WEEK 11

UNIT 4_5: CONTROL OF GENE EXPRESSION	**Where? How long?**	***Explanations /Questions***
Introduction All cells have similar set of genes , however in order to function properly , needed proteins must be synthesized at the proper time. All cells control or regulate the synthesis of proteins from information encoded in their DNA. The process of turning on a gene to produce RNA and protein is called **gene expression**. Control of gene expression is essential to all organisms. In prokaryotes, cells adapt to environmental changes by changing gene expression. In multicellular eukaryotes, it is critical for directing development and maintaining homeostasis. In fact, different cell types in a multicellular organism have distinct functions based on the proteins they express. All cells in a multicellular organism share a set of "housekeeping" genes that sustain the cell, but they also express a unique set of proteins that distinguish each cell type ***Expectation*** In this lesson , you will be able to learn different molecular mechanism involved in Gene regulation either in prokaryotes or Eukaryotes. ***Course outline*** Prokaryotic Regulation Eukaryotic Regulation **Learning outcomes** At the end of this unit, you should be able to: - Define what is gene regulation - Explain the different process of gene regulation in eukaryotic and prokaryotes - Compare and contrast prokaryotic and eukaryotic gene expression	10 minutes	Where is this from? Students must know that protein synthesis is complex and need to be regulated . Malfunctions in this process are d disadvantageous to the cell and can lead to the development of many diseases, including cancer. .

Activity Ahead Of Lesson: Prokaryotic Gene Regulation			To student
Purpose:	To understand different mechanisms involved in Prokaryotic Gene Regulation	30 minutes	
Over to you:	Using the laptop go to this link about prokaryotic gene regulation Note everything you do not understand.		
Activity:	Make a brief description (no more than 5 sentences) of the following processes: • A Repressible Operon • An Inducible Operon Then, look for definition of following terms:		

DEFINITION **Gene expression** is the process of turning on or off expression of gene to direct protein synthesis and produce the structures of the cell . Gene expression is subjected to regulation throughout the life span of prokaryotic and eukaryotic cells. Thus total DNA or whole genome is never expressed at any given time. **Gene regulation** is a cellular processes that control the rate and manner of gene expression. It involves interactions between genes, RNA molecules, proteins , TFs and other components. Gene regulation will determine when and where specific genes are activated and the amount of protein or RNA product produced. **PROKARYOTIC REGULATION** Prokaryotic genes are often organized into operons. An **operon** is a region of DNA that consists of one or more genes that encode the proteins needed for a specific function. The operon also includes a **promoter** and an **operator**. The **operator** is a region of the operon where regulatory proteins bind. It is located near the **promoter** and helps regulate transcription of the operon genes. There are three types of regulatory molecules that can affect the expression of operons: **Repressors** are proteins that suppress transcription of a gene in response to an external stimulus, **Activators** are proteins that increase the transcription of a gene in response to an external stimulus. **Inducers** are small molecules that either activate or repress transcription depending on the needs of the cell and the availability of substrate. Examples of operons: The proteins necessary for the utilization of lactose are encoded by the - ***lac* operon:** all of the genes needed to use lactose as an energy source are coded next to each other operon. - ***trp (tryptophan)* operon: all the genes** that codes for the components for production of tryptophan.	15 minutes	**Notes to tutor** Explain to students what is an operon

The *trp* Operon: A Repressible Operon

The *trp* operon consists of a series of genes that encode enzymes that synthesize the amino acid tryptophan .
The *E. coli* can either ingest tryptophan from the environment or synthesize it using enzymes that are encoded by five genes aligned next to each other. The genes are transcribed into a single polycistronic mRNA, which is then translated to produce all five enzymes. If tryptophan is present in the environment, then *E. coli* does not need to synthesize it and the *trp* operon is turn off.
When tryptophan is absent , the switch controlling the operon is turned on, the mRNA is transcribed, the enzyme proteins are translated, and tryptophan is synthesized.
The *trp* **operator** contains the DNA code to which the *trp* repressor protein can bind

