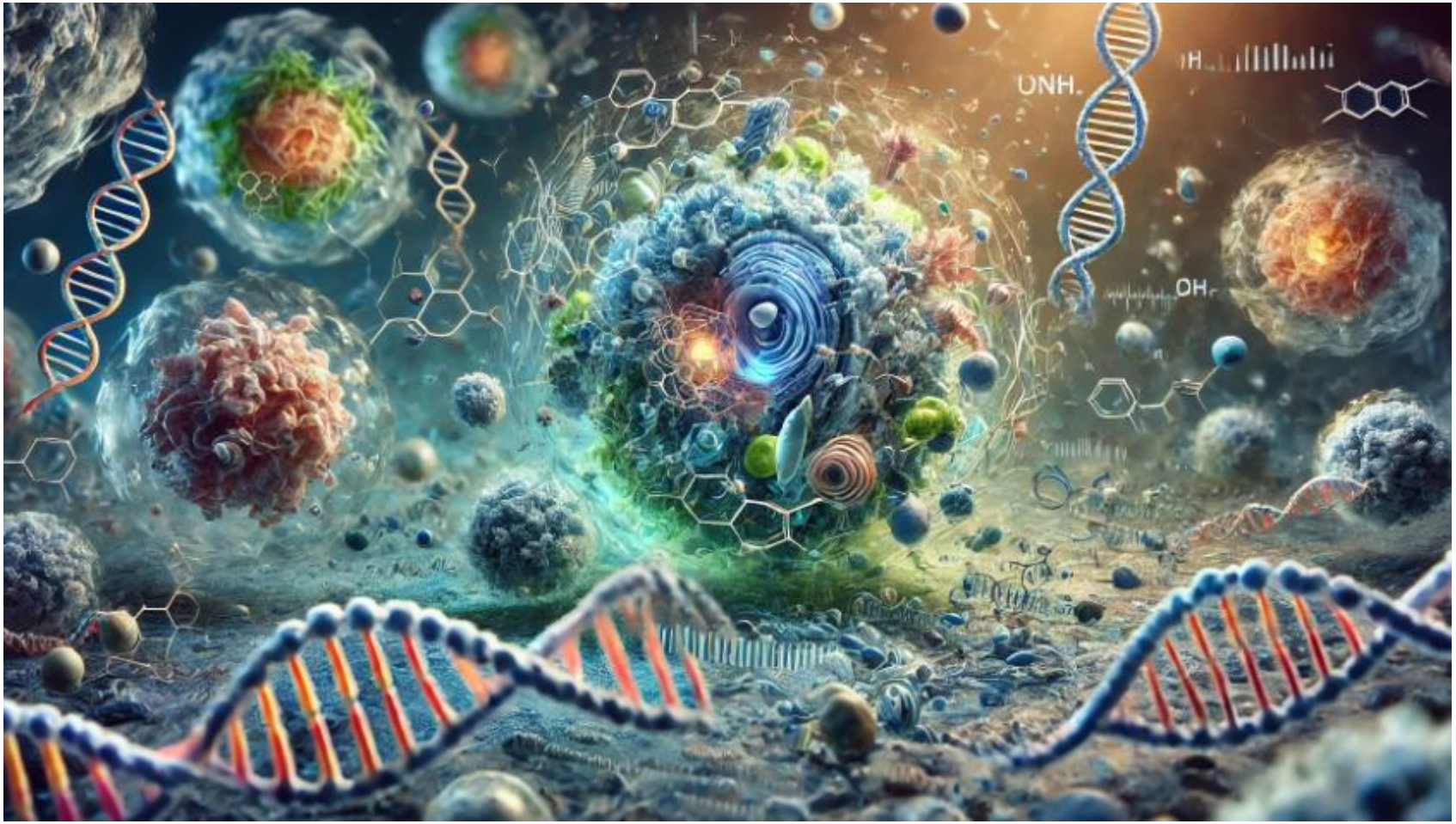
Transcription and Translation Regulation; Cell Differentiation



Ewa Jankowska-Steifer

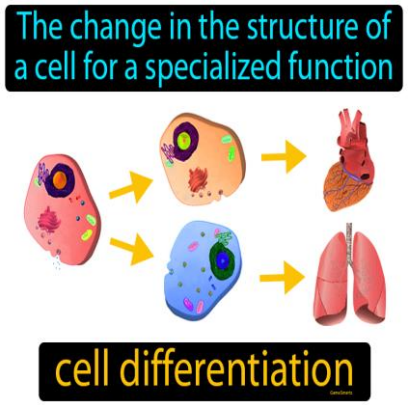
CELL DIFFERENTIATION

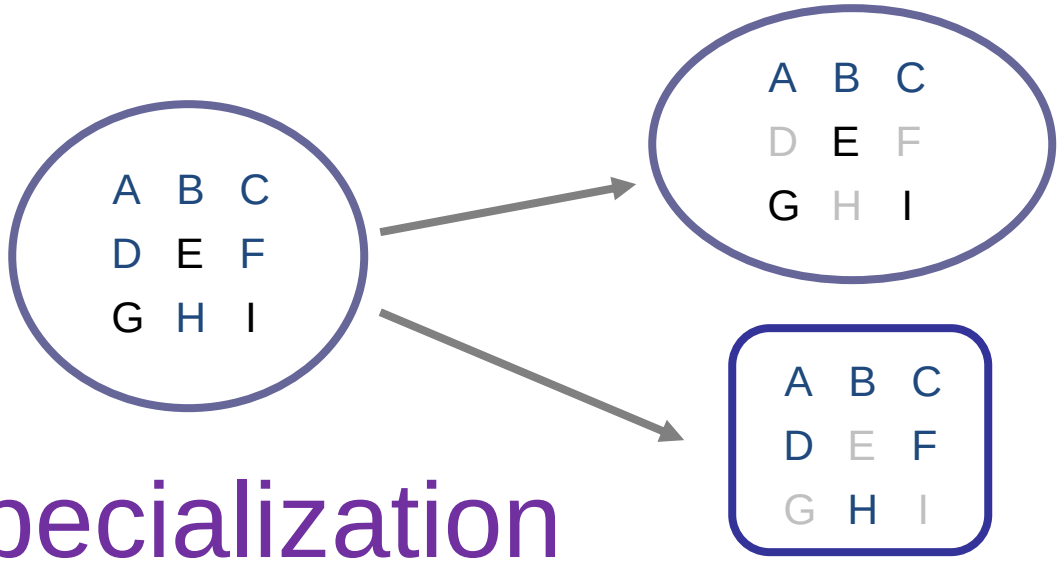
a cell changes from one cell type to another

