

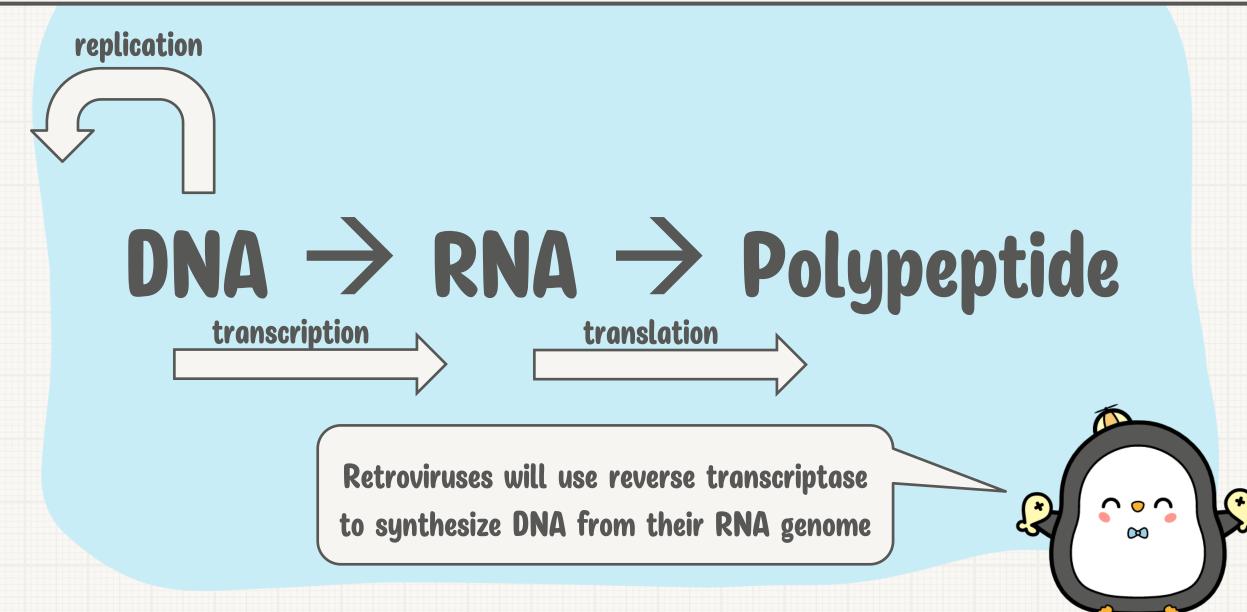
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Today's Plan Molecular Genetics Operons & BioTechnology Practice Questions Unit 6 Q&A **e** $\circ \circ \circ$

Central Dogma



<u>Location</u>

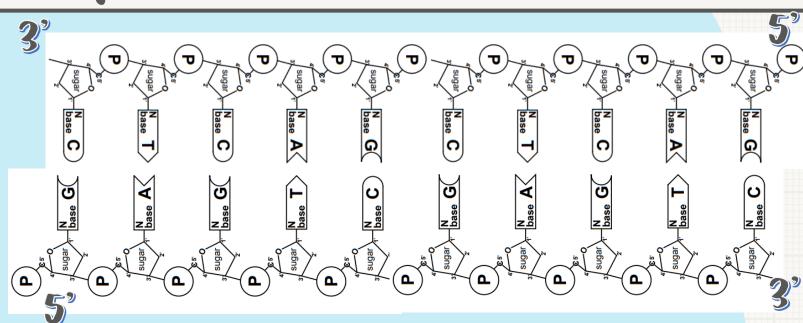
- Eukaryotes: nucleus
- Prokaryotes: nucleoid

<u>Structure</u>

- Eukaryotes: multiple linear
- Prokaryotes: single circular

Reminders about DNA:

- DNA made up of:
 - nitrogenous base (A, T, C, G)
 - pentose sugar (deoxyribose)
 - phosphate group
- Purine (A/G) have a double ring structure
- Pyrimidine (C/T) have a single ring structure
- Base Pair Rules
 - A & T with 2 H bonds
 - C & G with 3 H bonds



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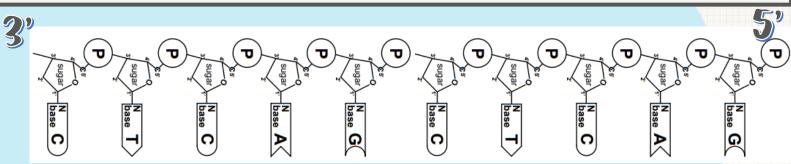
- Sidedness
 - 5' end: phosphate
 - 3' end: hydroxyl group
- Directionality
 - Read 3' to 5'
 - Synthesize 5' to 3'

(Remember ANTIPARALLEL)

