

MOLECULAR BIOLOGY
LECTURE 7
REGULATION OF GENE EXPRESSION
NOTES

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Slide: 2 "Gene Expression"

-As a simple example, DNA can be extracted from Somatic cells and Gametes, and the only difference between their DNA is whether it's a Haploid (23 Chromosomes) or a Diploid (46 Chromosomes).

-Note that you might read the number of genes located on DNA is 25,000 and in other reference it will be 20,000, it's an average number and you won't be asked about it anyhow.

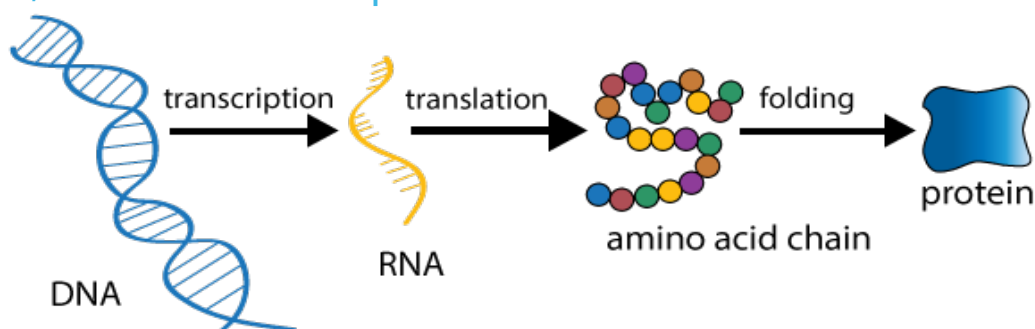
-As all our cells share the same genetic material, it would be quite surprising that the heart tissue differs from the hepatic tissue and so on, and this Completely refers to the difference in gene expression in our very own cells.

-Example to clarify the idea: Assume that we have 25,000 genes there are only 300 of them is activated in the process of building "synthesizing" Cerebral tissue.

- Gene expression: is the process by which information from a gene is used in the synthesis of a functional gene products: either protein or RNA such as tRNA and rRNA.

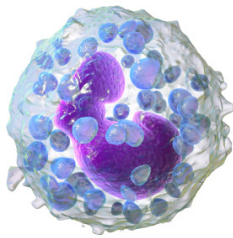
- The central dogma in genetics describes the flow of genetic information in cells from DNA to mRNA to protein.

-A misfolded amino acid in a protein will result in a non-functional protein, and the whole process would be useless.

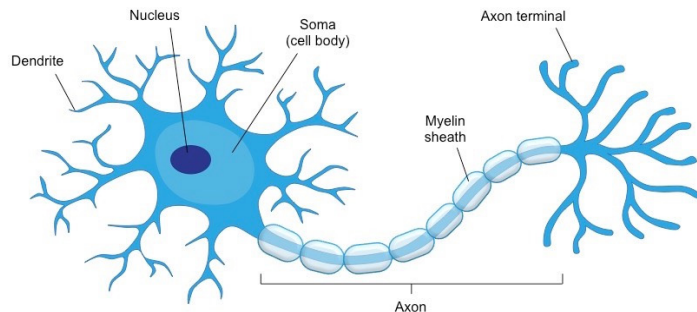


Slide: 3 "Gene Expression"

- Different cell types differ dramatically in both structure and function although they contain the same genome (e.g. basophil and neuronal cell).



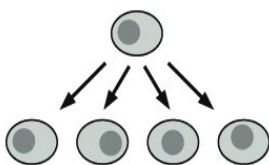
basophil



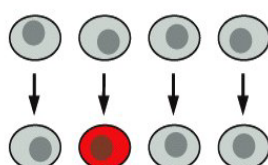
- Gene regulation controls cell structure and function. It is the basis for cellular division, differentiation and morphogenesis

-The difference in gene expression starts once the Zygote is formed, firstly, the genes which is responsible for cell division "Proliferation" is switched on and the entire left genes are switched off, so only cell's job is to divide "proliferate", when this process is done, these very particular genes will be switched off and the genes responsible for Cell Spiecilaization "Differentiation" will be switched on so the cells can specialize and when this process is done these genes will be switched off, and the genes of morphogenesis and cell movement will be switched on, this whole pocess is carried by Gene Expression Regulation.

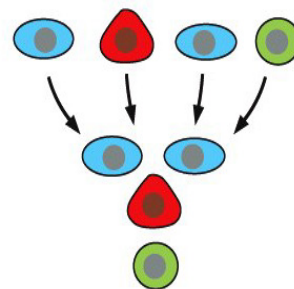
-Morphogenesis: The shaping of cells, tissue or organism.



Cell Proliferation



Cell Specialization



Cell movement and morphogenesis

Slide: 4 "Gene Expression"

- Different cell types synthesize and accumulate different sets of RNA and proteins (Hemoglobin in RBCs).

-Hemoglobin won't be expressed in any cell except those where the Hemoglobin is functional "RBCs".

- Also, the level of expression of almost every active gene varies from one cell type to another.

• Moreover, the level of expression is temporal and spatial.

-Spatial: Relating to occupying space "place", since there are common genes in cells (e.g. Histones) the level of expressions to these genes varies from place to place.

-Temporal: Relating to time, some gene expression level differs from day to night.

- Gene expression can be regulated at many steps:

1- Transcriptional control (the most efficient point of gene expression regulation).

2- RNA processing control.

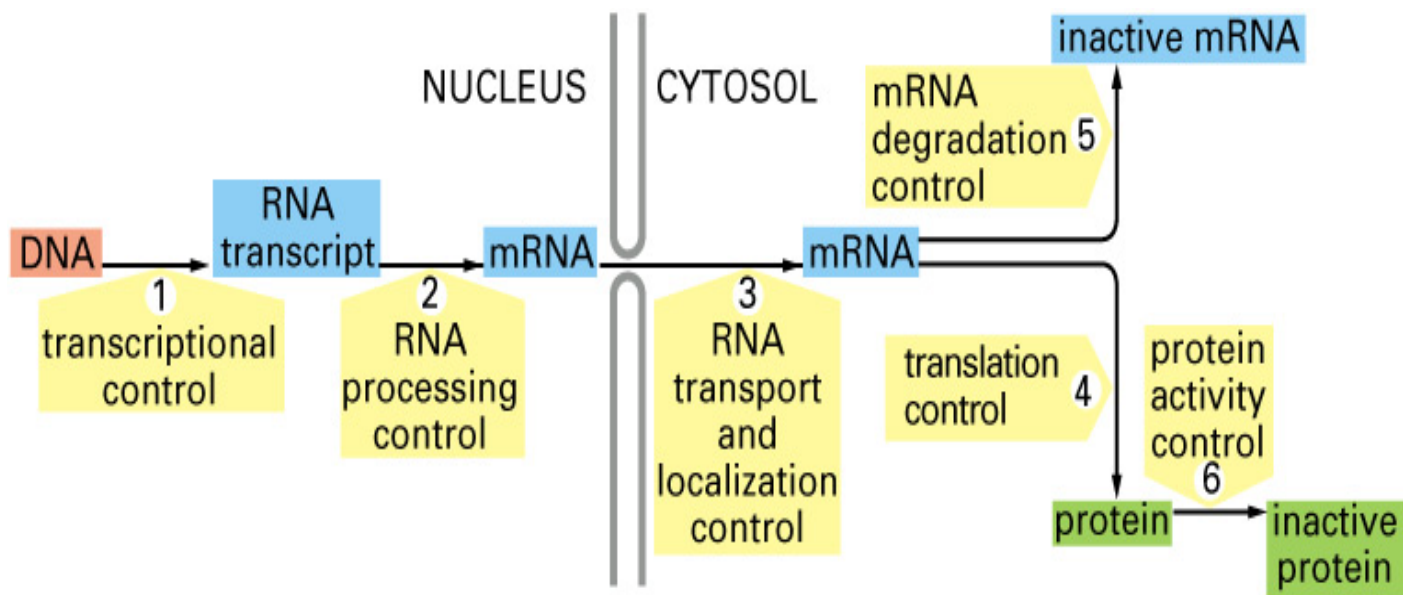
3- RNA transport and localization control.

4- Translational control.

5- mRNA degradation control.

6- Protein activity control.

Slide: 5 "Steps in Eukaryotic Gene Expression regulation"



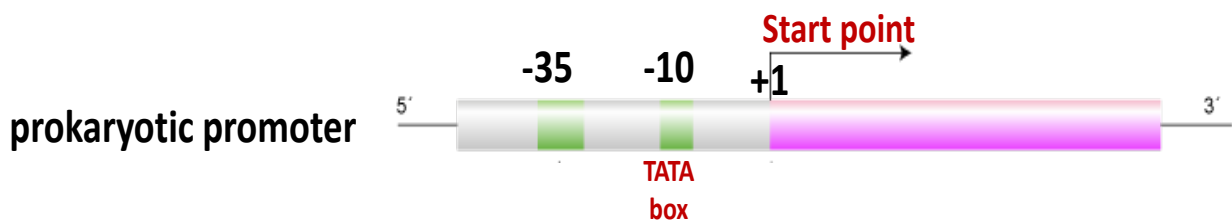
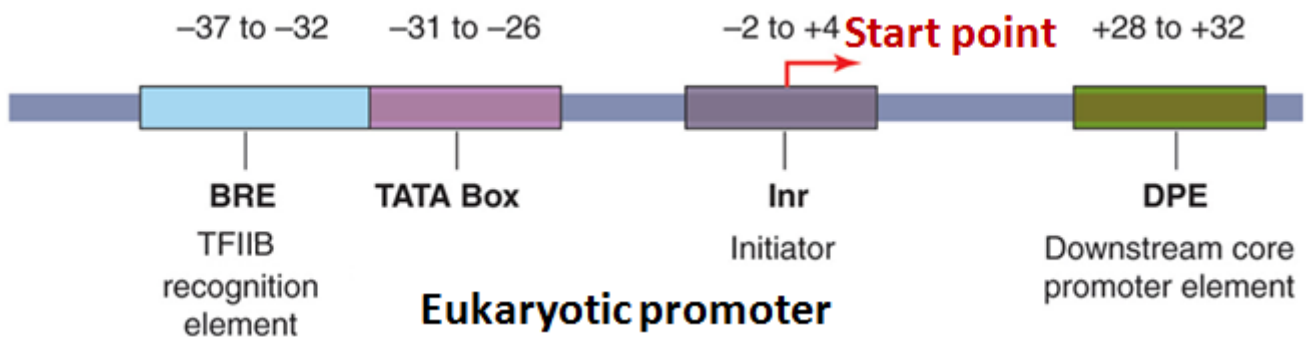
- DNA is transcribed into RNA, and this process occurs in both Eukaryotes and Prokaryotes, Prokaryote lacks a nucleus, the Transcription and Translation processes are made into the cytoplasm, hence there is no nuclear transportation of synthesized RNA, therefore there won't be a post-transcriptional modification.

