UNIT 3-MOLECULAR GENETICS

Focal points

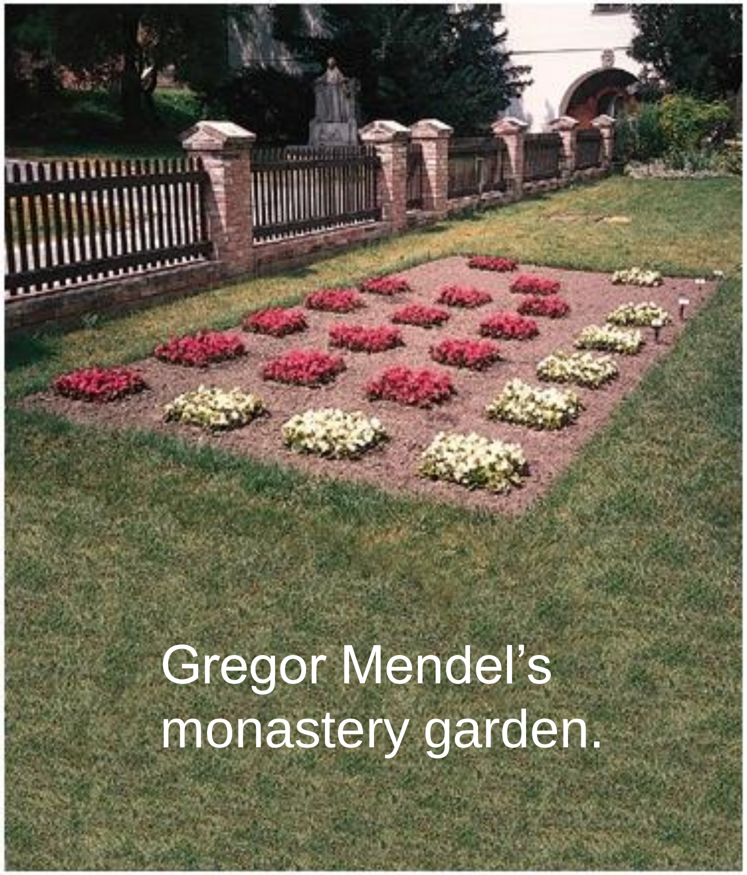
- Mendelians discovery
- DNA and RNA replication and repair
- Translation and the Genetic code

HEREDITY

- What genetic principles account for the transmission of traits from parents to offspring?
- One possible explanation of heredity is a "blending" hypothesis - The idea that genetic material contributed by two parents mixes in a manner analogous to the way blue and yellow paints blend to make green
- An alternative to the blending model is the "particulate" hypothesis of inheritance: the gene idea - Parents pass on discrete heritable units, genes.

GREGOR MENDEL

- Documented a particulate mechanism of inheritance through his experiments with garden peas



Gregor Mendel’s monastery garden.

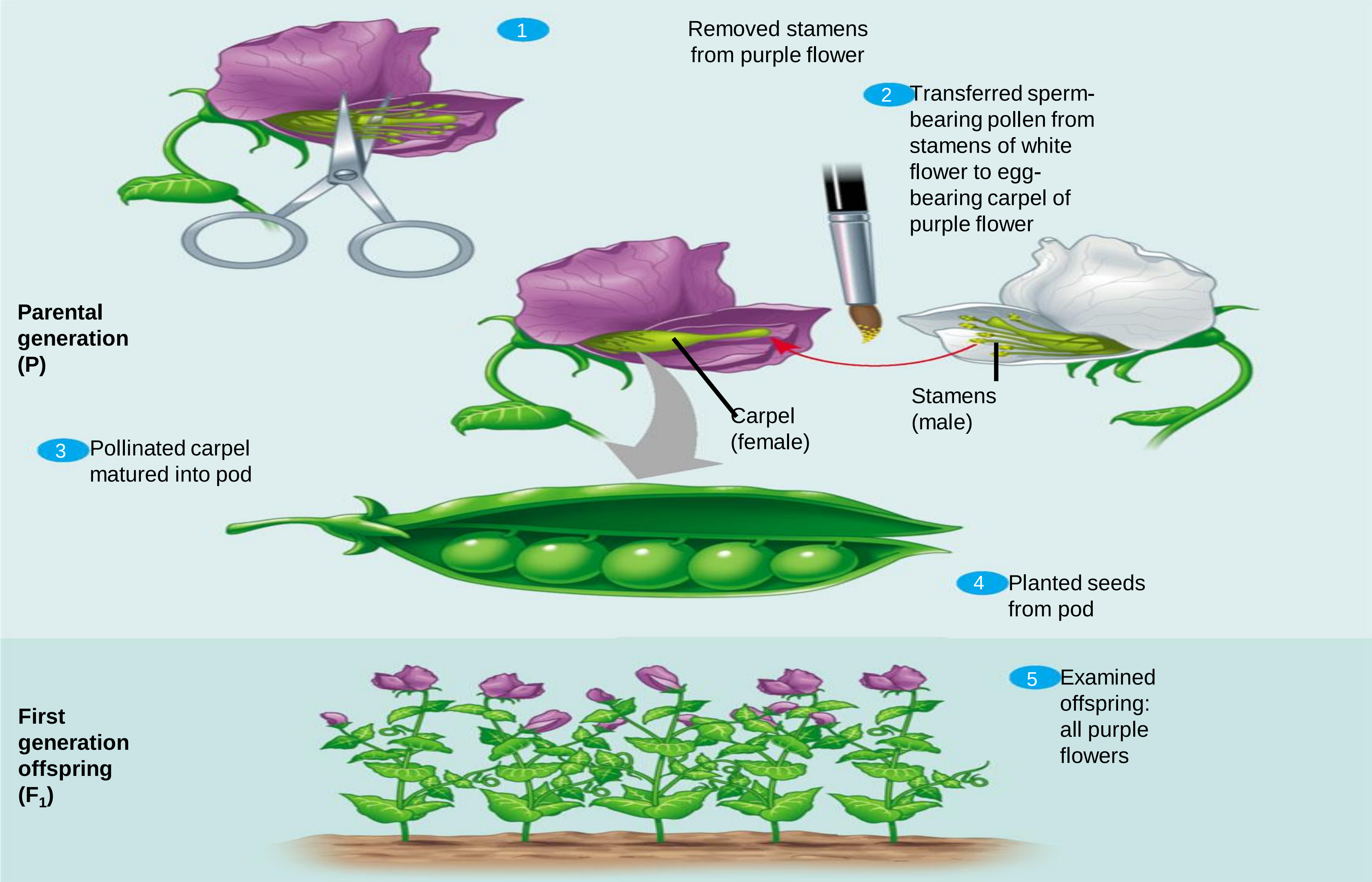
THEMES OF MENDEL'S WORK

- Variation is widespread in nature.
- Observable variation is essential for following genes.
- Variation is inherited according to genetic laws and not solely by chance.
- Mendel's laws apply to all sexually reproducing organisms.

MENDEL'S EXPERIMENTAL, QUANTITATIVE APPROACH

- Mendel used the scientific approach to identify two laws of inheritance
- Mendel discovered the basic principles of heredity by breeding garden peas in carefully planned experiments
- Mendel chose to work with the garden pea (Pisum sativum)
  - Because they are available in many varieties, easy to grow, easy to get large numbers
  - Because he could strictly control which plants mated with which

CROSSING PEA PLANTS



1 Removed stamens from purple flower

2 Transferred sperm-bearing pollen from stamens of white flower to egg-bearing carpel of purple flower

Parental generation (P)

Carpel (female)

Stamens (male)

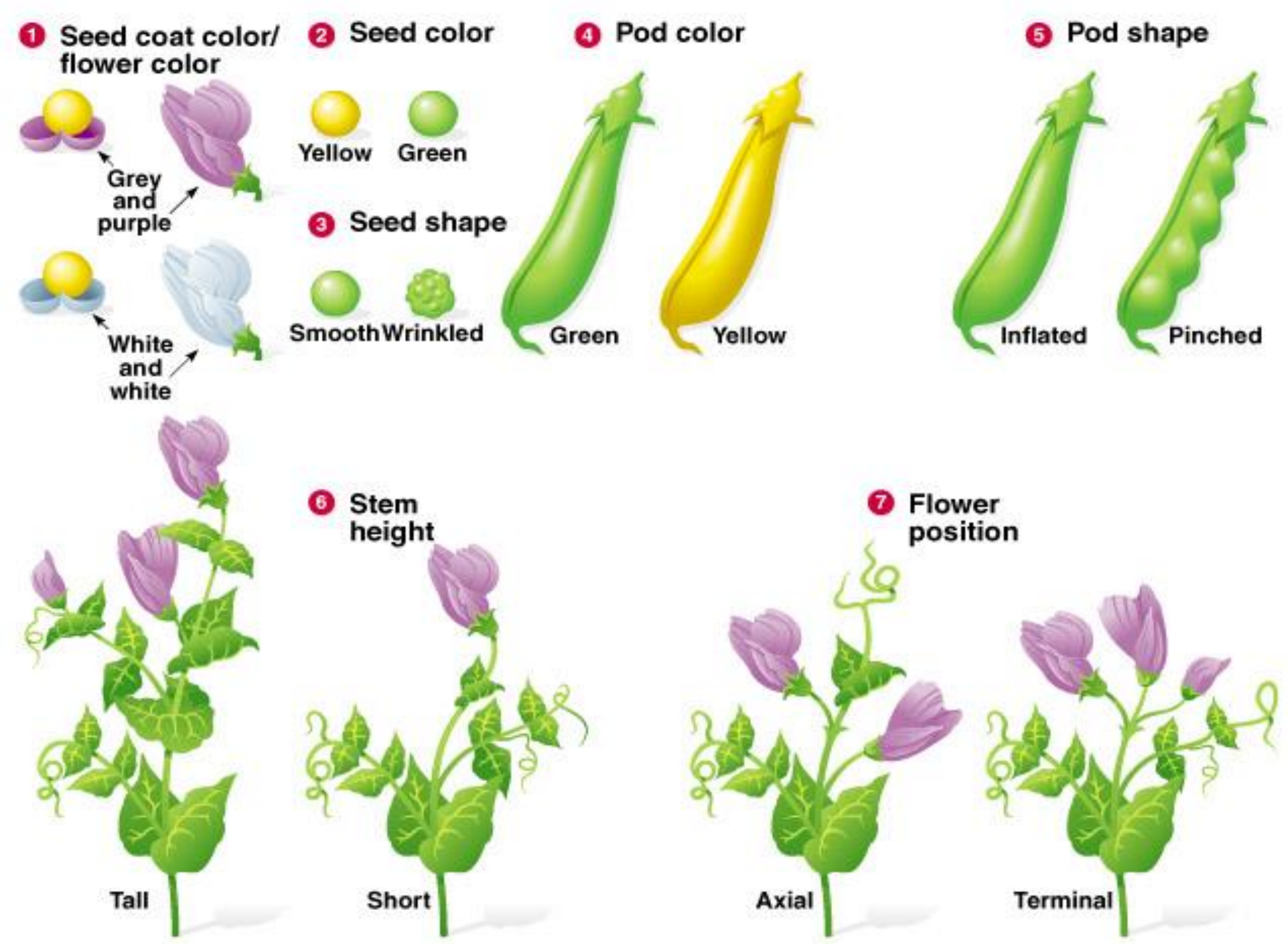
3 Pollinated carpel matured into pod

4 Planted seeds from pod

First generation offspring (F_1)

5 Examined offspring: all purple flowers

MENDEL'S STUDIED DISCRETE TRAITS



GENETIC VOCABULARY

- Character: a heritable feature, such as flower color
- Trait: a variant of a character, such as purple or white flowers
- Each trait carries two copies of a unit of inheritance, one inherited from the mother and the other from the father
- Alternative forms of traits are called **alleles.**

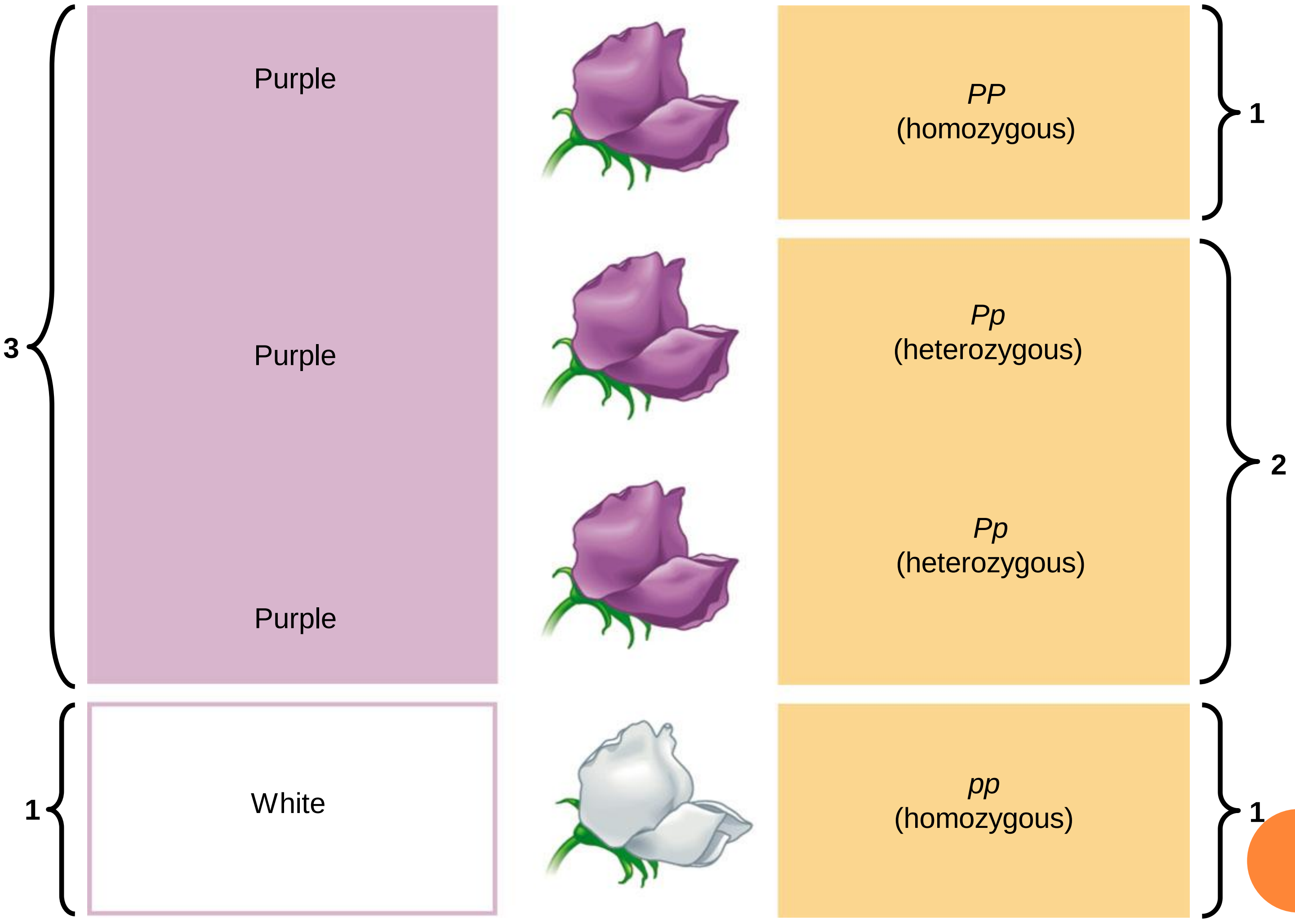
GENETIC VOCABULARY

- Phenotype - observable characteristic of an organism.
- Genotype - pair of alleles present in and individual.
- Homozygous - two alleles of trait are the same (YY or yy).
- Heterozygous - two alleles of trait are different (Yy).
- Capitalized traits = dominant phenotypes
- Lowercase traits= recessive phenotypes.

GENETIC VOCABULARY

- Generations:
  - P = parental generation
  - F1 = 1st filial generation, progeny of the P generation
  - F2 = 2nd filial generation, progeny of the F1 generation (F3 and so on)
- Crosses:
  - Monohybrid cross = cross of two different true-breeding strains (homozygotes) that differ in a single trait.
  - Dihybrid cross = cross of two different true-breeding strains (homozygotes) that differ in two traits.

PHENOTYPE VS GENOTYPE



Phenotype		Genotype	
3	Purple	*PP* (homozygous)	1
	Purple	*Pp* (heterozygous)	2
	Purple	*Pp* (heterozygous)	
1	White	*pp* (homozygous)	1

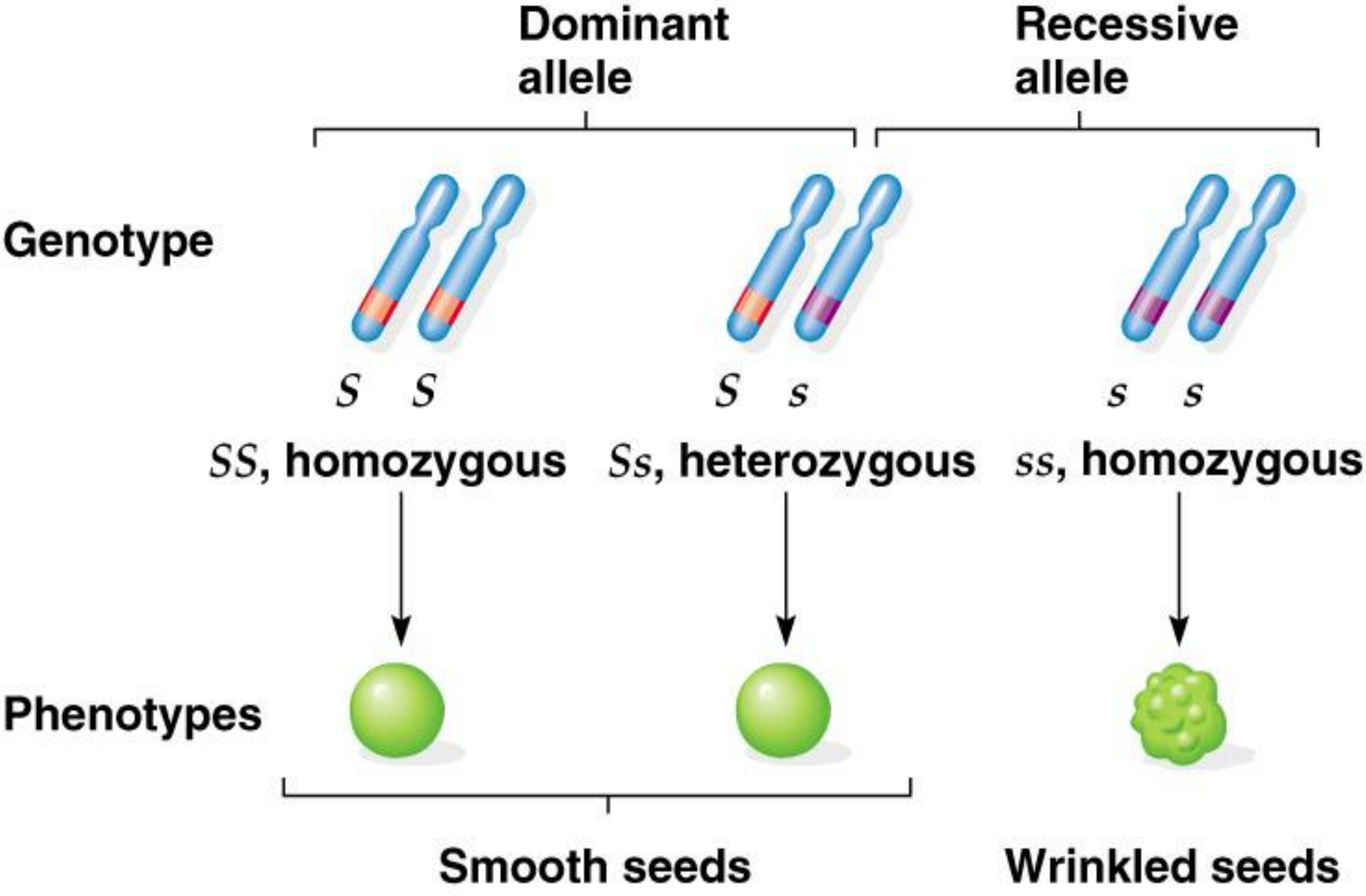
Ratio 3:1

Ratio 1:2:1

Figure 14.6

PHENOTYPE VS GENOTYPE

Dominant & recessive alleles

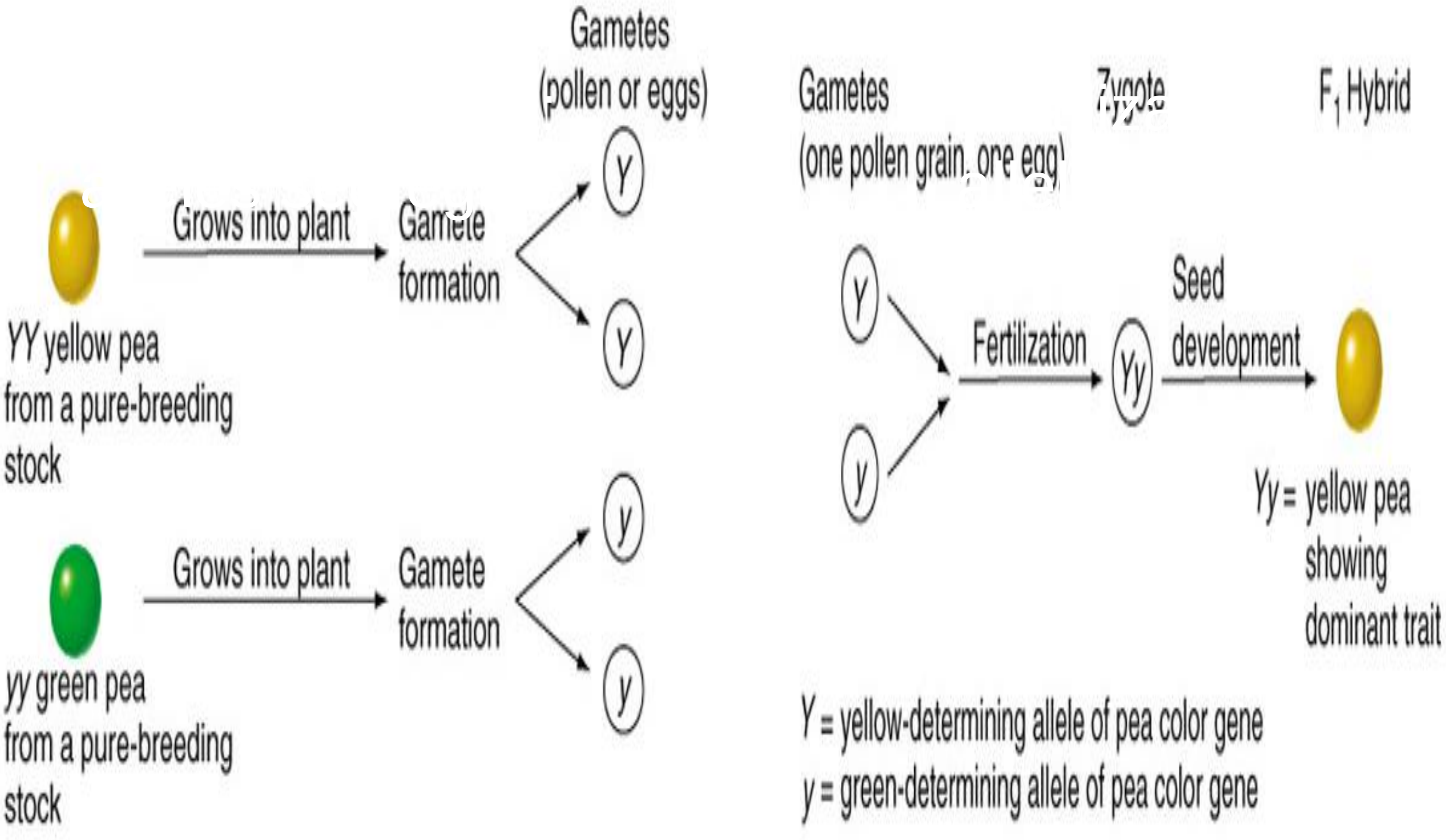


MENDEL'S EXPERIMENTAL DESIGN

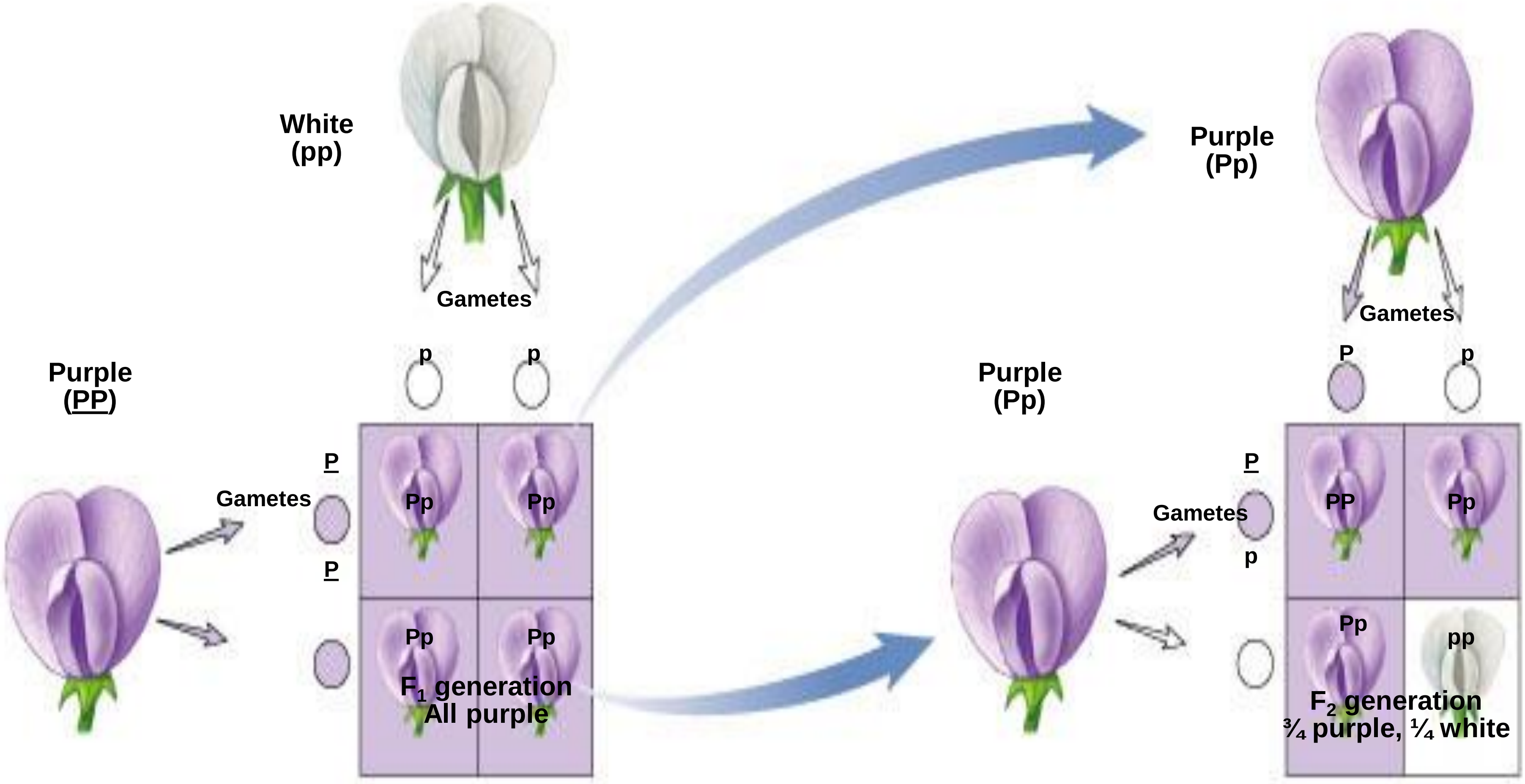
- In a typical breeding experiment Mendel mated two contrasting, true-breeding varieties, a process called hybridization.
- The true-breeding parents are called the P generation.
- The hybrid offspring of the P generation are called the F1 generation.
- When F1 individuals self-pollinate the F2 generation is produced.

