Molecular Biology (8)

Transcription-Regulation

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Second semester, 2019-2020

Resources

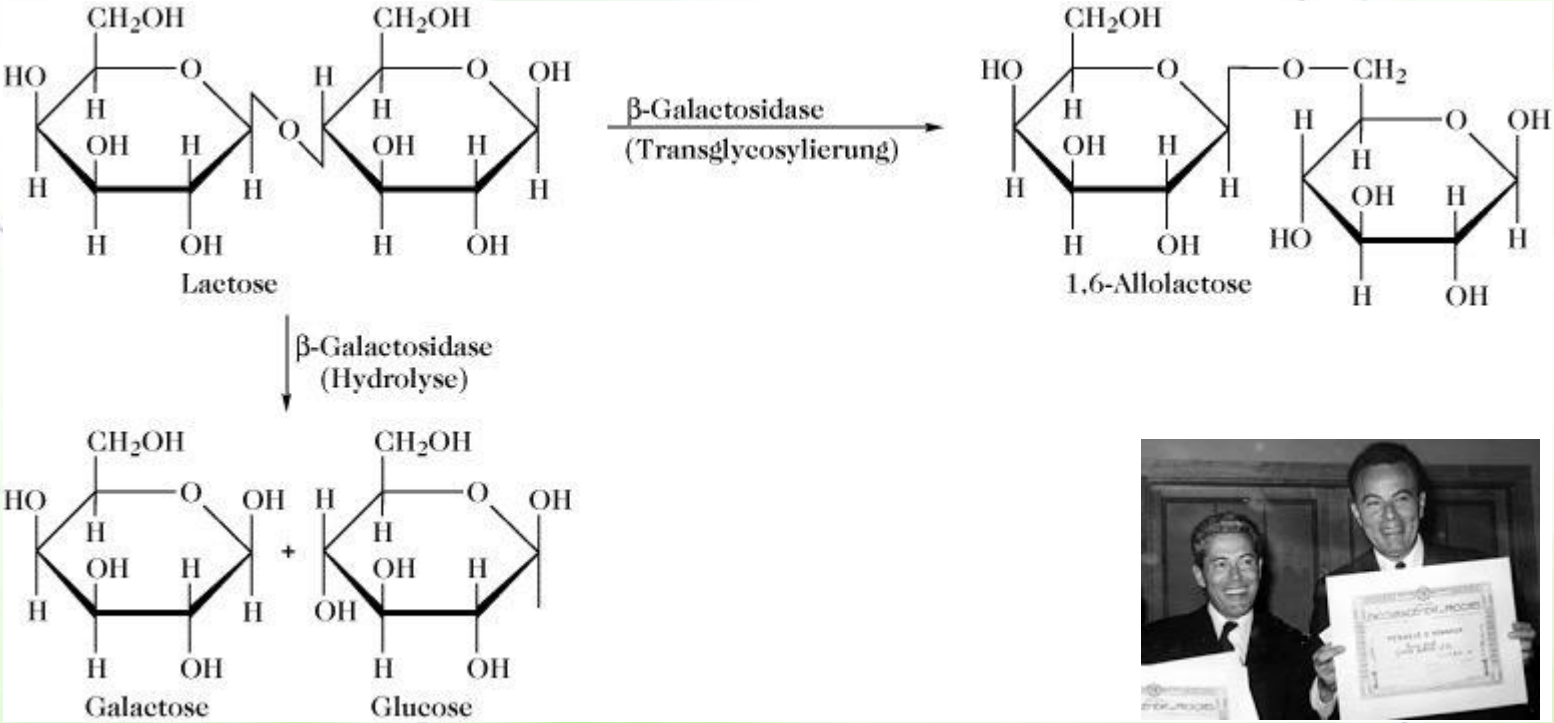
- This lecture
- Cooper, Chapter 8

Regulation of transcription in prokaryotes

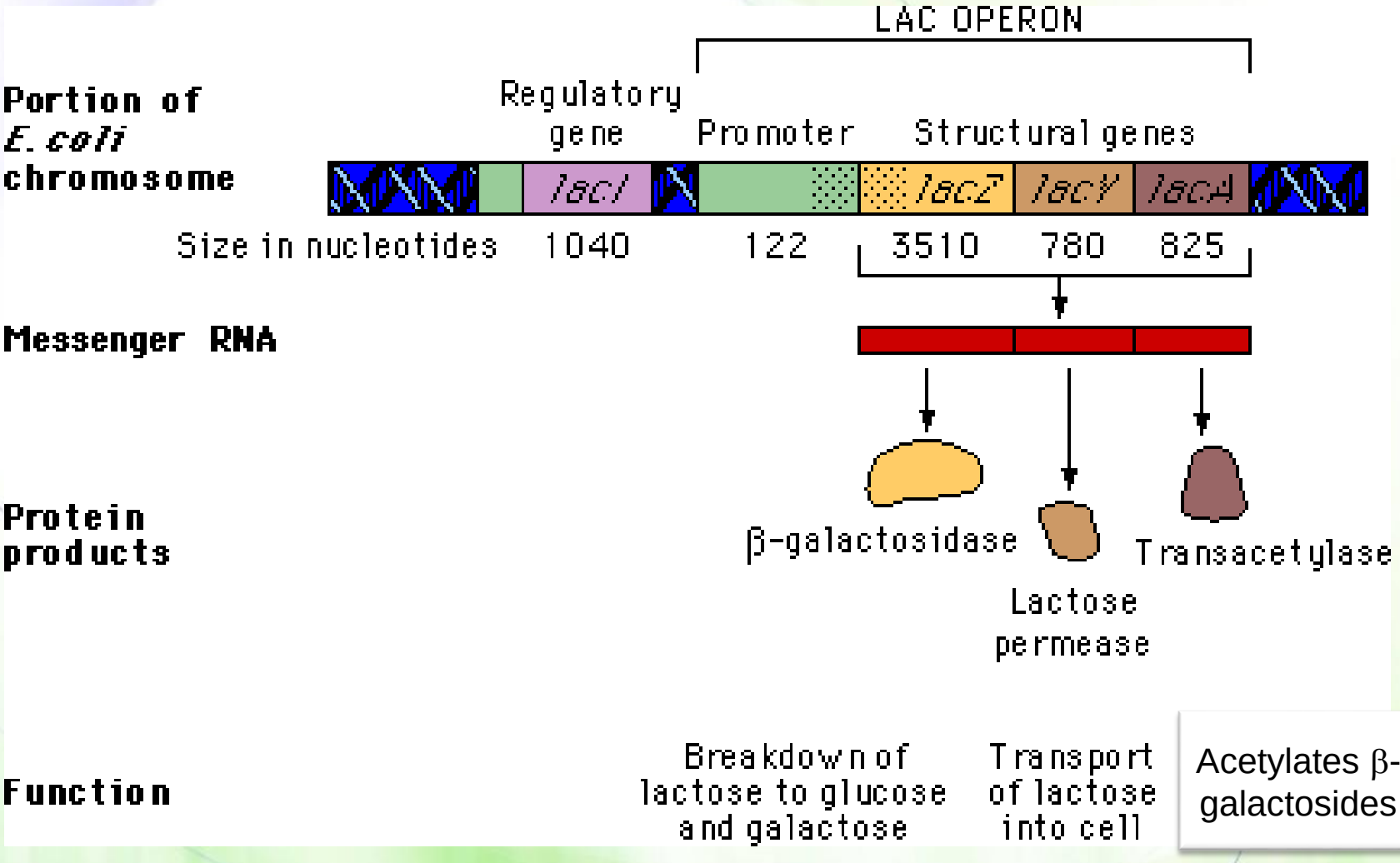
The lac operon

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.

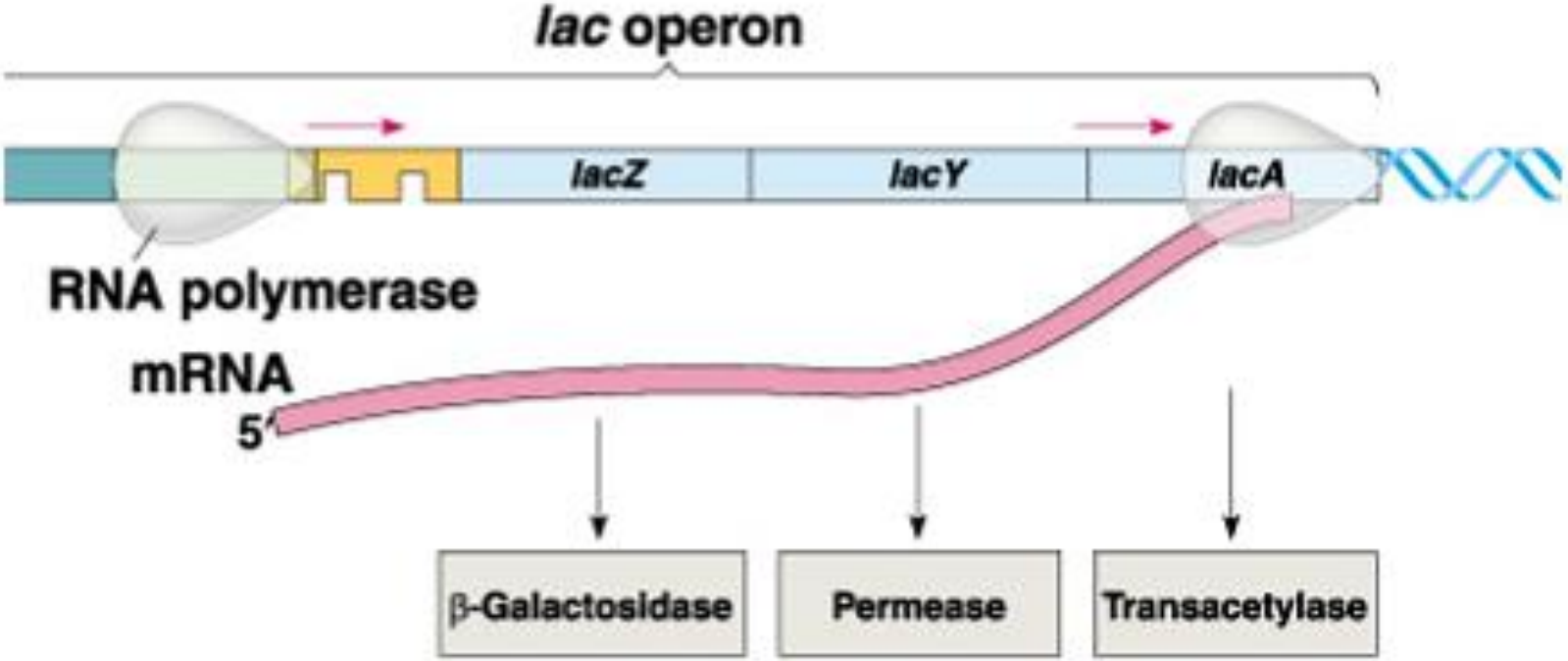


Components of the lac operon



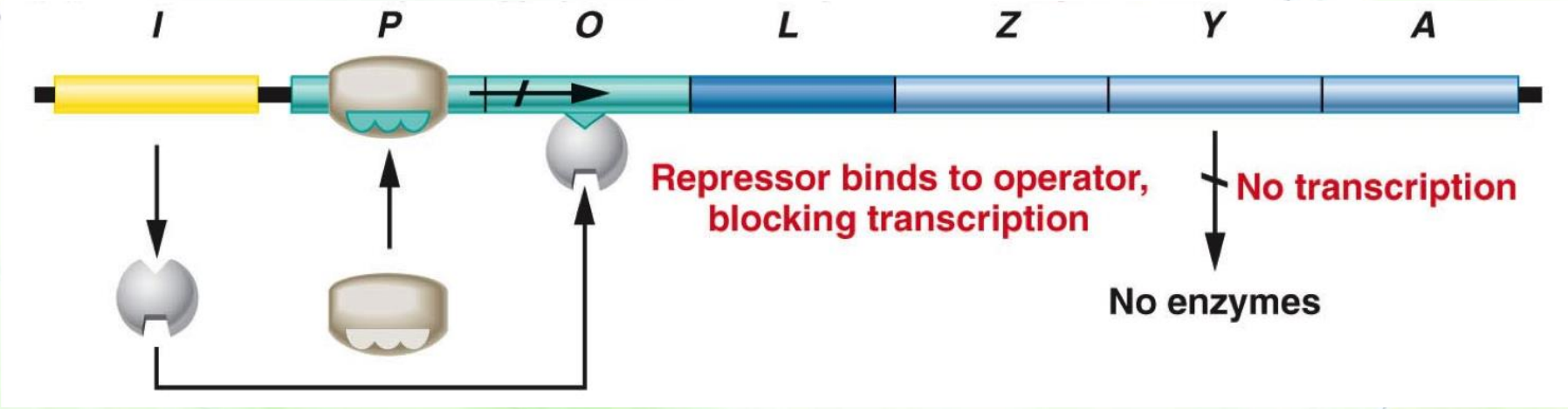
What is an operon?

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

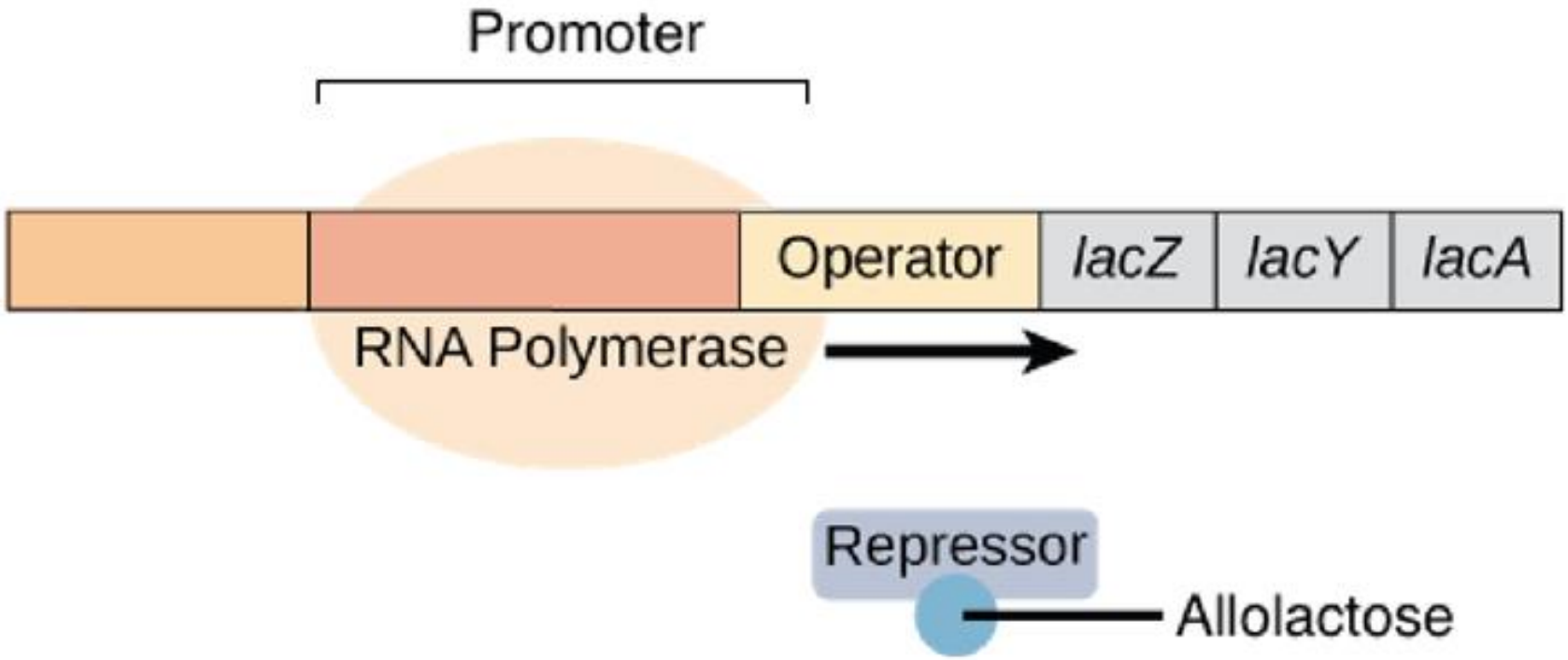


The operator

- 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.

