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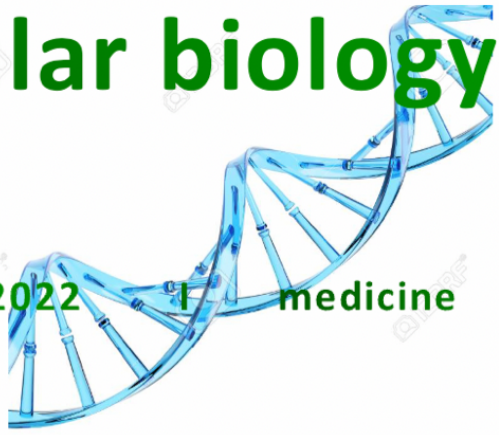
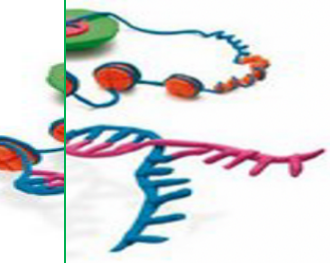
Molecular biology sheet





Doctor 2022 | medicine | MU

DOCTOR

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Gene Expression Regulation

Note that every single word in the original slides is included, however, the arrangement is different.

In today's lecture we are going to talk about regulation of gene expression.

Revision form last lectures:

- **What is the meaning of central dogma? It means a group of reactions occurs for expressing genes (which means producing a sequence of amino acids from DNA)**
- **Here, when we say "gene expression regulation" we mean regulation of two process :**
  1) **Transcription: which means producing mRNA from templet DNA (from specific one or more genes.**
  2) **Translation (protein synthesis): which means releasing mRNA from nucleus after being processed for synthesizing protein.**
- **What are the posttranscription modification processes that occur in mRNA?**

1) **capping 2) poly A tail (polyadenylation) and 3) Splicing: removing introns.**

- **What is the meaning of alternative splicing? That means not all exons (after splicing introns) will join each other, so every gene can produce multiple proteins because there are many options (possibilities) for joining exons.**
- **Regulation of transcription: means the control of transcription initiation; so, it includes control of transcription factors, enhancers (a non- promoter DNA sequence which interacts with some proteins [transcription factors] then join the RNA polymerase to initiate transcription) and silencers.**
- **Controlling gene expression is often accomplished by controlling transcription initiation. This process occurs by: Regulatory proteins bind to DNA to either (block or stimulate) transcription, depending on how they interact with RNA polymerase. When it makes stimulation, it means increasing the rate of transcription which is followed by increasing protein synthesis. When it makes inhibition or blocking: it means decreasing or completely shutting down of transcription (absence of transcription) which is followed by decreasing thetranslation to produce a little or no protein completely.**
- **Why does this regulation occur?**

1. **Prokaryotic organisms regulate gene expression in response to their environment.**

   So, thesurrounding environment makes changes then prokaryote cells respond to these changes by regulation. Such as some nutrients like lactose, increase the rate of transcription of genes which catabolize this nutrient so when the cell does not need enzymes which catabolize this food it will inhibit or block the rate of transcription and inhibit the protein synthesis.

   Shortly, in prokaryotic cells the surrounding environment says if there is an increase or a decrease in the rate of transcription.

2. **Eukaryotic cells regulate gene expression to maintain homeostasis in the organism.**

   So, regulation occur under the effect of one purpose; maintaining homeostasis, which is the maintenance of the internal environment stable. So, any disturbances will result in losinghomeostasis. Therefore, there should be gene expression regulation to keep the rate of transcription.

Regulatory Proteins:

- **The regulatory proteins are binding to specific DNA sequences then they interact with RNA polymerase to regulate gene expression.**
- **They gain access to the bases of DNA at the major groove by possessing DNA-binding motifs (models).**
- **DNA- binding motifs are regions of regulatory proteins which bind to DNA**

  1- Helix-turn-helix motif 2- Homeodomain motif

  3- Zinc finger motif 4- Leucine zipper motif

- **Shortly, regulatory proteins bind specific sequence of DNA in major groove in operators and it**

  makes interaction between this sequence and protein so it regulates gene expression.

Regulation of Gene Expression in Prokaryotes:

- **Control of transcription initiation can be:**
  1. **Positive control – increases transcription when activators bind to DNA**
  2. **Negative control – reduces transcription when repressors bind to DNA regulatory regions (operators).**

  So, the type of protein factor which will bind DNA will be either activator or repressor (inhibitor). Operator: is the first point of promoter of each gene.

- **Types of genes:**
  1. **Monocistronic: single gene producing single protein.**
  2. **Polycistronic: a single mRNA will be produced, which will be translated on ribosomes in the form of a single polypeptide chain, then it will be cleaved by proteases to give different types of proteins separated from each other. Note that polycistronic genes arecalled operons (those operons are a group of genes that usually participate in the same metabolic pathway with is related to one reagent like lac operon which is responsible for catabolizing lactose in case of bacteria). For example, if we have three enzymes that are represented by three genes (which means polycistronic) it will make one mRNA that includes the three genes then it will produce a single polypeptide chain which will be cleaved by proteases.**

     Shortly, collective genes in one metabolic pathway are called operons (which is under the effect of internal factors so operons don't work all the time).

- **Prokaryotic cells often respond to their environment by changes in gene expression.**
- **Genes involved in the same metabolic pathway are organized in operons.**
- **Some operons are induced when the metabolic pathway is needed and some operons are repressed when the metabolic pathway is no longer needed.**

Examples of Operons:

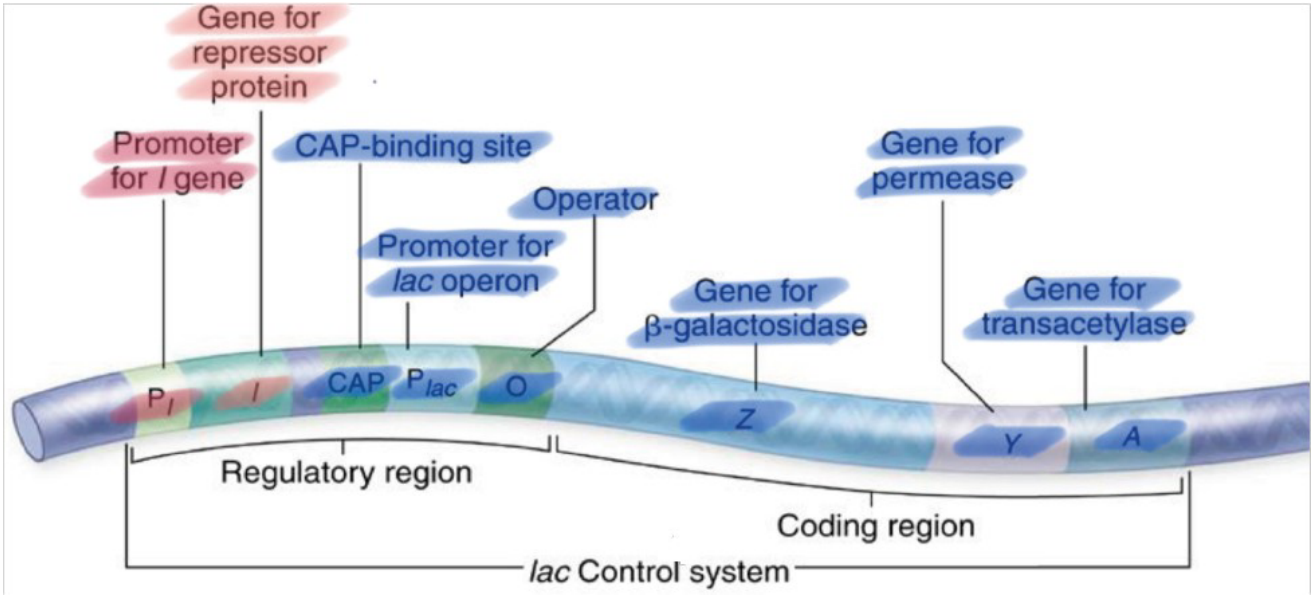
1) **Lac operon (lactose operon):**

   It involves the group of enzymes which are responsible for catabolizing lactose to produce energy. Bacteria use lactose to produce energy on in cases of absence in glucose; because glucose is easier to catabolize than lactose.

   Explanation, if glucose exists in the surrounding environment of bacteria it will use it as a source of energy and there will be no need for lactose. If the glucose is absent, bacteria will use glucose as a source of energy. So, as we said before: in prokaryotic cells the surrounding environments control gene expression (if glucose is present, lac operons will be repressed, negatively regulated) and the cell won't need the three enzymes to catabolize lactose. If glucose is absent, lac operons will be activated (so, the cell will increase the rate of transcription of lac operon to produce these three enzymes).