a less specialized type becoming a more specialized type

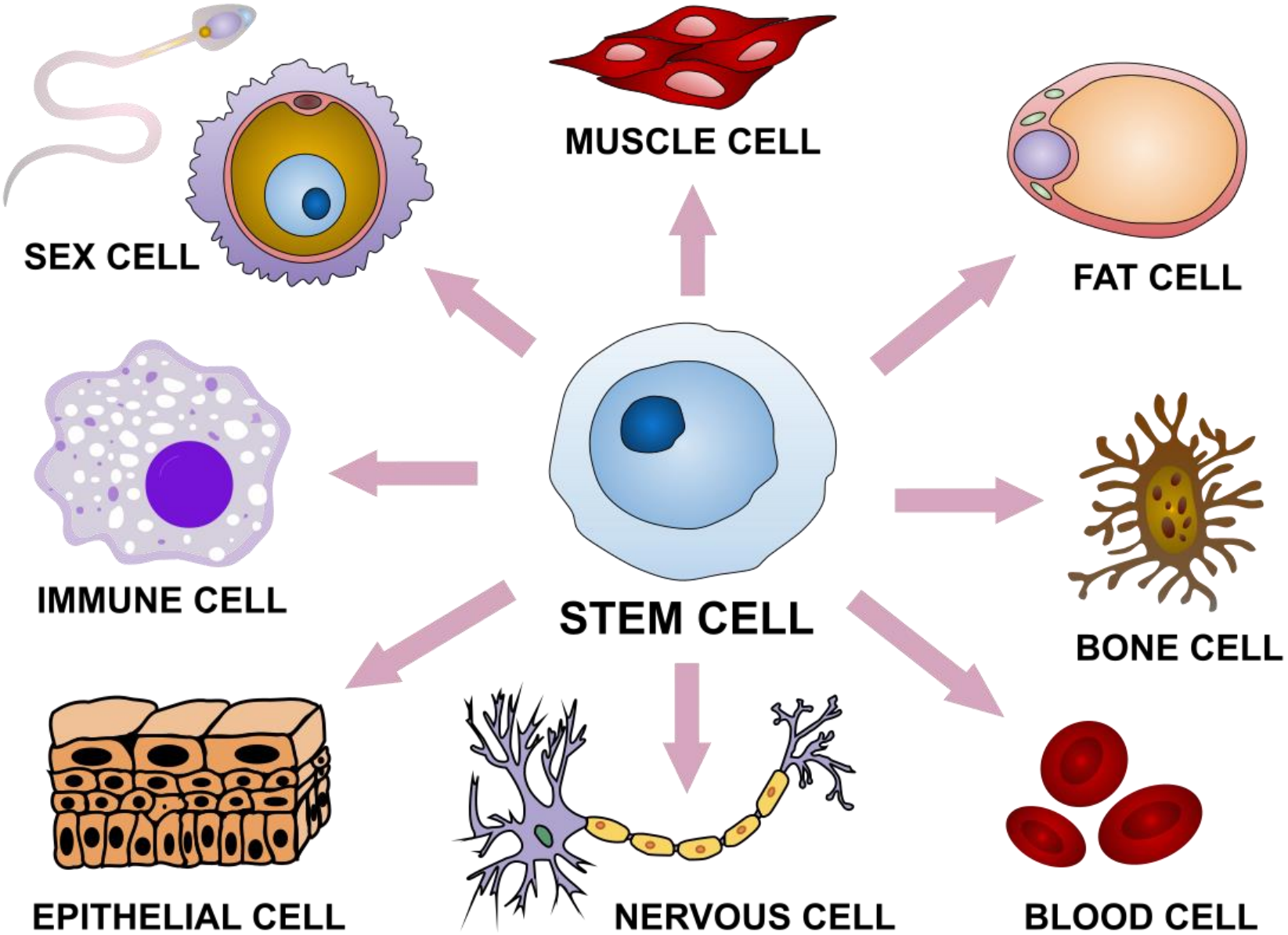
The phenomenon of gradual structural and functional specialization of cells resulting from changes in gene expression patterns of the progeny

The gradual reduction of the developmental ability (potency) subsequent generations of cells

