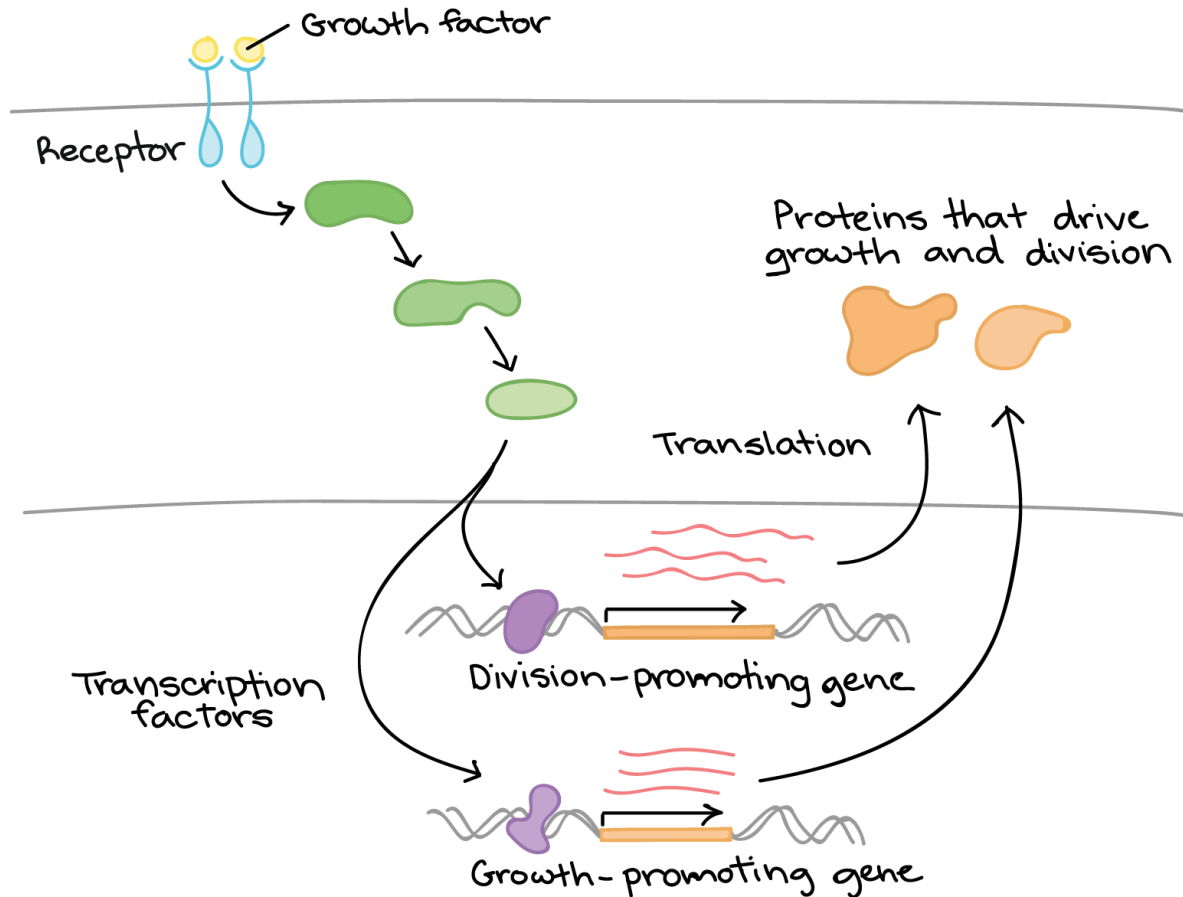


Regulation of gene expression in eukaryotes and cell signaling



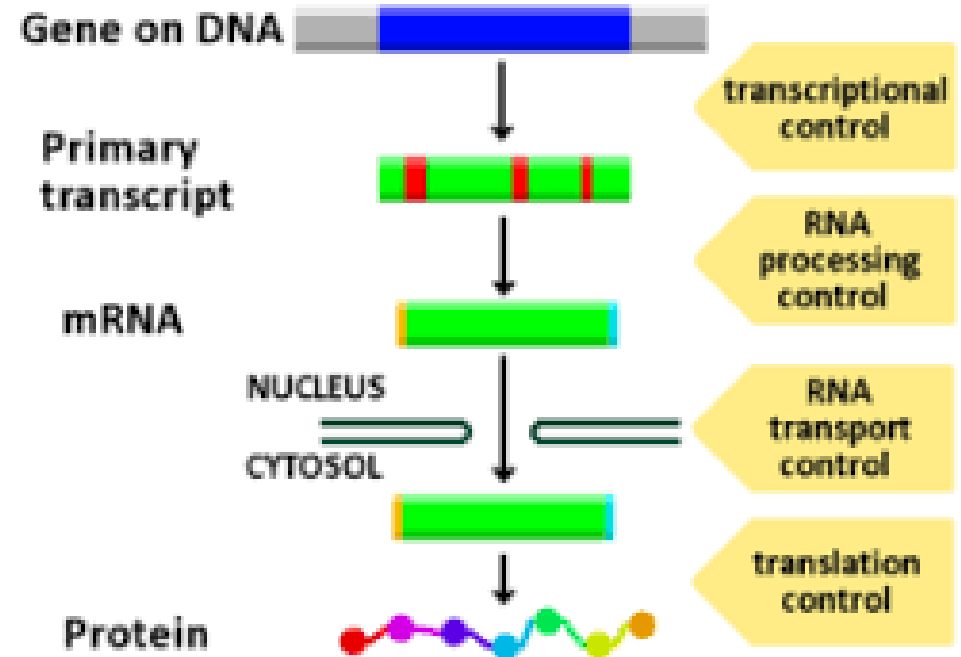
Gene regulation is the process of controlling which genes in a cell will be expressed.