LAW OF SEGREGATION

- Mechanism of gene transmission



MENDEL'S MONOHYBRID CROSS



MENDEL OBSERVED THE SAME PATTERN IN CHARACTERS

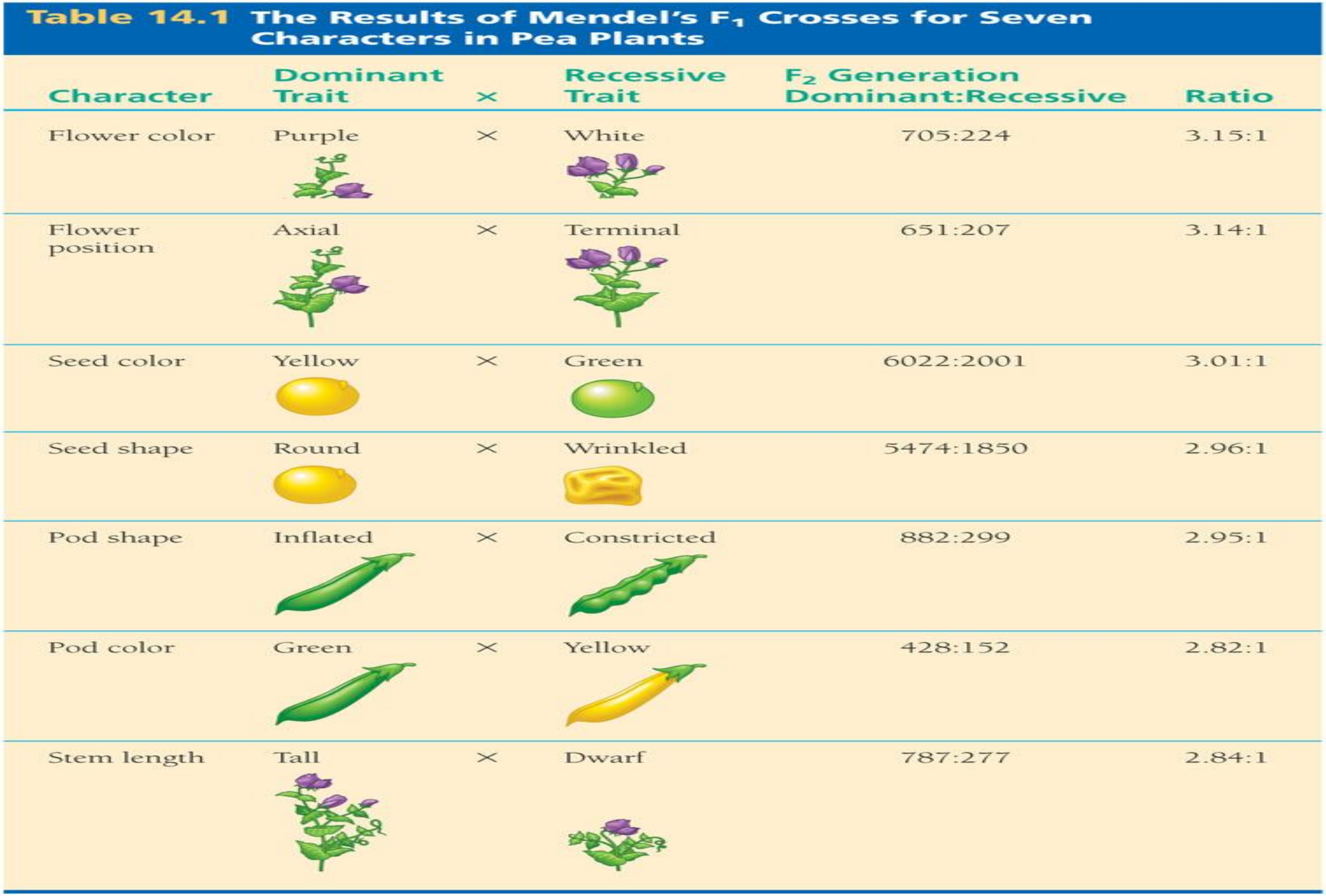


Table 14.1 The Results of Mendel's F_1 Crosses for Seven Characters in Pea Plants

Character	Dominant Trait	×	Recessive Trait	F_2 Generation Dominant:Recessive	Ratio
Flower color	Purple	×	White	705:224	3.15:1
Flower position	Axial	×	Terminal	651:207	3.14:1
Seed color	Yellow	×	Green	6022:2001	3.01:1
Seed shape	Round	×	Wrinkled	5474:1850	2.96:1
Pod shape	Inflated	×	Constricted	882:299	2.95:1
Pod color	Green	×	Yellow	428:152	2.82:1
Stem length	Tall	×	Dwarf	787:277	2.84:1

THE LAW OF INDEPENDENT ASSORTMENT

- Mendel derived the law of segregation by following a single trait
  - 2 alleles at a single gene locus segregate when the gametes are formed
  - The F1 offspring produced in this cross were monohybrids, heterozygous for one character
- Mendel identified his second law of inheritance by following two characters at the same time
  - Mendel was interested in determining whether alleles at 2 different gene loci segregate dependently or independently
  - Crossing two, true-breeding parents differing in two characters produces dihybrids in the F1 generation, heterozygous for both characters

DIHYBRID CROSS

- With his monohybrid crosses, Mendel determined that the 2 alleles at a single gene locus segregate when the gametes are formed.
- With his dihybrid crosses, Mendel was interested in determining whether alleles at 2 different gene loci segregate dependently or independently.

DIHYBRID CROSS

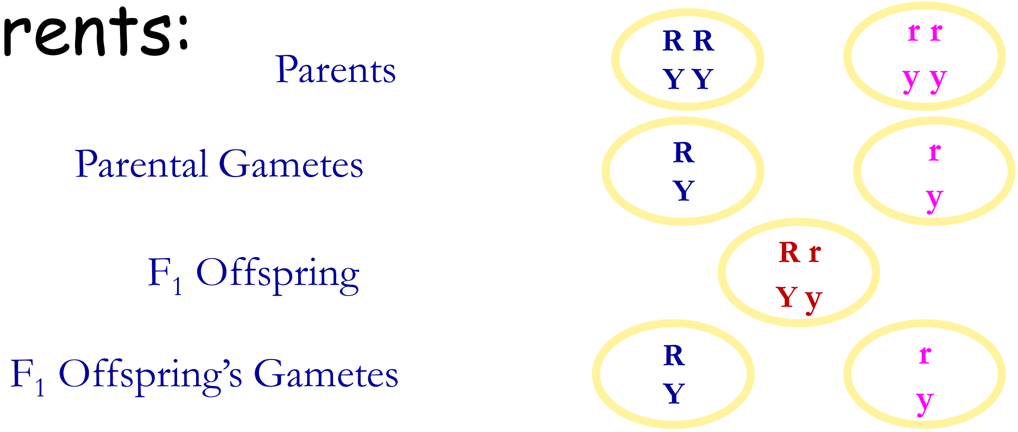
- For example, in pea plants seed shape is controlled by one gene locus where round (R) is dominant to wrinkled (r) while seed color is controlled by a different gene locus where yellow (Y) is dominant to green (y).
- Mendel crossed 2 pure-breeding plants: one with round yellow seeds and the other with green wrinkled seeds.

Dihybrid Cross

- For example, in pea plants seed shape is controlled by one gene locus where round (R) is dominant to wrinkled (r) while seed color is controlled by a different gene locus where yellow (Y) is dominant to green (y).
- Mendel crossed 2 pure-breeding plants: one with round yellow seeds and the other with green wrinkled seeds.

DEPENDENT SEGREGATION

- If dependent segregation (assortment) occurs:
  - Alleles at the 2 gene loci segregate together, and are transmitted as a unit.
  - Therefore, each plant would only produce gametes with the same combinations of alleles present in the gametes inherited from its parents:



Parents: R R Y Y; r r y y

Parental Gametes: R Y; r y

F_1 Offspring: R r Y y

F_1 Offspring's Gametes: R Y; r y

What is the expected phenotypic ratio for the F_2?

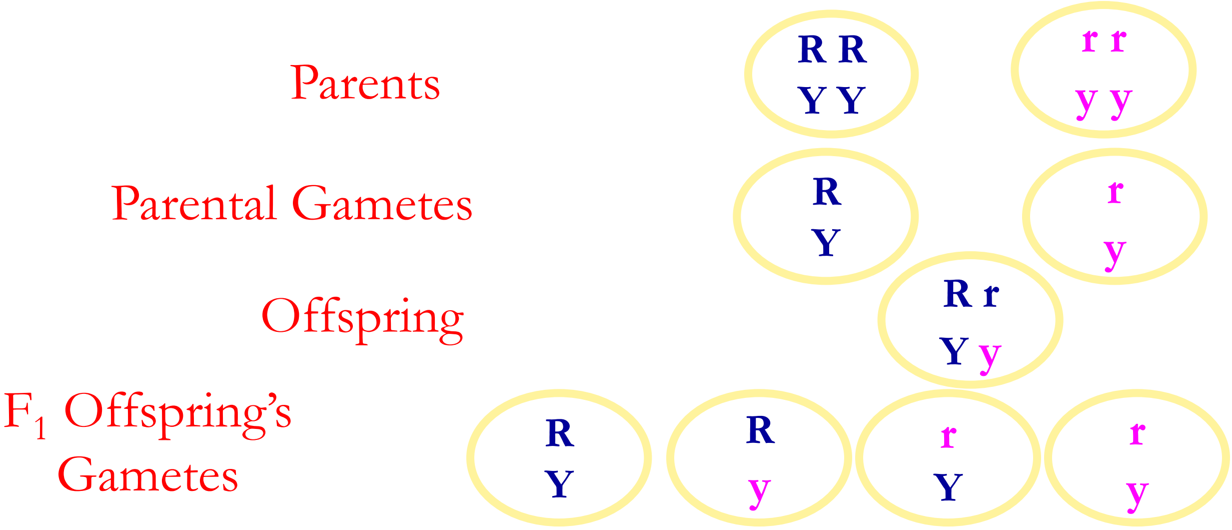
F_2 With Dependent Assortment:

	R Y	r y
R Y	R R Y Y	R r Y y
r y	R r Y y	r r y y

Ratio is 3 round, yellow : 1 wrinkled, green

INDEPENDENT SEGREGATION

- Alleles at the 2 gene loci segregate (separate) independently, and are NOT transmitted as a unit. Therefore, each plant would produce gametes with allele combinations that were not present in the gametes inherited from its parents:



Parents	R R Y Y	r r y y		
Parental Gametes	R Y	r y		
Offspring	R r Y y			
F_1 Offspring's Gametes	R Y	R y	r Y	r y

What is the expected phenotypic ratio for the F_2?

Mendelian Genetics

Dihybrid cross - parental generation differs in two traits
example-- cross round/yellow peas with wrinkled/green ones.
Round/yellow is dominant.

	RY	Ry	rY	ry
RY				
Ry				
rY				
ry				

What are the expected phenotype ratios in the F_2 generation?

round, yellow =

round, green =

wrinkled, yellow =

wrinkled, green =

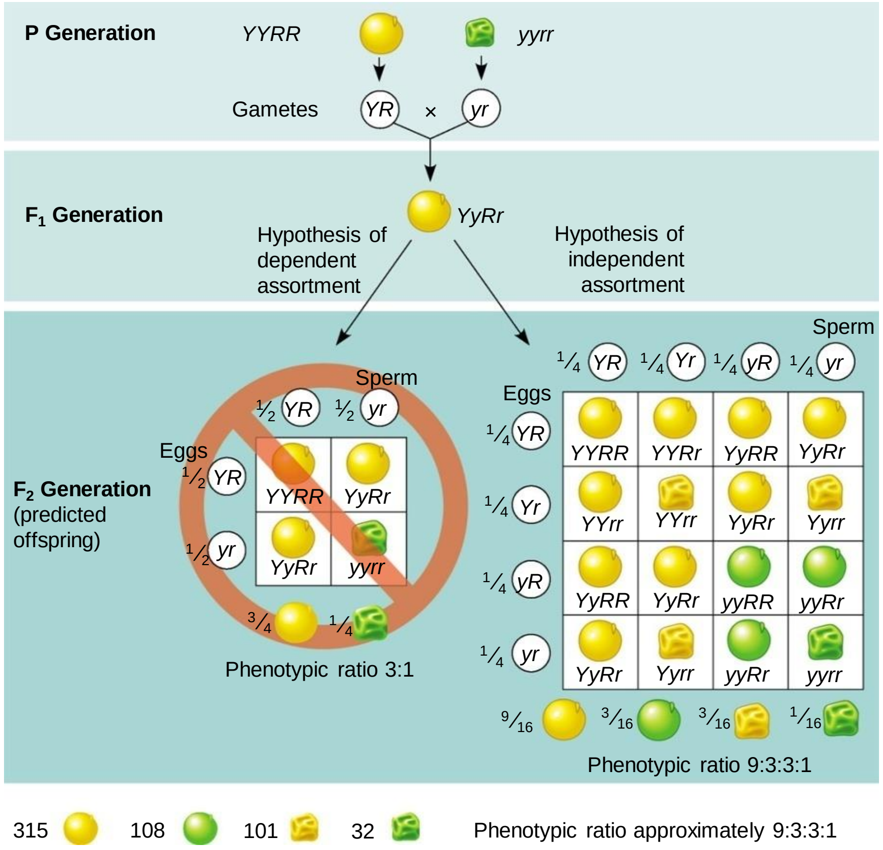
F2 WITH INDEPENDENT ASSORTMENT

	RY	Ry	rY	ry
RY	RR YY	RR Yy	Rr YY	Rr Yy
Ry	RR Yy	RR yy	Rr Yy	Rr yy
rY	Rr YY	Rr Yy	rr YY	rr Yy
ry	Rr Yy	Rr yy	rr Yy	rr yy

A DIHYBRID CROSS

- How are two characters transmitted from parents to offspring?
  - As a package?
  - Independently?
- A dihybrid cross
  - Illustrates the inheritance of two characters
  - Produces four phenotypes in the F2 generation

EXPERIMENT Two true-breeding pea plants—one with yellow-round seeds and the other with green-wrinkled seeds—were crossed, producing dihybrid F_1 plants. Self-pollination of the F_1 dihybrids, which are heterozygous for both characters, produced the F_2 generation. The two hypotheses predict different phenotypic ratios. Note that yellow color (*Y*) and round shape (*R*) are dominant.

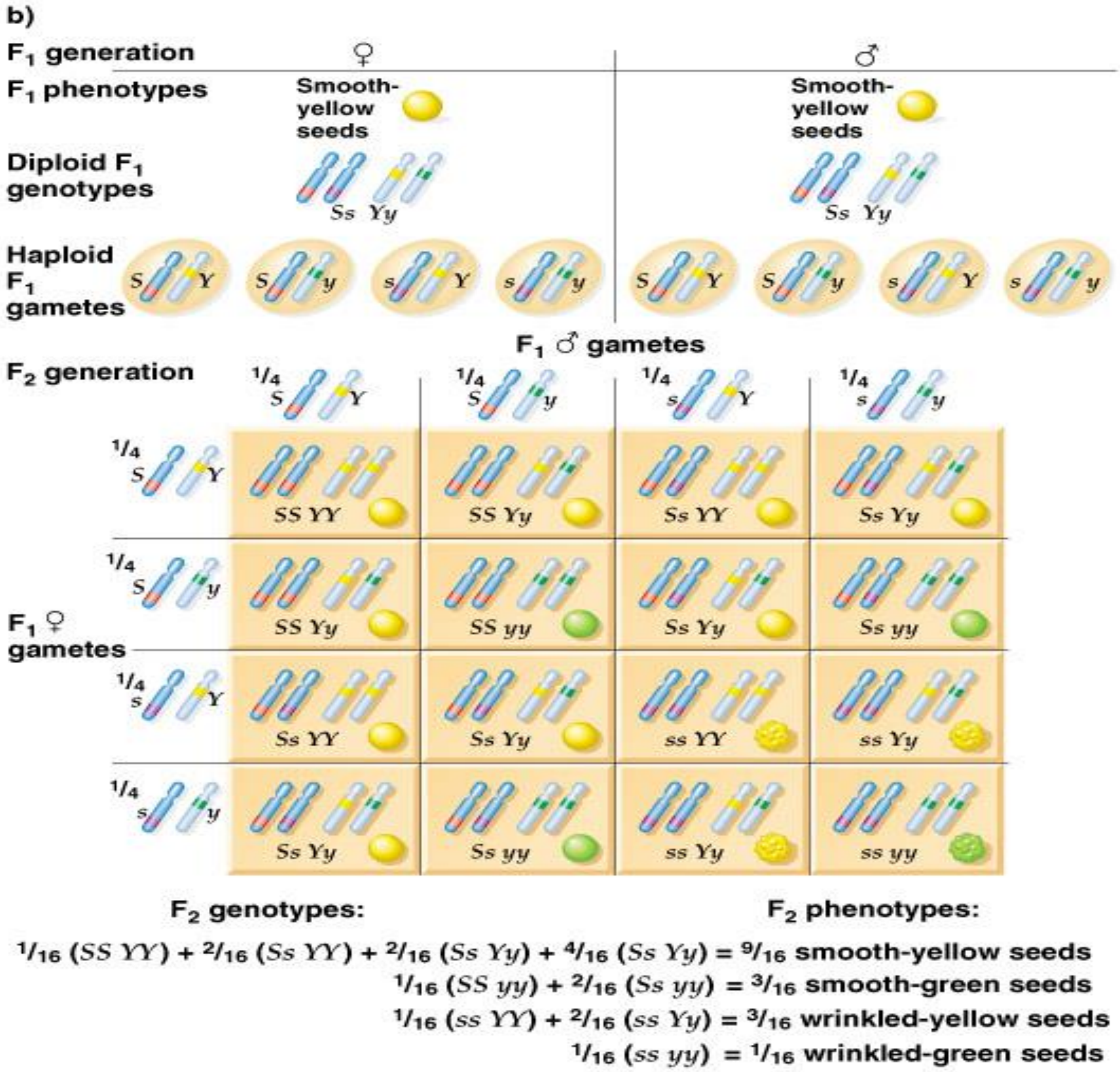


CONCLUSION The results support the hypothesis of independent assortment. The alleles for seed color and seed shape sort into gametes independently of each other.

DIHYBRID CROSS

- F_2 generation ratio:

  9:3:3:1



F_2 genotypes: F_2 phenotypes:

1/16 (SS YY) + 2/16 (Ss YY) + 2/16 (Ss Yy) + 4/16 (Ss Yy) = 9/16 smooth-yellow seeds
1/16 (SS yy) + 2/16 (Ss yy) = 3/16 smooth-green seeds
1/16 (ss YY) + 2/16 (ss Yy) = 3/16 wrinkled-yellow seeds
1/16 (ss yy) = 1/16 wrinkled-green seeds

LAW OF INDEPENDENT ASSORTMENT

- Mendel's dihybrid crosses showed a 9:3:3:1 phenotypic ratio for the F2 generation.
- Based on these data, he proposed the Law of Independent Assortment, which states that when gametes form, each pair of hereditary factors (alleles) segregates independently of the other pairs.

MENDEL'S CONCLUSIONS

- Genes are distinct entities that remain unchanged during crosses
- Each plant has two alleles of a gene
- Alleles segregated into gametes in equal proportions, each gamete got only one allele
- During gamete fusion, the number of alleles was restored to two

SUMMARY OF MENDEL'S PRINCIPLES

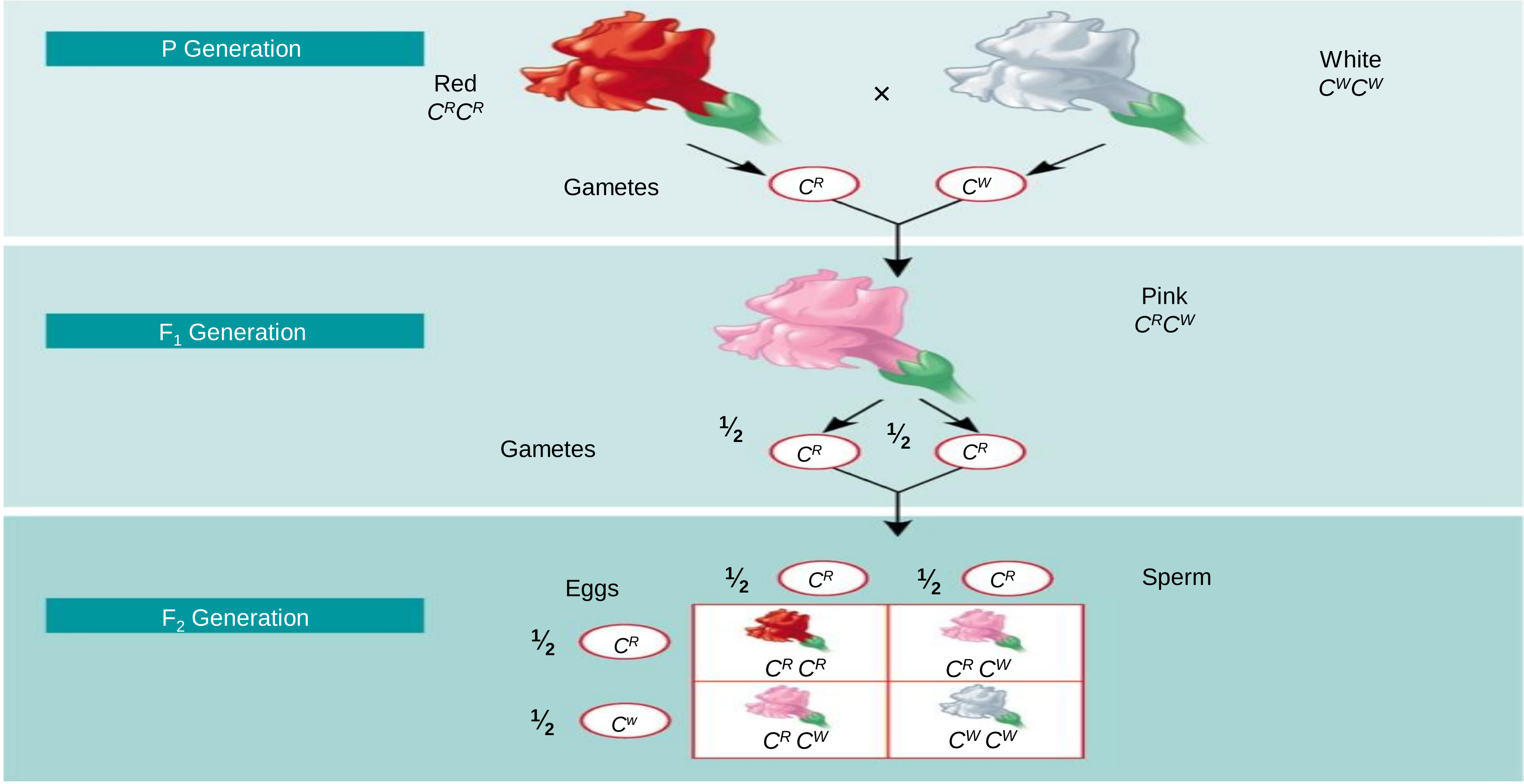
- Mendel's Principle of Uniformity in F1:
  - F1 offspring of a monohybrid cross of true-breeding strains resemble only one of the parents.
  - Why? Smooth seeds (allele S) are completely dominant to wrinkled seeds (alleles).
- Mendel's Law of Segregation:
  - Recessive characters masked in the F1 progeny of two true-breeding strains, reappear in a specific proportion of the F2 progeny.
  - Two members of a gene pair segregate (separate) from each other during the formation of gametes.
- Mendel's Law of Independent Assortment:
  - Alleles for different traits assort independently of one another.
  - Genes on different chromosomes behave independently in gamete production.

EXCEPTIONS TO MENDEL'S ORIGINAL PRINCIPLES

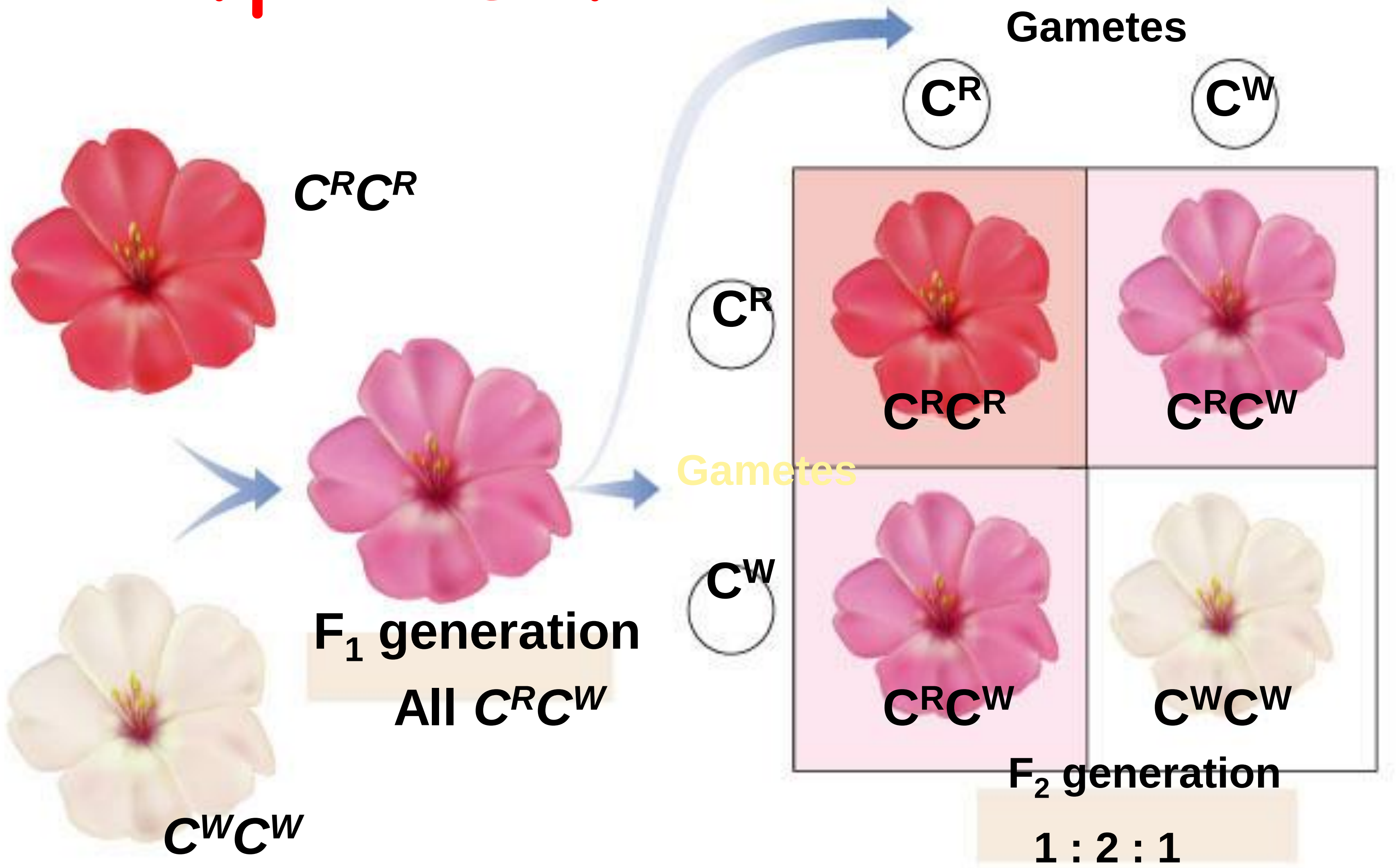
- Incomplete dominance
- Codominance
- Multiple alleles
- Polygenic traits
- Epistasis
- Pleiotropy
- Environmental effects on gene expression
- Linkage
- Sex linkage

INCOMPLETE DOMINANCE

- Neither allele is dominant and heterozygous individuals have an intermediate phenotype
- For example, in Japanese “Four o’clock”, plants with one red allele and one white allele have pink flowers:



Incomplete Dominance



C^RC^R

C^WC^W

F_1 generation

All C^RC^W

Gametes

Gametes

	C^R	C^W
C^R	C^RC^R	C^RC^W
C^W	C^RC^W	C^WC^W

F_2 generation

1 : 2 : 1

CODOMINANCE

- Neither allele is dominant and both alleles are expressed in heterozygous individuals
- Example ABO blood types

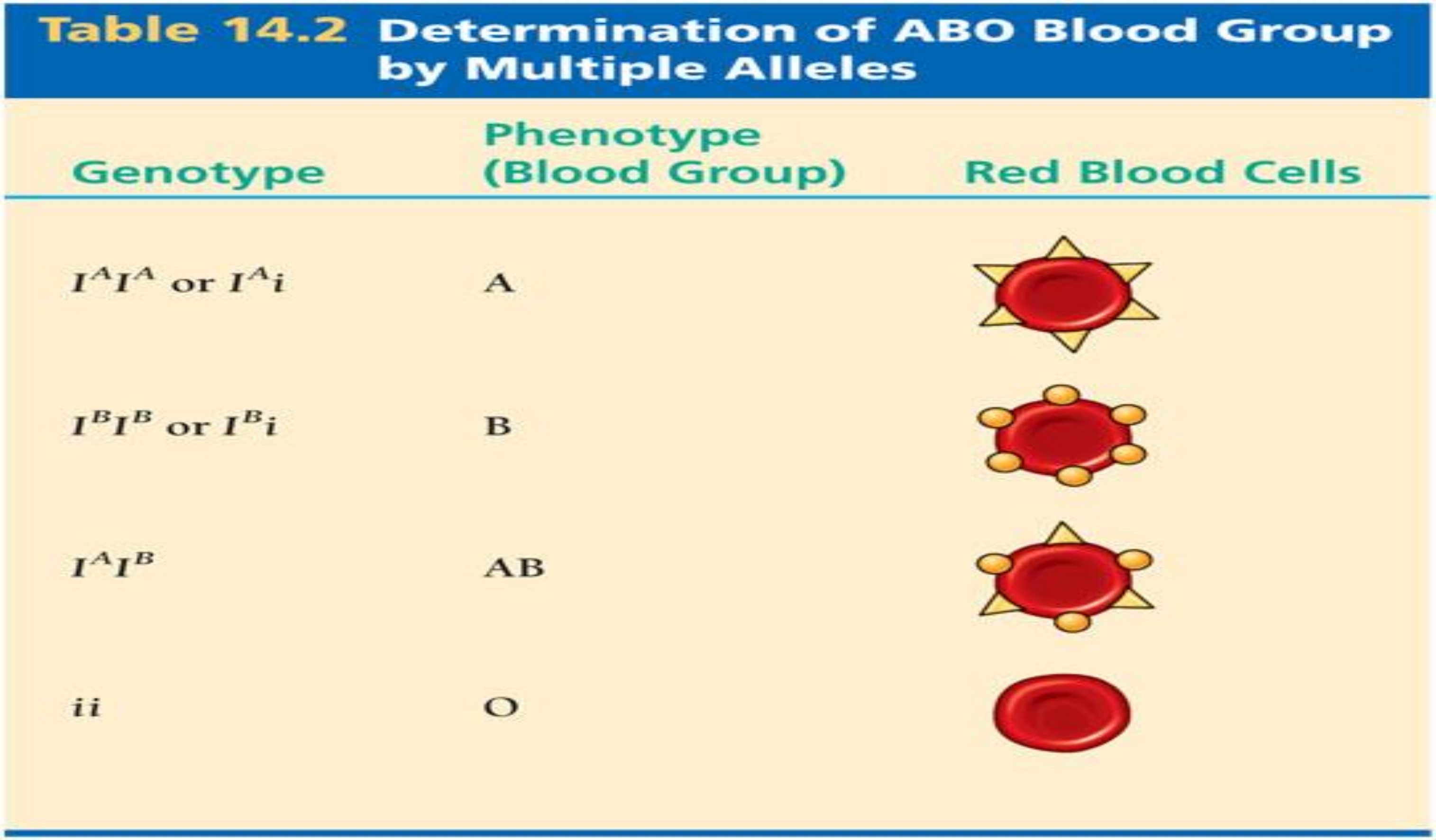


Table 14.2 Determination of ABO Blood Group by Multiple Alleles

Genotype	Phenotype (Blood Group)	Red Blood Cells
I^AI^A or I^Ai	A	
I^BI^B or I^Bi	B	
I^AI^B	AB	
ii	O	

EXCERCISE

1. In peas, tall is dominant over dwarf. If a plant homozygous for tall is crossed with one homozygous for dwarf:

a. What will be the appearance of F1 plants?

b. What will be the phenotypes of the F2, and what fraction of the offspring will have each phenotype?

c. What will be the phenotypes and fractions if an F1 plant is crossed with its tall parent?

d. What will be the phenotypes and fractions if an F1 plant is crossed with its short parent?

