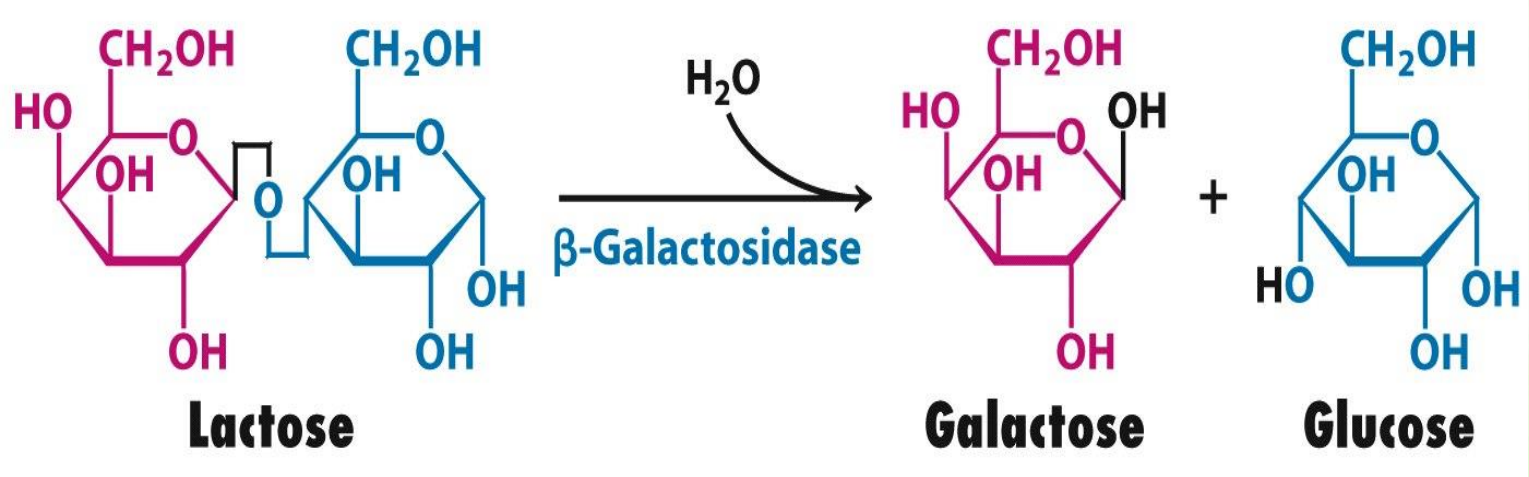
Transcription-regulation (1)

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

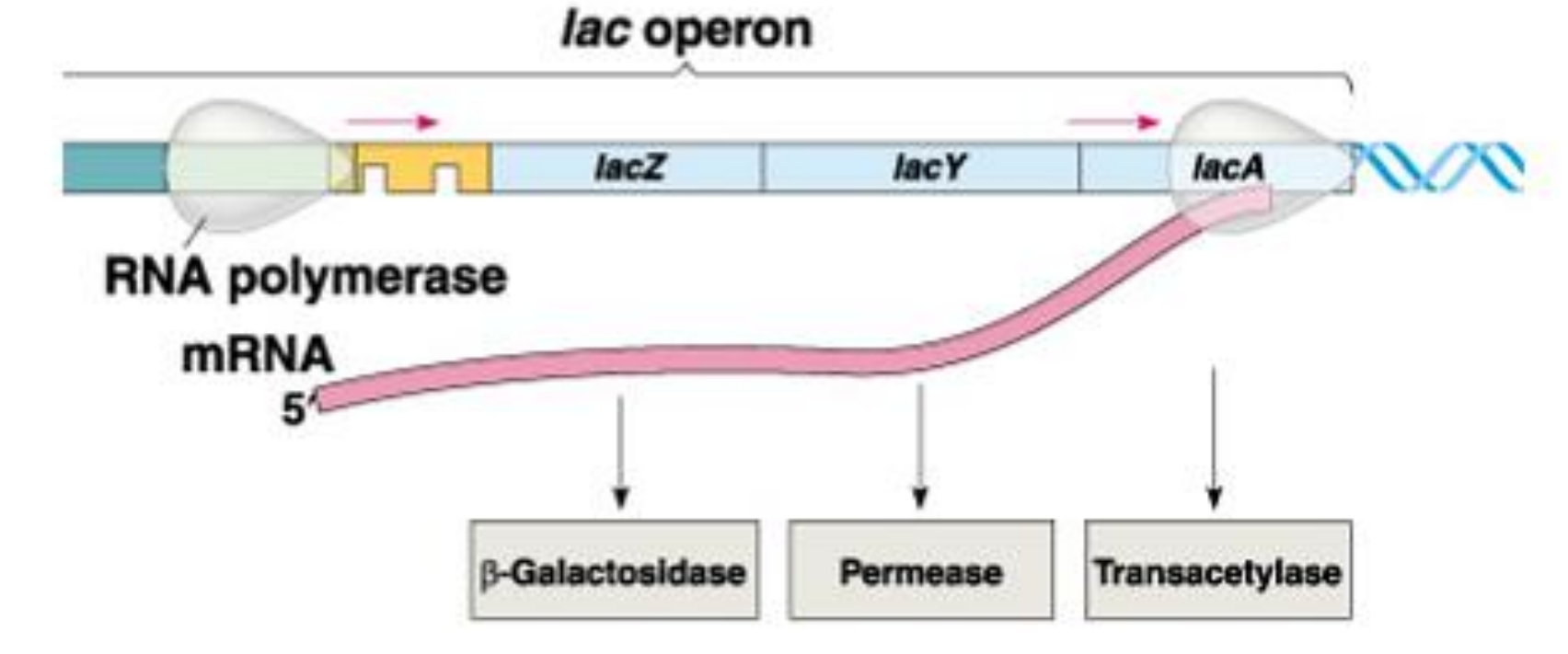


Components of the lac operon

- Lactose induces the synthesis of enzymes involved in its own metabolism including:
  - β-galactosidase: catalyzes the cleavage of lactose
  - lactose permease: transports lactose into the cell
  - a transacetylase: acetylates β-galactosides
- These genes are located in one operon known as the lac operon

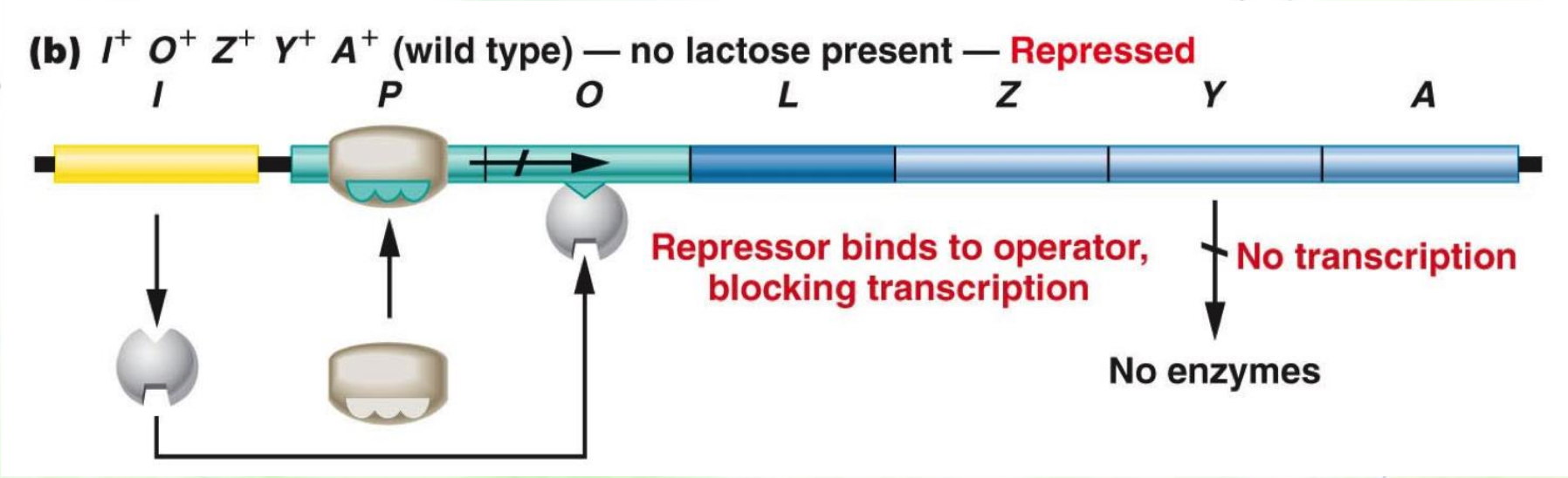
What is an operon?

- A cluster of genes transcribed from one promoter producing a polycistronic mRNA.



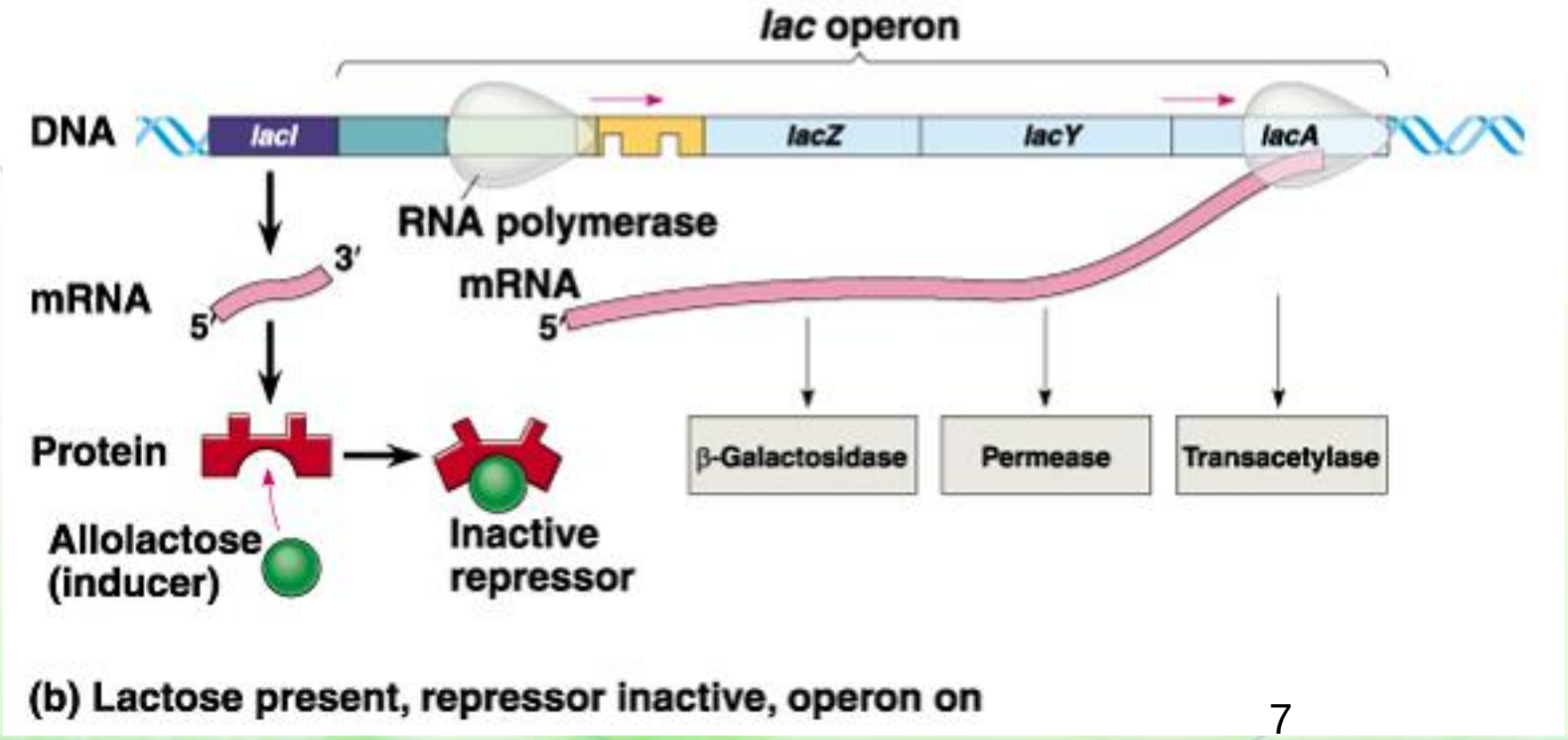
The operator

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



Regulation by lactose (positive)

- Lactose binds to the repressor, thereby preventing it from binding to the operator DNA.
- This is known as positive regulation.



