# CYTOLOGY

Premed 2018 - JU

◉ Sheet ○ Slides

Number

Molecular 17

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❖ In this sheet we will talk about **Regulation of transcription in eukaryotes**.

Regulation in eukaryotes is more complex than in prokaryotes because eukaryotic cells have multiple amounts of cis-acting elements and trans-acting factors (Transcriptional regulatory proteins).

## ❖Transcription in eukaryotic cells is controlled by:

**1. Cis-acting elements:** Sequences that include promoters, enhancers, proximal promoter elements (PPEs) and silencers.
**2. Transcriptional regulatory proteins** for example: Activators and Repressors.

**3. Chromatin Remodeling:** Changing the Structure of chromatin by changing the Interactions between DNA and histones.

(NOTE: Histones are NOT fixed molecules).

**4. DNA Modification**
Ex: Methylation of Cytosine.

**5. Non-coding RNA molecules.**

***Remember***: Methylation of Adenine ⟶ in bacteria.

*When do bacteria methylate Adenine?

In DNA repair

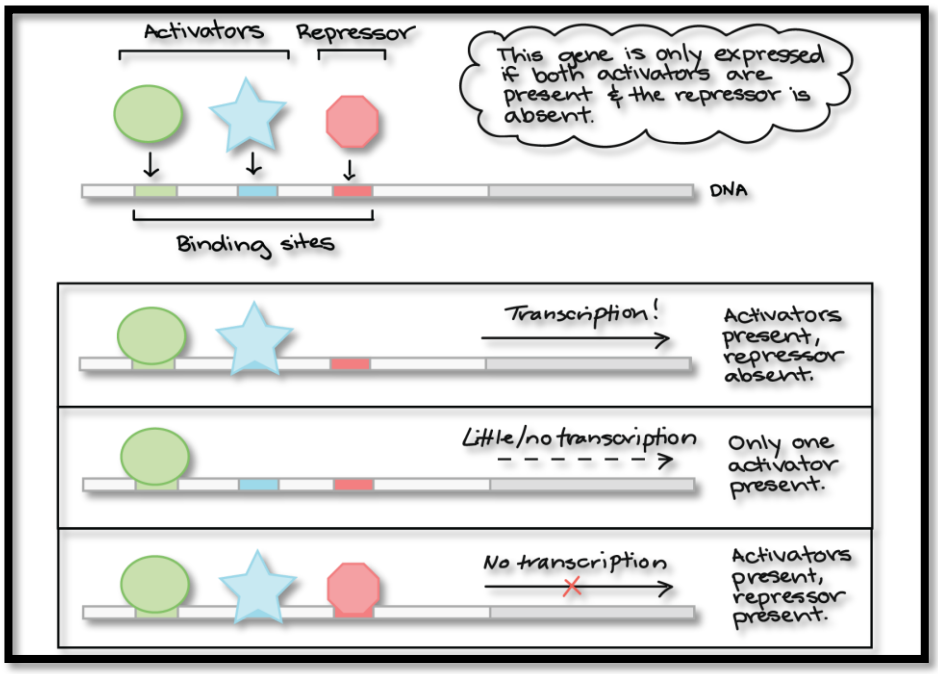
❖There are binding sites on DNA for activators and repressors and we can say that transcription has different levels according to the presence of these trans-acting factors on promoter region. So In **Transcription, there is always a gradient.**

⇩

✓ Lot of transcription: Activators bound to promoter region (**NO** repressor bound).

✓ Little/no transcription: Only one activator bound to promoter region (**NO** repressor bound).

✓ No transcription: If one or more repressor bound **even though the activators are bound too.**




## ❖ Now let's discuss more about how eukaryotes are controlled:

**1. Cis-acting elements** were discussed in previous sheets,but **Remember** that if we transfer them from one certain location to another, it will **NOT** function at all.

**How do transcriptional factors regulate gene expression?**

TFs cause **epigenetic/ epigenomic** changes in DNA. (>>**epi**: above or in addition to).

**Epigenetic** indicates genetic alterations in gene expression **WITHOUT** a change in DNA sequence. It is a higher level of regulation because this regulation doesn't happen by changing the base sequence of DNA.

What does this statement mean "**alterations in gene expression WITHOUT a change in DNA sequence?"**

It means that when we say ' genetic mutation', we are talking about a change in the sequence of DNA (ex: Changing A to T or to G whatever), but in this situation, there is a change in the shape of DNA and this affects gene expression by:

✓ Changing the chromatin packaging.

