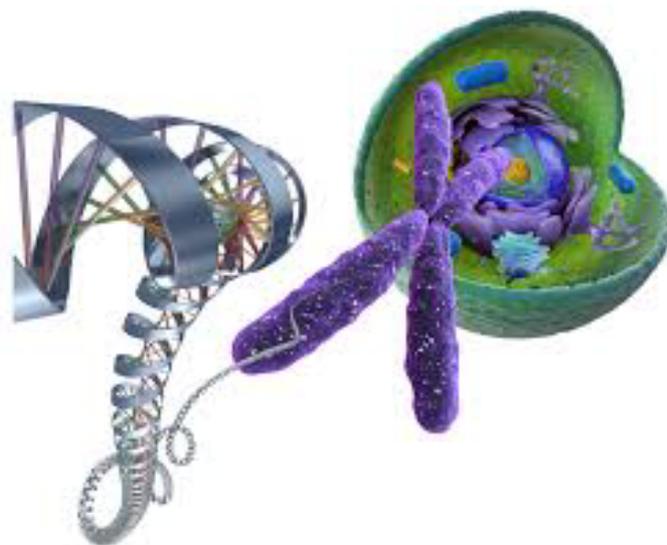




Regulation of Gene Expression



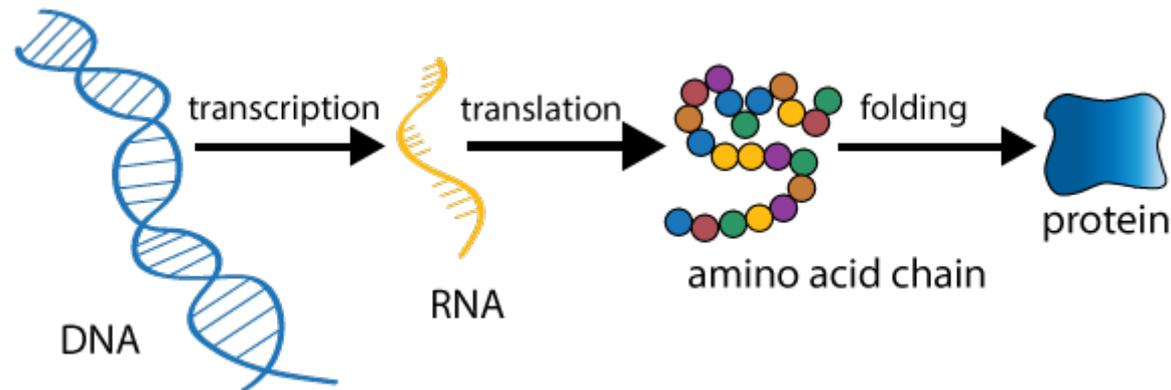
Dr. Nesrin Mwafi

Biochemistry & Molecular Biology Department
Faculty of Medicine, Mutah University

Gene Expression



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

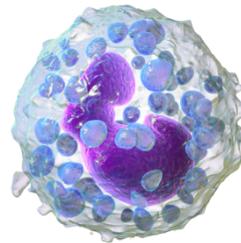


- Gene expression: is the process by which information from a gene is used in the synthesis of a functional gene products: either protein or RNA such as tRNA and rRNA

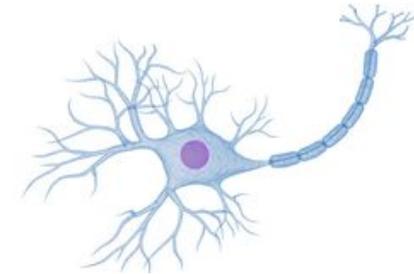
Gene Expression



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

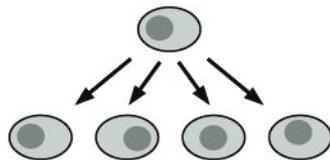


basophil

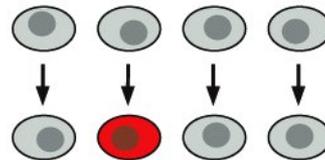


Neuronal cell

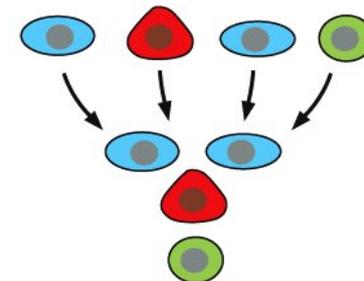
- Gene regulation controls cell structure and function. It is the basis for cellular division, differentiation and morphogenesis



