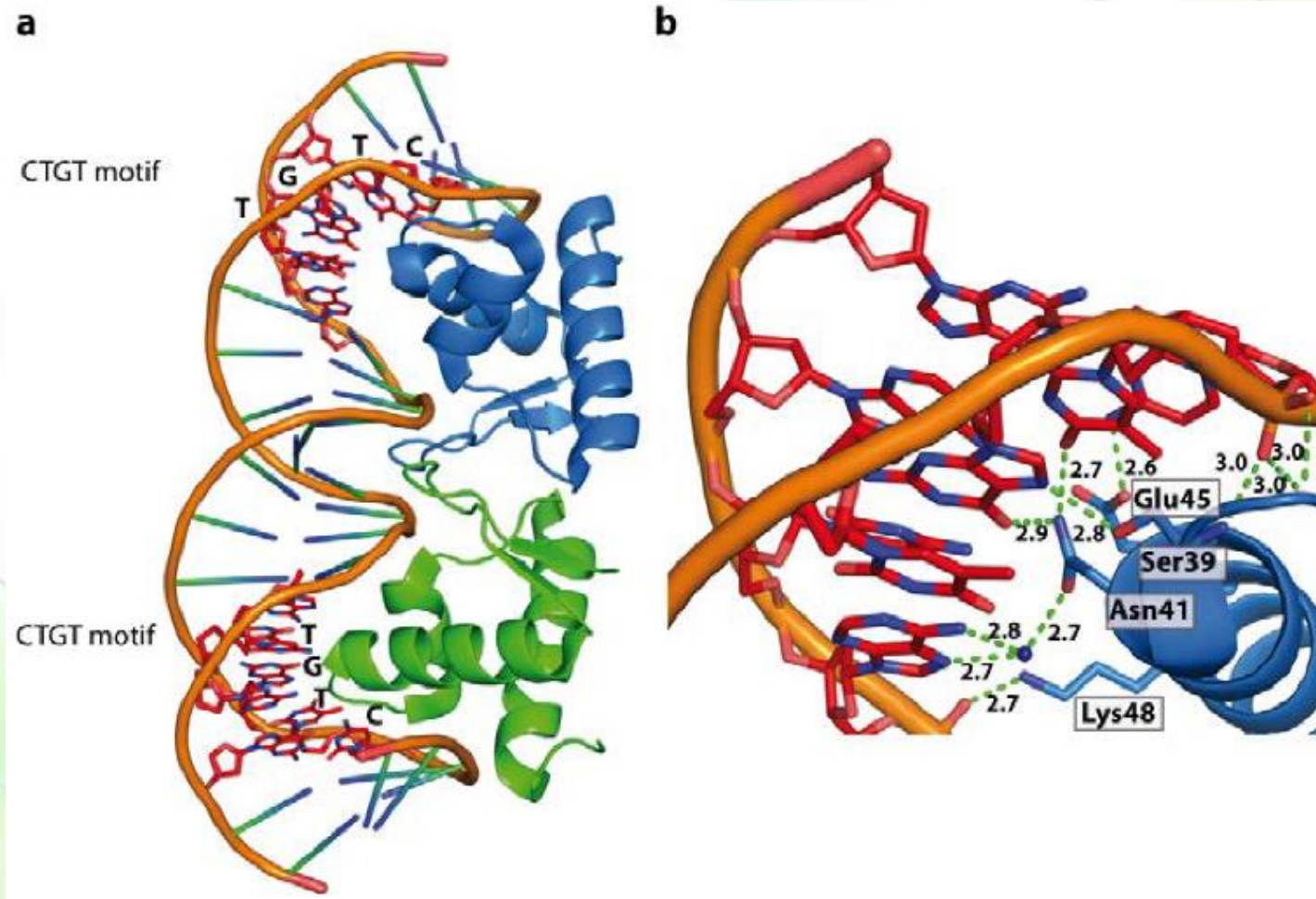




Transcription-Regulation

Prof. Mamoun Ahram
School of Medicine
Second year, First semester, 2024-2025

How do proteins recognize/interact with DNA sequences specifically?





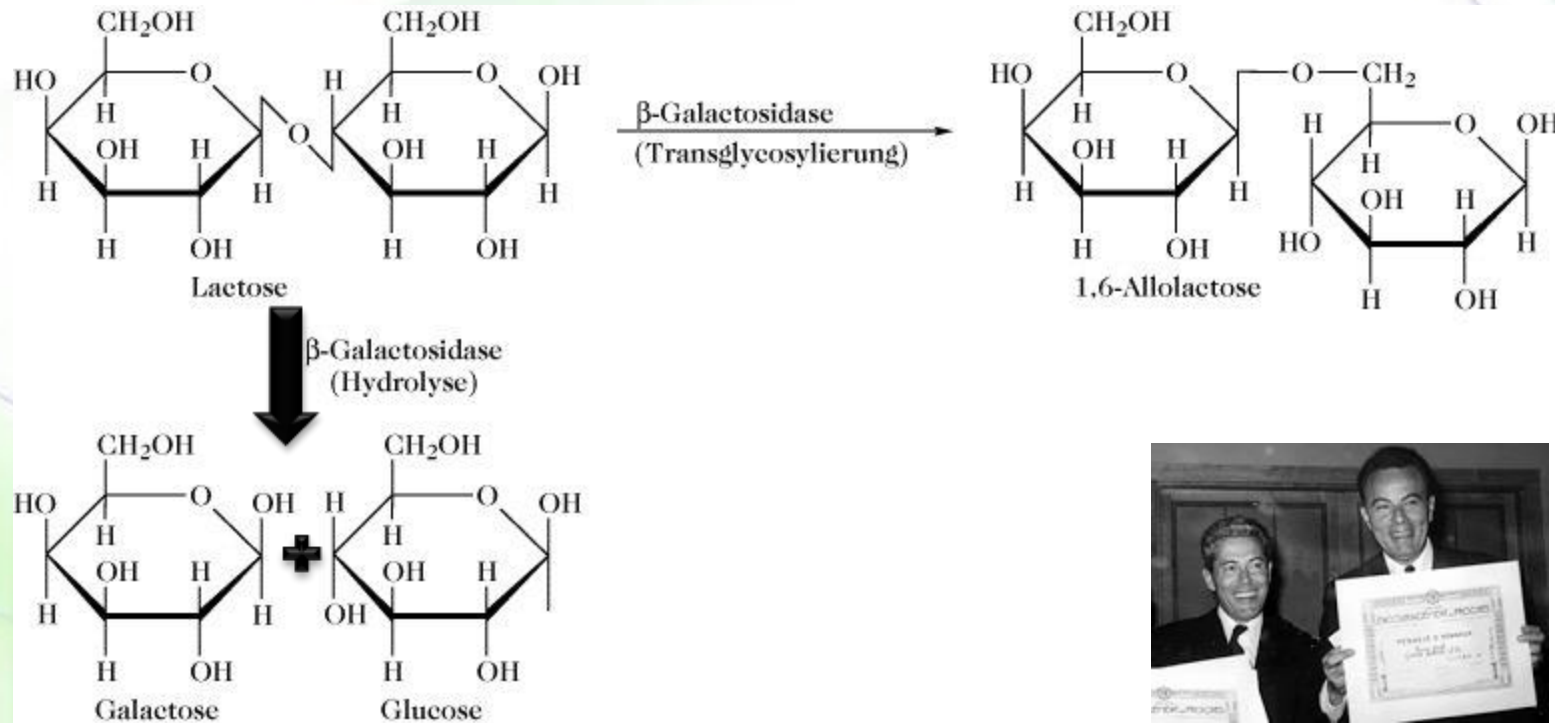
Regulation of transcription in prokaryotes

The lac operon

Metabolism of lactose



- In the 1950s, pioneering experiments were carried out by François Jacob and Jacques Monod who studied regulation of gene transcription in *E. coli* by analyzing the expression of enzymes involved in the metabolism of lactose.

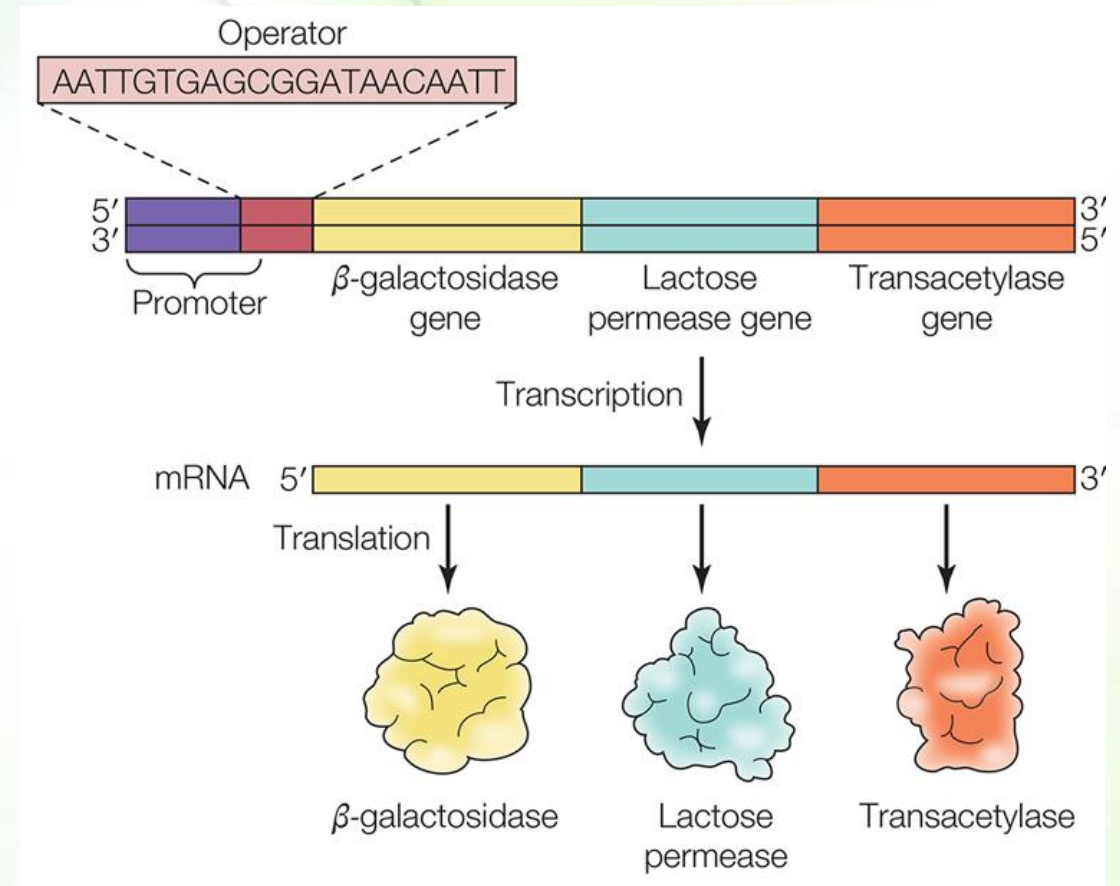


The lac operon



- A cluster of genes transcribed from one promoter producing a polycistronic mRNA that is used to make proteins that are different in structure and function, but they participate in the same pathway (purpose).