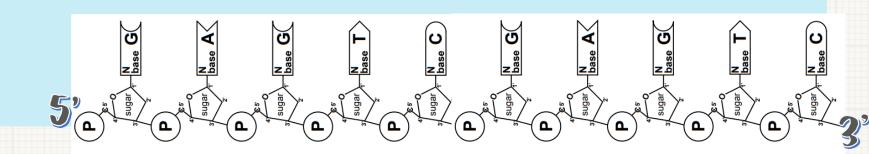
Important Enzymes

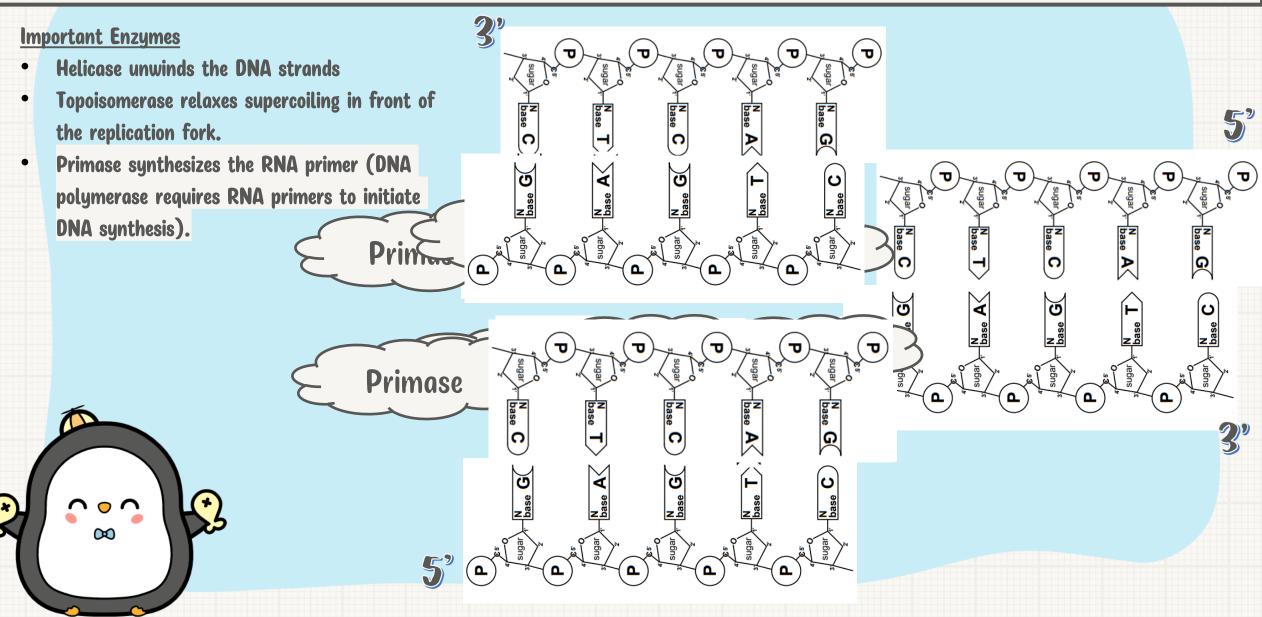
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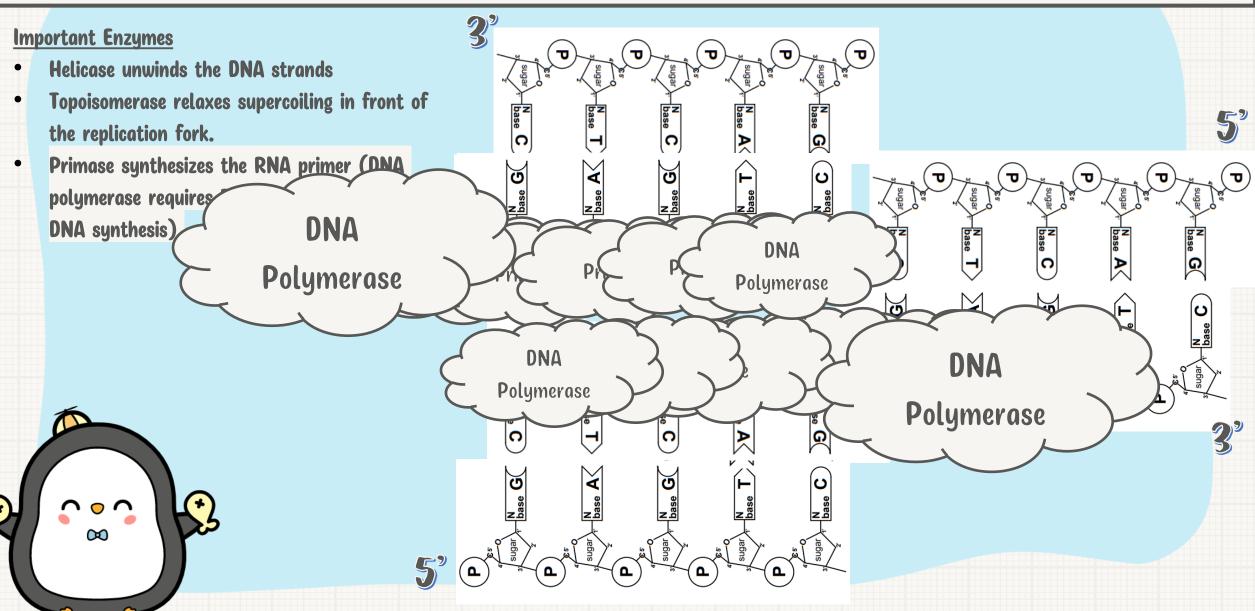
- Helicase unwinds the DNA strands
- Topoisomerase relaxes supercoiling in front of the replication fork.









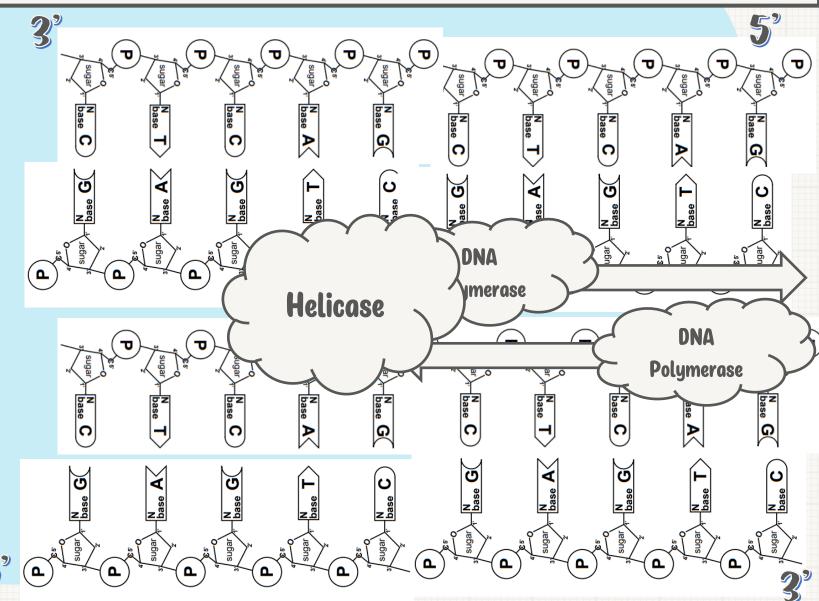


Important Enzymes

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- Helicase unwinds the DNA strands
- Topoisomerase relaxes supercoiling in front of the replication fork.
- Primase synthesizes the RNA primer (DNA polymerase requires RNA primers to initiate DNA synthesis).
- DNA polymerase synthesizes new strands of DNA continuously on the leading strand and discontinuously on the lagging strand.

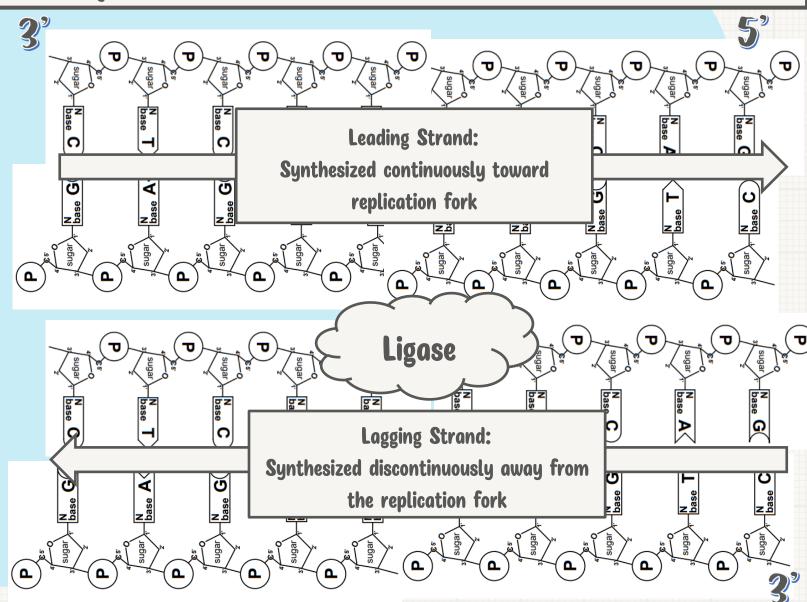


Important Enzymes

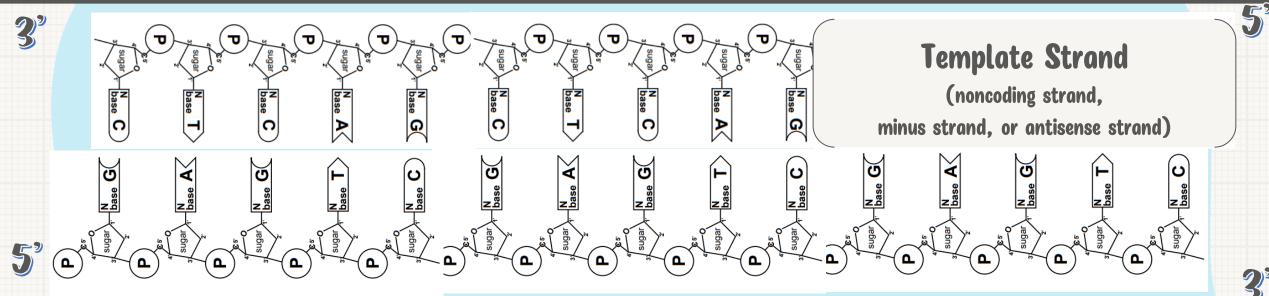
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- Helicase unwinds the DNA strands
- Topoisomerase relaxes supercoiling in front of the replication fork.
- Primase synthesizes the RNA primer (DNA polymerase requires RNA primers to initiate DNA synthesis).
- DNA polymerase synthesizes new strands of DNA continuously on the leading strand and discontinuously on the lagging strand.
- Ligase joins the fragments on the lagging strand.