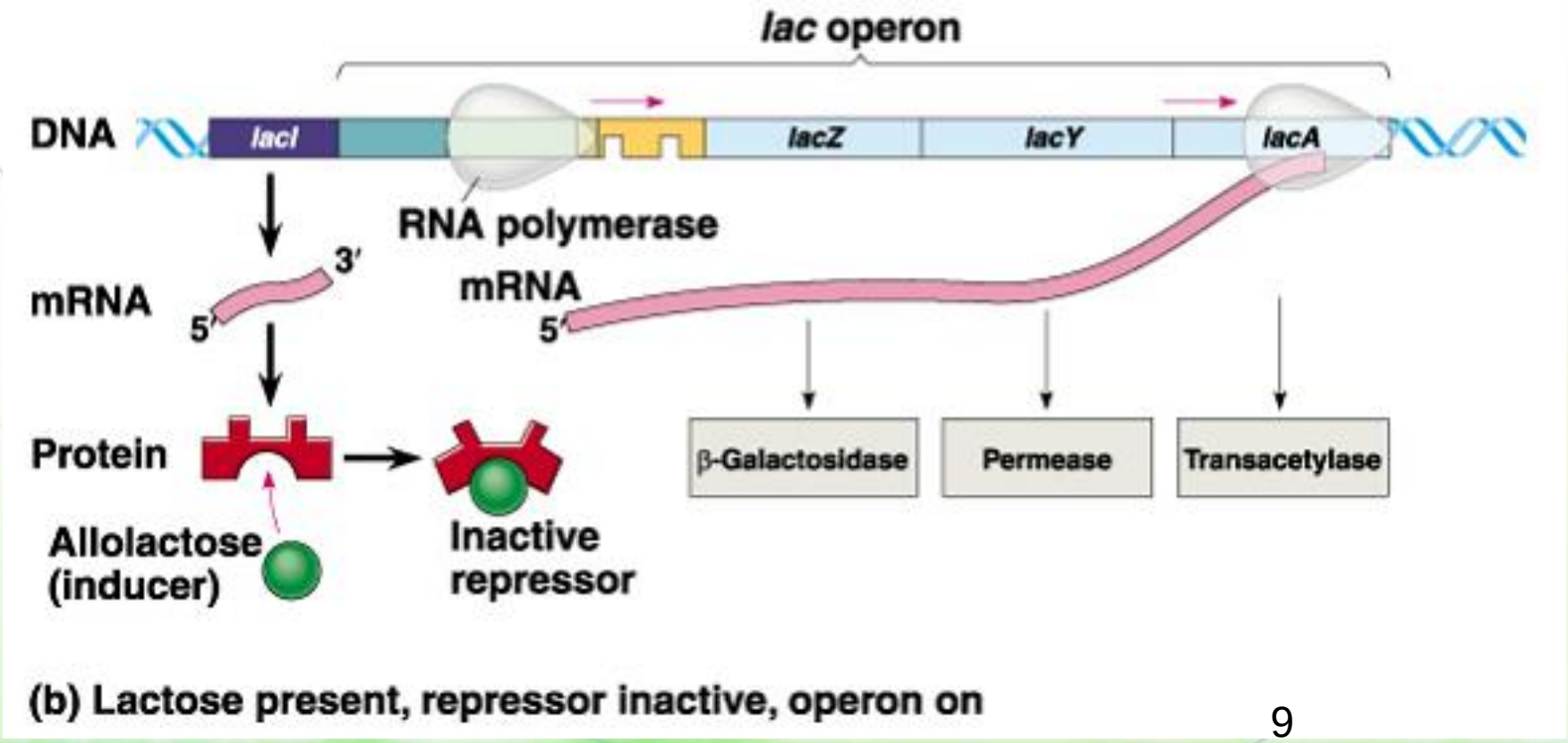


Glucose present, lactose present:



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

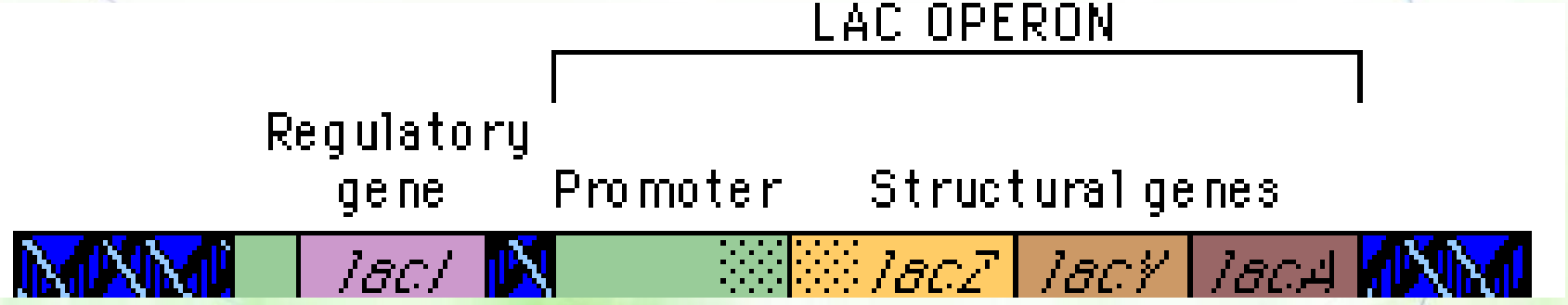
Cis vs. trans regulatory elements

- 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 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**.
  - Mention other examples of trans-acting elements

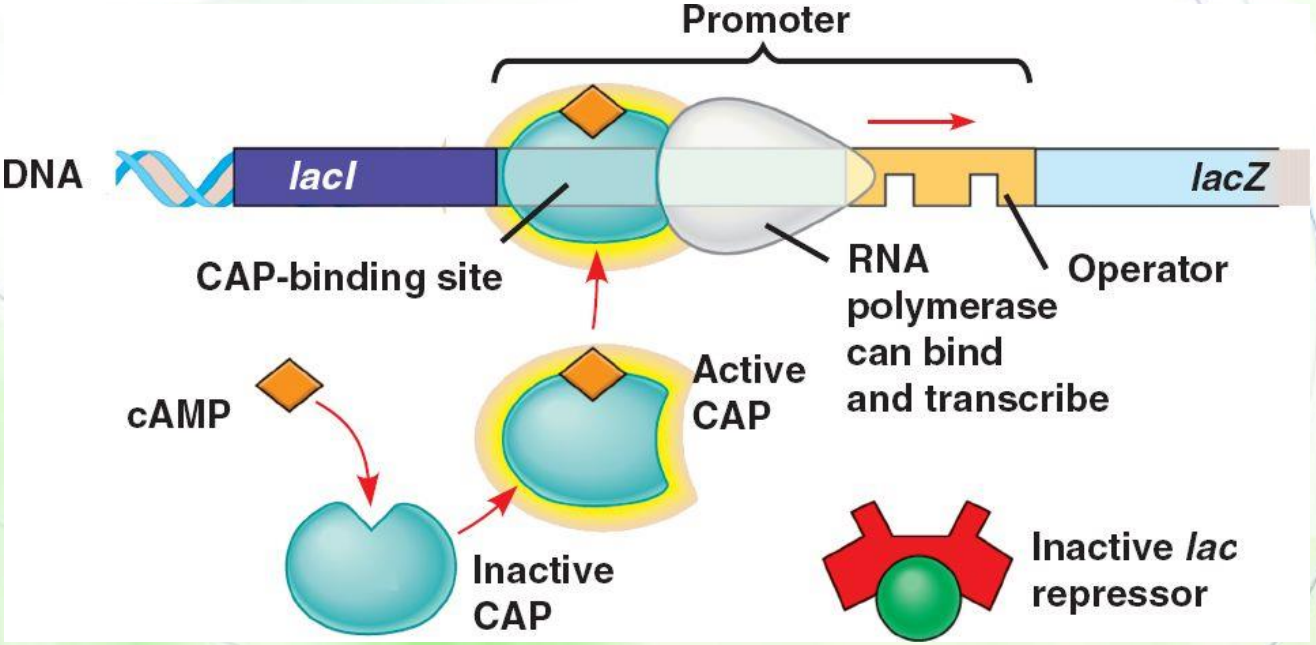
Effect of mutations

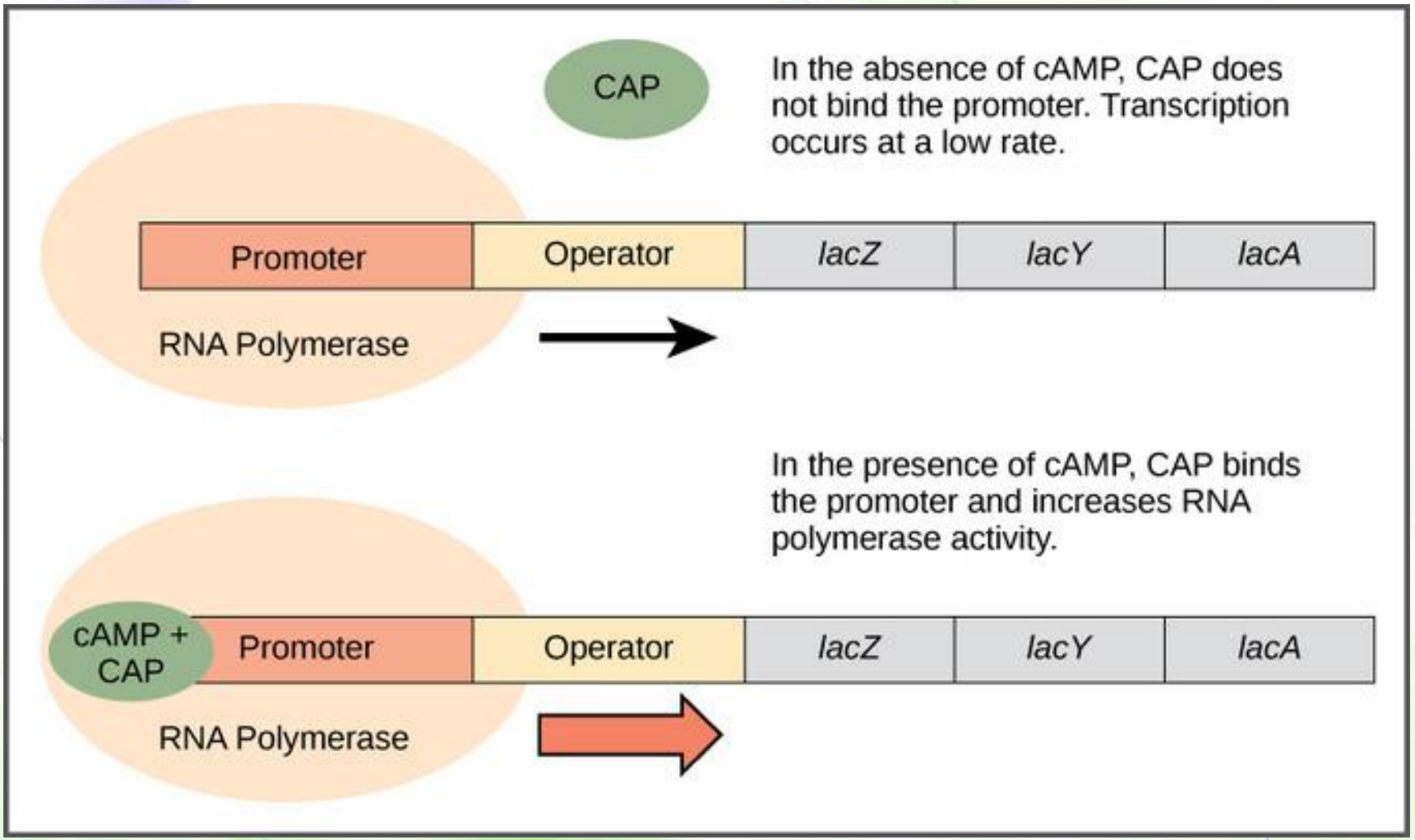
- Some mutations result in **constitutive** expression (always on).
  - Mention examples.
- Other mutations cause **non-inducible** or **repressed** expression (always off).
  - Mention examples



Another level of regulation

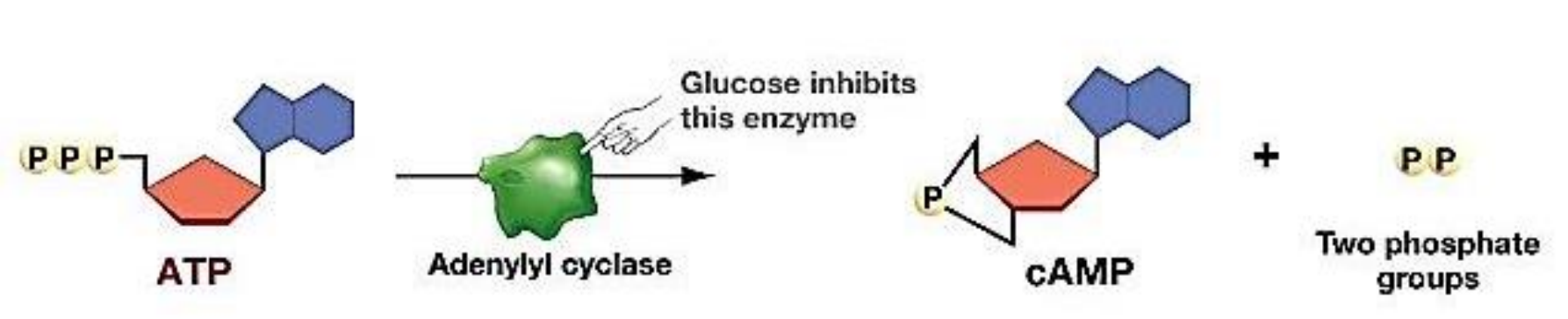
- Another regulator is cAMP, which binds to a protein known as catabolite activating protein (CAP) and stimulates its binding to regulatory sequences upstream of the promoter.
- CAP interacts with the RNA polymerase to facilitate the binding of polymerase to the promoter (P).




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

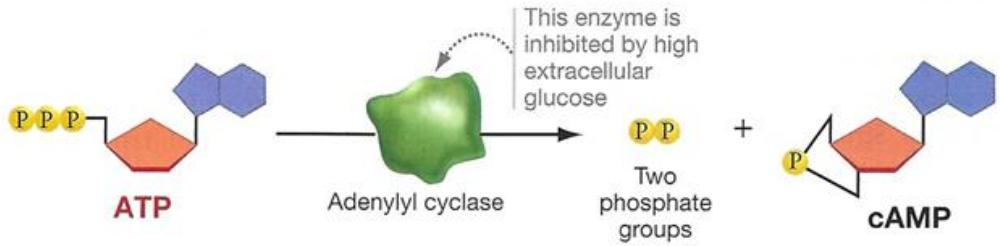
Regulation by glucose (negative)

- 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.



Glucose repression

(a) The enzyme adenylyl cyclase catalyzes production of cAMP from ATP.



(b) The amount of cAMP and the rate of transcription of the *lac* operon are inversely related to the concentration of glucose.

