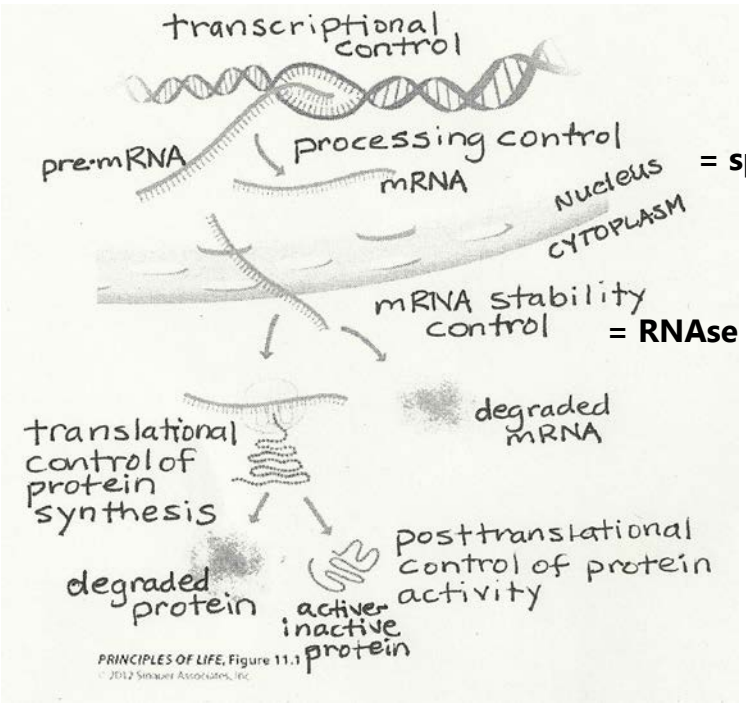
Chapter 11: Regulation of Gene Expression

Chapter Review

1. It has long been known that there is probably a genetic link for alcoholism. Researchers studying rats have begun to elucidate this link. Briefly describe the genetic mechanism found between alcoholism and rats.

Scientists have found that CREB, a protein that regulates gene expression, is a key element in the genetic propensity for alcoholism in rats. CREB, abundant in the brain, is activated by phosphorylation with the enzyme kinase A. Scientists found that alcoholic "P" rats have lower levels of CREB in their brains. When they consume alcohol, levels of CREB do not increase but levels of phosphorylated CREB do, and it is the latter that regulates gene transcription. This shows the molecular nature of a complex behavioral disease.

2. In the diagram below, label and identify the 5 potential points (arrows) for the regulation of gene expression.



3. Explain the primary difference between constitutive and inducible genes, and provide an example of each.

Constitutive genes are expressed most the time; genes coding the enzymes of glycolysis, which is an ongoing metabolic cellular process, are constitutive genes. Inducible genes are only expressed at certain times or in specific cells when their proteins are needed. Genes encoding for repair of damage to DNA by pathogens, including bacteria, are inducible genes. An example of this is recA, a key component in DNA repair.

4. Explain why viruses are not considered to be cellular organisms.

Viruses are not cells but are "acellular," as they do not carry independently out many of the processes characteristic of life, and they are dependent on living cells to reproduce. The DNA of viruses can be single-stranded.

5. Describe and explain the claim that the 4 types of viruses are distinguished by difference in their genetic material.

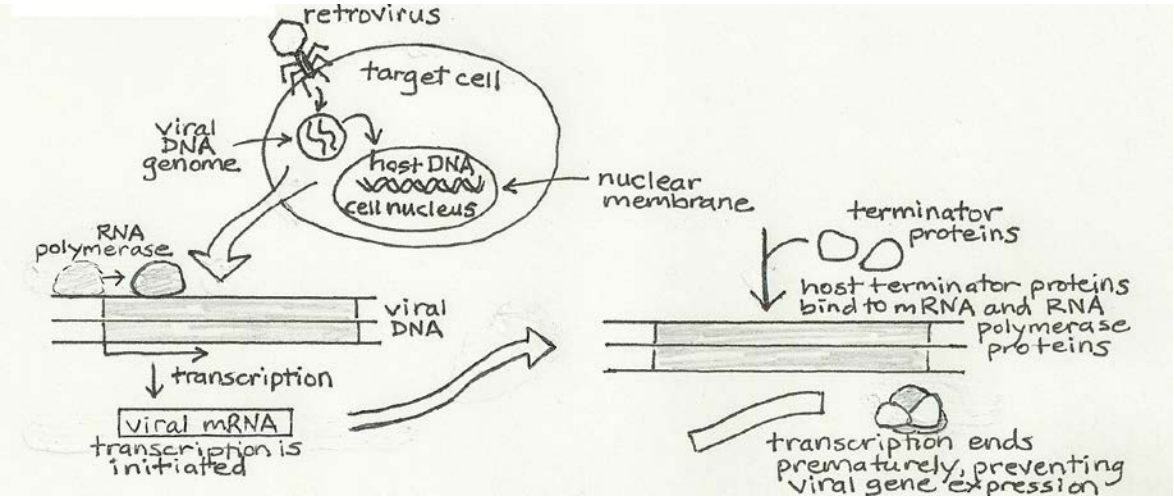
Four types of viruses are made of double-stranded DNA, single-stranded DNA, double–stranded RNA, and single-stranded RNA, the genetic material for the viruses.