✓ Chemical modification of DNA.

✓ Chemical modification of histones.

>>If we chemically modify histones, the chromatin packaging will change as well.

Also we have a term which is **' domain',** what does it mean?

**Protein's domain:** A three-dimensional structure that is part of protein's structure. It forms **INDEPENDENTLY** of the rest of the protein and usually has a function.

Ex: Steroid hormone receptors.

What do we mean by **INDEPENDENTLY**?

If we cut one of the domains, the other will still function. Also if we add another domain, the function and the shape of other domains will not change.

➢**The mechanism of steroid hormone receptor:**

✓ Steroids which are small molecules,like testosterone,progestrone and cortisone that produced by cholesterol can diffuse through plasma membrane without carriers or channels because they are lipophilic .

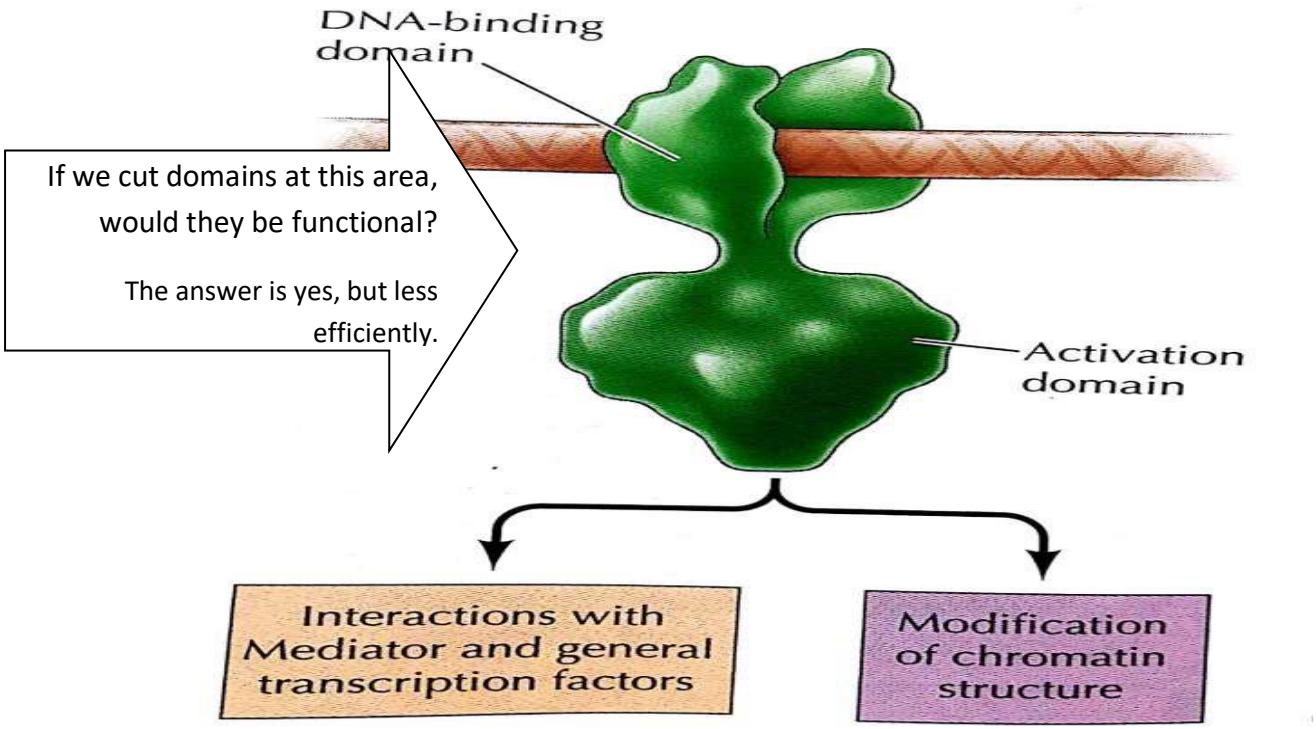
✓ Steroids bind to their receptors at ligand-binding domain ,then they can go inside the nucelus.

✓ After they enter the nucleus, they bind with DNA at specific sequence.

✓ Once they bind to DNA,the domain which is called "Activation domain" binds to activators and repressors.

