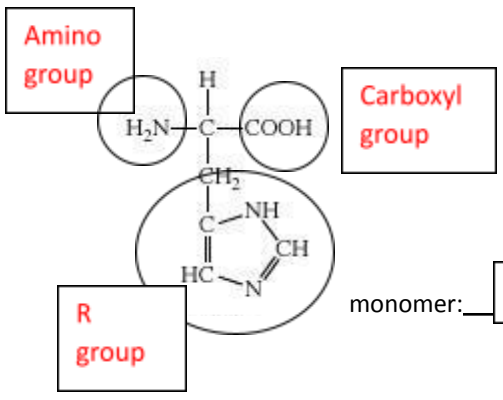
Name: Answer Key

Unit 6: Viruses, DNA Structure, DNA Replication, Protein Production, DNA Regulation, Biotechnology

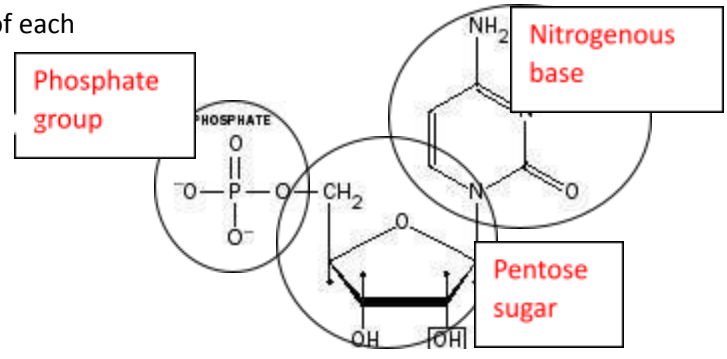
Organic Molecules Chart Review

	Proteins	Nucleic Acids		
elements	CHONS	CHONP		
Monomer	Amino acid	Nucleotide		
polymer	polypeptide		DNA	RNA
polymer structure	Bond types in: primary structure: covalent (peptide) secondary structure: hydrogen tertiary or quaternary structure: hydrogen, ionic, covalent, hydrophobic interactions	Sugar	deoxyribose	ribose
		Bases (letters)	ATCG	AUCG
		Number of strands typical	2	1
polymer function(s)	so many functions! All of the tools of cells, like enzymes, antibodies, receptors, many hormones, structural proteins, etc.	Function	genetic info	Regulates DNA

Identify each monomer and label the circled parts of each monomer:



monomer: Amino acid



monomer: Nucleotide

Virus Section

1. List the two structures that always make up a virus. What additional structure also often encloses a virus?

Protein and nucleic acid (either DNA or RNA). Additionally, they may have a lipid covering (taken from the cell membrane of the host).

2. The genetic material of viruses can be different from the genetic material of cells (which is always double stranded DNA). List TWO ways in which it can be different.

The genetic material may be RNA or DNA. It can be double stranded or single stranded. So, it can be SINGLE STRANDED DNA or double stranded DNA or single stranded RNA or DOUBLE STRANDED RNA.

3. What macromolecules are always found in viruses? What do viruses always inject into their hosts?

Protein and nucleic acid. Nucleic acid is always injected (instructions are really important). Protein may or may not make it in, depending on the virus type.

4. Fill out the chart below

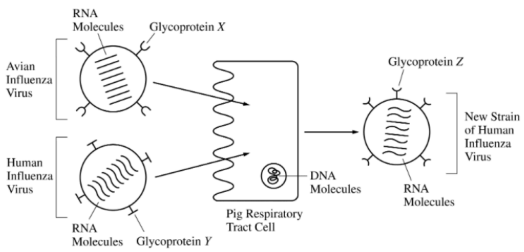
macromolecule	Elements (atoms) that make it up
Carbohydrate	CHO
Lipid	CHO
Protein	CHONS
Nucleic Acid	CHONP

5. When a bacteriophage injects DNA or RNA into a bacterial cell, which elements, if radiolabeled, would end up inside a host? Which would not?

CHONP ends up inside, but it's the Phosphorus that sets nucleic acids apart, so that's what will be radiolabeled if we want to follow the path of nucleic acids. Sulfur will NOT end up inside since it is not part of nucleic acids.

6. Describe what is going on in this diagram.

A new virus!



7. Describe the three types of horizontal gene transfer (from a past unit).

Conjugation – **bacteria get together and swap plasmids**

Transduction – **viruses accidentally bring bacterial DNA to a new bacterium**

Transformation **– bacteria pick up naked DNA from the environment**

8. How are the processes described above similar to meiosis and fertilization in eukaryotes?

They increase genetic variation/diversity

9. Viruses are very specific. Each infects only a small range of host cells. Describe the specificity between a virus that infects a particular animal cell and the animal cell membrane.

The virus takes advantage of some cell surface receptor. If another cell doesn't have that receptor, then the virus can't infect it. The mechanism that the virus uses to enter the cell must be present in all of the hosts that the virus uses.

10. Infection by a retrovirus is different from infection by a DNA virus. First, the virus injects its RNA and enzymes into the host. It uses its **reverse transcriptase** enzyme to convert RNA to cDNA.

The cDNA is then replicated to become **double** stranded.

Then the virus enzyme **integrase** is used to insert the viral DNA into the host's genome. This is copied whenever the cell goes through the **S (synthesis)** phase of the cell cycle.

A new retrovirus is formed when the host's enzymes are used to **transcribe** viral DNA into RNA in the nucleus and to **translate** viral mRNA into viral proteins at the host's **ribosomes**

11. What is a latent or dormant virus?

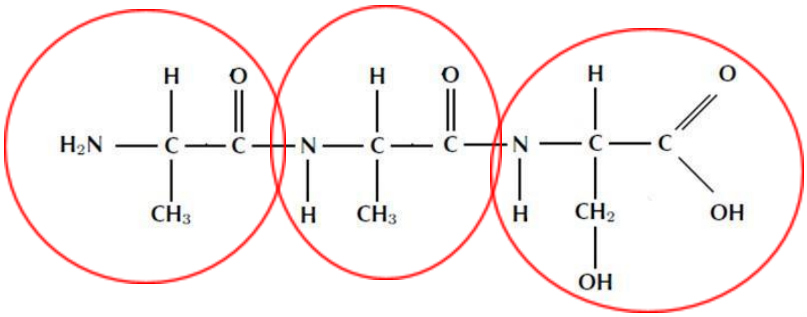
It quietly hangs out without making the host sick. It may have integrated into the host DNA, but is not presently being transcribed or translated.

12. What is a prion?

A protein that can cause disease by changing the shape of other proteins (e.g. mad cow disease).

Protein Review

1) Circle each amino acid in the figure.

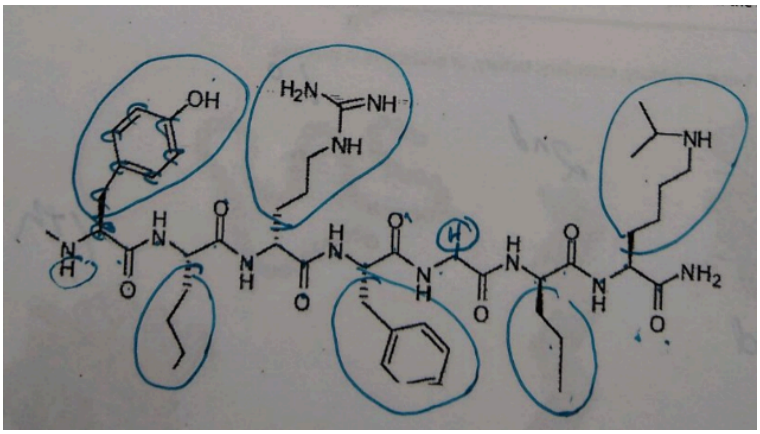


2) Is this a polypeptide or a nucleic acid?

This is an oligopeptide, so let's go with polypeptide.

3) Below is a polypeptide. The carbons are just represented as bends in the structure. Many hydrogen atoms are not shown.

a. Circle each R group.



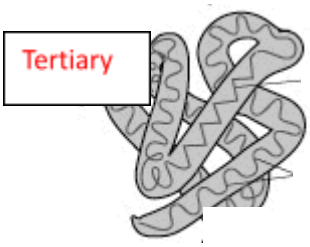
b. There are 20 amino acids. What makes each different?

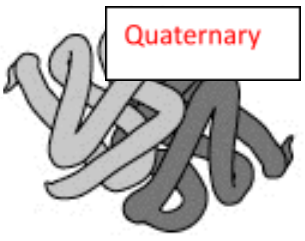
R group

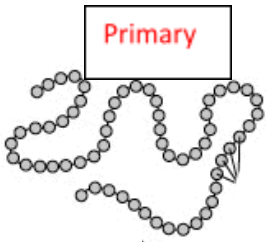
c. There are gazillions of proteins. What makes each different, structurally?

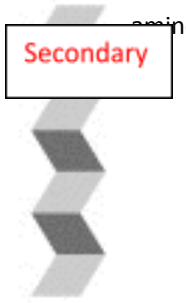
The order of amino acids determines the primary structure of the protein (which is coded for by DNA to RNA). From the primary structure, interactions between atoms in the backbone (N-C-C with an O) (secondary structure) and between atoms in the R groups with each other and with the environment (water, acids, etc.) (tertiary structure) and maybe with another protein (quaternary structure) determine the proteins shape. It's shape determines its function.

4) a. Label the proteins below as primary, secondary, tertiary, or quaternary in structure.
b. For each, list what produces this structure (i.e. R group interactions, backbone hydrogen bonds, order of amino acids, etc.)









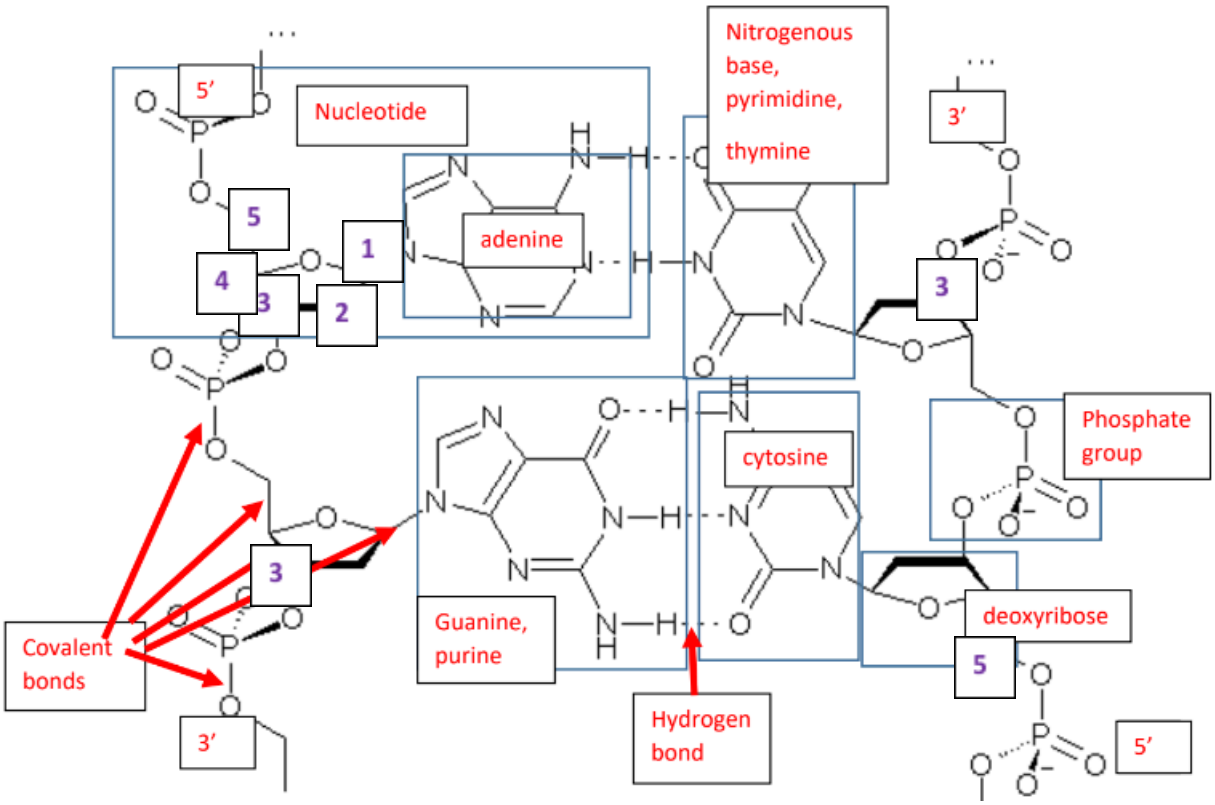
5. The function of a protein depends on its **shape**.

6. The structure of a protein is ultimately determined by **DNA (or maybe RNA if it's a viral protein)**.

DNA Structure

1. Label each of the following structures on the diagram below:

Nucleotide	Phosphate group	Cytosine
Deoxyribose	Nitrogenous base	Adenine
Thymine	Purine	Pyrimidine
Hydrogen bond	Covalent bond	Guanine



2. Number the carbon atoms of one of the sugar molecules. **Done in purple**

3. Label each end of each polynucleotide as 5' or 3' **Done in red**
4. Label a hydrogen bond. Why are hydrogen bonds located here? **So that DNA can be unzipped to copy it (DNA replication) or for protein synthesis (to make RNA).**
5. Which bases are purines? **Little word, big base (adenine, guanine); Just remember that purines pair with pyrimedines**
6. Which are pyrimidines? **big word, small base (thymine, cytosine, (uracil in RNA))**

7. Why do purines always pair with pyrimidines? **To keep the radius (diameter) of the double helix the same**

8. How many hydrogen bonds are shared between A and T? **2**

9. How many hydrogen bonds are shared between G and C? **3**

10. What does complementary base pairing mean? **A bonds with T, C bonds with G**
11. State Chargaff's rule and why it matters for DNA replication.
    Rule: In any organism, the % of A equals the % T and the % of G equals the % C. Also, the relative percentage of each base is different in different organisms. Why it matters: It suggests complementary base pairing and it suggests that the nitrogenous bases are the coding part of the DNA molecule.