- **The lac operon contains genes for the use of lactose as an energy source. (it includes genes encoding for three enzymes):**

a. lacZ: encodes β-galactosidase which cleaves lactose into glucose and galactose
b. lacY: encodes lactose permease to transport lactose into the cell
c. lacA: encodes galactoside O- acetyltransferase (transacetylase or transacylase) which plays a role in cell detoxification (which means removing any harmful things from

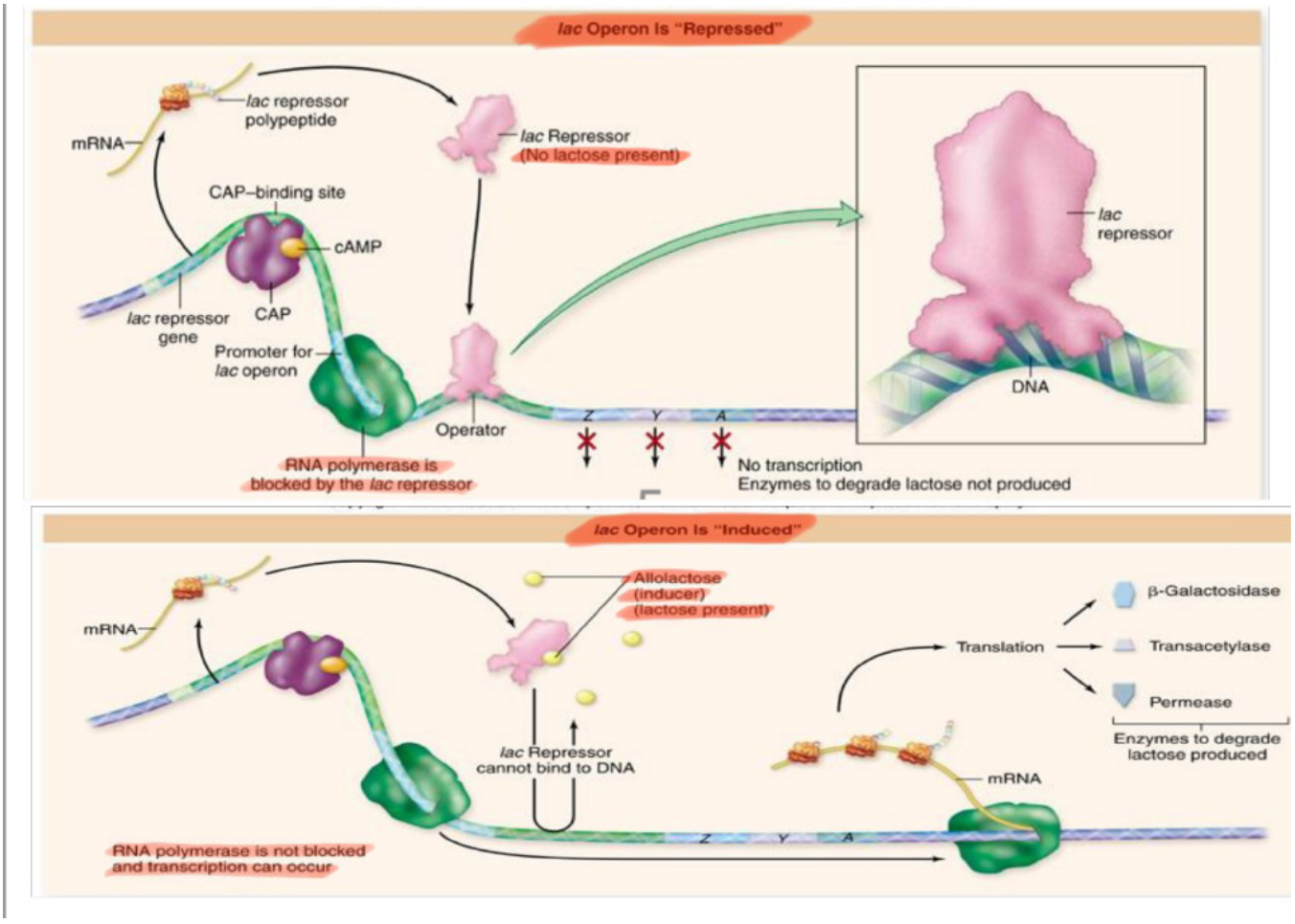


catabolites that are produced by cells reactions). At first, lactose passes from outside the bacteria into the cell by permease, then it is cleaved into glucose and galactose to produce energy by β-galactosidase, finally, detoxification occur by acetyltransferase.

- Regulatory regions of the operon include the CAP (Catabolite activator protein) binding site, promoter, and the operator. Moreover, in the regulatory region there is gene I (constitutive gene) that produces repressor protein which is used in case of presence of glucose.
- A summary for lac operon transcription:
  - ¾ If glucose is present: the gene I produces repressor protein to stop transcription of lac operon. How? By binding to operator (which is the site for binding RNA polymerase to initiate the transcription of lac operon) so when repressor binds it will prevent RNA polymerase from binding. So, there is no transcription of lac operon and no induction ofit.
  - ¾ If glucose is absent: there will be an inducer molecule (allolactose) which will bind repressor and makes on it conformational changes to prevent it from binding to operator.So, RNA polymerase finds an access to operator and binds it for initiating transcription of lac operon. So, there is an induction of lac operon. (allolactose is a part of lactose in surrounding environment converted to another form).

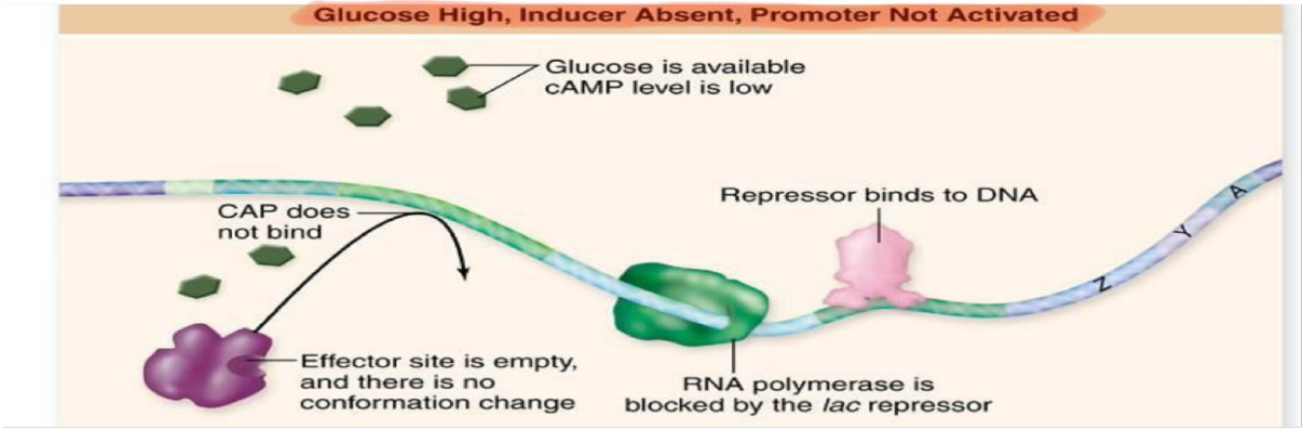
- The lac operon is negatively regulated by a repressor protein:
  - ¾ lac repressor binds to the operator to block transcription.

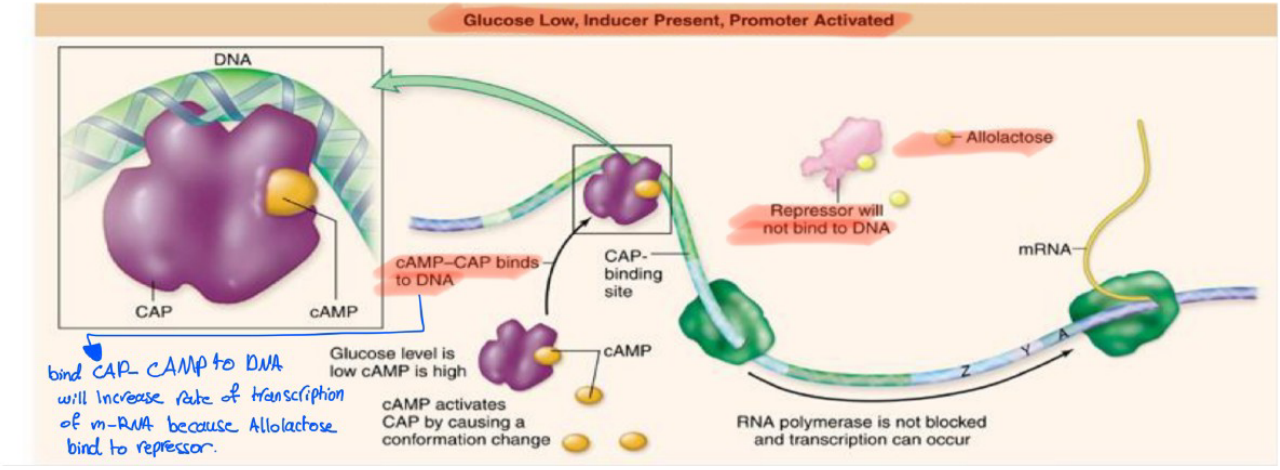
- ¾ **In the presence of lactose, an inducer molecule binds to the repressor protein.**
- ¾ **Repressor can no longer bind to operator.**
- ¾ **Transcription proceeds**



- In the presence of both glucose and lactose, bacterial cells prefer to use glucose.
- Glucose prevents induction of the lac operon: binding of CAP – cAMP complex to the CAP binding site is required for induction of the lac operon.
- High glucose levels cause low cAMP levels.
- High glucose levels cause low cAMP so, no induction
- Low glucose levels cause high cAMP (which binds to CAP). So, there is an induction of lac operon.
- Summary: Lac operon is active only in time, when the activator CAP- cAMP complex is attached to promotor (no glucose) and when is not present repressor on operator (lactose present).

