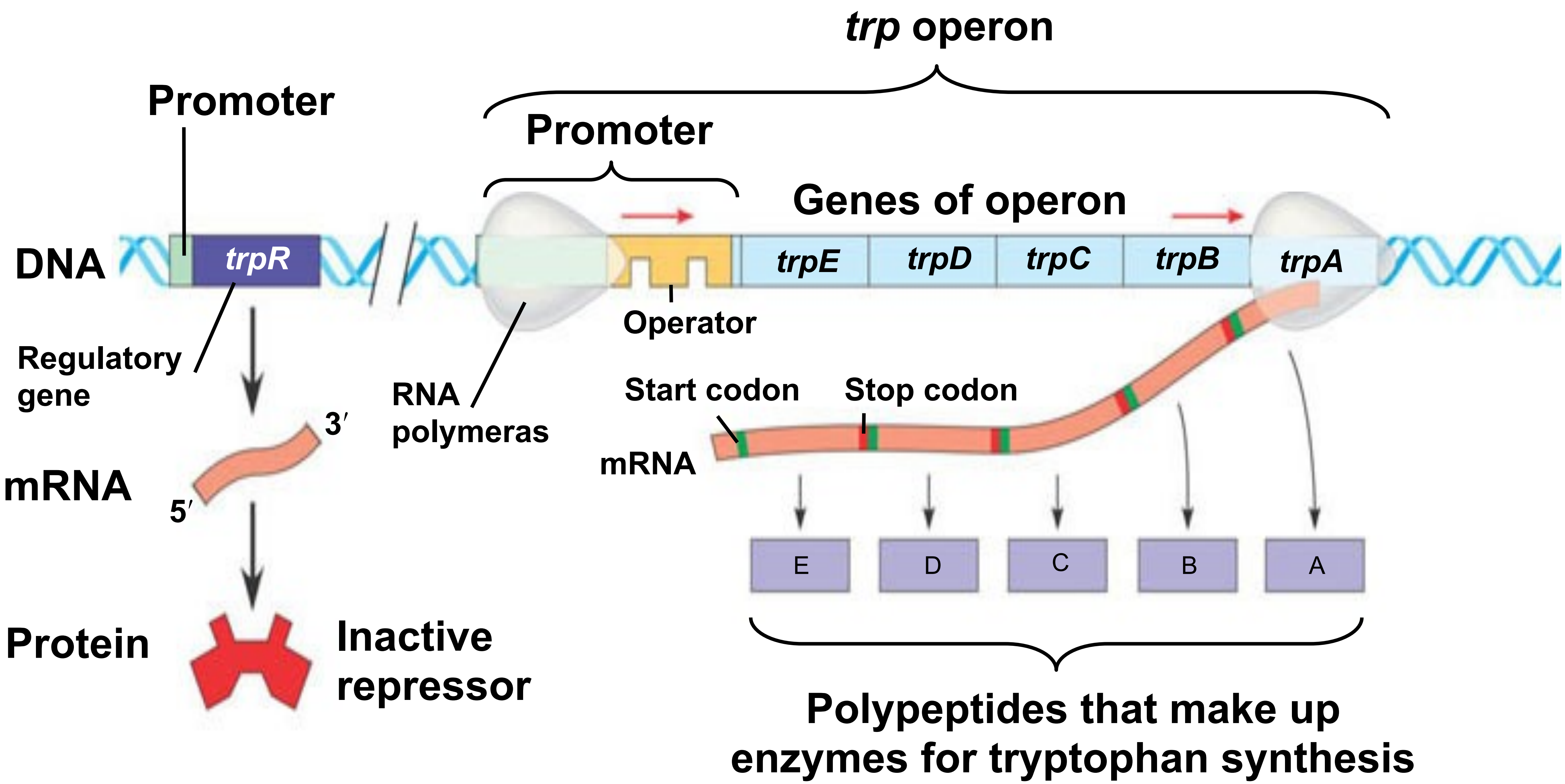


Tryptophan present, repressor active, operon off. As tryptophan accumulates, it inhibits its own production by activating the repressor protein.

Repressible Operons "turn-off-able"

Negative Gene Regulation

Normally Turned ON



Tryptophan absent, repressor inactive, operon on. RNA polymerase attaches to the DNA at the promoter and transcribes the operon's genes.