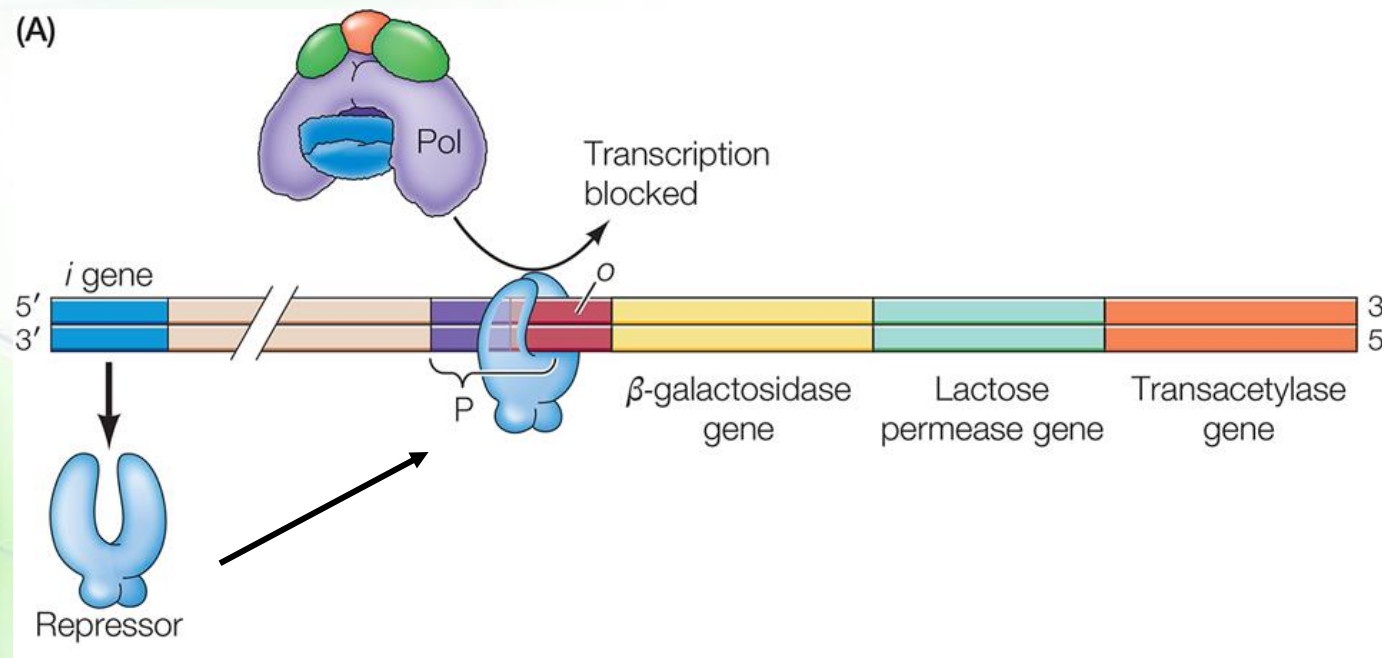
- β -Galactosidase: Cleavage of lactose into galactose and glucose
- Permease: Transport of lactose
- Transacetylase: Acetylation of toxic thiogalactosides



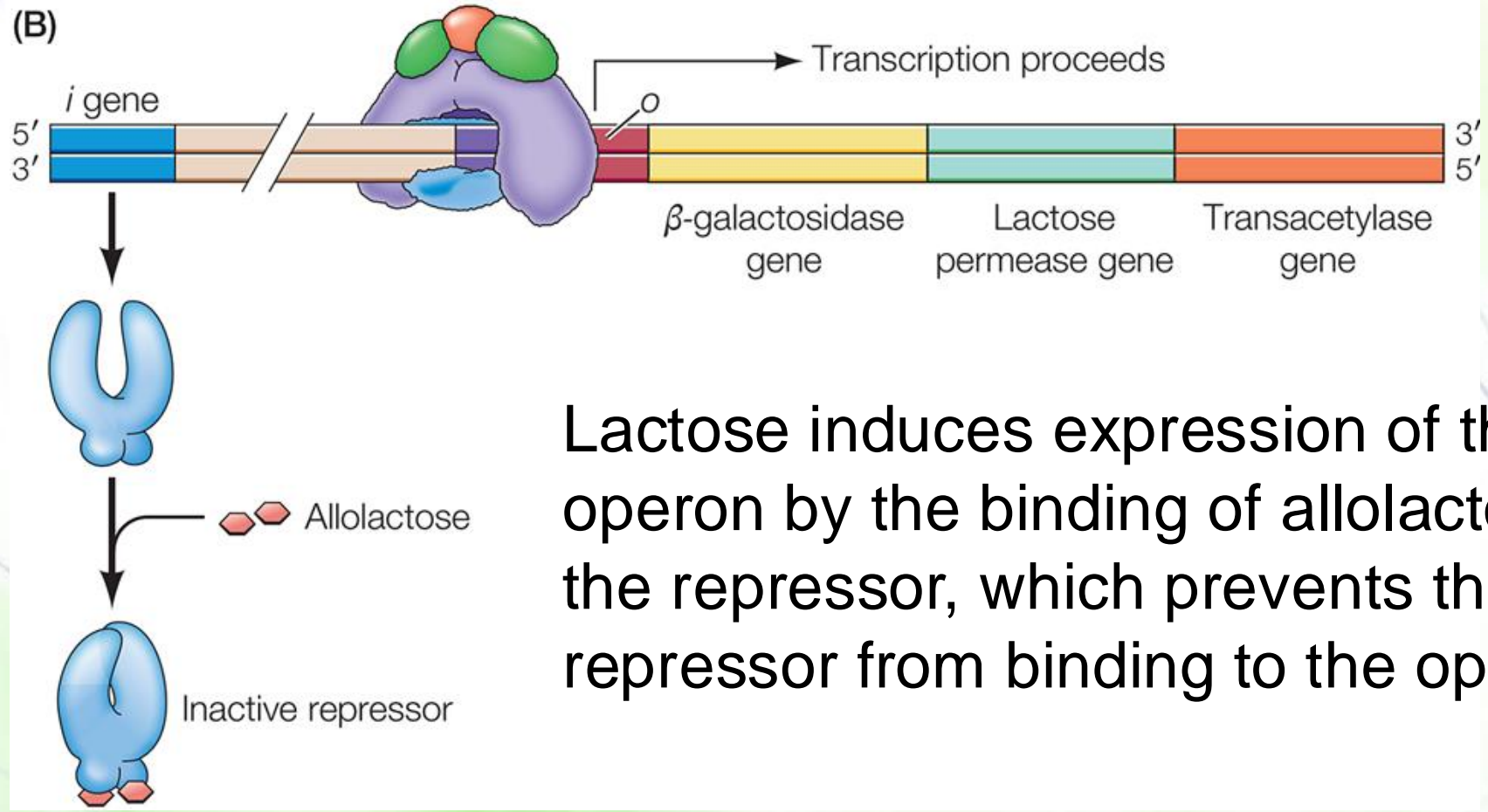
The operator



- The promoter region includes the operator region, which is a binding site of a protein called the lac repressor.
- The lac repressor blocks transcription by preventing the RNA polymerase from binding to the promoter.

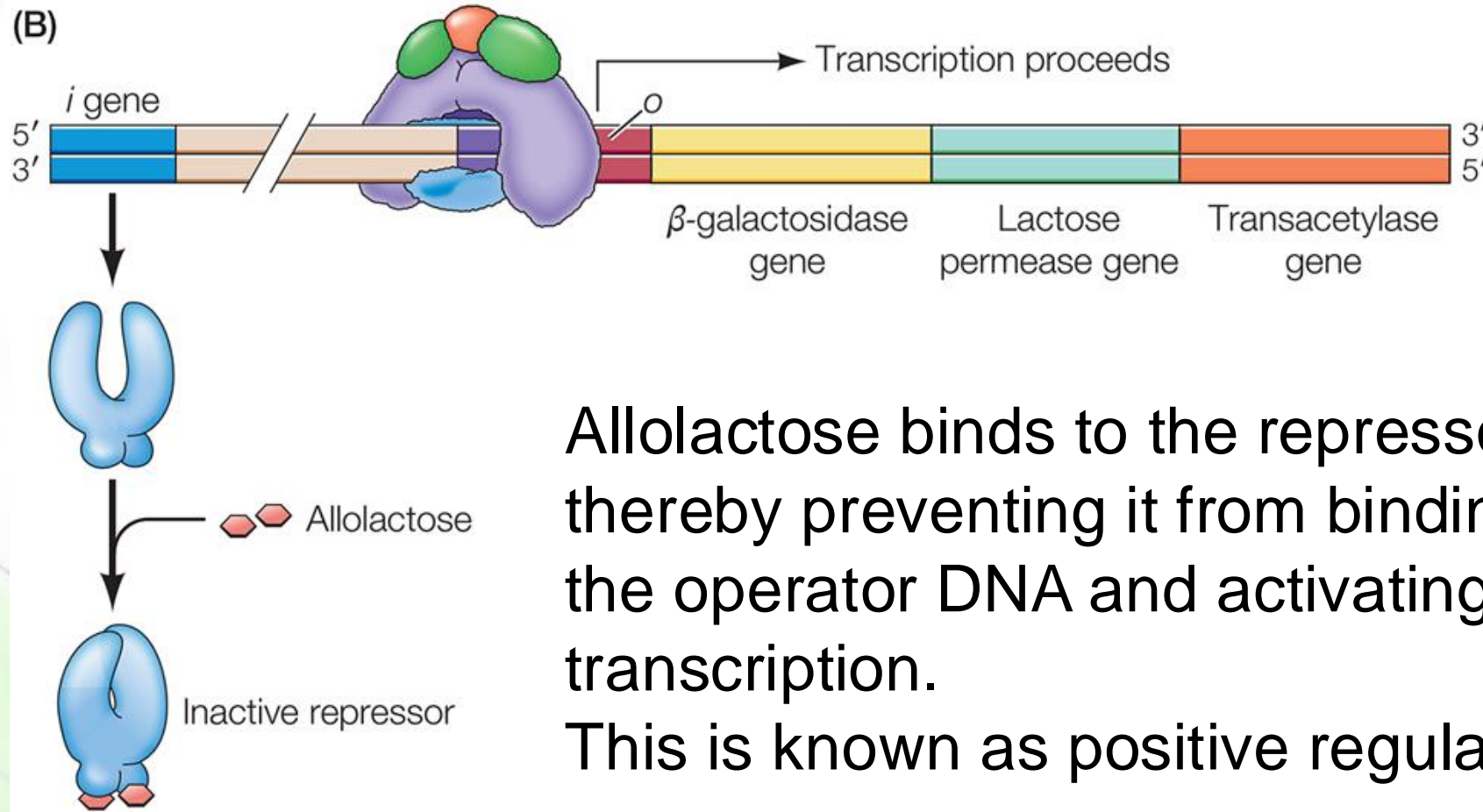


The role of allolactose



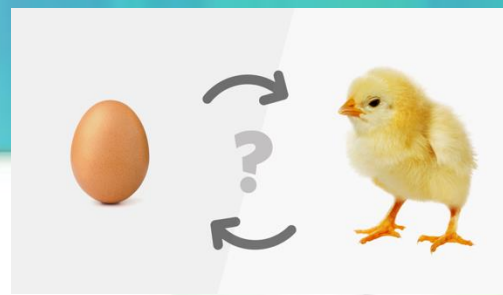
Lactose induces expression of the operon by the binding of allolactose to the repressor, which prevents the repressor from binding to the operator.