2. How many different kinds of gametes can the offspring of TtppRr produce? What will be the expected genotypes and phenotypes resulting from a cross between two of these individuals?

QUIZ 5%

a. If a pure-breeding purple-flowered pea plant is crossed with a pure-breeding white-flowered pea plant, all the offspring have purple flowers. Suppose two F1 plants are crossed, and 2400 offspring are obtained.

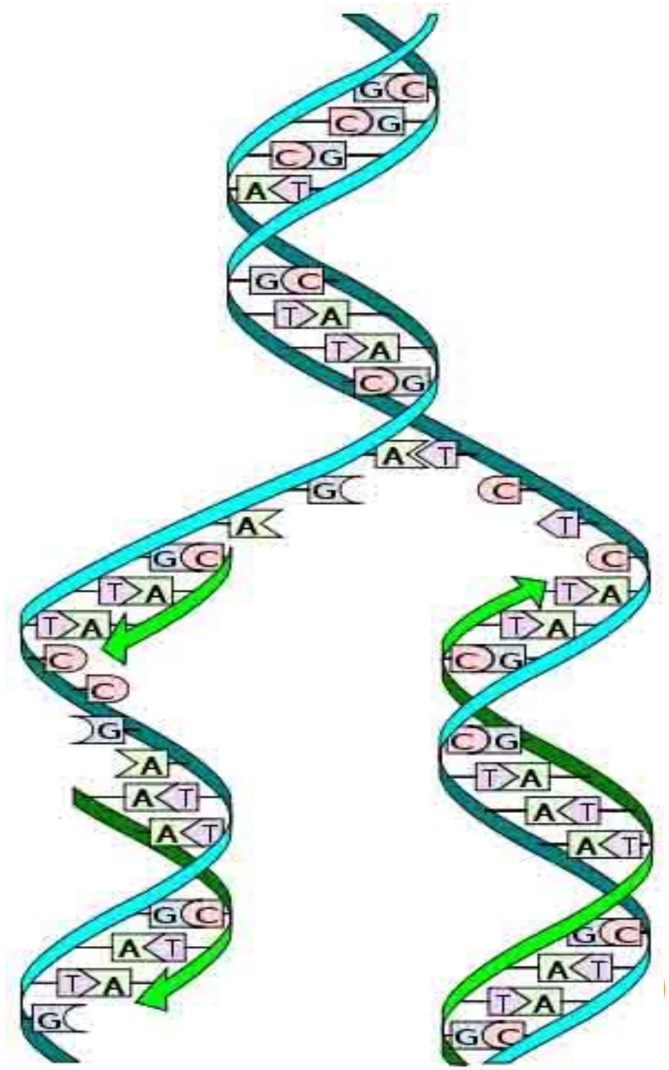
A. Use the punnet square and show the cross

B. How many white flowered plants will you expect?

C. How many purble flowered plants will you expect?

DNA REPLICATION

- **Copying** of a double-stranded DNA molecule.
- Each DNA strand holds the same genetic information, so each strand can serve as a template for the new, opposite strand.
- The **parent** strand is preserved and the **daughter** strand is assembled from nucleotides.
- This is called **semi-conservative** replication.
- Resulting double-stranded DNA molecules are identical.
- Q: **Why would a cell need to copy its DNA?**



DNA Replication

- Chromosomes
  - ✓ Strands of DNA that contain all of the genes an organism needs to survive and reproduce
- Genes
  - ✓ Segments of DNA that specify how to build a protein
    - genes may specify more than one protein in eukaryotes
  - ✓ Chromosome maps are used to show the **locus** (location) of genes on a chromosome

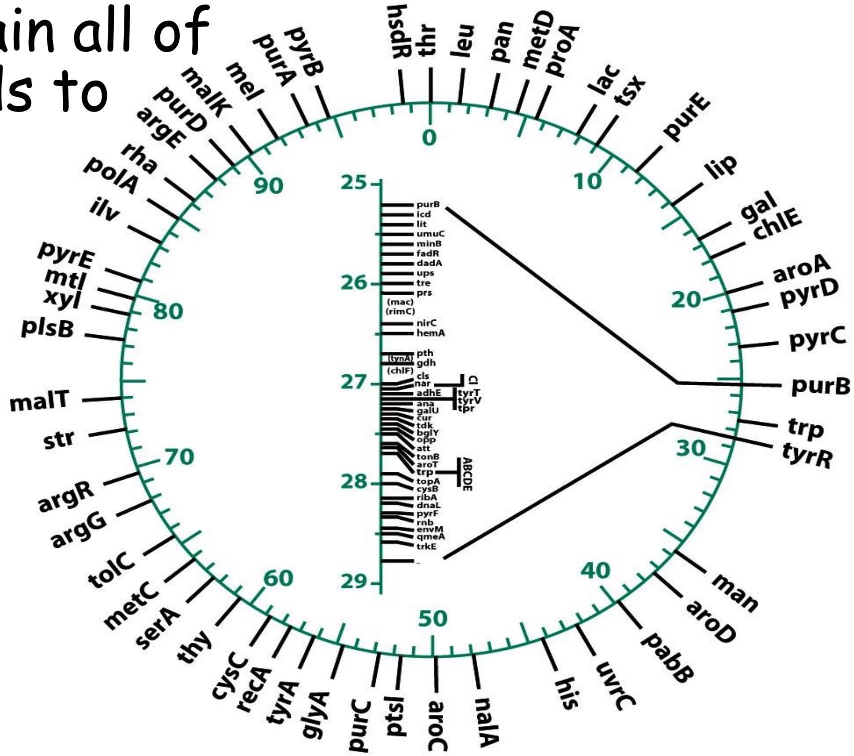


Figure 7-2a Microbiology, 7/e
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The E. Coli genome includes approximately 4,000 genes

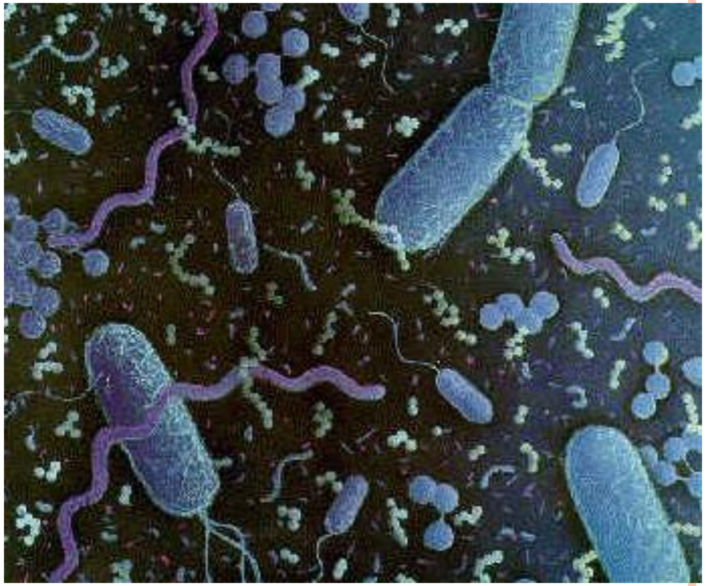
- In a cell, DNA replication must happen before cell division, because the new, daughter cell will also need a complete copy of cellular DNA

- Prokaryotes replicate their DNA throughout the interval between cell divisions.

- In eukaryotes, timing of replication is highly regulated.

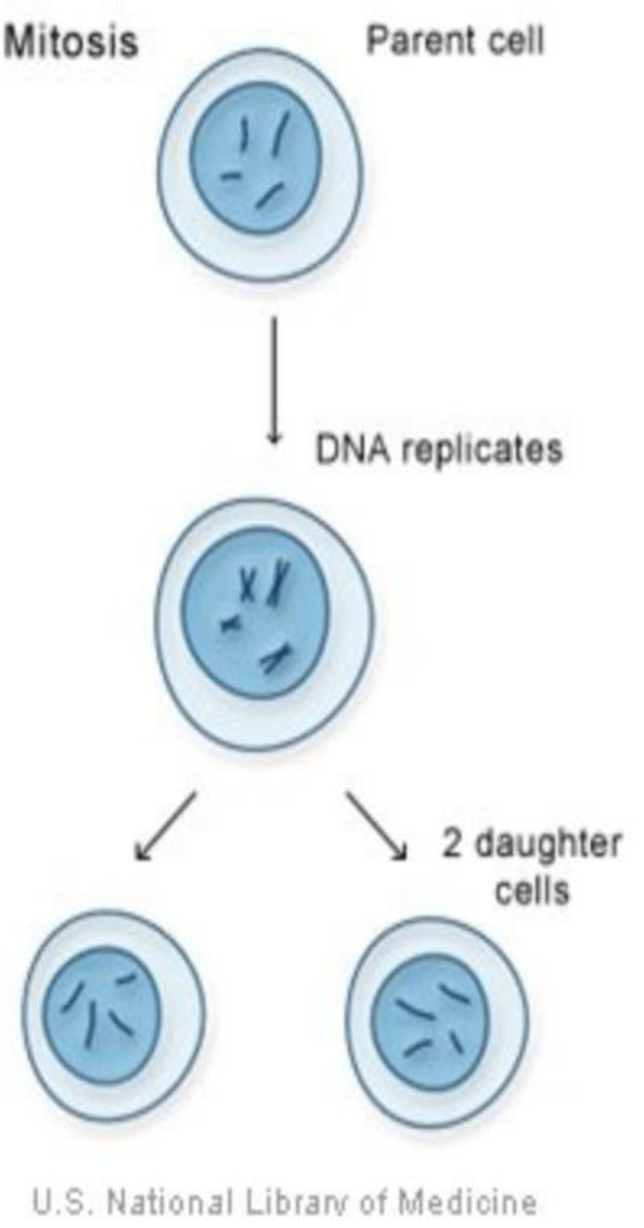
DNA Replication

- Genetic Variation
  - ✓ **Phenotypic** variation among organisms is due to **genotypic** variation (differences in the sequence of their DNA bases)
  - ✓ Differences exist between species and within a species
    - Different genes (**genomes**) → different proteins (**proteomes**)
    - Different versions of the same gene (**alleles**)
    - Differences in gene expression (**epigenetics**)



DNA Replication

- Cell Division (mitosis)
  - ✓ Cells must copy their chromosomes (**DNA synthesis**) before they divide so that each daughter cell will have a copy
  - ✓ A region of the chromosome remains uncopied (**centromere**) in order to hold the **sister chromatids** together
    - Keeps chromatids organized to help make sure each daughter cell gets exactly one copy
    - **Nondisjunction** is when sister chromatids do not assort correctly and one cell ends up with both copies while the other cell ends up with none



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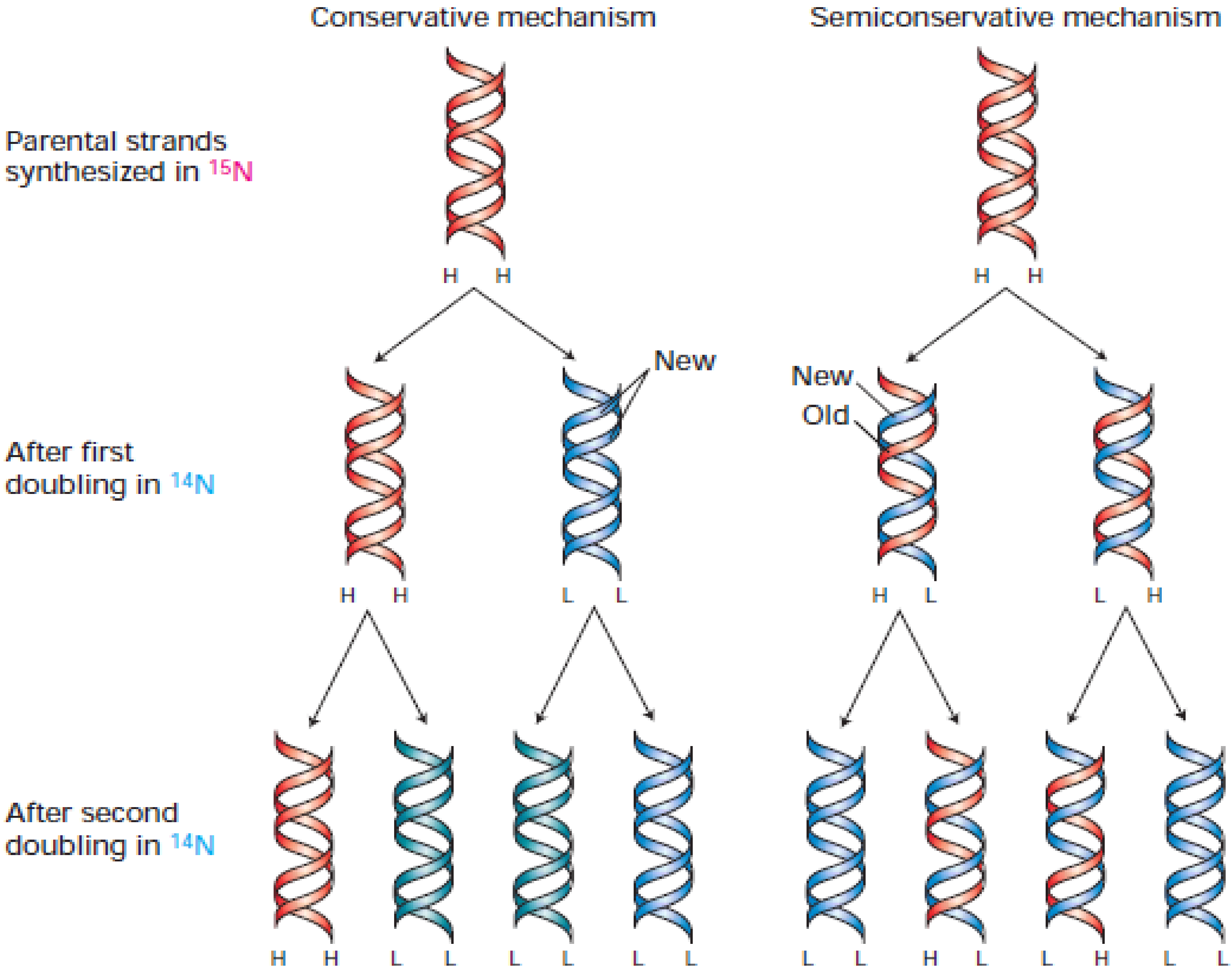
THE MESELSON-STAHI EXPERIMENT



Conservative mechanism

Semiconservative mechanism

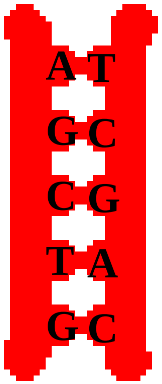
Parental strands synthesized in ^{15}N

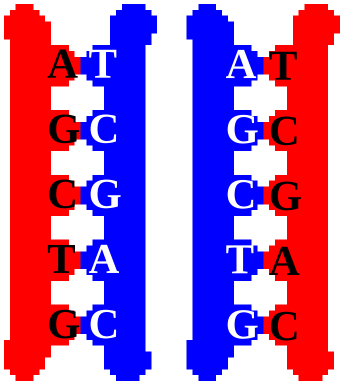
After first doubling in ^{14}N

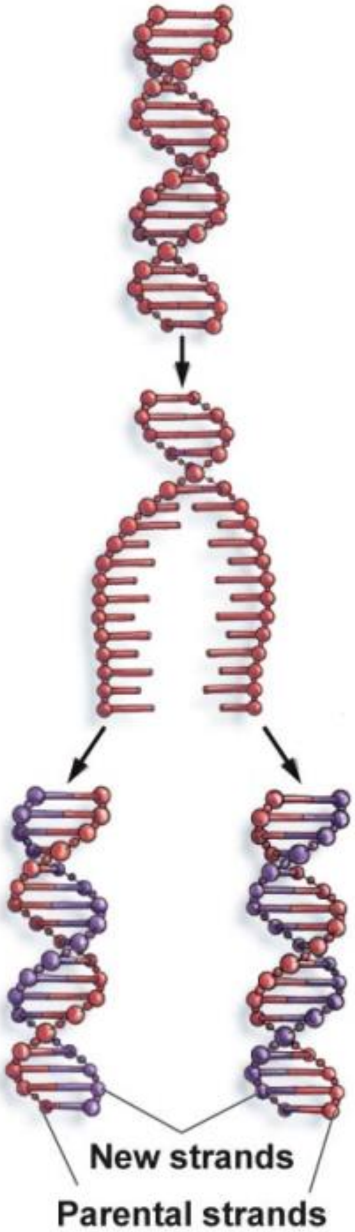
After second doubling in ^{14}N

DNA Replication

- DNA Synthesis
  - ✓ The DNA bases on each strand act as a template to synthesize a complementary strand
    - Recall that Adenine (A) pairs with thymine (T) and guanine (G) pairs with cytosine (C)
  - ✓ The process is **semiconservative** because each new double-stranded DNA contains one old strand (template) and one newly-synthesized complementary strand

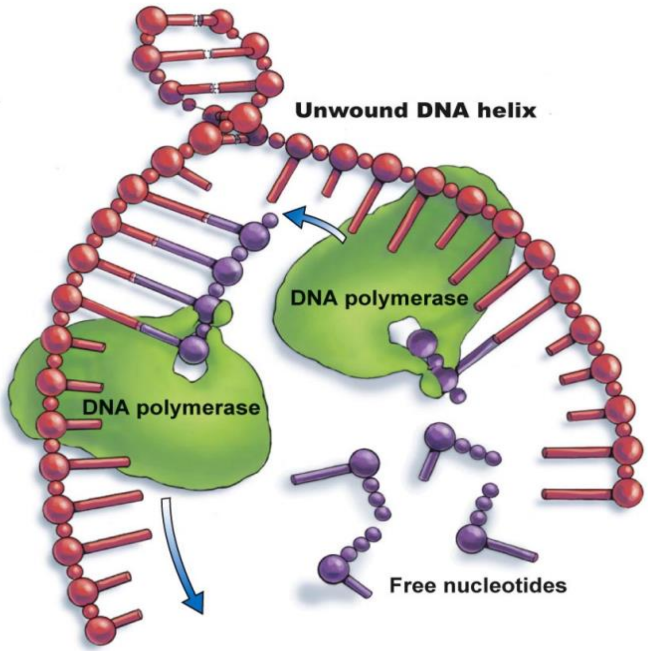




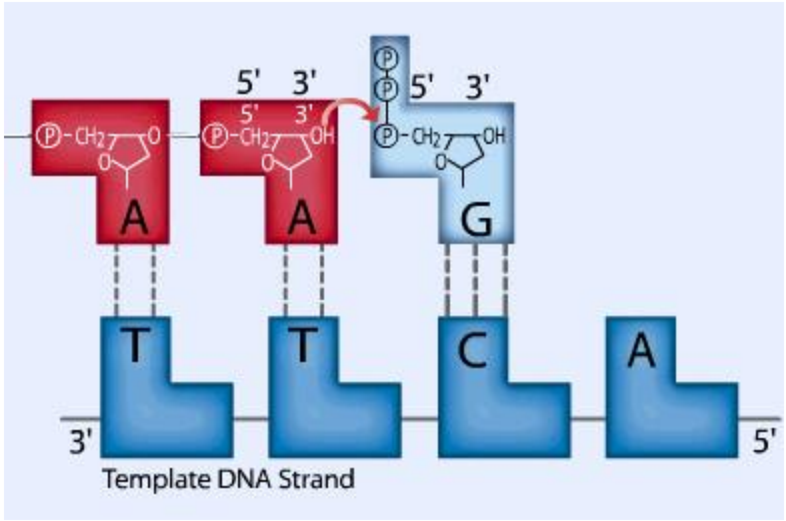


DNA Replication

- DNA Polymerase
  - ✓ Enzyme that catalyzes the covalent bond between the phosphate of one nucleotide and the **deoxyribose (sugar)** of the next nucleotide
  - ✓ ***Pol I*** *couples deoxynucleoside triphosphates on DNA templates in a reaction that occurs through the nucleophilic attack of the growing DNA chain's* 3' *-OH group on the -phosphoryl of an incoming nucleoside triphosphate.*



DNA Polymerization



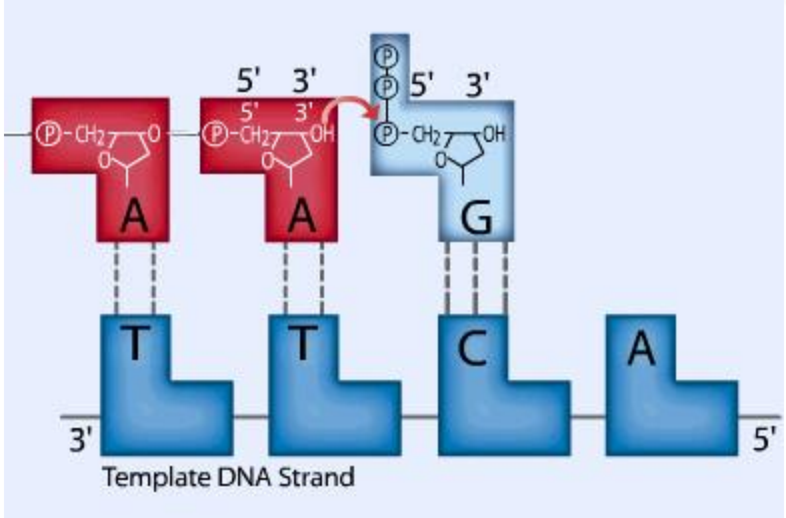
DNA Replication

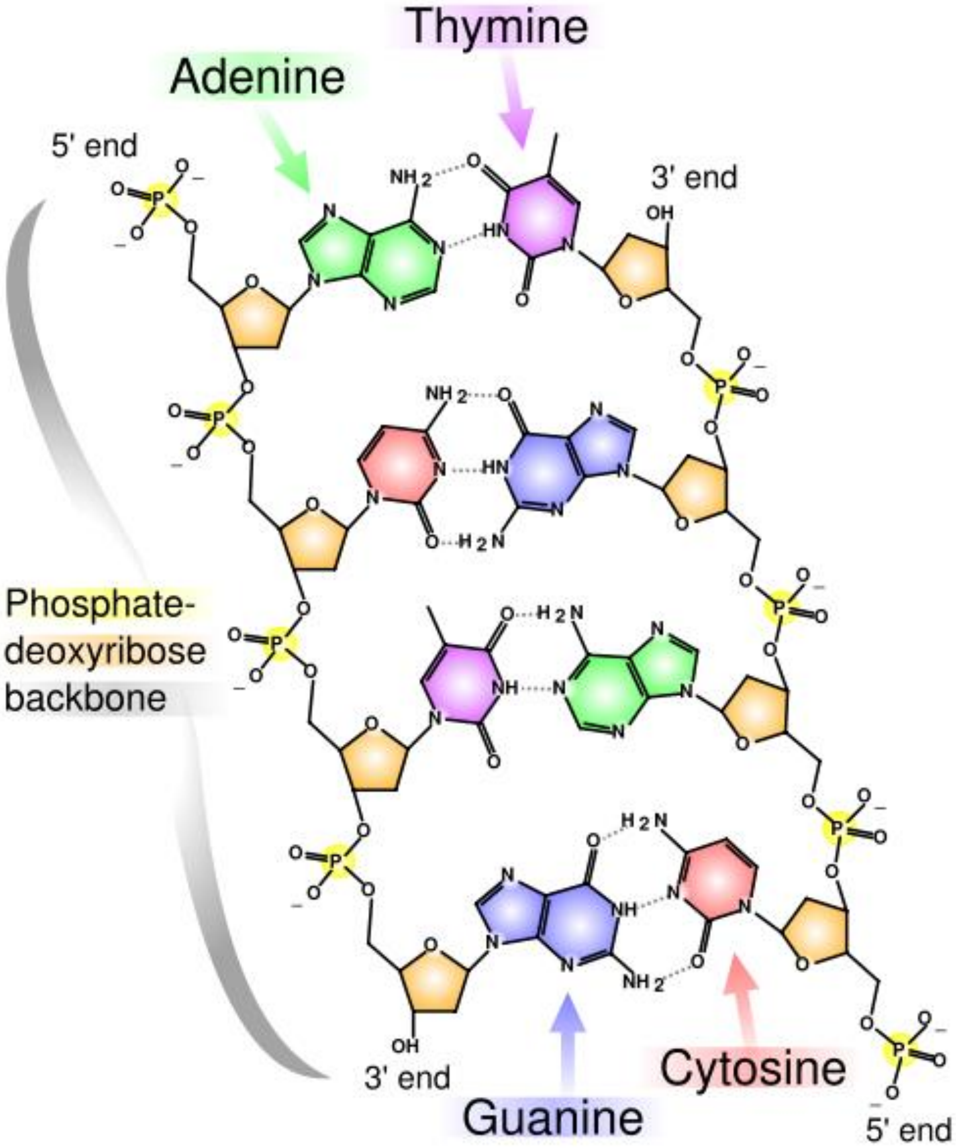
3′ end has a free deoxyribose

5′ end has a free phosphate

DNA polymerase:

- ✓ can only build the new strand in the 5′ to 3′ direction
- ✓ Thus scans the template strand in 3′ to 5′ direction

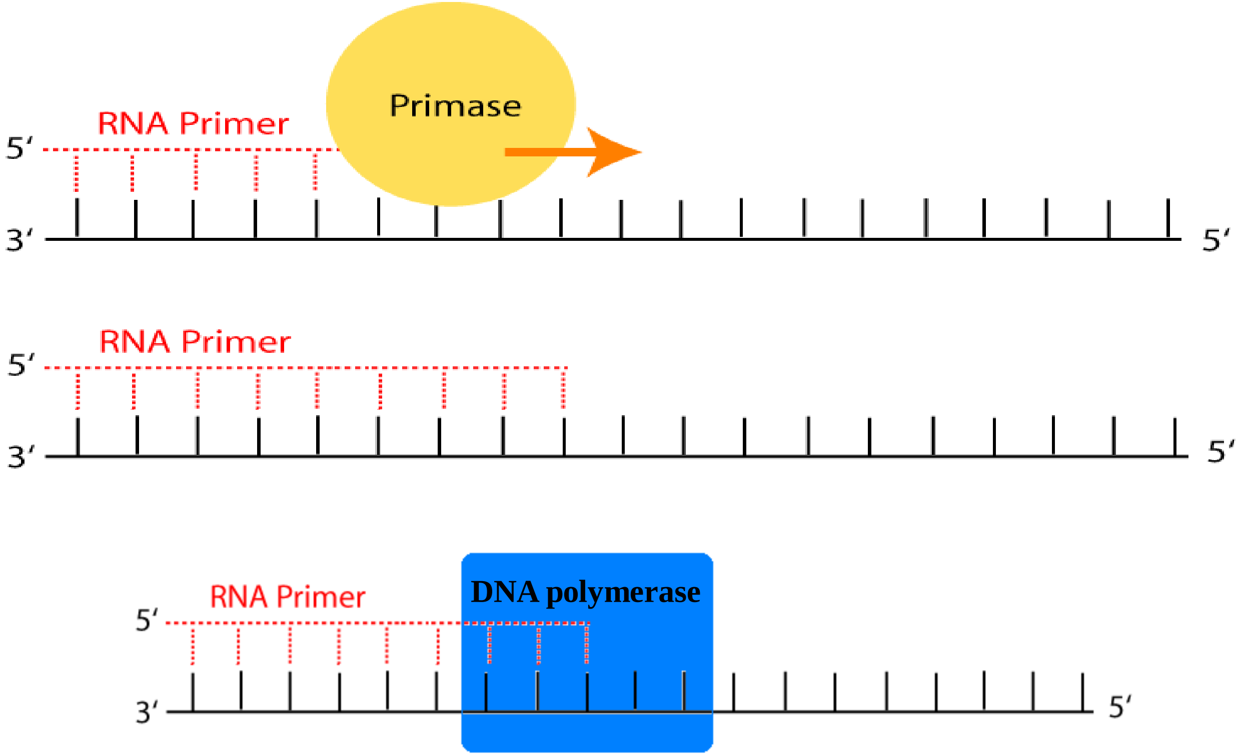




DNA Replication

Initiation

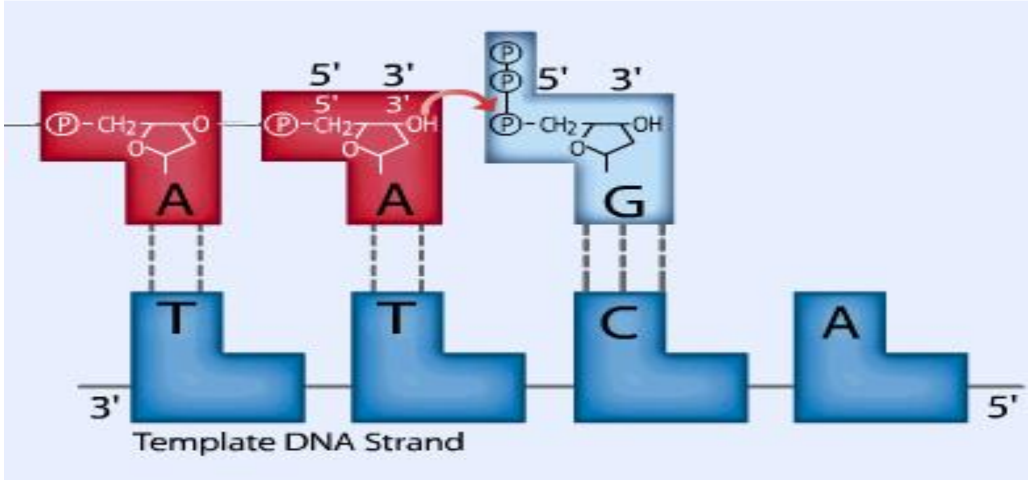
- **Primase** (a type of RNA polymerase) builds an **RNA primer** (Nucleotides for the starting point for DNA replication, Short strands of RNA)
- **DNA polymerase** attaches onto the 3′ end of the **RNA primer**

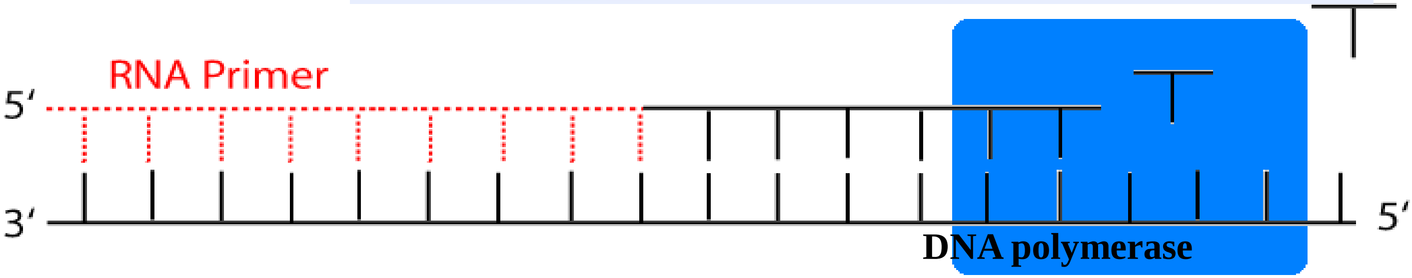


DNA Replication

Elongation

- **DNA polymerase** uses each strand as a template in the 3′ to 5′ direction to build a complementary strand in the 5′ to 3′ direction

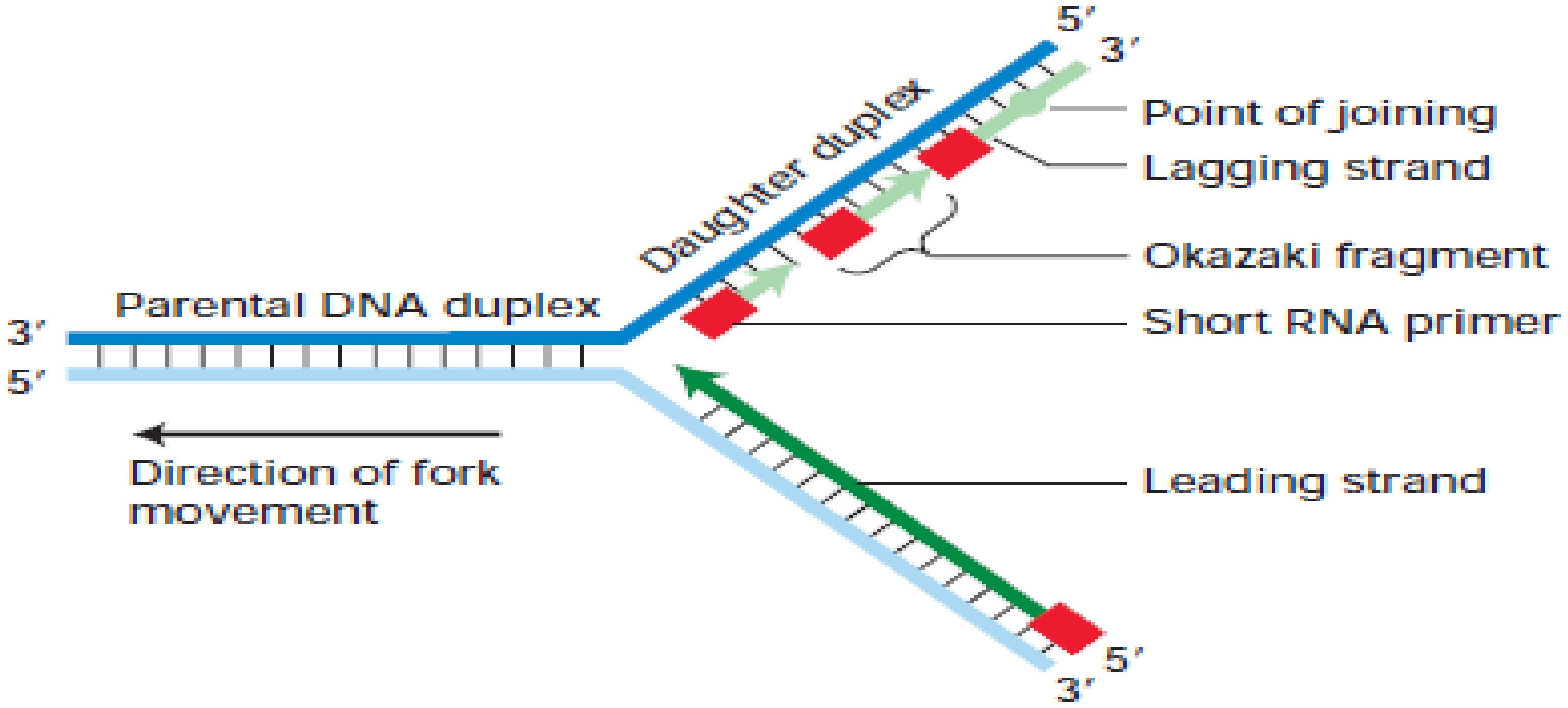




DNA Replication

Elongation

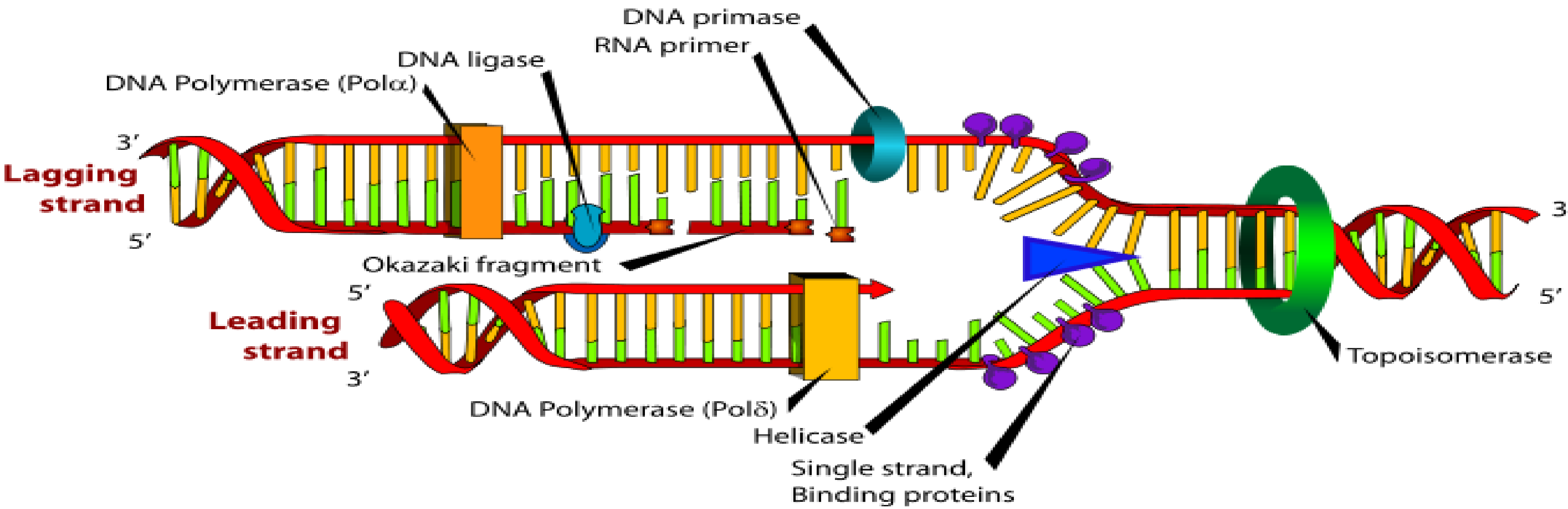
- **DNA polymerase** uses each strand as a template in the 3′ to 5′ direction to build a complementary strand in the 5′ to 3′ direction
  - ✓ results in a **leading strand** and a **lagging strand**



DNA Replication

Leading Strand

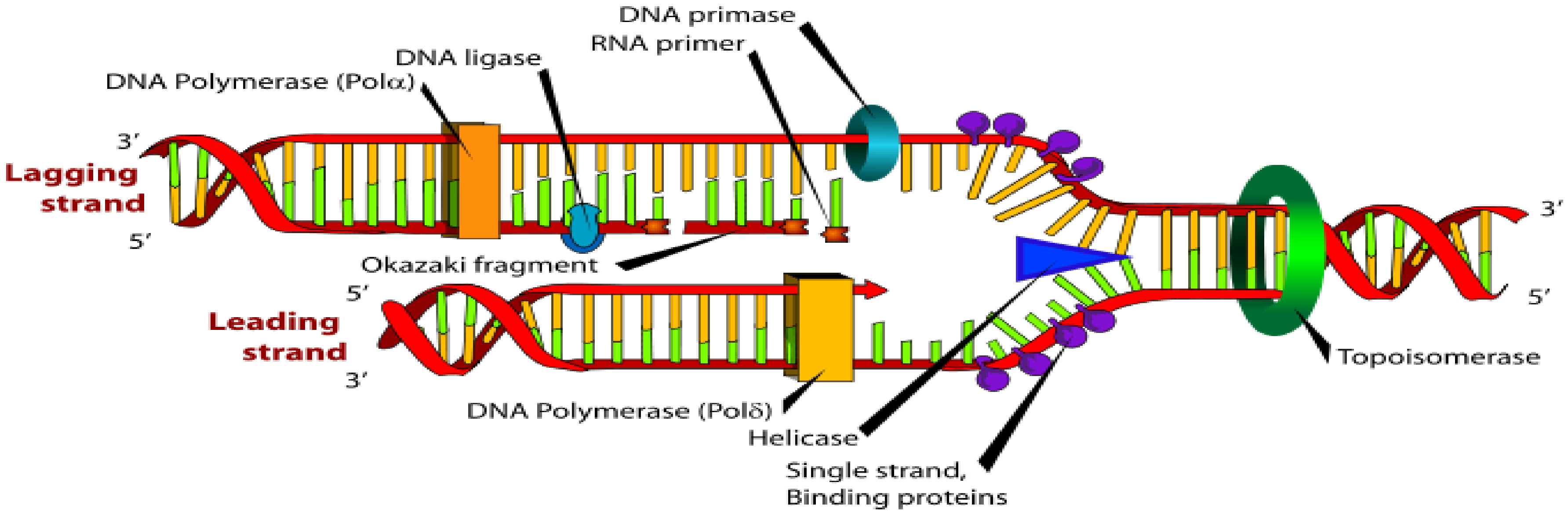
1. Topisomerase unwinds DNA and then Helicase breaks H-bonds
2. DNA primase creates a single RNA primer to start the replication
3. DNA polymerase slides along the leading strand in the 3′ to 5′ direction synthesizing the matching strand in the 5′ to 3′ direction
4. The RNA primer is degraded by RNase H and replaced with DNA nucleotides by DNA polymerase, and then DNA ligase connects the fragment at the start of the new strand to the end of the new strand (in circular chromosomes)

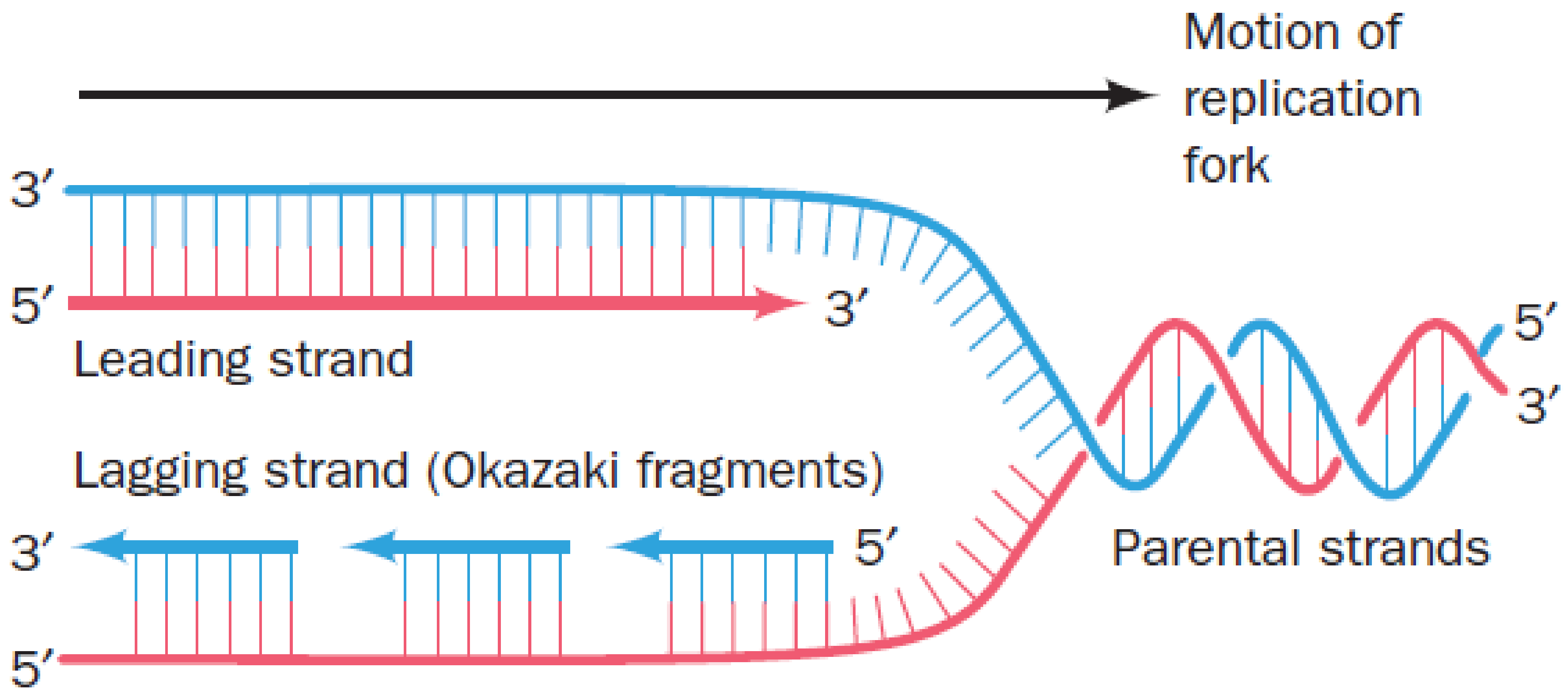


DNA Replication

Lagging Strand

1. Topisomerase unwinds DNA and then Helicase breaks H-bonds
2. DNA primase creates RNA primers in spaced intervals
3. DNA polymerase slides along the leading strand in the 3’ to 5’ direction synthesizing the matching Okazaki fragments in the 5’ to 3’ direction
4. The RNA primers are degraded by RNase H and replaced with DNA nucleotides by DNA polymerase
5. DNA ligase connects the Okazaki fragments to one another (covalently bonds the phosphate in one nucleotide to the deoxyribose of the adjacent nucleotide)





In DNA replication, both daughter strands (leading strand red, lagging strand blue) are synthesized in their 5′ to 3′ directions. The leading strand is synthesized continuously, whereas the lagging strand is synthesized discontinuously.

Enzymes in DNA Replication

1. **Topoisomerase** - unwinds DNA

2. **Helicase** – enzyme that breaks H-bonds

3. **DNA Polymerase** – enzyme that catalyzes connection of nucleotides to form complementary DNA strand in 5′ to 3′ direction (reads template in 3′ to 5′ direction)

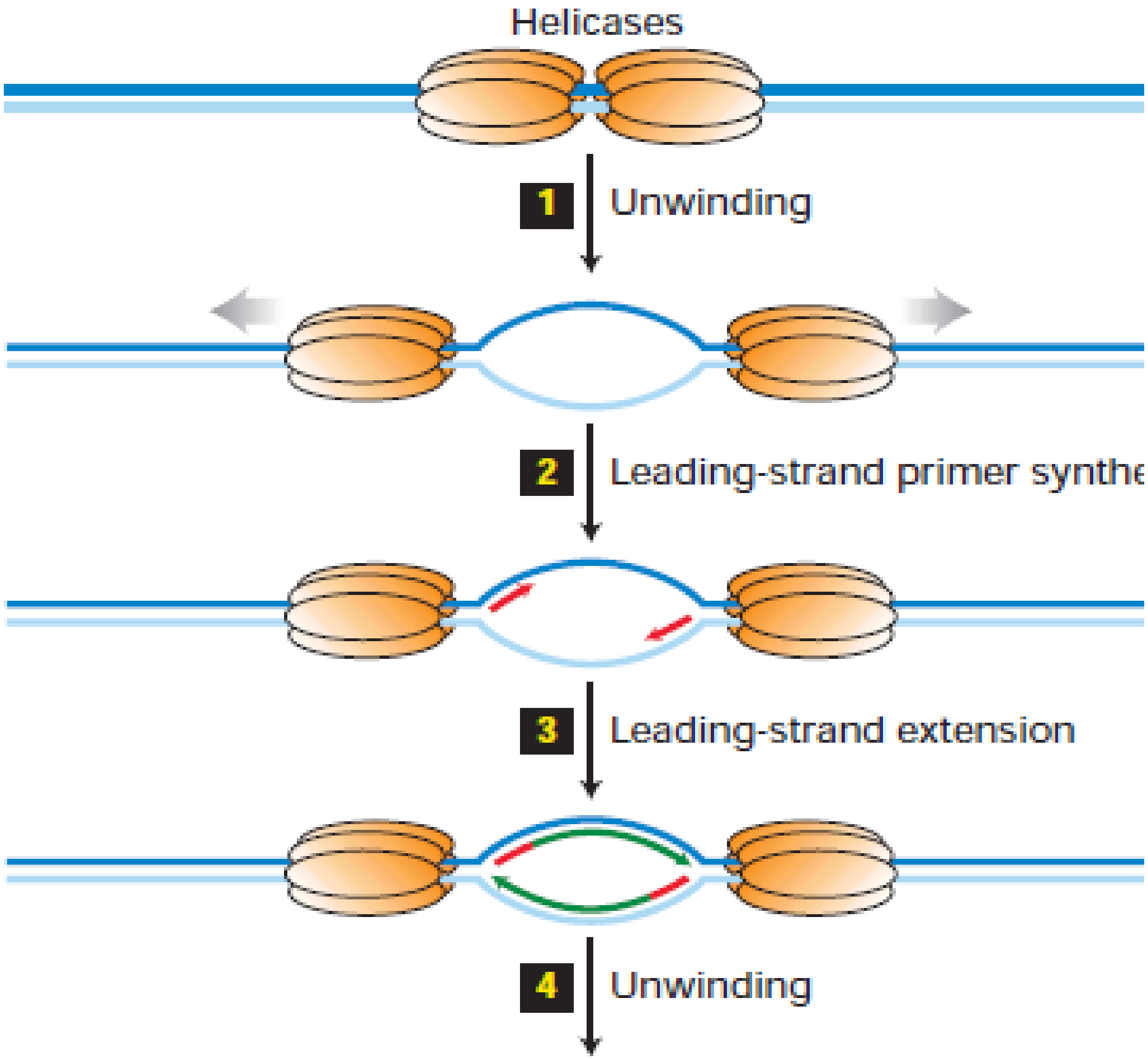
Leading Strand – transcribed continuously in 5′ to 3′ direction

Lagging Strand – transcribed in segments in 5′ to 3′ direction (**Okazaki fragments**)

4. **DNA Primase** – enzyme that catalyzes formation of RNA starting segment (**RNA primer**)

5. **DNA Ligase** – enzyme that catalyzes connection of two Okazaki fragments

DNA REPLICATION GENERALLY OCCURS BIDIRECTIONALLY FROM EACH ORIGIN



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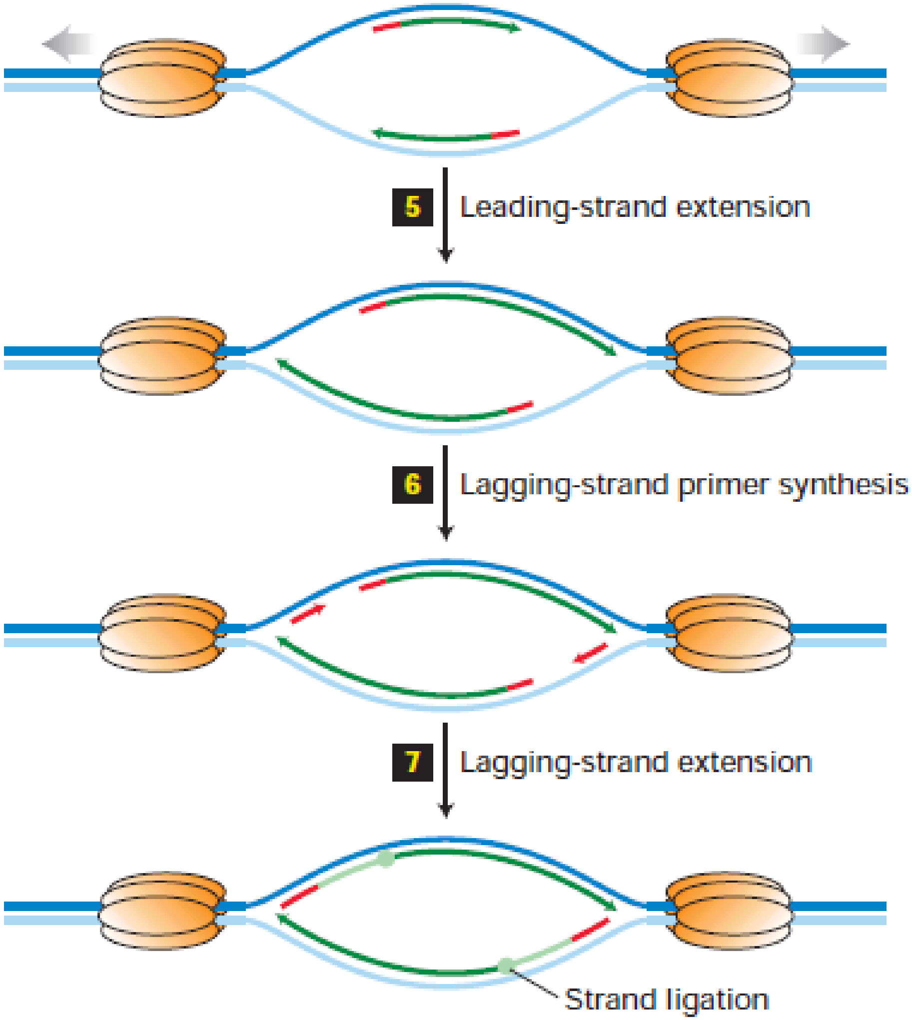

5
Leading-strand extension
6
Lagging-strand primer synthesis
7
Lagging-strand extension
Strand ligation

PROTEIN SYNTHESIS

- The order of bases along the DNA strand codes for the order in which amino acids are chemically joined together to form a polypeptide.
- Protein synthesis involves two types of nucleic acids:
  1. DNA (deoxyribonucleic acid)
  2. RNA (ribonucleic acid)
- RNA, like DNA, is a polymer formed by a sequence of nucleotides
- Three Types of RNA:
  1. messenger RNA (mRNA)
  2. transfer RNA (tRNA)
  3. ribosomal RNA (rRNA)

GENETIC INFORMATION COPIED FROM DNA IS TRANSFERRED TO 3 TYPES OF RNA:

- **messenger** (mRNA) **is like a**

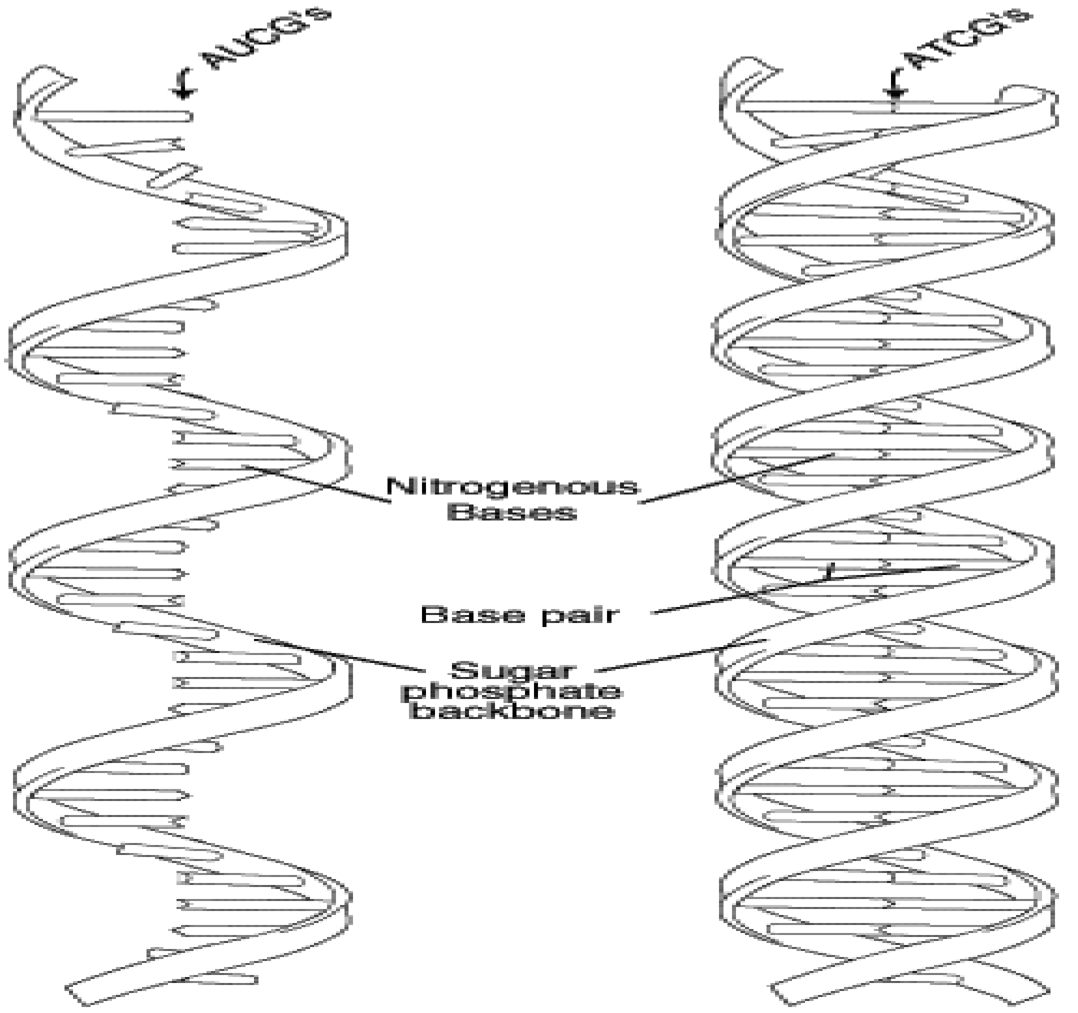
    Copy of information in DNA that is brought to the ribosome where the information is translated into a protein.

