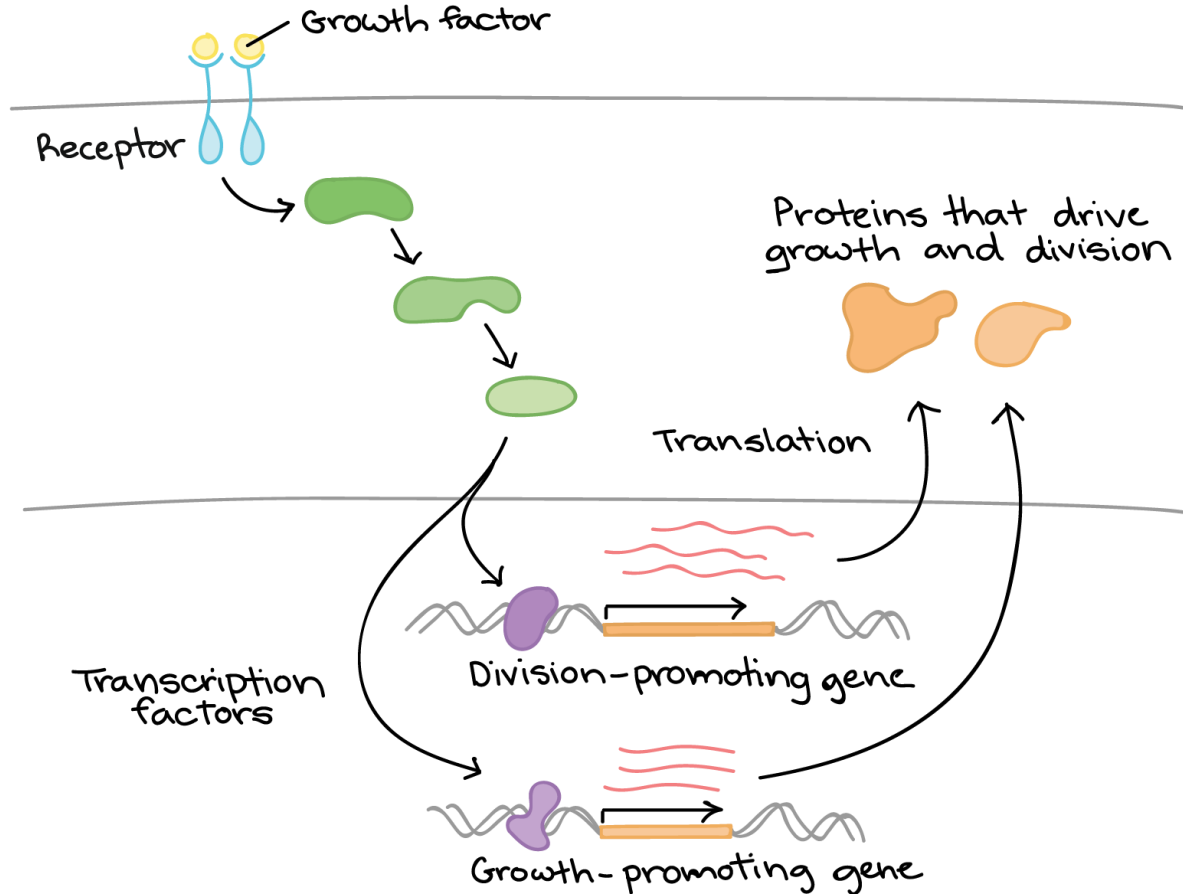


Regulation of gene expression in eukaryotes and cell signaling



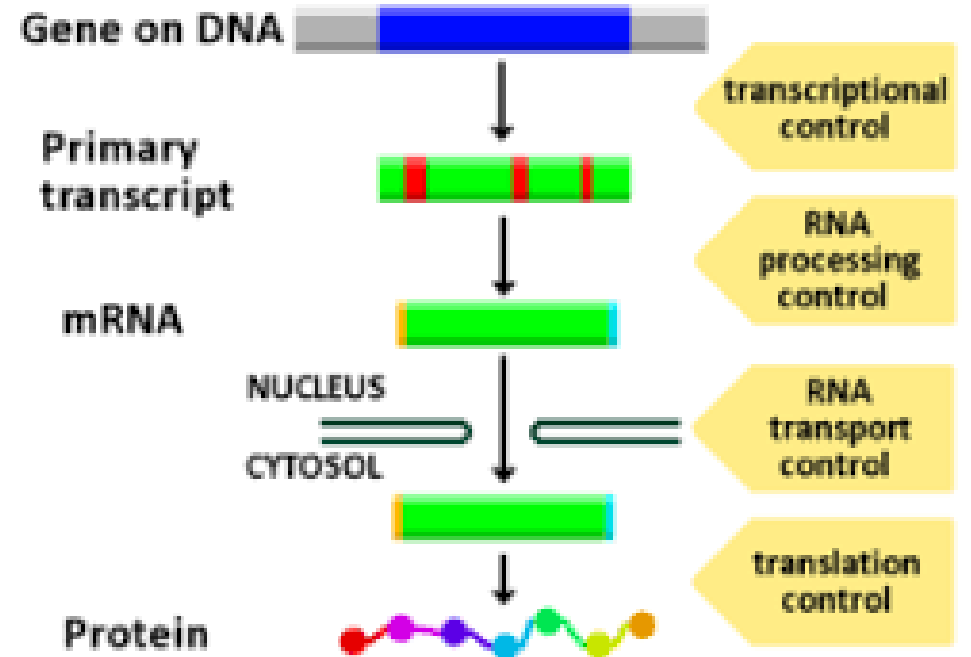
Gene regulation is the process of controlling which genes in a cell will be expressed.

- Different cells of a multicellular organism may express very different sets of genes, even if they contain the same DNA.
- A set of genes expressed in a cell identifies the set of proteins and functional RNAs it contains, giving it its unique properties.
- In eukaryotes, such as humans, gene expression involves many steps, and gene regulation may occur in any of these steps. However, many genes are regulated primarily at the transcriptional level.