6. Describe the lytic and lysogenic phases of viral reproduction.
Lytic phase - Typically, the host cell immediately begins to produce new viral particles called virions within minutes after the virus enters the cell. These virions are released as the cell breaks open or lyses.
Lysogenic phase – This is a dormant phase, which is included in some viral life cycles, where the viral genome becomes incorporated into the host cell genome and is replicated along with the host genome.

7. Explain how bacteriophages are an example of positive regulation and of postranscriptional regulatory mechanisms.

A bacteriophage exhibits positive regulation because its genome contains a regulatory promoter, which encodes proteins that inhibit the expression of the host's own genes, stimulate viral genome replication, and activate the transcription of viral genes. Expression of the host's genes are inhibited by a post-transcriptional mechanism: a virus-encoded enzyme degrades the most of the host's RNA molecules before they can be translated. Another viral enzyme digests the host's chromosome, providing nucleotides for synthesis of many copies of the viral genome.

8. Draw and label a diagram that shows how an infected cell attempts to utilize transcription terminator proteins to prevent a retrovirus from expressing itself. Include in your diagram: the nucleus, nuclear membrane, and provirus.



9. Explain how HIV counteracts the process you described in your answer to the previous question on retrovirus protection.

Shortly after infection into host cells, the HIV virus uses transcriptase to make a DNA strand that is complementary to the HIV's single-stranded RNA. As this is completed, the RNA is degraded and a second DNA strand, complementary to the first, is synthesized. The resulting double-stranded DNA becomes integrated into the host chromosome, where it resides as a provirus, sometimes dormant. Normally, host cell use negative regulatory systems as defense mechanisms to repress the expression of invading viral genes. However, HIV can counteract these negative regulation tactics with a virus-encoded protein called Tat, which binds to the viral mRNA along with associated proteins that allow RNA polymerase to transcribe the viral genome.

10. Describe the three primary parts of the *lac* operon.
Promoter – this is the DNA sequence to which RNA polymerase binds to initiate transcription. It is highly efficient, but mRNA synthesis can be shut down when the enzymes are not needed (negative regulation).

Operator – this is the region of an operon that acts as the binding site for the repressor and controls transcription of the structural genes.

Three structural genes – these genes encode the primary structures of proteins not involved in the regulation of gene expression.

11. Genetic mutations are useful in analyzing the control of gene expression. In the *lac* operon of *E. coli*, gene *i* codes for the repressor protein, *Plac* is the promoter, *o* is the operator, and *z* is the first structural gene. (+) means wild (or normal) type; (–) means mutant. Fill in the table below by writing "YES" or "NO" in box of the following table, describing the level of transcription in different genetic and environmental conditions.

Z TRANSCRIPTION LEVEL		
GENOTYPE	LACTOSE PRESENT	LACTOSE ABSENT
i– *Plac*+ *o*+ *z*+	Yes	Yes
i+ *Plac*+ *o*+ *z*–	No	No
i+ *Plac*– *o*+ *z*+	No	No
i+ *Plac*+ *o*– *z*+	Yes	Yes

12. The Trp operon is a repressible operon. Explain what is meant when the Trp operon is described as a repressible operon, and how this regulatory function is important to a bacterial cell.

A repressible operon is one that is switched off when its repressor is bound to its operator. However, Trp operons have a repressor that binds to the DNA *only* in the presence of a co-repressor. A co-repressor is a molecule that binds to the repressor, causing it to change shape and bind to the operator, thereby inhibiting transcription. It is important for a bacterial cell, as it conserves energy, allowing transcription only when necessary for the cell to function.

