DNA & Molecular Genetics in Eukaryotes

Molecular Basis of Inheritance

I.

Main Idea: After Mendel worked out the laws of inheritance, the race was on to find the "units of heredity", the molecular basis of inheritance.

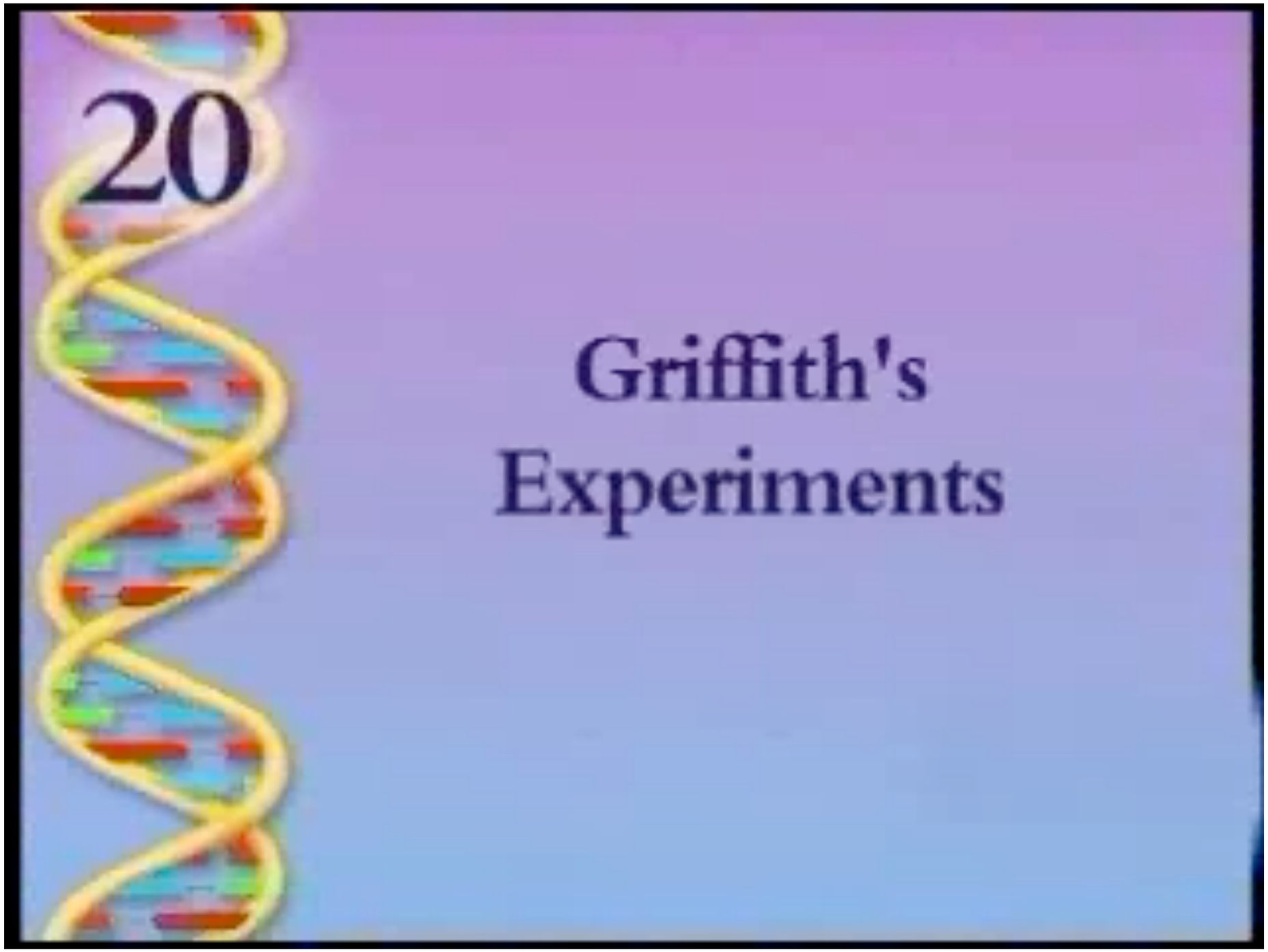
Recall…"The Story of DNA"

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

Recall…"The Story of DNA"

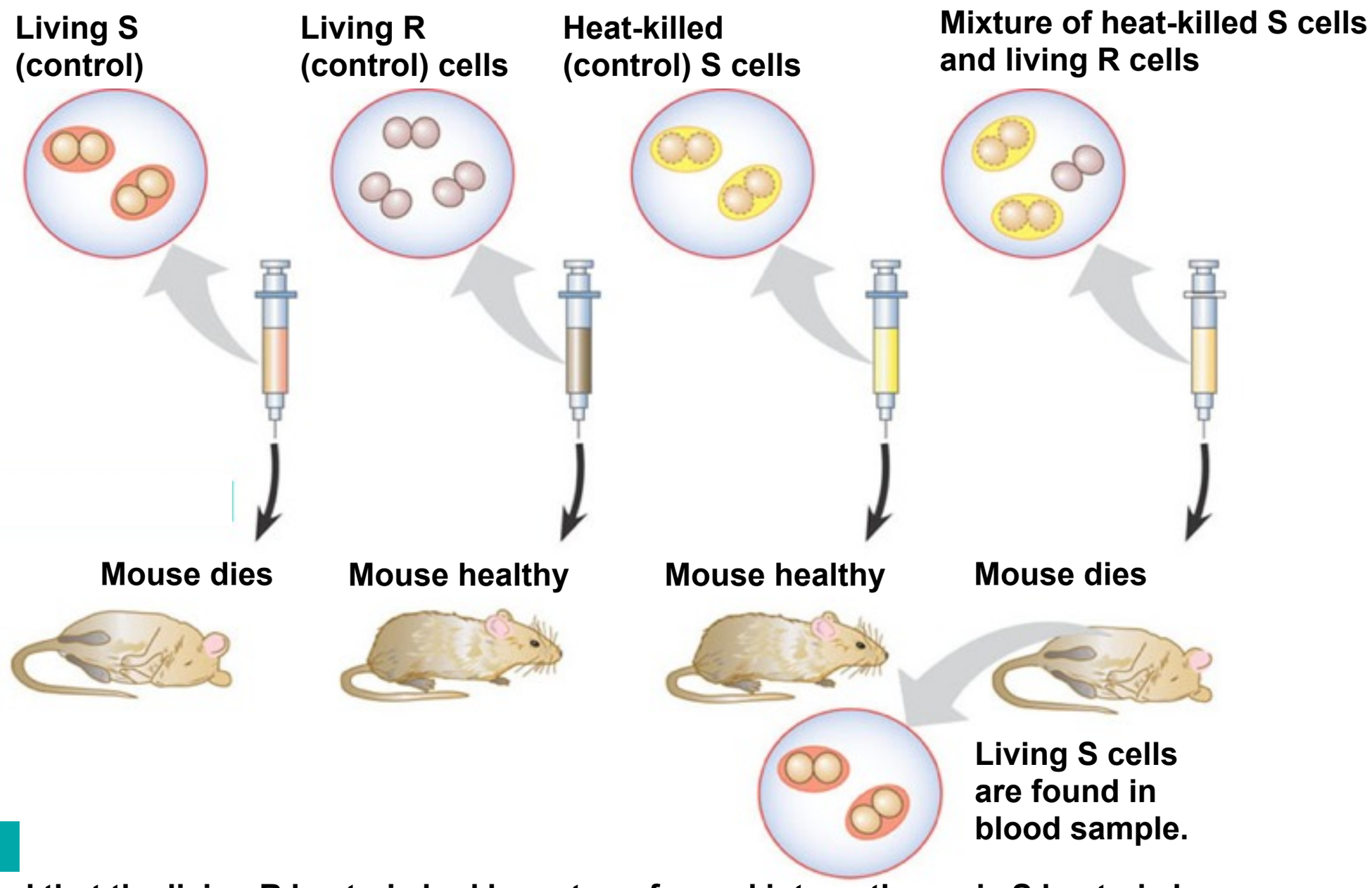
1928 Frederick Griffith



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:



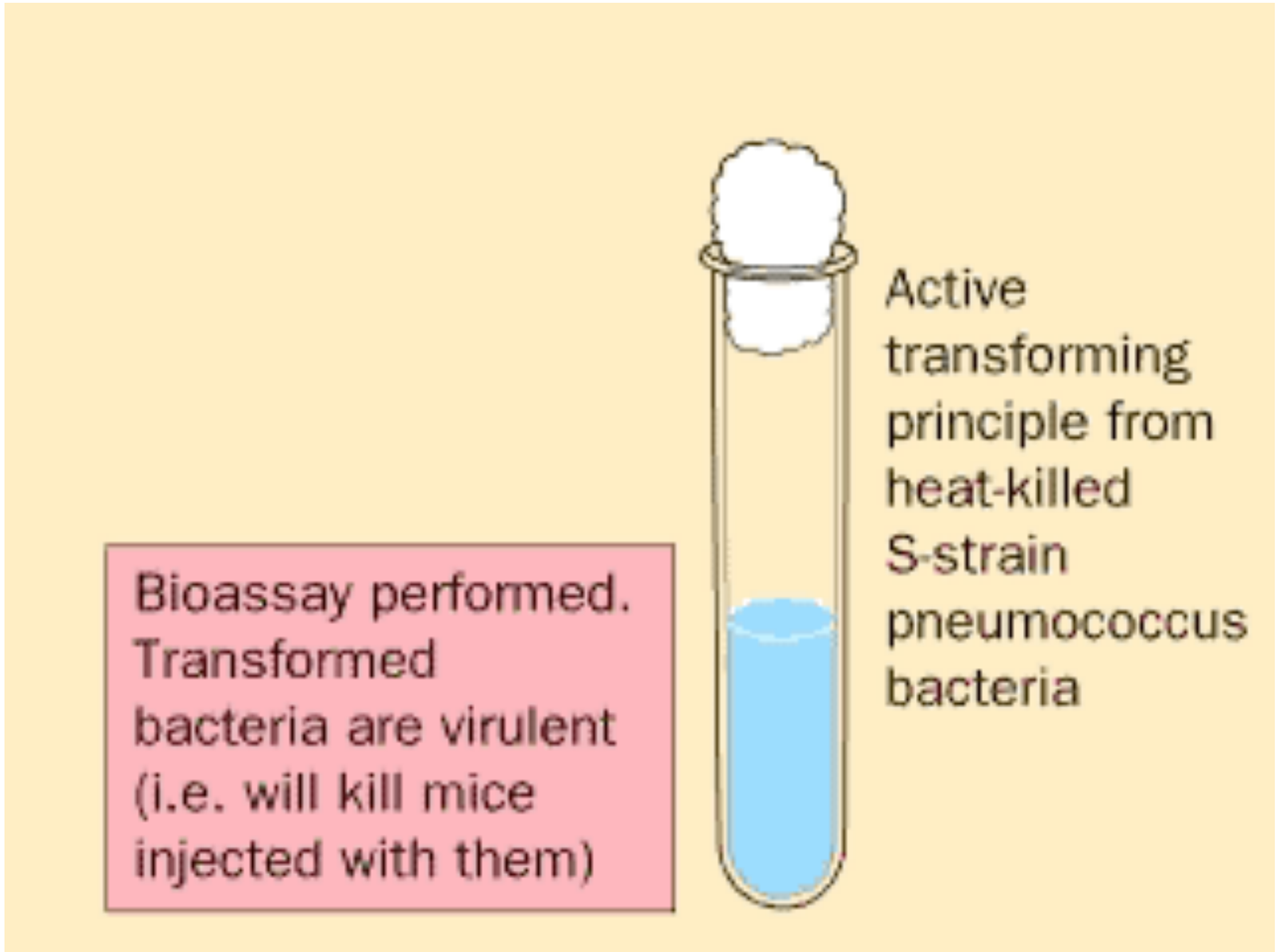
RESULTS

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.

Recall…"The Story of DNA"

1944 Avery, McCarty & Macleod



Recall…"The Story of DNA"

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.

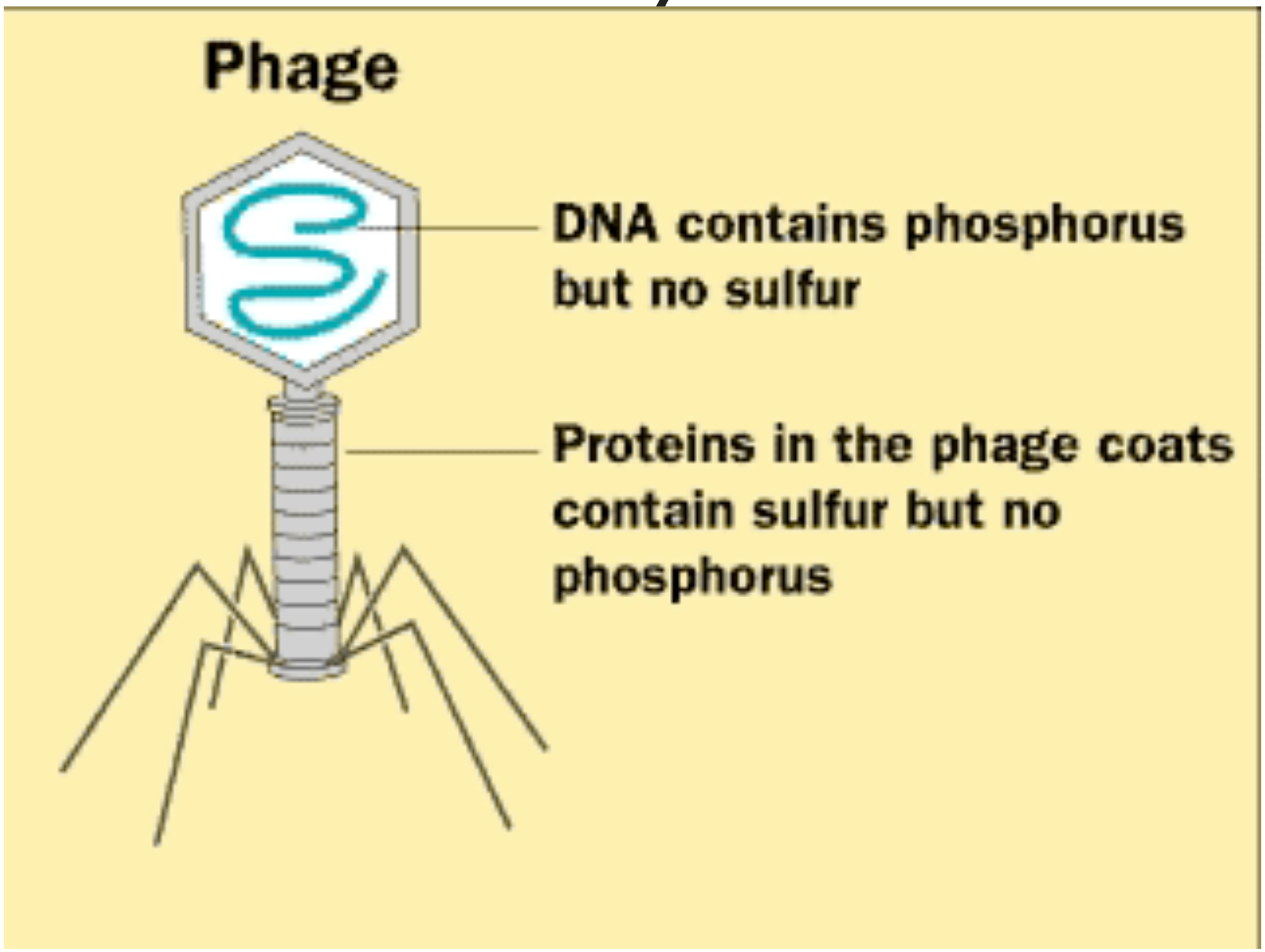
Recall…"The Story of DNA"

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**

Recall…"The Story of DNA"

1952 Alfred Hershey & Martha Chase



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

1952 Alfred Hershey & Martha Chase

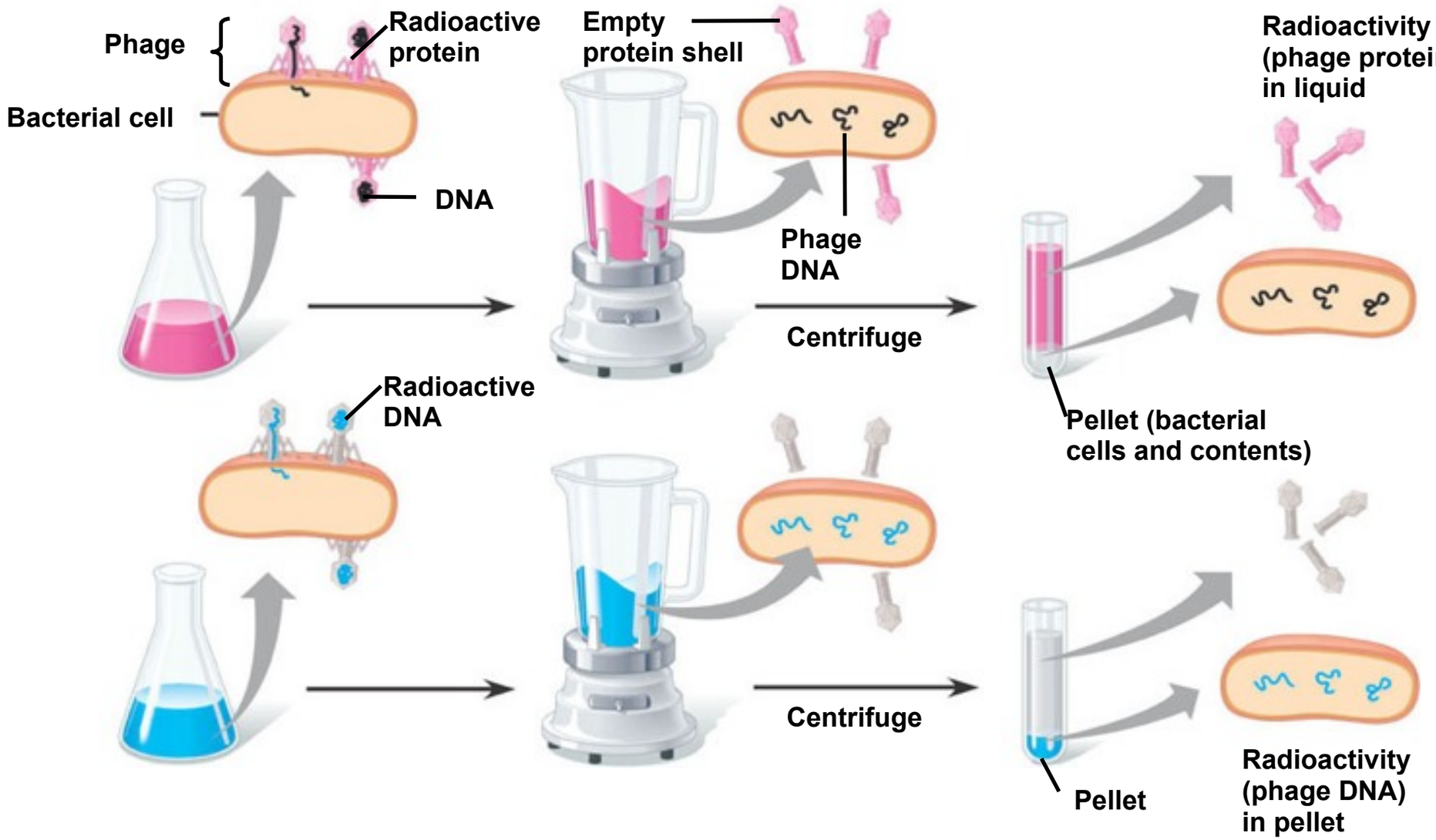
EXPERIMENT

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.

1. Mixed radioactively labeled phages with bacteria. The phages infected the bacterial cells.
2. Agitated in a blender to separate phages outside the bacteria from the bacterial cells.
3. Centrifuged the mixture so that bacteria formed a pellet at the bottom of the test tube.
4. Measured the radioactivity in the pellet and the liquid

Batch 1: Phages were grown with radioactive sulfur (^{35}S), which was incorporated into phage protein (pink).

Batch 2: Phages were grown with radioactive phosphorus (^{32}P), which was incorporated into phage DNA (blue).



RESULTS

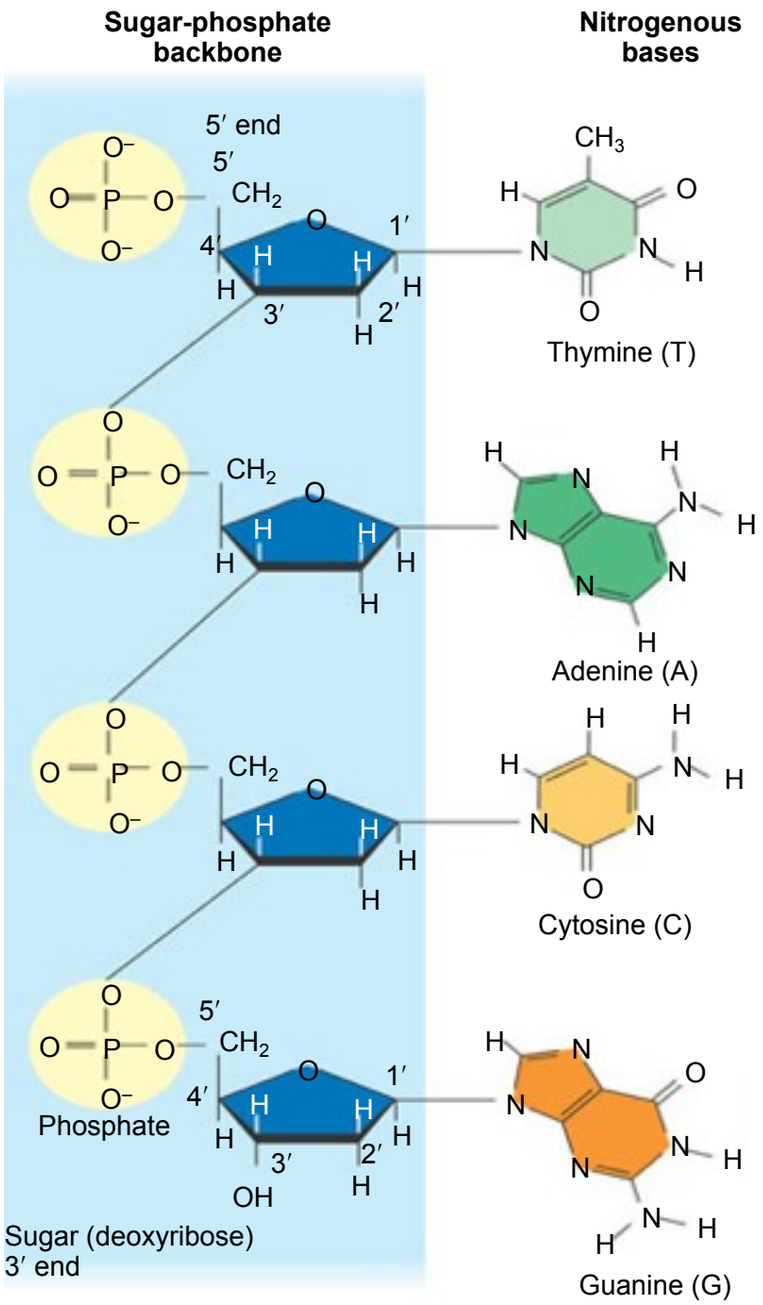
Phage proteins remained outside the bacterial cells during infection, while phage DNA entered the cells. When cultured, bacterial cells with radioactive phage DNA released new phages with some radioactive phosphorus.

CONCLUSION

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

Recall…"The Story of DNA"

1950's Rosalind Franklin, Linus Pauling, Maurice Wilkins



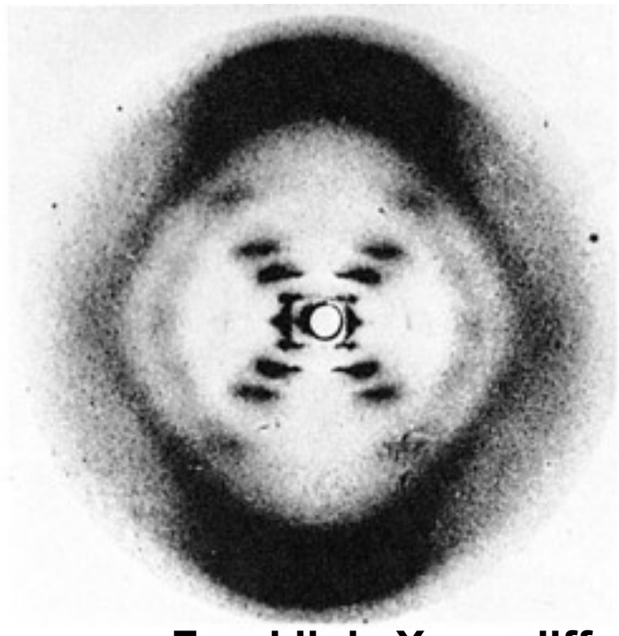
- By now many felt that DNA was the elusive "unit of heredity" and the next step would be to determine its structure.
- Prior to the 1950's chemists already knew that DNA is a polymer of nucleotides, each consisting of three components: a nitrogenous base, a sugar, and a phosphate group.

Recall…"The Story of DNA"

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

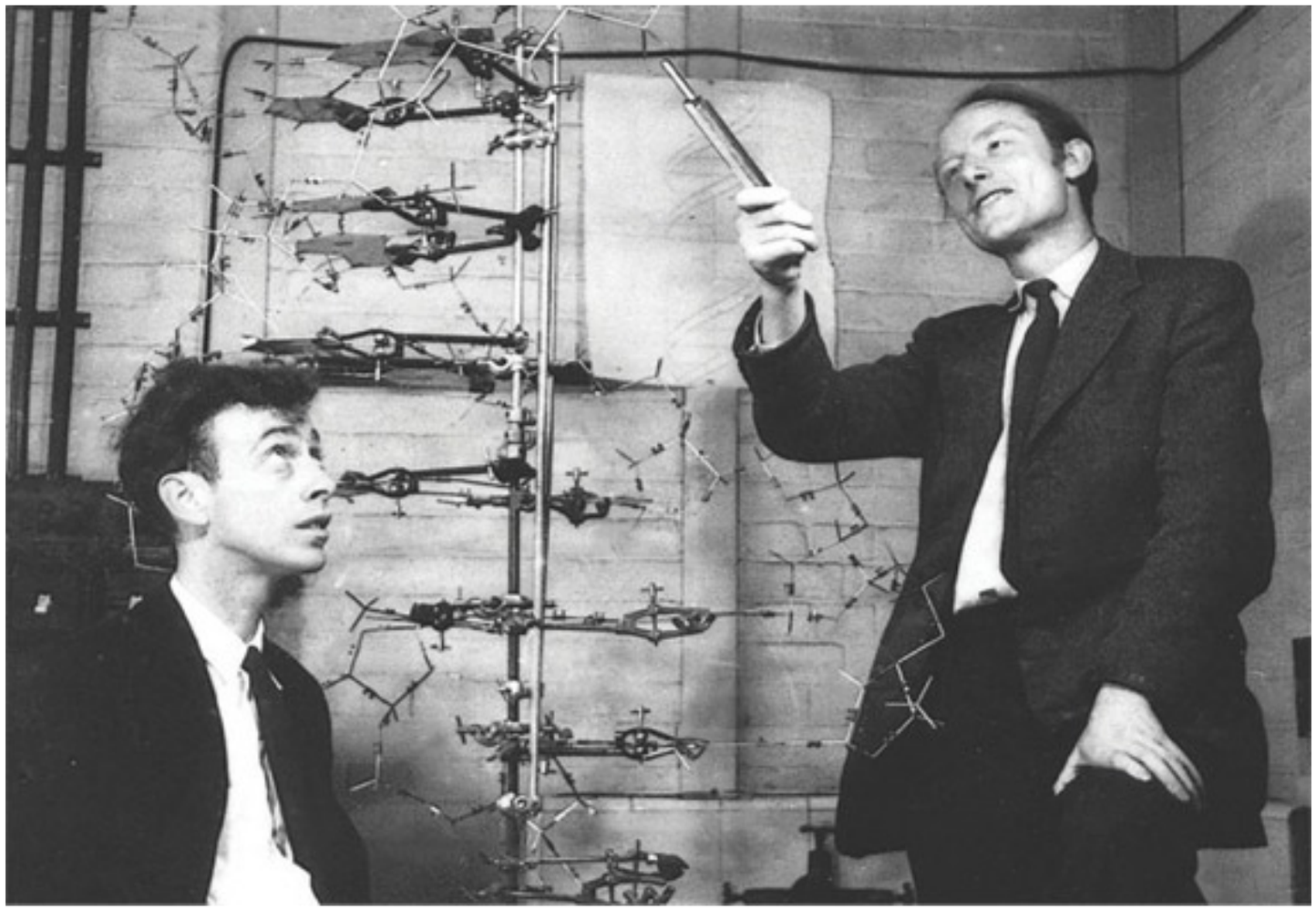


(b) Franklin's X-ray diffraction Photograph of DNA

Recall…"The Story of DNA"

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



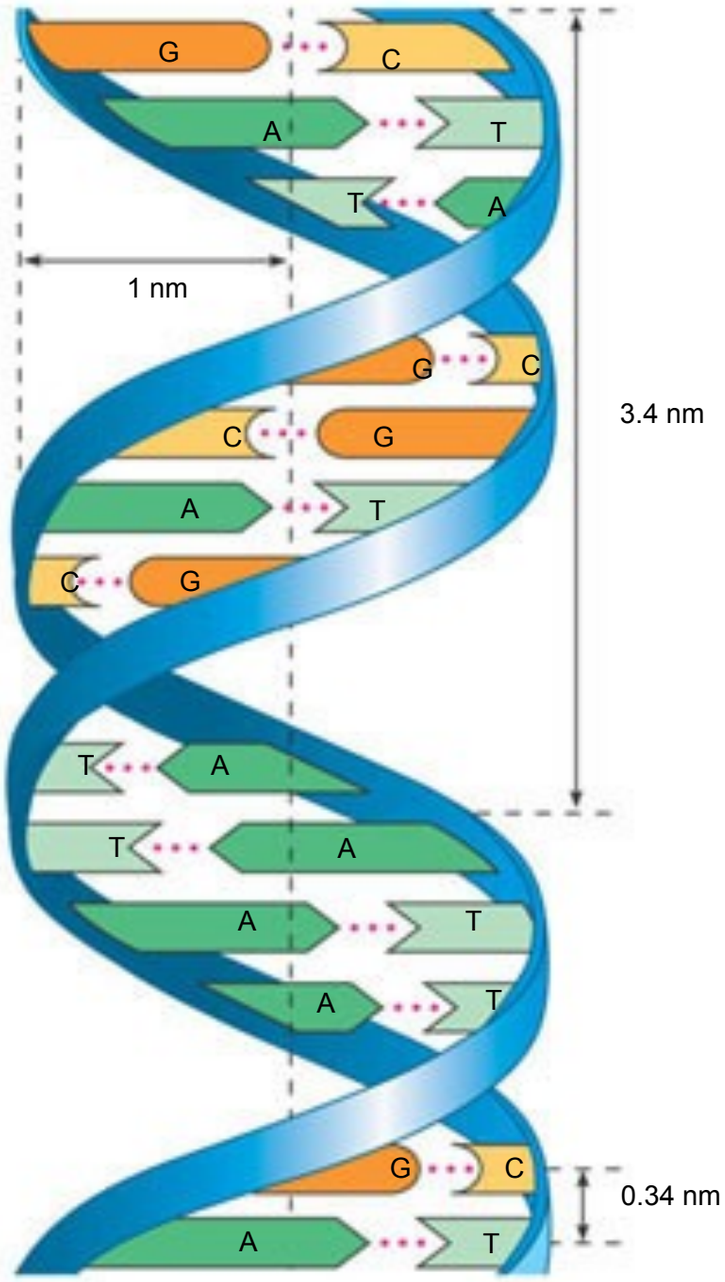
Deoxyribonucleic Acid…DNA

They knew…

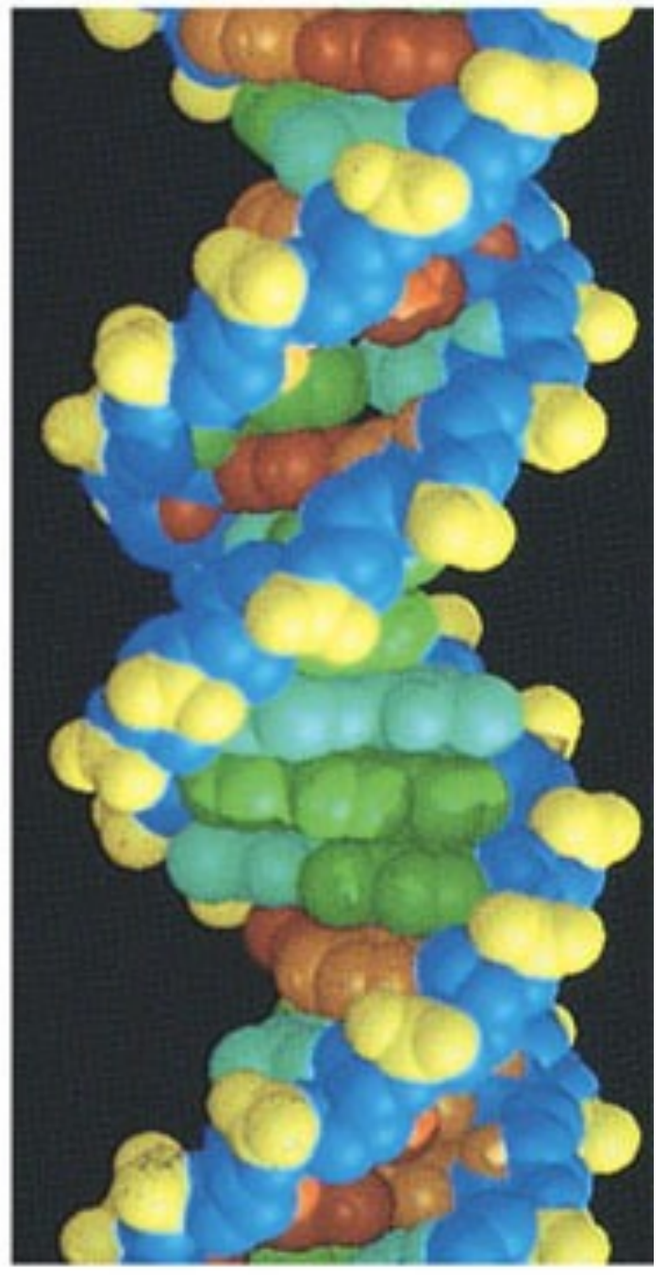
its width

its length

its shape



(a) Key features of DNA structure



(c) Space-filling model

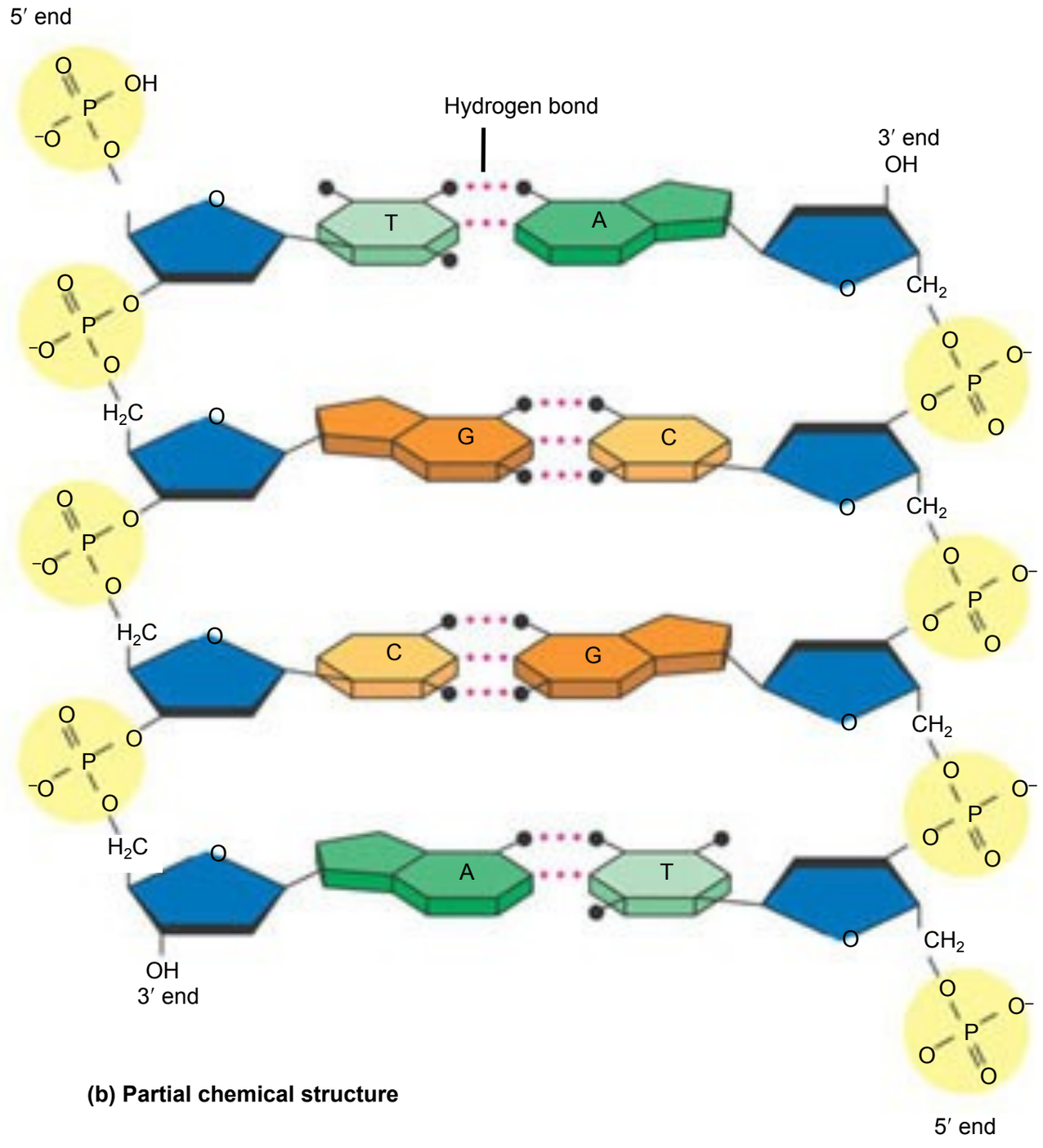
Deoxyribonucleic Acid...DNA

the structure of the backbone

its anti-parallel nature

in fact they even hypothesized a replicating mechanism

They knew...