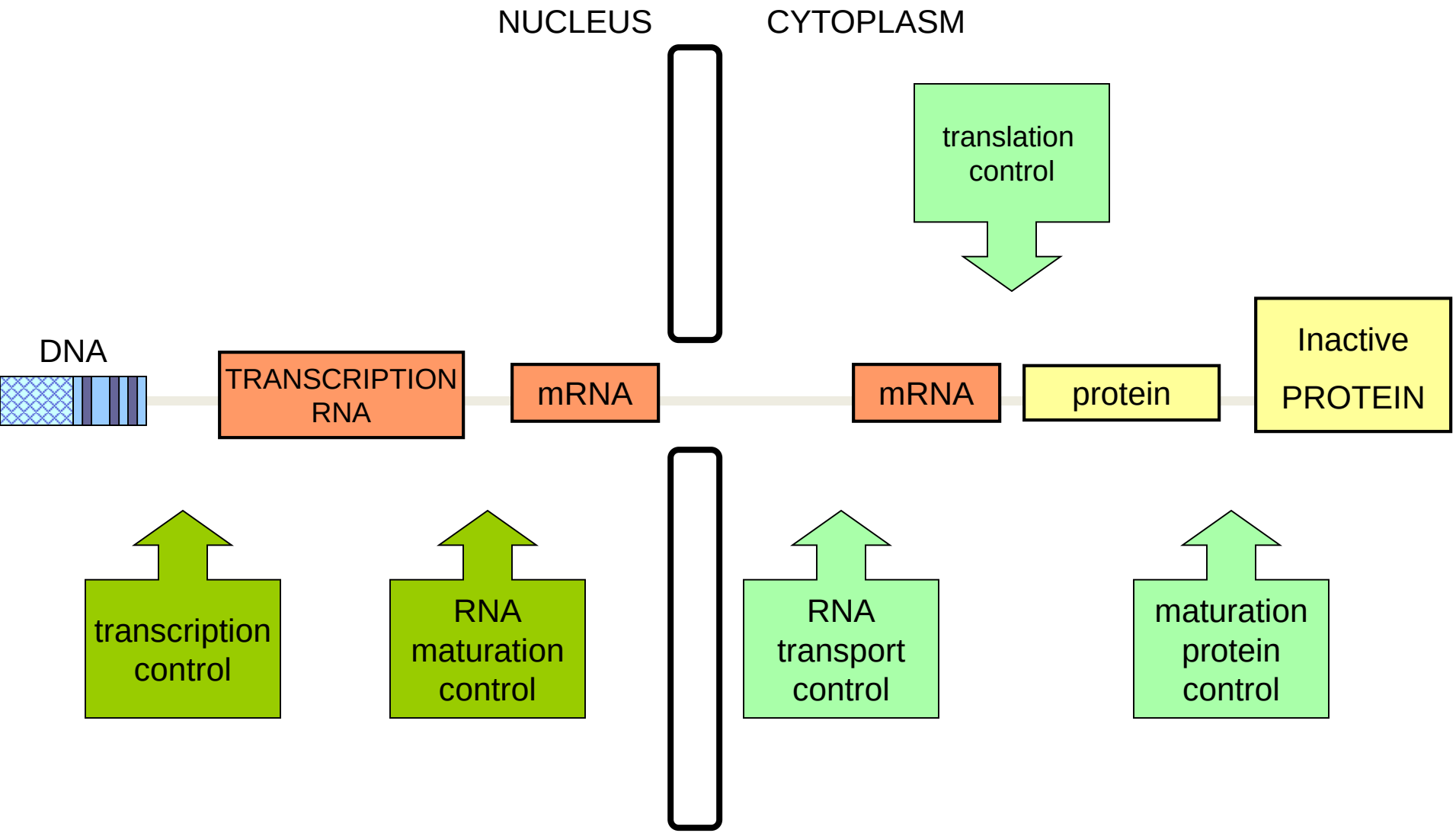




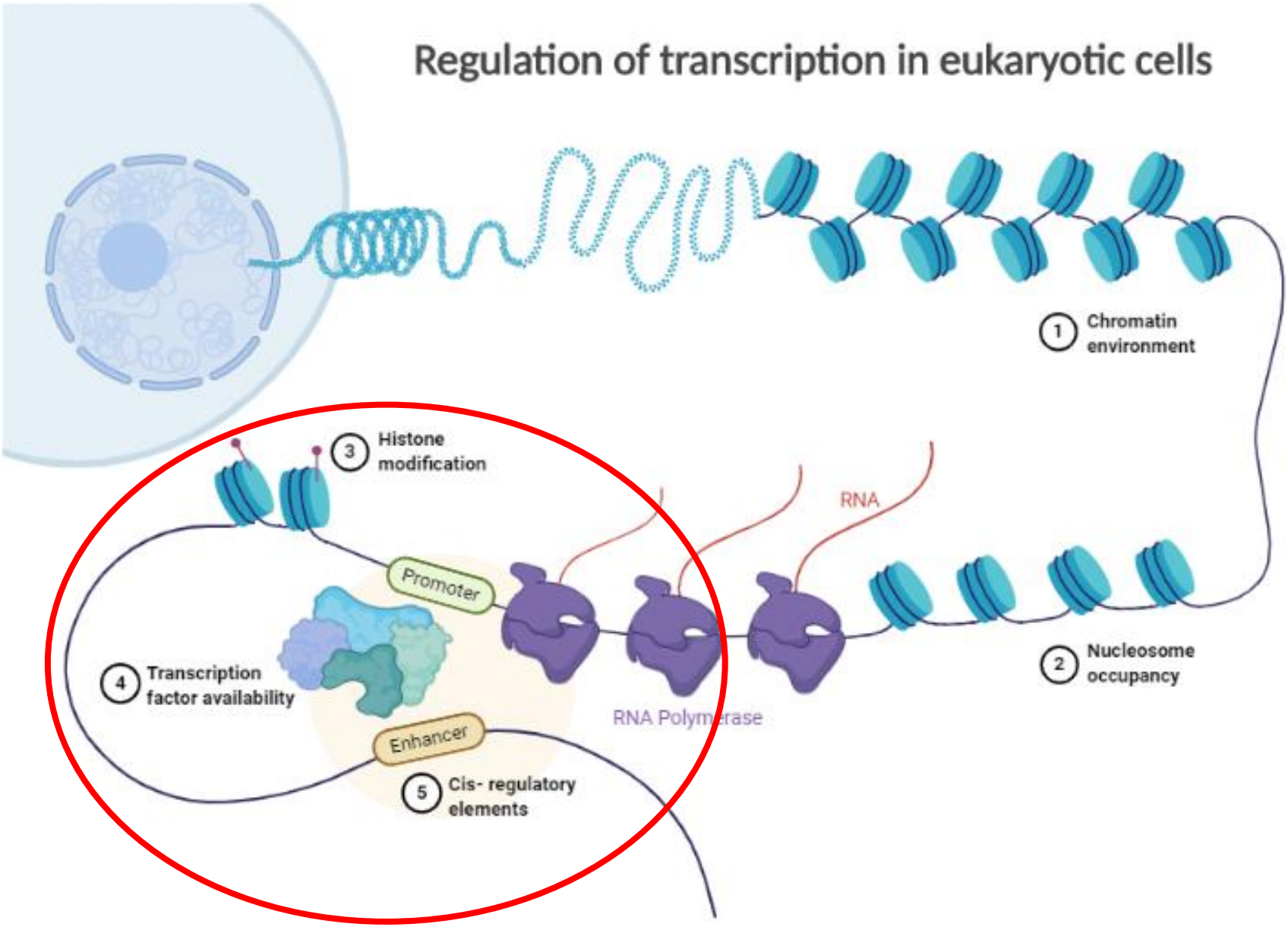
Cell specialization


SEX CELL
MUSCLE CELL
FAT CELL
IMMUNE CELL
STEM CELL
BONE CELL
EPITHELIAL CELL
NERVOUS CELL
BLOOD CELL

STEPS OF GENE EXPRESSION CONTROL

