AP* BIOLOGY

GENE REGULATION

Teacher Packet

Gene Regulation

Objective

To review the student on the concepts and processes necessary to successfully answer questions over prokaryotic and eukaryotic gene regulation.

Standards

Gene Regulation is addressed in the topic outline of the College Board AP Biology Course Description Guide as described below.

II. Heredity & Evolution
- A. Heredity
  - Meiosis and gametogenesis
  - Eukaryotic chromosomes
  - Inheritance patterns
- B. Molecular Genetics
  - RNA and DNA structure and function
  - Gene regulation
  - Mutation
  - Viral structure and replication
  - Nucleic acid technology and applications

The principles of gene regulation are tested every year on the multiple choice and occasionally make up portions the free response section of the exam. As with many AP Biology free response, these topics are often intertwined with other topics. It seems that the time is due to have an "all out" gene regulation question on the free response section of the exam. Each year there is typically one very difficult free response question. Gene regulation is one of the more confusing topics for teachers and students and could therefore be "tough question" on the free response. Extra emphasis should be placed on delineating prokaryotic and eukaryotic regulation mechanisms. The list below identifies free response questions that have been previously asked over this topic. These questions are available from the College Board and can be downloaded free of charge from AP Central http://apcentral.collegeboard.com.

Free Response Questions	
2005 Question #2	2003 B Question #1

Gene Regulation

Prokaryotes: Operon

An **operon** is a set of genes grouped together, transcribed together with one promoter for one function (we think).
At the essential level, operons consist of a promoter, operator, and coding genes.

→Promoter site- Sequence of DNA where RNA Polymerase binds for transcription. This is the beginning of a gene.
→Operator site- This site controls access to the promoter. A repressor protein may bind here. The operator is typically located within or very near the promoter. This is the "on/off" switch of a gene.

Prokaryotes: Negative Gene Regulation- Repressible Operons- *trp* operon

Major Players

DNA Sequences

Regulatory gene
→Code for Repressor

Operator
→Binding site for Represssor

Promoter
→Binding site for RNA Polymerase

Coding genes
→Actual genes produced

Proteins & other

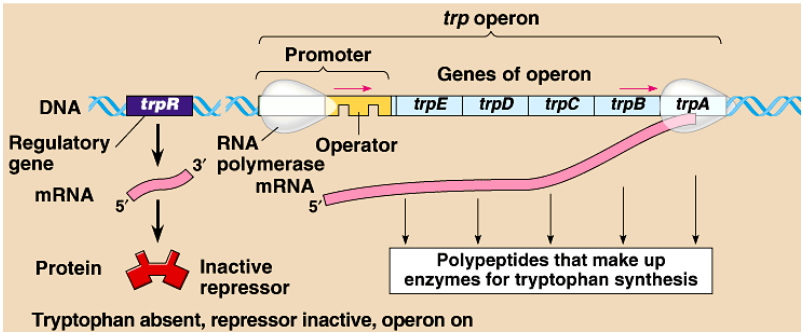
RNA Polymerase
→Transcribes operon genes

Repressor
→Blocks transcription by RNA Polymerase

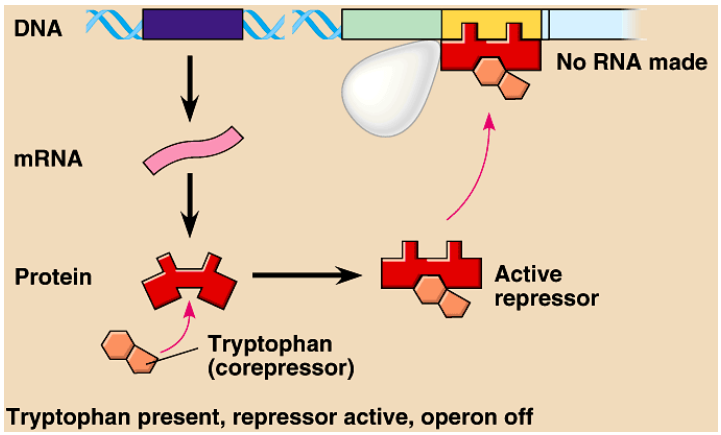
*Corepressor**
→*Activates the repressor*