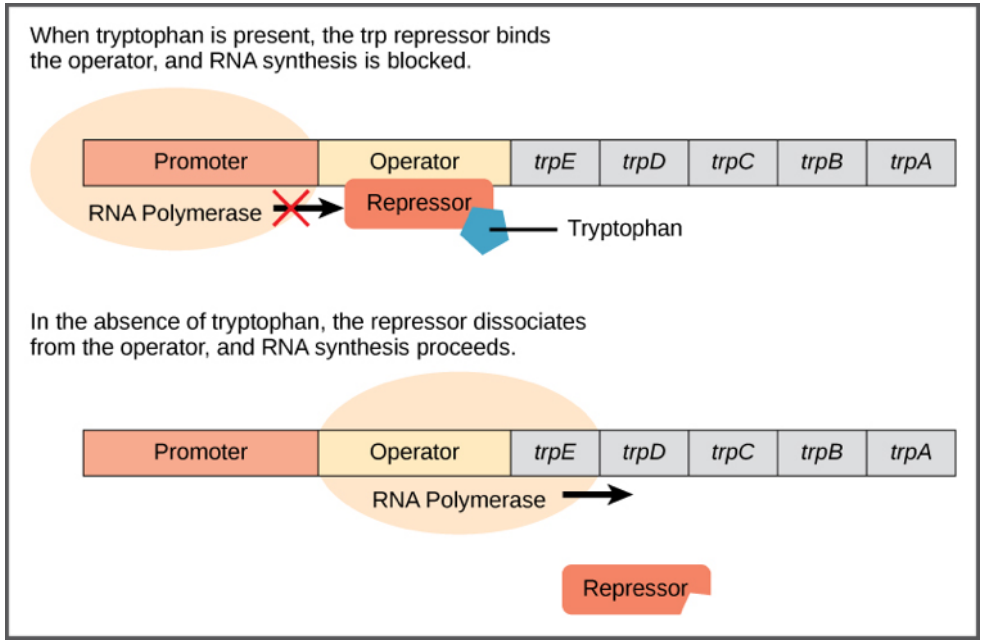

Figure 4.19 : The *trp* operon. By OpenStax, Biology. OpenStax CNX. Nov 7, 2018 , Accessed on March 7, 2019. https://cnx.org/contents/GFy_h8cu@11.6:drSgdNIj@6/Prokaryotic-Gene-Regulation

10 minutes

Notes to tutor

Polycistronic genes: Describing a type of messenger RNA that can encode more than one polypeptide separately within the same RNA molecule. Bacterial messenger RNA is generally polycistronic.

Introduce the tern of monocistronic genes (opposite of polycistronic).

When tryptophan is present in the cell, two tryptophan molecules bind to the *trp* repressor, which changes shape to bind to the *trp* operator. Binding of the tryptophan–repressor complex at the operator physically prevents the RNA polymerase from binding, and transcribing the downstream genes. When tryptophan is not present in the cell, the repressor by itself does not bind to the operator; therefore, the operon is active and tryptophan is synthesized. Because the repressor protein actively binds to the operator to keep the genes turned off, the *trp* operon is negatively regulated and the proteins that bind to the operator to silence *trp* expression are **negative regulators**.	10 minutes	**Notes to tutor** Coding sequences are DNA sequence that codes for protein Project this video to help student to understand the course about Tryptophan repressor

In Class Activity: *Trp* Operon		30 minutes	
Purpose:	To understand the prokaryotic gene regulation through *trp* operon		
Over to you:	Sit in group and answer following question		
Activity:	Suppose nature took a different approach to regulating the *trp* operon. Design a method for regulating the expression of the *trp* operon with a positive regulator instead of a negative regulator.		

THE *LAC* OPERON: AN INDUCIBLE OPERON

The *lac* operon of *E. coli* contains genes involved in lactose metabolism. It's expressed only when lactose is present and glucose is absent.