- 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

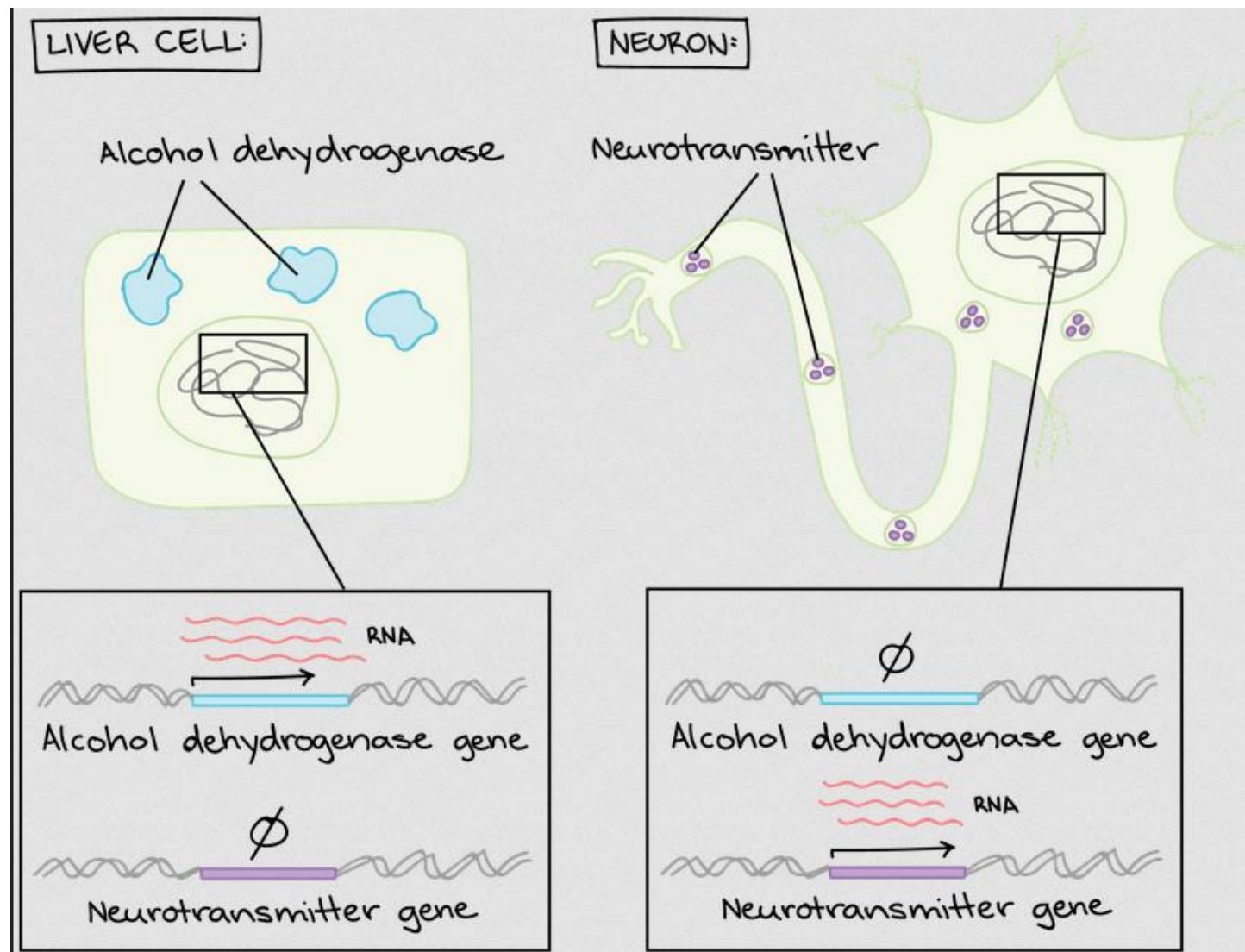
<https://www.khanacademy.org/science/biology/gene-regulation/gene-regulation-in-eukaryotes/a/overview-of-eukaryotic-gene-regulation>

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

Regulace exprese u eukaryot:

- komplikovaný proces mnoha faktorů působících v závislosti **na čase a místě**
- produkty téhož genu mají v různých tkáních **jinou funkci**
- v různých **fázích ontogenetického** vývoje jsou exprimovány různé geny kódující podobné produkty



Naše úžasné tělo obsahuje stovky různých typů buněk, od imunitních buněk přes kožní buňky až po neurony. Téměř všechny vaše buňky obsahují stejnou sadu pokynů pro DNA - tak proč vypadají tak odlišně a dělají tak odlišnou práci? Odpověď: odlišná regulace genů!