2)

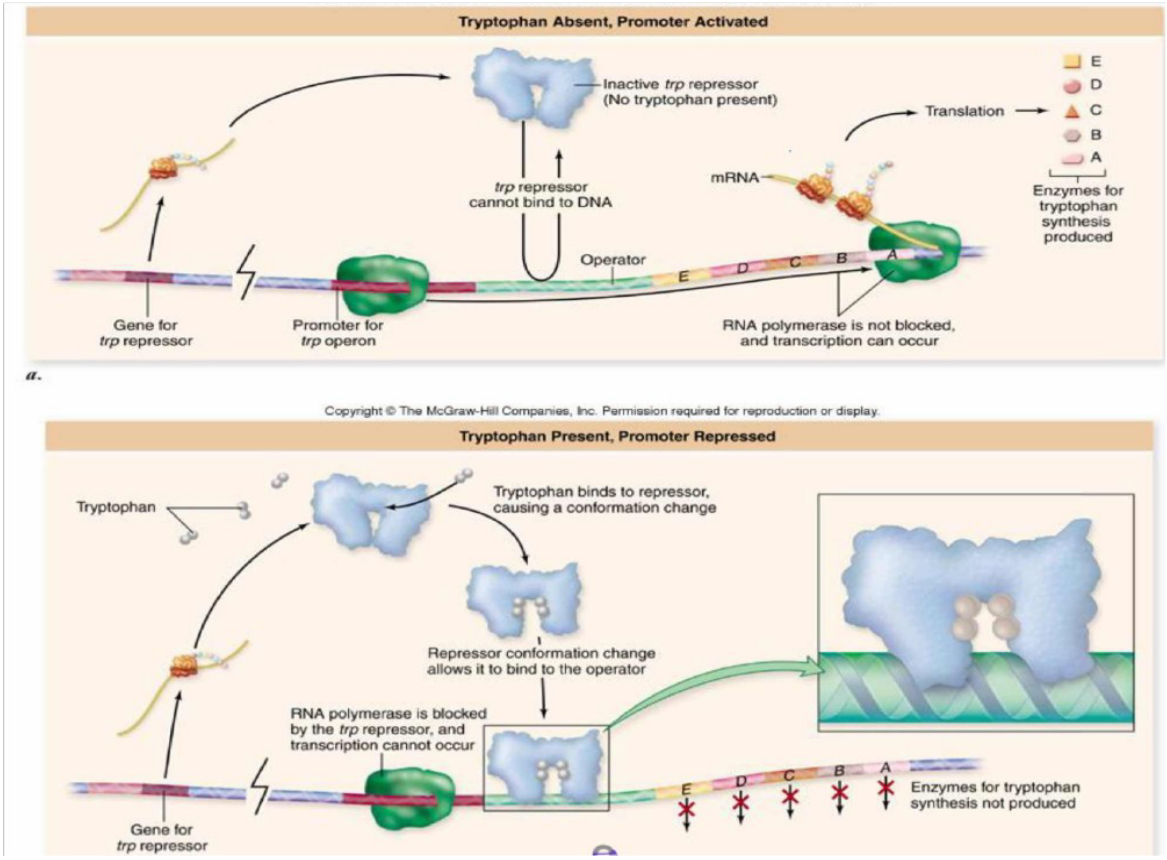




trp operon (tryptophan operon):

- The trp operon encodes genes for the biosynthesis of tryptophan. Tryptophan is an essential amion acid. So, regulation of transcription of trp operon depends on the amount of tryptophan that is found in the cell.
- The operon is not expressed when the cell contains sufficient amounts of tryptophan, no need for inducting tryptophan operon.
- The operon is expressed when levels of tryptophan are low.

- The trp operon is negatively regulated by the trp repressor protein. The exact same idea of lac operon.
- Trp repressor binds to the operator to prevent RNA polymerase to bind operator to block transcription.
- Binding of repressor to the operator requires a corepressor which is tryptophan (corepressor makes conformational changes in repressor to activate it).
- Low levels of tryptophan prevent the repressor from binding to the operator (in case of lac operon: allolactose will prevent repressor from binding to operator for making transcription. While in case of trp operon; the presence of repressor alone without tryptophan makes inactivation, but if there is sufficient amount of tryptophan the repressor will be activated by corepressor and bind to operator to prevent transcription of trp operon. So, prevent production of excess tryptophan).



This process occur in prokaryotic cell so in Eukaryotic cell the process will be more complex

Regulation of Gene Expression in Eukaryotes:

There are 6 levels of regulation of gene expression in eukaryotic cells (check the figure below before reading the following)

- Controlling the expression of eukaryotic genes requires transcription factors. Which transcription factors can recognize promoter element?

1) TFIID- TBP: recognizes TATA box.
2) TFIID- TAF: recognizes non- TATA box elements; CAAT and GC boxes.

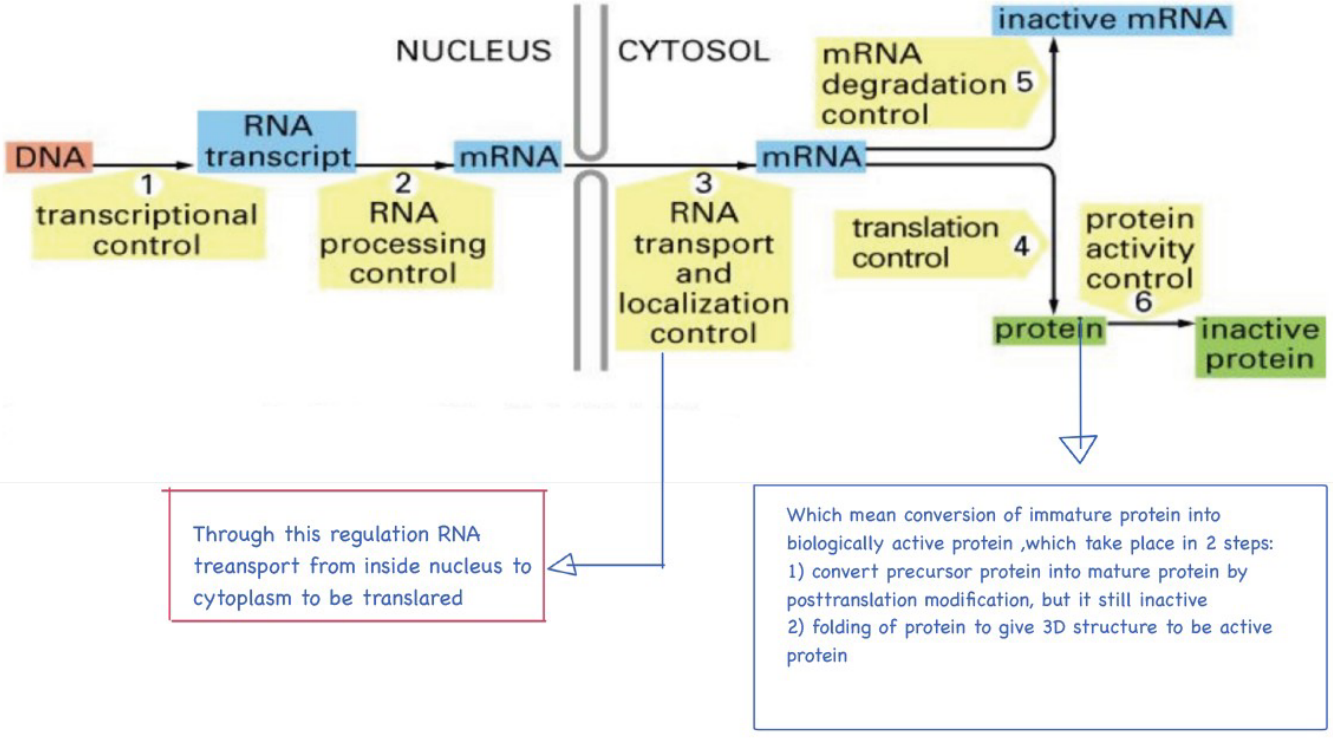
- There are two types of transcription factors:

1) General transcription factors are required for transcription initiation (for proper binding of RNA polymerase to the DNA). They are 7 types we have talked about them in transcription lecture (TFIID, TFIIB… etc.). Their function: recognition of promoter elements, recruitment of other factors, recruitment of RNA polymerase II, clearance and opening of promoter with helicase activity

