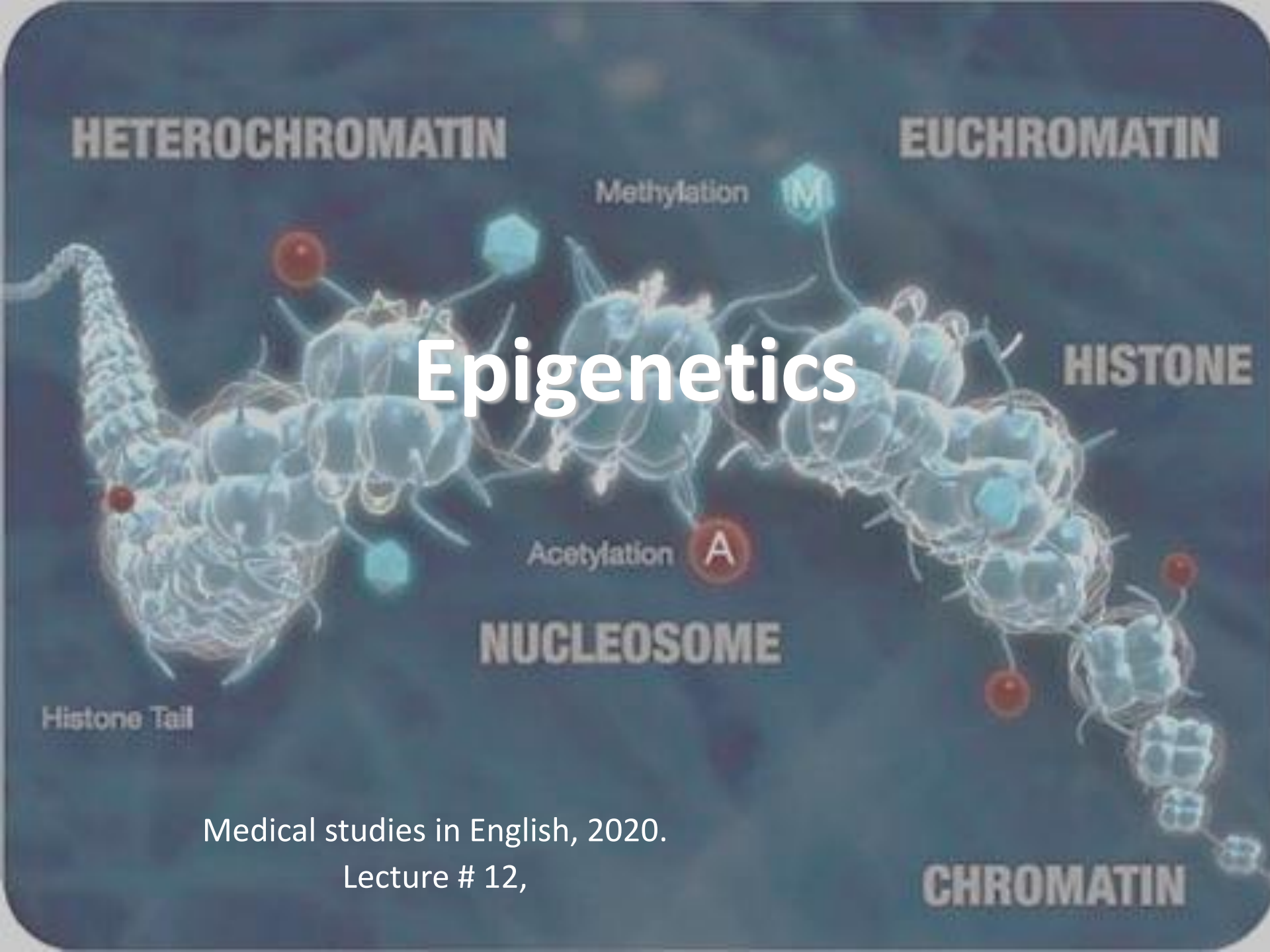


Epigenetics

Medical studies in English, 2020.

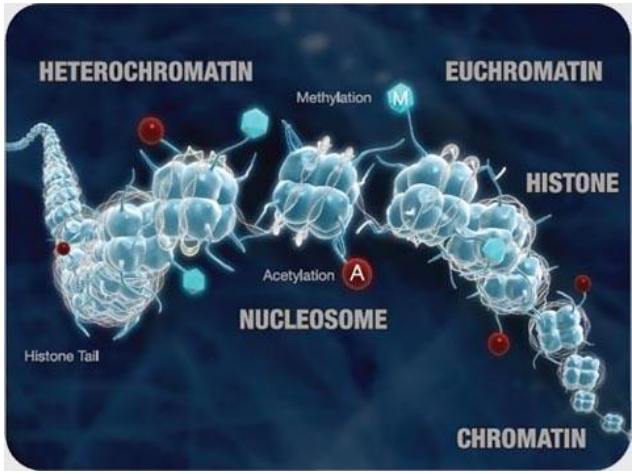
Lecture # 12,

Epigenetics

- Regulation of gene activity in eukaryotes
- Correlation of chromatin structure with transcription

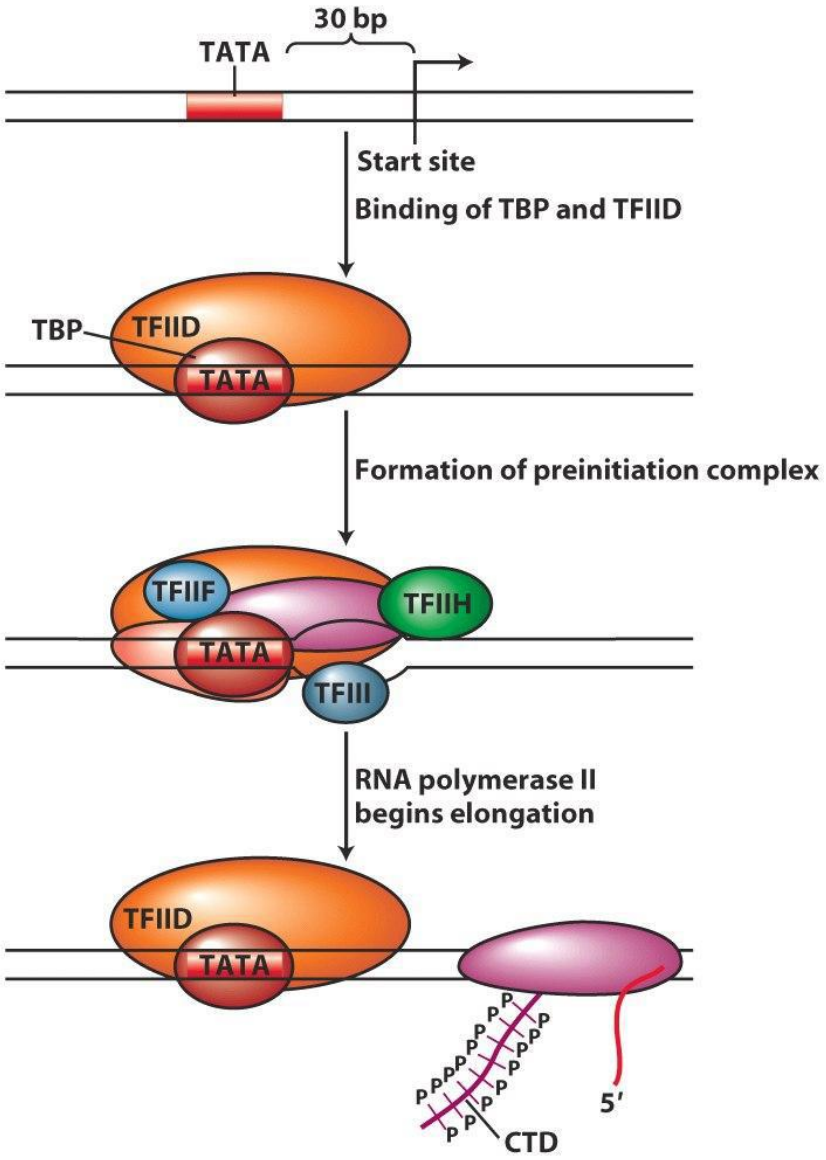
- Molecular mechanisms of epigenetics
  - DNA methylation
  - Histone modification – „Histone code”
  - RNA interference

- Influence of epigenetics in humans
  - Twins
  - Barr body
  - Genomic imprinting