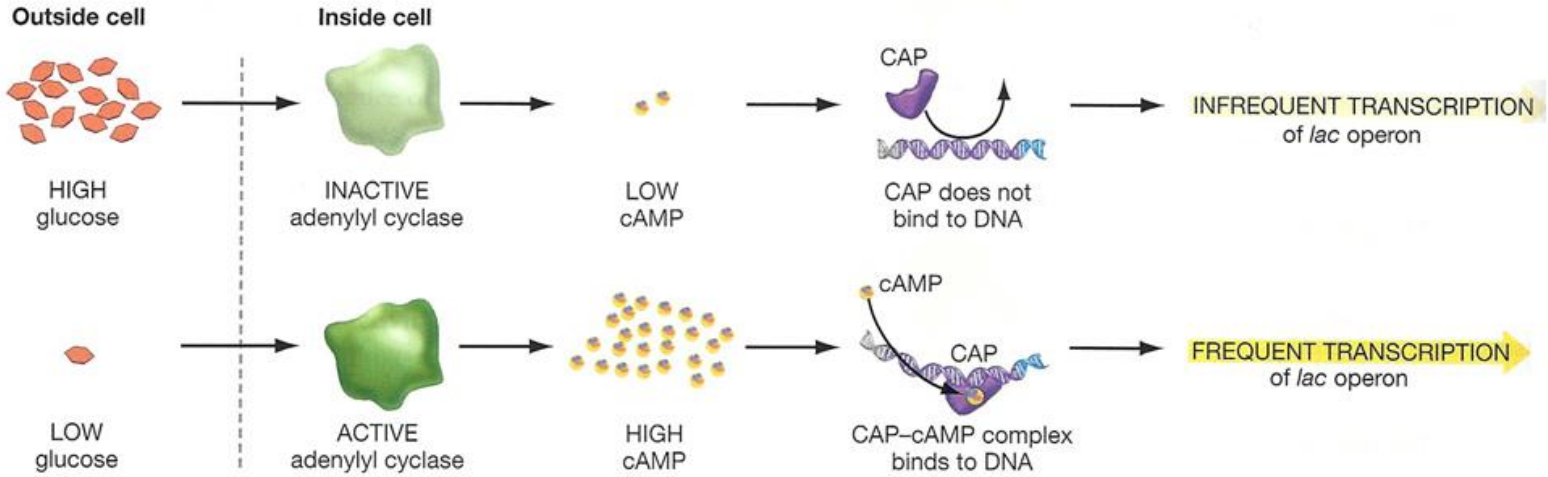
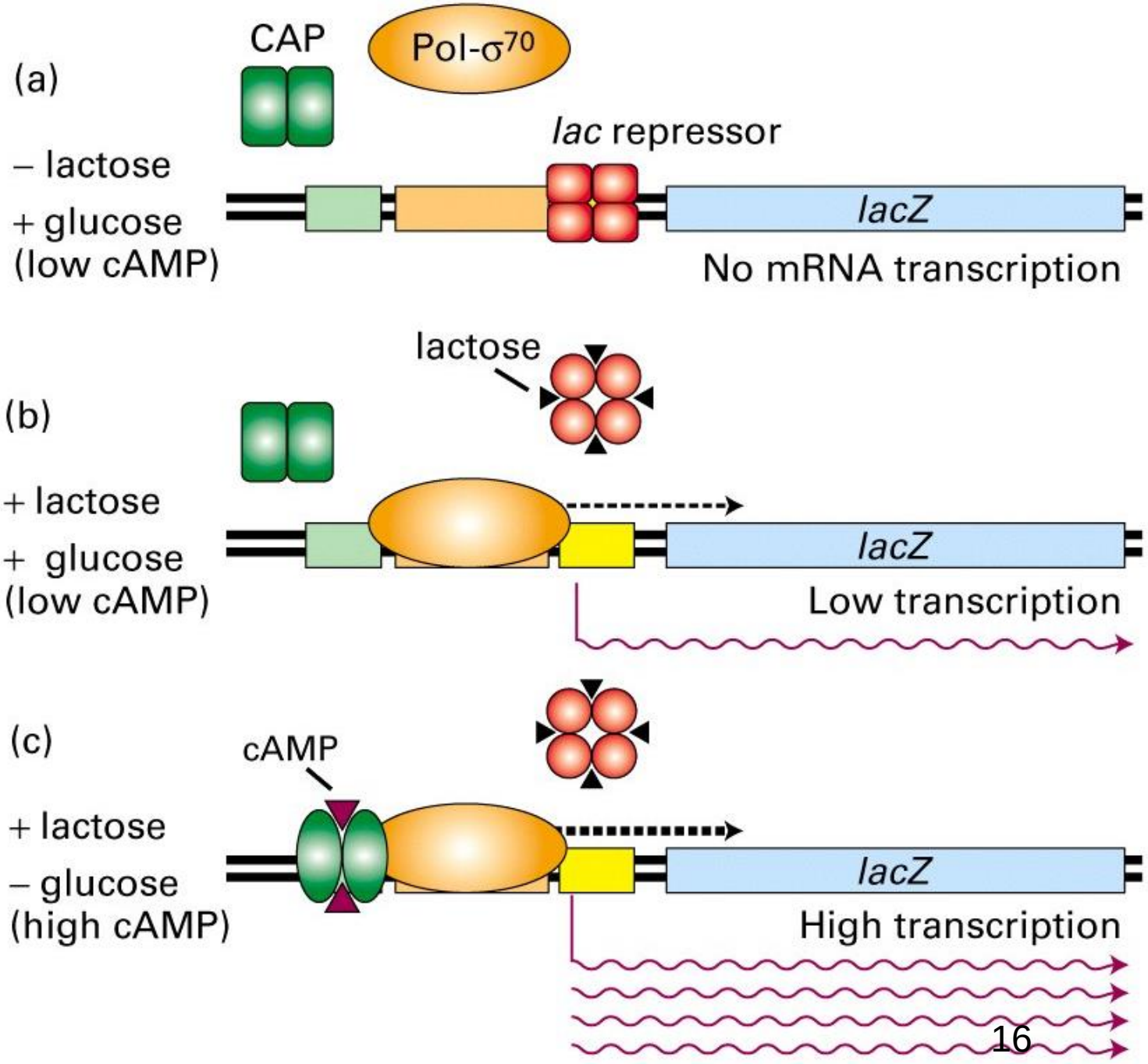


FIGURE 17.11 Cyclic AMP (cAMP) Is Synthesized When Glucose Levels Are Low.


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

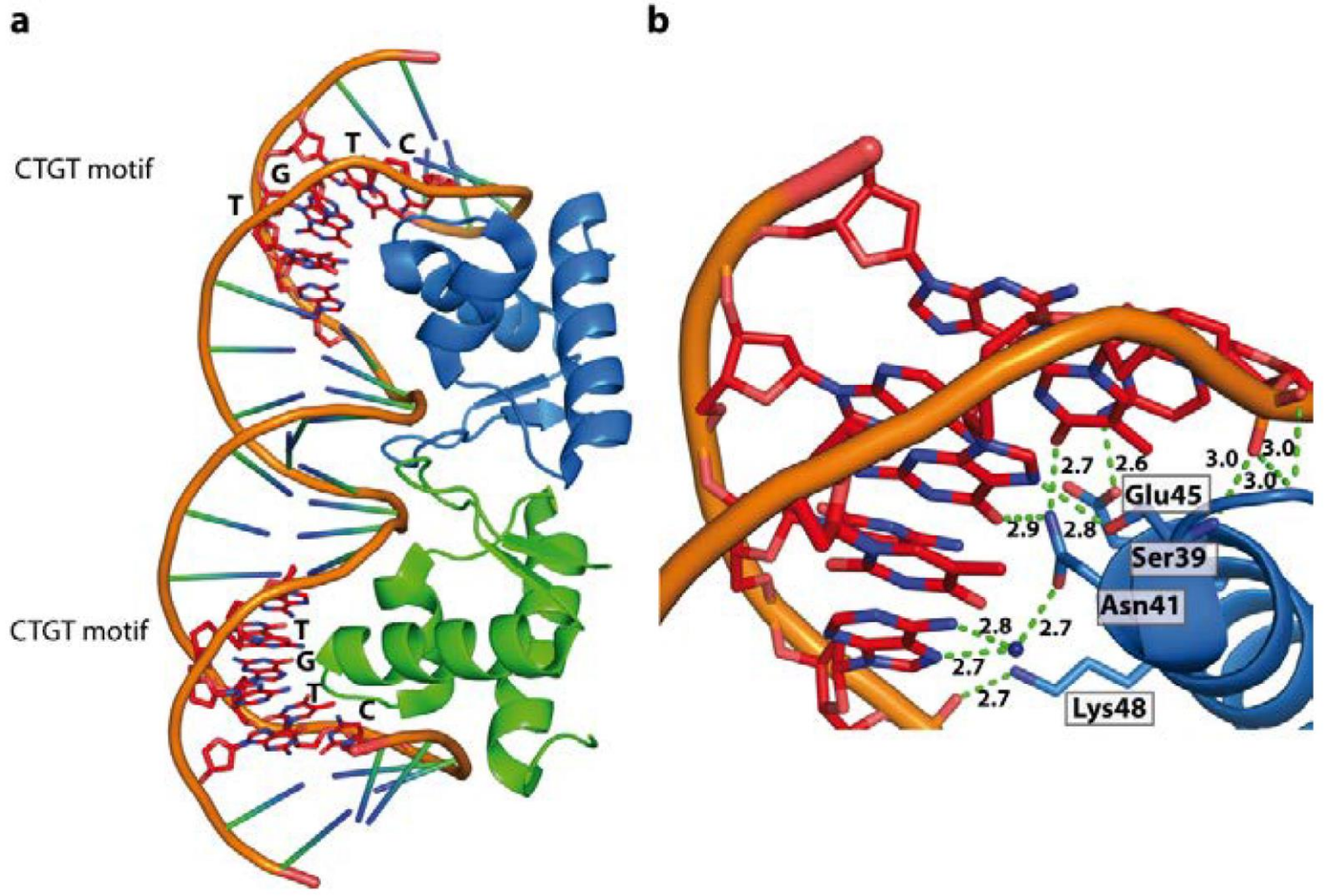
Take-home message

- Gene expression is regulated by regulatory proteins that would ultimately:
  - Guide the RNA polymerase (or other regulatory proteins) to the promoter
  - Strengthen/stabilize RNA polymerase binding to the promoter
  - Activate the RNA polymerase
  - Open up the DNA for the RNA polymerase

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.

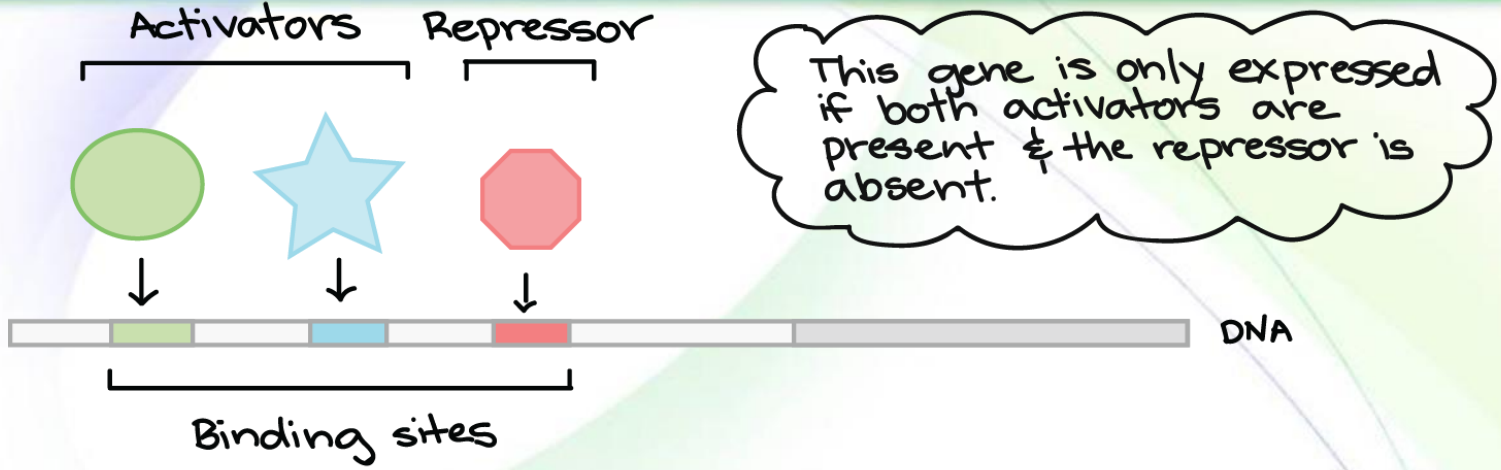
How do proteins recognize/interact with DNA sequences specifically?



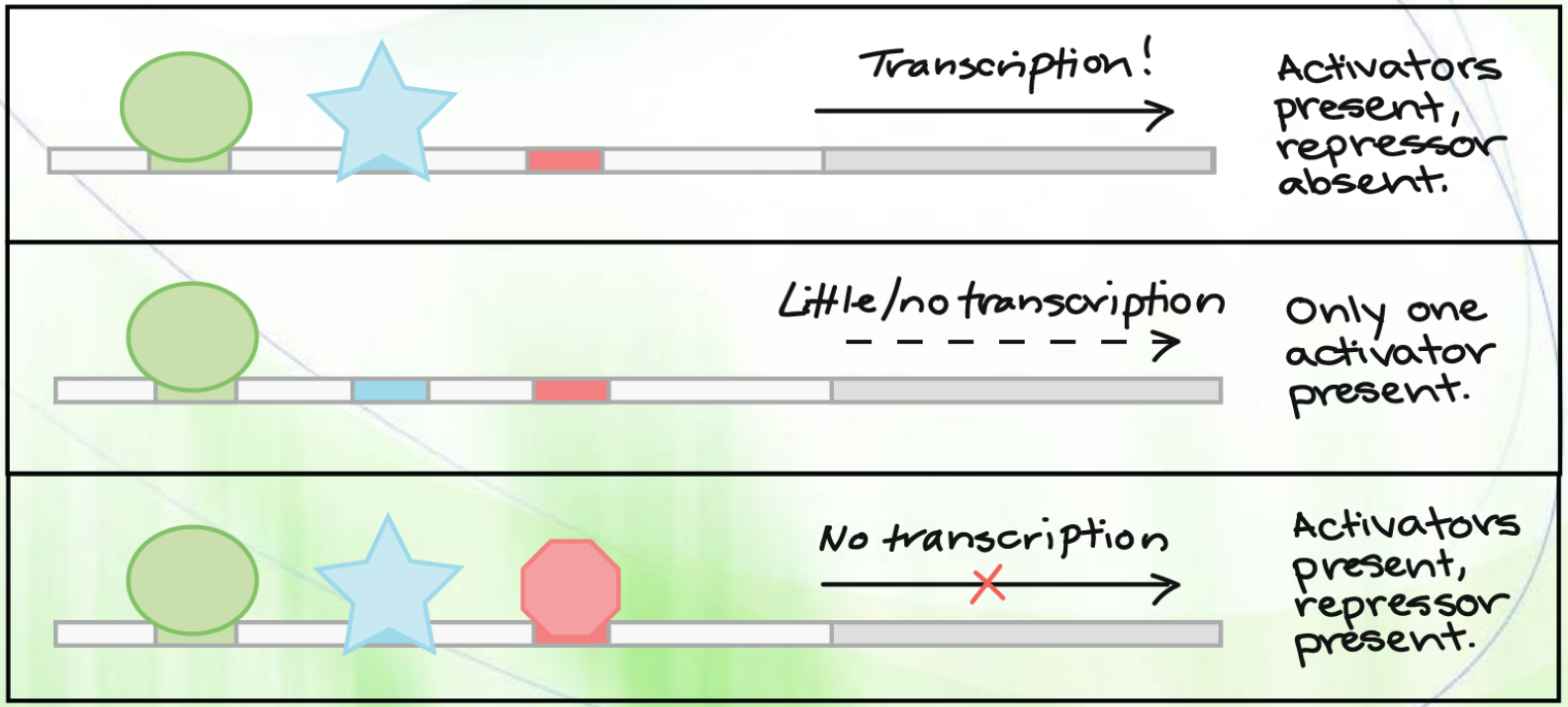
Regulation of transcription in eukaryotes

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, proximal promoter elements, enhancers, and silencers
  - Transcriptional regulatory proteins
    - Activators, repressors
      - Chromatin remodeling
      - DNA modification (example: methylation of cytosine)
  - Noncoding RNA molecules



This gene is only expressed if both activators are present & the repressor is absent.



How do TFs regulate gene expression?

- Transcription factors cause epigenetic/epigenomic changes in DNA.