Cell Proliferation



Cell Specialization



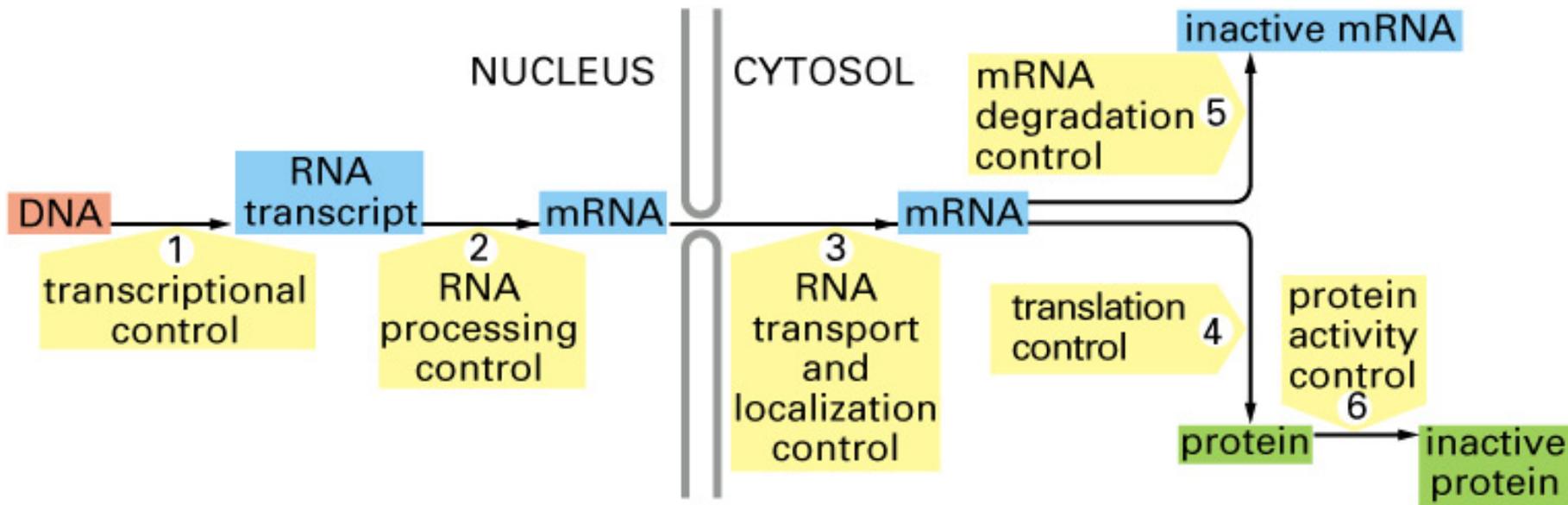
Cell movement and morphogenesis

Gene Expression



- 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

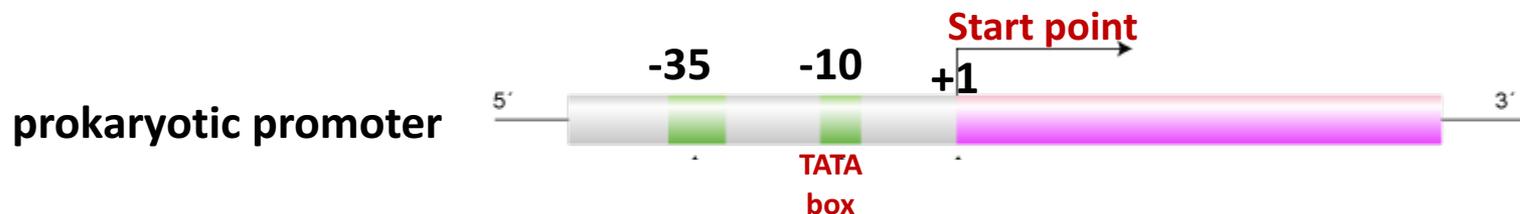
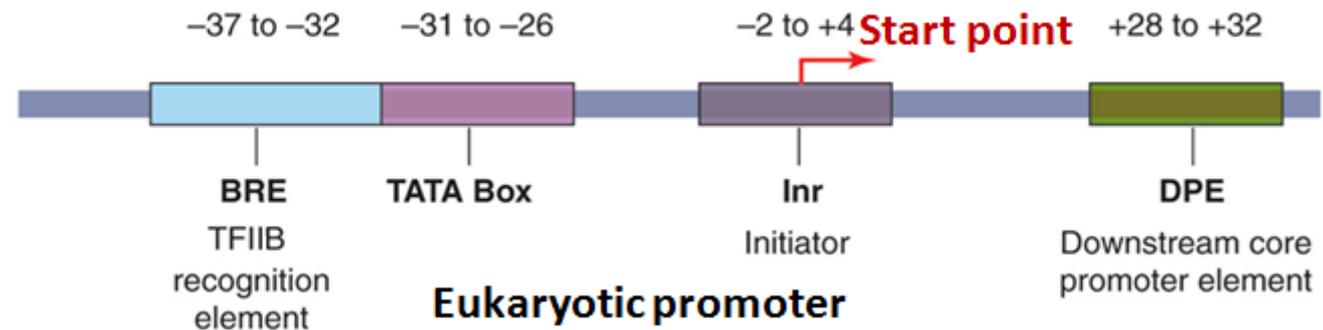
Steps in Eukaryotic Gene Expression regulation





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.