Let's Review so far

- **Inducible Operons ("turn-on-able")**
  - inducer binds to innately active repressor there by inactivating the repressor and turning operon on
  - usually operates in catabolic pathways
- **Repressible Operons ("turn-off-able")**
  - repressor binds to innately inactive repressor there by activating the repressor and turning operon off
  - usually operates in anabolic pathways

Let's Review so far

- **Negative Gene Control**
  - both repressible and inducible operons operate as negative control
    - because operons are switched off by an "active" form of the repressible protein
- **Positive Gene Control**
  - some operons operate under positive control
  - these use activator proteins that have a stimulatory effect

Preface to Positive Gene Control

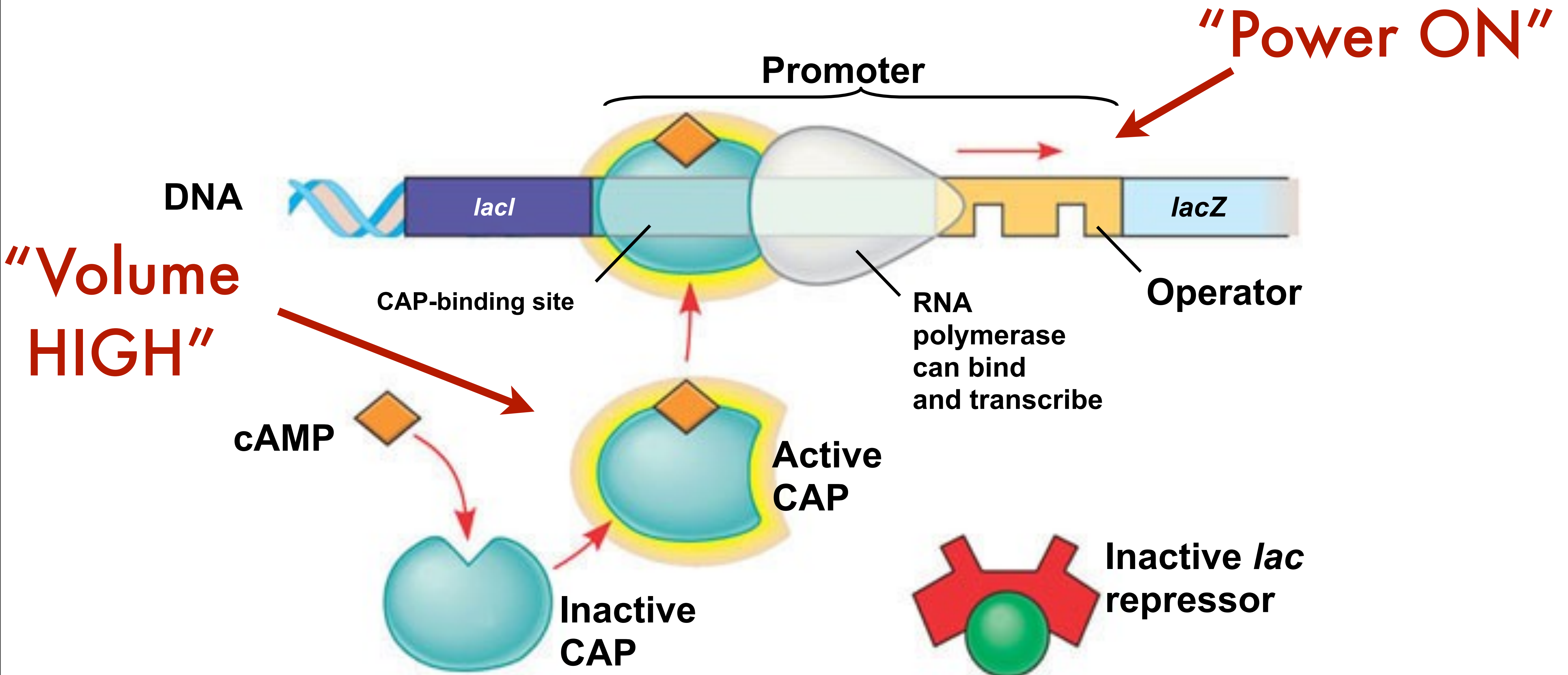
- **Negative Gene Control-** uses “repressors” to turn operons on/off
- **Positive Gene Control-** uses “activators” to turn the volume up/down
- Bacteria prefer glucose to produce ATP via glycolysis but they can use other sugars (ex. lactose)
  - Enzymes used to catabolize glucose are always present
  - How does cell “know when glucose is plentiful or scarce?
  - If glucose is plentiful then ATP>cAMP
  - But if glucose is scarce then ATP<cAMP