Transcription



Location

- Eukaryotes: nucleus
- Prokaryotes: nucleoid

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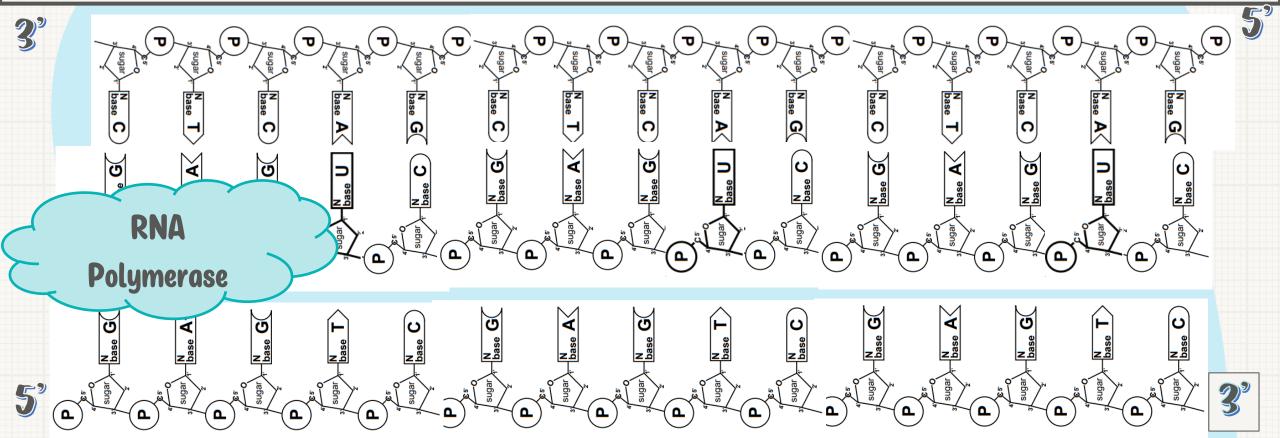
(cytosol)

Reminders about RNA:

- DNA made up of:
 - nitrogenous base (A, U, C, G)
 - pentose sugar (ribose)
 - phosphate group
- Purine (A/G) have a double ring structure
- Pyrimidine (C/U) have a single ring structure •

- Base Pair Rules
 - A & T(DNA)/U(RNA) with 2 H bonds
 - C & G with 3 H bonds
- Sidedness
 - 5' end: phosphate
 - 3' end: hydroxyl group
- Directionality
 - Read 3' to 5'
 - Synthesize 5' to 3'

Transcription



Important Enzyme & Components

- RNA polymerase synthesizes mRNA molecules in the 5' to
 3' direction by reading the template DNA strand in the 3'
 to 5' direction.
- Promoter: site where RNA polymerase binds to start transcription
- Transcription Factors: activators/inhibitors to turn on/off gene expression

Post-Transcriptional Modifications

5' Guanine Cap

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- Signals the "start" of the mRNA transcript for ribosome to bind
- Facilitates export from nucleus

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N base

Poly-A Tail

C

N base

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• Inhibits degradation from hydrolytic enzymes in cytosol

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N base

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Splicing

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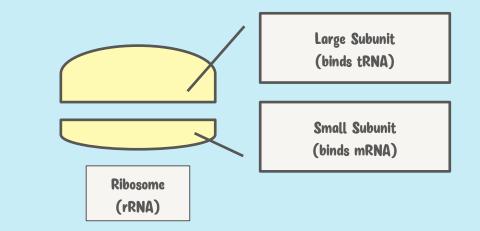
• Removal of introns from premRNA transcript