Regulace u eukaryot

složitější než u prokaryot

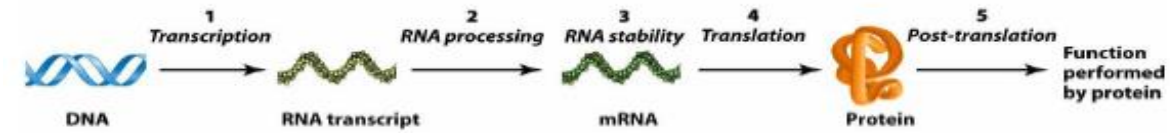
vysoký počet genů, které jsou různě exprimovány v různých tkáních

- nepřístupnost DNA heterochromatinu transkripci
- exprese genu vyžaduje přítomnost několika aktivátorů
- problém: DNA v jádře, **transkripční faktory a proteinové regulátory** vznikají v cytoplazmě

- pozitivní a negativní regulace

- **pozitivní**: gen může být exprimován, pokud dostane určitý **pozitivní signál-aktivátor**
- **negativní**: exprese genu je tlumena **represorem** a může být zahájena jen po jeho odstranění - závislém na přijetí signálu
- pozitivní i negativní regulace je tak závislá na malé molekule - **induktoru**, který se váže k regulačnímu proteinu
- signálů ovlivňujících expresi určitého genu může být větší počet
- společné pro prokaryota i eukaryota