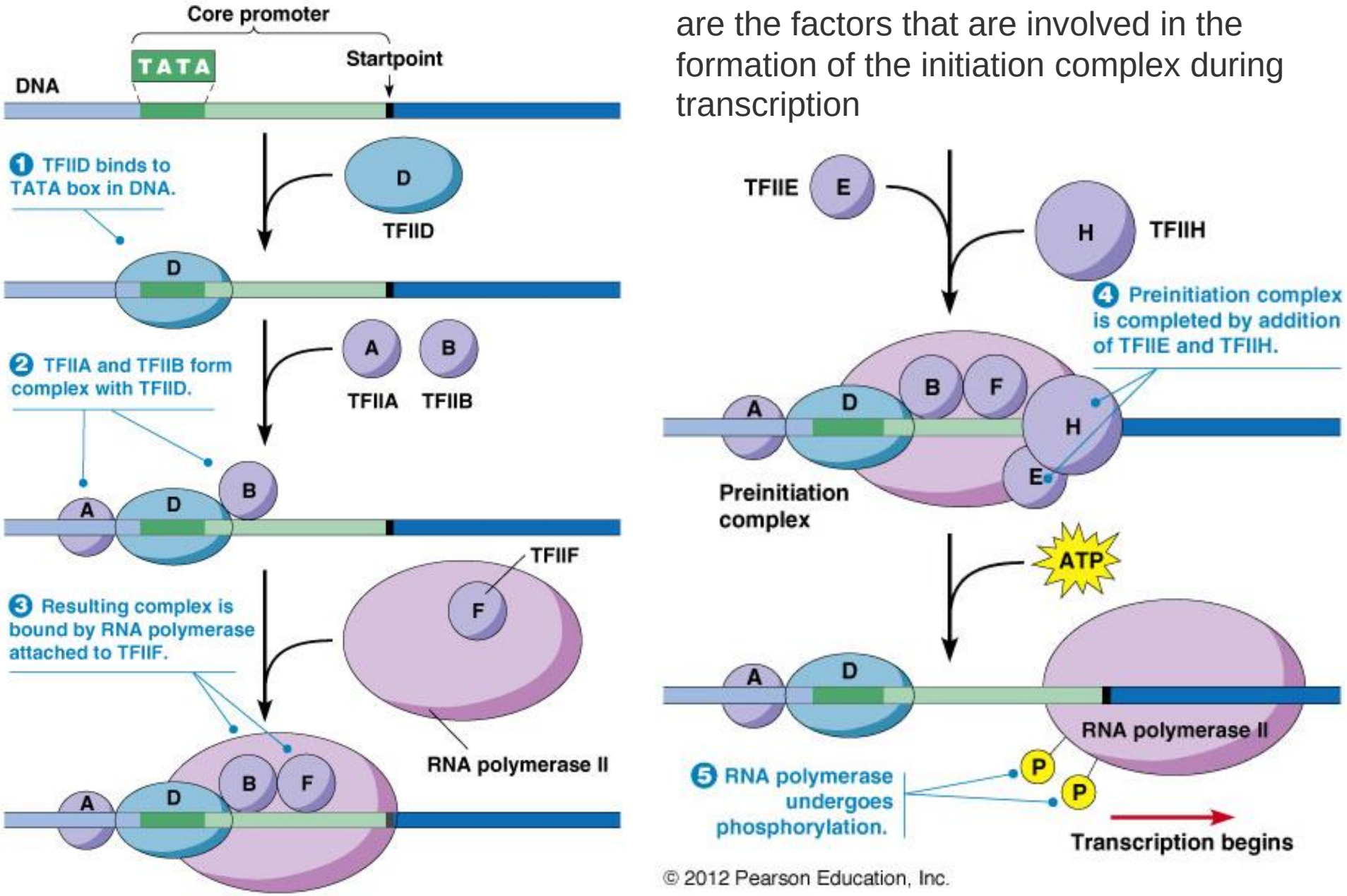


Regulation of transcription in eukaryotic cells

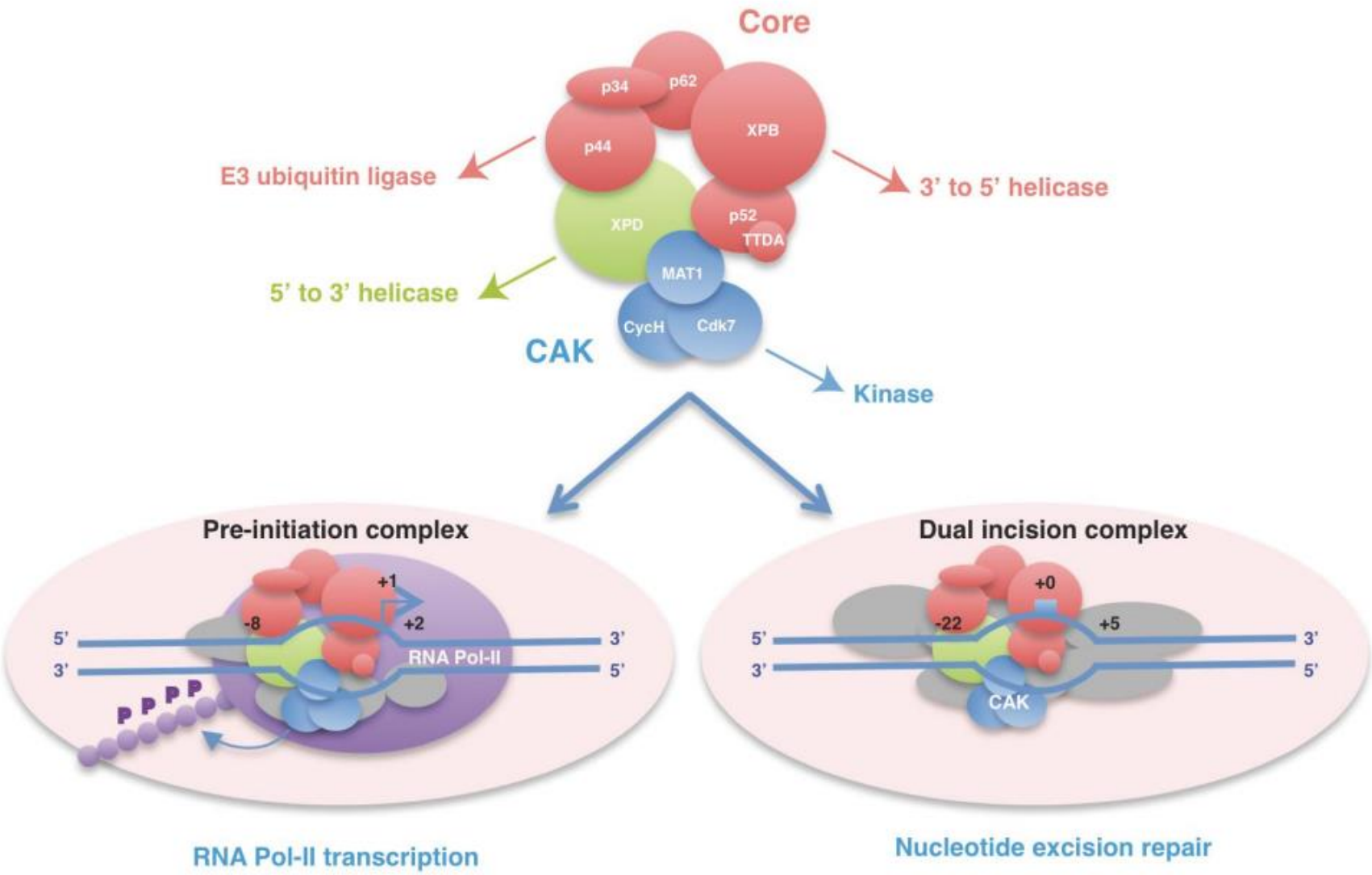


General transcription factors

are the factors that are involved in the formation of the initiation complex during transcription