Relationship between cell expression and signaling.

Regulation of gene expression - in general

Products of all genome genes are not necessary at every point in a cell's life
conditions and variability of the environment play a significant role
the complexity of the gene expression process is mostly energetic
cell variability during the cell cycle



- in unicellular: reactions to environmental changes (temperature, osmotic pressure, nutrient availability, etc.)
- - in multicellular: reactions to changes in the environment + communication between cells of the same organism + developmental processes within the organism

<https://www.khanacademy.org/science/biology/gene-regulation/gene-regulation-in-eukaryotes/a/overview-of-eukaryotic-gene-regulation>

Constitutive and regulatable genes

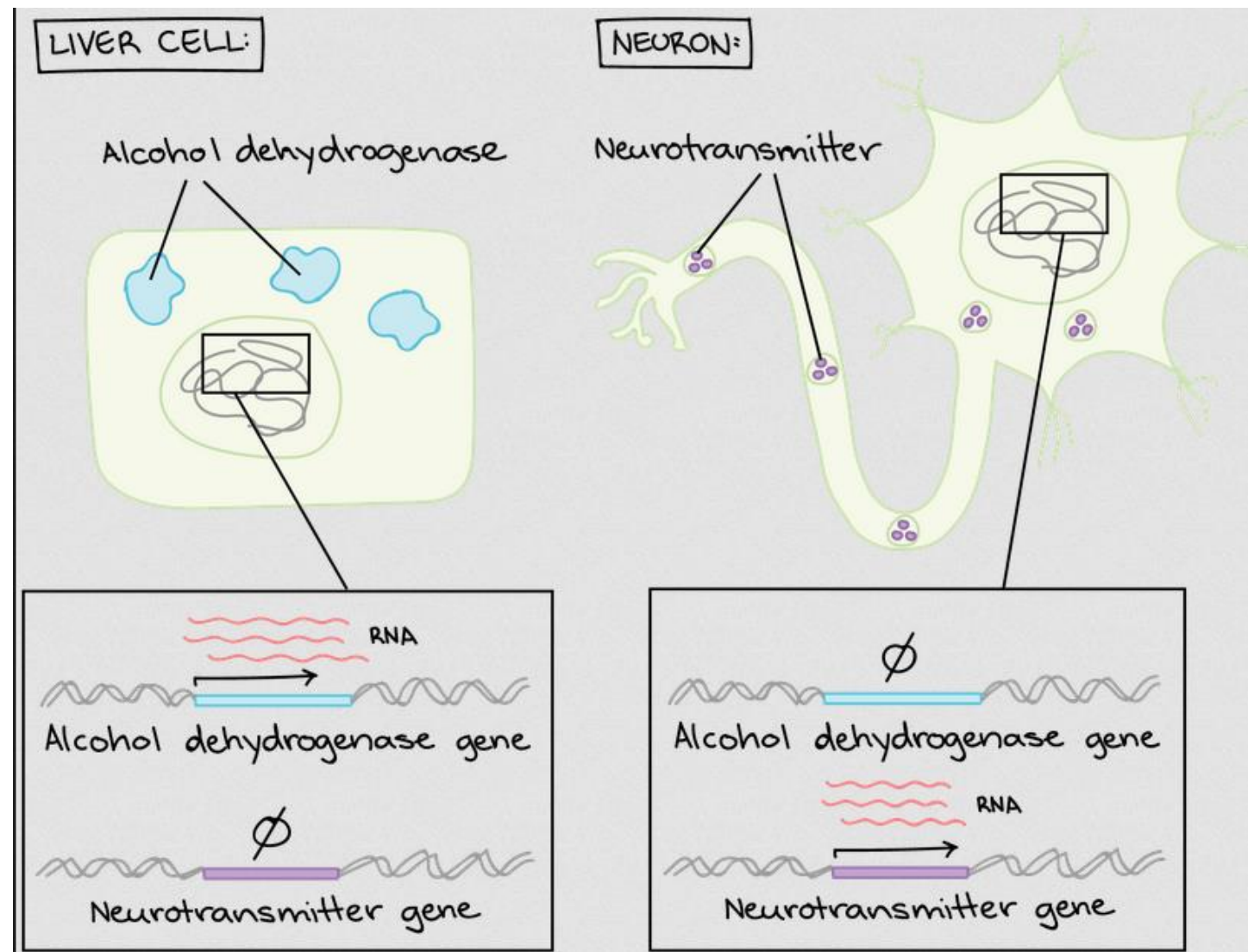
- there are different types of genes (constitutive, regulated)
- **constitutive genes** are expressed in most cells
- ensure stable (continuous) expression of genes that encode the components of cells necessary to maintain normal - operational - functions ("housekeeping functions")
- eg expression of genes for rRNA, tRNA, ribosome proteins, RNA polymerases, proteins involved in proteosynthesis, enzymes catalyzing operational functions
- the expression of **regulatable (inducible / repressible) genes** increases or decreases as needed
- refers to (inducible / repressible) genes whose products
- they are only needed under certain conditions
- the synthesis of these genes is under the control of special regulatory systems
- constitutive expression of these genes would mean an unnecessary energy load on the cell,
- evolutionary advantages - regulation
- common for both prokaryotes and eukaryotes

Regulation of expression in eukaryotes:

complicated process of many factors acting depending on time and place

Products of the same gene have a different function in different tissues

At different stages of ontogenetic development, different genes coding for similar products are expressed



Our amazing body contains hundreds of different types of cells, from immune cells to skin cells to neurons. Almost all of your cells contain the same set of instructions for DNA – so why do they look so different and do such a different job? Answer: different gene regulation!