Regulatory levels

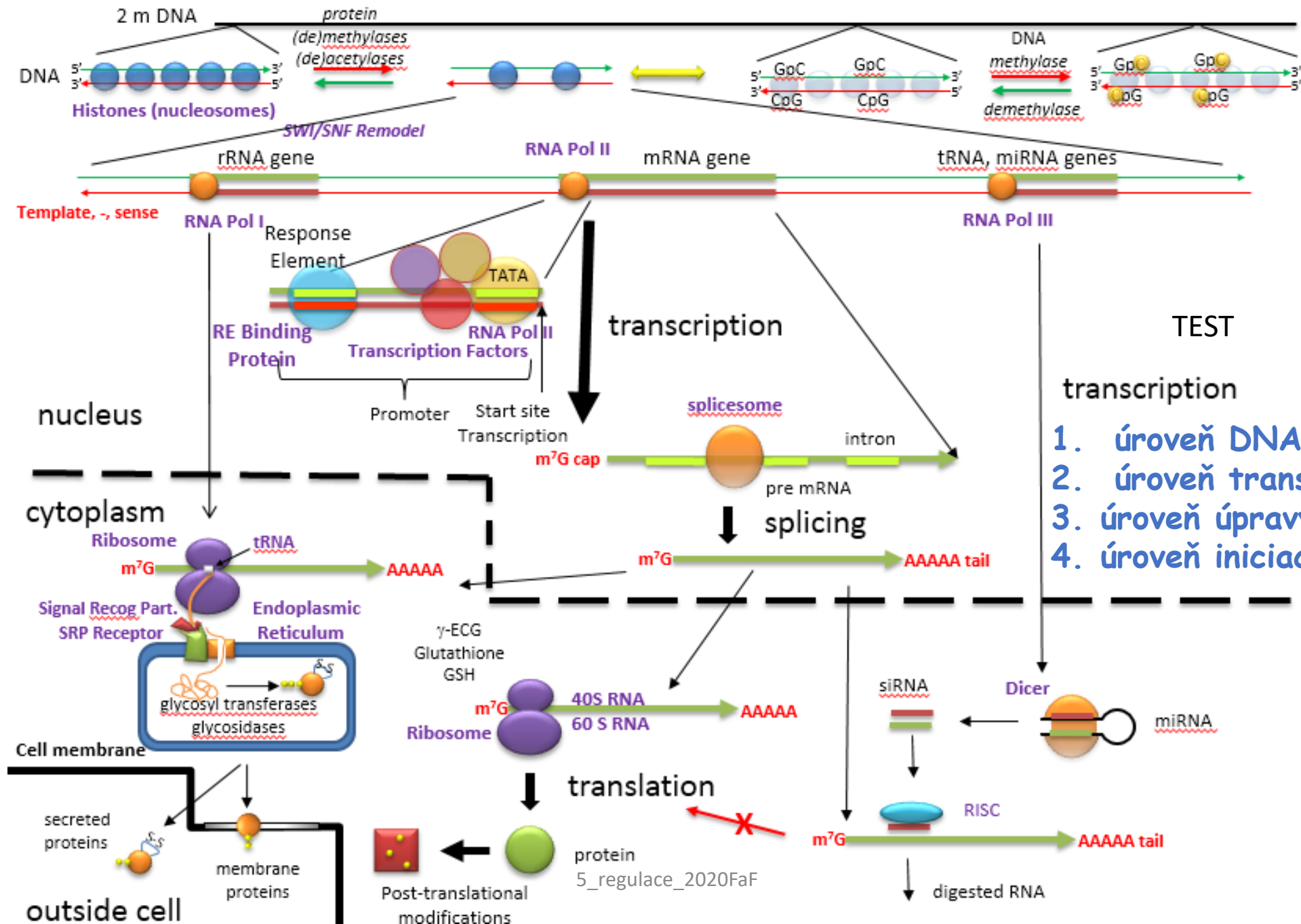


SUMMARY TABLE 18.1 Regulating Gene Expression in Bacteria and Eukaryotes

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



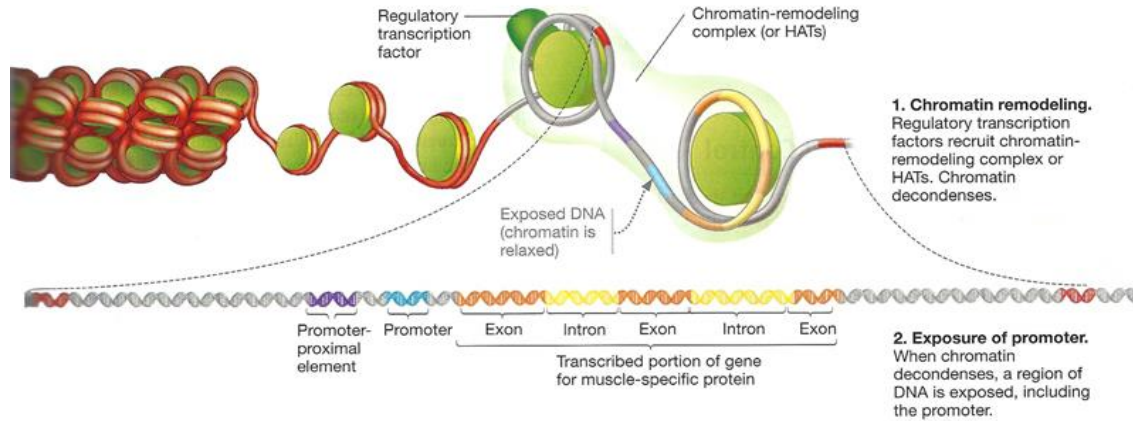
- TEST
1. úroveň DNA a chromozomů
 2. úroveň transkripce
 3. úroveň úpravy transkriptů
 4. úroveň iniciace translace

https://employees.csbsju.edu/h.jakubowski/classes/ch331/bind/olbindtranscription.html

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

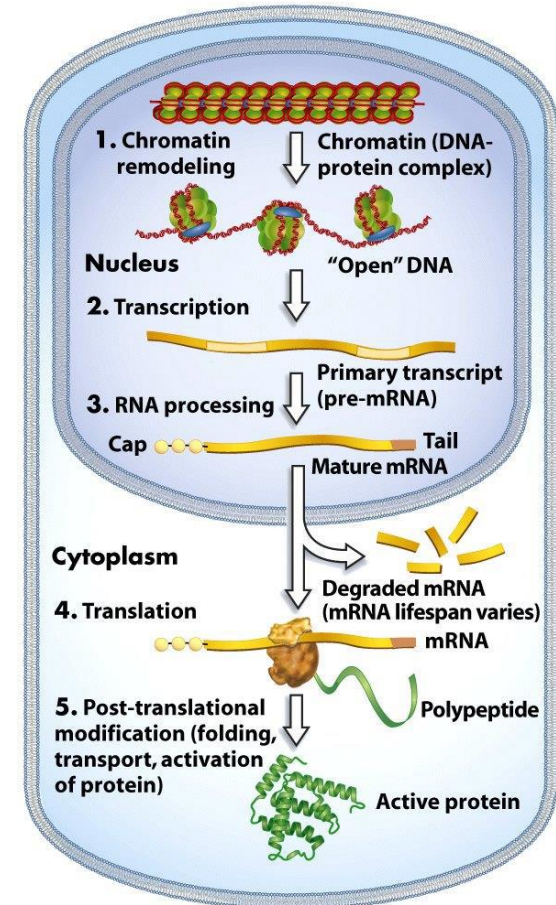
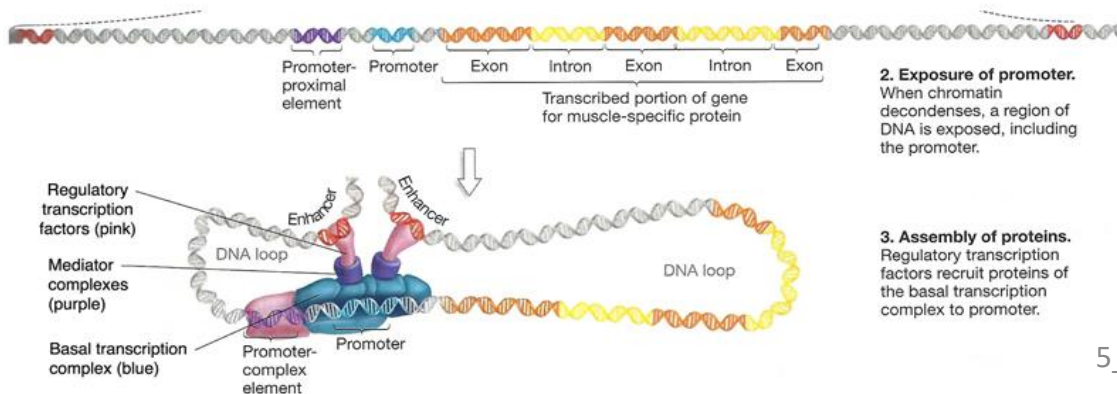


Figure 18-1 Biological Science, 2/e © 2005 Pearson Prentice Hall, Inc.