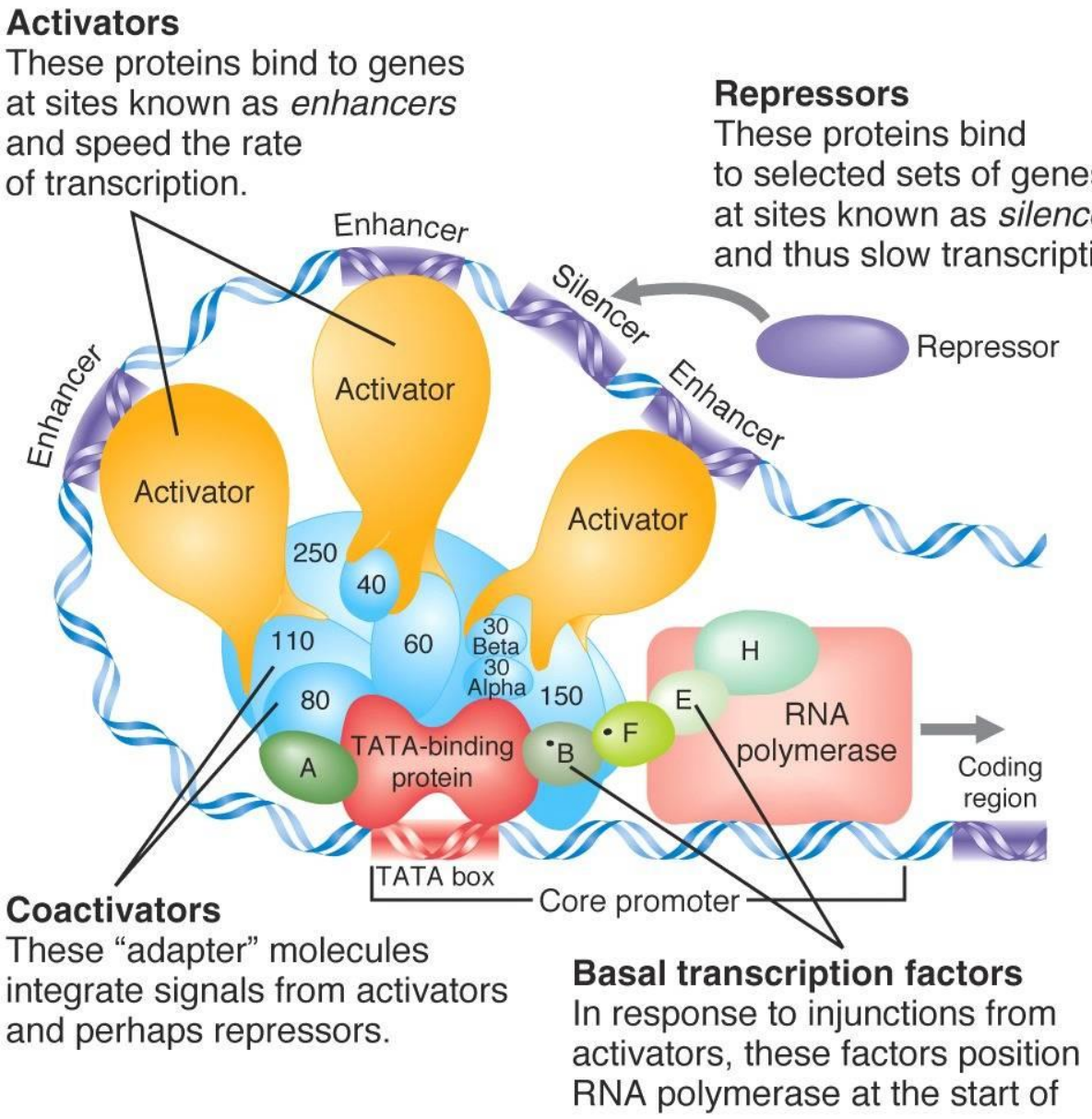
TFIIH



The transcription direction is 5' → 3'

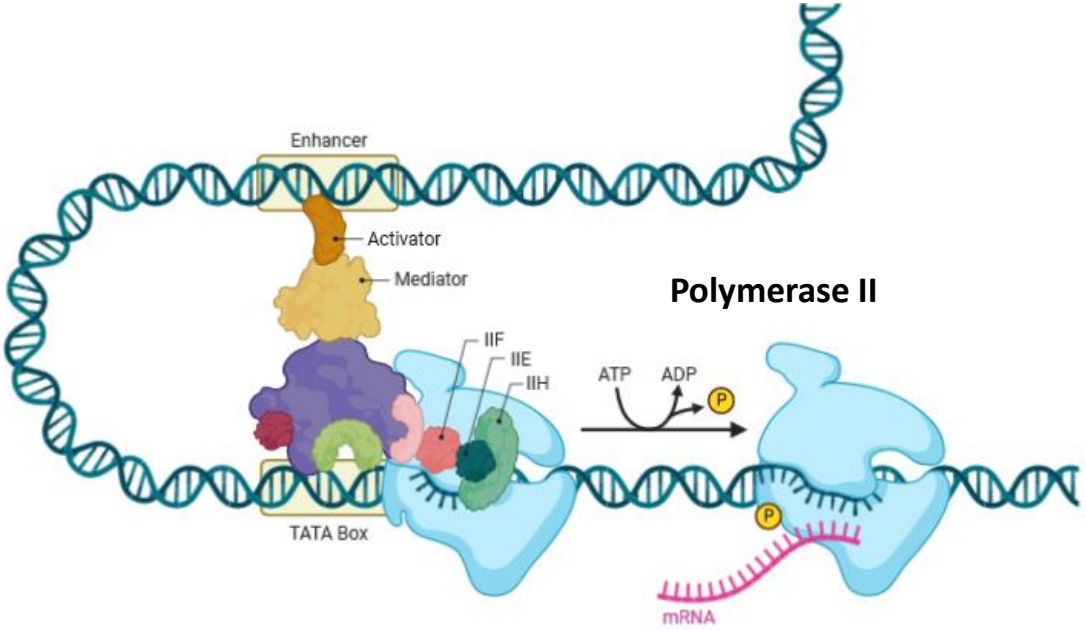
Cancer 2016; 7(15):2258-2265. doi:10.7150/jca.16966

Specific TF activate or repress transcription initiation depending on their precise position



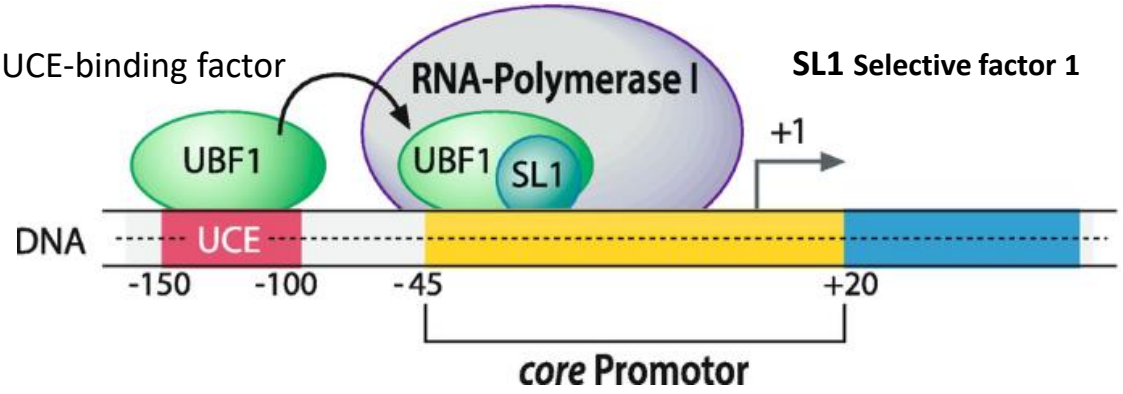
Types of RNA polymerases and their functions