The role of allolactose



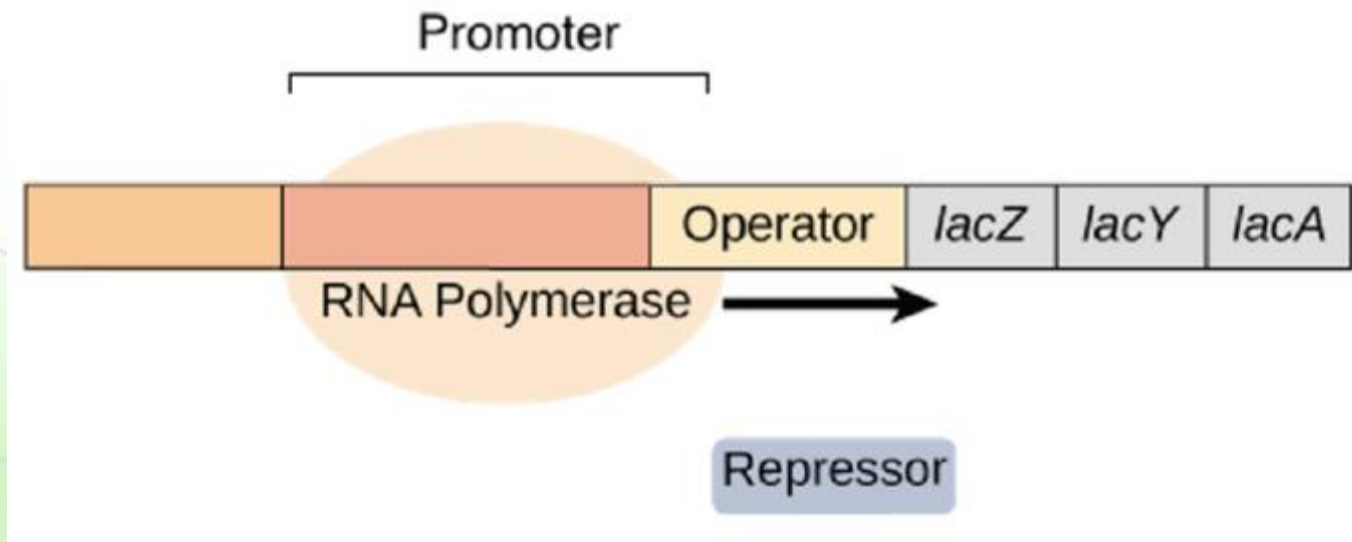
Allolactose binds to the repressor, thereby preventing it from binding to the operator DNA and activating transcription. This is known as positive regulation.

Wait...



- So, we need allolactose to make β -galactosidase, but we need β -galactosidase to make allolactose. Which one comes first?

- ANSWER: some promoters are leaky.



Cis vs. trans regulatory elements

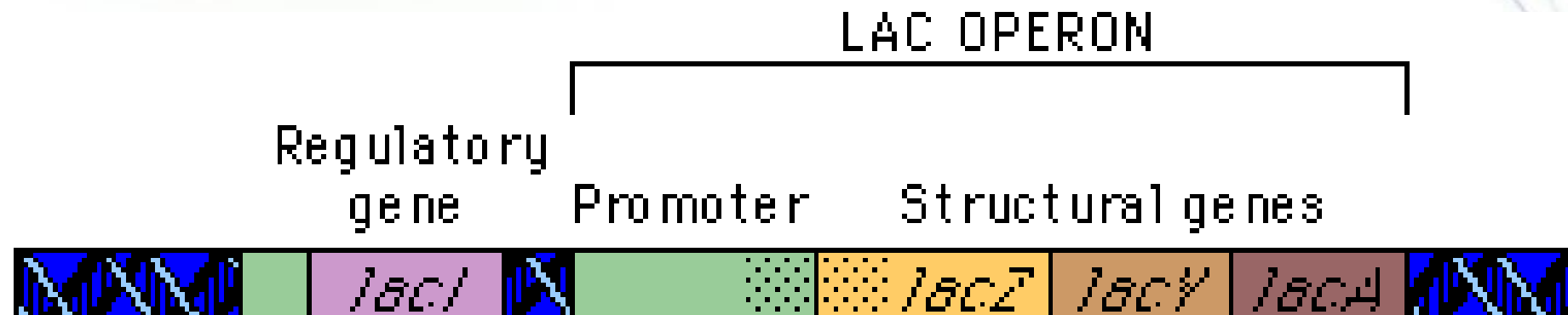


- DNA regulatory sequences like the operator are called cis-acting elements because they affect the expression of only genes linked on the same DNA molecule or close-by.
 - Mention other examples of cis-acting elements.
- Proteins (usually) like the repressor are called transacting factors because they can affect the expression of genes located on other chromosomes within the cell. They are produced from trans-acting elements (that is, genes).
 - Mention other examples of trans-acting elements.

Effect of mutations

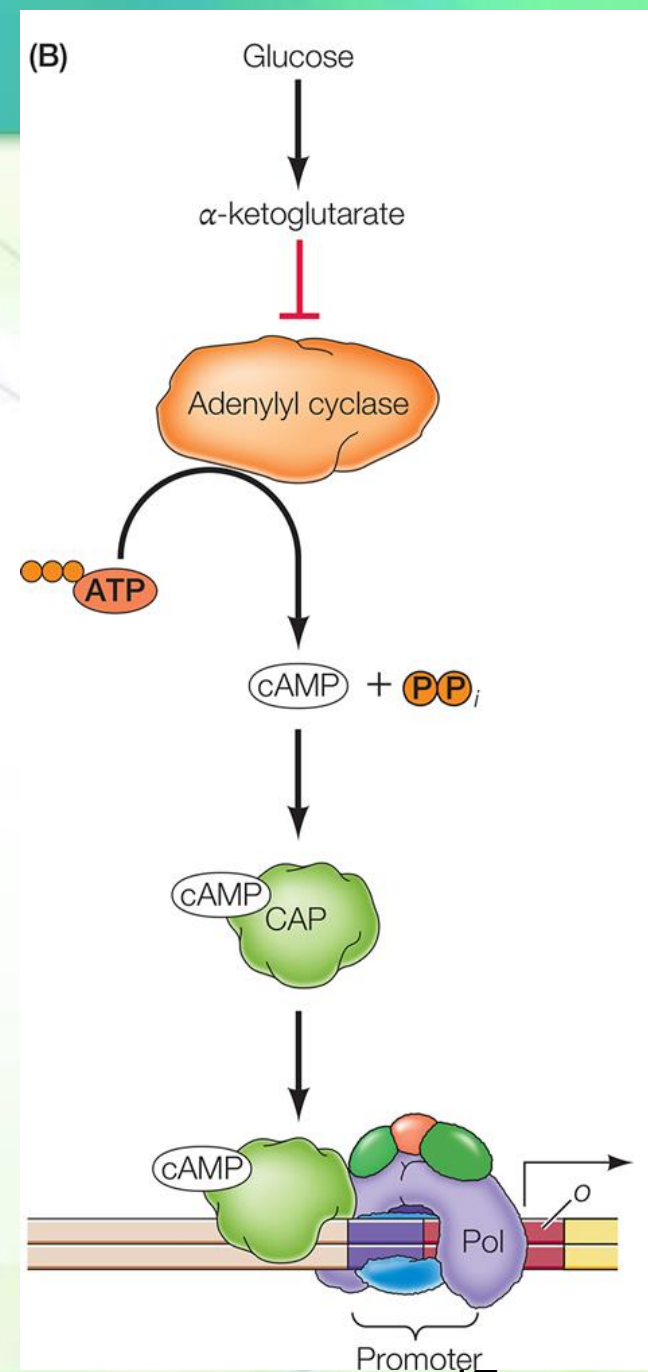


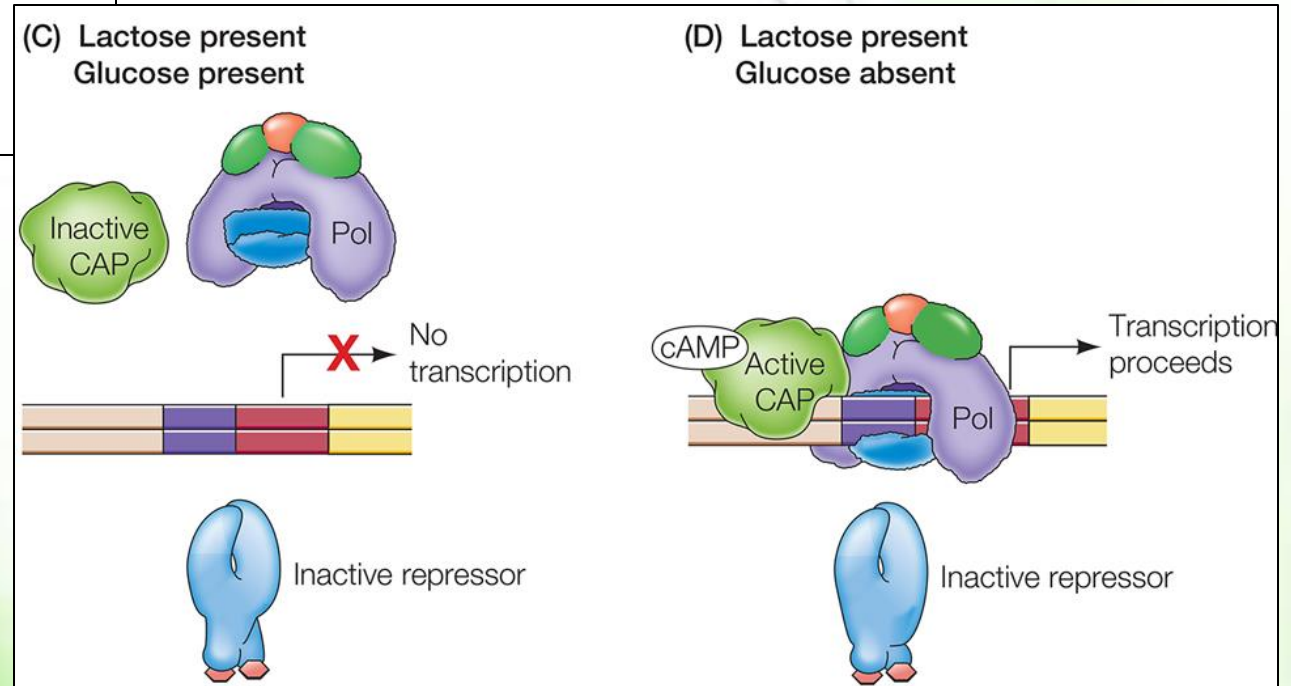
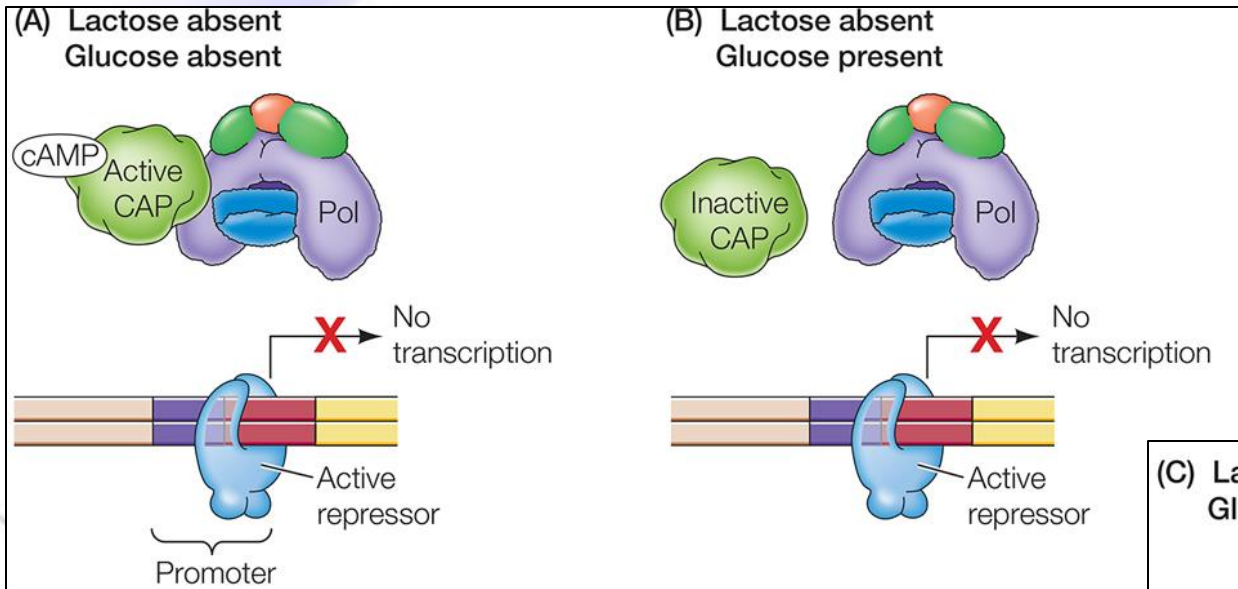
- Some mutations result in constitutive expression (always on).
 - Mention examples.
- Other mutations cause non-inducible or repressed expression (always off).
 - Mention examples



Another level of regulation

- Another regulator is catabolite activator protein (CAP) which binds to regulatory sequences upstream of the promoter.
- CAP can then interact with the RNA polymerase to facilitate its binding to the promoter (P).
- CAP binding to DNA is influenced by cAMP, which is produced by adenylyl cyclase, which is inhibited by high level of glucose.
- If glucose is present, it is preferentially utilized by bacterial cells and it represses the lac operon even in the presence of the normal inducer (lactose).
- This is known as negative regulation.