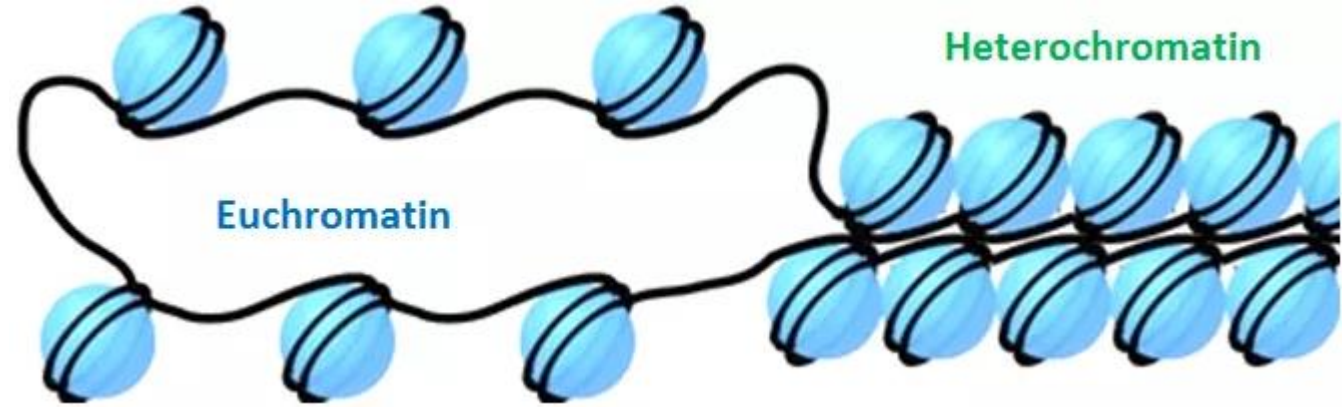
Regulation of gene transcription availability - chromatin

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

Chromatine in nucleus:

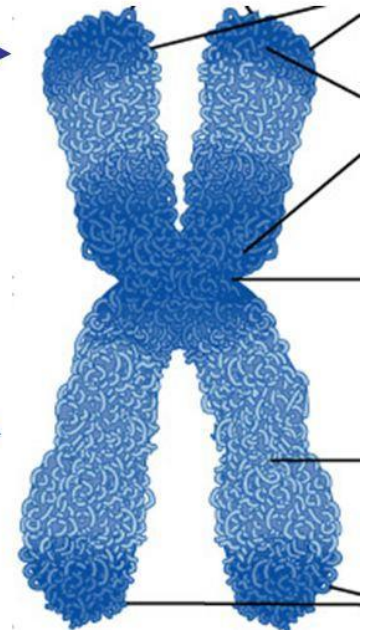
- Condensed (heterochromatin) - genes are inactive
- Diffuse (euchromatin) genes produce mRNA