Regulation in eukaryotes more complex than in prokaryotes

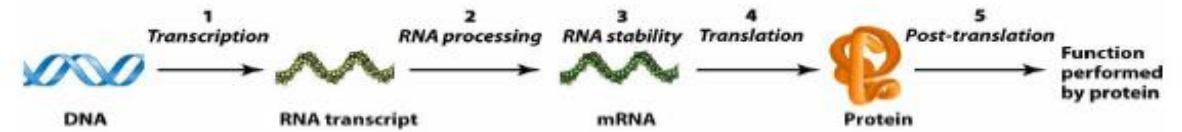
high number of genes that are variously expressed in different tissues

- inaccessibility of heterochromatin DNA by transcription
- gene expression requires the presence of several activators
- problem: DNA in the nucleus, transcription factors and protein regulators are formed in the cytoplasm

- positive and negative regulation

- **positive:** the gene can be expressed if it receives a certain positive signal-activator
- **negative:** gene expression is suppressed by a repressor and can only be initiated after its removal - dependent on the reception of the signal
- Positive and negative regulation is thus dependent on a small molecule - an inducer that binds to the regulatory protein
- signals affecting the expression of a particular gene may be greater
- Common to both prokaryotes and eukaryotes

Regulatory levels



SUMMARY TABLE 18.1 Regulating Gene Expression in Bacteria and Eukaryotes

Level of Regulation	Bacteria	Eukaryotes
Chromatin remodeling	<ul style="list-style-type: none"> Limited packaging of DNA Remodeling not a major issue in regulating gene expression. 	<ul style="list-style-type: none"> Extensive packaging of DNA Chromatin must be opened for transcription to begin.
Transcription	<ul style="list-style-type: none"> Positive and negative control by regulatory proteins that act at sites close to the promoter Sigma interacts with promoter. 	<ul style="list-style-type: none"> Positive and negative control by regulatory proteins that act at sites close to and far from promoter Large basal transcription complex interacts with promoter. Mediator complex required.
RNA processing	<ul style="list-style-type: none"> None documented 	<ul style="list-style-type: none"> Extensive processing: alternative splicing of introns addition of 5' cap and 3' tail
mRNA stability	<ul style="list-style-type: none"> Some RNA interference documented 	<ul style="list-style-type: none"> For many genes, RNA interference limits life span or translation rate.
Translation	<ul style="list-style-type: none"> Regulatory proteins bind to mRNAs and/or ribosome and affect translation rate. 	<ul style="list-style-type: none"> Regulatory proteins bind to mRNAs and/or ribosome and affect translation rate.
Post-translational modification	<ul style="list-style-type: none"> Folding by chaperone proteins Chemical modification (e.g., phosphorylation) may change activity. 	<ul style="list-style-type: none"> Folding by chaperone proteins Chemical modification (glycosylation, phosphorylation) Ubiquitination targets proteins for destruction by proteasome