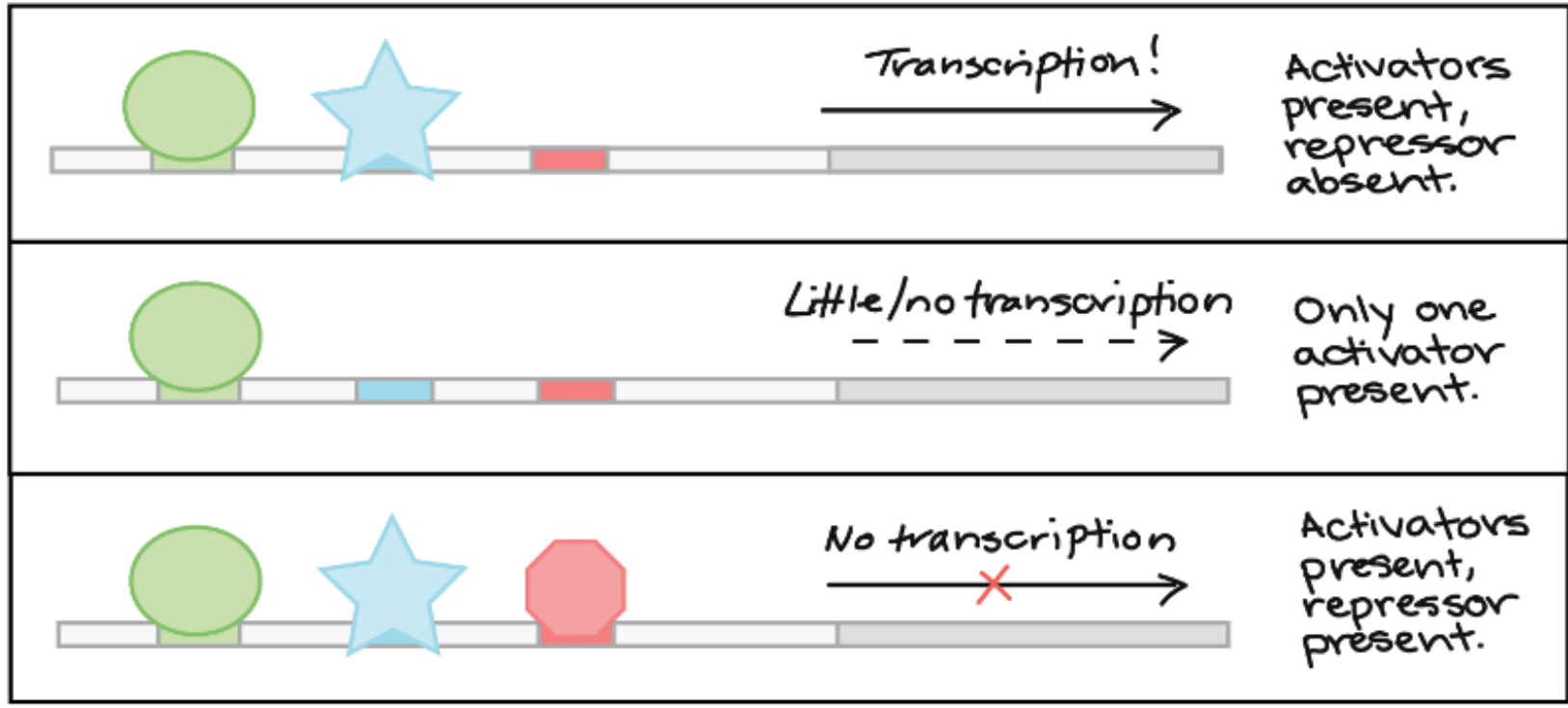
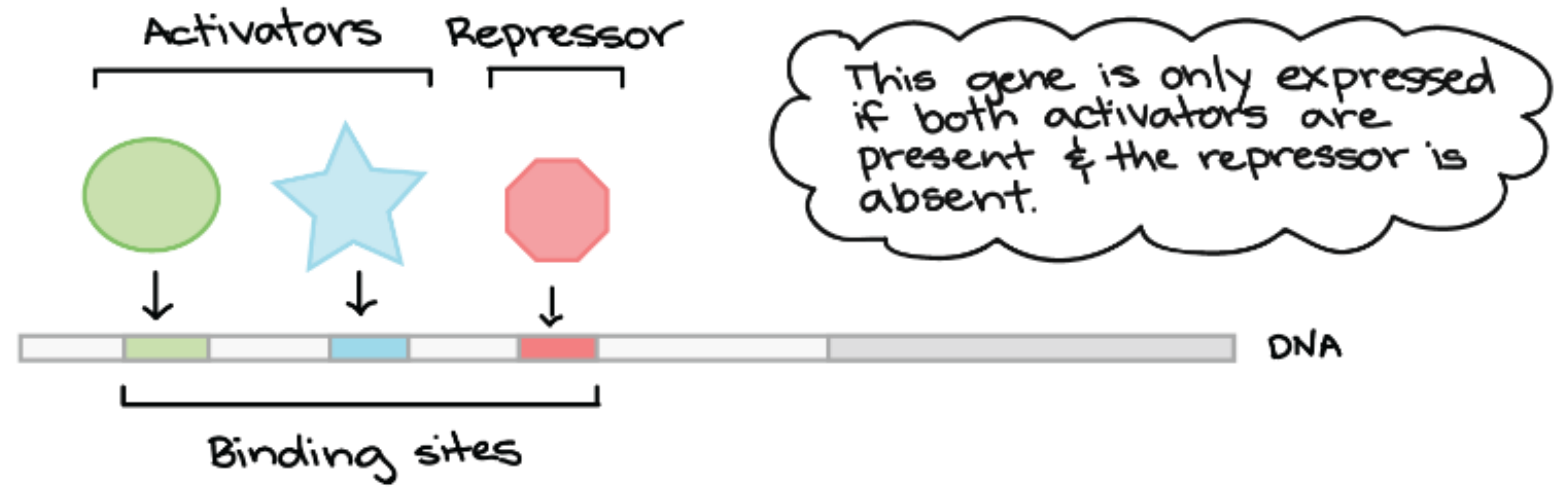


Regulation of transcription in eukaryotes

Regulatory mechanisms



- Although the control of gene expression is far more complex in eukaryotes than in bacteria, the same basic principles apply.
- Transcription in eukaryotic cells is controlled by:
 - **Cis-acting elements**
 - Promoters, promoter proximal elements, enhancers, and silencers
 - **Trans-acting factors**
 - transcriptional regulatory proteins (activators, repressors)
 - DNA and chromatin structural modification
 - DNA chemical modification (example: methylation of cytosine)
 - **Noncoding RNA molecules**



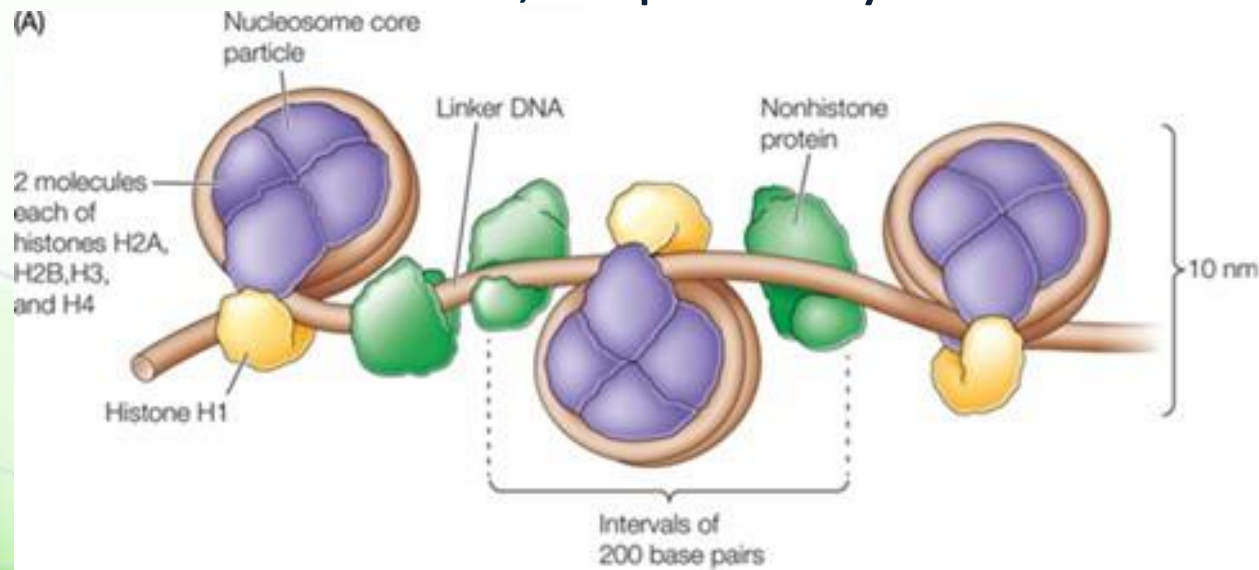
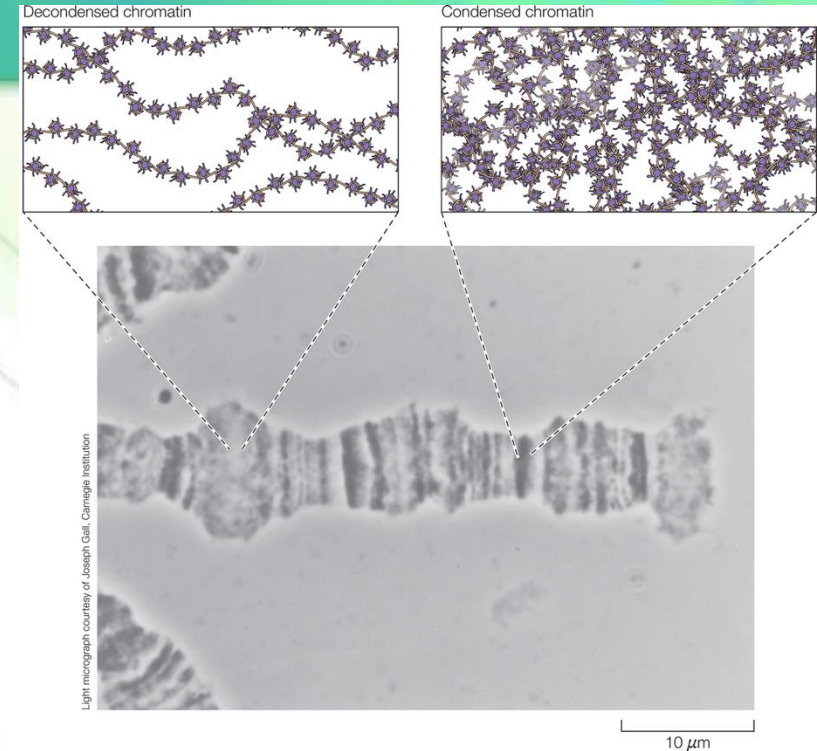
How do TFs regulate gene expression?



- Transcription factors cause epigenetic/epigenomic changes in DNA and chromatin.
- What is epigenetics?
 - Epi: “above” or “in addition to”
 - It indicates alterations in gene expression without a change in the DNA sequence, but through DNA modification via internal or external factors.