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

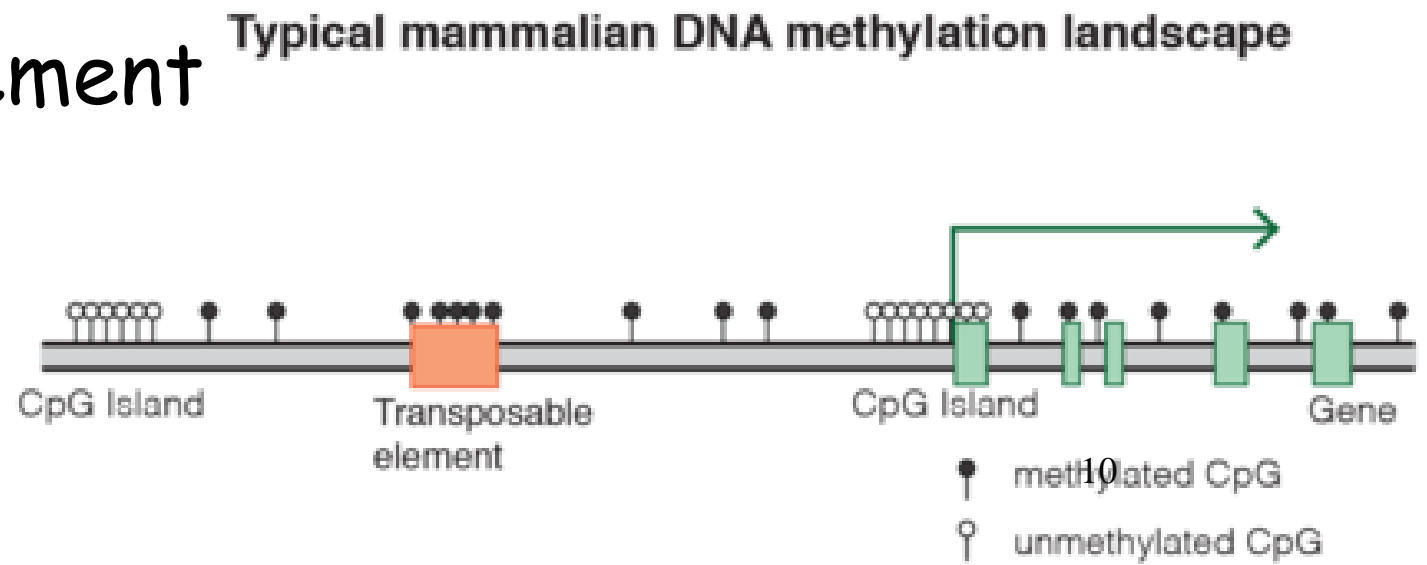


Influencing gene expression at the level of chromosome structure

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

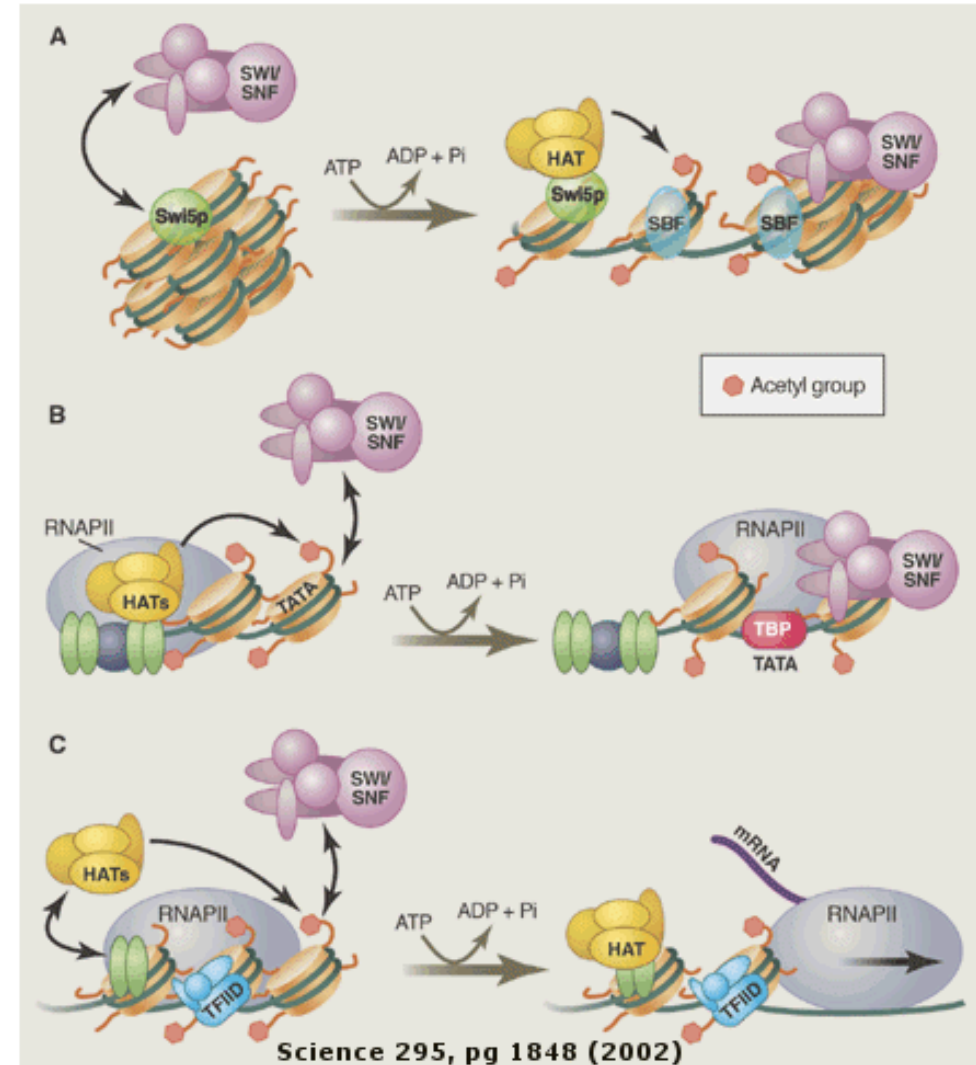
Epigenetic modifications

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



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 ϵ -amino group in the lysine side chain at the N-termini of histones H2A, H2B, H3 and H4).