- What is epigenetics?
  - Epi: “above” or “in addition to”
  - It indicates genetic alterations in gene expression without a change in DNA sequence.
    - Chromatin packaging
    - Chemical modification of histones
    - Chemical modification of DNA

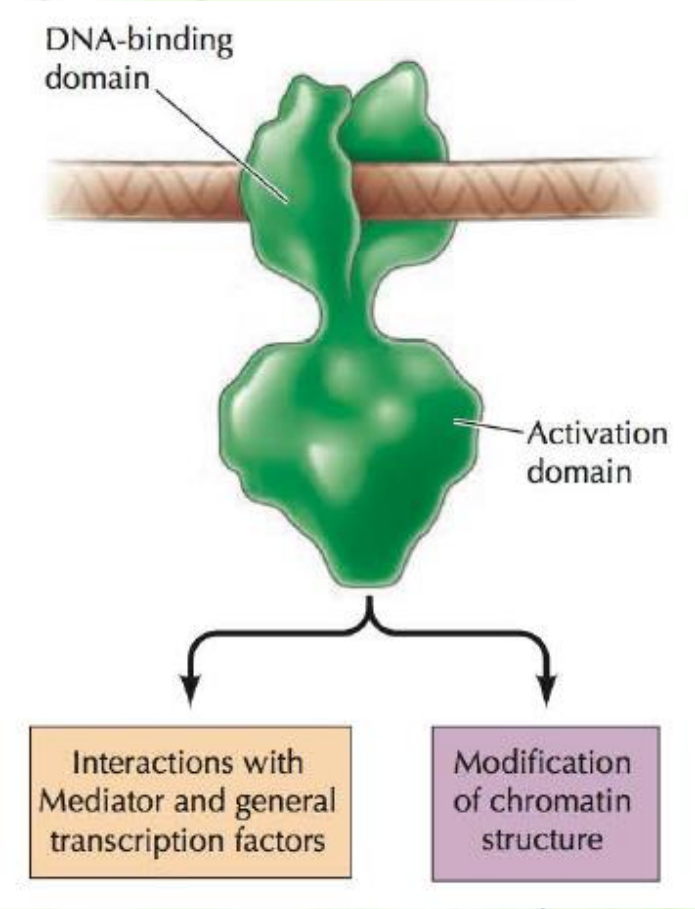
General structure of TFs

- Positive transcription factors have at least two domains:
  - DNA binding domain
  - Activation 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 word, it can be separated from the protein and till be functional

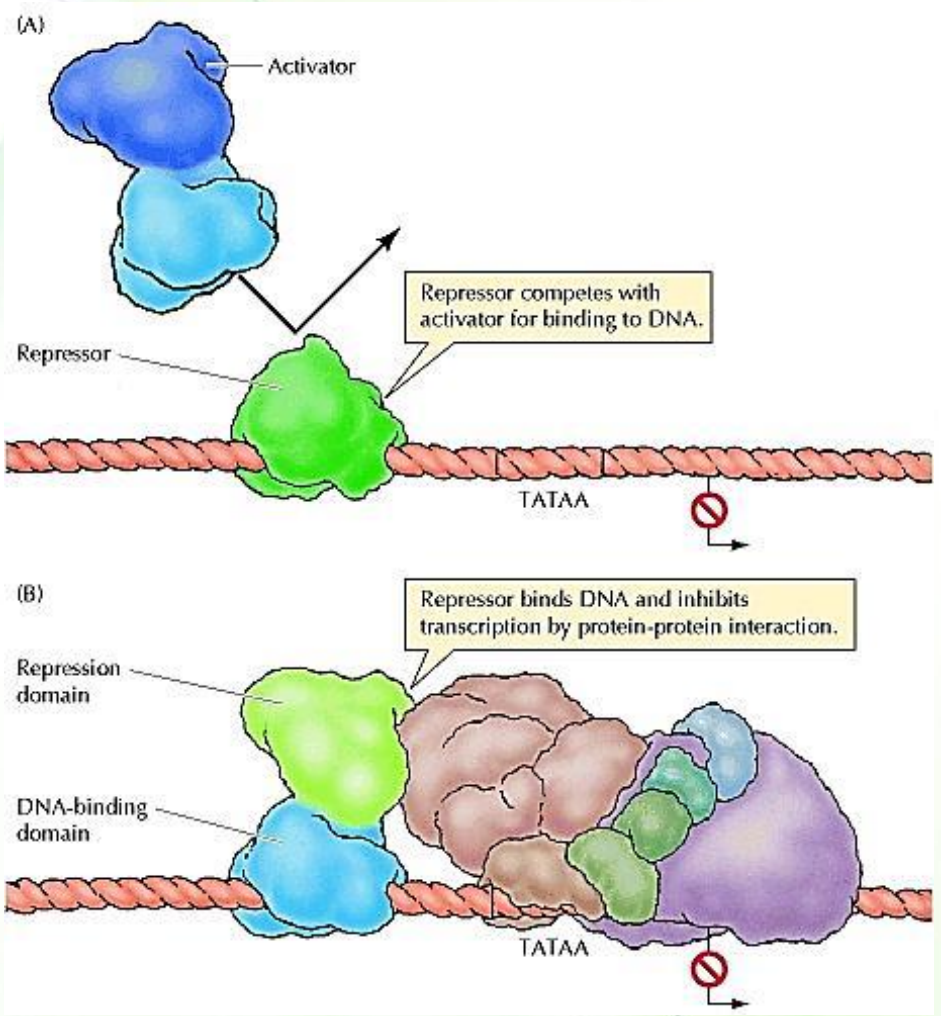
The activation domains

- Activation domains stimulate transcription by
  - interacting with general transcription factors, facilitating the assembly of a transcription complex on the promoter,
  - modifying the chromatin.



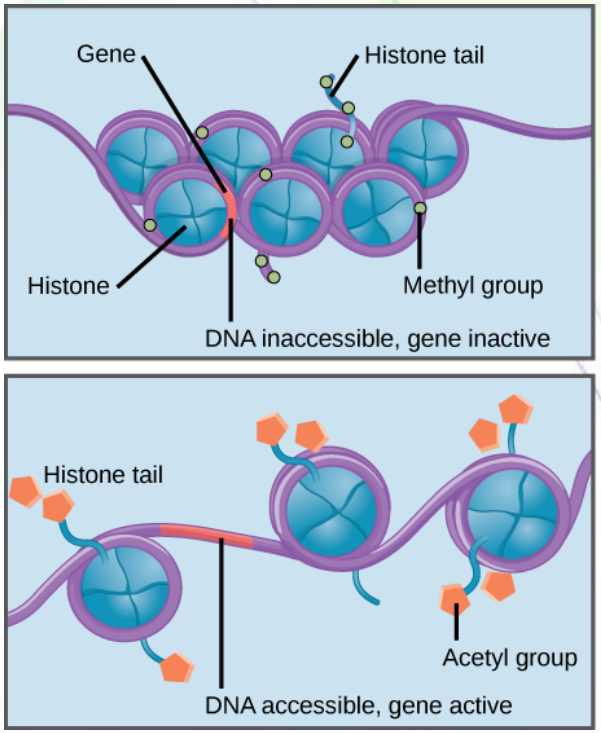
Eukaryotic Repressors

- 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



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)
  - Inactive genes are located in highly packed heterochromatin.
- Regulatory proteins switch between the two structures of chromatin.

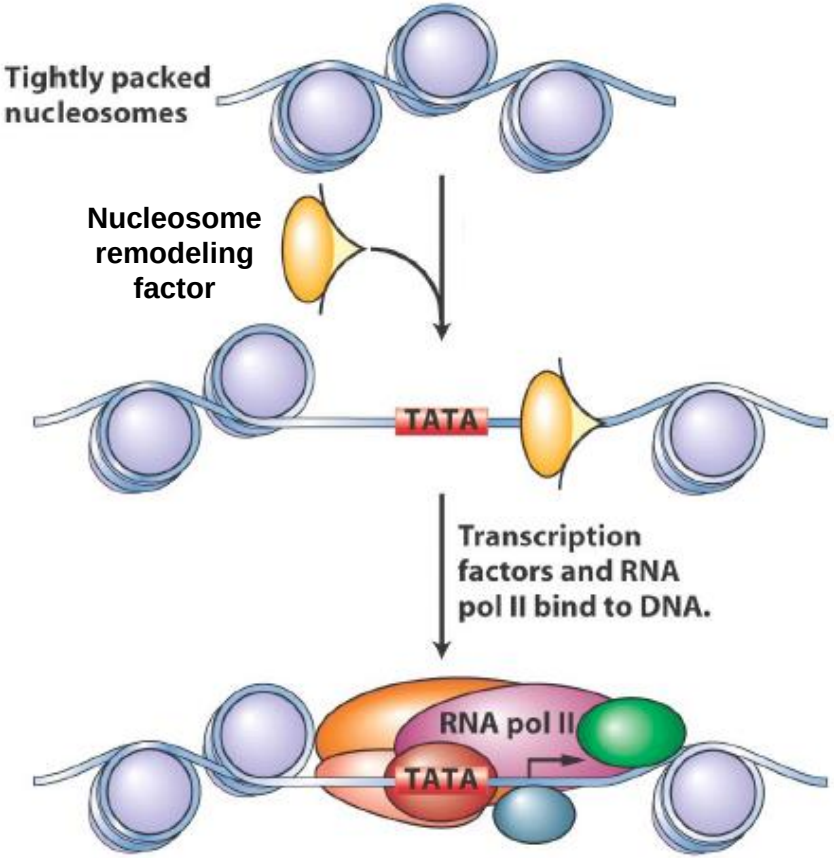


Methylation of DNA and histones causes nucleosomes to pack tightly together. Transcription factors cannot bind the DNA, and genes are not expressed.

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

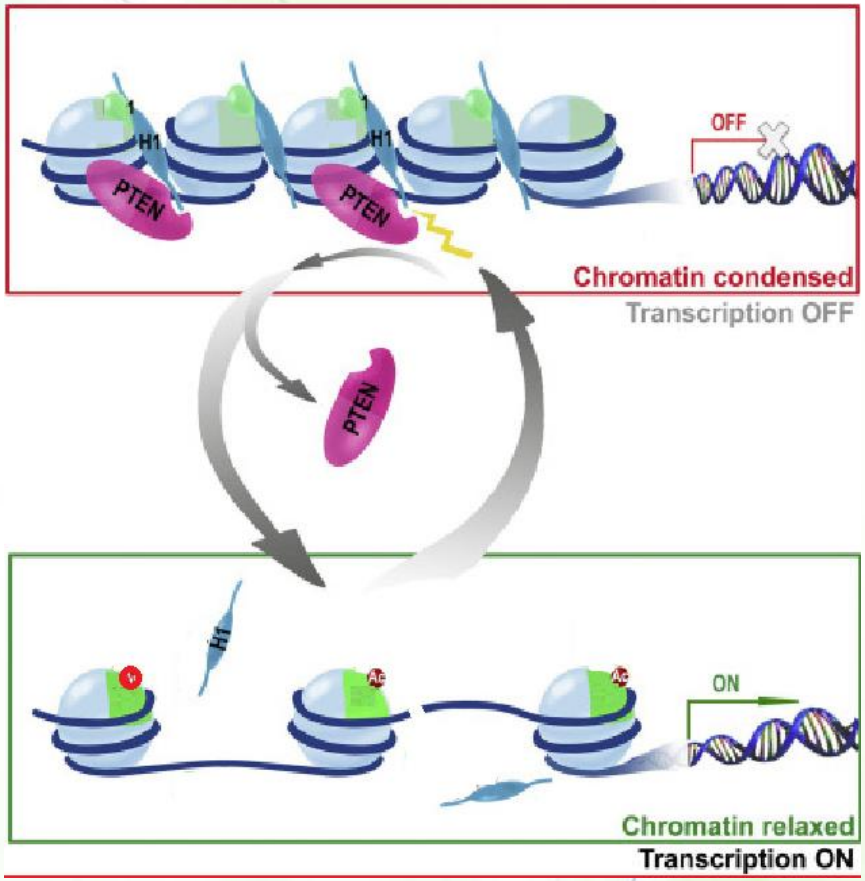
Chromatin remodeling factors

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



Changing nucleosome structure by histone 1

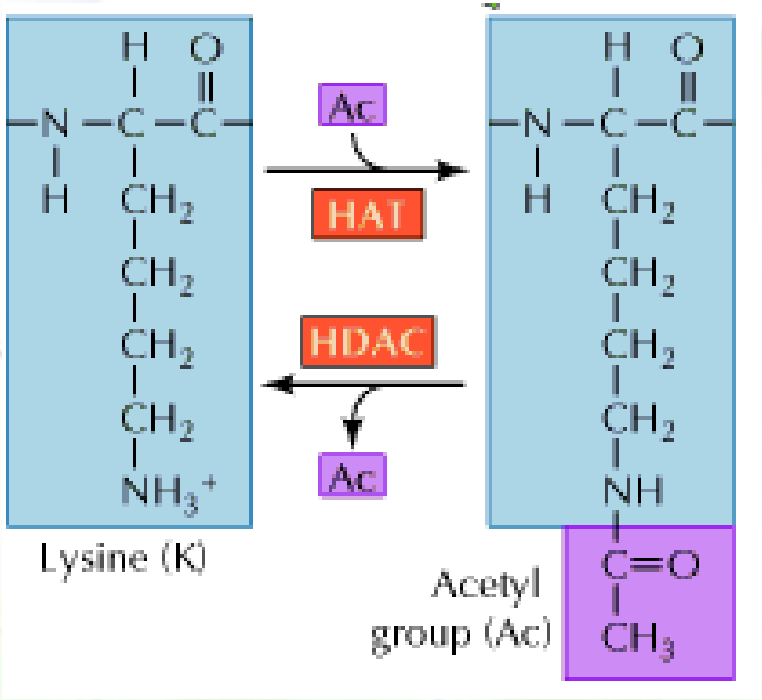
- Transcriptional regulatory proteins either release Histone 1 (H1) from DNA or facilitate its binding.

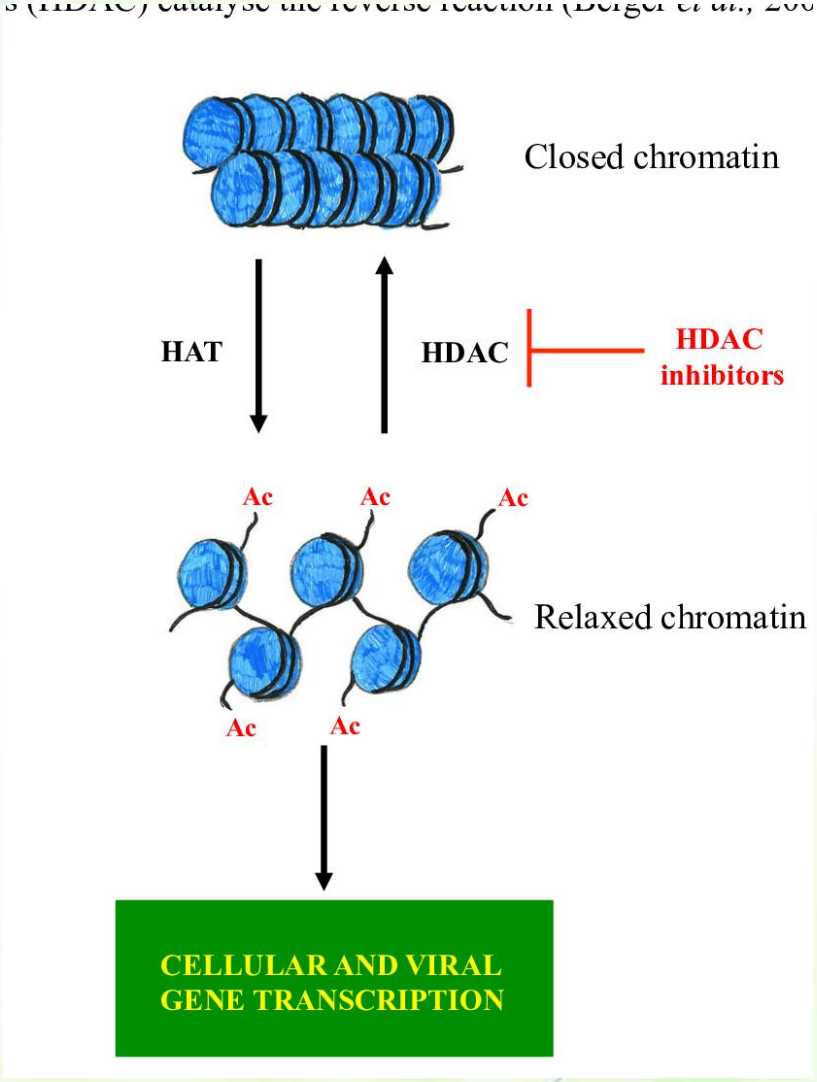


How else are chromosomal structures altered?