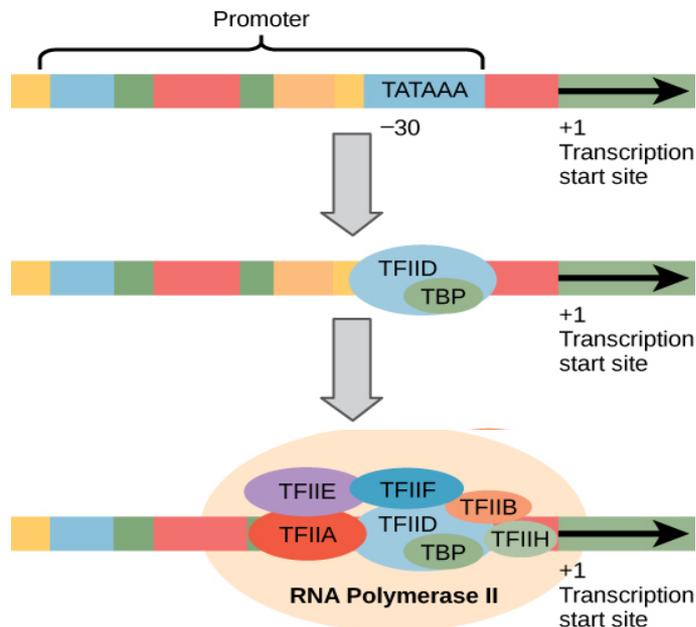


1. Transcription Initiation



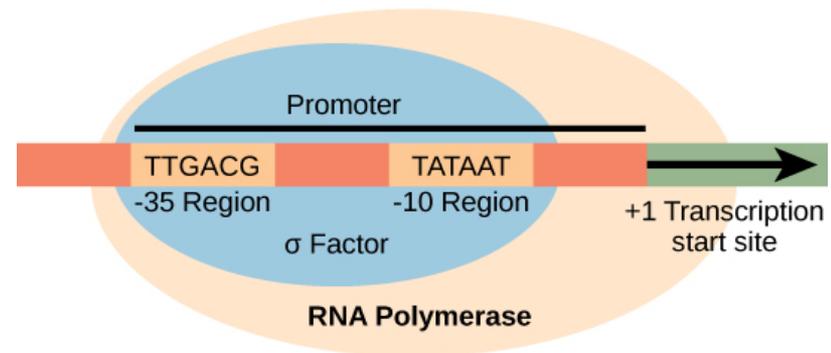
Transcription initiation in eukaryotes

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

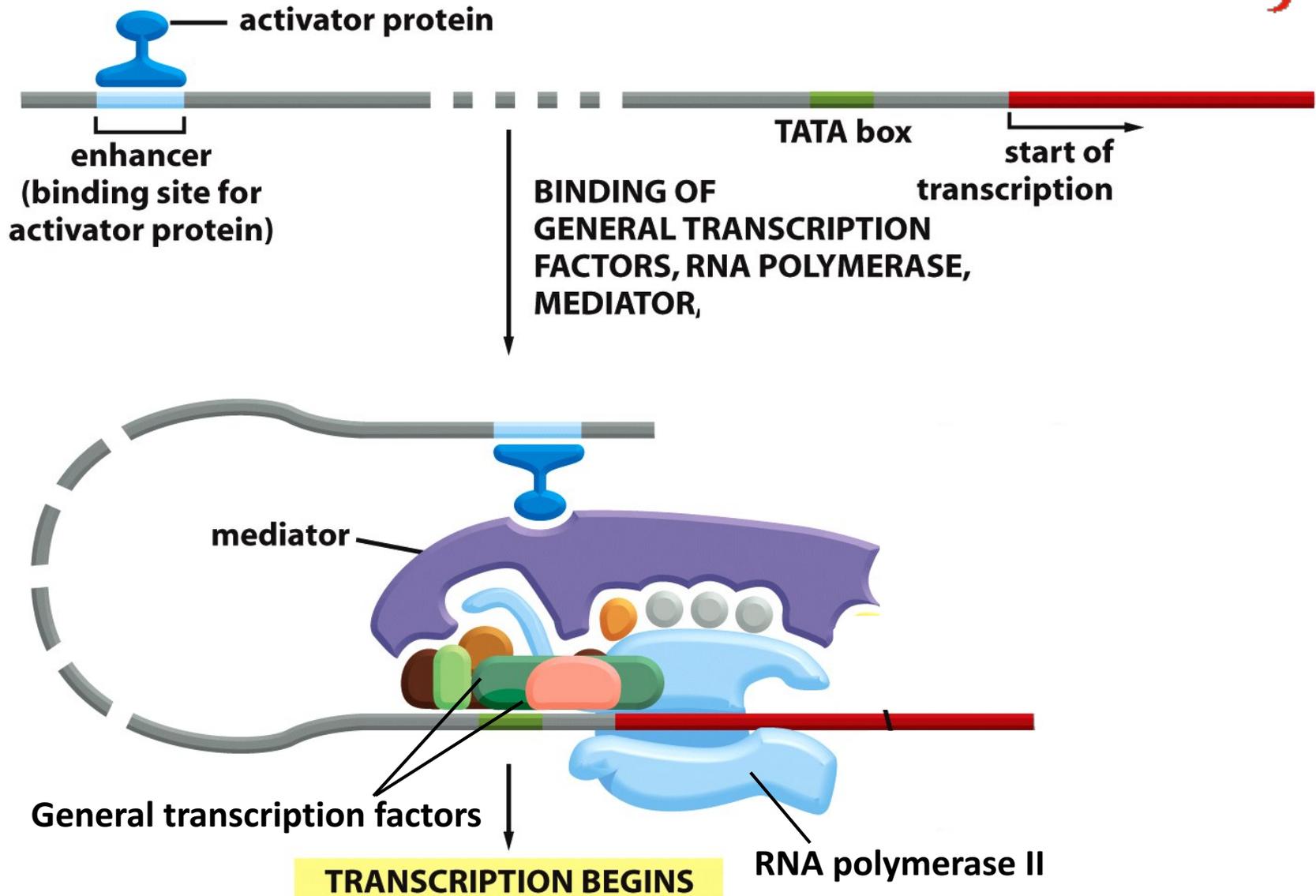


Regulation of Transcription Initiation



- 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***

Regulation of Transcription Initiation

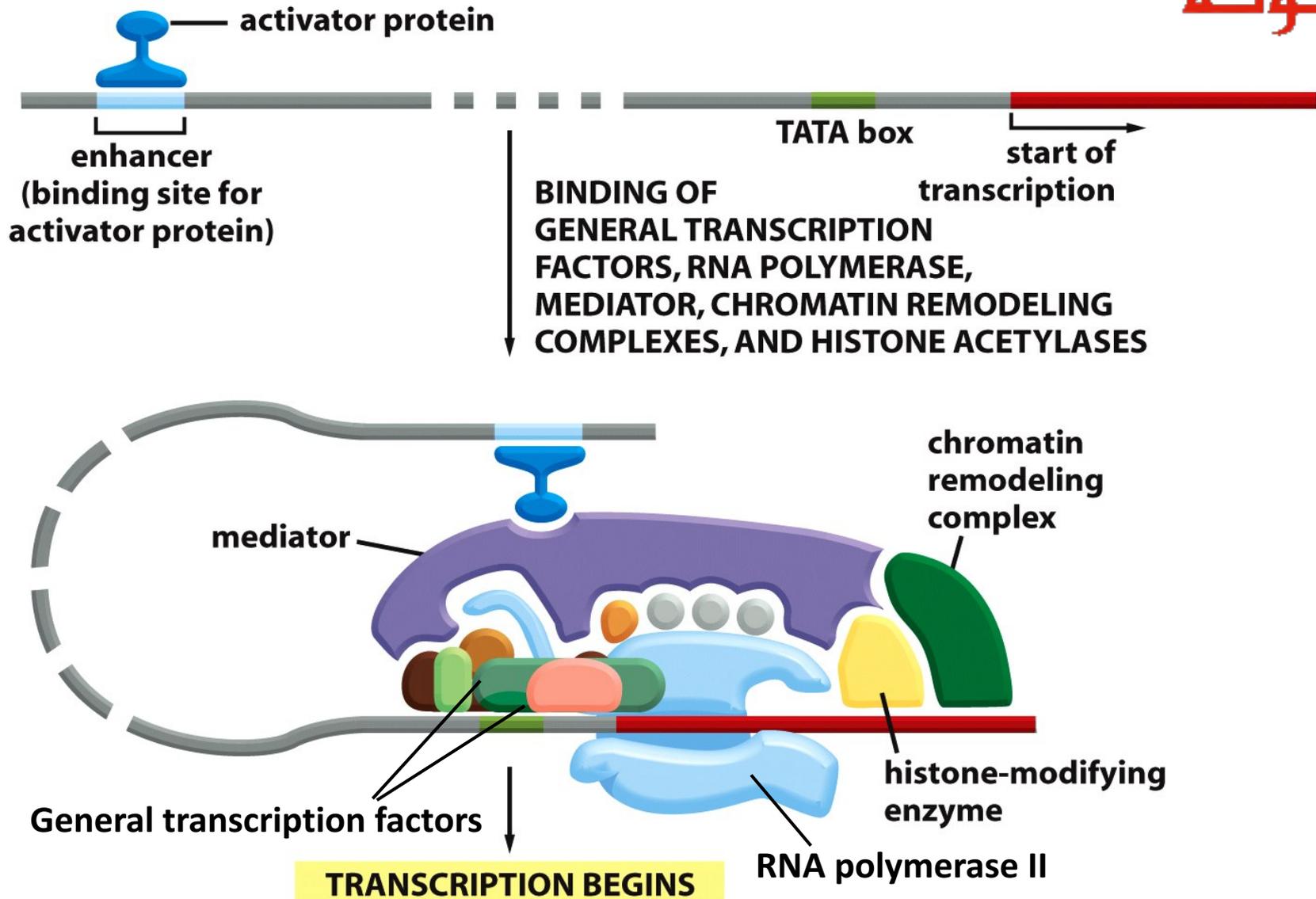


Regulation of Transcription Initiation

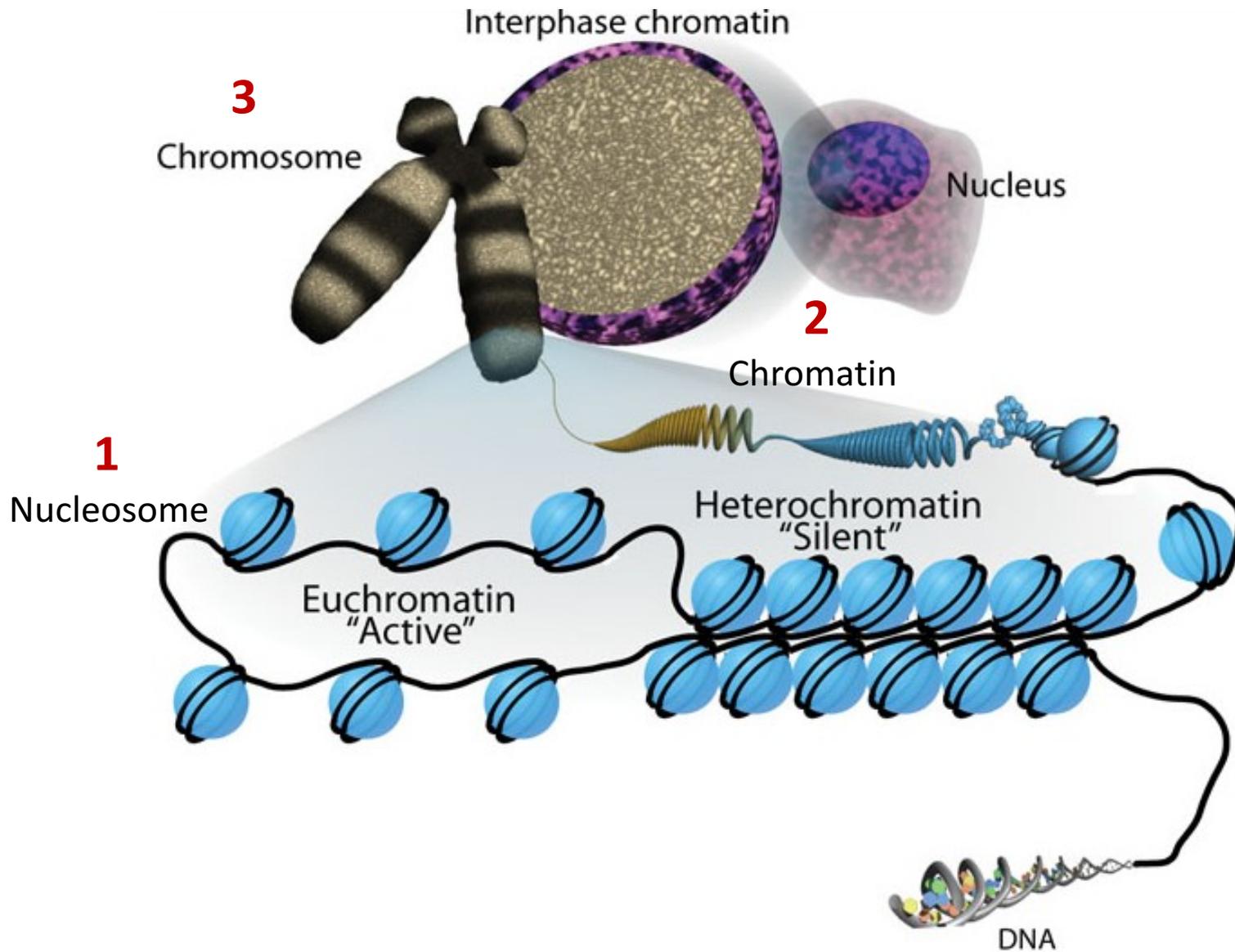


- Cis-regulatory elements (**CREs**): are regions of non-coding DNA which regulate the transcription of nearby genes. These include promoters, enhancers and silencers
- Gene regulatory proteins act at distance: DNA looping allows them to interact with the assembled proteins at the promoter
- Mediator is a protein complex recruited to the promoter via specific transcription factors. It provides extended contact area for the gene regulatory proteins

