Molecules and mechanisms of epigenetics

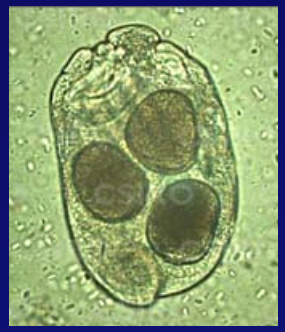
Piotr A. Ziolkowski

Department of Genome Biology

The diversity of life comes from differences in genetic information





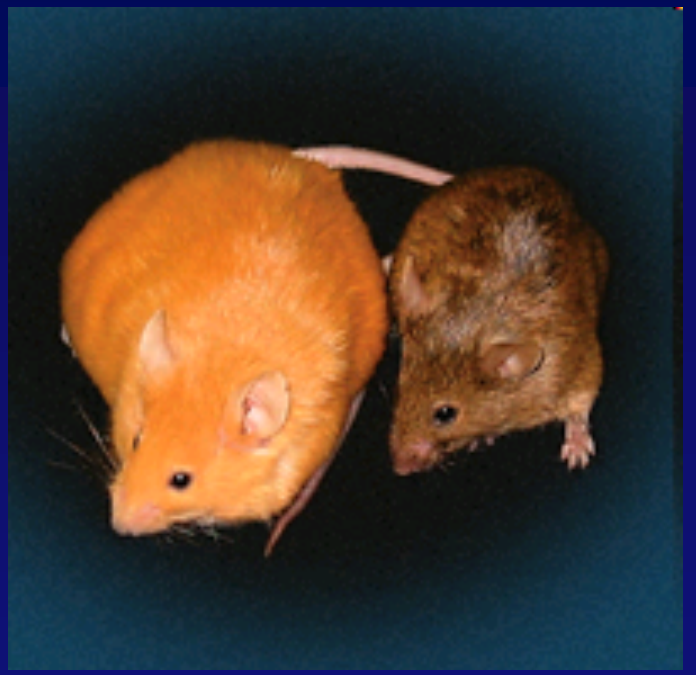





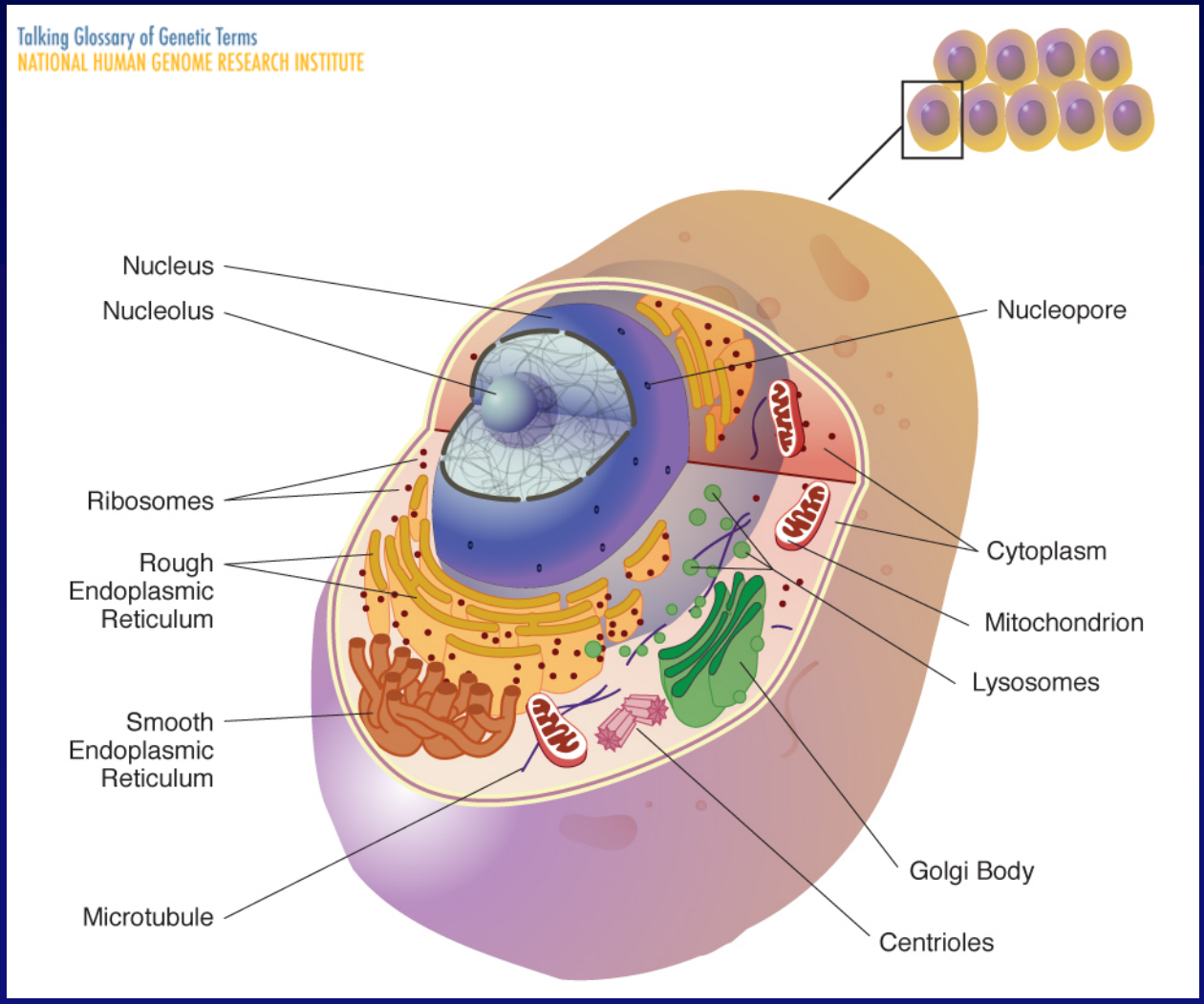


In case of identical tweens, there is no difference in DNA sequence

However, the differences can exist at epigenetic level

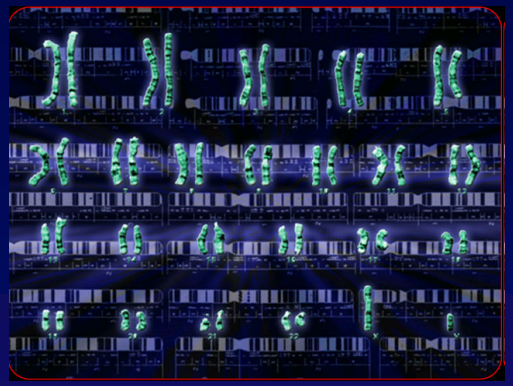


Cells can be defined as ever-working factories



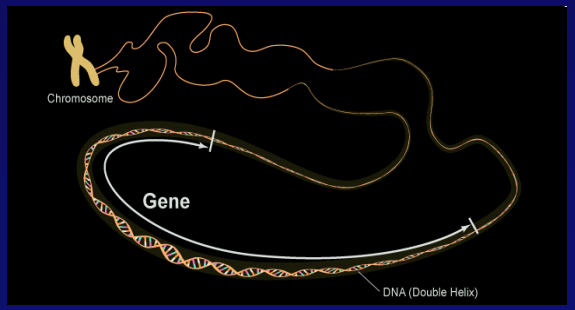
The information, how the cel should work, is encoded in the genome