DNA and chromosome levels

level of transcription

level of transcript editing

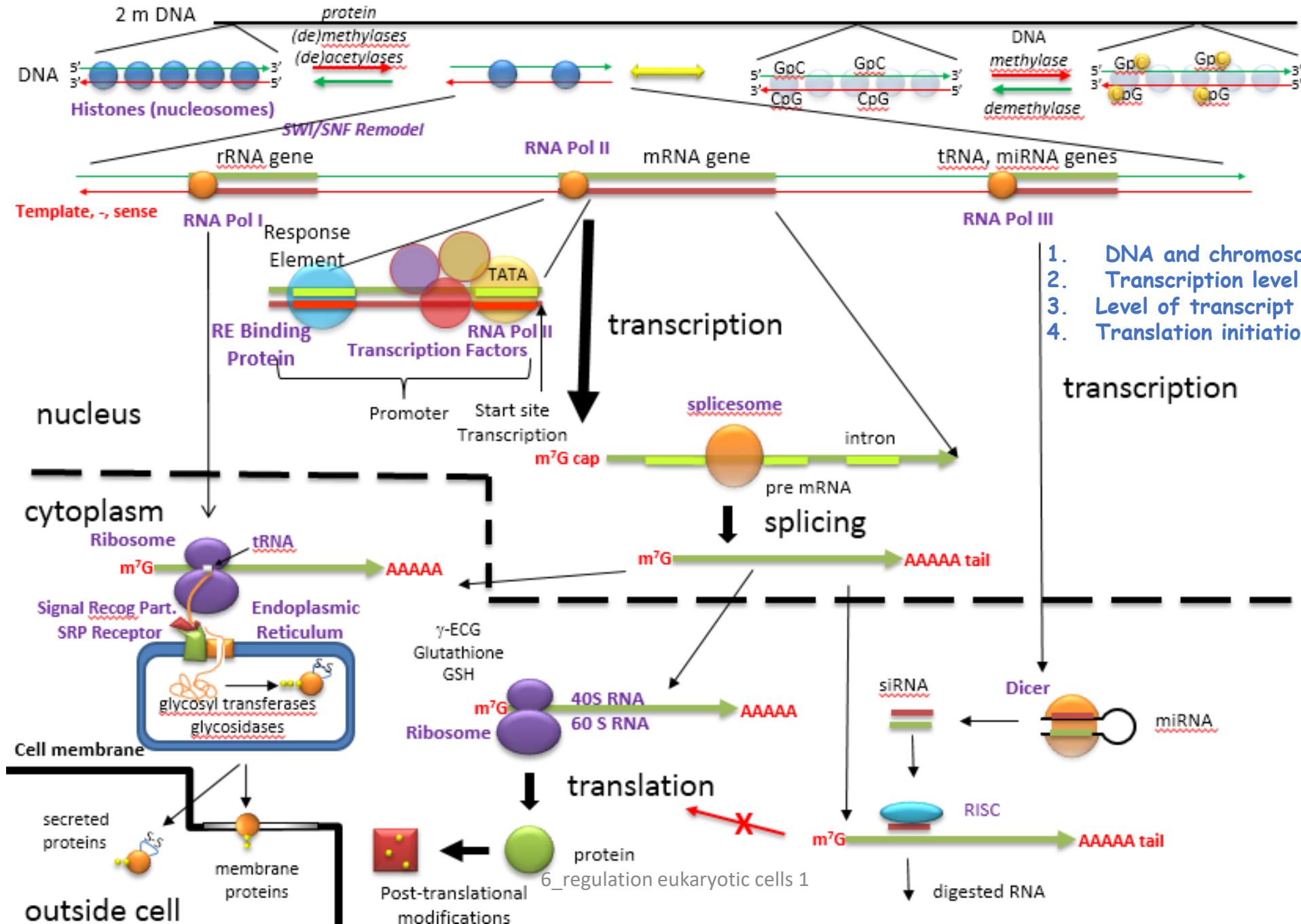
translation initiation level

Proteins

Transcription factors

Eukaryotic Gene Expression: An Overview

TEST



1. DNA and chromosome levels
2. Transcription level
3. Level of transcript editing
4. Translation initiation level

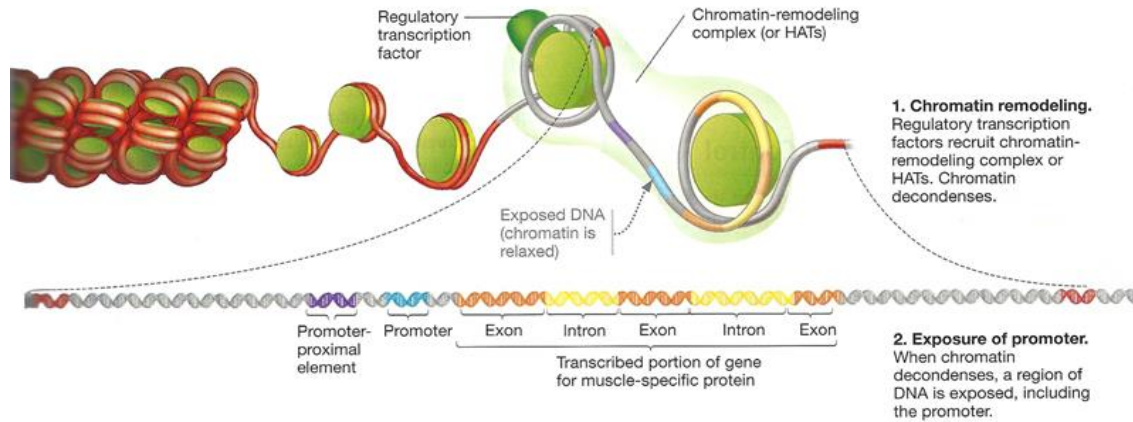
<https://employees.csbsju.edu/h.jakubowski/classes/ch331/bind/epigenetics/transcription.html>

TEST

Regulatory levels:

- there are many changes in the chromatin structure at the transcription site
- positive mechanisms regulate transcription much more often than negative ones.
- transcription and translation occur at spatially and temporally different places and times.

Chromatin remodeling exposes the promoter



Assembly of basal transcription complex

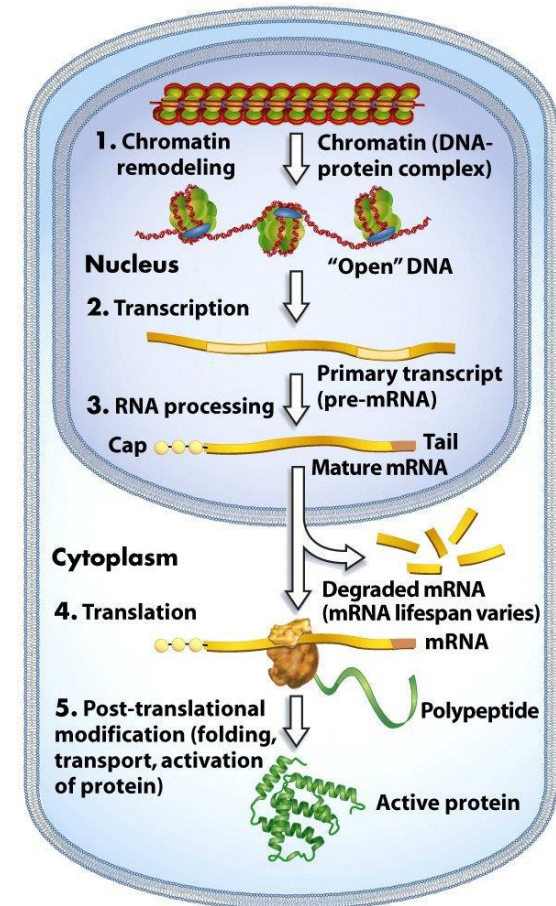
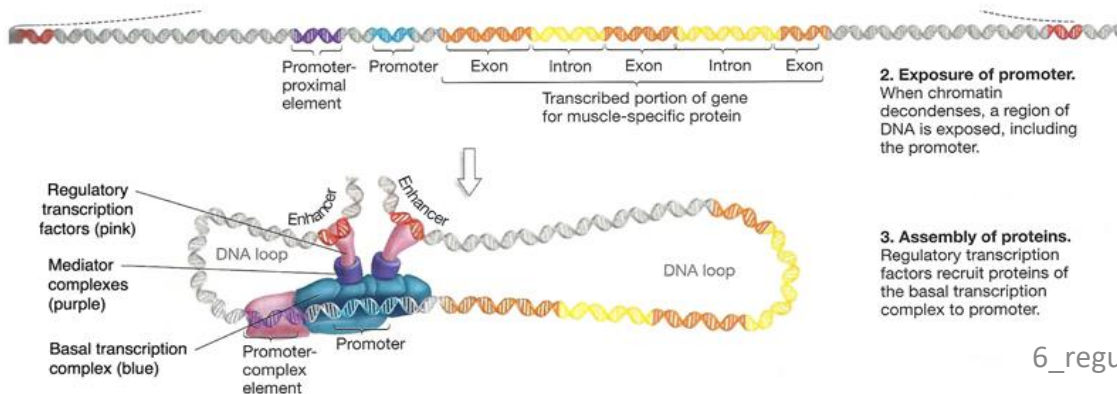


Figure 18-1 Biological Science, 2/e © 2005 Pearson Prentice Hall, Inc.

