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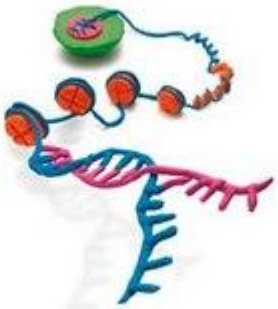
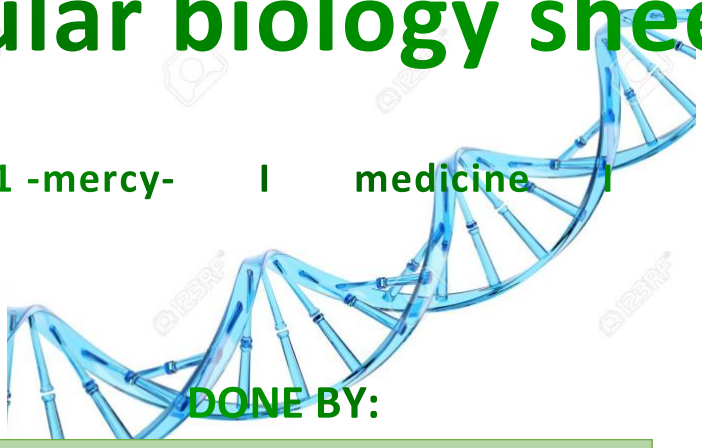
Molecular biology sheet

Doctor 2021 -mercy- I medicine I MU





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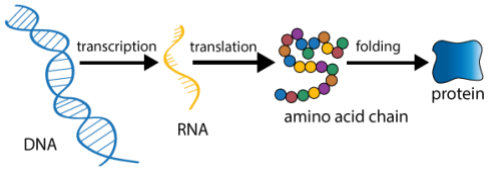
Regulation of Gene Expression

***All body cells have the exact same genetic material however; cells differ from each other due to gene expression**

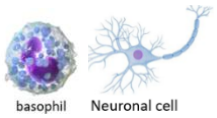
كل نوع من الخلايا يتم تفعيل جينات معينة واهمال التعبير عن باقي الجينات، مما يؤدي الى تمايز وظائف الخلايا على الرغم من تطابق المادة الوراثية

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

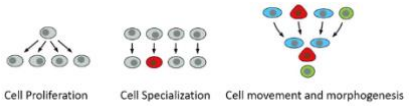
Gene expression: is the process by which information from a gene is used in the synthesis of a functional gene products:
either protein or functional RNA such as tRNA and rRNA



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



Gene regulation controls cell structure and function. It is the basis for cellular division (proliferation), differentiation (specialization) and morphogenesis



***Both cells: neuron and basophil contain the same genetic material.**

***Regulation of gene expression being once the zygote has been formed.**

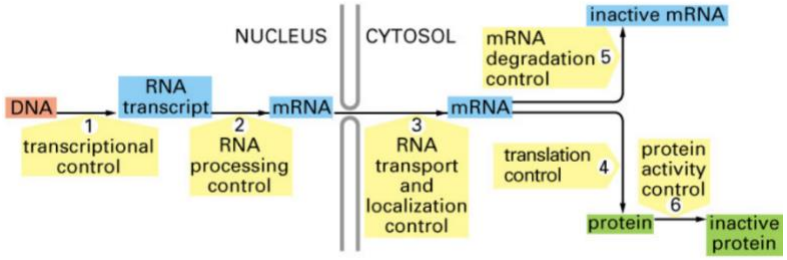
***morphogenesis=shaping of 1-cell 2-tissue 3-organism**

Different cell types synthesize and accumulate different sets of RNA and proteins (Hemoglobin in RBCs)
Also, the level of expression of almost every active gene varies from one cell type to another 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**

***Hemoglobin gene is silenced in other cells rather than RBCs.**
***In addition, level of gene expression depends on timing.**
***Gene expression is spacial temporal process عملية تتأثر بالوقت و المكان.**

Steps in Eukaryotic Gene Expression regulation



Prokyrotic gene expression regulation is done in steps 1&4.