-In Eukaryotes, the post-transcriptional modification is done to the mRNA in order to convert it into MATURE mRNA, and only mature mRNA are transported from the nucleus to cytoplasm, but before the translation of mRNA it must be localized "Localization".

-Localization: Localizing the mature mRNA in the place where it is translated into a protein and this protein will be functional.

Slide: 6 "1. Transcription Initiation"

- Steps: Initiation, elongation and termination.
- RNA polymerase catalyzes the synthesis of RNA strand from DNA template.
- The promoter is a regulatory DNA region (100-1000 bp). In eukaryotes, it consists of consensus sequences such as TATA box, BRE, INR and DPE . In prokaryotes, two consensus sequences at -10 and -35.

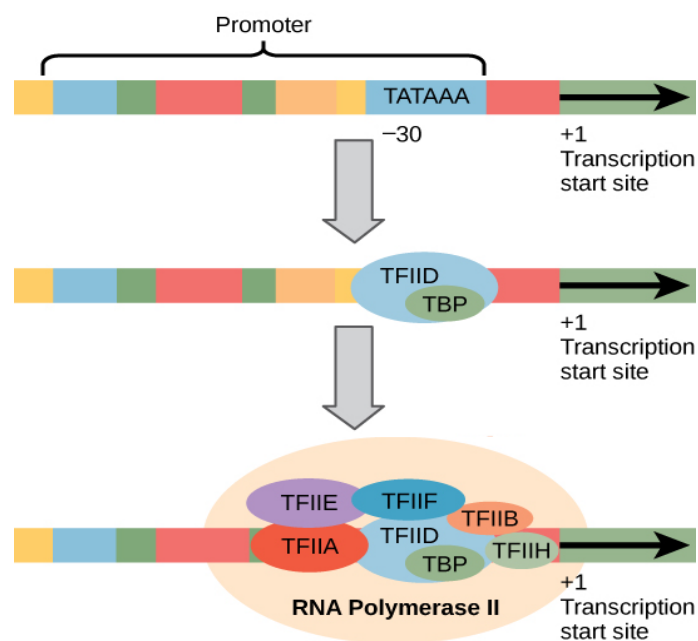


- Slide (6,7) are to help you revise the Transcription process, and it's highly recommended for you to study the Transcription lecture very well.

Slide: 7 "1. Transcription Initiation"

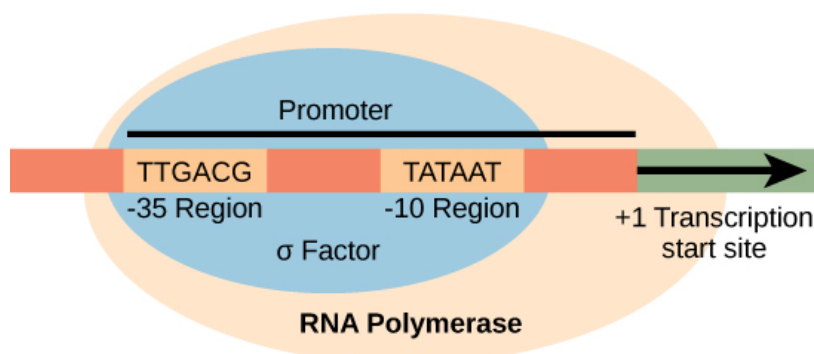
Transcription initiation in eukaryotes

1. It requires general transcription factors which assemble together with RNA polymerase at the promoter to form pre-initiation complex (PIC).
2. TFIID binds first at the TATA box via its TBP subunit.



Transcription initiation in prokaryotes

1. "Sigma" factor recognizes the -35 region in the promoter and binds to it
2. Once the RNA polymerase starts the transcription, "Sigma" factor then dissociates to guide another enzyme to the initiation site.



Slide: 8 "Regulation of Transcription Initiation"

- Gene regulatory proteins called specific transcription factors (activators or repressors) bind DNA specific sequences called gene regulatory regions (enhancers or silencers) to control the expression of various genes.

- Enhancers bind to Activators, whereas Silencers bind to repressors.

- These 2 regions are separated from the promoter by thousands of base pairs, and they are mainly upstream.

- Every cell has its very unique kinds of Specific Transcription Factors, because the genes which will be switched on in a tissue won't be switched on in another tissue. -Remember The example in slide 2 and the Spatial conception-

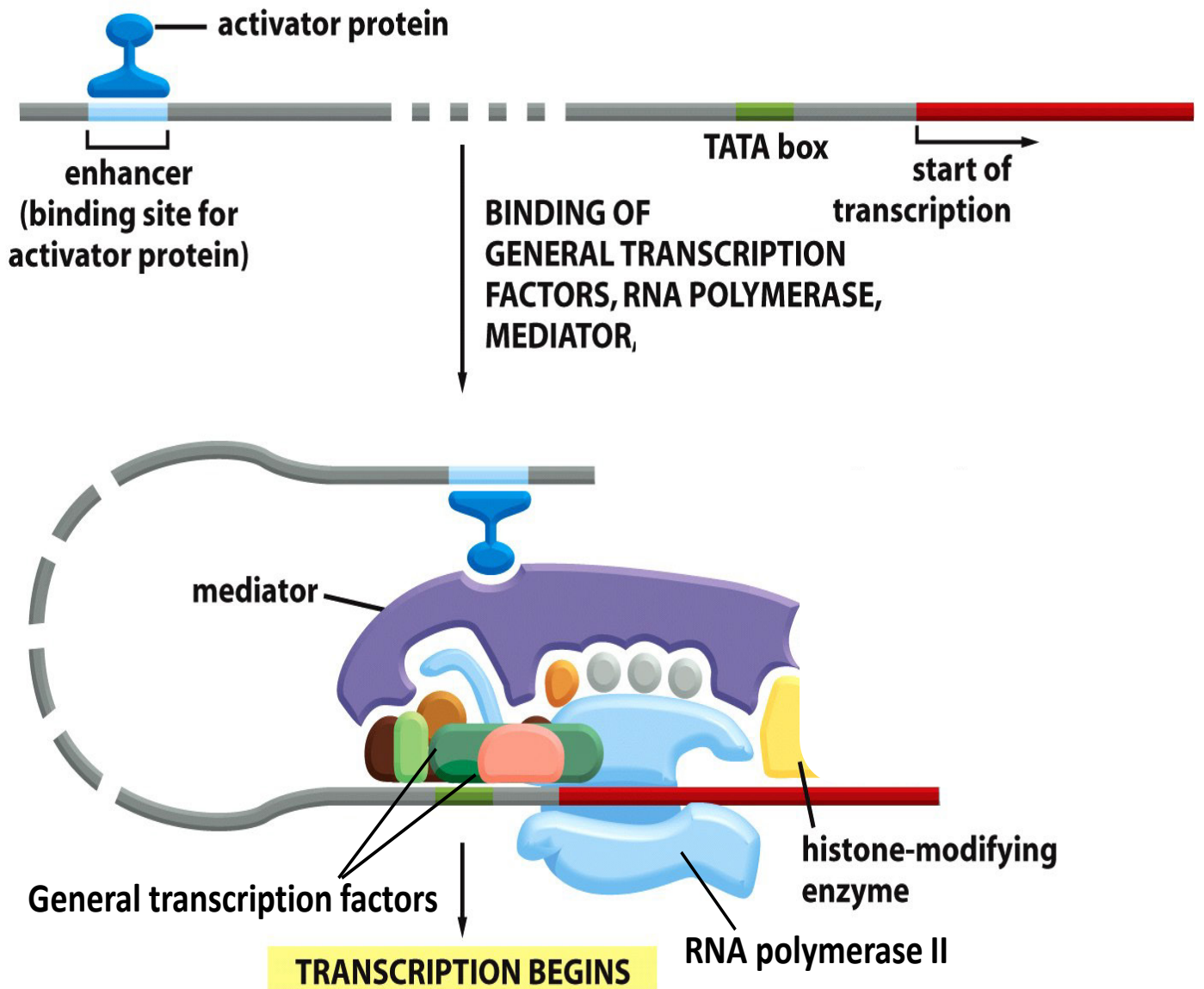
- Specific transcription factors (regulatory proteins) are different from general transcription factors which are involved in the transcription initiation process.

- General factors are 5 or 6, whereas Specific are thousands.

- General are needed by all cells, whereas specific need varies from cell to cell.

- General bind to Promoters to facilitate the assembly of Pre-initiation complex, by recruiting Polymerase, whereas Specific bind to gene regulatory regions to regulate transcription initiation.

Slide: 9 "Regulation of Transcription Initiation"



- The specific transcription factors act at a distance by the DNA looping.
- Specific factors have a domain site that binds to the gene regulatory regions, and the other site "Catalytic site" used to activate or repress.
- The specific factors either act directly or indirectly "through a mediator" and indirectly is way better since it allows them to act at more than one part.
- Mediator protein is formed by 24 polypeptide chains, and extends the contact area for the specific factor.


Slide: 10 "Regulation of Transcription Initiation"

- Cis-regulatory elements (CREs): are regions of non-coding DNA which regulate the transcription of nearby genes. These include promoters, enhancers and silencers.