(b) Partial chemical structure

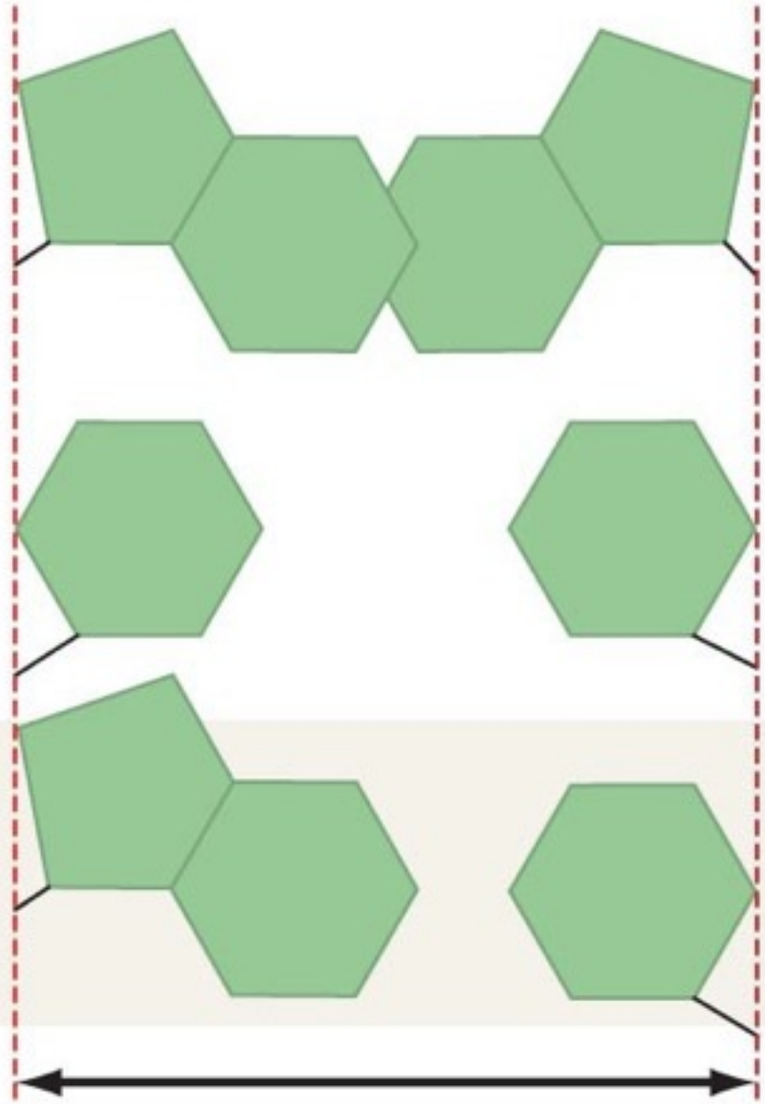
Deoxyribonucleic Acid...DNA

They knew... **Only purine-pyrimidine pairs fit inside the double helix.**

Purine-purine pair
NOT ENOUGH SPACE

Pyrimidine-pyrimidine pair
TOO MUCH SPACE

Purine-pyrimidine pair
JUST RIGHT



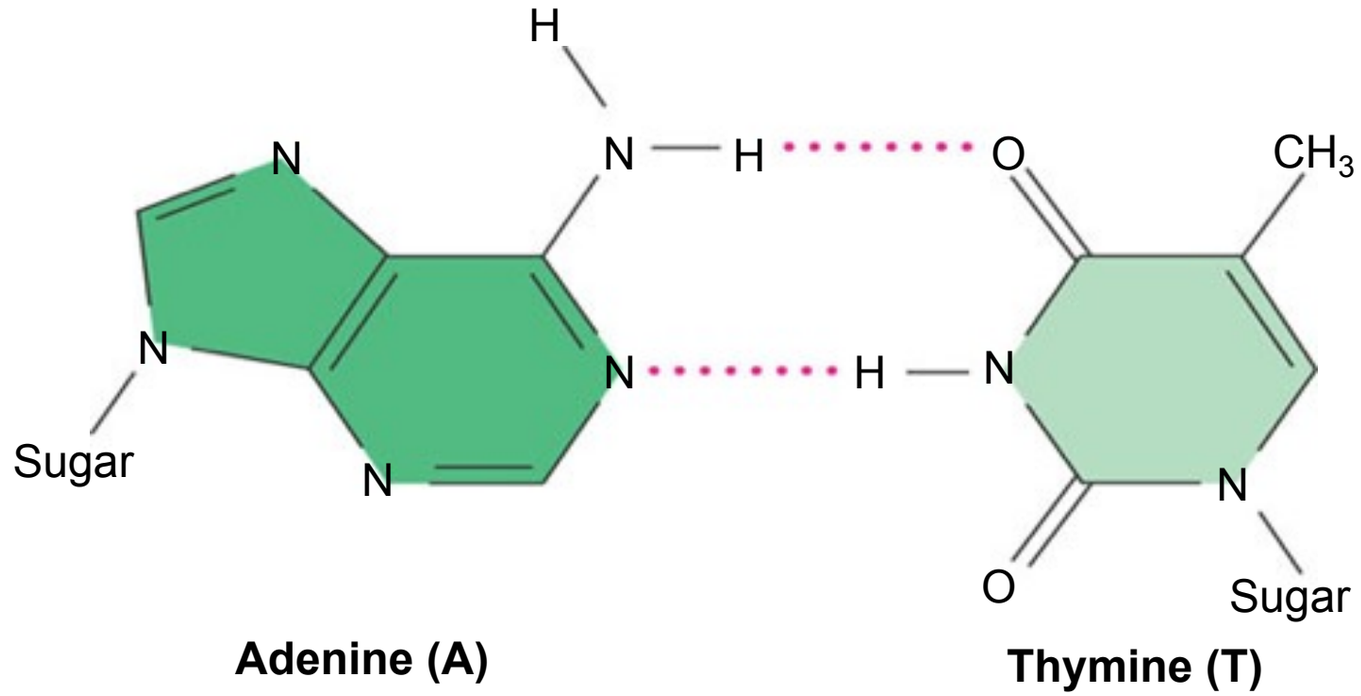
Space inside sugar-phosphate backbones

Deoxyribonucleic Acid...DNA

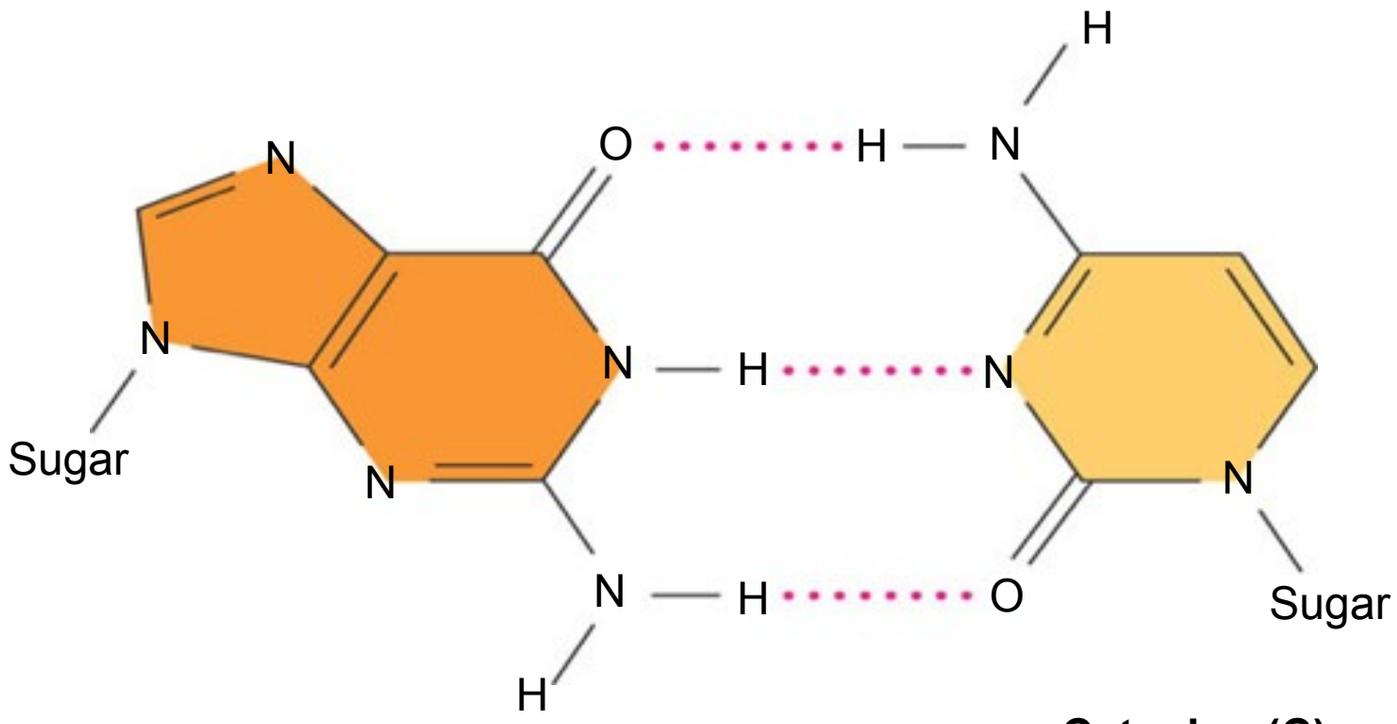
They knew...

base pairing rules

bonds between the bases



Adenine (A) **Thymine (T)**



Guanine (G) **Cytosine (C)**

Molecular Basis of Inheritance

II.

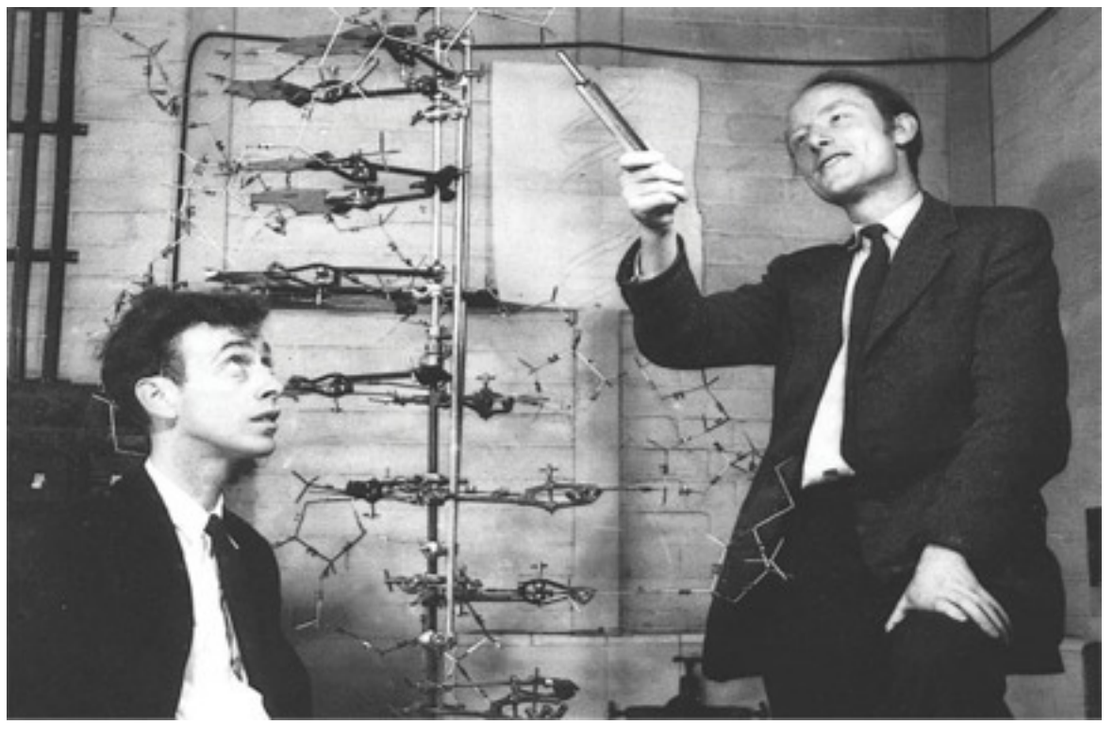
Main Idea: Once the structure of DNA came to light, the next problem to solve was its mechanism replication.

DNA Replication

1953 James Watson & Francis Crick

- Watson and Crick ended their classic paper with the following "It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material".

- Watson and Crick wrote a second paper describing their hypothesis for DNA replication.
- Their basic explanation can see on the next slide...
- Their model however remained untested for years!



DNA Replication

The basic principle behind DNA replication is that each of the two complimentary strands serves as a template for the replication of new strands

(a) Before replication, the parent molecule has two complementary strands of DNA. Each base is paired by hydrogen bonding with its specific partner, A with T and G with C.

(b) The first step in replication is separation of the two DNA strands.

(c) Each "old" strand now serves as a template that determines the order of nucleotides along "new" complementary strands. Nucleotides plug into specific sites along the template surface according to the base-pairing rules.

(d) The nucleotides are connected to form the sugar-phosphate backbones of the new strands. Each DNA molecule now consists of one "old" strand and one "new" strand. We have two DNA molecules identical to the one molecule with which we started.

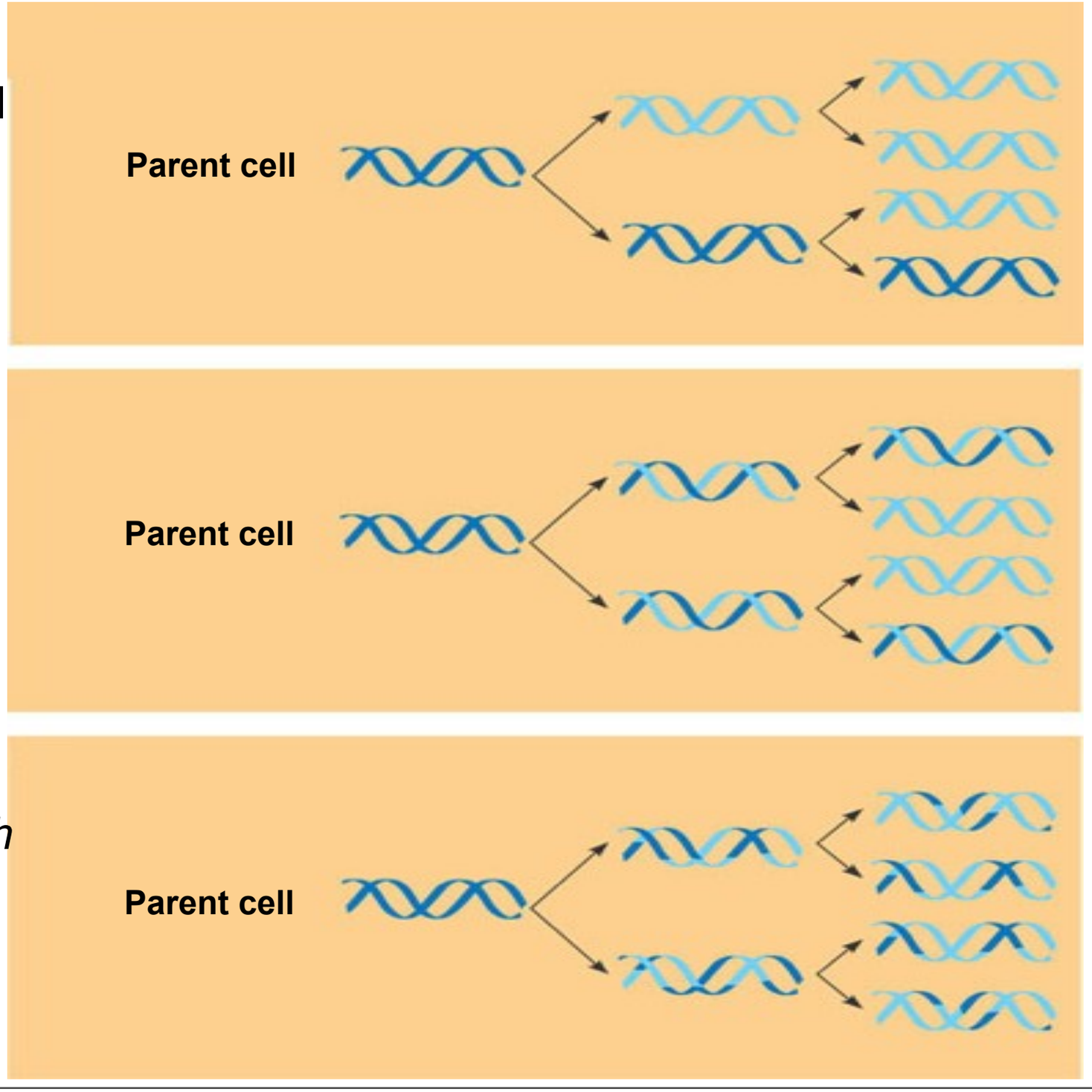
Additional Models DNA Replication

3 possible copying mechanisms

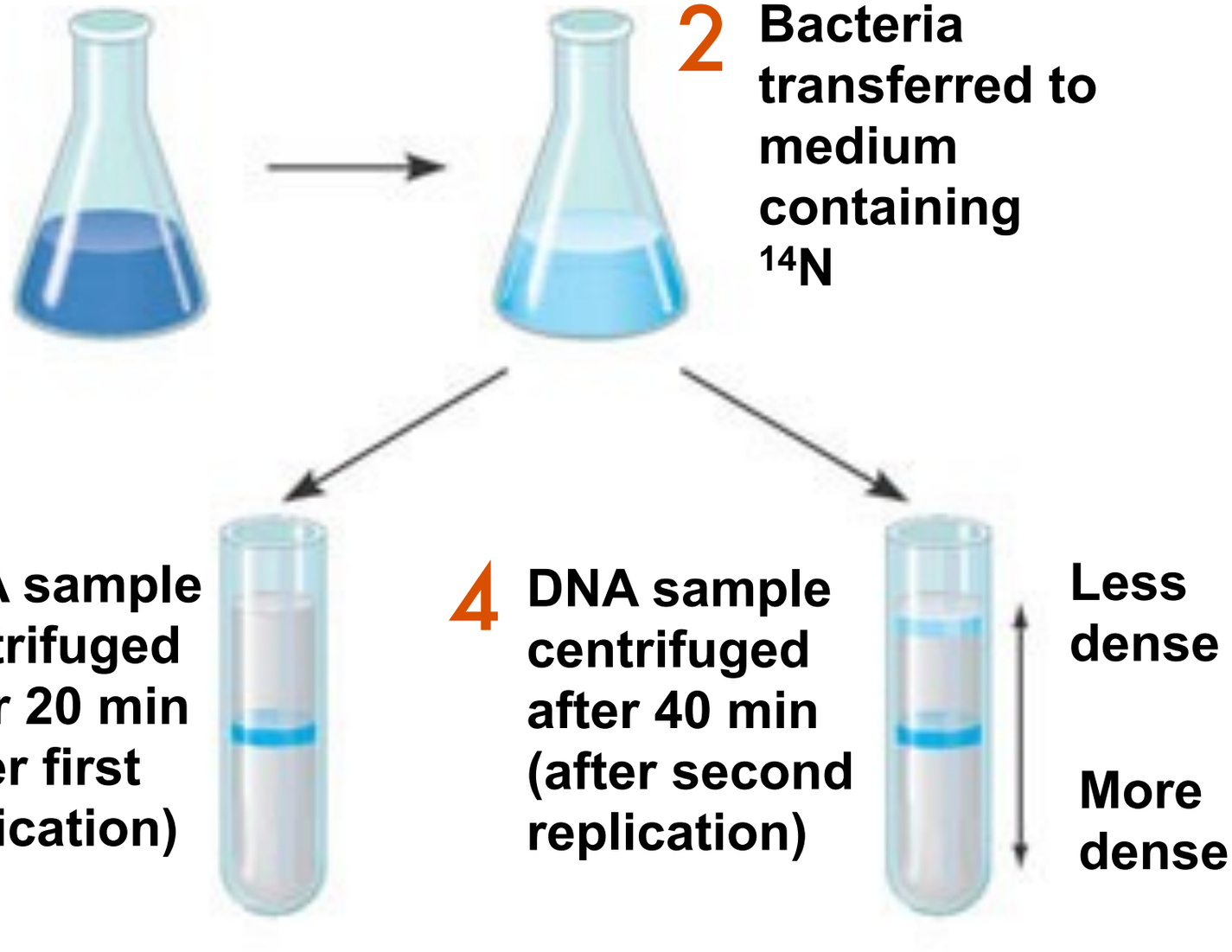
Conservative model. The two parental strands reassociate after acting as templates for new strands, thus restoring the parental double helix.

Semiconservative model. The two strands of the parental molecule separate, and each functions as a template for synthesis of a new, complementary strand.

Dispersive model. Each strand of *both* daughter molecules contains a mixture ofold and newly synthesized DNA.



EXPERIMENT Matthew Meselson and Franklin Stahl cultured *E. coli* bacteria for several generations on a medium containing nucleotide precursors labeled with a heavy isotope of nitrogen, ^{15}N. The bacteria incorporated the heavy nitrogen into their DNA. The scientists then transferred the bacteria to a medium with only ^{14}N, the lighter, more common isotope of nitrogen. Any new DNA that the bacteria synthesized would be lighter than the parental DNA made in the ^{15}N medium. Meselson and Stahl could distinguish DNA of different

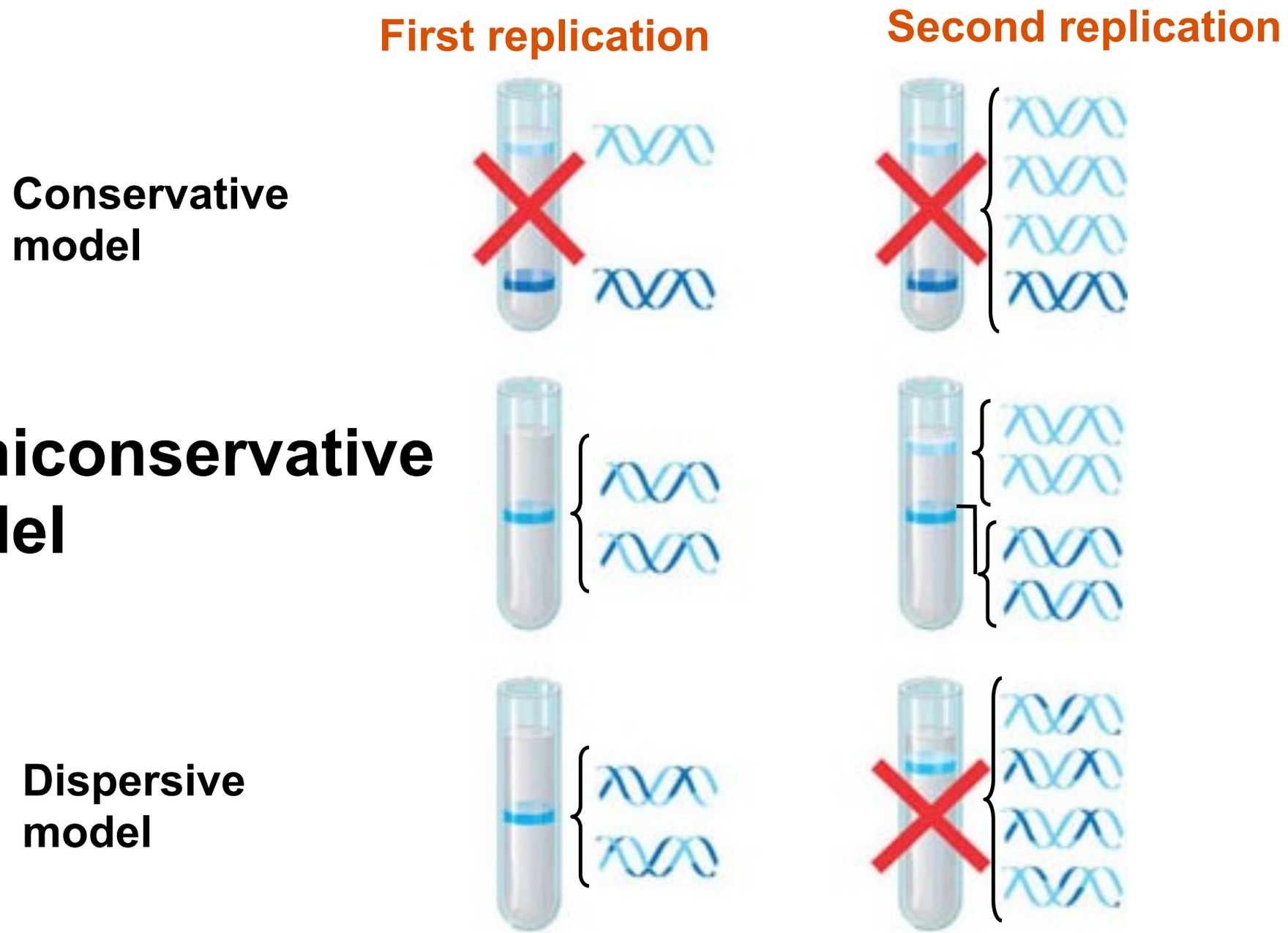


RESULTS

The bands in these two centrifuge tubes represent the results of centrifuging two DNA samples from the flask

CONCLUSION

Meselson and Stahl concluded that DNA replication follows the semiconservative model by comparing their result to the results predicted by each of the three models. The first replication in the ^{14}N medium produced a band of hybrid (^{15}N–^{14}N) DNA. This result eliminated the conservative model. A second replication produced both light and hybrid DNA, a result that eliminated the dispersive model and supported the semiconservative model.



DNA Replication

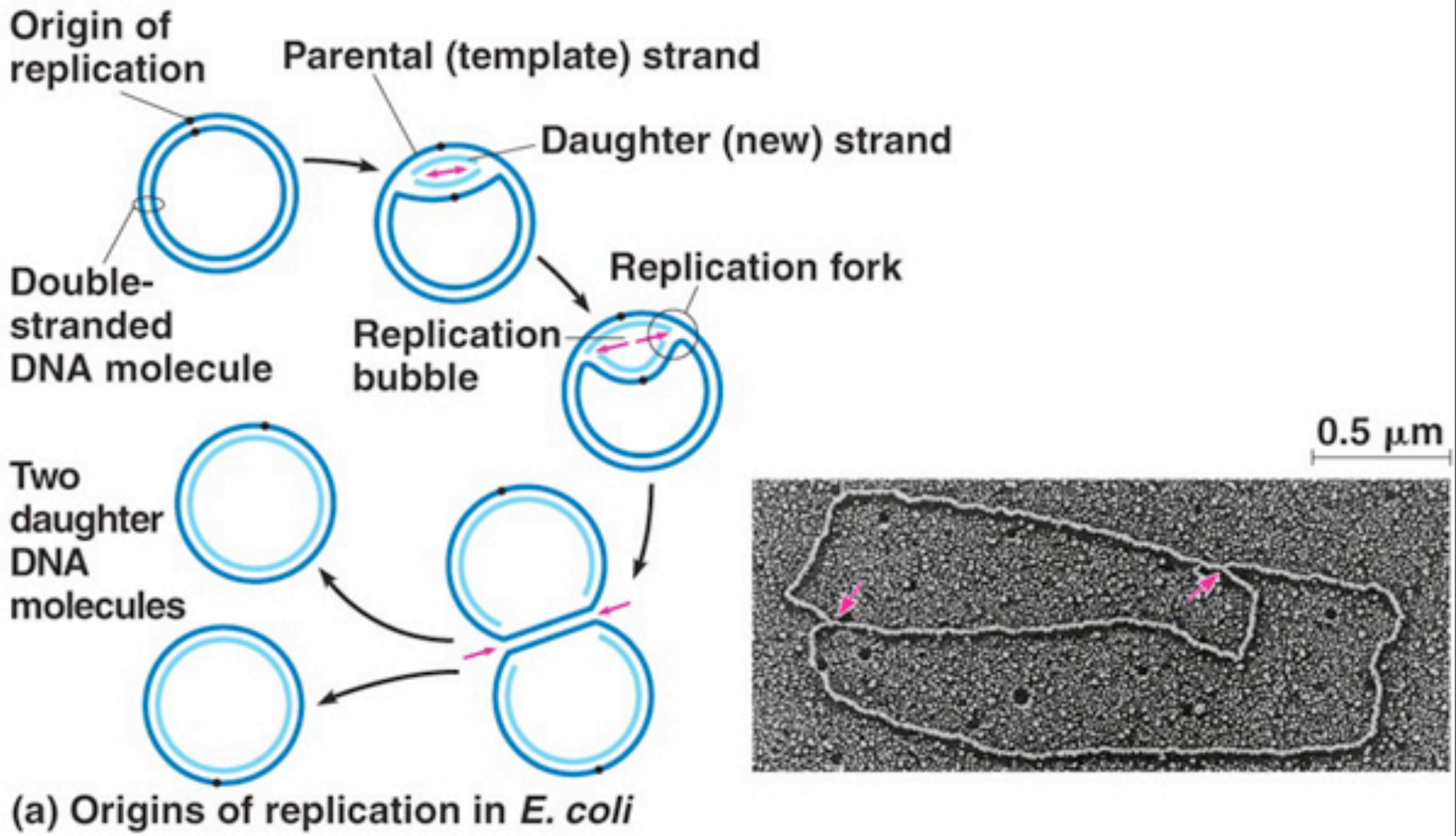
- Many proteins work together in DNA replication and repair
- The parent molecule unwinds, and two new daughter strands are built based on base-pairing rules
- Copying DNA Is done with remarkable in its speed and accuracy
- The replication of DNA begins at special sites called **origins of replication**, where the two strands are separated

Please note even though this powerpoint's focus lies on eukaryotes, we know much more about prokaryotic replication. As a result we focus primarily on prokaryotic replication since it is fundamentally the same. Along the way I will point that important distinctions between the two.

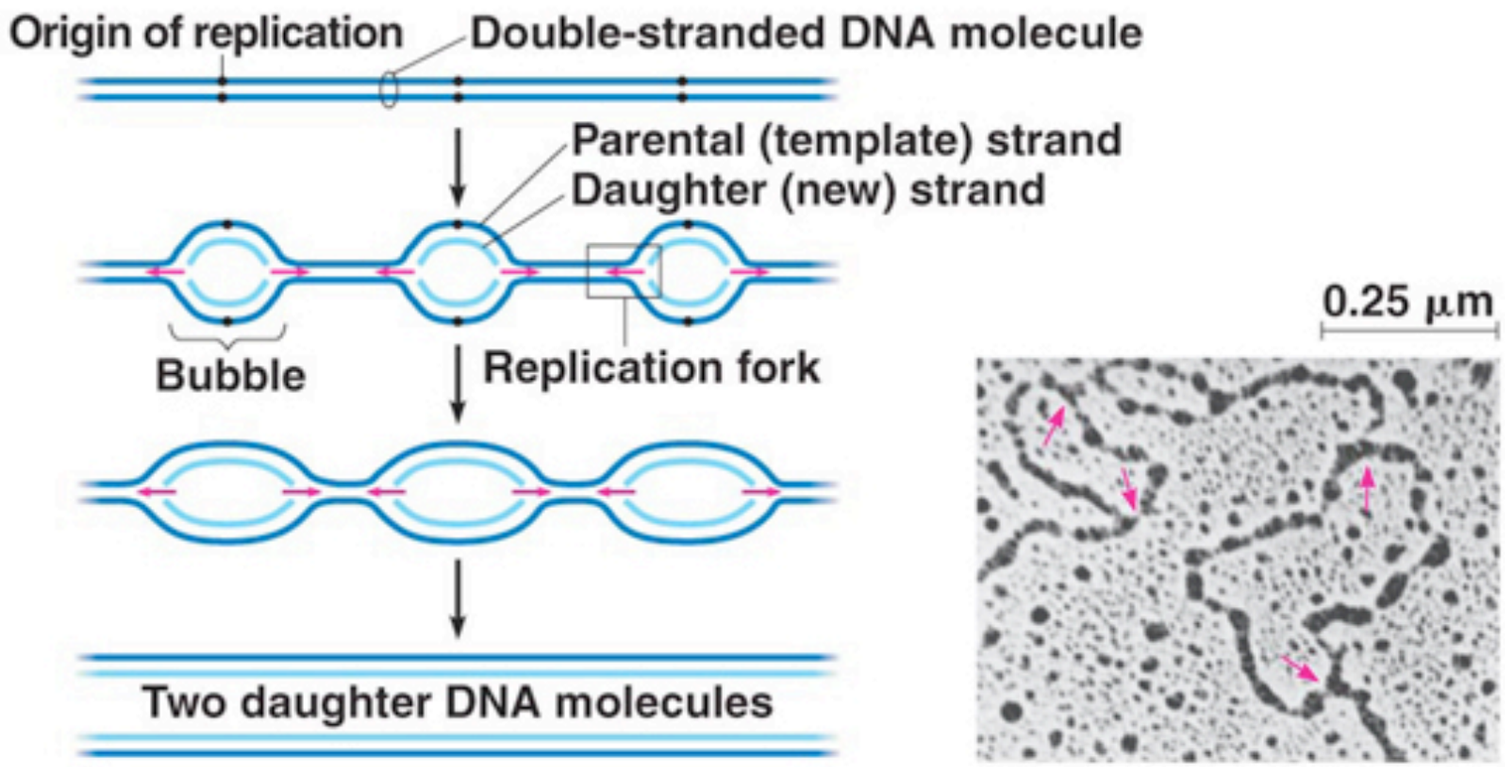
DNA Replication

Our first important distinction-

prokaryotes have only one origin of replication, where eukaryotes have hundreds or even thousands