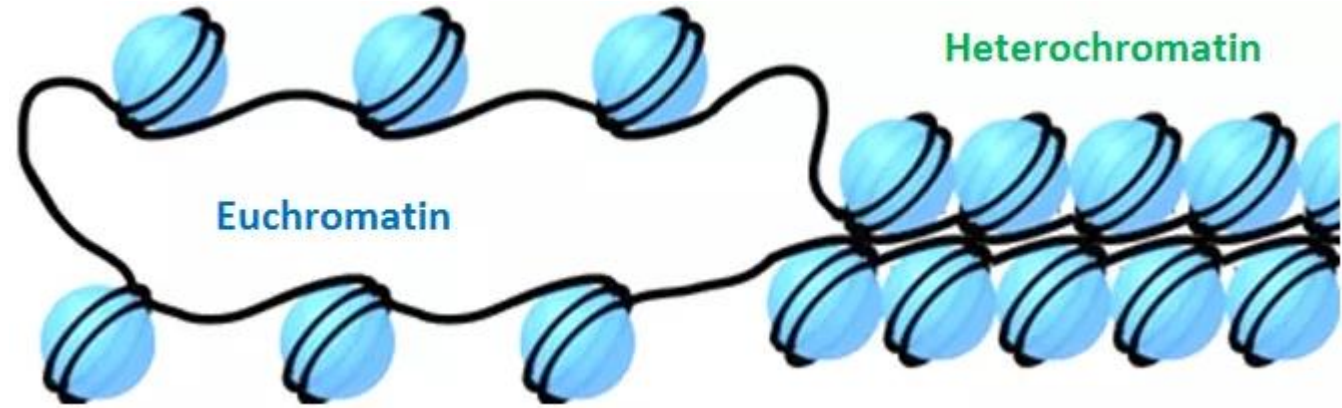
Regulation of gene transcription availability - chromatin

In cells of differentiated tissues, only those genes that play a role in a given cell are manifested

Chromatine in nucleus:

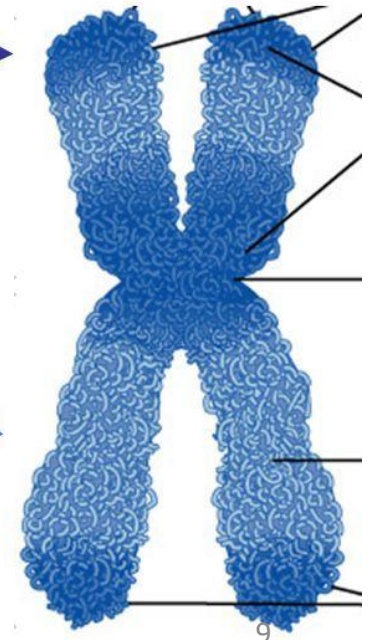
- Condensed (heterochromatin) - genes are inactive
- Diffuse (euchromatin) genes produce mRNA

During development, there are changes in the activity of genes, chromatin changes from the condensed form to the diffuse and vice versa.



Chromosome Parts:

- **Heterochromatin:**
 - More condensed
 - Silenced genes (methylated)
 - Gene poor (high AT content)
 - Stains darker
- **Euchromatin:**
 - Less condensed
 - Gene expressing
 - Gene rich (higher GC content)
 - Stains lighter



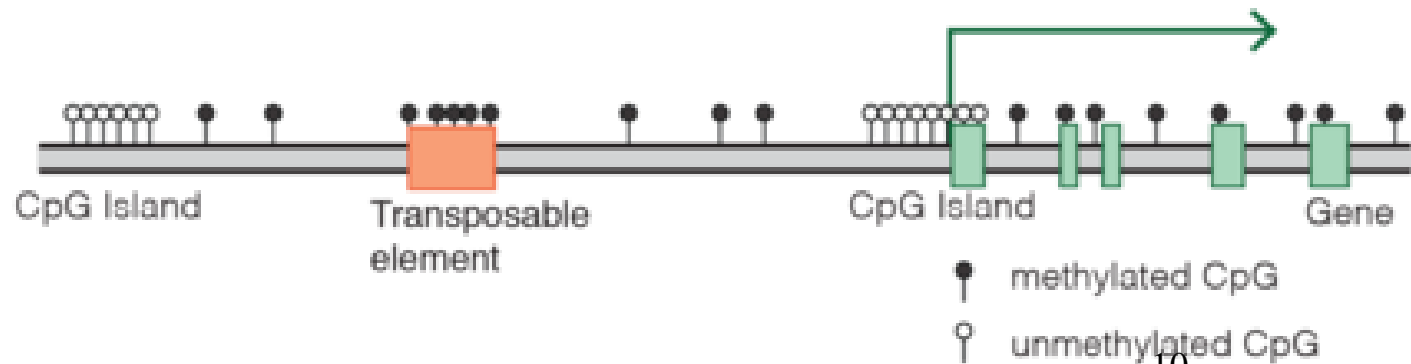
Influencing gene expression at the level of chromosome structure

- DNA accessibility in chromatin
- histone acetylation regulators
- chromatin remodeling complexes
- DNA methylation
- gene DNA rearrangement
- gene amplification
- gene deletion