- Genome

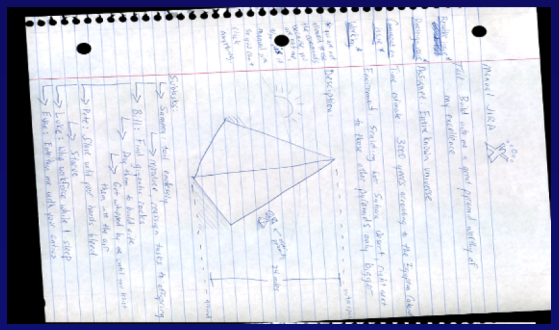


- Library

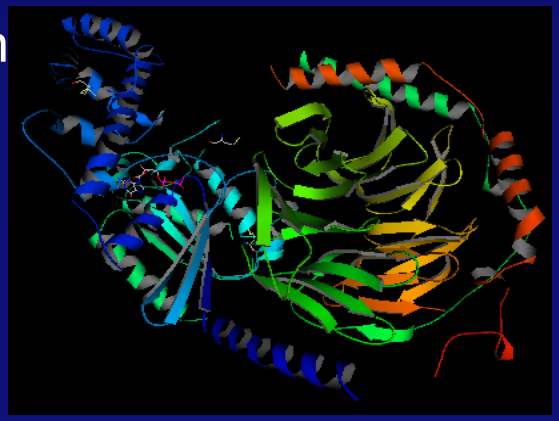
- Gene



- Instruction

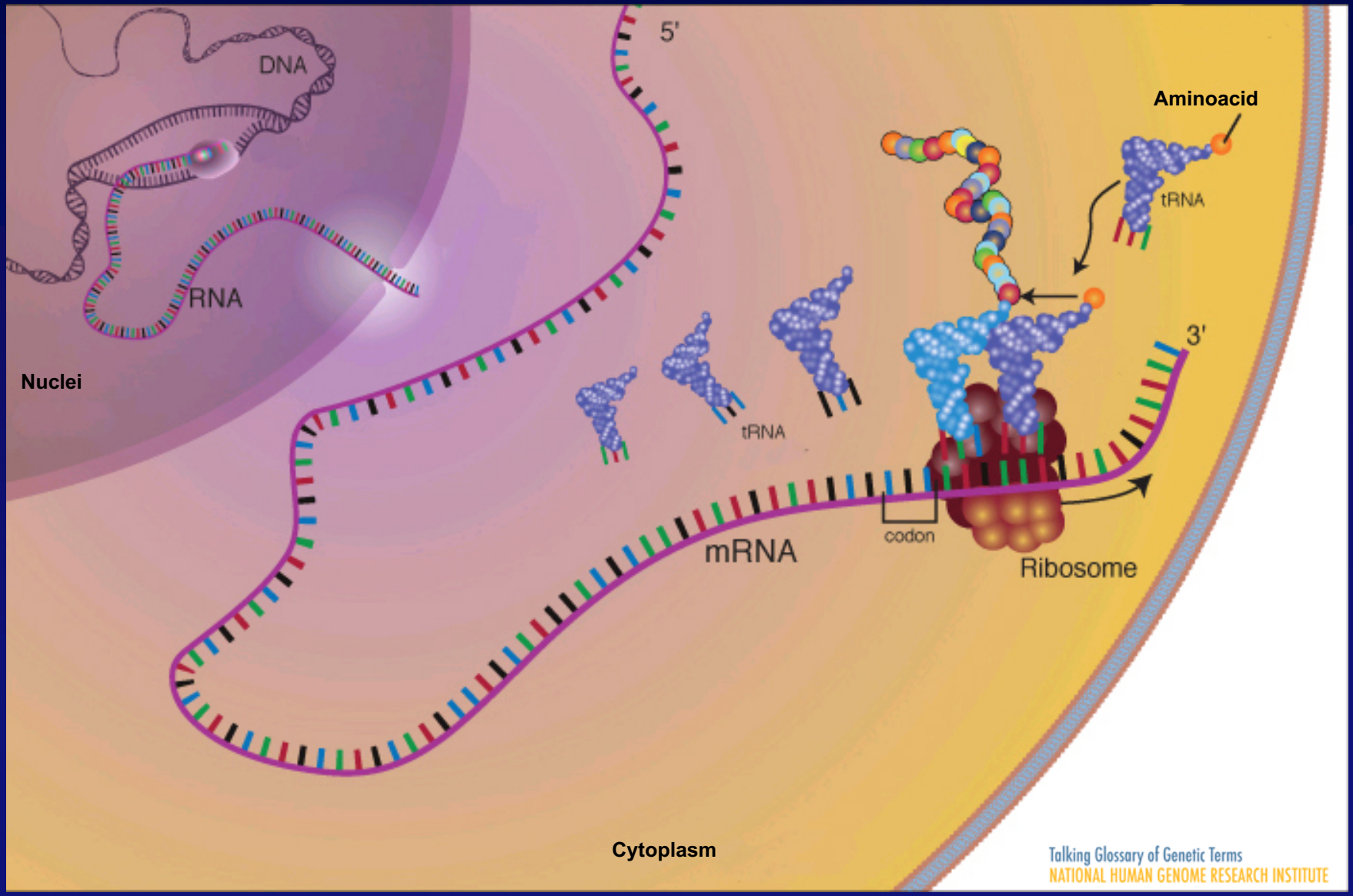


- Protein

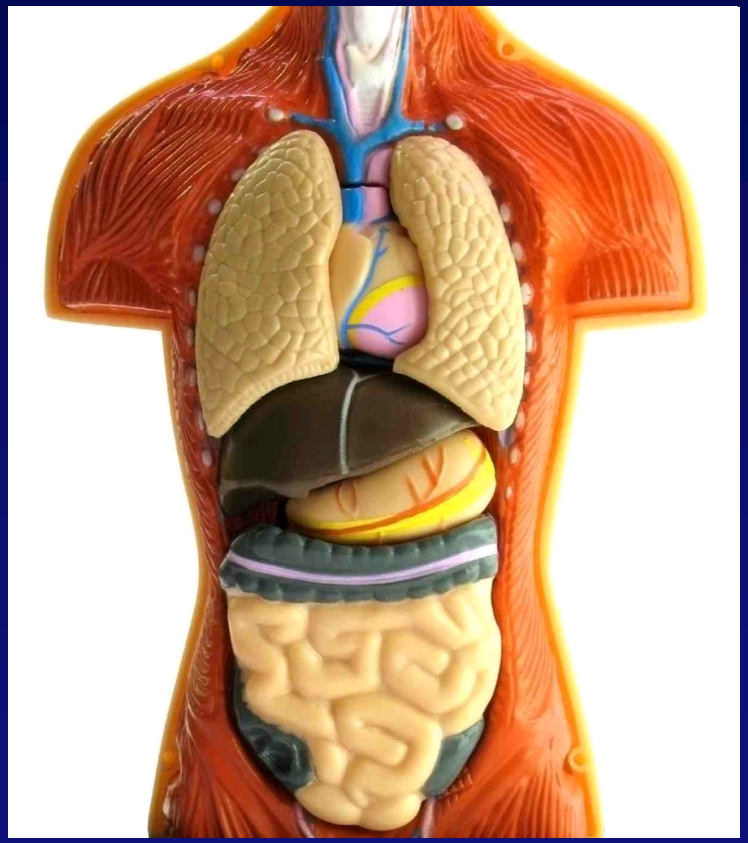


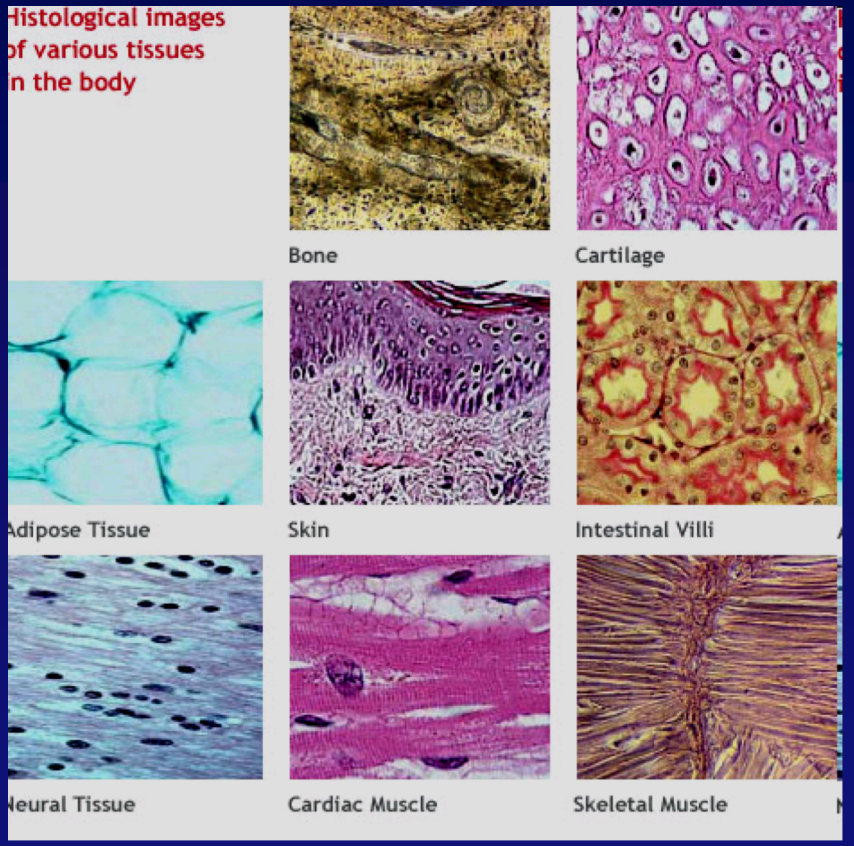
- Product

Information encoded in DNA is translated into proteins

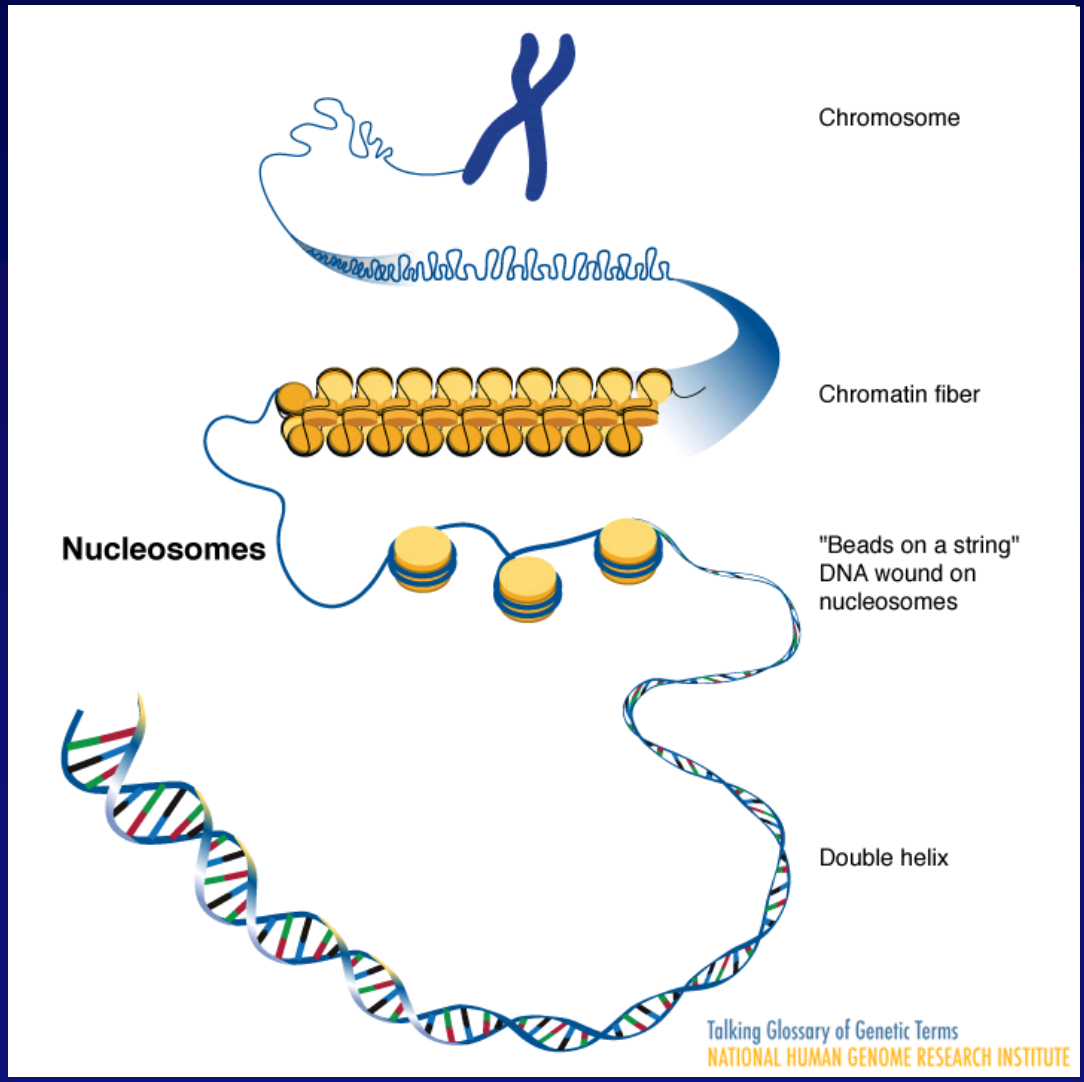


During development and cell specialization the genome stays unchanged while epigenome can be modified





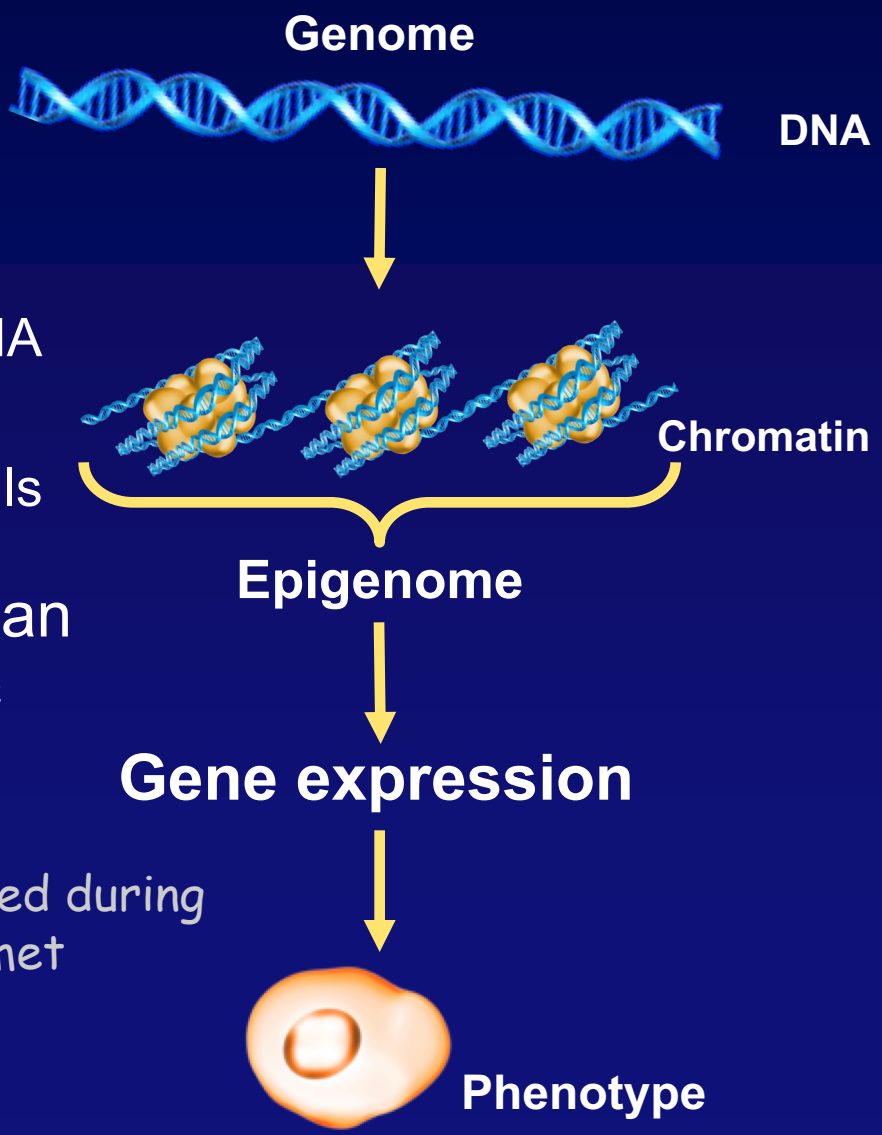
DNA is wrapped on nucleosomes forming chromatin



Defining epigenetics

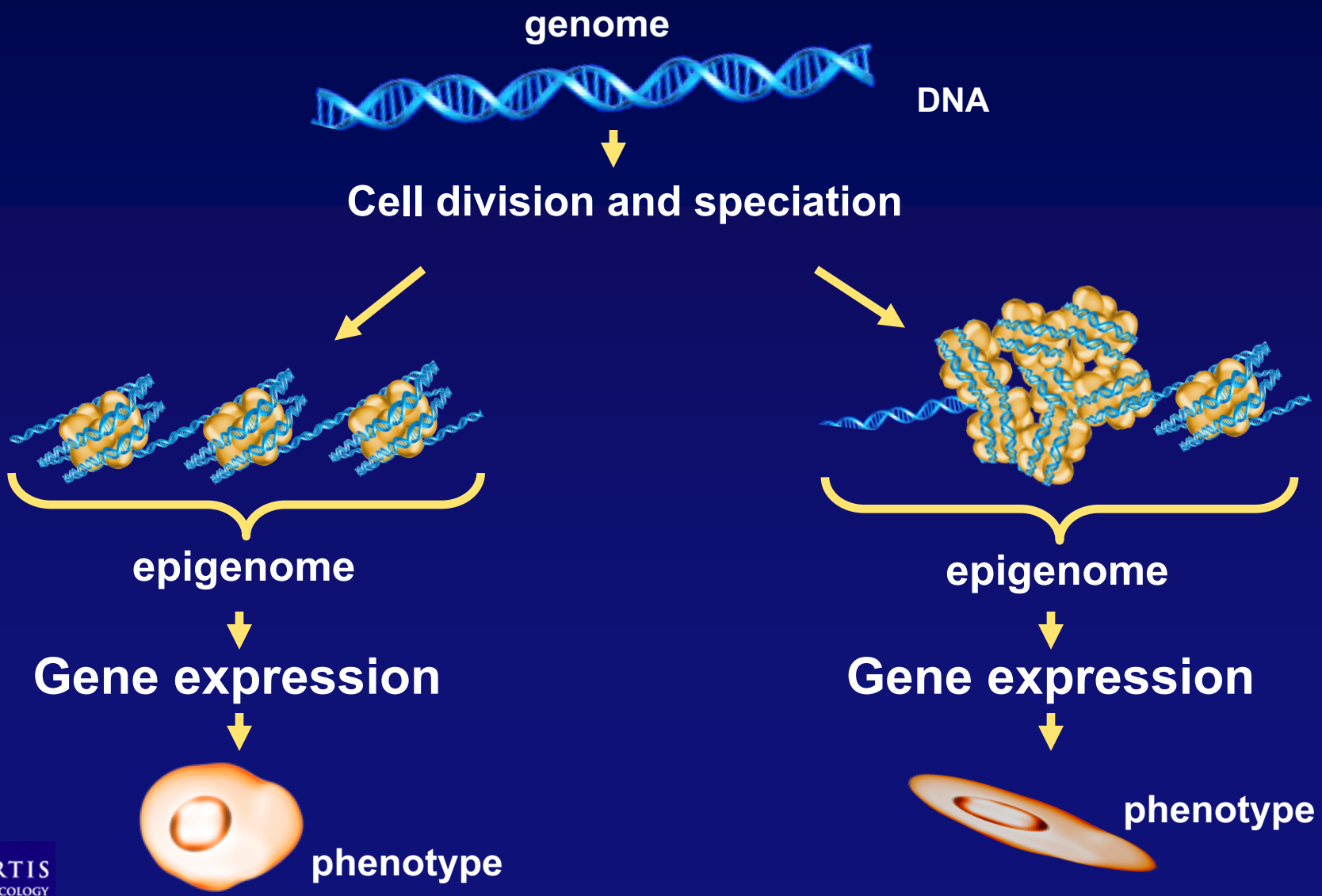
Epigenetics:

- Reversible changes in gene expression
  - which occur without changes of DNA sequence
  - which can be inherited between cells
- Genetic information is therefore an important addition to the genetic information genetycznej

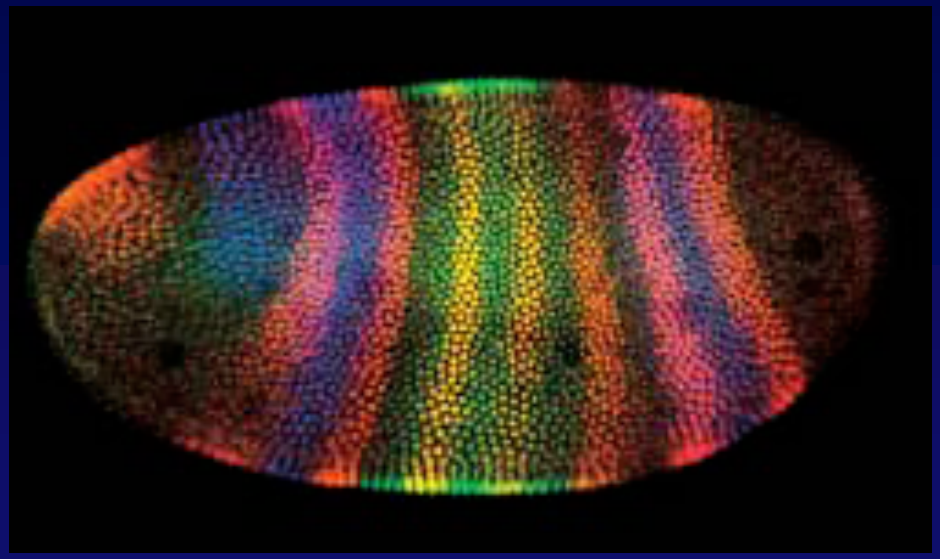


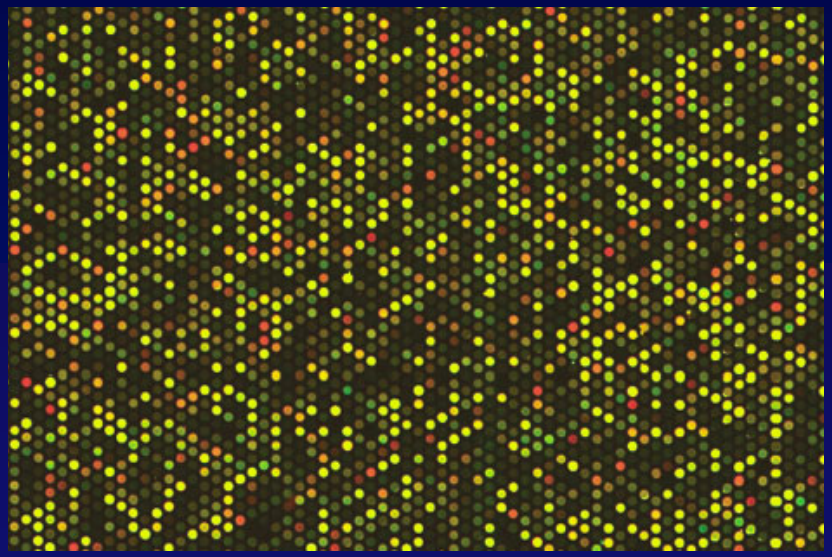
Majority of epigenetic changes is tranmitted during mitosis, but removed in early stages of gamet formation at meiosis

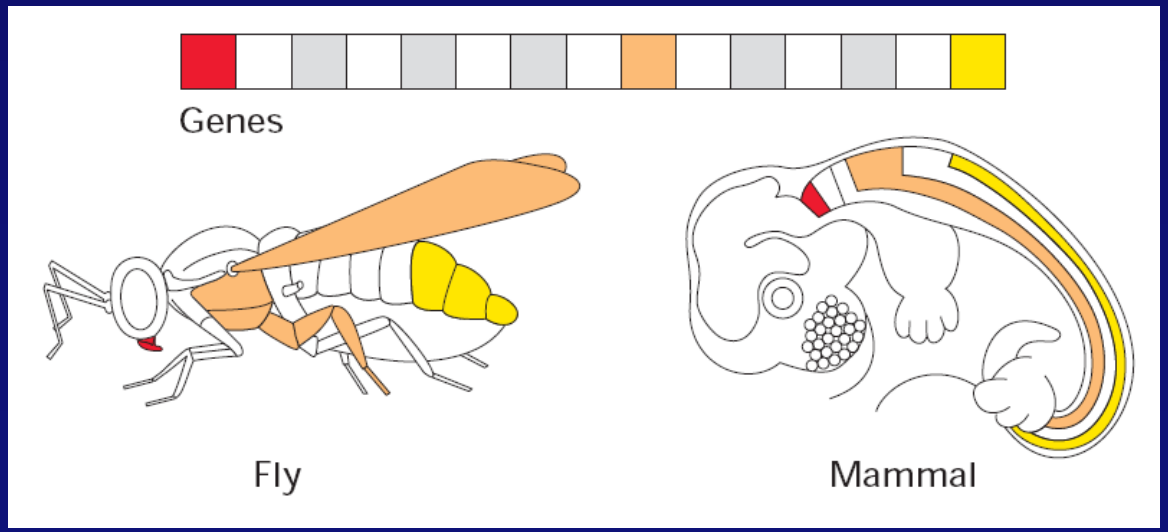
During development and cell specialization the genome stays unchanged while epigenome can be modified



Differences between cells of the organism are due to differences in the pattern of gene expression

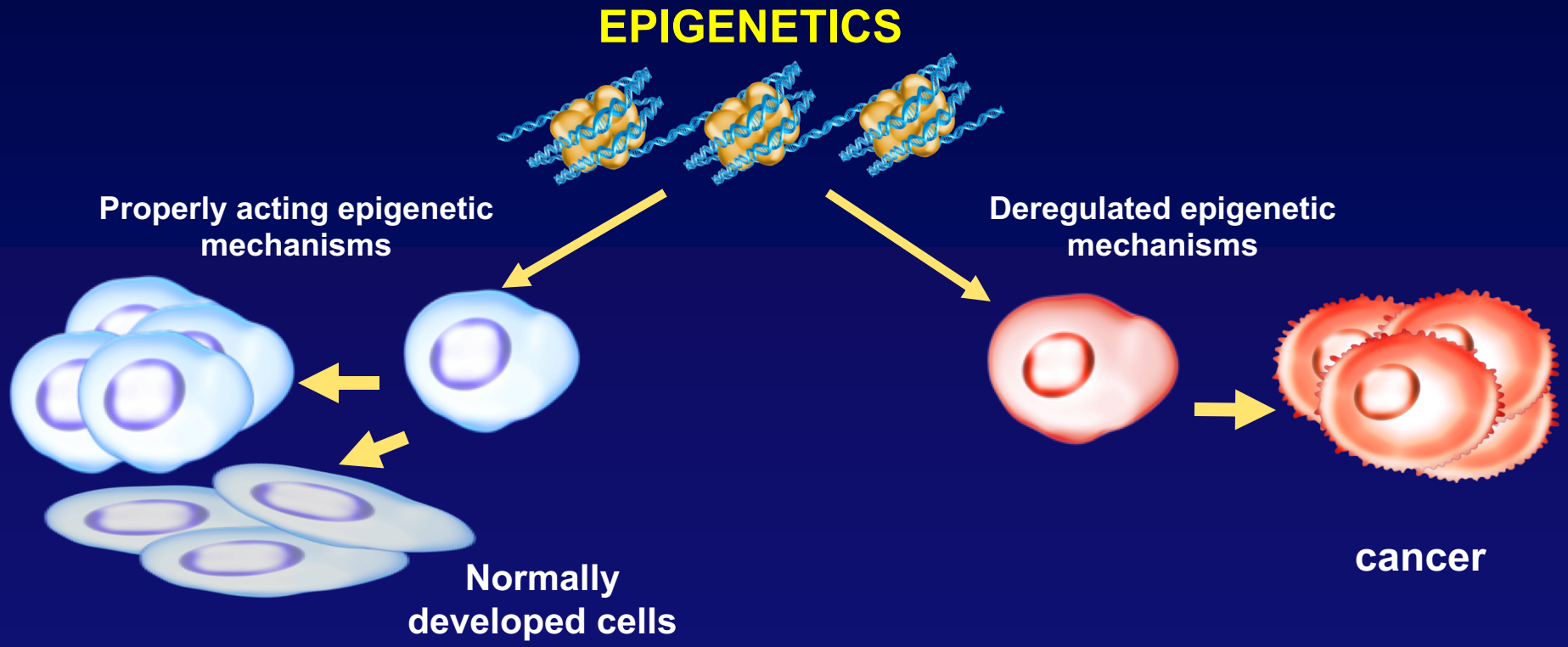






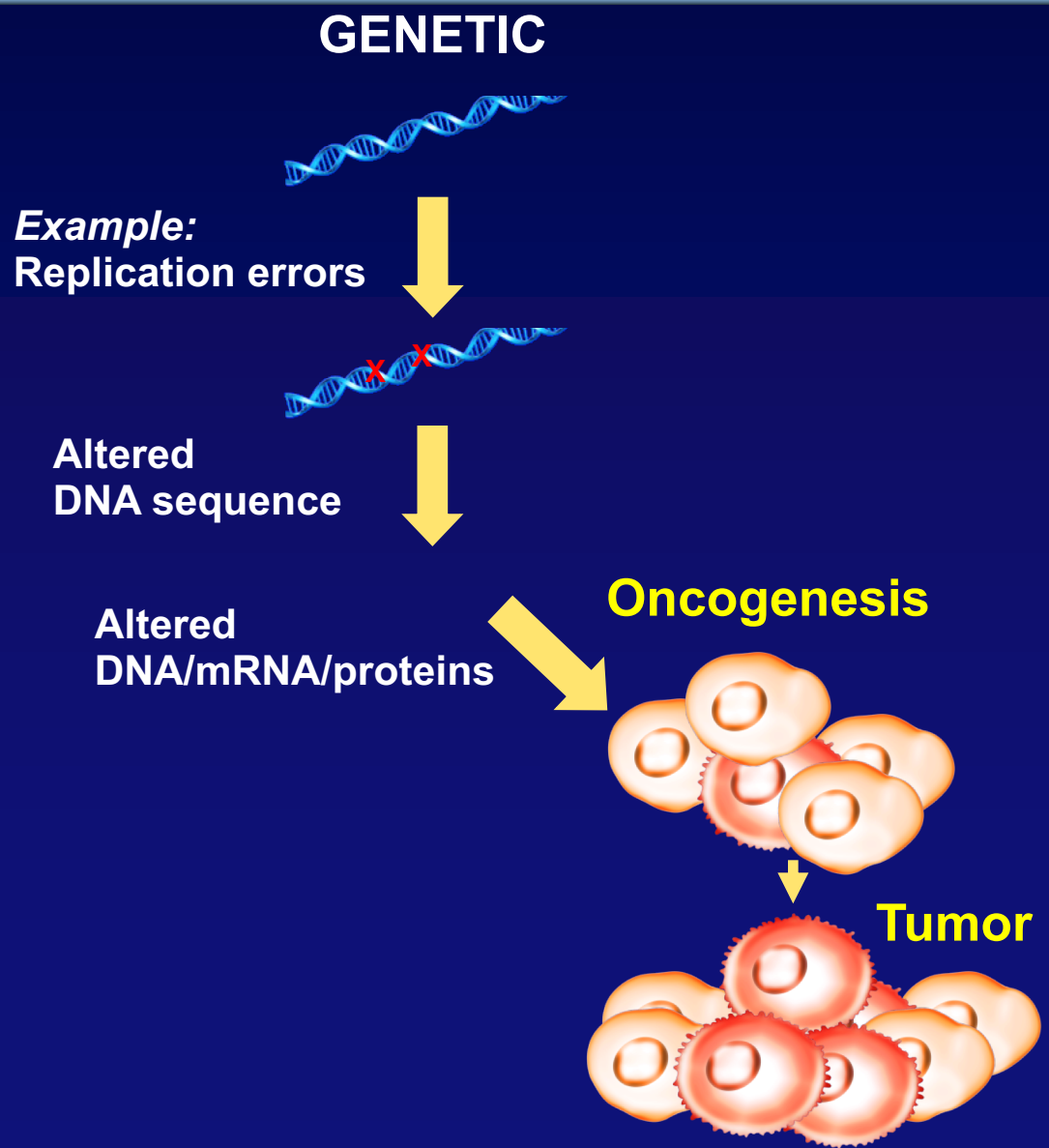
Courtesy of Sean Carroll
University of Wisconsin