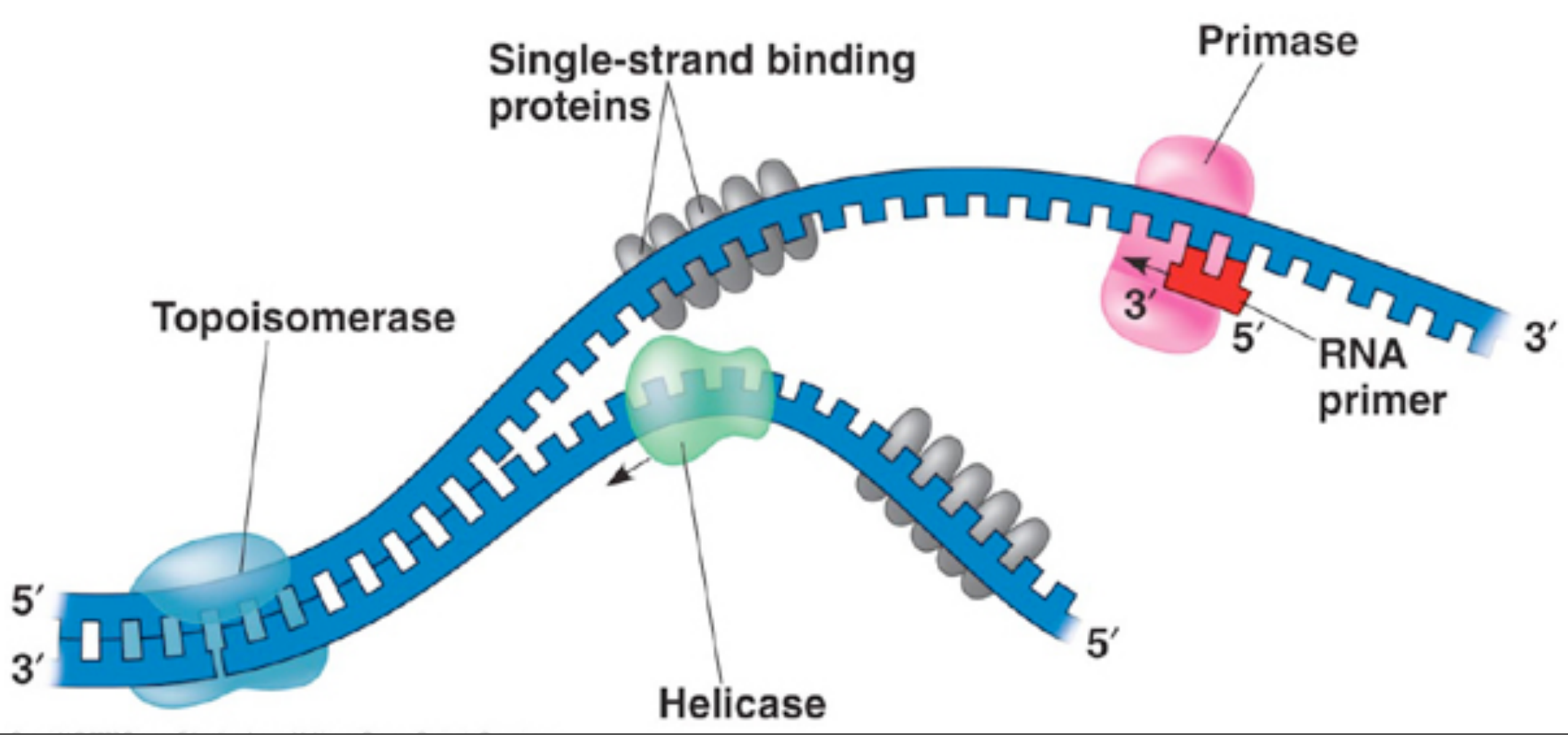
(a) Origins of replication in *E. coli*



(b) Origins of replication in eukaryotes

DNA Replication

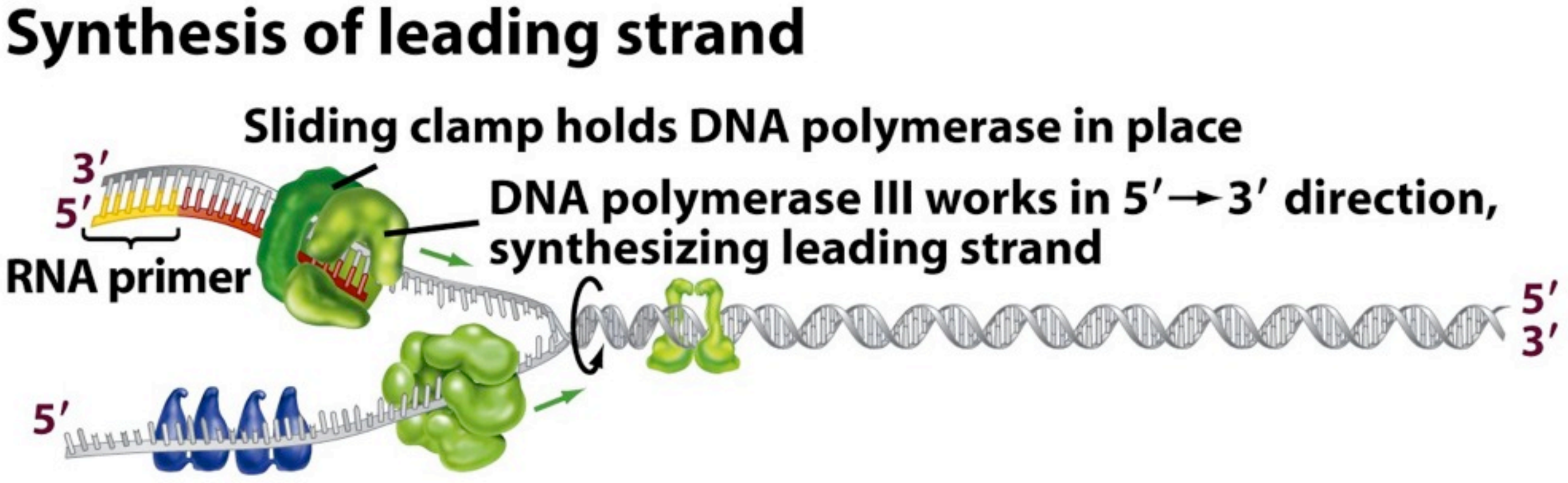
Topoisomerase- *relaxes the supercoils and unwinds DNA*
Helicase- *separates DNA strands (requires ATP)*
Single Strand Binding Proteins- holds the two strands apart
Primase- *special RNA polymerase that lays down 15-50 nucleotides, that serve as a starting point for replication*



DNA Replication

DNA Polymerase III- *large protein/enzyme complex that synthesizes new DNA strands from the template strands by adding one nucleotide at a time according to base pair rules*

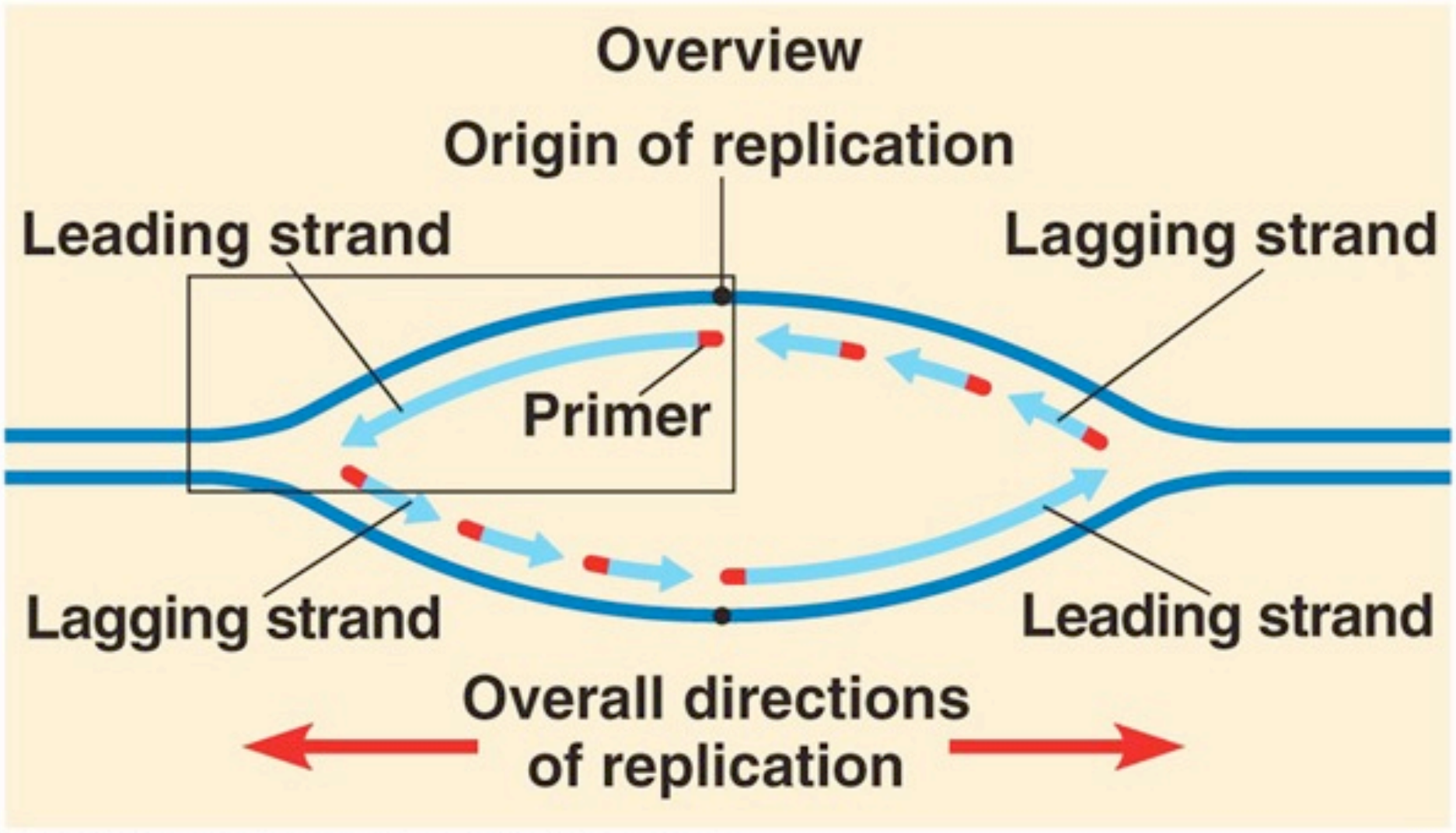
DNA Replication- always adds nucleotides to the 3' end of the growing DNA strand



DNA Replication

Leading Strand- *works towards the replication fork*

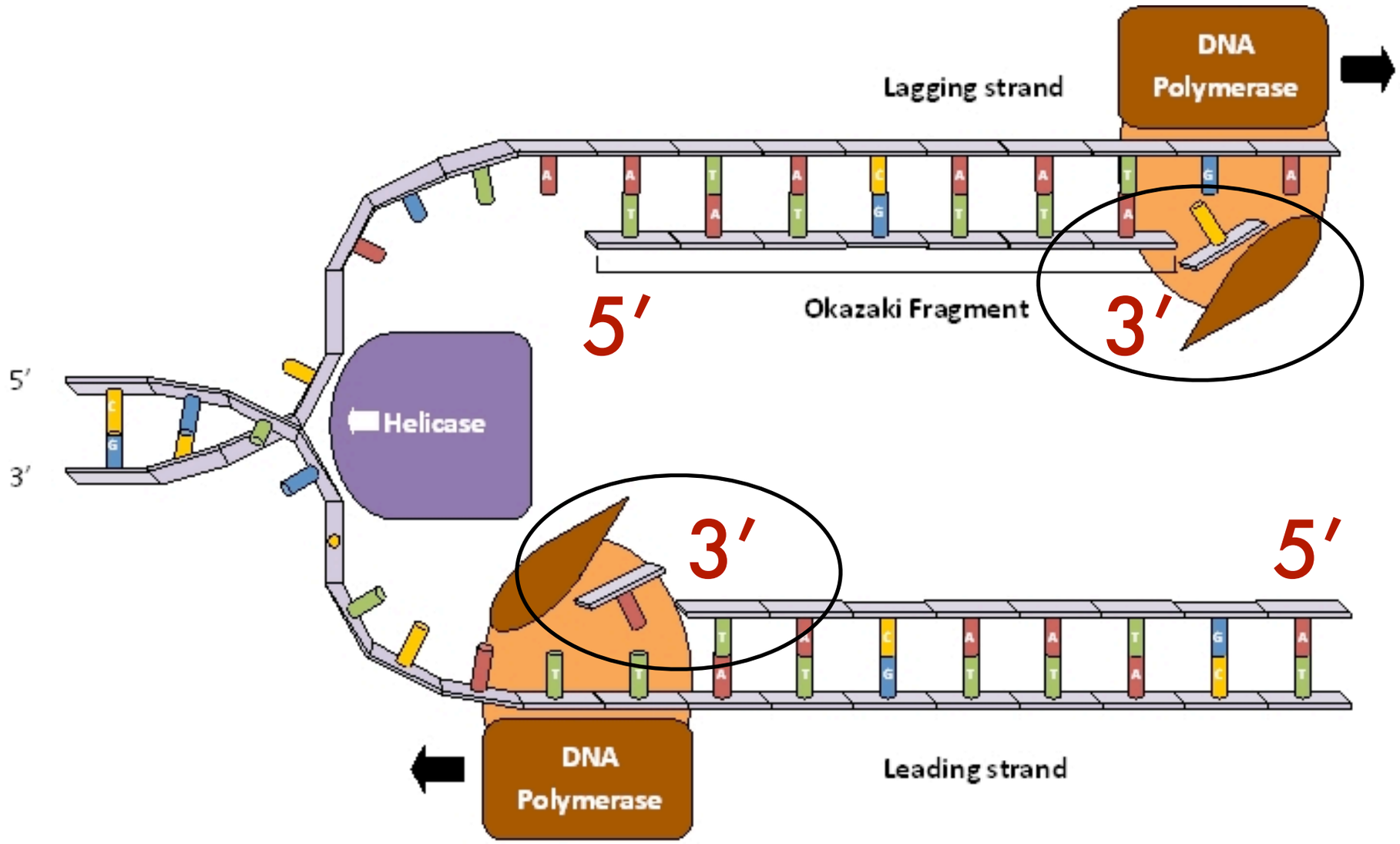
Lagging Strand- *works away from the replication fork*



DNA Replication

Leading Strand- *is produced continuously*

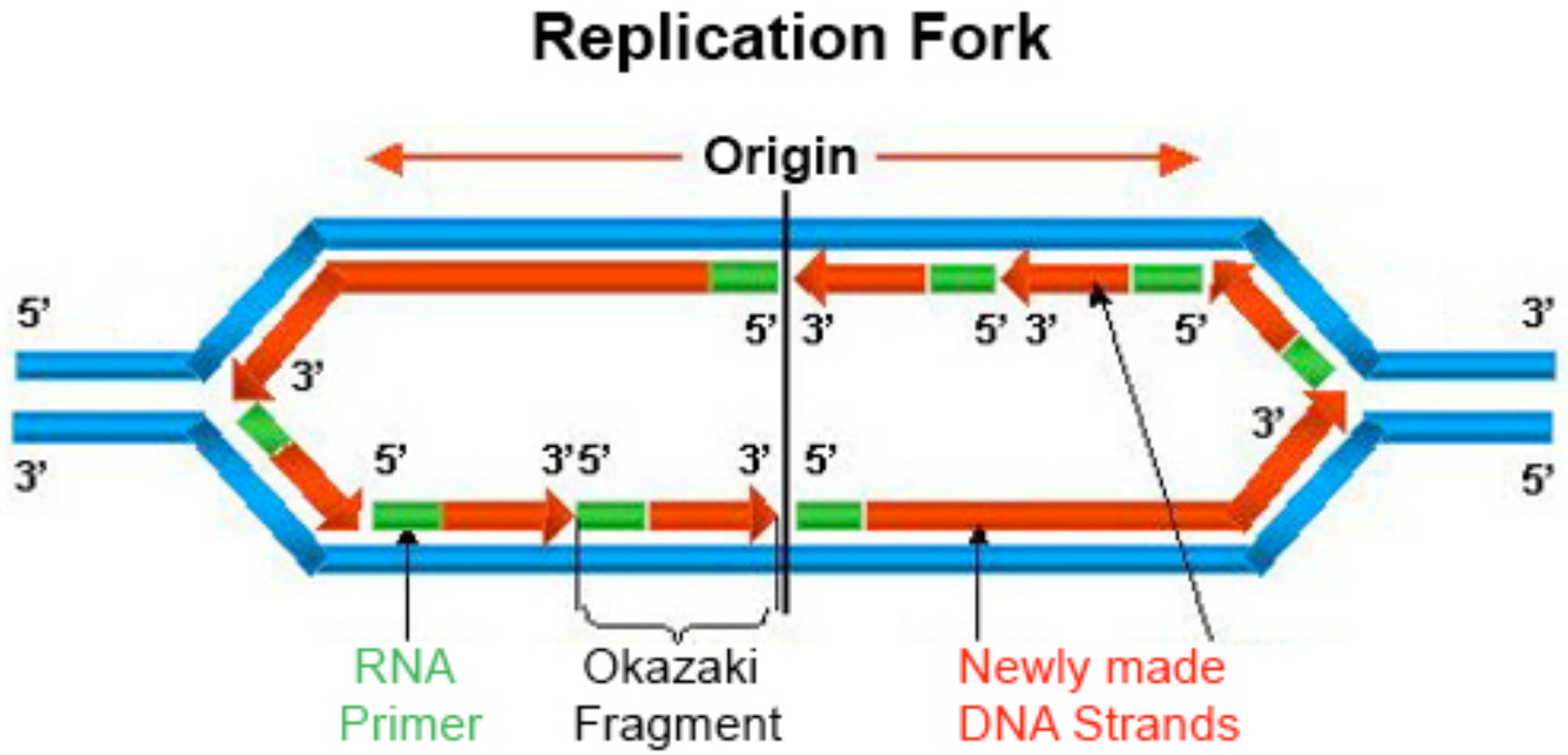
Lagging Strand- *is produced in pieces called Okazaki Fragments*



DNA replication always adds nucleotides to the 3′ end of the growing DNA strand

DNA Replication

DNA Polymerase I- *replaces RNA primers with DNA nucleotides*



Ligase- *forms covalent bonds between Okazaki fragments in the lagging strand*

SYNTHESIS OF LAGGING STRAND

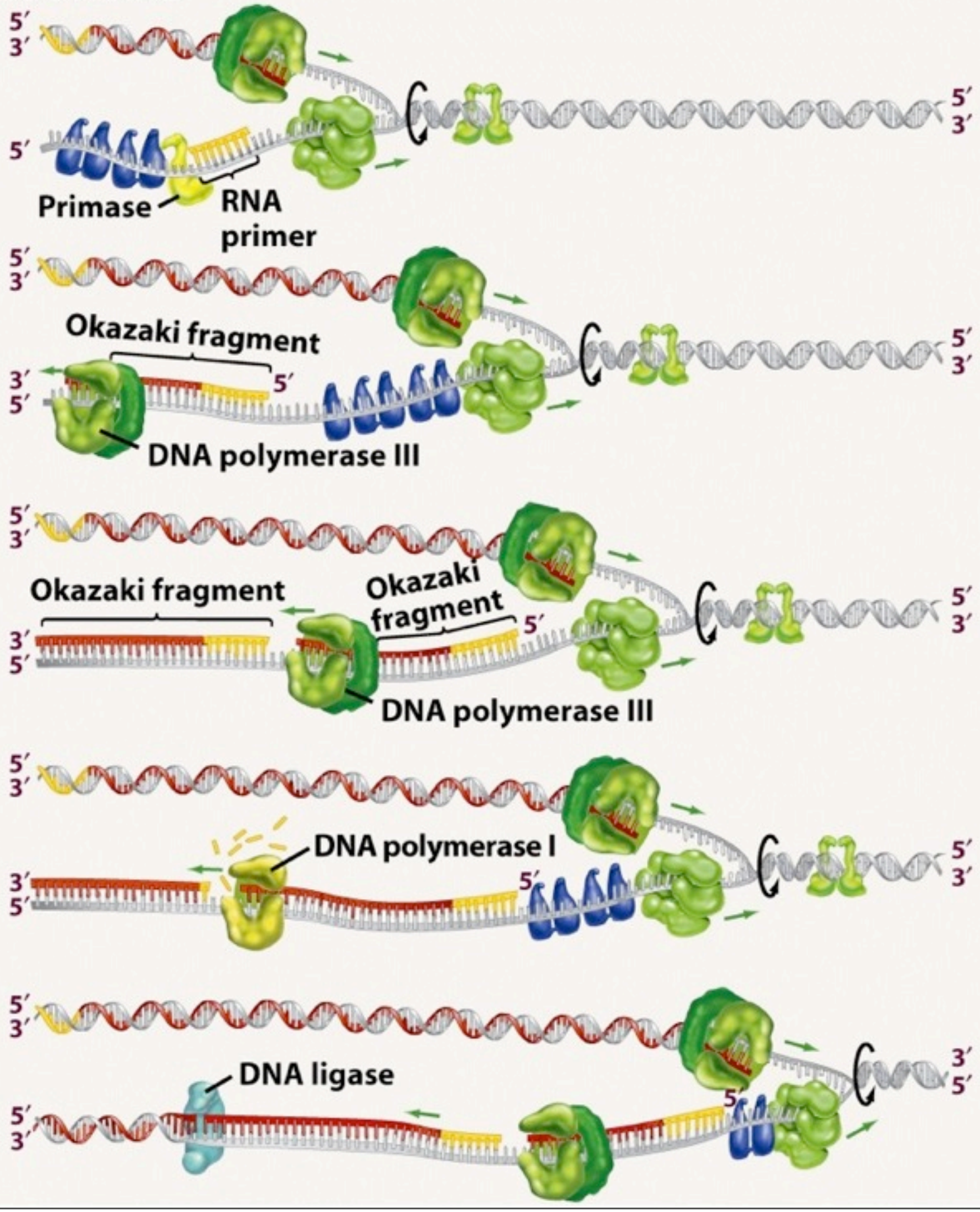
1. Primase synthesizes RNA primer.

2. DNA polymerase III works in 5′→3′ direction, synthesizing lagging strand.

3. DNA polymerase III synthesizes another fragment.

4. DNA polymerase I removes ribonucleotides of primer, replaces them with deoxyribonucleotides in 5′→3′ direction.

5. DNA ligase closes gap in sugar-phosphate backbone.



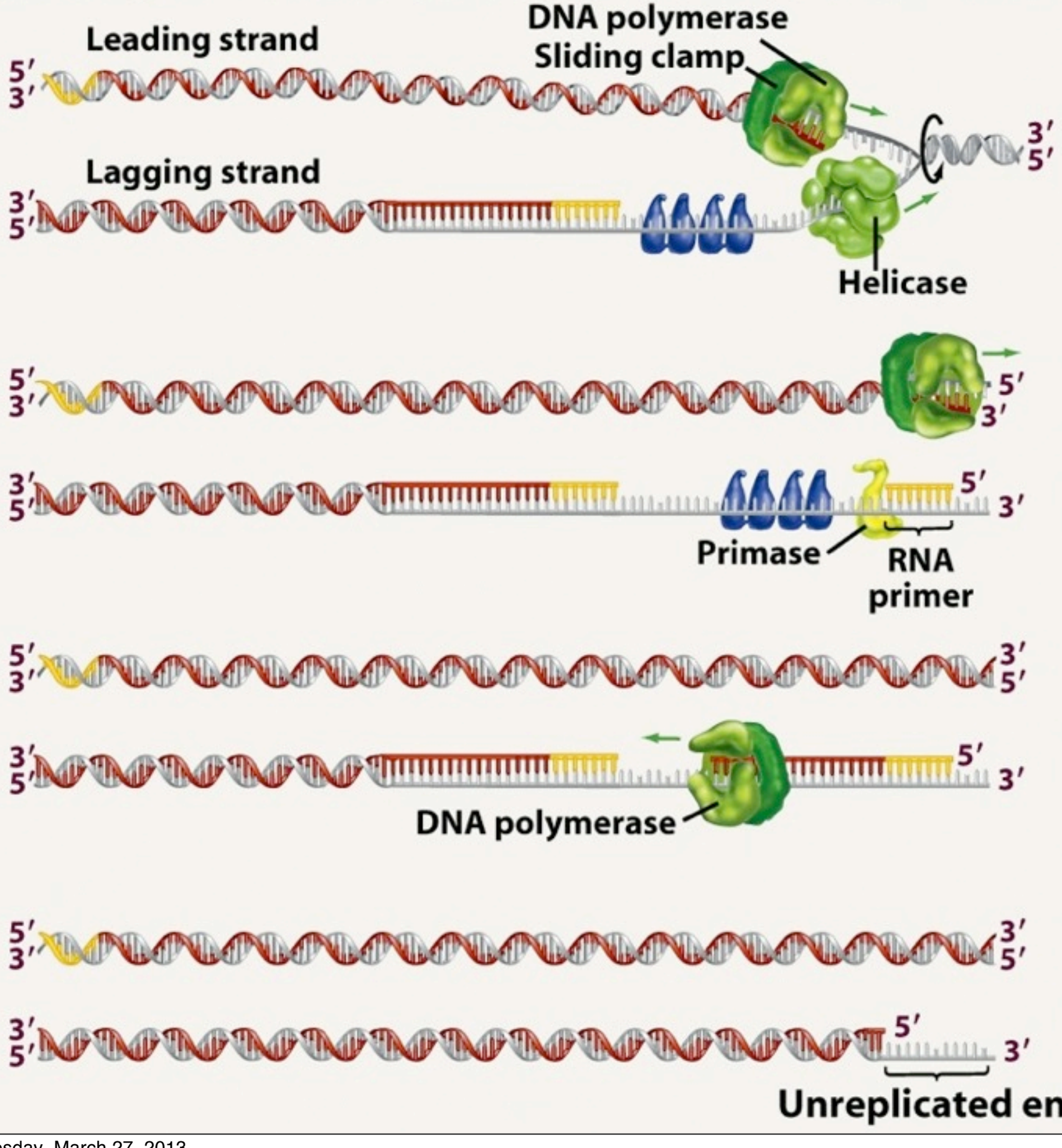
DNA Replication

Another important distinction- *prokaryotes have circular chromosomes, while eukaryotes have linear chromosomes.*

Replicating the ends of linear eukaryotic DNA presents its own unique set of problems.

The next slide illustrates the problem more clearly.

CHROMOSOME SHORTENING DURING NORMAL DNA REPLICATION



1. Helicase unwinds end of DNA helix (at end of chromosome).

2. DNA polymerase completes the leading strand. Primase synthesizes RNA primer at end of lagging strand.

3. DNA polymerase synthesizes the last Okazaki fragment in lagging strand.

4. No DNA synthesis occurs after primer is removed (no free 3′ end for DNA polymerase); chromosome is shortened.

As you can see one strand of DNA remains unfinished and the over hanging end will be removed, which completes replication.

This will however result in the DNA becoming shorter over time and eventually “eating away” important sequences that code for polypeptides.

Eukaryotes avoid this problem because the ends of their DNA has has about 6 nucleotides repeated hundreds of times, this creates long noncoding sequences called **telomeres** at the ends of linear chromosomes.

Of course, the length of the telomere will limit the number of times a molecule of DNA can be replicated.

DNA Replication

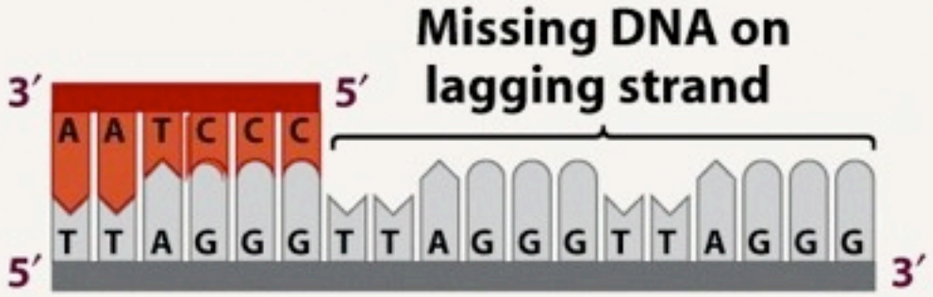
But what about germ cells, whose genome must persist virtually unchanged over the generations?

Think about it...If germ cells DNA got shorter with each replication the gametes would eventually lose all their DNA.

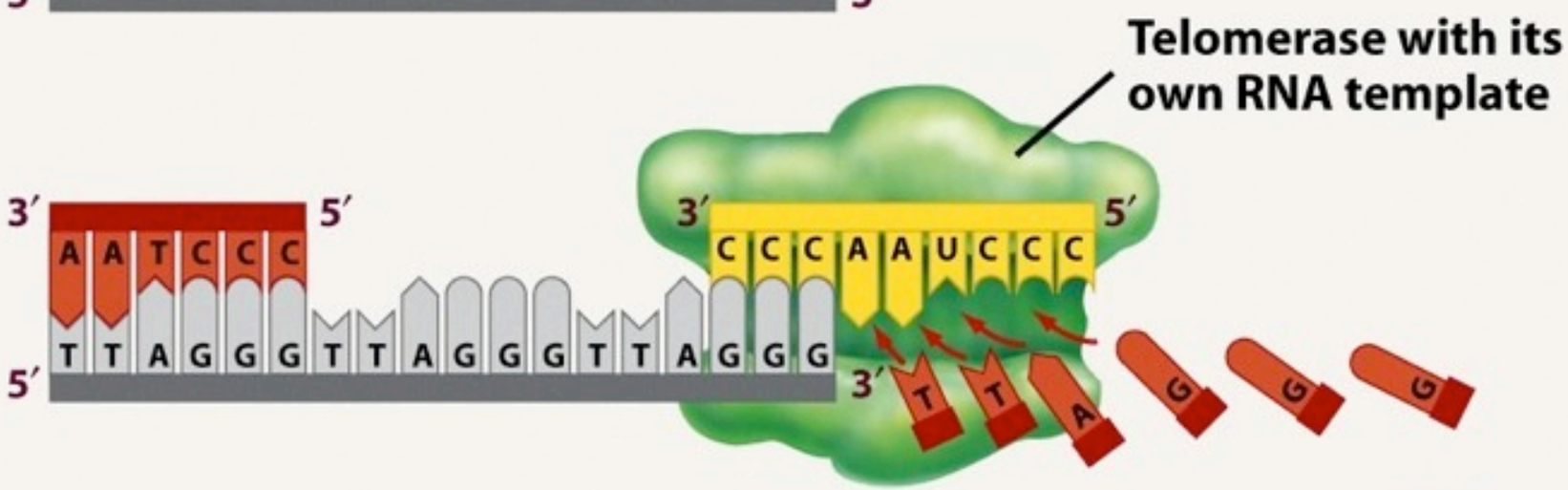
Germ cells have an enzyme called telomerase, that rebuilds the telomeres so that the length of the DNA remains constant over time.

Telomerase is not active in most somatic cells, in fact this may play a protective role in preventing cells that become cancerous from replicating indefinitely.

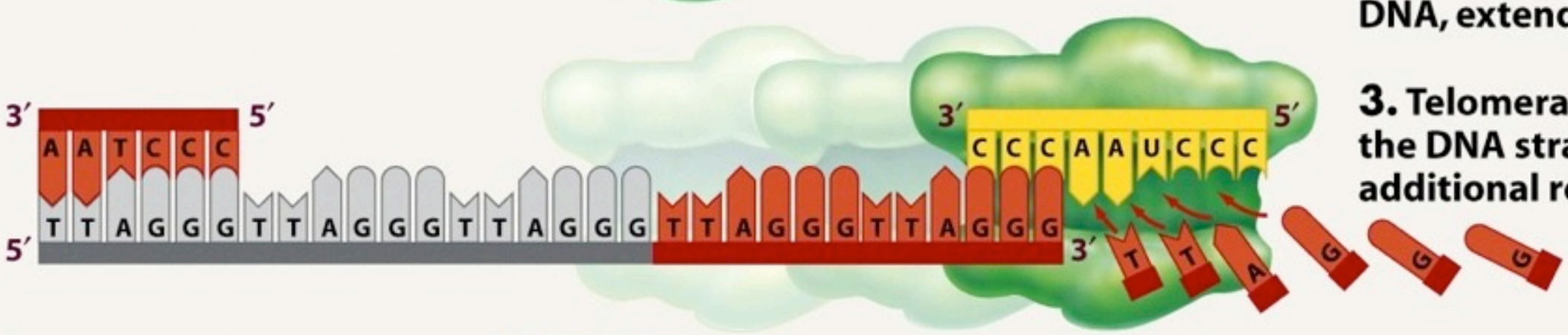
TELOMERE REPLICATION



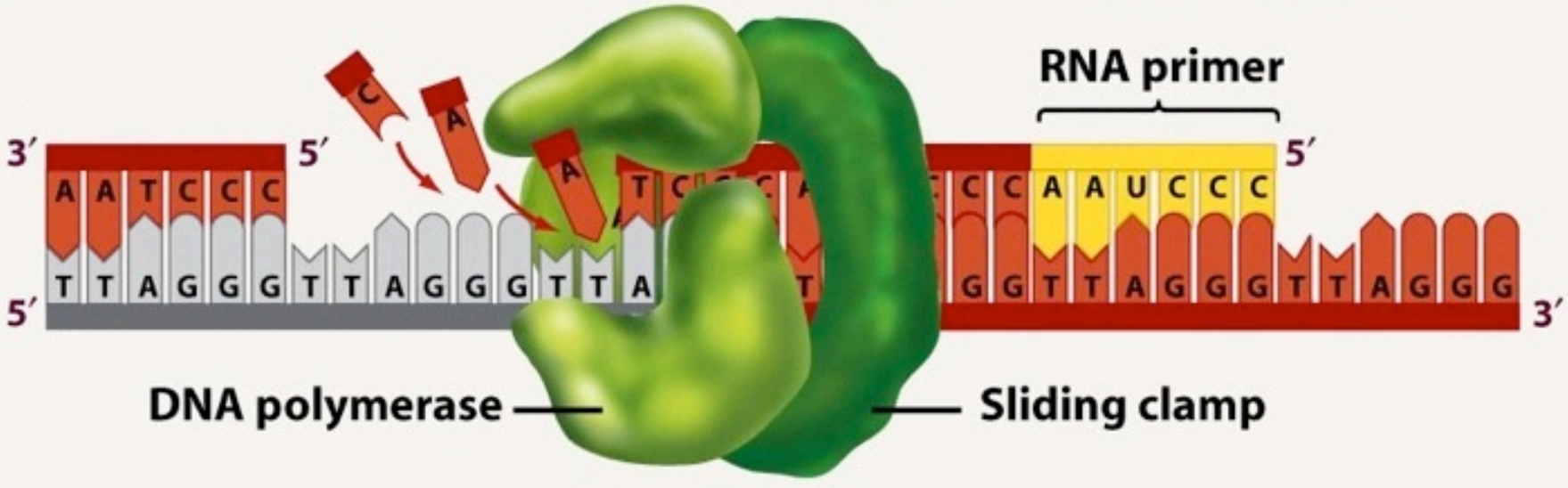
1. When the RNA primer is removed from the 5′ end of the lagging strand (see Figure 14.14), a strand of parent DNA remains unreplicated.



2. Telomerase binds to the "overhanging" section of single-stranded DNA. Telomerase adds deoxyribonucleotides to the end of the parent DNA, extending it.



3. Telomerase moves down the DNA strand and adds additional repeats.



4. Primase, DNA polymerase, and ligase then synthesize the lagging strand in the 5′→3′ direction, restoring the original length of the chromosome.