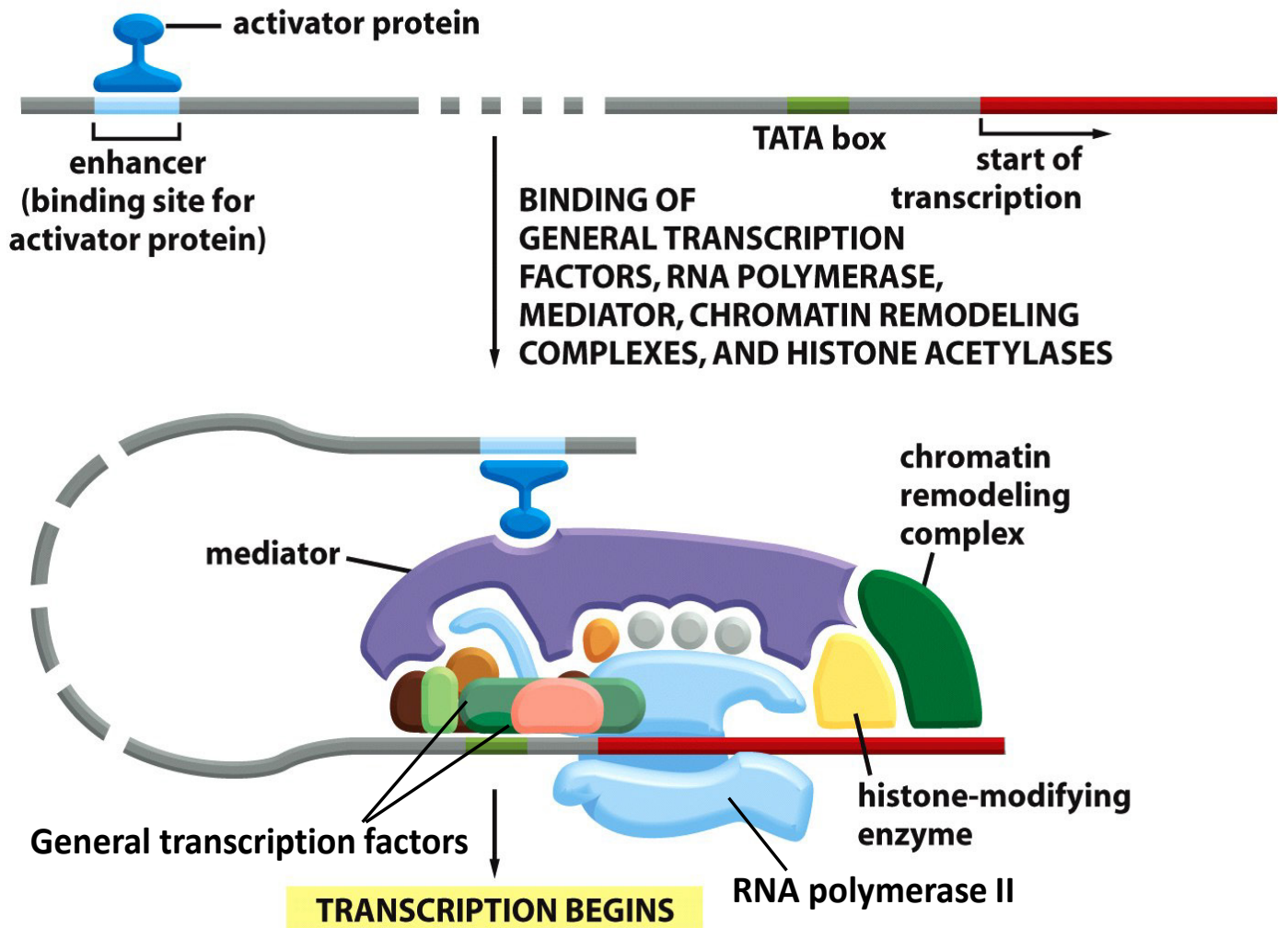
- These regions are mainly found upstream, in case they are downstream they will be present only in Pre-mRNA not in mature mRNA.

- Cis does mean near.

- Gene regulatory proteins act at distance: DNA looping allows them to interact with the assembled proteins at the promoter.

- Mediator is a protein complex recruited to the promoter via specific transcription factors. It  provides extended contact area for the gene regulatory proteins.

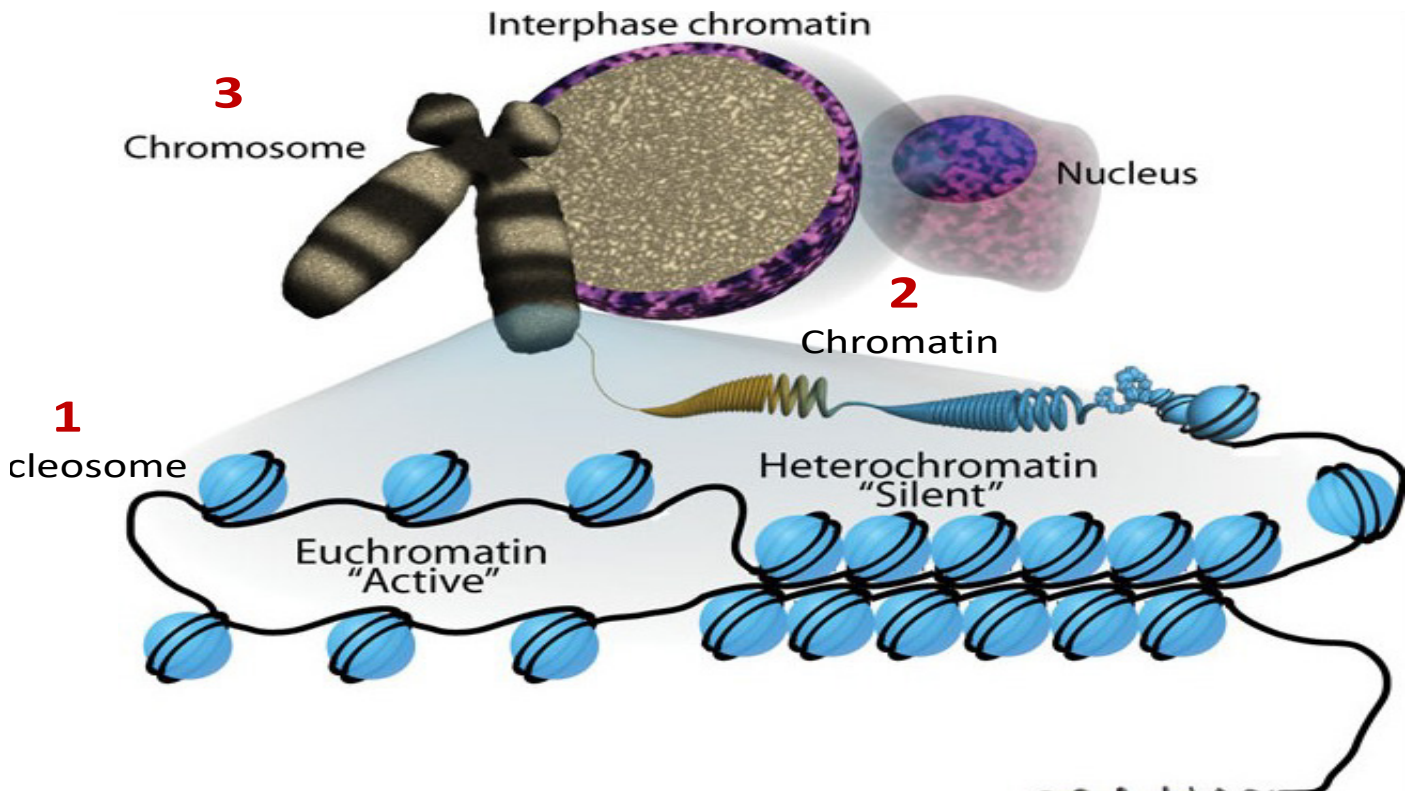
Slide: 11 "Regulation of Transcription Initiation"



- Here note the addition of Chromatin remodeling complex (CRC) & (HME) Histone modifying complex.
- (CRC), (HME) and Mediator, differs from the recruiter "activator or repressor", the mediator recruited by the activator varies from the one by repressor.

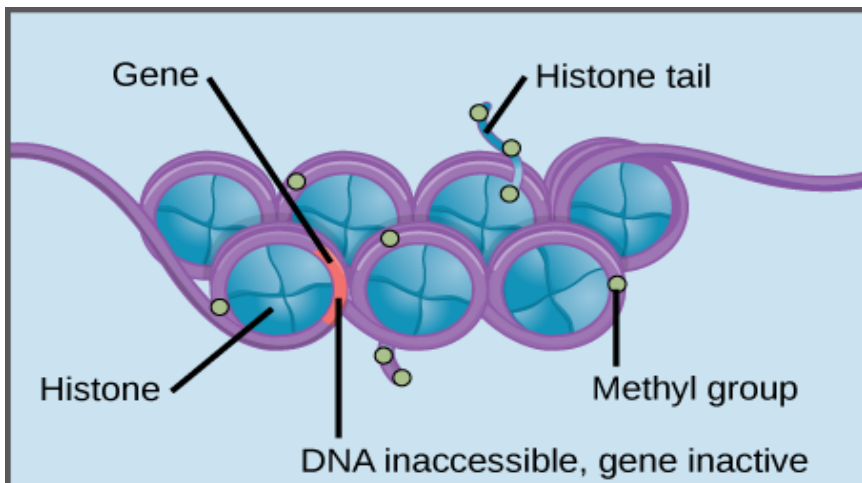
♥ مساء الخير يا باشا، ترى الاختصارات من عندي "جدعنة"، عشان زهقت وأنا بييض، تصبح ع خير.