2) Specific transcription factors increase transcription in certain cells or in response to signals

which tells the cell: produce more of specific gene

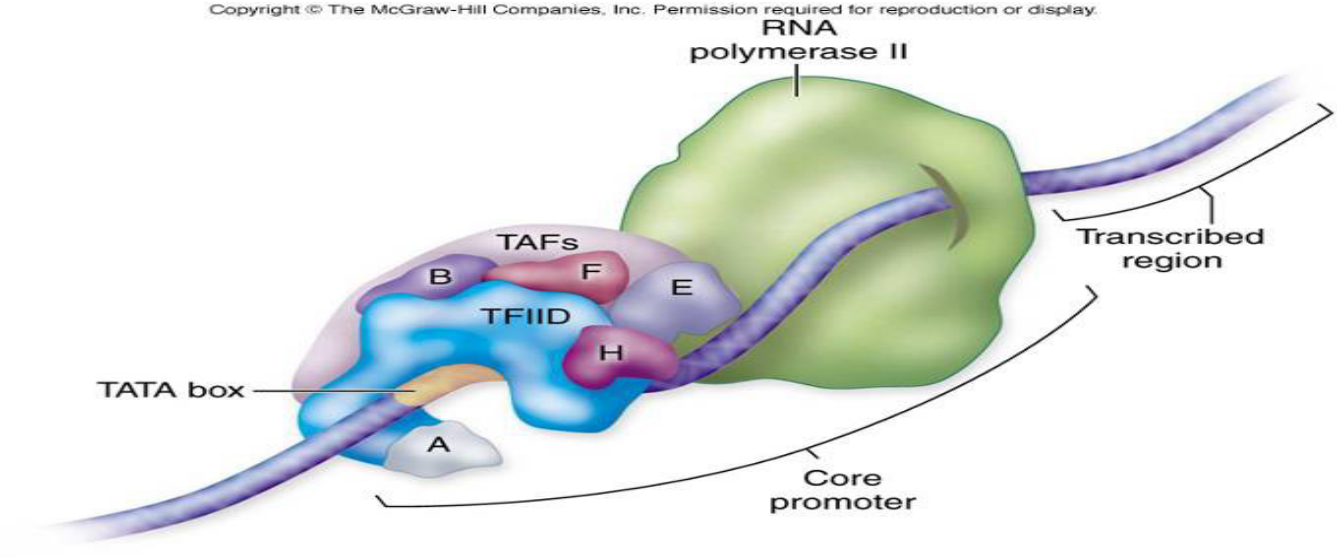
Regulation of gene expression in eukaryotes

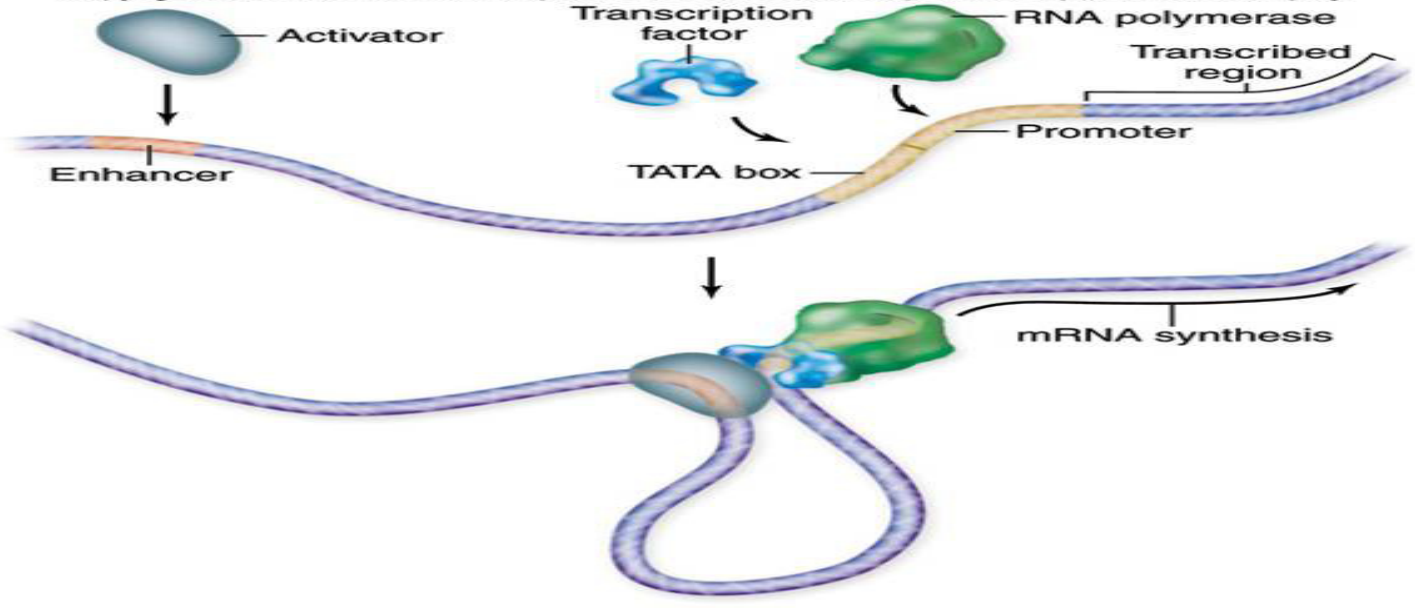


- General transcription factors bind to the promoter region of the gene.
- RNA polymerase II then binds to the promoter to begin transcription at the start site (+1). Aswe said before, transcription factors guide RNA polymerase to bind promoter because it is a blind enzyme. We have already learned that termination in eukrayotic cells is still unknown

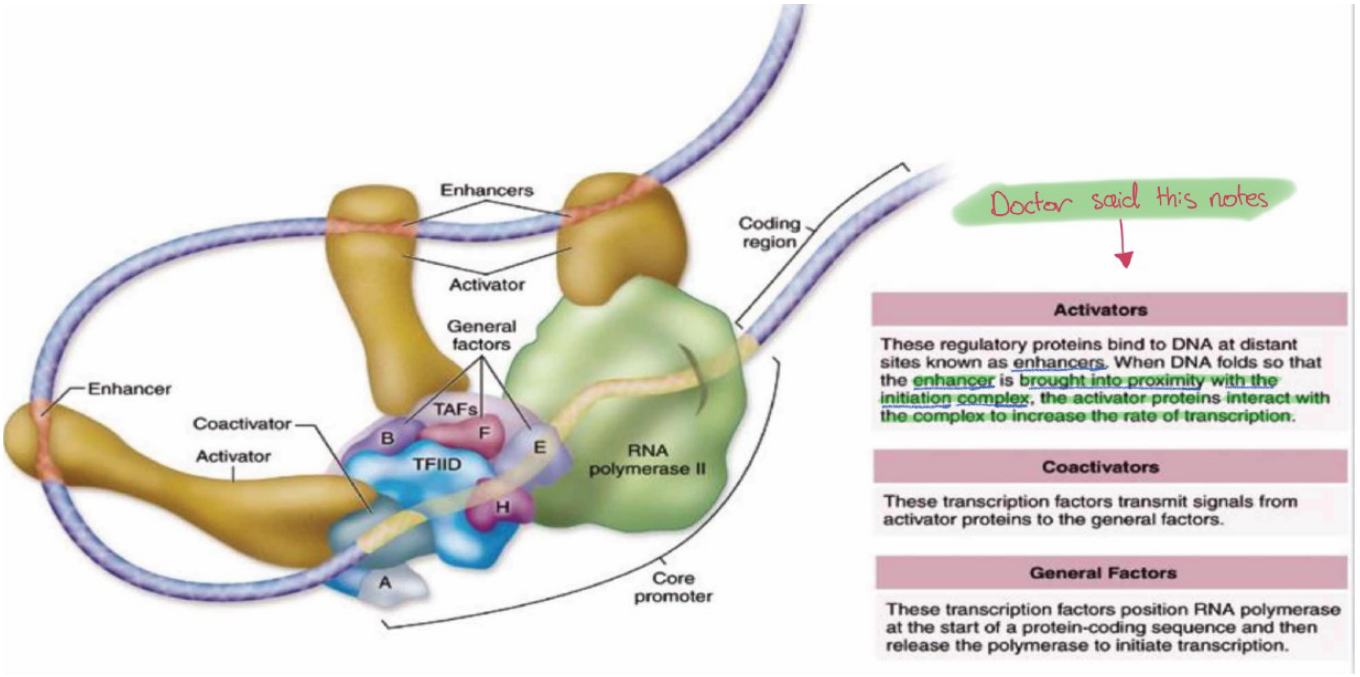
but in prokaryotic cells it is either an Rho depedent or Rho independent termination.

- **Enhancers are DNA sequences to which specific transcription factors (activators) bind to increase the rate of transcription. Now, instead of enhancers/ silencers we will use activators/ repressors (which control rate of transcription). They are non-promoter DNA sequence, as a result of binding these proteins to RNA polymerase they can increase the rate of transcription or decrease it, so some of them act as enhancers (activators) and others as silencers (repressors).**





- **<u>Coactivators and mediators</u> are also required for the function of transcription factors.**
- **Coactivators and mediators bind to transcription factors and bind to other parts of the transcription apparatus**



Eukaryotic Chromosome Structure:

- **Eukaryotic DNA is packaged into chromatin which 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.**

Explanation, chromatin also acts in regulation of gene expression. There are 2 types of chromatin:

a. **Euchromatin: which is the active DNA**
b. **Heterochromatin: which is the inactive DNA (in this form of DNA, it is very hard for polymerases (DNA or RNA) to enter and begin their work. So, when we talk about increasingthe rate of trnascription we go toward euchromatin and for decreasing it we go toward heterochromatin.**

In replication lecture, DNA after synthesis will be organized on histone molecule (octameric molecule
that consists of 8 subunits [4 types of H2A, H2B, H3 and H4. 2 molecules of each]). So, DNA is wrapped around it to be kept in the nucleus. The reason behind this wrapping is to decrease the DNA size so it can fit inside the nucleus when it is packaged; as its original length is 2 meters.