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Translation



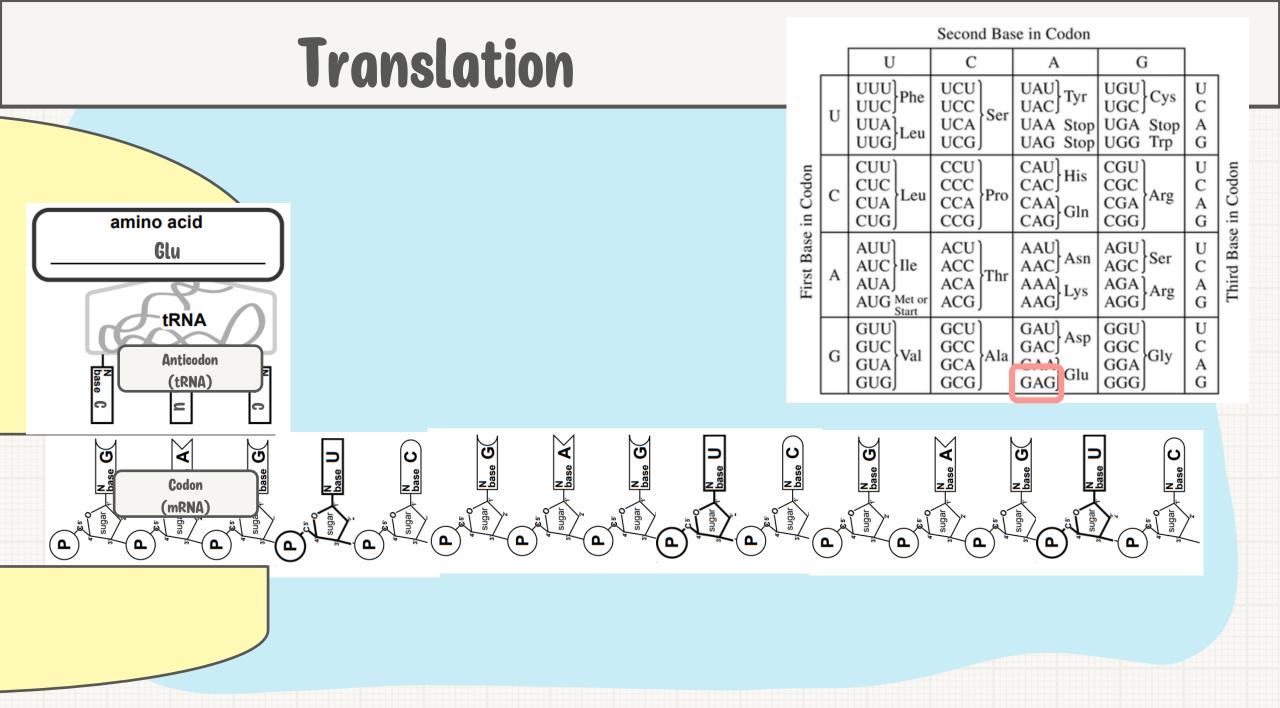
Location

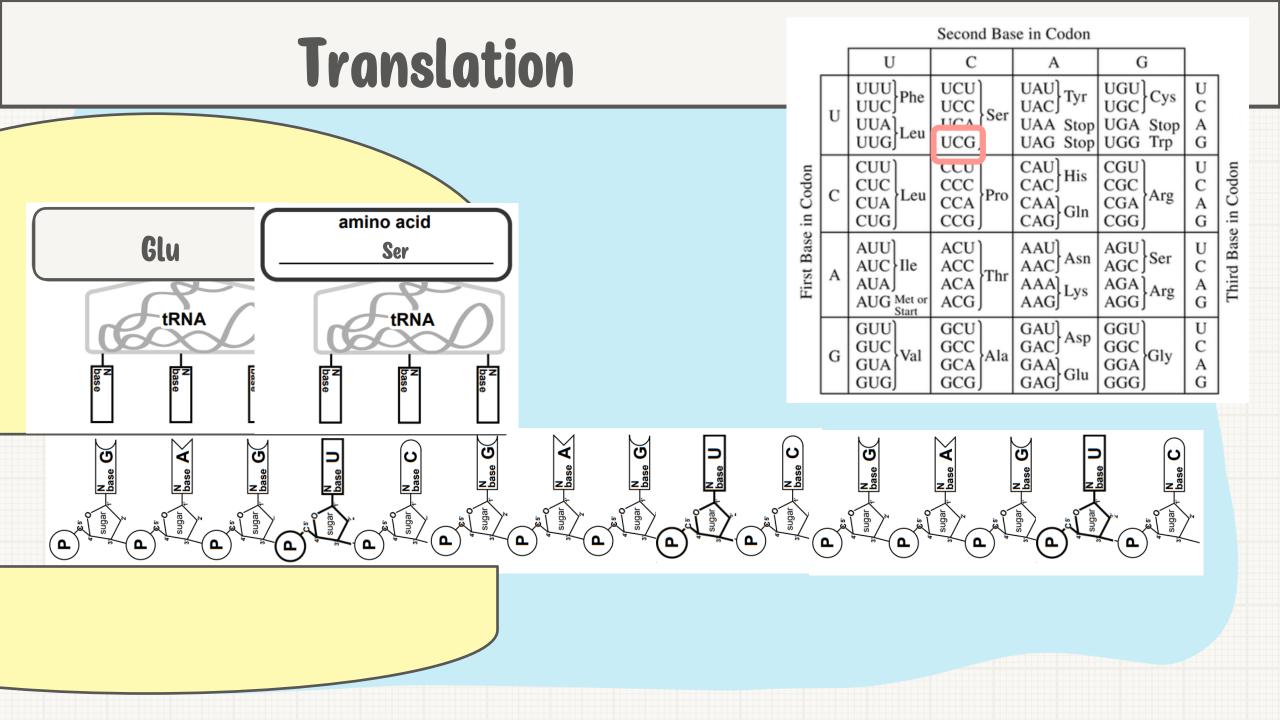
- Eukaryotes: cytosol/rough ER •
- **Prokaryotes:** cytosol •