Polymerase RNA	MAIN PRODUCTS	FUNCTION	LOCATION IN CELL
Polymerase I	rRNA (28S, 18S, 5.8S)	Ribosomal RNA synthesis	Nucleolus
Polymerase II	mRNA, snRNA, miRNA, LncRNA	Transcription of protein-coding genes and regulatory RNAs	Nucleoplasm
Polymerase III	tRNA, 5S rRNA, snRNA	Synthesis of transfer RNA and ribosomal 5S RNA, small nuclear RNA	Nucleoplasm



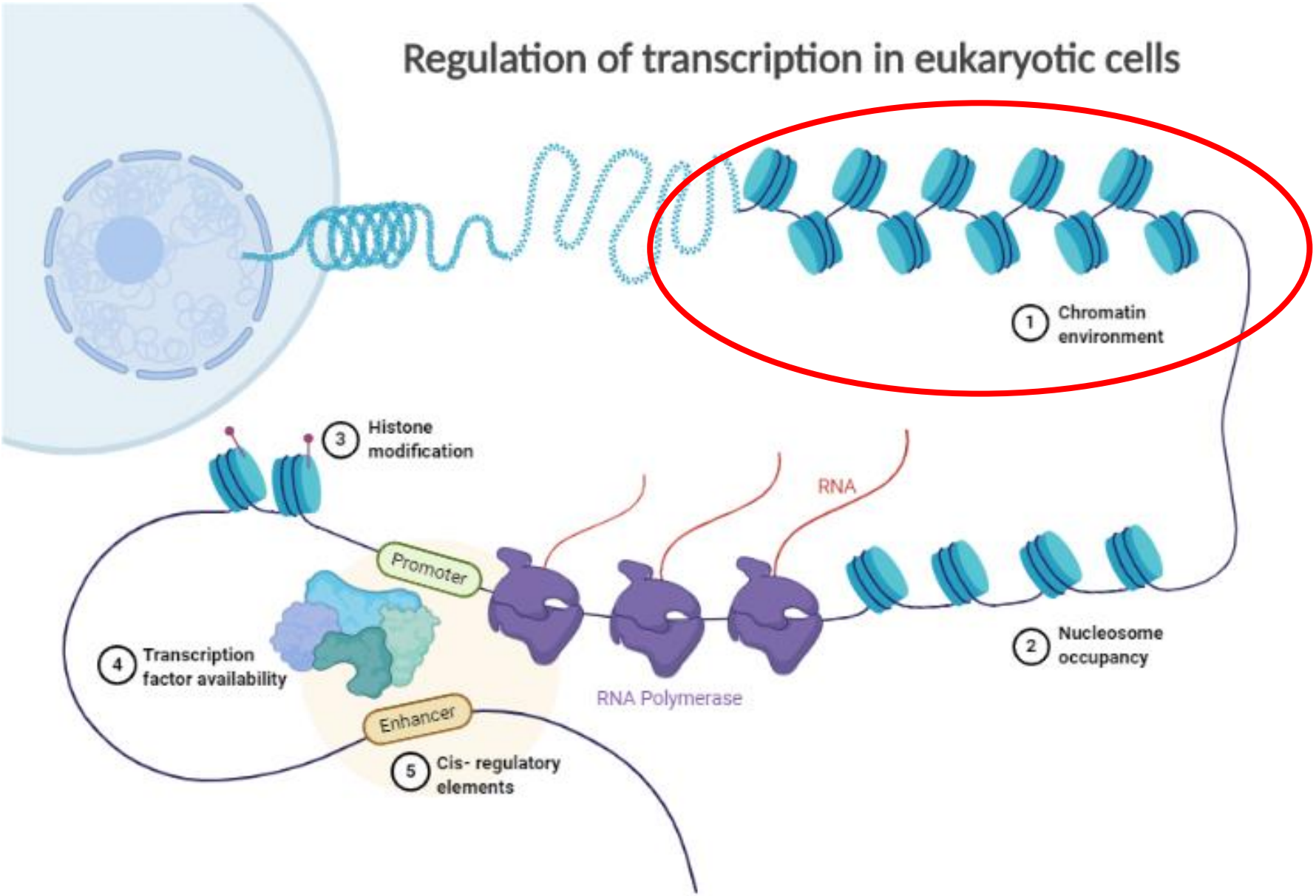
TBP - TATA-binding protein

selectivity factor SL1 is an important target for the regulation of pol I transcription during cell differentiation