13. Describe two features of bacteria that make them especially useful for studying the mechanisms of gene regulation.

Bacteria are useful for studying the mechanisms of gene regulation because functionally related genes are often clustered in operons, there is only one RNA polymerase, there are a limited number of promoters and other regulatory sequences, and inscription is always initiated by the binding of RNA polymerase. This arrangement is not as complex as that found in eukaryotes.

14. Explain why transcription factors are found more commonly in eukaryotes than in prokaryotes.

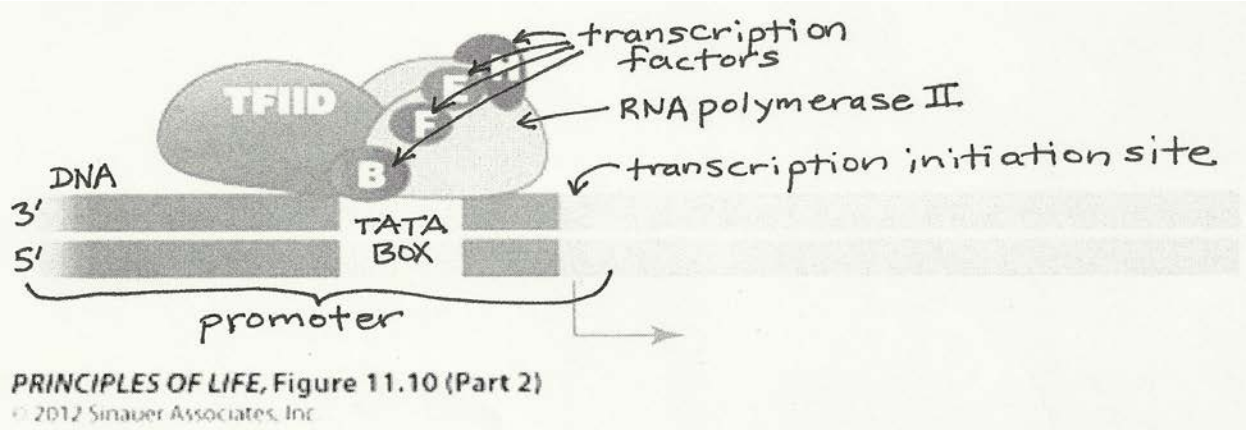
There are more transcription factors in eukaryotes than in prokaryotes because the regulation of transcription is more complex in eukaryotes. Many eukaryotic genes require several transcription factors before they are expressed, and there are several different types of regulation (instead of just one in prokaryotes). Eukaryotes also utilize more ways of initiating transcription to fine tune gene expression.

15. Describe all the necessary components that must be present before RNA polymerase II can transcribe a segment of DNA.

The first transcription factor, TFIID, binds to the promoter at the TATA box. Then another transcription factor binds. RNA polymerase II binds only after several transcription factors are bound to other proteins in the

complex (not to the DNA), so the RNA polymerase cannot initiate transcription until numerous transcription factors have become attached.

16. In the diagram below, label: DNA, TATA box, transcription initiation site, promoter, transcription factors, and RNA polymerase II.



PRINCIPLES OF LIFE, Figure 11.10 (Part 2)

17. Describe the chemical matching between a transcription factor and the DNA sequence to which it can bind.

DNA bases contain atoms that are available for hydrogen bonding but not involved in base pairing. These play an important role in the chemical interactions between an NFAT and DNA, as the atoms of these bases are exposed within the major or minor grooves of DNA and can interact via hydrogen bonding with the transcription factors. In addition, there are hydrophobic interactions between the ring in the DNA bases and some amino acid R groups in the protein.

18. When a transcription factor binds to DNA, there is an induced fit. What is an induced fit and how does it occur?

For an enzyme and its substrate, such as transcription factors and DNA, the protein undergoes a conformational change after binding begins. This is called an induced fit, which means that the shape of the enzyme is changed by binding to its substrate, and this exposes the active site of the enzyme. The binding of transcription factors leads to induced fit of other parts of the transcription-regulating complex.

19. Discuss the functional importance of coordinated gene expression.

The expression of genes can be coordinated with shared regulatory sequences that bind the same transcription factors. This type of coordination is used by organisms to respond to stress. An example is the response by plants to drought. Under environmental conditions such as drought, the plant organism must simultaneously synthesize a number of proteins whose genes are scattered throughout the genome. The synthesis of these proteins comprises the stress response. To coordinate expression, each of these genes shares a specific regulatory sequence near its promoter.