(b) Lactose present, repressor inactive, operon on

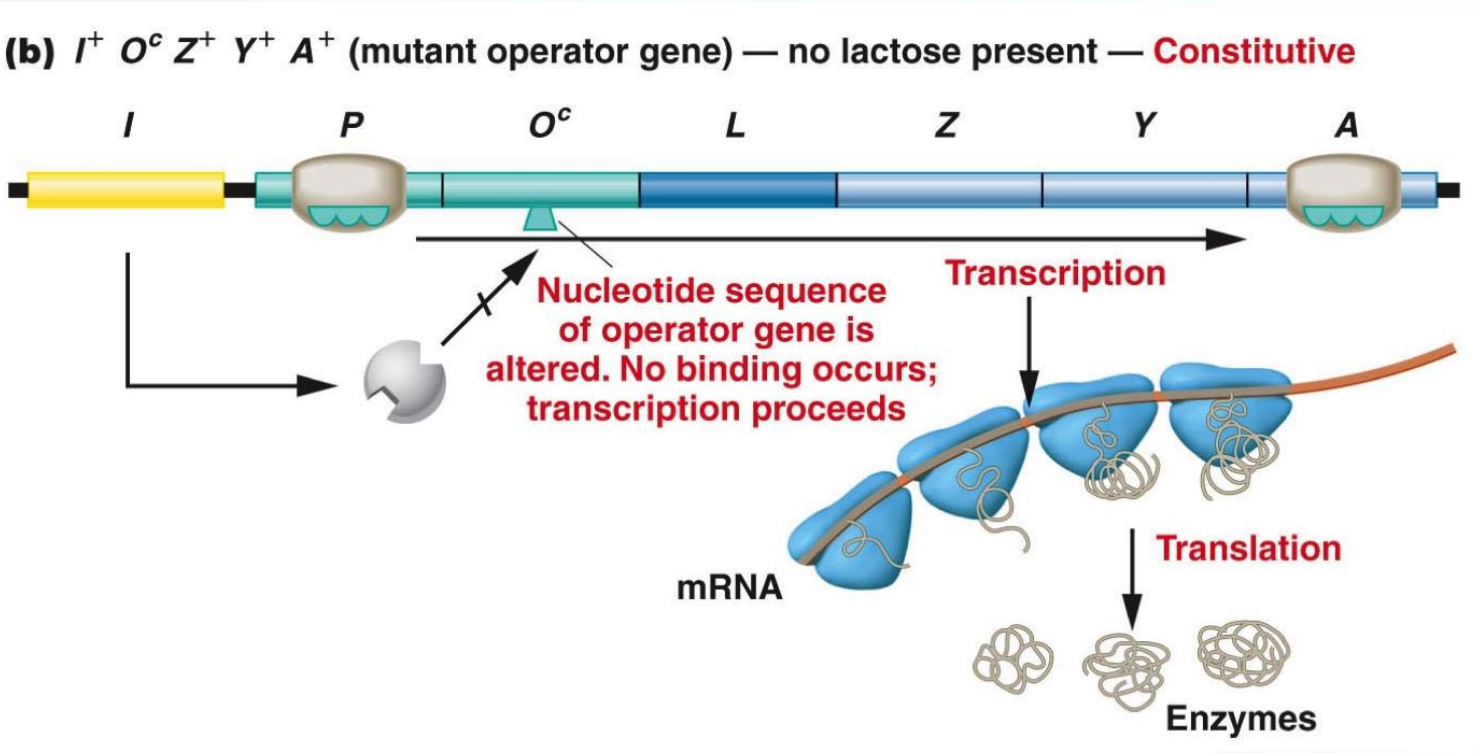
Cis vs. trans regulatory elements

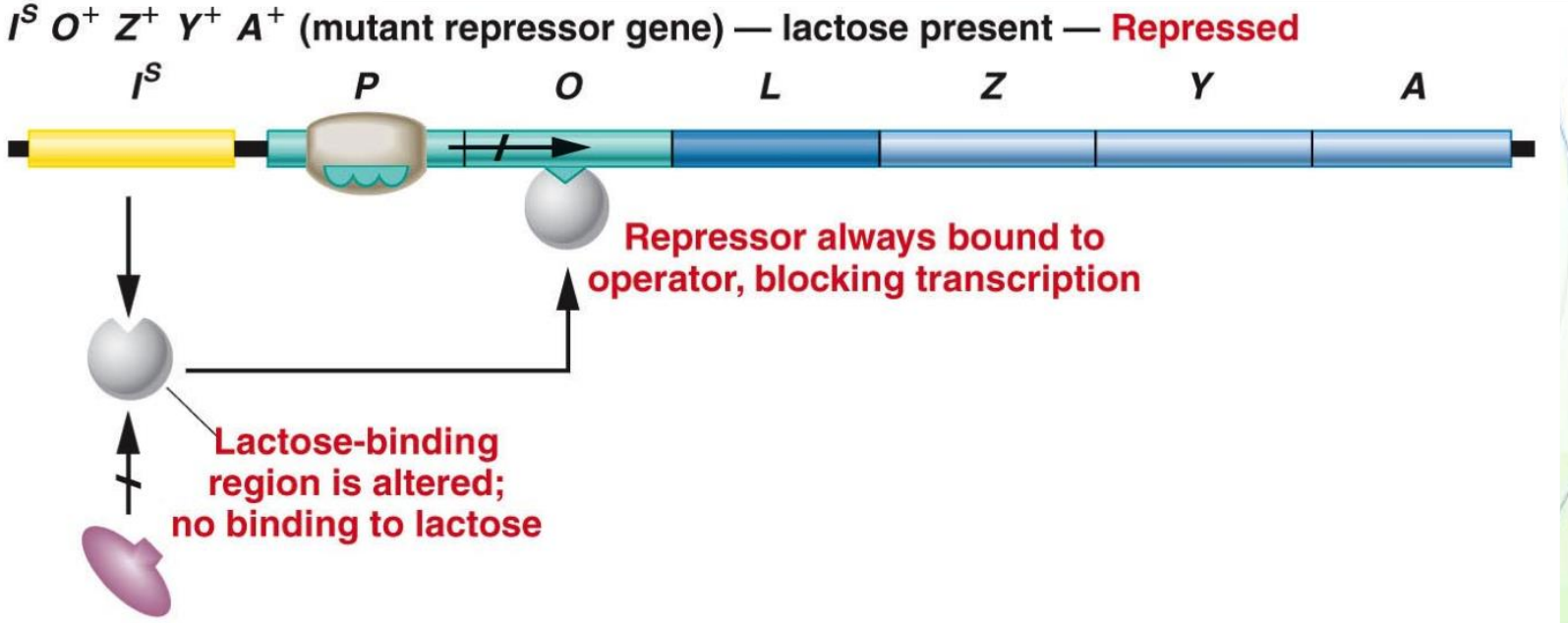
- Regulatory sequences like the operator are called cis-acting control elements, because they affect the expression of only linked genes on the same DNA molecule.

- Proteins like the repressor are called transacting factors because they can affect the expression of genes located on other chromosomes within the cell.

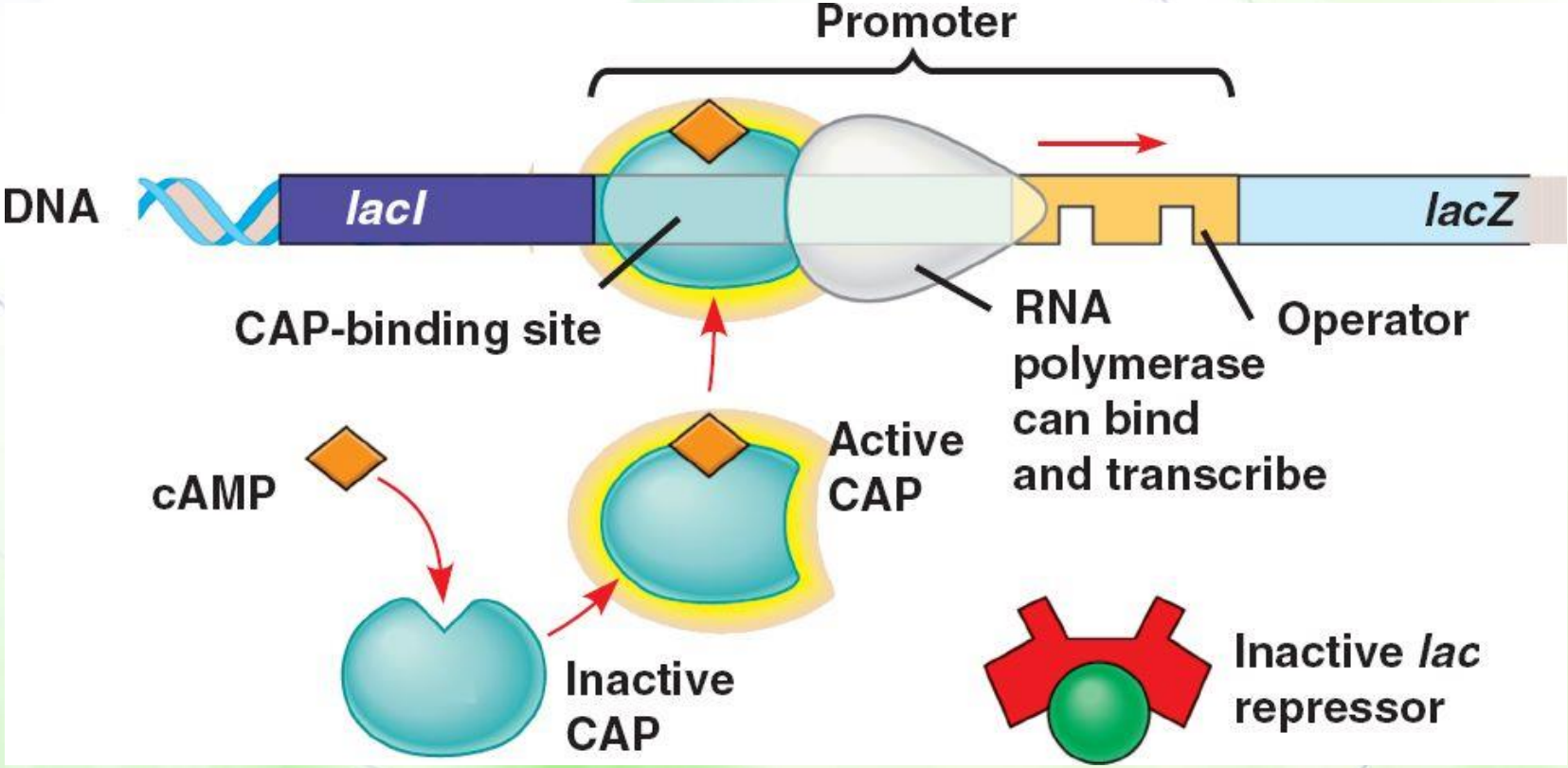
Effect of mutations

- Mutations affecting o result in constitutive expression (always on) since these mutations prevent i from binding to the operator.
- Mutants of i are either constitutive or noninducible (always off).
- In constitutive i mutants, i always binds lactose, so expression of the operon is always induced.
- In noninducible i mutants, the repressor binds to the operator very tightly even in the presence of lactose.


(b) I^+ O^c Z^+ Y^+ A^+ (mutant operator gene) — no lactose present — Constitutive
I
P
O^c
L
Z
Y
A
Nucleotide sequence of operator gene is altered. No binding occurs; transcription proceeds
Transcription
mRNA
Translation
Enzymes

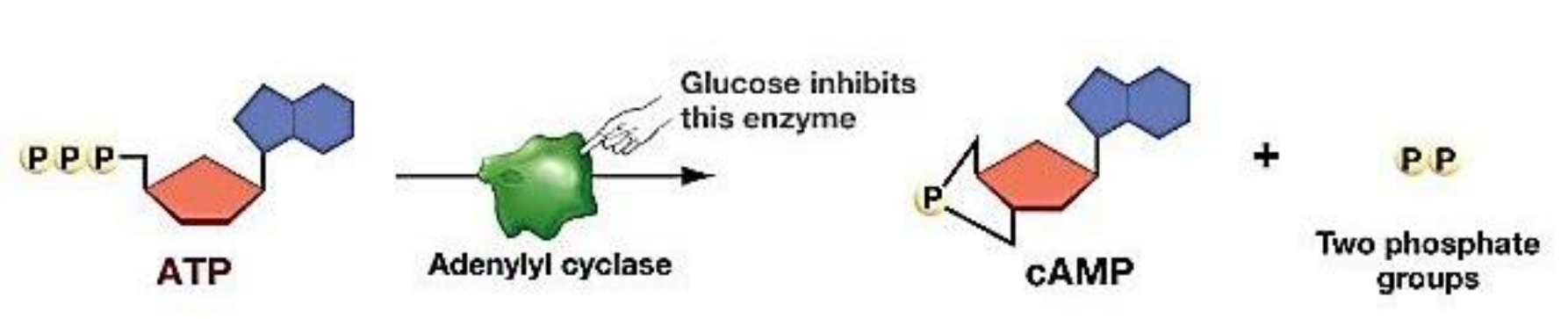

I^S O^+ Z^+ Y^+ A^+ (mutant repressor gene) — lactose present — Repressed
I^S
P
O
L
Z
Y
A
Repressor always bound to operator, blocking transcription
Lactose-binding region is altered; no binding to lactose