Regulation of gene activity in eukaryotes

- gene expression is controlled primarily on the level of transcription

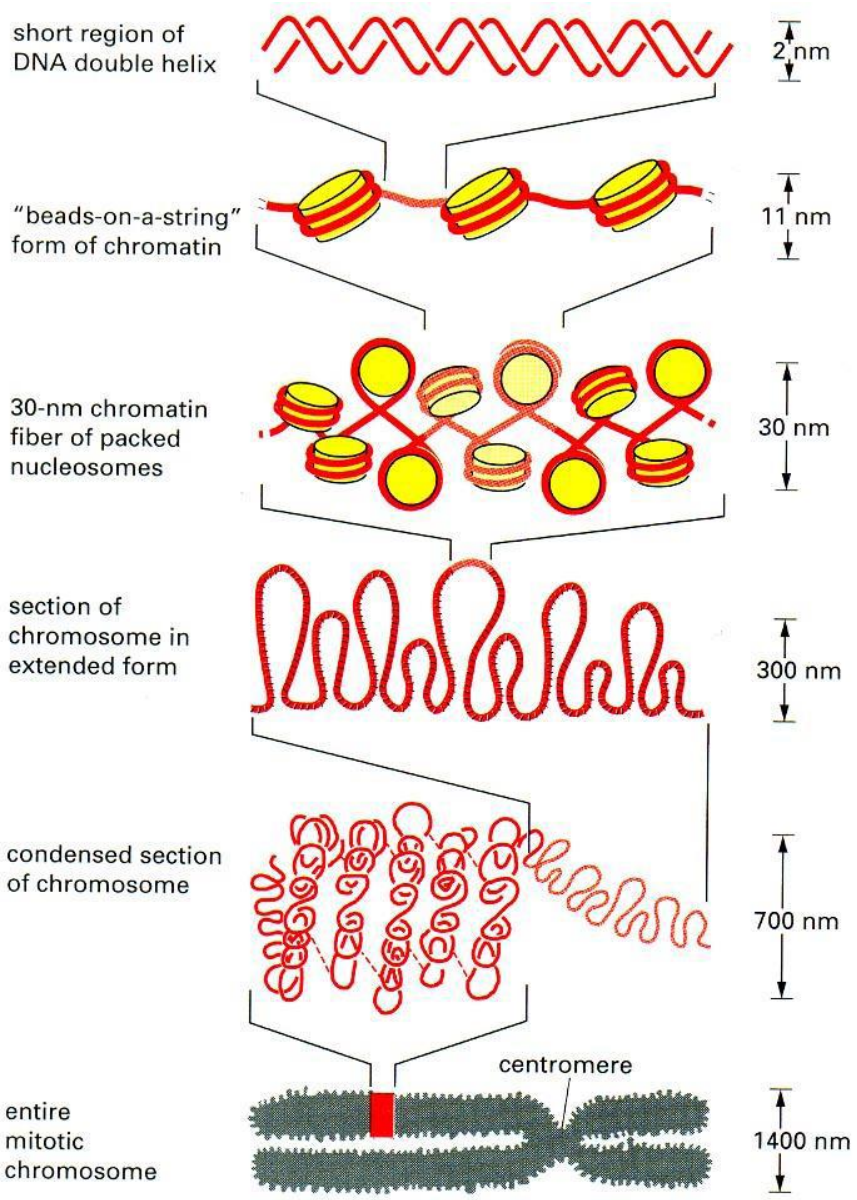


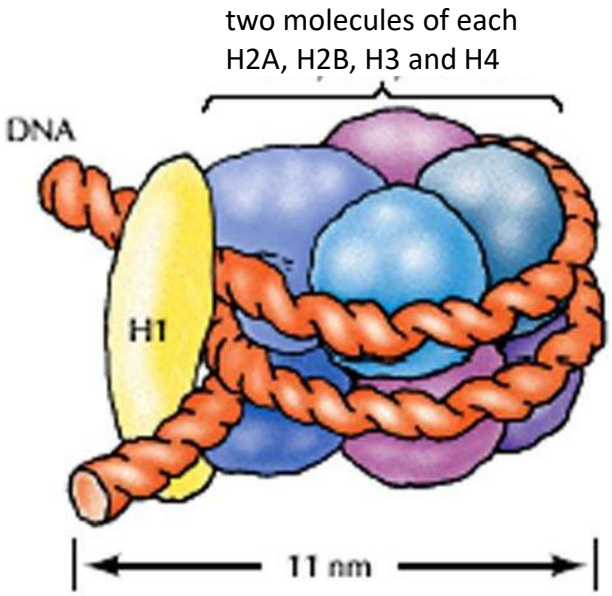
TBP – TATA binding protein
TFIID – transcription factor 2 D

Regulation of gene activity in eukaryotes

- Specificity of gene regulation in eukaryotes:
  - several RNA polymerases (only one in prokaryote)
  - Polymerase needs interaction with transcription factors, activators and repressors to interact with promoters
  - DNA is packed into chromatin – reduced accessibility of DNA for transcription
- Chromatin modification – key role in gene transcription control

Chromatin structure

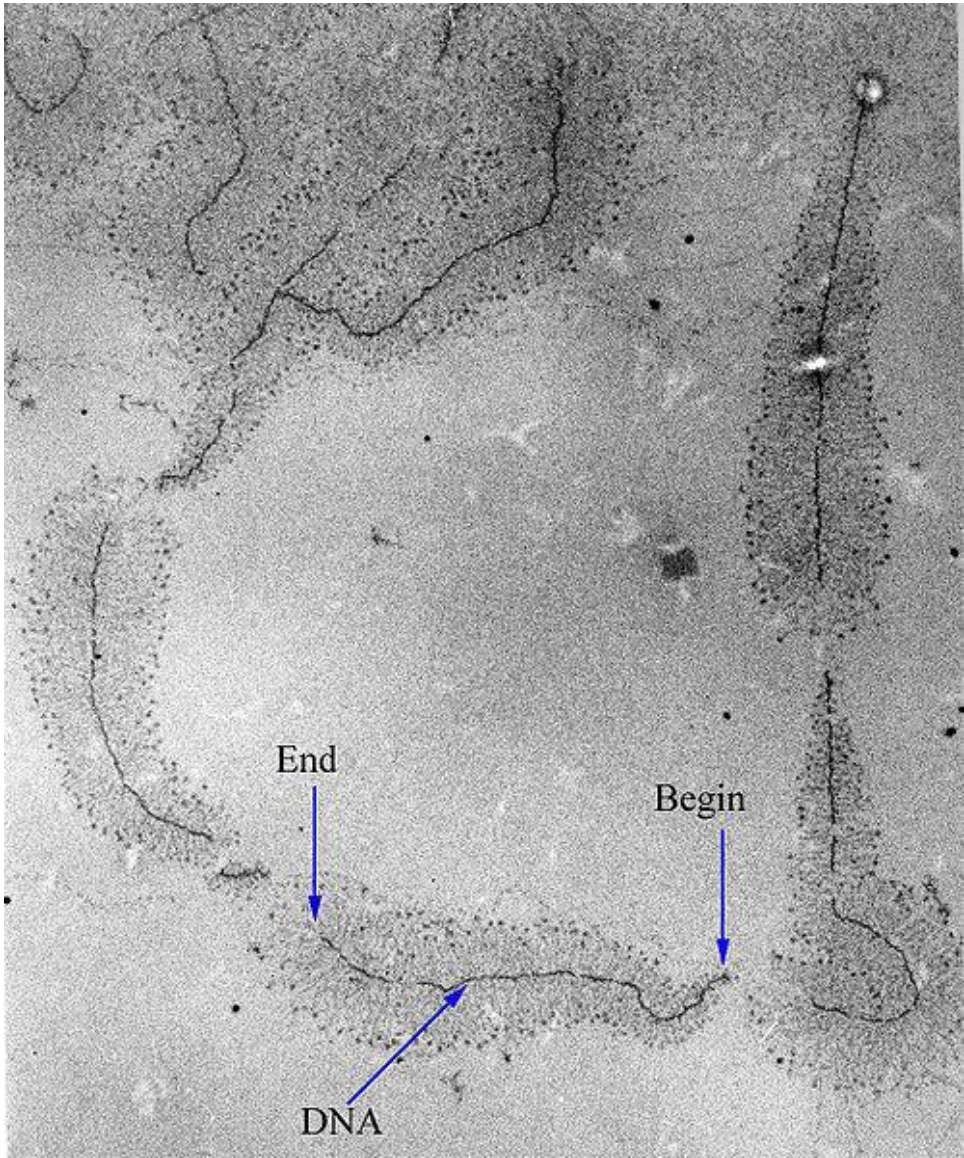




- Nucleosom – basic structural unit of cromatin
- 146 bp of DNA wraped arround octamer histone core
- Histons 2x(H2A, H2B, H3, H4) + H1 on the outer side
- 60 – 80 bp „linker" DNA

Chromatin structure and transcription

- Transcription happens in interphase
- Only euchromatin is transcribed (not heterochromatin)
- Multiple mRNA chains form simoultaneously



Epigenetics

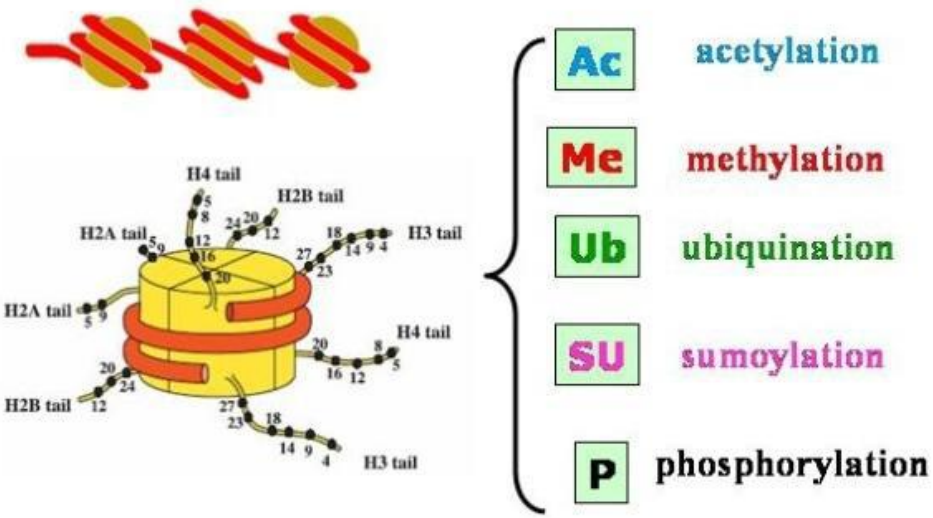
- *Epi* (Greek) – „on top of" – additional genetic information
- **Epigenetics** – the study of heritable changes in gene expression and phenotype, caused by mechanisms other than changes in the DNA sequence