DNA Replication: Enzymes

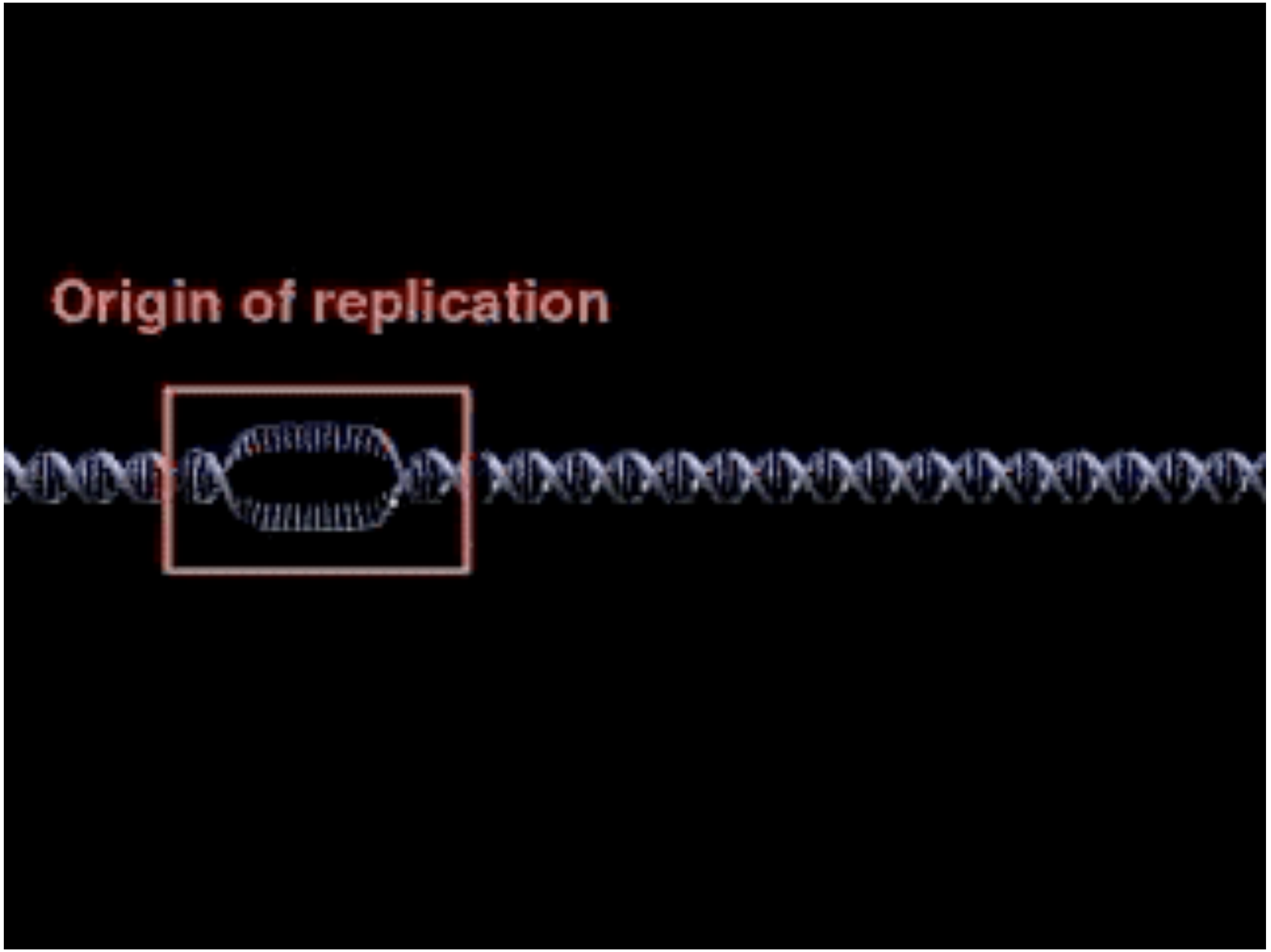
Bacterial DNA replication proteins and their functions

Protein	Function for Leading and Lagging Strands
Helicase	Unwinds parental double helix at replication forks
Single-strand binding protein	Binds to and stabilizes single-stranded DNA until it can be used as a template
Topoisomerase	Corrects "overwinding" ahead of replication forks by breaking, swiveling, and rejoining DNA strands

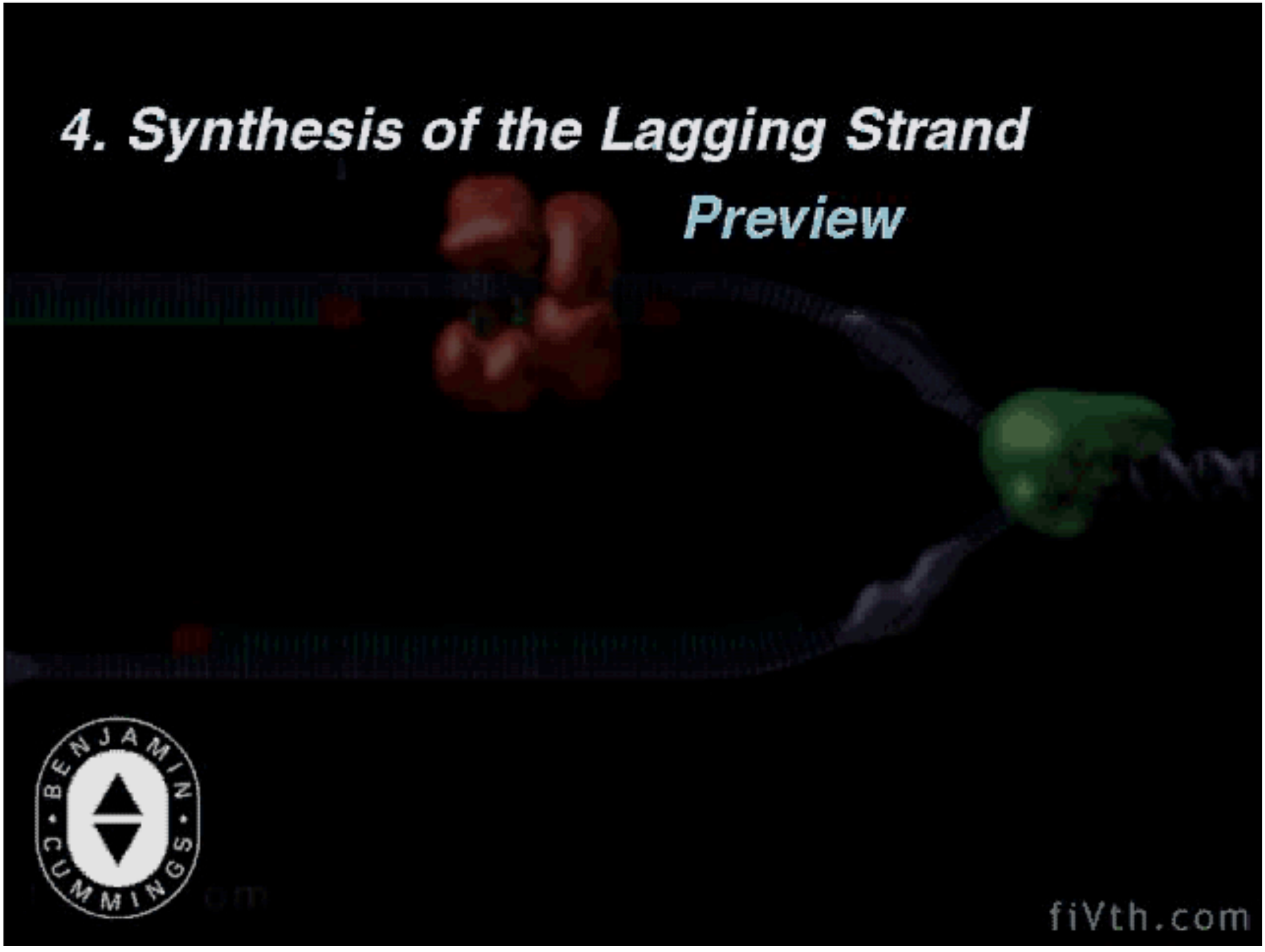
	Function for Leading Strand	Function for Lagging Strand
Primase	Synthesizes a single RNA primer at the 5′ end of the leading strand	Synthesizes an RNA primer at the 5′ end of each Okazaki fragment
DNA pol III	Continuously synthesizes the leading strand, adding on to the primer	Elongates each Okazaki fragment, adding on to its primer
DNA pol I	Removes primer from the 5′ end of leading strand and replaces it with DNA, adding on to the adjacent 3′ end	Removes the primer from the 5′ end of each fragment and replaces it with DNA, adding on to the 3′ end of the adjacent fragment
DNA Ligase	Joins the 3′ end of the DNA that replaces the primer to the rest of the leading strand	Joins the Okazaki fragments

Another important distinction- Eukaryotes have a least 11 different DNA polymerases involved in replication

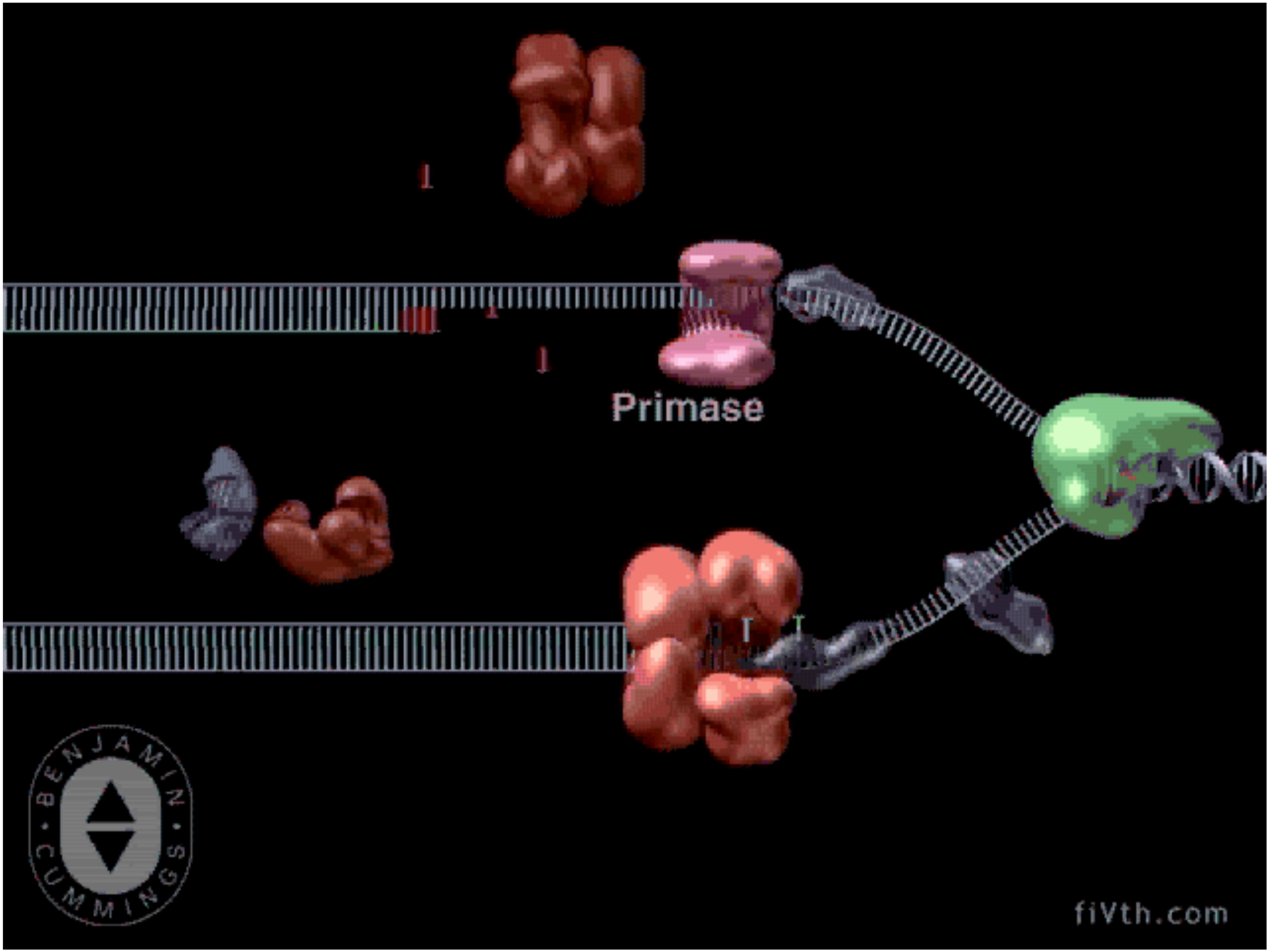
DNA Replication: Leading Strand



DNA Replication: Lagging Strand



DNA Replication: Overall



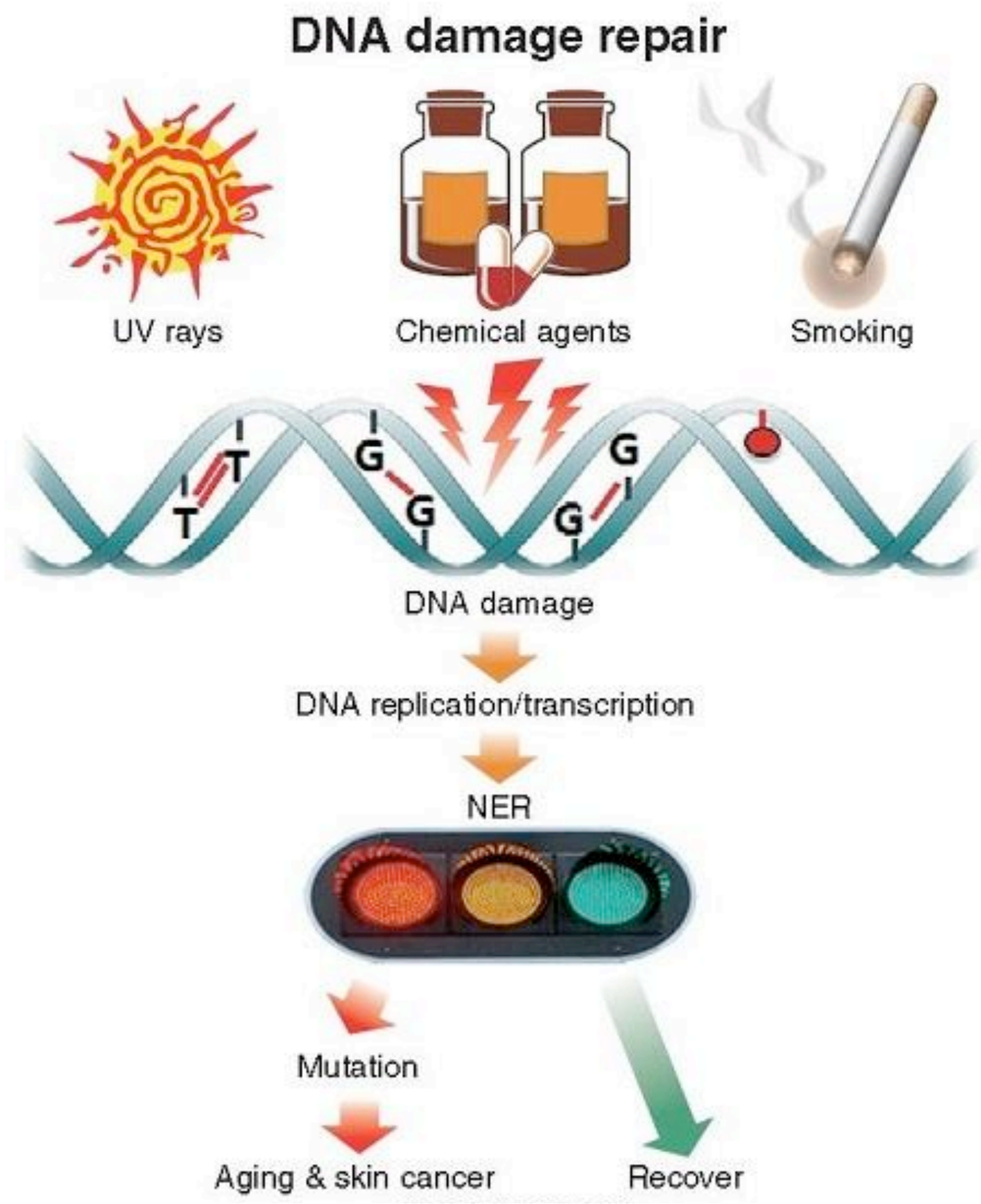
Proofreading & Repairing DNA

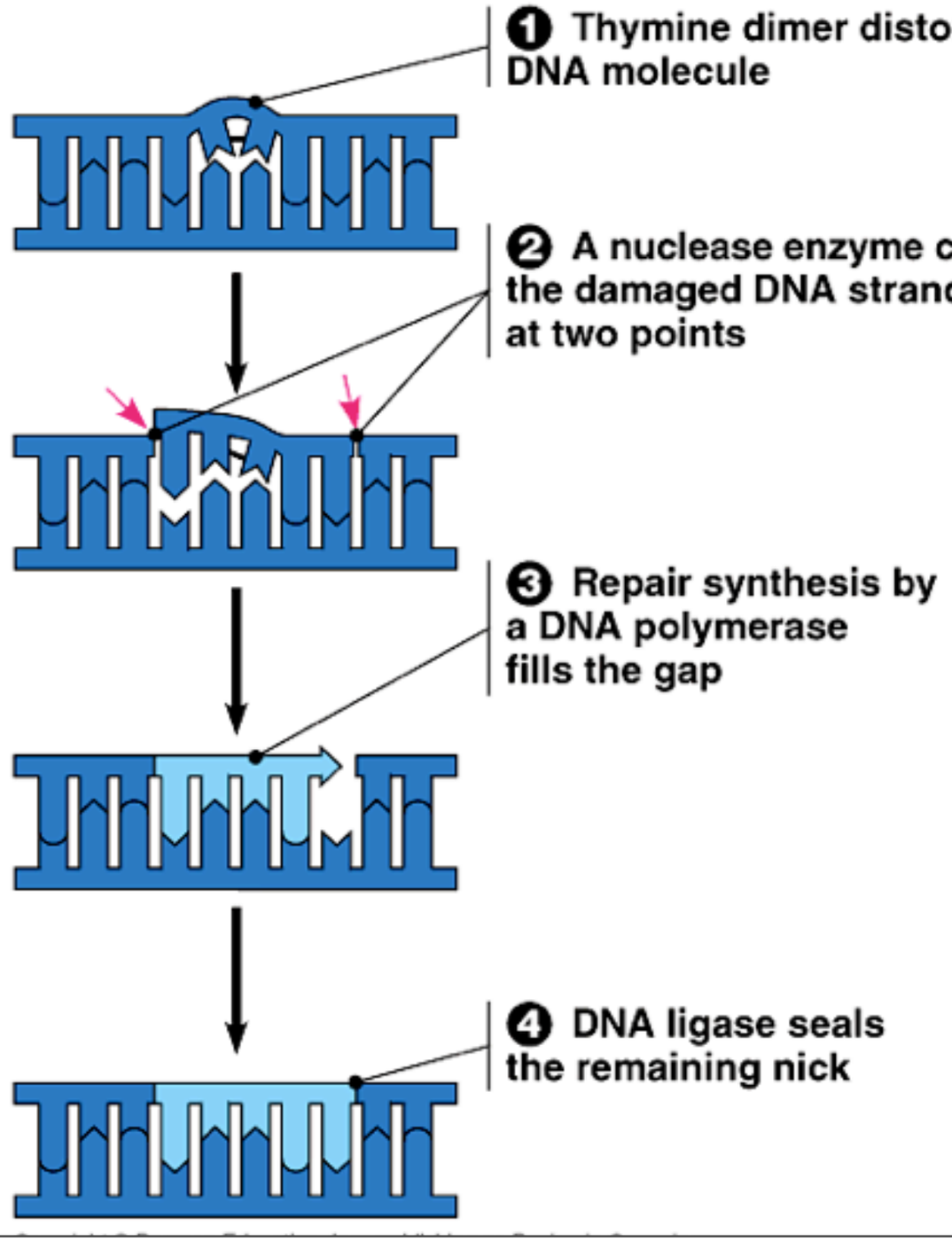
- DNA replication is extremely accurate, but because of the base pairing rules alone.
- The rate of base pairing errors is ~ 1 in 10,000
- Additional DNA polymerases and other proofreading enzymes search out and repair most of these mismatches resulting in a final error rate of ~ 1 in 10 billion!
- As you might expect errors in the genes that produce these proofreading enzymes might have serious consequences.
- *For example lack of the necessary proofreading enzymes in colon cells have been implicated in a particular type of colon cancer .*

Proofreading & Repairing DNA

- Alterations and errors in DNA can also after replication.
- **Mutagens**, chemical or physical agents such as cigarette smoke or x-rays increase the rate of DNA alterations
- Over 130 repair enzymes that correct these mutations have been identified in humans.
  - *Unfortunately these enzymes are not infallible, as result some of these mutations may develop into cancer. Mutagens that increase the rate of cancer are called* ***carcinogens****.*

Proofreading & Repairing DNA



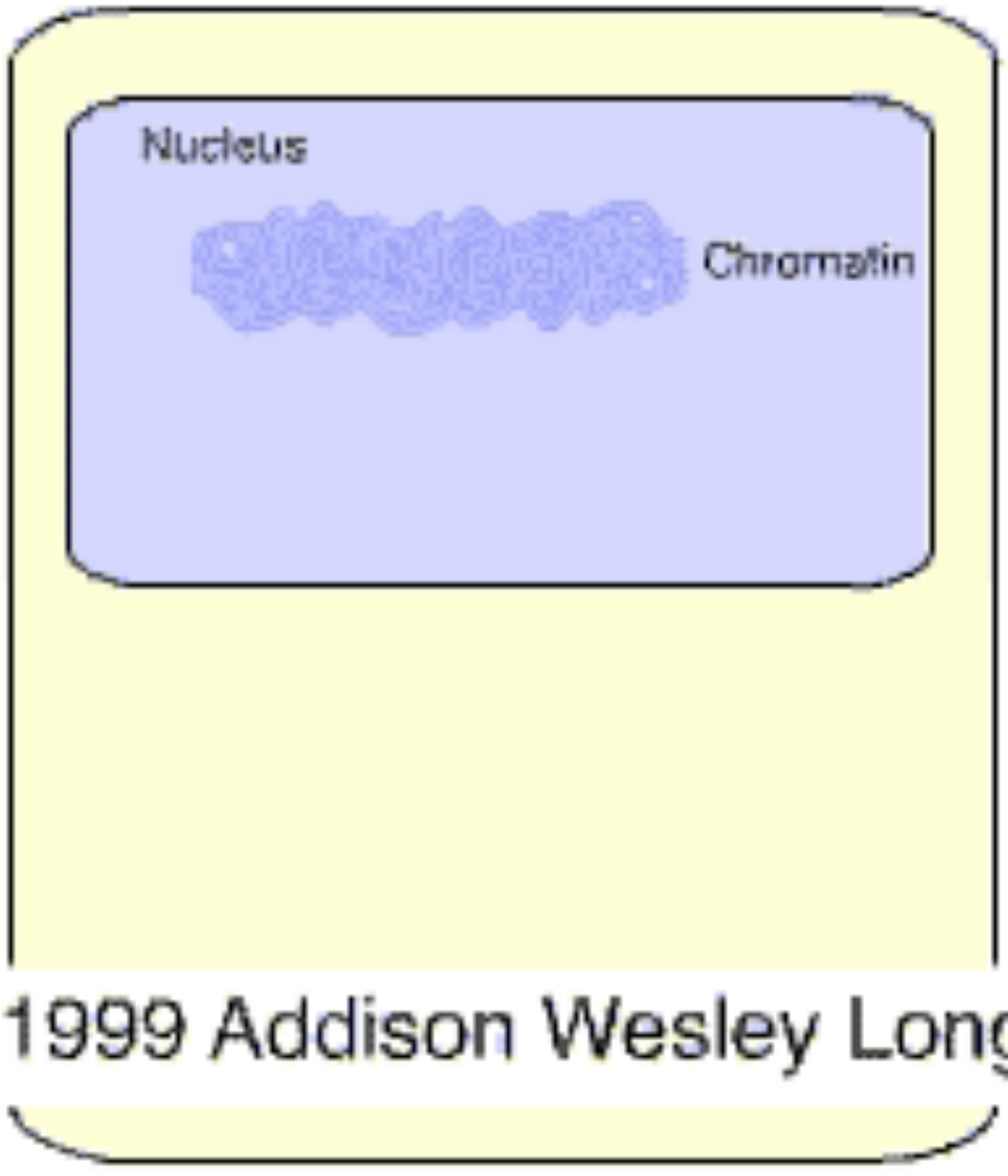


Molecular Basis of Inheritance

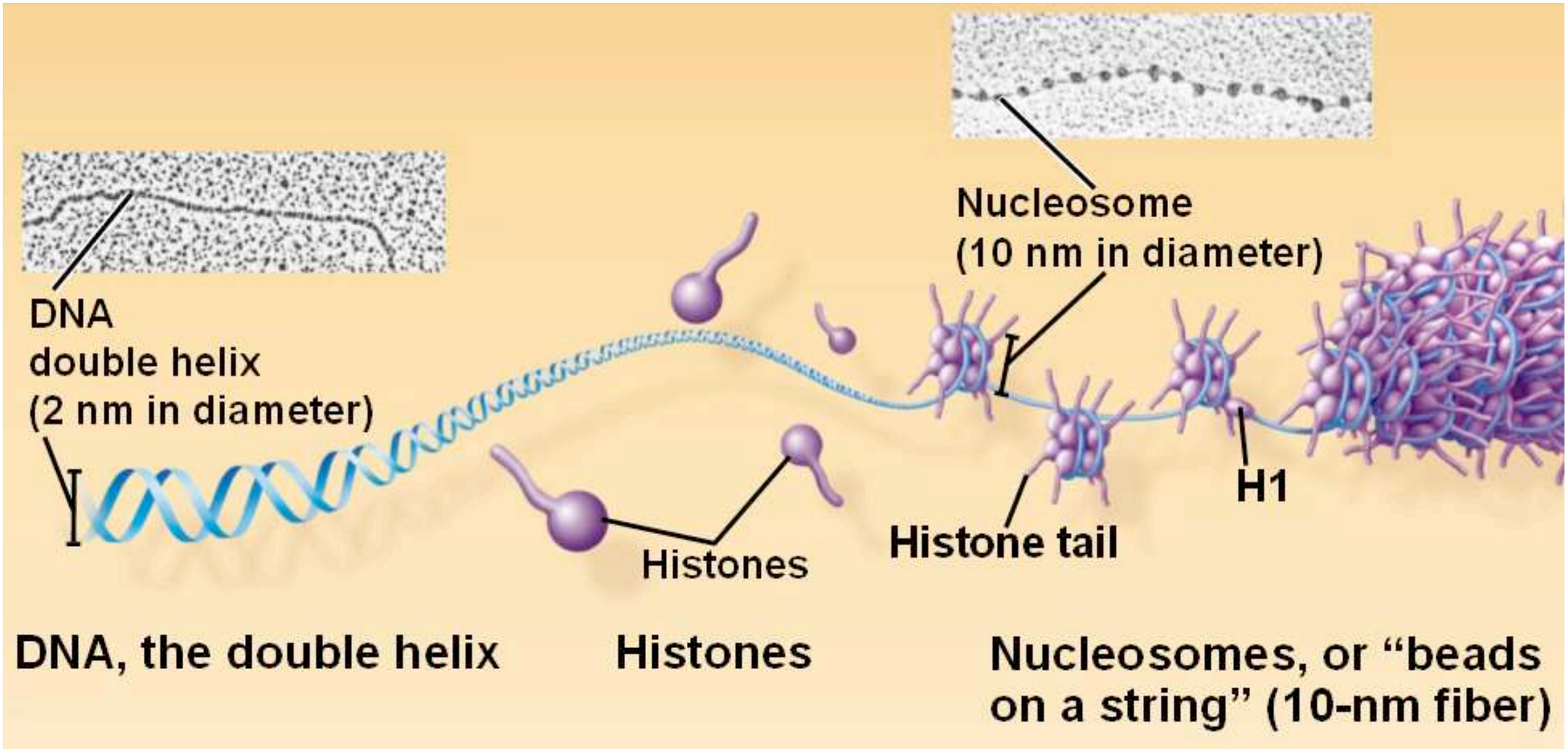
III.

Main Idea: DNA is packaged around proteins in cells, in varying forms, some forms more accessible than others.

Chromatin & Chromosomes

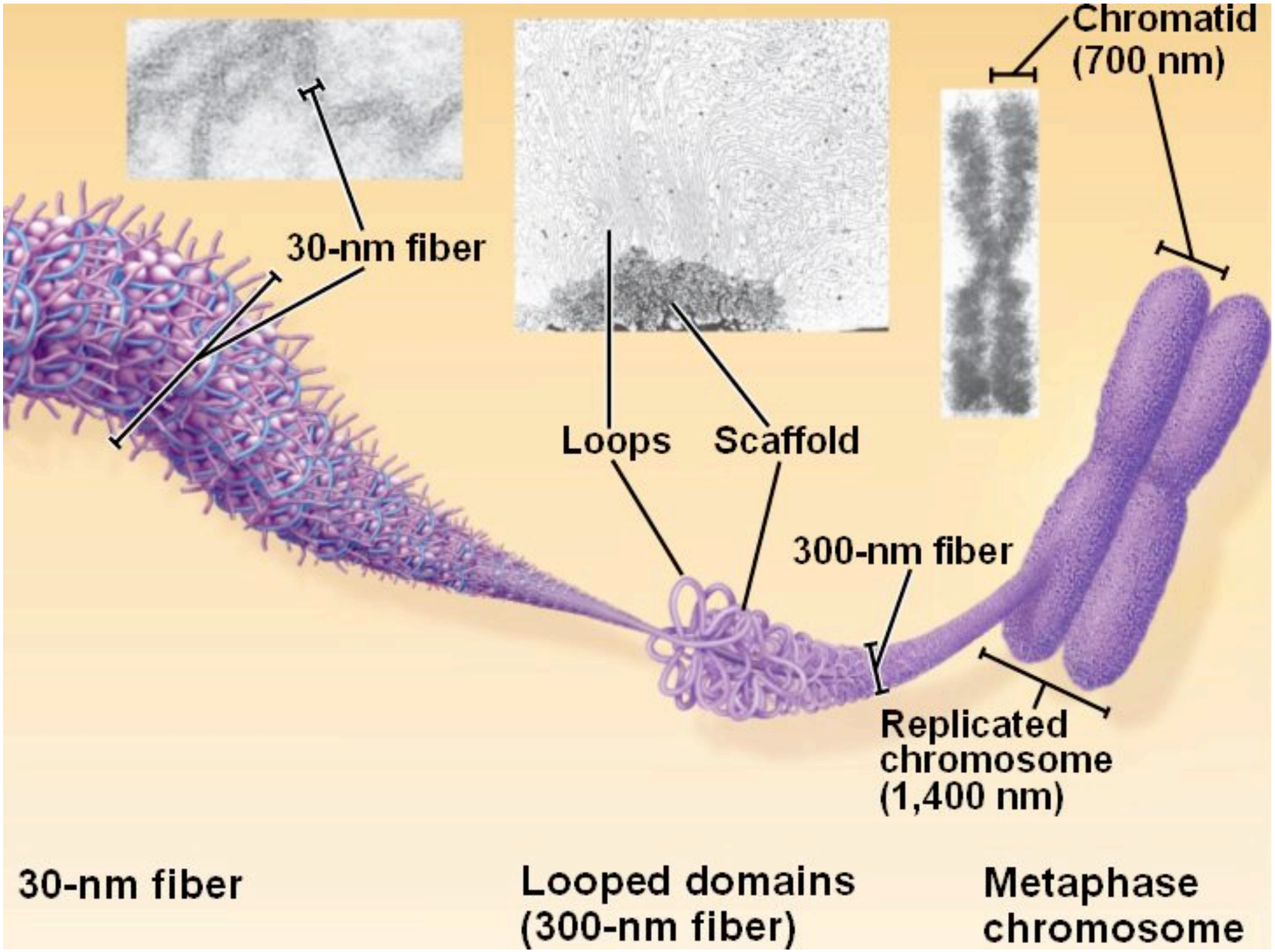


DNA & Chromatin



All the DNA in one cell, when stretched out would be 4cm in length, thousands of times longer than the cells diameter. It fits only because it folded over and over.

Chromatin & Chromosomes



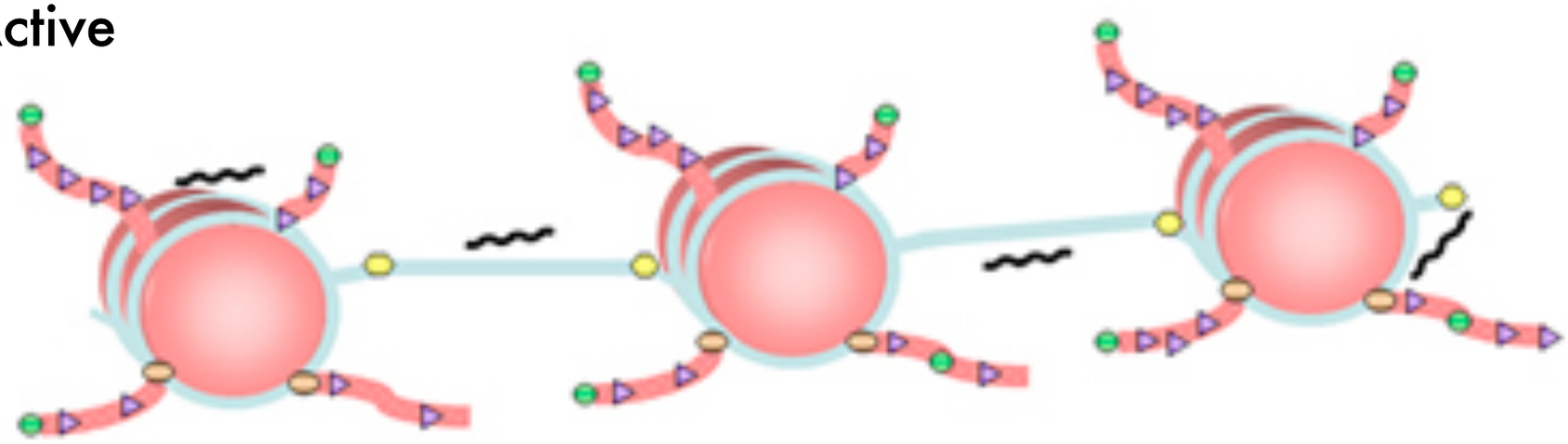
Euchromatin & Heterochromatin

Euchromatin

Gene Rich, Transcriptionally Active

Dispersed Appearance

Unique DNA sequences



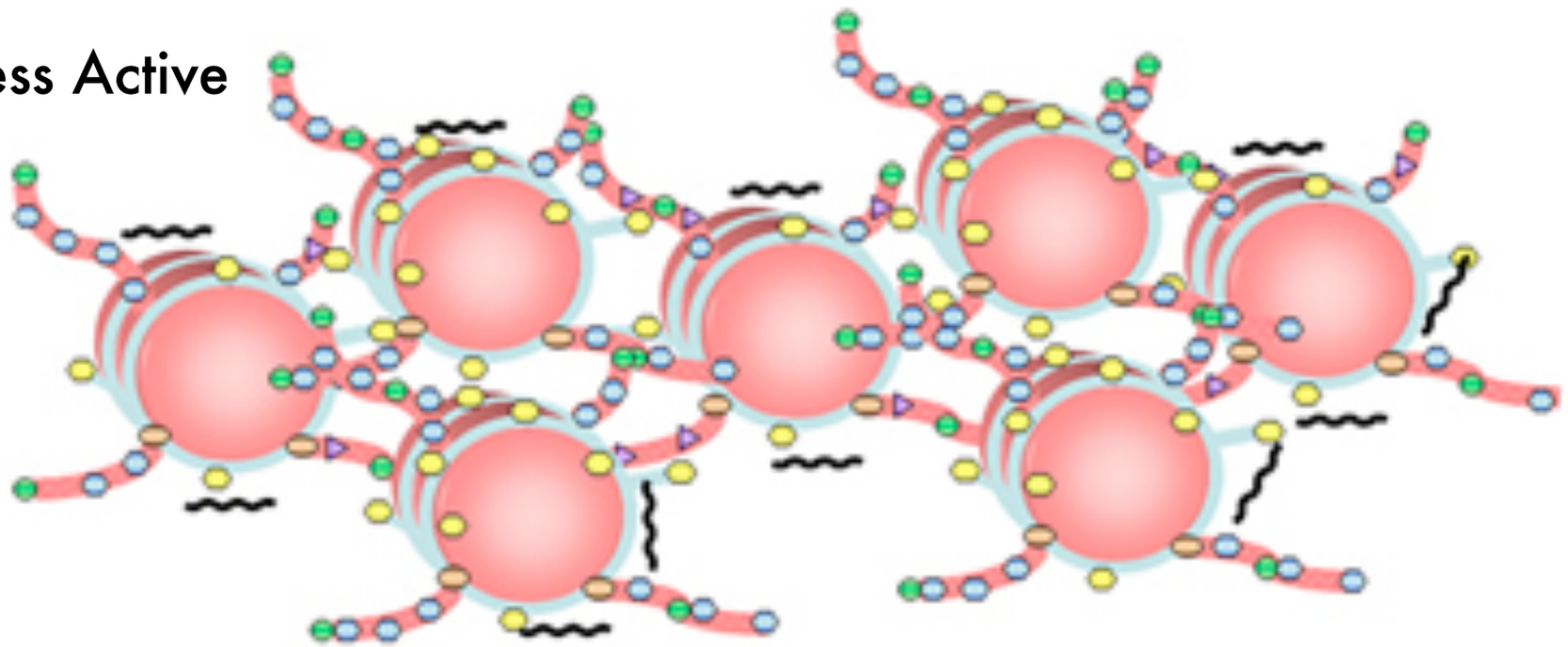
Interphase DNA

Heterochromatin

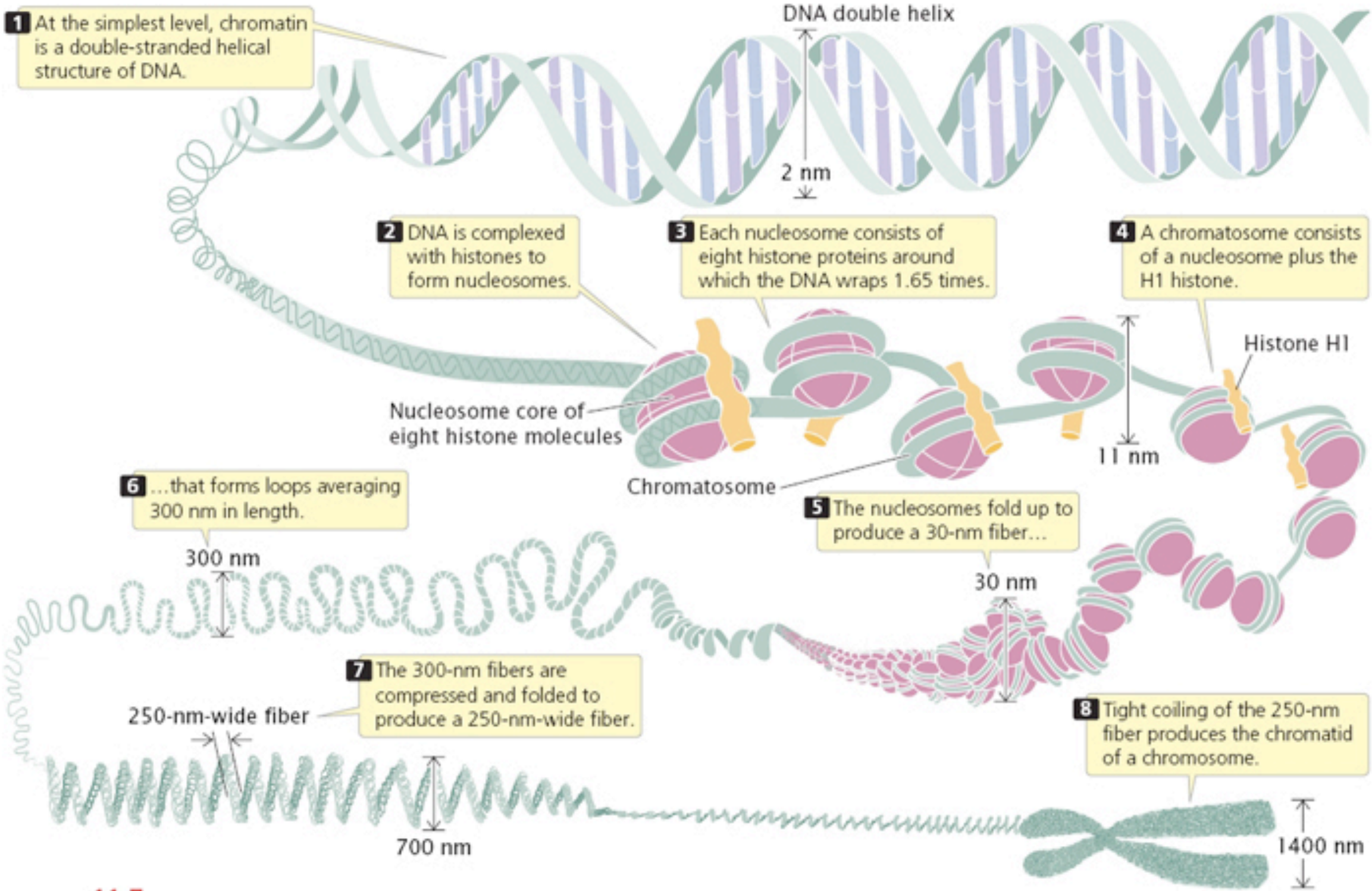
Gene Poor, Transcriptionally Less Active