Nucleosome

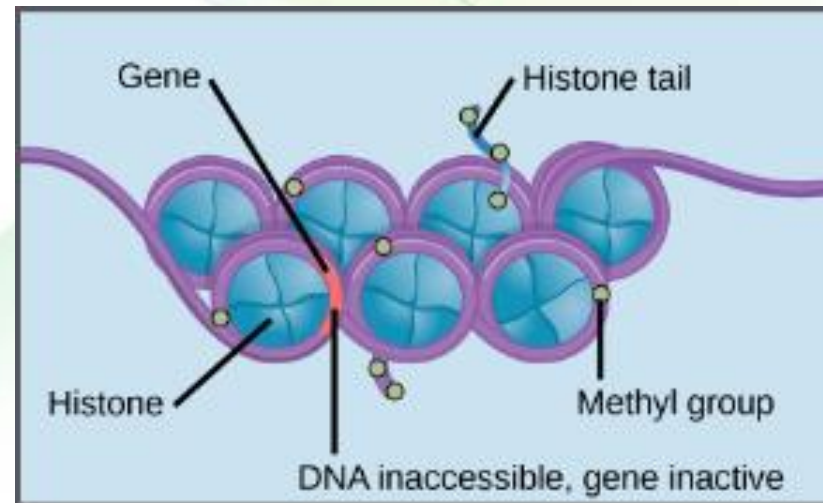
- DNA exists as chromatin, which is DNA wrapped around an octamer of histone proteins (H2A, H2B, H3, and H4) as a nucleosome core particle. Histone 1 can also bind to the DNA outside the nucleosome core. There is a free linker DNA between every two nucleosome core particles.
- DNA can either be loosely or tightly condensed, that is as euchromatin or heterochromatin, respectively.



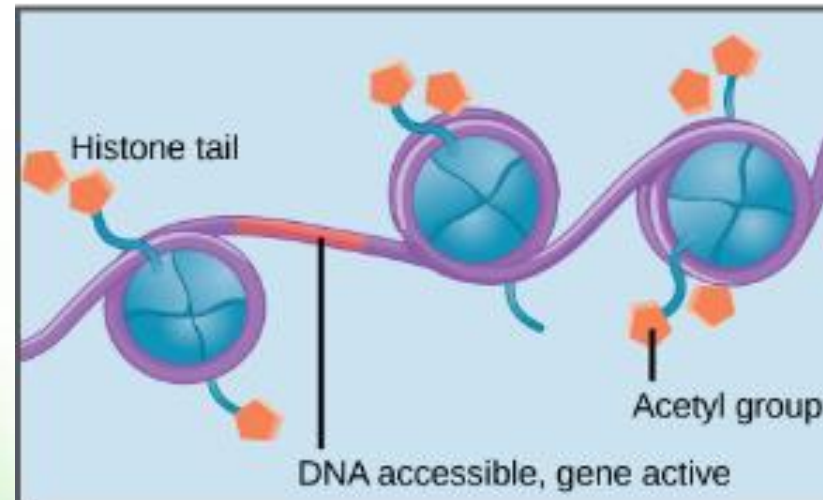
Modulation of chromosomal structure



- Active genes exist in euchromatin.
- Inactive genes exist in heterochromatin.
- The packaging of eukaryotic DNA in chromatin can regulate transcription.
- Regulatory proteins switch between the two structures of chromatin.



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



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

How are chromosomal structures altered?



- Change of compactness of the chromatin by:
 - Change the structure and position of nucleosomes
 - Chemically modify histones
 - Acetylation, methylation, and phosphorylation
 - Chemically modify cytosine
 - Binding of noncoding RNAs to DNA

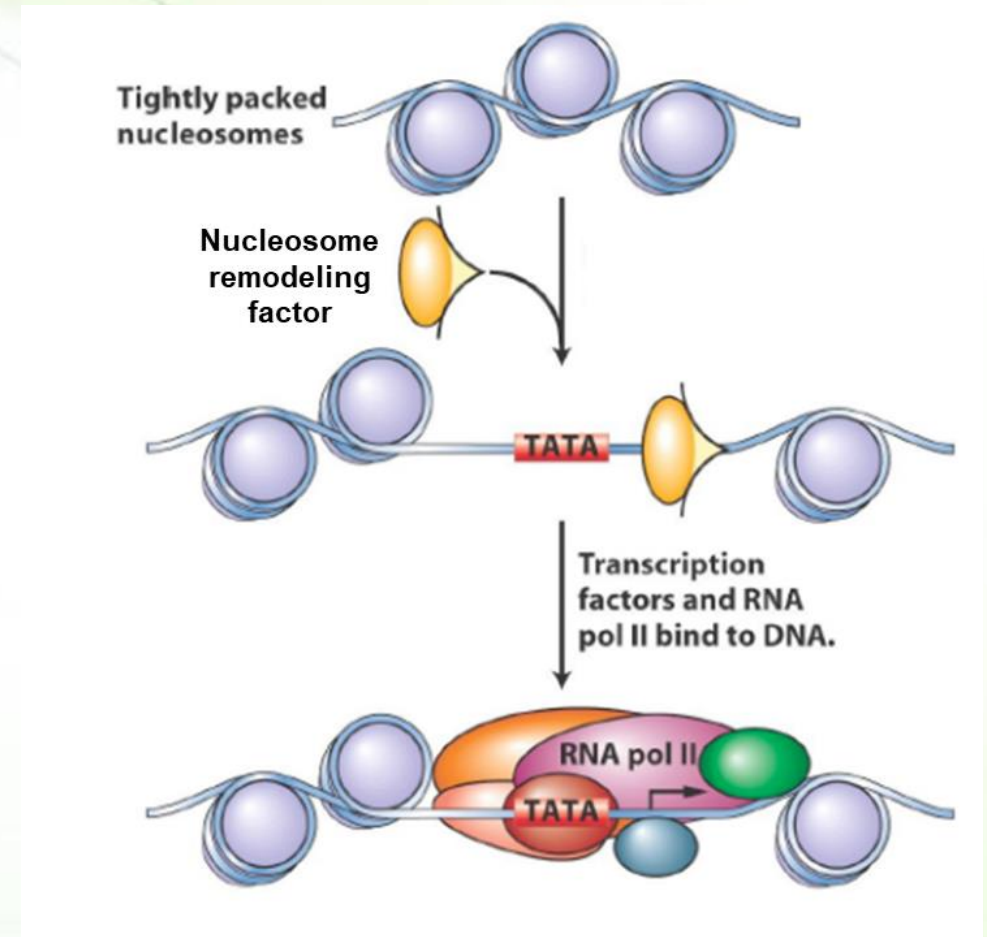


Change the structure and position of nucleosomes

Chromatin remodeling factors



- They facilitate the binding of transcription factors by
 - Removing histones from DNA
 - Repositioning nucleosomes making DNA sequences accessible
 - Altering nucleosome structure allowing protein binding to DNA
- Chromatin remodeling factors can be associated with transcriptional activators and repressors.



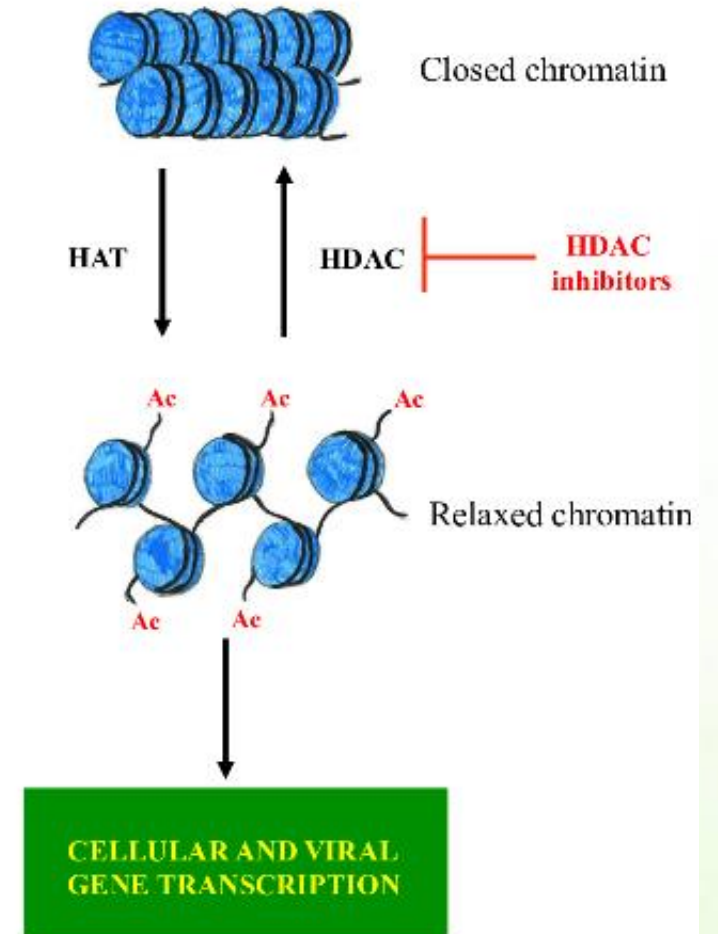
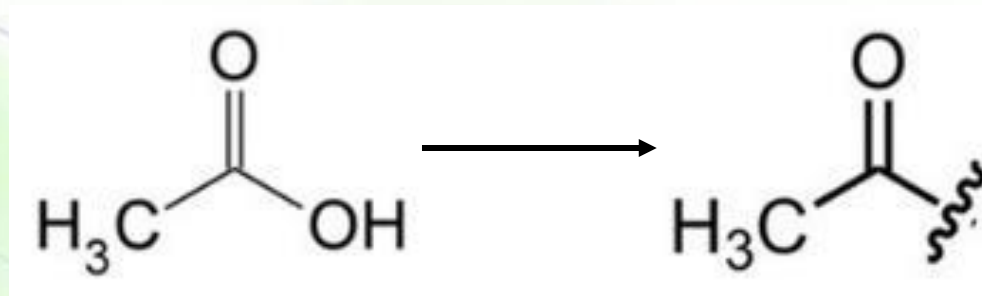