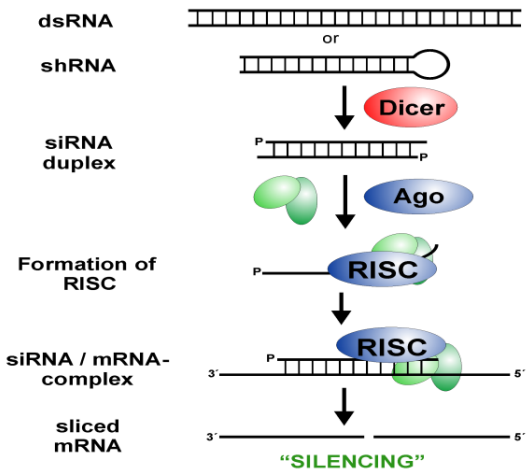
- Studies the environmental influence on genetic structure, and transgeneration transfer of that influence

- Estimate is that 20% of genes is under influence of epigenetic modifications

Molecular mechanisms of epigenetics

1. **DNA methylation** –
   methyl group addition
2. **Histone modification** – („Histone code") addition of different molecules on histons – activation or suppression
3. **RNA interference** – transcription regulation via noncoding RNA molecules

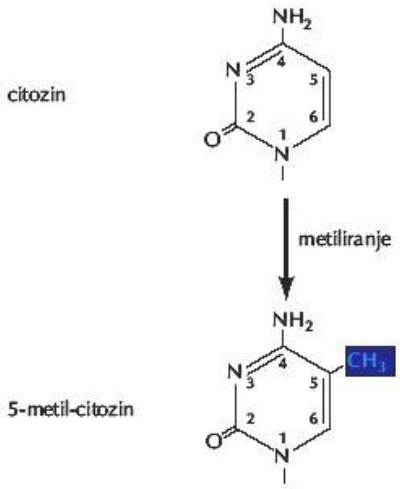


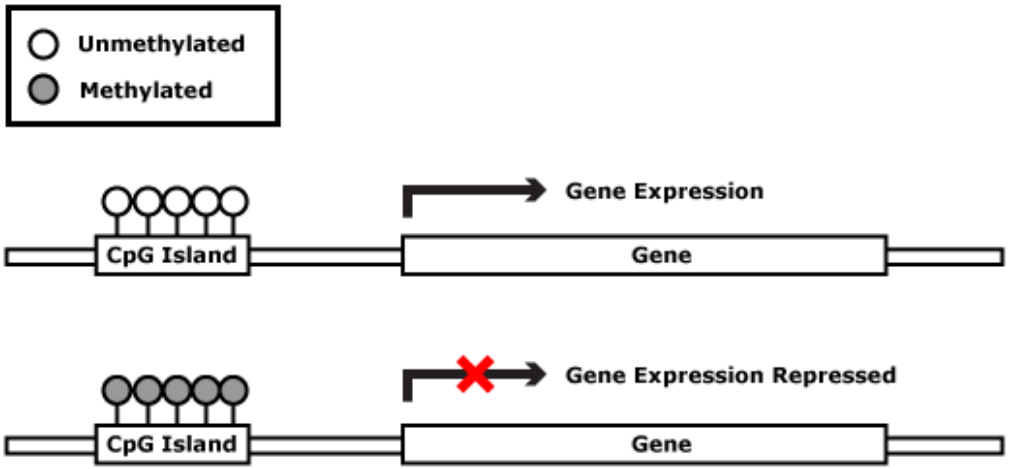


modification of chromatin - tutorial

1. DNA Methylation

- General mechanism of relating chromatin structure to transcription
- Cytosines in proximity to guanines are methylated (CpG islands)
- Addition of methyl groups in promoter region causes transcription reduction

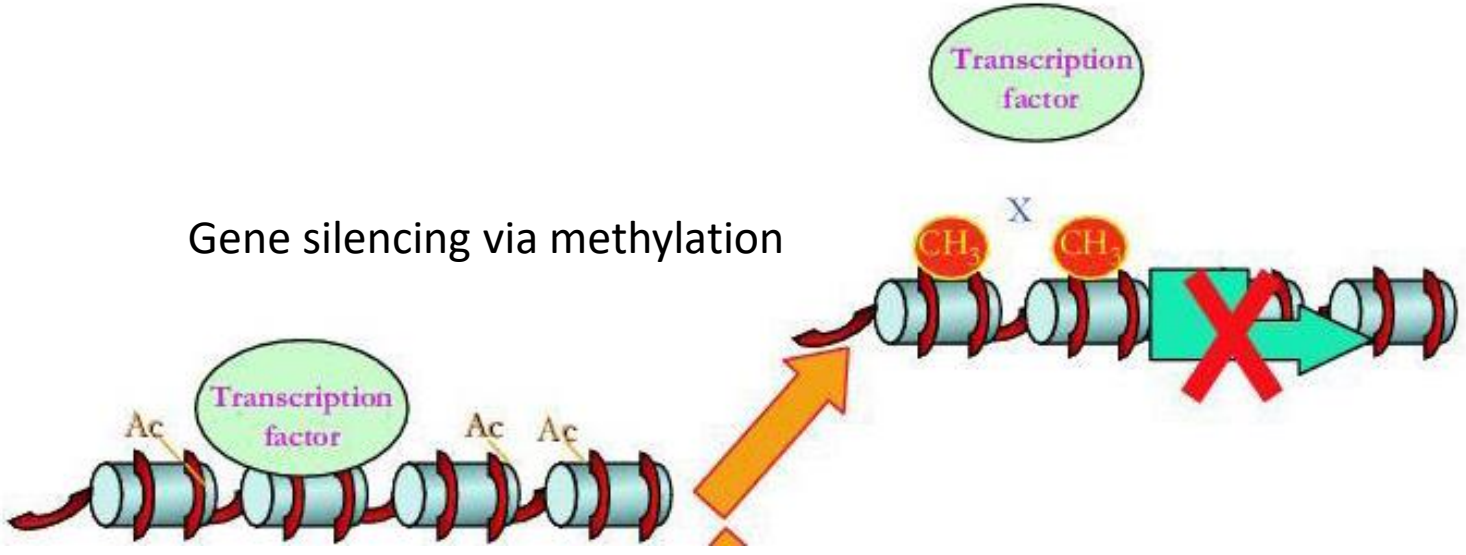


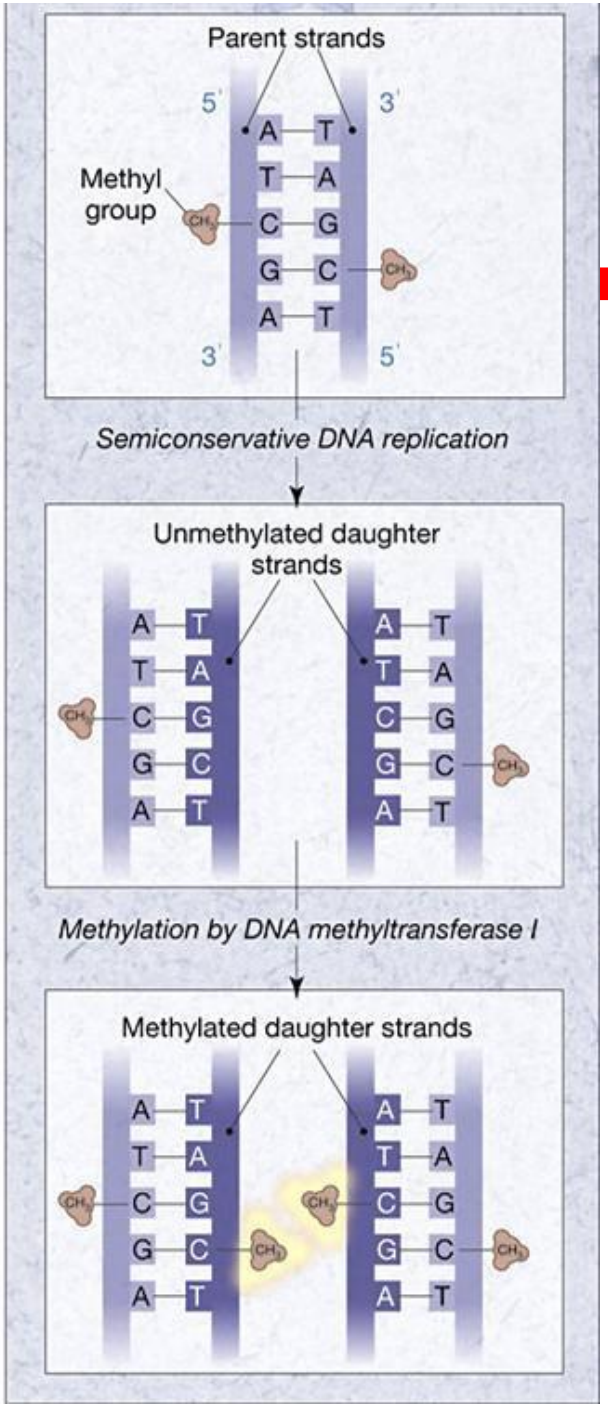


DNA methylation

- Methylated CpG is target for transcription repressors – transcription repression and cromatine inactivation
- Constantly active genes do not have methylated CpG dinucleotides in promoter region
- Methylation does not interfere with replication