Epigenetic modifications

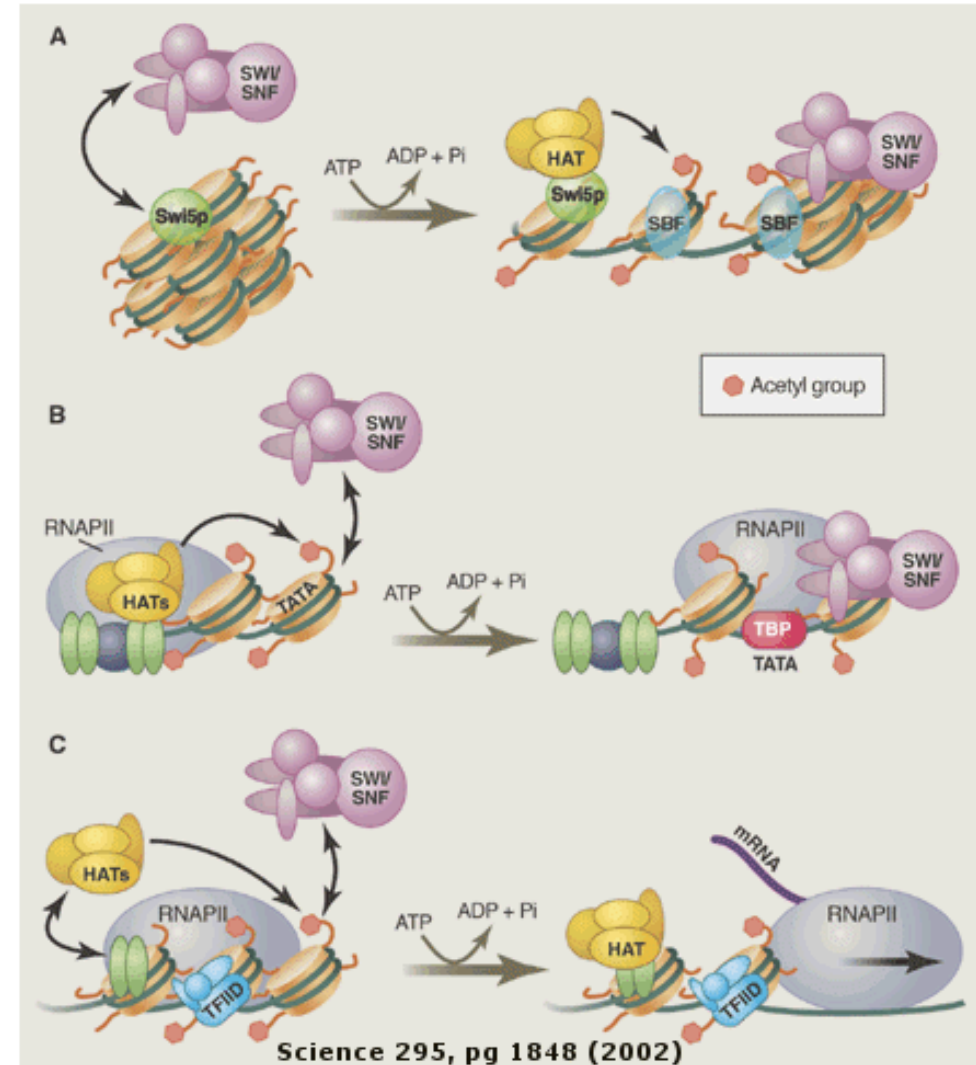
they regulate genome function by altering the local structure of chromatin - primarily by regulating availability and compactness

Typical mammalian DNA methylation landscape



DNA accessibility in chromatin, chromatin remodeling

- densely composed DNA in chromatin cannot be transcribed; RNA polymerase does not have access to the promoter
- histone acetylation affects chromatin compactness: non-acetylated histones form highly condensed chromatin, acetylated histones - less condensed chromatin
- the **degree of acetylation is determined by the enzymes: histone acetyltransferases (HAT) and histone deacetylases (HDAC)** a change in chromatin status that leads to the activation of transcription
- release of the nucleosome from the chromatin
- Development of a section of DNA from a nucleosome using ATP cleavage
- Covalent modification of histone ends by acetylation (acetylation of the γ -amino group in the lysine side chain at the N-termini of histones H2A, H2B, H3 and H4).



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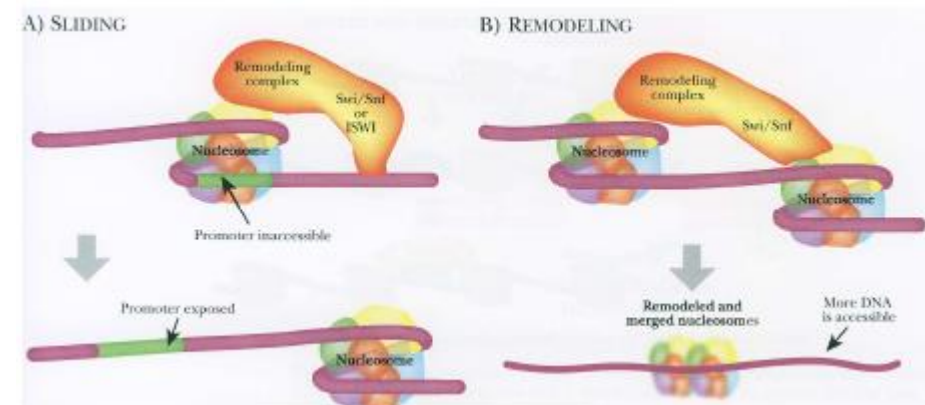
Regulators changes acetylation of histones

- coactivators of transcription are HAT (histone acetyltransferases) eg CBP / p300 proteins
- transcription corepressors are HDAC (histone deacetylase)
- coactivators and corepressors do not bind to DNA, but interact with transcription factors
- - binding of the transcription factor to DNA
- - HAT binding to transcription factor
- - HAT acetylates surrounding histones and releases them
- binding of nucleosomes to DNA
- - chromatin remodeling complexes change nucleosome organization - accessibility
- DNA is elevated
- - binding of other transcription factors
- - RNA polymerase binding to DNA

Complexes for chromatin remodeling

they complement nucleosome changes resulting from histone acetylation
they move nucleosomes across

Activation of eukaryotic transcription gene - sequence of events



ATP-dependent chromatin remodeling complexes

multiprotein complexes that alter the conformation of histones and DNA (in promoter regions) use energy from ATP hydrolysis

- the substrate is not a mononucleosome, but rather a chain of nucleosomes - it changes the position of nucleosomes on DNA and forms "nucleosome-free" regions