Epigenetics plays an important role in normal cell development, but can also lead to development of cancer

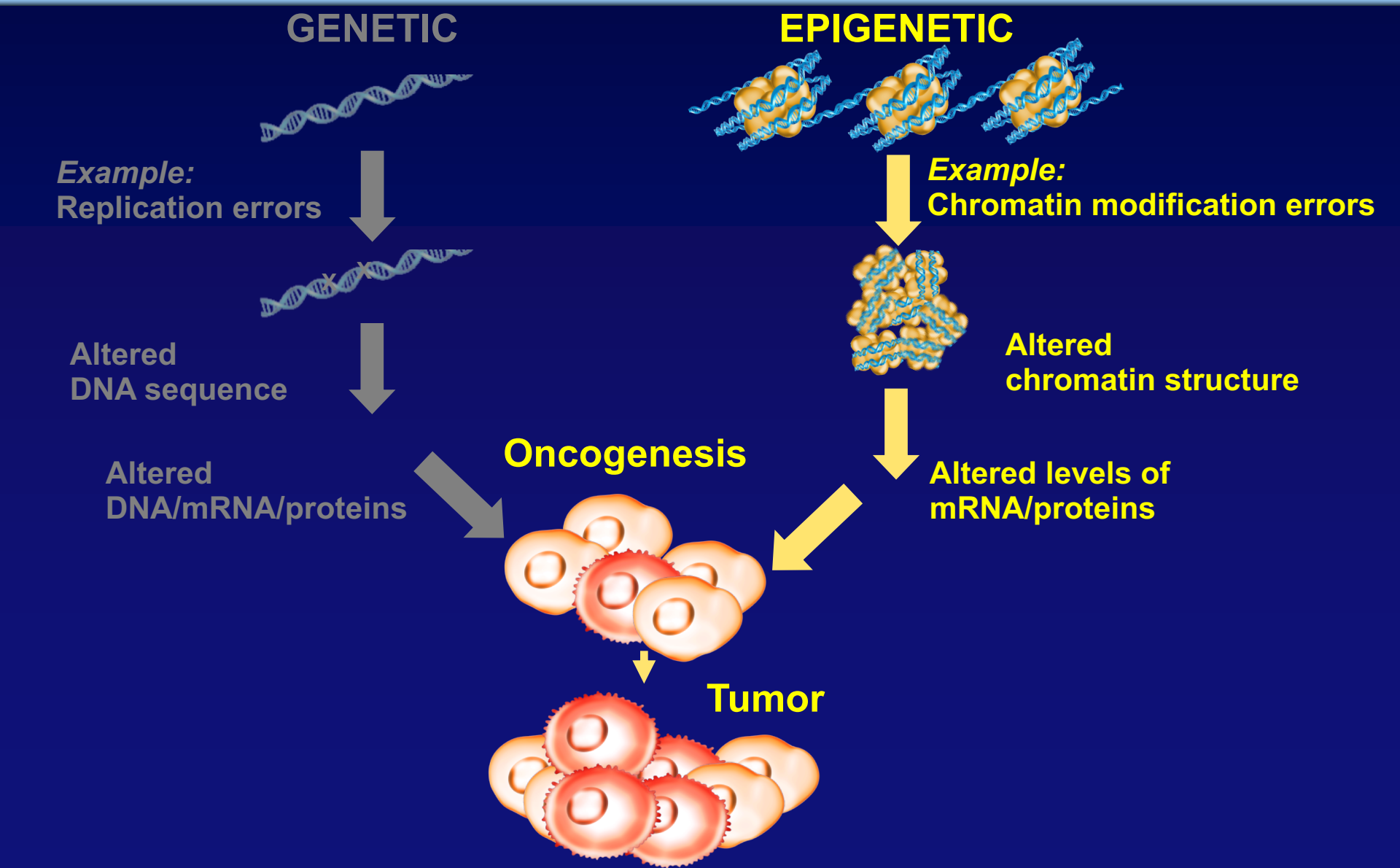


- Epigenetic mechanisms regulate activity of genes involved in cell proliferation, differentiation, cell cycle and apoptosis
- Deregulation of epigenetic mechanism effects in alterned gene expression, which in turn may cause development of cancer

Historically, Cancer Was Considered to be Driven Mostly by Genetic Changes



Recent Evidence Shows that Epigenetic Changes are Also Important in Causing Cancer



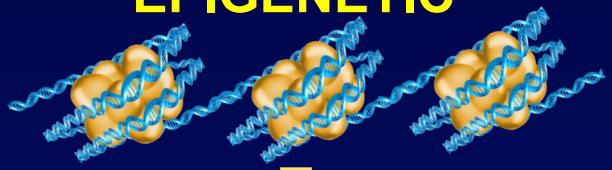
Epigenetics Can Cooperate With Genetic Mutations to Promote Oncogenesis

GENETIC

↑ Oncogene function

↓ Tumor suppressor function

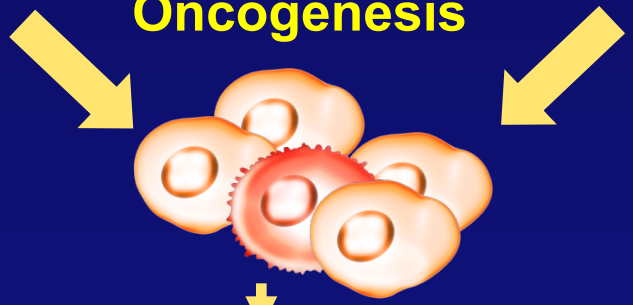
EPIGENETIC



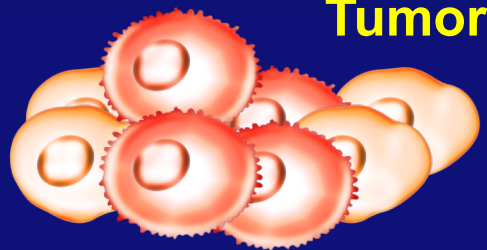
↑ Oncogene levels

↓ Tumor suppressor levels

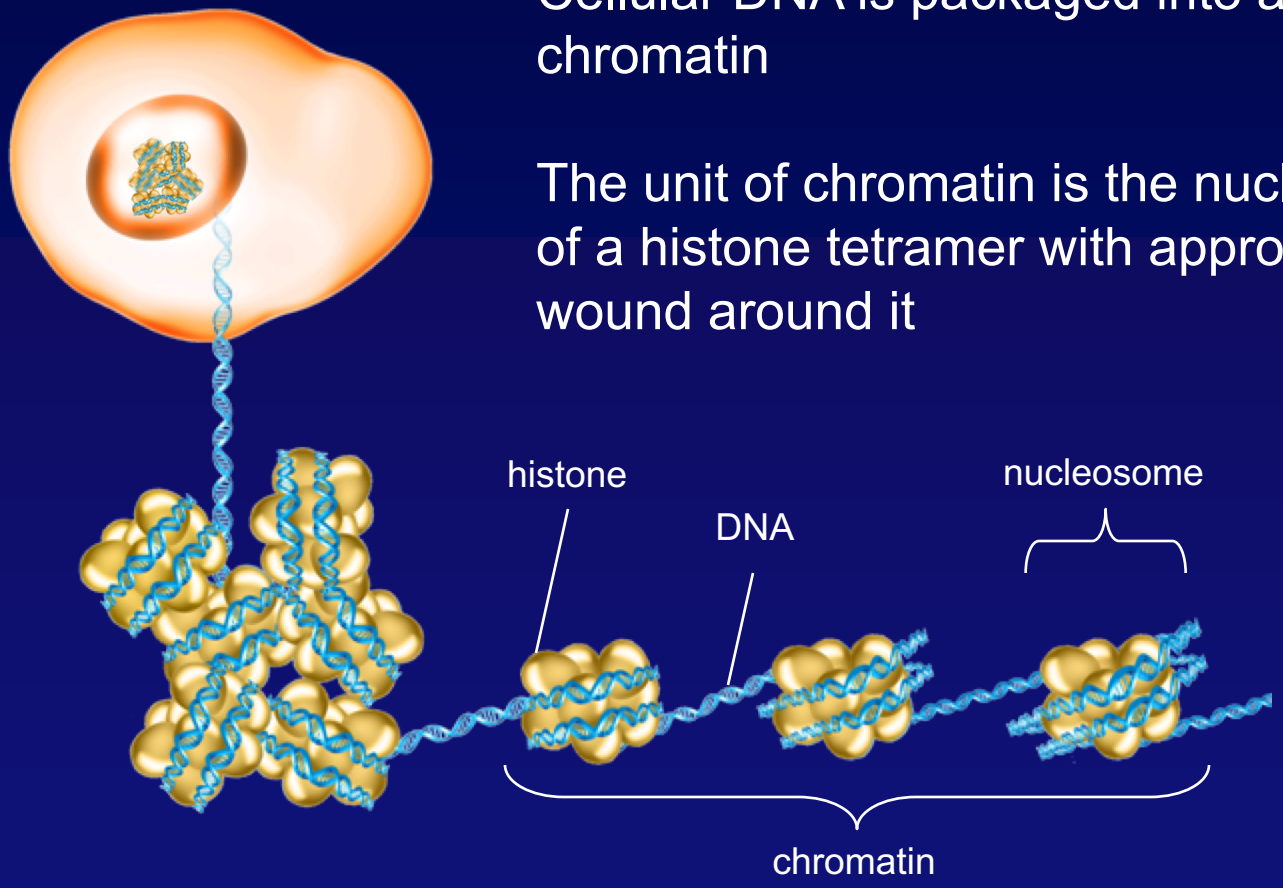
Oncogenesis



Tumor



Chromatin is a Key Component of Epigenetic Mechanisms

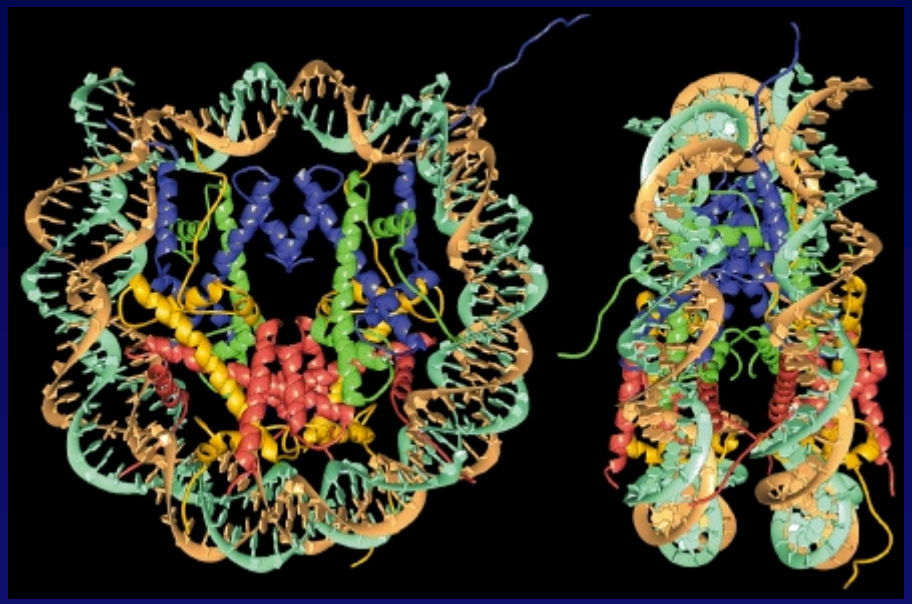


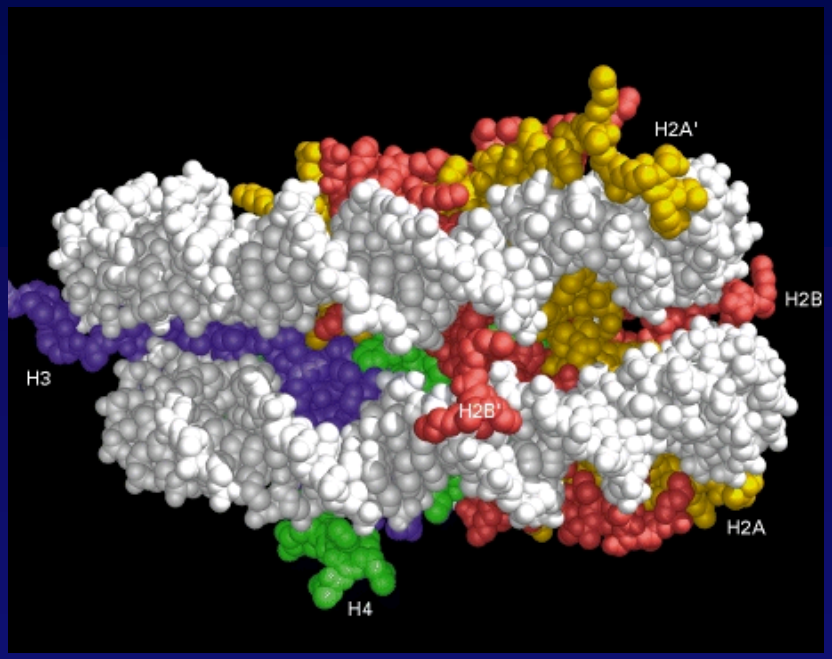
Cellular DNA is packaged into a structure called chromatin