Gene silencing via methylation





DNA methylation happens after replication

- Methyltransferase I – active on hemimethylated DNA – maintains the methylation patter through cell divisions
- Methylation pattern is transferred to daughter cells
- Other methyltransferases can perform methylation „de novo”

2. ”Histone code” – covalent histone modifications

- Histones have:
  - Core, on which the DNA is wrapped around
  - Tails stick out the nucleosome and are rich with lysines (K)

- Tails can be covalently modified with different groups, which makes them active (decondensed) or inactive (condensed)
- Combination of such modification is called „Histone code” – regulation of gene expression by association with other different regulatory proteins

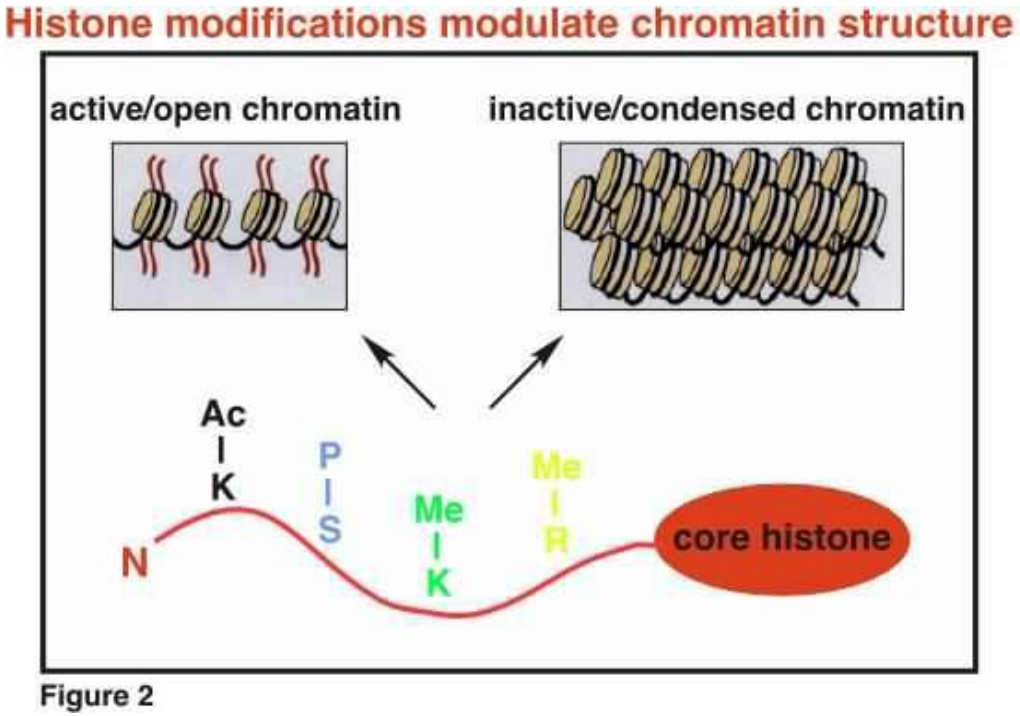
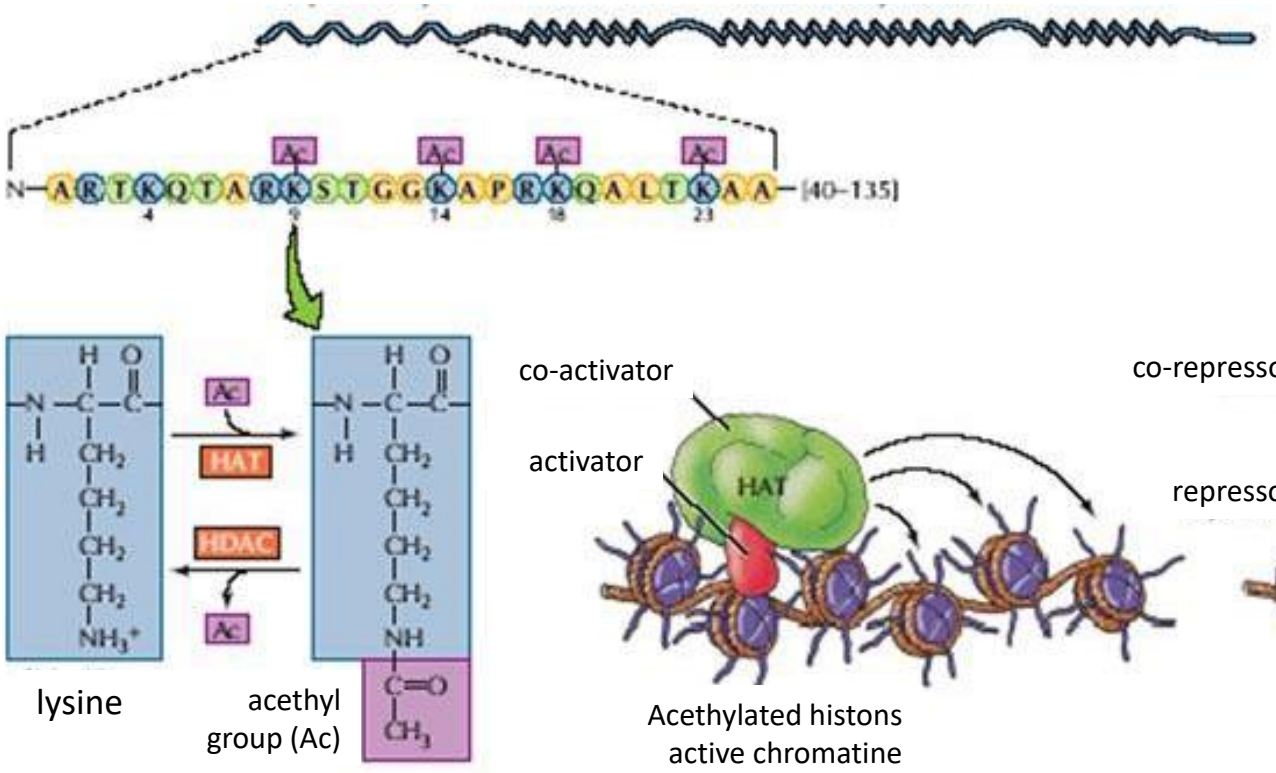