And when we look to one of these receptors, we will see these three domains :

| Domain | Function |
|---|---|
| 1. Ligand-binding domain | Binds to steroid hormone |
| 2. DNA-binding domain | Binds to DNA and directs Activation domain specifically. |
| 3. Activation domain | Stimulates transcription by interacting with general transcription factors, facilitating the assembly of a transcribtion complex on the promoter. Also it modifies the chromatin structure. |




If we cut the linkage above, activation domain will still be functioning, but not specifically because it will work in different regions of DNA and they will not affect the particular gene.

**mediator: Co- activator binds with activator from one side and with pre-initiation complex from the other side.

## 2. Transcriptional regulatory proteins

**As we know, regulatory proteins are activators and repressors. Now we will talk about repressors.**

>>Repressors may have:

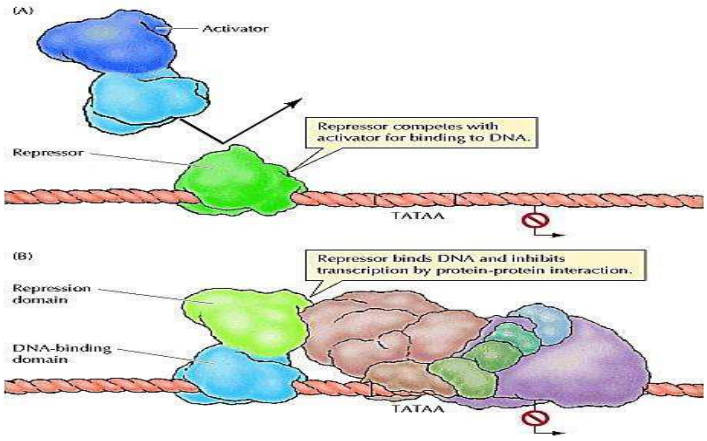
**Both DNA-binding domain and repressor domain.

**Only DNA –binding domain.

**What do repressors do?**

When they bind to DNA, they prevent activators from binding as well as they interact with RNA polymerase or general TFs to prevent them from moving forward. So, they inhibit transcription.

Additional note: It is not essential for domains to be formed by the same polypeptide.




## 3. Chromatin Remodeling

We know that chromatin is formed of DNA+Histones. According to the packaging of chromatin, we will able to find:

➢**Heterochromatin**: Chromatin that is really packed and DNA is actually condensed (DNA is not accessible to TFs).

Why DNA is not accessible to TFs?

In this case, TFs go to DNA and they try to find the sequence where they usually bind with. This sequence is hidden within the structure, so TFs will not see it.

➢**Euchromatin**: Loose chromatin, DNA sequence is exposed and can be seen by activators, so they can bind to them starting transcription.

And according to chromatin packaging, you can have:

❖ **Actively transcribed genes in euchromatin.**

❖**Inactive genes in Heterochromatin.**

And this is how we have cells specific transcription of certain genes. Because in neurons, the genes that they need are presented in euchromatin structure. Also the genes that they don't need, they packed them in Heterochromatin structure.

Liver cells have another active genes that exist in euchromatin and inactive genes that exist in heterochromatin and the active genes in liver cells are **NOT** the same active ones in neurons.

So it depends on the cell's needs.

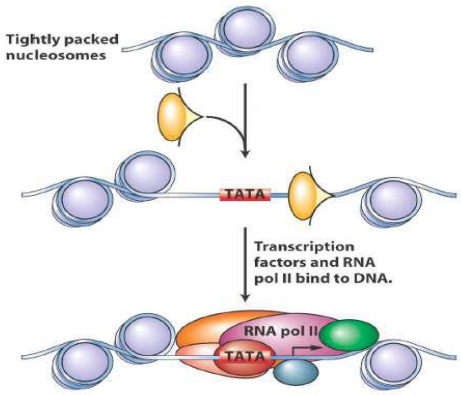
**How can we change chromatin from euchromatin to heterochromatin and vice versa?**

Well,we have **" Chromatin Remodeling Factors".**

**Chromatin Remodeling Factors**: Proteins that change the position of nucleosomes to facilitate the binding of TFs. Also they can be associated with transcriptional activators and repressors.

The mechanism:

Chromatin Remodeling Factors bind to DNA at a certain sequence. (This sequence may be the promoter sequence or even PPE sequence) ⟶
Reposition of nucleosomes ⟶
This reposition makes the sequence of DNA exposed, allowing TFs to bind with.
Also, they can alter the nucleosome's strucure as well as they can remove histones from DNA.
In other words,these factors loosing chromatin and releasing DNA to start transcription.




