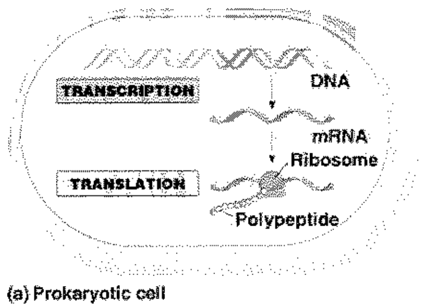
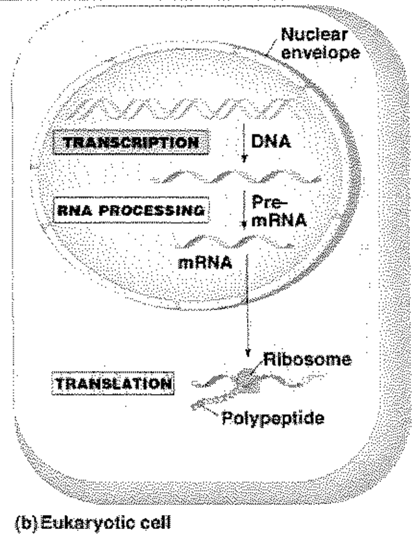
Transcription and Translation

Overview: Prokaryote vs. Eukaryote



(a) Prokaryotic cell



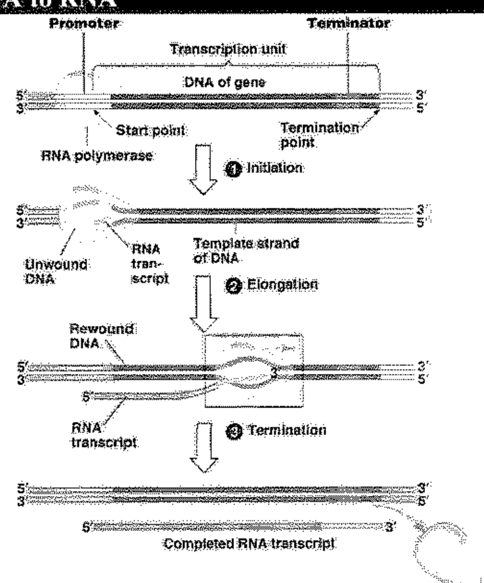
(b) Eukaryotic cell

→Transcription: synthesizing RNA from a DNA template.
→Translation: synthesizing protein from RNA created in transcription.

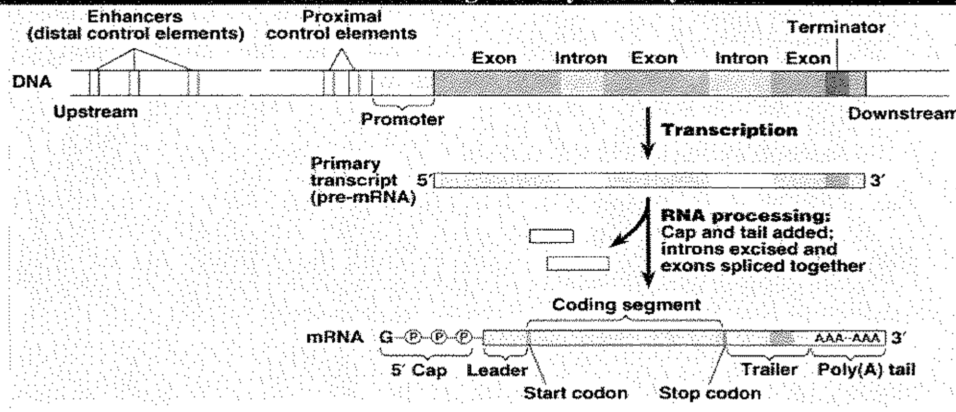
Prokaryotes	Eukaryotes
→Both transcription and translation take place in the cytosol since prokaryotes do not contain nuclei.	→Transcription takes place in the nucleus while translation takes place in the cytoplasm. This compartmentalization of events allows for additional control and modification of products (RNA splicing for example).

Transcription: DNA to RNA

→Promoters signify the beginning of the sequence to be transcribed; terminators or transcription stop sights signify the end.
→RNA Polymerase is guided into place by transcription factors as it latches onto and unzips the appropriate segment of DNA creating a transcription bubble.
→RNA Polymerase will then attach appropriate nucleotides (A,U,G,C) creating a complementary strand from one side of DNA until it reaches the terminator.
→RNA Polymerase will transcribe both <u>ex</u>pressed (<u>ex</u>ons) genetic material that codes for protein and the intervening introns.