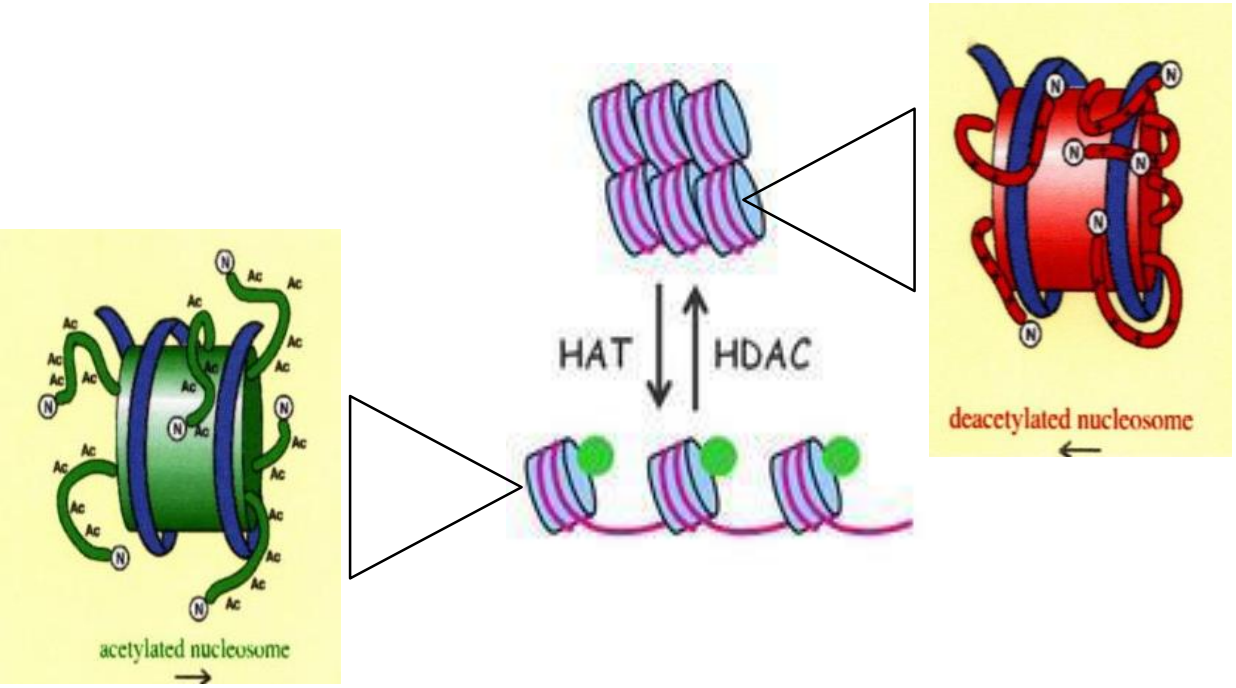
Figure 2

”Histone code” – lysine acetylation

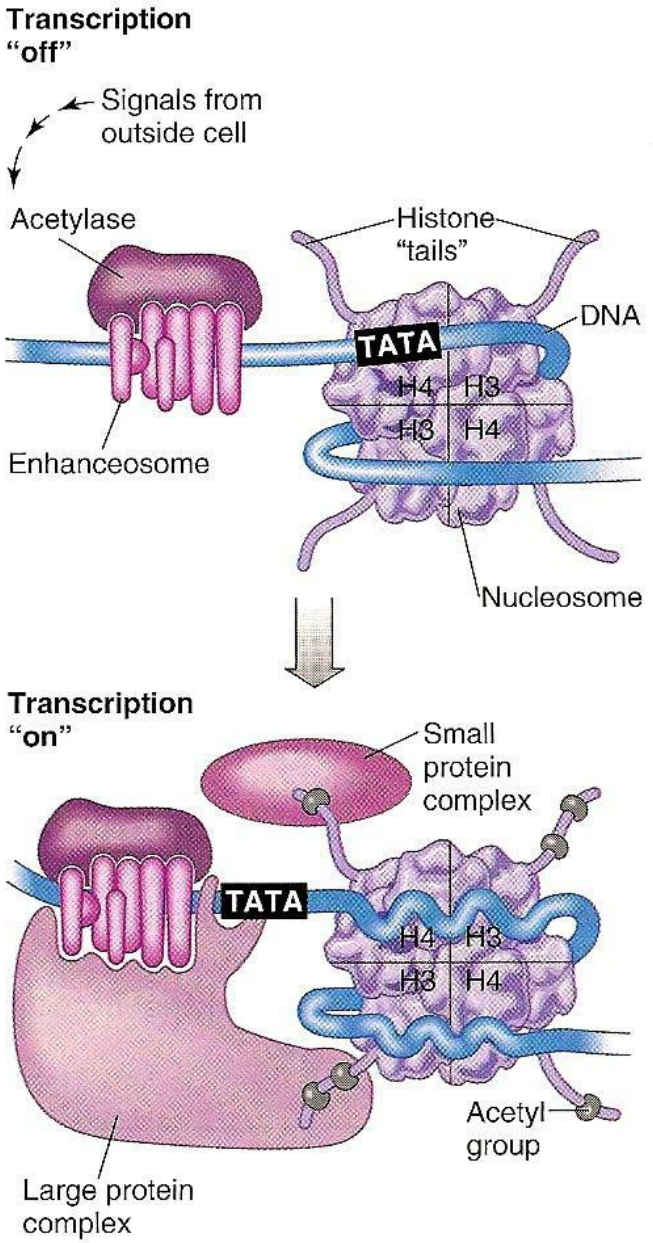
- Lysine acetylation reduces positive charge – reduces DNA binding – typical for active chromatin
- Histone acetyl transferase (HAT) – binds with transcription activators
- Histone deacetylase (HDAC) – binds with transcription repressors



Chromatin remodeling by histone acetylation

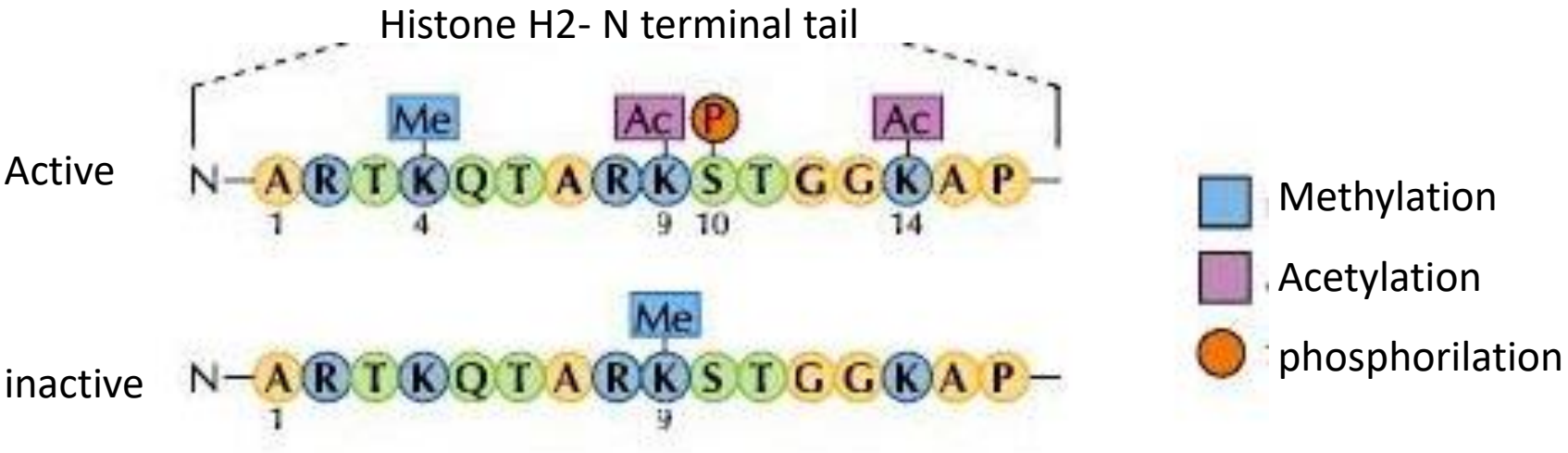


- Histone acetylation – chromatin remodeling – promoter TATA sequence becomes accessible to transcription factors

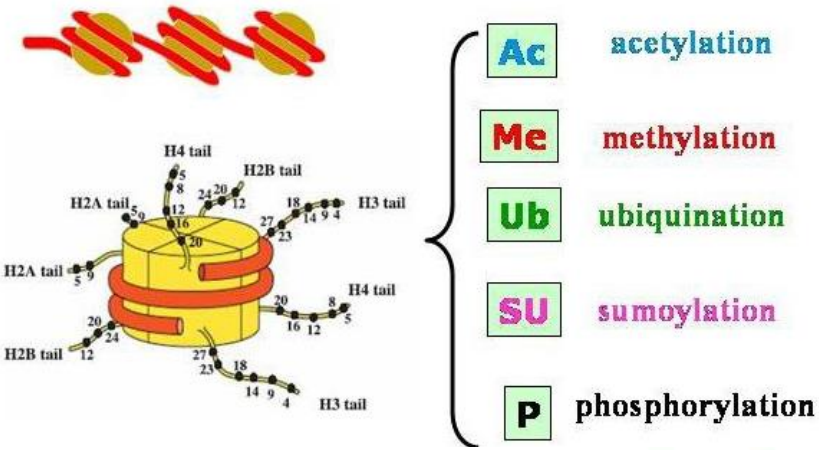


Other covalente histon modifications

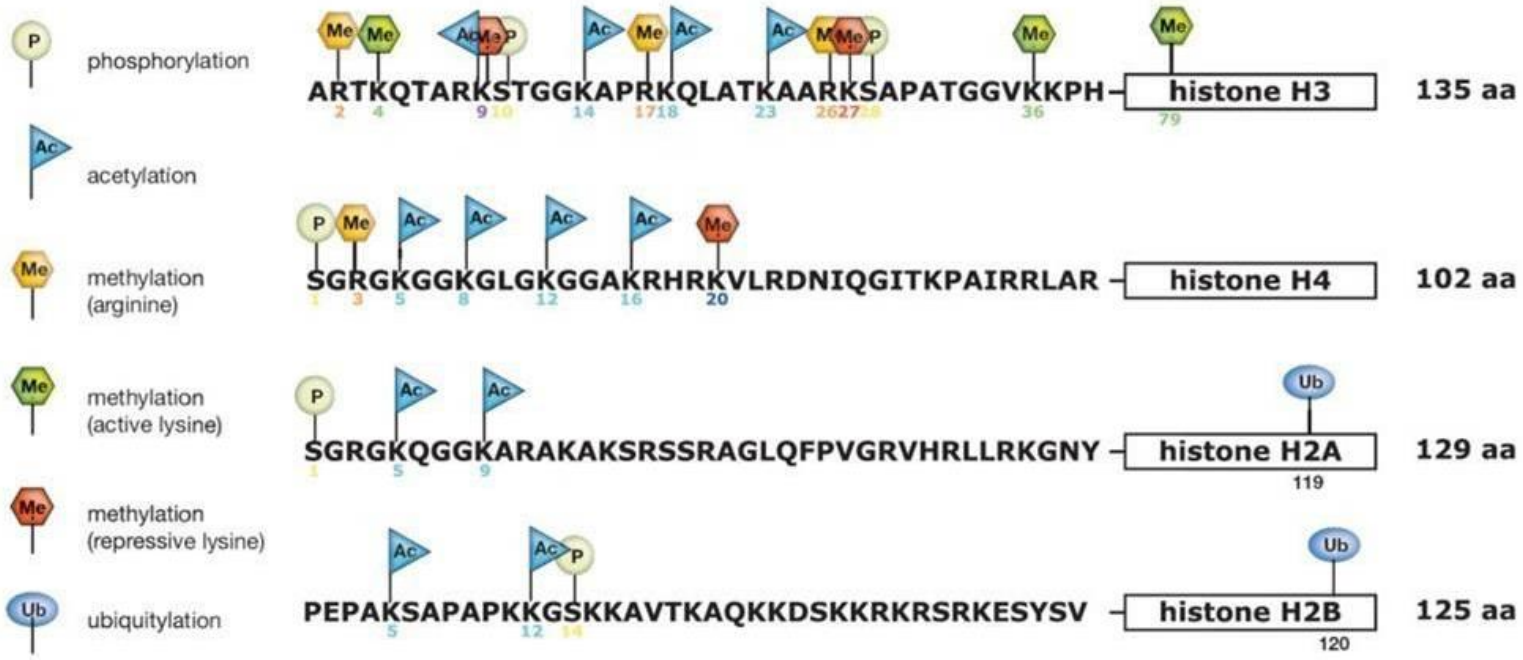
- Asside of lysine (K) acetylation, there are:
  - Serine (S) phosphorylation
  - Lysine (K) and arginine (R) methylation
  - Lysine (K) ubiquitilation