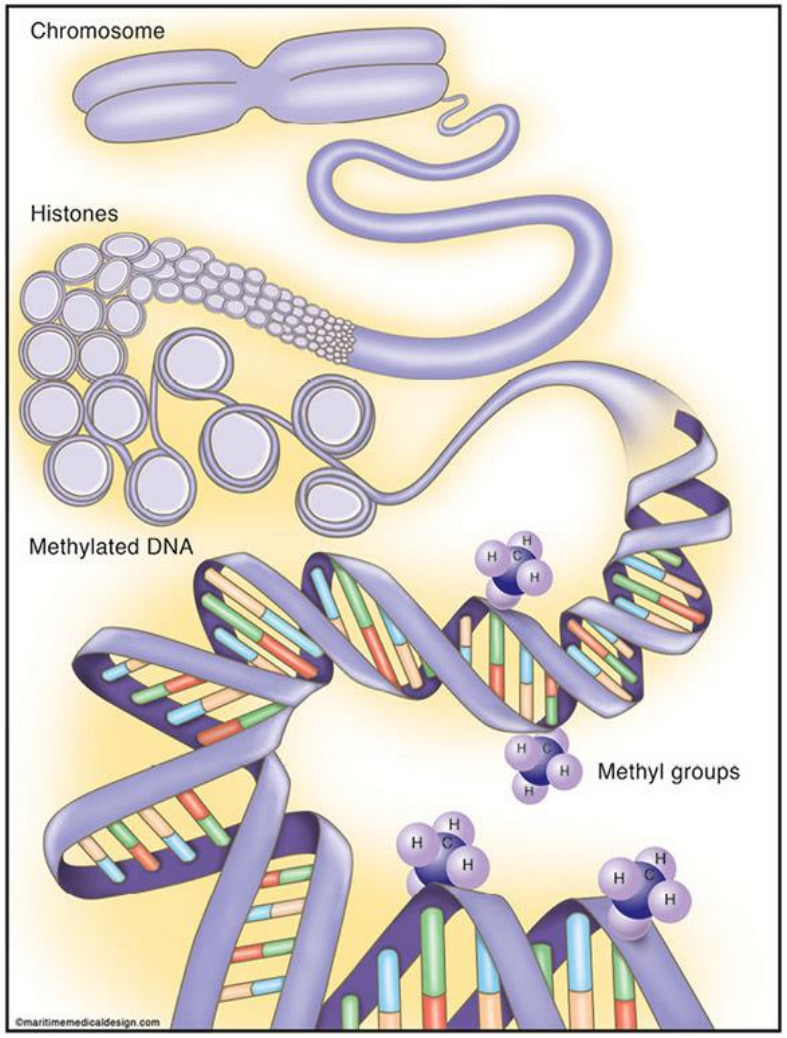


Upstream control element

Regulation of transcription in eukaryotic cells



EPIGENETIC



♦ Changes in gene expression resulting from the modification of chromatin without change in the DNA sequence

♦ The hereditary nature of the modification - does not change change after DNA replication

Cellular memory

Epigenetic memory

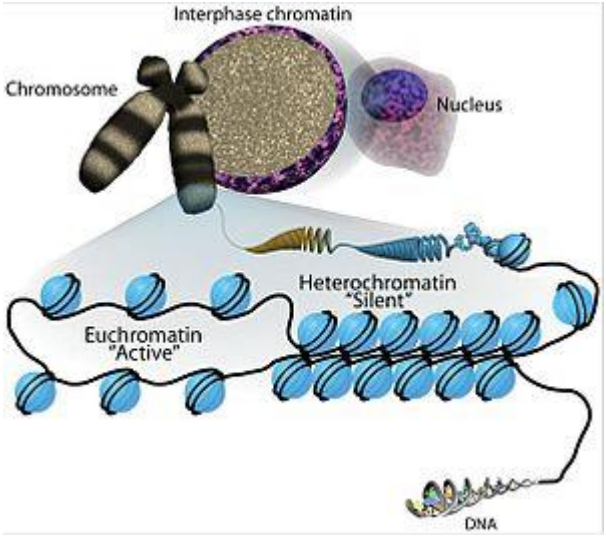
♦ mechanisms responsible for maintenance fixed pattern of gene expression

♦involving e.g:
protein complexes
♦TRITHORAX - Transcriptional activators
POLYCOMB - Transcriptional inhibitors

Heterochromatin protein 1 - HP1

Biochemical basis of epigenetic memory

Memory storage - DNA and proteins interacting with DNA



- DNA metylation
- Modification of histone proteins
  - methylation ME
  - acetylation AC (low stable)
  - phosphorylation (low stable)
  - ubiquitination
  - sumoylation

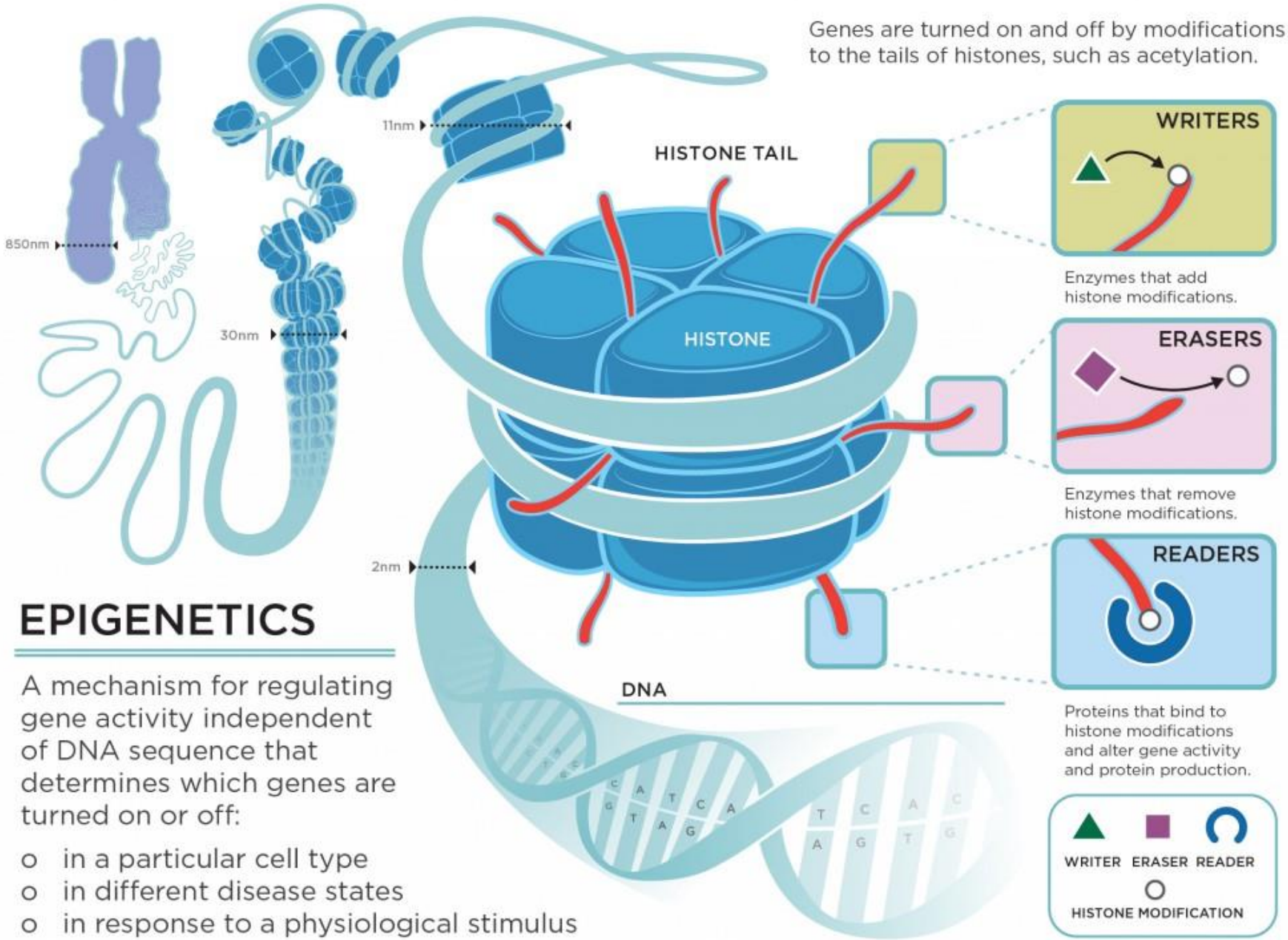
Modification meaning: structural
informational

epigenetic code

Various modifications of histones comprising the collection of information managing the various manifestations of the activity of the genome