RNA Processing: Eukaryotes only	

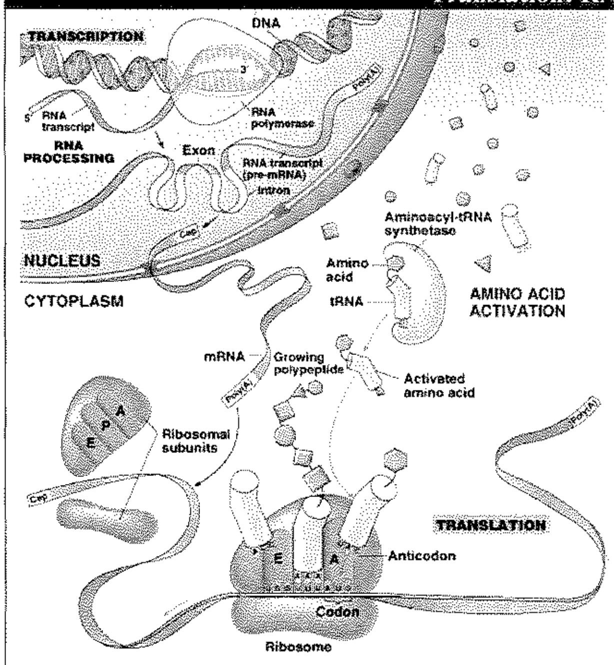


5' CAP	Poly (A) Tail
This unique bonding arrangement added to the 5' end of a pre-mRNA results in increased length of survival time for the mRNA when the cap is added...increased survival time means more translated protein. The 5' cap also acts to guide the ribosome into place during translation.	This "many adenine" tail allows for the exit of mRNA from the nucleus. Not all RNA is destined to be transcribed in the cytoplasm (histone proteins, etc.). It is thought that the Poly (A) tail is the "secret handshake" that allows the RNA to pass by proteins in the nuclear pore and out into the cytoplasm for translation.

Alternative Splicing

Splicing mRNA exons together in different combinations to produce different proteins. Example: The original sequence ABCD may be spliced into ABC, ABD, ACD, etc. Research suggests that nearly 60% of all human DNA is expressed as alternatively spliced RNA. Alternative splicing is pervasive in the creation of antibodies.

Translation: RNA to Protein



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

Translation

Initiation

→The small subunit of the ribosome begins protein synthesis at a start codon (most always AUG) of mRNA with the help of intitiation factors. This is usually the 1st AUG closes to the 5' end. This is important in insuring that the protein is read with the correct reading frame.

→The appropriate tRNA carrying the amino acid methionine will pair its anticodon via hydrogen bonds with the mRNA codon according to base pairing rules.

→Once the initiation complex is formed, the large subunit of the ribosome attaches "on top" of the small subunit.

Elongation

→The ribosome moves (translocation) one codon at a time attaching complementary mRNA codons with tRNA anticodons. Because anticodons are associated with specific amino acids, the appropriate amino acids are in the appropriate order (assuming no mutation).

→Before each tRNA is released by the ribosome, the amino acid is joined via peptide bond to the growing chain of amino acids.

Termination

→Elongation continues until 1 of 3 stop codons is reached. Stop codons do not code for an amino acid, but instead "call for" the binding of release factors. Once release factors are bound the mRNA, ribosomal subunits, etc. all disengage.

→The string of amino acids (protein) will fold into the proper 3-dimensional shape with the aid of chaperonin proteins.

Recycling of materials and dditional information about translation…

Recycling of materials

→The mRNA will be degraded and the nucleotides used again in the next message.
→The "used" tRNA that have left behind amino acids can also be "recharged" with their appropriate amino acid and used again as well.

→ Multiple ribosomes may read an mRNAstrand at a time. This is structure of mRNA bound by multiple ribosomes is referred to as polyribosomes.

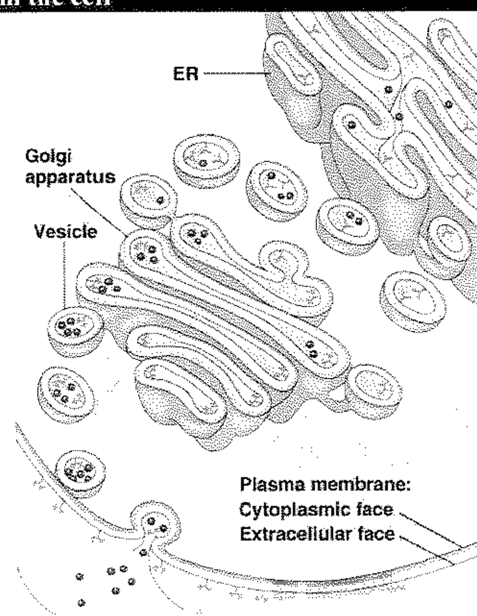
→The genetic code is degenerate or redundant due to the fact that there are 64 possible mRNA codons and only 20 coding amino acids (actually 22 aminos can be made). When observing the genetic code or codon chart above it should be noted that the 3rd nucleotide in a codon is the least important in determining the corresponding amino acid. This phenomenon is called the "wobble effect."