Step 2 is mainly splicing

Step 5 is done by microRNAs

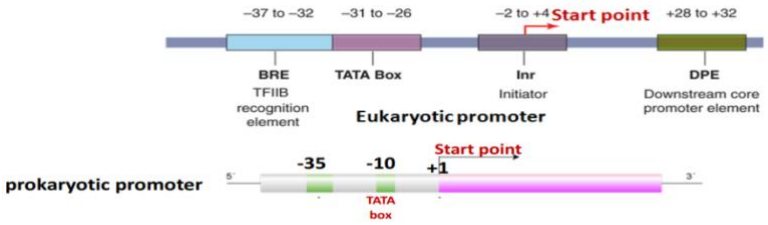
***Folding of protein is required to synthesize a functional protein.**

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.



***The best stage to silent a specific gene is transcriptional control.**

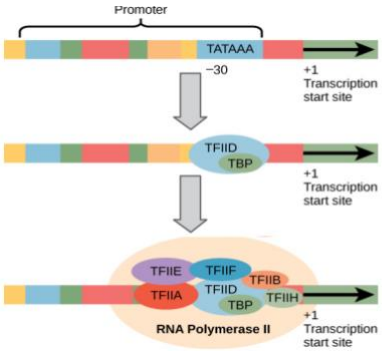
***RNA polymerase = 3types in eukaryote and 1 type in prokaryote.**

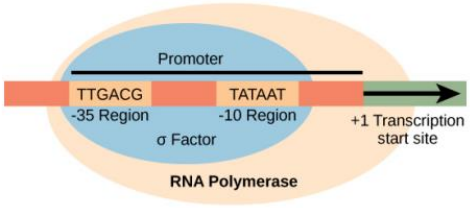
***Promoter can be downstream the genes such as DPE.**

***DPE may be present or absent in a certain promoter.**

***DPE is located in intronic sequences so it is found in primary transcript but not in mature mRNA.**

Transcription initiation in eukaryotes	Transcription initiation in prokaryotes
1. It requires general transcription factors which assemble together with RNA polymerase at the promoter to form pre-initiation complex (PIC)	1. σ factor recognizes the -35 region in the promoter and binds to it
2. TFIID binds first at the TATA box via its TBP subunit	2. Once the RNA polymerase starts the transcription, σ factor then dissociates to guide another enzyme to the initiation site





***TFIIA is not found with all genes.**

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

***activators activate the gene**

Repressors switch off a gene

Regulatory region is found along **noncoding DNA**

Enhancers for activator

Silencers for repressors

***Enhancers and silencers are located upstream the gene prior to promoter by thousands of bases pare.**

*Specific transcription factors are DNA regulatory proteins (not RNA regulatory proteins)

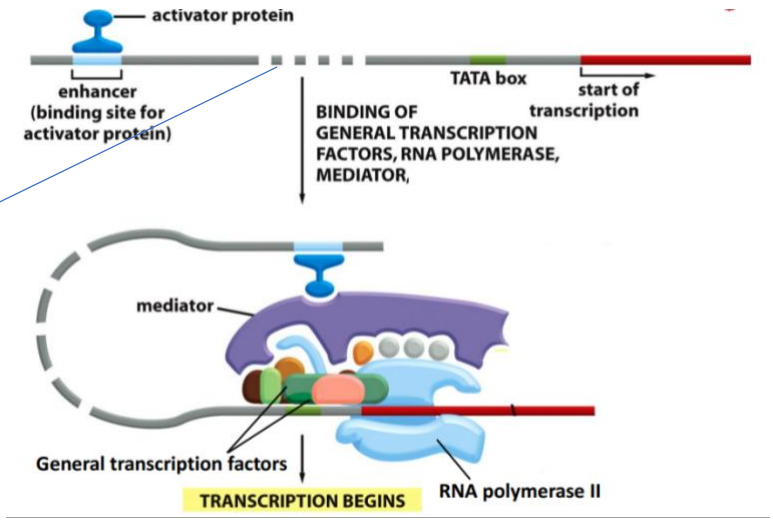
* specific transcription factors are:

1-numerous in number

2-differ from one cell to another

Indicates thousands of b.p

*Although enhancers and repressors are located away from the gene, they regulate gene expression because of DNA looping (shortens the real distance between enhancer and gene.)