Slide: 12 "Regulation of Transcription Initiation"



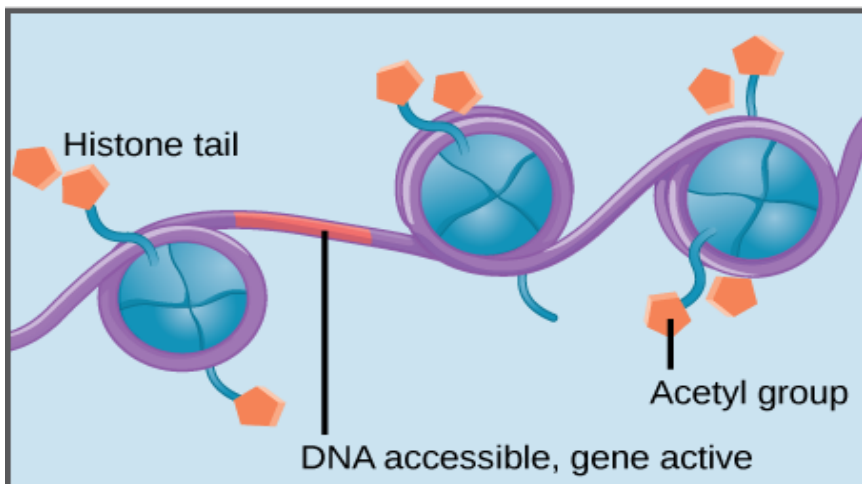
- Why is Euchromatin active and Heterochromatin is not?
Due to the condensed shape of Heterochromatin, so there is no space for enzymes and proteins to bind.
- If the present chromatin in Promoter is Euchromatin the RNA polymerase enzyme and General factors can access the area and initiate the transcription process, but if it's Heterochromatin the area won't be accessible and it will be Transcription inhibition.

Slide: 13 "Regulation of Transcription Initiation"



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

recruited by repressor

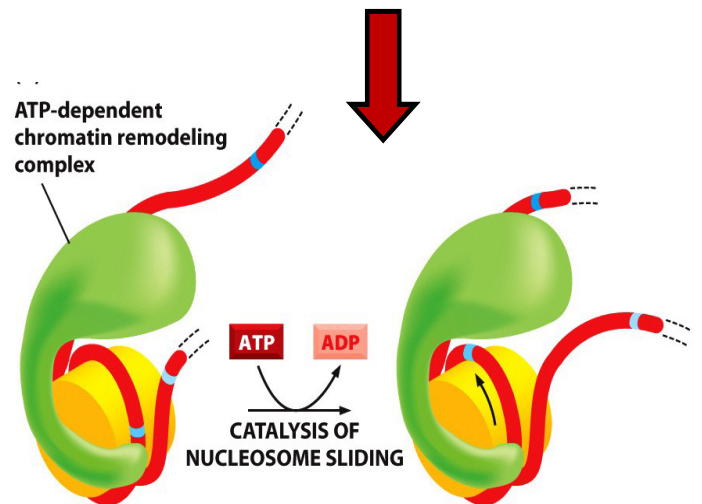
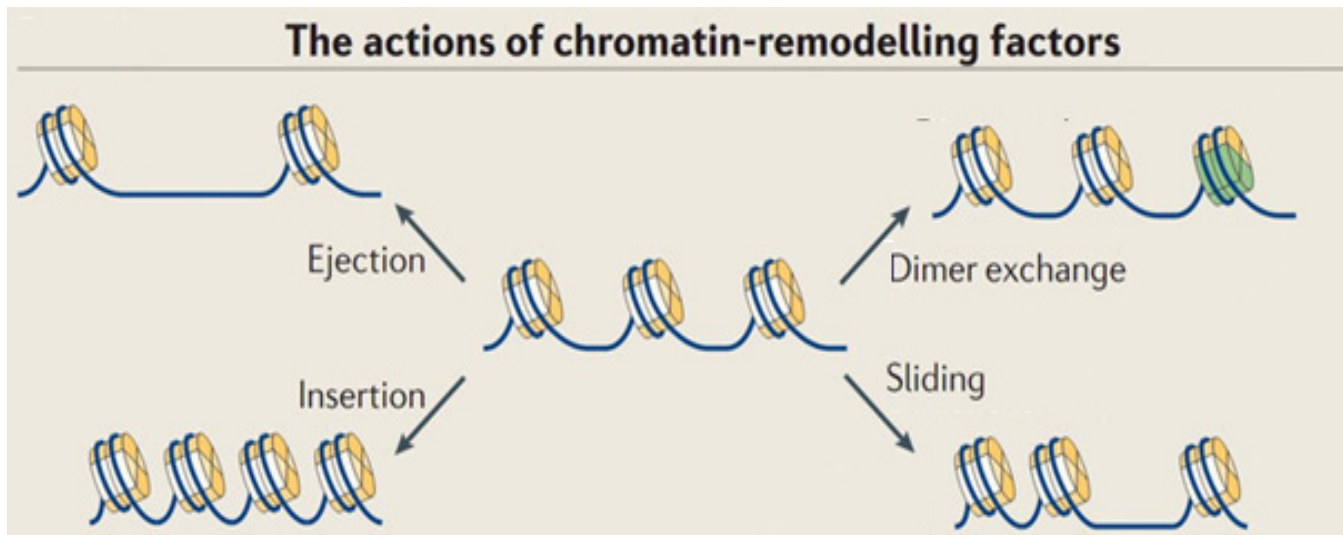


Histone acetylation results in loose packing of nucleosomes. Transcription factors can bind the DNA and genes are expressed.

recruited by activator

- Histone Methylation enzyme adds methyl group.
- Histone Acetylating enzyme adds Acetyl group.
- Sure you know that this is a Histone Modifying Complex.

Slide: 14 "Regulation of Transcription Initiation"



- Ejection by deleting a histone, so the chromatin will be less condensed.
- Insertion by adding a histone, which results in Heterochromatin.
- Dimer Exchange, by replacing a Histone variant according to the present tag on the it like Methyl or Acetyl and you know the rest :).
- Sliding by repositioning or moving the gene place from Euchromatin to Heterochromatin and vice versa, and this does require ATP.
- Sure you know that this is a Chromatin-remodeling factor.

Slide: 15 "Regulation of Transcription Initiation"

- Beside the mediator, other proteins are recruited by specific transcription factors to the promoter such as: histone modifying enzymes and chromatin remodeling complexes.
 - Epigenetic factors: gene expression is affected by changes in chromatin structure (Heterochromatin/ Euchromatin).
- Epi: Outside, and the epigenetic means outside the DNA.

Slide: 16 "Regulation of Transcription Initiation in Prokaryotes"

- The expression of many genes is regulated according to the available food in the environment.
 - Operon: DNA unit consists of a cluster of related genes controlled by single promoter and transcribed together into single mRNA strand (bicistronic or polycistronic transcript).
 - Operator: a segment of regulatory DNA to which a repressor can bind to regulate the transcription of downstream target genes.
- All operons does contain Operator, and the Operator is the same to the silencer region in Eukaryotes.

- The three basic DNA components of operon:

1- Promoter

2- Operator

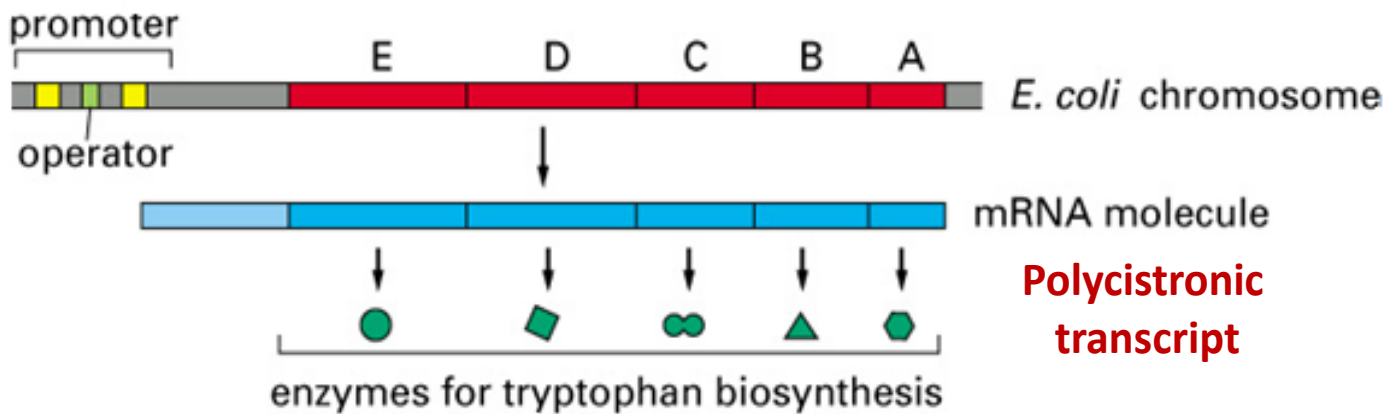
3- Structural genes

- Examples in E-coli bacteria: Trp operon and Lac operon

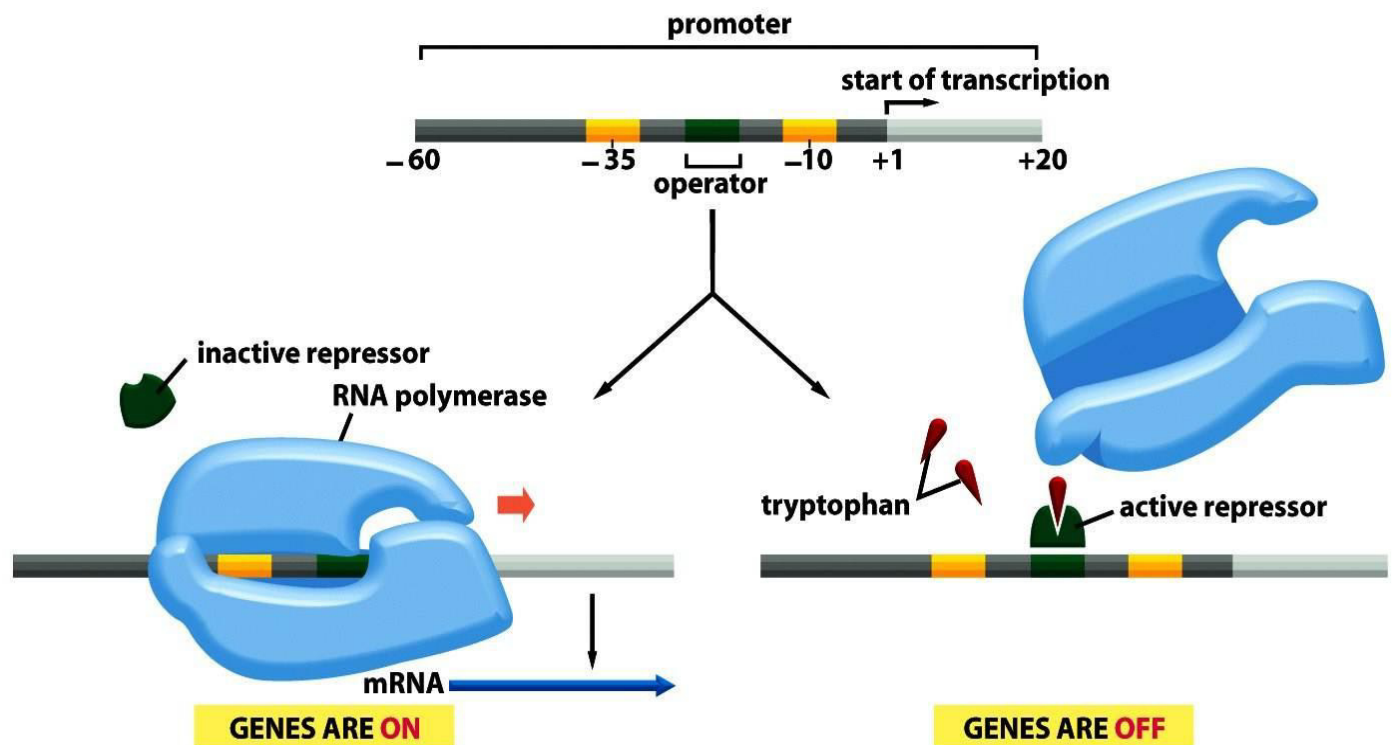
Slide: 17 "Trp Operon"

- Trp operon consists of five structural genes required for the biosynthesis of the amino acid tryptophan.

- Trp: Tryptophan.



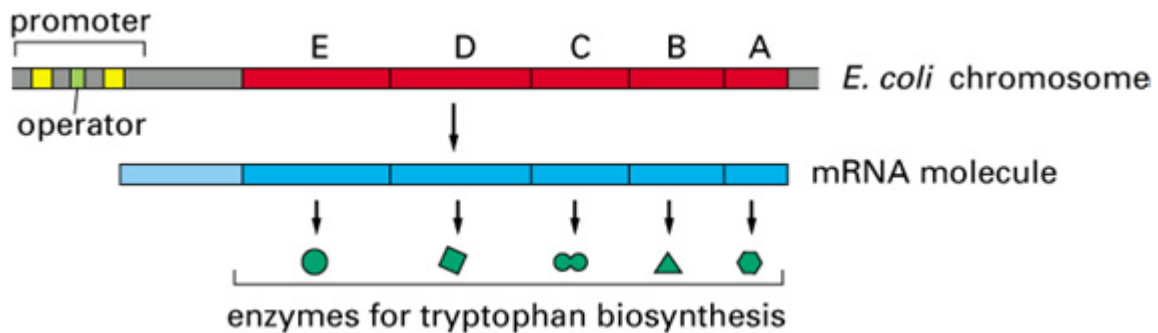
Slide: 18 "Trp Operon"



- When the medium contains Tryptophan the cell won't synthesize Tryptophan, and that happens because the Tryptophan in the medium will bind to repressor "which is located in the middle of promoter" and when binds to the repressor it will leave no space for the RNA polymerase to bind to the promoter and start transcription. Note that the Trp is called Co-Repressor.

Slide: 19 "Trp Operon"

- Trp operon consists of five structural genes required for the biosynthesis of the amino acid tryptophan.



- In the presence of tryptophan in the growth medium, trp repressor (a gene regulatory protein) binds the operator and blocks the access of RNA polymerase (negative control).
- In the absence of tryptophan, the repressor is in the inactive form so cannot bind the operator and the enzymes are transcribed as single polycistronic mRNA.

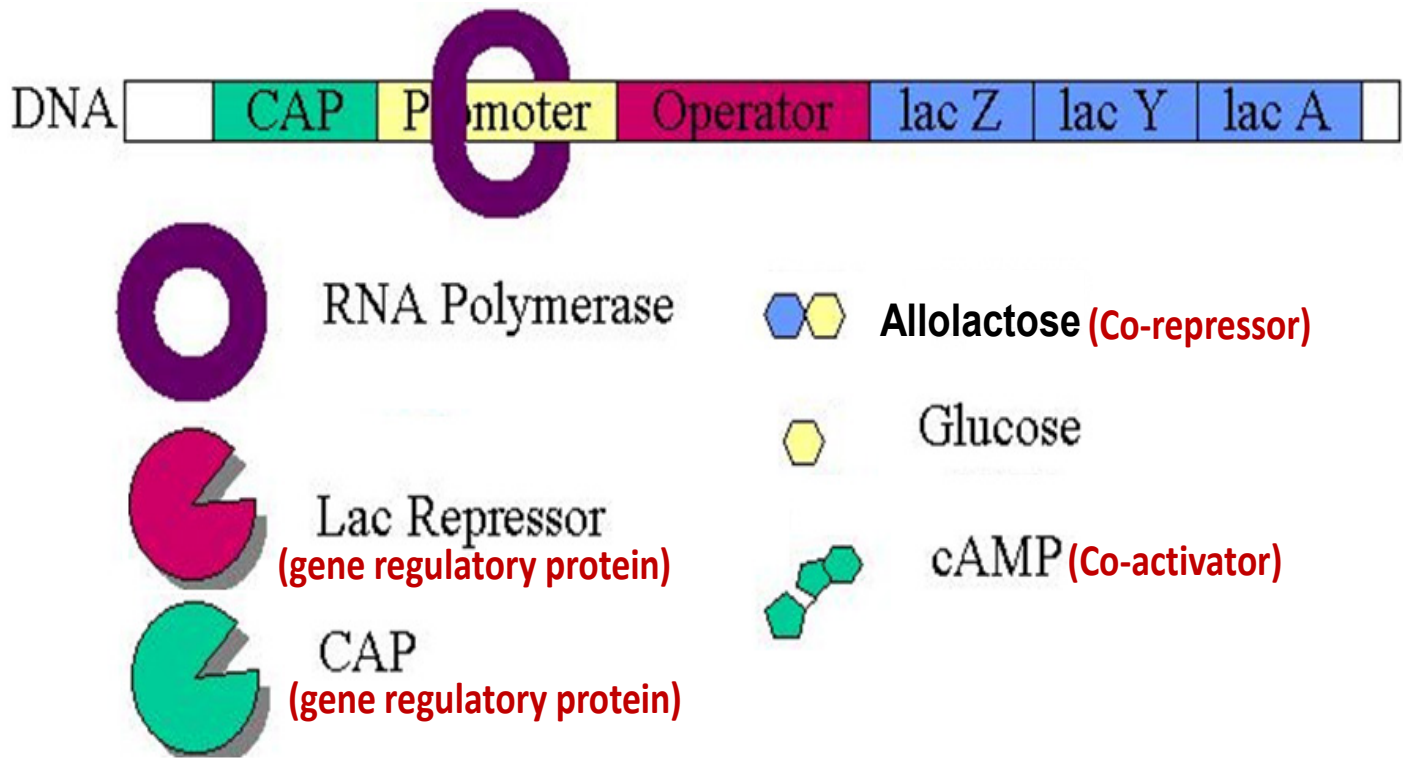
Slide: 20 "Lac Operon"

- Lac operon consists of three structural genes required for the transport and metabolism of lactose as an alternative carbon source to glucose:

- 1,2 and 3 don't memorize them, DON'T.

1. lacZ : encodes Beta-galactosidase which cleaves lactose into glucose and galactose
2. lacY : encodes lactose permease to transport lactose into the cell
3. lacA : encodes galactoside O-acetyltransferase which plays a role in cell detoxification

Slide: 21 "Lac Operon"



- The promoters in Bacteria are weak and they are poorly recognized by RNA Polymerase, this results in weak "Low-level" Transcription, but the presence of CAP the Transcription process will be way better.

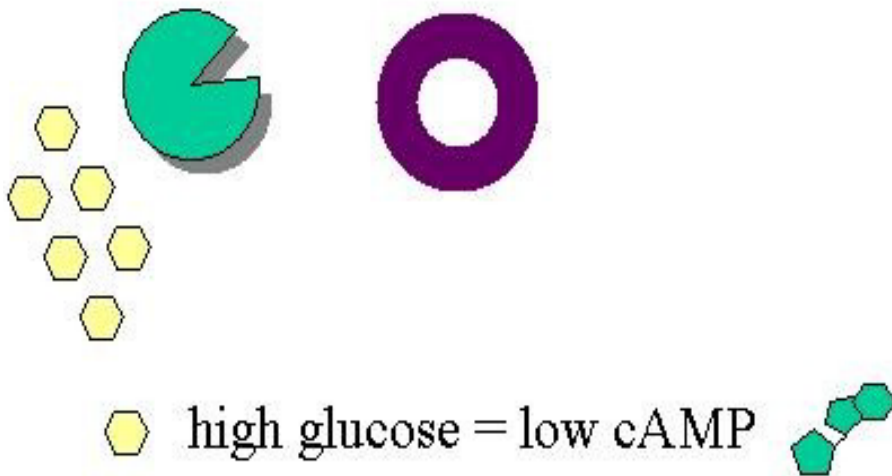
- When does the CAP binds? When there is cAMP.