Chromatin Structure:

- **In eukaryotes, the DNA is organized into nucleosomes: about 200 bp of DNA wrapped around a protein core.**
- **The protein core consists of 8 histone proteins.**
- **Histones are basic (i.e. alkaline): they contain positively charged amino acids that bind to the negative charges on the DNA (backbone phosphate groups).**
- **DNA tightly wrapped around histones is inaccessible to RNA polymerase.**

- **Thus, one important event in preparing a gene for transcription is "chromatin remodeling": sliding the nucleosomes along the DNA to expose the promoter region.**

The effects of chromatin structure changes on genes expression

1. **DNase I hypersensitivity:**

- **DNase I hypersensitive sites: more open chromatin configuration site, upstream of the transcription start site. When DNA hypersensitivity occurs, a breakdown of some hydrogen bonds between nucleotides in both strands of DNA occurs, this allows RNA polymerase to access into promoter. Note that polymerases (DNA and RNA) can't work on double strands.So, these strands has to be opened by DNase then helicase breaks more hydrogen bonds.**

2. **Histone modification:**

- **Addition of methyl groups to the histone protein tails**
- **Addition of acetyl groups to histone proteins by acetylase enzyme to convert heterochromatin to euchromatin to be accessbile for:**
  1) **RNA polymerase in case of transcription**
  2) **DNA polymerase in case of replication**

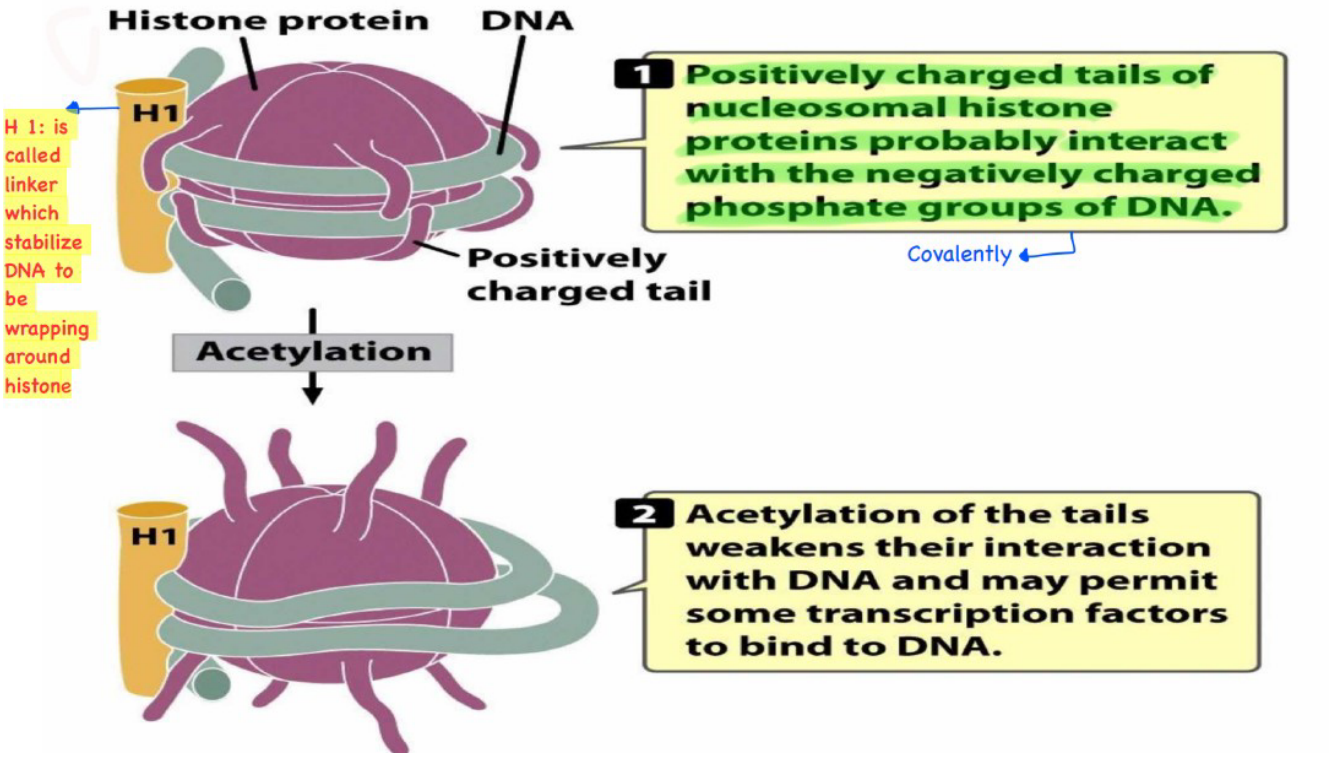
  This is called chromatin remodelling. The idea of using acetyl group is because it is an acidic group that detaches histone (a basic molecule that contains basic amino acids like lysine) from DNA (an acidic molecule) by acetylase enzyme which provides acetate group for histone in the place of DNA.

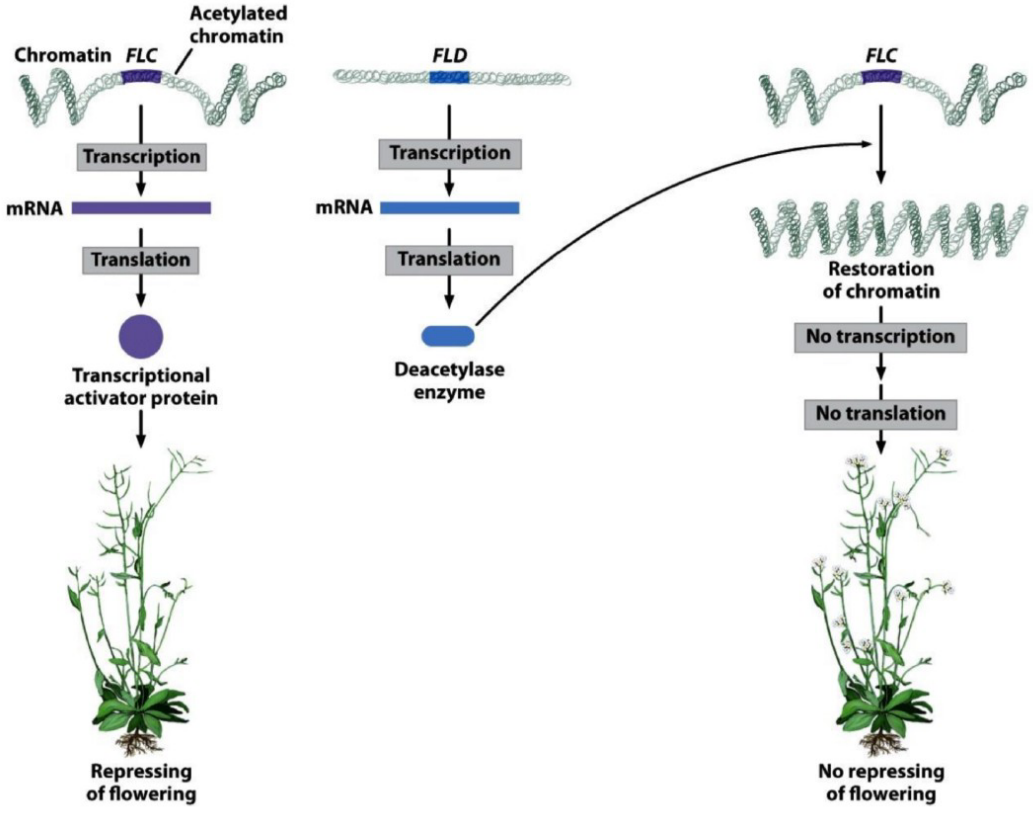
Histone Acetylation:

- **A second event needed for transcription affects large regions of the chromosome instead of individual genes.**
- **DNA is normally tightly wrapped around the histones and is inaccessible to transcription factors.**
- **The structure can be loosened by acetylating the histones.**
- **Acetyl groups are added to lysines, which removes their positive charge.**
- **The binding of the DNA to the histones is lessened, and the DNA structure opens up by**

helicase, allowing access to transcription factors.

- Conversely, deacetylation tightens the chromatin structure, preventing transcription throughout that region of the chromosome . Deacetylation occurs after transcription orreplication by deacetylase enzyme so DNA returns to make wrapping around histone.