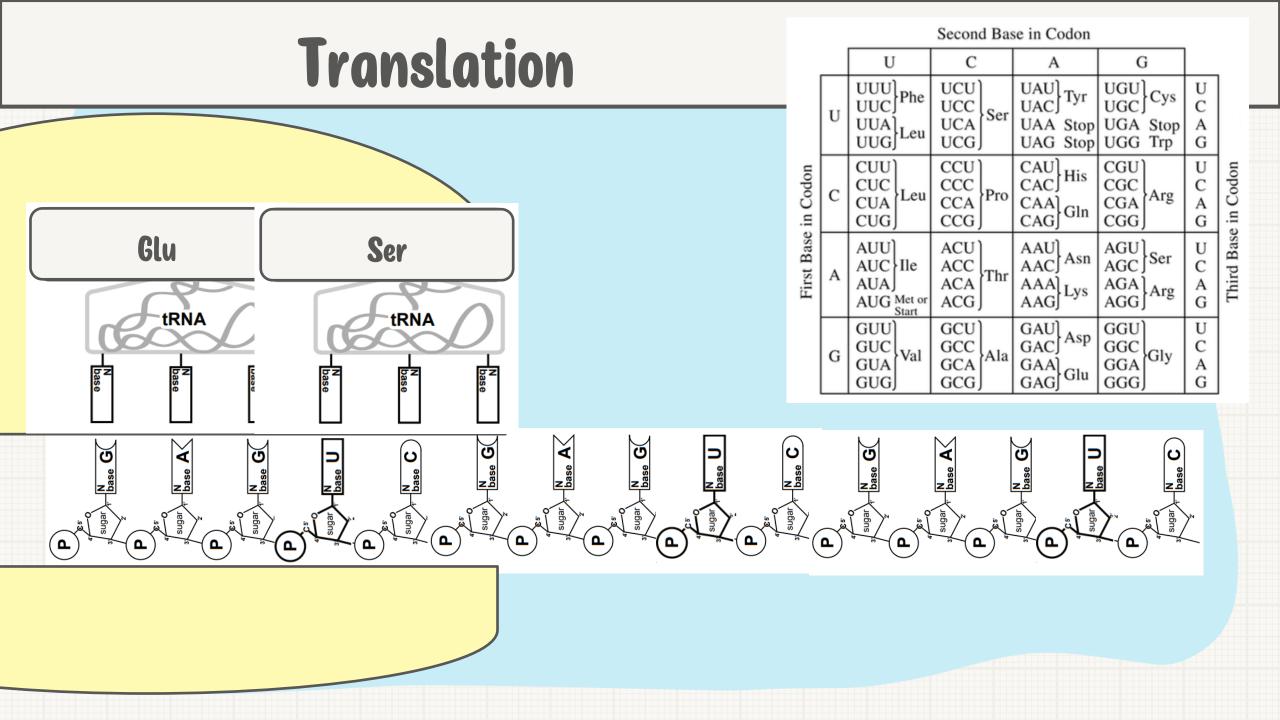
Steps of Translation

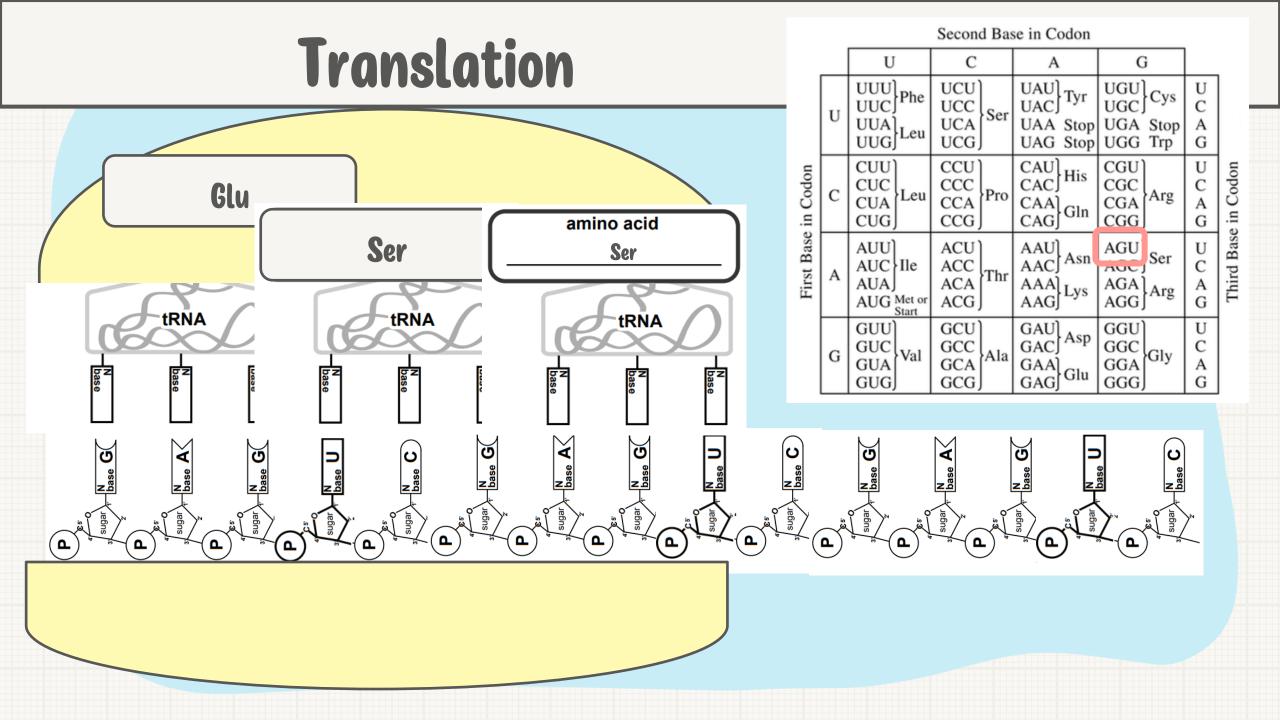
- Initiation: start codon (AUG) ۲
- Elongation: base pair between tRNA/mRNA with • amino acid added
- Termination: stop codon (UAG, UAA, UGA) •

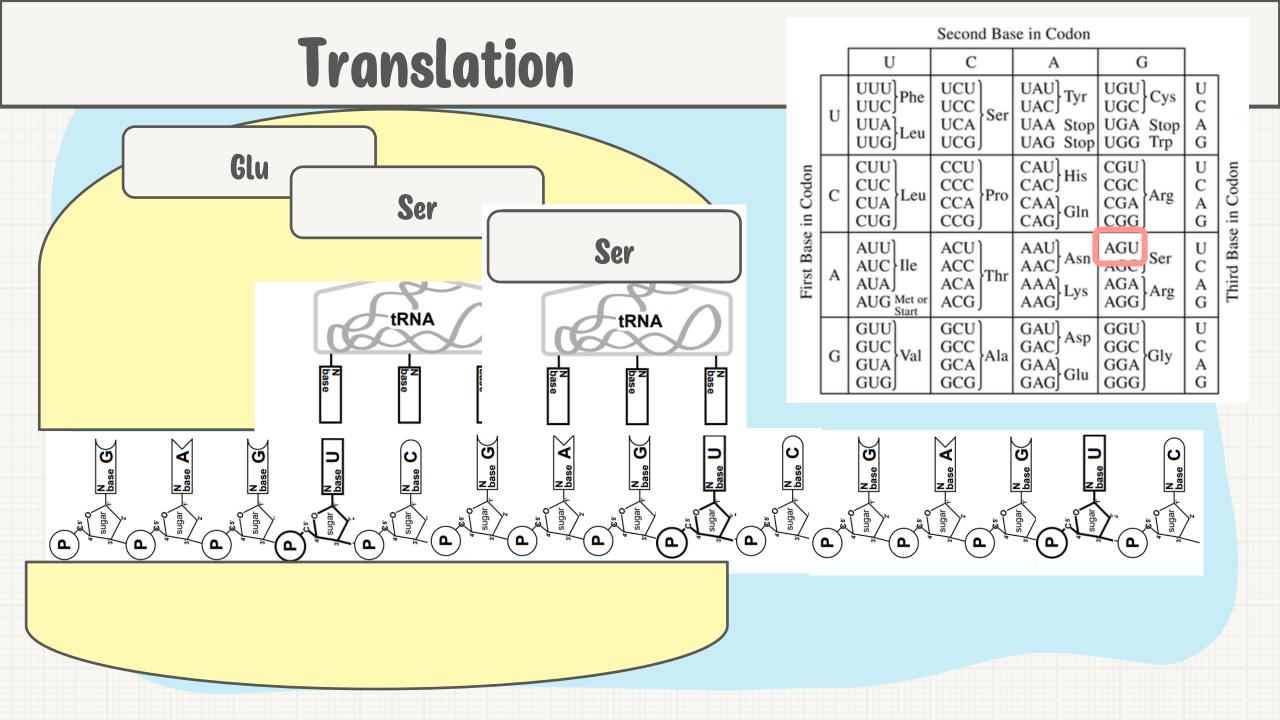
Second Base in Codon								
		U	С	А	G			
First Base in Codon	U	$\begin{bmatrix} UUU \\ UUC \end{bmatrix}$ Phe $\begin{bmatrix} UUA \\ UUG \end{bmatrix}$ Leu	$\left. \begin{matrix} UCU \\ UCC \\ UCA \\ UCG \end{matrix} \right\} Ser$	UAU UAC UAA Stop UAG Stop	UGU UGC UGA Stop UGG Trp	U C A G		
	с	CUU CUC CUA CUG	CCU CCC CCA CCG	CAU CAC CAA CAA CAG Gln	CGU CGC CGA CGG	U C A G	e in Codon	
	A	AUU AUC AUA AUG Met or Start	$\left. \begin{array}{c} ACU \\ ACC \\ ACA \\ ACG \end{array} \right\}^{Thr}$	AAU AAC AAA AAA AAG	$\left. \begin{array}{c} AGU \\ AGC \end{array} \right\}$ Ser $\left. \begin{array}{c} AGA \\ AGG \end{array} \right\}$ Arg	U C A G	Third Base in	
	G	GUU GUC GUA GUG	GCU GCC GCA GCG	GAU GAC GAA GAA GAG Glu	GGU GGC GGA GGG	U C A G		