20. Epigenetic changes to DNA alter gene expression without changing DNA sequences, and these changes are passed to offspring. Describe two different ways that epigenetic changes to DNA can occur, and describe how these changes can be inherited.

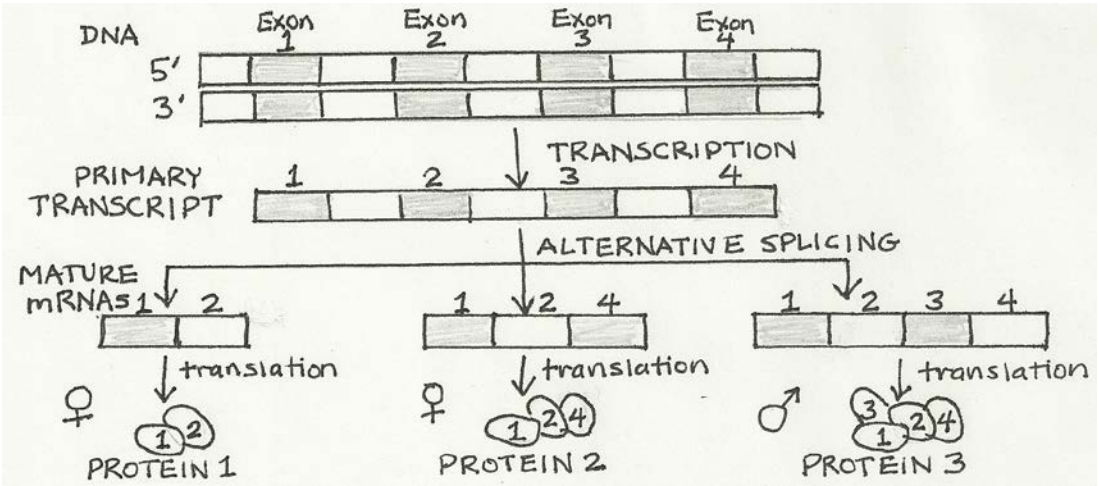
Epigenetic changes are different than mutations, because they are reversible and occur when the expression of a gene is altered without a change in the DNA sequence. One mechanism for epigenetic change is DNA methylation, which involves the addition of a methyl group in the new DNA strand, which can be passed along to offspring after mitosis or meiosis. Methylated DNA binds to specific proteins that are involved in the

repression of transcription; thus heavily methylated genes tend to be inactive (silenced). Another mechanism for epigenetic gene regulation is the alteration of chromatin structure, or chromatin remodeling. The basic unit of DNA packaging within the nucleus in eukaryotes is the nucleosome, a core of positively charged histone proteins around which the DNA is wound. Each histone protein has a positively charged "tail" of about 20 amino acids that juts out of the compact structure. The addition of acetyl groups to these amino acid tails neutralizes these charges, thus reducing the affinity of histones for DNA, loosening the compact nucleosome, and making the activation of transcription more likely. Conversely, other types of histone modifications can repress transcription. Both types of epigenetic changes can result in phenotypic changes in daughter cells.

21. Early in life, identical twins are often difficult to tell apart and behave very similarly. As they age, subtle differences begin to appear and as they reach middle age, they often have distinct differences. Explain how this can occur at a molecular level with their DNA.

Monozygotic twins share identical genomes. In the first years of their lives, their epigenomes are also virtually identical. But by age 50, when the twins have usually been living separately in different environments for many years, the patterns are very different. This indicates that the environment plays an important role in the epigenetic modification of gene expression and, therefore, in the regulation of the phenotype. Stress is an example of an environmental factor that could lead to an epigenetic change.