Gene expression depends on epigenetics

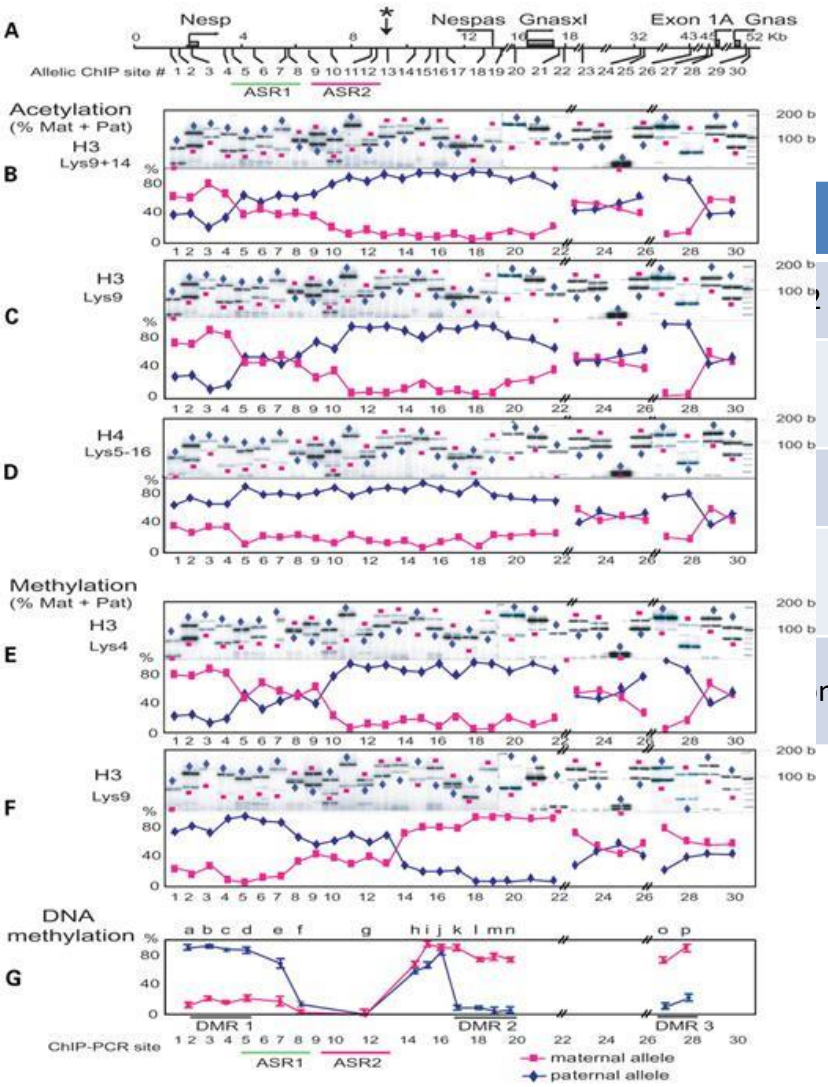


Histone tails modification

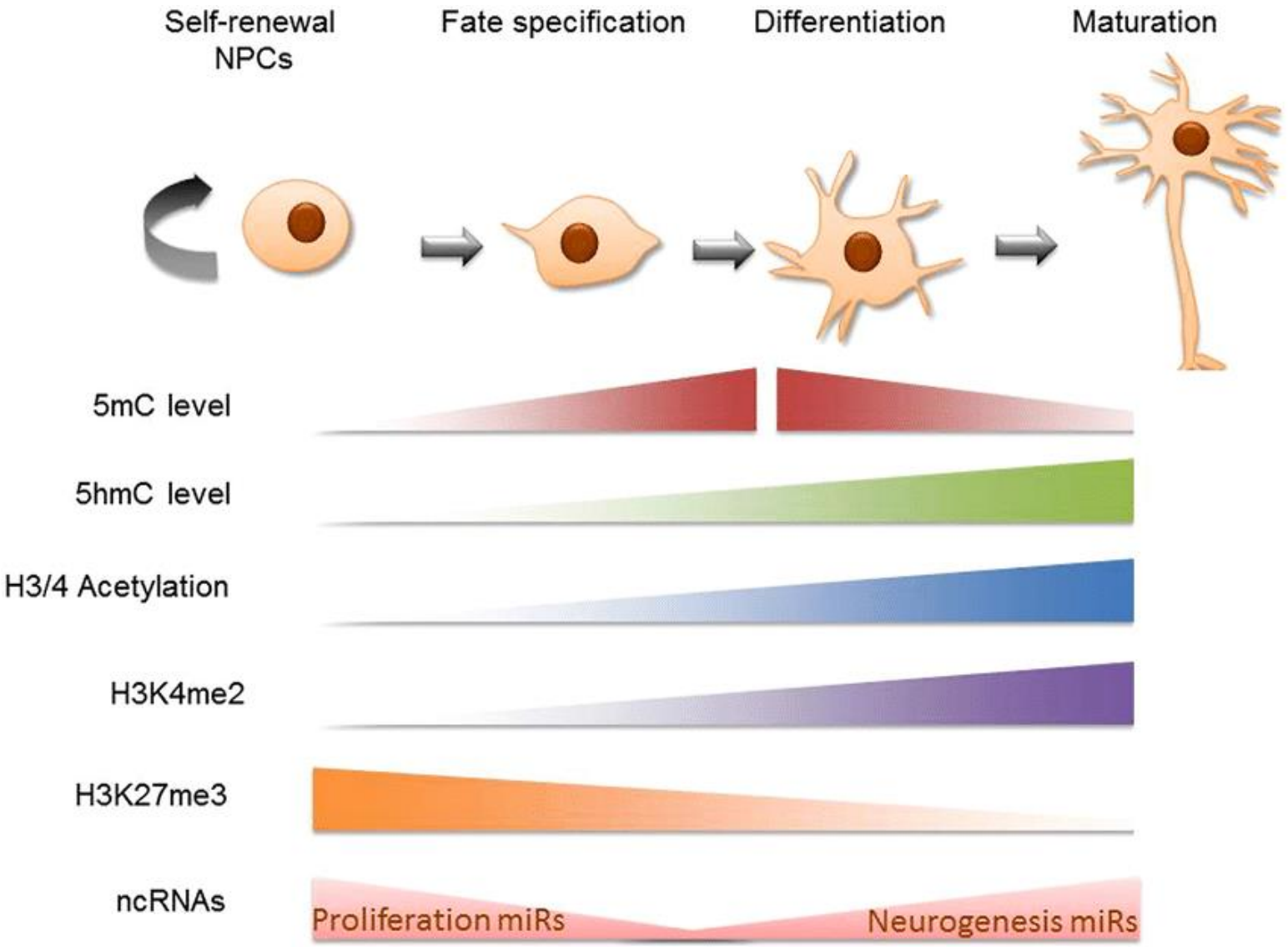


Examples of epigenetic effects

Type of modification						
	H3K4				H4K20	H2BK5
mono-methylation	activation	ac			activation	activation
di-methylation	activation	rep				
tri-methylation	activation	rep				repression
acetylation		ac		n		