Preface to Positive Gene Control

- **Here are scenarios bacteria might deal with**
  - **high glucose/high lactose**
    - **glucose enzymes active, volume low, lac operon turned on**
  - **high glucose/low lactose**
    - **glucose enzymes active, volume low, lac operon turned off**
  - **low glucose/high lactose**
    - **glucose enzymes inactive, volume high, lac operon turned on**
  - **low glucose/low lactose**
    - **glucose enzymes inactive, volume high, lac operon turned off**

Inducible "turn-on-able" *lac* operon

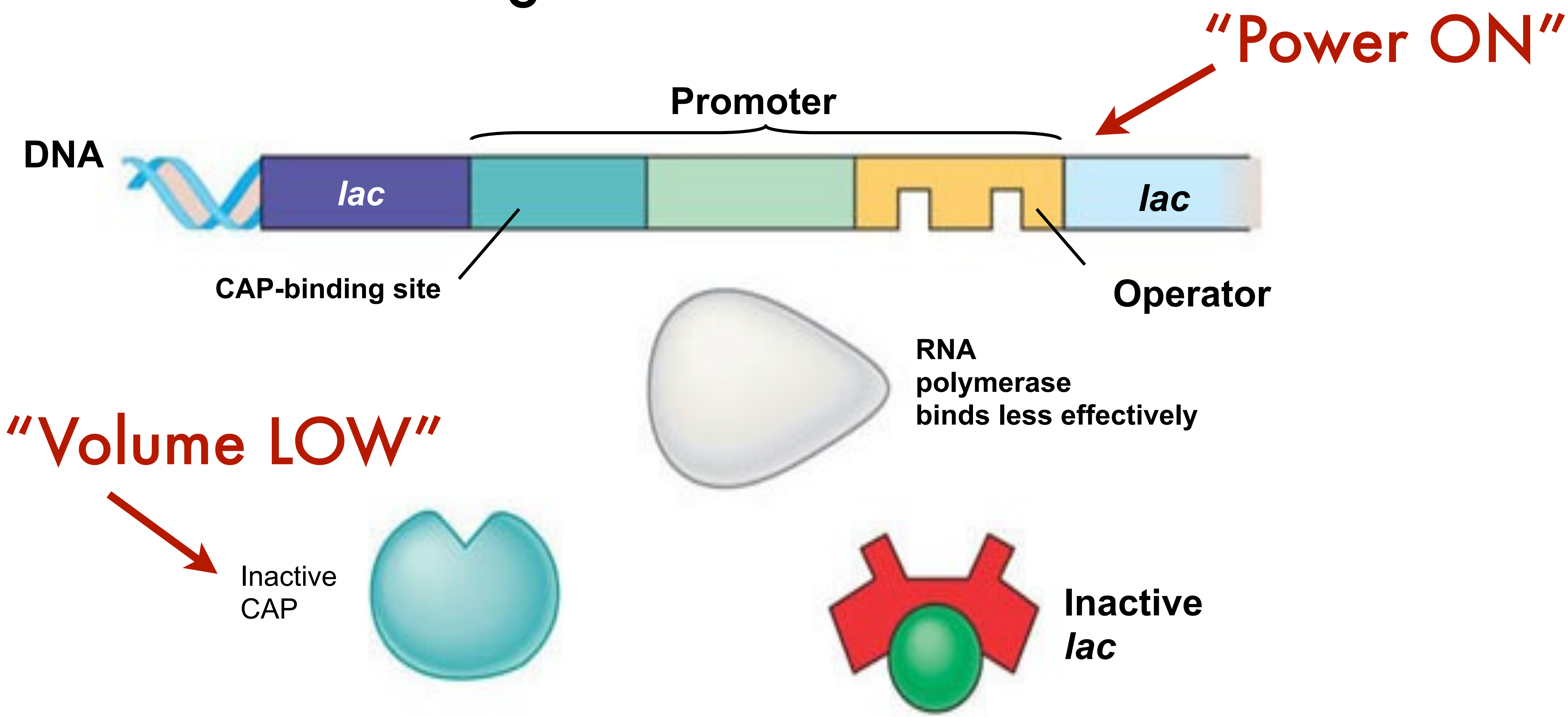
Negative Gene Regulation- on/off switch
Positive Gene Regulation- volume control



Lactose present, glucose scarce (cAMP level high): abundant *lac* mRNA synthesized. If glucose is scarce, the high level of cAMP activates CAP, and the *lac* operon produces large amounts of mRNA for the lactose pathway.

Inducible "turn-on-able" *lac* operon

Negative Gene Regulation- on/off switch
Positive Gene Regulation- volume control



Lactose present, glucose present (cAMP level low): little *lac* mRNA synthesized. When glucose is present, cAMP is scarce, and CAP is unable to stimulate