DNA Structure Practice Questions

1. Researchers studying new viruses analyzed the genetic material found in four different virus samples to determine the percent nitrogen base composition of each virus. The data are shown in the table.

Nitrogen Base	Percent in Sample 1	Percent in Sample 2	Percent in Sample 3	Percent in Sample 4
Adenine	27	33	21	22
Cytosine	23	17	33	28
Guanine	23	17	27	28
Thymine	0	33	0	22
Uracil	27	0	19	0

Which of the samples most likely contains a double-stranded RNA virus? **Sample 1** (uracil shows it is RNA, equal amounts of U/A and of C/G show its doubles stranded)

2. Antibiotics can be used to kill the specific pathogenic bacterium, Mycobacterium tuberculosis, that causes tuberculosis. The appearance of antibiotic-resistant strains has made it more difficult to cure M. tuberculosis infections. These antibiotic-resistant bacteria survive and pass on the genes to their offspring, making the resistant phenotype more common in the population. DNA analysis indicates that the genes for antibiotic resistance are not normally present in bacterial chromosomal DNA
   Which of the following statements best explains how the genes for antibiotic resistance can be transmitted between bacteria without the exchange of bacterial chromosomal DNA?
   A. The antibiotic-resistant bacteria release a hormone that signals neighboring bacteria to become resistant.
   B. The genes for antibiotic resistance are located on a plasmid that can be passed to neighboring bacteria.
   C. The antibiotic-resistant bacteria are the result of bacteria that specifically modify their own chromosomal DNA to neutralize the antibiotics.
   D. The antibiotic alters the bacterial genome of each bacterium, which results in an antibiotic-resistant population.

DNA Experiments

1. In what ways is DNA like other codes, like the English language or Morse code? In what ways is it different?

Similar because it has variation (four nitrogenous bases); Different because its chemical

2. Working with pneumonia and mice, Fred Griffith was the first scientist to discover the process of transformation (a type of horizontal gene transfer in which bacteria pick up genetic molecules from the environment).

a. What happens to a mouse exposed to rough pneumococcus bacteria?

They live because their immune systems can recognize the foreign antigens (carbohydrate and/or protein surface markers) and produce antibodies and white blood cells to attack the foreign antigens and kill the rough bacteria.

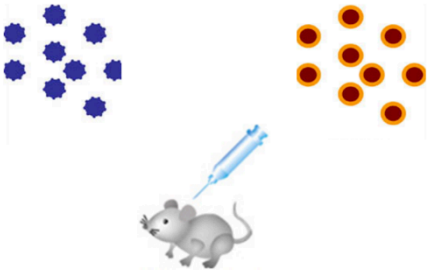
b. What happens to a mouse exposed to smooth pneumococcus bacteria?

They due because there is a carbohydrate coating over the cell membrane, covering the foreign antigens, making it very difficult for the mouse immune system to recognize and mount an immune response.

c. What happened to the mice in the experiment below? Why?

The mice died because the living rough bacteria picked up DNA released from the dead smooth bacteria. This DNA coded for making the polysaccharide coating and the rough bacteria became smooth, thus they were able to evade the mouse immune systems and kill the mouse.

Keep these bacteria alive.

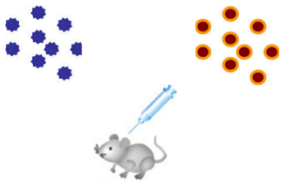


Kill these bacteria then mix the two strains together.

3. Later, Avery, MacLeod, and McCarty continued his work, but isolated different possible "transforming factors" to add to the harmless bacteria. They did this by treating virulent bacteria with various enzymes. What kinds of enzymes do you think they used?? Why?

Nucleases would destroy DNA and RNA, thus the experiment would not result in mouse death. Proteases would destroy protein, thus the experiment would result in mouse death. This would support the hypothesis that nucleic acids are the genetic material.

Keep these alive.



Kill these and add enzymes to the mix.

4. How is horizontal gene transfer advantageous to bacteria? **Increases genetic variation (to improve survival). If a population has more genetic diversity, then it is more likely that some members of the population will survive a changing environment)**

5. What is antibiotic resistance? How does it form initially? How does it spread?
   Antibiotic resistance is the ability of bacteria to survive and reproduce when exposed to a medicine designed to kill them. Good for bacteria, bad for people. It forms initially because bacteria are exposed to antibiotics and the naturally occurring resistance is selected for/advantageous to the bacteria. Over use of antibiotics and the use of antibiotics in farming increases the chances of developing antibiotic resistant bacteria. It can spread more quickly when bacteria swap plasmids through conjugation or any time horizontal gene transfer takes place. This produces "super bugs," which are bacteria that are resistant to many types of antibiotics.
6. Alfred Hershey and Martha Chase used radioactive elements (sulfur and phosphorus) in their virus experiments.
   a. What macromolecule has sulfur in it?

      protein

   b. What macromolecule has phosphorus in it?

      DNA/RNA (phospholipids do, too, but were not considered likely candidates for genetic info)

   c. Which ended up inside the cells?

      phosphorus

   d. What path were these elements tracing?

   DNA/RNA

7. Erwin Chargaff found that the amount of thymine equals the amount of adenine and the amount of cytosine equals the amount of guanine for any organism. However, these amounts vary from species to species. What two things does this imply?
   Sorry for the repeat question: In any organism, the % of A equals the % T and the % of G equals the % C. Also, the relative percentage of each base is different in different organisms. Why it matters: It suggests complementary base pairing and it suggests that the nitrogenous bases are the coding part of the DNA molecule.
8. Rosalind Franklin's DNA pictures show what about the shape of DNA?
   It is a double helix and it has a regular radius (width).

9. What implications does the Watson and Crick model of DNA structure imply for the process of DNA replication?

That it must unzip in order to use it (make RNA or replicate it) because the genetic information is inside.

DNA Experiments Practice Questions

In the 1940's, Avery MacCleod, and McCarty transformed nonencapsulated bacteria into encapsulated forms by growing the nonencapsulated cells in a culture containing an extract made from dead encapsulated cells. The transformed cells produced colonies of encapsulated bacteria. Three different procedures and their results are outlined below.

Procedure I:
Extract made from dead encapsulated cells added to culture medium.
Nonencapsulated bacteria added to culture medium.
Results: Both nonencapsulated and encapsulated bacteria grow.

Procedure II:
Extract made from dead encapsulated cells treated with protein-degrading enzymes before adding extract to culture medium.
Nonencapsulated bacteria added to culture medium.
Results: Both nonencapsulated and encapsulated bacteria grow.

Procedure III:
Extract made from dead encapsulated cells treated with DNAse (an enzyme that selectively destroys DNA) before adding extract to culture medium.
Nonencapsulated bacteria added to culture medium.
Results: Only nonencapsulated bacteria grow.

1. A reasonable conclusion to draw from the results of the experiment is that
A. DNA is the genetic material
B. DNA replication is semiconservative
C. DNA is a double helix
D. DNA is translated into protein
E. mutation is a change in the genetic material

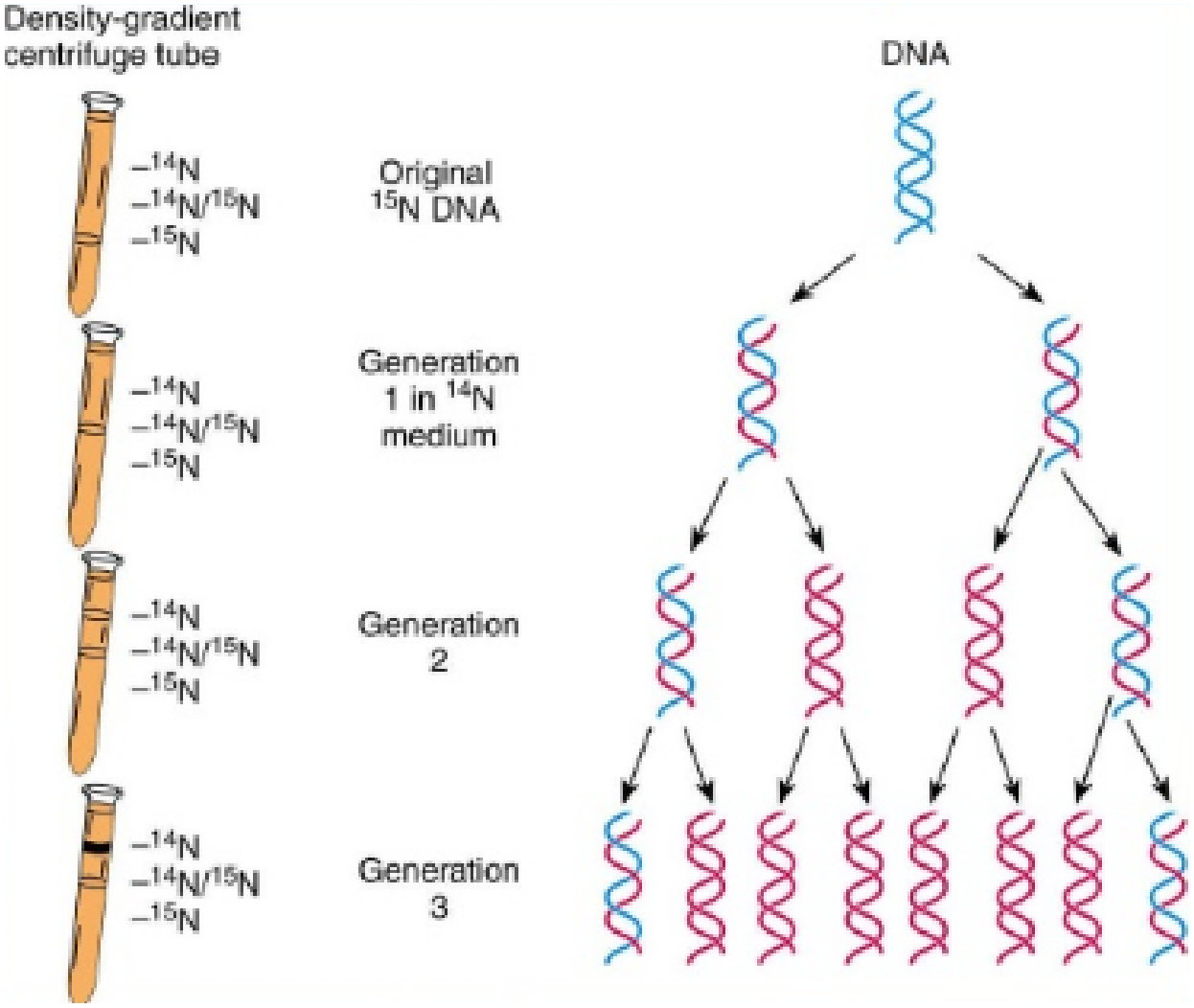
2. What was the purpose of treating the extract with protein-degrading enzymes in Procedure II?
A. To demonstrate that the transforming factor is an enzyme
B. To demonstrate that the transforming factor is not a protein
C. To destroy nuclei acids in the extract
D. To destroy any capsules in the extract
E. To prevent the extract from being contaminated by nonencapsulated bacteria

3. What was the purpose of treating the extract with DNAse in Procedure III?
A. To remove the encapsulated bacteria from the extract
B. To serve as a positive control by demonstrating that a protein in the extract is the transforming factor
C. To serve as a negative control by demonstrating that transformation does not occur without DNA
D. To destroy enzymes in the extract
E. To destroy any capsules that might be in the extract

DNA Replication Section

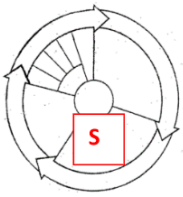
In 1958, Meselson and Stahl grew *E. coli* for several generations in a medium containing ^{15}N (a heavy isotope of nitrogen). When DNA is extracted from these cells and centrifuged on a salt density gradient, the DNA separates out at the point at which its density equals that of the salt solution. *E. coli* cells with only ^{15}N in their DNA were transferred to a ^{14}N medium and were allowed to divide; the progress of cell division was monitored by microscopic cell counts and by colony assay. DNA was extracted periodically, centrifuged, and compared to DNA from *E. coli* grown on pure ^{14}N and to DNA from *E. coli* grown on pure ^{15}N.

1. Draw the DNA bands in the test tubes that would be found from spinning a sample from each Erlenmeyer flask shown below.
2. Using colored pencils, draw heavy and light DNA for each cycle.



3. During what part of the cell cycle does DNA replication occur?

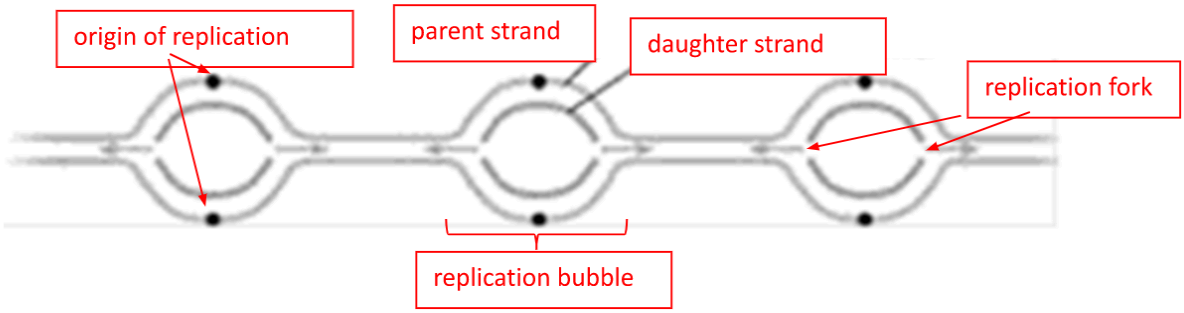
S (synthesis in interphase)



4. Is the DNA coiled or uncoiled during DNA replication?

Uncoiled so that enzymes can get to the DNA!