Exporting protein from the cell

→Protein will be manufactured by ribosomes on the rough endoplasmic reticulum (RER). From here the protein will be injected into the RER for packaging.

→The protein will then be transported to the Golgi apparatus via vesicle for further modification (addition of sugars, etc.).

→The tagged protein will now travel to the cell membrane, fuse with the cell membrane, and export the protein via exocytosis.



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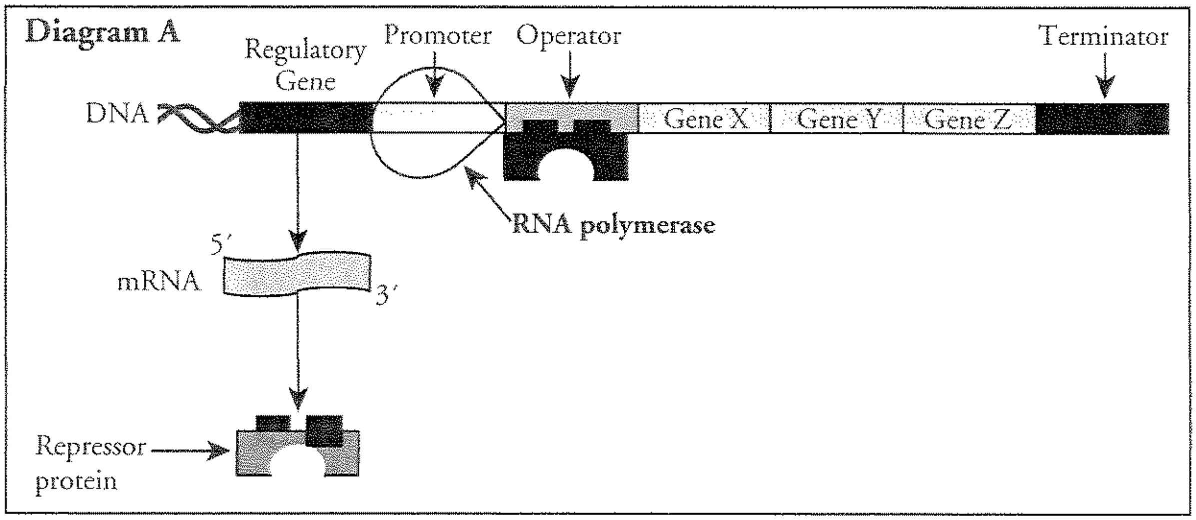
Control of Gene Expression in Prokaryotes

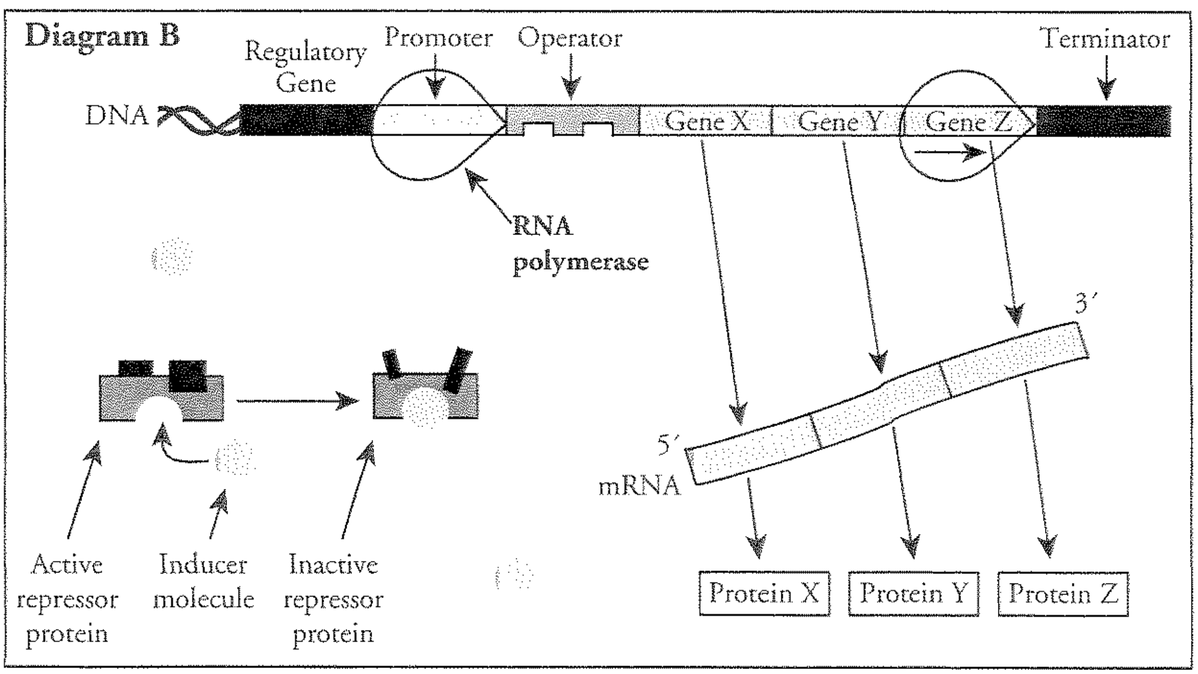
How do prokaryotes use operons to control gene expression?