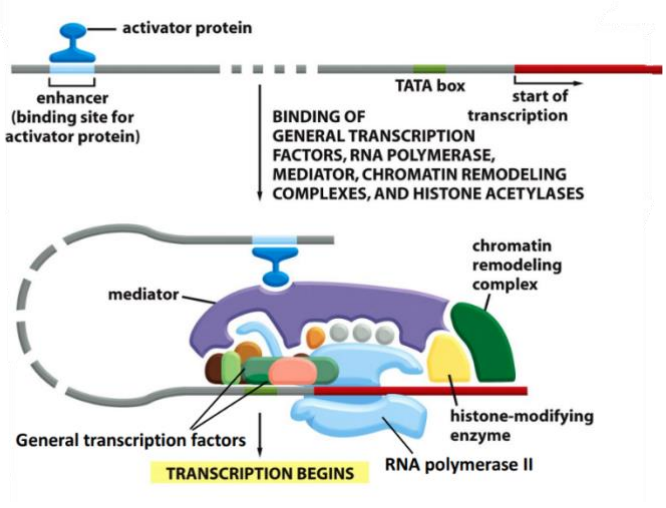


• **Cis-regulatory elements (CREs): are regions of non-coding DNA which regulates the transcription of nearby genes. These include promoters, enhancers, and silencers**

• **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** with pre-initiation complex.

*All CRE may contain parts downstream the gene.



- Chromatin remodling complex and hisone modifying enzyme Affect the structure of chromatin→ making the gene less or more condensed

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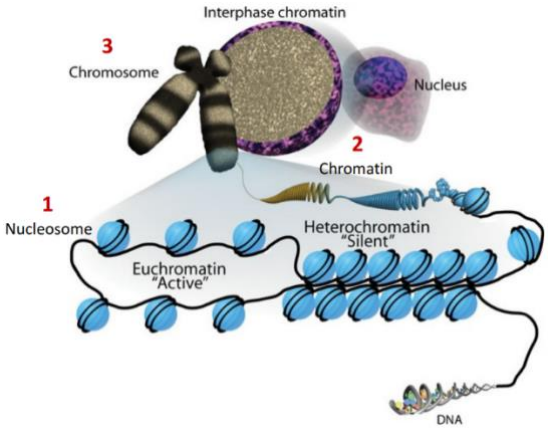
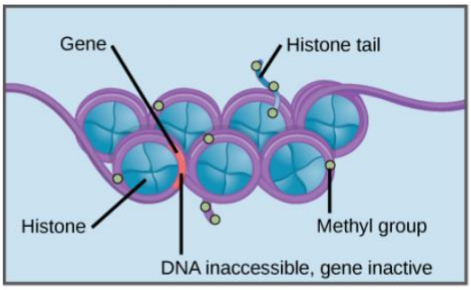


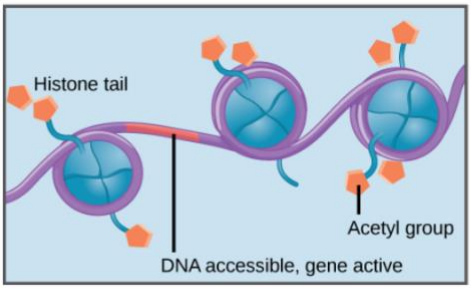
Euchromatin = accessible for RNA polymerase



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

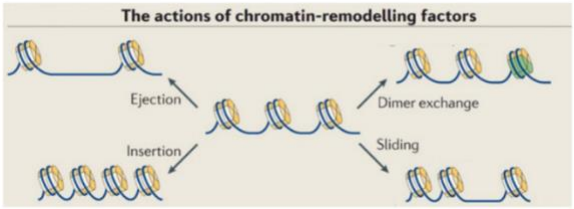
***such as: histone methylation enzyme**