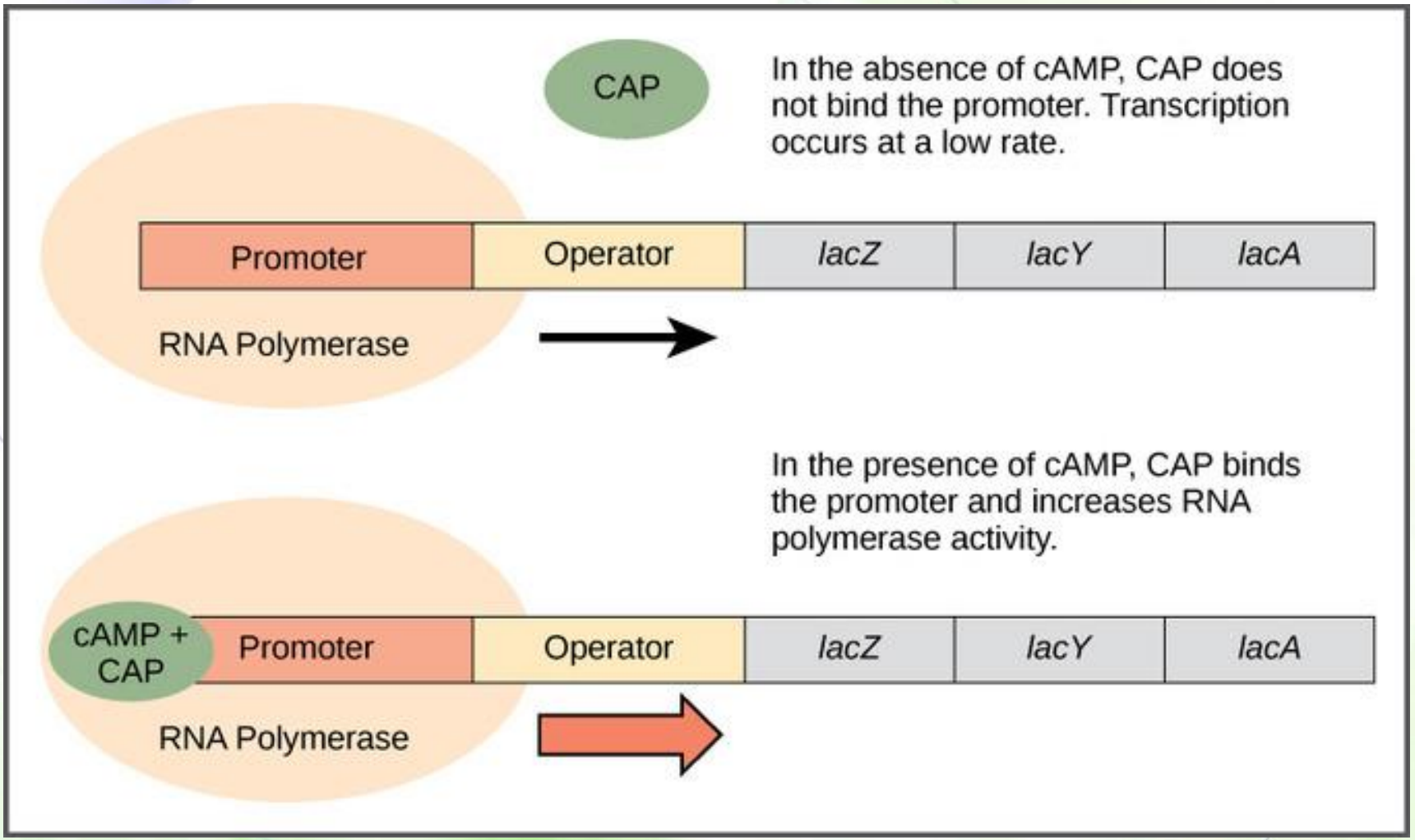
- Another regulator is cAMP, which binds to a protein known as catabolite activating protein (CAP).

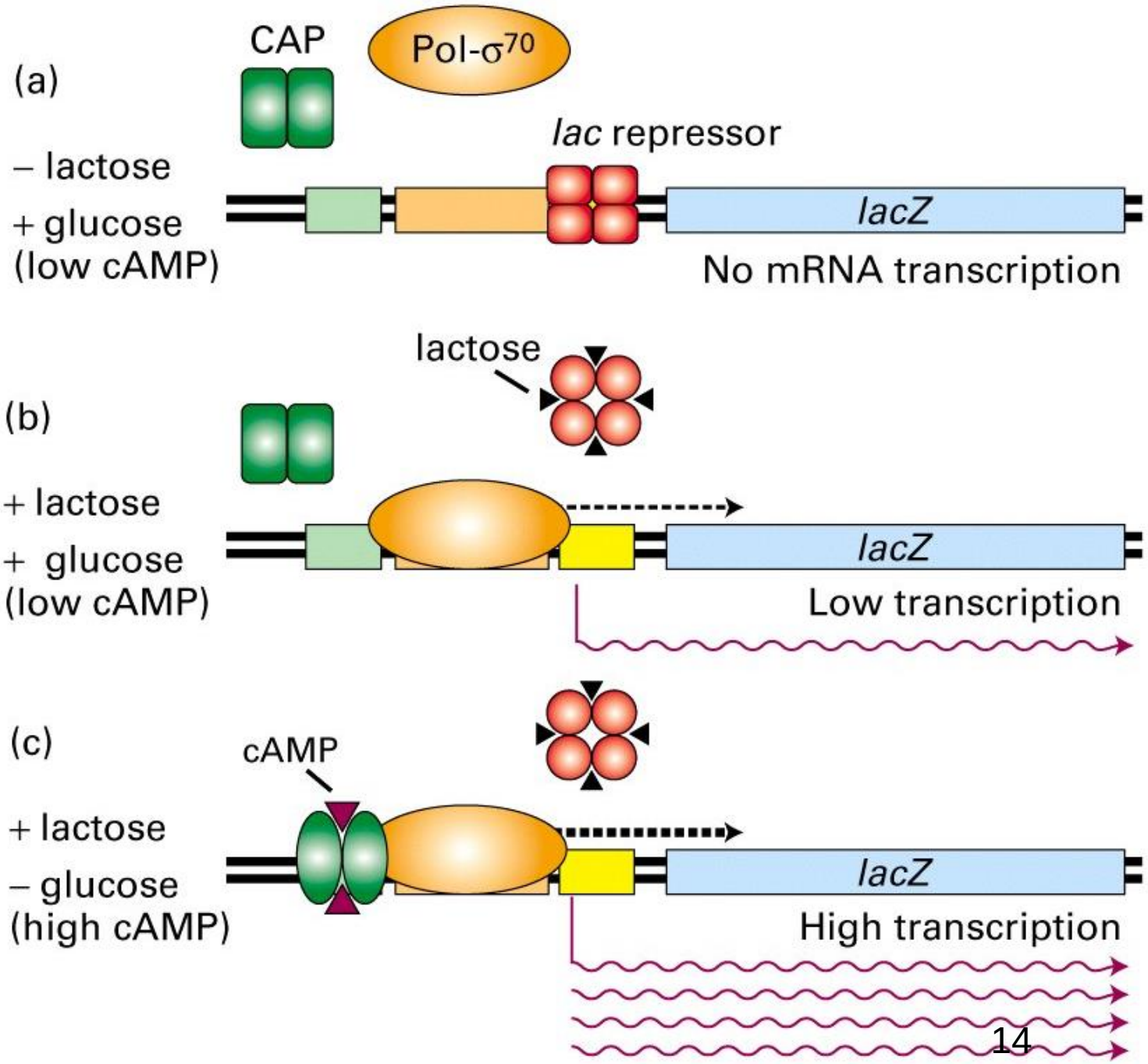


Regulation by glucose (negative)

- The ability of CAP to bind to the promoter is influenced by how much cAMP is in the cell is produced by adenylyl cyclase, which is inhibited by high level of glucose.
- Glucose 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.




CAP
In the absence of cAMP, CAP does not bind the promoter. Transcription occurs at a low rate.
Promoter
Operator
lacZ
lacY
lacA
RNA Polymerase
In the presence of cAMP, CAP binds the promoter and increases RNA polymerase activity.
cAMP + CAP
Promoter
Operator
lacZ
lacY
lacA
RNA Polymerase


CAP
Pol-σ70
(a)
lac repressor
– lactose
+ glucose
(low cAMP)
lacZ
No mRNA transcription
lactose
(b)
+ lactose
+ glucose
(low cAMP)
lacZ
Low transcription
(c)
cAMP
+ lactose
– glucose
(high cAMP)
lacZ
High transcription

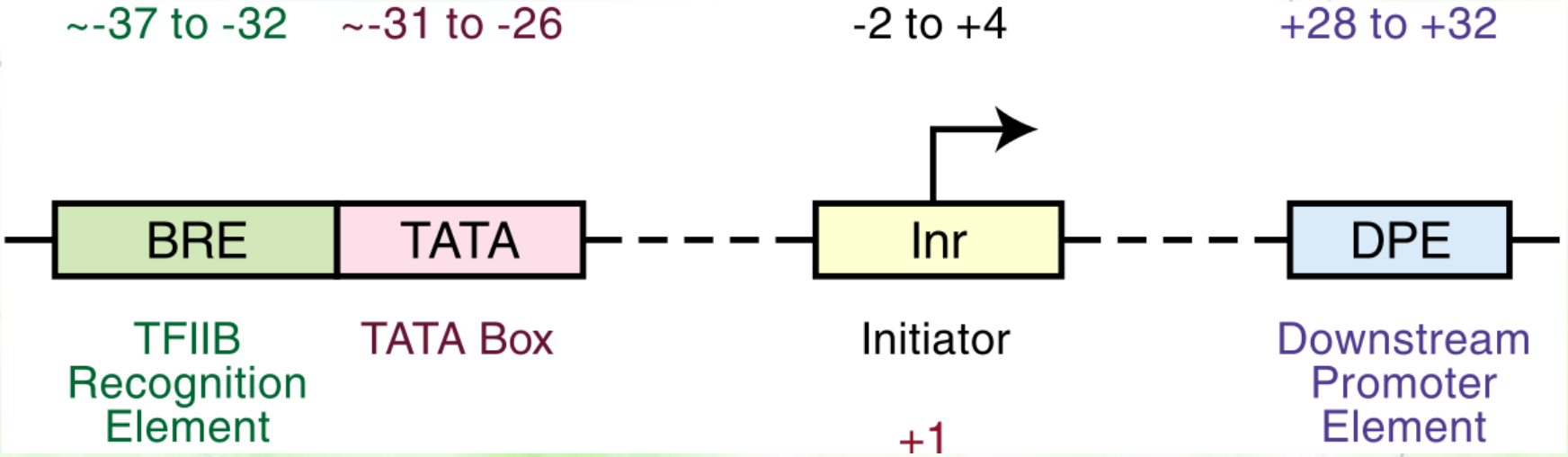
Regulation of transcription in eukaryotes

Regulatory mechanisms

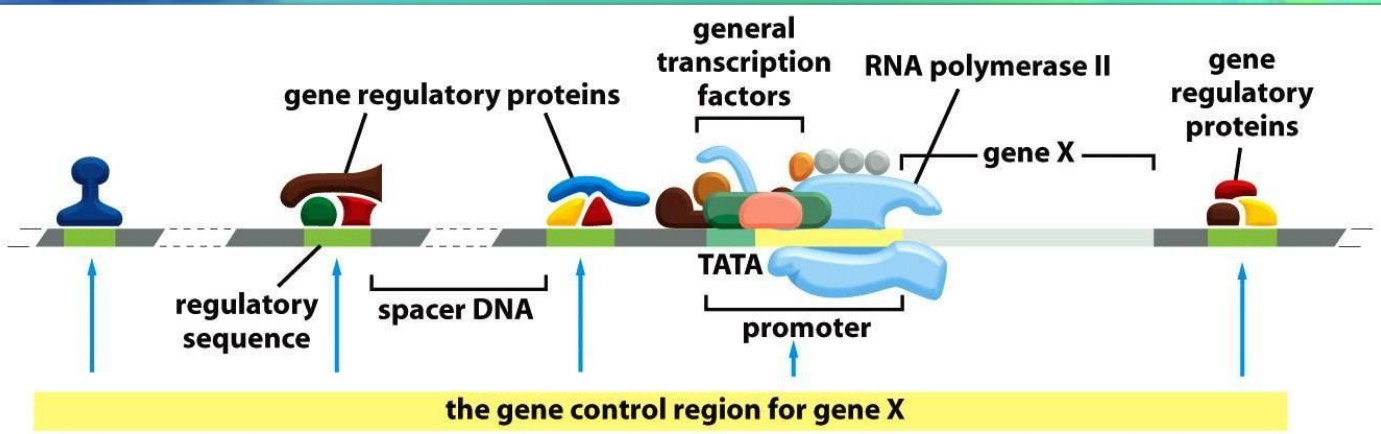
- Although the control of gene expression is far more complex in eukaryotes than in bacteria, the same basic principles apply
- Transcription in eukaryotic cells is controlled by:
  - Cis-acting DNA sequences
    - Promoters and enhancers
  - Transcriptional regulatory proteins
  - Repressor proteins
  - Modification of DNA and its packaging into chromatin

General components of promoters

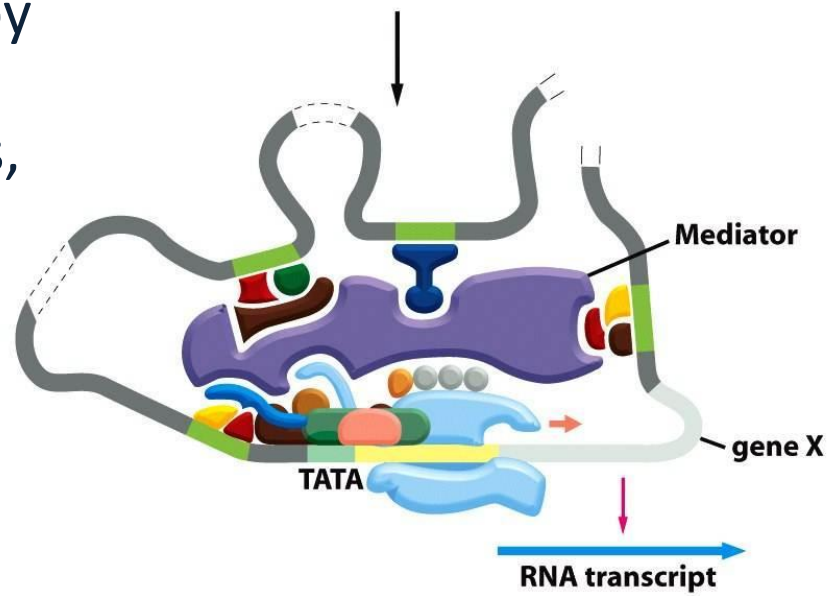
- An upstream element (BRE) that is binding site of TFIIB.
- The TATA box, which is binding site of TFIID.
- The initiator element (*Inr)*, which surrounds the +1 site.
- Multiple downstream elements.