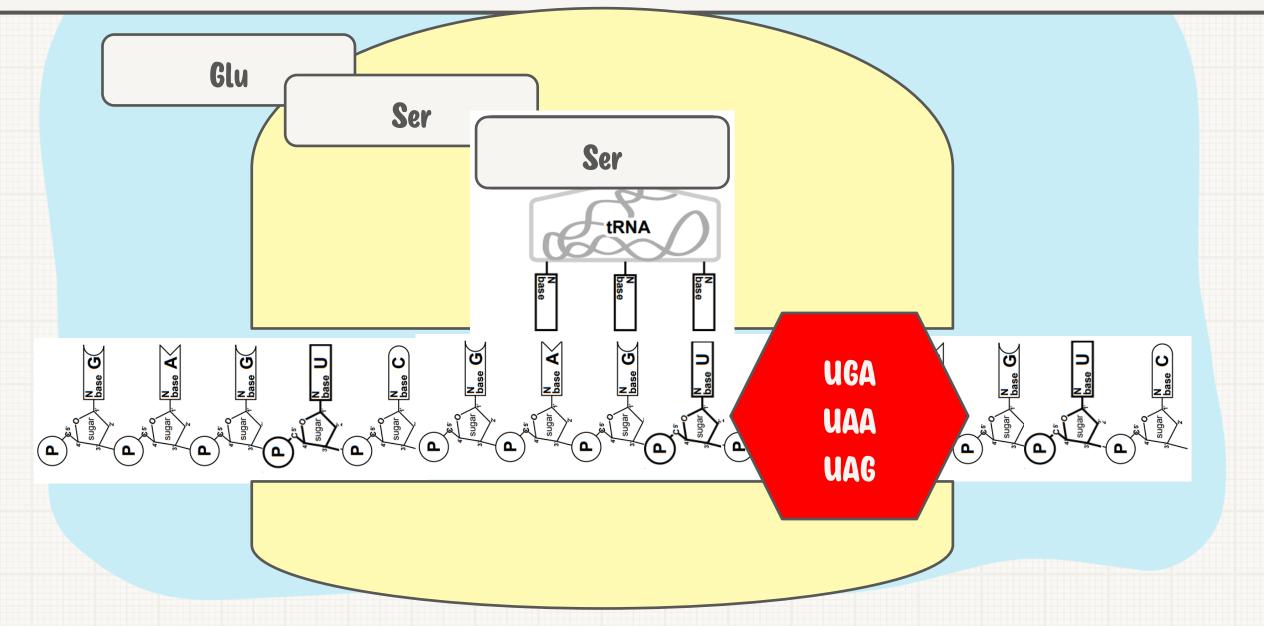








Translation

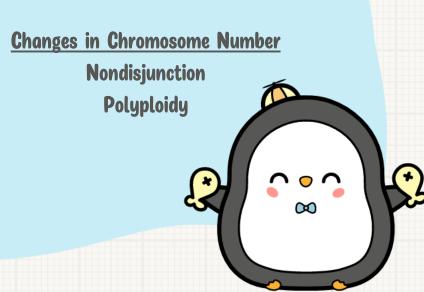


Mutations

Point Mutations Mutation at one nucleotide base pair Silent no change in Frameshift amino acid (AA) insertion/deletion of 1 or 2 nucleotide Missense base pairs change from one AA shifts the reading frame for codons to another AA Nonsense change from AA to STOP codon ORIGINAL DNA: corresponds to the amino acid leucine. PRAMESHIFT NUTATION SILENT MUTATION **HISSENSE HUTATION** NONSENSE MUTATION every emino acid that leucine valine step coden. follows will be altered.

Chromosomal Mutations Rearrangement of chromosome parts or changes in chromosome numbers

> Rearrangement Insertion Deletion Duplication Inversion Translocation



Operons

Gene Regulation found in prokaryotes

<u>Promoter</u> Site when RNA polymerase binds

Repressible Operon

Example: Trp Operon synthesizes tryptophan

Starts: ON Repressor: INACTIVE <u>Operator</u> Site when repressor binds

<u>Genes</u> DNA

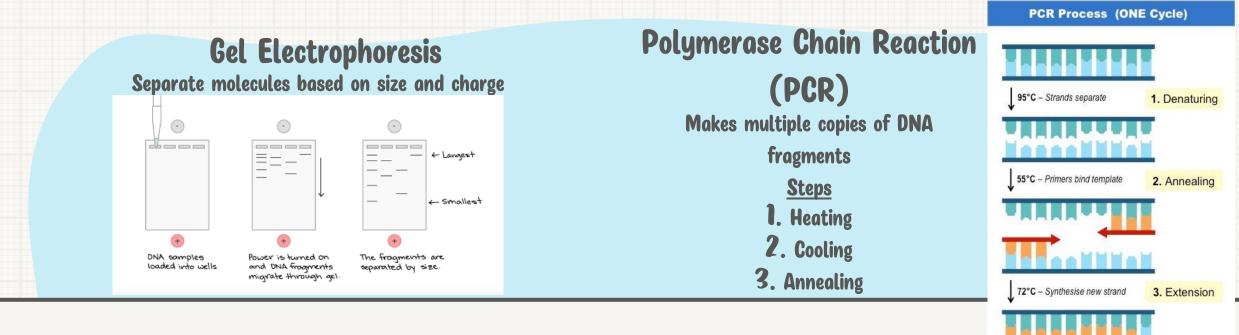
Inducible Operon

Example: Lac Operon synthesizes enzymes to break down lactose

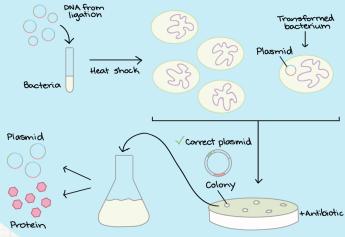
> Starts: OFF Repressor: ACTIVE

If trp is present... Trp binds to <u>repressor</u> to ACTIVATE Repressor binds to operator to turn the <u>operon</u> OFF If lactose is present... lactose binds to <u>repressor</u> to INACTIVATE Repressor no longer binds to operator to turn the <u>operon</u> ON





BioTechnology



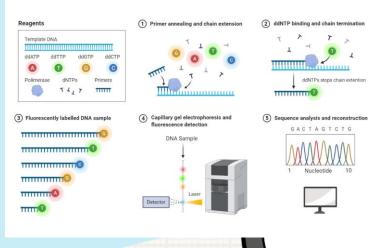
Bacterial Transformation

Introduce genetic material (plasmid) to bacteria

DNA Sequencing

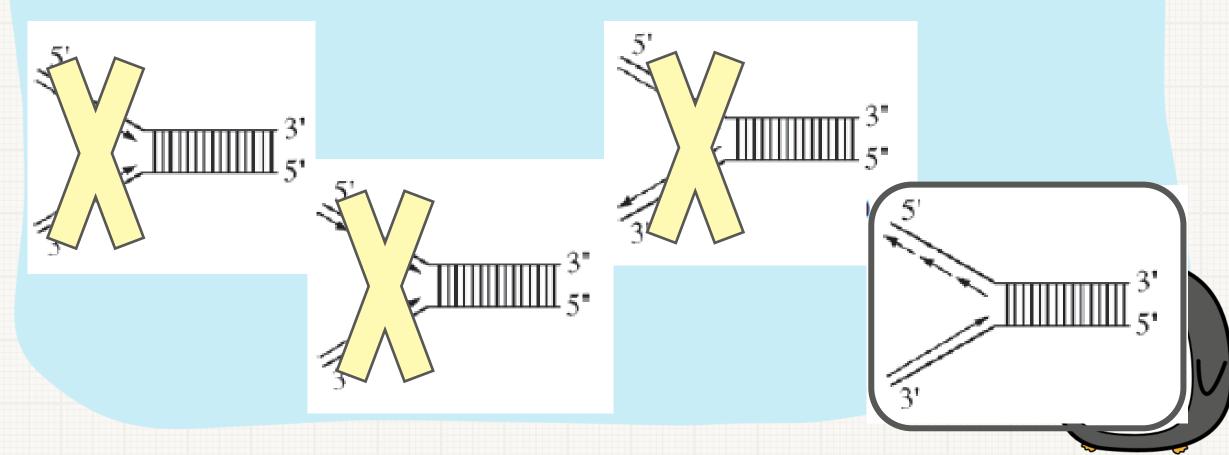
Use radioactive nucleotides to determine the sequence of a DNA

strand



Multiple Choice Practice:

When DNA replicates, each strand of the original DNA molecule is used as a template for the synthesis of a second, complementary strand. Which of the following figures most accurately illustrates enzyme-mediated synthesis of new DNA at a replication fork?