(Fry and Peterson)

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Regulators changes acetylation of histones

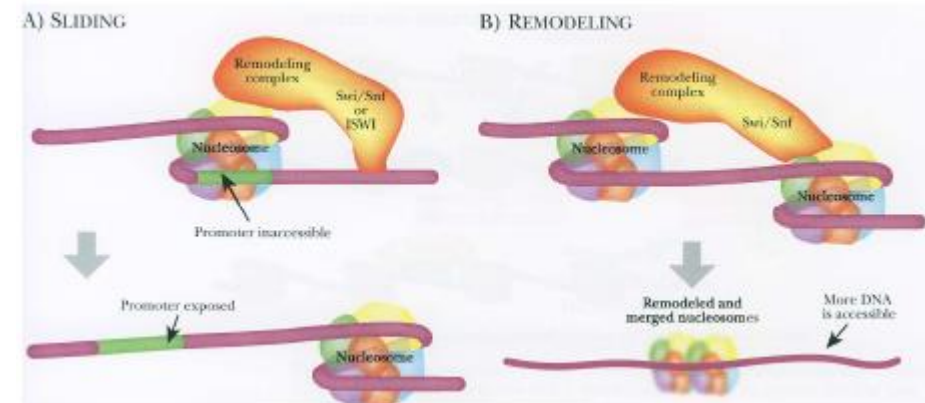
- coactivators of transcription are HAT (histone acetyltransferases) eg CBP / p300 proteins
- transcription corepressors are HDAC (histone deacetylase)
- coactivators and corepressors do not bind to DNA, but interact with transcription factors

Activation of eukaryotic transcription gene - sequence of events

- - 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

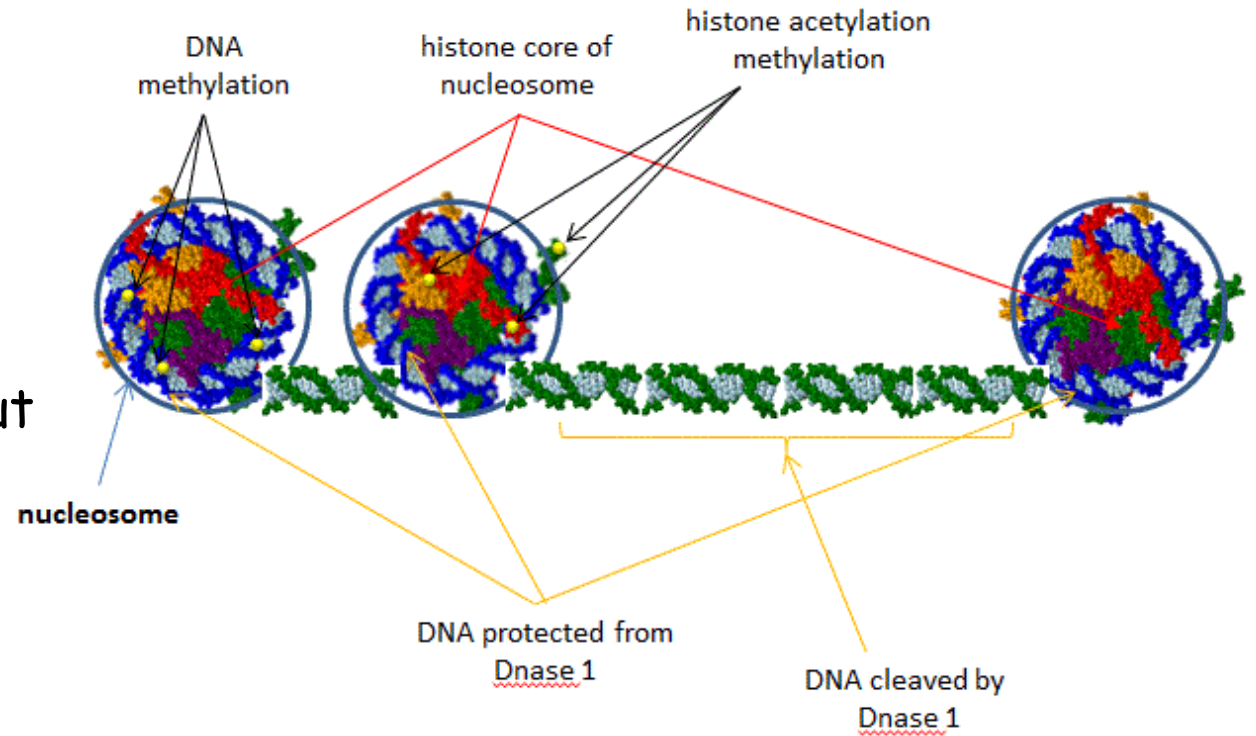
they complement nucleosome changes resulting from histone acetylation
they move nucleosomes across



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 \rightarrow 5-methylcytosine

- catalyzed by methyltransferase

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

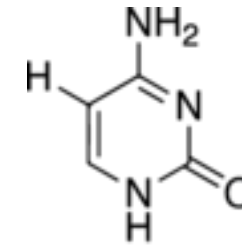
- Methylation of DNA weakens gene expression

- constitutive operational genes do not have GC methylated islands

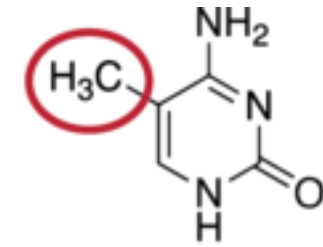
- Tissue-specific genes do not have GC islands methylated only if their products are needed in that tissue