Why?

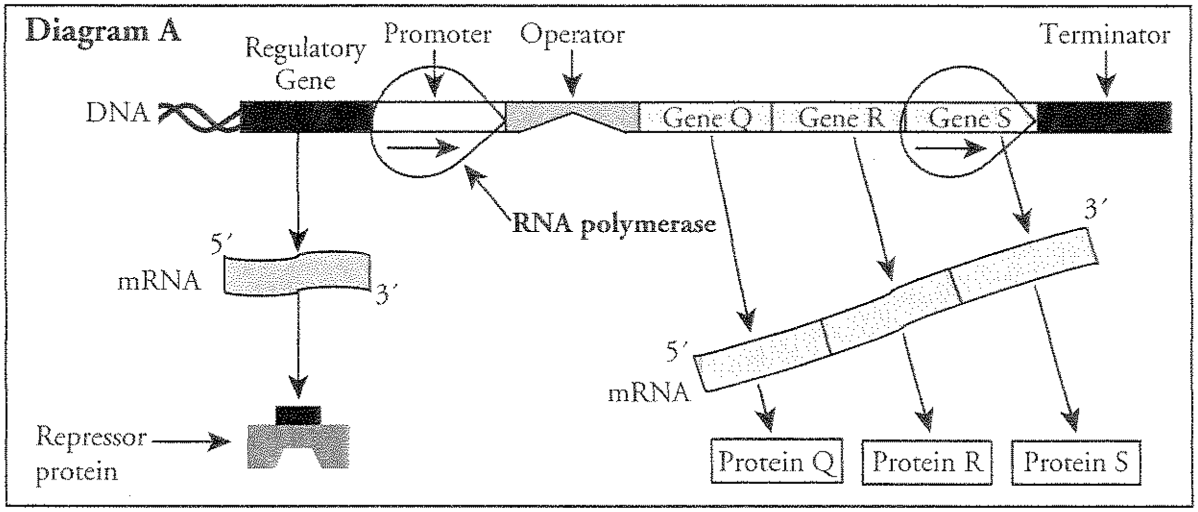
Houses usually have a light source in every room, but it would be a waste of energy to leave every light on all the time, so there are switches to turn off the lights in rooms that are not in use. Sometimes one switch controls several lights in the same room. Likewise, prokaryotic cells can turn genes on and off based on environmental factors. Sometimes related genes are grouped together with one switch. This group of genes, along with the sections of DNA that regulate them, is called an **operon**.