The ***lac* operon** encodes the genes necessary to acquire and process the lactose from the local environment. It consists of 3 genes :

- ***lacZ,*** which encodes the enzyme β-galactosidase (which splits lactose into glucose and galactose);
- ***lacY,*** which encodes lactose permease
- ***lacA,*** which encodes a lactose transacetylase.

In addition, there is a binding site for the **catabolite activator protein** (CAP), which affects RNA polymerase binding to the promoter.

The ***Lac I gene*** : repressor encoding gene , which can bind the operator and block transcription of the *lac* operon

Two regulators turn the operon "on" and "off" in response to lactose and glucose levels: the *lac* repressor and catabolite activator protein (CAP).

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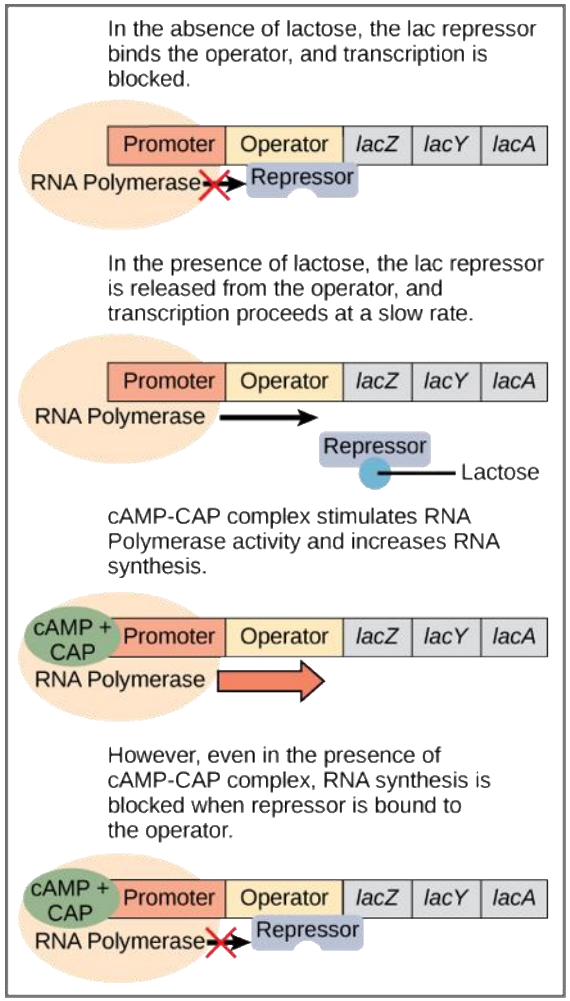


Figure 4.20 : The *lac* operon. By OpenStax, Biology. OpenStax CNX. Nov 7, 2018 , Accessed on March 7, 2019. https://cnx.org/contents/GFy_h8cu@11.6:drSgdNIj@6/Prokaryotic-Gene-Regulation

20 minutes

Notes to tutor

Inducible operons: are operon which have proteins that bind to activate or repress transcription depending on the local environment and the needs of the cell.

enzyme β-galactosidase: splits lactose into glucose and galactose.

Negative control The ***lac* repressor** • When lactose is absent the repressor is bound to the operator preventing RNA polymerase binding → no transcription. • The *lac* repressor senses lactose indirectly, through its isomer **allolactose**. **Positive Control** **CAP** activates transcription of the operon, but only when glucose levels are low. This protein binds to a site on the DNA within the promoter region and increases the rate of RNA polymerase binding; then initiate transcription. It only does this when complexed to cAMP.	10 minutes	**Notes to tutor** Project this video to help student to understand the course of lac operon

In Class Activity: *Lac* Operon	15 minutes	
Purpose: To understand the prokaryotic gene regulation through *lac* operon **Over to you:** Use a laptop and go to the following link **Activity:** Attempt the MCQ , then read associated tutorials		

Activity ahead of Lesson: Eukaryotic Gene Regulation		30 minutes	To student
Purpose:	To understand different mechanisms involved in Eukaryotic Gene Regulation		
Over to you:	Using the laptop go to these link 1 and link 2 about eukaryotic gene regulation Note everything you do not understand.		
Activity:	List different level of eukaryotic gene regulation Define Cis and trans acting elements.		