**Note that the italicized "corepressor" is the difference between inducible and repressible operons.*

"On"



"Off"



Summary→ By default, a repressible operon is "on" and is thus often involved in anabolic processes. When the operon is on, the concentration of the product of the operon is being produced in increasing quantity. Once the concentration is high enough, the product will act as a corepressor, bind to the repressor, and result in a conformational change and thus activate the repressor. The active repressor will bind to the operator site turning off the operon. This is, of course, an example of negative feedback. As product (corepressor) concentrations decrease, the represssors become inactive and leave the operator site. RNA Polymerase is now able to latch on to the promoter site and transcribe. Repressible operons are ideal for keeping a consistent amount of product since a rise above the desired range reduces production and a fall below the desired range results in synthesis.

Prokaryotes: Negative Gene Regulation- Inducible Operons- *lac* operon

Major Players

DNA Sequences

Regulatory gene
→Code for Repressor

Operator
→Binding site for Represssor

Promoter
→Binding site for RNA Polymerase

Operon Genes
→Actual genes produced

Proteins and other

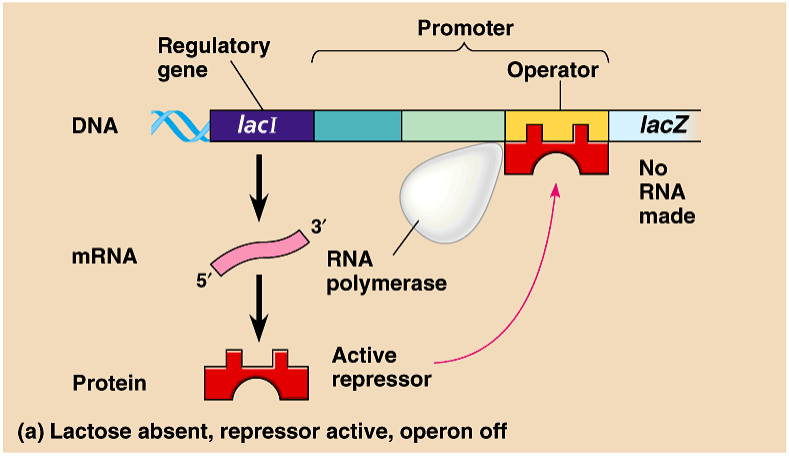
RNA Polymerase
→Transcribes operon genes

Repressor
→Blocks transcription by RNA Polymerase

*Inducer**
→*Inactivates the repressor*

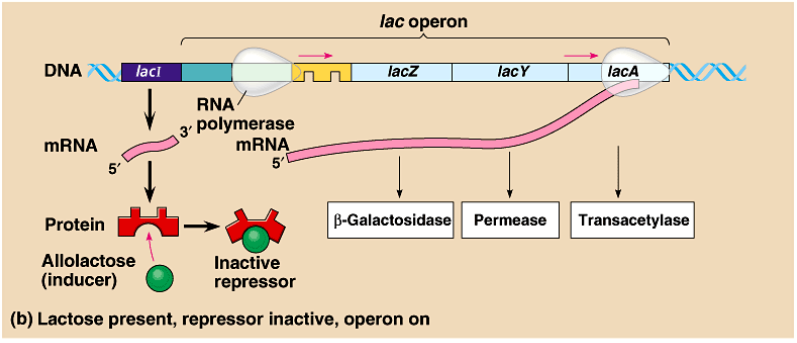
**Note that the italicized "inducer" is the difference between inducible and repressible operons.*

"Off"



(a) Lactose absent, repressor active, operon off

"On"