- There is an interverse relationship between the presence of Glucose in the cell and the presence of the cAMP.

- The Allolactose is a metabloite of Lactose.

Slide: 22 "Lac Operon"

Glucose, No Lactose



- The absence of Lactose means the absence of Allolactose, and this keeps the Lac Repressor binded to the operator, so there will be no transcription.

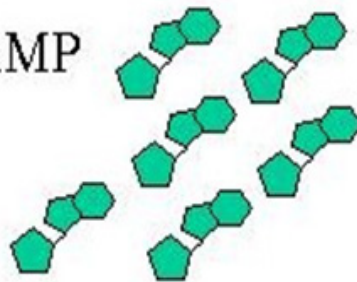
- cAMP does bind to their binding site, since they are very low.

Slide: 23 "Lac Operon"

No Glucose, No Lactose.



⬡ low glucose = high cAMP



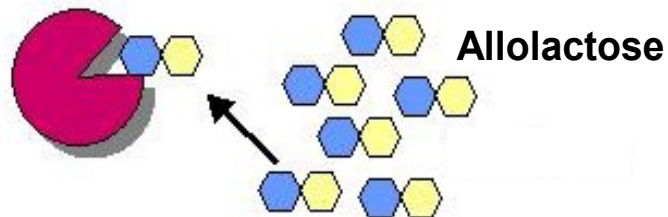
- The absence of Lactose means the absence of Allolactose, and this keeps the Lac Repressor bound to the operator, so there will be no transcription.

- cAMP will bind, but it has no meaning for them to bind, since the whole transcription process is switched off!!!

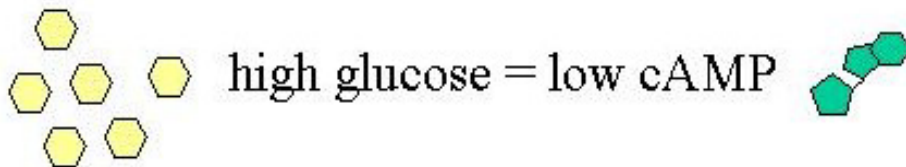
Slide: 24 "Lac Operon"

Glucose and Lactose

Low Transcription



Allolactose

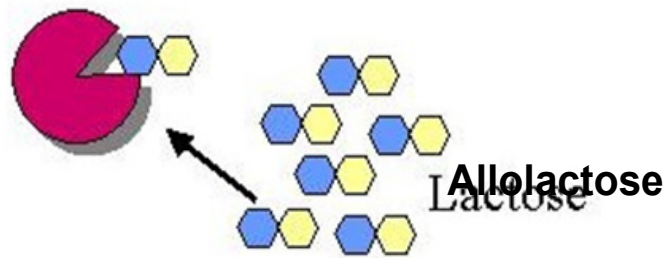
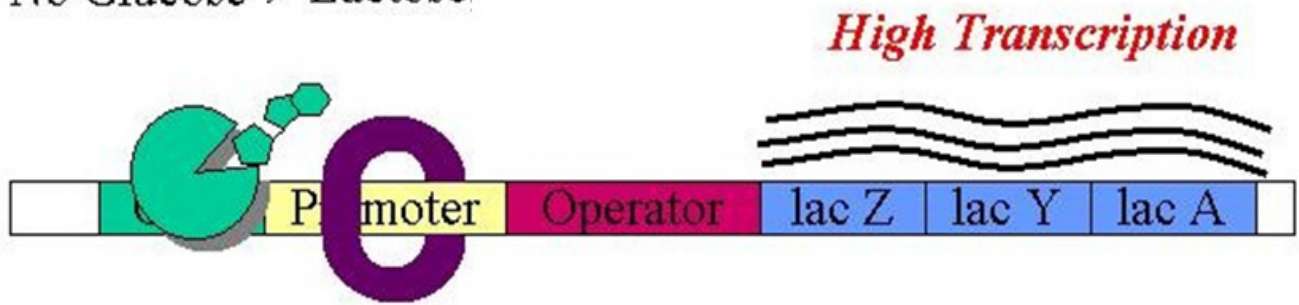


high glucose = low cAMP

- The presence of Lactose means the presence of Allolactose, and this release the Lac Repressor from the operator "By binding allolactose to Lac repressor", so there will be transcription.
- cAMP will not bind, so the transcription process is weak somehow.

Slide: 25 "Lac Operon"

No Glucose , Lactose



low glucose = high cAMP



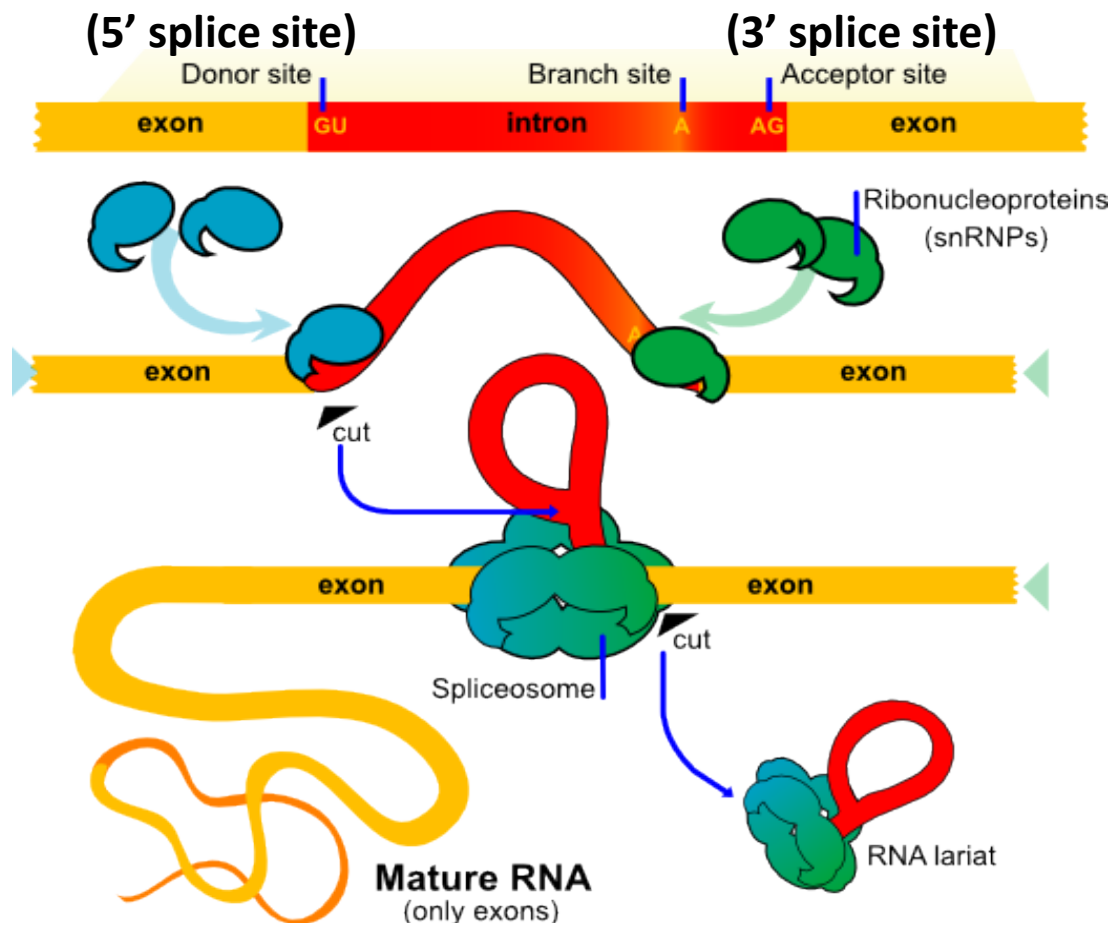
- The presence of Lactose means the presence of Allolactose, and this release the Lac Repressor from the operator "By binding allolactose to Lac repressor", so there will be transcription.
- cAMP will bind, so the transcription process is high.

Slide: 26 "Lac Operon"

- It is under both negative and positive transcriptional controls (dual control): the lac repressor and the CAP activator (catabolite activator protein) respectively.
- In the absence of lactose, lac repressor binds lac operator and inhibits RNA polymerase binding so genes are switched off (regardless of glucose level).
In the absence of glucose, cAMP level is high. cAMP is co-activator of CAP.
- In presence of lactose, the lac repressor is inactivated by the binding to lactose metabolite "allolactose" so the repressor dissociates from the operator with the genes are weakly transcribed in the presence of glucose but extensively transcribed in the absence of glucose.
- Allolactose is an inducer of lac operon. It acts as co-repressor of lac repressor protein