Enhancers

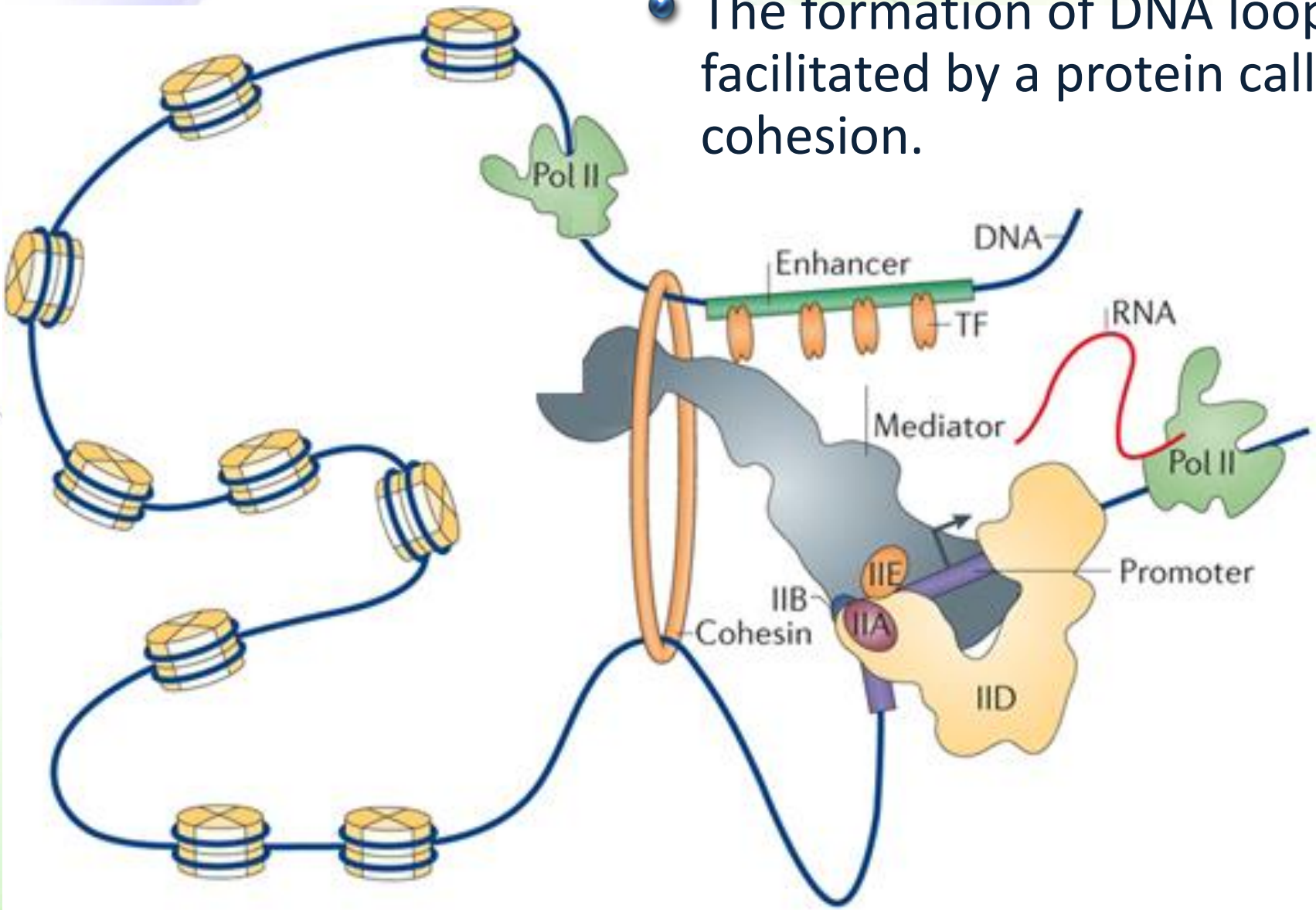


- Many genes are regulated by cis-acting regulatory sequences called enhancers, which are binding sites for gene-specific transcription factors that regulate RNA polymerase II.
- They can regulate transcription regardless of orientation or location due to DNA looping.

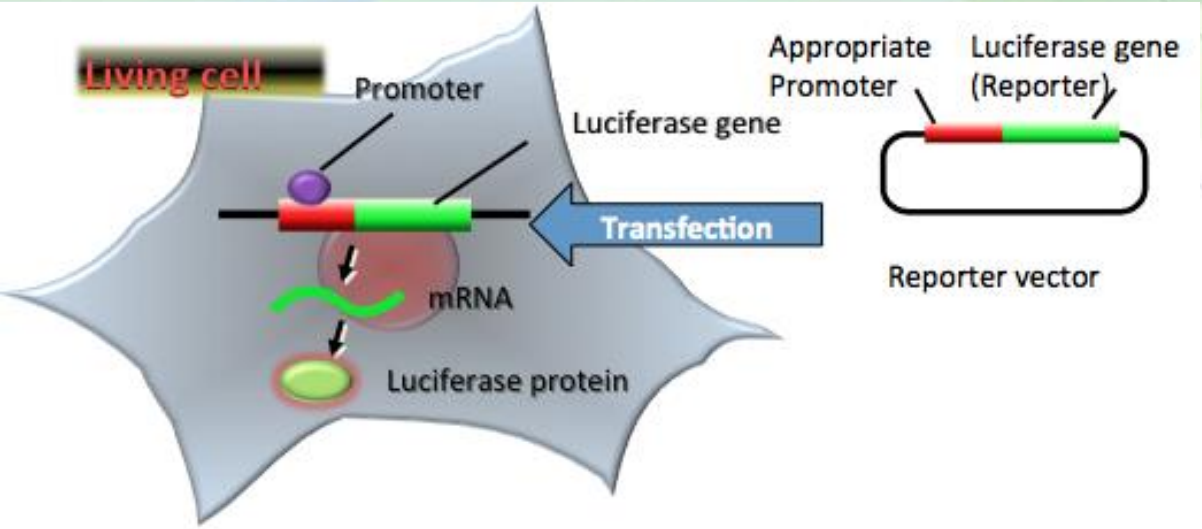


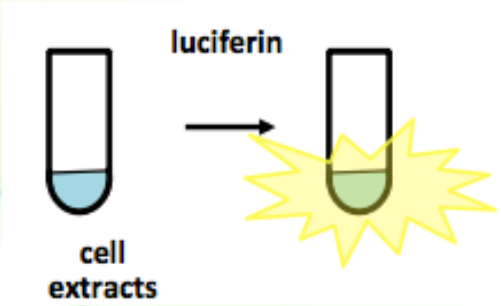
Cohesion

- The formation of DNA loops is facilitated by a protein called cohesion.

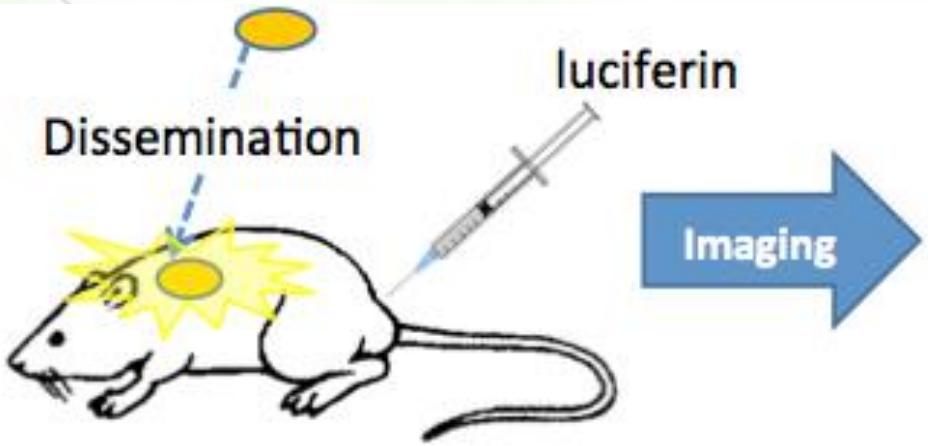


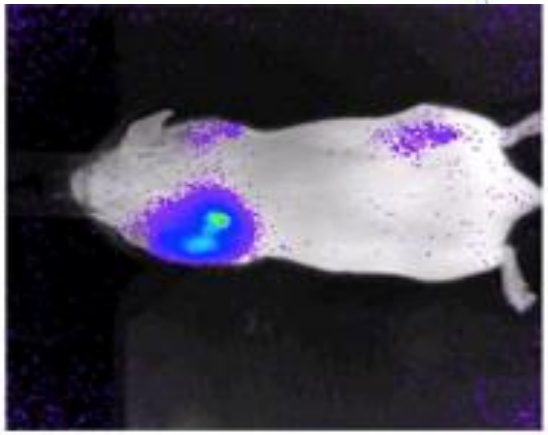
Gene transfer assay (Reporter gene)





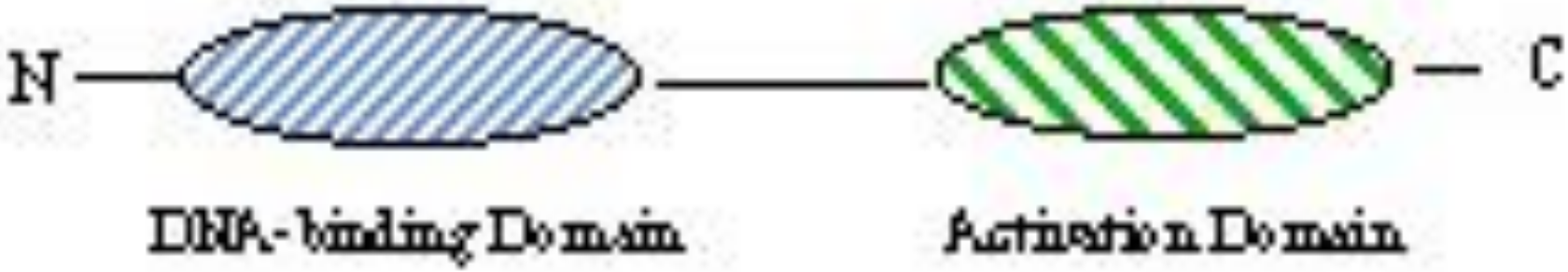
- A DNA sequence suspected to be a regulatory sequence is placed upstream of a reporter gene such as luciferase in a plasmid.
- If positive, a signal is detected.