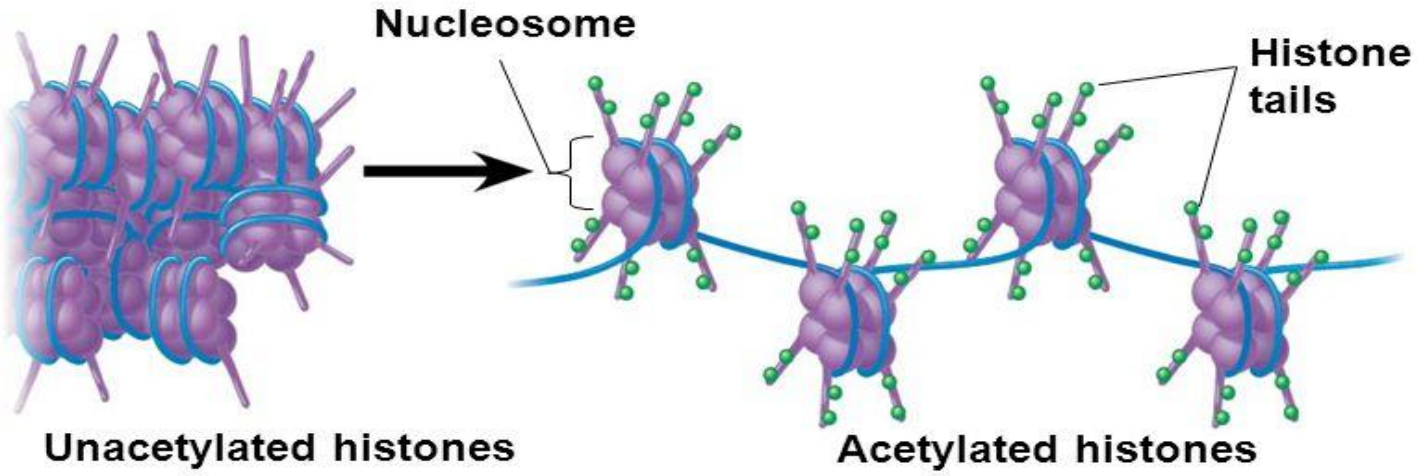
- Change of compactness of the chromatin by:
  - Chemical modification of histones
    - Acetylation, methylation, and phosphorylation
  - Binding of noncoding RNAs to DNA

Histone acetylation



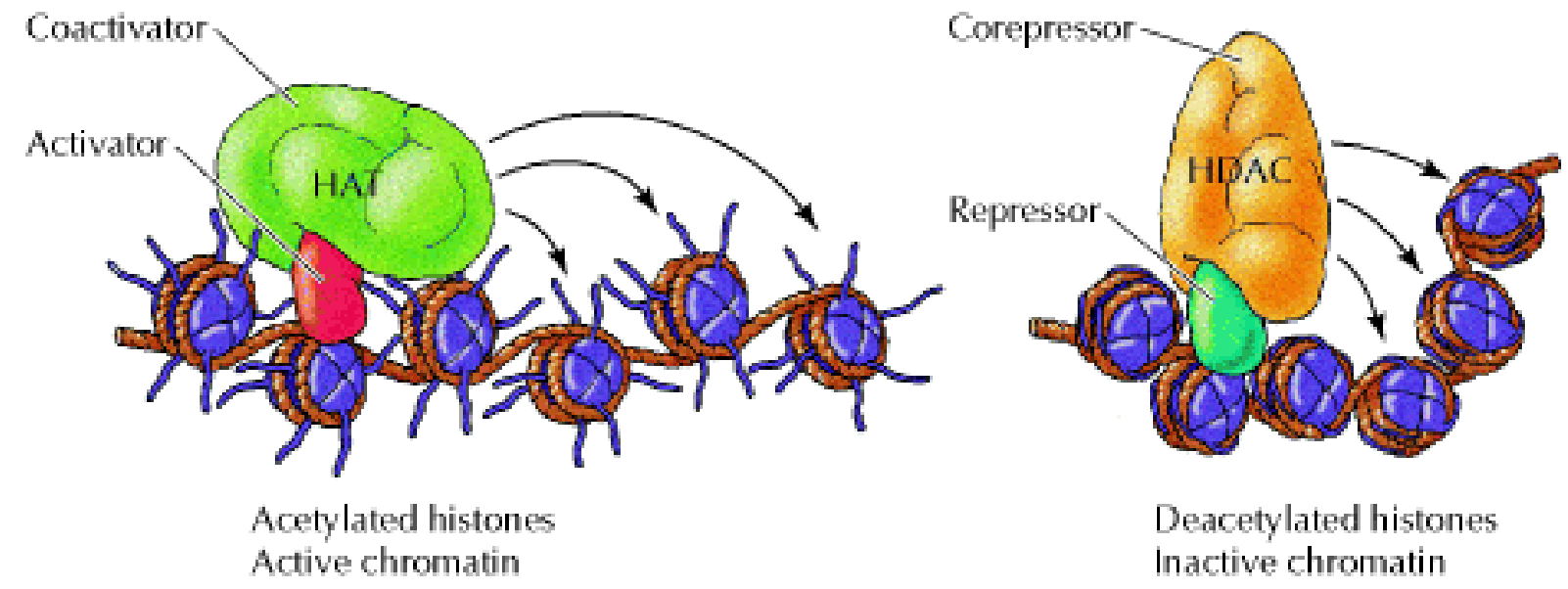


In **histone acetylation**, acetyl groups are attached to positively charged lysines in histone tails. This generally loosens chromatin structure, promoting the initiation of transcription.



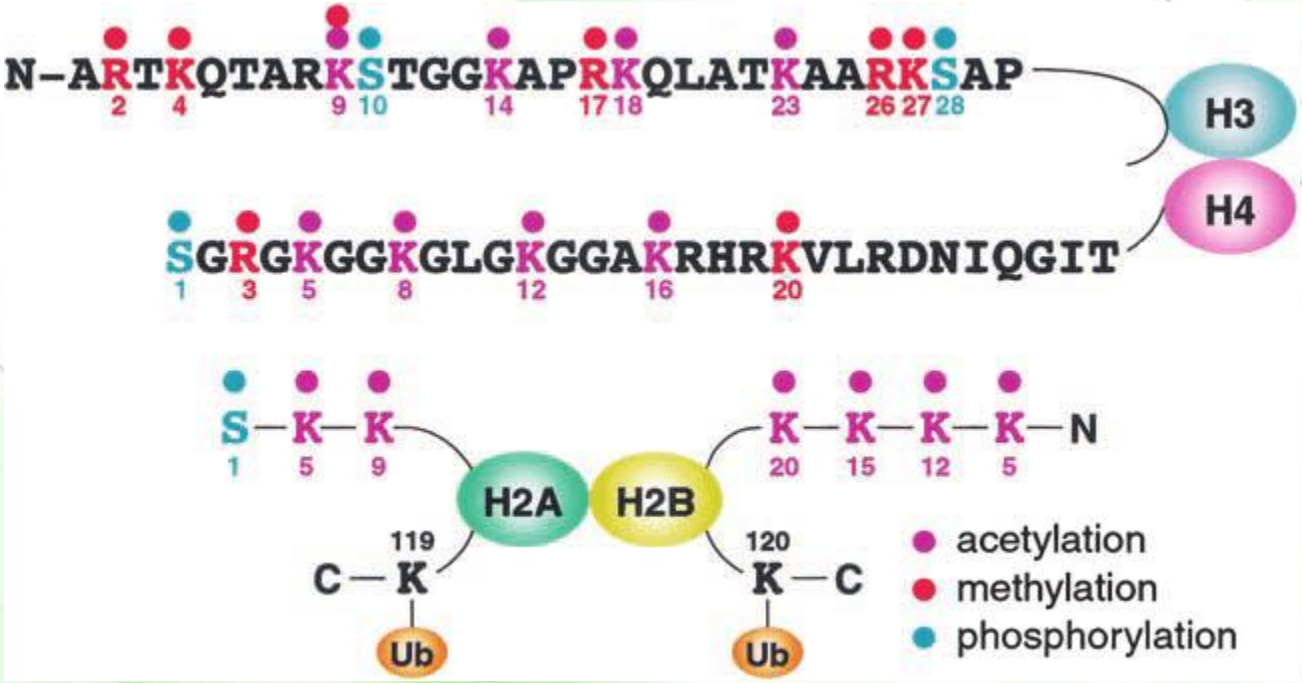
Enzymatic association

- Transcriptional activators and repressors are associated with histone acetyltransferases and deacetylases, respectively
  - TFIID associates with histone acetyltransferases.



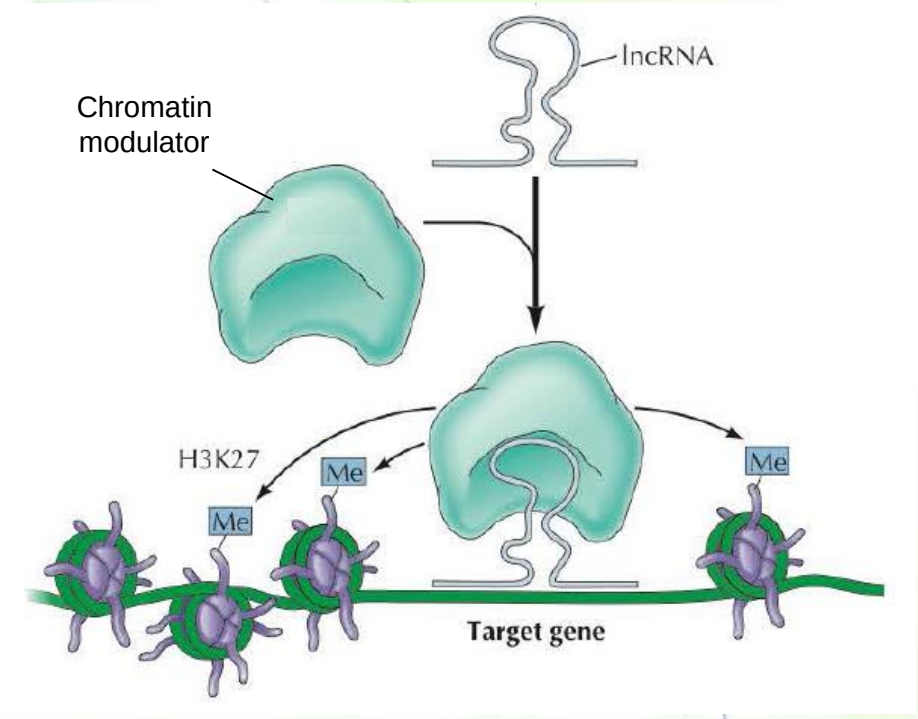
Other modifications of histones

- Histone can also be methylated or phosphorylated.
- Effect is dependent on sites of modification.



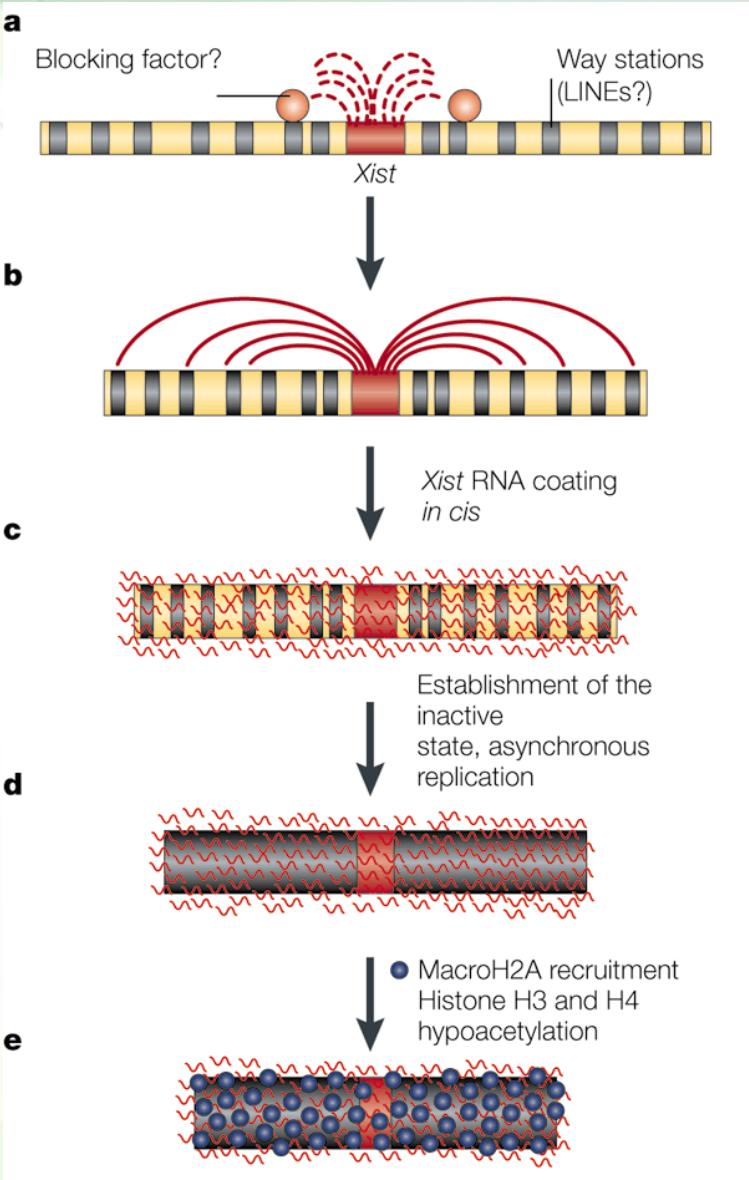
Role of noncoding RNAs

- RNA molecules that are homologous to the DNA or to the growing mRNA sequences of certain genes induce chromatin condensation and histone methylation.