## 4. DNA Modification

We can alter the structure of chromosme by two ways:
1.Changing compactness of the chromatin through chemical modification of histones.
2.Binding of non codinf RNAs to DNA.
Chemical modifications of histones can be by acetylation,methylation and phsphorylation.
The effect is dependent on sites of modification:

✓ It depends on which histone is modified( Remember: The histone protein core is an octamer (two molecules of histones H2A, H2B, H3, and H4). NOT all of them get acetylated).

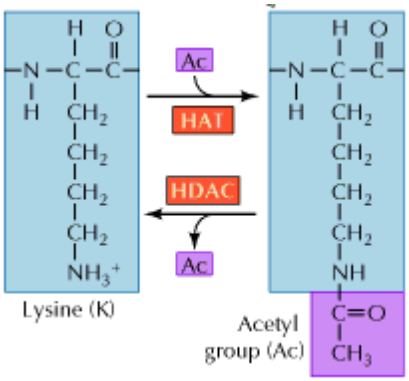
✓ Also it depends on which lysine is modified.

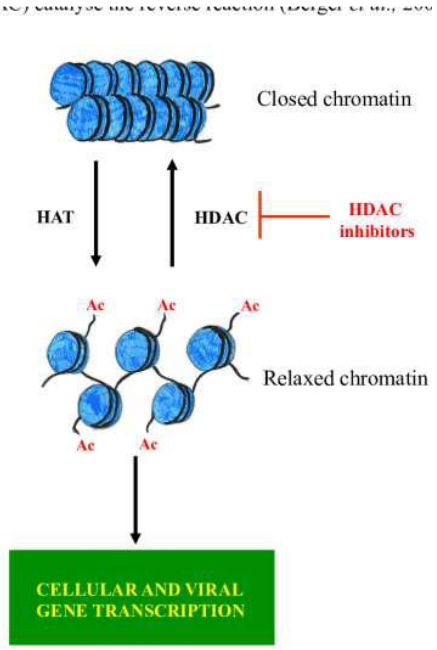
>>Activation domain acetylates histone's tails.

Histone Acetylation:Adding the acetyl group for histones.

Mainly, Lysine can be acetylated. Activators bind to DNA at specific sequence and the activation domain acetylates histones,loosen the DNA and making the interaction between DNA and hisones weaker, so DNA sequence becomes exposed for TFs.

Remember: Histones are positively charged proteins since they are made of positively charged amino acids (Arginine and Lysine).

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**Two strands of DNA are walking down the street.**
**One says to the other:**
**"Do these genes make me look fat?"**

❖ **Let's talk about Enzymatic Association.**

When we talk about activators and repressors, we have to know that they have enzymatic activity. Activator has Acetyltransferase activity.On the other hand, repressor has the opposite enzymatic activity which is Deacetylase.

➢What is the difference between the above enzymes?

Acetyltransferase catalyses the acetylation of histones while Deacetylase removes acetyl groups, converting lysine into +ve amino acid and strengthening the linkage between histones and DNA, so DNA becomes really packed and this prevents transcription.

>>There is an example of TFs and how they function:

TFIID which is the first one that binds to the promoter. It has acetyltransferase activity, it exposes the DNA sequence and allows to RNA polymerase to bind, activating transcription.

## 5. Non-coding RNA molecules.

**How do non coding RNAs function?**

The non coding RNA has a sequence that is complementary to a certain gene(Just like a probe). It will bind to it and attracts proteins to bind too at this region. These proteins can acetylate, deacytelate,phosphorylate, whatever. So these non coding RNA molecules are to activate and inactivate transcription. It depends on which protein they bind with.

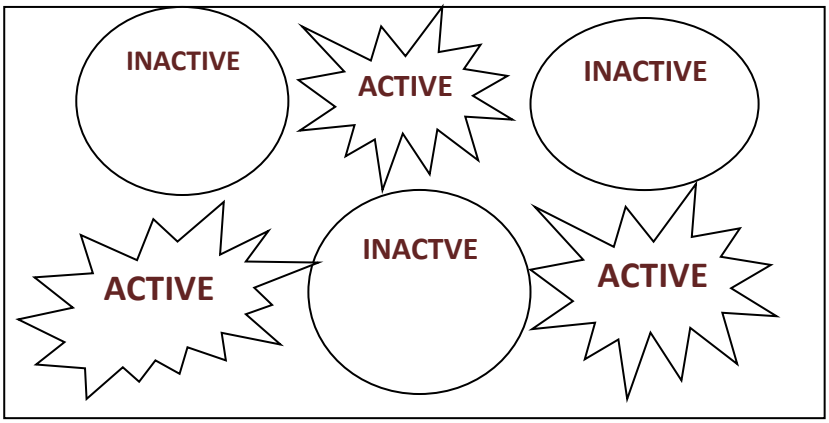
*Ex: X Chromosome inactivation*

Males have one X chromosome while females have two. This means that females express doubled number of genes and will have doubled number of proteins,but this is un fair! Females and males must have the same amount of genes.