(b) Lactose present, repressor inactive, operon on

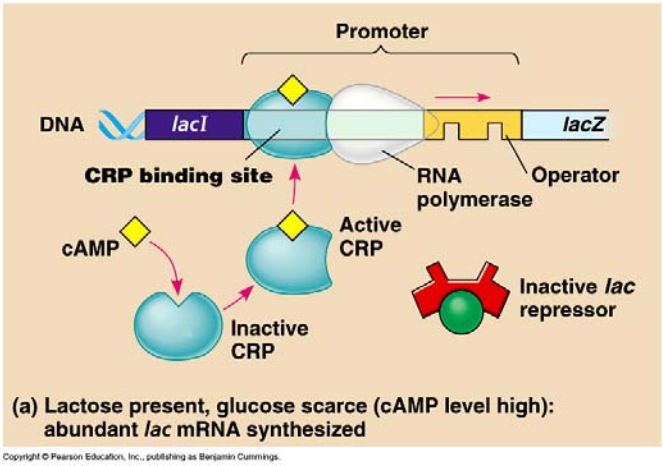
Summary→ By default, an inducible operon is "off" and is thus often involved in catabolic processes. Observe the instance of the *lac* operon above. It would energetically inefficient to produce lactase when the substrate lactose (allolactose) is absent. The operon is therefore off until the substrate lactose is present. Note that lactose will act as inducer as it inactivates the repressor.

Note→ Notice that the above system is still considered a negative control mechanism as it is still involving repressors.

Prokaryotes: Positive Gene Regulation- *lac* operon, a closer look

"To catabolize or not to catabolize" is not always a simple yes/no (inactive repressor/active repressor) question. In this example, glucose is a "first choice" food source while lactose is a "second choice" food source. The inclusion of an activator (CRP) in this more detailed view of the *lac* operon creates an additional step in the "decision making" process. If the 1st choice food source is scarce, and the 2nd choice food source is present, the operon for the second food choice (lactose/lactase) is on.

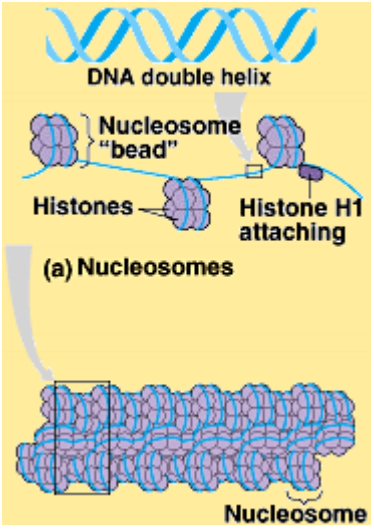
The essential idea→ Each protein is an additional "yes/no" step of regulation. If it is necessary to account for the presence of both glucose and lactose, it is possible to do so by have two different regulatory proteins.



Eukaryotes: Gene Regulation prior to transcription

Histone Acetylation (-$COCH_3$)
→Histones allow for ~2m of DNA to be packed into the nucleus of a human somatic cell in an organized manner. In addition, the degree to which the DNA is packed plays a role in gene expression.
→Acetylated histones hold DNA less tightly and vice versa. Tightly compacted DNA is unable to be unzipped and transcribed and is therefore "off"
→Acetylation/deacytlation enzymes may be closely related to transcription factors

DNA Methylation (-CH_3)
→Highly methylated DNA is more likely to be "off" and vice versa. Some speculate that the methyl groups attach to promoter sites blocking access to RNA Polymerase.
→Methylation patterns are reproduced after DNA replication resulting in genomic imprinting.



The main idea→ Histone acetylation and DNA methylation are both pre-transcription control mechanisms because they control access off RNA Polymerase to promoter sites.

Eukaryotes: Gene Regulation During Transcription

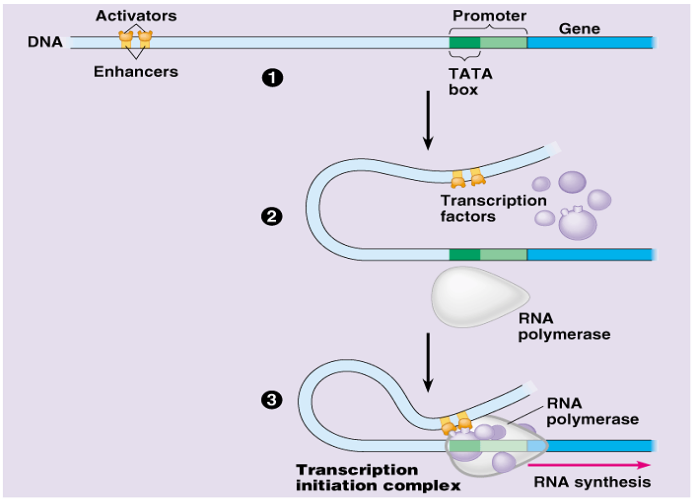
Transcription Factors

→Transcription Factors bind to specific sites near the promoter (like the TATA box in Eukaryotes).