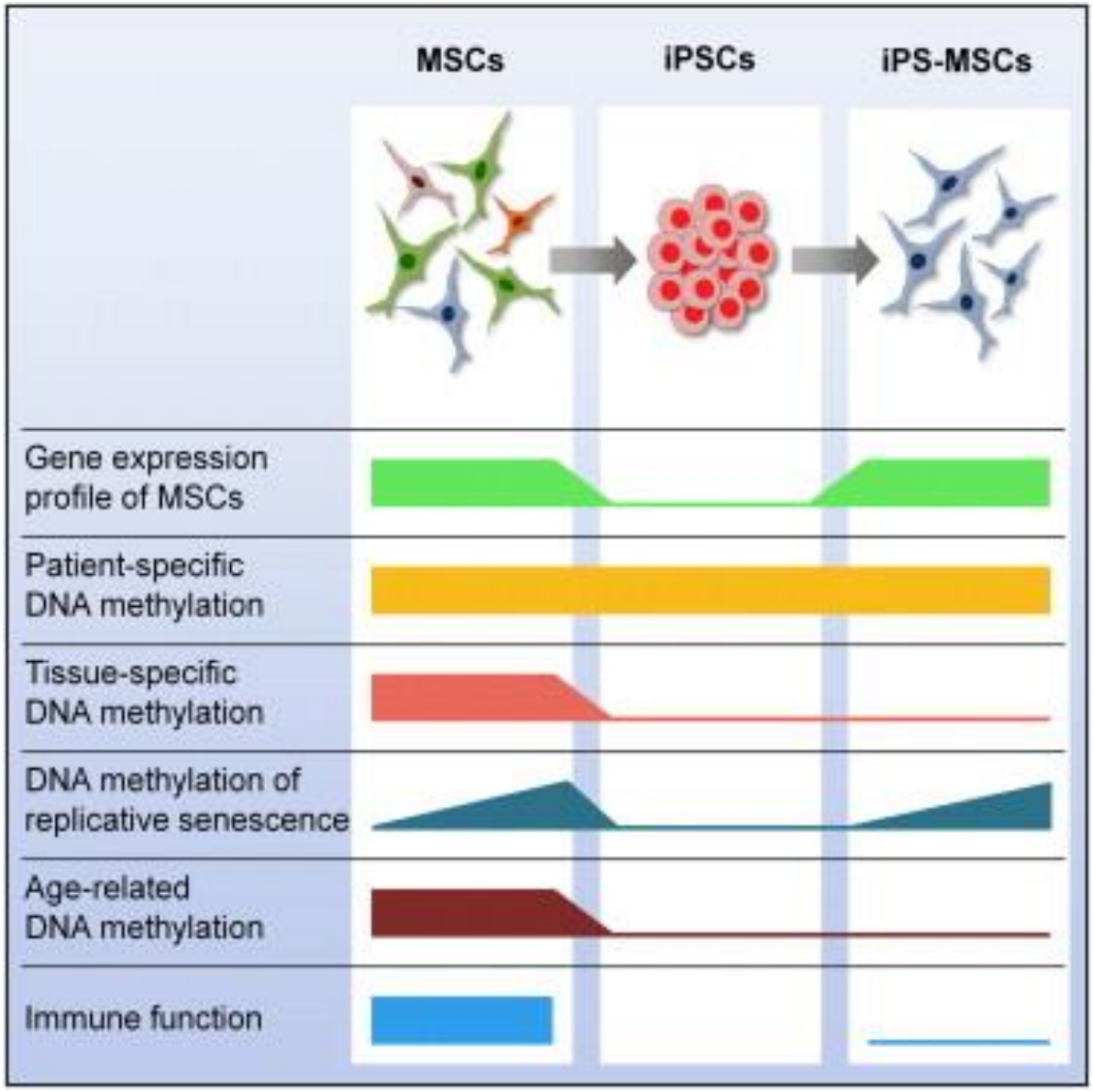


Epigenetics and cell differentiation



DOI: 10.3389/fgene.2014.00285

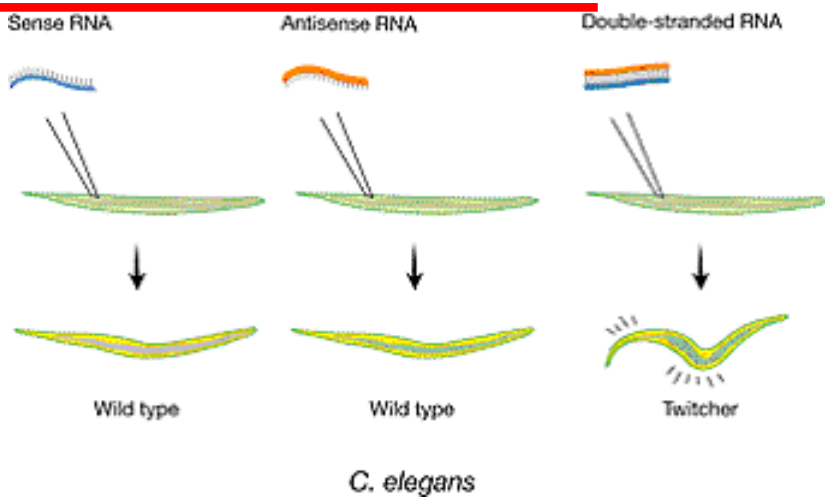
Epigenetics and Induced pluripotent stem cells



https://doi.org/10.1016/j.stemcr.2014.07.003

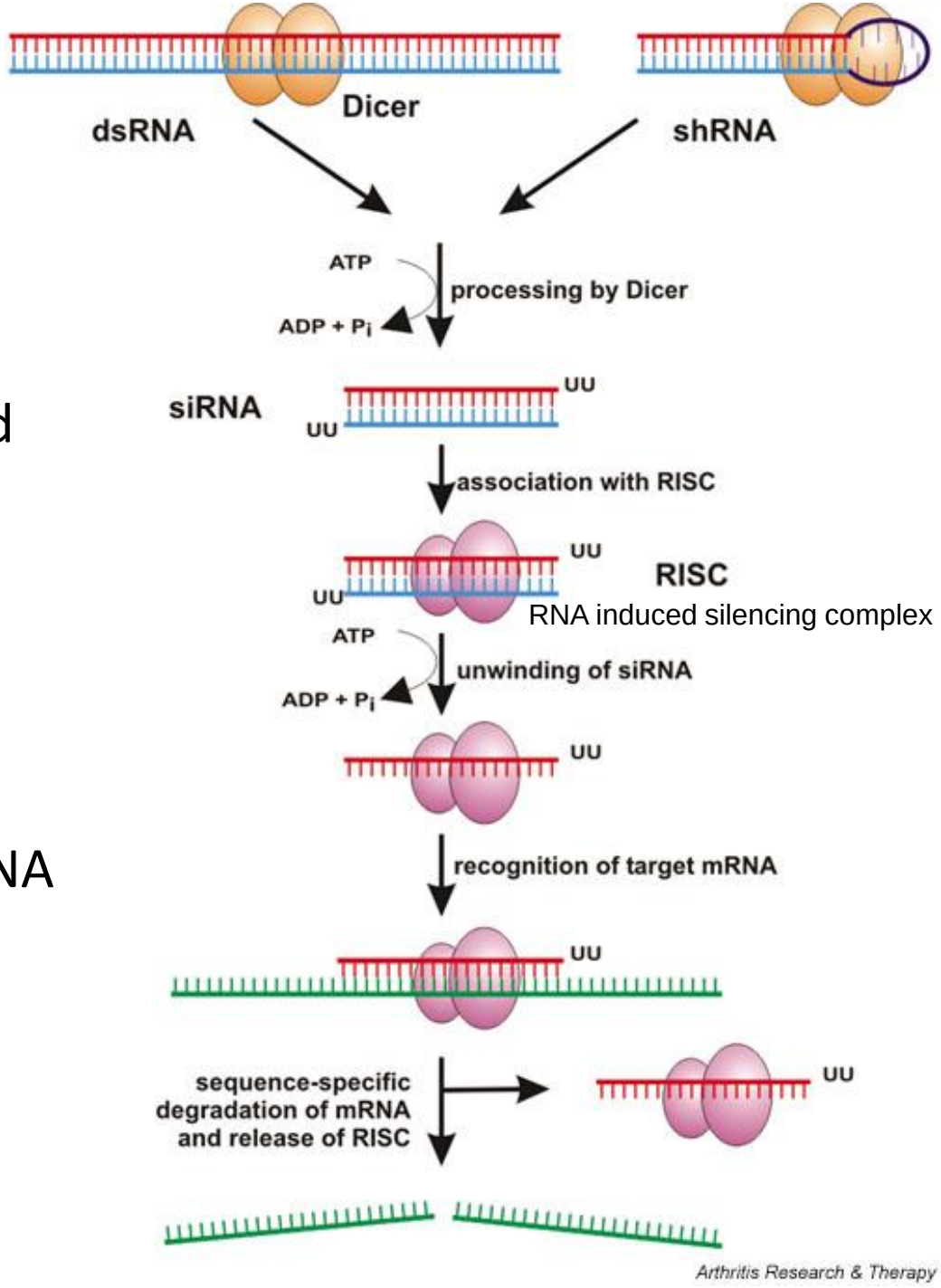
3. RNA interference

- RNA interference = posttranscription gene silencing
- Short double stranded RNA (dsRNA) cause degradation of homologue mRNA – gene silencing
- Andrew Fire/Craig Mello – 2006. Nobel price
- Where does dsRNA come from?
  - Endogenous – result of both strand transcription (miRNA, shRNA)
  - Exogenous – viral dsRNA, synthetic (shRNA ili siRNA)



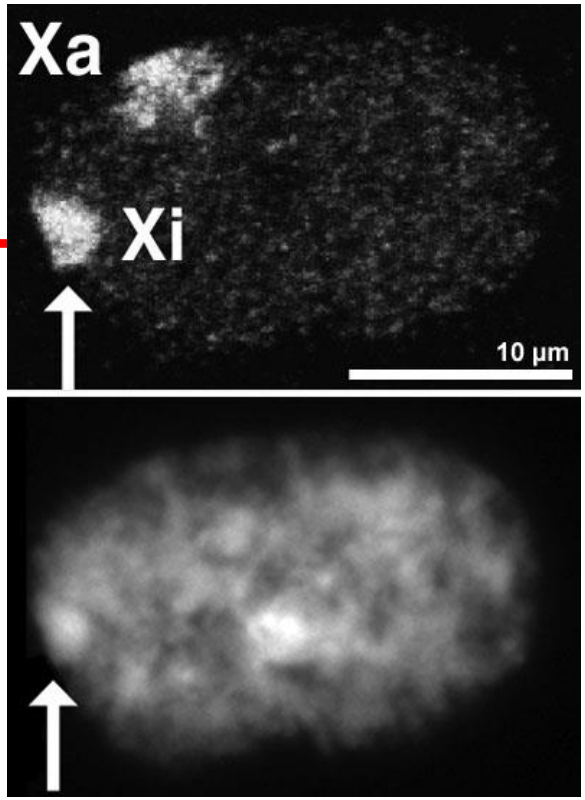
Miofilament gene unc22 in C. elegans can be knocked down by injection of double stranded complement RNA

- DICER cuts double stranded RNA into siRNA
- siRNA binds to RISC
- Single stranded RNA is produced
- ssRNA/RISC bind to homologous mRNA
- RISC ribonuclease cuts mRNA