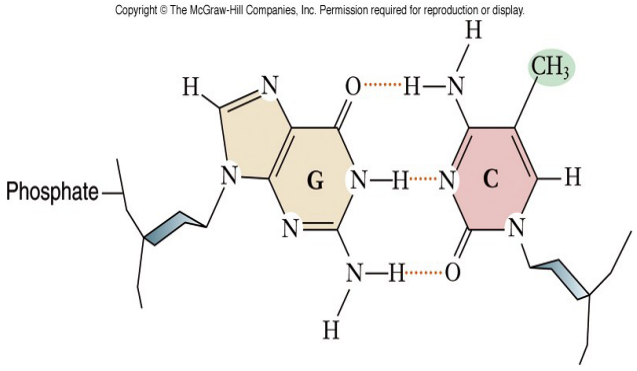


Methylation:

- It is used for inhibiting the rate of transcription; it converts large parts of chromatin to be inactive
- Addition of methyl group to DNA or histone proteins is associated with the control of gene expression.
- If it binds to DNA: clusters of methylated cytosine nucleotides will inhibit transcription because it bindsto a protein that prevents activators from binding to DNA.
- If it binds to histone: methylated histone proteins are associated with inactive regions of chromatin. So, acetate group bind to basic amino acid in histone and it binds for initiating transcription but methyl group binds to cytosine in DNA or to histone and it binds for inhibiting transcription.

Chromatin Remodelling:

- It is for regulating transcription by acetylation or methylation
- Chromatin-remodeling complexes: binddirectly to DNA sites and reposition nucleosomes
- DNA methylation of cytosine bases adjacent to guanine nucleotides (CpG)–CpG islands



Shortly, what factors participate in regulation of transcription? We have talked about general transcription factors, interactions between enhancers and silencers with different proteins to increase or decrease transcription and remodelling of chromatin.

Posttranscriptional Regulation:

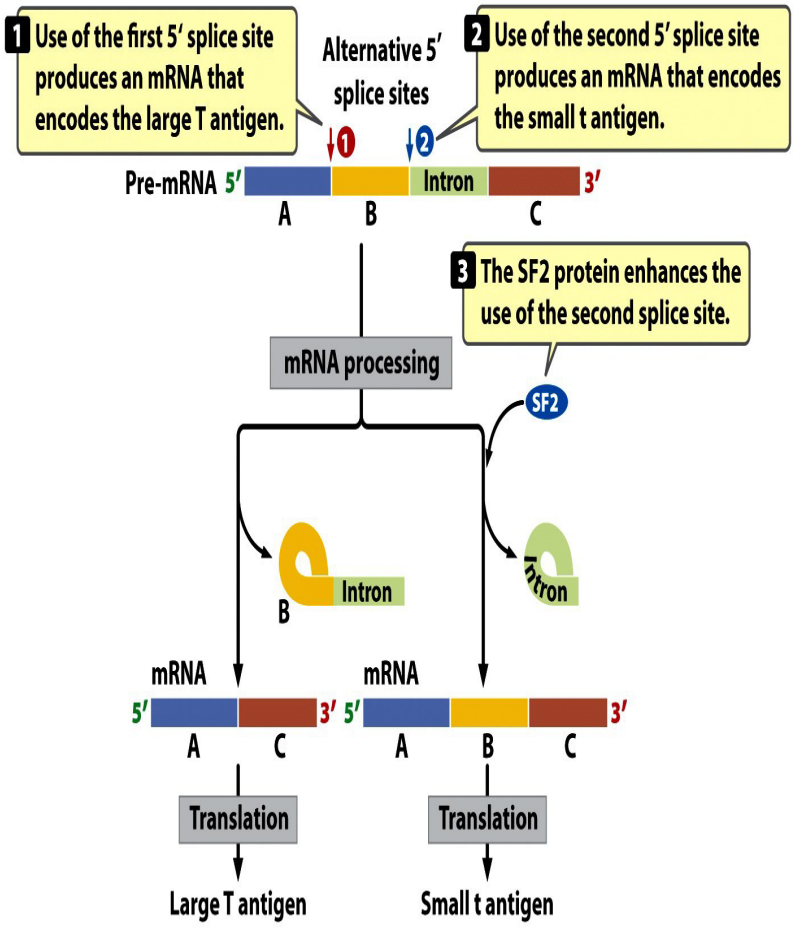
- Control of gene expression usually involves the control of transcription initiation.
- But gene expression can be controlled after transcription, with mechanisms such as:
  A- RNA interference B- alternative splicing
  C- RNA editing D- mRNA degradation

A- RNA interference:

- Involves the use of small RNA molecules : micro RNA and small interfering RNA. These 2 molecules are for regulating the rate of transcription.
- 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
- This process occur by Dicer and RISC

B- Alternative splicing

- It is optional splicing which means it is not necessary that all exons that produced after removing introns to be jointedtogether. So, it will produce different types of proteins related to exons joining.
- Introns are spliced out of pre- mRNAs to produce the maturemRNA that is translated.
- The spliceosome recognizes different splice sites in differenttissue types.
- The mature mRNAs in each tissue possess different exons,resulting in different polypeptide products from thesame gene, for this reason, thenumber of proteins in our cellsis more than the number of genes.



C- RNA editing:

- creates mature mRNA that are not truly encoded by the genome.
- Editing in the coding region to produce isoforms of the same protein and pre- and post- coding region affecting pre-mRNA splicing, mRNA transport to cytosol or RNA translation effectiveness, there is apoprotein B and it has 2 types: 1) apo B48 which exists in compartments of chylomicron (lipoprotein) and 2) apo B100 which exists in very low density and intermediate density lipoproteins (VLDL and IDL). In this care, the same gene produces 2 different proteins as a result of RNA editing not alternative splicing.
- For example:
  - Apolipoprotein B exists in 2 isoforms.

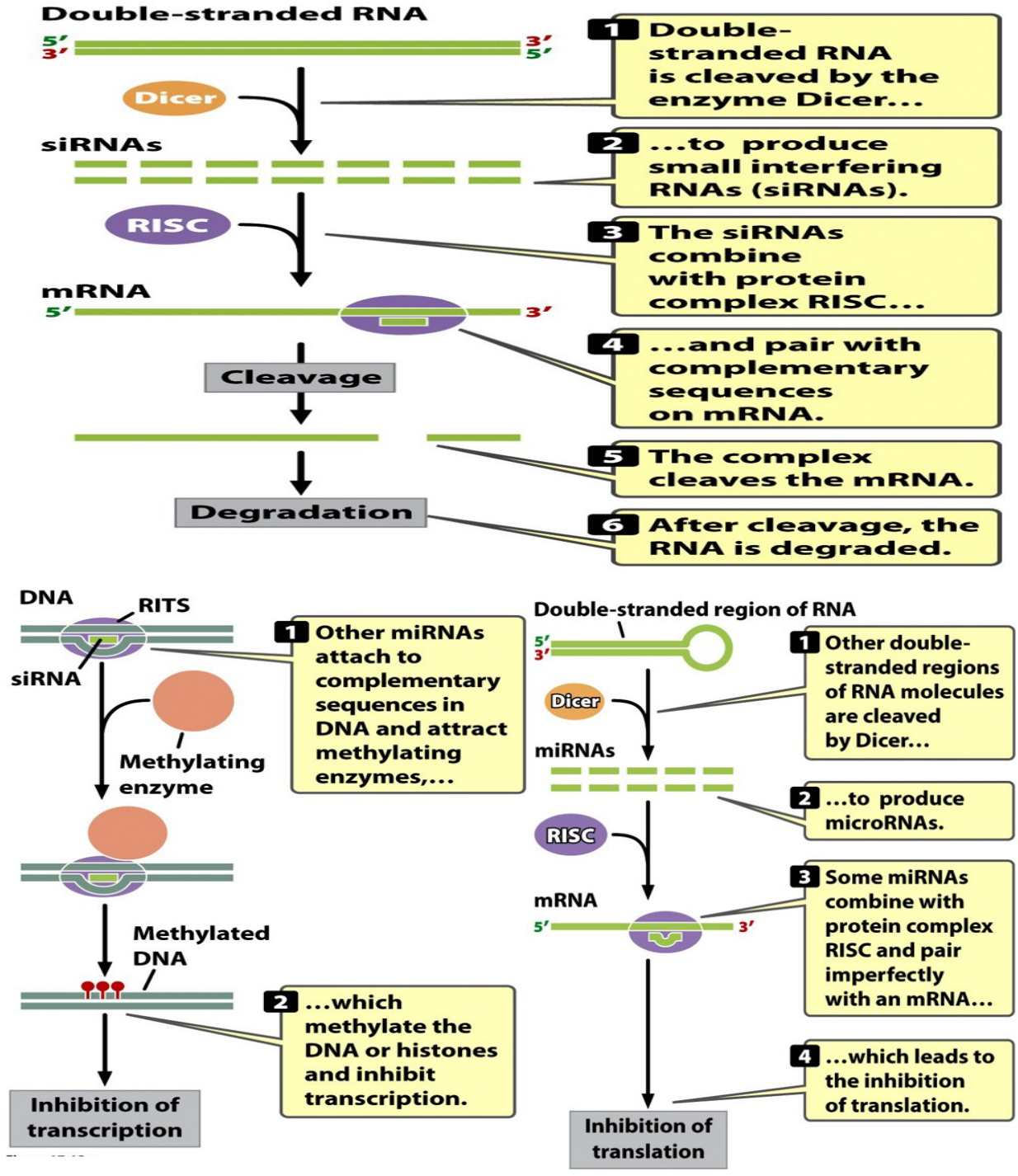
- One isoform is produced by editing the mRNA to create a stop codon instead of glutamine codon (Apo B48 in intestine) which is produced by intestinal mucosal cells and (Apo B 100 in liver) which will complete transcription.
- This RNA editing is tissue-specific. Which means it does not occur in all cells.
- Mature mRNA molecules have various half-lives depending on the gene and thelocation (tissue) of expression and in turn it is affecting the amount of the polypeptide produced.
- RNA editing is very important to produce different types of proteins from the same gene not by alternative splicing.