The unit of chromatin is the nucleosome, a complex of a histone tetramer with approx. 125 bp of DNA wound around it

- Chromatin organizes genes to be accessible for transcription, replication, and repair

Chromatin condensation is determined by histone proteins





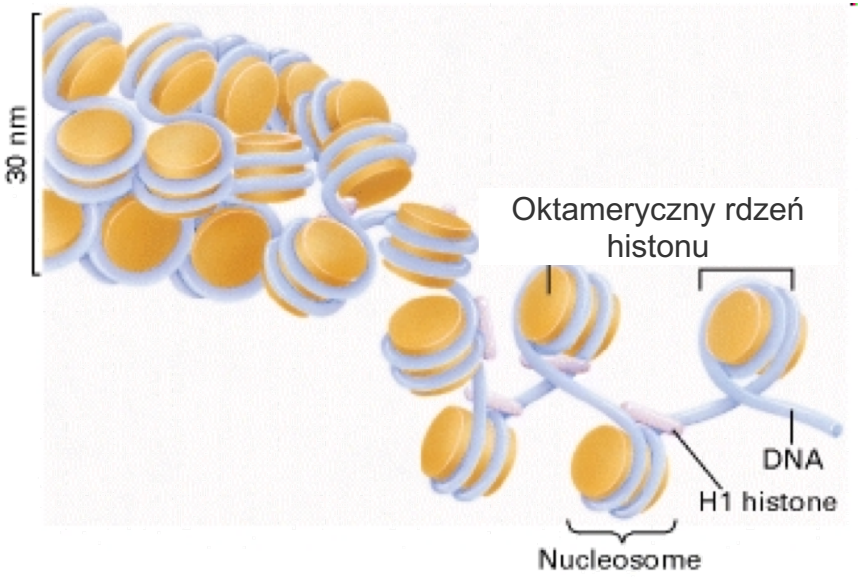
- DNA is nagatively charged, while histones – are positively charged. However, the charge of histones may be modified to some extend (especially in their tails)

Chromatin is based on nucleosomes

- Molecular studies show that DNA in chromatin is associated with histone proteins;
- Histones are organised into disc-like structures and DNA is wrapped around these structures;
- The nucleosome core consists of 2 copies each of the core histones H2A, H2B, H3, and H4;
- Core particles are connected by stretches of linker DNA, which can be up to about 80 bp long.
- Histones are evolutionary highly conserved;

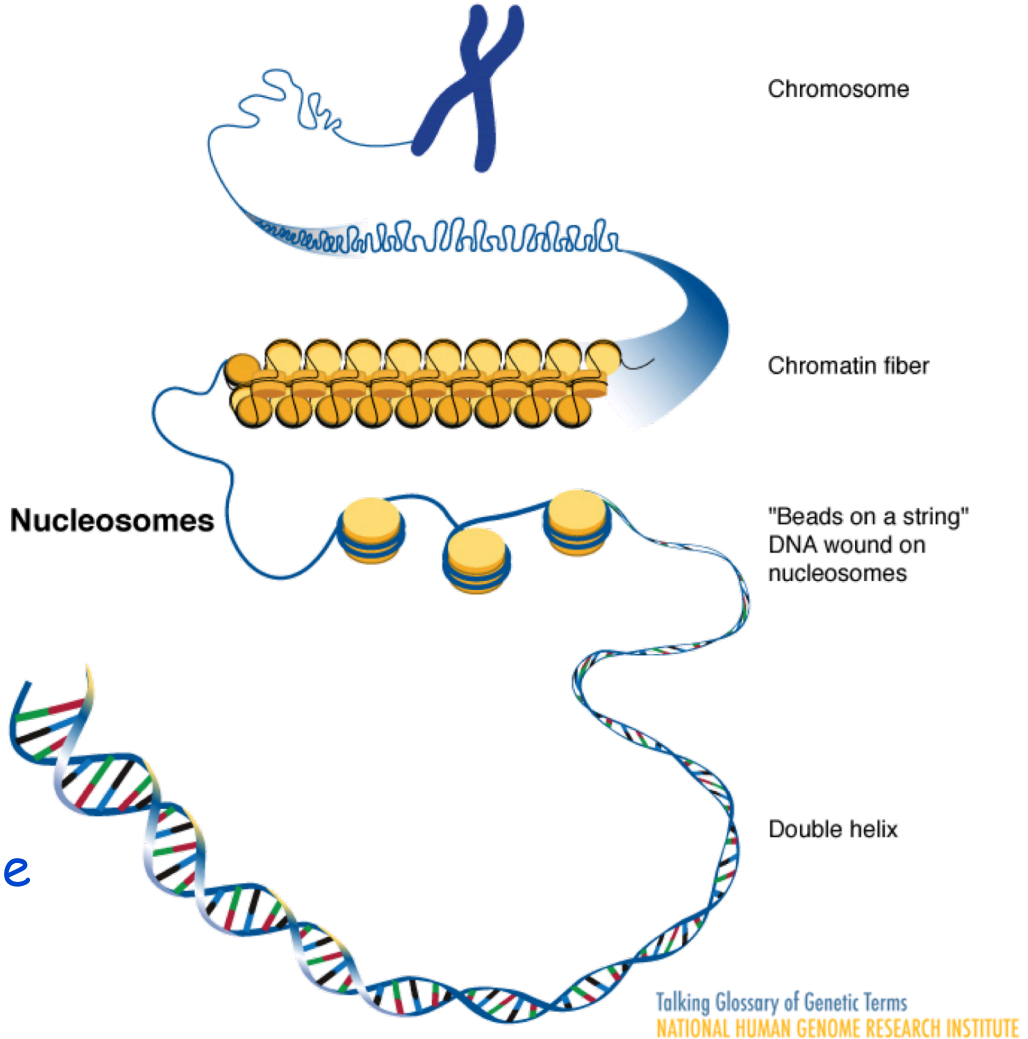
Chromatin is based on nucleosomes

- Additional histone H1 is present outside the nucleosome core and stabilizes its structure;
- Approximately 146 base pairs (bp) of DNA wrapped in 1.67 left-handed superhelical turns around the histone octamer;



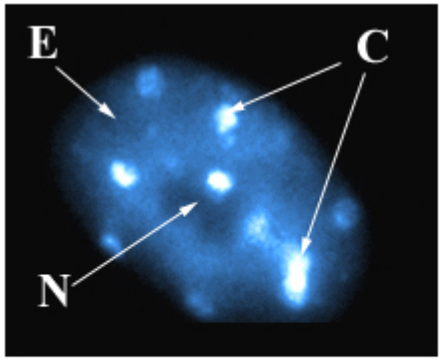
Chromatin is based on nucleosomes

- Transcriptionally active chromatin is extended (openned) and has a structure described as „beads on a string" or 10-nm fibre;
- Inactive regions form solenoid structure, so called 30-nm fibre;
- During cell division 30-nm fibre is further coiled with the aid of non-histone proteins;



Euchromatin and heterochromatin are two forms of chromatin

- Chromatin can take a form of euchromatin and heterochromatin; both forms can be. easily distinguished using cytology;
- Euchromatin forms chromosomal regions which are weakly packed;
  in interphase they exist as 10-nm fibres and transcriptionally active;
- Heterochromatin consists of transcriptionally inactive regions,
  as it exists in a form of highly condensed 30-nm fibre;
- Heterochromatin encompasses satellite regions (centromeres and telomeres); heterochromatin blocks are enriched in transposons;



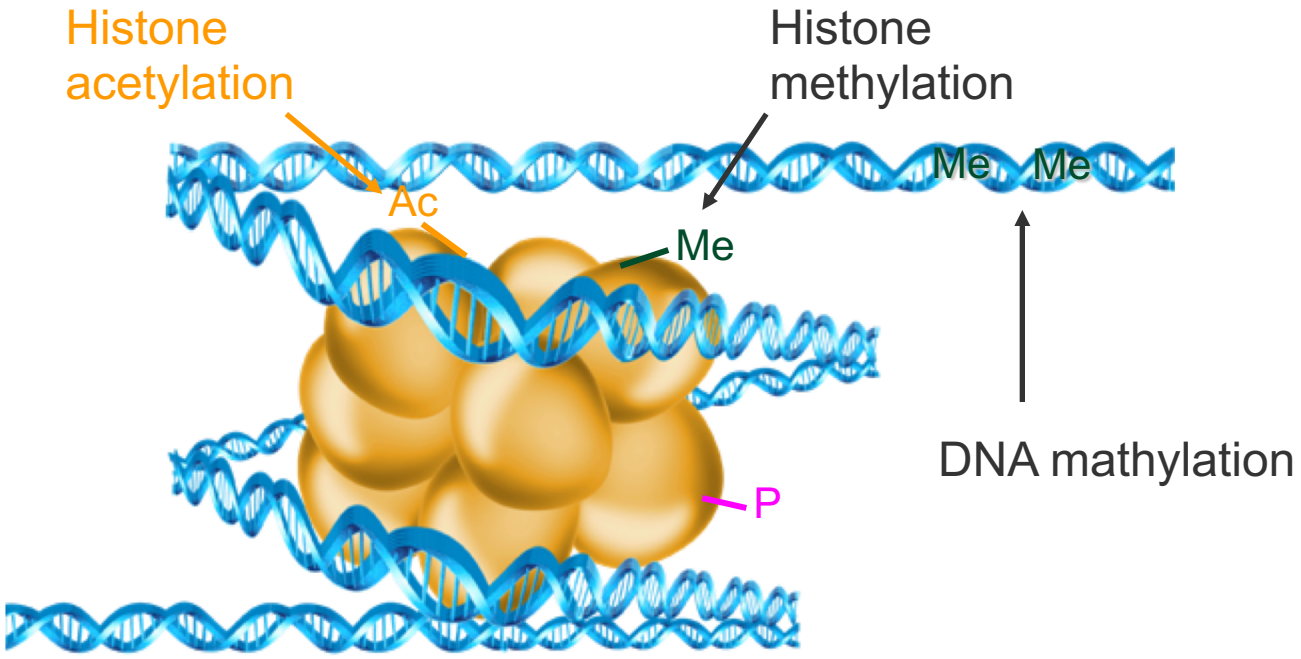
C – heterochromatin (chromocentre)
E – euchromatin
N - nucleolus