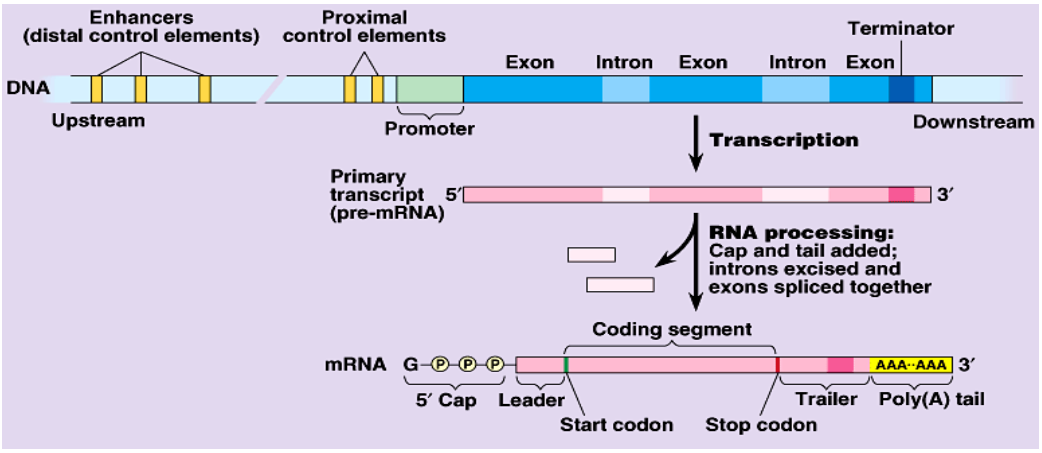
→These transcription factors bind to double helix DNA and act as a call to begin transcription.

Enhancer Sites & Activator Proteins

→Specific sites known as enhancer sites bind activator proteins and are then able to interact with a promoter 1000's of nucleotides downstream due to the bending of DNA. The Activator proteins work in tandem with transcription factors to aid in RNA Polymerase binding.



Eukaryotes: Post transcription regulation: 5' Cap, Poly (A) Tail, Alternative Splicing



5' CAP

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.

Poly (A) Tail

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.

Contrasting DNA & Gene Regulation in Eukaryotes & Prokaryotes
Prokaryotes organize genes via operons while eukaryotic genes are spread out…even on different chromosomes. Prokaryotes usually have 1 promoter for multiple genes. Eukaryotes usually have 1 promoter for 1 gene (that's what biologists think at the moment). Prokaryotes don't typically have introns, eukaryotic genomes are made up of many. The human genome for example is primarily made up of introns. Prokaryotes have 1 copy of DNA (haploid). Eukaryotes are diploid. Though prokaryote chromosomes can coil and may be associated with protein, eukaryotic DNA is able to become greatly condensed as chromatin. Prokaryotes have no nucleus, so translation can start once transcription has started. Eukaryotes have additional control mechanisms available since transcription and translation take place in separate locations (nucleus and cytoplasm).

Multiple Choice

Questions 1-4 refer to the following proteins and gene sequences

(A) Reverse transcriptase
(B) DNA polymerase
(C) DNA helicase
(D) Operator
(E) Promoter

1. Binds a repressor protein

D	The operator site is the portion of an operon that is responsible for binding a repressor protein

2. Binds RNA Polymerase

E	The promoter region is found at the beginning of an operon and is responsible for binding RNA Polymerase for transcription.