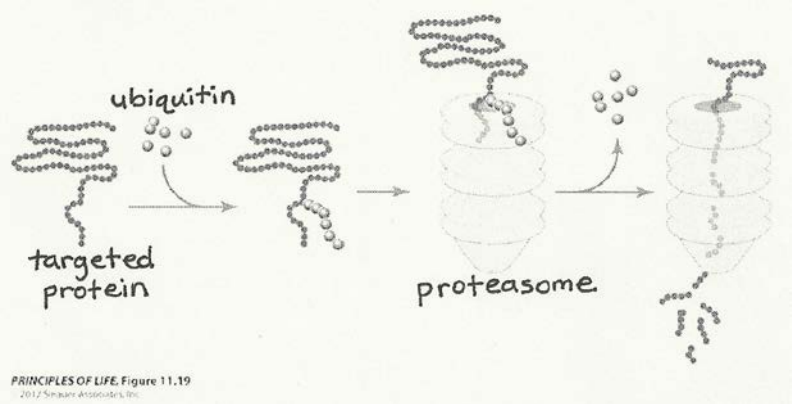
22. In *Drosophila*, sex is determined by a gene that has four exons, which we will designate 1, 2, 3, and 4. In the female embryo, splicing generates two active forms of the protein, containing exons 1 and 2, and 1, 2, and 4. However, in the male embryo, the protein contains all four exons (1, 2, 3, and 4) and is inactive. Draw a diagram similar to Figure 11.16 that represents this process.



23. Explain how humans have approximately 24,000 genes, yet have at least 85,000 mRNA's.

The fact that there are many more mRNAs than genes can be primarily attributed to alternative splicing. Recent studies show that more than 80 percent of all human genes are alternatively spliced during different patterns of expression.

24. In the figure below, label: targeted protein, ubiquitin, and proteasome.



25. Explain the role of ubiquitin in the figure in the preceding question.

Proteins that bind ubiquitin are thus targeted for degradation, as ubiquitinated proteins readily bind to proteasomes. The proteasome is a complex structure where proteins are digested by several powerful proteases. In the figure above, the protein to be targeted for breakdown is first bound to ubiquitin, and this complex is then bound to a proteasome. Ubiquitin is released and recycled, and the proteasome hydrolyzes the target protein.

26. Explain why miRNA's are considered to be a gene silencing mechanism.

Each relatively short miRNA segment is transcribed as a longer precursor that is cleaved through a series of steps to double-stranded miRNAs. A protein complex guides the miRNA to its target mRNA, where translation is inhibited and the mRNA is degraded. This inhibition of translation is, in effect, a silencing mechanism. The remarkable conservation of this mechanism in eukaryotes indicates that it is evolutionarily ancient and may be an important "fine tuning" factor in the genesis of complex organs.

27. Complete the table below for the different types of gene regulation:

	Location in cell	Molecule(s) acted on	Example (others possible)
operons	Prokaryotes-cytoplasm; eukaryotes-nucleus	DNA	lacOperon in E. coli
transcription factors	Prokaryotes-cytoplasm; eukaryotes-nucleus	DNA	tumor suppressors or oncogenes
translational	ribosomes	RNA	tobacco horn-worm moth – fertilized eggs
epigenetics	nucleus	DNA	DNA methylation
chromatin remodeling	nucleus	nucleosomes	histone acetylation, such as CREB that mediates addiction
miRNA	ribosomes	mRNA	early stage of breast cancer – high levels in blood serum
alternative splicing	nucleus	pre-mRNA	HIV
translational repressors	ribosomes	mRNA	translation of ferritin increases with higher levels of free iron ions in humans
proteasome	eukaryote cytoplasm	ubiquitin tagged cellular proteins	Breakdown of proteins targeted for degradation bound by ubiquitin

Science Practices & Inquiry

28. In an experiment, fruit flies showed unusual outgrowths on their eyes. This trait lasted for 11 - 13 generations of offspring until breeding of fruit flies resulted in normal fruit flies.

a. Could this change have occurred due to a mutation or change in the DNA sequence? Why or why not?
b. Explain how this change can occur and then result in normal fruit flies.

a. This change was not caused by mutations or changes in the DNA sequence. It was most likely caused by epigenetic changes that affected gene expression, since the normal trait reappeared many generations later. If this change had been instigated by a mutation or change in the DNA sequence, it would have been permanent, and the original eye condition would not have returned.

b. Epigenetic changes, usually by DNA methylation or histone modification, can be induced by the environment. These changes can be retained during subsequent cell divisions and can be passed along through germline cells to succeeding generations. However, since they are not permanent changes to the DNA structure, they can continue to cause phenotypic modifications without permanent changes to the genome. In this example of Drosophila with an abnormal pattern of outgrowths on the eye, the trait appears in as many as 13 subsequent generations. Eventually, the eye phenotype returns to normal because there was no change in the genotype. One mechanism for this return to normal could be the reversal of "gene silencing," i.e. the epigenetic modification in gene expression that "silenced" normal development of the eye for several generations.

Reference: http://www.time.com/time/magazine/article/0,9171,1952313,00.html#ixzz1atmFPU4N