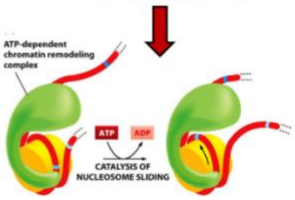


Histone acetylation results in loose packing of nucleo-somes. Transcription factors can bind the DNA and genes are expressed.

recruited by activator

***Such as histone acetylation enzyme**





Ejection=removal of histone

Insertion=adding histone

Dimer exchange= exchange apart of histone

to modify it is structure

Sliding= repositioning or moving certain histone

• 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)

***epigenetic factors are Proteins able to change chromatin structure.**

***Regulation of transcription initiation in eukaryotes is done by contact of eithers enhancers or repressors with pre: initiation complex: -directly -via mediator -via mediator beside of epigenetic factors.**

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 (bicistronicity or polycistronic transcript)**

• **Operator: a segment of regulatory DNA to which a repressor can bind to regulate the transcription of downstream target genes**

• **The three basic DNA components of operon:**

1. Promoter

2. Operator

3. Structural genes

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

***Operator=resemble silencer in eukaryotes.**

***Absence of certain nutrient in the surrounding environment of prokaryote activates specific genes to produce that nutrient.**

***Operator--→ downstream the promoter and upstream the gene.**

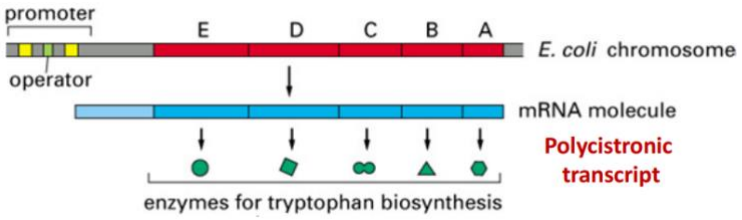
***Operator→ can be found sometimes within the promoter.**

***Trp=tryptophan**

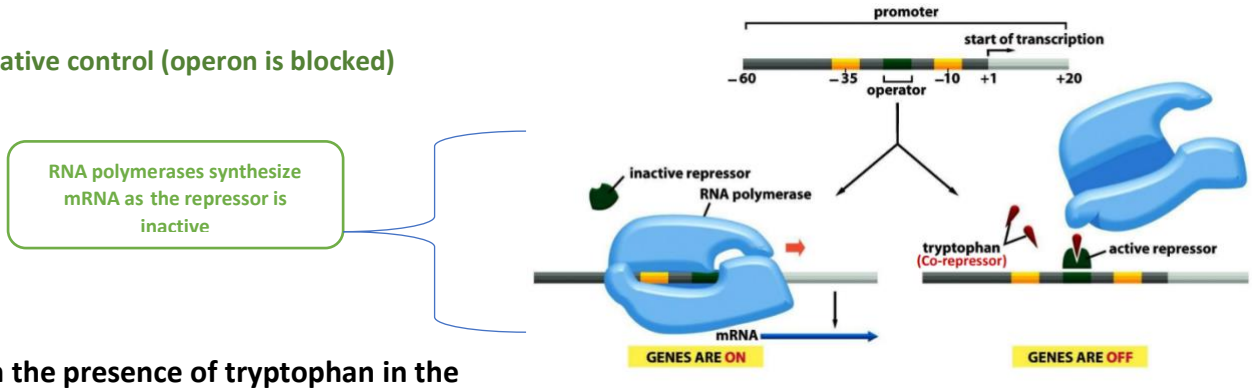
***Lac=lactose.**

Trp Operon

• **Trp operon consists of five structural genes required for the biosynthesis of the amino acid tryptophan**



negative control (operon is blocked)