- SWI / SNF, RSC, NURF, CHRAC, ACF, FACT

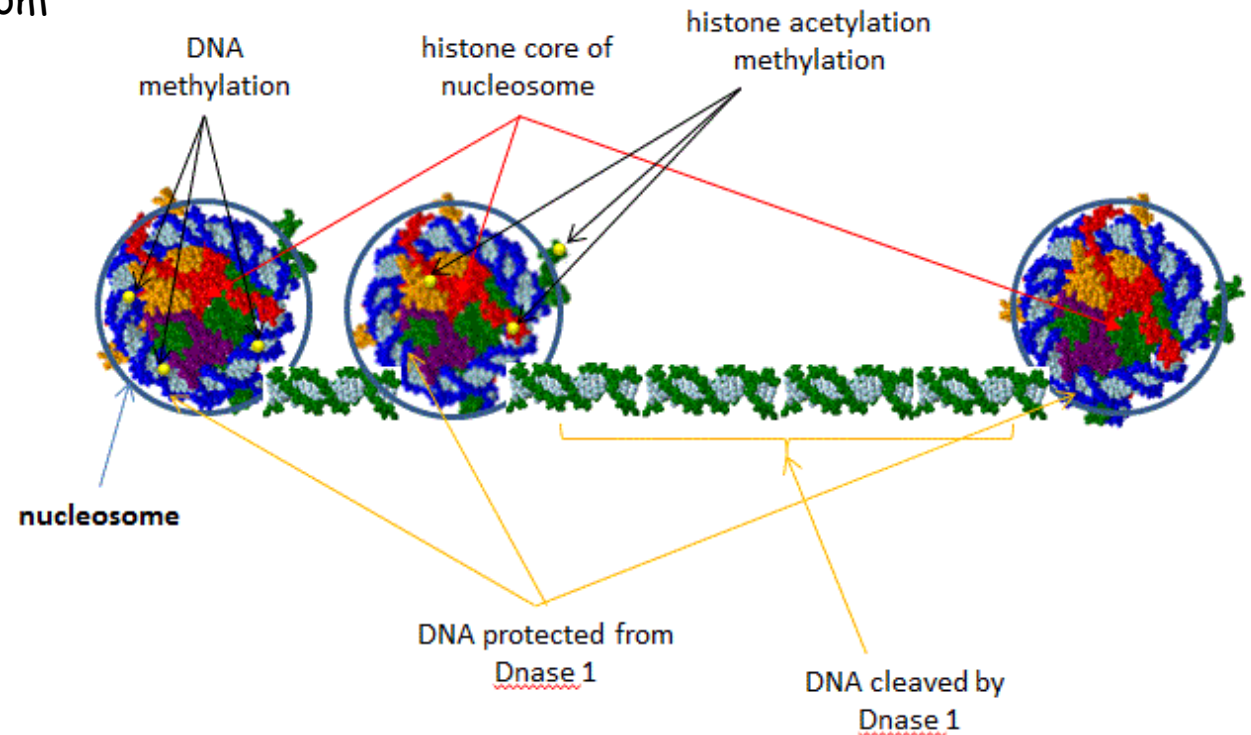
- classification according to ATPase subunit:

SWI2 / SNF2

ISWI

Mi-2 (+ deacetylase subunit) (CHD complexes)

- their cooperation necessary for activators and repressors



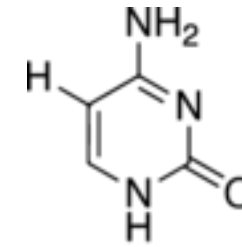
Gene expression in eukaryotes is affected by DNA methylation

- methylation of cytosine residues in DNA **5-methylcytosine**
- catalyzed by **methyltransferase**

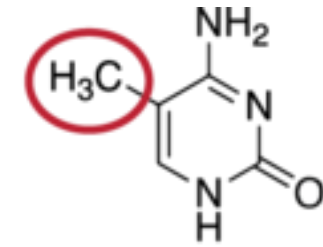
recognition sequences are short: *GC* in animals and *GNC* in plants (*GC* islands)

- **methylation of DNA weakens gene expression**
- constitutive operational genes do not have *GC* methylated islands
- tissue-specific genes do not have *GC* islands methylated only if their products are needed in that tissue
- methyl groups protrude into a large groove of DNA and thus prevent proper binding of transcription factors

Ex .: genes for globin are methylated in non-erythroid cells (hemoglobin synthesis does not take place here), in erythroblasts and reticulocytes (erythrocyte precursors) these genes are not methylated

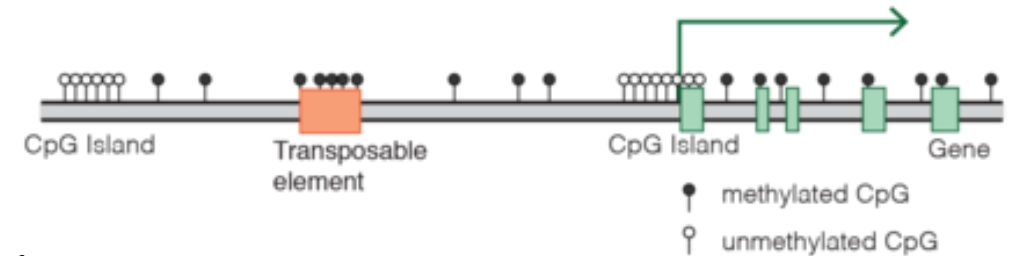


Cytosine



methylated Cytosine

Typical mammalian DNA methylation landscape



DNA methylation

covalent modification of cytosine at position 5' in the **CpG dinucleotide**

- cytosine methylation takes place by transfer of a methyl group from the donor: S-adenosylmethionine with the participation of DNA methyltransferases
- DNMT1 - "maintenance" - 10x higher affinity for semimethylated DNA, much more active than DNMT3a and 3b
- DNMT3a and DNMT3b - de novo - methylate unmethylated DNA
- Deletion of DNMT in mice is embryonally lethal
- The pattern of DNA methylation is relatively stable in adult cells, significant changes are described in connection with aging
- . gene silencing

The DNA methylation landscape of vertebrates is very particular compared to other organisms. In mammals, around 75% of CpG dinucleotides are methylated in [somatic cells](#),^[15] and DNA methylation appears as a default state that has to be specifically excluded from defined locations.^{[12][16]} By contrast, the genome of most plants, invertebrates, fungi, or protists show "mosaic" methylation patterns, where only specific genomic elements are targeted, and they are characterized by the alternation of methylated and unmethylated domains.^{[17][18]}

Methylation patterns can account **for gene silencing (in which one gene in a pair of identical chromosomes is not expressed)** and inactivation of one entire X chromosome in a female (who has 2 X chromosomes). In general, transcription from genes that are methylated is inhibited.