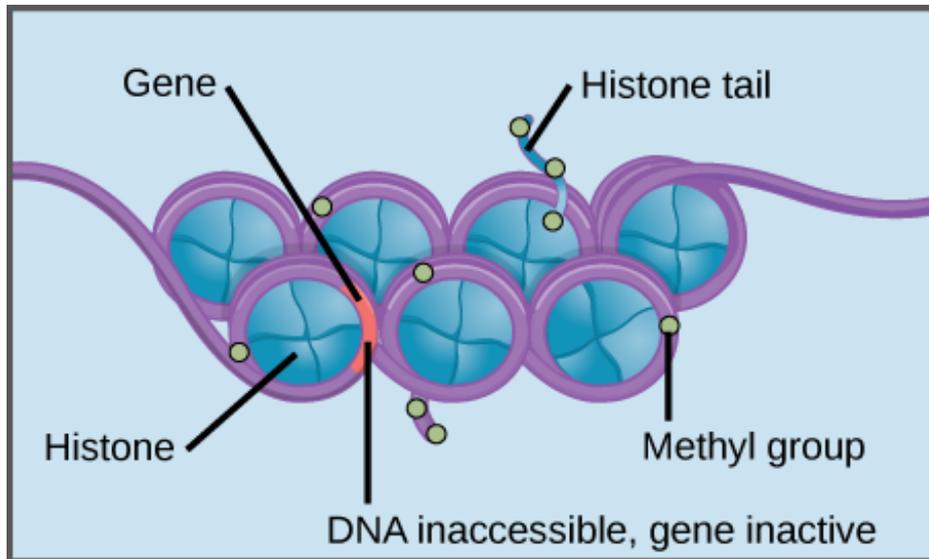
Regulation of Transcription Initiation



Regulation of Transcription Initiation

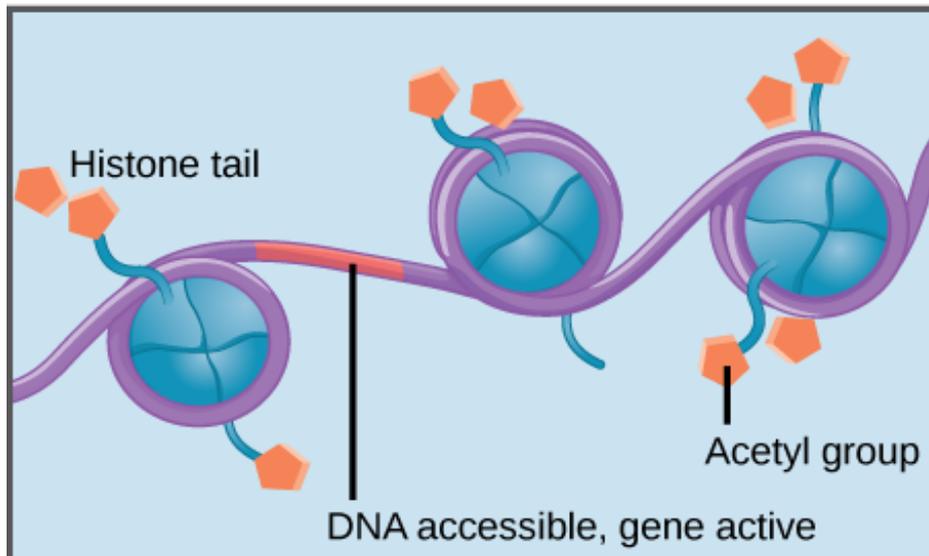


Regulation of Transcription Initiation



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

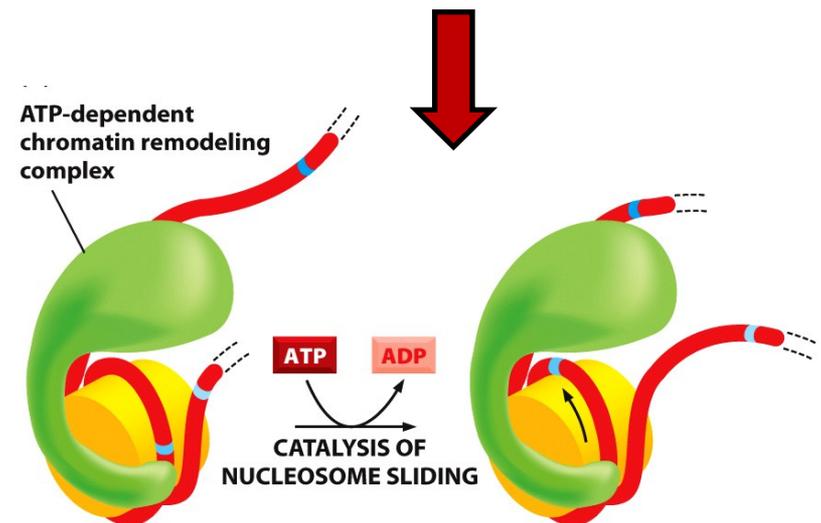
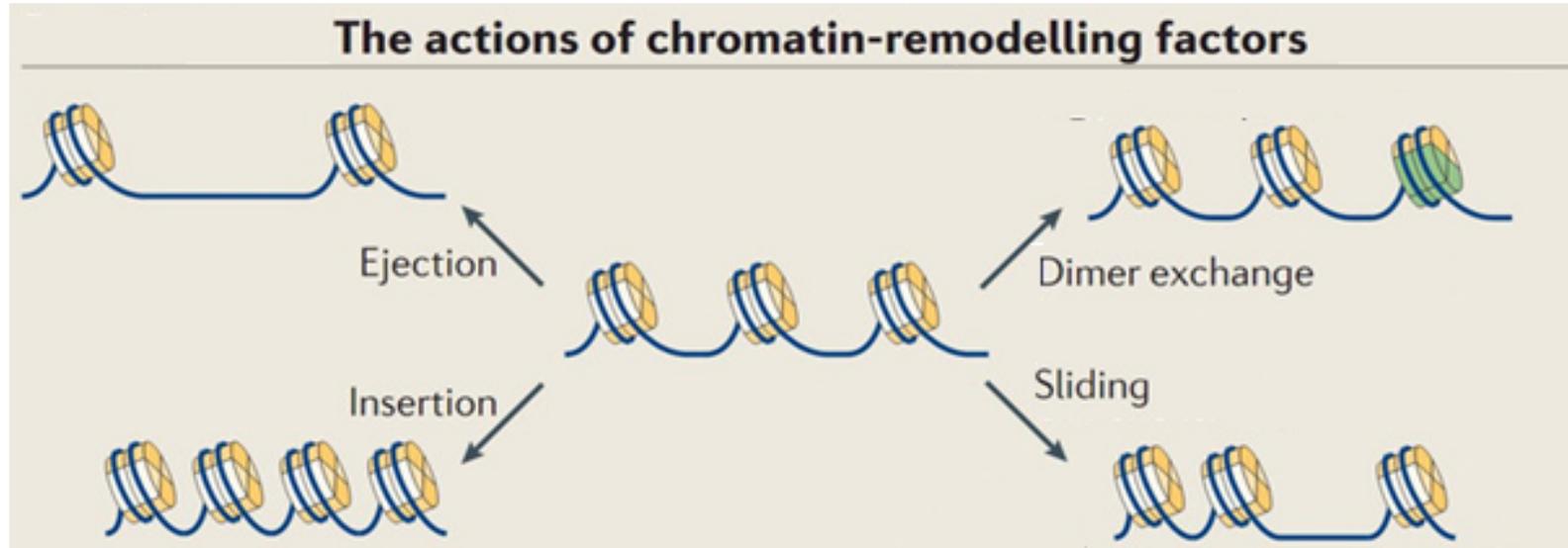
recruited by repressor



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

recruited by activator

Regulation of Transcription Initiation



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)