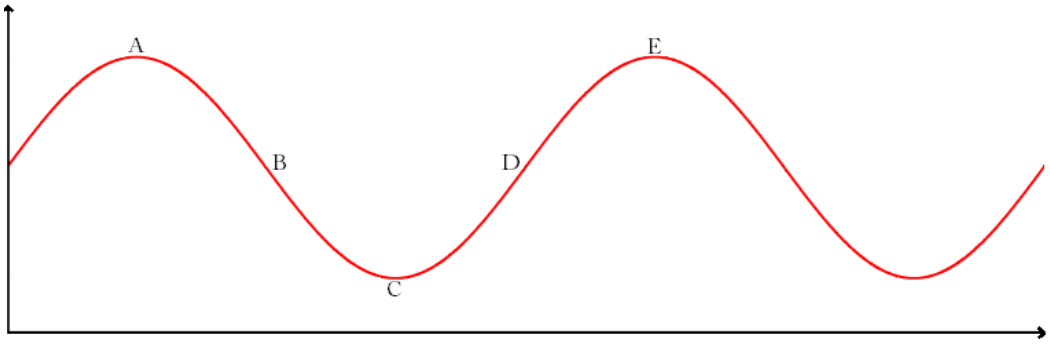
3. Responsible for synthesizing DNA from a DNA template.

B	DNA Polymerase is an enzyme (-ase) that acts on and makes polymers of DNA from a DNA template during replication during the S phase of the cell cycle.

4. Breaks hydrogen bonds during replication

C	DNA helicase is an enzyme (-ase) that acts on the DNA double helix. DNA helicase "unzips" DNA by breaking the hydrogen bonds that join complementary base pairs.

Questions 5-6 refer to the following graph of cellular activity in E. Coli.



5. If the graph represents a change in tryptophan production over time, which of the following statements is most correct? Recall that the *trp* operon is a repressible operon.

(A) The graph is following patterns typical of a negative feedback loop.
(B) The highest rate of tryptophan production occurs in the region around points A and E.
(C) Repressors become active at point C.
(D) The presence of tryptophan will result in an increased rate of tryptophan production.
(E) The presence of an operon will decrease production of tryptophan.

A	Repressible operons typically function to maintain a narrow range of product, in this case tryptophan. This graph is a classic example of a negative feedback mechanism. The highest rate of tryptophan production actually occurs at point D (also point B if negative slope is considered). The presence of tryptophan at say point A or E actually results in a decrease in the level of tryptophan.

6. If the graph were to represent the activation of *trp* operon repressors over time, which of the following would be true assuming that the actions of a repressor were immediate?

I. The highest concentration of tryptophan would be found at points A and E.
II. The highest concentration of tryptophan would be found at point C.
III. The concentration of tryptophan would be decreasing from point A to C.

(A) I only
(B) II only
(C) III only
(D) I and III only
(E) II and III only

B	The production of tryptophan would be completely out of sync with the production of repressor. As the concentration of repressor increases, the amount of tryptophan will decrease.

7. Which of the following is a true statement regarding gene regulation in prokaryotes?

(A) Nuclear DNA may be modified prior to transcription
(B) RNA is modified in the nucleus before translation
(C) Repressors may act to turn off operons in both inducible and repressible operons
(D) Genes are likely "on" when histone proteins are methylated.
(E) Genes are likely "on" when DNA is tightly condensed around histone proteins

C	Prokaryotes do not contain a nucleus and are generally thought to not contain histone proteins. Both inducible and repressible operons can work with repressor proteins. Inducible operons contain repressors that are active by default. Repressible operons contain repressors that are inactive by default.

8. Which of the following statements regarding eukaryotic splicing is correct?

I. Exons are excised before transcription.
II. Introns are excised before translation.
III. Introns are excised before leaving the nucleus.

(A) I only
(B) II only
(C) III only
(D) I and III only
(E) II and III only

E	Exons are portions of DNA that code for protein and therefore are expressed. Introns are transcribed in the nucleus, but removed by splicesomes before exiting the nucleus for translation. Introns are intervening sequences.