Chemical modification of histones

Histone acetylation



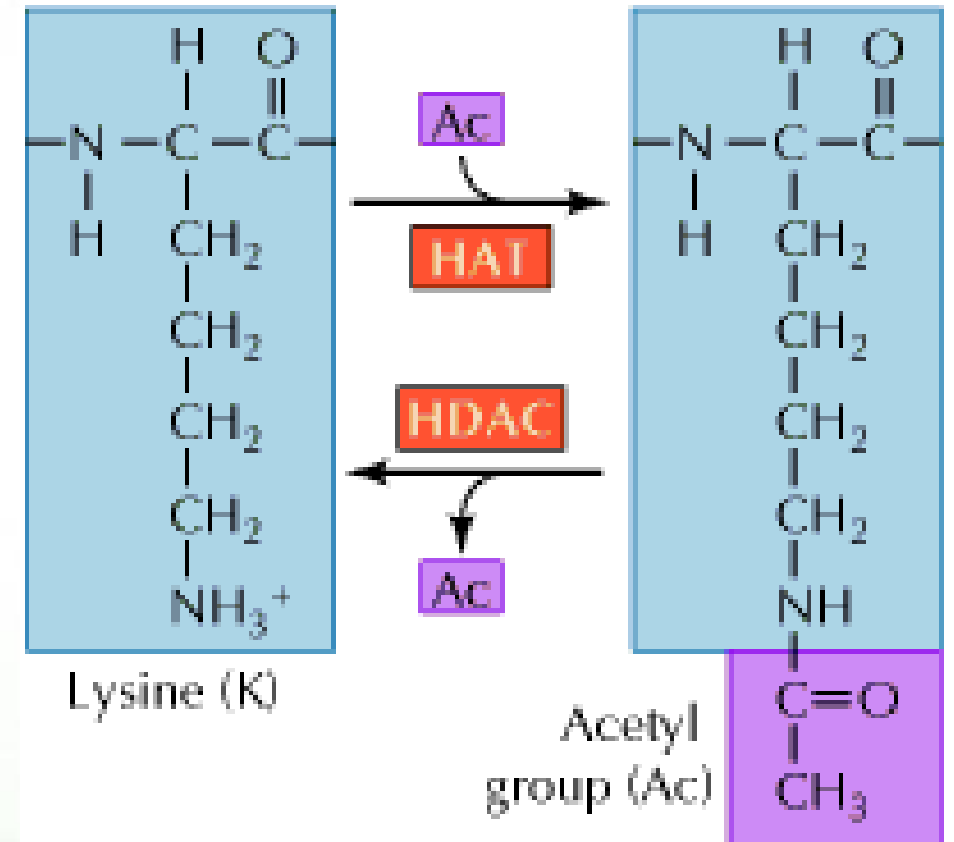
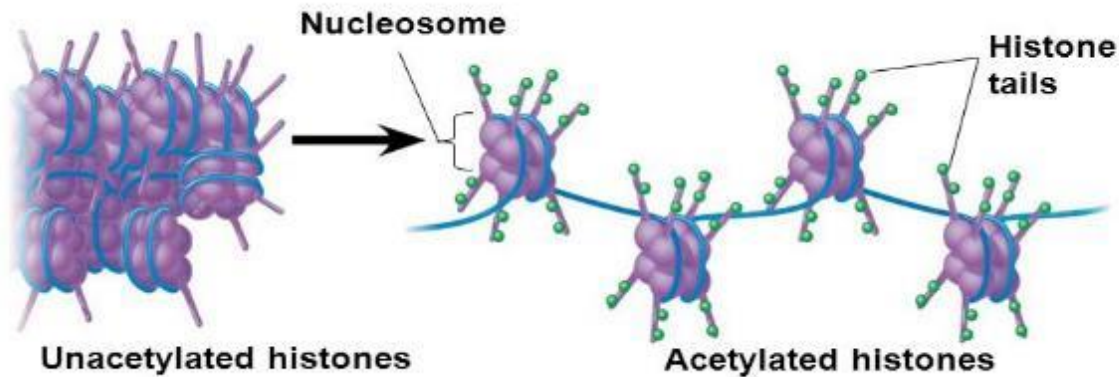
- The core histones (H2A, H2B, H3, and H4) have two domains (internal 3-dimensional structures):
 - A histone-fold, which is involved in interactions with other histones and in wrapping DNA around the nucleosome core particle.
 - An amino-terminal tail, which extends outside of the nucleosome, and is rich in lysine.



Acetylation of lysine



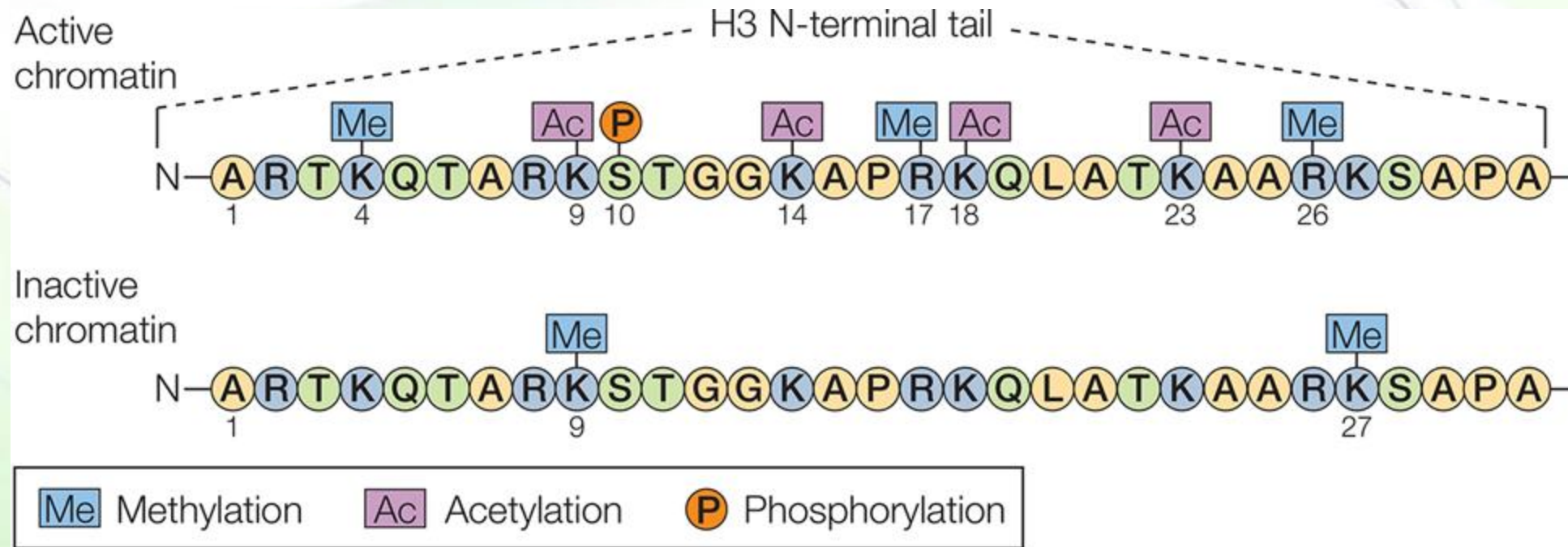
In **histone acetylation**, acetyl groups are attached to positively charged lysines in histone tails. This generally loosens chromatin structure, promoting the initiation of transcription.



Other modifications of histones



- Histone can also be methylated or phosphorylated.
- The effect, whether transcriptional activation or repression, depends on the modification sites.
- Histone modifications can: (1) alter chromatin structure and (2) provide binding sites for other proteins that can either activate or repress transcription.



General structure of TFs

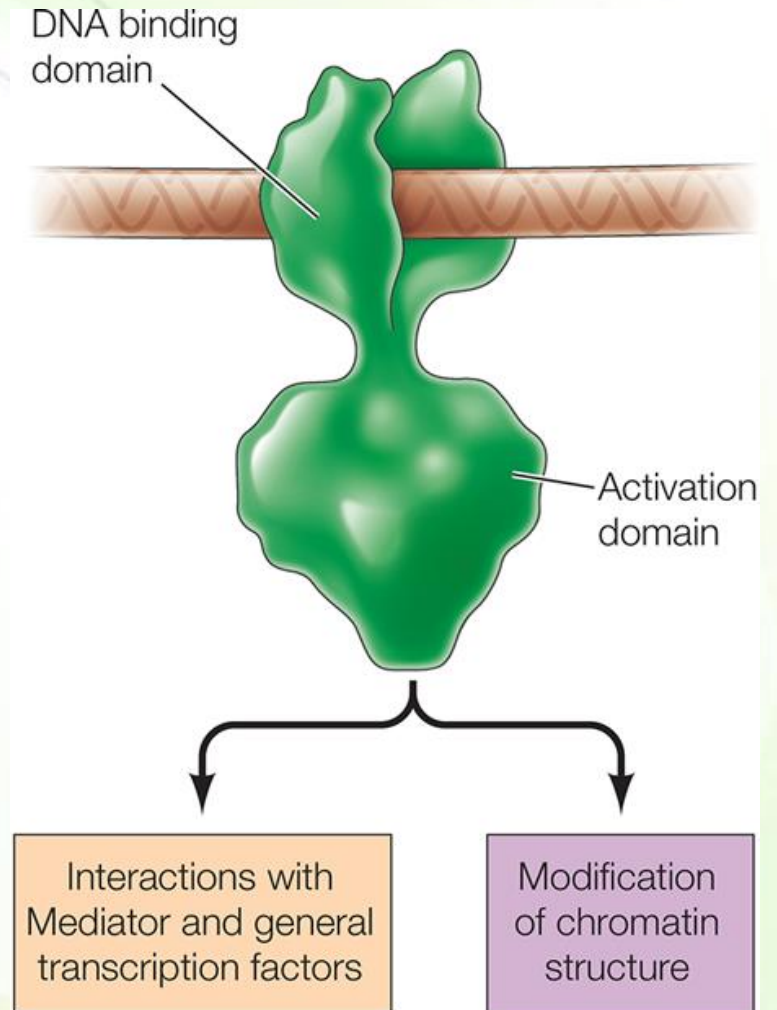


- There are about 2000 transcription factors encoded in the human genome, that is 10% of protein-coding genes.
- Positive transcription factors have at least two independent domains:
 - DNA-binding domain
 - Activation domain
- What is a domain?
 - A three-dimensional structure that is part of a protein's structure. It forms independently of the rest of the protein and usually has a function.
 - In other words, it can be separated from the protein and still be functional.

The activation domains



- Activation domains stimulate transcription by
 - interacting with Mediator proteins and general transcription factors, such as TFIID, to recruit the RNA polymerase and facilitate the assembly of a transcription complex on the promoter,
 - modifying the chromatin.

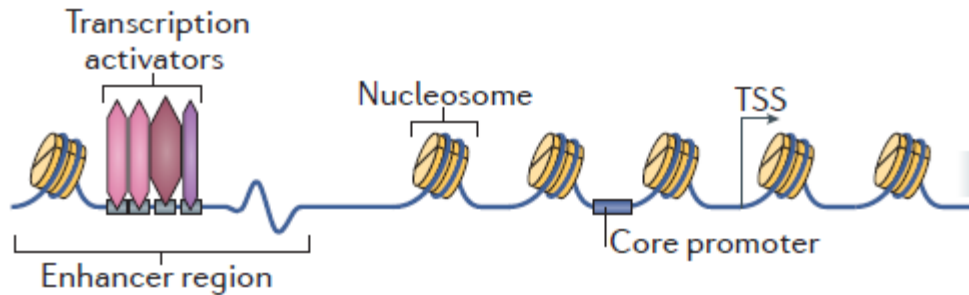


A model of transcriptional activation



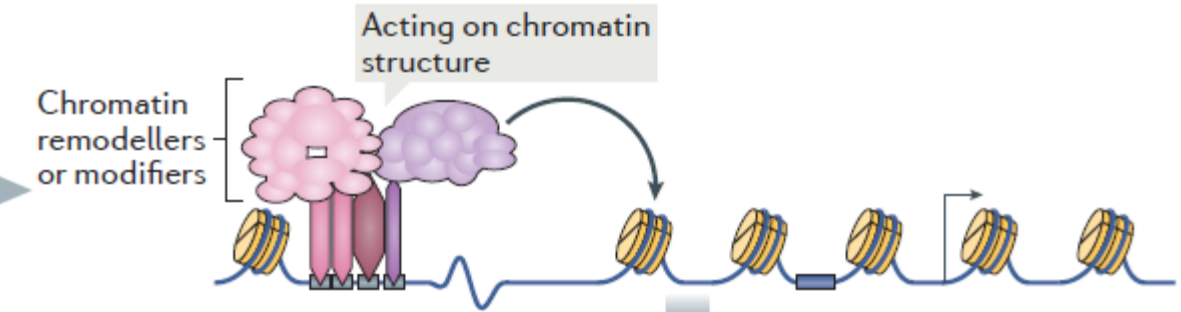
1) Binding of transcriptional activators to the enhancer region