- **ribosomal** (rRNA) **is like a**

    The protein factories of the cells.

- **transfer** (tRNA) **are like a**

    Brings the amino acid to the ribosome



RNA

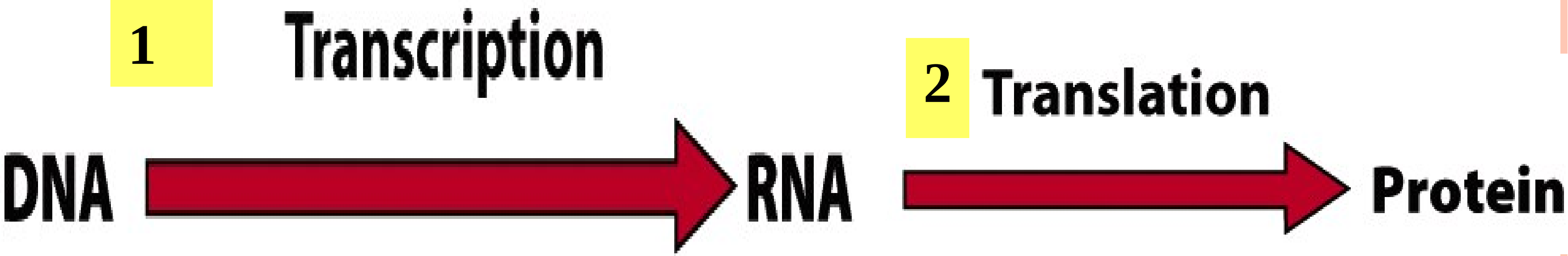
Ribonucleic acid

DNA

Deoxyribonucleic acid

Protein Synthesis

- Protein synthesis occurs in two primary steps



mRNA (**messenger RNA**) copy of a gene is synthesized

✓Cytoplasm of prokaryotes
✓Nucleus of eukaryotes

mRNA is used by ribosome to build protein

(Ribosomes attach to the mRNA and use its sequence of nucleotides to determine the order of amino acids in the protein)

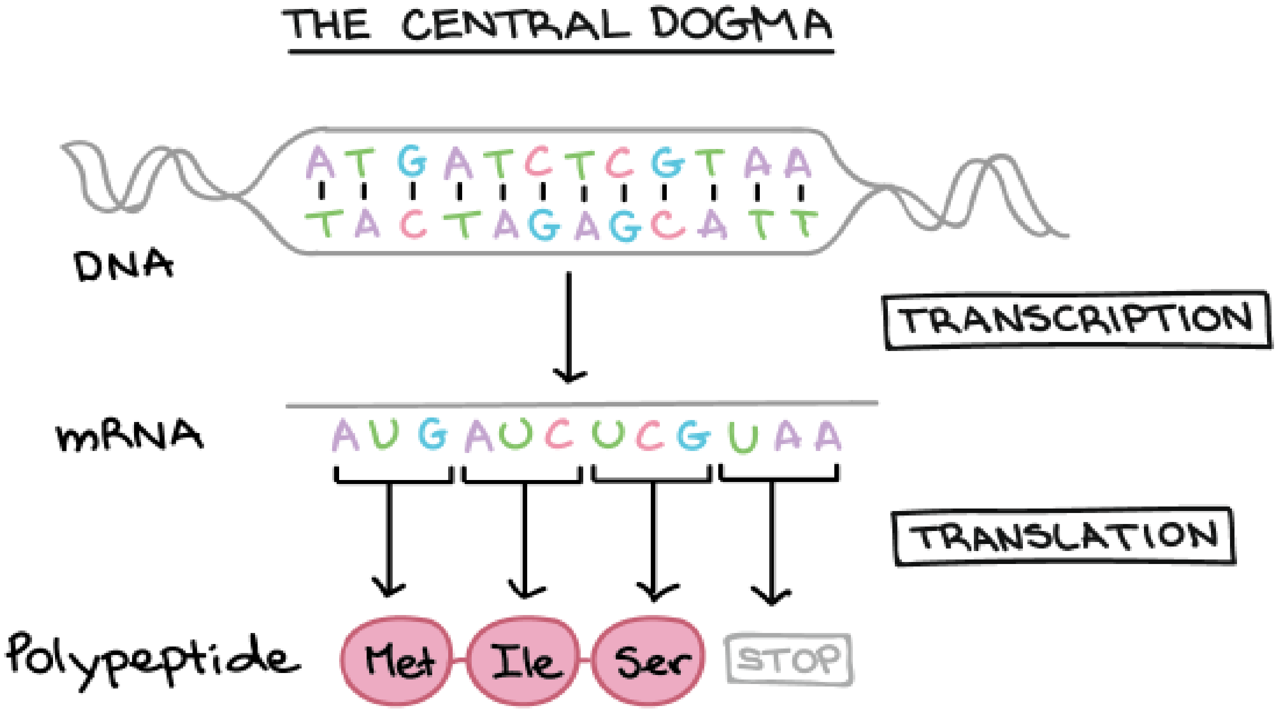
✓Cytoplasm of prokaryotes and eukaryotes

✓Some proteins feed directly into rough ER in eukaryotes

- In **transcription**, the DNA sequence of a gene is copied to make an RNA molecule.
- This step is called *transcription* because it involves rewriting, or transcribing, the DNA sequence in a similar RNA "alphabet."
- In eukaryotes, the RNA molecule must undergo processing to become a mature **messenger RNA (mRNA)**.

- In **translation**, the sequence of the mRNA is decoded to specify the amino acid sequence of a polypeptide.
- The name ***translation*** reflects that the nucleotide sequence of the mRNA sequence must be translated into the completely different "language" of amino acids.

- Thus, during expression of a protein-coding gene, information flows from DNA→ RNA → protein. This directional flow of information is known as the **central dogma** of molecular biology. Non-protein-coding genes (genes that specify functional RNAs) are still transcribed to produce an RNA, but this RNA is not translated into a polypeptide. For either type of gene, the process of going from DNA to a functional product is known as **gene expression**



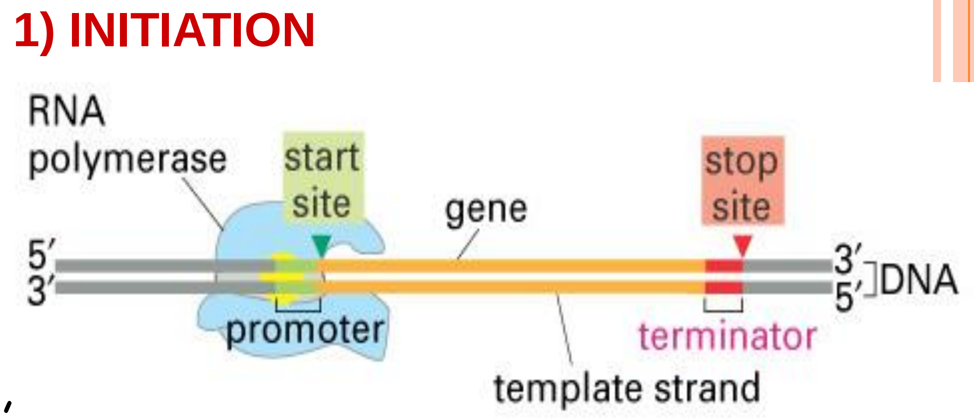
- **Transcription** is the process of RNA synthesis, controlled by the interaction of promoters and enhancers. Several different types of RNA are produced, including **messenger RNA (mRNA)**, which specifies the sequence of amino acids in the protein product, plus **transfer RNA (tRNA)** and **ribosomal RNA (rRNA)**, which play a role in the translation process.
- **Transcription involves four steps:**
- ***1) Initiation***. The DNA molecule unwinds and separates to form a small **open complex**. RNA polymerase binds to the promoter of the **template strand** (also known as the 'sense strand' or 'coding strand'). The synthesis of RNA proceeds in a 5' to 3' direction, so the template strand must be 3' to 5'.

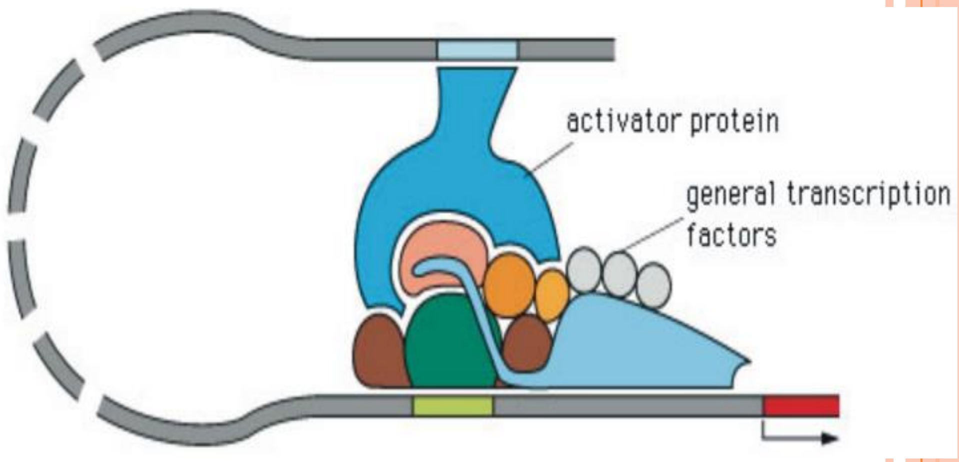
Protein Synthesis

- Transcription

Initiation

- ✓ **RNA polymerase** binds to a region on DNA known as the ***promoter***, which signals the start of a gene
- ✓ RNA polymerase (RNAP), the enzyme responsible for the DNA-directed synthesis of RNA, which couples together the ribonucleoside triphosphates ATP, CTP, GTP(guanosine triphosphate), and UTP(Uridine triphosphate) on DNA templates in a reaction
- ✓ **Promoters** are specific to genes
- ✓ RNA polymerase does not need a primer
- ✓ **Transcription factors** assemble at the promoter forming a *transcription initiation complex* – activator proteins help stabilize the complex





(eukaryotes)

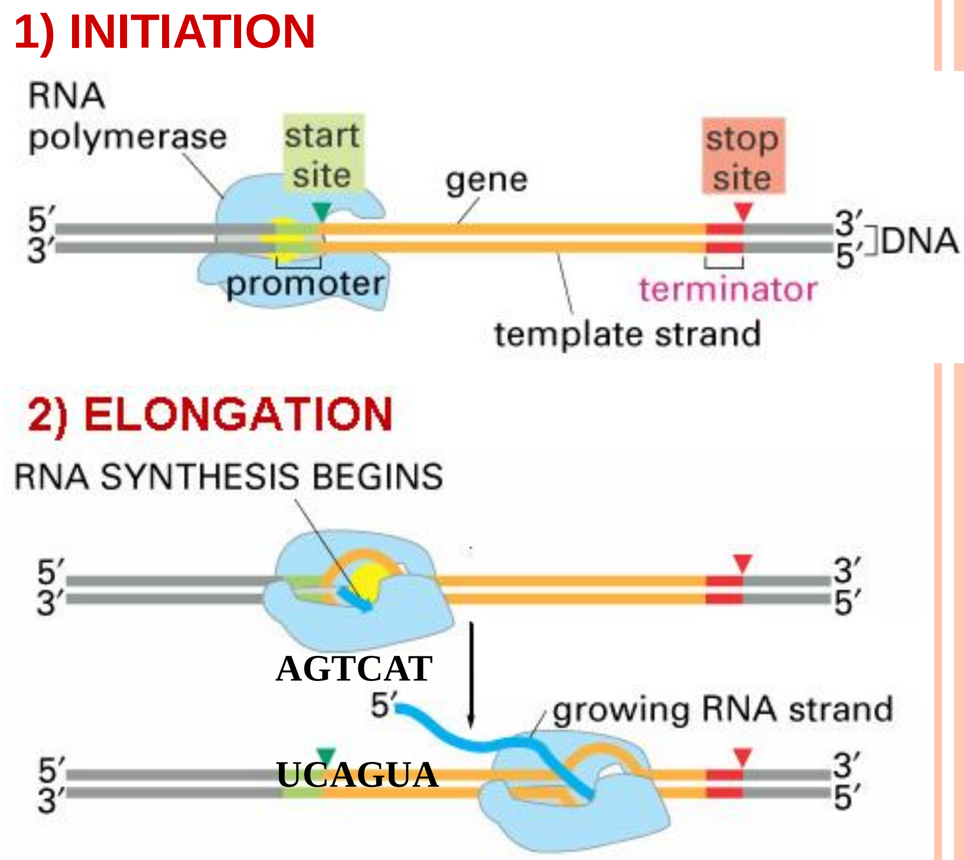
- 2. Elongation.
- Is addition of nucleotides to mRNA
- RNA polymerase moves along the template strand, synthesising an mRNA molecule. In prokaryotes RNA polymerase is a holoenzyme consisting of a number of subunits, including a **sigma factor** (transcription factor) that recognises the promoter. In eukaryotes there are three RNA polymerases: I, II and III. The process includes a proofreading mechanism.

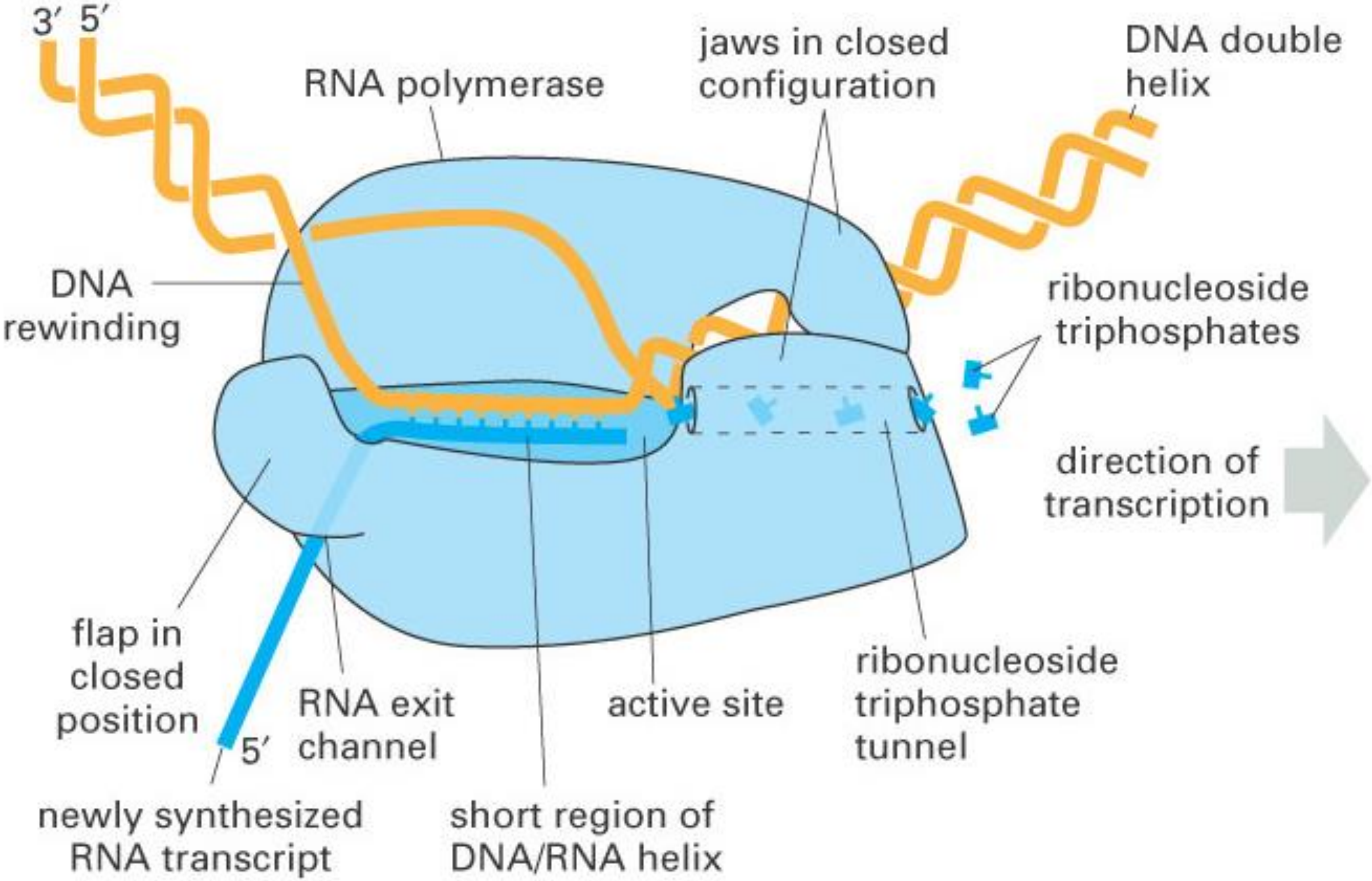
Protein Synthesis

- Transcription
  Elongation

  ✓ **RNA polymerase** unwinds the DNA and breaks the H-bonds between the bases of the two strands, separating them from one another

  ✓ Base pairing occurs between incoming RNA nucleotides and the DNA nucleotides of the gene (template)
    - recall RNA uses uracil instead of thymine



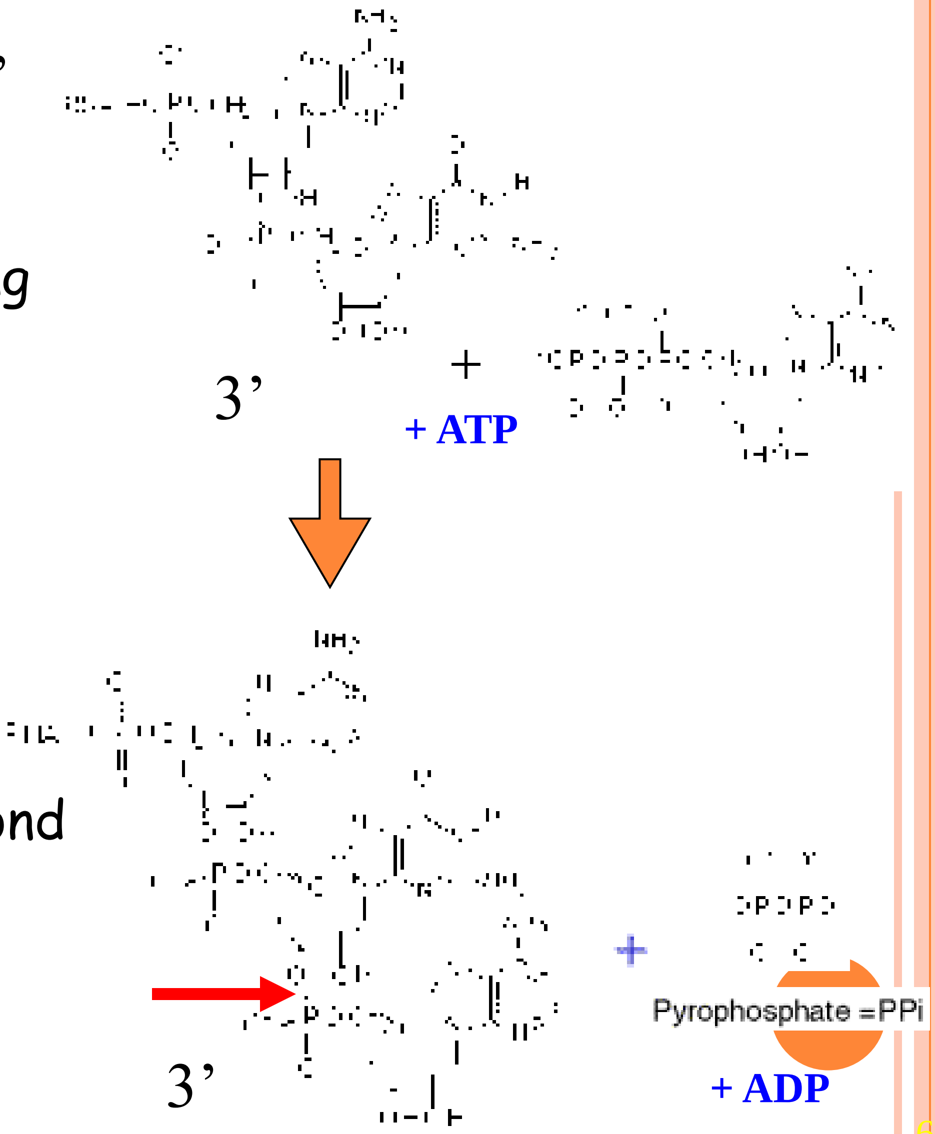

3′ 5′
RNA polymerase
jaws in closed configuration
DNA double helix
DNA rewinding
ribonucleoside triphosphates
direction of transcription
flap in closed position
RNA exit channel
active site
ribonucleoside triphosphate tunnel
5′
newly synthesized RNA transcript
short region of DNA/RNA helix

Protein Synthesis

- Transcription

Elongation

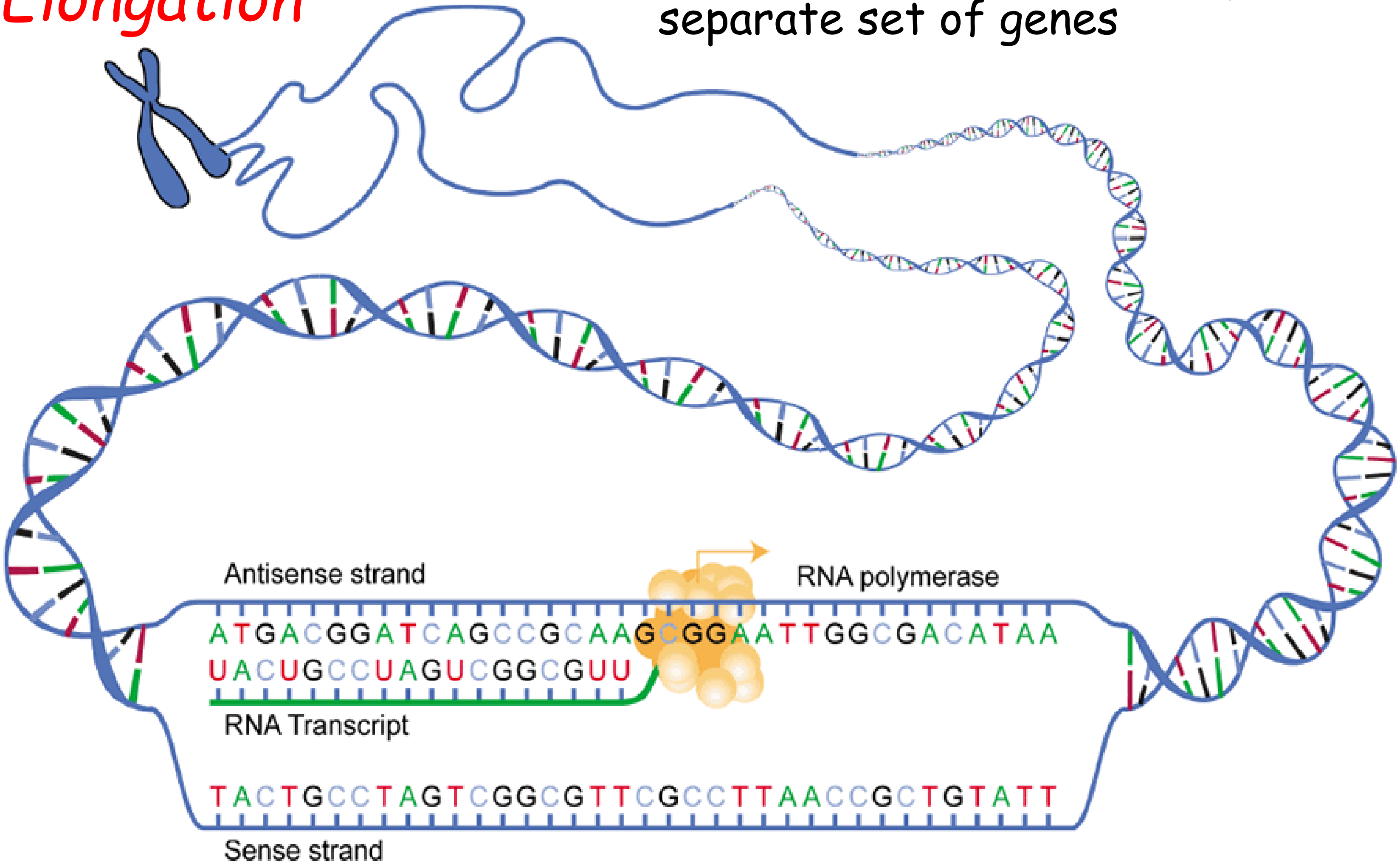
- ✓ **RNA polymerase** unwinds the DNA and breaks the H-bonds between the bases of the two strands, separating them from one another.
- ✓ Base pairing occurs between incoming RNA nucleotides and the DNA nucleotides of the gene (template)
  - recall RNA uses uracil instead of thymine
- ✓ RNA polymerase catalyzes bond to form between ribose of 3' nucleotide of mRNA and phosphate of incoming RNA nucleotide



Protein Synthesis

- Transcription
  Elongation

The gene occurs on only one of the DNA strands; each strand possesses a separate set of genes