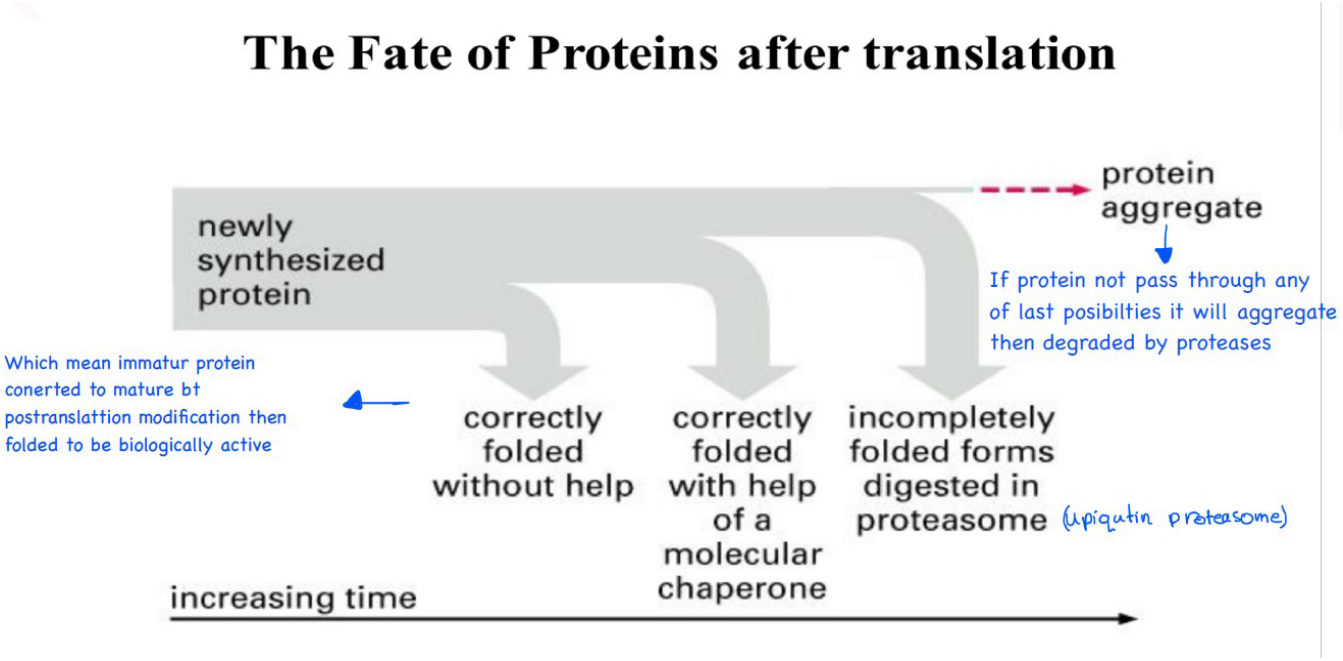
D- Control of RNA Transport and intracellular Localization:

- The control of transporting nuclear mRNAs to cytosol by the presence of protein calledlocalization element and their localization to specific cellular compartment which is affected by the post-transcriptional processing of hn-RNA
- The intracellular localization is under effect of elements (localization elements) specified by cis-acting elements (mostly found in 3'UTR)
- Localization elements are recognized by trans-acting factors (RNA- binding proteins: another name for localization element). This protein is needed after processing of RNA to be transported to cytosol using it.

E- RNA processing and degradation can regulate some genes:

- The degradation of RNA:
  - 5'-cap removal: 5' cap is 7- methyl GTP which has 2 functions: 1) to be recognized by specific protein synthesizing machinary for translation (ribosomes) and 2) to protect mRNA from 5' exonucleases; so, when 5' cap is removed, the 5' end will beexposed to attacks by 5' exonucleases.
  - Shortening of the poly (A) tail : poly A tail is located in the 3' end of mRNA for protection against 3' exonucleases. So, when shortening occurs, the 3' end will be exposed to attacks by 3' exonucleases.
  - Degradation of 5' UTR, coding sequence, and 3' UTR
- Mechanisms of Gene regulation by RNA interference RNA cleavage:
  - RISC (RNA Induced Silencer Complex) containing an siRNA (small interfering RNA), pair with mRNA molecules and cleavage to the mRNA. RISC is incorporated with micro RNA and siRNA for degradation of mRNA when it is no longer needed; as in cases of massive increments in rates of transcription which leads to decreasing the rates of translation as the amount of mRNA is limited.
  - Inhibition of translation
  - Transcriptional silencing: altering chromatin structure
  - Silencer-independent degradation of mRNA

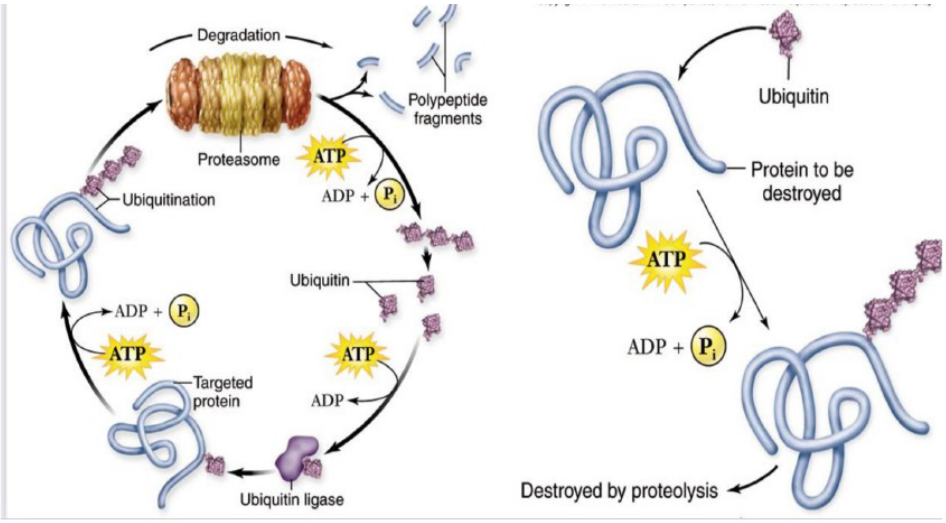

Double-stranded RNA
5′
3′
3′
5′
Dicer
siRNAs
RISC
mRNA
5′
3′
Cleavage
Degradation
1 Double-stranded RNA is cleaved by the enzyme Dicer...
2 ...to produce small interfering RNAs (siRNAs).
3 The siRNAs combine with protein complex RISC...
4 ...and pair with complementary sequences on mRNA.
5 The complex cleaves the mRNA.
6 After cleavage, the RNA is degraded.
DNA
RITS
siRNA
Methylating enzyme
Methylated DNA
Inhibition of transcription
1 Other miRNAs attach to complementary sequences in DNA and attract methylating enzymes,...
2 ...which methylate the DNA or histones and inhibit transcription.
Double-stranded region of RNA
5′
3′
Dicer
miRNAs
RISC
mRNA
5′
3′
Inhibition of translation
1 Other double-stranded regions of RNA molecules are cleaved by Dicer...
2 ...to produce microRNAs.
3 Some miRNAs combine with protein complex RISC and pair imperfectly with an mRNA...
4 ...which leads to the inhibition of translation.



Protein Degradation:

- **Degradation occurs to the protein which is not correctly folded or if there is excess production of a specific protein or a specific cell is going to control the production of specificgene.**
- **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.**

- **Explanation, ubiquitin is a protein in nature that is a small molecule (it has a molecular weight of about 7KDa). When it binds to protein it makes a signal to degrade this protein by proteosome. There are a group of enzymes that will bind ubiquitin with the particular protein**