Condensed Appearance

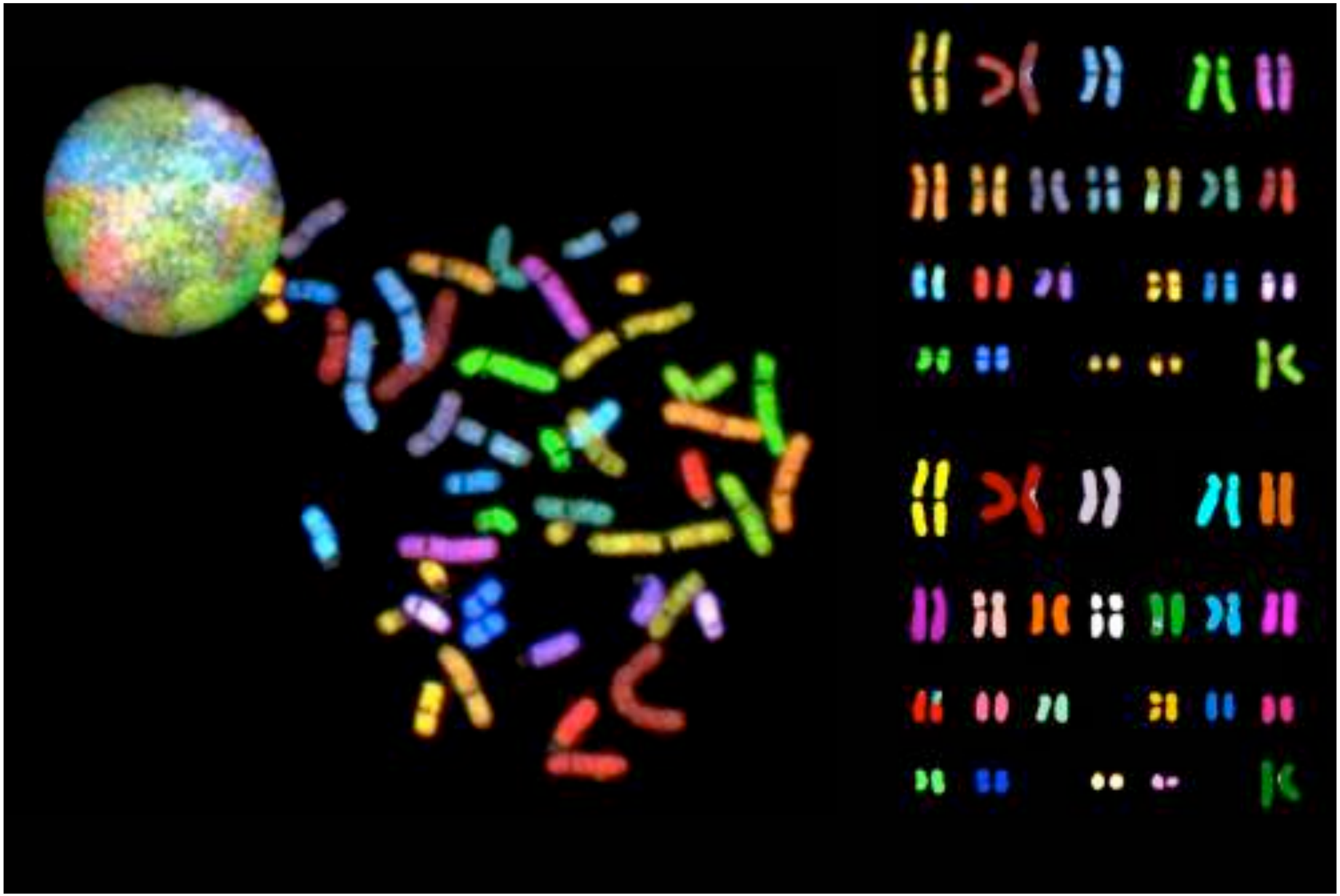
Repetitive DNA sequences



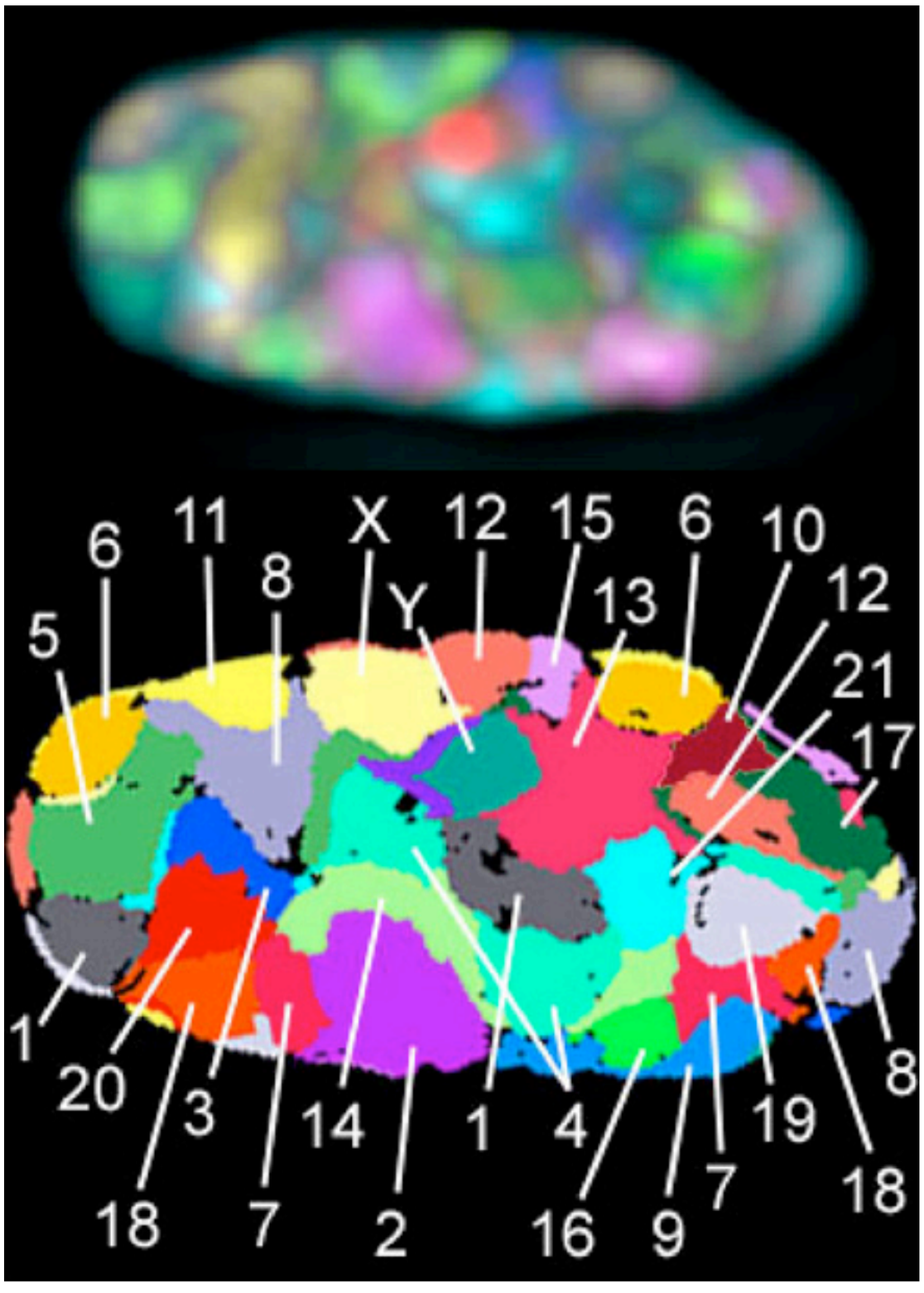
Chromatin & Chromosomes



Painting Chromosomes



Chromatin & Chromosomes



Allows researchers to see how chromosomes are arranged in the interphase nucleus.

- each chromosome appears to occupy a specific space

- homologous chromosome appear to be separate from each other

Molecular Basis of Inheritance

IV.

Main Idea: This section explores the discovery of the relationship between genes and their products .

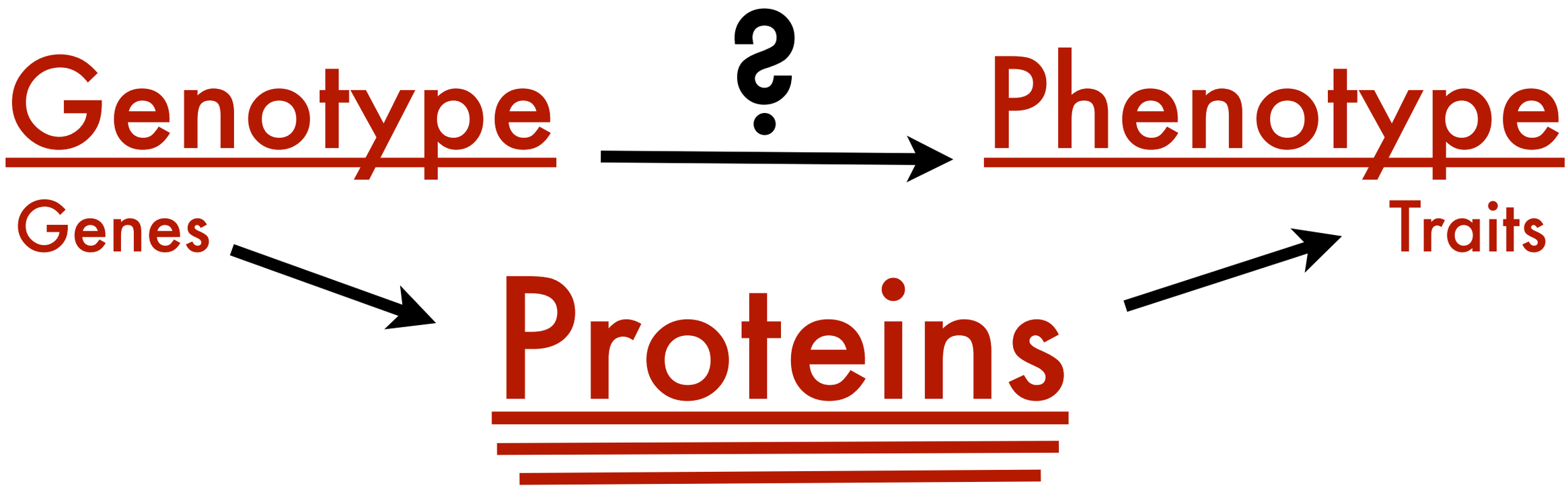
Now Lets Revisit this idea…

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?



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.

Recall Gene Expression…"The Story"

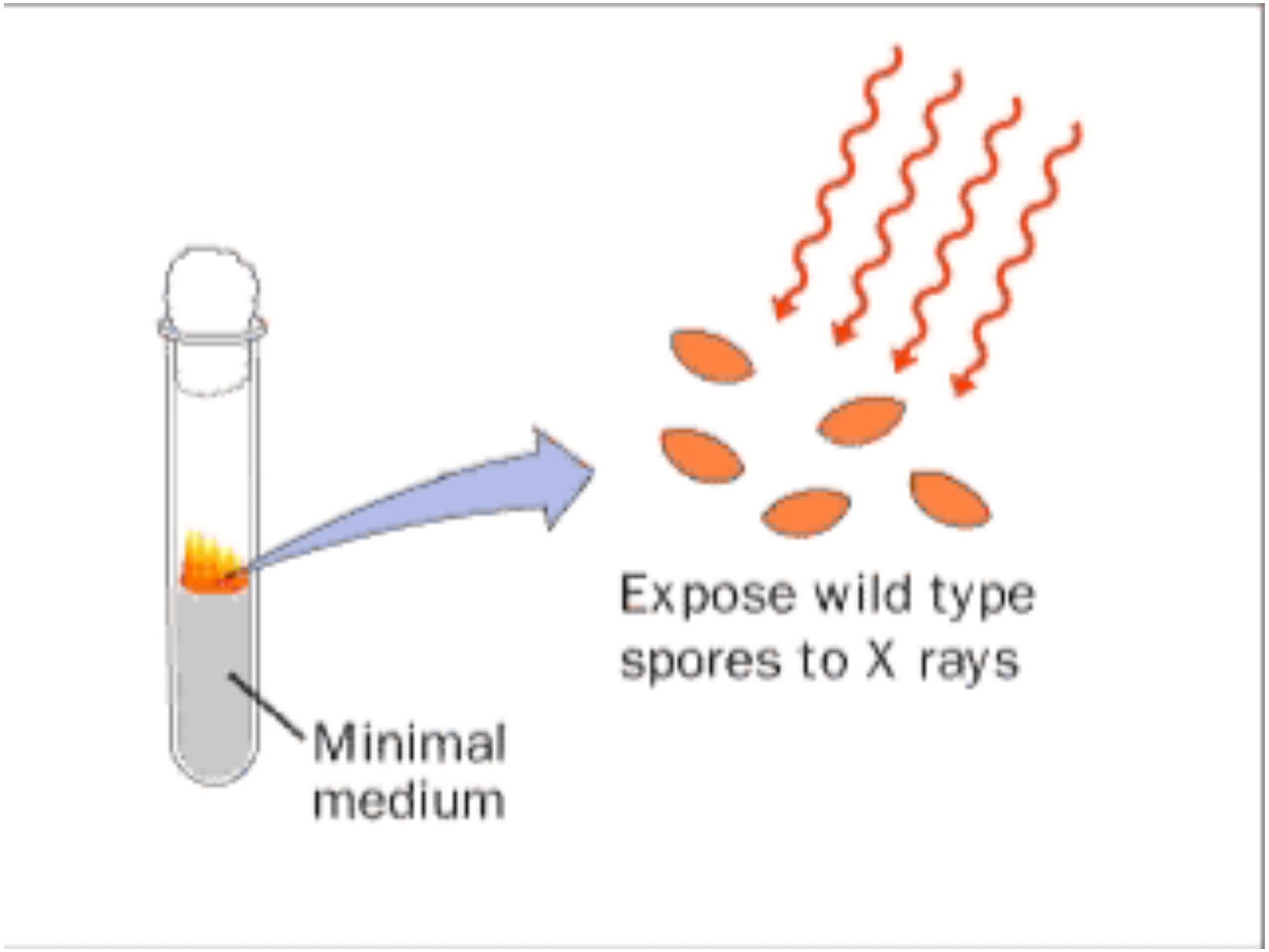
Early 1940's George Beadle & Edward Tatum

- The work of Beadle and Tatum supported the claims made decades earlier by Archibold 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

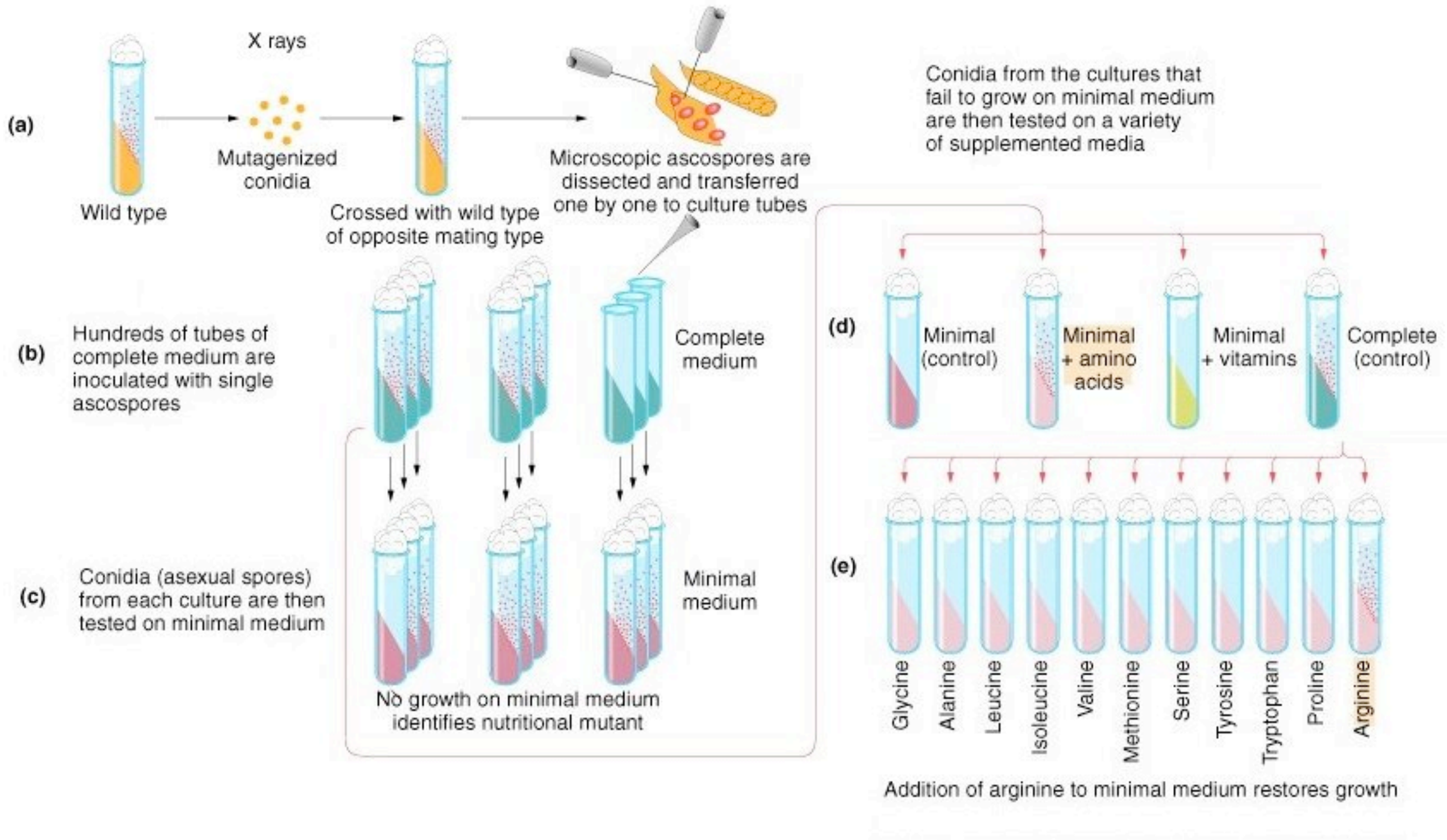
Beadle & Tatum's Experiment is described on the next slide

Recall Gene Expression…"The Story"

Early 1940's George Beadle & Edward Tatum



George Beadle & Edward Tatum Experiment



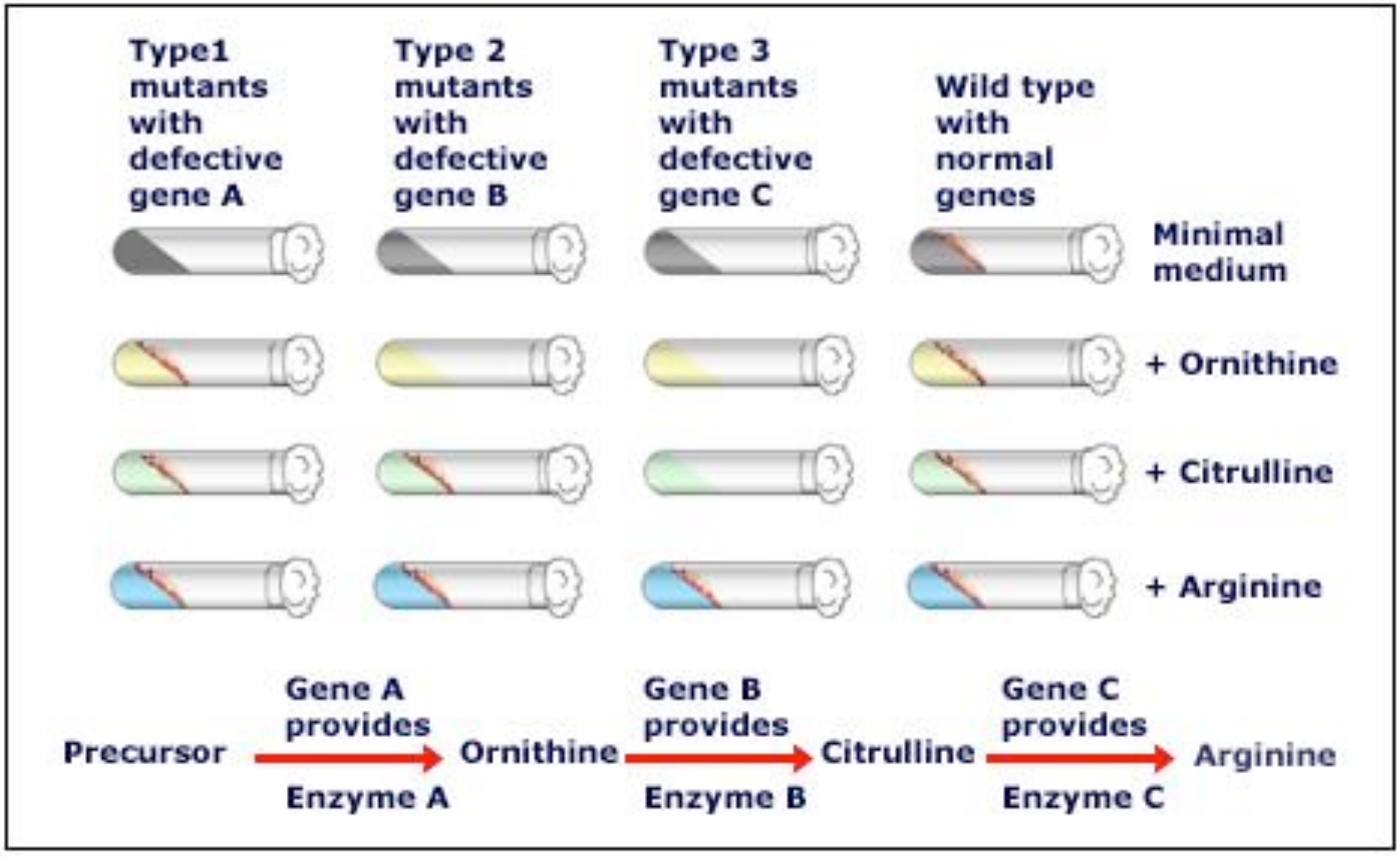
Recall Gene Expression…"The Story"

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



Molecular Basis of Inheritance

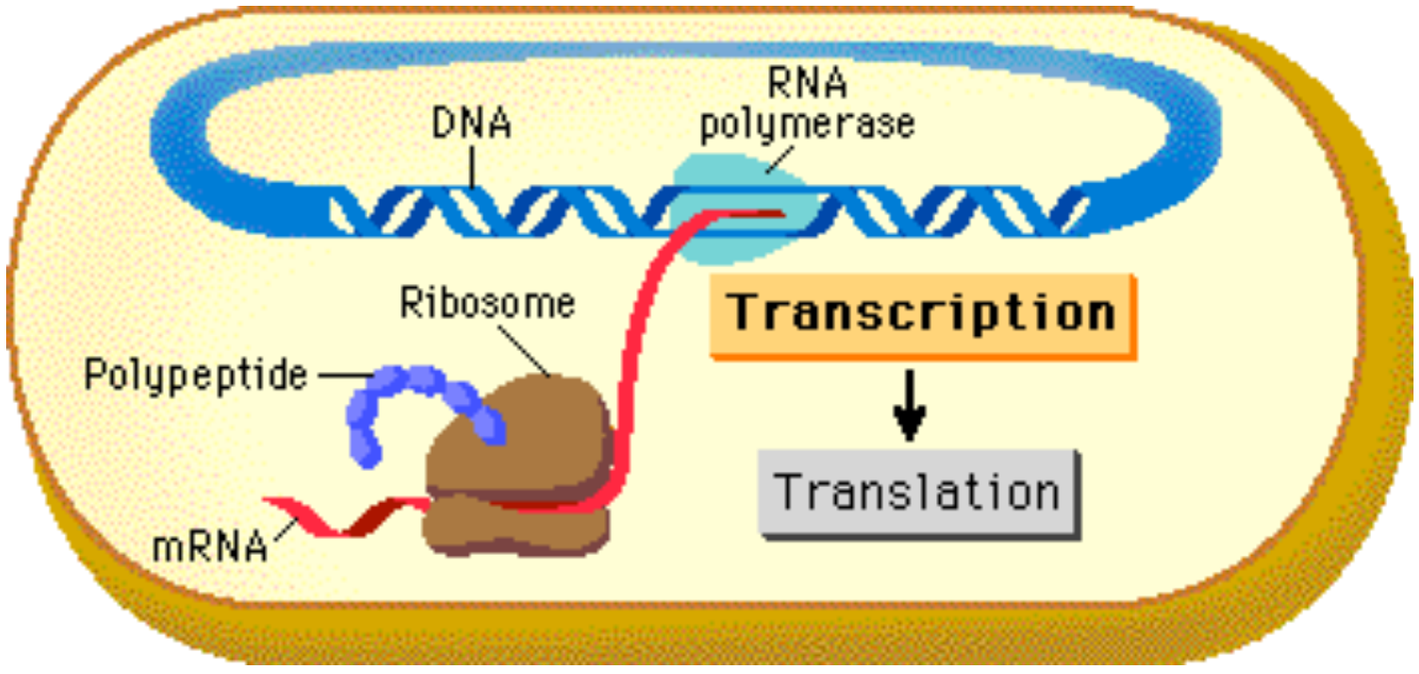
V.

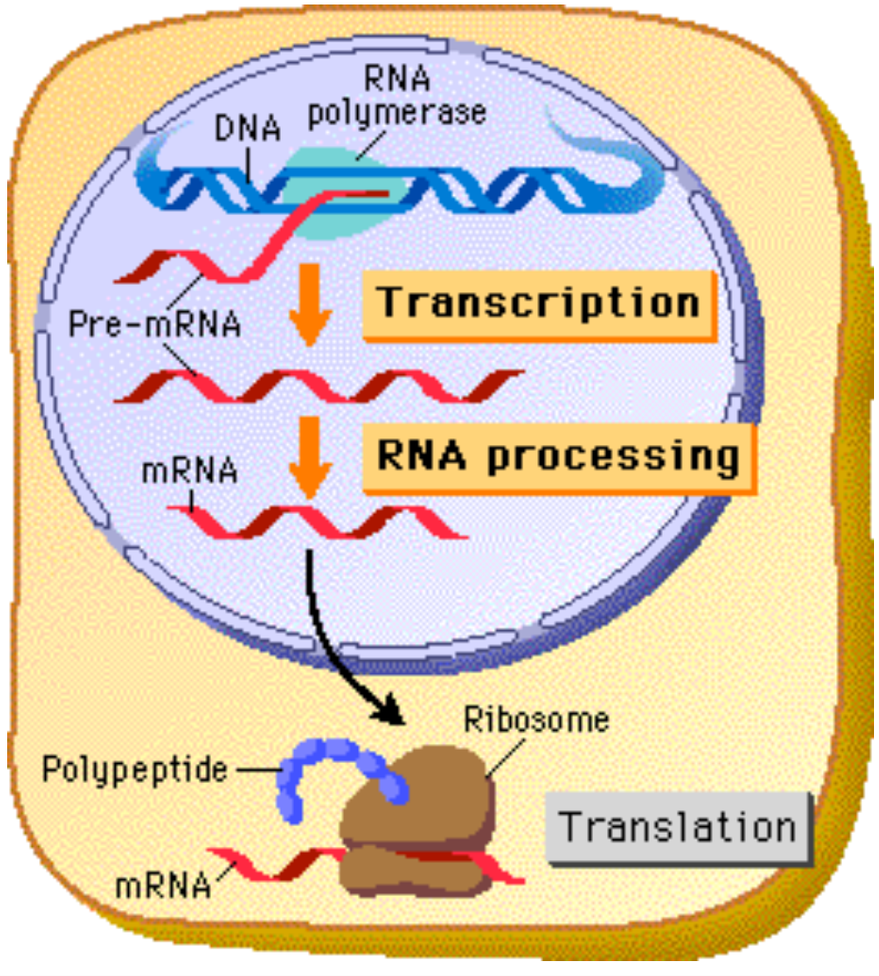
Main Idea: Understanding the basic principles of protein synthesis and an examination of the genetic code.

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***





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

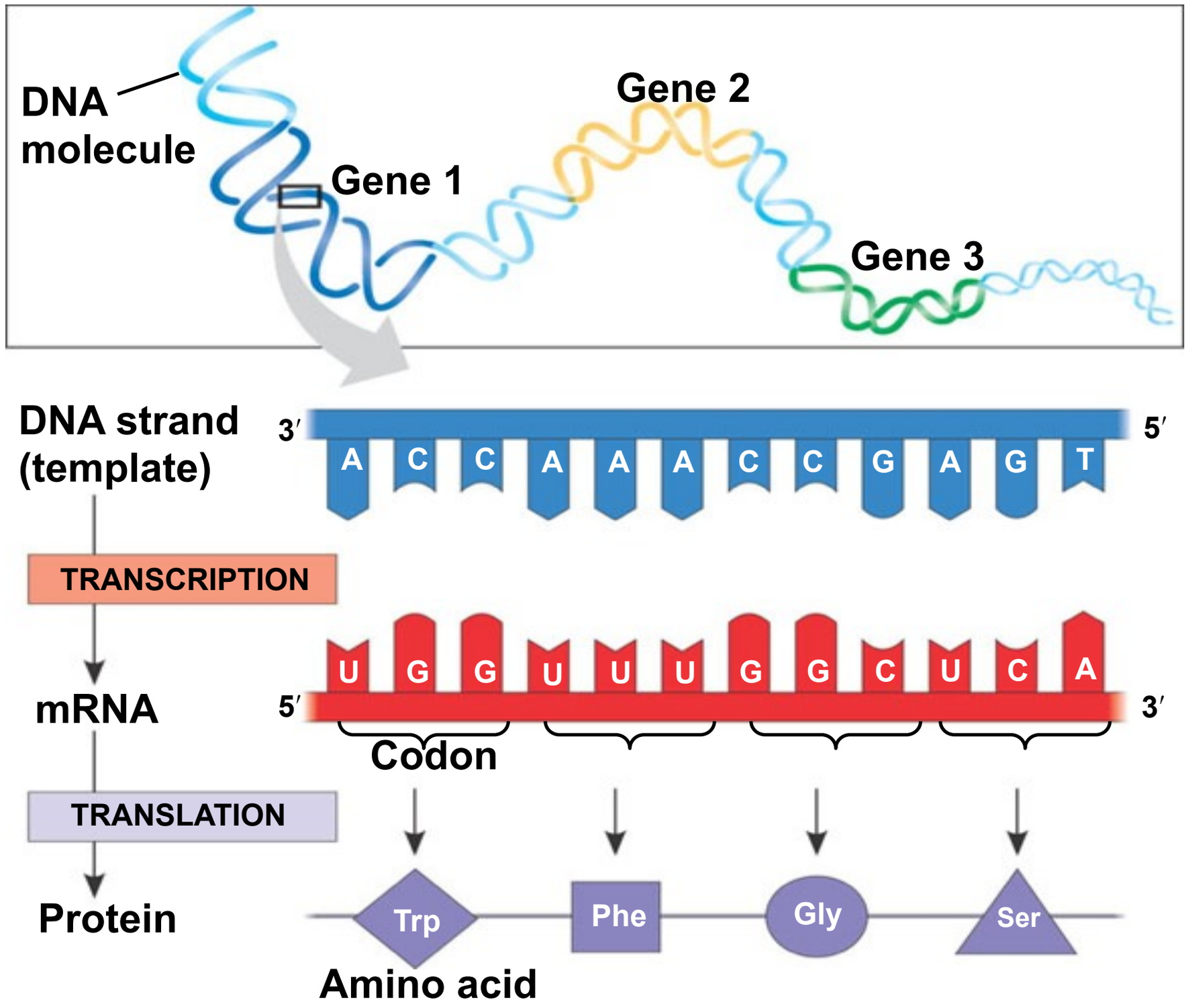
Recall The Genetic Code

- We know that the language of life (nucleic acids) is written in a ***triplet code***.

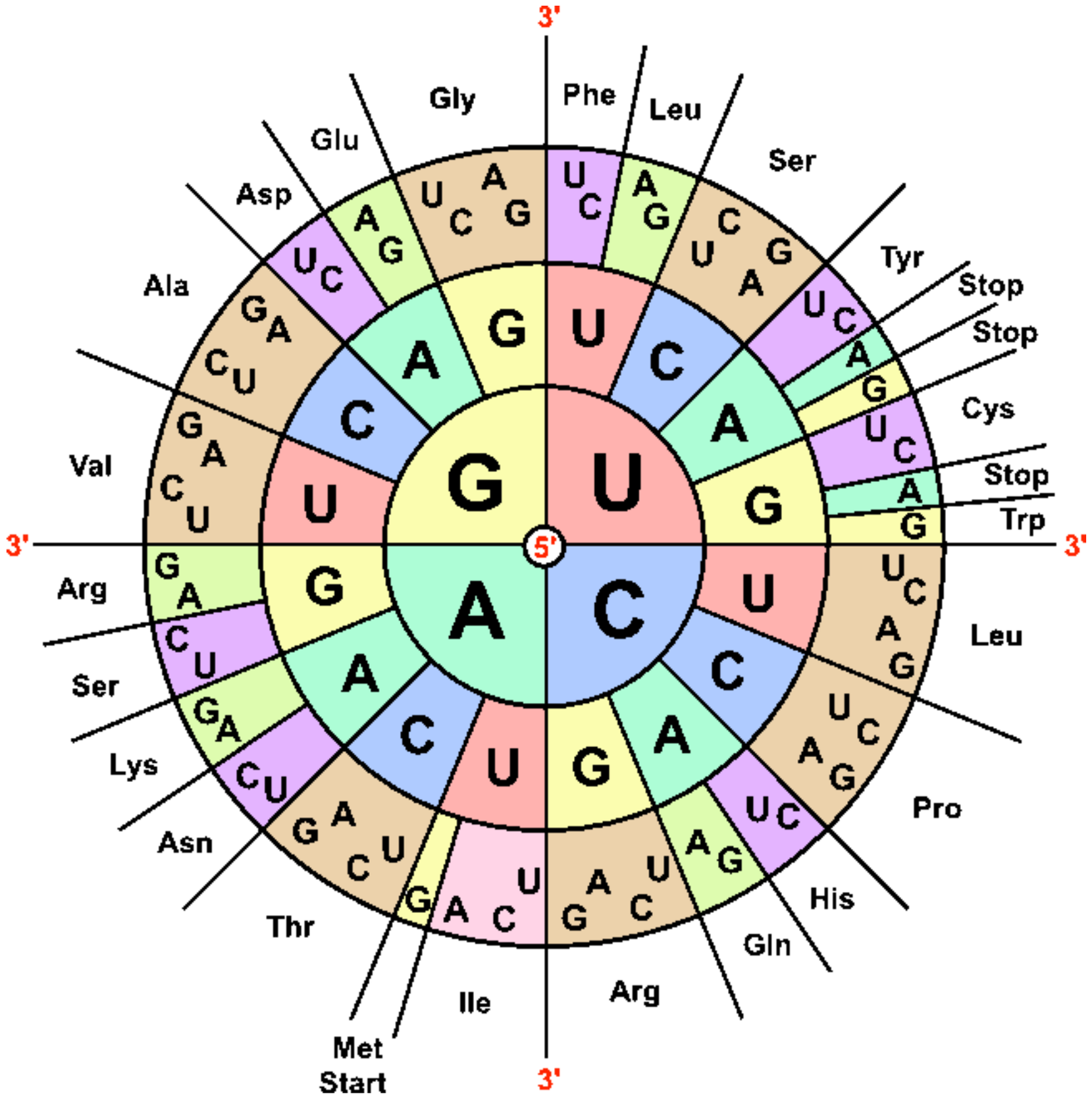
- ***DNA uses three non-overlapping nucleotides to code for three non-overlapping nucleotides (codons) of mRNA which in turn codes for a single amino acid.***

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

Recall The Genetic Code



Another Amino Acid Look Up Table



Recall 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!

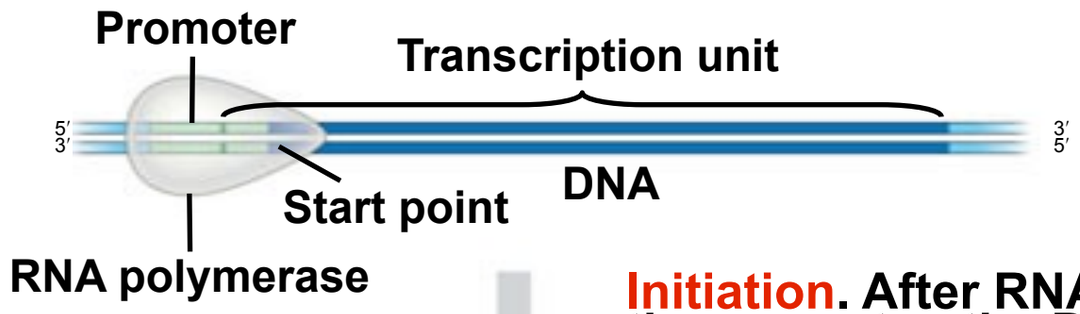
*A shared genetic code supports the idea common ancestry among all living organisms

Molecular Basis of Inheritance

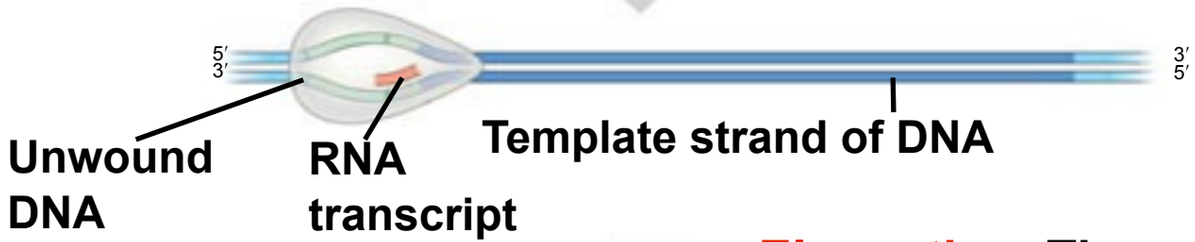
V.