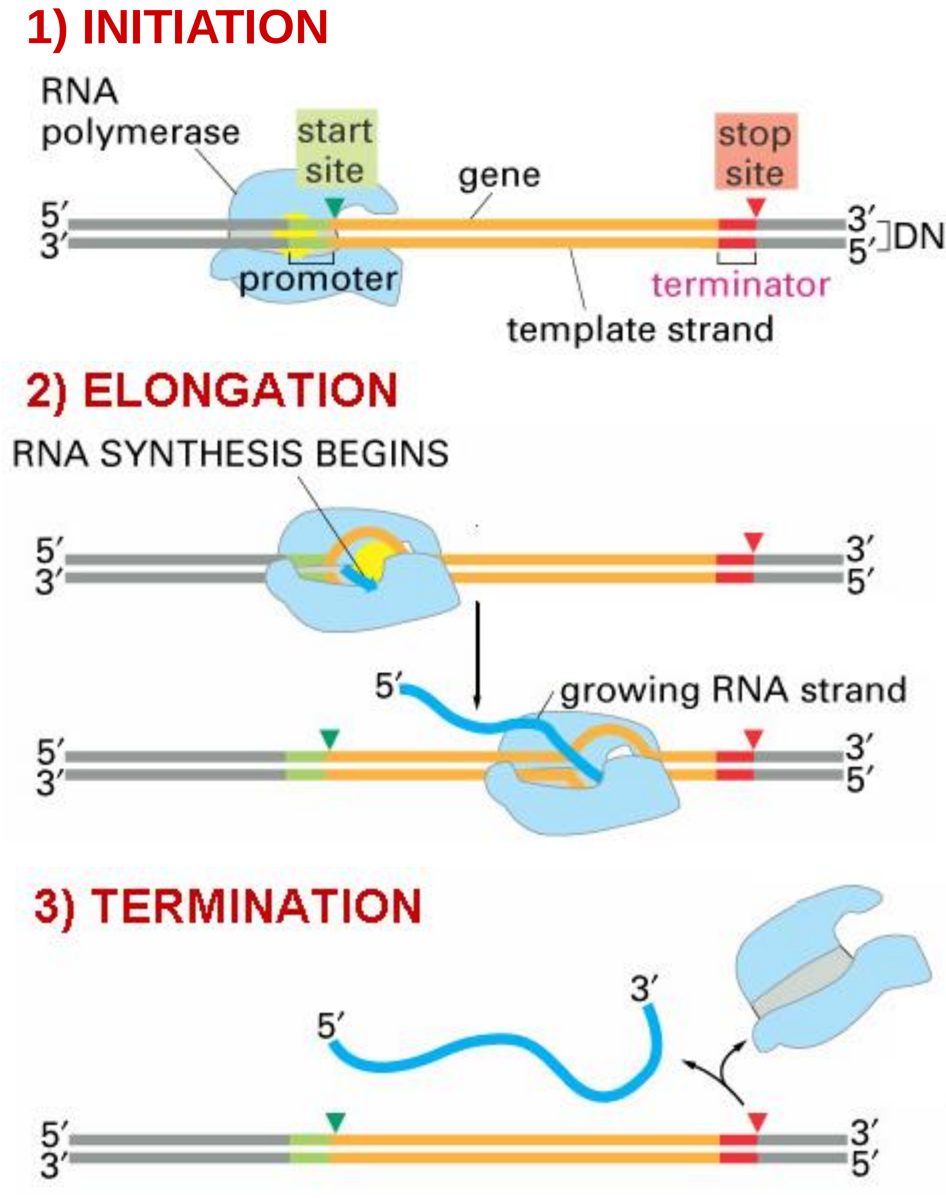


Protein Synthesis

- Transcription

  3. ***Termination***

  - ✓ Is the ending of transcription and occurs when Rna polymerase crsses a stop (termination) sequence in gene. The mRNA strand is complete and it detaches from DNA
  - ✓ A region on DNA known as the ***terminator*** signals the stop of a gene
  - ✓ **RNA polymerase** disengages the mRNA and the DNA

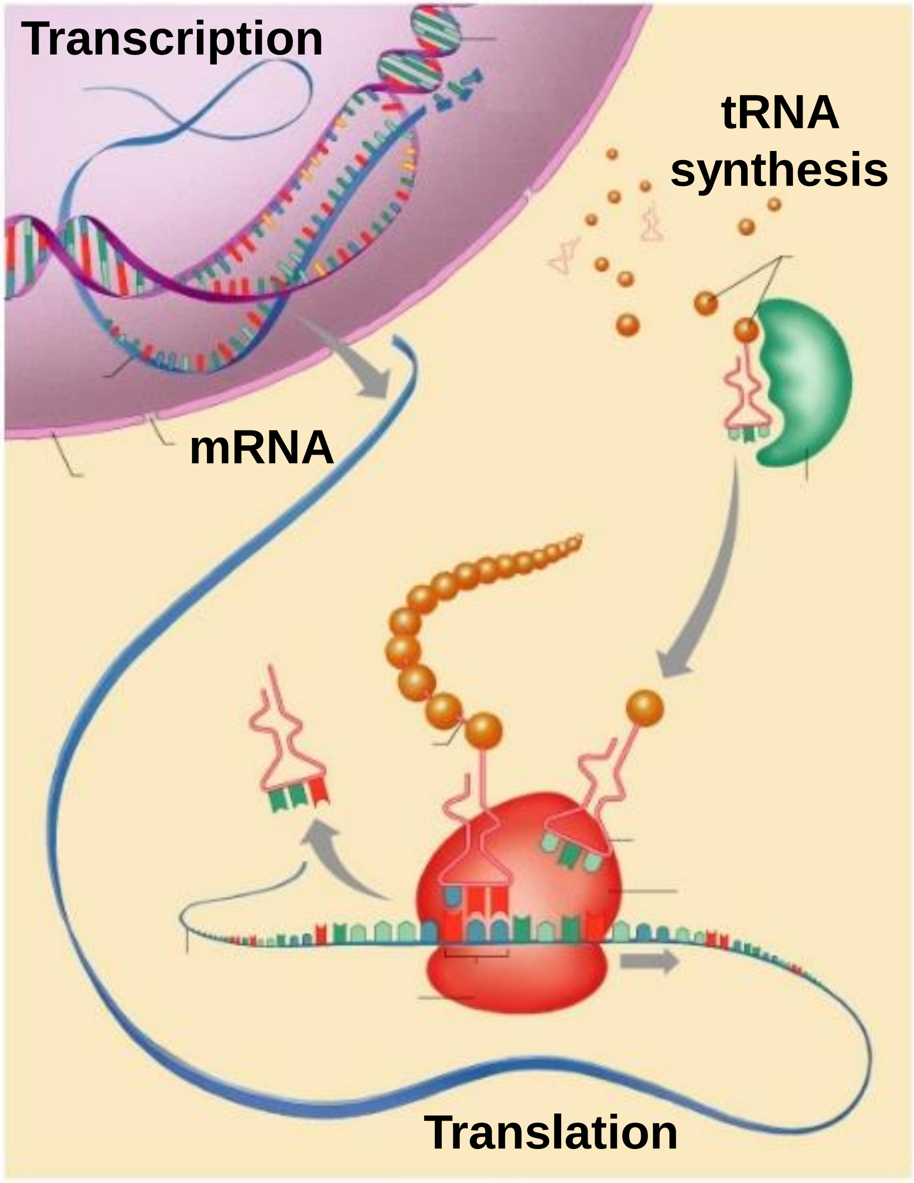


- ***4. Processing***. After transcription the RNA molecule is processed in a number of ways: introns are removed and the exons are spliced together to form a mature mRNA
- mRNA which has been transcribed upto this point is reffered to as Pre-mRNA. Processing must be occur to convert this into **mature** mRNA. This process include capping, polyadenylation / tailing and splicing.

NOTE; IN TRANSCRIPTION AND RNA PROCESSING

- *In bacteria*, the primary RNA transcript can directly serve as a **messenger RNA**, or **mRNA**.
- Messenger RNAs get their name because they act as messengers between DNA and ribosomes.
- Ribosomes are RNA-and-protein structures in the cytosol where proteins are actually made.
- *In eukaryotes* (such as humans), a primary transcript has to go through some extra processing steps in order to become a mature mRNA.
- During processing, caps are added to the ends of the RNA, and some pieces of it may be carefully removed in a process called **splicing**. These steps do not happen in bacteria

Protein Synthesis



2 Translation

mRNA → **Protein**

mRNA is used by ribosome to build protein

(Ribosomes attach to the mRNA and use its sequence of nucleotides to determine the order of amino acids in the protein)

- ✓Cytoplasm of prokaryotes and eukaryotes
- ✓Some proteins feed directly into rough ER in eukaryotes

Translation

- After transcription , an mRNA molecule is ready to protein synthesis . The process of using information in an mRNA to build a polypeptide is called Translation.

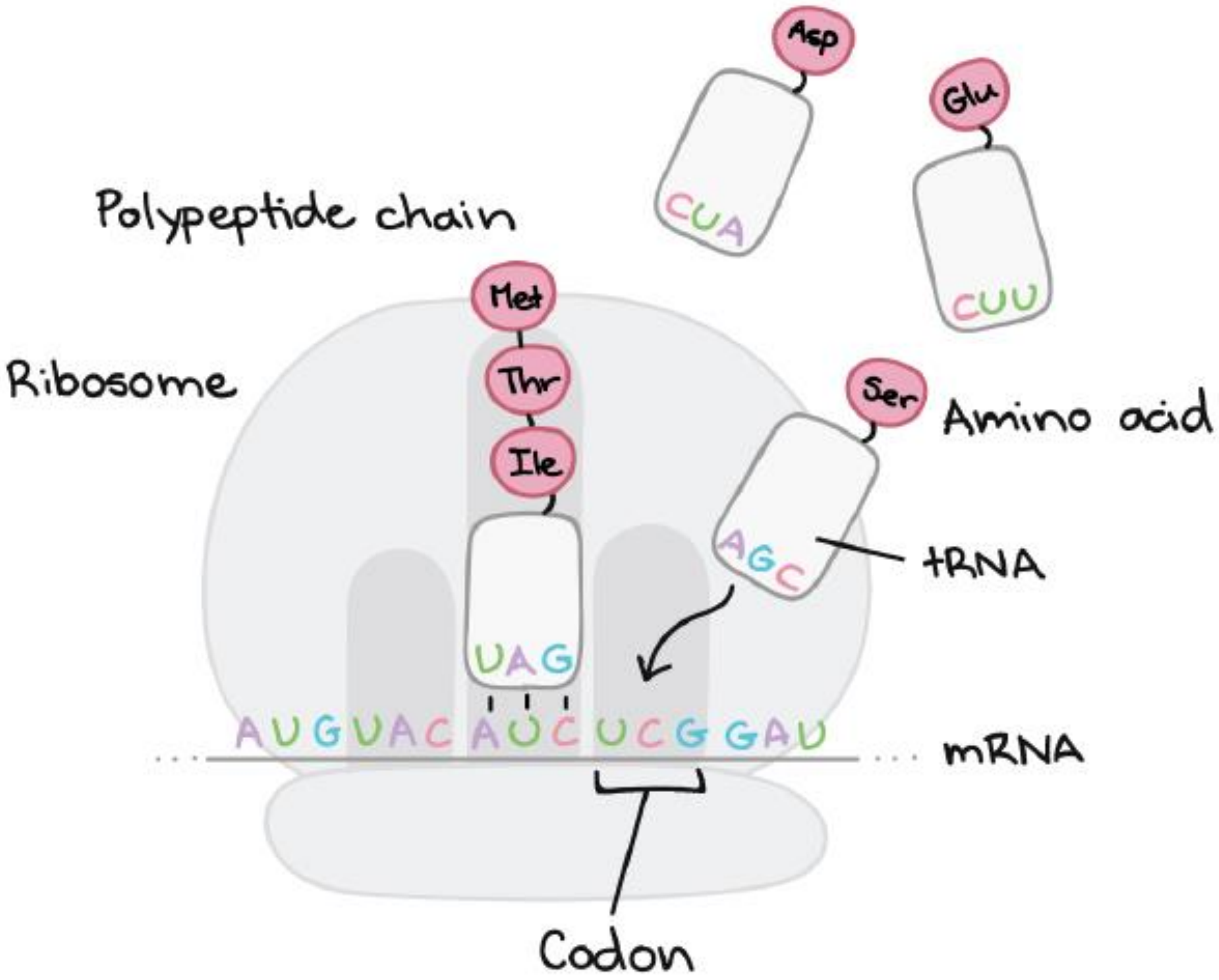
Genetic Code

- During the translation, the nucleotide sequence of mRNA is translated into amino acid sequence of polypeptide .
- Specifically nucleotide of mRNA are read in triplets (group of three) called **codons**.
- There are 61 codons that specify amino acids. One codon is start codon that indicates where to start translation. Three other stop codons signals the end of a polypeptide. These relationship between the codons and amino acids are called genetic code.

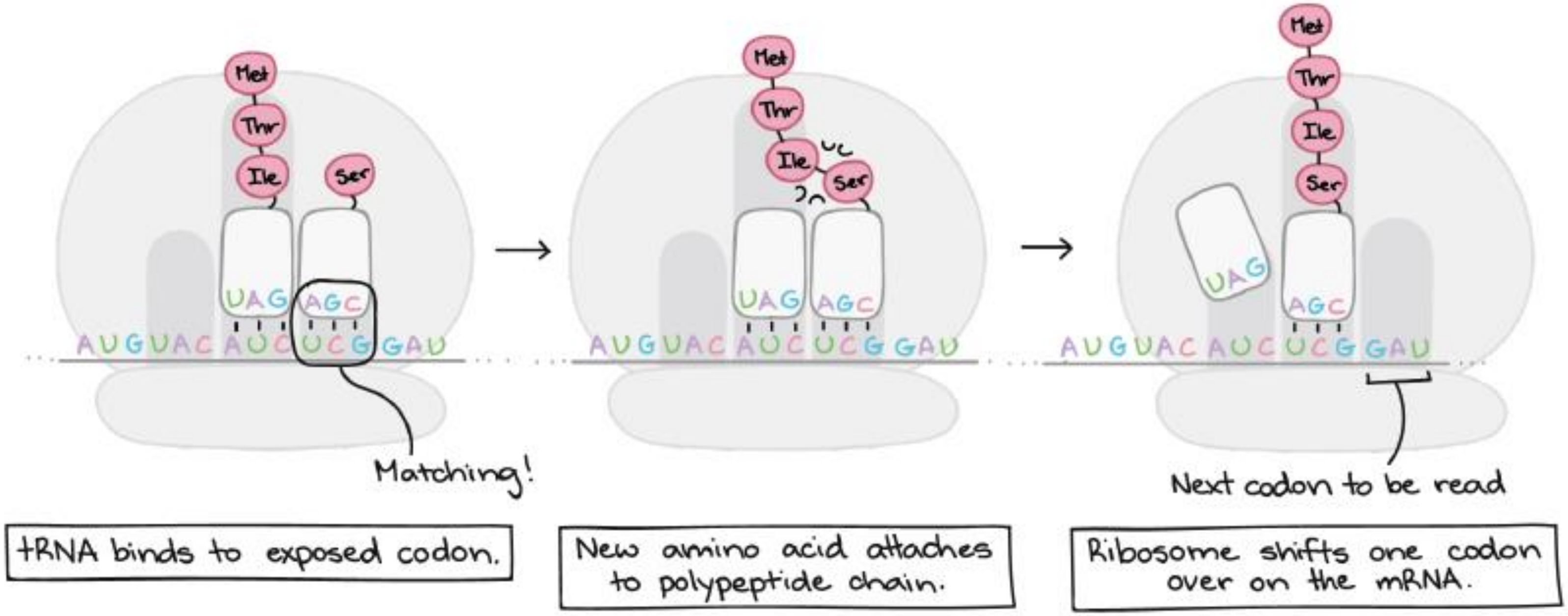
STEPS OF TRANSLATION

- Translation takes place inside of structures known as **ribosomes**. Ribosomes are molecular machines whose job is to build polypeptides.
- Once a ribosome latches on to an mRNA and finds the "start" codon, it will travel rapidly down the mRNA, one codon at a time.
- As it goes, it will gradually build a chain of amino acids that exactly mirrors the sequence of codons in the mRNA.

- Q; How does the ribosome "know" which amino acid to add for each codon?
- As it turns out, this matching is not done by the ribosome itself. Instead, it depends on a group of specialized RNA molecules called **transfer RNAS (tRNAs**).
- Each tRNA has a three nucleotides sticking out at one end, which can recognize (base-pair with) just one or a few particular codons.
- At the other end, the tRNA carries an amino acid –specifically, the amino acid that matches those codons.


Polypeptide chain
Ribosome
Met
Thr
Ile
Asp
CUA
Glu
CUU
Ser
Amino acid
AGC
tRNA
UAG
AUGUACAUCUCGGAU
mRNA
Codon

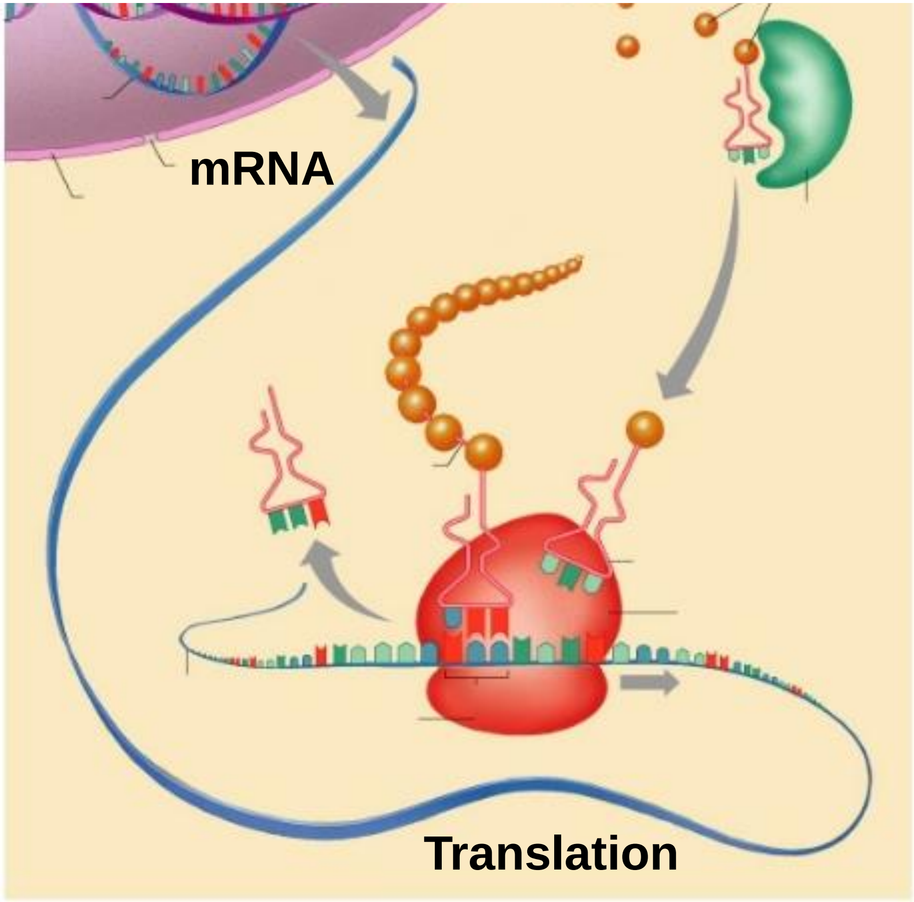
- There are many tRNAs floating around in a cell, but only a tRNA that matches (base-pairs with) the codon that's currently being read can bind and deliver its amino acid cargo.
- Once a tRNA is snugly bound to its matching codon in the ribosome, its amino acid will be added the end of the polypeptide chain

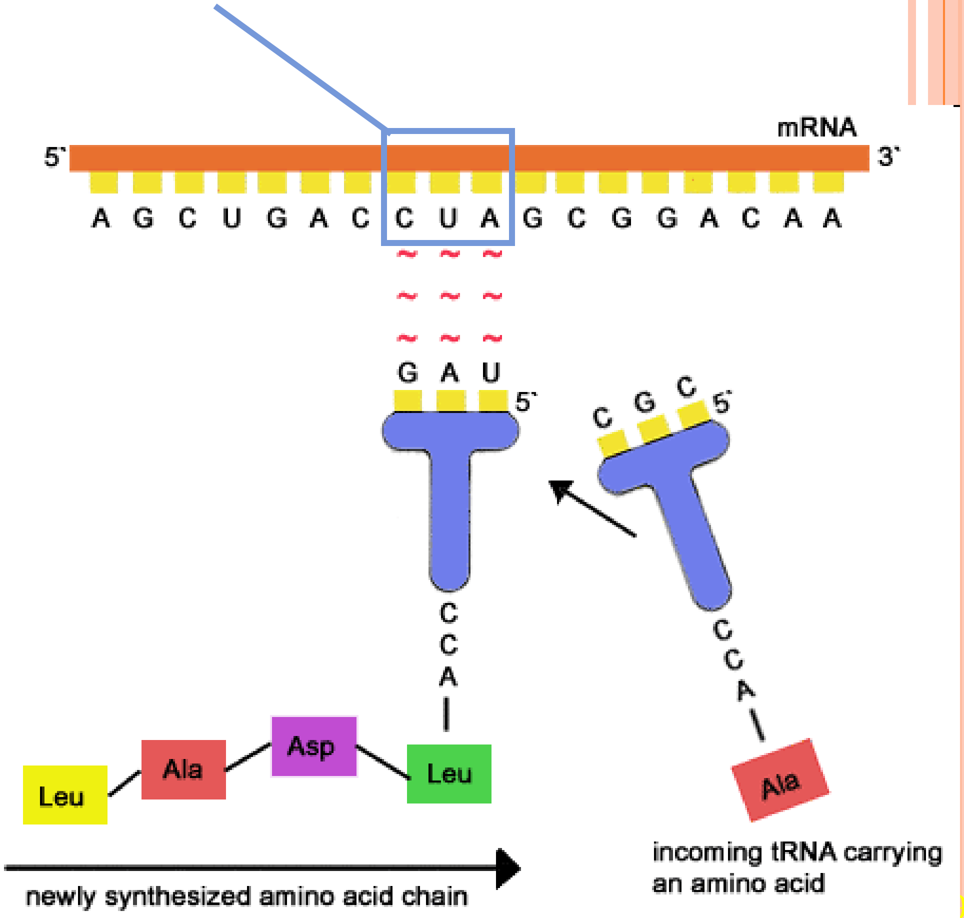


- This process repeats many times, with the ribosome moving down the mRNA one codon at a time.
- A chain of amino acids is built up one by one, with an amino acid sequence that matches the sequence of codons found in the mRNA.
- Translation ends when the ribosome reaches a stop codon and releases the polypeptide

Protein Synthesis

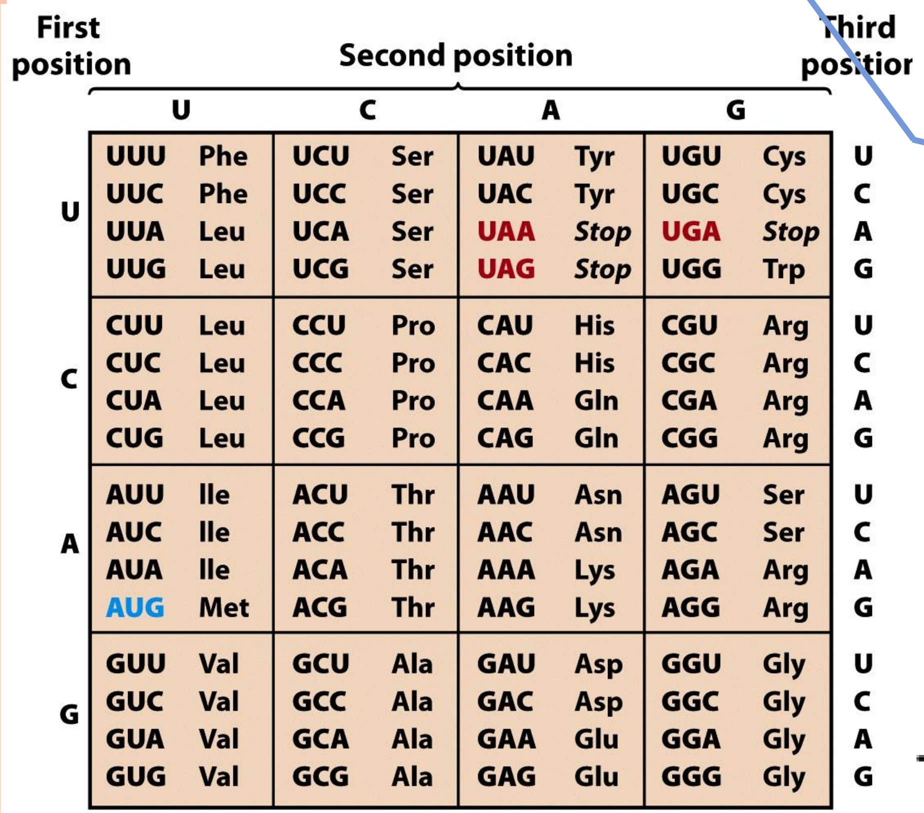
- Translation
  - ✓ Every three mRNA nucleotides (**codon**) specify an amino acid





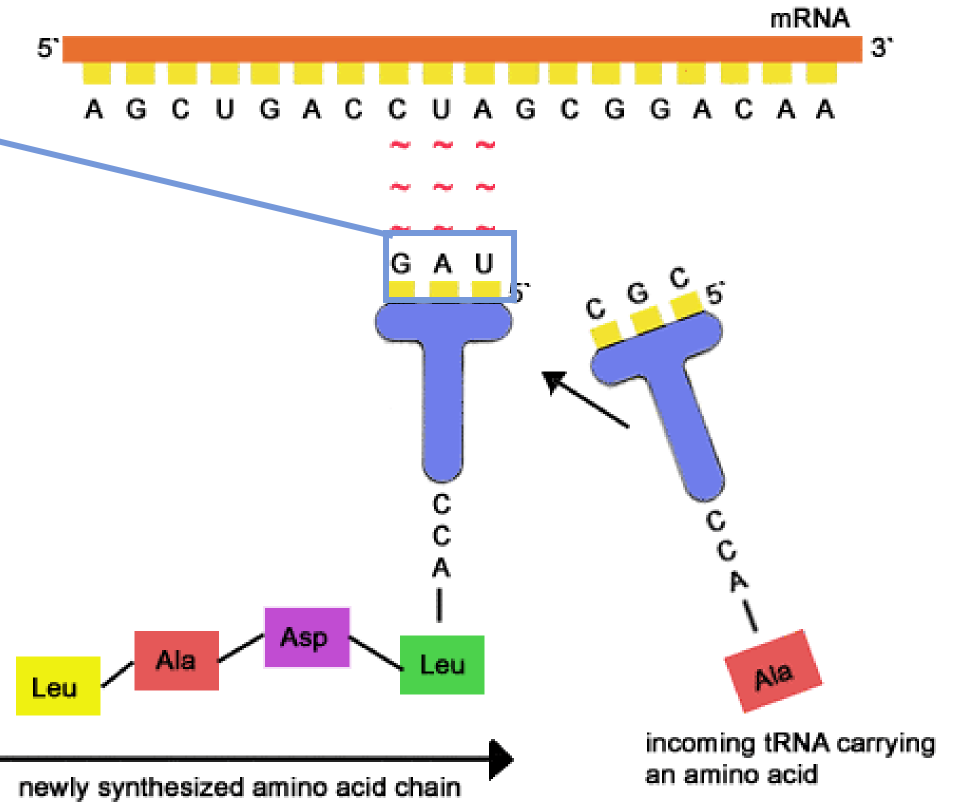
Protein Synthesis

- Translation
  - ✓ tRNA have an **anticodon** region that specifically binds to its codon