EUKARYOTIC GENE EXPRESSIONS Eukaryotes have : - Variety of cell types - All have same genome - Which genes get expressed & when - Protein have different functions: in development, cell differentiation and metabolic regulation Eukaryotic cells can regulate gene expression at many different levels.	10 minutes	**Note to tutor** Explain that Eukaryotic gene expression is more complex than prokaryotic gene expression because the processes of transcription and translation are physically separated. Refers to the unit of DNA structure and Genome organization

EUKARYOTIC CHROMOSOME STRUCTURE

Chromatin accessibility (EPIGENETIC control)

- The human genome encodes over 20,000 genes
- Eukaryotic DNA is packaged into chromatin.
- Chromatin structure is directly related to the control of gene expression.
- Chromatin structure begins with the organization of the DNA into nucleosomes.
- Nucleosomes may block RNA polymerase II from gaining access to promoters.

Explain epigenetics

Methylation is the addition of $–CH_3$

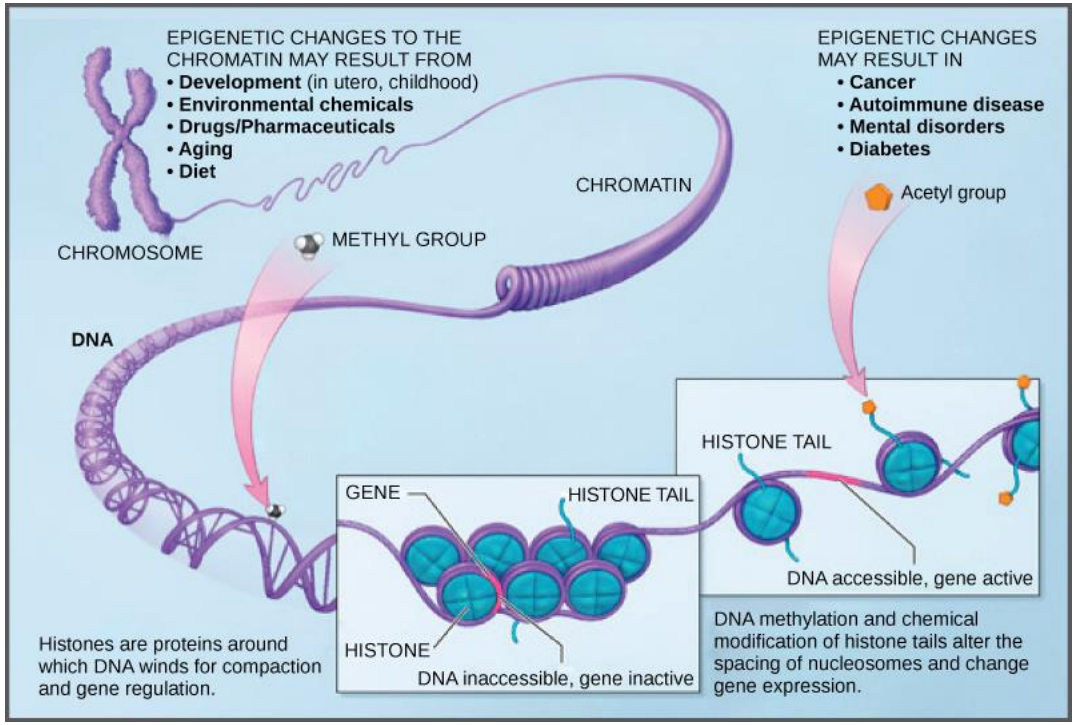
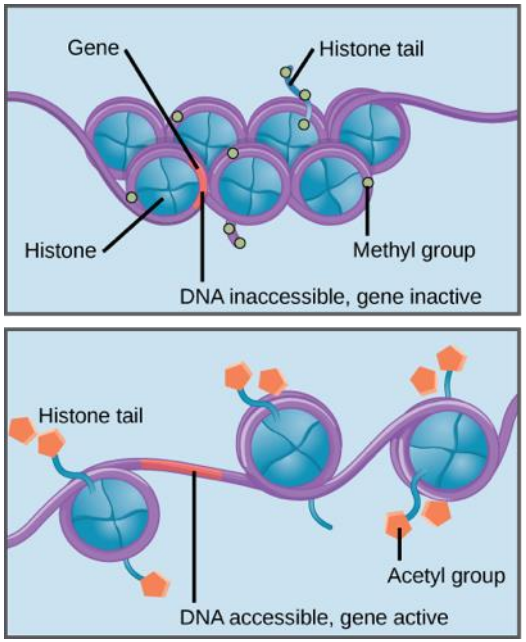