Regulation of Transcription Initiation in Prokaryotes

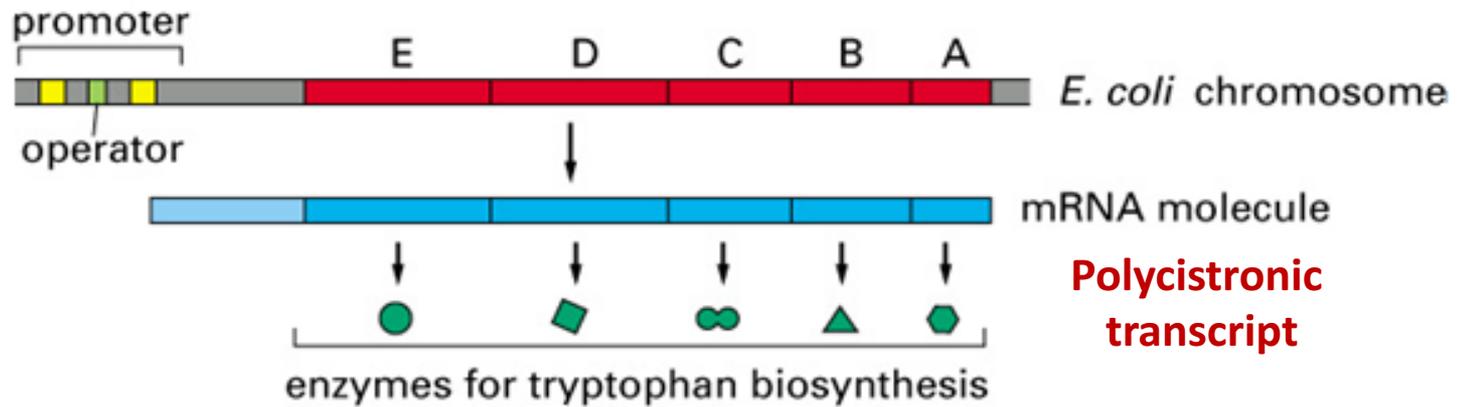


- The expression of many genes is regulated according to the available food in the environment
- **Operon:** DNA unit consists of a cluster of related genes controlled by single promoter and transcribed together into single mRNA strand (bicistronic or polycistronic transcript)
- **Operator:** a segment of regulatory DNA to which a repressor can bind to regulate the transcription of downstream target genes
- The three basic DNA components of operon:
 1. **Promoter**
 2. **Operator**
 3. **Structural genes**
- Examples in *E-coli* bacteria: **Trp operon** and **Lac operon**

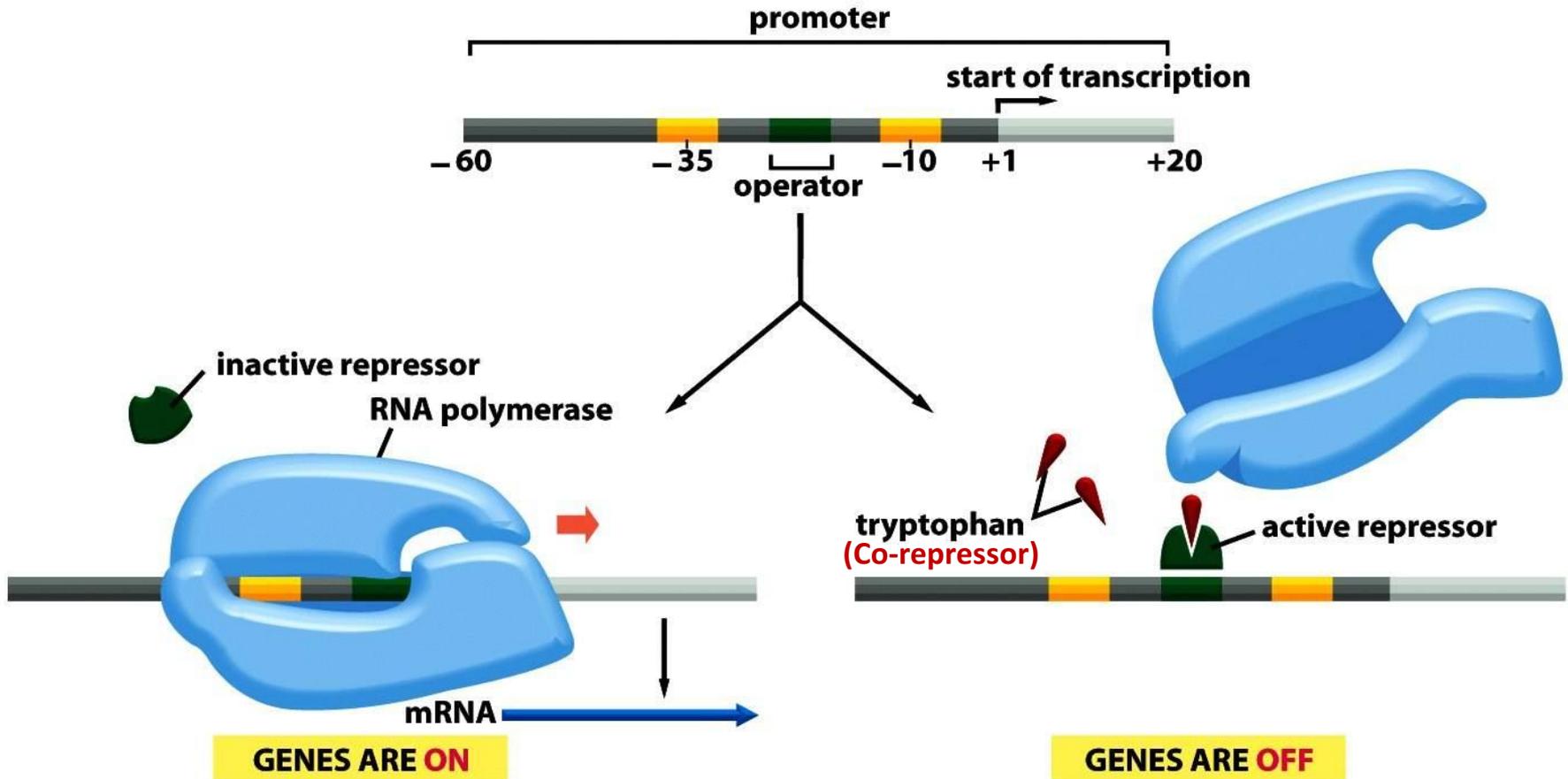
Trp Operon



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



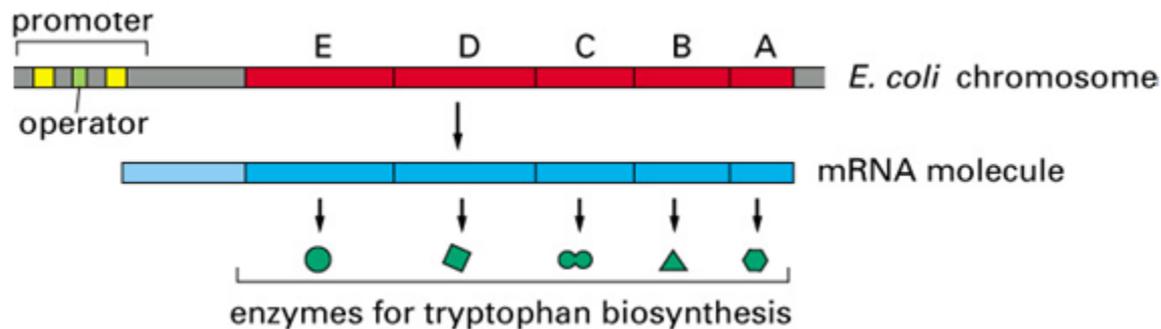
Trp Operon



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

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

Lac Operon



RNA Polymerase



Allolactose (Co-repressor)



Lac Repressor
(gene regulatory protein)



Glucose



CAP
(gene regulatory protein)

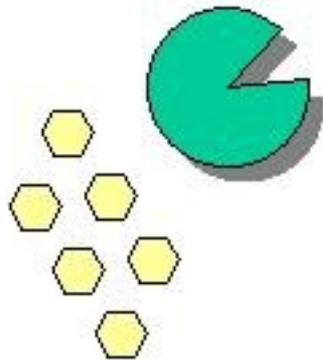


cAMP (Co-activator)

Lac Operon



Glucose, No Lactose



high glucose = low cAMP



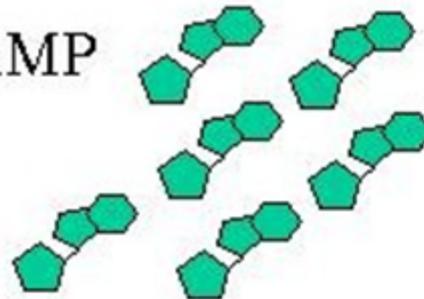
Lac Operon



No Glucose, No Lactose.