5. Label the replicating DNA image below with the following terms.



6. What is the function of each of the following enzymes in DNA replication?

A) helicase – **unwinds/separates DNA strands**

B) topoisomerase - **relaxes supercoiling in front of the replication fork. (Relieves stress on DNA as it gets separated by clipping, spinning, and reattaching the sugar-phosphate backbone).**

C) primase – **makes an RNA primer so that DNA polymerase can work**

D) DNA polymerase - **synthesizes new strands of DNA continuously on the leading strand and discontinuously on the lagging strand. (Adds DNA nucleotides to the 3' end of a growing DNA polymer).**

E) ligase - **joins the Okazaki fragments on the lagging strand.**

7. What type of bond does helicase break?

Hydrogen bond

8. What keeps the unwound DNA from reannealing? What does reannealing mean?

Single stranded binding proteins. Reannealing means complementary pieces bind/go back together.

9. Why does eukaryotic DNA replication use multiple origins of replication?

To speed up replication (it would take a really long time if there were not multiple origins of replication because chromosomes are each so long in eukaryotes).

10. Why does prokaryotic DNA replication use just one origin of replication?

They don't have a lot of DNA.

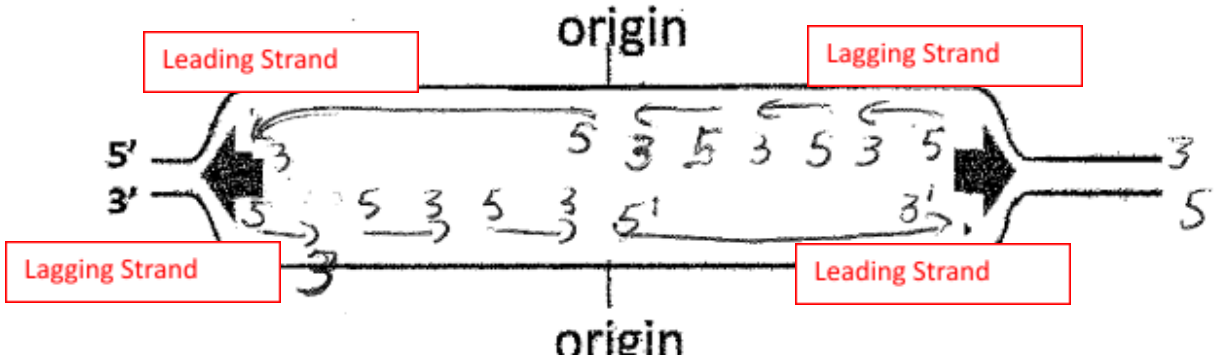
11. What is the function of a primer? What type of molecule is a primer?

DNA polymerase requires some nucleotides to work from. It cannot start from nothing. The primer is a small molecule of RNA.

12. Why does the DNA strand grow only in the 5' to 3' direction?

DNA polymerase is a real, physical object. It is an enzyme, and, like all enzymes, it has a special three dimensional shape. DNA polymerase is the right shape to add to the 3' end of DNA, but not to the 5'end.

13. Draw in the DNA being replicated below and label each section as a leading or lagging strand.



14. What is an Okasaki fragment?

A section of DNA on the lagging side that is being replicated non-continuously.

15. What is a mutation? Mutations in which types of cells can be passed on to offspring?

A mutation is a change in DNA. Only mutations in gametes/germ cells can be passed on to offspring.

16. What problem occurs at the ends of linear chromosomes when they are replicated?

DNA polymerase has to start from somewhere, so the chromosomes keep getting shorter every time they are replicated, as the RNA primers are degraded and no DNA replaces them.

17. What is the function of a telomere?

Telomeres are noncoding/nonregulatory ends of each chromosome that give the chromosome the ability to replicate without eroding coding portions of the chromosome. They protect the coding and regulatory parts of the chromosome, while still allowing replication.

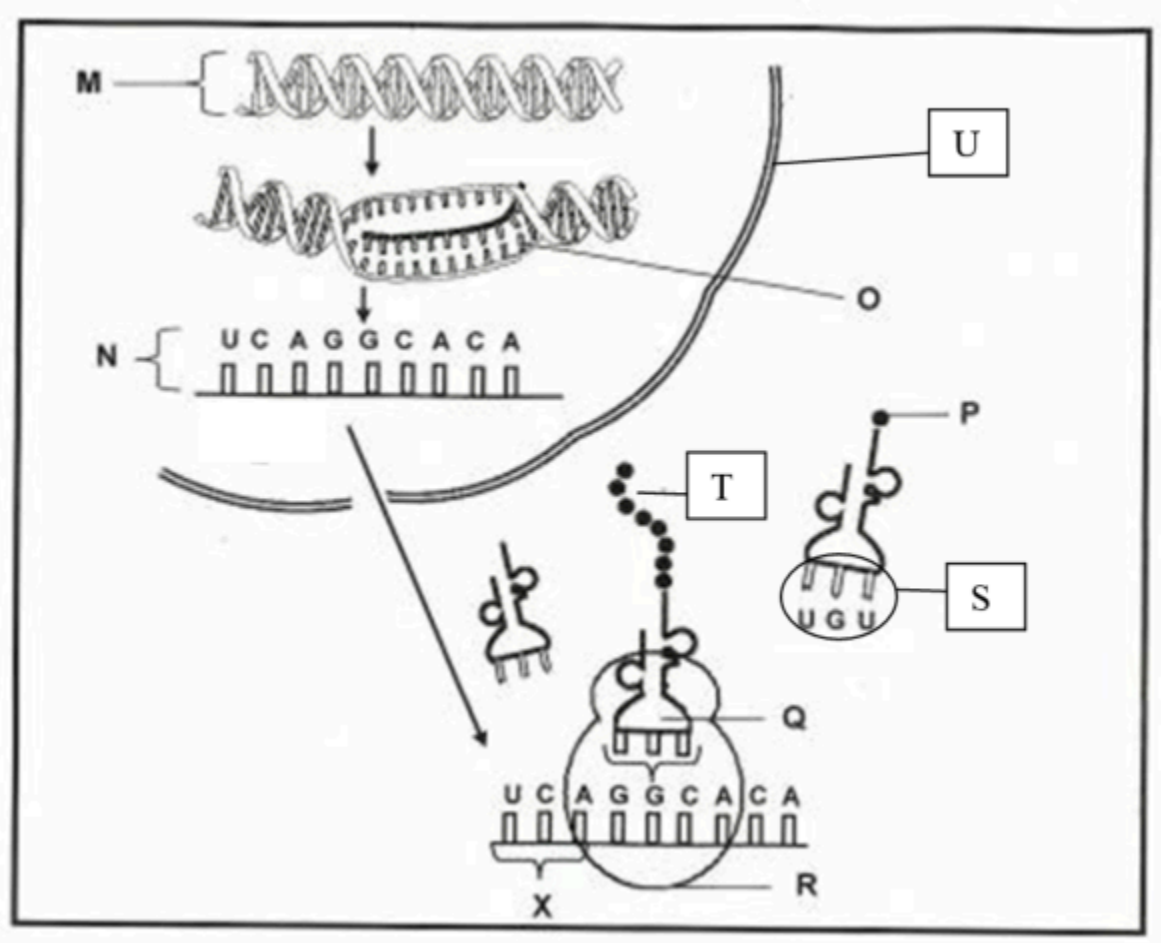
18. What is the function of telomerase?

Telomerase is an enzyme that produces/resynthesizes telomeres in germ cells so that babies are born with normal telomeres.

Protein Synthesis Section

1. Label each term with a letter from the diagram below:

Anticodon S ribosome R amino acid P
Codon X tRNA Q mRNA N
nucleus U DNA M transcription O
growing polypeptide T



2. The glycolipids on the surface of my liver cells are really important in cell identification (Hey – I'm a liver cell!!!). How does the liver cell make glycolipids??

Glycolipids are part carbohydrate and part lipid. They are put together by enzymes. Enzymes are proteins, which are made with directions from mRNA which is made with directions from DNA. So, your glycolipids may be different from mine, because our DNA is a little different. Cool, huh?

3. Why are the glycolipids on the surface of your liver cells different from the glycolipids on the surface of your neurons?? Do your liver cells and your neurons have different DNA??

No! Your liver cells and your neurons have the same DNA. Your different cells just use or read different sections of your DNA. So liver cells will read/transcribe different genes than nerve cells. Even though

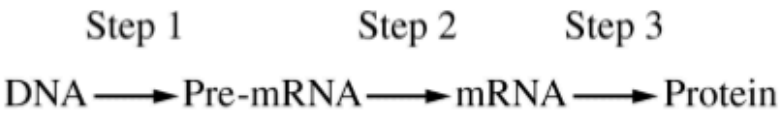
your nerve cells have the same DNA, they don't use the directions meant just for liver cells. They don't express the same genes that liver cells might express.

4. What does it mean for a gene to be "expressed?"
It is transcribed, and, if it codes for a protein, it is translated. (If it codes for tRNA, piwiRNA, etc., it will not be translated).

5. Where do transcription and translation occur in prokaryotes compared to eukaryotes?

	Location of transcription	Location of translation
Prokaryotes	**cytoplasm**	**cytoplasm (ribosome)**
Eukaryotes	**nucleus**	**cytoplasm (ribosome)**

6. Identify each step listed below and explain what happens at each step.

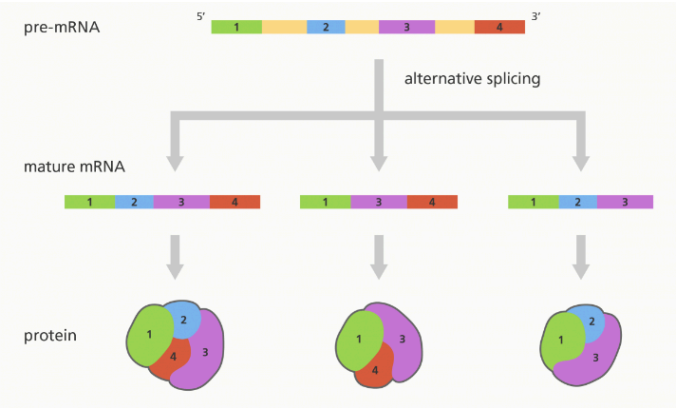


Step 1: transcription – DNA is unwound and RNA polymerase reads the template strand and produces a complementary RNA molecule (remember to use uracil, not thymine!)
Step 2: mRNA processing – a 5' GTP cap is added to the beginning of the RNA transcript, and a 3' poly-A tail is added to the other end. Introns are spliced out and exons are put together.
Step 3: translation – a polypeptide is produced at a ribosome from the mRNA code (each codon determines the amino acid)

7. Explain the evolutionary significance of a nearly universal genetic code.
We share a common ancestor. We are all related. OMG!!!

8. Describe the functional and evolutionary significance of introns.
Eukaryotes can shuffle exons and make different proteins so eukaryotes have a greater variety of proteins than would be predicted by the number of expressed genes. It also gives eukaryotes the ability to regulate which exons are expressed in particular cells.

9. What is wobble? How does it affect translation? Why do we say that the genetic code is redundant?
There are 64 possible combinations of the 4 codons in groups of 3. So… There are 64 codons. However, there are only 20 amino acids. The genetic code is somewhat redundant, meaning that there is more than one codon specifying certain amino acids. Part of this is made possible by "wobble." The third nucleotide of an anticodon of tRNA might not have to be complementary to its codon base.



10. What is RNA splicing? How does it increase protein variation in eukaryotes?
Certain sequences (introns) are spliced out (or taken out). The process of splicing

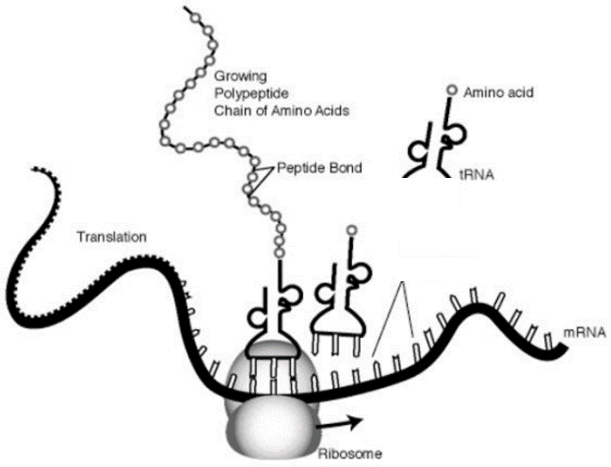
can create different variations of the same mRNA by keeping different combinations of exons. This is called alternative splicing.

11. Identify the roles of the following molecules in translation:

Molecule	Function
mRNA	**Carries the code for a particular polypeptide**
tRNA	**Carries the correct amino acid to the ribosome for each mRNA code**
rRNA	**Makes up the ribosome's structure (with protein)**
amino acids	**The monomer of a protein**

12. Distinguish between codon and anticodon.

Codon – mRNA
Anticodon- tRNA
They are complementary to eachother



13. With regard to the "RNA world" hypothesis on the origin of life:

a. Define the hypothesis.

The original genetic material (when life started on this planet) was RNA (not DNA).

b. Explain the evidence.

RNA can work as genetic material and as enzymes. So, RNA is very versatile. Life started as simple, so it makes sense that RNA was the genetic material.

c. Why, today, are more enzymes made of protein?

There are more possible variations. Proteins can make many more, nuanced shapes, so there are more types of enzymes.

d. Why, today, is DNA the genetic material, rather than RNA?

DNA is more stable.

e. What is the modern role of RNA (in today's cells)?