• 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

Lac Operon

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

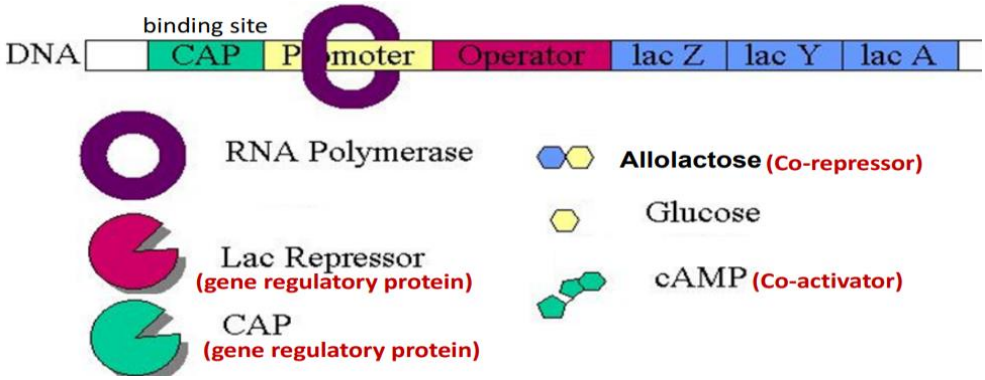
1. lacZ: encodes β-galactosidase which cleaves lactose into glucose and galactose

2. lacY: encodes lactose permease to transport lactose into the cell

3. lacA: encodes galactoside O-acetyltransferase which plays a role in cell detoxification

النقاط 3 غير مطلوب

*Glucose is the major food source of bacteria. however, lactose work as alternative of glucose if glucose is absent.



*Lac operon is under negative and positive control (dual control)

*Lac operon activity depends on both –glucose and lactose.

- Lac Repressor is active repressor binds with operator.

-low glucose levels raise level of cAMP.

- CAP resembles activators in eukaryotes

*Generally, promoters in prokaryotes are weak, therefore, high levels of transcription require activators to stabilize binding of RNA polymerase with the promoter.

-cAMP activates CAP to bind with CAP binding site

الخطوات العامة

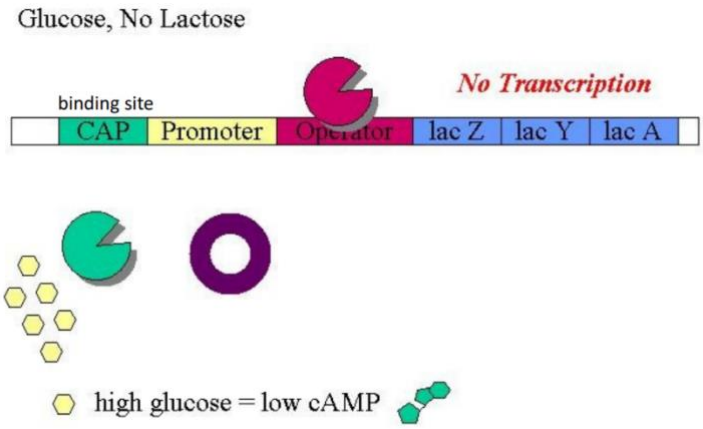
1-presence of lactose in media

2-alteration of some lactose moieties into allolactose (isomer of lactose)

3-allolactose binds with lac repressor (inactivation of lac repressor)

4-inactive lac repressor cannot bind with operator.

5-operon is expressed …



الخطوات

1- No lactose-→active lac repressor

2- Binding of repressor with operate

3- High glucose→low cAMP

4- Low cAMP -→inactive CAP

5- No binding of CAP with CAP binding site

6- No transcription--→no metabolism for lactose

7-Using glucose as main source of energy.