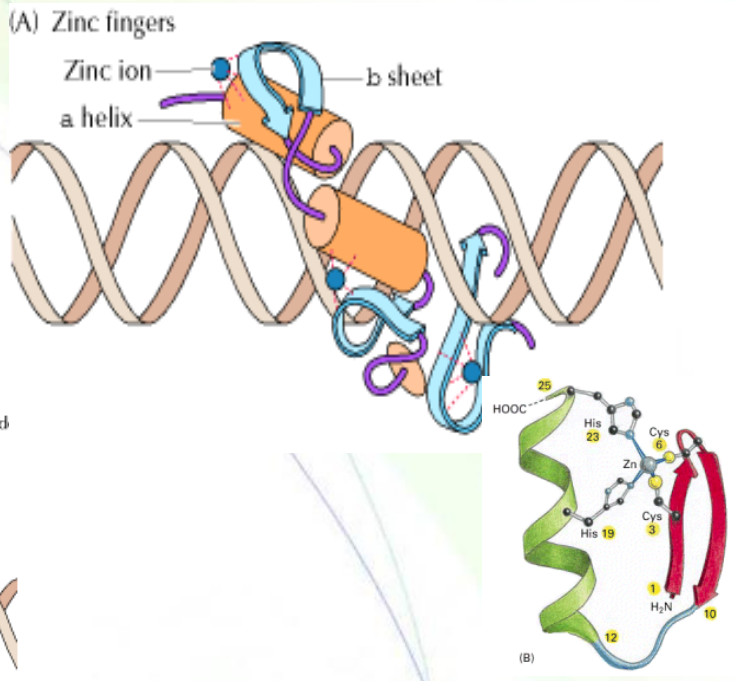
Transcriptional regulatory proteins

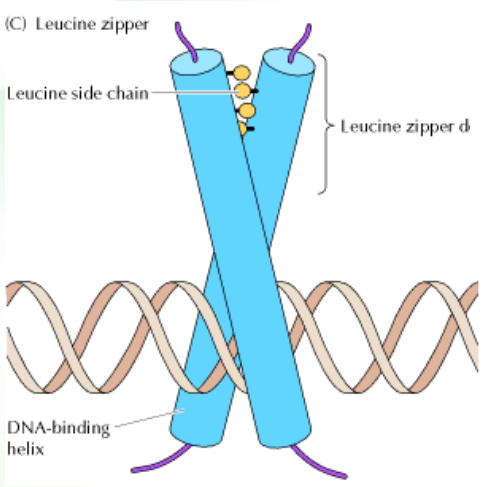
- These proteins to consist of two domains:
  - One region of the protein specifically binds DNA (DNA-binding domain)
  - the other activates transcription by interacting with other components of the transcriptional machinery (regulatory or activation domain)
- Both activities are independent and can be separated from each other

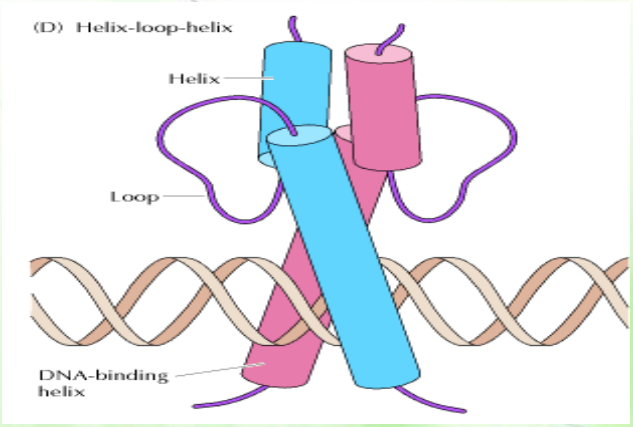


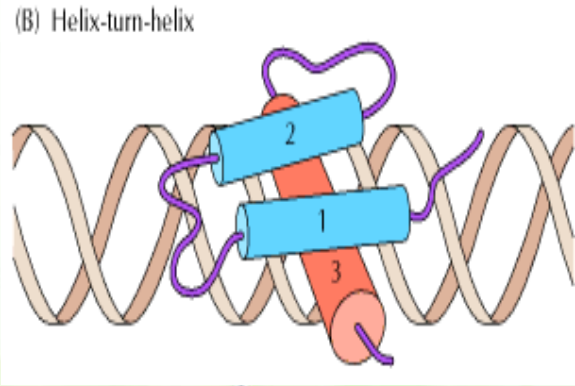
DNA-binding domains

- Zinc finger domains (Steroid receptors)
- helix-turn-helix motif
- leucine zipper (CREB)
- helix-loop-helix



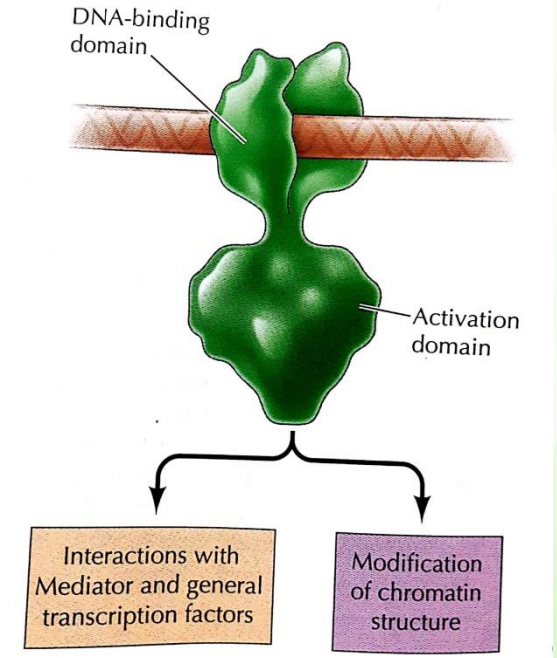






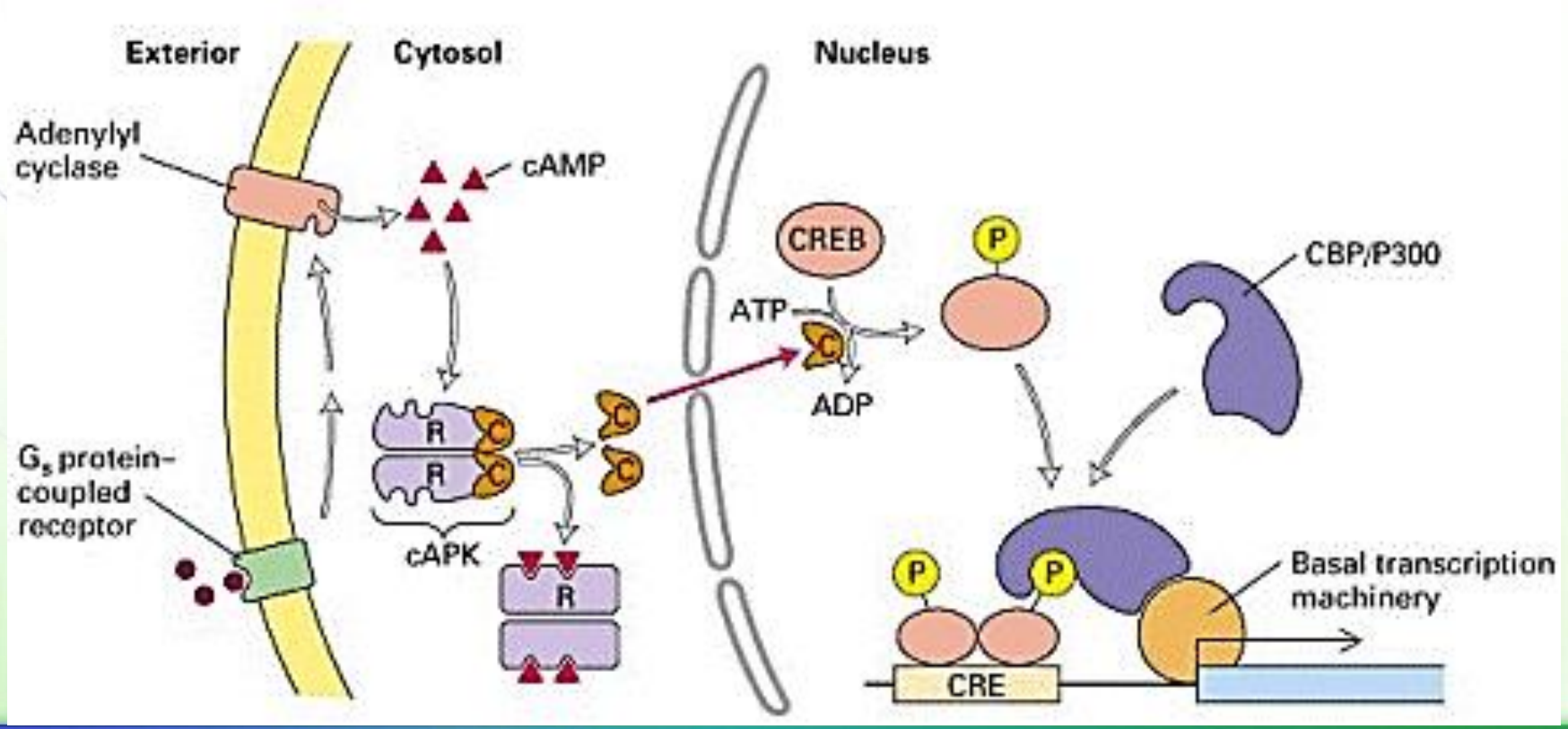
The activation domains

- Activation domains are stimulate transcription by interacting with general transcription factors, facilitating the assembly of a transcription complex on the promoter or modifying the chromatin.
- Composition of domains:
  - Acidic domains
  - Glutamine-rich domains
  - Proline-rich domains



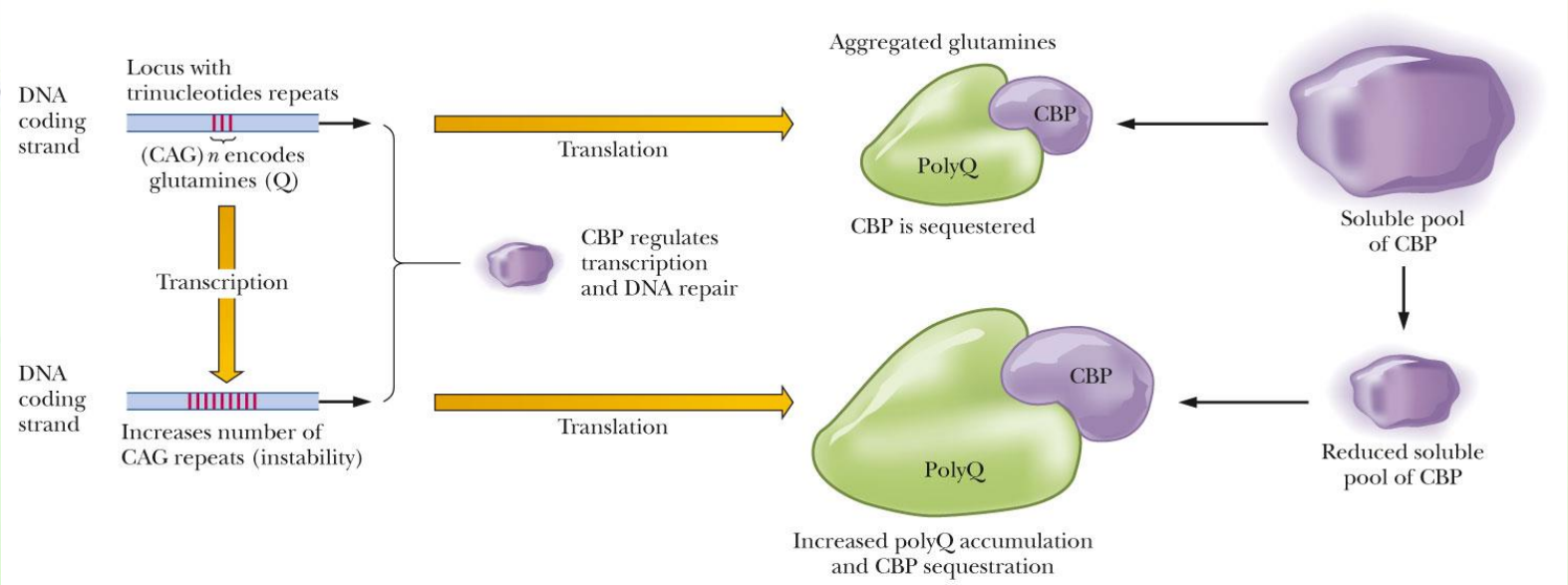
cAMP-response element (CRE) Binding protein (CREB)

- In the presence of cAMP, protein kinase A is activated phosphorylating CREB, which binds to CRE. The dimer can then form a complex with the RNA polymerase and, thereby, activating transcription.