RNA is regulatory and it brings the code out to ribosomes.

post chrom. ppt
abnormal meiosis

DNA Mutations Practice Worksheet

DIRECTIONS: Transcribe and translate the original DNA sequence. Then, do the same for each mutated DNA sequence. Then, determine the consequence, if any, for each mutation, by circling your choice for each question. **You will need a Genetic Code Chart.**

template

Original DNA sequence:	TAC	ACC	TTG	GCG	ACG	ACT
mRNA transcript:	AUG	UGG	AAC	CGC	UGC	UGA
amino acids:	met-	trp-	Asn	-Arg-	Cys-	Stop

template

Mutated DNA sequence #1:	TAC	ATC	TTG	GCG	ACG	ACT
mRNA transcript: (Circle any changes)	AUG	UAG	AAC	CGC	UGC	UGA
amino acids:	met-	Stop				

Type of mutation (Circle one.)	Point ⇨ Substitution			Frameshift ⇨ Insertion or Deletion		
How did the mutation affect the amino acid	No change	1 amino acid	Premature	No stop	1 amino acid added/	All the amino acids changed after the

Mutations Section: Transcribe the original DNA sequence. Then, do the same for each mutated DNA sequence. Then, determine the consequence, if any, for each mutation, by circling your choice for each question.

Mutated DNA sequence #2:	TAC GAC CTT GGC GAC GAC T					
mRNA transcript: *(Circle any changes)*	AUG CUG GAA CCG CUG CUG A					
amino acids:	met - Leu - Glu - Pro - Leu - Leu -					
Type of mutation (Circle one.)	Point ⇨ Substitution			Frameshift ⇨	Insertion	or Deletion
How did the mutation affect the amino acid sequence (protein)? (Circle one.)	No change	1 amino acid changed	Premature stop signal	No stop signal	1 amino acid added/ deleted	All the amino acids changed after the point of mutation

frameshift mutation

Mutated DNA sequence #3:	TAC ACC TTA GCG ACG ACT					
mRNA transcript: *(Circle any changes)*	AUG UGG AAU CGC UGC UGA					
amino acids:	Met-trp-asn-arg-cys-stop					
Type of mutation (Circle one.)	Point ⇨ Substitution			Frameshift ⇨ Insertion or Deletion		
How did the mutation affect the amino acid sequence (protein)? (Circle one.)	No change	1 amino acid changed	Premature stop signal	No stop signal	1 amino acid added/ deleted	All the amino acids changed after the point of mutation

Mutated DNA sequence #4:	TAC ACC TTG GCG ACT ACT					
mRNA transcript: *(Circle any changes)*	AUG UGG AAC CGC UGA UGA					
amino acids:	Met-trp-asn-arg-stop					
Type of mutation (Circle one.)	Point ⇨ Substitution			Frameshift ⇨ Insertion or Deletion		
How did the mutation affect the amino acid sequence (protein)? (Circle one.)	No change	1 amino acid changed	Premature stop signal	No stop signal	1 amino acid added/ deleted	All the amino acids changed after the point of mutation

Mutated DNA sequence #5:	TAC ACC TTG GGA CGA CT					
mRNA transcript: *(Circle any changes)*	AUG UGG AAC CCU GCU GU					
amino acids:	Met-trp-asn-pro-ala					
Type of mutation (Circle one.)	Point ⇨ Substitution			Frameshift ⇨ Insertion or Deletion		
How did the mutation affect the amino acid sequence (protein)? (Circle one.)	No change	1 amino acid changed	Premature stop signal	No stop signal	1 amino acid added/ deleted	All the amino acids changed after the point of mutation

1. A geneticist found that a particular mutation had no effect on the protein coded by a gene. How could this occur?

The change could have coded for the same amino acid, since some amino acids have more than one codon that codes for them. It could have been a mutation in an intron (which was then spliced out).

2. Examine your genetic code chart. Name one amino acid that has more than one codon. Name an amino acid that has only one codon.

Every amino acid except methionine and tryptophan have more than one codon.

3. Look at the following sequence: THE FAT CAT ATE THE RAT. Delete the first H and regroup the letters in groups of three- write out the new groups of three. Does the sentence still make sense? What type of mutation is this?

TEF ATC ATA TET HER AT
This sentence does not make sense. It is like a frameshift mutation (or a reading frame mutation).

4. Given the following three mRNA sequences, determine which two code for the same protein. Circle them.

	mRNA #1	mRNA #2	mRNA #3
Transcript	AGU UUA GCA ACG AGA UCA	UCG CUA GCG ACC AGU UCA	AGC CUC GCC ACU CGU AGU
Translate	Ser-Leu-Ala-Thr-Arg-Ser	Ser-Leu-Ala-Thr –Ser-Ser	Ser-Leu-Ala-Thr-Arg-Ser

5. You have a DNA sequence that codes for a protein and is 105 nucleotides long. A frameshift mutation occurs at the 85th base - how many amino acids will be correct in this protein?

84/3=28
Twenty eight amino acids should be correct. The rest should be different.

6. Write the mRNA produced and the polypeptide produced from the following **coding** (**non-template**) strand from a gene. This sequence was taken from the middle of a gene, so there is no punctuation. Use your codon chart.

5’ – GTA CTG TAC TGT CAC GTG GAC GAC TGA -3’

GUA CUG UAC UGU CAC GUG GAC GAC UGA

val-leu-tyr-cys-his-val-asp-asp-stop

7. What would be the effect of a mutation that deletes the second T in **the coding (non-template) strand**? List the new DNA sequence, and the resulting polypeptide produced.

5’ – GTA CGT ACT GTC ACG TGG ACG ACT GA -3’

GUA CGU ACU GUC ACG UGG ACG ACU GA

val-arg-thr-val-thr-trp-thr-thr

This is a frameshift mutation in which the entire amino acid sequences is different. We now have an entirely different protein. If the original protein was essential, this mutation is catastrophic.

8. Write the mRNA produced and polypeptide produced from the following **non-coding (template)** strand from a gene. Use your codon chart.

5' – GAG GTA TCC CCA CAC TGA TCC CCG ACT -3'

CUC CAU AGG GGU GUG ACU AGG GGC UGA

Leu-his-arg-gly-val-thr-arg-gly-stop

9. What would be the effect of a mutation that substitutes the last T with a C in the strand above? List the new DNA sequence, and the resulting polypeptide produced. Explain how it will be different.

DNA: ACT changes to ACC

RNA: UGA (stop) changes to UGG (tryp)

The stop codon will become a tryptophan (an amino acid) and the polypeptide will be longer. If the original protein was essential, this mutation is catastrophic.

AP Practice Questions

1. A tobacco plant can be made to express a gene from fireflies, resulting in the emission of light. Which of the following is the basis for this phenomenon?
A. Chloroplasts can be made to produce light if firefly proteins are injected into plant cells.
B. Fireflies and tobacco plants share a recent common ancestor.
C. Fireflies and tobacco plants are infected by the same kinds of bacteria.
D. Transcription and translation are fundamentally similar in both fireflies and tobacco plants.
E. Most enzymes in fireflies have the same amino acid sequence as the enzymes in tobacco plants.

2. Analysis of DNA sequences from two individuals of the same species results in a greater estimate of genetic variability than does analysis of amino acid sequences from the same individuals because
A. different DNA sequences can code for the same amino acid
B. some amino acid variations cannot be detected by protein electrophoresis
C. DNA sequencing is a more reliable technique than protein electrophoresis
D. proteins are more easily damaged than is DNA
E. DNA is more heat-sensitive and therefore varies more

3. Enzyme found in retroviruses that produce DNA from an RNA template
Reverse transcriptase
4. Enzyme used during replication to attach Okazaki fragments to each other
Ligase

This group of questions refers to the following enzymes.
(A) DNA ligase
(B) DNA polymerase
(C) RNA polymerase
(D) Restriction enzyme
(E) Reverse transcriptase

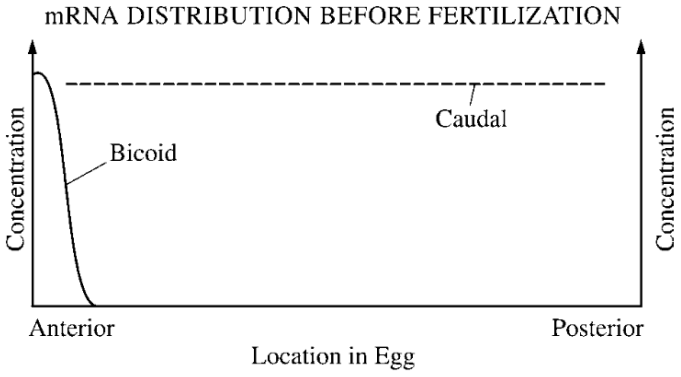
5. Enzyme used in the synthesis of mRNA

RNA polymerase

6. Enzyme used to position nucleotides during DNA replication

DNA polymerase

The first diagram below shows the levels of mRNA from two different genes (bicoid and caudal) at different positions along the anterior-posterior axis of a Drosophila egg immediately before fertilization. The second diagram shows the levels of the two corresponding proteins along the anterior-posterior axis shortly after fertilization.



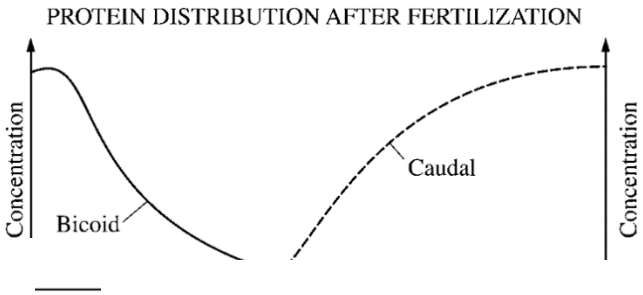
7. Which of the following conclusions is best supported by the data?

A. Bicoid protein inhibits translation of caudal mRNA.

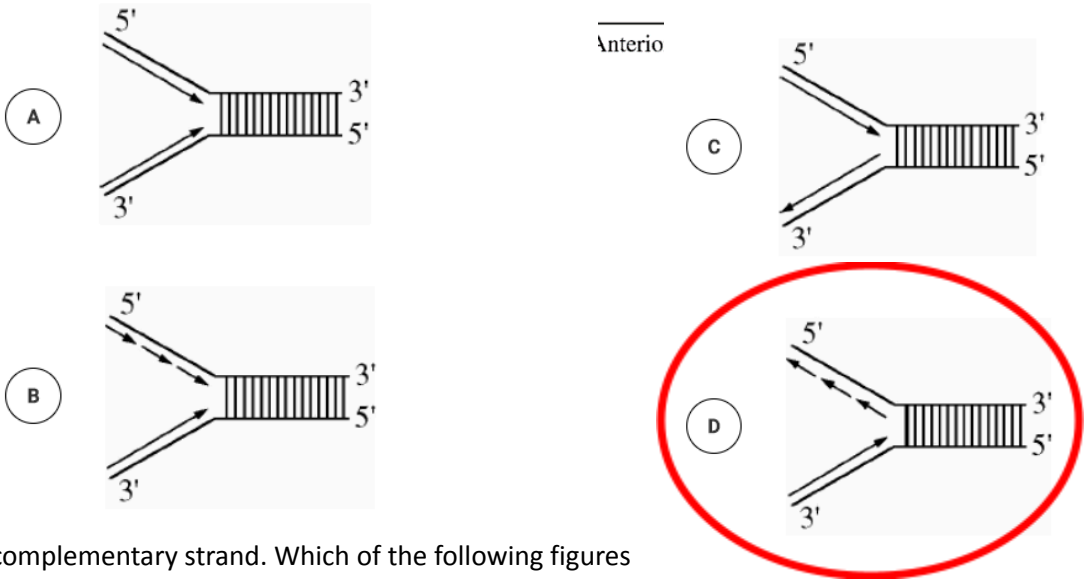
b. Bicoid protein stabilizes caudal mRNA.

C. Translation of bicoid mRNA produces caudal protein.

D. Caudal protein stimulates development of anterior structures.



8. 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?

9. Which of the following normally leads to the production of functional messenger RNA in eukaryotic cells?

A. A decrease in the rate of ribosome synthesis

B. The removal of portions of RNA known as intervening sequences (introns)

C. A decrease in RNA polymerase activity

D. The replication of new messenger RNA molecules from existing messenger RNA molecules

E. The formation of peptide bonds between adjacent nucleotides

10. Which of the following statements concerning a gene is correct?

A. A gene can code for a specific protein.

A. A gene can exist in alternate forms called introns.

B. A gene undergoes crossing-over during DNA replication.

C. A gene that is very similar in sequence in a human and in a bacterium is probably a recent mutation.

D. A gene that is expressed in every offspring of every generation is recessive.

5′-GACCGCAUGGUGACGAAAUUUGGCCAUUAA-3′

11. Based on the universal genetic code, which of the following represents the correct polypeptide that will result from translation of the mRNA molecule shown above, beginning with the first available start codon?