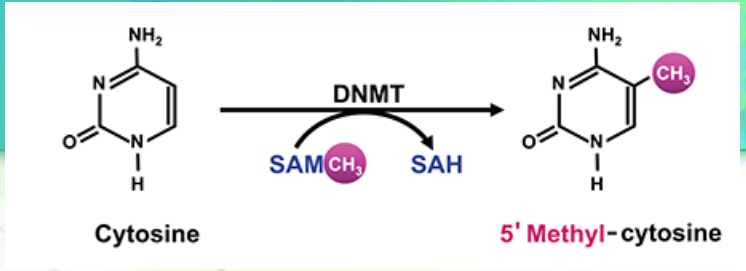


X chromosome inactivation

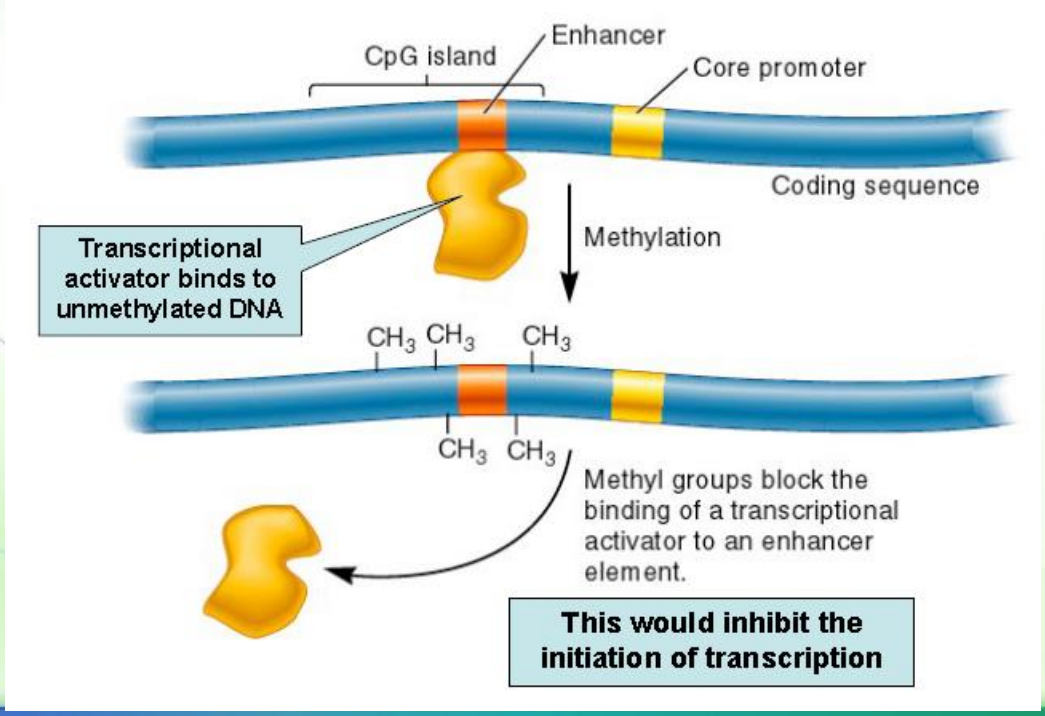
- A long noncoding RNA (lncRNA) is transcribed from *Xist* gene located on the inactive X chromosome.
- The Xist RNA coats the inactive chromosome and promotes the recruitment of a protein complex that methylates histone 3 leading chromosomal condensation.



DNA methylation

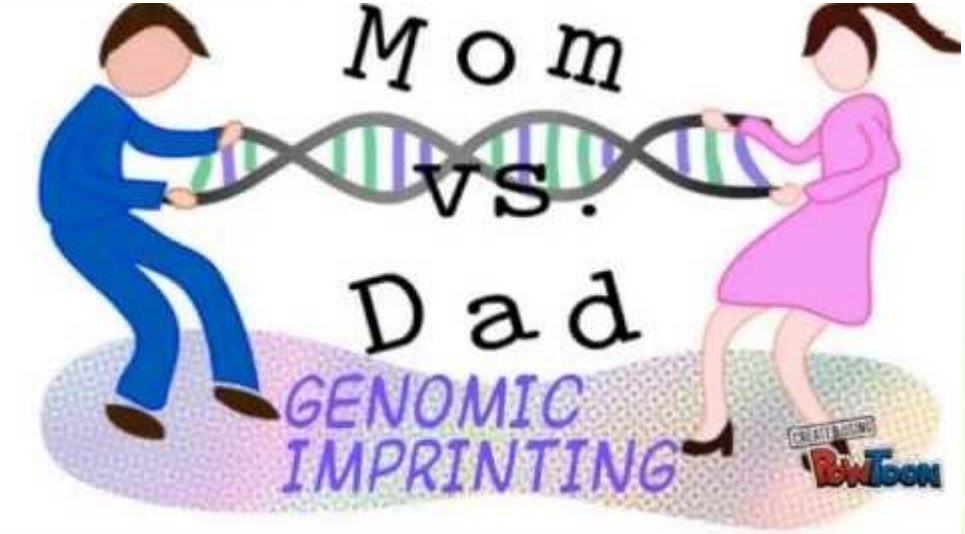


- Cytosine residues can be methylated groups at the 5’-carbon position specifically at CG sequences (called CpG isalnds near promoters).
- DNA methylation reduces gene transcription by blocking of activator binding to DNA and inducing heterochromatin formation.



Genetic imprinting

- Methylation is maintained following replication and is inherited.
- Methylation is a mechanism of genomic imprinting (either the paternal gene or the maternal gene is active).



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

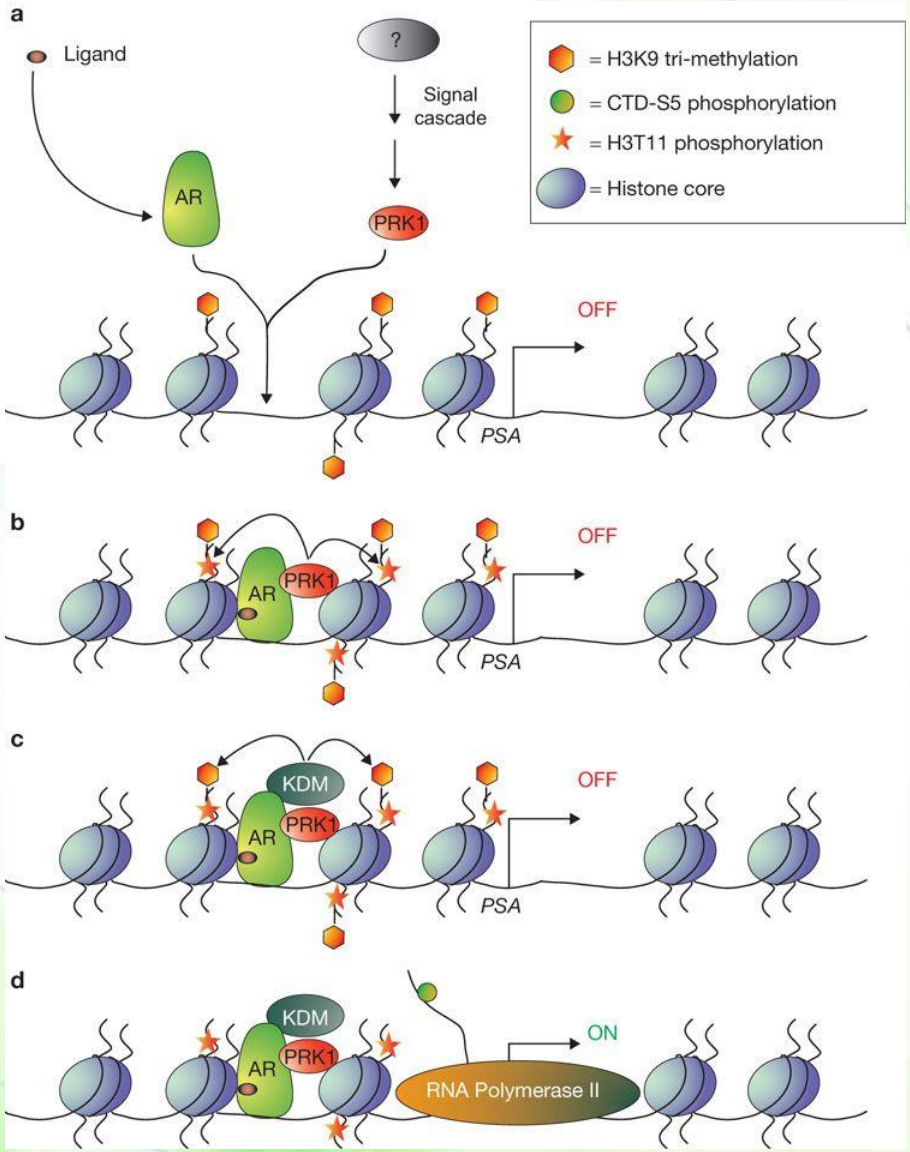
Stress-induced gene expression and behavior are controlled by DNA methylation and methyl donor availability in the dentate gyrus

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

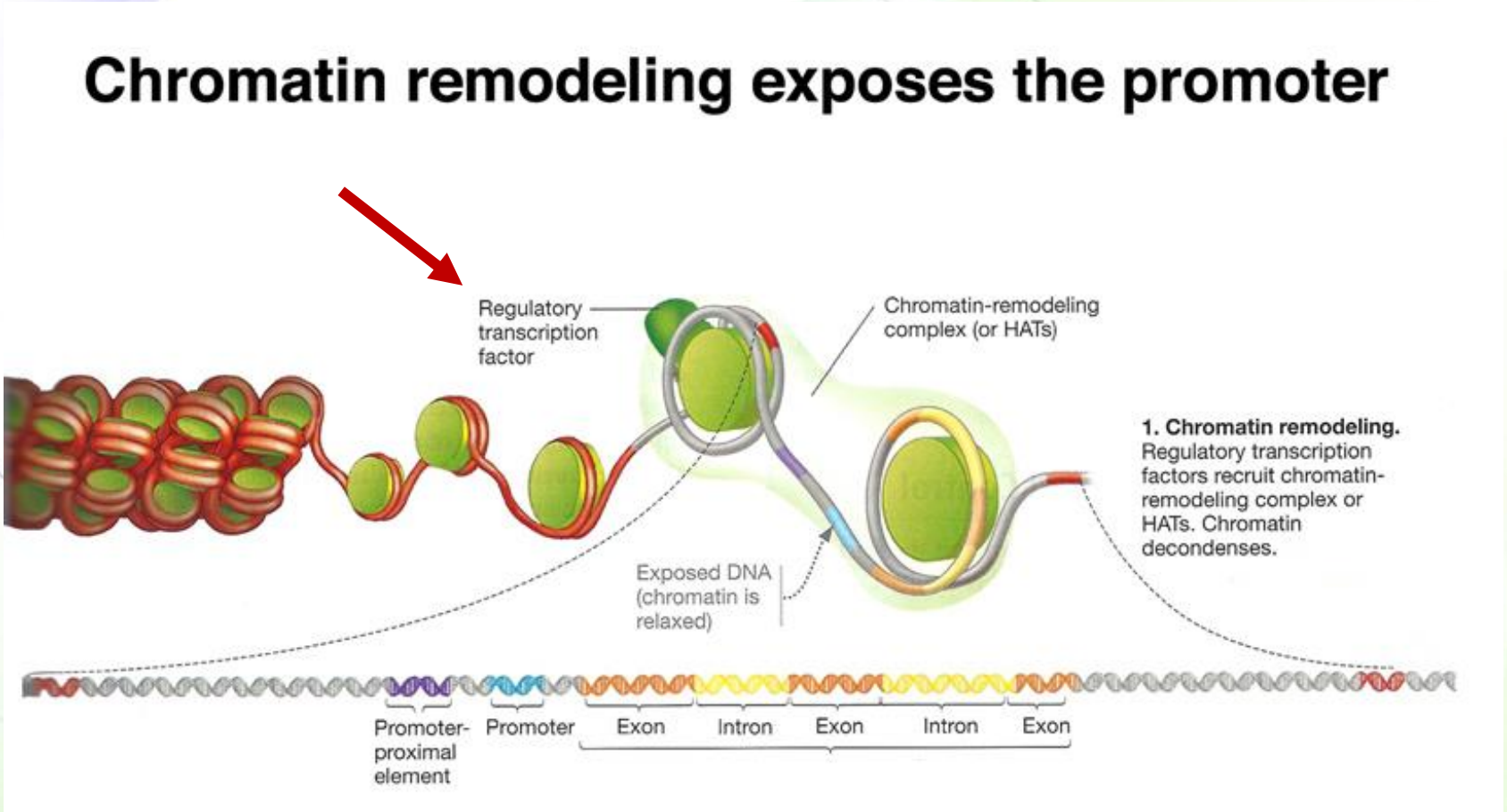
[a]Neuro-Epigenetics Research Group, University of Bristol, Bristol BS1 3NY, United Kingdom; [b]Institute of Psychiatry, King's College London, London United Kingdom; and [c]University of Exeter Medical School, University of Exeter, Exeter EX2 5DW, United Kingdom

Scenario

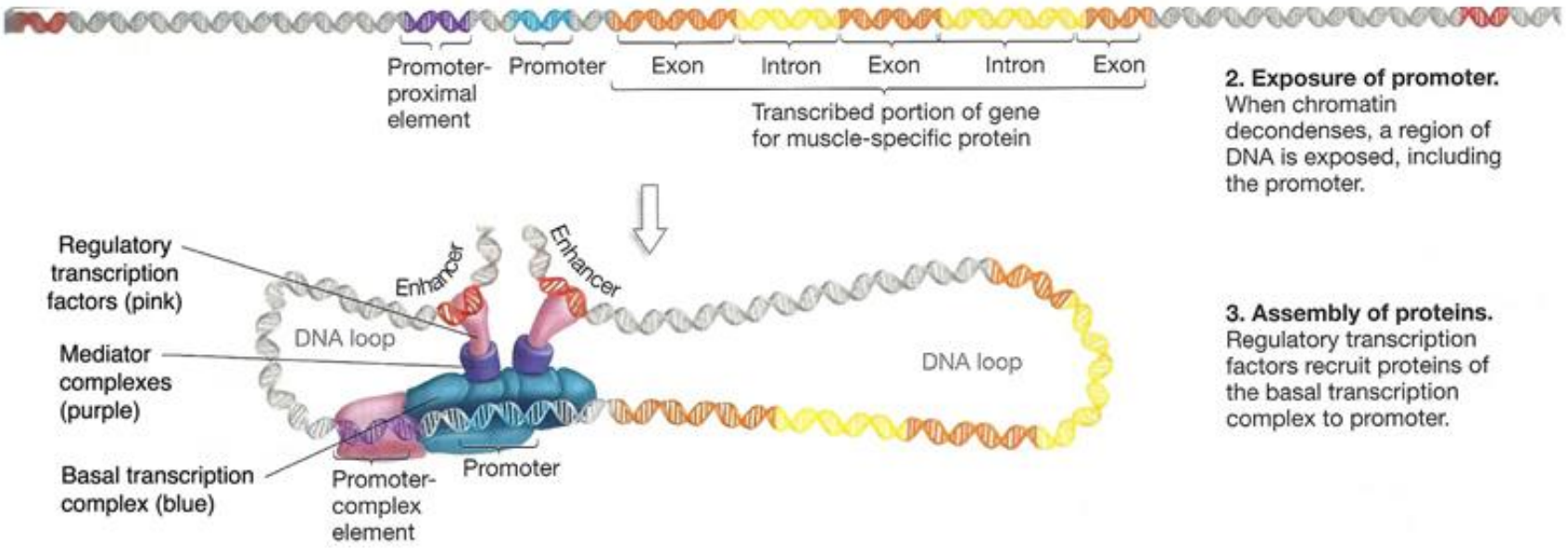
Sequence of events



A little more detailed process



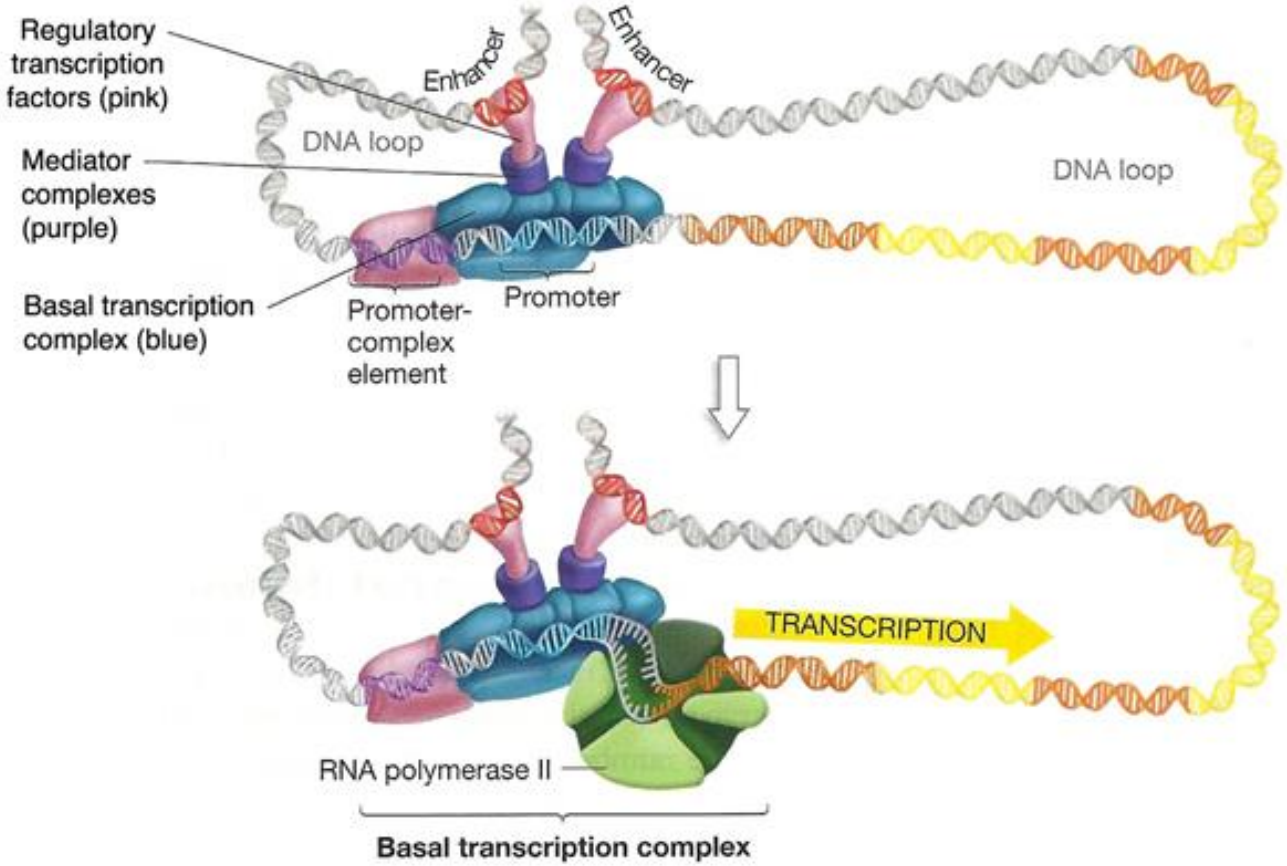
Assembly of basal transcription complex



2. Exposure of promoter. When chromatin decondenses, a region of DNA is exposed, including the promoter.

3. Assembly of proteins. Regulatory transcription factors recruit proteins of the basal transcription complex to promoter.