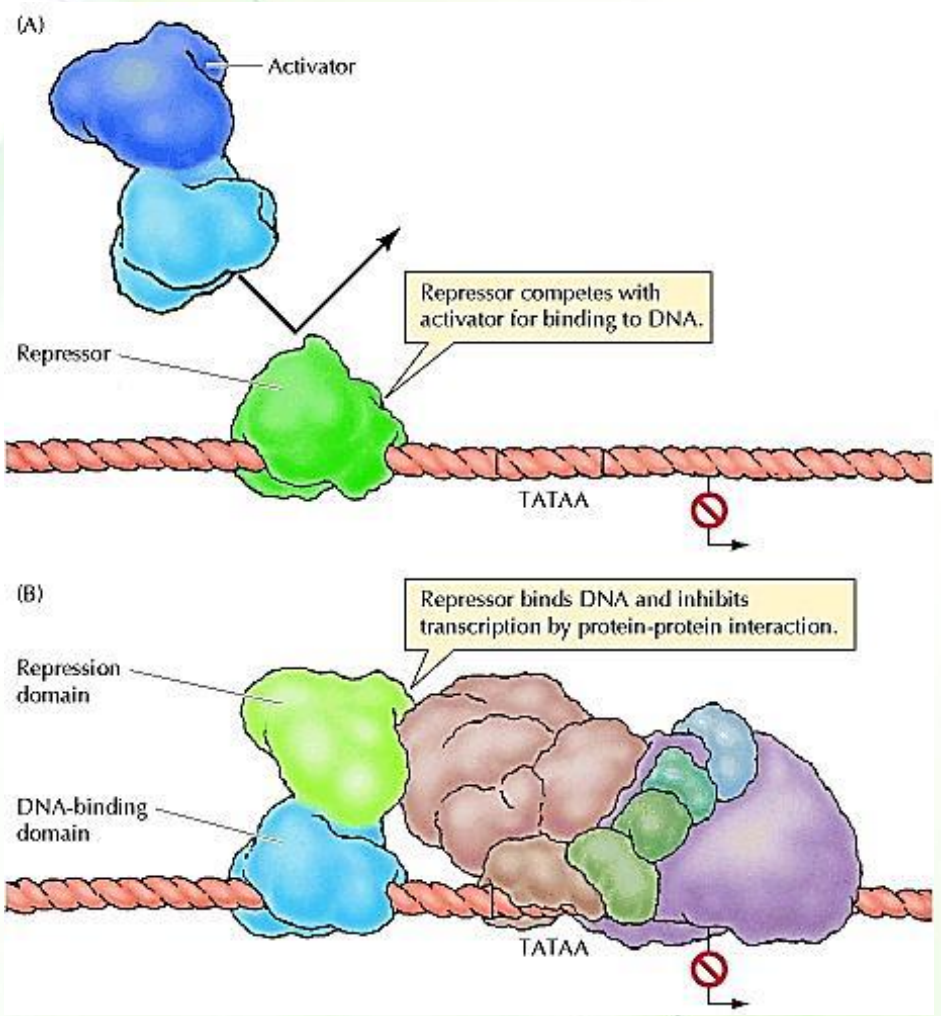
Huntington's disease

- It is a disease caused by a mutation in a transcription protein known as *huntingtin*. The mutation is an increase in the number of a trinucleotide repeat of CAG (it encodes polyglutamine).
- The polyglutamine product sequesters CBP, making less of it available for molecular processes, such as transcription and DNA repair.
- The loss of the DNA repair leads to a propagation of the CAG repeats and leads to the disease becoming worse in successive generations.

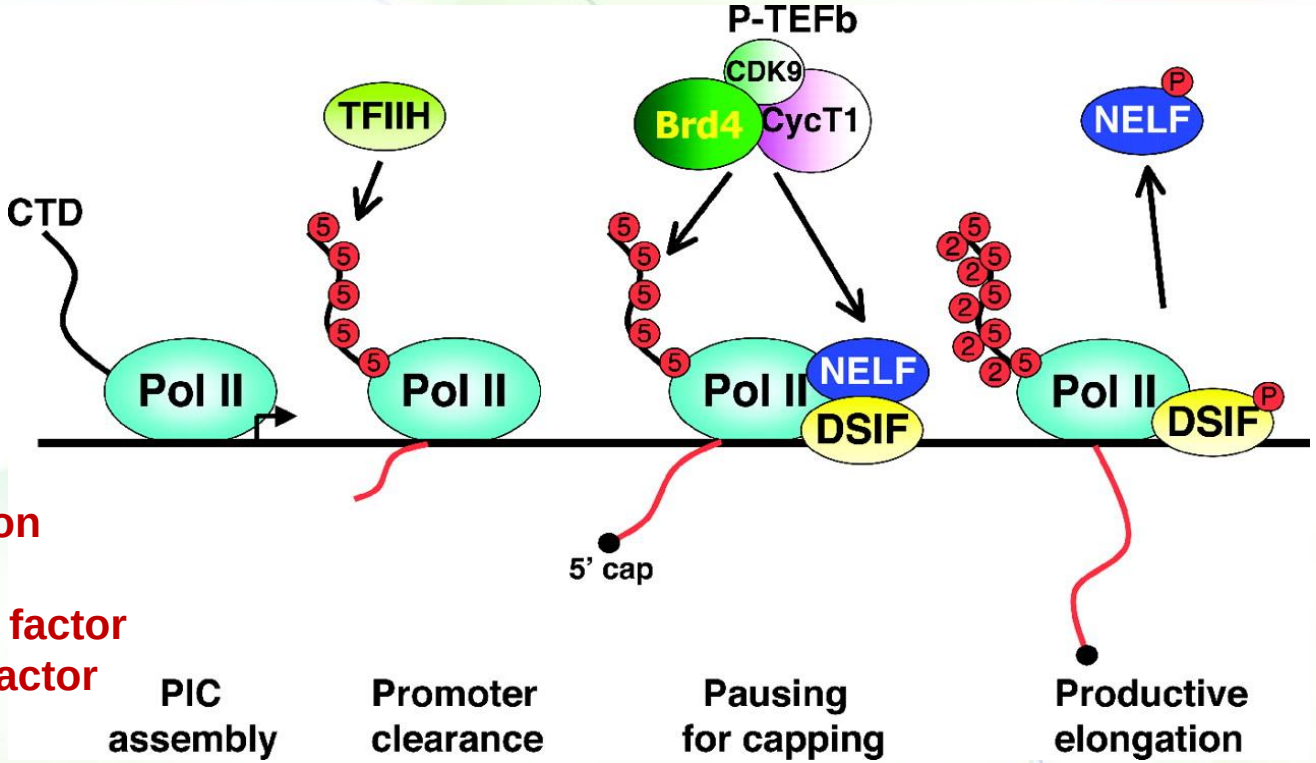


Eukaryotic Repressors

- Repressors bind to specific DNA sequences and inhibit transcription.
- Repressors may have
  - both DNA-binding and protein-binding domains
  - DNA-binding domains, but not protein-interaction domains



Regulation of elongation

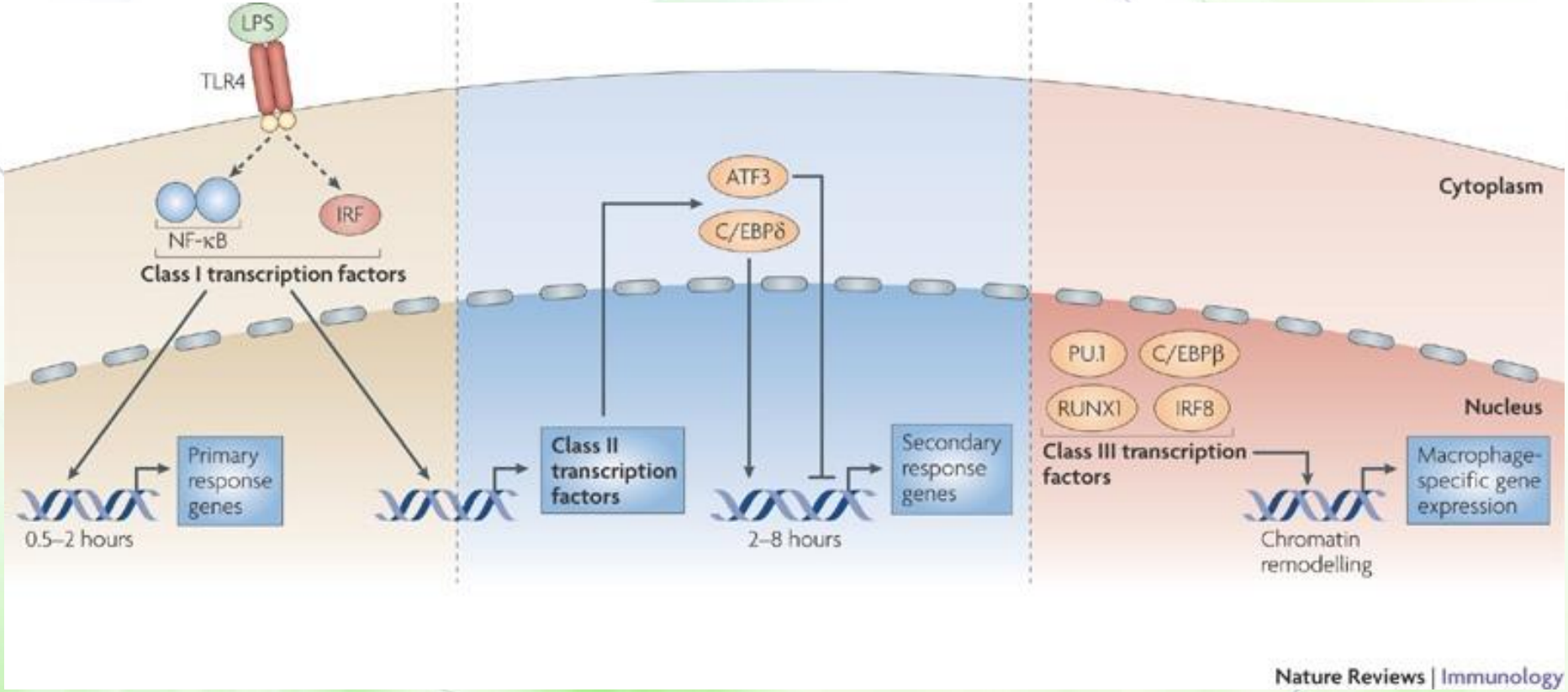


P-TEFb: Positive transcription elongation factor b
DSIF: *D* sensitivity-inducing factor
NELF: negative elongation factor

- Shortly after initiation, the progression of Pol II is stalled by two negative elongation factors, DSIF and NELF.
- This facilitates capping of the nascent pre-mRNA.
- P-TEFb phosphorylates DISF, NELF, and and RNA pol II promoting the dissociation of NELF, converting DSIF into a positive elongation factor, and allowing productive elongation.

Trancriptional regulatory network

- primary, secondary,…etc transcription regulation

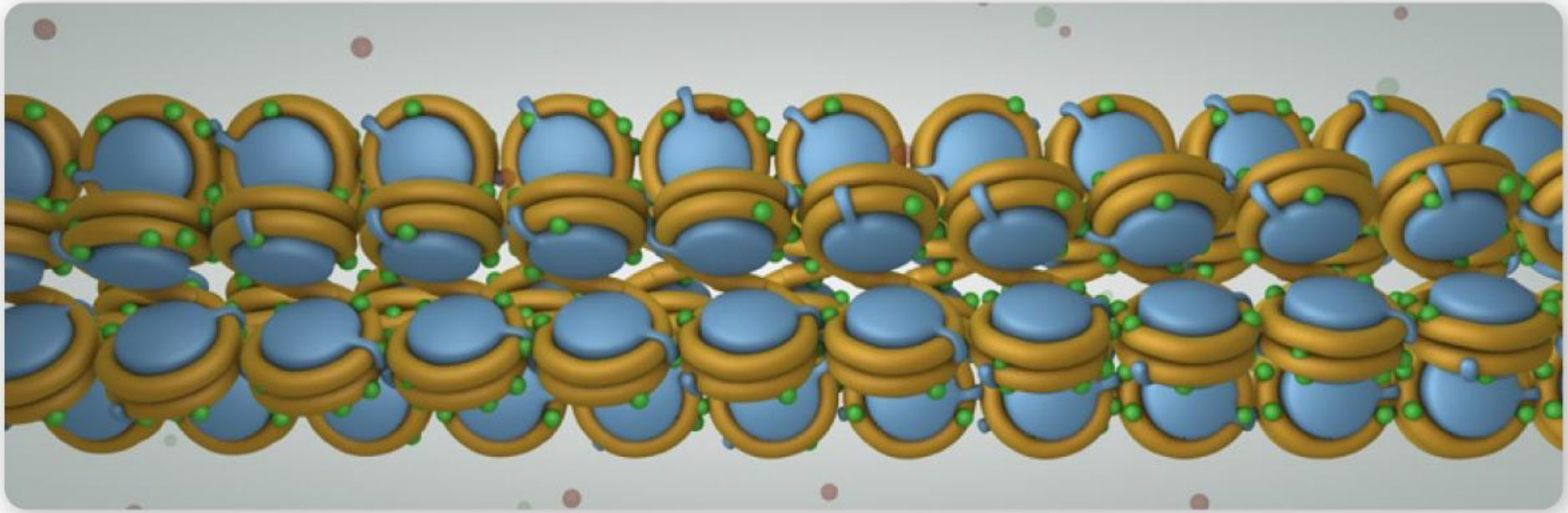


Epigenomics

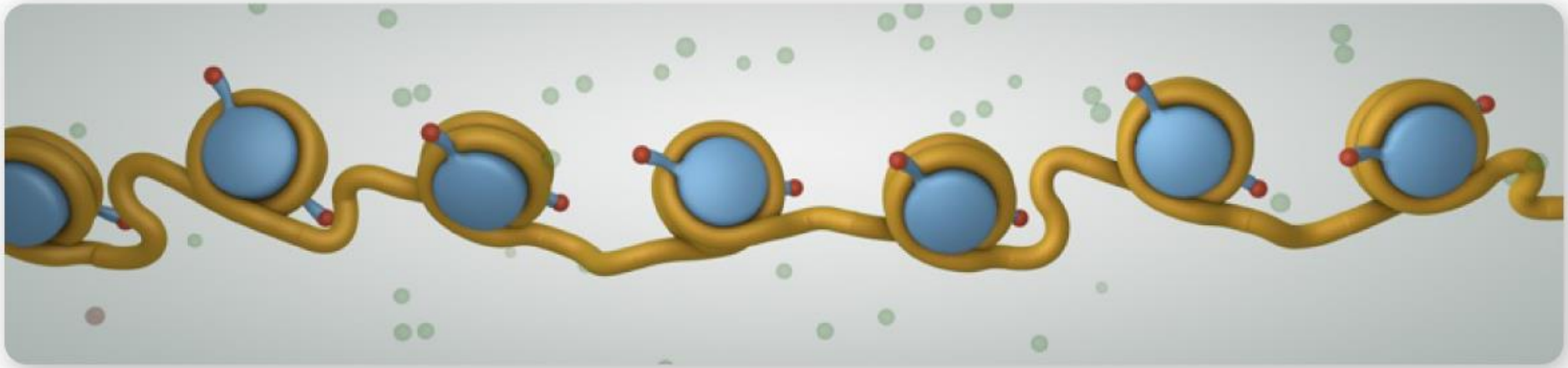
- Epi: “above” or “in addition to”
- It indicates genetic alterations in gene expression without a change in DNA sequence
  - Can be caused by the pattern of chromosomal packaging and modification

Modulation of chromosomal structure

- The packaging of eukaryotic DNA in chromatin has important consequences in terms of its availability as a template for transcription
  - Actively transcribed genes are found in loose chromatin (euchromatin), and vice versa (heterochromatin).
  - Even in loose chromatin, the tight winding of DNA around the nucleosome core particle can prevent transcription factors from binding DNA and the RNA polymerase to transcribe through a chromatin template.