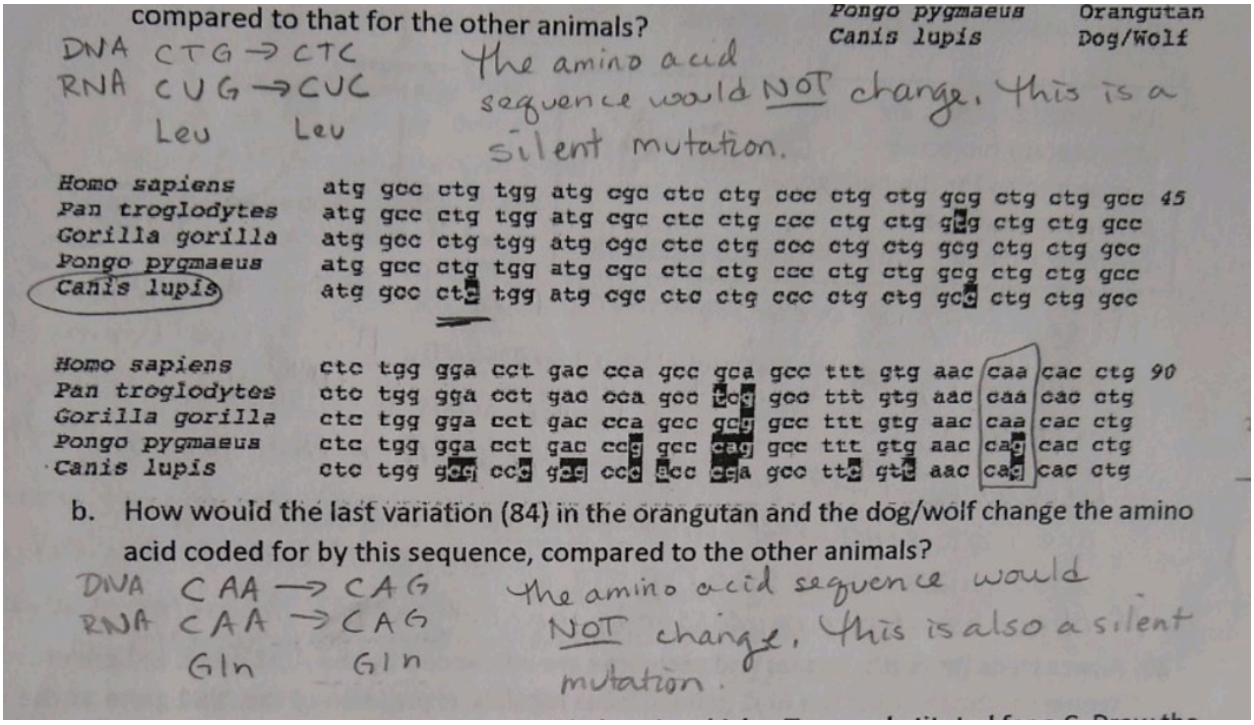
A. Asp–Arg–Met–Val–Thr–Lys–Phe–Gly–His

B. Met–Arg–Asp–Stop–His–Gly–Phe–Lys–Thr–Val

C. Met–Val–Thr–Lys–Phe–Gly–His

D. Val–Thr–Lys–Phe–Gly–His

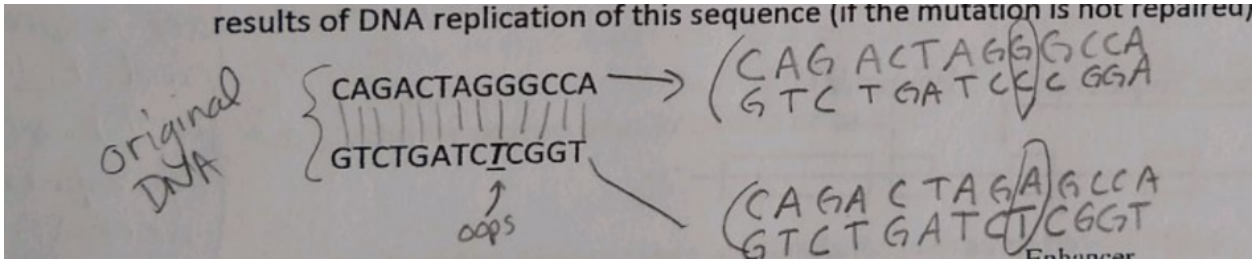
12. The following is the coding (non-template) portion of a particular gene. Differences from the human gene are highlighted in black.



13. A point mutation is shown in the sequence below, in which a T was substituted for a C. Draw the results of DNA replication of this sequence:

CAGACTAGGGCCA

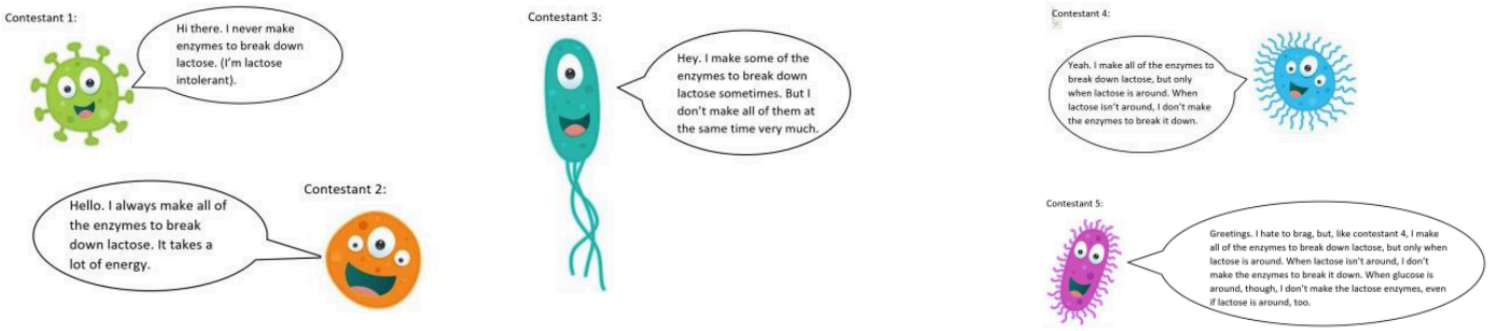
GTCTGATC***T***CGGT



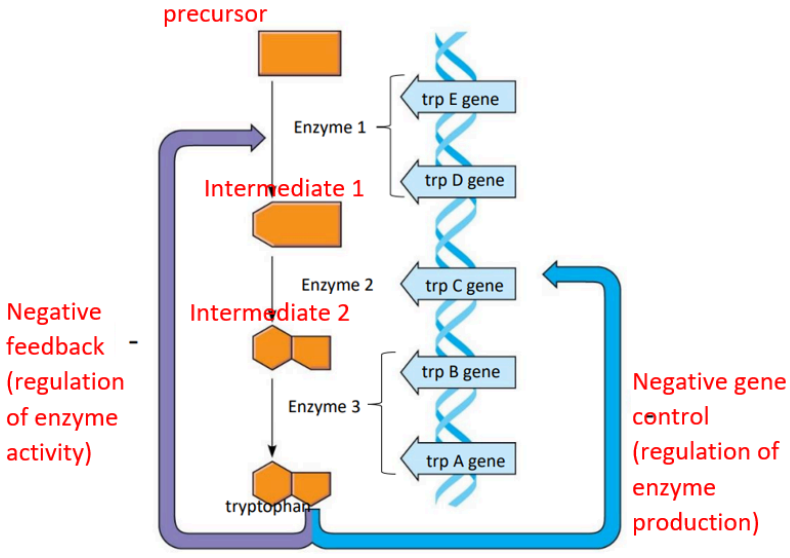
There's a test question that looks like this (not promising that it will make it onto the test this year). Students struggle with it, but it really is just seeing the two different DNA strands that are made – one with the mutation and one without the mutation.

Operons (Prokaryotic Gene Regulation) Section

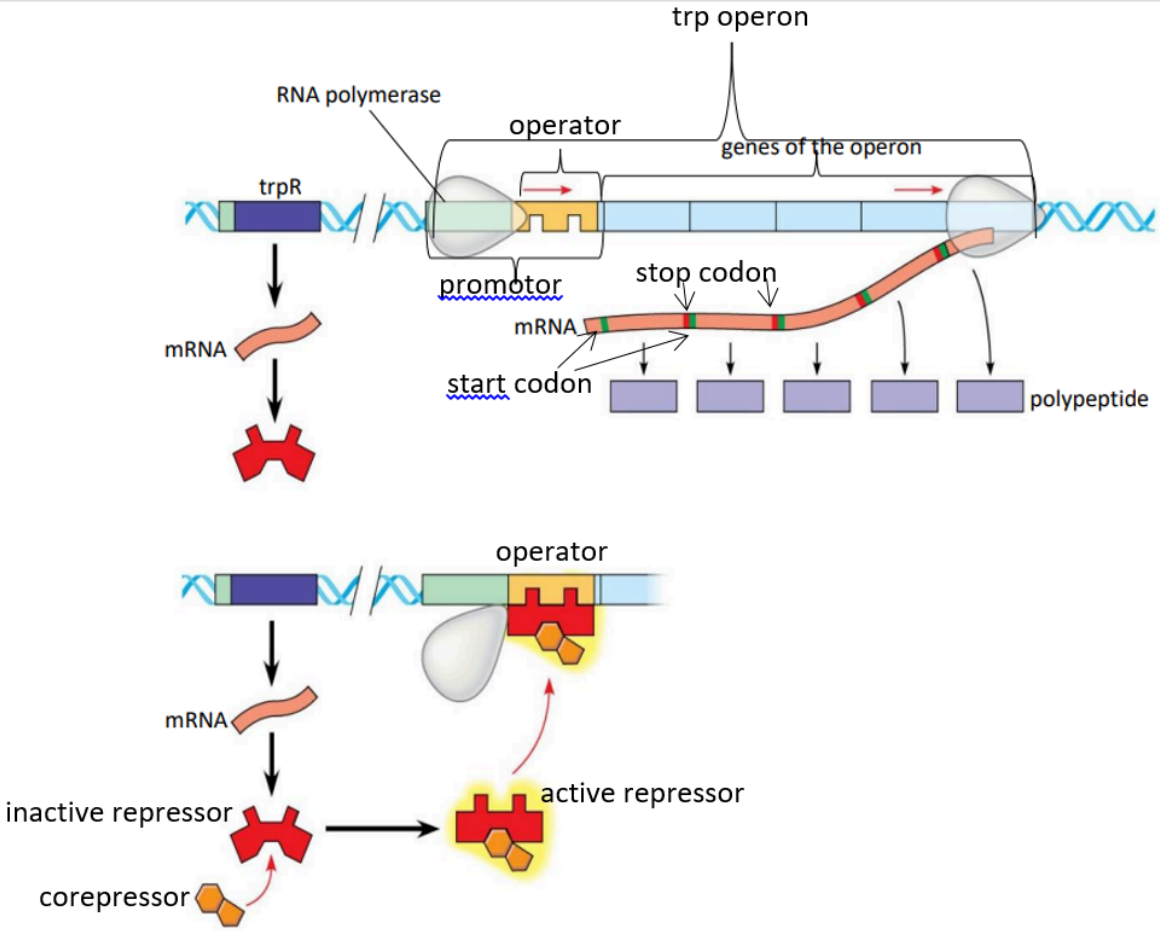
1. Why do bacteria regulate gene expression? To increase efficiency, thereby spending more energy on survival and reproduction.



2. *E. coli* regulates the production of the amino acid tryptophan both by regulating enzyme activity and by regulating the production of the enzymes.
    a. Label the diagram below with the following terms:
    precursor, intermediate 1, intermediate 2.
    b. What is the product?
    Tryptophan (the final molecule made)
    c. Distinguish between positive feedback and negative feedback.
    Positive feedback – changes away from a set point trigger more changes away from the set point
    Negative feedback – changes away from a set point trigger changes in the opposite direction, bringing the factor back to the set point (homeostasis).
    d. Label each side of the diagram with:
        i. negative gene control or negative feedback
        ii. regulation of enzyme production or regulation of enzyme activity



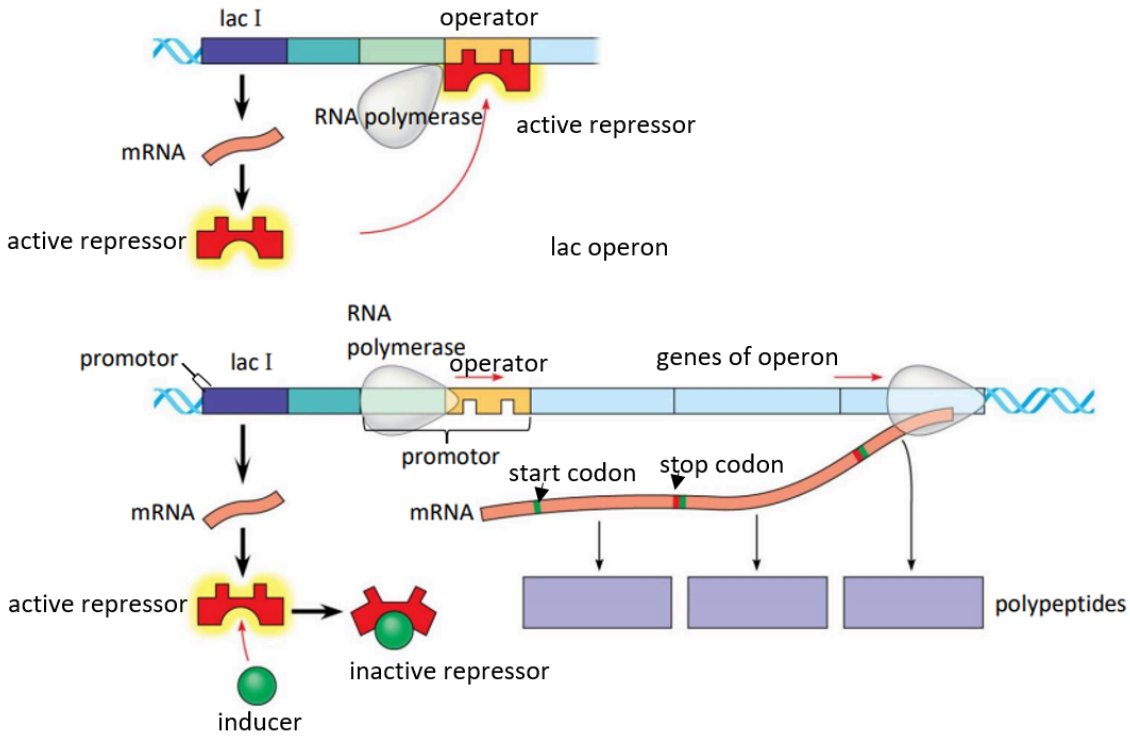
3. Below is the *trp* operon. Label the following terms.