low glucose = high cAMP

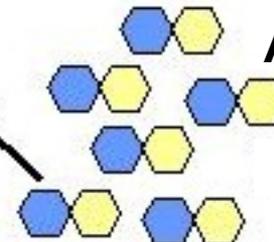
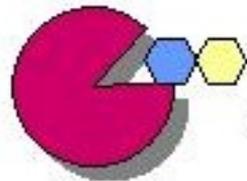


Lac Operon

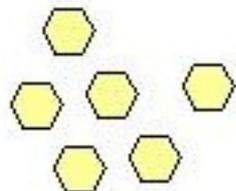


Glucose and Lactose

Low Transcription



Allolactose



high glucose = low cAMP

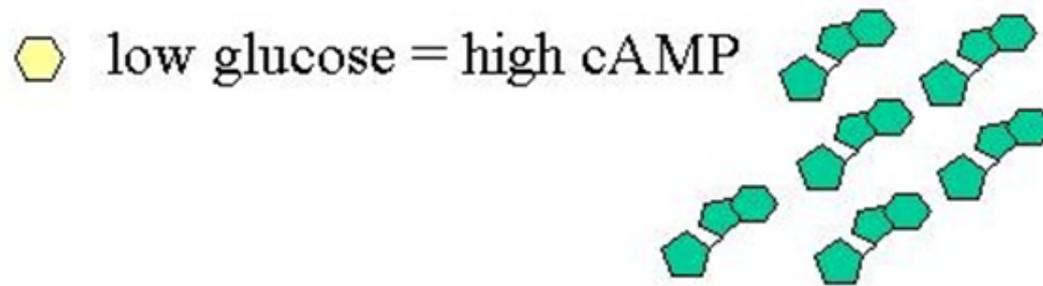
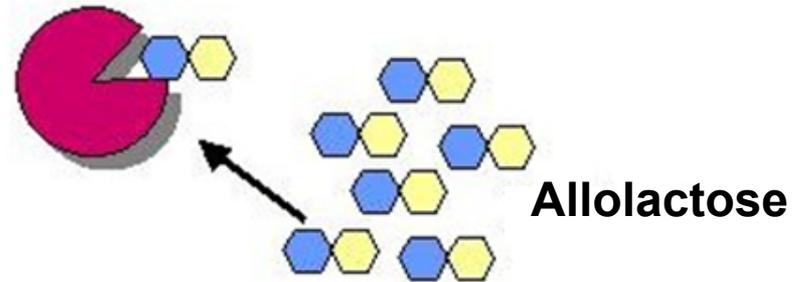


Lac Operon



No Glucose , Lactose

High Transcription



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 and it acts as **inducer** of lac operon Only in presence of lactose

Lac Operon



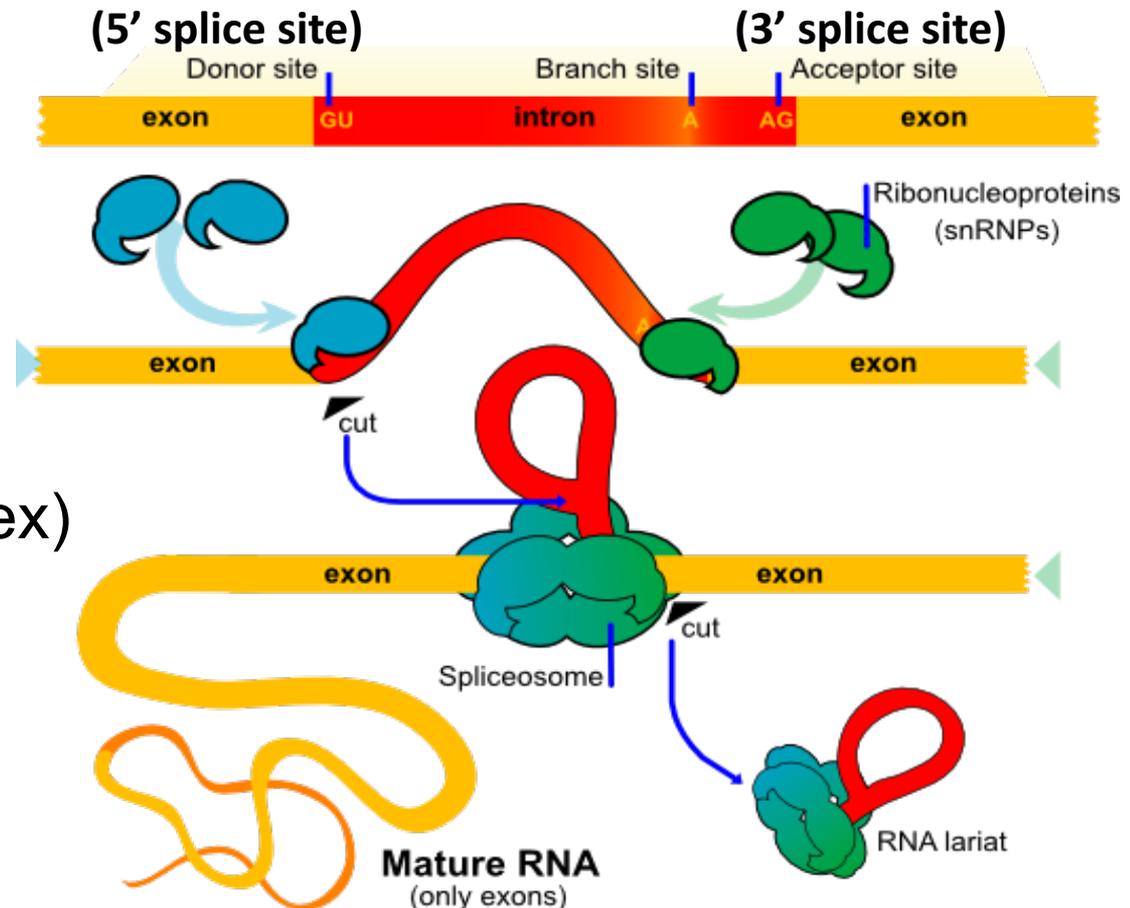
- In presence of lactose, the lac repressor is inactivated by the binding to lactose metabolite “allolactose” so the repressor dissociates from the operator with the genes are weakly transcribed in the presence of glucose but extensively transcribed in the absence of glucose
- Allolactose is an **inducer** of lac operon (regardless of presence or absence of glucose). It acts as co-repressor of lac repressor protein

2. RNA Splicing Control



- The post transcriptional modification (5'-capping, splicing and 3' polyadenylation) transfers **pre-mRNA** into the mature transcript **mRNA (Eukaryotes only)**