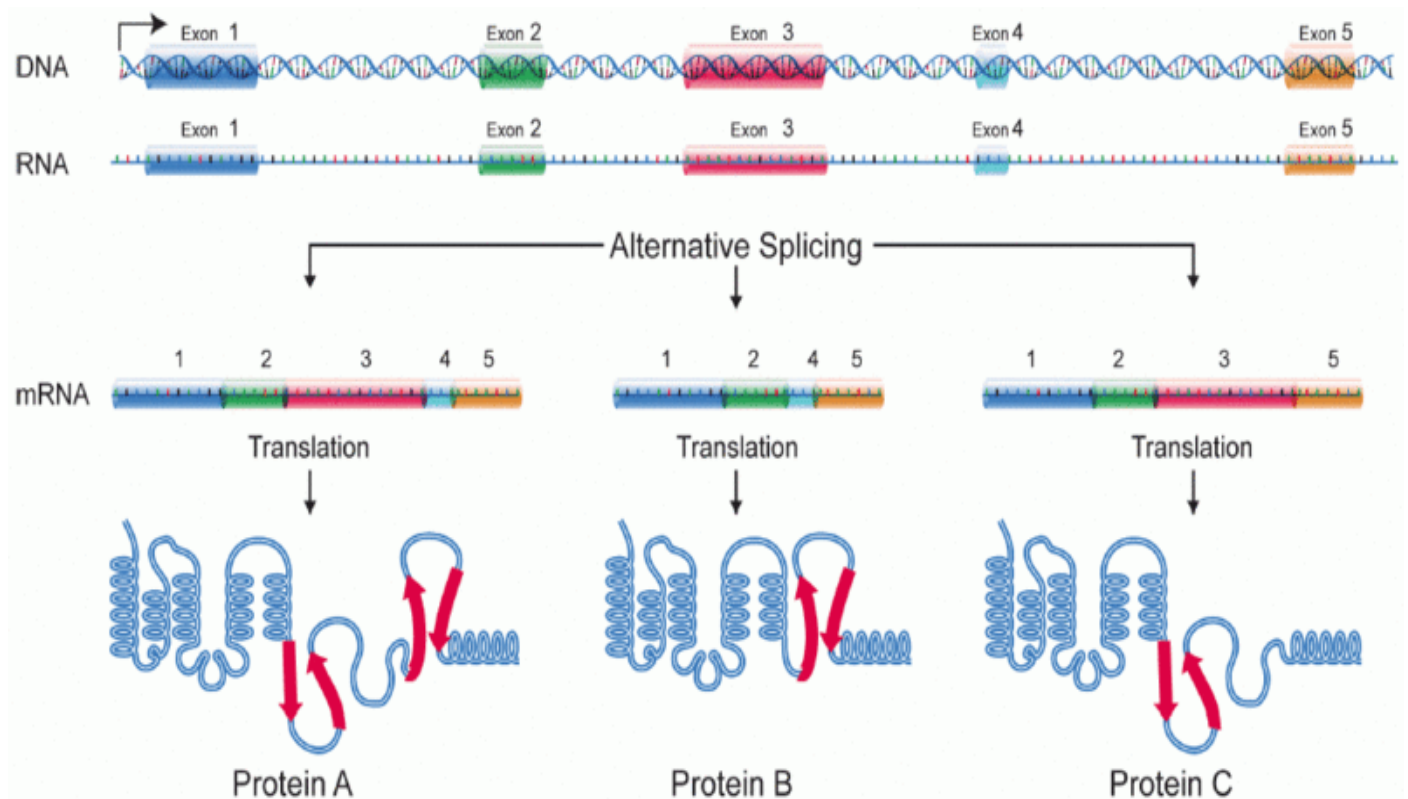
Slide: 27 "2. RNA Splicing Control"

- The post transcriptional modification (5'-capping, splicing and 3' polyadenylation) transfers pre-mRNA into the mature transcript mRNA (Eukaryotes only).
- RNA splicing is the removal of introns and the joining of exons catalyzed by spliceosome (RNA-protein complex).



Slide: 28 "2. RNA Splicing Control"

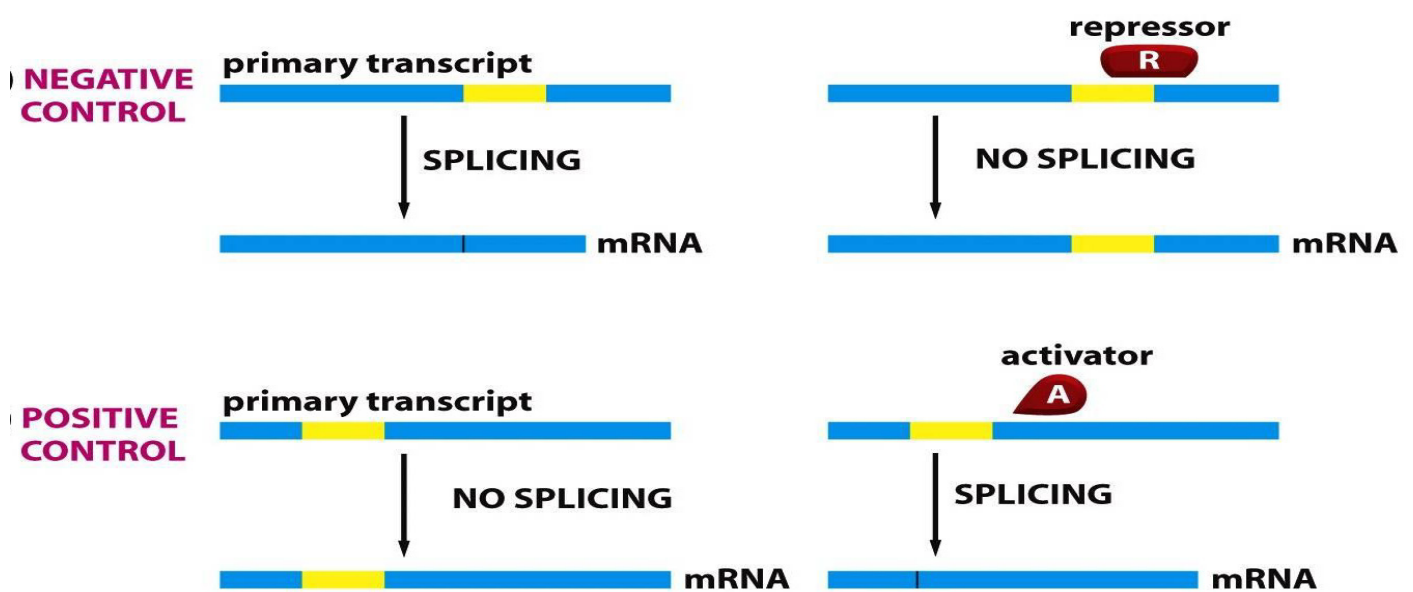
- Alternative RNA splicing (splicing at different junctions) produces different forms (isoforms) of a protein from the same gene.



- Isoforms: Proteins from the same gene, but found in different tissues.

Slide: 29 "2. RNA Splicing Control"

- Many genes undergo alternative splicing in tissue-specific manner and/or under specific cellular conditions (a protein is non-functional in one cell type but functional in another cell type).
- Alternative splicing of pre-mRNA transcripts is regulated by proteins (activators/repressors) that bind specific RNA sequences (regulatory elements) on the primary transcript itself.



- Negative control: a repressor inhibits the spliceosome from splicing.

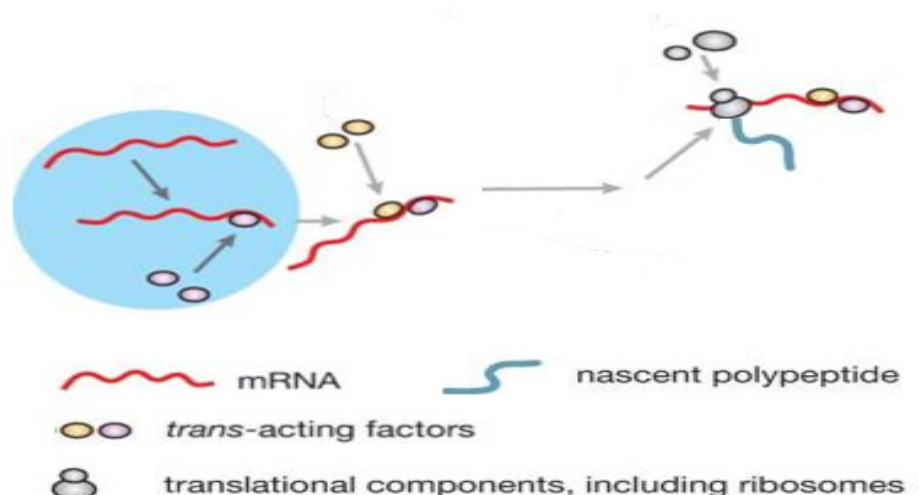
-Positive control: an activator induces the spliceosome to splice.

Slide: 30 "2. RNA Splicing Control"

- Alternative RNA splicing is a phenomenon refers to the process by which a single pre-mRNA of a given gene is spliced into different mRNA molecules called splice variants.
- The translation of these splice variants will results in different forms of proteins in different tissues called isoforms.
- Activators and repressors affect the recognition of different splice sites either by exposing or covering a specific splice site, respectively.

Slide: 31 "3. RNA Transport & Localization Control" "SKIP THIS SLIDE, SKIP THIS SLIDE!"

- Gene expression can be regulated by controlling the nuclear transport of mRNAs and their localization to specific cytoplasmic domains.
- Processing of pre-mRNA in the nucleus is critical for the cytoplasmic localization.
- The cellular address is specified by cis-acting elements (called localization elements) mostly found in 3'UTR.
- Localization elements are recognized by trans-acting factors (RNA-binding proteins).

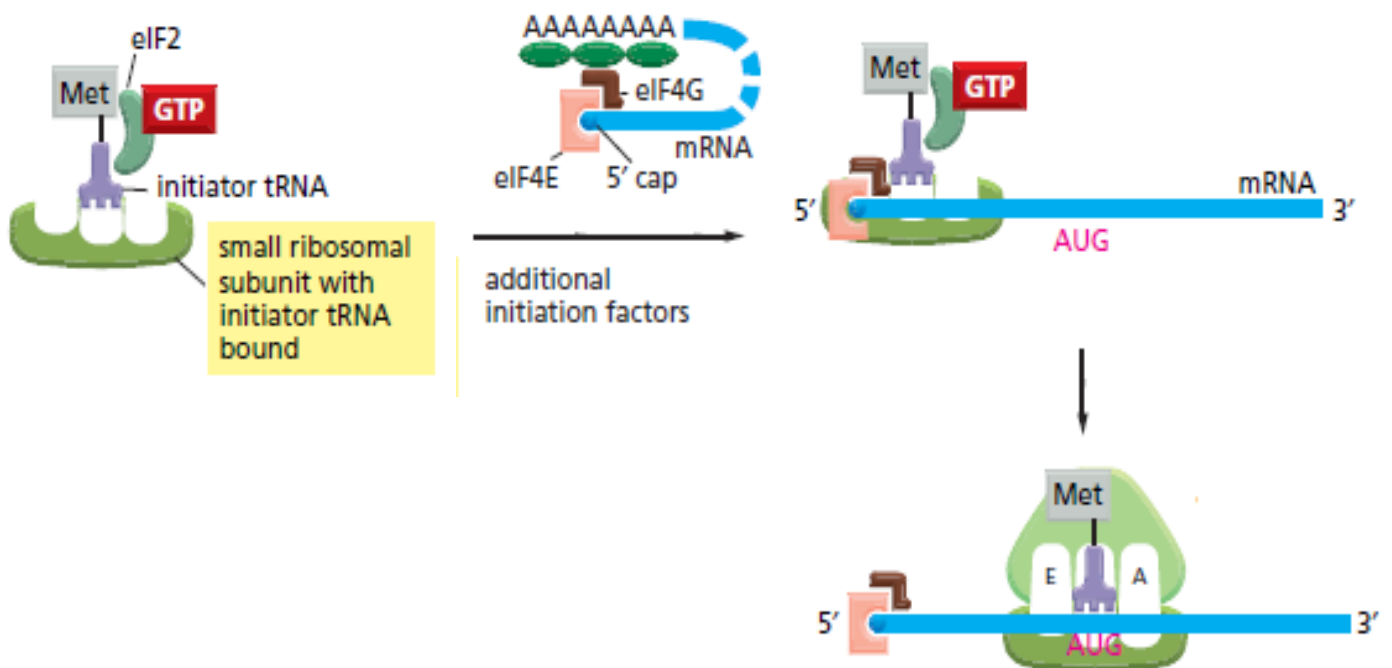


Slide: 32 "4. Translation Control" "Revision!"