4. Explain the role of tryptophan in regulating this operon

Tryptophan acts as a co-repressor, making the repressor active. The active repressor then binds to the operator, preventing the binding of RNA polymerase. This prevents transcription and translation of the operon's structural genes. So tryptophan stops more tryptophan from being made. Repressible operon (the operon is on and the repressor is inactive unless the corepressor is around, in which case the repressor becomes active and inactivates the operon).

5. Below is the *lac* operon. Label the following terms and explain their function in the operon.



6. Explain the role of allolactose in regulating this operon.

Allolactose (an isomer of lactose) binds to the active repressor, making it inactive. Then the operon is no longer stopped, so it produces the enzyme to break down lactose.

7. Distinguish between positive gene control and negative gene control.

Positive gene control is a volume control. It increases the rate of gene expression. CAP, with cAMP, helps RNA polymerase to bind better and transcribe more!

Negative gene control is an on/off switch. It uses a repressor that binds to an operator to stop transcription. Both inducible and repressible operons use negative gene control. If there is a repressor, it is negative gene control.

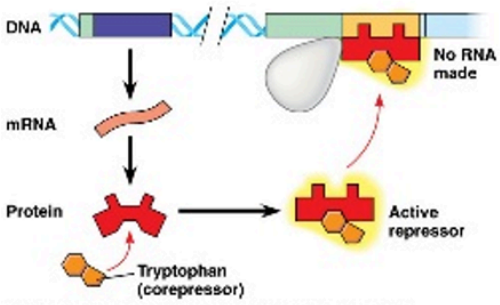
Operons Summary

Negative Gene Regulation in Prokaryotes

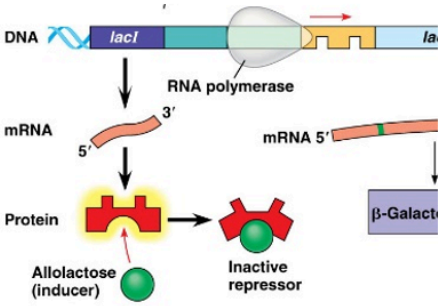
The operons are switched off by the active form of the repressor protein.

(Stops gene expression)

Repressible Operon	Inducible Operon
ex. Tryptophan synthesis	ex. Lactose digestion
Operon is usually on, but it can be turned off	Operon is usually off, but it can be turned on
Repressor protein is inactive by itself	The repressor protein is active by itself
Tryptophan (corepressor) activates the repressor	Inducer (small molecule) inactivates the repressor
Anabolic (build/synthesize molecules)	Catabolic (breaks down molecules)
The end product of the anabolic pathway becomes a corepressor and stops the production of the end product.	The substrate (allolactose) of the catabolic pathway inactivates the repressor, so the repressor can't bind to the operator, and the digestive enzymes are produced



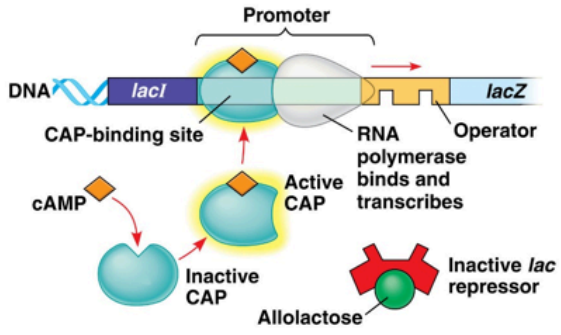
(b) Tryptophan present, repressor active, operon off



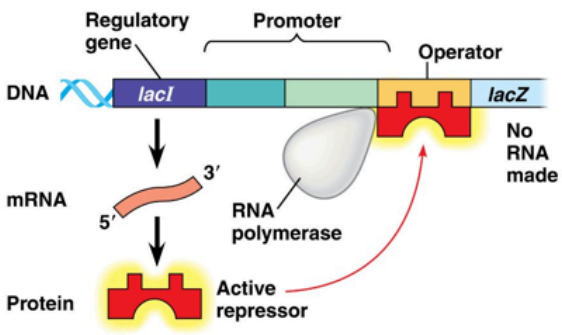
(b) Lactose present, repressor inactive, operon on

Prokaryotic Gene control

Positive Gene Control	Negative Gene Control
Stimulates gene expression	Stops gene expression
An activator protein binds to the upstream portion of the promotor and facilitates the binding of RNA polymerase to the promotor, increasing the rate of transcription	The active form of a repressor molecule binds to the operator (in the promotor), preventing RNA polymerase from binding to the promotor and preventing transcription.
Volume control	**On/Off switch**



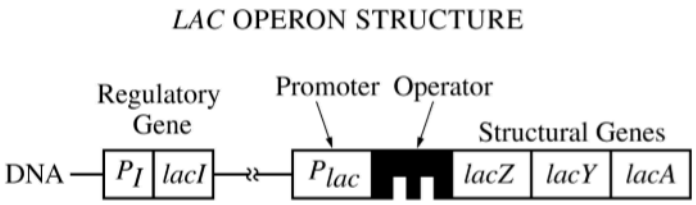
(a) Lactose present, glucose scarce (cAMP level high): abundant *lac* mRNA synthesized

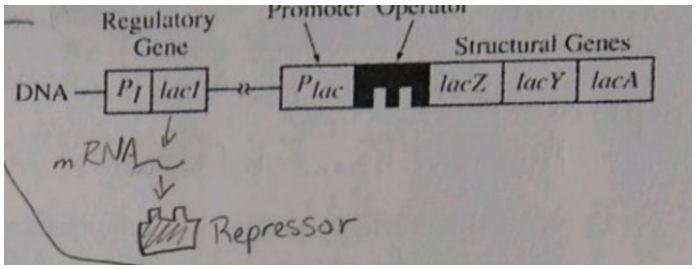


(a) Lactose absent, repressor active, operon off

Operons Practice

1. Identify the role of each section of DNA shown in the diagram of the lac operon.





Regulatory gene - codes for a repressor protein that can bind to the operator to stop transcription of the structural genes. This is always transcribed at a low level. It is not repressed. RNA polymerase always has access to this gene's promotor.

Promotor - DNA segment before a gene; RNA polymerase binds here to start transcribing a gene. This is found just upstream of the structural genes

Pi - this is the promotor of the regulatory gene; it is always transcribed at a low level. It is not repressed. RNA polymerase always has access to this particular promotor.

Plac - this is the promotor for structural genes of the operon. If the repressor is bound to the operator, RNA polymerase can't bind to this promotor.

Operator - Think of this as the on/off switch. It is a DNA segment to which the repressor binds. If the repressor is bound, RNA polymerase is blocked, and structural genes are not transcribed.

Structural genes of the lac operon (lacZ, LacY, lacA) - DNA that codes for enzymes that break down lactose. All three gene products (proteins) are needed to break down lactose.

2. Describe the negative feedback system involved in the lac operon.

Negative gene control means that something stops gene expression. In this operon, the repressor (a protein) binds to the operator (part of DNA), blocking RNA polymerase from binding to the promoter, thus stopping transcription of the genes.

3. If there is a mutation in the promoter of the operon, would the operon most likely be always on or always off?

Maybe RNA polymerase wouldn't be able to bind to the promoter, so the operon would be off.

4. If there is a mutation in the operator operon, would the operon most likely be always on or always off??

Maybe the repressor wouldn't be able to bind to the operator, so the operon would be on.

5. If there is a mutation in the lacZ gene, what is the most likely outcome?

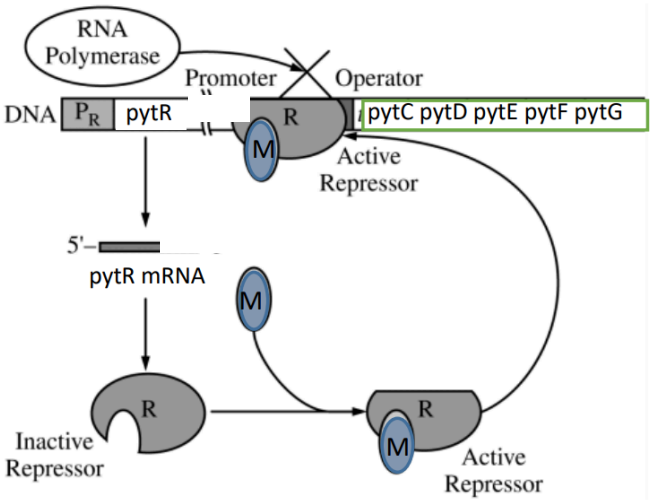
The lacZ gene product (an enzyme) might be nonfunctional, but the other enzymes (lacY and lacA) would probably work. You need all of them to work, though, so the bacterium would not be able to utilize lactose as a food source.

6. If there is a mutation in the lacI gene, would the operon most likely be always on or always off???

If there is a mutation in the lacI gene, a functional repressor would probably not be made, so the repressor wouldn't be able to bind to the operator, and the operon would most likely be on always.

7. The pyt operon is a coordinately regulated group of genes (pytC-G) that are required for silky cilia in *E. lash* bacteria. Describe how the presence or absence of M (mascara) affects the expression of the silky cilia proteins.

R = repressor molecule
M = mascara molecule
P_R = promotor for the pytR gene
*pyt*R = repressor gene
operator = binding site for repressor molecule



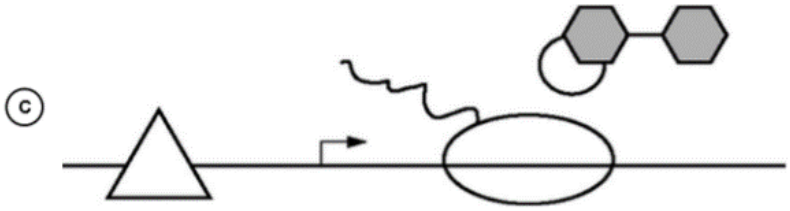
M (mascara) is a co-repressor that binds to the inactive repressor, activating the repressor. The active form of the repressor can then bind to the operator (DNA) of the operon, thus blocking RNA polymerase. Therefore, the genes of this operon are **not** expressed, and E. lash bacteria do not make the enzymes necessary to produce their silky cilia. Bummer.

If M (mascara) is absent, the repressor is inactive, so the promoter is available to RNA polymerase so the genes of the operon are expressed and E. lash will have the enzymes necessary to make silky cilia.

This problem is, of course, made up. Prokaryotes don't make cilia! That's a eukaryotic organelle!

8. Which option shows the correct operon when lactose is present and glucose is NOT present?

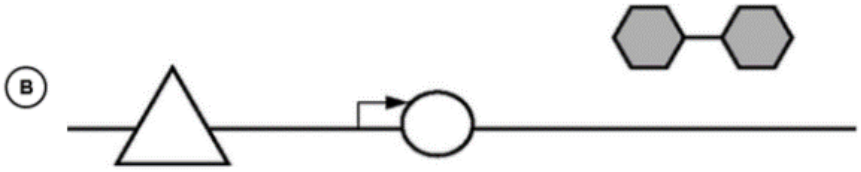
C. In picture C, lactose is present and has attached to the repressor, removing it from the operon. RNA polymerase is transcribing the gene (producing RNA).



CAP is present and has bonded to the operon, which increased the affinity (binding) of RNA polymerase for the promoter. When glucose is scarce, CAP increases the transcription of genes for enzymes that are used to break down other food sources (since there's no glucose around).

9. Which option shows the correct operon when lactose is NOT present and glucose NOT is present?

Sorry - this question is a bit of a fail on my part. B is the closest, since the repressor is bonded to the operon (as it would be if there were not lactose around). And CAP is also bonded to the operon (at the promoter, where it would help RNA polymerase bind to the promoter, if the repressor weren't there. However, it does show lactose around, so it's not a great option. Sorry about that. I guess you can see how I tried to modify an AP question and failed. The question above is the real one.



Eukaryotic Gene Regulation Section

1. Explain the ways in which gene expression can be regulated at each of the following steps:

a. Chromatin - Chromatin (DNA) can be condensed or de-condensed by modifying the DNA and/or the histone proteins with various functional groups (methyl, phosphate, acetyl, etc.). Condensed DNA is all coiled up, and RNA polymerase can't get to it, so the DNA is not used/transcribed. The functional groups and the order in which they were added seem to play a role in turing on or off genes.

b. Transcription factors (activators and repressors) - These can make it easier or harder for RNA polymerase to bind to the promoter. Activators (special proteins) bind to enhancers (special DNA segments) to promote transcription. Repressors (special proteins) bind to silencers (special DNA segments) to decrease or block transcription.
Transcription factors can be general (all transcription requires them) or specific to that particular gene.

c. Primary transcript processing - A primary transcript is the single-stranded RNA molecule made directly by transcription of DNA. If it is going to be mRNA, it is also called pre-mRNA.
It is processed (or modified) in several ways. A poly-A tail is added to the 3' side of the RNA, a GTP cap is added to the 5' side of the RNA, and RNA is spliced.
Splicing can be done in different ways (which is called alternative RNA splicing). This tends to produce a greater variety of gene products (usually proteins) than we would have without this processing because of exon shuffling. Exon shuffling means that not every mature mRNA made from this pre-mRNA will have the same exons. (Exons are expressed (translated) and introns are intervening sequences/not expressed).