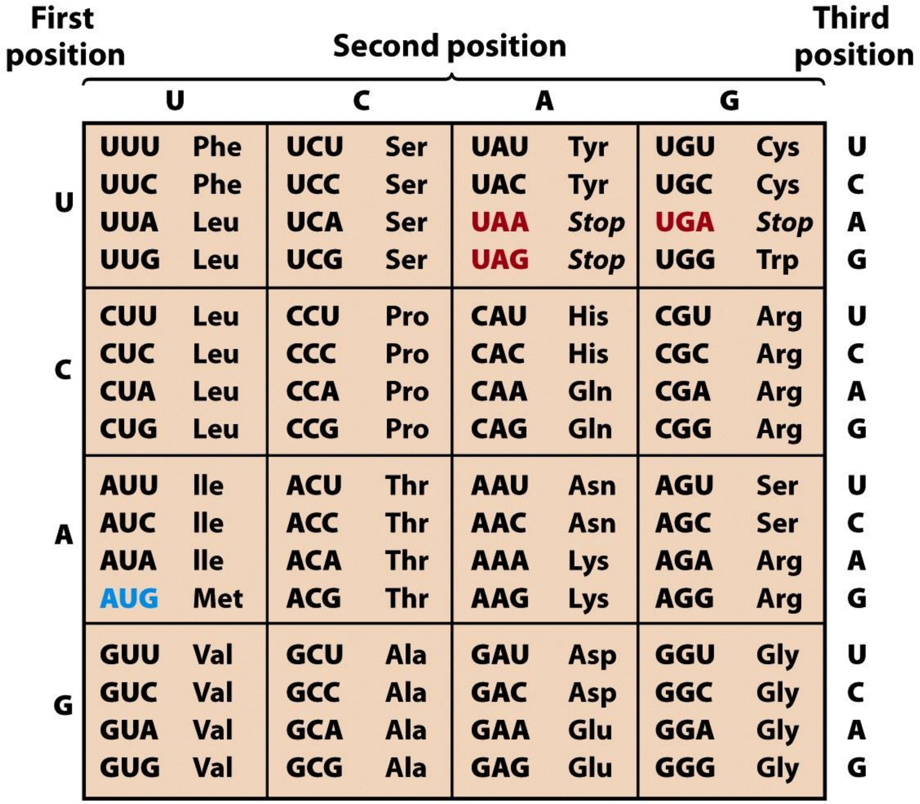
First position	Second position: U		C		A		G		Third position
U	UUU	Phe	UCU	Ser	UAU	Tyr	UGU	Cys	U
	UUC	Phe	UCC	Ser	UAC	Tyr	UGC	Cys	C
	UUA	Leu	UCA	Ser	UAA	*Stop*	UGA	*Stop*	A
	UUG	Leu	UCG	Ser	UAG	*Stop*	UGG	Trp	G
C	CUU	Leu	CCU	Pro	CAU	His	CGU	Arg	U
	CUC	Leu	CCC	Pro	CAC	His	CGC	Arg	C
	CUA	Leu	CCA	Pro	CAA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

Figure 7-8 Microbiology, 7/e



Protein Synthesis

- Translation
  - ✓ Each tRNA carries a specific amino acid



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

Figure 7-8 Microbiology, 7/e
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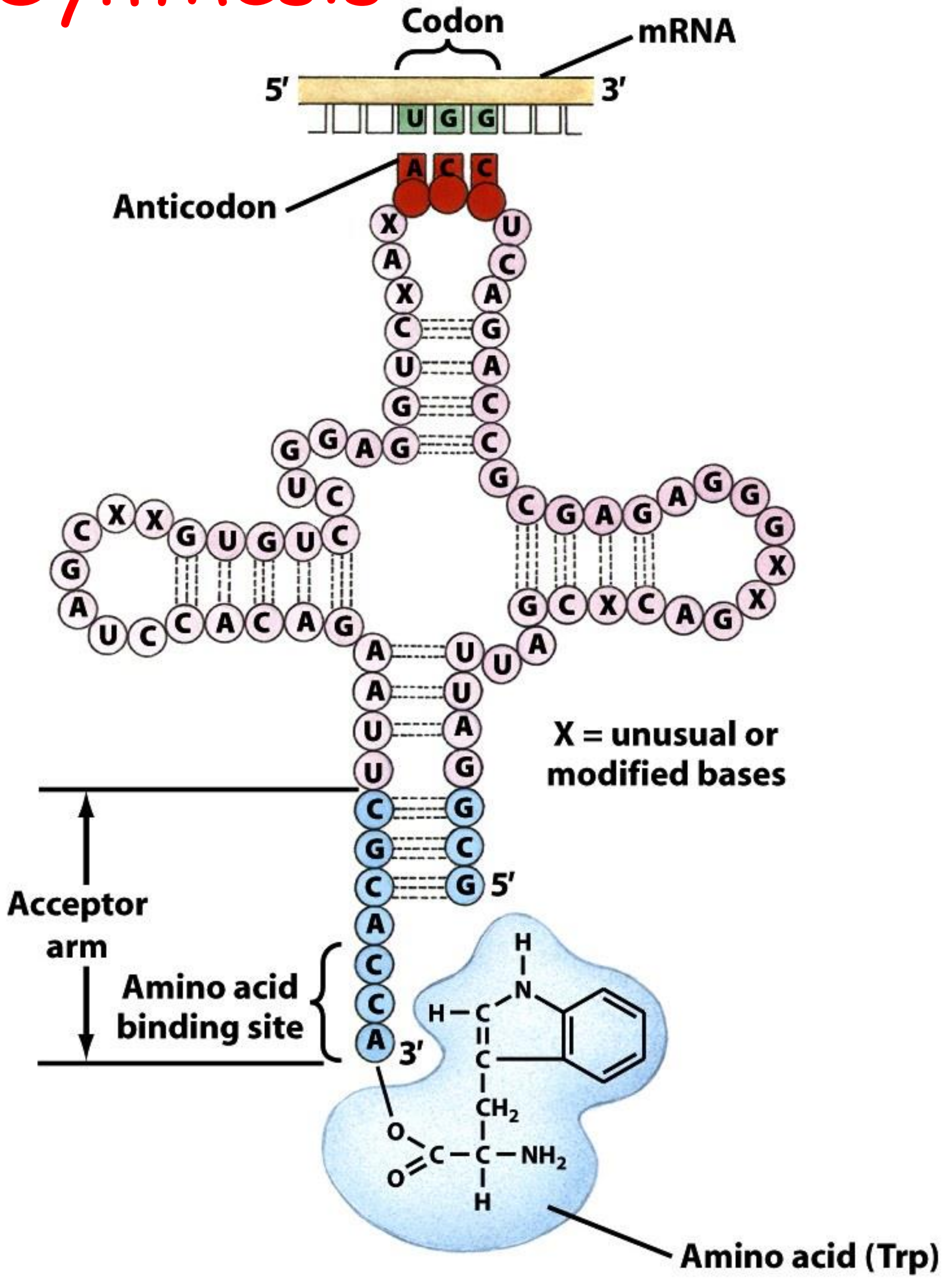

Figure 7-9a Microbiology, 7/e
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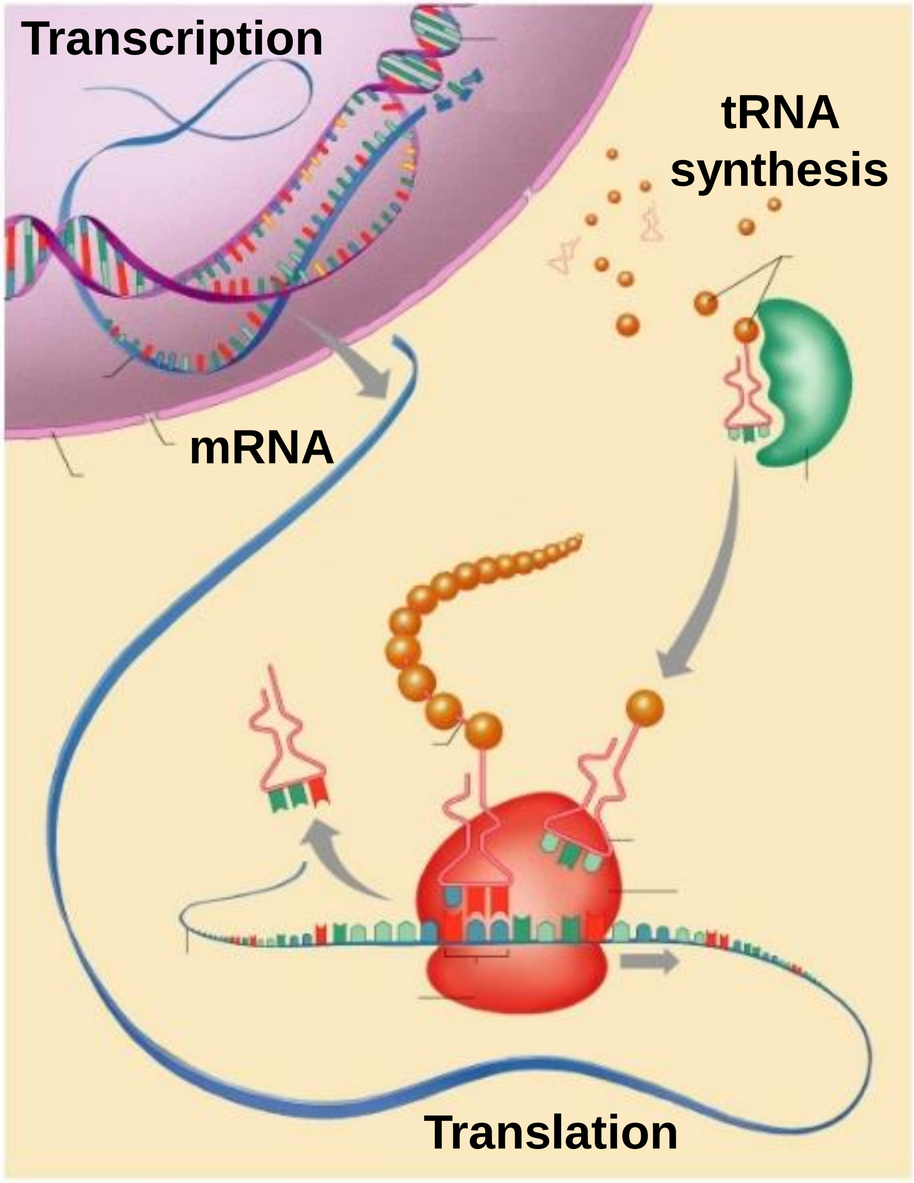
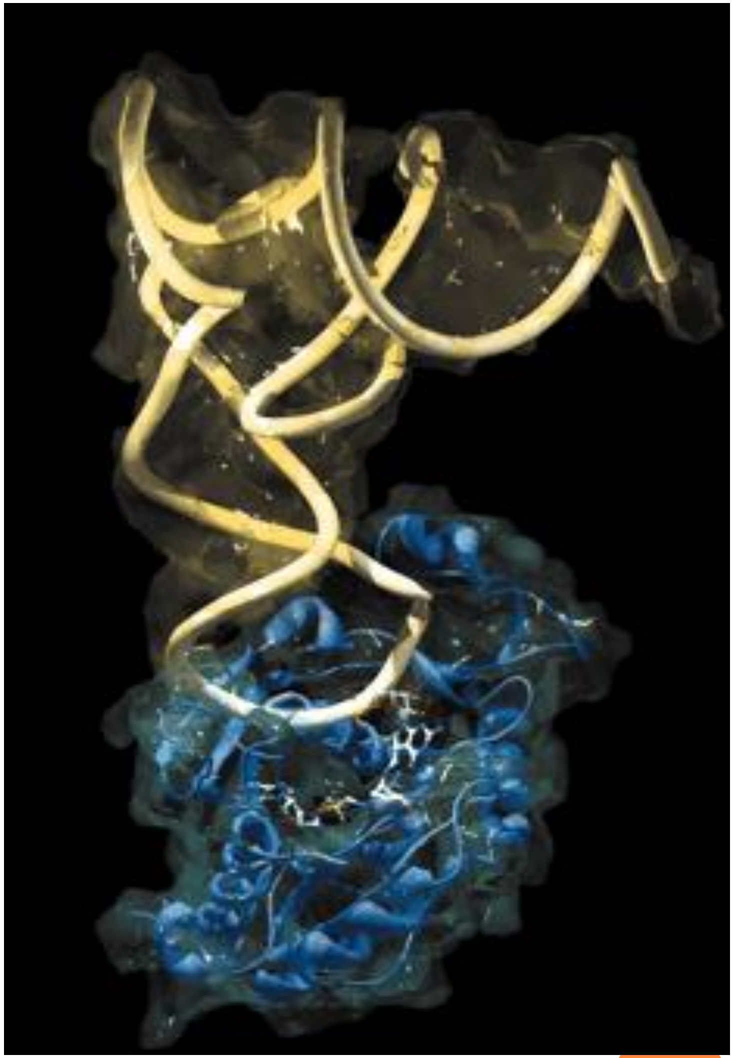
Protein Synthesis





Aminoacyl tRNA synthetases attach amino acids to their specific tRNA

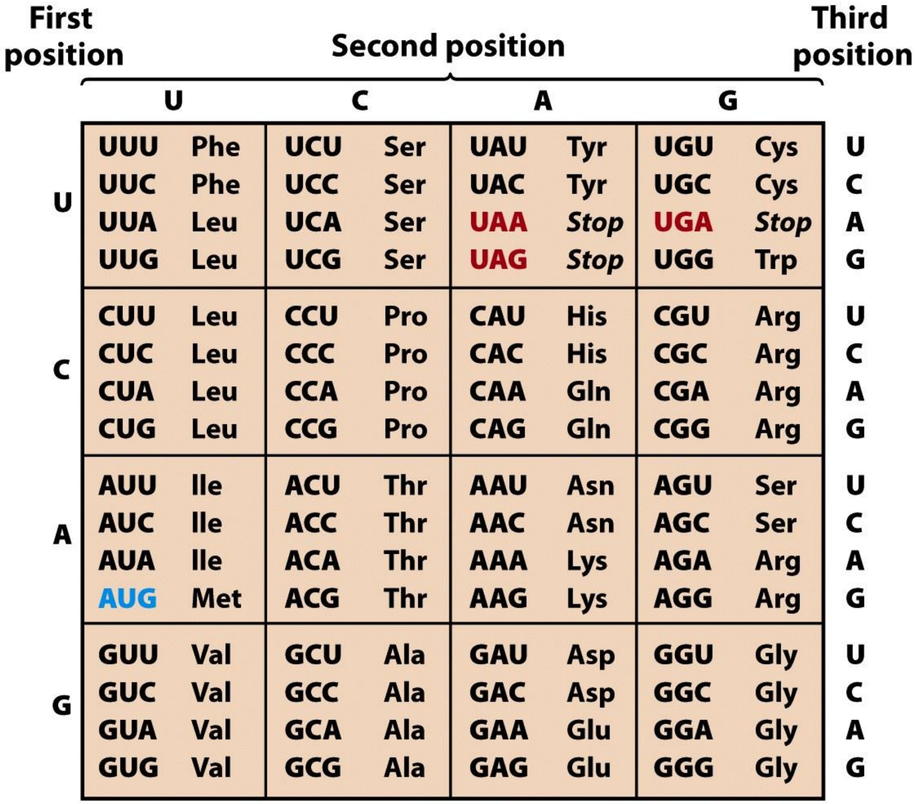
TRANSLATION INVOLVES FOUR STEPS

- In translation the mature mRNA molecule is used as a template to assemble a series of amino acids to produce a polypeptide with a specific amino acid sequence.
- The complex in the cytoplasm at which this occurs is called a **ribosome**. Ribosomes are a mixture of ribosomal proteins and ribosomal RNA (rRNA), and consist of a large subunit and a small subunit

- ***1. Initiation.*** The small subunit of the ribosome binds at the 5' end of the mRNA molecule and moves in a 3' direction until it meets a start codon (AUG). It then forms a complex with the large unit of the ribosome complex and an initiation tRNA molecule.

Protein Synthesis

- Translation
  Initiation
  - ✓ Start codon signals where the gene begins (at 5’ end of mRNA)



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

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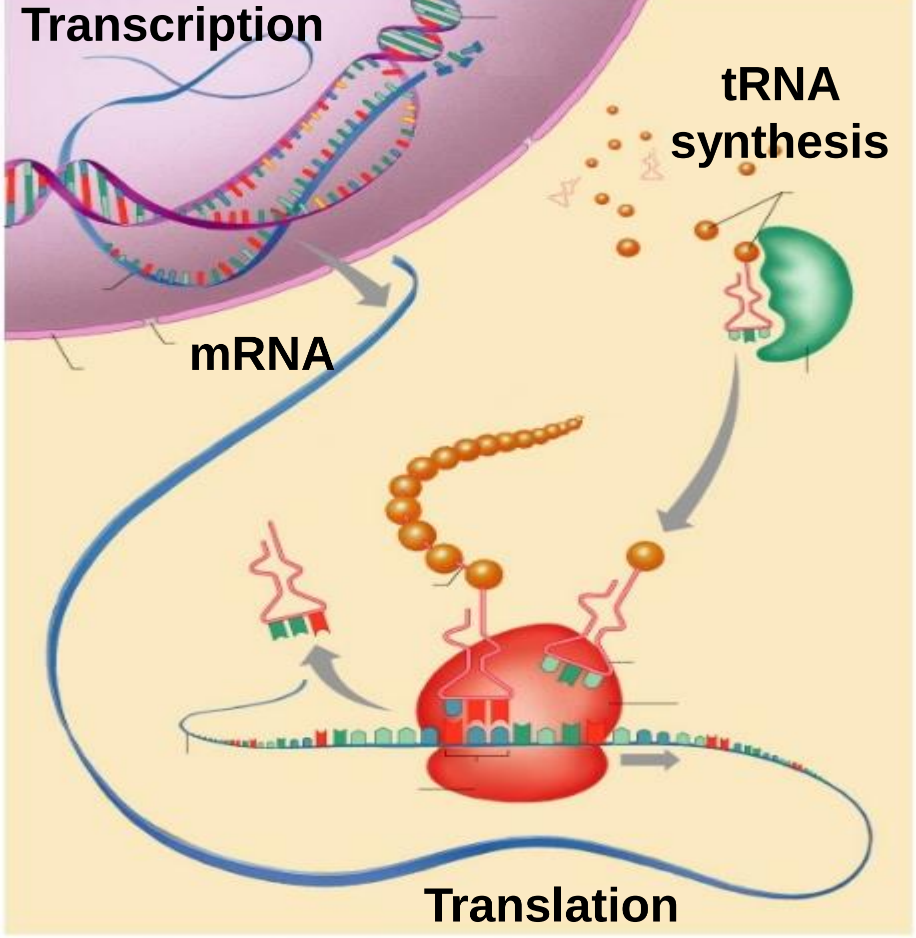

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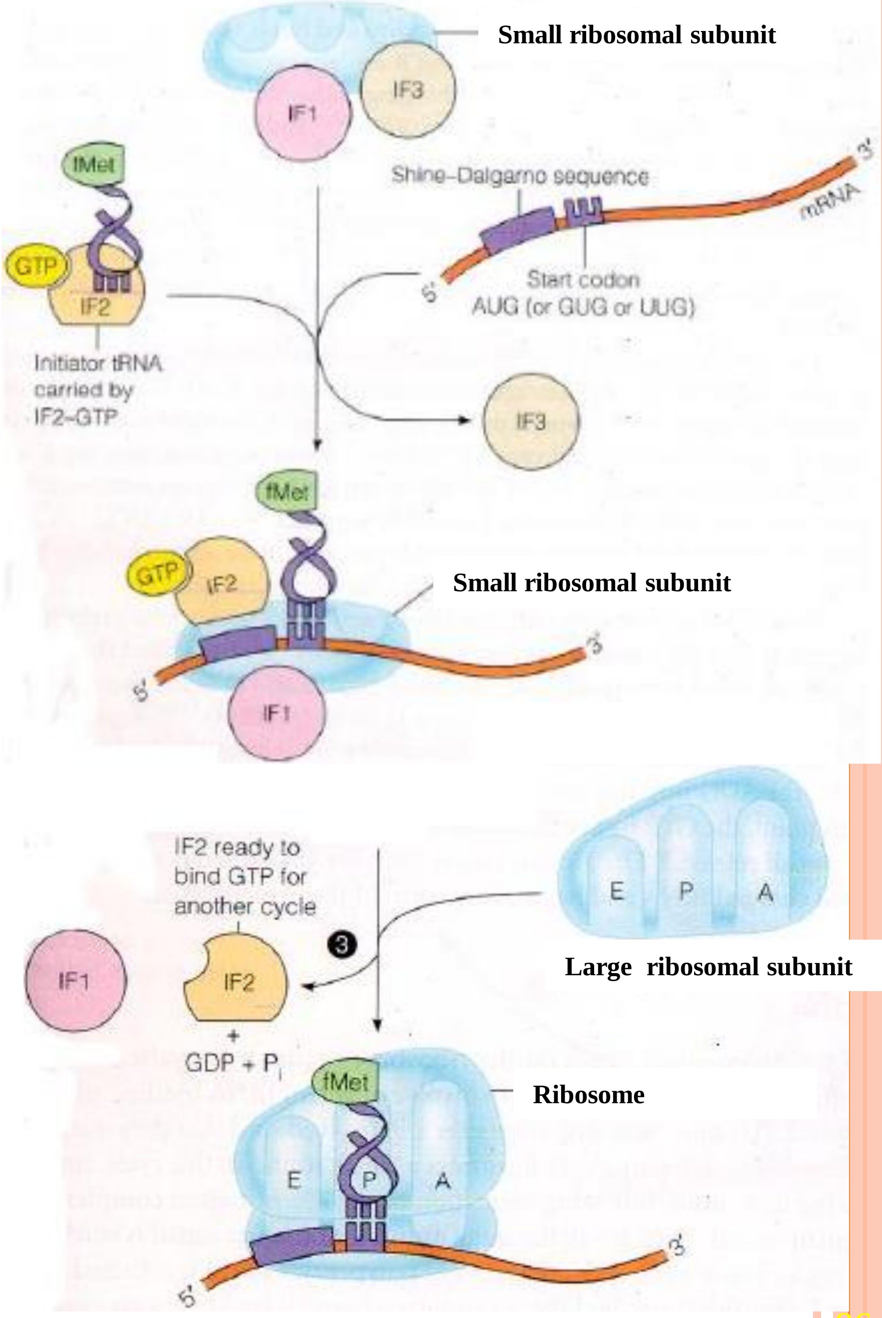
5’ 3’

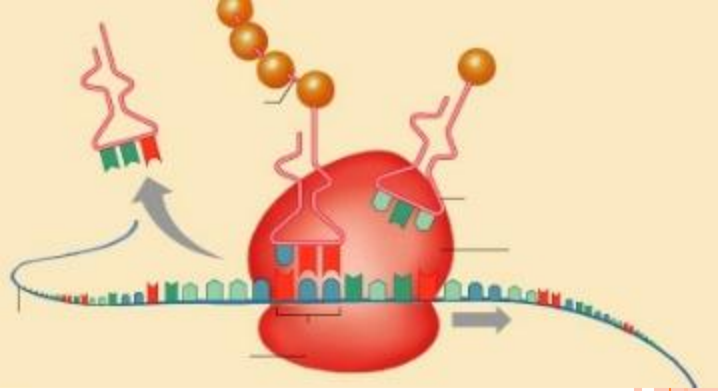
$\underbrace{\text{AUG}}_{\text{start codon}}\text{GACAUUGAACCG…}$

Protein Synthesis

- Translation
  Initiation
  - ✓ Start codon signals where the gene begins (at 5’ end of mRNA)
  - ✓ **Ribosome binding site** (*Shine Dalgarno sequence*) upstream from the start codon binds to small ribosomal subunit
    - then this complex recruits the large ribosomal subunit



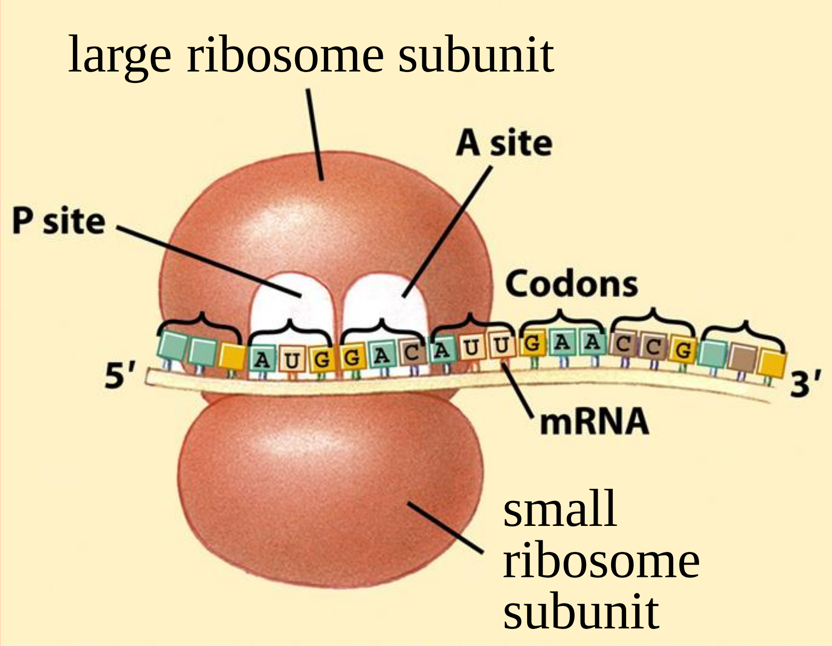
Protein Synthesis

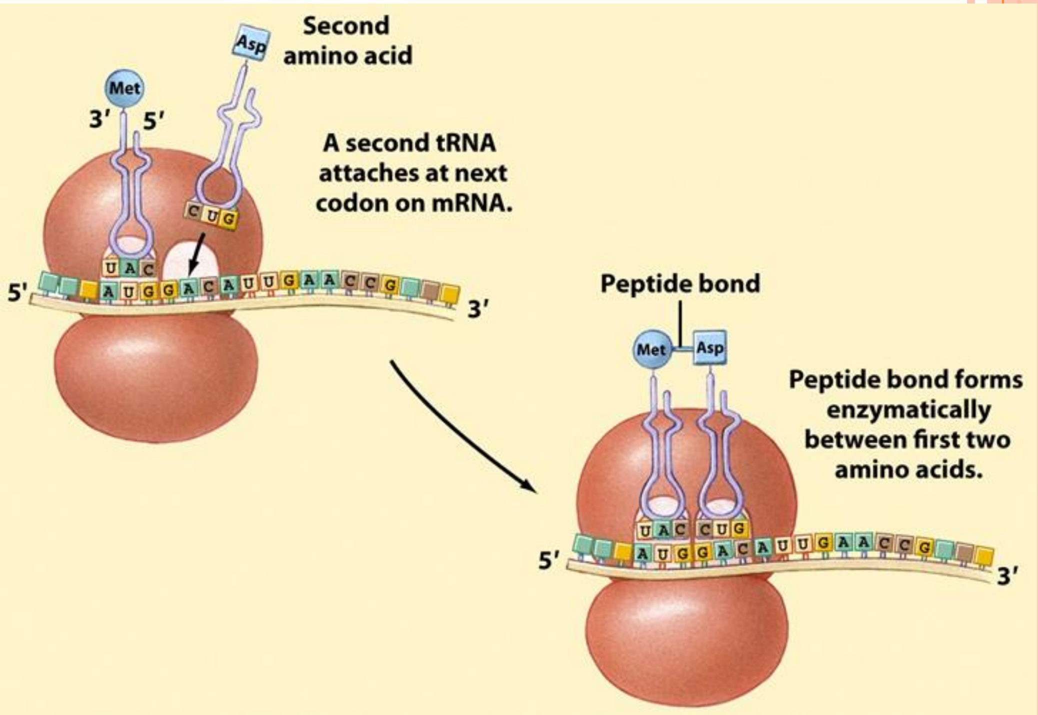


- Translation

  2.*Scanning / elongation*

  ✓ The ribosome moves in 5’ to 3’ direction “reading” the mRNA and assembling amino acids into the correct protein

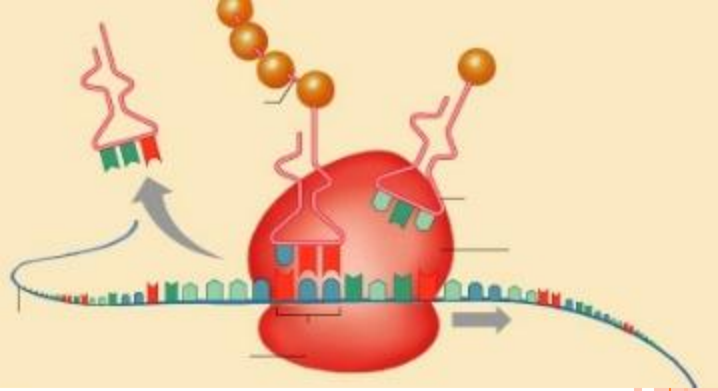




A second tRNA attaches at next codon on mRNA.

Peptide bond forms enzymatically between first two amino acids.