Nukleosomes influence gene transcription

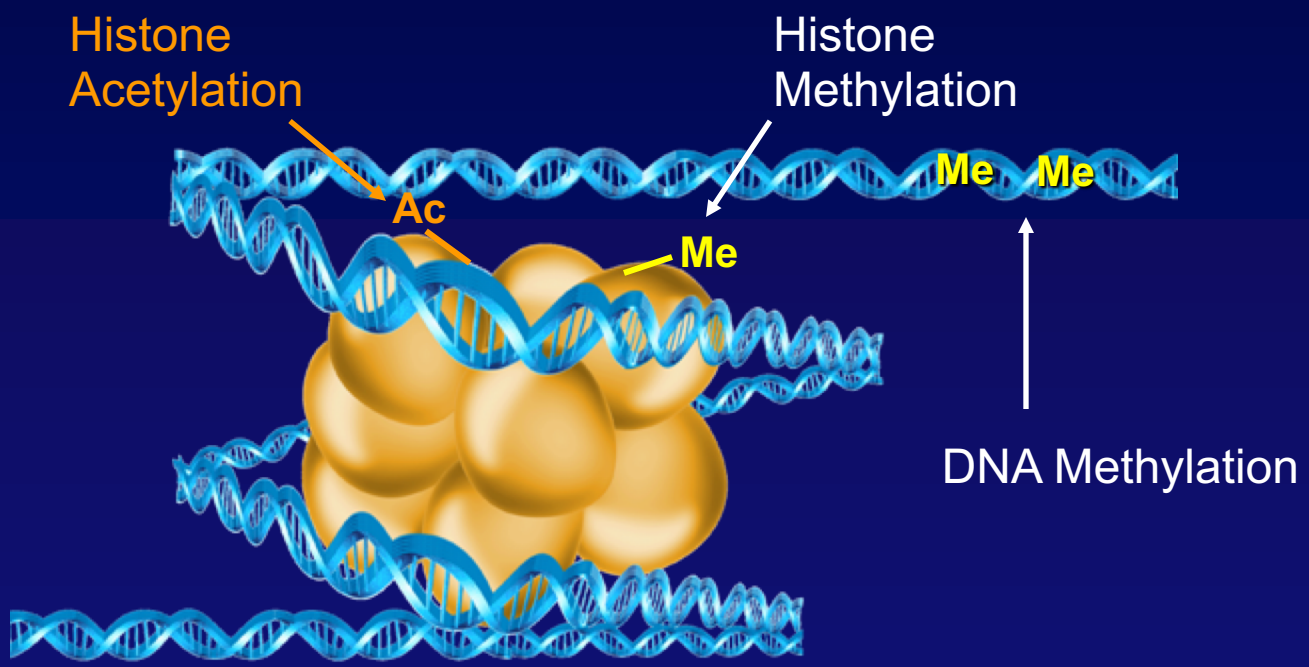
- Besides their structural functions nucleosomes adopt important roles in the regulation of gene expression by modulating access of transcription machinery to DNA;
- Regulation is connected with nucleosome distribution along DNA molecule, but also withnumerous chromatin modifications;

Nukleosomes influence gene transcription

- The most important modification of chromatin are posttranslational histone modifications such as acetylation and methylation that change affinity of histones to DNA or attract proteins influencing gene expression;
- Besides histone modifications, DNA methylation plays a crucial role;



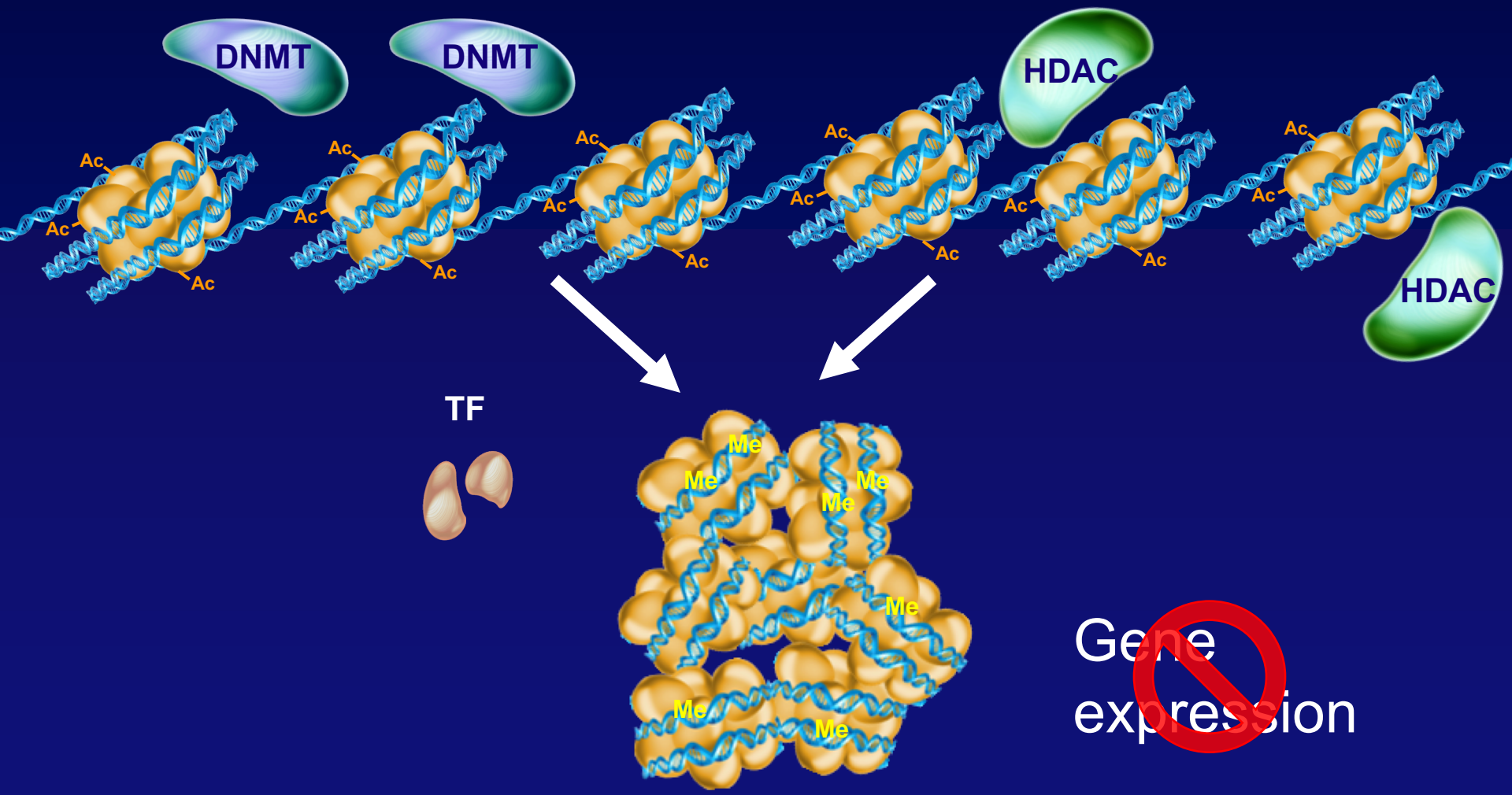
Basic Epigenetic Mechanisms: Post Translational Modifications to Histones and Base Changes in DNA



- Chromatin epigenetic modification include:
  - Histone modification (acetylation, methylation, phosphorylation, ubiquitination etc.)
  - Histone variant exchange
  - DNA methylation
  - RNAi

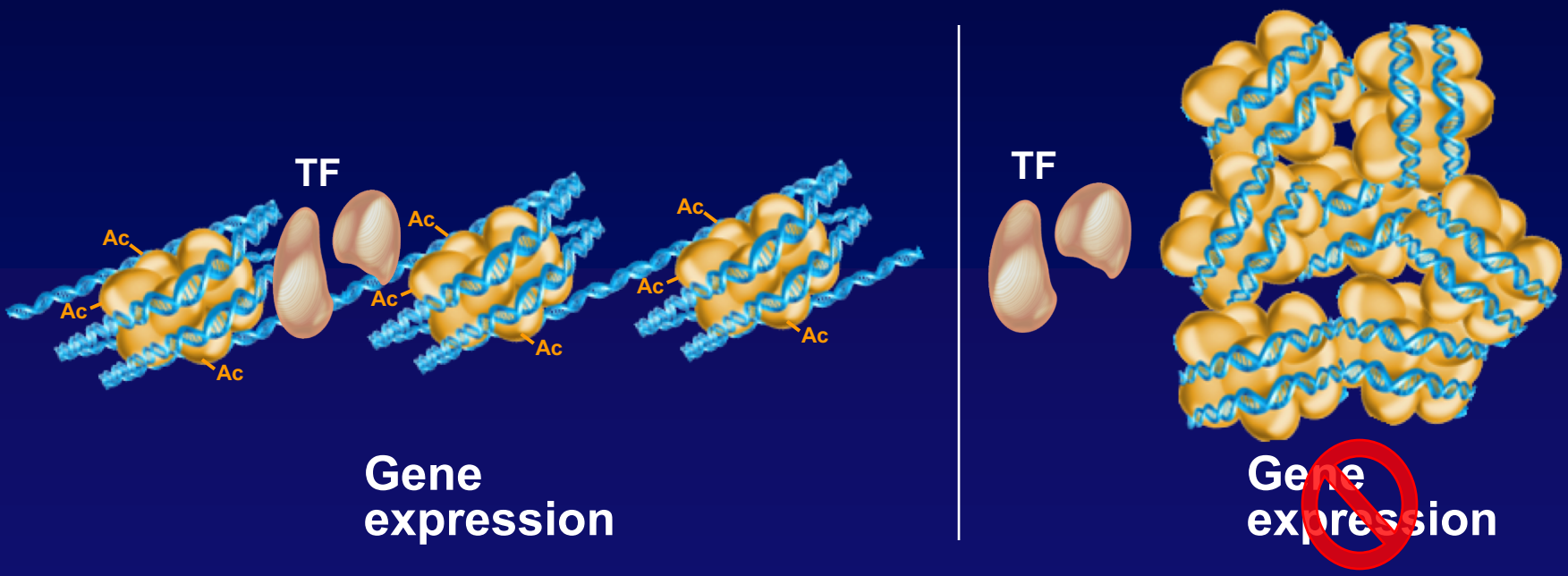
} 'histone code'

Epigenetic Modifications to Histones and DNA Can Cooperate to Silence Gene Expression



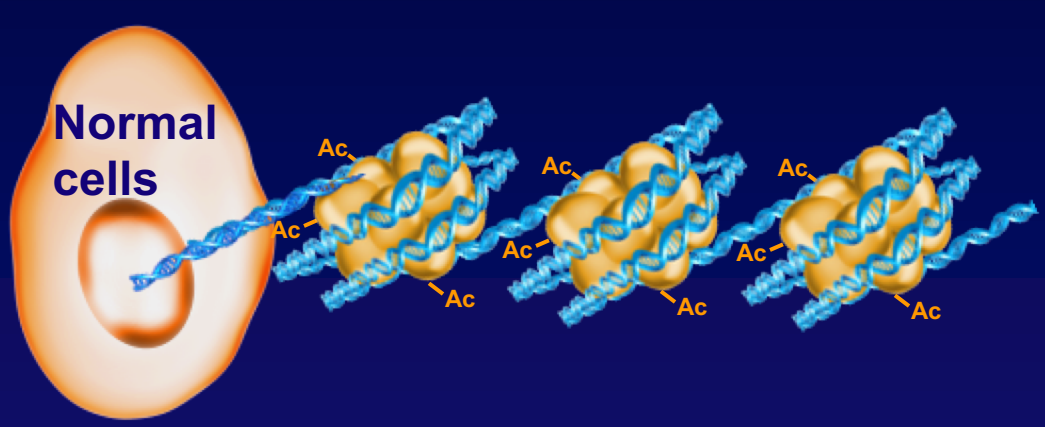
- Epigenetic modifications can cooperate to silence gene expression

Epigenetic Changes can Alter Chromatin Structure and Regulate Gene Expression

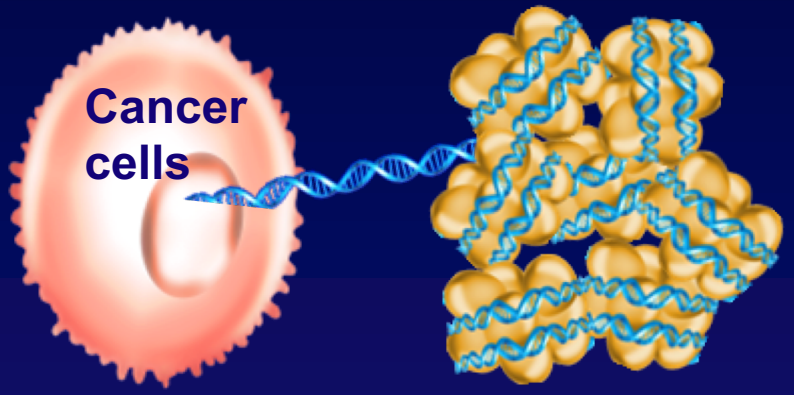


- Gene expression (transcription) requires DNA to be physically accessible to transcription factors (TF)
- Epigenetic changes alter the structure of the chromatin, which determines whether DNA is accessible
  - Open chromatin allows gene expression
  - Closed chromatin prevents gene expression

In Cancer, Changes in Chromatin Structure Can Silence Tumor Suppressor Genes



Tumor suppressor gene expression



Tumor suppressor gene expression

- Silencing of tumor suppressor genes, a major process in tumorigenesis, may result from epigenetic changes that condense chromatin structure