الخطوات

1-binding of lac repressor with operator

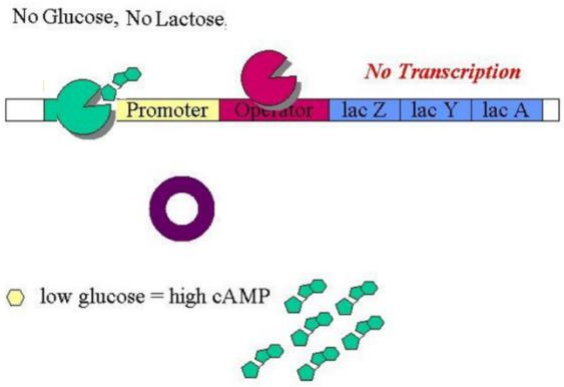
2-low glucose-→high cAMP

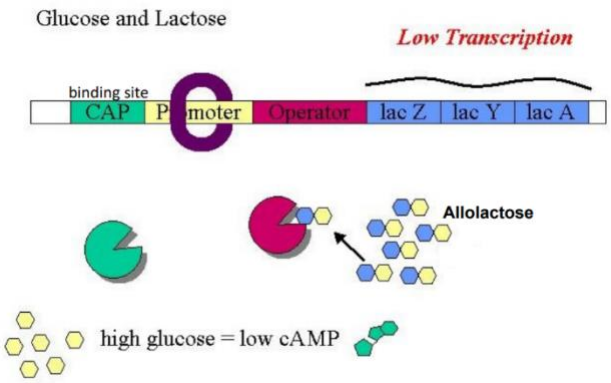
3-high cAMP-→active CAP

4-binding of CAP

5-No transcription

6-no metabolism for both glucose or even lactose.





الخطوات

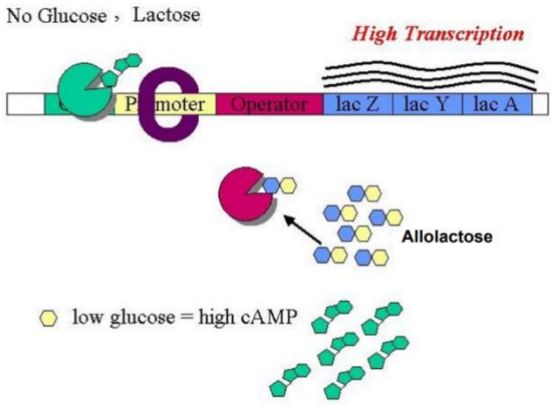
1-binding of allolactose with lac repressor

2-inactive lace repressor

3-low cAMP -→inactive CAP

4-low transcription as CAP is not bounded prior to promoter

5-metabolism of both in glucose and lactose



*Optimum condition for expression of lac operon.

*الخطوات

1-inactive lac repressor

2-free operator

3-high cAMP -→ binding of CAP

4-high levels (CAP is attached to CAP binding site) of transcription (operator is free)

5-no glucose to be utilized lactose is metabolism

• 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, and it acts as inducer of lac operon Only in presence of lactose.

• 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

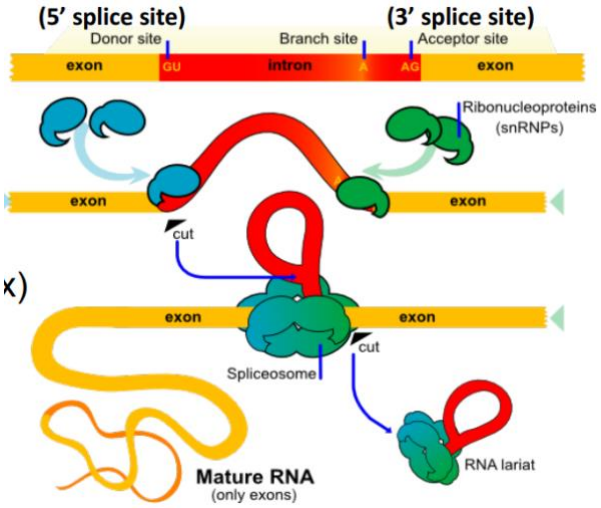
• Allolactose is an inducer of lac operon (regardless of presence or absence of glucose). It acts as corepressor of lac repressor protein

*Isoforms are the different forms of proteins which result from alternative splicing in different tissues, yet they are synthesized from the same gene.

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).