<https://employees.csbsju.edu/hjakubowski/classes/ch331/bind/olbindtranscription.html>

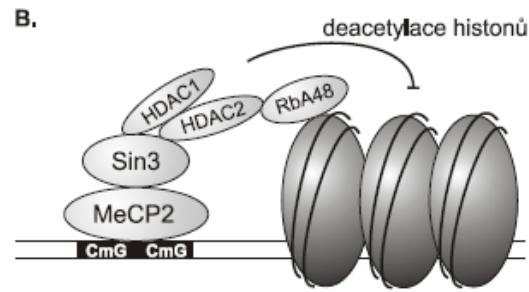
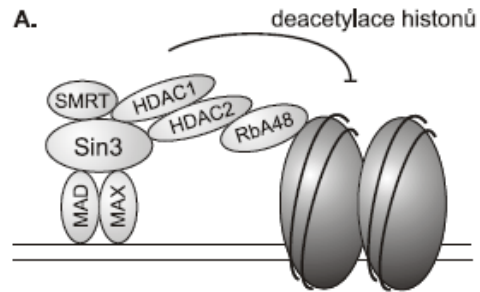
DNA methylation and histone deacetylation

CpG DNA methylation is associated with repression of transcription - with maintaining a more stable state of chromatin

□ methylated DNA regions are recognized by the MeCP2 protein (its methyl-DNA binding domain) and specifically interact (transcriptional repressor domain) with the Sin3 corepressor, which brings HDACs to the methylated regions

Other histone modifications:

- phosphorylation of histones is functionally linked to acetylation
- methylation of histones (lysines and arginines can be methylated; H2B, H3 (lys 4, 9, 27, 36) and H4 (lys 20))
- monoubiquitination, at the C-terminus of histones: lysine 119 histone H2A and lysine 123 histone H2B
- associated with a transcriptionally active or, conversely, repressed (H2A K119) chromatin state
- Histone polyubiquitination is also an integral part of DNA repair



transient repression

stable repression



Gene amplification

In gene amplification, a region of a chromosome undergoes repeated cycles of DNA replication

Newly synthesized DNA is excised to form small, unstable chromosomes (double minutes)

These integrate into other chromosomes and the corresponding gene is thus amplified

Normally, amplification is caused by errors in DNA replication and cell division - under certain circumstances, they may be encoded in the genome.

Ex .: Patients treated with methotrexate (a dihydrofolate reductase inhibitor) developed drug resistance (the drug ceased to be effective).

The reason is an increase in the number of dihydrofolate reductase genes due to amplification.

Gene rearrangement

DNA segments can rearrange and associate with other genes within the genome

Ex .: Gene rearrangement in antibody-producing cells (immunoglobulins)

II. Regulation at the transcriptional level

promoter in eukaryotes

Basic regulation of transcription (common to all genes)

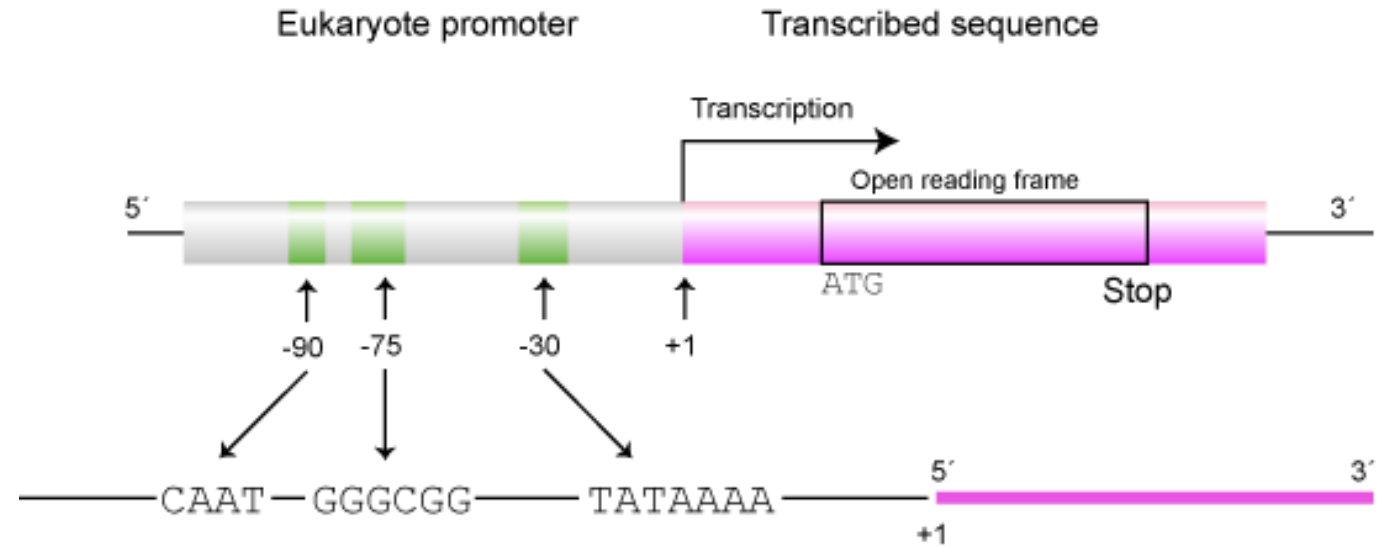
Regulation by components of the "basal transcription complex" (RNA polymerase binding to the TATA box, TATA binding proteins and other "basal" transcription factors binding to the RNA polymerase or in the promoter region)

Genes regulated only in this way:

Constitutively expressed genes

Specific effects on gene expression:

Through regulatory sequences in DNA and **specific transcription factors.**



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- binding of **basal transcription factors**
- regulatory sequences in DNA and **specific transcription factors.**