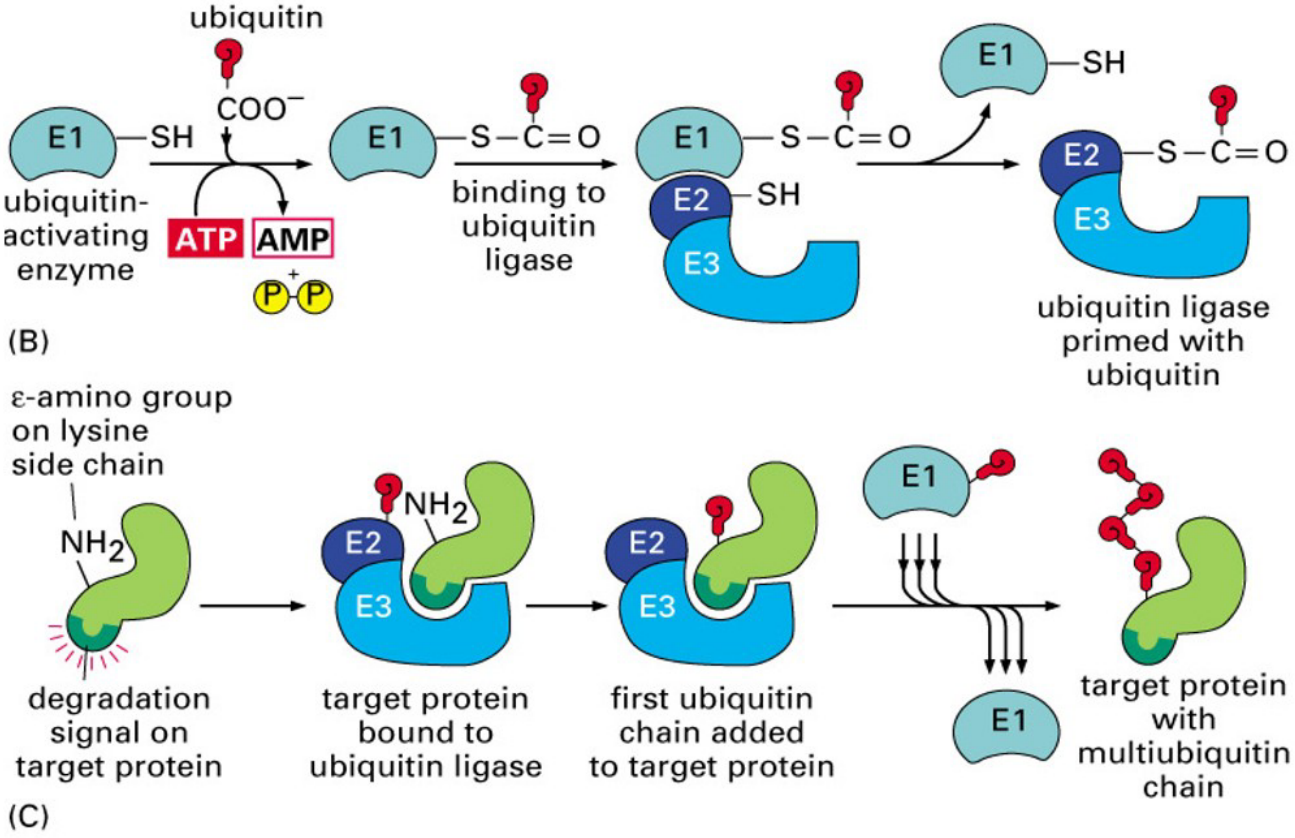


The process of degradation in general

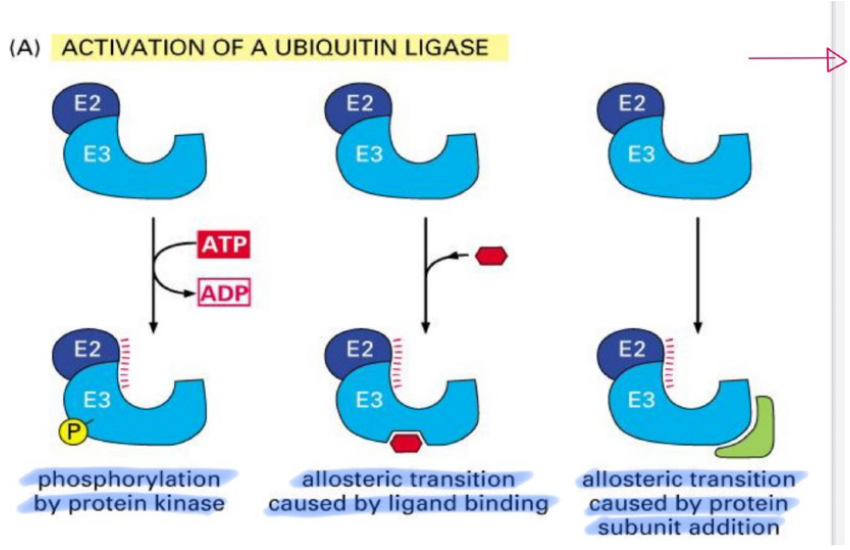
1) upiquitin bind to the target protein (using ATP)
2) poly upiquitination (adding more than one upiquitin molecule
3)then it degraded by proteasome to small fragment
4) but the process not end because the protein should be converted to indivudual amino acid by proteases which convert small fragments of protein into amino acids

- **The following figure(s) talk about the process of degradation in details:**

E1: ubiquitin activating enzyme; E2/3: ubiquitin ligase



1) **Ubiquitin molecule binds E1 (ubiquitin activating enzyme) by binding ubiquitin to SH (sulfhydryl or thiol) group in cystine amino acid in E1 with the use of ATP to result in theactivation of ubiquitin molecule.**
2) **After activation, the complex with bind to E2 and E3 (ubiquitin ligase).**
3) **Ubiquitin group will now be transferred from E1 to ubiquitin ligase. Note that target protein has degradation signal which occurs through amino group on lysine side chain.**
4) **Target protein will bind ubiquitin group.**
5) **Ubiquitin group will transfer from ubiquitin ligase to the target protein.**
6) **Poly ubiquitination occur to be attacked by proteosome with degrade it into small fragments.**



Activation of a upiquitin ligase occur by using ATP tnen ligases bind to ligand which followed by binding the target protein protein

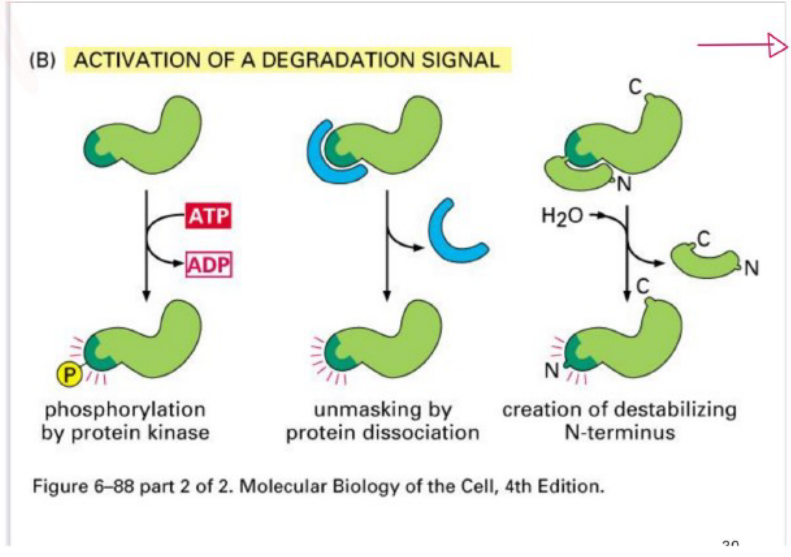


Figure 6–88 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

Degradation signal activated by using ATP so it should be a kinase enzyme to transfer phosphate group from ATP to the target protein then followed by binding the upiquitin molecule which result in creation of destabilizing N terminus
So present of degradation signal and poly upiquitination will give the chance for proteolytic enzyme belonging to proteasome system to degrade the target protein

Prokaryotics Vs. Eukaryotics:

All of these differences we took in details in transcription and translation lectures

- **Bacterial genetics are different.**

- **Prokaryote genes are grouped in operons.**
- **Prokaryotes have one type of RNA polymerase for all types of RNA**
- **mRNA is not modified**
- **The existence of introns in prokaryotes is extremely rare.**
- **To initiate transcription in bacteria, sigma factors bind tRNA polymerases. RNA polymerases/**
  sigma factors complex can then bind to promoter prior to the gene coding region.
- **In prokaryotes, the newly synthesized mRNA is polycistronic (polygenic) (code for more than one polypeptide chain).**
- **In prokaryotes, transcription of a gene and translation of the resulting mRNA occur simultaneously, so many polysomes are found associated with an active gene.**

¾ **Prokaryotic Cell:**

1. **Its genes are grouped in operons**
2. **It has one type of RNA polymerase which consists of 4 subunits (a2BB'), it is helped (guided) by sigma factor which can recognize the initiation site by the presence of promoter boxes of prokaryotic genes.**
3. **Transcription and translation occur simultaneously.**
4. **mRNA in prokaryotes is polycistronic**

¾ **Eukaryotic Cell:**

1. **It has 3 types of RNA polymerase: 1) produces 3 types of rRNA, 2) produces snRNA andmRNA and 3) produces one type of rRNA, tRNA and snRNA (so, RNA polymerase 3 isthe least specialized one because it produces 3 different types of RNA).**
2. **Its genes are monocistronic**
3. **Transcription and translation are separated because it has nuclear membrane. mRNA should be processed before translation and it is transferred by localization element tothe cytosol.**

كُتب لك في قدرك أن تكون في هذا المكان وان تكون ذا أثر علم يساوي بحارا ما زلت لا تملك منه إلا قطرة لكنك ستنجو بعلمك في يوم ما فمعاركك اليوم هي أرباحك غدًا

هدئ من روعك ليس عليك أن تعيش المعركة بقلبٍ مرتجف خذها بقلبٍ سعيد وافهم جيدا مقولة استمتع بالرحلة لا في الوصول فقط وتذكر همك الصغير يخدم أمة كن ذا اثر