RNA polymerase joins transcription complex



3. Assembly of proteins. Regulatory transcription factors recruit proteins of the basal transcription complex to promoter.

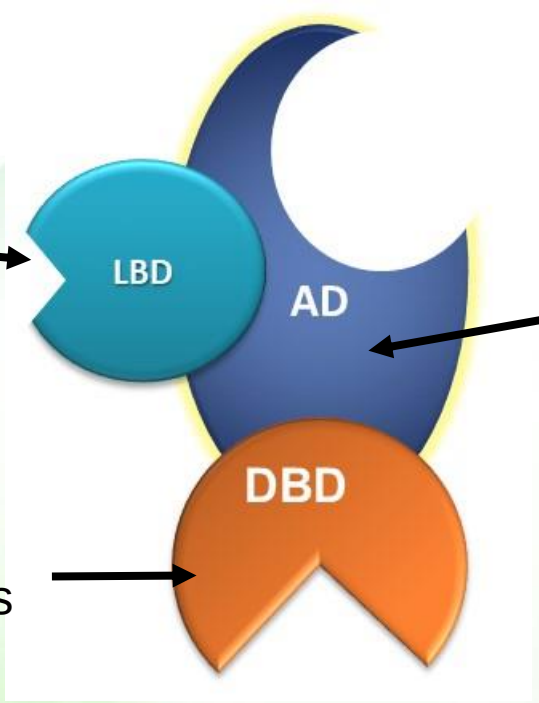
4. Attachment of RNA polymerase. RNA polymerase II completes the basal transcription complex; transcription begins.

Example

Nuclear steroid receptor

General structure of SNRs

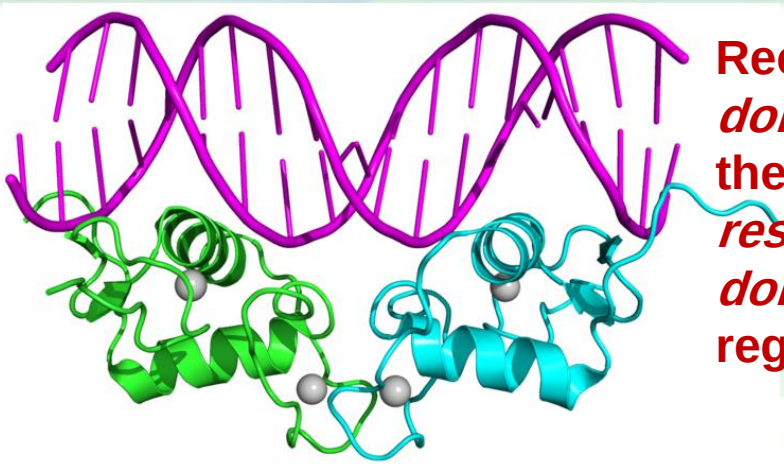
Ligand-binding domain: binds to steroid hormones



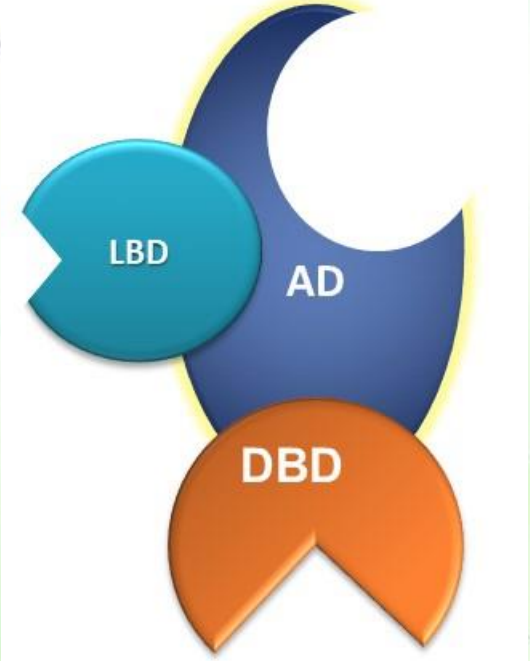
Activation domain: binds to transcriptional regulatory proteins known as co-activators and co-repressors

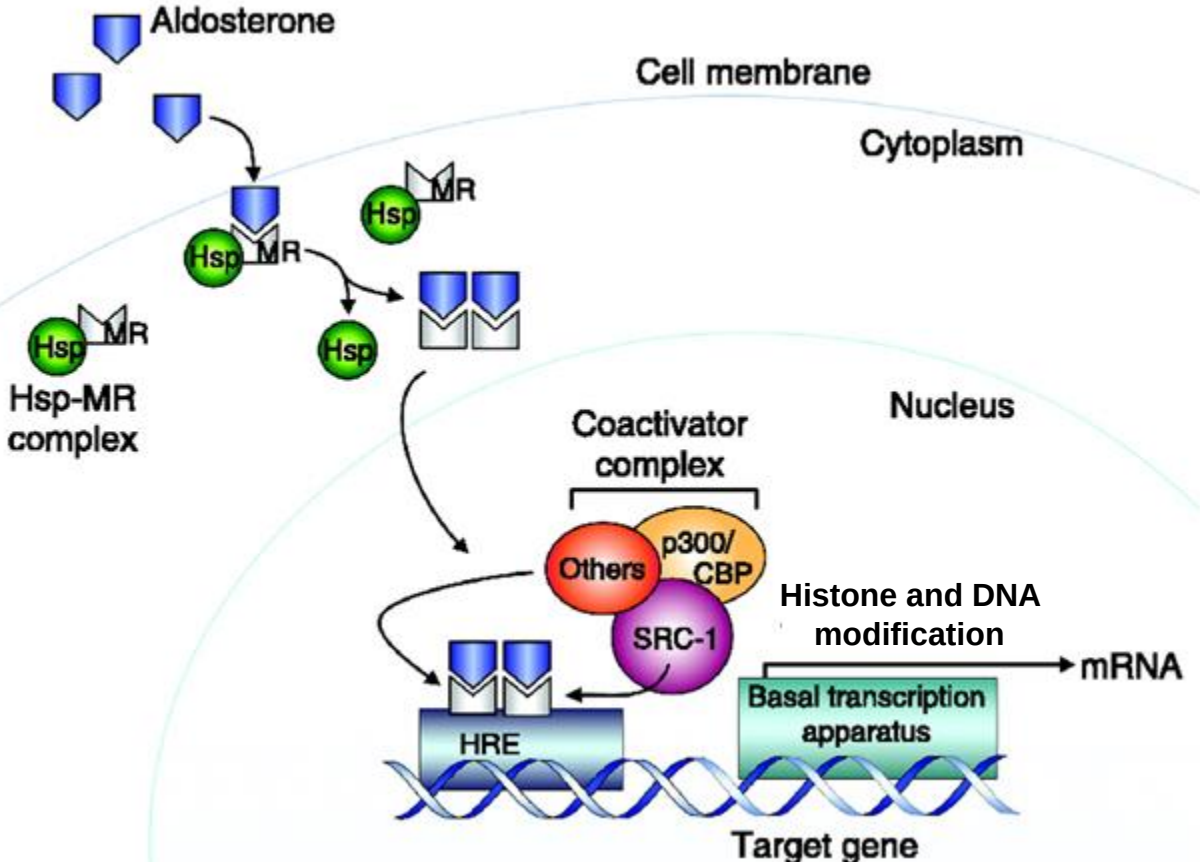
DNA-binding domain: binds to DNA elements

Steroid hormone receptors

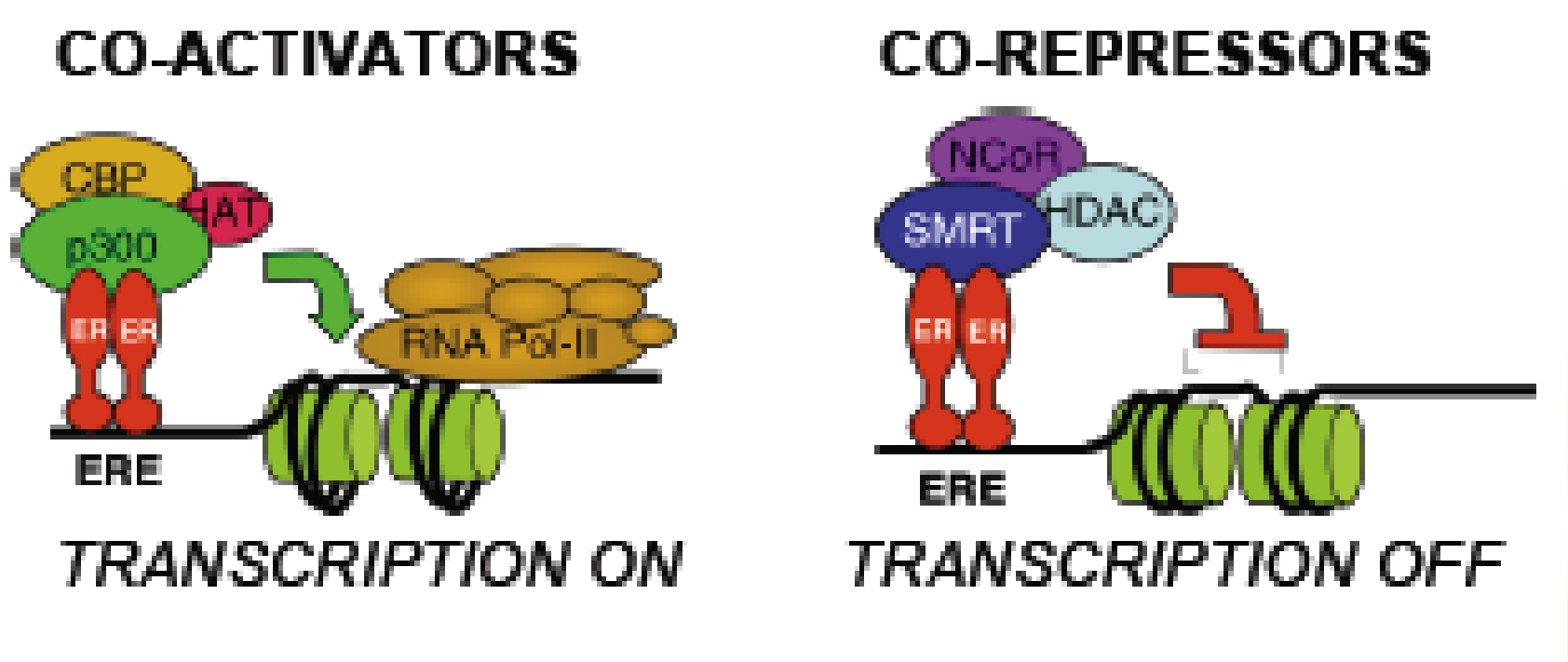


Receptors bind steroid hormones at *ligand-binding domain*, then translocate into the nucleus where they bind specific DNA sequences called *hormone response element (PPE)* via *their DNA-binding domain*, and recruit and bind transcriptional regulatory proteins using their *activation domain*.