- 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

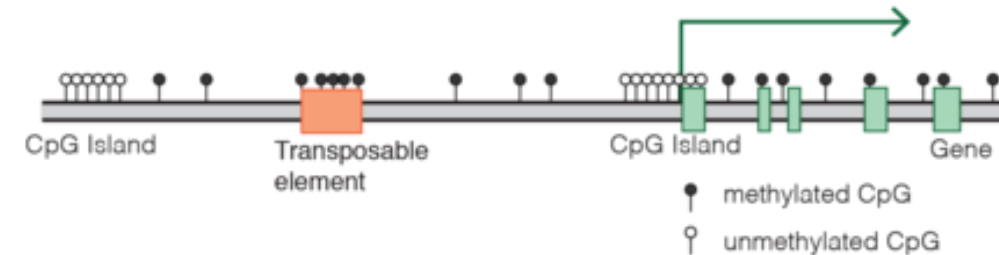


Cytosine



methylated Cytosine

Typical mammalian DNA methylation landscape



Methylace DNA

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>

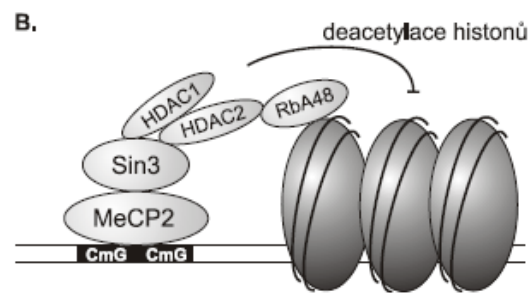
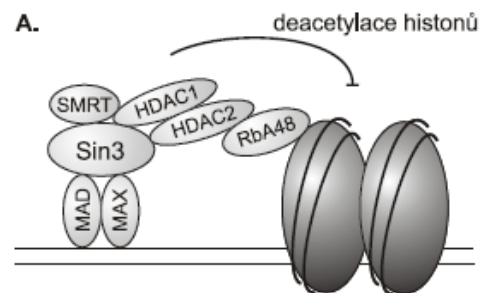
Metylace DNA a deacetylace histonů

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



Přechodná represe

stabilní represe

Prof. Šmardová – přednáška epigenetika



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

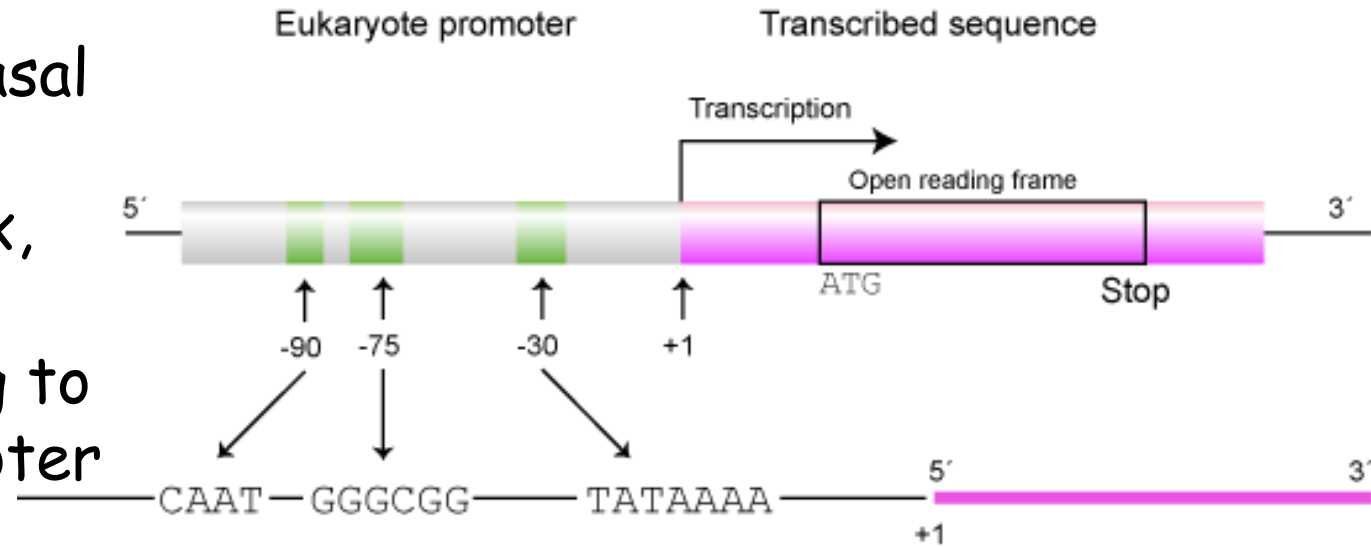
Ex .: Gene rearrangement in antibody-producing cells (immunoglobulins)

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)



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Genes regulated only in this way:

Constitutively expressed genes

Specific effects on gene expression:

Through regulatory sequences in DNA and specific transcription factors.

Binding of basal transcription factors