Main Idea: To understand the details of protein synthesis, and to illustrate similarities and differences between prokaryotic and eukaryotic gene expression.

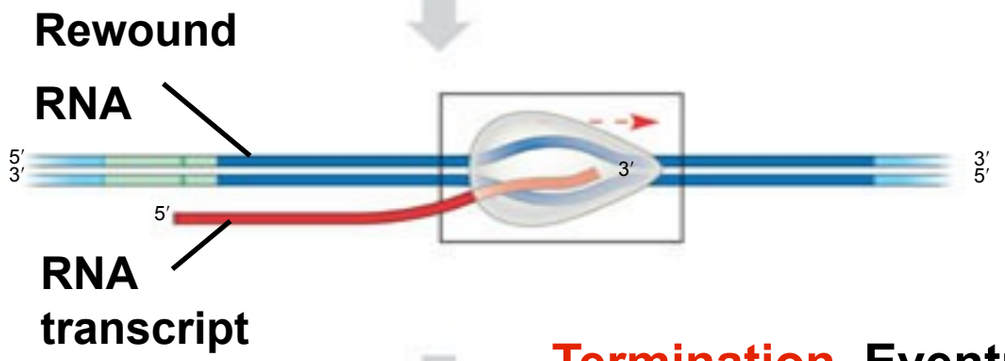
Recall Prokaryotic Transcription



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.



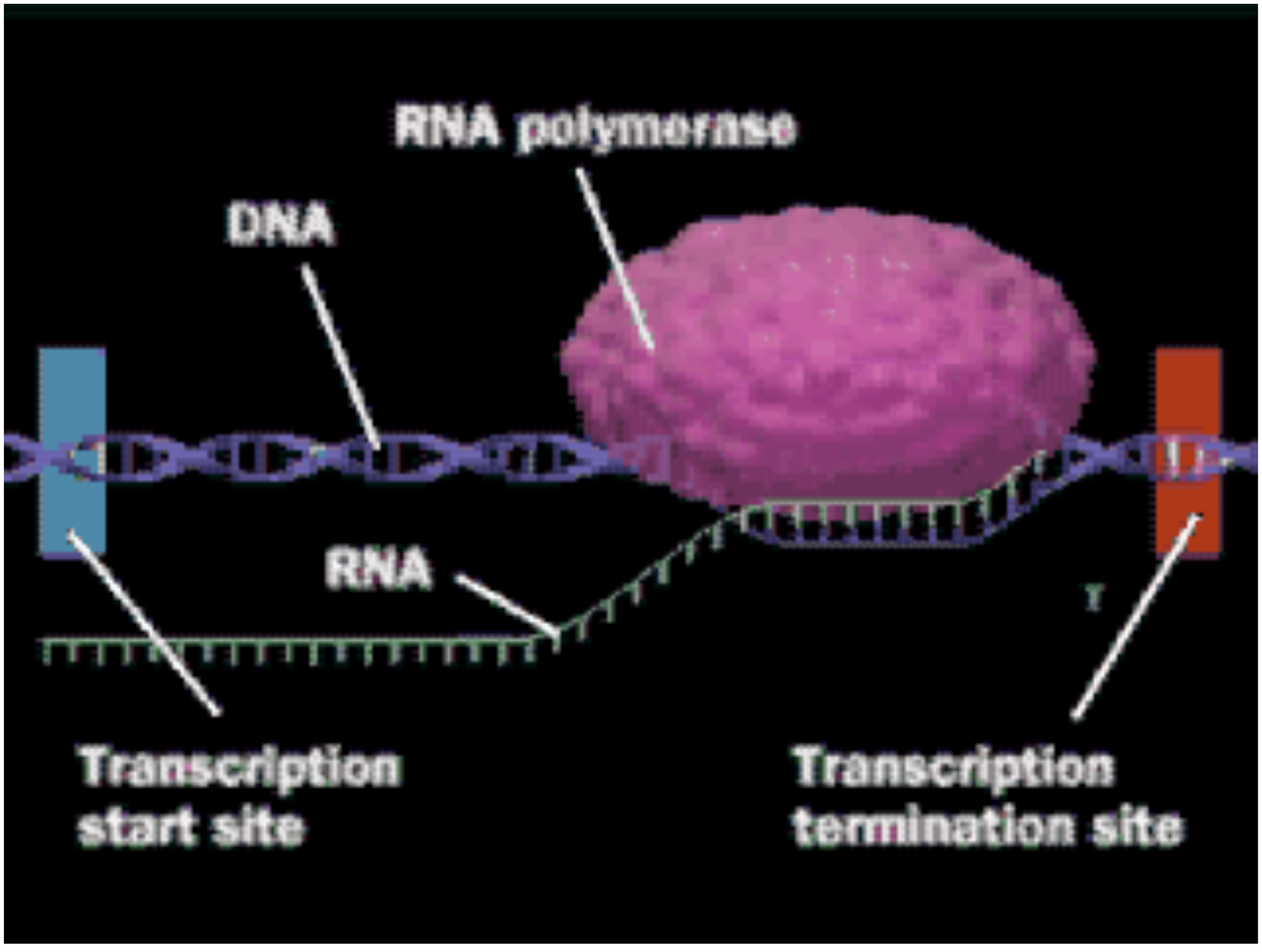
Elongation. The polymerase moves downstream, unwinding the DNA and elongating the RNA transcript 5′ → 3′. In the wake of transcription, the DNA strands re-form a double helix



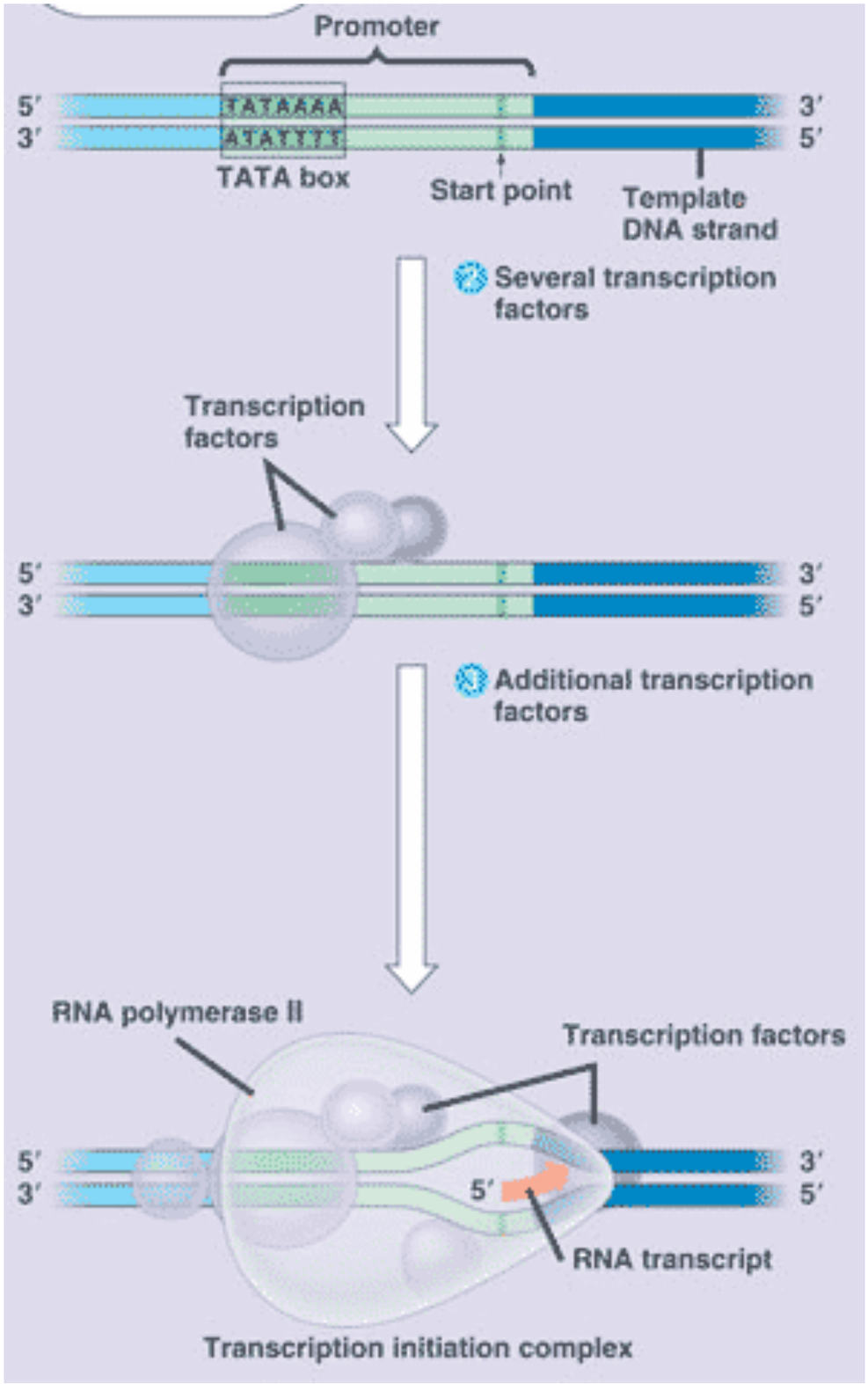
Termination. Eventually, the RNA transcript is released, and the polymerase detaches from the DNA.

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

Transcription



Eukaryotic Transcription (Initiation)



Eukaryotic Promoter- *includes TATA box a nucleotide sequence of TATA about 25 nucleotides upstream from the transcriptional starting point*

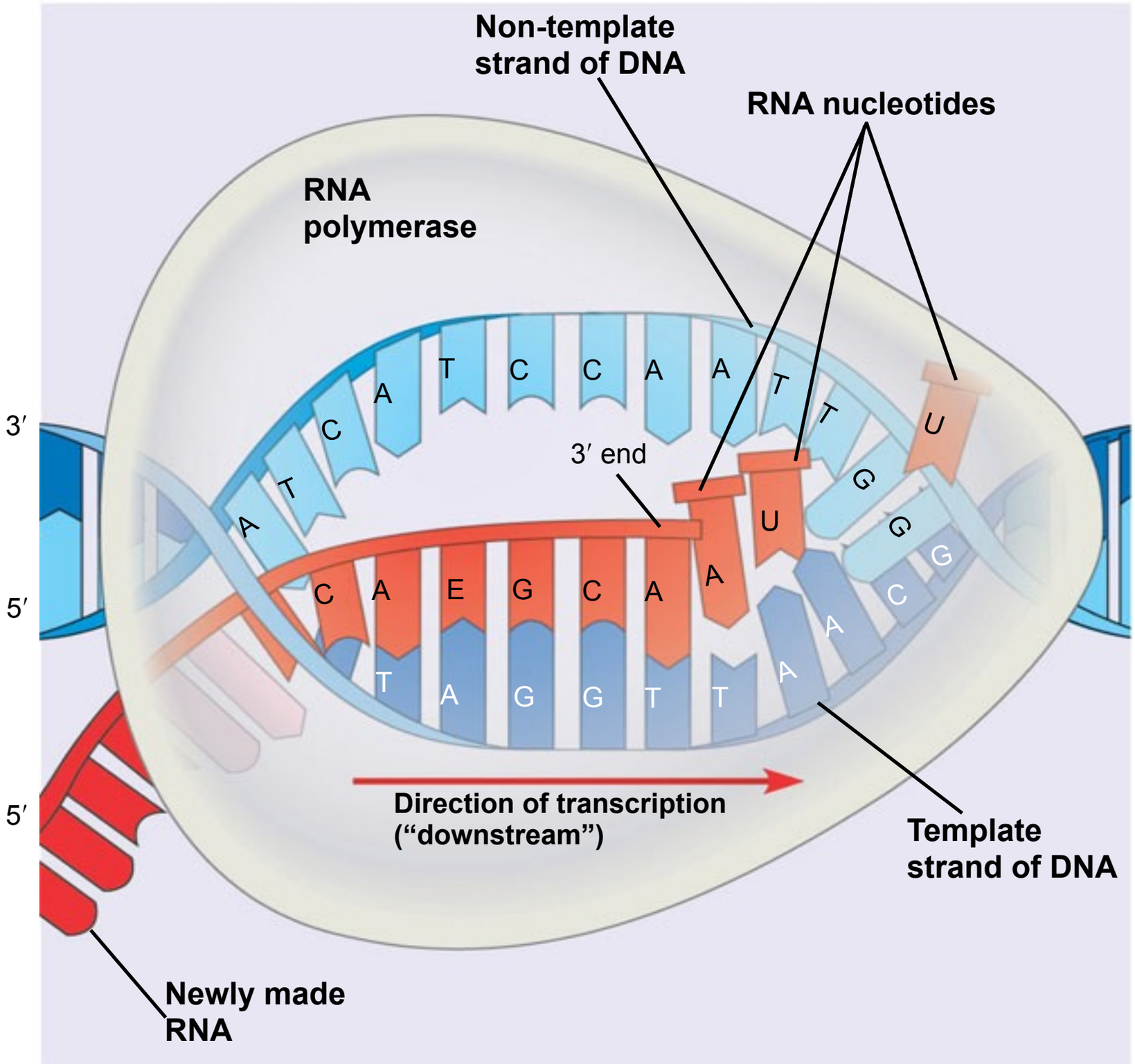
Several Transcription Factors- *one recognizing the TATA, must bind to the DNA before RNA polymerase II can bind correctly*

Additional Transcription Factors- *bind to DNA along with RNA polymerase II, forming the initiation complex, only then can RNA polymerase II begin to do its work.*

Transcription (eukaryotic)

Transcription (Elongation)

No significant differences between prokaryotic and eukaryotic elongation



RNA polymerase

-uncoils DNA

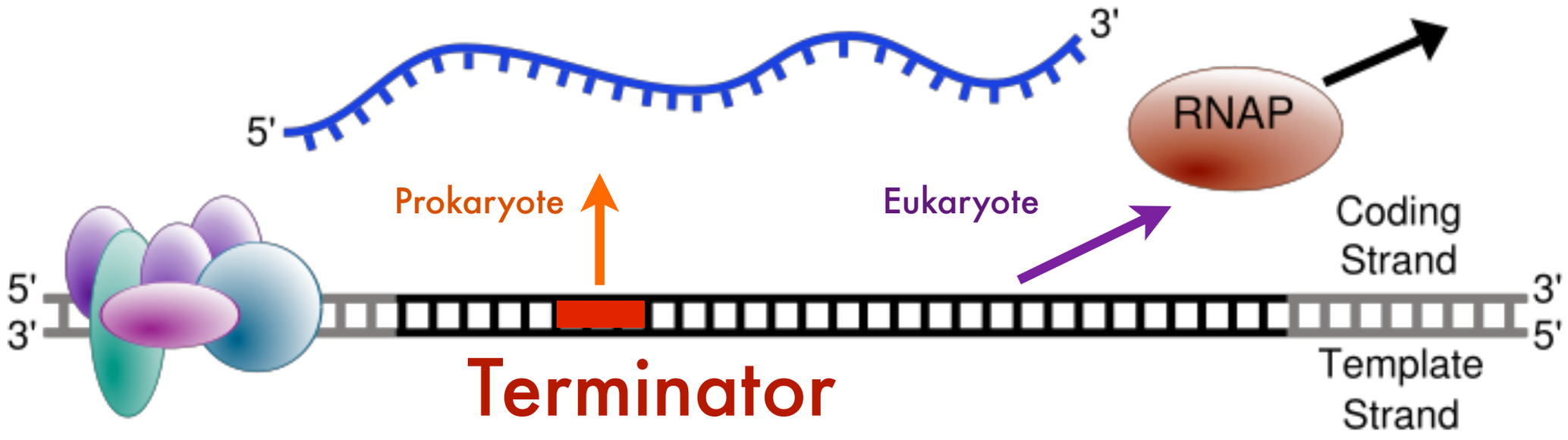
-splits DNA

-holds DNA open

-adds RNA nucleotides

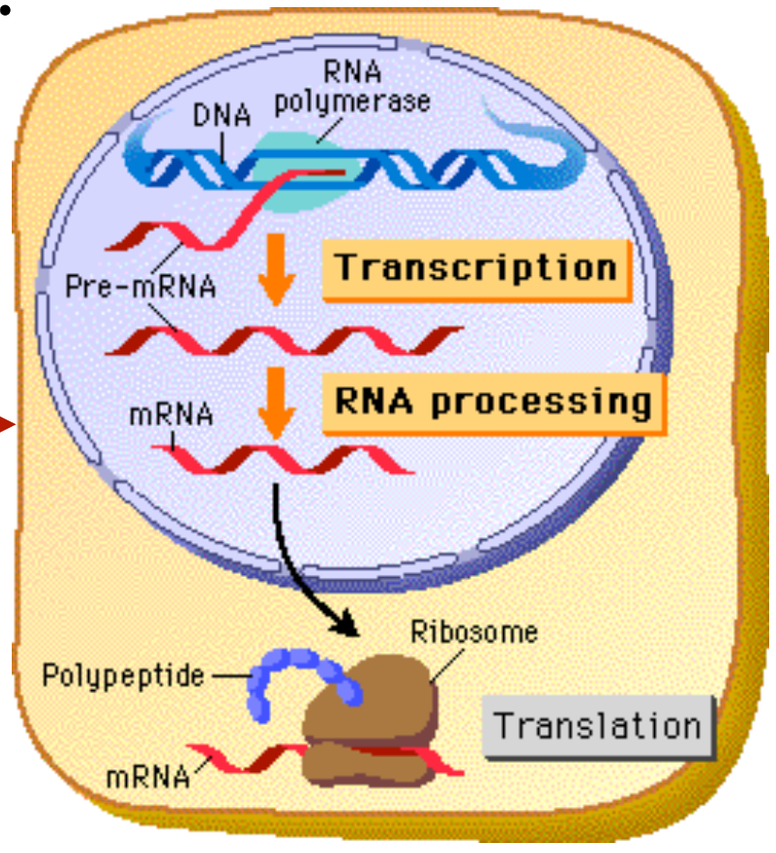
Transcription (Termination)

- Transcription stops when the RNA polymerase reaches a region on the DNA known as the terminator.
  - *This is generally true in both prokaryotes and eukaryotes.*
  - **The difference lies in the details, in prokaryotes the RNA polymerase falls off at the terminator.**
- **In Eukaryotes the RNA polymerase proceeds past the terminator for about 10-35 nucleotides downstream from the terminator and then it falls off.**



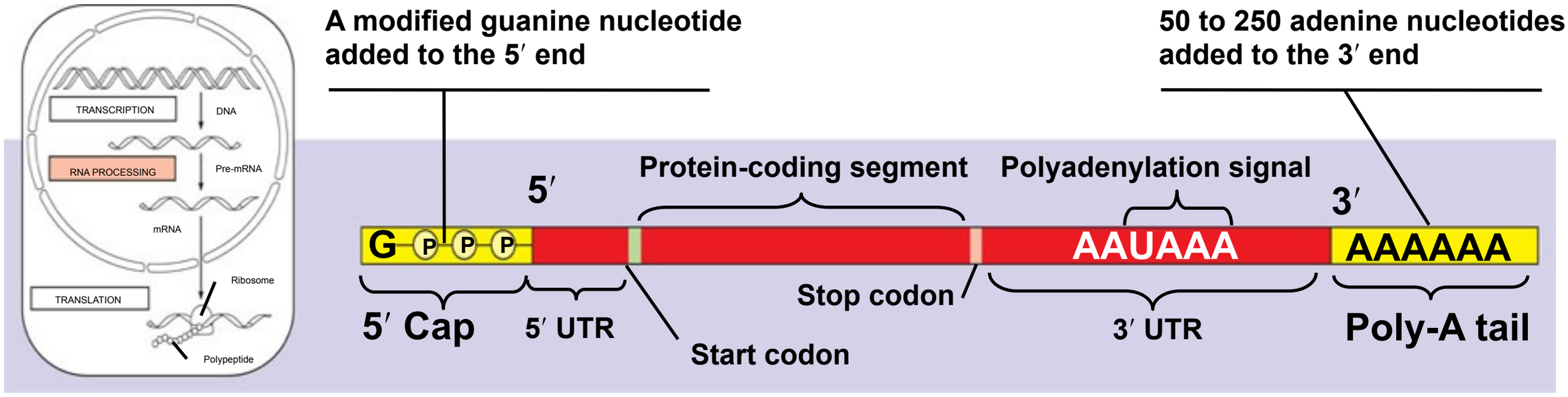
RNA Processing

- The mRNA transcribed in eukaryotes must be modified before translation.
  - *Both ends of the mRNA need to modified and the internal portion of the mRNA must be cut and spliced.*
  - These modifications that ready eukaryotic mRNA for translation are called **RNA Processing**.



RNA Processing

- **Alteration of the Ends**
  - The 5′ prime end of mRNA receives a **5′ cap,** a modified form of guanine (G).
  - The 3′ end receives a **poly-A tail**. Several enzymes add a string of 50-250 adenines (A) after the polyadenylation signal that terminated transcription.



RNA Processing

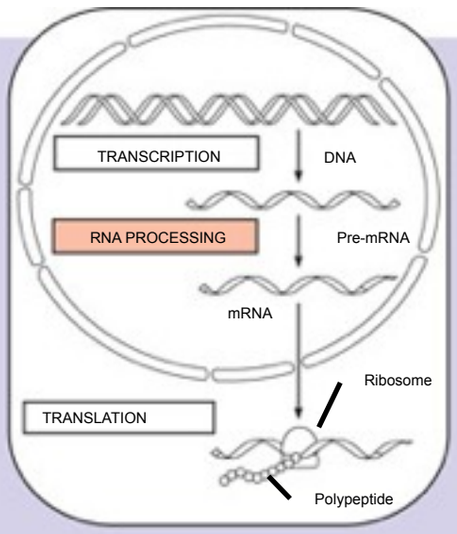
- **Alteration of the Ends**

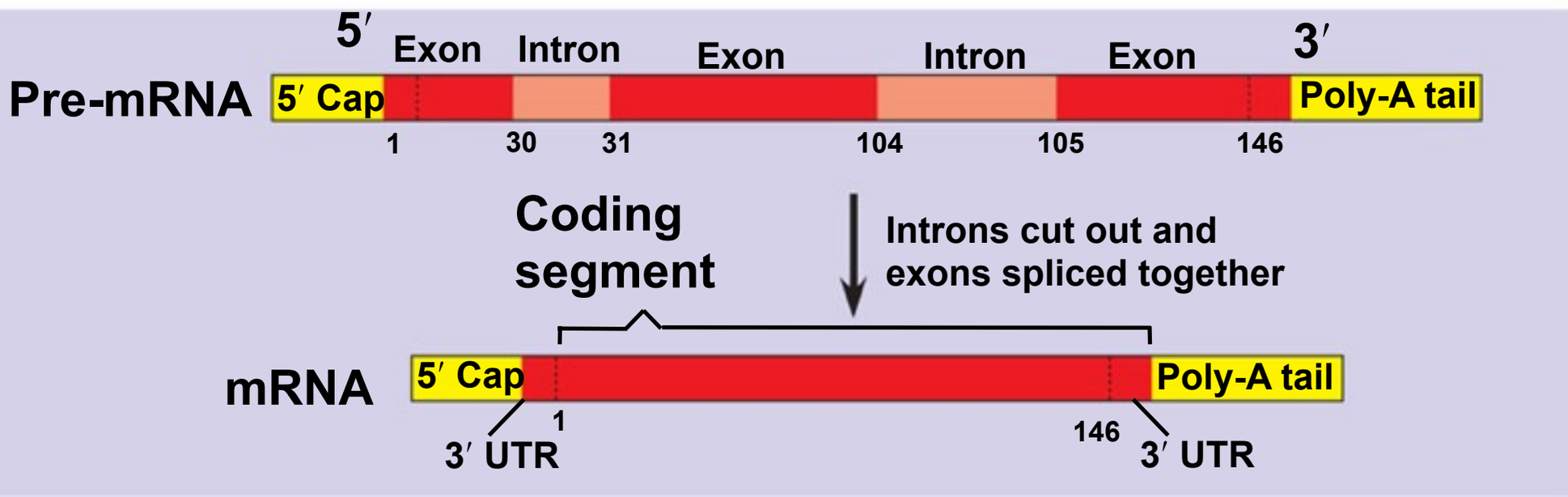
RNA Processing

- **Important Functions in the Alteration of Ends**
  - First, both *5′ cap* and *poly-A tail* facilitate the export of the mRNA from the nucleus.
  - Second, *5′ cap* and *poly-A tail* protect mRNA from the degradative enzymes found in the cytosol.
  - Third, the *5′ cap* helps orient ribosomes to the proper starting point for translation, while UTR regions help facilitate enzyme binding.

RNA Splicing

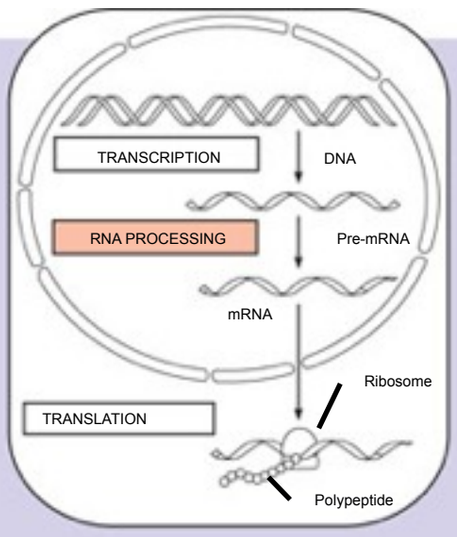
- **Gene Splicing**
  - The original mRNA that was transcribed is now highly modified in a "cut & paste" job called **RNA splicing**.
  - *The original transcript averages some 27,000 base pairs, however the average-sized protein requires only 1,200 base pairs. Most of eukaryotic mRNA is non-coding!*

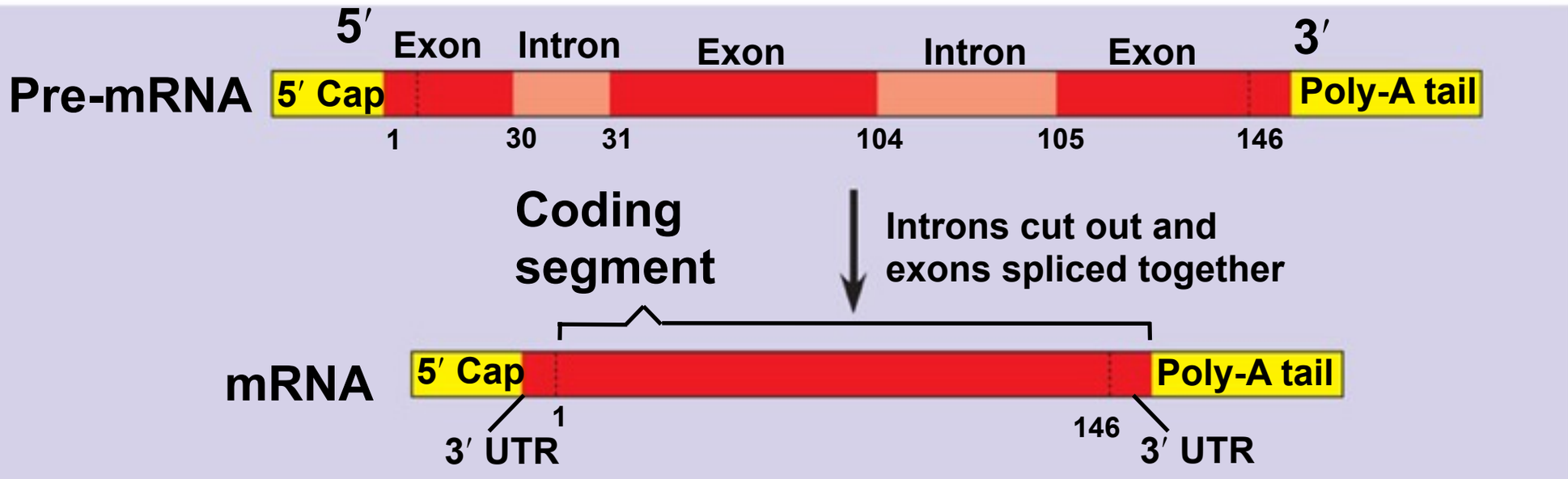




RNA Splicing

- The non-coding, "intervening" segments that lie between the coding regions are **introns**.
- The coding segments are called **exons**, because these regions are "expressed" as they get translated into amino acid sequences.
- *The exception are the UTR's which are necessary for translation but they not part of the polypeptide product.*





RNA Splicing

- **Gene Splicing**

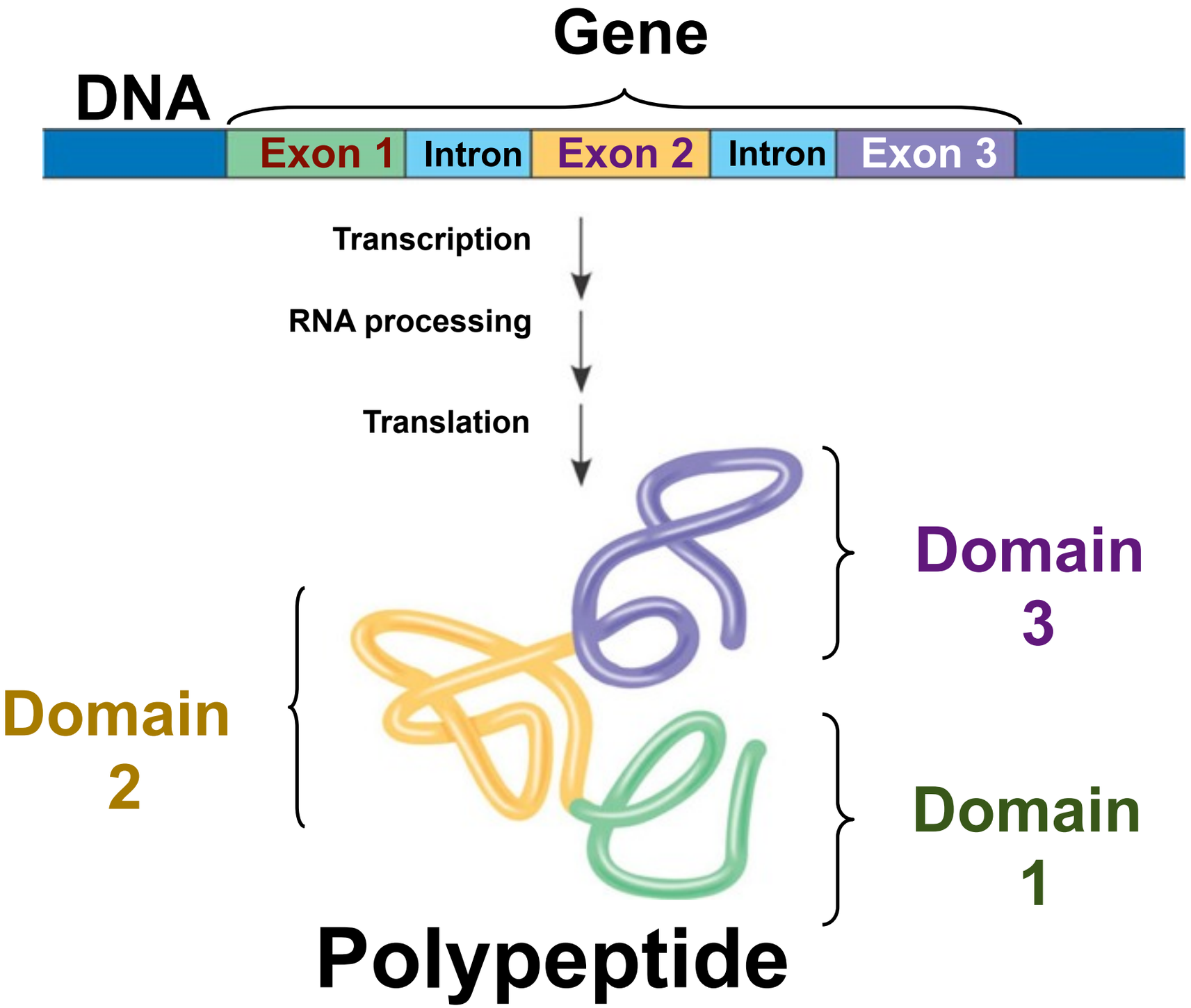
Ribozymes

- **Ribozymes**, RNA molecules that function as enzymes.
  - *This discovery made the idea that only proteins could be biological catalysts obsolete.*
- Three Properties are responsible for its ability to catalyze reactions:
  - *1. since RNA is single stranded it can base pair with itself and thus fold into unique 3-dimensional shapes*
  - *2. some of the bases can have functional groups that participate in the catalysis*
  - *3. the ability of RNA to base pair (form hydrogen bonds) with other nucleic acids adds specificity to its catalytic activity*

Functional & Evolutionary Role of Introns

- One important consequence of introns, is that a single gene can code for multiple polypeptides, depending on which segments are treated as exons during RNA processing.
  - *This alternative splicing may explain why humans get by with the same number of genes as a nematode.*
- Proteins are often built in modular architecture, consisting of discrete structural and functional regions called **domains**.
  - *In many cases each exon codes for a different domain.*

Functional & Evolutionary Role of Introns

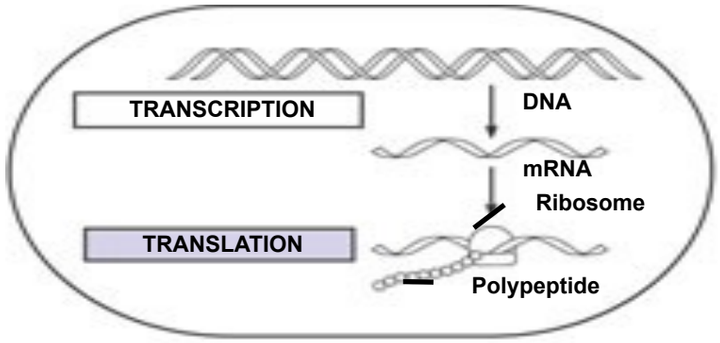


Exon Shuffling

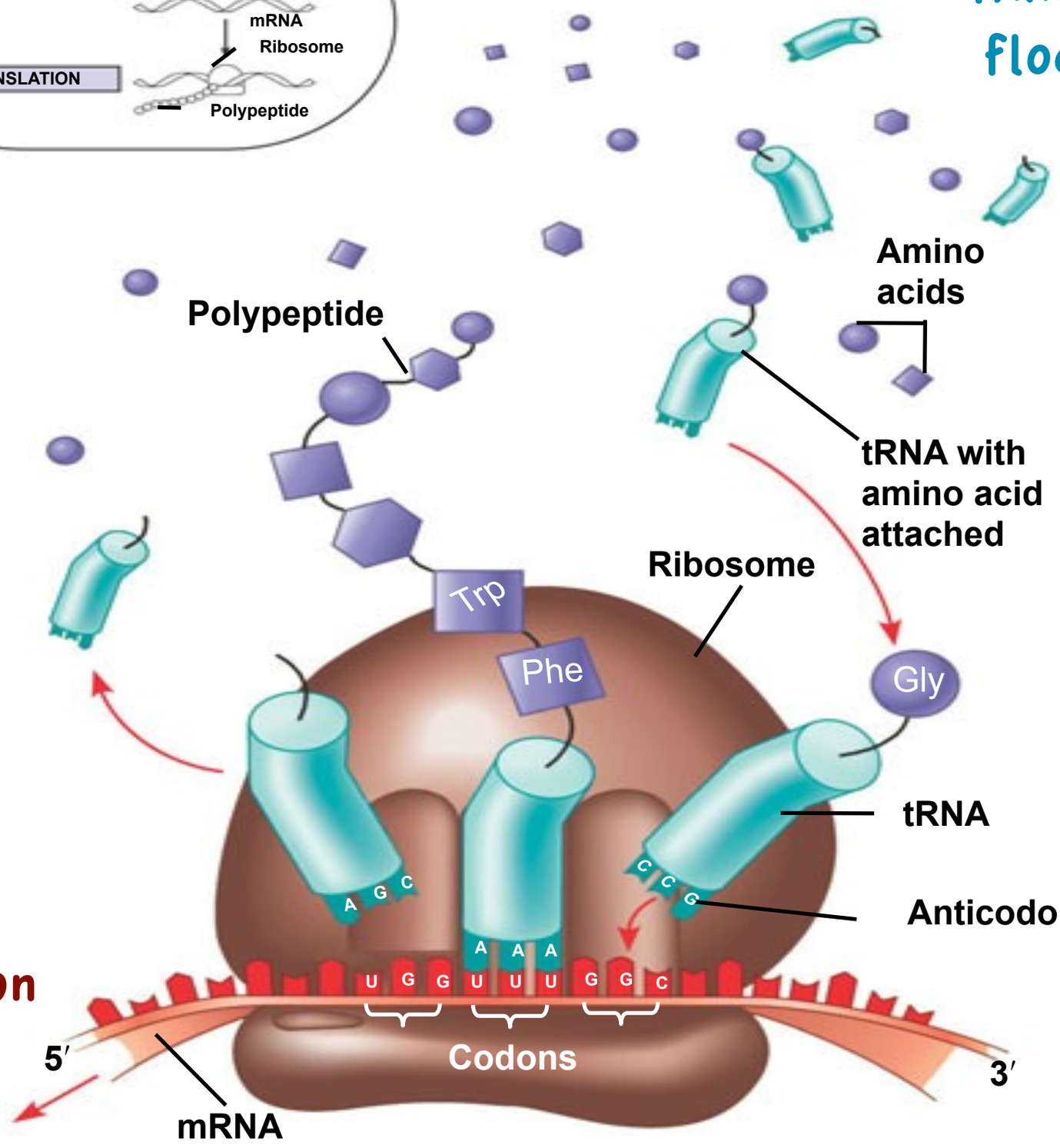
- generates combinations resulting in new and novel proteins