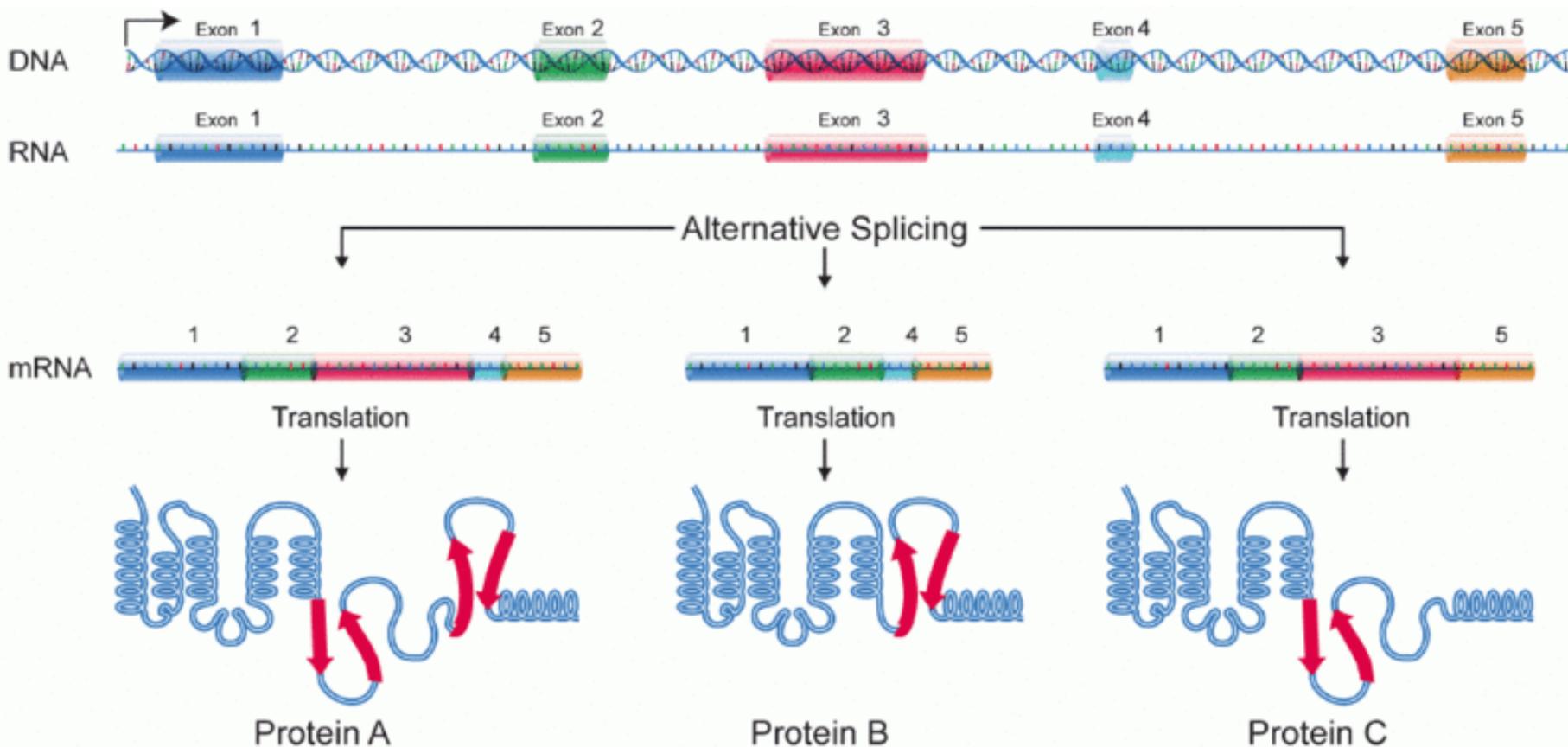
- RNA splicing is the removal of introns and the joining of exons catalyzed by spliceosome (RNA-protein complex)



2. RNA Splicing Control



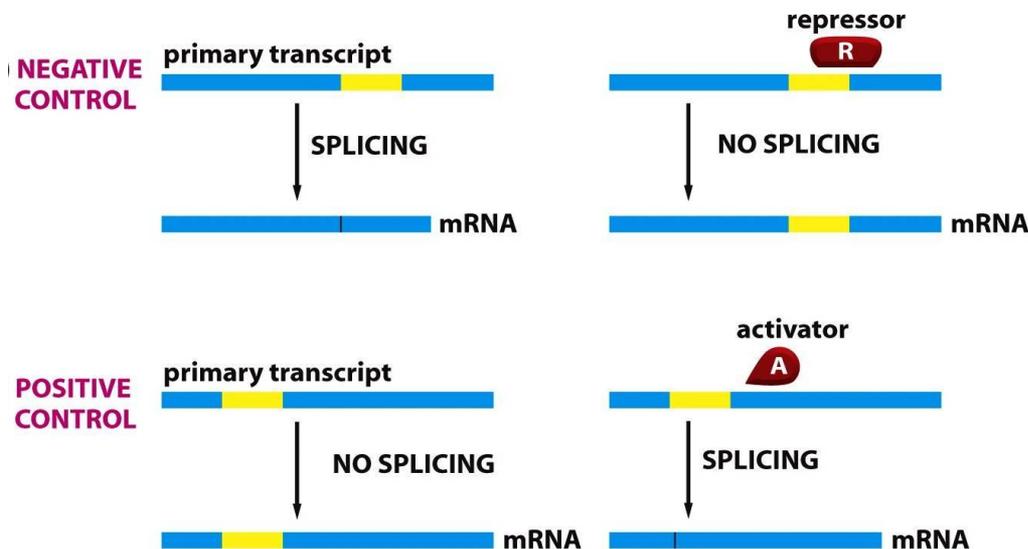
- Alternative RNA splicing (splicing at different junctions) produces different forms (isoforms) of a protein from the same gene



2. RNA Splicing Control



- 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



2. RNA Splicing Control

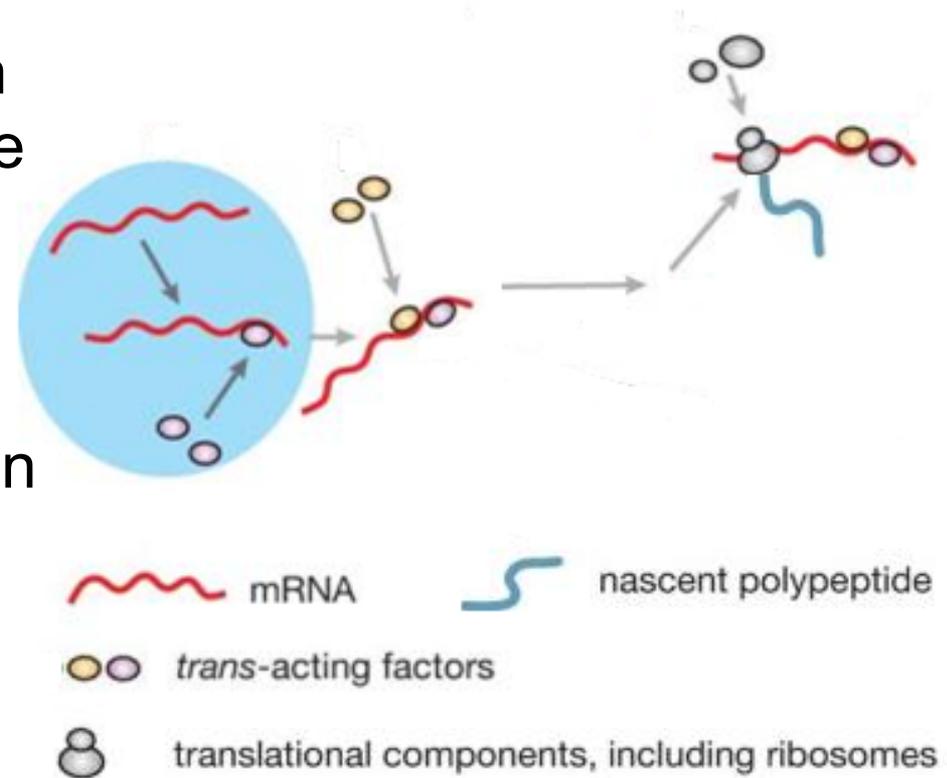


- Alternative RNA splicing is a phenomenon refers to the process by which a single pre-mRNA of a given gene is spliced into different mRNA molecules called splice variants
- The translation of these splice variants will results in different forms of proteins in different tissues called isoforms.
- Activators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