Protein Synthesis

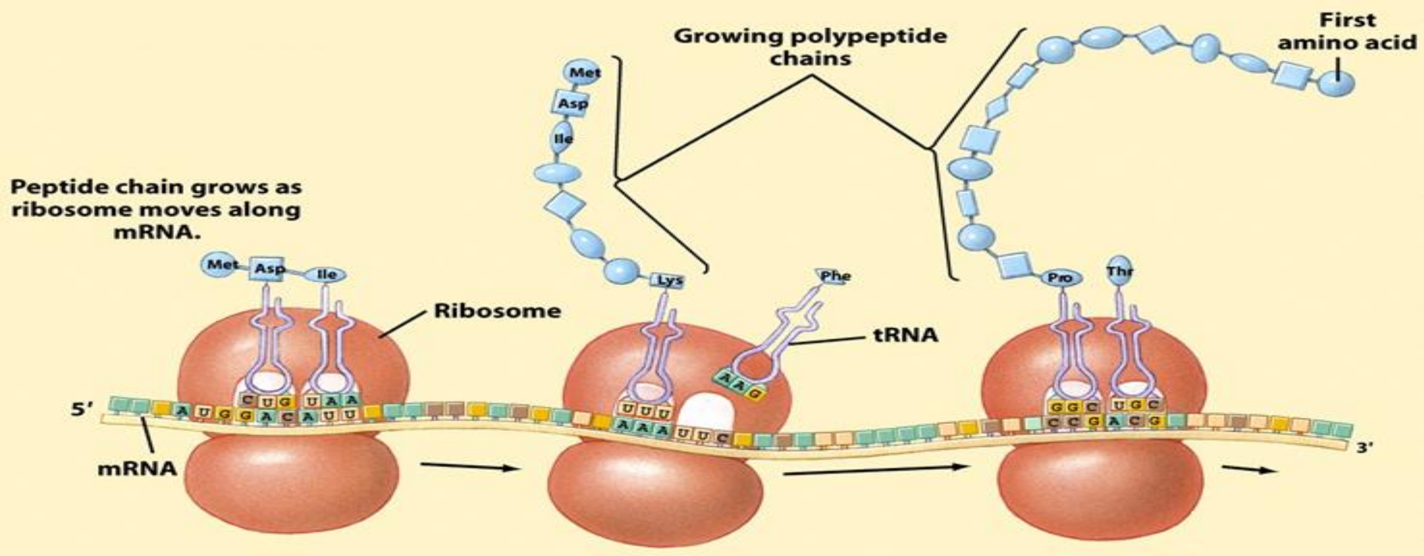


- Translation

  2.*Scanning / elongation*

Subsequent codons on the mRNA molecule determine which tRNA molecule linked to an amino acid binds to the mRNA. An enzyme peptidyl transferase links the amino acids together using peptide bonds. The process continues, producing a chain of amino acids as the ribosome moves along the mRNA molecule

✓ The ribosome moves in 5’ to 3’ direction “reading” the mRNA and assembling amino acids into the correct protein



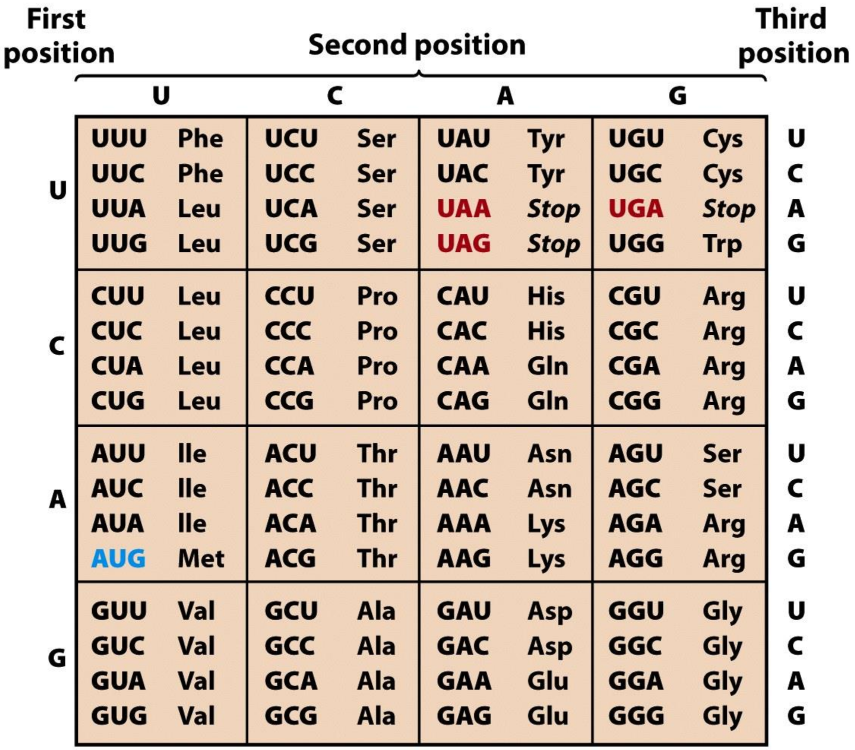
Protein Synthesis

- Translation

  3. *Termination*

Translation in terminated when the ribosomal complex reached one or more stop codons (UAA, UAG, UGA)

- ✓ Ribosome disengages from the mRNA when it encounters a stop codon



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

Figure 7-8 Microbiology, 7/e
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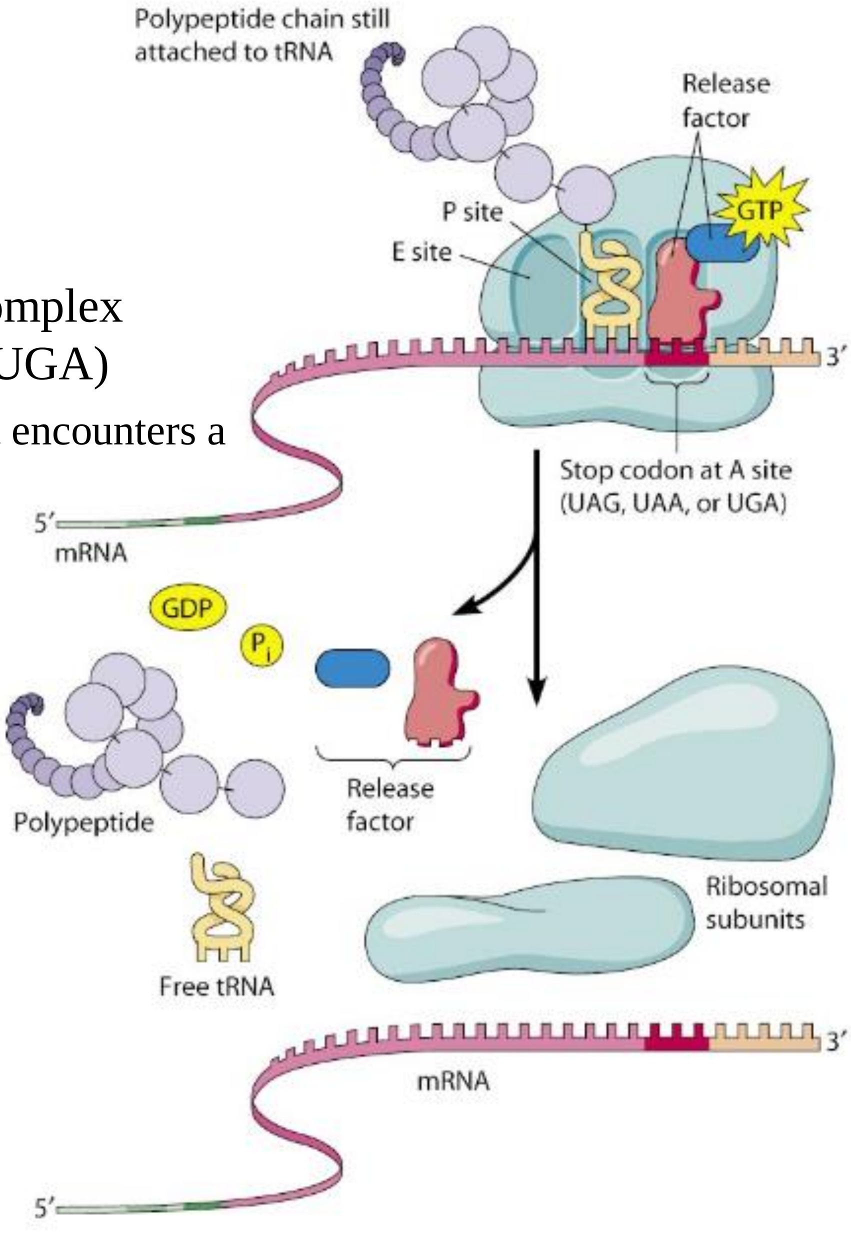

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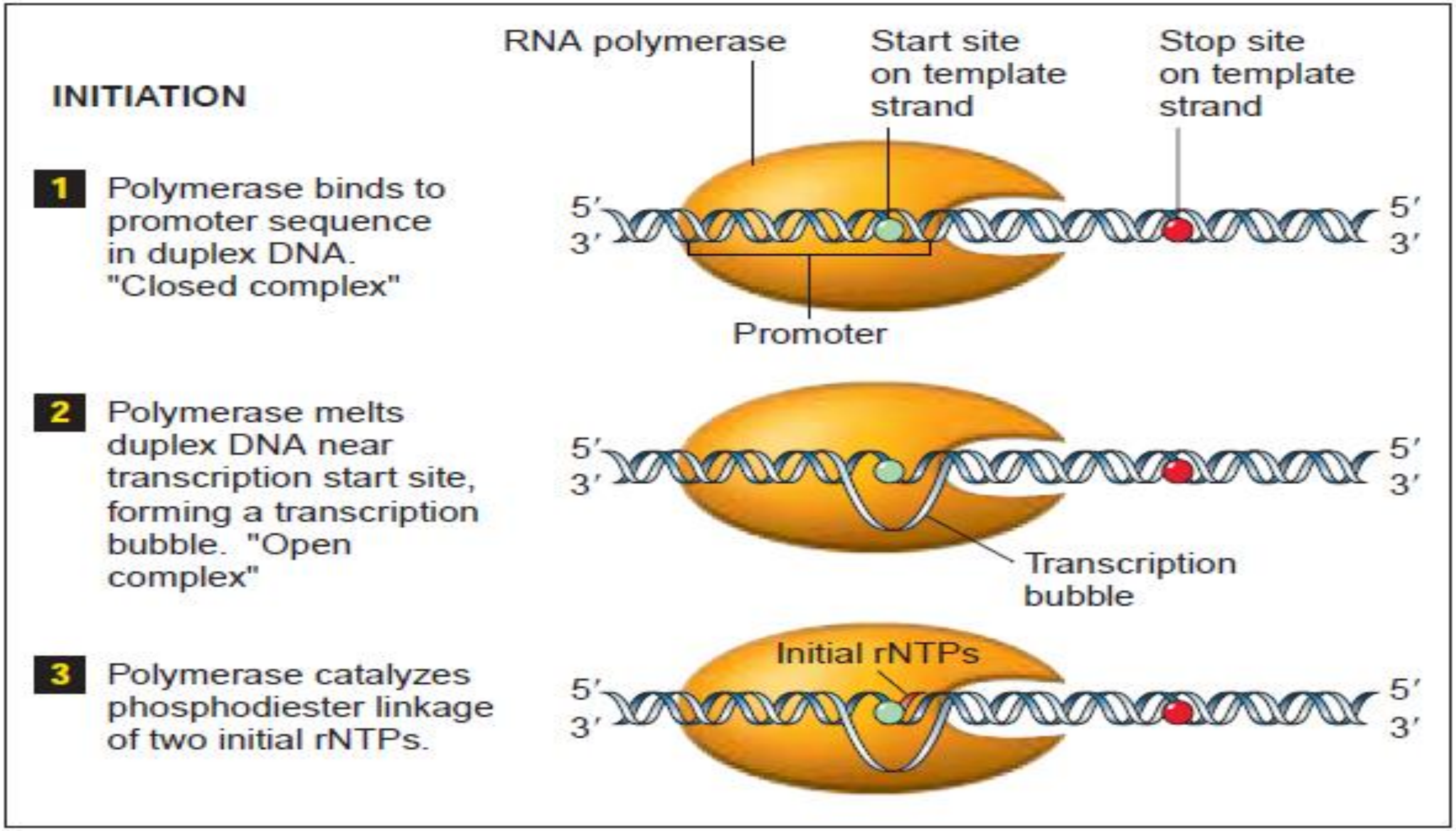
- **4. Post-translation processing of the protein**
- It refers to covalent and generally enzymatic modification of protein following protein biosynthesis
- Any of various enzymatically catalyzed post translational modification of peptides or proteins in cell of origin This modification involves carboxylation, hydroxylation, acetylation, phosphorylation, methylation , glycosylation etc

SUMMARY OF PROTEIN SYNTHESIS

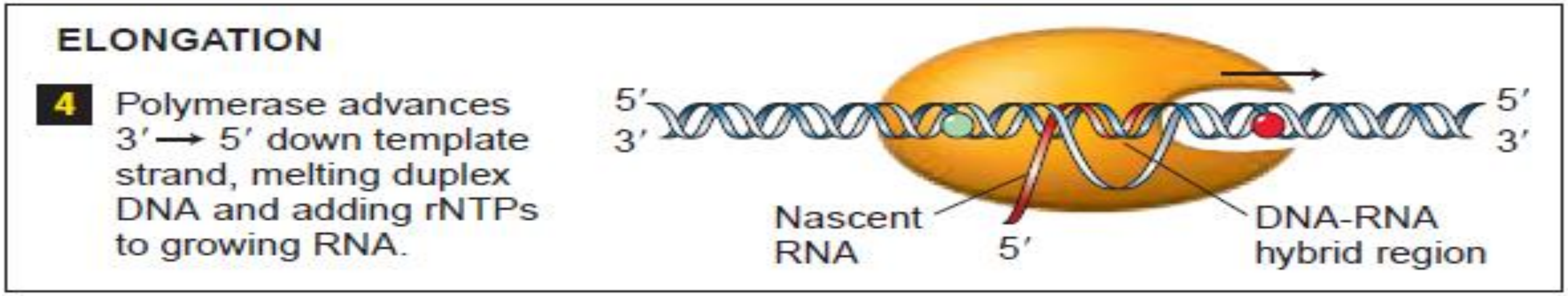


INITIATION

1 Polymerase binds to promoter sequence in duplex DNA. "Closed complex"

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

3 Polymerase catalyzes phosphodiester linkage of two initial rNTPs.



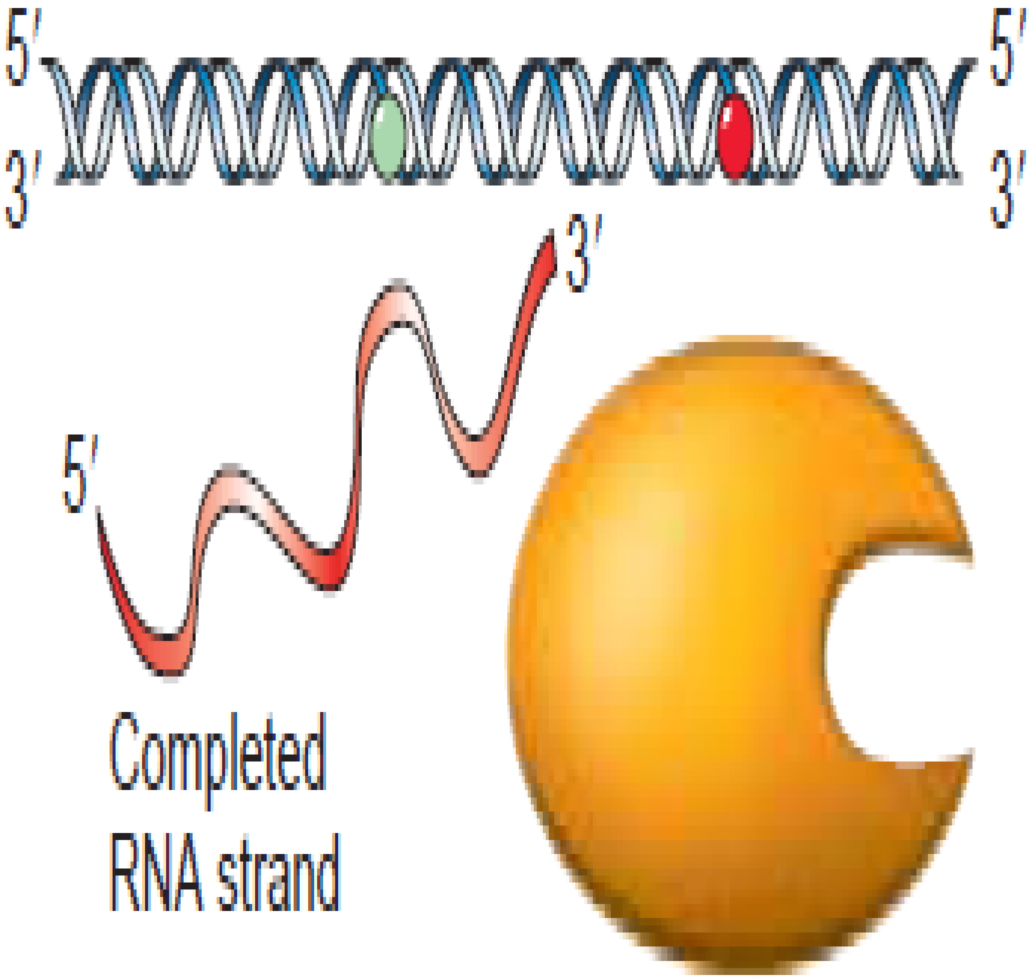
ELONGATION

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

CONT…

TERMINATION

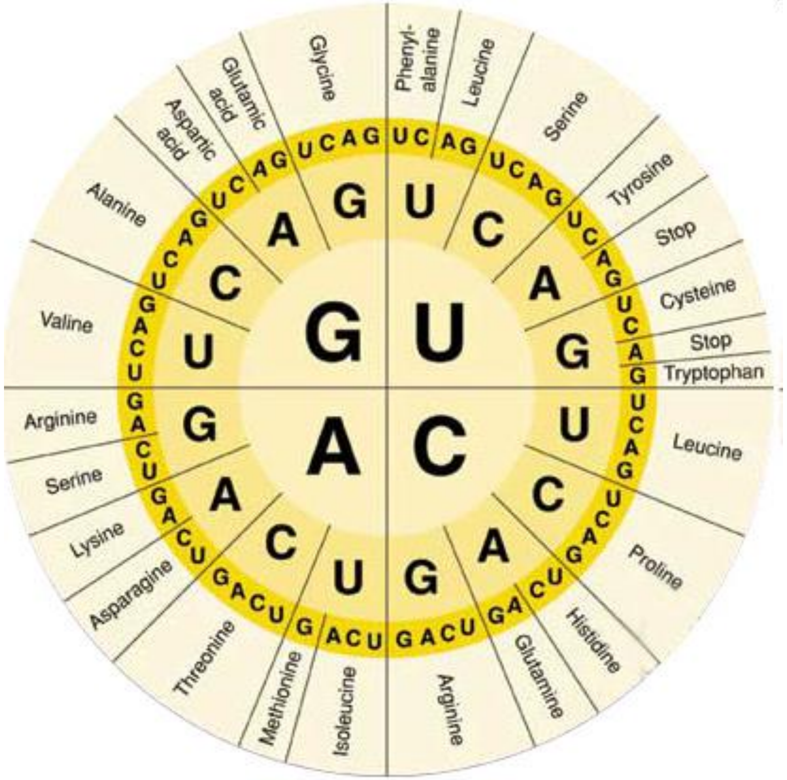
5 At transcription stop site, polymerase releases completed RNA and dissociates from DNA.



PRACTICE QUESTION

Translate the following mRNA sequence

AGCUACCAUACGCACCCGAGUUCUUCAAGC



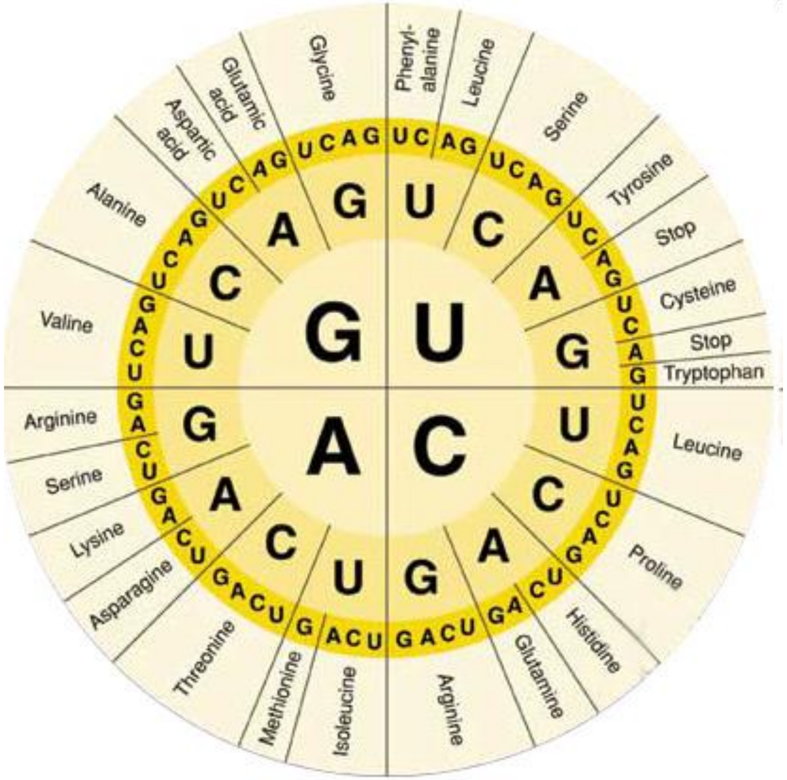
single-letter code	abbreviation	full name
A	Ala	Alanine
R	Arg	Arginine
N	Asn	Asparagine
D	Asp	Aspartic acid
C	Cys	Cysteine
Q	Gln	Glutamine
E	Glu	Glutamic acid
G	Gly	Glycine
H	His	Histidine
I	Ile	Isoleucine
L	Leu	Leucine
K	Lys	Lysine
M	Met	Methionine
F	Phe	Phenylalanine
P	Pro	Proline
S	Ser	Serine
T	Thr	Threonine
W	Trp	Tryptophan
Y	Tyr	Tyrosine
V	Val	Valine

PRACTICE QUESTION

Translate the following mRNA sequence

AGCUACCAUACGCACCCGAGUUCUUCAAGC

Serine – Tyrosine – Histidine – Threonine – Histidine – Proline – Serine – Serine – Serine - Serine



single-letter code	*abbreviation*	*full name*
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I	Ile	Isoleucine
L	Leu	Leucine
K	Lys	Lysine
M	Met	Methionine
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S	Ser	Serine
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W	Trp	Tryptophan
Y	Tyr	Tyrosine
V	Val	Valine

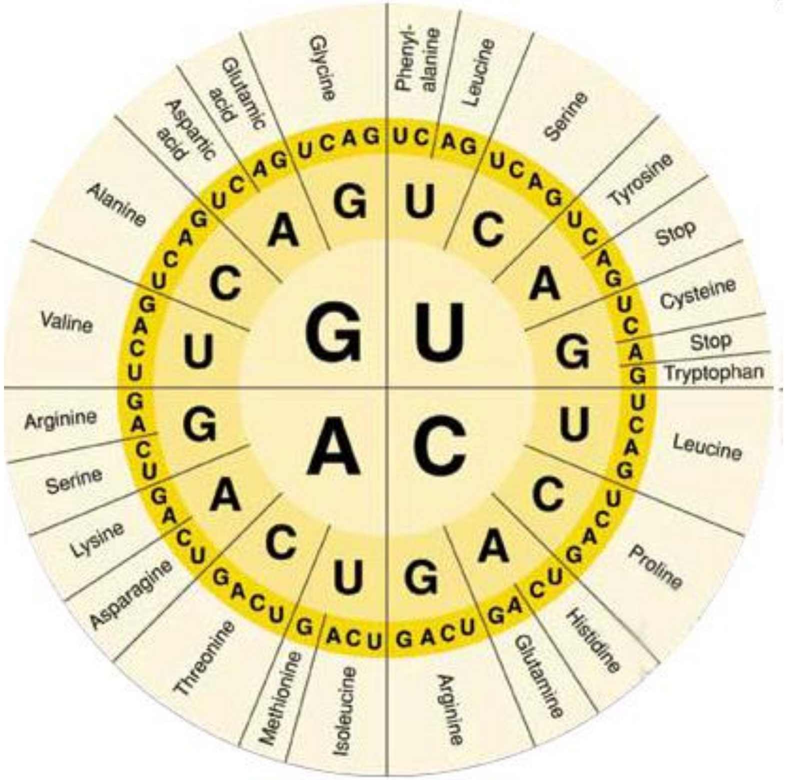
PRACTICE QUESTION

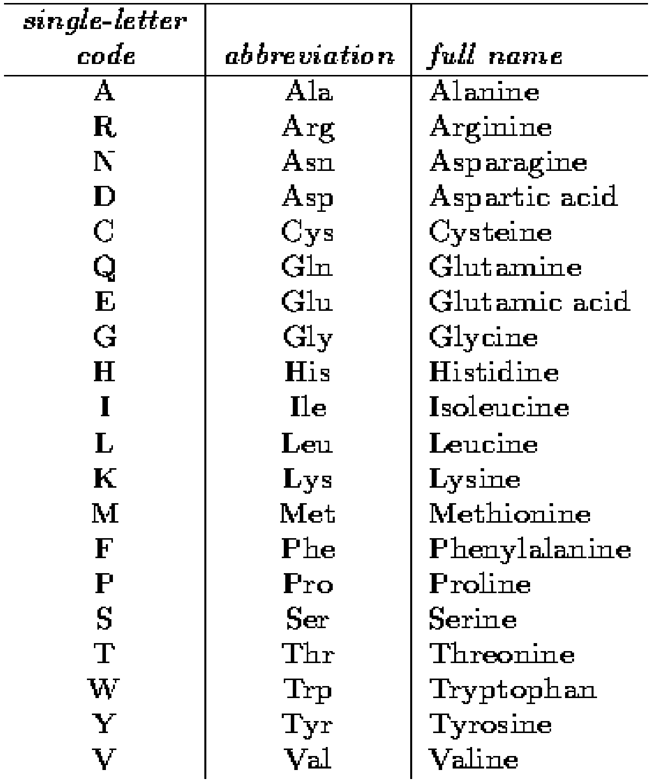
Translate the following mRNA sequence

AGCUACCAUACGCACCCGAGUUCUUCAAGC

Serine – Tyrosine – Histidine – Threonine – Histidine – Proline – Serine – Serine – Serine - Serine

Ser – Tyr – His – Thr – His – Pro – Ser – Ser – Ser - Ser





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PRACTICE QUESTION

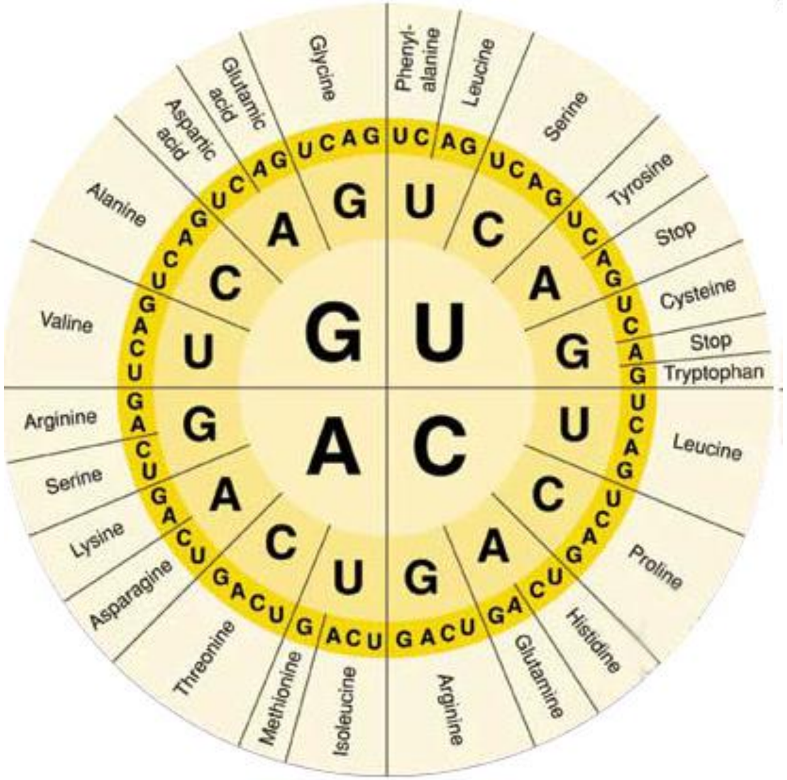
Translate the following mRNA sequence

AGCUACCAUACGCACCCGAGUUCUUCAAGC

Serine – Tyrosine – Histidine – Threonine – Histidine – Proline – Serine – Serine – Serine - Serine

Ser – Tyr – His – Thr – His – Pro – Ser – Ser – Ser - Ser

S – Y –H– T – H – P – S – S – S - S



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