The epigenome tightly wraps **inactive** genes, making them unreadable.



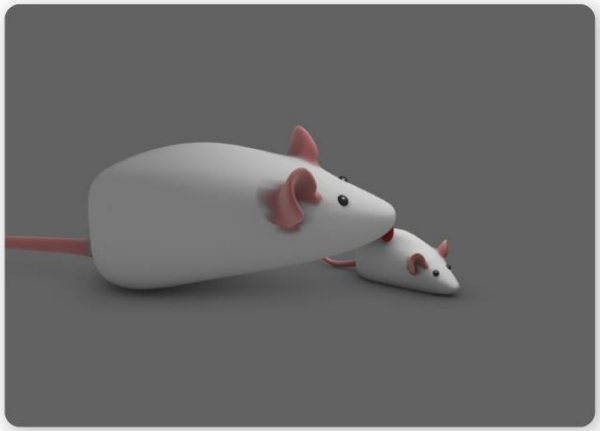
The epigenome relaxes **active** genes, making them easily accessible.

Identical twins have the exact same genetic information

But their epigenomes become increasingly different over time

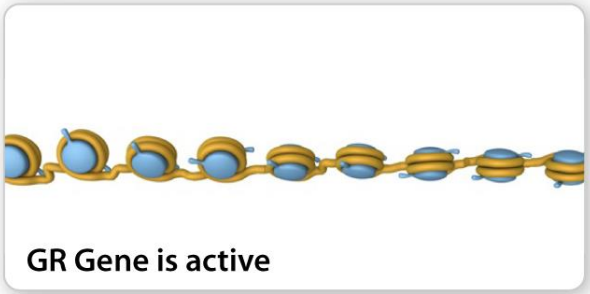
- Epigenetic changes can cause dramatic differences between twins, including many cases where one twin develops a disease and the other does not.

Maternal care affects the epigenome

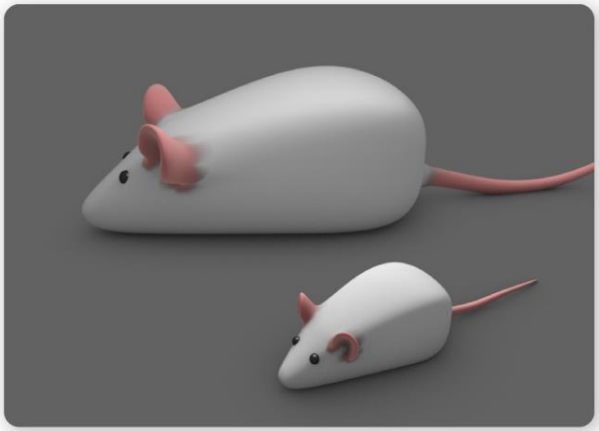


Highly nurtured rat pups tend to grow up to be calm adults.

High Nurtured

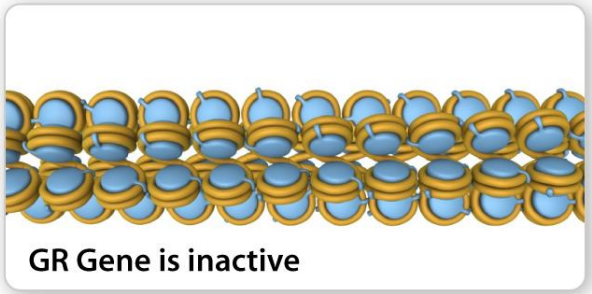


In high nurtured rat pups, the GR gene is active. These rats have an easy time relaxing after stress.



Rat pups who receive little nurturing tend to grow up to be anxious adults.

Low Nurtured



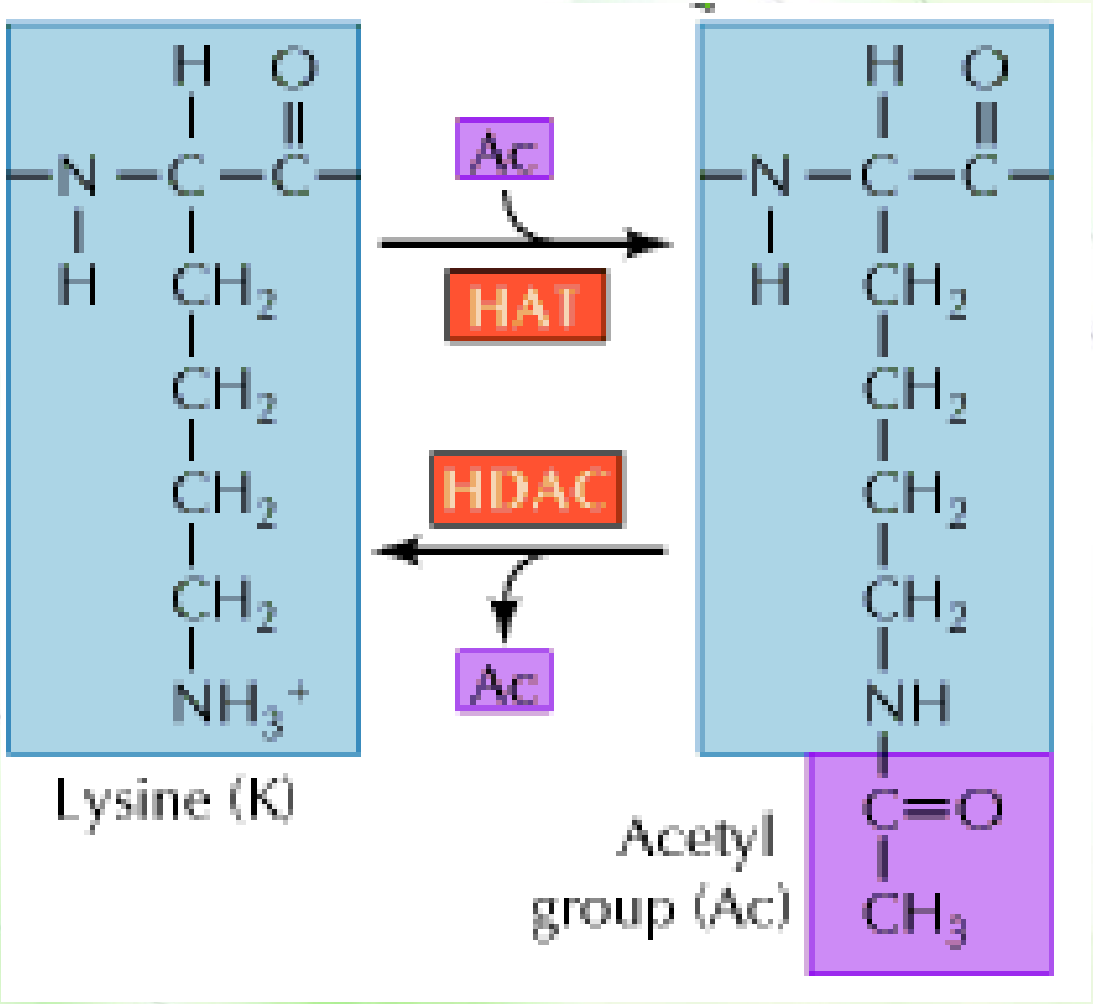
In low nurtured rat pups, the GR gene is epigenetically silenced. These rats have a hard time relaxing after stress.

When it's active, the GR gene produces a protein that helps the body relax after stress. Mom's nurturing during the first week of life shapes her pups' epigenomes.

How are chromosomal structures altered?

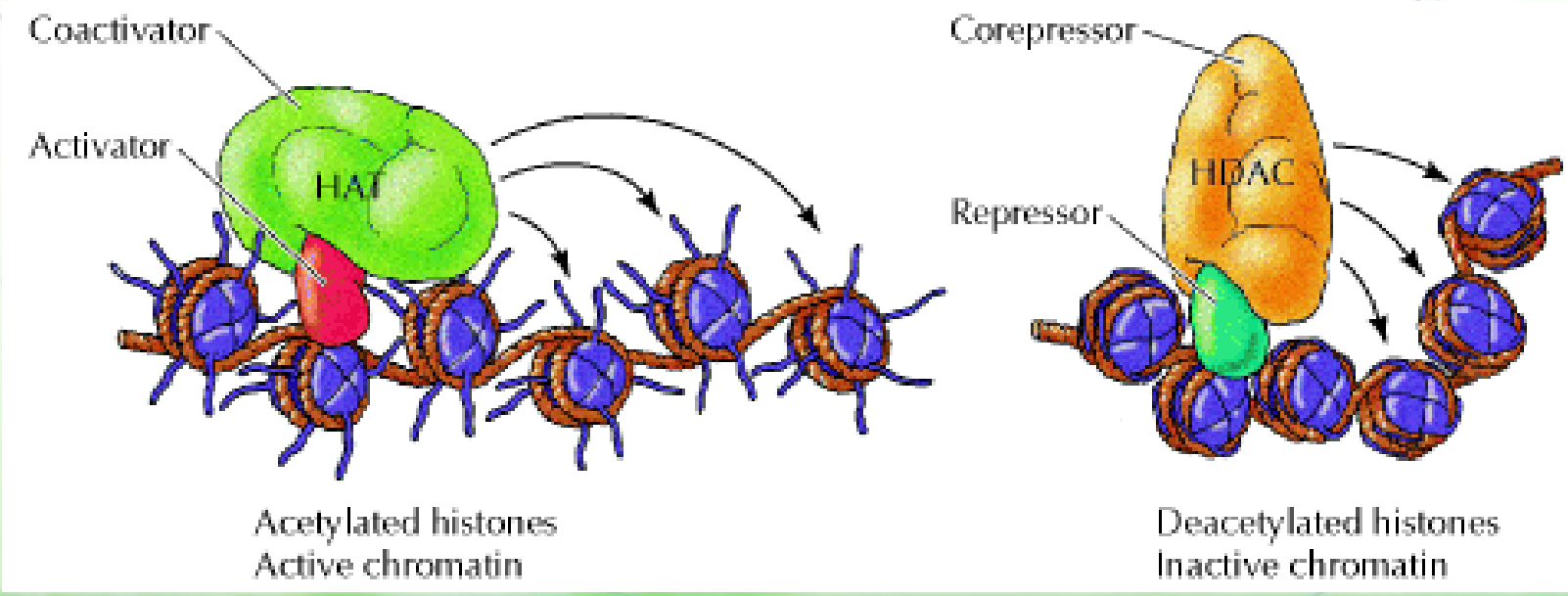
- Decrease in the compactness of the chromatin by:
  - Chemical modification of histones
    - Acetylation, methylation, phosphorylation, and SUMO (small ubiquitin-related modifier)
  - Complexing of transcription factors with nucleosome remodeling factors.
  - Binding of noncoding RNAs to DNA