- increases crossing over frequency by pushing exons farther apart

Eukaryotic Translation



tRNA's are also floating freely



the cytosol is stocked with free floating amino acids

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

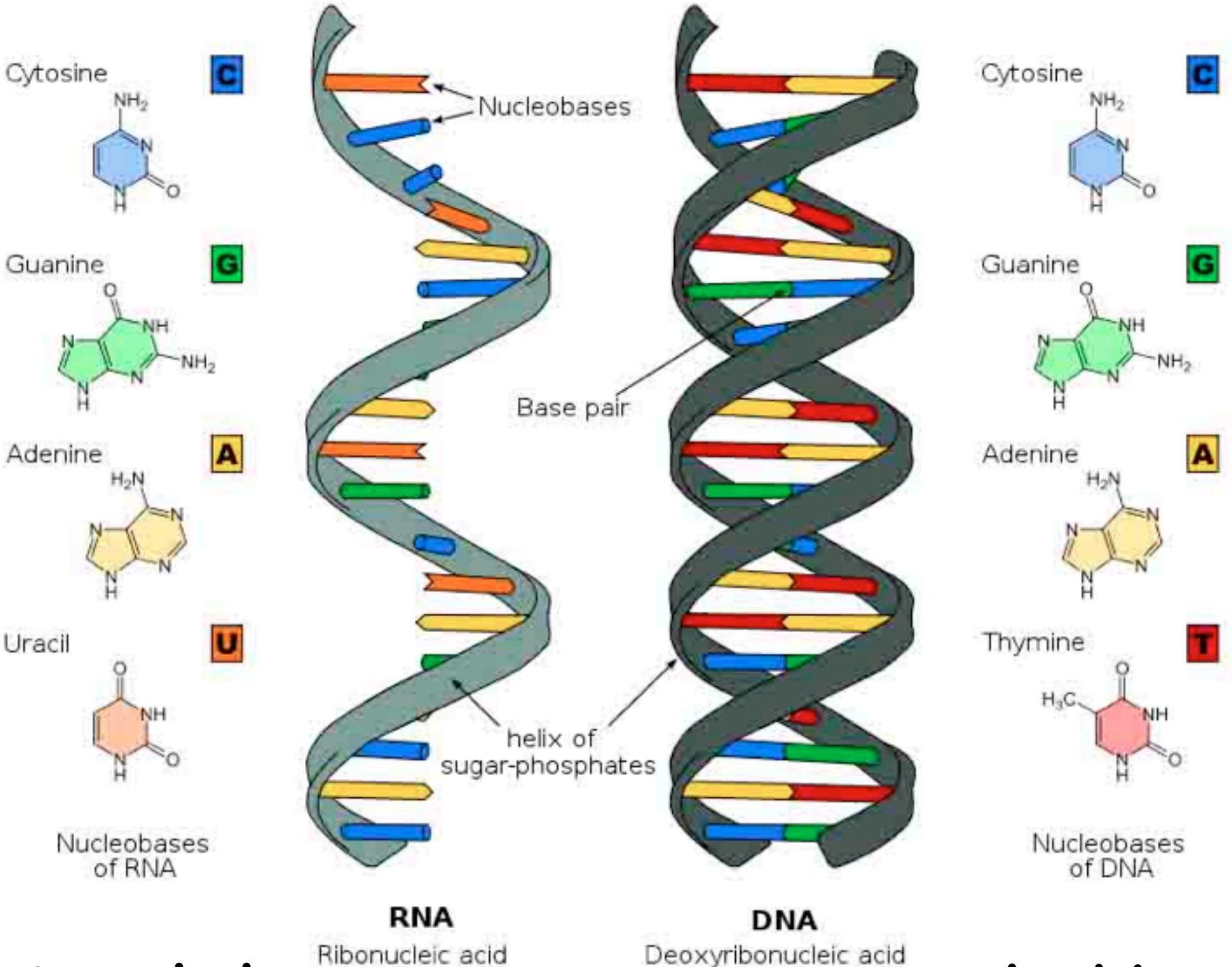
No significant differences between prokaryotic and eukaryotic elongation

Eukaryotic 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 (vs DNA)

"Our Cast of Characters"



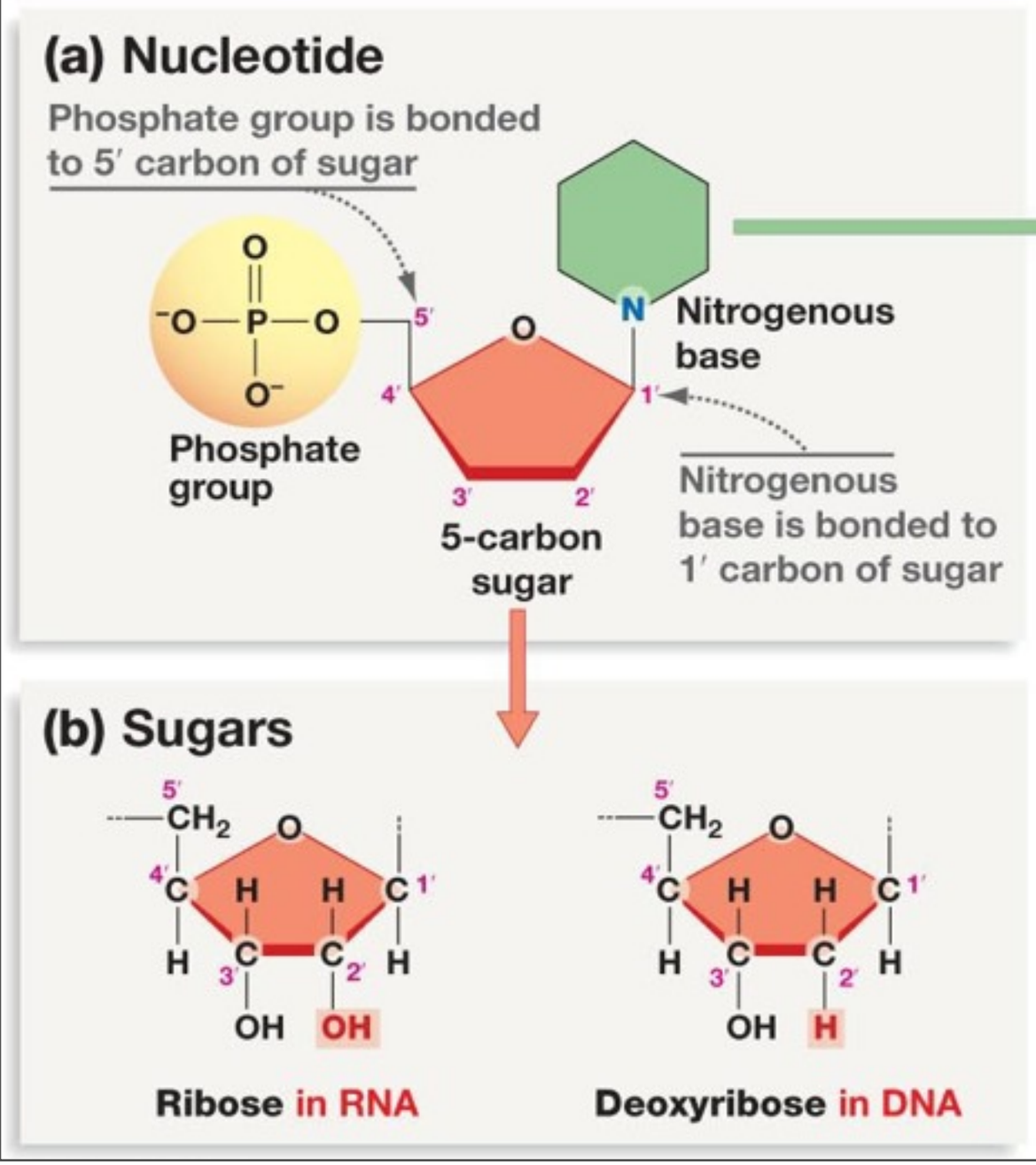
single stranded

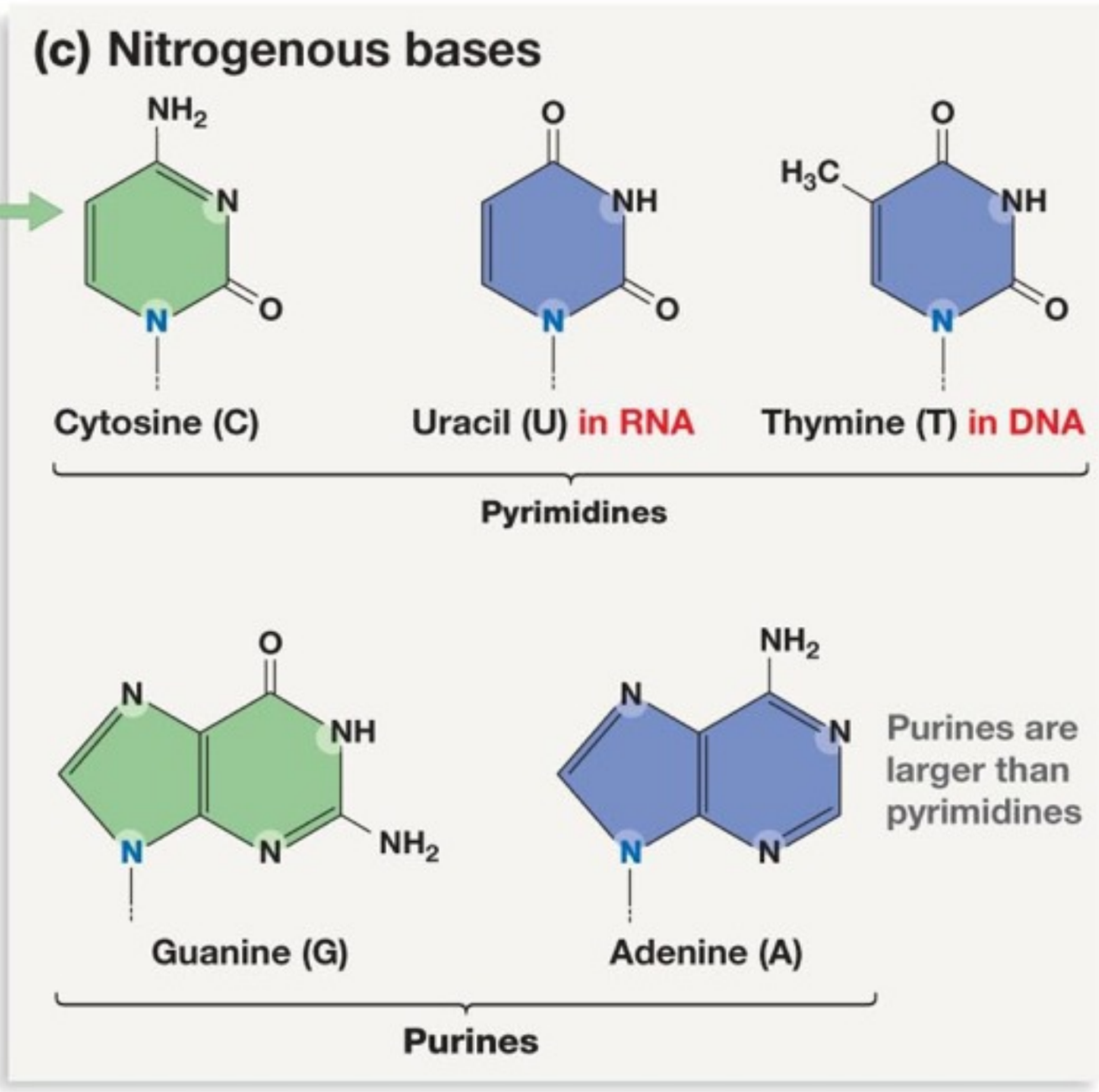
double stranded

mRNA (vs DNA)

different sugars in the backbone

Uracil instead of thymine



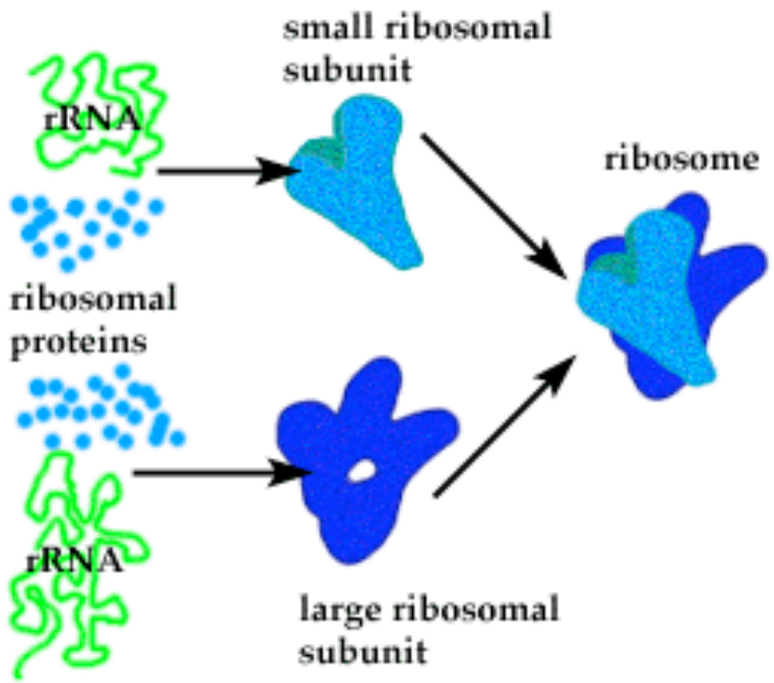


Ribosomes

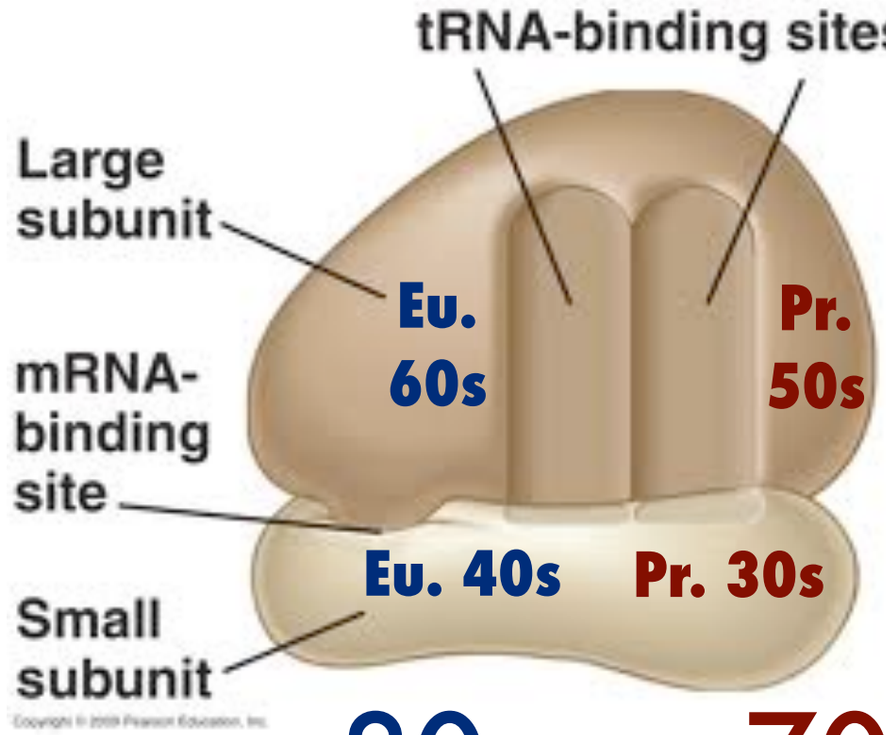
"Our Cast of Characters"

Translation :

the ribosome



eukaryotic ribosomes are larger than prokaryotic ribosomes



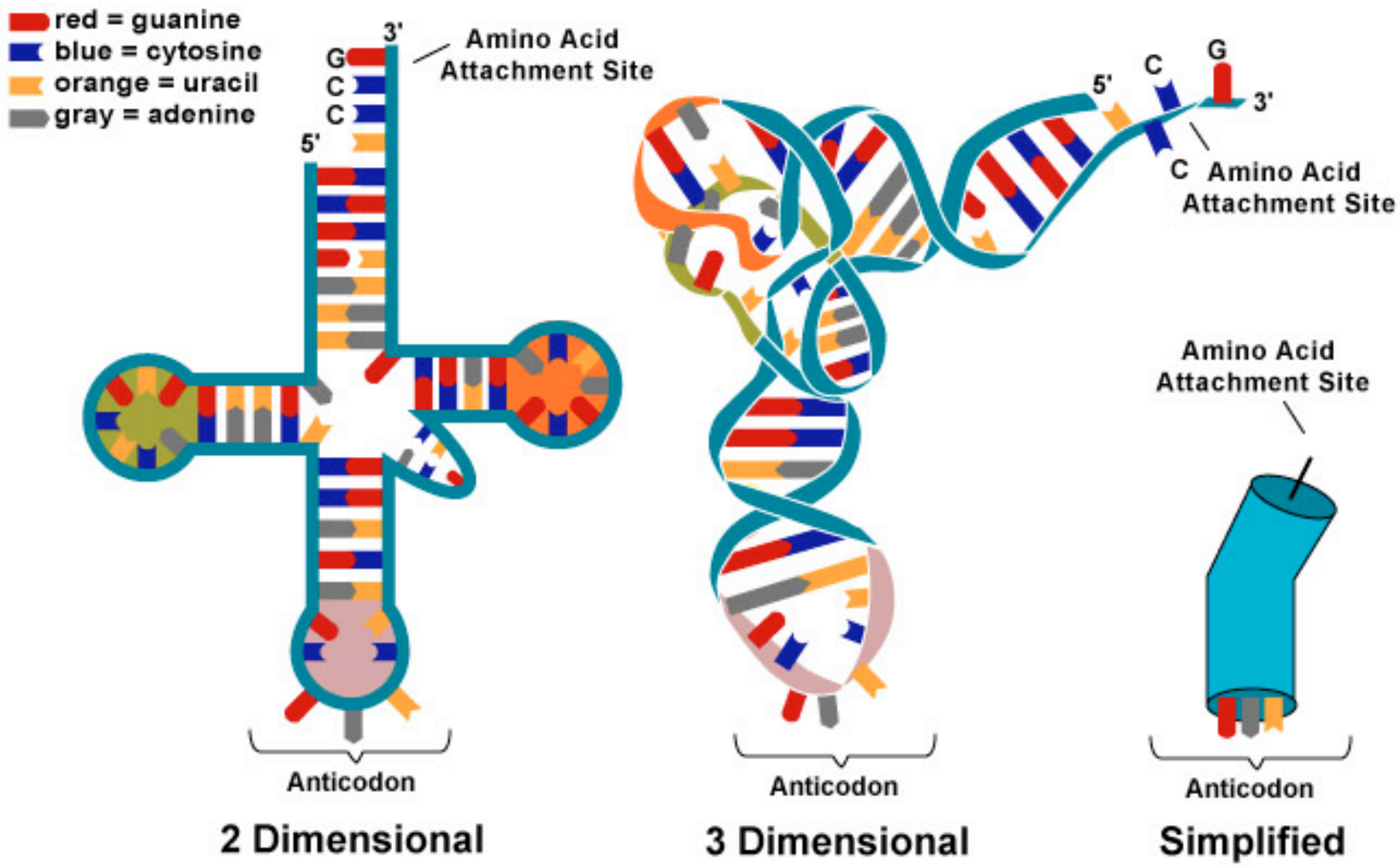
80s
Eukaryotes

70s
Prokaryotes

rRNA is the most abundant type of cellular RNA

tRNA

"Our Cast of Characters"



~80 nucleotides

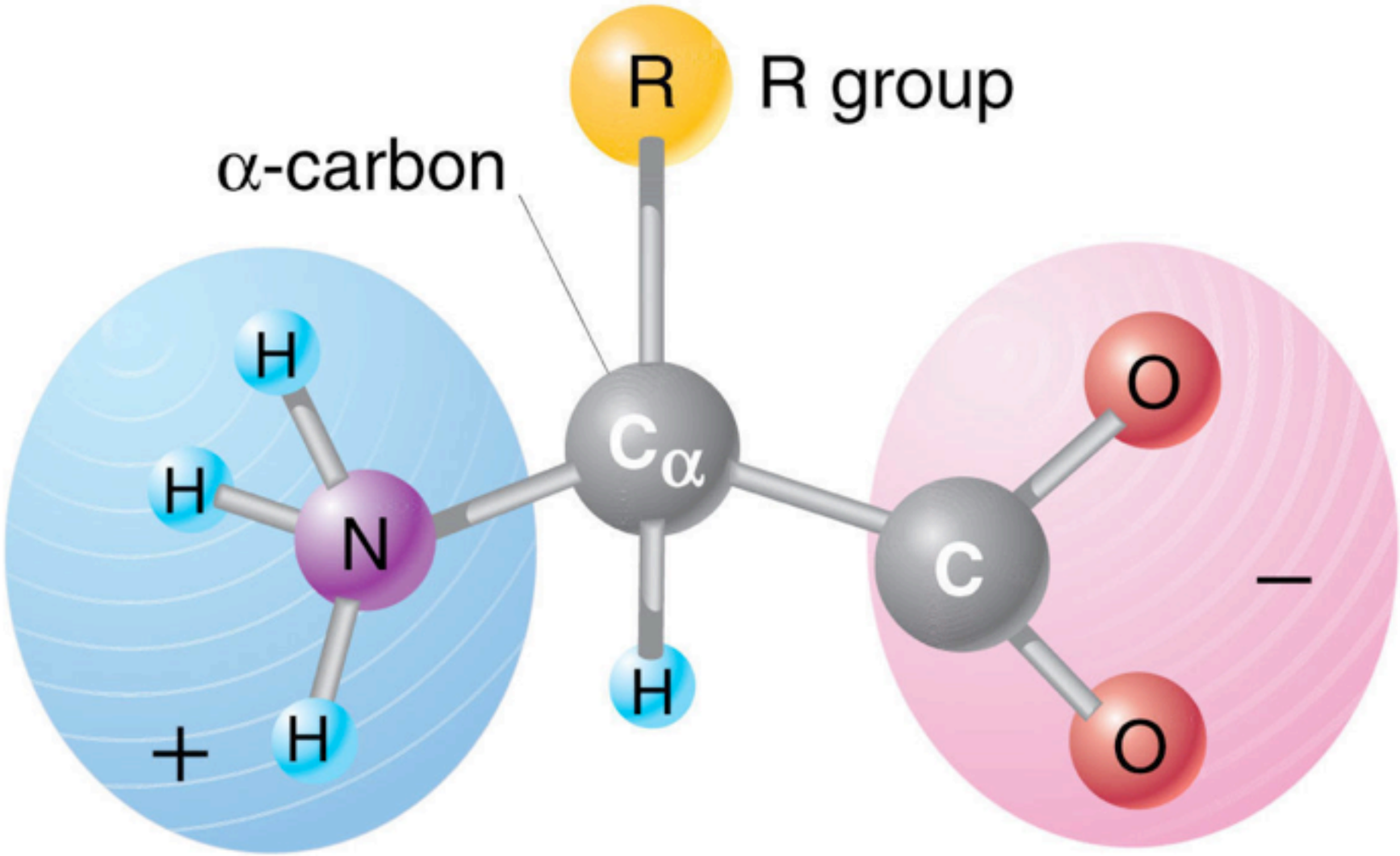
"L" shaped

tRNA

"Our Cast of Characters"

Amino Acids

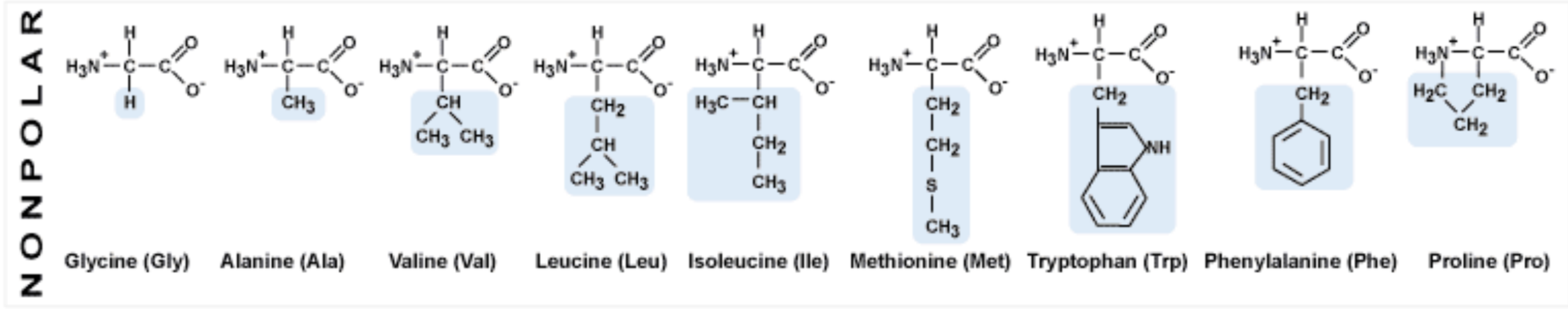
"Our Cast of Characters"

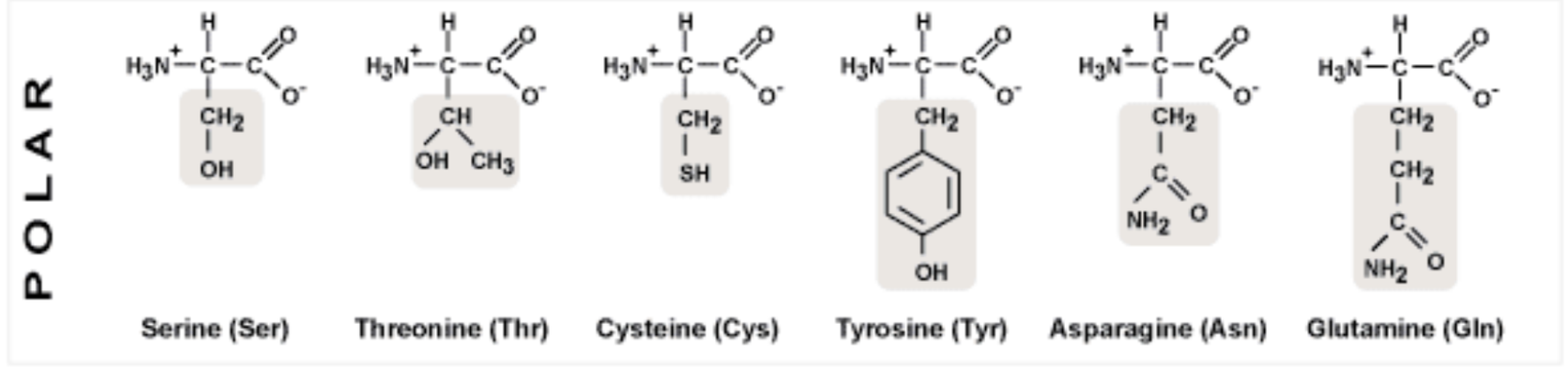


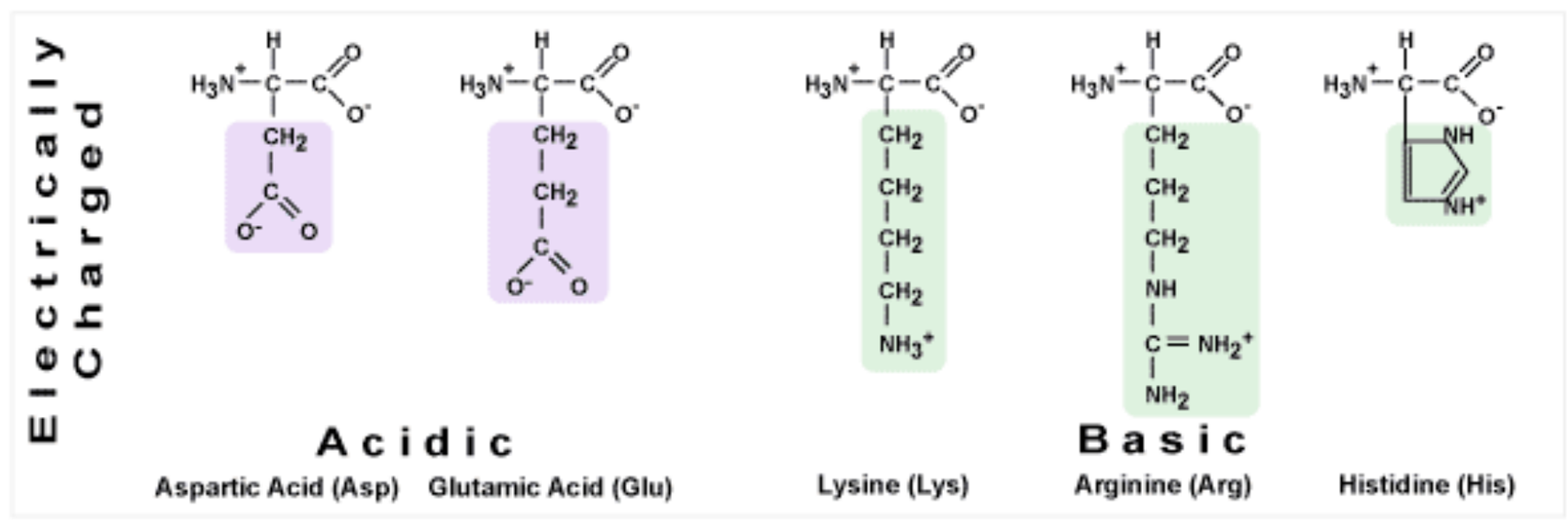
General Amino Acid Structure in Solution

Amino Acids

"Our Cast of Characters"

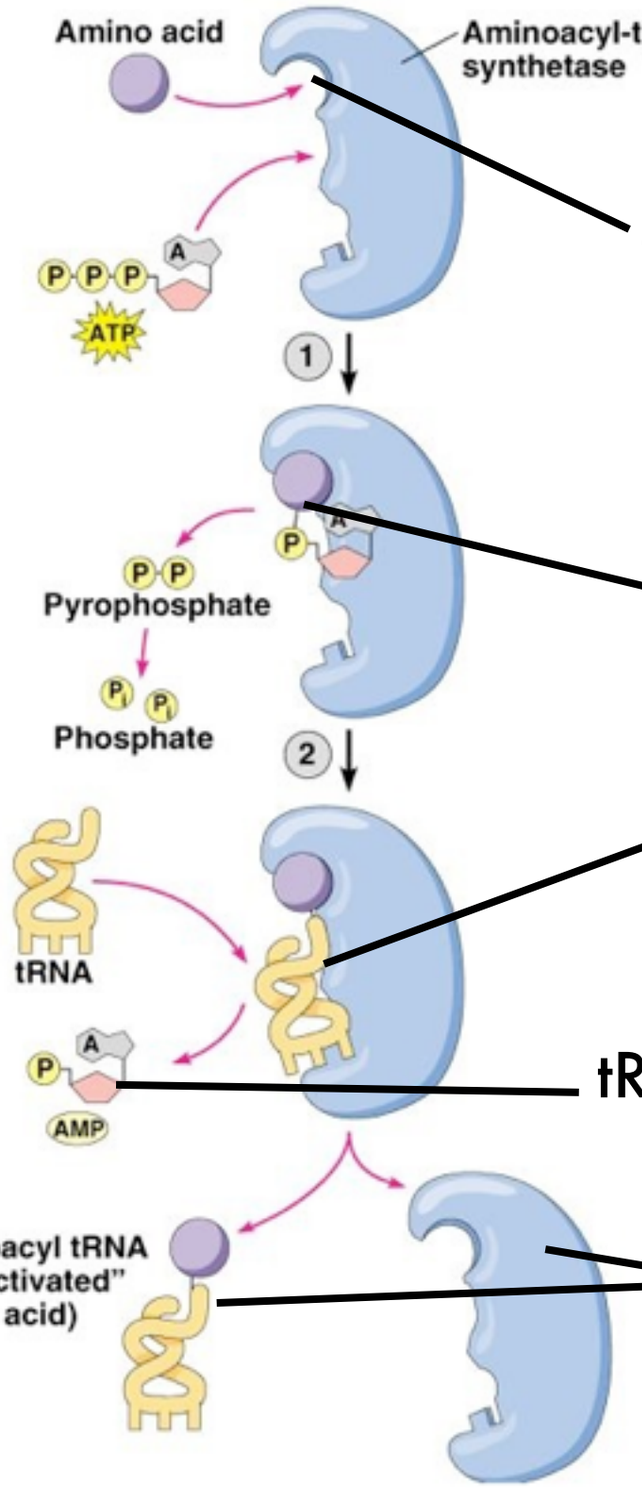






tRNA

"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.

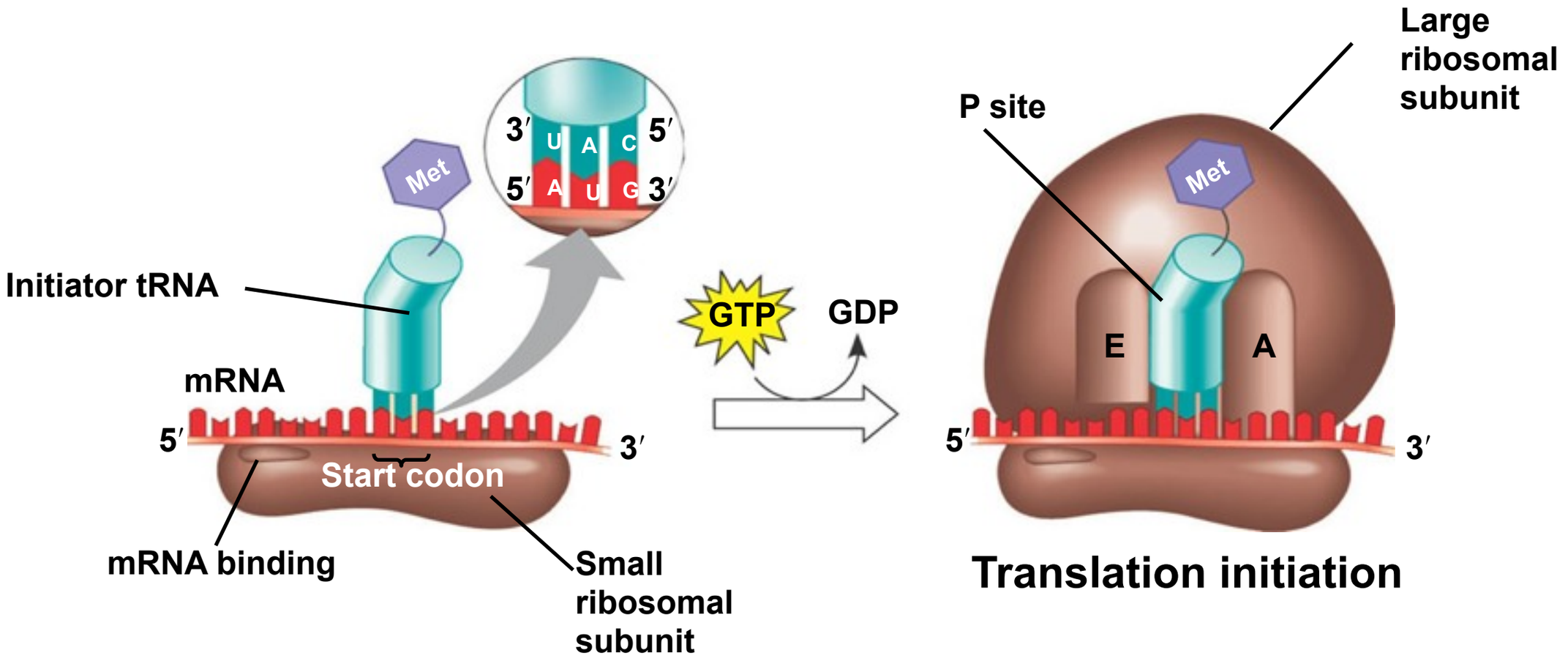
This flexibility, called "wobble" occurs at the third position of the codon/anticodon

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"



Translation initiation

1.