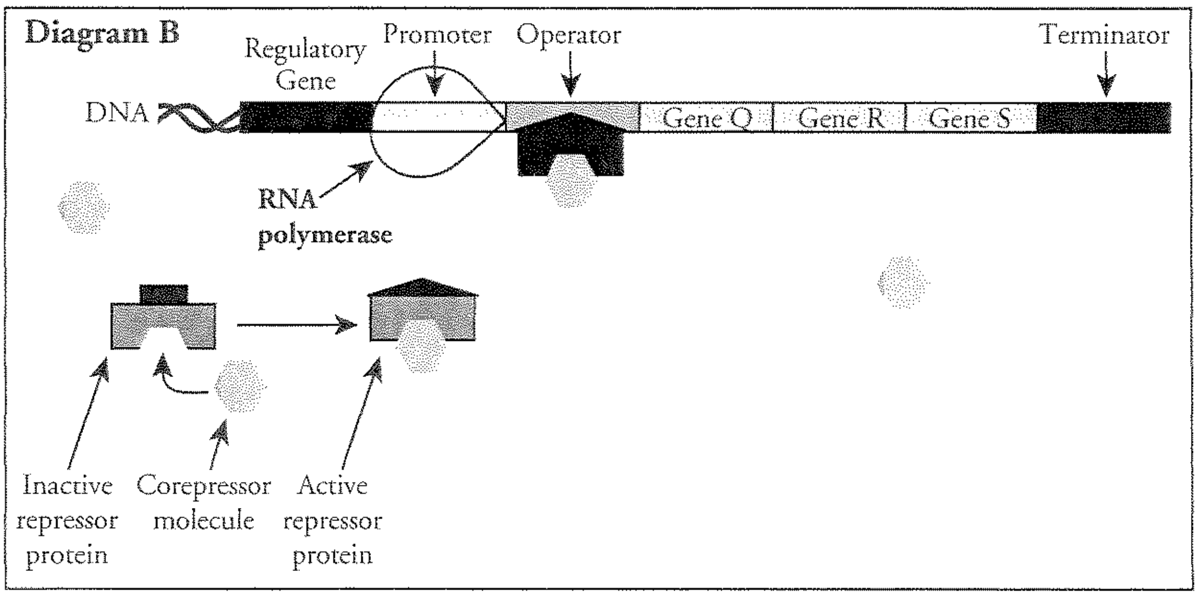
Model 1 – An Inducible Operon





Model 2 – A Repressible Operon





10. In Model 2, where on the DNA strand does RNA polymerase bind to start transcription?

11. In which diagram of Model 2 is transcription and translation occurring successfully, diagram A or diagram B? Justify your answer with evidence from Model 2.

12. Does the regulatory gene in Model 2 produce a protein that is an active or inactive repressor naturally?

Genetic Mutations

What mistakes can occur when DNA is replicated?

Why?

The genes encoded in your DNA result in the production of proteins that perform specific functions within your cells. Various environmental factors and spontaneous events can lead to changes in genes. These changes, called **mutations**, can lead to alterations in the structure and activity of the proteins your cells use in their daily activities. In other words, changes to your genotype can result in changes to your phenotype. We all have mutations in most of our body cells—yet we are, for the most part, normal and functional human beings. How can that be?

Model 1 – Gene Mutations

Sequence 1 (normal)

DNA sequence ...T A C G T A G T C A C C T A A T G G A T C...

mRNA sequence **A U G C A U C A G U G G A U U A C C U A G**

Amino acid sequence Met His Gln Trp Ile Thr stop

Sequence 2 (substitution)

DNA sequence ...T A C G T A G T C A G C T A A T G G A T C...

mRNA sequence **A U G C A U C A G U C G A U U A C C U A G**

Amino acid sequence Met His Gln Ser Ile Thr stop

Sequence 3 (insertion)

DNA sequence ...T A C G T A T G T C A C C T A A T G G A T C...

mRNA sequence **A U G C A U A C A G U G G A U U A C C U A G...**

Amino acid sequence Met His Thr Val Asp Tyr Leu...

Sequence 4 (deletion)

DNA sequence ...T A C G T A G T C C C T A A T G G A T C...

mRNA sequence **A U G C A U C A G G G A U U A C C U A G...**

Amino acid sequence Met His Gln Gly Leu Pro...

1. How many nucleotides are present in the "normal" DNA sequence in Model 1?

2. How many codons are contained in the mRNA that is produced by the "normal" DNA in Model 1?

3. How many amino acids will be in the polypeptide produced by the normal DNA/mRNA sequence?

Model 2 – Comparing Substitution Mutations

Original DNA:	…T A C C C T A G G A A T A T C A A A…
mRNA:	**A U G G G A U C C U U A U A G U U U…**
Amino acid:	Met Gly Ser Leu stop
Mutation A:	…T A C C C T A G G A A A A T C A A A….
mRNA:	**A U G G G A U C C U U U U A G U U U…**
Amino acid:	Met Gly Ser Phe stop
Mutation B:	…T A C C C T A G C A A T A T C A A A….
mRNA:	**A U G G G A U C G U U A U A G U U U…**
Amino acid:	Met Gly Ser Leu stop
Mutation C:	…T A C A C T A G G A A T A T C A A A…
mRNA:	**A U G U G A U C C U U A U A G U U U…**
Amino acid:	Met stop
Mutation D:	T A G C C T A G G A A T A T C A A A…
mRNA:	**A U C G G A U C C U U A U A G U U U…**
Amino acid:	No protein will be translated because there is no start codon.

15. For each of the mutations A – D in Model 2, circle the substitution that occurred by comparing the mutated DNA with the original DNA.
16. As a group, describe the range of changes in the amino acid sequence that can result from this type of mutation.