9. The amount of expressed mRNA present in the eukaryotic cytoplasm may be influenced by all of the following EXCEPT:

(A) the presence of a poly (A) tail
(B) the average length of introns
(C) the methylation of DNA
(D) the presence of activator proteins
(E) the acetylation of hisone proteins

B	Introns are spliced out by splicesomes before mRNA exits the nucleus. The average length of introns therefore has no impact on the amount of mRNA present for translation.

10. Which of the following best explains why a human kidney cell is different from a human pancreatic cell?

(A) Pancreatic cells contain DNA that is different from kidney cells.
(B) Pancreatic cells contain different RNA polymerases when compared to kidney cells.
(C) Pancreatic cells contain additional chromosomes when compared to kidney cells.
(D) Different ribosomes are active in pancreatic cells when compared to a kidney cell.
(E) Different sets of genes are "on" in a pancreatic cell when compared to a kidney cell.

E	Both cell types contain the same 23 pairs of chromosomes. The polymerases and ribosomes are identical in both cells. Pancreatic genes are "on" in the pancreas while genes for all other organs, tissues, etc. (including kidneys) are "off".

Free Response

1. Gene regulation is important for proper cellular function.

   A. Describe how the prokaryotic operon works. Relate operons to homeostasis by including descriptions of events that may turn genes "on" and "off."

1 point for a valid description (not just a mention of) of each of the following. (6 pt. max)
_Operon is a functional set of genes with all regulatory mechanisms _Operator function- bind repressor _Promoter function- bind RNA Polymerase OR sight of the beginning of transcription _RNA Polymerase function to create an RNA strand _Repressor bind to operator to turn "off" OR lack of bound repressor at operator site = "on" _set of genes that code for protein _terminator or termination sequence ends transcription _valid description of repressible operon like *trp* operon resulting in homeostasis _correct application of a corepressor…MUST mention that the product of the operon is the corepressor _valid description of an inducible operon like *lac* _correct application of lactose or similar acting to turn on the operon _valid description of having multiple proteins…repressors and activators

   B. Thoroughly describe 4 mechanisms of eukaryotic gene regulation.

1 point for a valid description of (not just a mention of) the following. Acceptable answers must include WHY the mechanism is important. (5 pt. max)
_5'cap- retards degradation of mRNA transcript _poly A tail- allows exit through nuclear pores _ proteins in pores allow for exit _alternate splicing of introns _specific mention that splicesomes do this _methylating DNA….must must mention that methylating turns off or that removing methyl groups turns genes on _"state" of chromatin…must mention that tightly condensed chromatin increases the likelihood that DNA genes are off of inaccessible OR that less condensed chromatin results in more accessible DNA…DNA that is "on" _acetylating histones…must mention that acetyl turns DNA on and vice versa OR that DNA is less condensed when histones are acetylated.

Free Response

2. Feedback loops and other control mechanisms are important to the homeostasis of a cell.

A. The hypothetical enzyme xyzase is able to break down the hypothetical sugar xyzose. Thoroughly describe the way in which the *xyz* operon might exhibit negative feedback regulation in order to maintain homeostasis in a bacterium. Be sure to comment on the significance of all of the functional portions of the operon and the associated proteins.

1 point for each of the following (6 pt. max)
_ the *xyz* operon is an inducible operon _the presence of xyzose will be able to turn on the *xyz* operon _xyzose is a corepressor _xyzose will bind to and change the shape of a repressor protein to turn on the gene _the repressor protein will be removed from the operator site _removal of the repressor will allow access to the promoter site by RNA Polymerase _the absence of xyzose will result in the default off state of the operon _general idea that this entire process results in increased efficiency since xyzase is only manufactured in the presence of its substrate xyzose

B. It is possible to regulate the activity of the enzyme xyzase once it has been translated into a functional protein. Explain how noncompetitive inhibition might work on the enzyme xyzase.

1 point for each of the following
_A noncompetitive inhibitor will bind at an allosteric site _Binding at an allosteric site will result in conformational change of the active site of the enzyme. _The active will no longer be complementary to the substrate, essentially turning the enzyme off. _Noncompetitive inhibition is reversible. _Contrasting competitive and noncompetitive inhibitors: must mention that competitive inhibitors bind the active site.