So that, in females, one of X chromosomes becomes inactive and it is random, some of these cells will have this X chromosome is inactivated,other cells will have the other X chromosome is inactivated like 50% chance for each X chromosome.

It can be like a mosaic. If you look at a certain tissue like liver for example and you look to the cells, you will see a combination of cells includes groups that have this X chromosome is active and the other one is inactive.

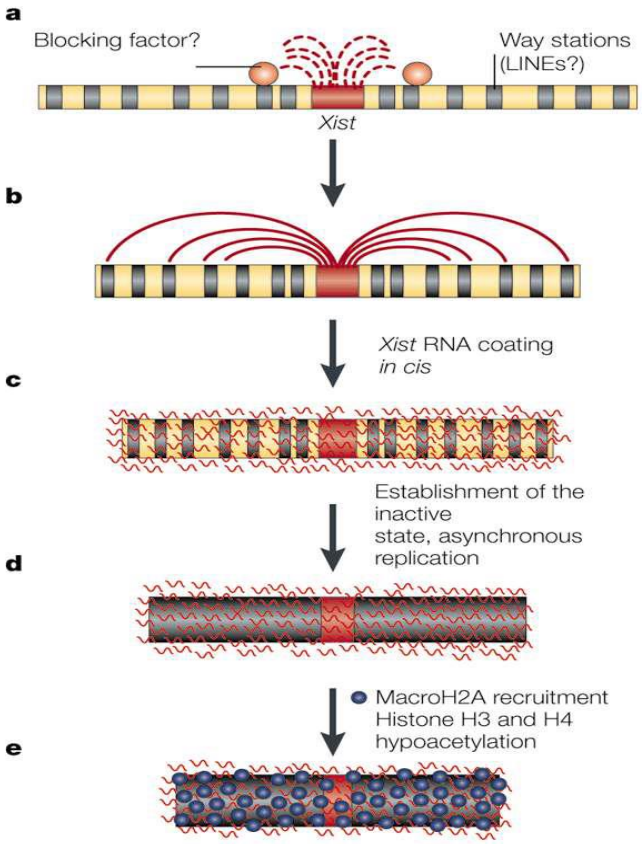
It is a normal situation.




### How does the inactivation of X chromosome take place?

There is a gene called **" Xist"** loacated on X chromosome that it will become inactive and this gene produces a long non-coding RNA that coats X chromosome,attracting different proteins(Recruitment of a protein complex)

leading to methylate Histone 3 and the chromosome will **shrink**. It leads to chromosomal condensation(**Bar Body**).




➢Let's say that there is a mutation on X chromosome, and the X chromosome that has the detective gene is inactivated, what does it mean? It means that females will escape the disease.

➢On the other hand, let's say that the X chromosome that has the good gene is inactivated even though the female is heterozygous, she will have the disease because the bad copy is expressed.
Remember that we are talking about mosaic system, basically it depends on the poportion or percentage of cells that have this X chromosome active or not.

➢If there is a mutation in (Xist gene) on the transcriptional form of X chromosome or deletion for it, it will be a disease because there will not be RNA to coat X chromosome,making it inactive.

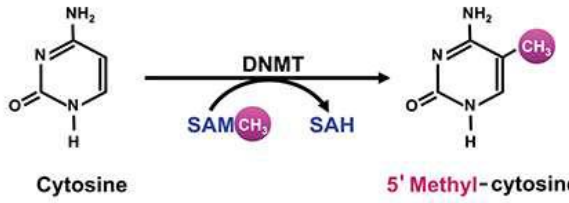
So, the two X chromosomes will be active,producing double amount of proteins this is an abnormal condition(disease).