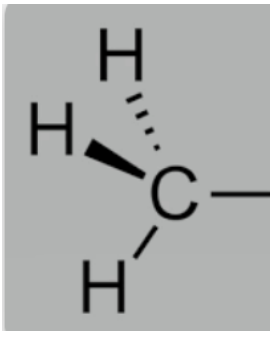
d. mRNA degradation or blockage - the life span of mRNA depends on the untranslated region (UTR) at the 3' end of the molecule. Some last longer in the cell, so they are translated more (thus we get more gene product/protein from them. Others are degraded quickly and produce very little protein. Sometimes an mRNA is blocked from a ribosome by another protein until it is needed, at which point some regulatory cascade will remove the regulator. Cool, huh?

e. protein processing - modification to a protein after it's been made. This could be cutting part of it off, making it active (for example, pepsinogen is an inactive protein enzyme made in the stomach lining. It becomes activated inside the stomach lumen - space inside the organ - by acid cutting part of it off). Other forms of modification could be adding a carb (glycoprotein) or adding a lipid (lipoprotein) in the endoplasmic reticulum, for example.

f. protein degradation - proteosomes break down proteins (after they've been tagged with ubiquitin). If a protein is degraded quickly, this is really a form of gene expression regulation since the gene product is only around for a little while.

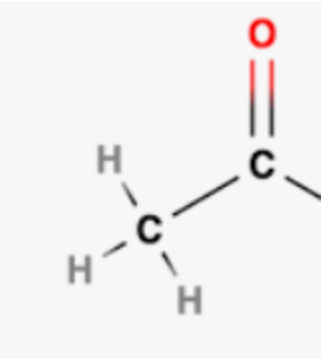
g. cofactor/coenzyme function - these are needed for some enzymes to work. If the enzyme/protein is inactive, this is a form of gene product regulation.

2. Draw a methyl group. What does DNA methylation do?



The addition of methyl groups to certain bases in DNA is associated with reduced transcription and/or gene inactivation. The addition of methyl groups (methylation) to histones can condense chromatin. Just know that modification to DNA and/or histones can change the level of transcription.

3. Draw an acetyl group. What happens when histones are acetylated? What happens when DNA is acetylated?



Acetylated histones tend to push away from each other and deconcense DNA, making it more available for RNA polymerase (thus more easily translated). Just know that modification to DNA and/or histones can change the level of transcription.

4. How is mRNA different from all other types of RNA?

mRNA codes for proteins. Other types of RNA are noncoding. For example, tRNA and rRNA (housekeeping) support transcription, but do not, themselves, code for a polypeptide. Another example of noncoding RNA are regulatory RNAs, such as microRNA, snoRNA, siRNA, snRNA, piRNA, IncRNA.

5. Are all gene products translated?

No! Some code for noncoding RNA, like tRNA, rRNA, piwiRNA, etc. Still others do code for mRNA, but are broken down or blocked before they can be translated.

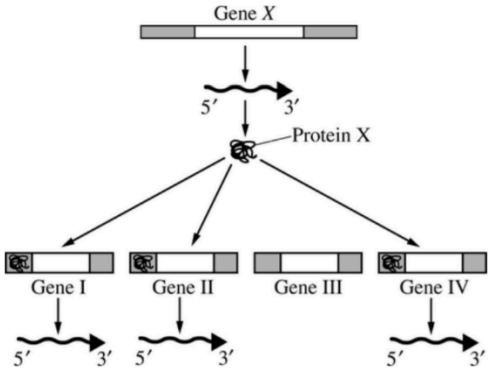
6. What are some of the roles of noncoding RNA (such as piwiRNA, snRNA, siRNA, snoRNA, and microRNA)?

These are regulatory RNAs. They may increase or decrease transcription or translation. For example:

piRNA blocks the transcription of transposons (jumping genes which could damage our DNA by jumping into a necessary gene).

siRNA and miRNA bond to specific mRNA and tag them to prevent translation. This might be to block viral translation, for example.

7. What does protein X do in this illustration?

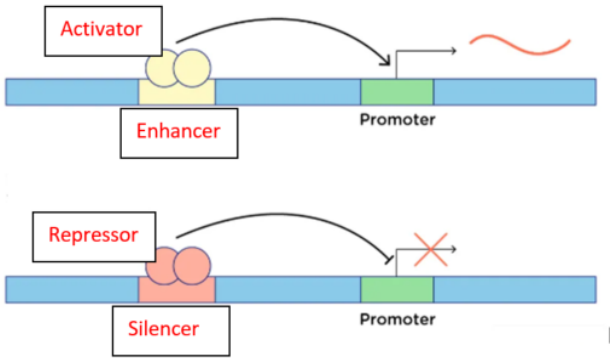


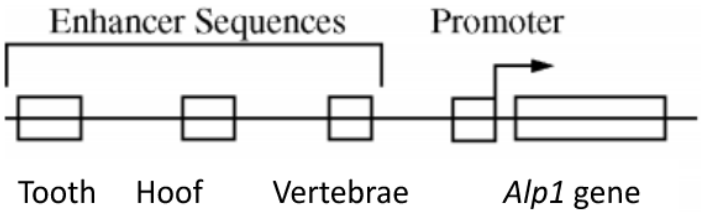
It looks like its protein product works as a specific transcription factor (activator in this case) to promote the transcription of particular genes that might all need to be transcribed together.

8. Fill in this chart to explain the terms.

	Is this DNA or protein?	Promotes or Inhibits transcription?
Activator	protein	Promotes transcription
Repressor	protein	Inhibits transcription
Enhancer	DNA	Promotes transcription
Silencer	DNA	Inhibits transcription

9. Identify the terms above on the diagram here. The long rectangle is DNA. The double circles are proteins. The squiggly line is RNA.





10. Alpaca traits for teeth, hooves and vertebrae are influenced by the *Alp1* gene. Enhancer sequences upstream of the Alp1 genetic locus regulate expression of the Alp1 gene at the appropriate times and in the appropriate tissues during development.

a. A mutation in the promoter sequence is most likely to affect which tissue types?

All (tooth, hoof, vertebra)

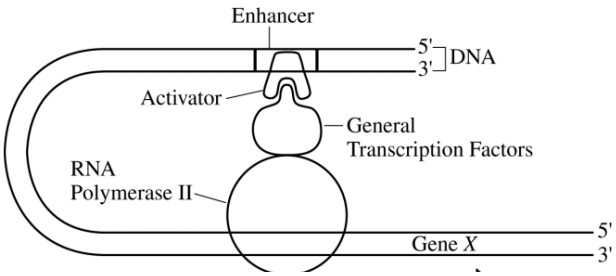
b. A mutation in the hoof enhancer sequence is most likely to affect which tissue types?

hoof only

11. How is the protein crystallin produced in the lens of the eye in humans, but it is not produced in our liver cells? Do liver cells have the DNA instructions to make this protein?

The crystallin gene is present in **all** of our cells, but it is only transcribed and translated in our lens cells because the specific transcription factors (activators) necessary for its transcription are produced only in the lens cells. Liver cells have a different developmental history. The crystallin gene was inactivated in those cells during differentiation and/or the regulatory genes which produce activators for the crystallin gene were not activated in liver cells during differentiation (they were probably inactivated).

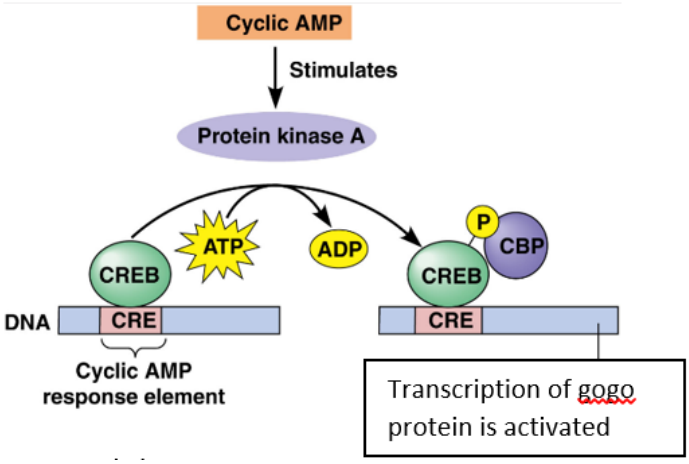
12. Define the role of each part of the diagram below in the transcription of gene X. Also, which parts are DNA and which parts are protein?



Activator (protein) binds to a specific enhancer (DNA) which increases gene expression. The activator also usually binds to general transcription factors.
General transcription factors are proteins needed to help RNA polymerase bind to the promoter.
RNA polymerase II is an enzyme (a protein) that transcribes DNA to make mRNA.
Gene X is just DNA that codes from something needed.

Eukaryotic Gene Regulation Practice Questions

The cAMP response element binding protein (CREB) controls gene expression when cAMP levels increase. Genes activated by cyclic AMP possess an upstream cyclic AMP response element (CRE) that binds a transcription factor called the CREB protein. In the presence of cyclic AMP, cytoplasmic protein kinase is activated and its activated catalytic subunit then moves into the nucleus, where it catalyzes phosphorylation of the CREB protein, thereby stimulating its activation domain. Then it interacts with CBP (**CREB-binding protein**), which acts as a scaffold to stabilize additional protein interactions with the transcription complex. The most immediate consequence of CREB activation is initiation of new gene transcription for the protein gogo. Gogo appears to stimulate neuron growth by activating CDKs (cyclin dependent kinases) required for mitosis. CDKs then deactivate cAMP. (Note: Most details in this example are made up).



A

1. Based on the model of gogo synthesis presented in the figure, which of the following best describes the role of feedback on the control of gogo synthesis?

A. A decrease in cAMP activates protein kinase A. Protein kinase A in turn activates the CREB proteins, thereby stimulating neuron growth and activating cAMP.

B. A decrease in cAMP activates CBP. CBP in turn activates the CREB proteins, thereby stimulating neuron mitosis and deactivating cAMP.

C. An increase in cAMP activates protein kinase A. Protein kinase A in turn phosphorylates the CREB protein, thereby stimulating neuron growth and **deactivating cAMP. (this part is key to the feedback part of the question)**

D. An increase in cAMP activates CBP. CBP in turn activates the CREB proteins, thereby stimulating neuron growth and activating cAMP.

2. Based on the model of gogo synthesis presented in the figure, which of the following best describes the mechanism whereby cAMP most likely regulates gogo production?

A. Transcription occurs under low intracellular cAMP concentration when phosphorylated CREB acts as a transcription factor at the cAMP response element

B. Transcription occurs under low intracellular cAMP concentration when ADP dephosphorylates the CBP protein, blocking the transcription of the cAMP response element

C. Transcription occurs under high intracellular cAMP concentration when phosphorylated CREB acts as a transcription factor at the cAMP response element

D. Transcription occurs under high intracellular cAMP concentration when ADP dephosphorylates the CBP protein, blocking the transcription of the cAMP response element

3. After a search of nucleotide sequence databases, researchers identified a cAMP response element in the 5' untranslated region of a gene encoding melanopsin, a protein involved in light detection in the retina of the eye. Which of the following pieces of experimental evidence best supports the claim the synthesis of melanopsin is controlled by a mechanism similar to gogo protein regulation regulation?
   A. CREB binds to a melanopsin promotor in the presence of cAMP
   B. The relative amount of melanopsin protein increases in the presence of high levels of cAMP (true, but doesn't address the mechanism)
   C. Light stimulation of retinal cells increases in the presence of high levels of cAMP
   D. Light stimulation of retinal cells decreases in the presence of high levels of cAMP
   For C and D, the question doesn't specify what role melanopsin plays in light detection. It could increase or decrease detection).

4. CREB downregulation is implicated in the pathology of Alzheimer's disease. A possible therapeutic target for Alzheimer's disease might include:
   A. decreasing CBP expression
   B. increasing the expression of CREB
   C. blocking the cAMP response element
   D. increasing the dephosphorylation of CREB

5. Explain how the protein (Iron Response Protein) regulates translation in the diagram below.

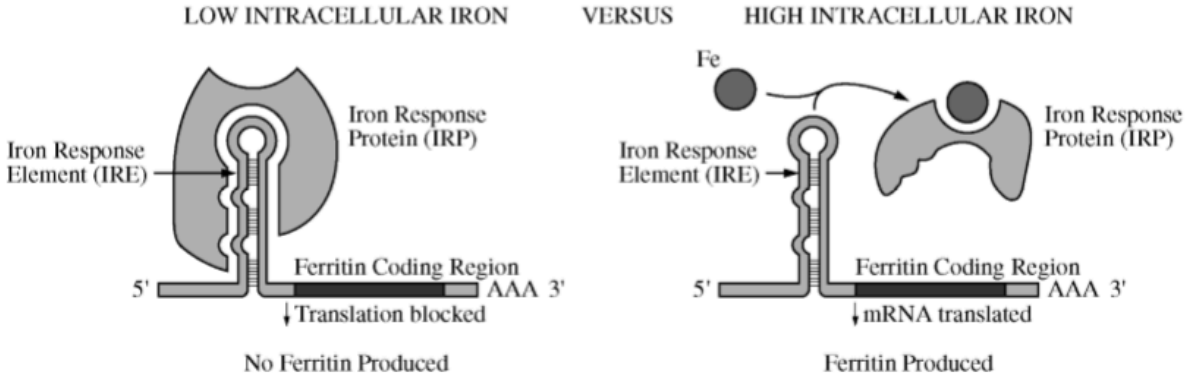


Figure 2. Model of regulation of ferritin synthesis by iron

RNA has already been produced. We are looking at regulating translation (RNA to protein). Cool, huh?

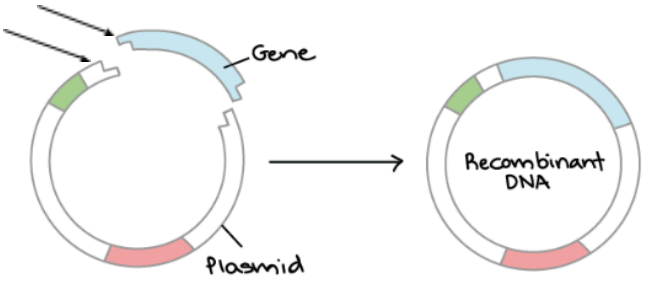
IRP (iron response protein) binds to IRE IRE (iron response element) and gets in the way of the ribosome, so translation is blocked.