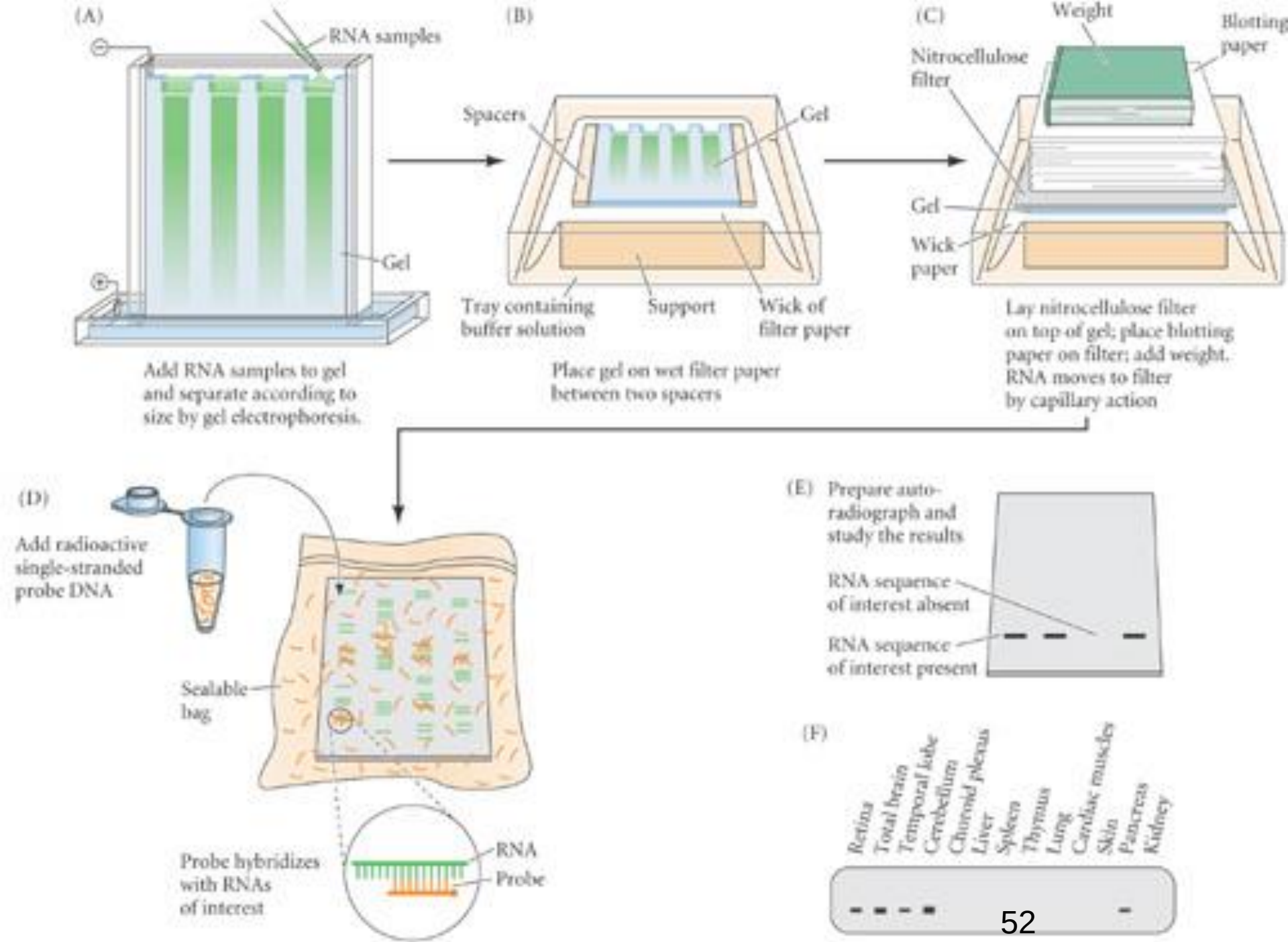
Co-repressors can also bind



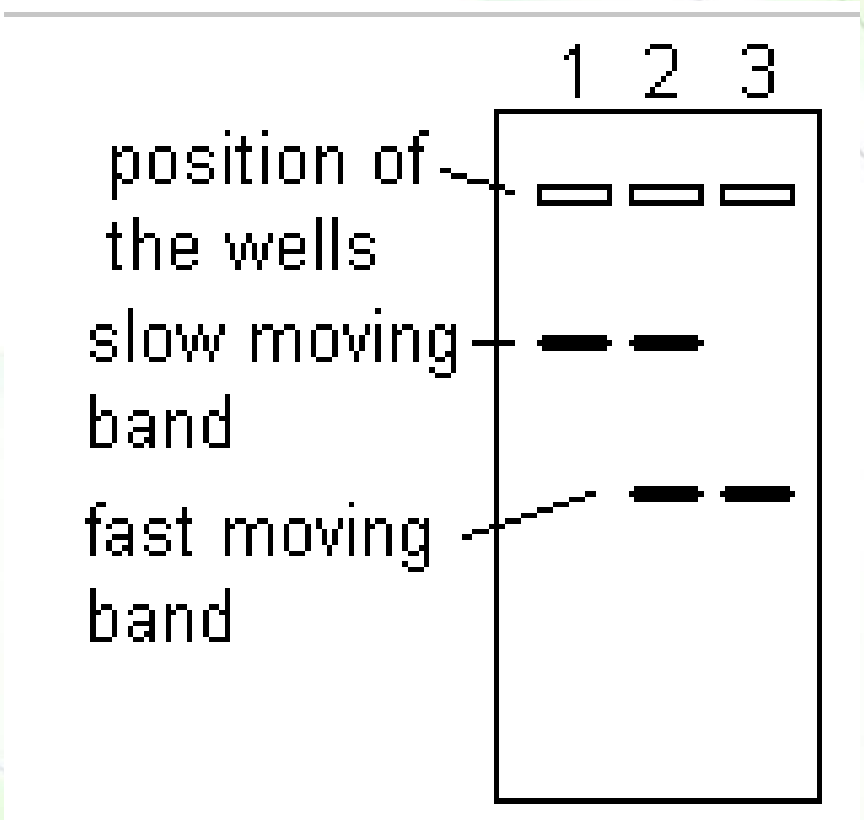
How can we measure RNA levels and site of expression?

Northern blotting

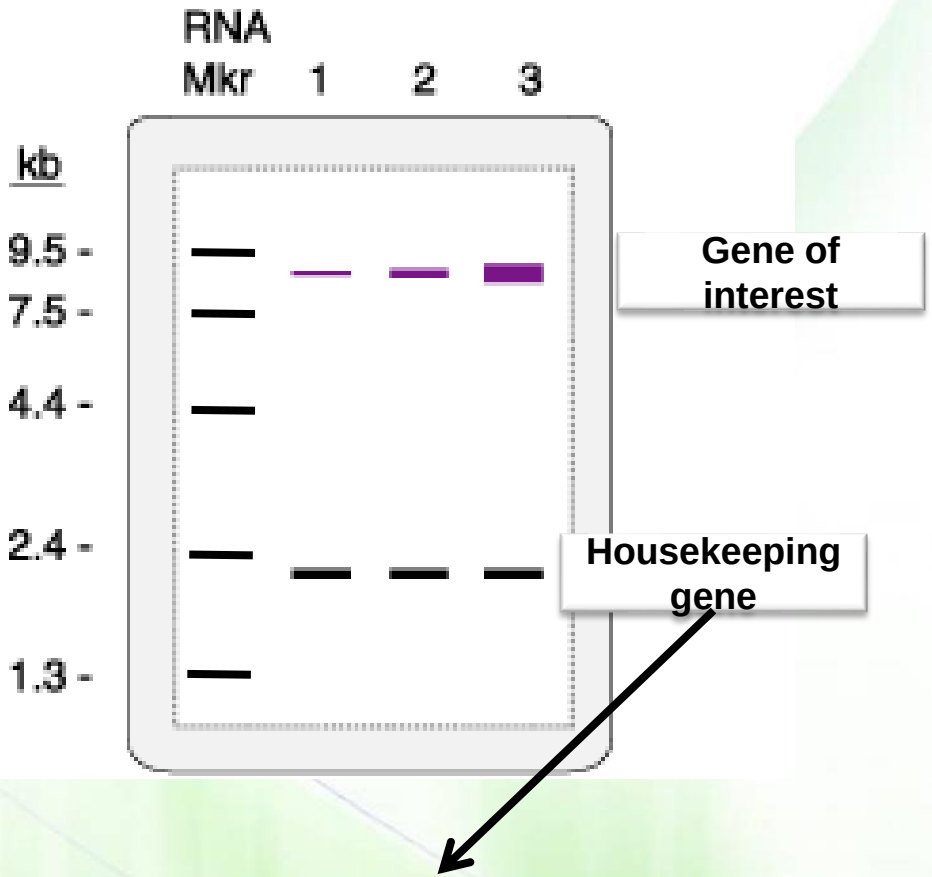
- This is done exactly like Southern blotting except that RNA from cells is isolated instead of DNA.
- RNA molecules are fractionated based on size by gel electrophoresis.
- The fractionated RNA molecules are transferred onto a membrane.
- RNA molecules are targeted by a labeled DNA probe with sequence that is complementary to a specific RNA molecule.
- What information can you deduce from it?

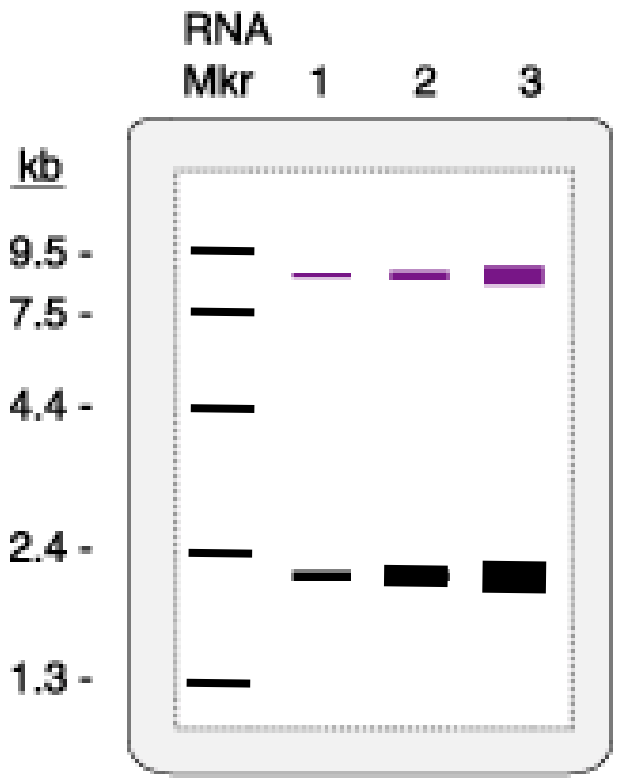

(A)
RNA samples
Gel
Add RNA samples to gel and separate according to size by gel electrophoresis.
(B)
Spacers
Gel
Tray containing buffer solution
Support
Wick of filter paper
Place gel on wet filter paper between two spacers
(C)
Weight
Blotting paper
Nitrocellulose filter
Gel
Wick paper
Lay nitrocellulose filter on top of gel; place blotting paper on filter; add weight. RNA moves to filter by capillary action
(D)
Add radioactive single-stranded probe DNA
Sealable bag
Probe hybridizes with RNAs of interest
RNA
Probe
(E) Prepare auto-radiograph and study the results
RNA sequence of interest absent
RNA sequence of interest present
(F)
Retina
Total brain
Temporal lobe
Cerebellum
Choroid plexus
Liver
Spleen
Thymus
Lung
Cardiac muscles
Skin
Pancreas
Kidney

What are your interpretations?



What are your interpretations?



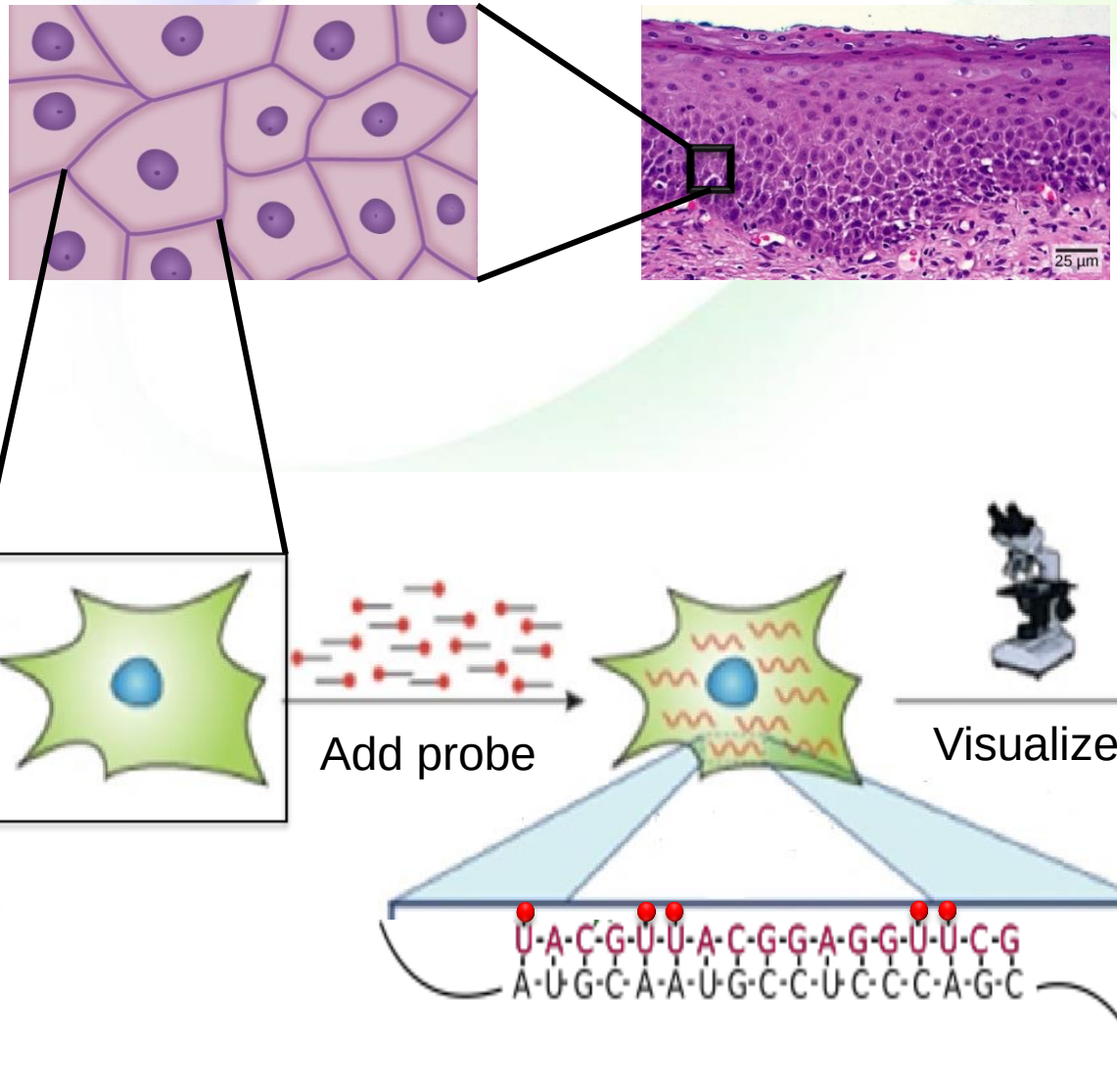


A gene with constant expression
(examples: actin, tubulin)

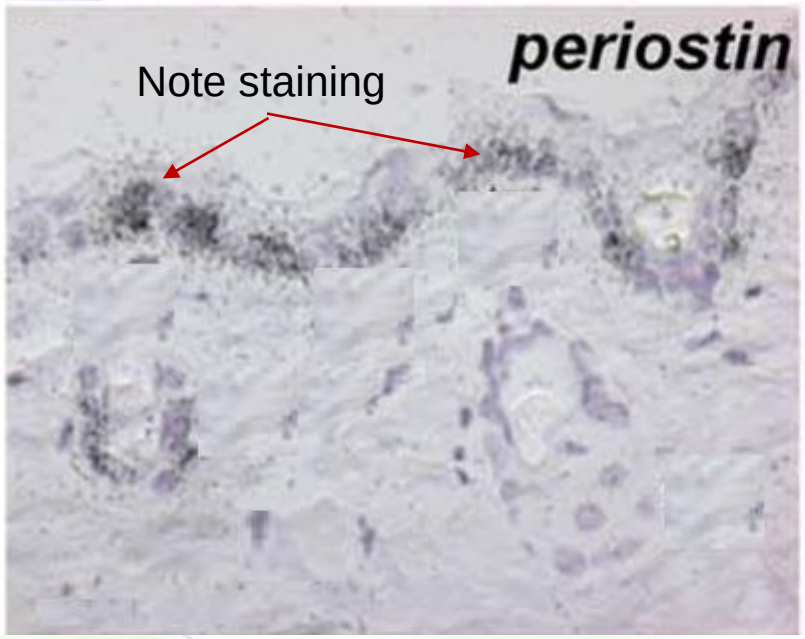
In situ hybridization

- In situ hybridization methods reveals the distribution of specific RNA molecules in cells in tissues.
- RNA molecules can hybridize when the tissue is incubated with a complementary DNA or RNA probe.
- In this way the patterns of differential gene expression can be observed in tissues, and the location of specific RNAs can be determined in cells.

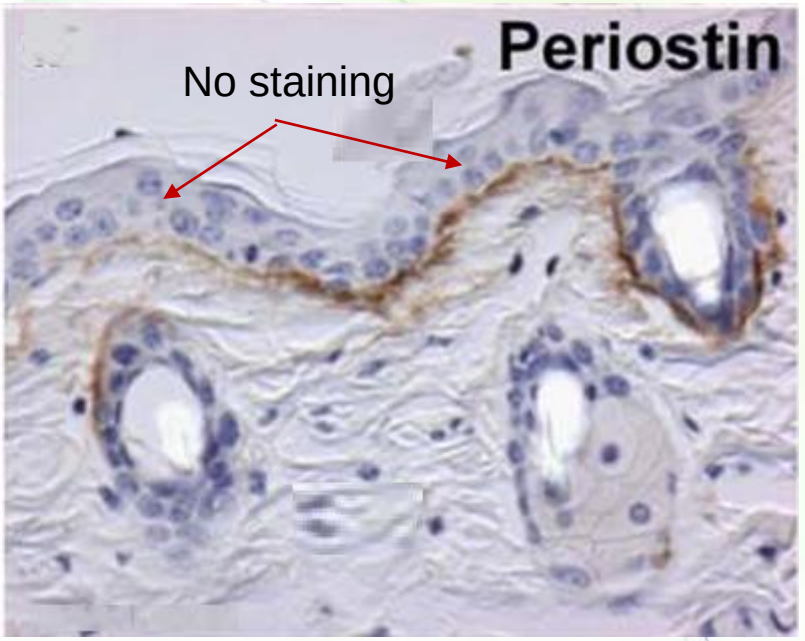
Procedure of in situ hybridization



ISH (RNA)



IHC (protein)



RNA and protein molecules do not coexist and are present in different places.

mRNA: inside cells along the basement membrane

Protein: outside cells in the basement membrane