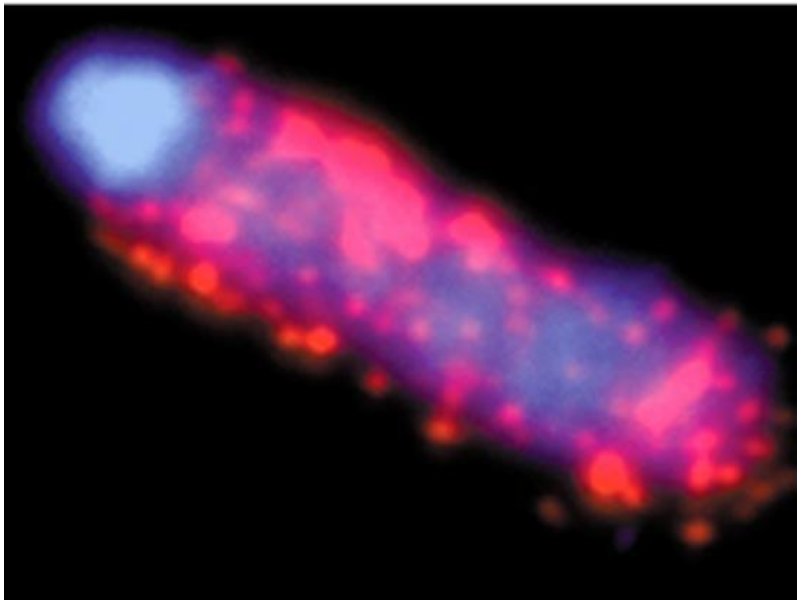


http://www.youtube.com/watch?v=2dL7Sh_udKs

RNA interference – inactivation of X chromosome and Barr body formation

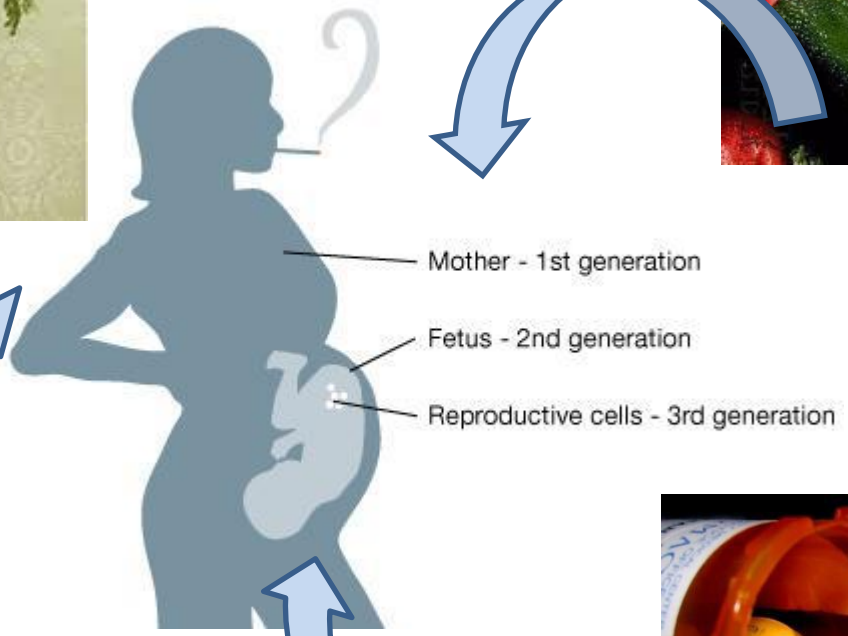


- Example of transcriptional gene silencing
- Noncoding RNA from regulatory Xist gene
- Xist RNA remains on inactivated X chromosome and binds transcription repressor proteins



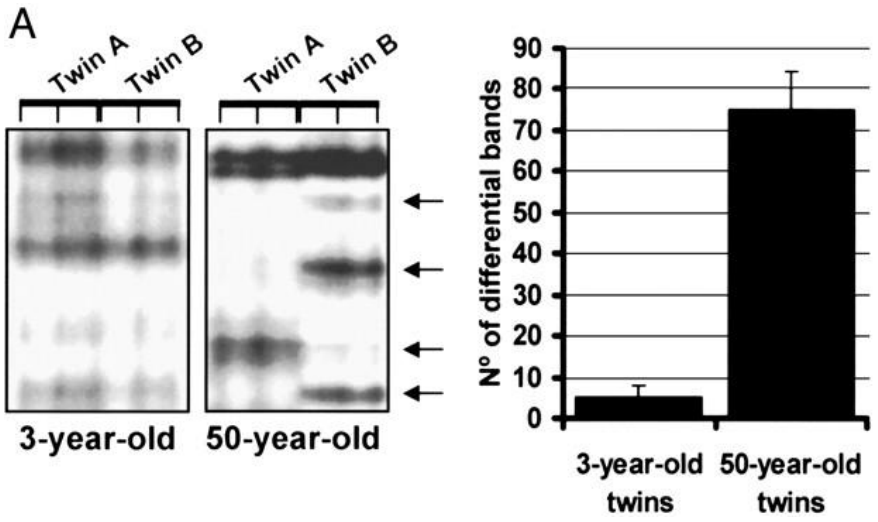
Inactive X chromosome (blue) coated with Xist RNA (red)

Environment and epigenetics



Three generations are influenced by the same environmental conditions at the same time (food, toxins, hormones, pollution)

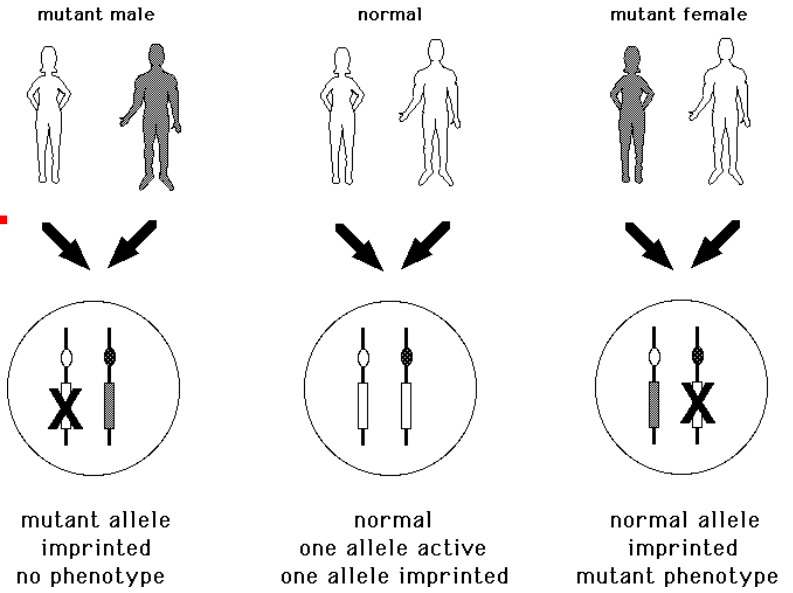
Epigenetics can explain the differences in monozygotic twins

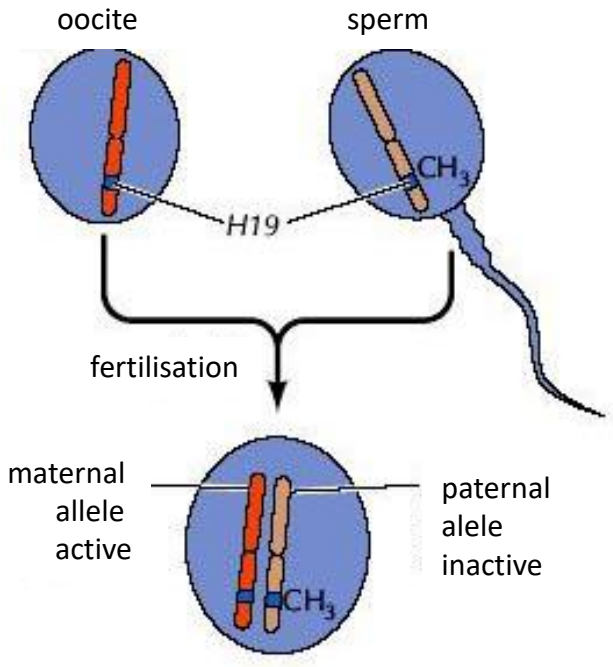


- Monozygotic twins – identical genotype, very similar epigenome during the first years of life
- Older monozygotic twins – substantial differences in methylation and acetylation of chromatin
- Different predisposition to diseases (twin study on 30 pairs of monozygotic twins)
- Environment: food, physical activity, smoking – long term epigenetic effects

Genomic imprinting

- ”parental imprinting”- gene activity depends on the parent it came from
- Genes ”remember” if they came from mother or father
- Aprox. 30 genes in human are inherited by imprinting
- Inactivation mechanism – methylation and histon modifications
- Example: H19 gene (long noncoding RNA) is methylated during spermatogenesis – paternal gen is inactivated





Diseases asociated with genomic imprinting

- Non-Mendelian inheritance
- Disease can develop in:
  - deletion of gene from one parent, or
  - Duplication of gene from one parent, and consequent inactivation (metilation of other parental copy (third copy) – uniparental disomy
- Examples:
  - Angelman (lack of functional maternal gene copy)
  - Prader Willy (lack of functional paternal gene copy)
  - Gene located on 15q12 – UBE3A – only maternal copy is active in the brain