Multiple Choice Practice:

Sickle-cell anemia results from a point mutation in the HBB gene. The mutation results in the replacement of an amino acid that has a hydrophilic R-group with an amino acid that has a hydrophobic R-group on the exterior of the hemoglobin protein. Such a mutation would most likely result in altered

a.) properties of the molecule as a result of abnormal interactions between adjacent hemoglobin molecules

b. DNA structure as a result of abnormal hydrogen bonding between nitrogenous bases
c. fatty acid structure as a result of changes in ionic interactions between adjacent
fatty acid chains

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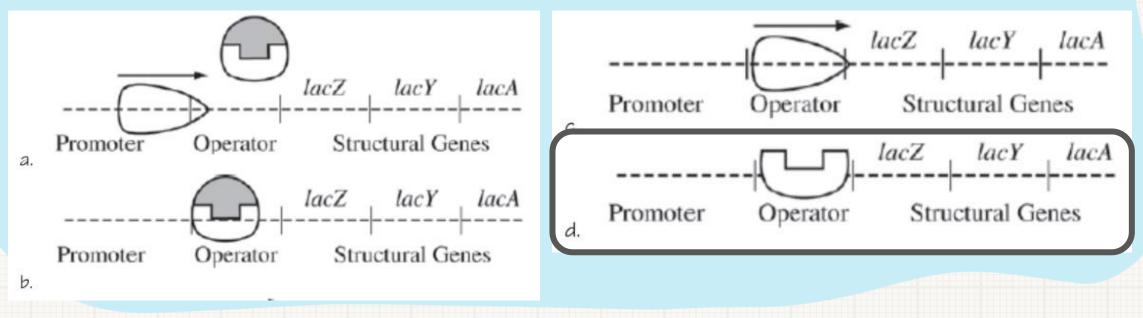
d. protein secondary structure as a result of abnormal hydrophobic interactions between R-groups in the backbone of the protein

Multiple Choice Practice:

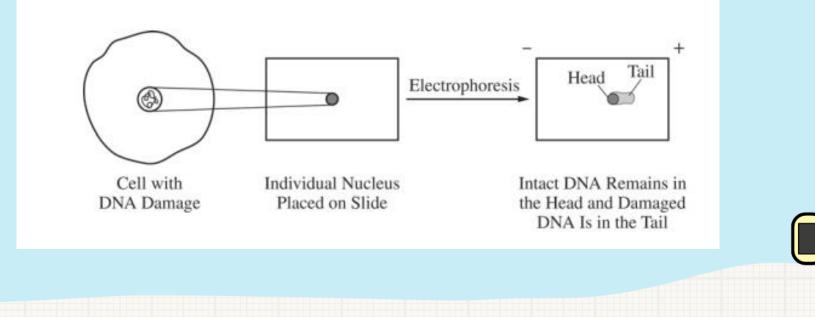
Lactose digestion in E. coli begins with its hydrolysis by the enzyme b-galactosidase. The gene encoding b-galactosidase, lacZ, is part of a coordinately regulated operon containing other genes required for lactose utilization.



Which of the following figures correctly depicts the interactions at the lac operon when lactose is **NOT** being utilized? (The legend above defines the shapes of the molecules illustrated in the options.)

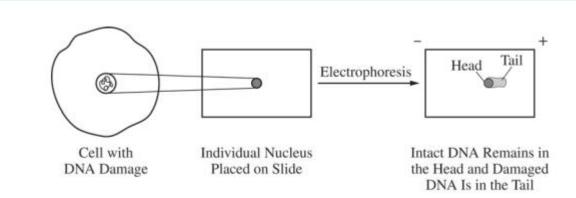


A comet assay is a technique used to determine the amount of double-strand breaks in DNA (DNA damage) in cells. The nucleus of an individual cell is placed on a microscope slide coated with an agarose gel. An electric current is applied to the gel that causes DNA to move (electrophoresis), and the DNA is stained with a fluorescent dye. When viewed using a microscope, undamaged DNA from the nucleus appears as a round shape (the head), and the fragments of damaged DNA extend out from the head (the tail). The length of the tail corresponds to the amount of the damage in the DNA (see Figure 1)



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(a) To explain the movement of DNA fragments in the comet assay, <u>identify</u> one property of DNA and <u>provide</u> <u>reasoning</u> to support how the property contributes to the movement during the comet assay technique.



Identification (1 point)	Reasoning (1 point)	
DNA has a (negative) charge.	DNA moves toward the positive/oppositely charged pole.	
DNA can be different sizes.	(Different size DNA fragments) move at different rates.	

(b) In a different experiment, cells are treated with a chemical mutagen that causes only nucleotide substitutions in DNA. <u>Predict</u> the likely results of a comet assay for this

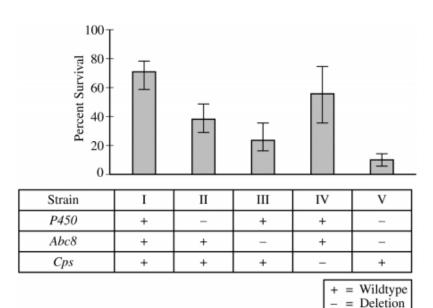
treatment.

When viewed using a microscope, undamaged DNA from the nucleus appears as a round shape (the head), and the fragments of damaged DNA extend out from the head (the tail).

Prediction (1 point)

- Head (only) OR (head with) no tail.
- Tail will be shorter than a cell with double-stranded breaks in DNA.

The common bedbug (*Cimex lectularius*) is a species of insect that is becomingly increasingly resistant to insecticides. Bedbugs possess several genes suspected of contributing to the resistance, including P450, Abc8, and Cps. To investigate the role of these genes in insecticide resistance, researchers deleted one or more of these genes in different strains of bedbugs, as indicated in Figure 1, and treated the strains with the insecticide beta-cyfluthrin. Each strain was genetically identical except for the deleted gene(s) and was equally fit in the absence of beta-cyfluthrin. The percent survival of each strain following beta-cyfluthrin treatment is shown in Figure 1.





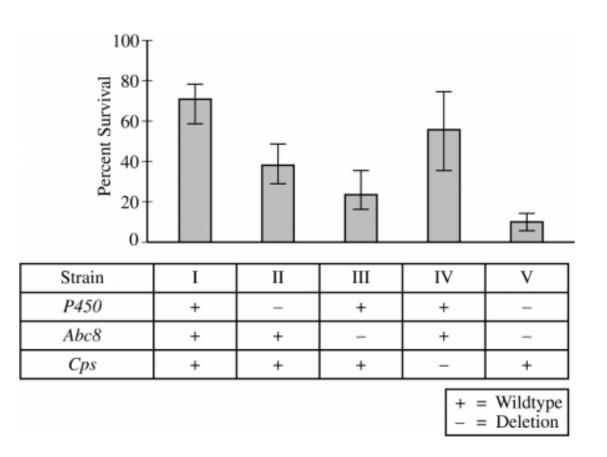
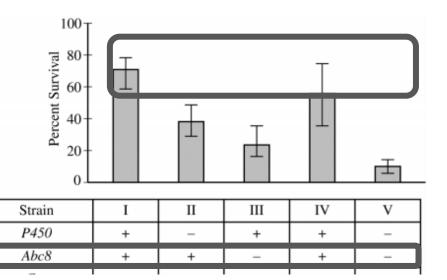


Figure 1. Percent survival of five strains of bedbugs treated with betacyfluthrin. A (+) indicates the gene is present; a (-) indicates the gene is deleted. Error bars represent the 95% confidence interval.