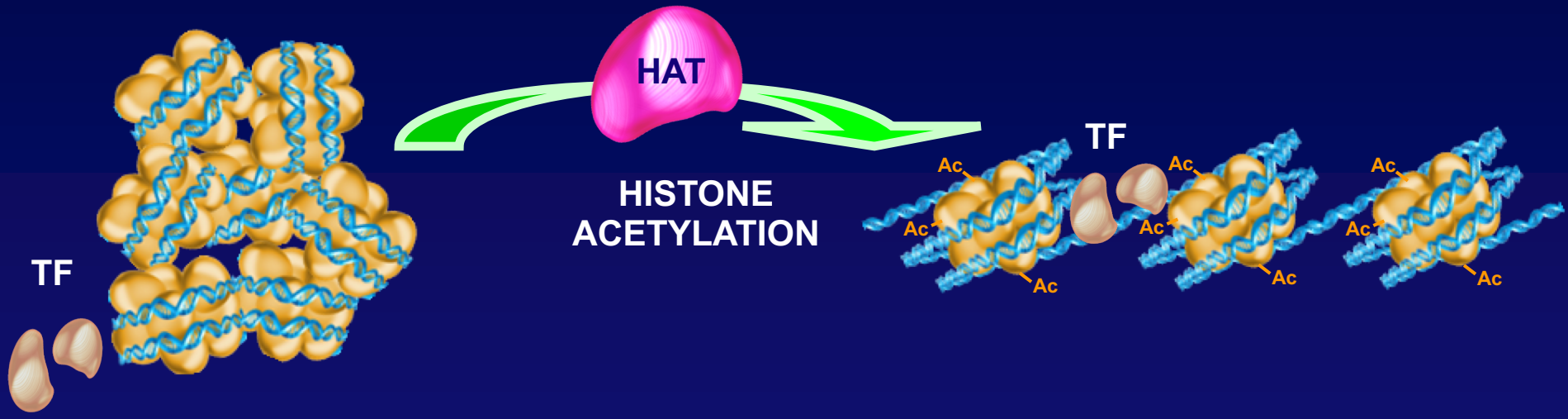
Posttranslational histone modifications

- Following incorporation into a nucleosome, the core histones are modified in order to change their properties and strength of interaction with DNA;
- Modyfications are reversible and are not random, but targeted;
- The crosstalk between particular modifications is very complex, scientists use to call them 'histone code'
- The pattern of histone modifications may be transduced during cell divisions;

„Histone code"

- The ‚histone code' idea is based on the assumption that the most important effect of histone modifications is specific, targeted recruitment of some proteins and not alteration of the strength of DNA-nucleosome interaction;
- Lysine and Arginine methylation may cause both activation and repression of gene transcription dependently whichg residue is modified. H3K4 and H3K36 methylation correlates with transcriptional activation, while H3K9 trimethylation (H3K9me3) and H3K27 correlates with repression.
- Histone acetylation leads usually to open chromatin structure, which is accessible for transcriptional machinery, because acetylated histones don't bind DNA so strongly.

Balance of Histone Acetylation is a Key Factor in Transcriptional Regulation in Normal Cells



Deacetylated Histones

Closed chromatin
Transcription factors cannot access DNA

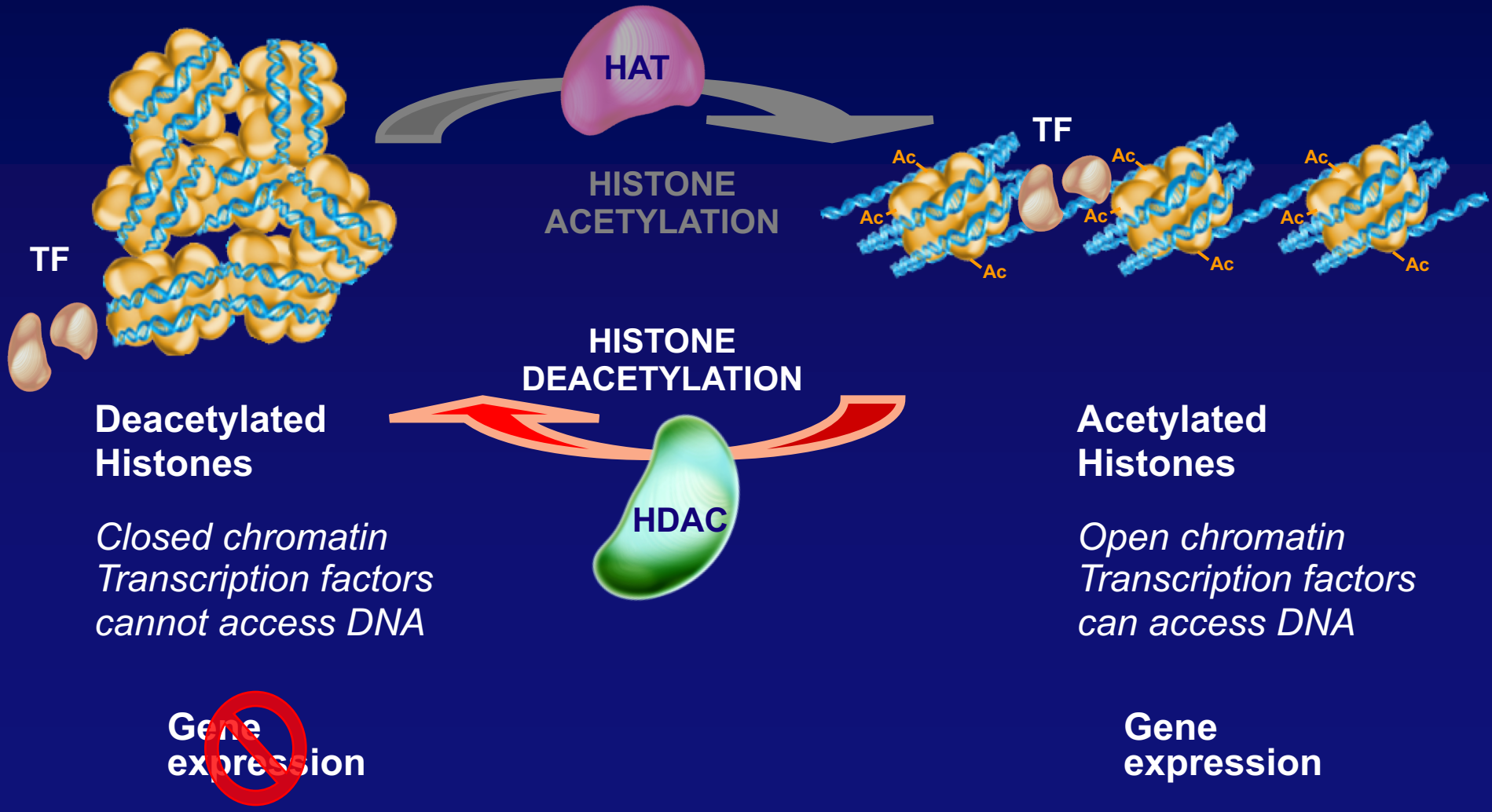
Gene expression

Acetylated Histones

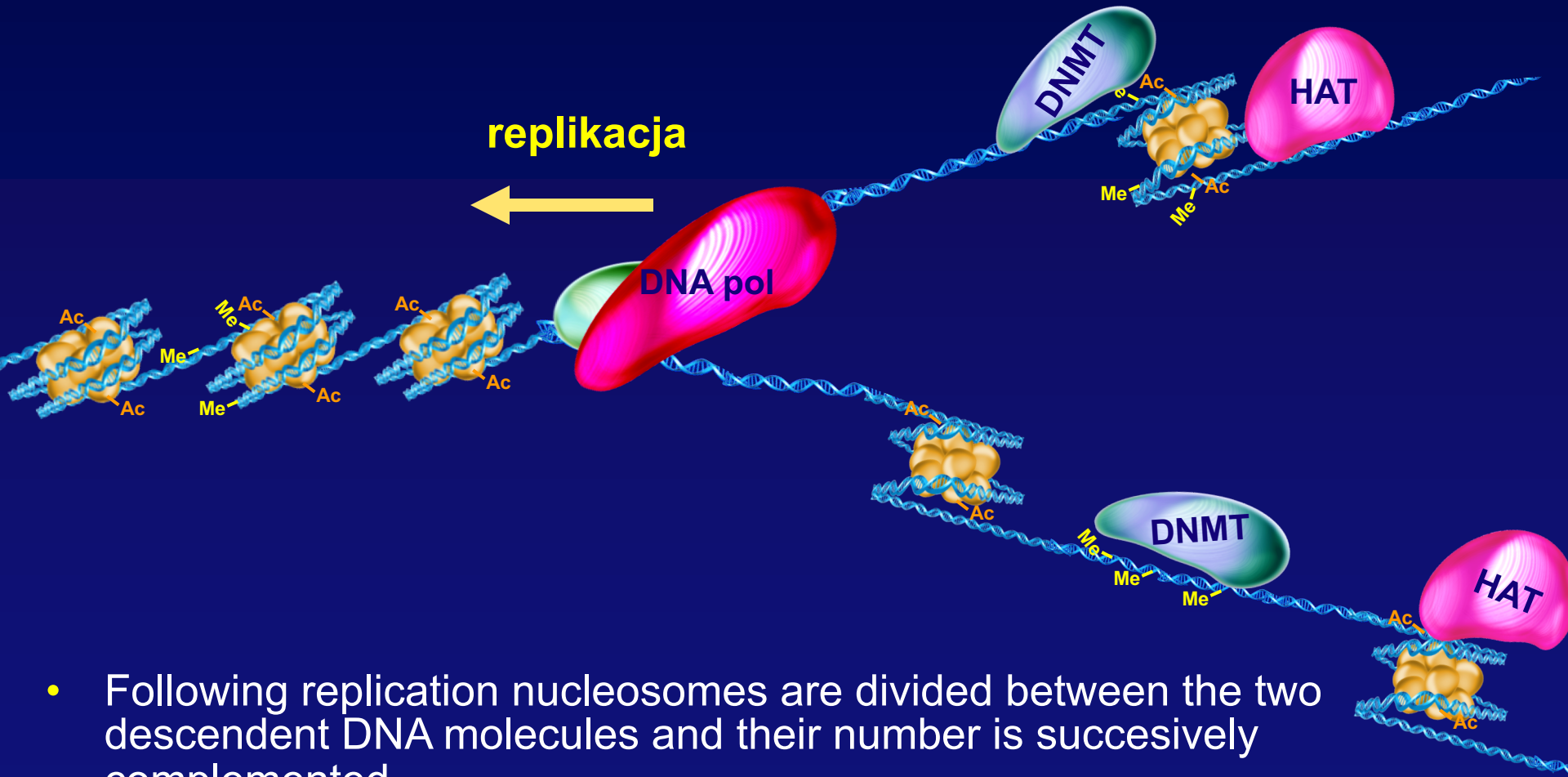
Open chromatin
Transcription factors can access DNA

Gene expression

Balance of Histone Acetylation is a Key Factor in Transcriptional Regulation in Normal Cells



Epigenetic changes may be inherited during DNA replication



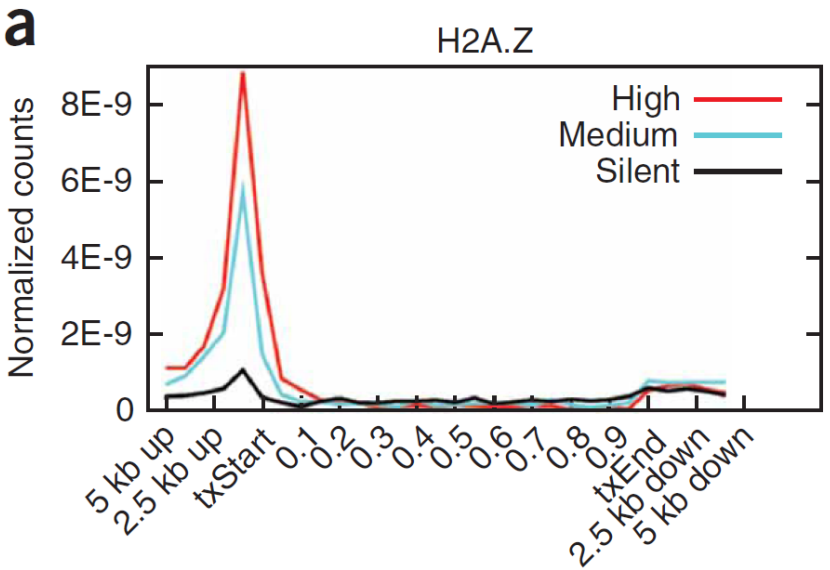
- Following replication nucleosomes are divided between the two descendent DNA molecules and their number is succesively complemented
- Histone modification pattern is also copied

Histone variants

- Besides canonical core histones there are also specified forms called histone variants;
- Histone variants possess specific properties that affect functional properties of the chromosomal region
- MacroH2A is a variant specific for vertebrates and is common in inactivated 'X' chromosome
- H3.3 is present in transcriptionally active regions where it replaces H3 during elongation
- CenpA is an H3 variant associated with centromeric chromatin; it forms more compact and rigid nucleosomes and by this affects DNA compaction in centromeres

Histone variants

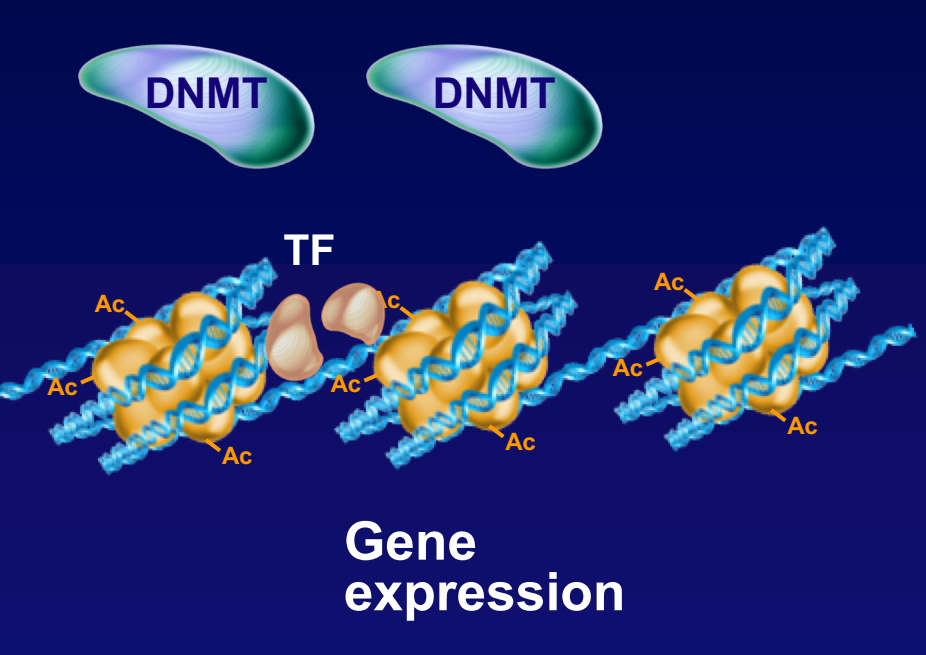
- H2A.X (phosphorylated form) bind to regions containing DSBs labelling them for the repair
- H2A.Z is a crucial variant of H2A with a specific location in TSS surrounding nucleosomes. It is believed it inhibits transcription and labels TSS. Its removal enables transcription start.