Figure 4.21 : Histone proteins and DNA nucleotides can be modified chemically. Modifications affect nucleosome spacing and gene expression. By OpenStax Biology 2nd Edition, Biology 2e. OpenStax CNX. Feb 13, 2019. Accessed March 10, 2019.https://cnx.org/contents/jVCgr5SL@15.3:5cz8bfb2@10/16-3-Eukaryotic-Epigenetic-Gene-Regulation

Methylation of DNA or histone proteins is associated with the control of gene expression

- Clusters of methylated cytosine nucleotides bind to a protein that prevents activators from binding to DNA.
- Methylated histone proteins are associated with inactive regions of chromatin.
- Methylation results in a human disease called fragile X syndrome; FMR-1 gene is silenced by methylation.



Methylation of DNA and histones causes nucleosomes to pack tightly together. Transcription factors cannot bind the DNA, and genes are not expressed.

Histone acetylation results in loose packing of nucleo-somes. Transcription factors can bind the DNA and genes are expressed.

Figure 4.22 : Methylation of DNA and Histone acetylation. By OpenStax Biology 2nd Edition, Biology 2e. OpenStax CNX. Feb 13, 2019. Accessed March 10, 2019.https://cnx.org/contents/jVCgr5SL@15.3:5cz8bfb2@10/16-3-Eukaryotic-Epigenetic-Gene-Regulation

20 minutes

For tutors

Explain the fragile X syndrome. For more information you can go to the following link.

EUKARYOTIC TRANSCRIPTION REGULATION Controlling the expression of eukaryotic genes requires **transcription factors (TF)**. - **General transcription factors** are required for transcription initiation and also required for proper binding of RNA polymerase to the DNA - **Specific transcription factors** increase transcription in certain cells or in response to signals General TFS bind to the **promoter** region of the gene. RNA polymerase II then binds to the promoter to begin transcription at the **start site (+1)**. **Enhancers** are DNA sequences to which specific transcription factors (**activators**) bind to increase the rate of transcription. **Transcriptional repressors** is a DNA binding protein that can bind to promoter or enhancer regions and block transcription. **Eukaryotic Post-transcriptional Gene Regulation** Gene expression can be controlled after transcription, with mechanisms such as: ➨ **RNA interference** (RNAi) involves the use of small RNA molecules - The enzyme **Dicer** chops double stranded RNA into small pieces of RNA - **micro-RNAs** bind to complementary RNA to prevent translation - **small interfering RNAs** degrade particular mRNAs before translation	20 minutes	**Note to tutor** **For tutors** **Explain** - Dicer - Micro-RNA - Small-interfering RNA

➨ **Alternative splicing**

- Introns are spliced out of pre-mRNAs to produce the mature mRNA that is translated.
- **Alternative splicing** recognizes different splice sites in different tissue types.
- The mature mRNAs in each tissue possess different exons, resulting in different polypeptide products from the same gene.