- Initiation, elongation and termination.
- Regulation of translation initiation is one determinant of the rate at which any protein is synthesized.
- The mechanism of translation initiation is different between eukaryotes and prokaryotes.

Slide: 33 "4. Translation Control" "Revision!"

1. Assembly of 43S pre-initiation complex (PIC) facilitated by eukaryotic initiation factors (eIFs).
2. Activation of 5' end of mRNA by eIF4E and eIF4G.
3. Binding of PIC to start scanning for initiating AUG codon.
4. Dissociation of eIFs and binding of large ribosomal subunit.

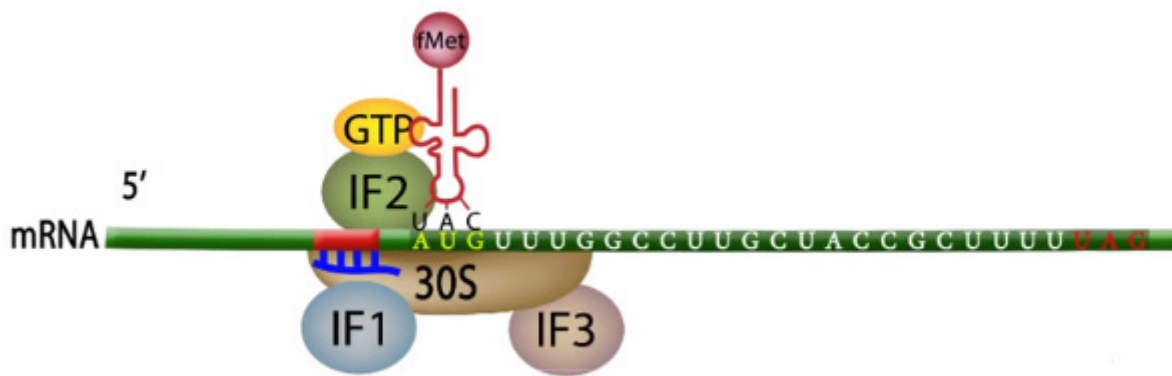


Slide: 34 "4. Translation Control" "Revision!"

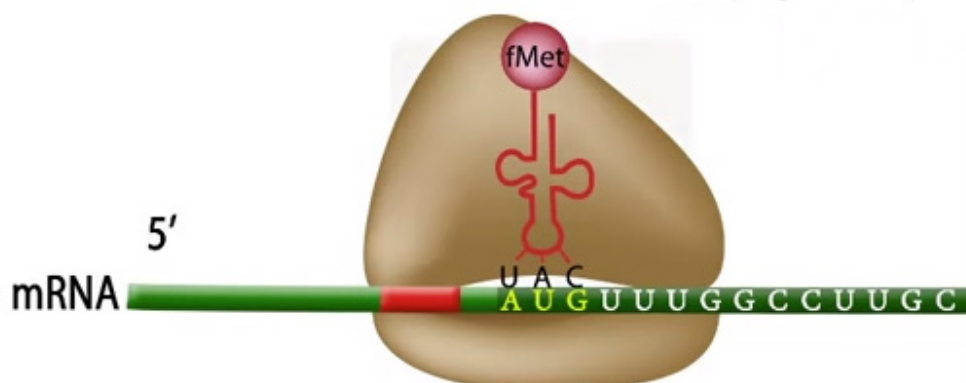
1. Binding of the small ribosomal subunit to the Shine Dalgarno sequence.



2. Recruitment of initiator tRNA carrying formylmethionine.



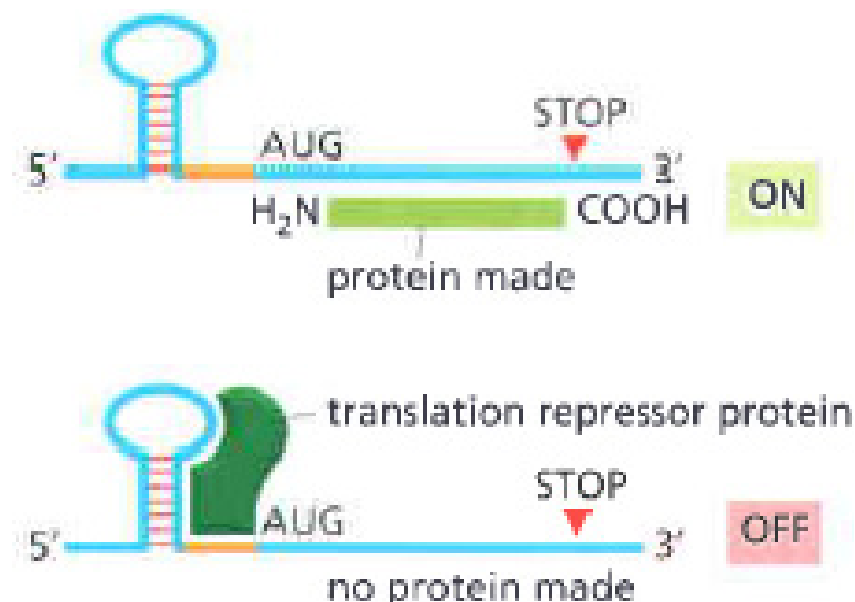
3. Dissociation of IFs and binding of large ribosomal subunit.



Slide: 35 "4. Translation Control"

- There are several mechanisms of translation initiation regulation:

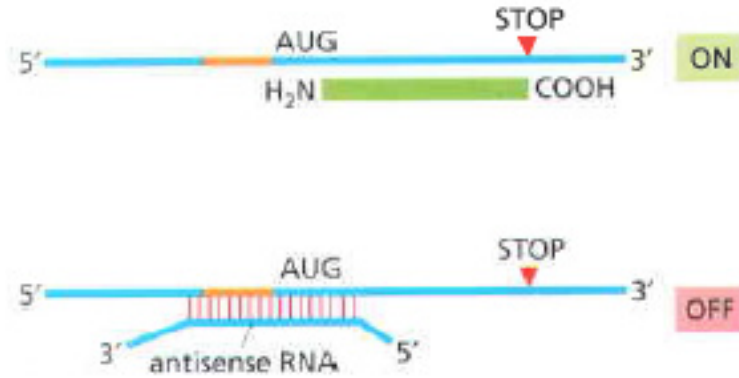
1. Translational repressor proteins: RNA-binding proteins which bind to and block the Shine-Dalgarno sequence. Similarly in eukaryotes, translational repressors bind 5' end of mRNA or 3' end and thus inhibit the translation initiation by interfering with the communication step between 5' cap and 3' poly A tail required for efficient translation initiation.



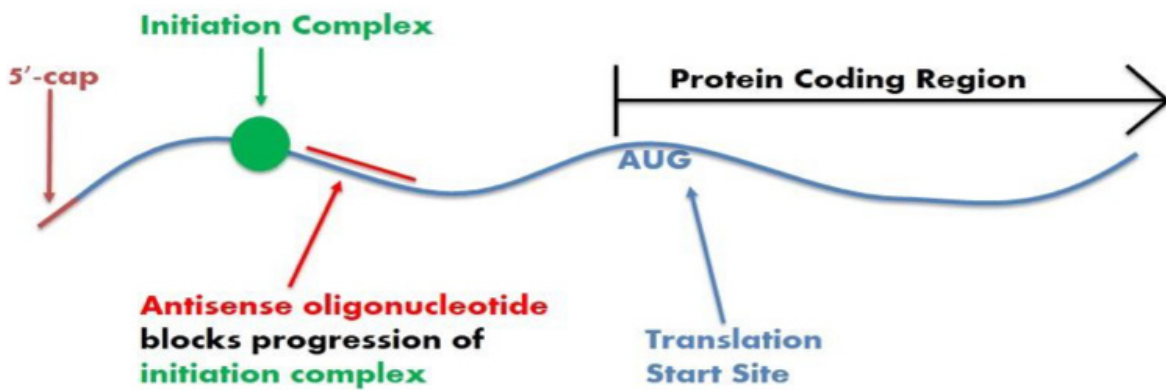
Slide: 36 "4. Translation Control"

2. Antisense RNA: a short RNA sequence (miRNAs) which binds specific complementary sequence of mRNA (near the AUG start codon) and blocks the translation initiation.

- miRNA varies from cell to cell "Tissue to Tissue".



• Morpholinos (synthetic molecules) are widely used in research as a technique for gene silencing.



طالما وصلت لهون، يعطيك ألف عافية بتمنى يكون الشرح تمام...

وع الأغلب ضيِّعت كل الوقت والامتحان بكرا وظايلك 15 محاضرة، فبالتوفيق يا رب 😂