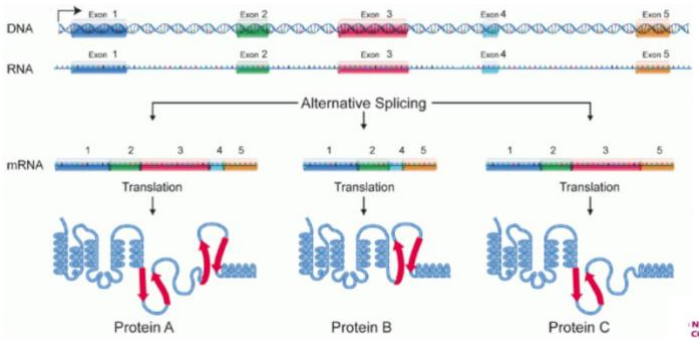


*Alternative splicing may miss an intron which results in pre-mature termination of protein synthesis forming a truncatedمقطوع inactive protein.

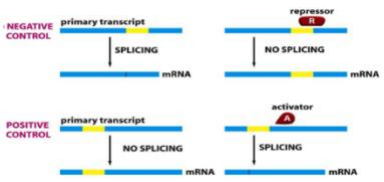
*(activators/repressors)→ RNA binding proteins

*Repressor → cover the splicing site

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



• Many genes undergo alternative splicing in tissue-specific manner and/or under specific cellular conditions (a protein is nonfunctional 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.

• 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 result 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.

3.RNA Transport & Localization Control

• Gene expression can be regulated by controlling the nuclear transport

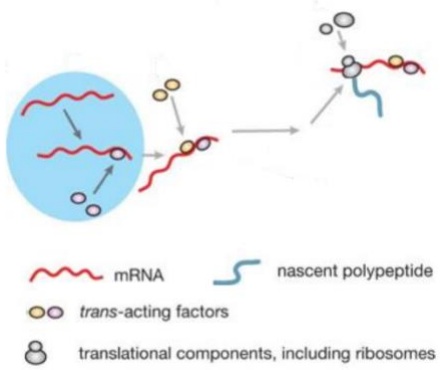
of mRNAs and their localization to specific cytoplasmic domains

• 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)

4. Translation Control

• 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

Translation Initiation in Eukaryotes

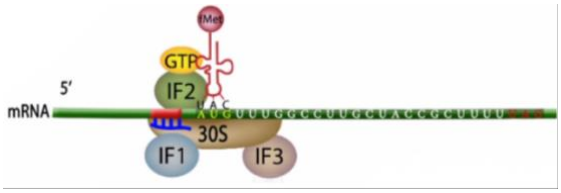
*The most controlled stage of translation in gene expression.

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

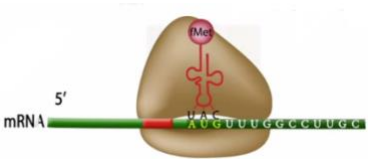
Translation Initiation in Prokaryotes

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

2. Recruitment of initiator tRNA carrying formyl methionine



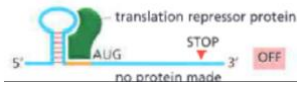
3. Dissociation of IFs and binding of large ribosomal subunit



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 thus inhibit the translation initiation by interfering with the communication step between 5’ cap and 3’ poly A tail required for efficient translation initiation**



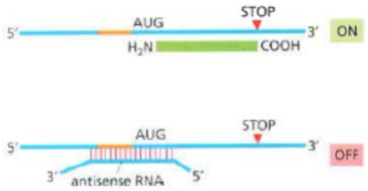
mRNA is sense sequence

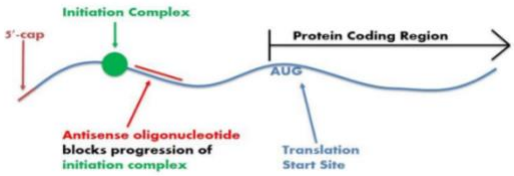
***miRNAs length extends from 21→27 nucleotide**

***Each cell type contains distance miRNA from other cell type.**

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**

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





"التوكل قوّة؛ ولو جُمعت للمرء أشكال المواساة وألوانها فلن يجد شيئًا يمسح على قلبه ويقوّي أركان طمأنينته مثل تفويض أمره لله واستشعاره أنه في ظلال معيّة الله وأن الله كافيه أمره "