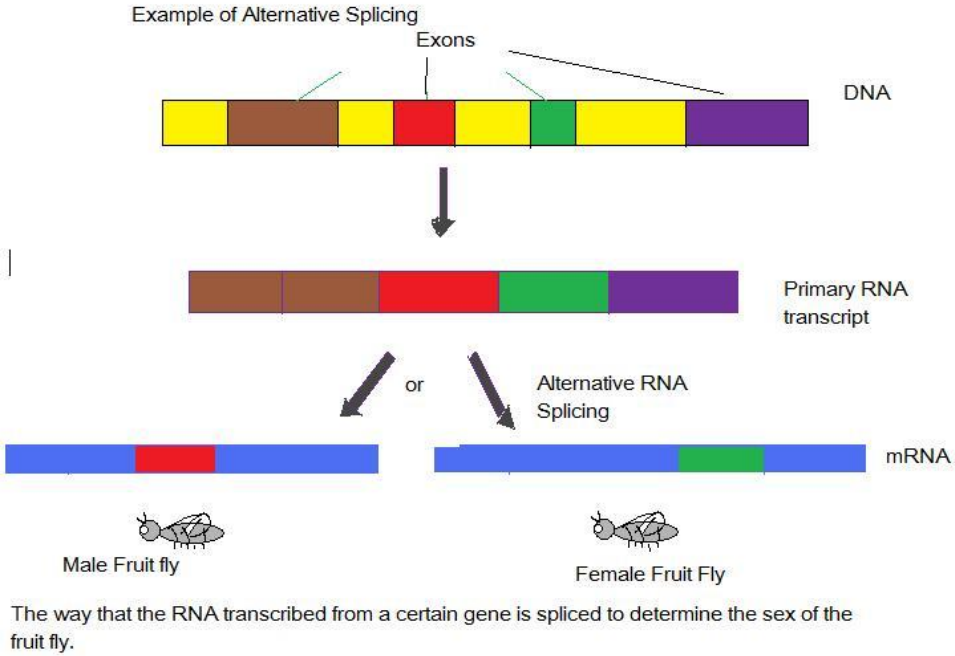


Figure 4.23 :RNA alternative splicing in drosophila. By Mei Huang (2008). Accessed March 10, 2019. https://commons.wikimedia.org/wiki/File:RNA_alternative_splicing.jpg

20 minutes

➨ **RNA editing**

- **RNA editing** creates mature mRNA that are not truly encoded by the genome.
- For example : apolipoprotein B exists in 2 isoforms
  one isoform is produced by editing the mRNA to create a stop codon, this RNA editing is tissue-specific
- Mature mRNA molecules have various half-lives depending on the gene and the location (tissue) of expression.
- The amount of polypeptide produced from a particular gene can be influenced by the half-life of the mRNA molecules.

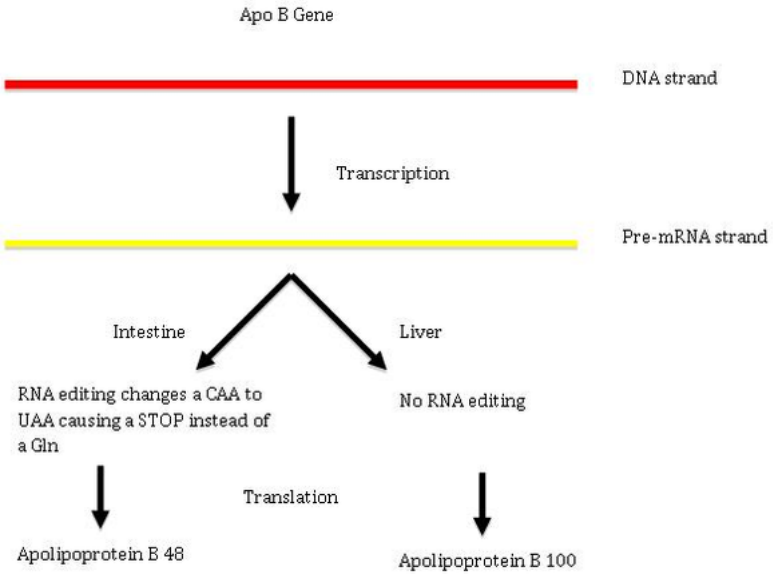


Figure 4.24 :RNA editing in Apolipoprotein B. By **Sryuu** (2009). Accessed March 10, 2019. https://en.wikipedia.org/wiki/RNA_editing#/media/File:Apobgene.PNG