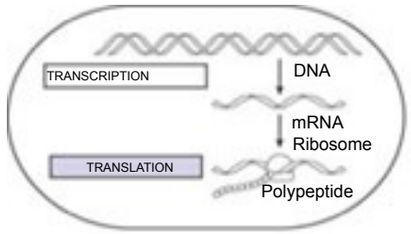
A small ribosomal subunit binds to a molecule of mRNA. In a prokaryotic cell, the mRNA binding site on this subunit recognizes a specific nucleotide sequence on the mRNA just upstream of the start codon. An initiator tRNA, with the anticodon UAC, base-pairs with the start codon, AUG. This tRNA carries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

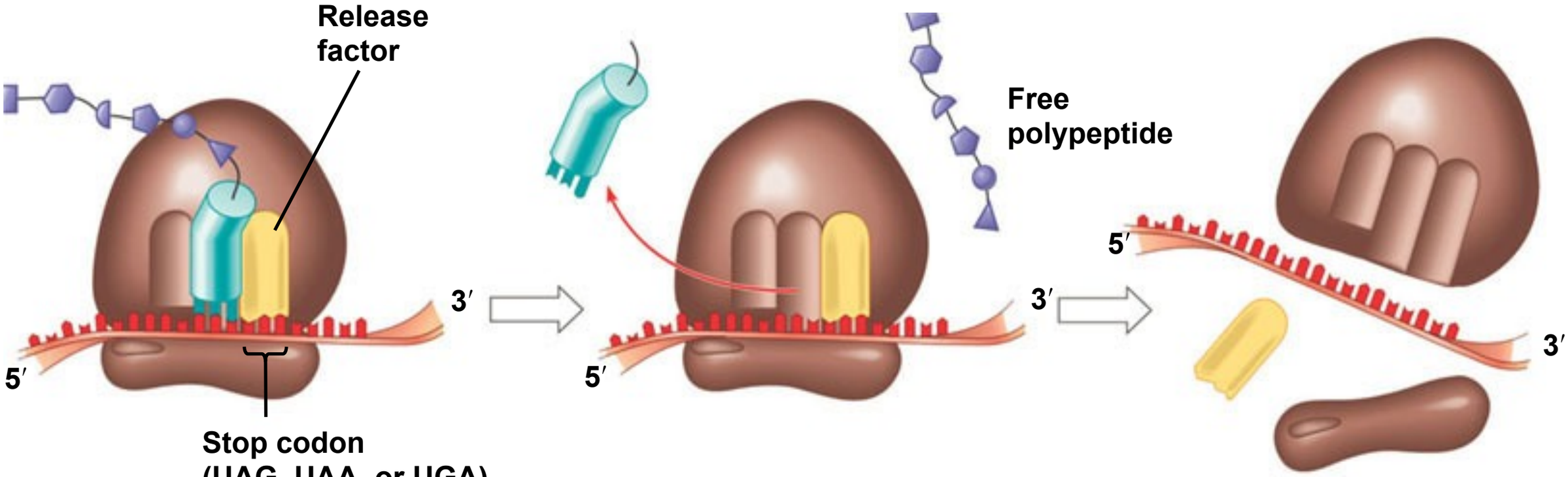
3.

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.

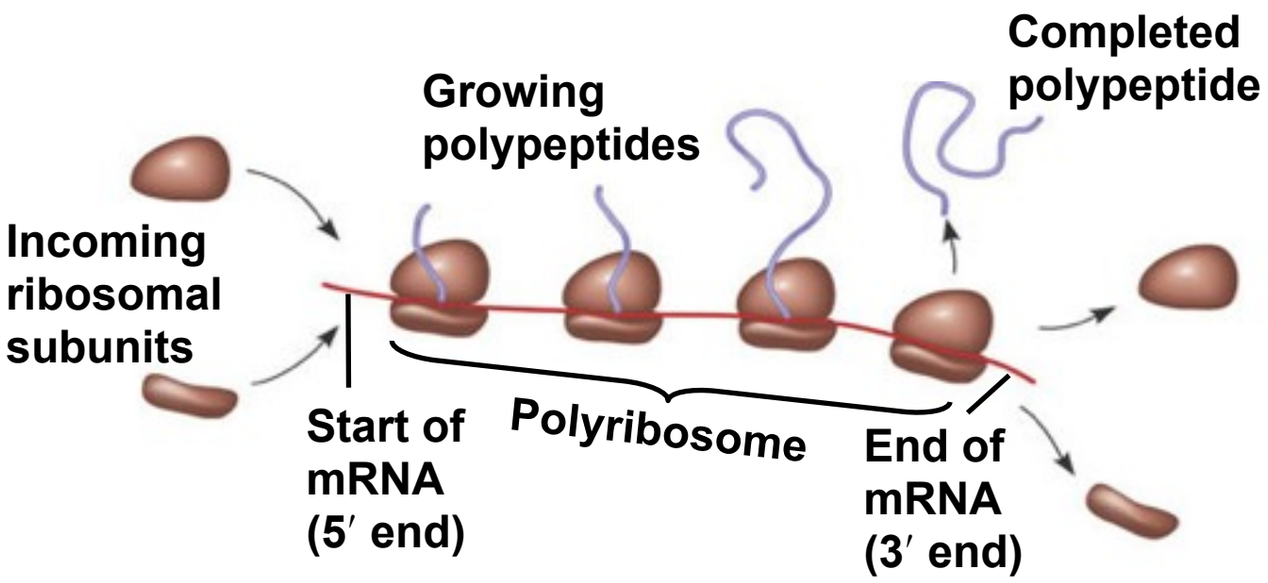
2.

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

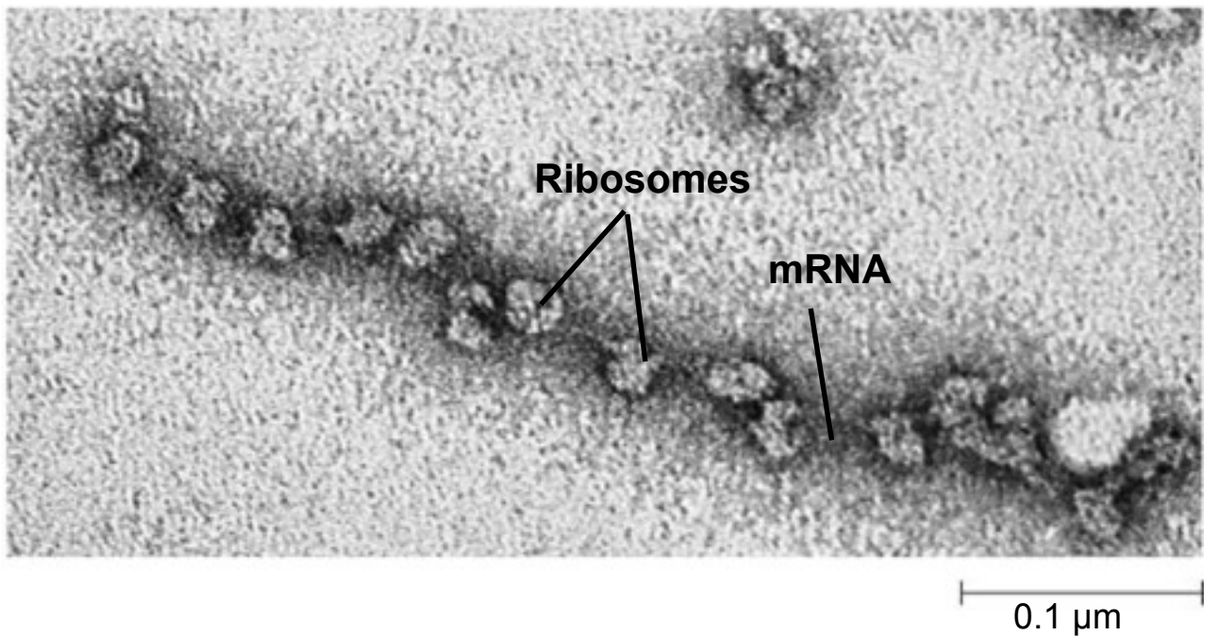
3.

Polyribosomes: Protein Synthesis

"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

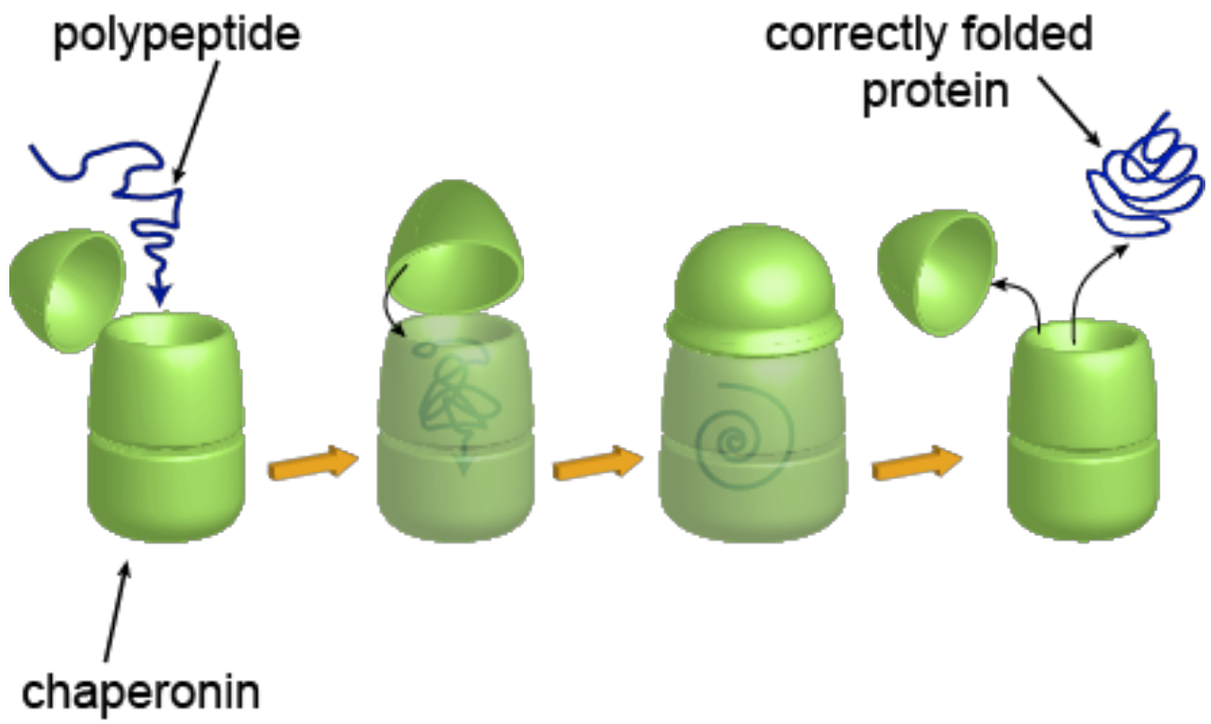
Recall- As soon as one RNA polymerase moves off of promoter another one can bind and so forth like a convoy of trucks

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 prokaryotic or a eukaryotic can make per unit time!

Completing a Functional Protein

- **Chaperone Proteins**
- The process of translation alone is often not enough to make a functional protein.
  - Although the amino acid sequence predicates a proteins 3-dimensional shape, chaperone proteins often help a protein fold into its proper shape.



Completing a Functional Protein

- **Post Translational Modifications**
- Many proteins require slight modifications after translation.
  - *Additions*- the attachment of functional groups, lipids, sugars or phosphate groups
  - *Cleavage*- trimming the ends of the polypeptide
  - *Splitting*- cutting the polypeptides in half to produce 2 functional polypeptides (ex. insulin)
  - *Combining*- putting two different non functional polypeptides together to produce one functionalprotein.

Targeting Protein Locations

- **Free Ribosomes & Bound Ribosomes**
- Cells make proteins for two general reasons, they need them or other cells need them.
  - *Free Ribosomes*- (in cytosolic) produce proteins for the cell itself
  - *Bound Ribosomes*- (attached to the endoplasmic reticulum) produce proteins for export

How does the cell determine if the protein is for itself or for export?

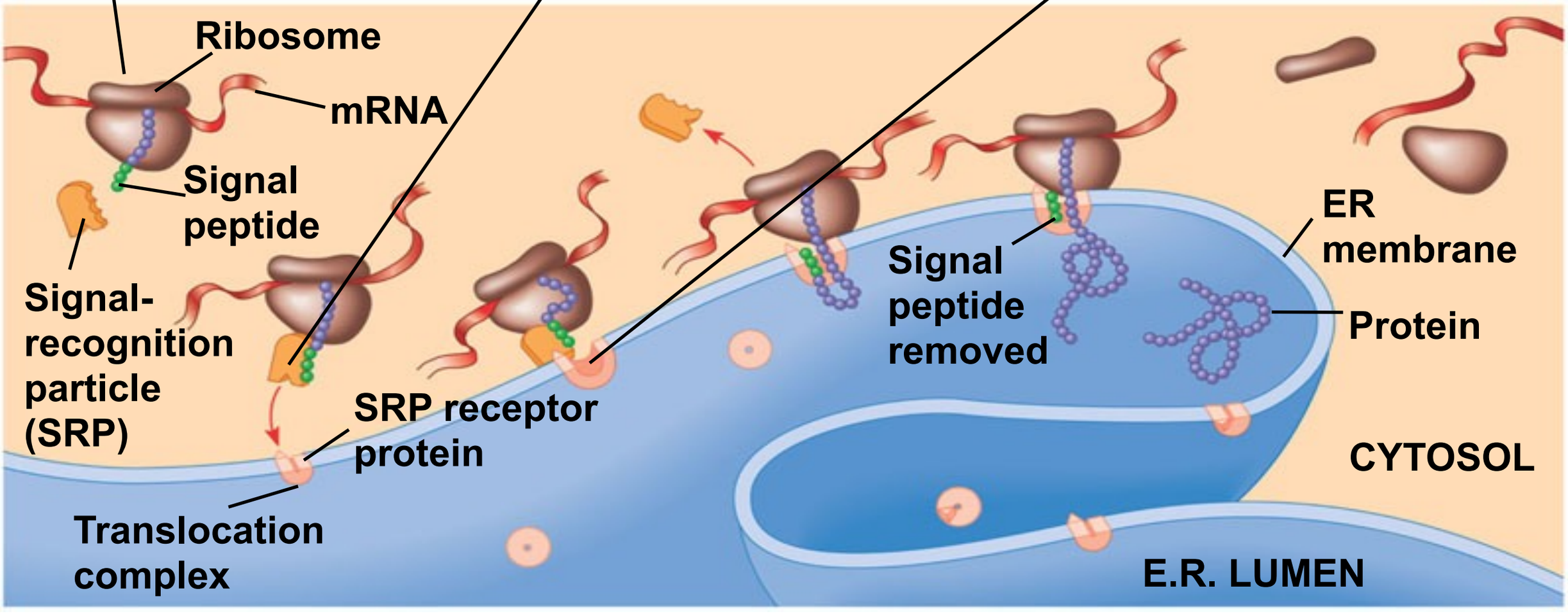
Targeting Protein Locations

Part I.

1. Polypeptide synthesis begins on a free ribosome in the cytosol.

2. An SRP binds to the signal peptide, halting synthesis momentarily.

3. The SRP binds to a receptor protein in the ER membrane. This receptor is part of a protein complex(a translocation complex) that has a membrane pore and a signal-cleaving enzyme.



Targeting Protein Locations

Part II.

**bacteria can do this as well*

The SRP leaves, and the polypeptide resumes growing, meanwhile translocating across the membrane. (The signal peptide stays attached to the membrane.)

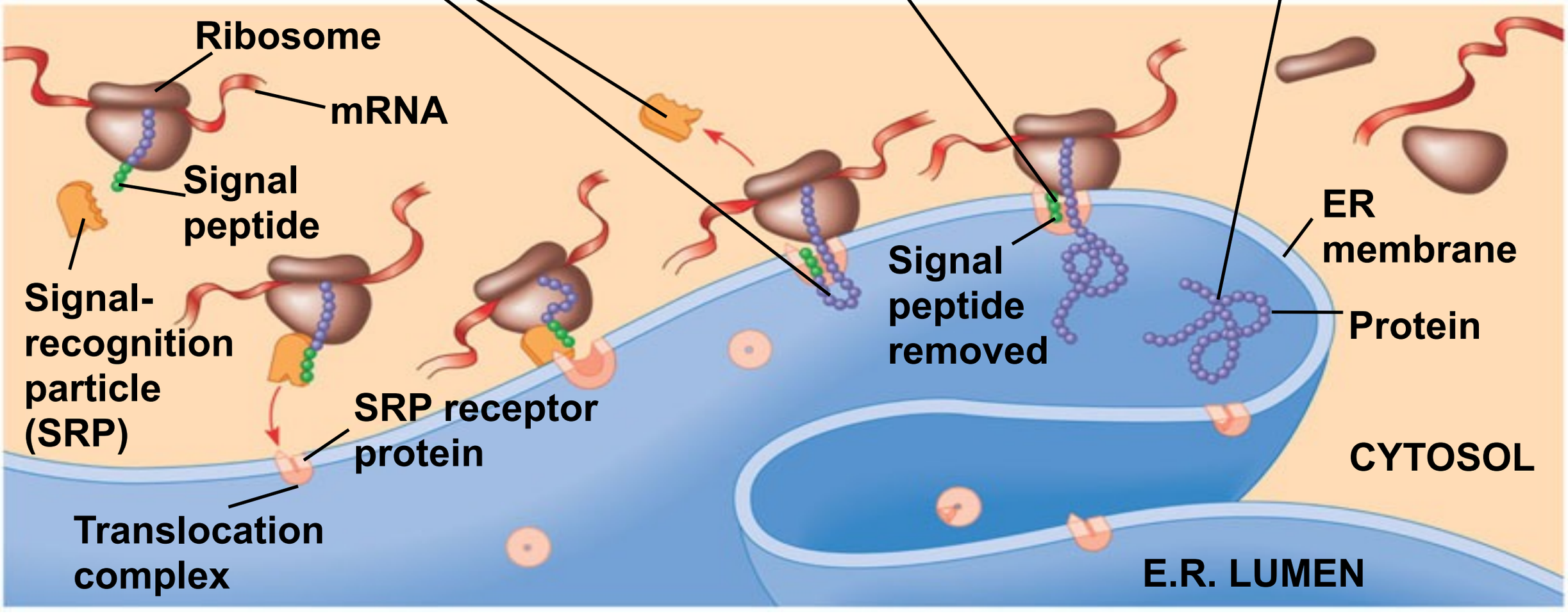
4.

The signal-cleaving enzyme cuts off the signal peptide.

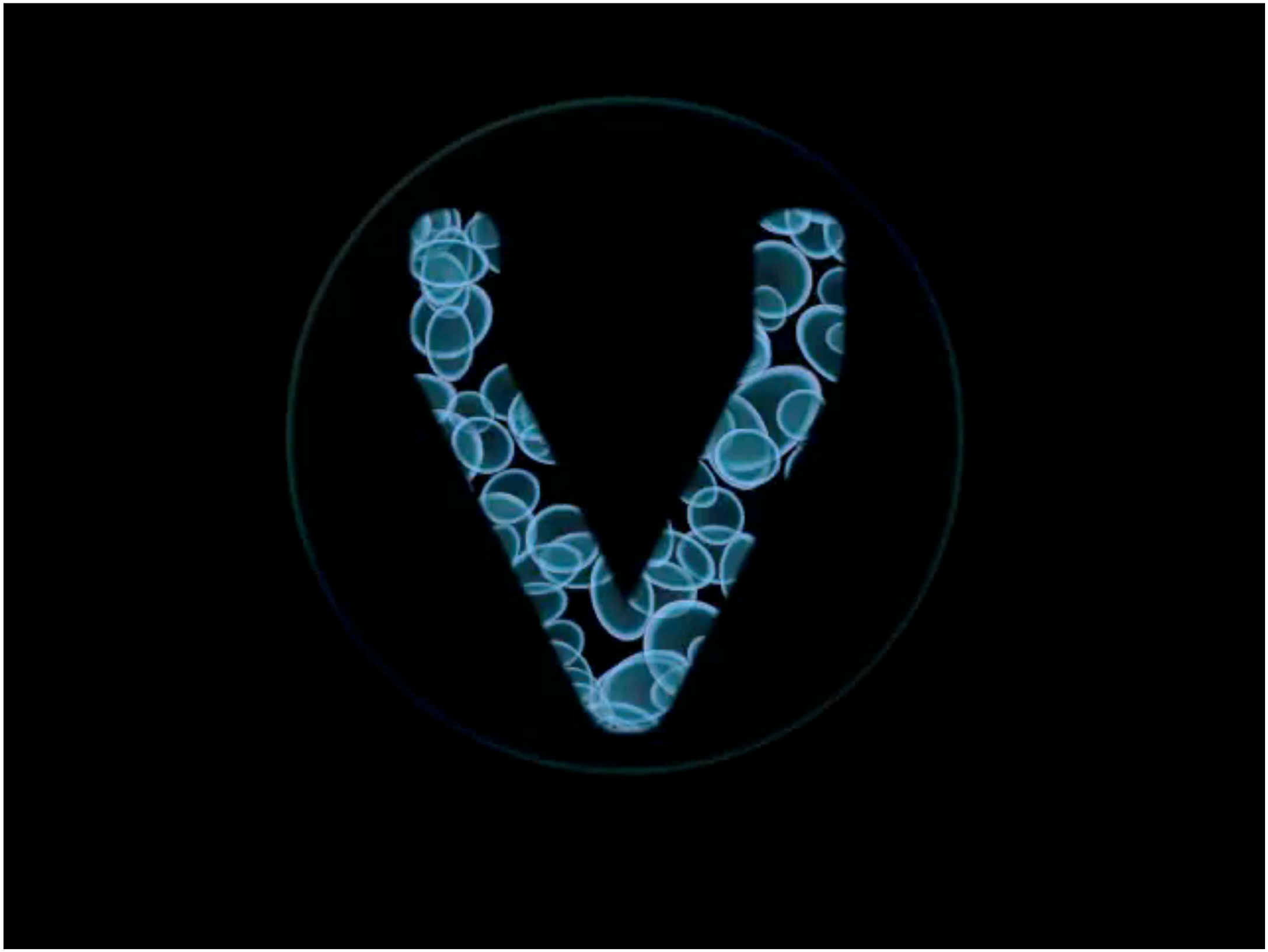
5.

The rest of the completed polypeptide leaves the ribosome and folds into its final confirmation.

6.



Golgi Regulated Secretion

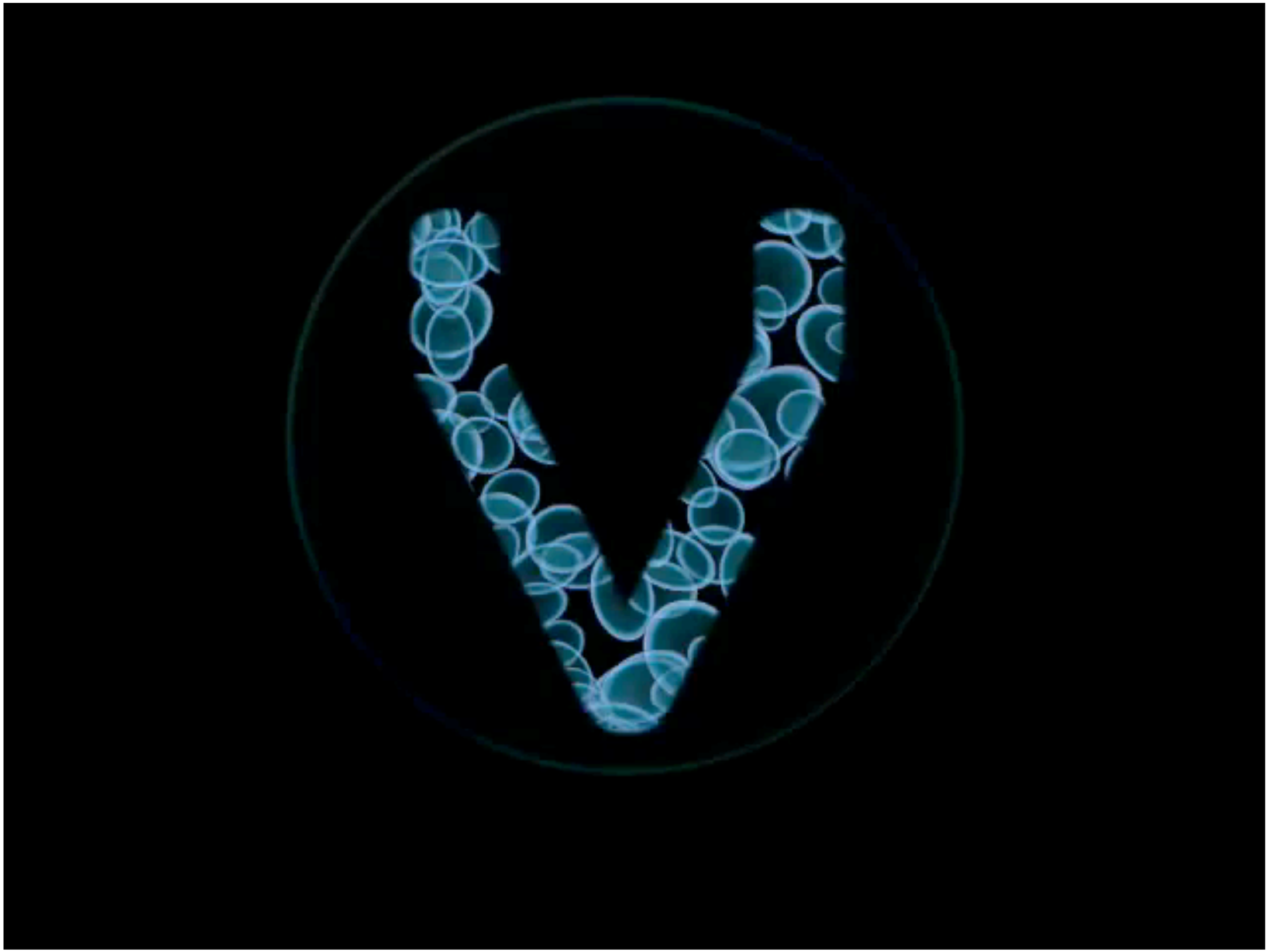


Protein Transport (mitochondria)

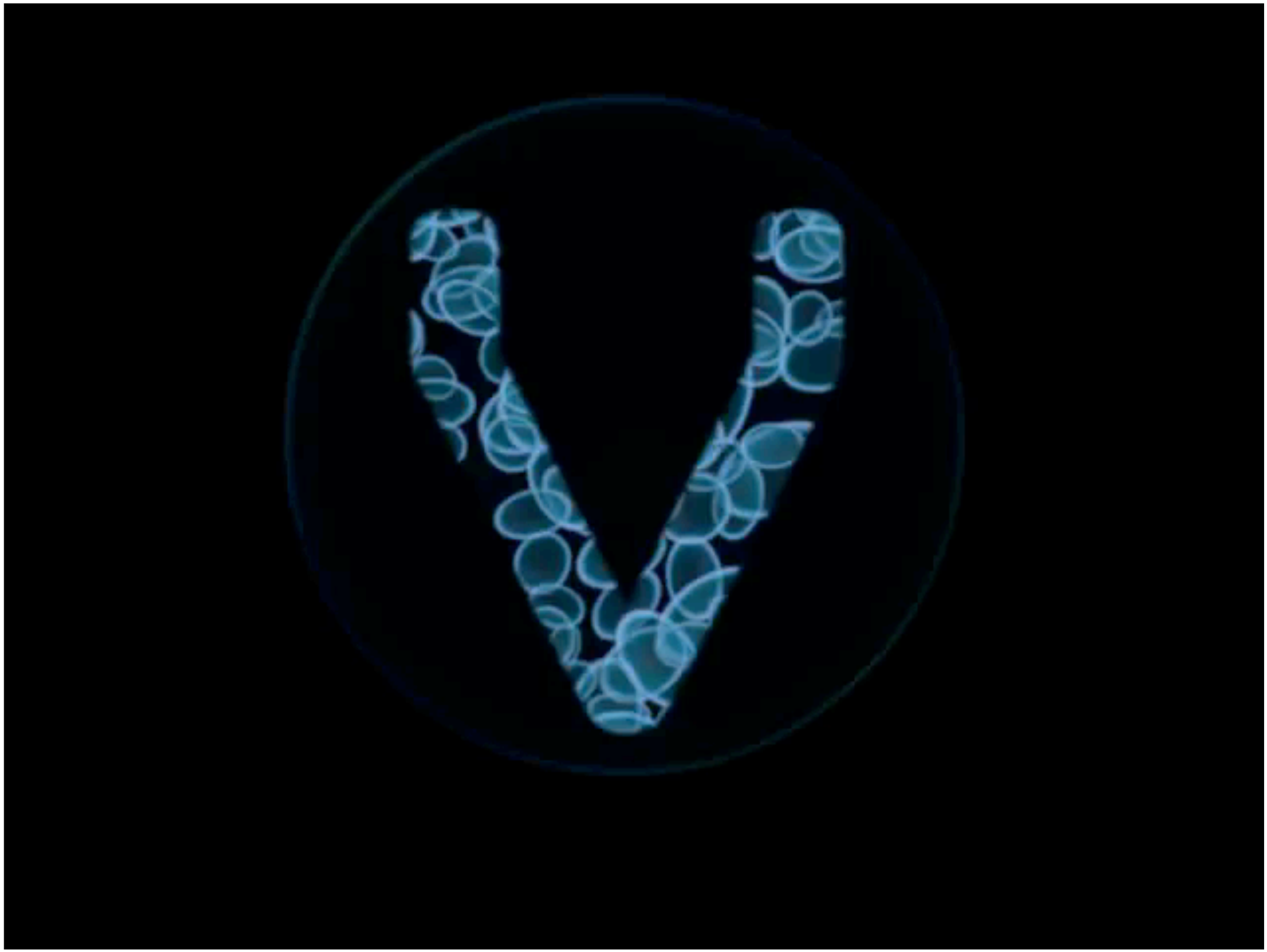
Protein Trafficking (golgi)

Protein Modification (golgi)

Constitutive Secretion



Protein Recycling



Final Summary

TRANSCRIPTION

RNA is transcribed from a DNA template.

DNA

3′

Poly-A

5′ RNA transcript

RNA polymerase

RNA PROCESSING

In eukaryotes, the RNA transcript (pre-mRNA) is spliced and modified to produce mRNA, which moves from the nucleus to the cytoplasm.

Exon

RNA transcript (pre-mRNA)

Intron

Cap

NUCLEUS

CYTOPLASM

FORMATION OF INITIATION COMPLEX

After leaving the nucleus, mRNA attaches to the ribosome.

mRNA

Growing polypeptide

Poly-A

Ribosomal subunits

Cap

5′

A C C

E

A

U A C

A A A

U G G U U U A U G

Anticodon

Codon

Ribosome

Aminoacyl-tRNA synthetase

Amino acid

tRNA

Activated amino acid

AMINO ACID ACTIVATION

Each amino acid attaches to its proper tRNA with the help of a specific enzyme and ATP.

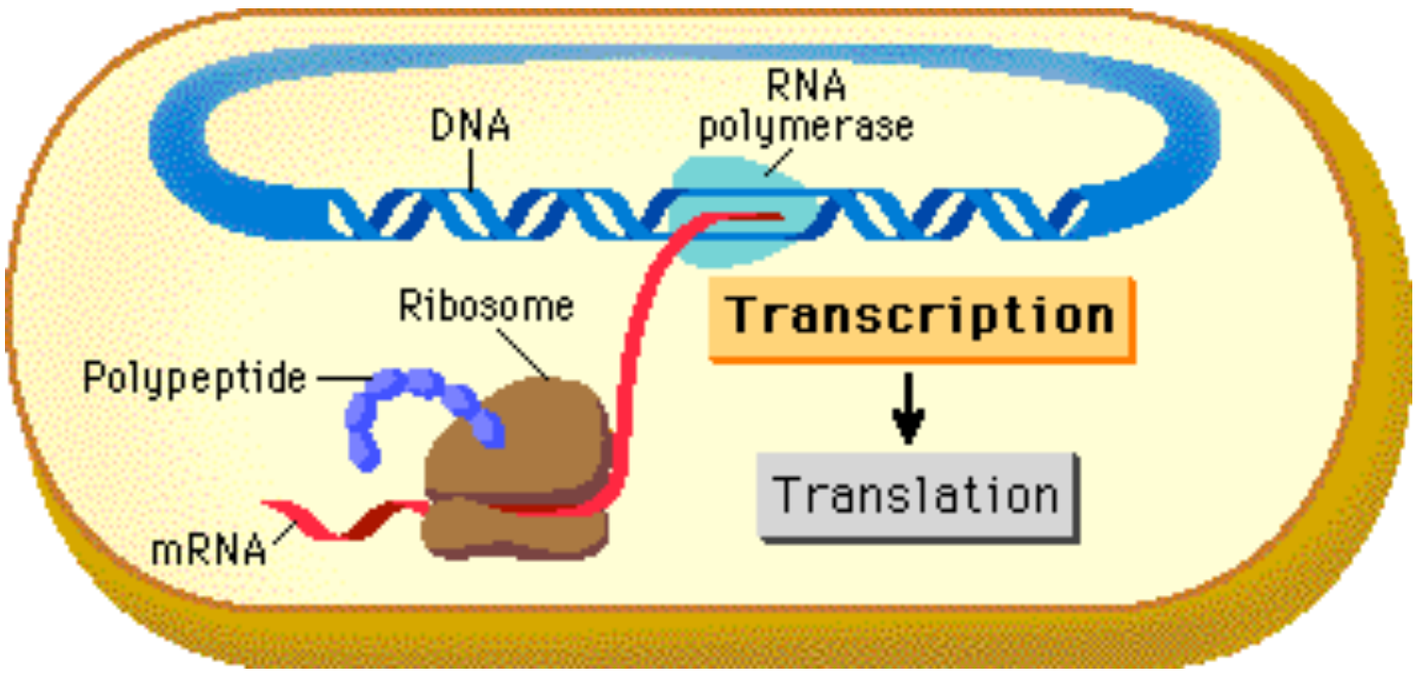
Poly-A

TRANSLATION

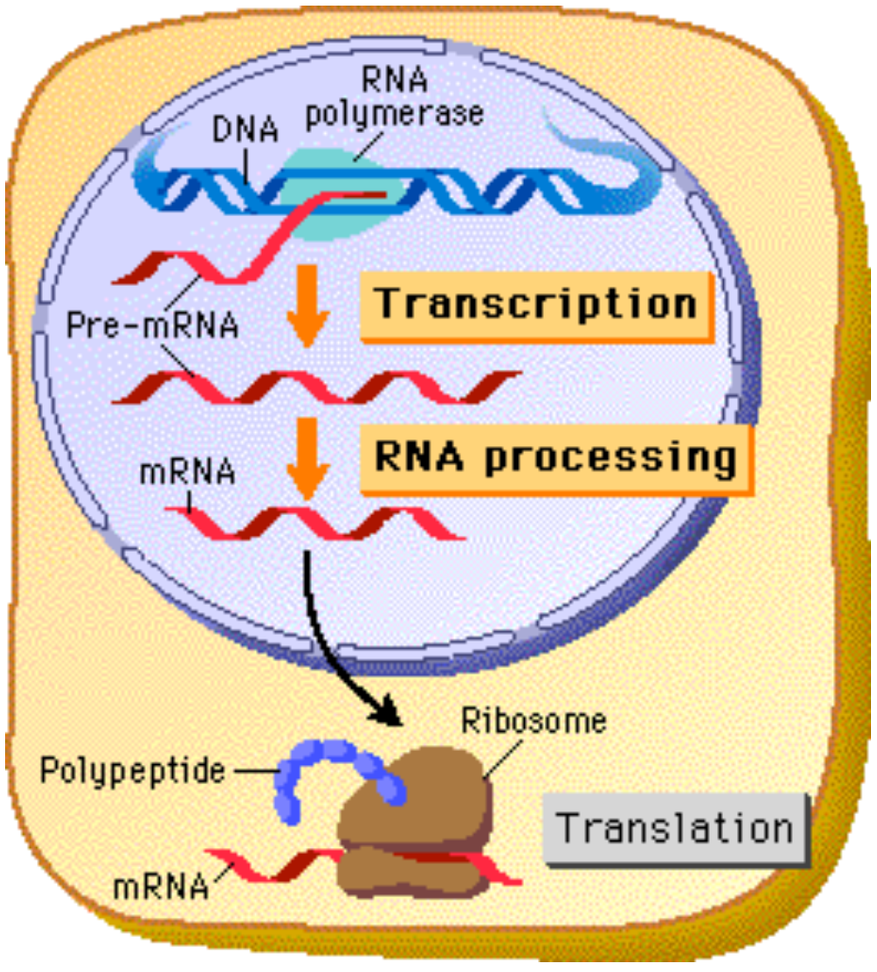
A succession of tRNAs add their amino acids to the polypeptide chain as the mRNA is moved through the ribosome one codon at a time. (When completed, the polypeptide is released from the ribosome.)

Final Comparisons

- **Transcription & Translation occurs in every organism.**
  - ***However, one very important difference exists between prokaryotes and eukaryotes***



Prokaryotic T&T are not separated by time and space



Eukaryotic T&T separated by time and space

Final Comparisons

Feature	Bacteria	Archaea	Eukaryotes
Chromosome Structure	circular	circular	linear
RNA polymerase type	I	II	I, II, III
Transcription Factors	few, simple ones	complex set	complex set
Transcription Termination	falls off at terminator	continues past terminator	continues past terminator
mRNA introns	absent	absent	present
RNA processing	absent	absent	present
Ribosome size	70s	70s	80s

A Review of Different RNA's

Type of RNA	Functions
Messenger RNA (mRNA)	Carries information specifying amino acid sequences of proteins from DNA to ribosomes.
Transfer RNA (tRNA)	Serves as adapter molecule in protein synthesis; translates mRNA codons into amino acids.
Ribosomal RNA (rRNA)	Plays catalytic (ribozyme) roles and structural roles in ribosomes.
Primary transcript	Serves as a precursor to mRNA, rRNA, or tRNA, before being processed by splicing or cleavage. Some intron RNA acts as a ribozyme, catalyzing its own splicing.
Small nuclear RNA (snRNA)	Plays structural and catalytic roles in spliceosomes, the complexes of protein and RNA that splice pre-mRNA.
SRP RNA	Is a component of the signal-recognition particle (SRP), the protein-RNA complex that recognizes the signal peptides of polypeptides targeted to the ER.
Small nucleolar RNA (snoRNA)	Aids in processing of pre-rRNA transcripts for ribosome subunit formation in the nucleolus.
Small interfering RNA (siRNA) and microRNA (miRNA)	Are involved in regulation of gene expression.