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

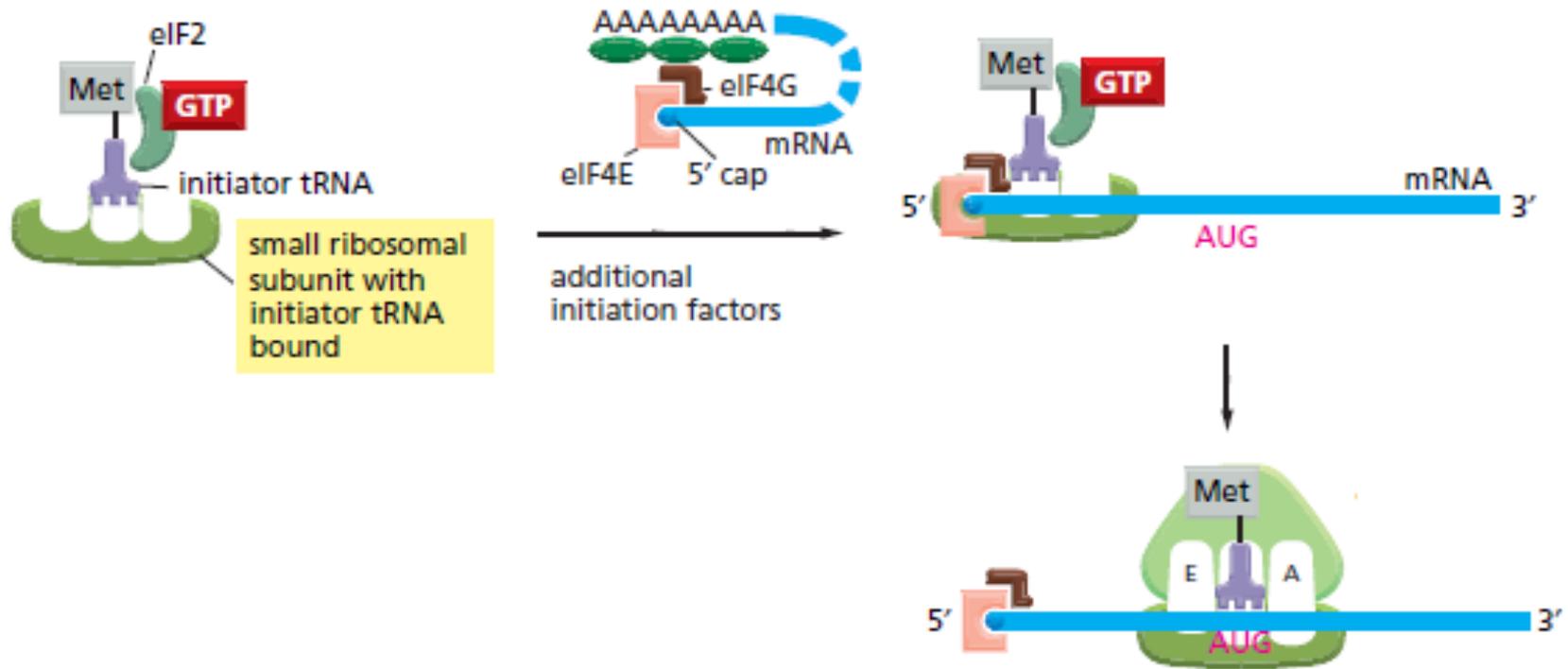


- Initiation, elongation and termination
- Regulation of translation initiation is one determinant of the rate at which any protein is synthesized
- The mechanism of translation initiation is different between eukaryotes and prokaryotes

Translation Initiation in Eukaryotes



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



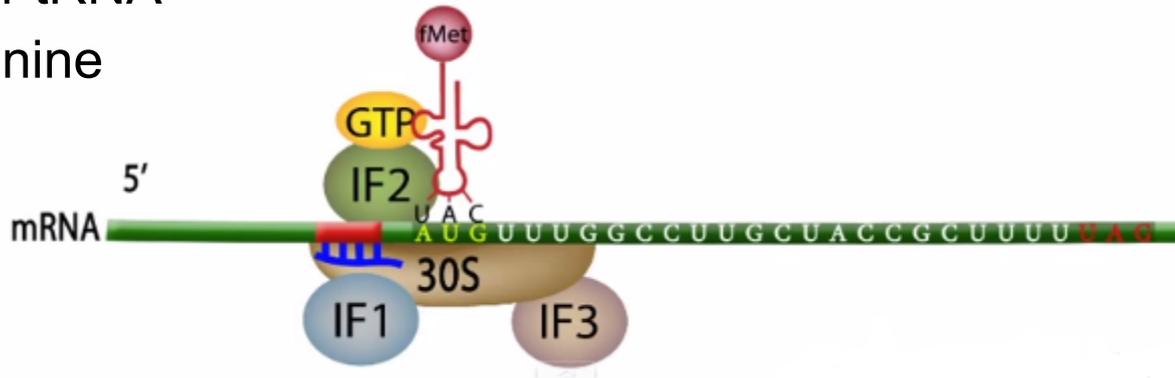
Translation Initiation in Prokaryotes



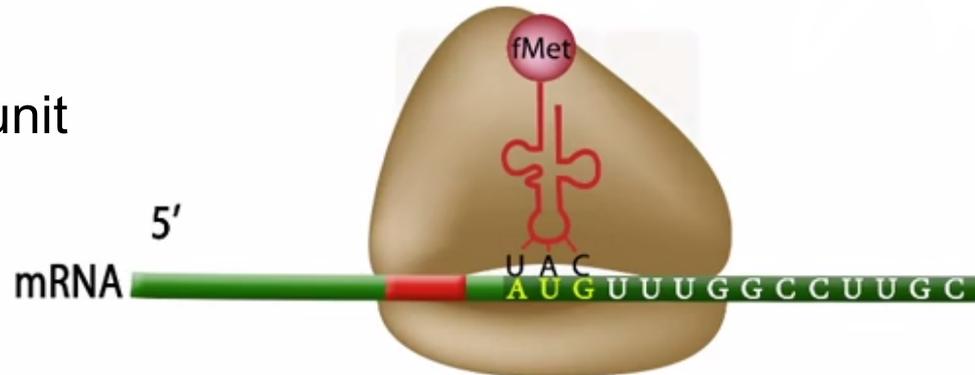
1. Binding of the small ribosomal subunit to the Shine Dalgarno sequence



2. Recruitment of initiator tRNA carrying formylmethionine



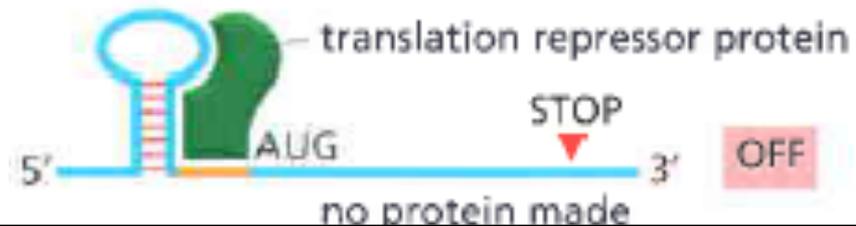
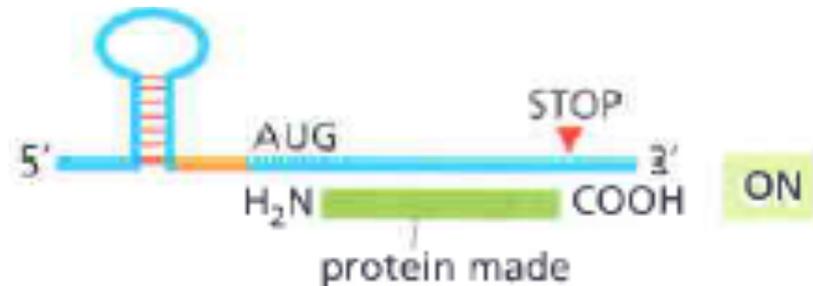
3. Dissociation of IFs and binding of large ribosomal subunit



4. Translation Control



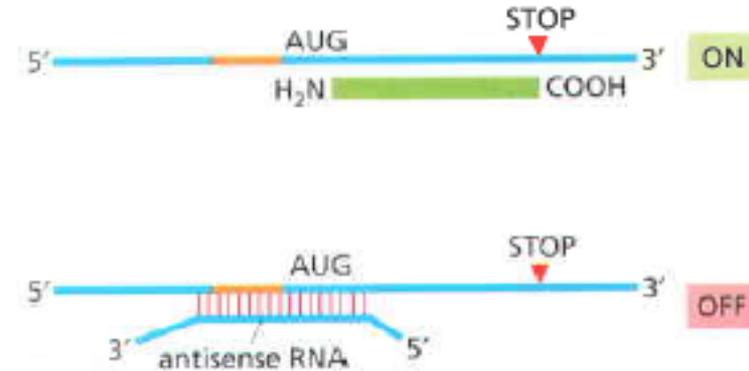
- There are several mechanisms of translation initiation regulation:
 1. Translational repressor proteins: RNA-binding proteins which bind to and block the Shine-Dalgarno sequence. Similarly in eukaryotes, translational repressors bind 5' end of mRNA or 3' end thus inhibit the translation initiation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



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



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