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20 minutes

➨ **Initiation of translation regulation**

In order for translation to begin, a protein called eukaryotic initiation factor-2 (eIF-2) must bind to a part of the ribosome called the small subunit. Binding of eIF-2 is controlled by phosphorylation, or addition of a phosphate group to the protein.
When eIF-2 is phosphorylated, it is turned "off" then it undergoes a shape change and can no longer play its role in initiation, so translation cannot begin.
When eIF-2 is not phosphorylated, in contrast, it is "on" and can carry out its role in initiation, allowing translation to proceed.

➨ **Proteins can be regulated after translation**

- **Phosphorylation**

A phosphate group is attached to a protein.
The effect of phosphorylation varies from protein to protein: some are activated by phosphorylation, while others are deactivated, and others yet simply change their behavior (interacting with a different partner, or going to a different part of the cell).

➨ **Protein Degradation**

- Proteins are produced and degraded continually in the cell.
- Proteins to be degraded are tagged with **ubiquitin**.
- Degradation of proteins marked with ubiquitin occurs at the **proteasome**.

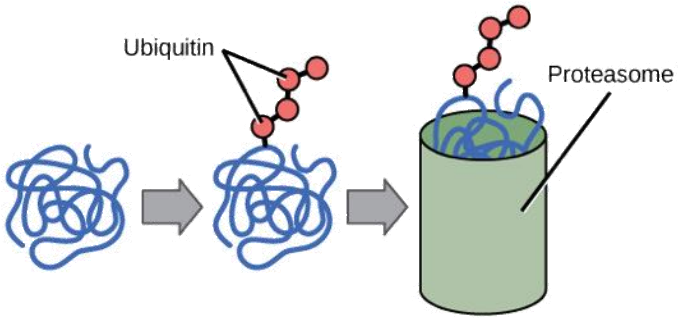

Figure 4.25: Proteins with ubiquitin tags are marked for degradation within the proteasome. By OpenStax, Biology. OpenStax CNX. Nov 7, 2018. Accessed March 10, 2019. https://cnx.org/contents/GFy_h8cu@11.6:8ZPIxfRy@5/Eukaryotic-Translational-and-Post-translational-Gene-Regulation

20 minutes

In Class Activity: Eukaryotic Regulation			
Purpose:	To understand the eukaryotic gene regulation	30 minutes	
Over to you:	Sit in groups and discuss about the questions below.		
Activity:	1. A mutation within the promoter region can alter transcription of a gene. Describe how this can happen. 2. What could happen if a cell had too much of an activating transcription factor present? 3. In cancer cells, alteration to epigenetic modifications turns off genes that are normally expressed. Hypothetically, how could you reverse this process to turn these genes back on? 4. Describe how RNA Binding Proteins can prevent miRNAs from degrading an RNA molecule. (for this question go to the internet and look for RNA binding Protein activity).		

Laboratory Practicals : Laboratory Report Review 1			
Purpose:	This is a to provide feedback of all laboratory report done from week 8 up to now	120 minutes	To tutors : Make sure that students have submitted their laboratory report on time. Report must have been reviewed then feedback will be provided during this time.
Over to you:	Lab tutors will be available to discuss specific questions about students report, general areas of confusion and offer advice offer general advice to the lab experiments.		
Activity	A global correction of the report will be organized as a special lecture and will be focused on the observed errors repeatedly present in submitted reports.		

Anticipated time required for Unit 4.5 activities :

Theory : 2h all activities + 2h self-learning

Practical : 2h