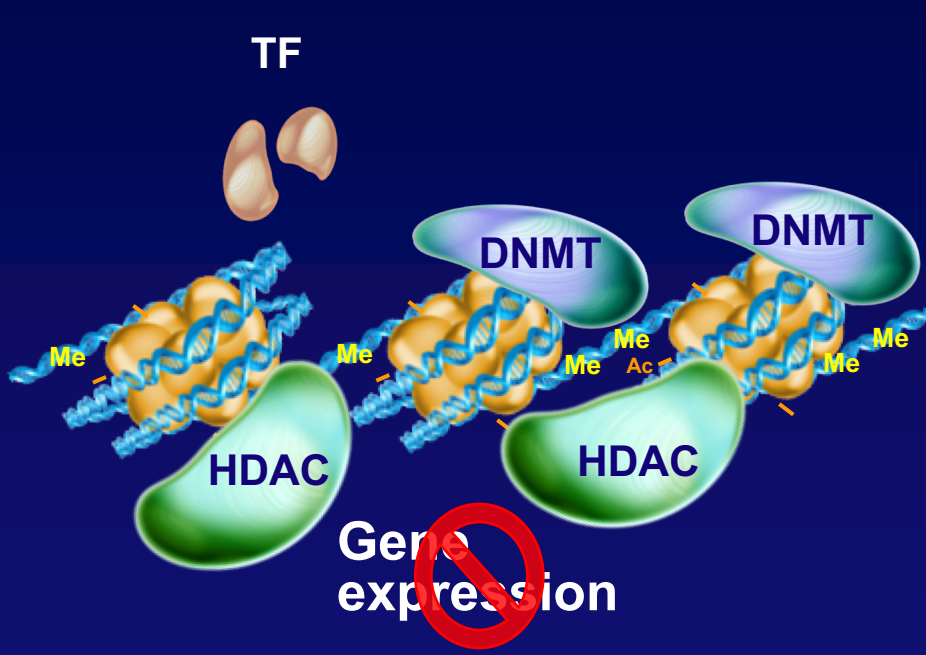


DNA methylation

- DNA methylation is necessary for normal development and crucial in such processes as imprinting, X chromosome inactivation, transposone suppression or oncogenesis;
- DNA methylation occurs on Cytosine, which is more easily mutated in the methylated form; therefore methylated regions are error-prone;
- DNA methylation may cause transcriptional silencing by two means:
  - Directly, by limiting affinity of TFs to the methylated region,
  - Indirectly, by binding methyl-CpG-bining-domain (MBD) proteins, which recruits HDAC

DNA Methylation can Prevent Gene Expression



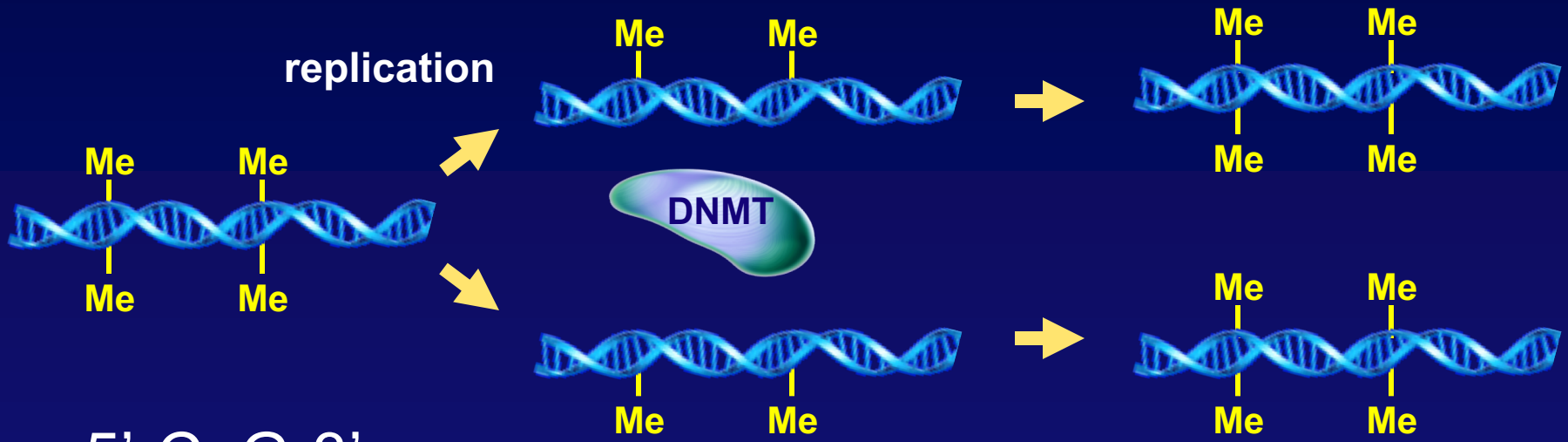


- DNA methylation involves the transfer of methyl groups to cytosine residues in DNA by DNA methyltransferases (DNMTs)
  - May prevent transcription factors from binding to DNA
  - May serve as binding site for methylated DNA-binding proteins, such as MECP2, which then recruit HDACs

DNA methylation in animals

- In animals DNA methylation occurs exclusively in CpG context and is catalized by two classes of DNA methyltransferases (DNMT): „maintenance" and „*de novo*"
- „Maintenance" DNM is responsible for preservation of methylation in its original place after each round of replication. In mammals this is done by DNMT1 methyltransferase – its knocking-out is embryo-lethal;
- „*de novo*" DNMT defines the pattern of DNA methylation in the early developmental stages. In mammals this process is executed by DNMT3a and DNMT3b

During replication epigenetic changes may be inherited



5’-CpG-3’
3’-GpC-5’

- Following replication each DNA strand contains preserved methylation pattern
- Its copying is automatic because DNA sequences are palindromic
- After fertilization the whole pattern of DNA methylation is removed and form de novo in successive generations

DNA methylation in plants

- In plants DNA methylation happens both on CpG dinucleotides but also on CpHpG and CpHpH (H is any nucleotide but G),
- The most important methyltransferase in plantsare MET1 (maintenance), DRM2 (de novo) and CMT3 (mainly maintenance);
- Mathyltransferase mutants in plants are not lethal, but have a severe phenotypes

RdDM – RNA-directed DNA methylation

- In plants de novo DNA methylation is directed by RdDM;
- This process is based on utilizing the products of viral RNA degradation (based on RNAi process, mostly siRNA and microRNA) – this degraded RNA is used as a template to silenced chromatin

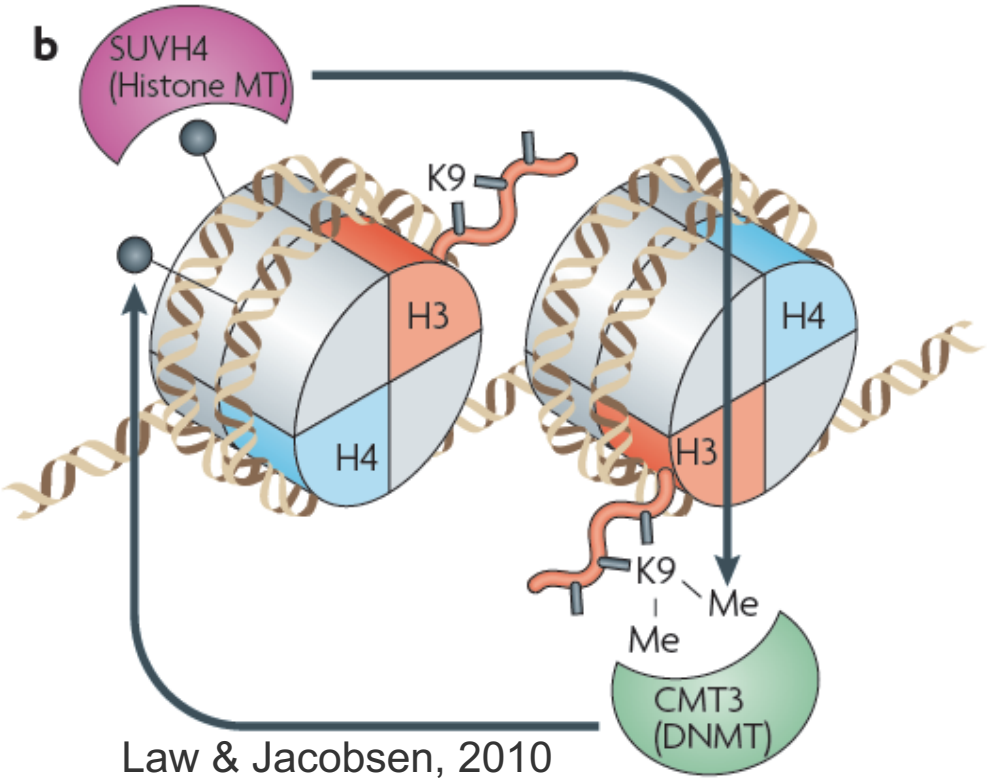
Genomic imprinting

- This process is based on selective expression of particular genes from an allele derived from one or the other parent, while the other allele is silenced;
- The basic mechanism responsible for this process involves DNA methylation;
- During gametogenesis the pattern of methylation is removed and the new pattern is formed dependently on the sex;
- In some cases the parental allele is silenced, in some other the maternal allele is silenced;
- In human 1% of genes is subject to imprinting.

DNA methylation and histone modifications cooperate to maintain gene silencing in other-than CpG contexts

- Epigenetic mechanisms are interlinked to support proper chromatin states;
- The best known example includes a cross-talk between DNA methylation and H3 and H4 deacetylation:
- DNA methylation in gene promoters caused by RdDM causes recruitment of HDA6 histone demethylase, which in turn deacetylases H3 and H4; Following HDA6 knocking-out, DNA methylation is no longer maintained;
- Similarly, maintenance of CpHpG and CpHpH requires H3K9 methylation by Kryptonite methyltransferase; Kryptonite recognizes specifically methylated DNA from „*de novo*" methylation

DNA methylation and histone modifications cooperate to maintain gene silencing in other-than CpG contexts

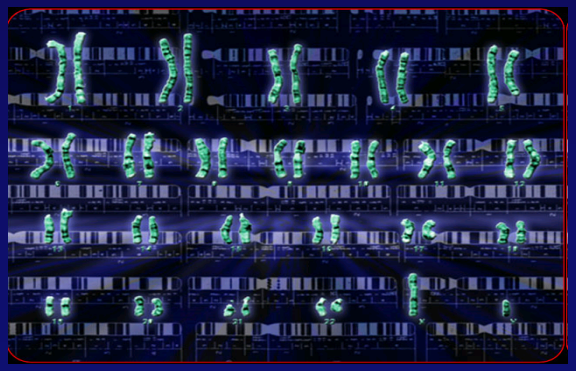


Law & Jacobsen, 2010

Conclusion

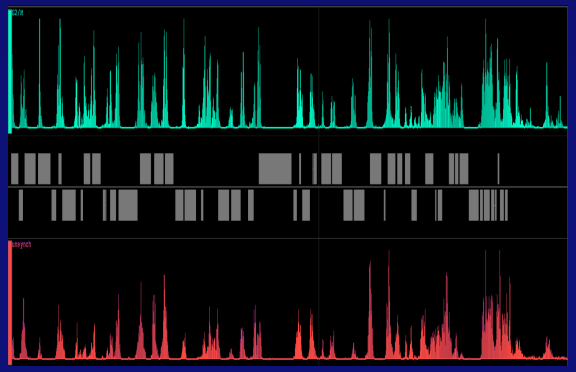
Epigenetics is necessary for proper gene expression and forms an additional source of information (besides genetic information)

- Genome



- Library

- Epigenome



- Folder