a Transcription factor binding

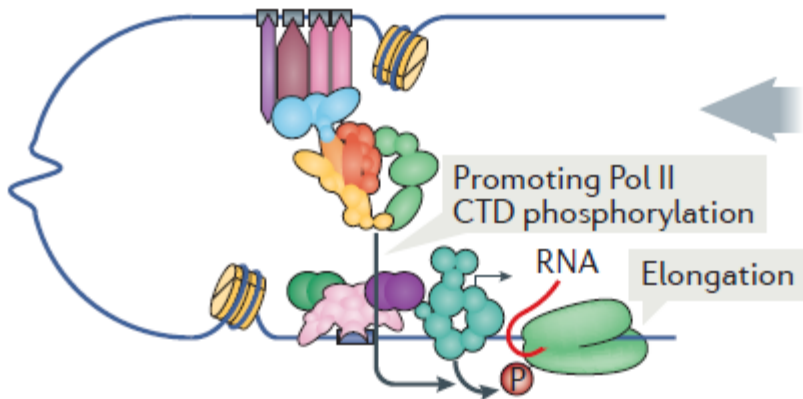


2) Recruitment of chromatin remodeling factors

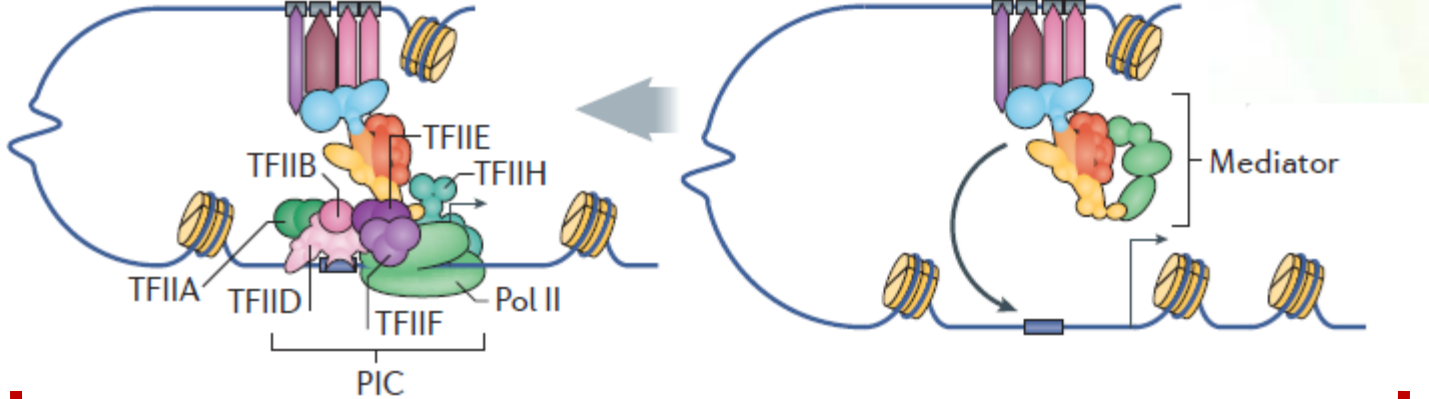
b Recruitment of co-activators



d Promoter escape



c PIC formation



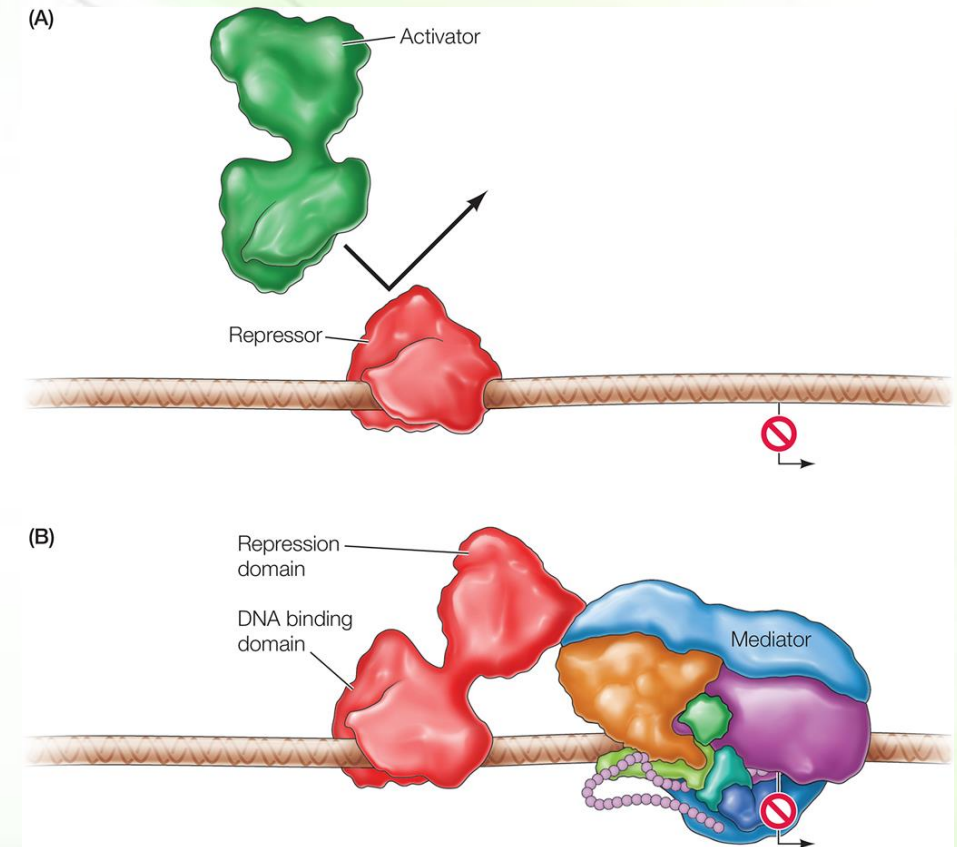
4) Phosphorylation of RNA polymerase II

3) Recruitment of pre-initiation complex

Eukaryotic repressors



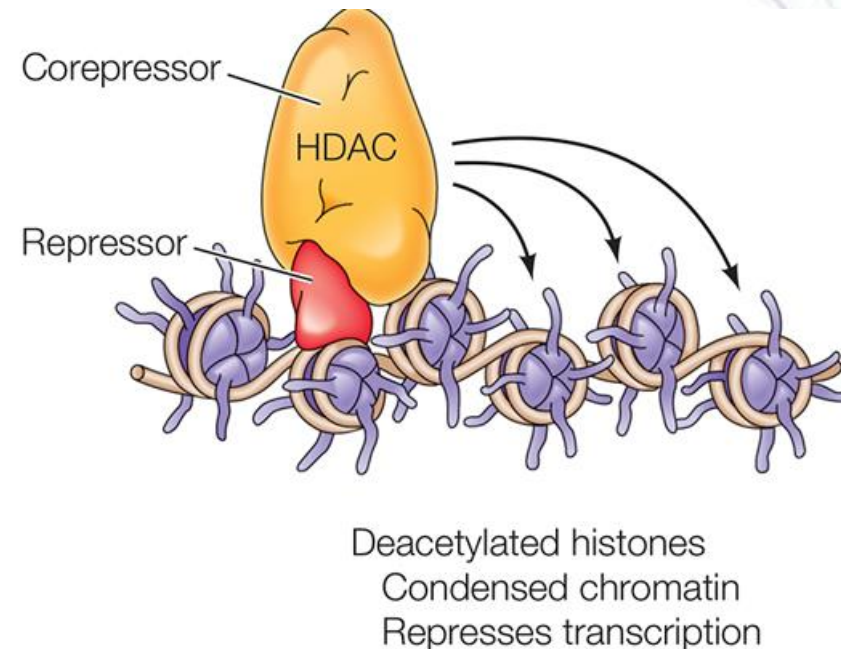
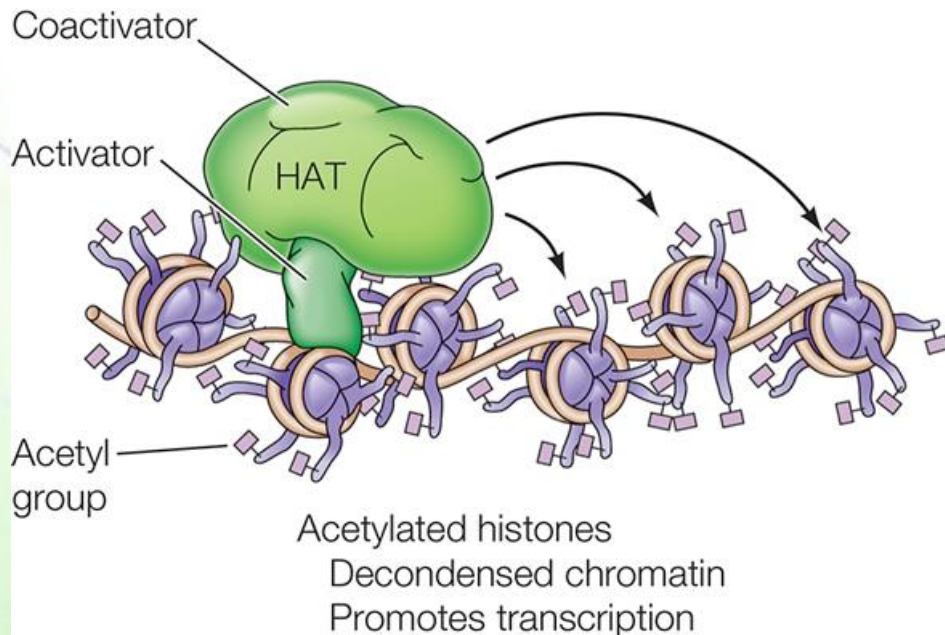
- (A) Some repressors block the binding of activators to regulatory sequences.
- (B) Other repressors have active repression domains that inhibit transcription by interactions with Mediator proteins or general transcription factors, as well as with corepressors that act to modify chromatin structure.



Enzymatic association



- Transcriptional activators and repressors are associated with coactivators and corepressors, which have histone acetyltransferase (HAT) and histone deacetylase (HDAC) activities, respectively.
 - Histone acetylation is characteristic of actively transcribed chromatin.
 - **TFIID associates with histone acetyltransferases.**



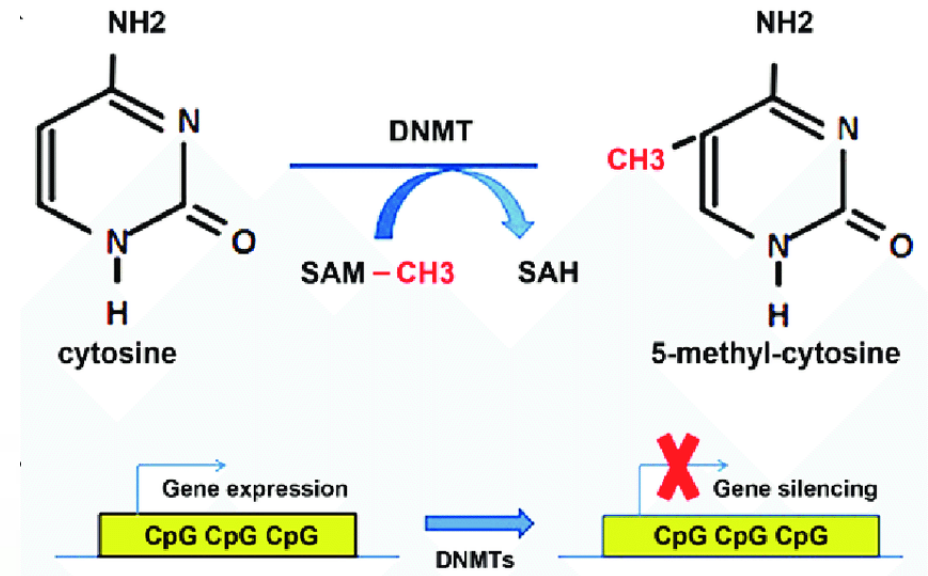


Chemical modification of cytosine

DNA methylation



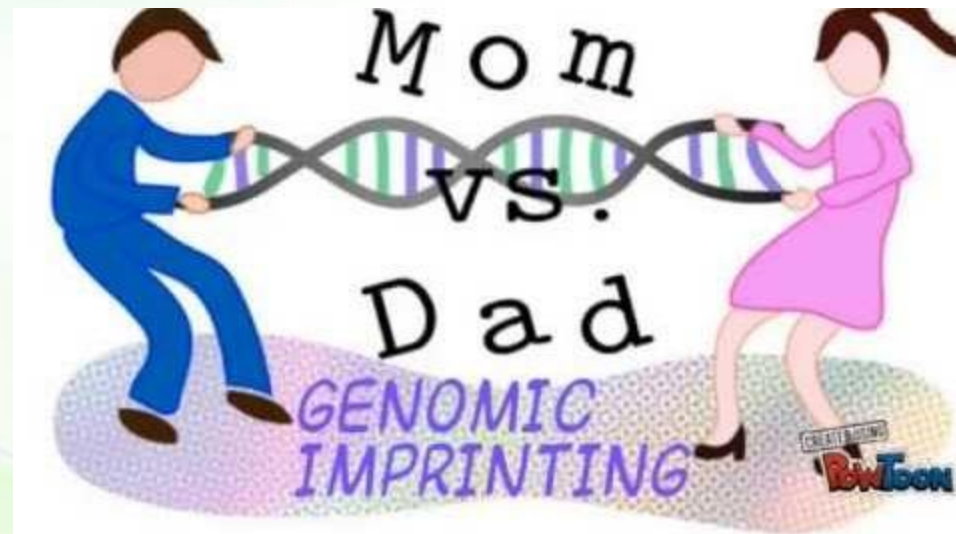
- Cytosine residues can be methylated at the 5'-carbon position specifically at CG sequences (called CpG islands near promoters).
- DNA methylation reduces gene transcription by blocking of activator binding to DNA and inducing heterochromatin formation.