Fe (iron) binds to IRP (iron response protein), and removes it from IRE (iron response element) so now the ribosome can get to the mRNA, and translation proceeds.

Biotechnology Questions

1. Plasmids are often used in recombinant DNA technology. What is a plasmid?

A small piece of circular DNA found in bacteria. They can be swapped during conjugation. They do not have a lot of DNA - they may code for traits like antibiotic resistance. There will always be an origin of replication.



2. What enzyme type is used to cut the DNA?

Restriction enzymes (aka endonucleases)

3. Are the ends considered blunt or sticky ends?

Sticky ends are shown in the diagram.

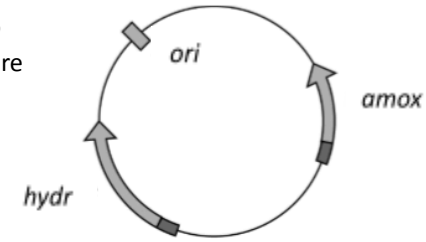
4. Why must the donor DNA containing the desired gene and the plasmid DNA be cut with the same restriction enzyme?

So they can hybridize (join together)/anneal at the sticky ends. They use ligase to join them.

5. What is the purpose of using a plasmid containing an antibiotic resistance gene in the transformation process?

There needs to be some way to select for the bacteria that have taken up the plasmid. Many times it is antibiotics that scientists use to do this because any bacteria that have not taken up the plasmid should be killed by the antibiotics (hopefully). There are other methods of selection.

6. The AMOX-HYDR plasmid carries an origin of replication (*ori*), *amox* gene (conferring resistance to amoxicillin), and *hydr* gene (conferring the ability to digest aliphatic hydrocarbons in oil). Cultures of *Alcanivorax borkumensis* were transformed with the plasmid. Next, the bacterial cells were cultured in the presence of amoxicillin overnight.



   a. Why are the bacterial cells cultured in the presence of amoxicillin?

To kill the cells that didn't get transformed with the plasmid. This selects for those bacteria that did take up the plasmid (and express the protective antibiotic resistance).

   b. Predict the outcome of culturing transformed *A. borkumensis* in the presence of amoxicillin.

Only those bacteria that took up the plasmid will survive and reproduce and produce a colony (many cells produced from one living cell). (cultures are visible, but individual bacterial cells are not visible). This question is really sort of a repeat of the one above it…..

   c. A human gene was inserted into the plasmid in the middle of the origin of replication. Predict the consequences of this insertion.

The plasmid will not replicate when the bacterium does binary fission, so it will be useless. One cell with cool DNA doesn't matter - we need it to copy itself.

   d. A gene for green fluorescent protein was inserted into the plasmid in the middle of the hydr gene. Predict the consequences of this insertion.

When exposed to the hydrocarbons (and uv light), the bacteria will glow because the RNA polymerase is able to bind to the promoter. Instead of transcribing the hydr gene, however, the green fluorescent protein is transcribed.

The bacteria will glow, but they won't digest the hydrocarbons (since that gene was interrupted by the glowy gene).

7. Polymerase Chain Reaction (PCR) is a process that amplifies DNA.
    a. What does amplify mean in this context?

To make many more copies (it creates lots of DNA from a small source).

    b. What are the raw materials (enzymes, etc.) needed for this technique?

Taq DNA polymerase (the specific DNA polymerase from the thermophilic bacterium. This is a heat stable enzyme!)

primers that match the beginning of the DNA sequence that we want to copy.

nucleotides

thermal cycler (a machine that heats DNA to break the hydrogen bonds/open up DNA and cools DNA to allow DNA polymerase to add nucleotides. Then repeat, repeat, repeat this cycle).

    c. Why is the DNA polymerase used in this process so special?

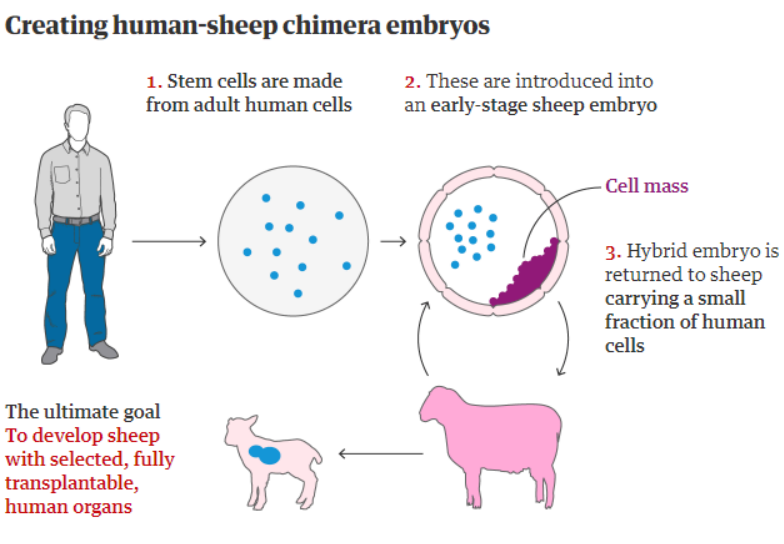
It can withstand the high temperatures required by this process so that DNA can be unwound (by breaking the hydrogen bonds between the nitrogenous bases). It was isolated from nature - a thermophilic bacterium!! cool, huh?

https://www.theguardian.com/science/2018/feb/17/breakthrough-as-scientists-grow-sheep-embryos-containing-human-cells

Growing human organs inside other animals has taken another step away from science-fiction, with researchers announcing they have grown sheep embryos containing human cells.

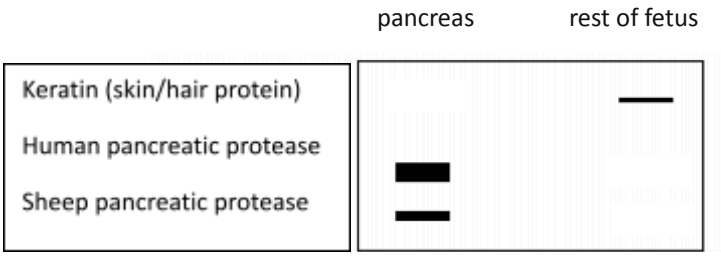
Scientists say growing human organs inside animals could not only increase supply, but also offer the possibility of genetically tailoring the organs to be compatible with the immune system of the patient receiving them, by using the patient's own cells in the procedure, removing the possibility of rejection.

According to NHS Blood and Transplant, almost 460 people died in 2016 waiting for organs, while those who do receive transplants sometimes see organs rejected.



"Even today the best matched organs, except if they come from identical twins, don't last very long because with time the immune system continuously is attacking them," said Dr Pablo Ross from the University of California, Davis, who is part of the team working towards growing human organs in other species..... Bruce Whitelaw..... said that while there was a long way to go before human organs could be grown in other animals, the latest research is "an important step forward through starting to explore whether sheep offer an option for the exciting 'chimeric' project."

In a fake experiment (which scientists are not allowed to do), the embryo was allowed to grow to a 2 month fetus. The pancreas was then separated from the rest of the fetus. Protein was extracted from each fragment and separated by electrophoresis.



8. Why is human protein found in the sheep fetus?

Because human cells were added to (injected into) a sheep embryo.

9. Why are sheep pancreatic proteases found in a different band from human pancreatic proteases?

Because they are a different size and/or charge. (This question is just asking you about electrophoresis and how molecules are separated).

10. What would have happened if human pancreatic protease mRNA were inserted into the embryo, instead of human stem cells?

The mRNA would probably be translated in whatever cells they ended up in, since sheep have ribosomes that recognise mRNA from humans (or any other mRNA).

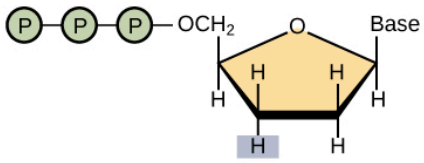
11. Why can a mouse eye gene cause eyeless fruit flies to develop eyes?

https://www.youtube.com/watch?v=OHR5K2o7uQU (video that we watched in class)

Because there is a common ancestor (of mice and fruit flies) that had this regulatory gene and both organisms can recognise it.

12. How and for what purpose are animals cloned?

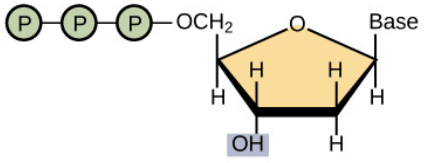
Varying reasons - one is that it takes a lot of money and effort to make a transgenic animal. Maybe it's a goat that produces human growth hormone in its milk. It might actually be easier to clone that goat rather than to produce more transgenic goats.



Dideoxynucleotide (ddNTP)

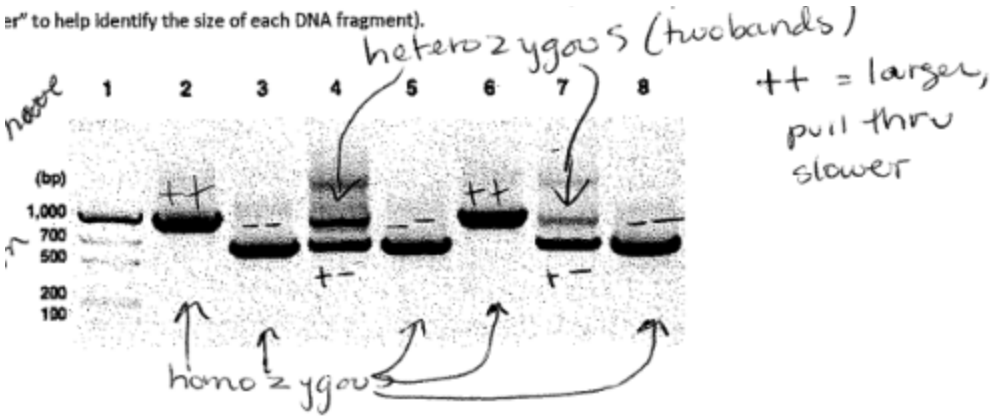
13. How is dideoxynucleotide different from deoxynucleotide? Why is this difference useful in sequencing DNA?

It has one more missing hydroxyl (-OH) group, so it can't be joined to another nucleotide. This means that no more nucleotides can be added after it. It is terminal. It stops DNA replication. So, this is useful because it will produce replicated fragments of different lengths which can be separated and analyzed. If a fluorescent tag is added to each dideoxynucleotide type (pink for adenine, blue for cytosine, whatever), then we can figure out the order/sequence of nucleotides in a DNA molecule. cool, huh?



Deoxynucleotide (dNTP)

14. PV92, a human-specific Alu insertion on chromosome 16 is detectable by electrophoresis. The presence of this insertion makes the gene larger; its absence makes the gene smaller. There are only two alleles possible, and people can have the genotype ++, +- or --. The blood 92 allele, amplify it, then run it on a gel. Identify the genotypes of each person from lanes 2 through 8. (Lane one is a ladder to help identify the size of each DNA fragment).



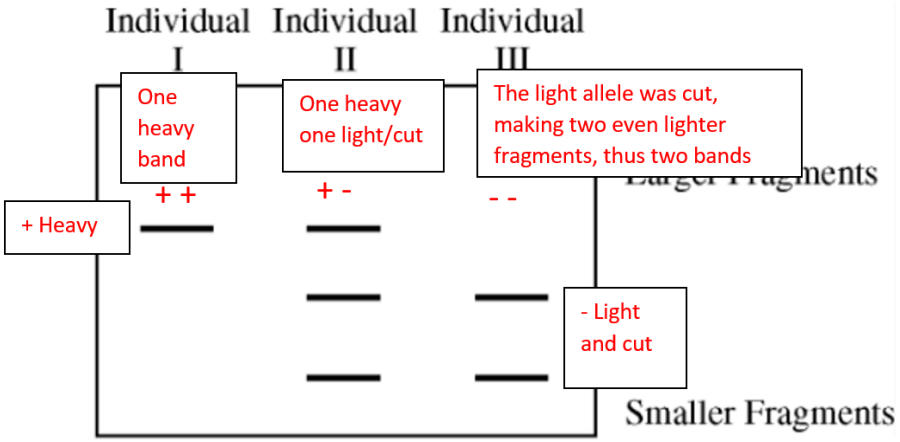
Note: Some of the extra shadows are hard to interpret. Each well/column should have one or two bands only. No restriction enzymes were used here.

15. A restriction endonuclease recognizes a particular sequence present only in the (-) allele of the PV92 system. Below is an image of a gel following electrophoretic separation of DNA fragments of the PV92 gene from three individuals. Identify the genotypes of each individual.

Individual one: ++

Individual two: +-

Individual three: --



Individual two is heterozygous. The + allele DNA is not cut by the restriction enzyme and moves slowly through the gel because it is big.. The - allele is cut, so two small fragments are made, both are small and travel further through the gel.

16. mRNA from a frog embryo is extracted and separated using gel electrophoresis. How can specific mRNA be identified?

You could use a probe (DNA or RNA that is complementary to the mRNA of interest. The probe should also have a fluorescent molecule (tag) attached to it, or be radioactive, or something that can be detected.

The probe would be spread onto the mRNA in the gel, then washed off. Only hybridized/annealed/joined probes would stay put. This would show that the RNA of interest is there.

You could also sequence it, I guess….

17. Define hybridization in the context of nucleic acids.

This just means they are complementary - the As match with Ts, the Cs match with Gs.

18. Probes:
    a. What is a probe?

Sorry - I already asked this….

DNA or RNA that is complementary to the mRNA of interest.

    b. How do we use probes to find specific mRNA or specific stretches of DNA?

The probe would be spread onto the mRNA in the gel, then washed off. Only hybridized/annealed/joined probes would stay put. This would show that the RNA of interest is there.

    c. How can we “see” probes?

The probe should also have a fluorescent molecule (tag) attached to it, or be radioactive, or something that can be detected.