Histone acetylation

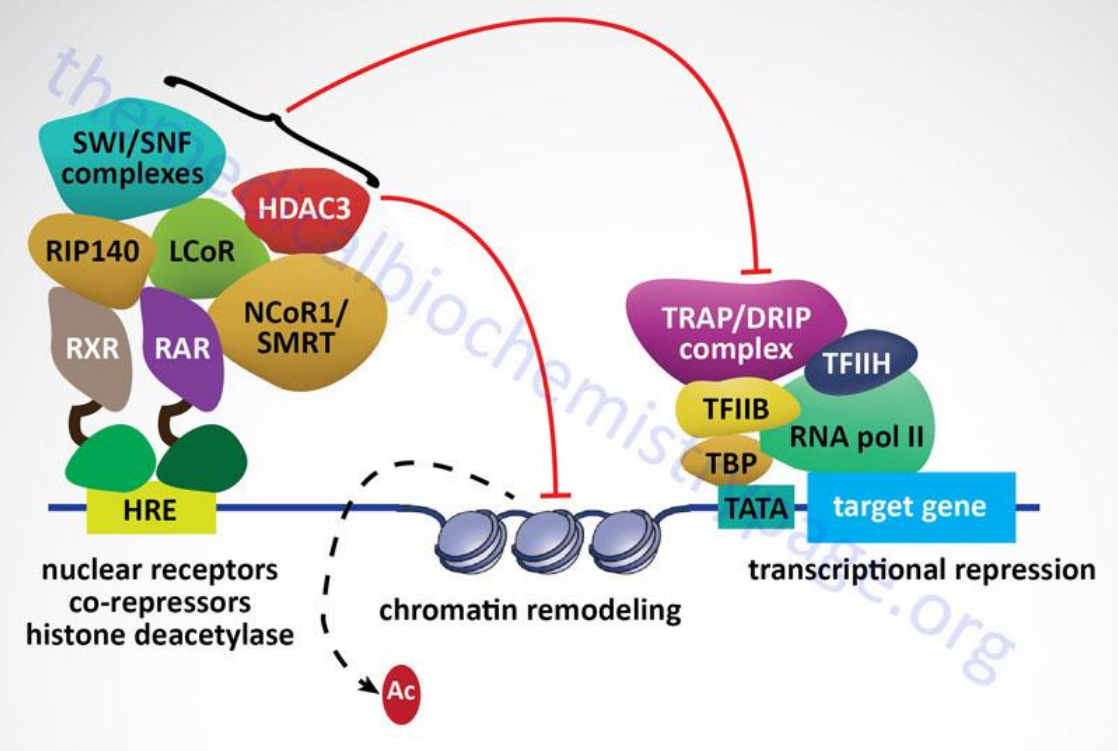


Enzymatic association

- Transcriptional activators and repressors are associated with histone acetyltransferases and deacetylases, respectively
  - A component of TFIID has been found to be a histone acetyltransferases



An example of transcriptional repression



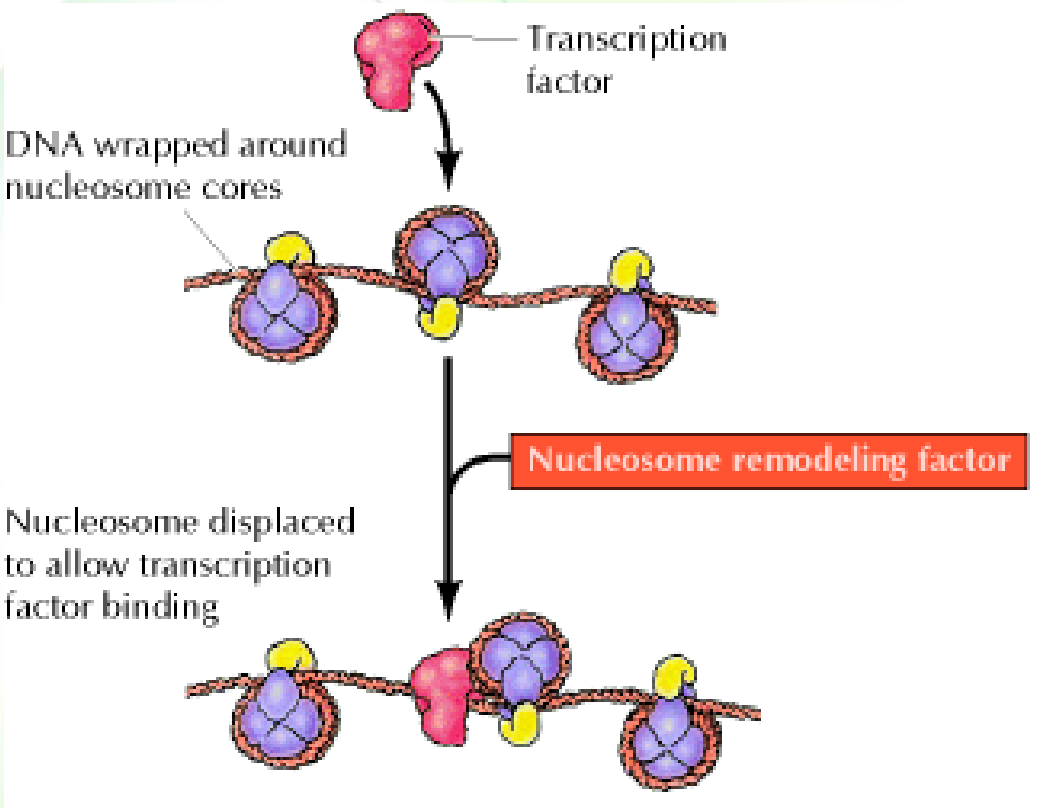
- A transcription co-repressor complex is associated with the retinoic acid receptor (RAR) and retinoid X receptor (RXR) transcription factor complex at the hormone response element (HRE) and are complexed with co-repressor proteins that include a histone deacetylase (HDAC).

Histone modifications are co-regulated

- Histone phosphorylation induces other modifications that stimulate transcription.
- Histone methylation recruit proteins associated with histone deacetylase.
- Histone phosphorylation (transcriptional activation) leads to histone acetylation (also activation) and inhibits methylation (repression)

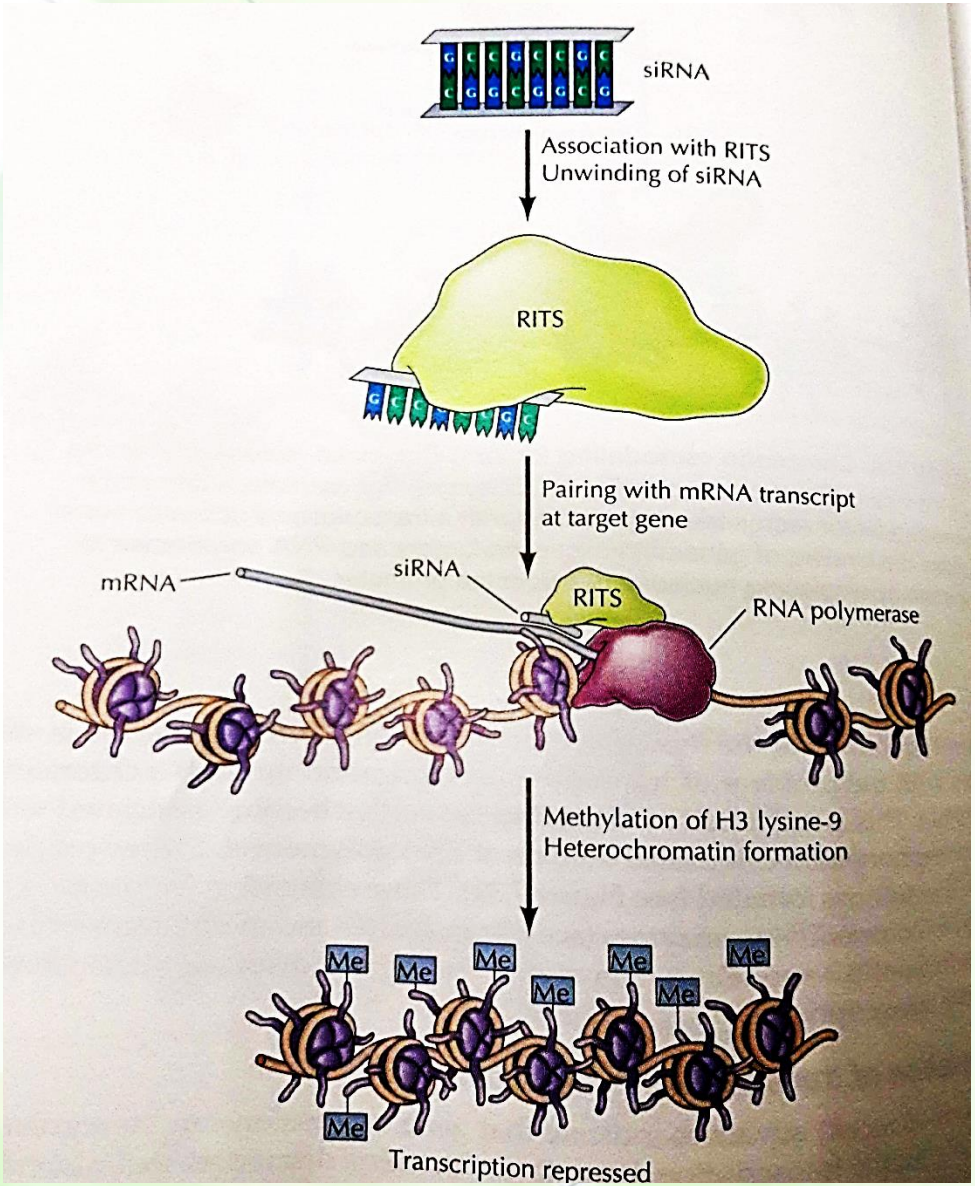
Nucleosome remodeling factors

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



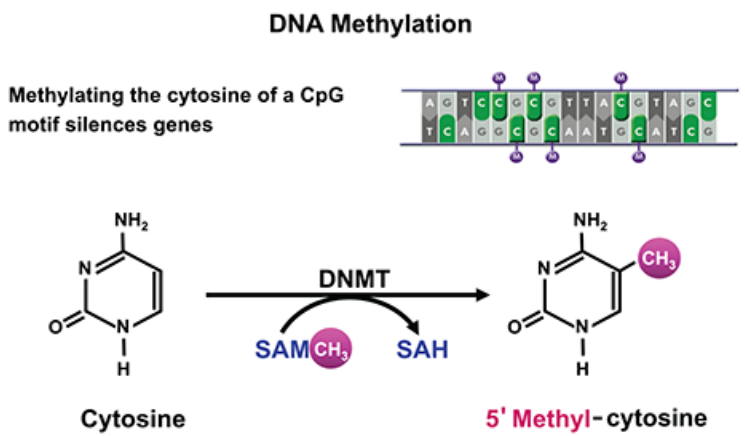
Role of noncoding RNAs

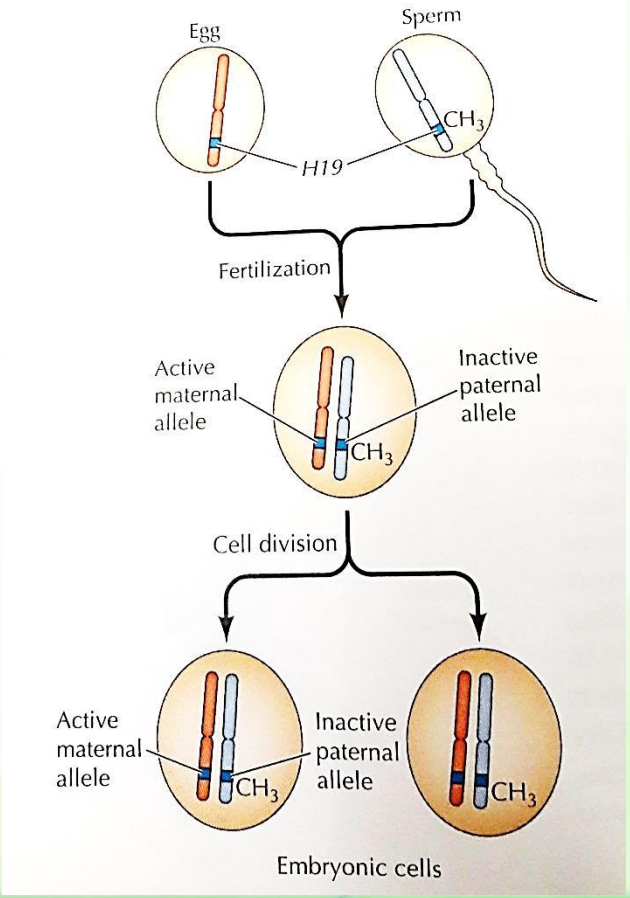
- RNA molecules that are homologous to the DNA sequences of certain genes can base pair with a growing mRNA to induce chromatin condensation and histone methylation.



DNA Methylation

- Cytosine residues in DNA can be modified by the addition of methyl groups at the 5-carbon position specifically at CG sequences.
- DNA methylation is correlated with reduced transcriptional activity of genes.
- Methylation is maintained following replication.
- Methylation is a mechanism of genomic imprinting (either the paternal gene or the maternal gene is active).





X chromosome inactivation

- A loong noncoding RNA (lncRNA) is transcribed from *Xist* gene located on the inactive X chromosome.
- The Xist RNA coats the inactive chromosome and promotes the recruitment of a protein complex that methylates histone 3 leading chromosomal condensation.