Genetic imprinting



- Methylation is a mechanism of genomic imprinting (either the paternal genes or the maternal genes are active).
 - This is the case for 75 genes.
- Methylation is inherited following DNA replication.



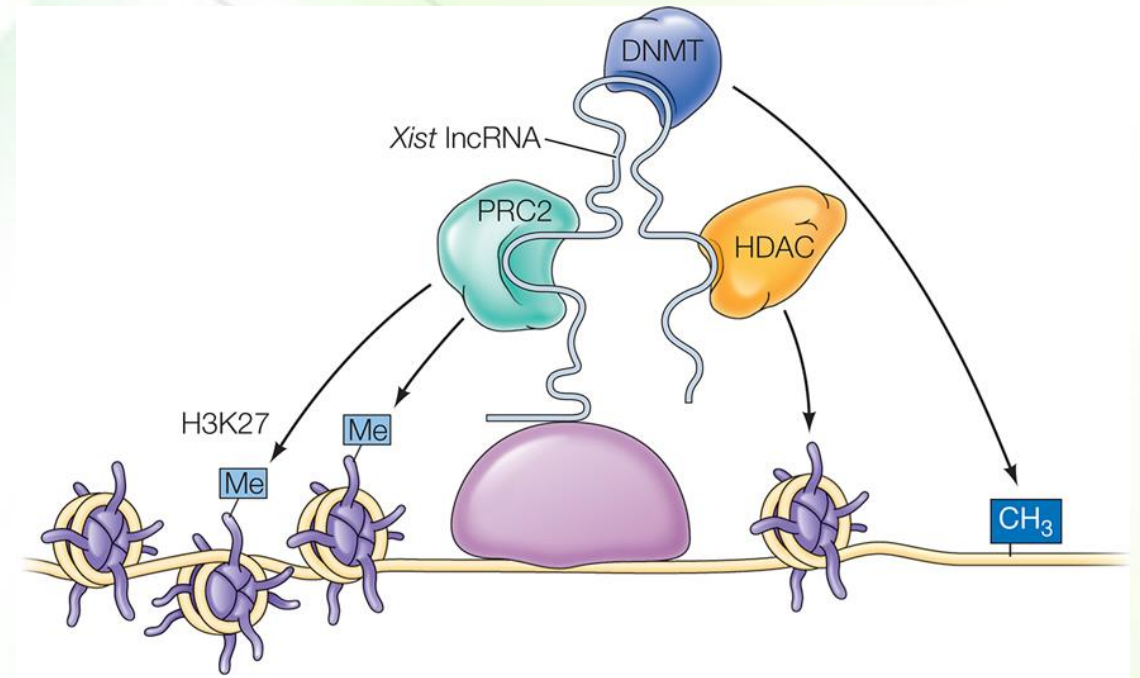


Binding of noncoding RNAs to DNA

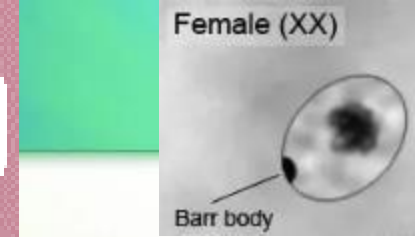
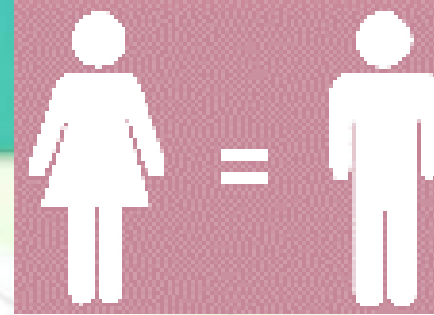
Role of noncoding RNAs



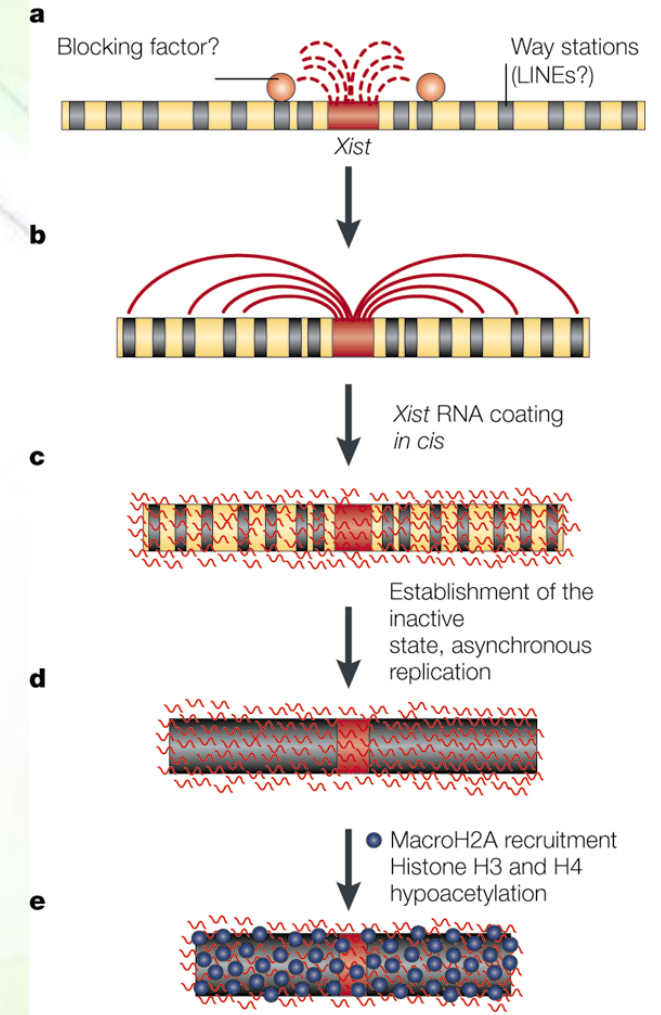
- More than 50,000 long noncoding RNAs (lncRNA), which are >200 nucleotides long, are encoded by the human genome.
- LncRNAs can be homologous to certain DNA sequences and form complexes with chromatin and DNA modifiers to activate or repress gene expression via chromatin modification and histone methylation.
- LncRNAs can complex with general or specialized transcription factors (e.g. TFIIB), Mediator, or RNA processing proteins.
- LncRNA can act in cis or trans.



X chromosome inactivation



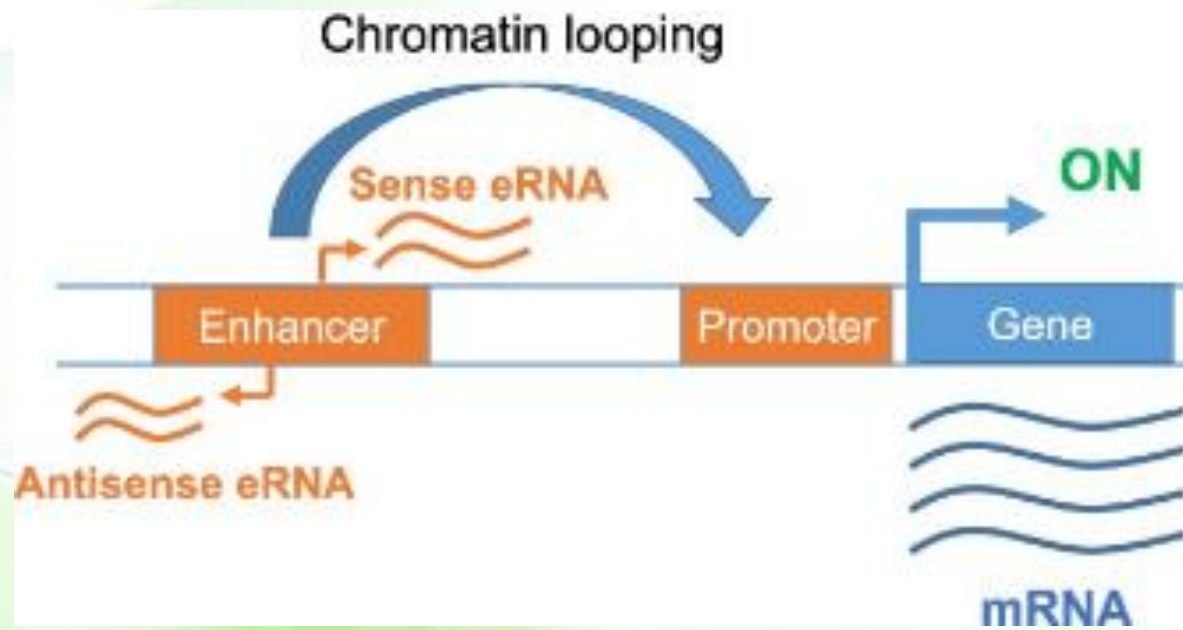
- A long noncoding RNA (lncRNA) is transcribed from Xist gene located on one of the two X chromosomes in females.
- The Xist RNA coats the X chromosome and promotes the recruitment of a protein complex that methylates histone 3 leading to chromosomal condensation.
- This results in X-chromosome inactivation in a phenomenon called **dosage compensation** to equate the number (and activity) of X chromosomes between males and females.



Enhancer RNA (eRNA)



- Some enhancers can be transcribed into RNA, hence called eRNA, that can regulate transcription of adjacent genes.



Identical twins have the exact same genetic information



But their epigenomes become increasingly different over time

- Epigenetic changes can cause dramatic differences between twins, including many cases where one twin develops a disease and the other does not.



The power of epigenetics



- Non-sequence dependent inheritance



Epigenetics is significant and heritable



PNAS

Stress-induced gene expression and behavior are controlled by DNA methylation and methyl donor availability in the dentate gyrus

Emily A. Saunderson^{a,1}, Helen Spiers^b, Karen R. Mifsud^a, Maria Gutierrez-Mecinas^{a,2}, Alexandra F. Trollope^{a,3}, Abeer Shaikh^a, Jonathan Mill^{b,c}, and Johannes M. H. M. Reul^{a,4}

^aNeuro-Epigenetics Research Group, University of Bristol, Bristol BS1 3NY, United Kingdom; ^bInstitute of Psychiatry, King's College London, London United Kingdom; and ^cUniversity of Exeter Medical School, University of Exeter, Exeter EX2 5DW, United Kingdom

According to the CDC -
Center for Disease Control -
75% of all chronic disease is
caused by modifiable, poor
lifestyle habits

Cell-Being.com



Take-home message



- Gene expression is regulated by regulatory proteins that would ultimately:
 - Guide the RNA polymerase (or other regulatory proteins) to the promoter
 - Strengthen/stabilize the RNA polymerase (or other regulatory proteins) binding to the promoter
 - Activate the RNA polymerase (or other regulatory proteins)
 - Create the open promoter complex for the RNA polymerase (or other regulatory proteins)
 - OR the opposite of the above in case of repressors.
 - All of the above effects are mediated via modulating non-covalent interactions between the amino acids of proteins and specific sequences of DNA.