(a) Identify the control strain in the experiment. Use the means and confidence intervals in Figure 1 to justify the claim that Abc8 is effective at providing resistance to beta-cyfluthrin.



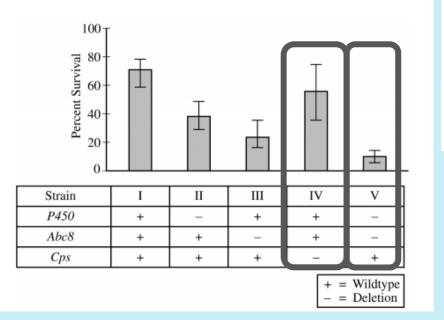
Identification (1 point) Strain I

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Justification (1 point)

- Error bars/CIs from strain I/control/WT do not overlap with strain III/Abc8 deleted strain.
- Mean % survival of strain III/Abc8 deletion falls outside the 95% confidence interval of strain I/control/WT.
- Strain III/Abc8 deletion shows a statistically significant difference from strain I/control.

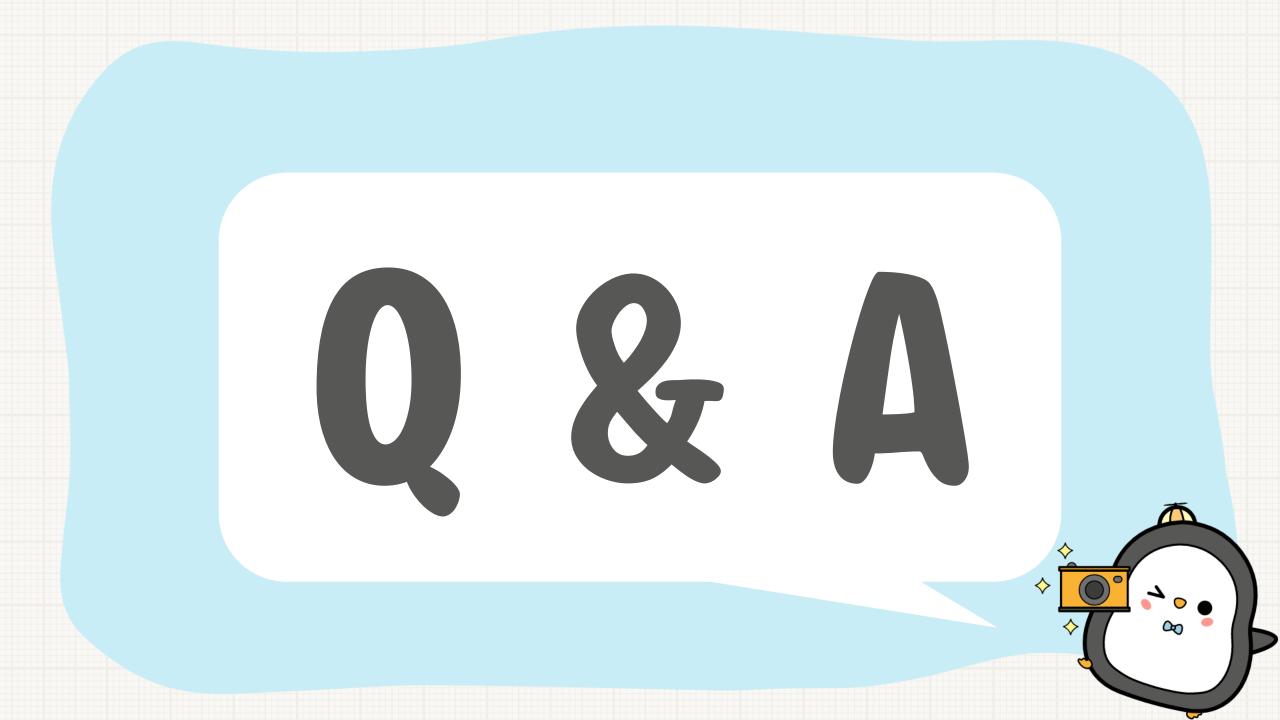
(b) P450 encodes an enzyme that detoxifies insecticides. Abc8 encodes a transporter protein that pumps insecticides out of cells. Cps encodes an external structural protein located in the exoskeleton that greatly reduces the absorption of insecticides. Based on this information and the data in Figure 1, explain how a deletion of both P450 and Abc8 results in lower survival in bedbugs compared with a deletion of Cps only.

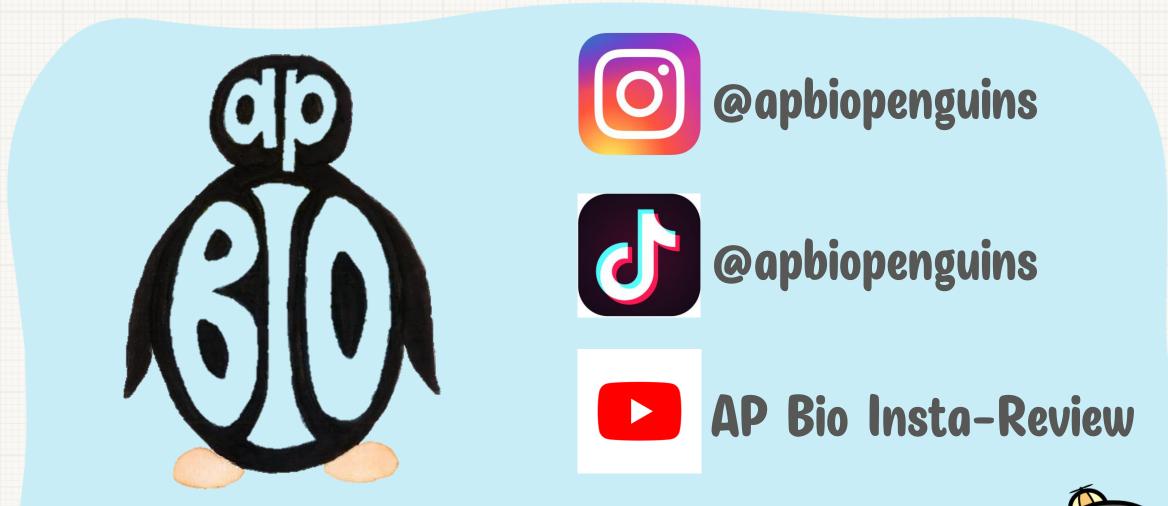


Explanation (1 point per row; 2 points maximum)

Strain	P450 and Abc8	Cps only	Explanation	
v	Deleted	Present	Bedbugs can neither detoxify nor pump out insecticide, which results in a lower chance of bedbug survival.	
IV	Present Deleted wh		Bedbugs can detoxify and pump out insecticide, which results in a higher chance of bedbug survival.	

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