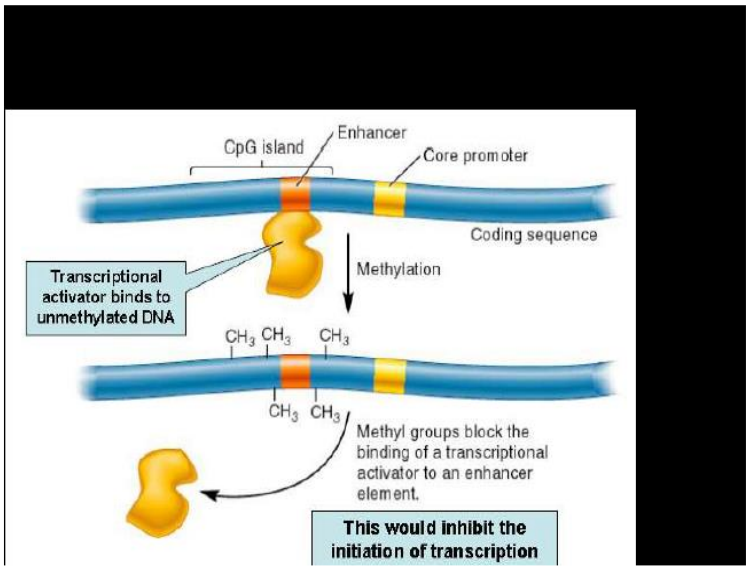
## DNA Methylation

Cytosine would become methylated and this happens in regions that are rich in Cs and Gs. The idea here is they are called **" CPG Islands"**. So these isalands are areas that are fulled with a lot of Cs followed by Gs and they are connected with phosphodiester bonds. These Cs become methylated in promoter region,then the genes become inactivated. DNA methylation reduces gene transcription by blocking the activators from binding to DNA,inducing heterochromatin formation.

In order to activate the genes, it can be done just by removing the methyl groups.

CPG Islands exist within enhancers or in promoter regions as well.




❖**Genetic Imprinting** is regulated by methylation of cytosine.This regulation is really important, it determines which genes should be expressed maternal or paternal.

In cetain situations, paternal genes must be expressed not the maternal genes. So once the paternal genes must be expressed, the maternal genes are inactivated.If the maternal genes expressed instead of paternal genes there

will be a disease even though the gene produces normal proteins. Also if both of maternal and paternal genes expressed there will be a disease too.


Genetic imprinting is reversible. Cells can methylate cytosine, demethylate cytosine, acetate histones and deacetate histones.

## YOUR DNA IS NOT YOUR DESTINY

Lifestyle would affect other generations,the external environment,the way you live,what you eat and how you behave can affect the DNA and the activity of genes. You can change gene expression by working well, reading,etc.
So, if there is a mutation, it doesn't mean that there is a disease because you can modify your DNA.
Ex: If you look to twins brothers with the same exact DNA sequence,but it does not mean that thay will have the same fate.
Also if we look to two twin sisters, one of them can develop breast cancer and the other one not, even though they have the same mutation.


## ❖Short Quiz

**Q1) Deacetylation of histones has which of the following effects?**

a.Uncoiling of histone structure,preventing it from being accessed by transcriptional machinery.
b. Uncoiling of histone structure,allowing it to be accessed by transcriptional machinery.
c.Coiling of histone structure,preventing it from being accessed by transcriptional machinery.
d.Coiling of histone structure,allowing it to be accessed by transcriptional machinery.

**Q2) Control of gene expression by preventing a gene from being expressed:**

a.transcription

b.translation

c.repression

d.replication

**Q3)What changes occur in the chromosome to make it inactive?**

a.acetylation

b.glycosylation

c.methylation

d.phosphorylation.

**Q4)Gene imprinting involves:**

a.phosphorylation

b.oxidation

c.DNA methylation

d.glycosylation

**Q5)Acetylation of histones increases transcription of the gene due to:**

a.Increase in the DNA-histone interaction.

b.Increased in blending which is recognized by RNA polymerase.

c.Because it loosens the DNA-histone complex thus making it accessible to RNA polymerase.

d. Acetyl groups that are recognizable by RNA polymerase.

**Q6) Histones have an abundance of which of the following amino acids:**

a. Arginine and Glycine.

b. Glycine and Glutamine.

c. Arginine and Lysine.

d.Arginine and Glutamine.

Answers:

1.c 2.c 3.c 4.c 5.c 6.c

سَتكُونُ الشَّدَائِدُ التّي تَعتَرِي طَرِيقَك اليَوم تِلك الفُصول الشَّيّقَة فِي قِصّتِك التِّي سَتَروِيهَا غدًا.