The code is read by specific protein domains
leading to differential expression of a particular gene

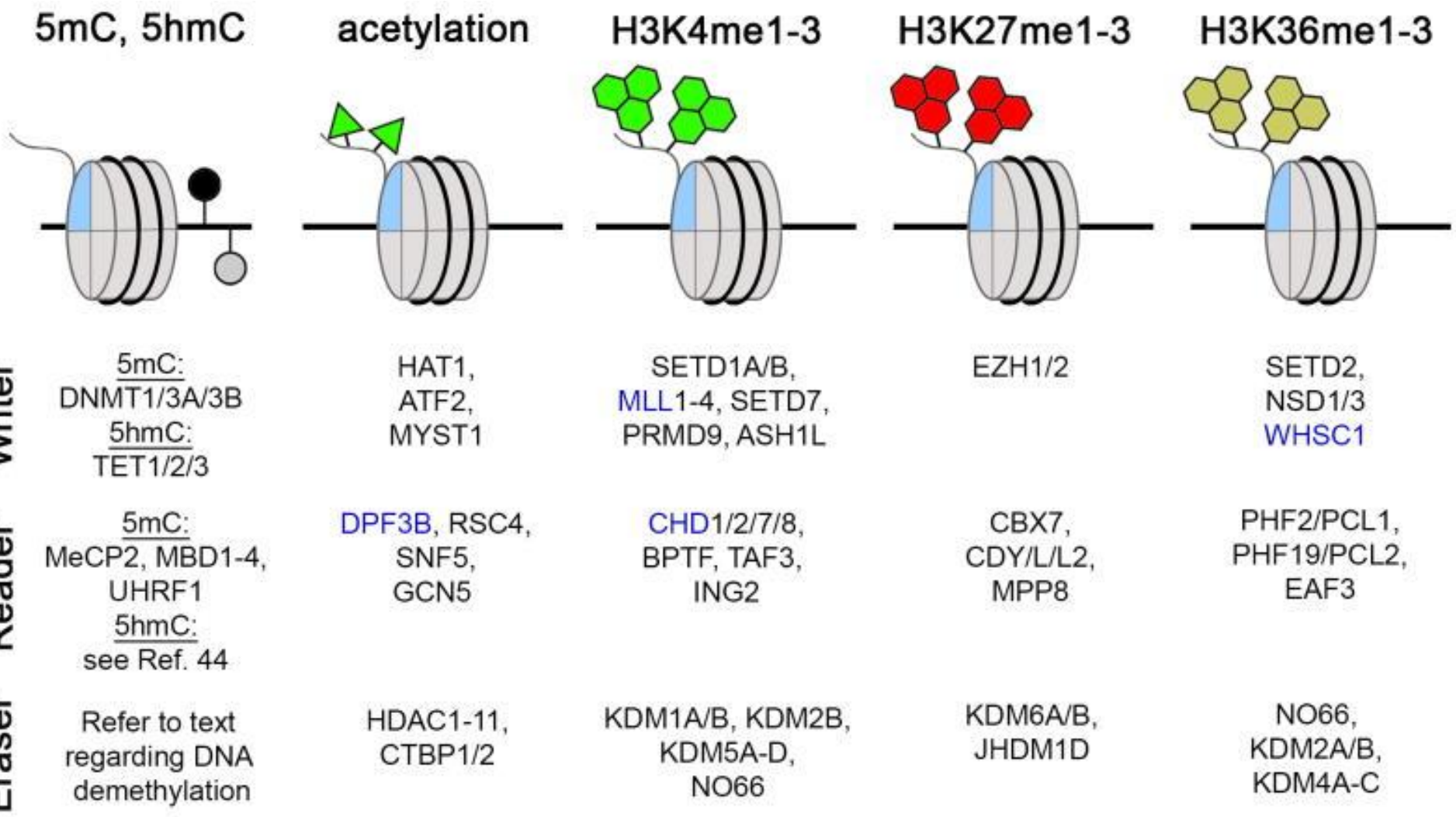
CHROMOSOME | CHROMATIN FIBRE | NUCLEOSOME



EPIGENETICS

A mechanism for regulating gene activity independent of DNA sequence that determines which genes are turned on or off:

- in a particular cell type
- in different disease states
- in response to a physiological stimulus



Proteins denoted in blue text exhibit heart defects when mutated.
DPF3B – Tetralogy of Fallot, MLL2- Kabuki Syndrome,
CHD7 - CHARGE Syndrome, WHSC1 - Wolf-Hirschhorn Syndrome.

Circ Res. 2014 Jul 7; 115(2): 311–324.
doi: 10.1161/CIRCRESAHA.115.301517

DNA methylation

- covalent addition of a methyl group at the 5-carbon of cytosine ring
resulting in 5-methylcytosine (5-mC)
In human 5-mC is found in approximately 1.5% of genomic DNA

- DNA Methyltransferase 1 (DNMT1) replication fork component,
copies the methylation pattern after replication (methylation maintenance)

- DNA Methyltransferase 3a (DNMT3a) and 3b (DNMT3b)
DNA methylation *de novo* -
set up DNA methylation patterns early in development and during cell differentiation

- Demethylation: mechanism ?

with methyl CpG sequences interact proteins containing binding domains
Me-CpG
eg: MECPs (Methyl-CpG binding Proteins)
leading to the modification of chromatin structure surrounding the methylated DNA

histones methylation

- Methylotransferase (HMT) - specific for histone H3

- lysine residues (K) of histon H3 can be methylated position K4, K9, K27 and K36

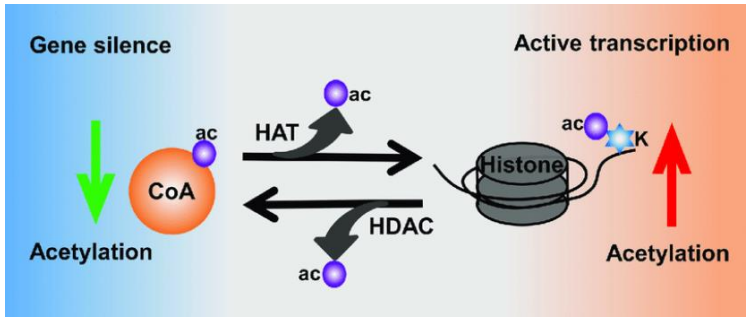
- K4 and K36 Methylation is connected to the activation of transcription
  promoters currently being transcribed genes

- K9 and K27 methylation is connected to the repression of transcription
  areas of heterochromatin in the centromeres and telomeres,
  inactive X chromosome, silenced gene promoters

- The methylation of H3-K9 allows the connection of proteins containing chromodomein heterochromatin protein 1 (HP1) responsible for the creation of a stable heterochromatin

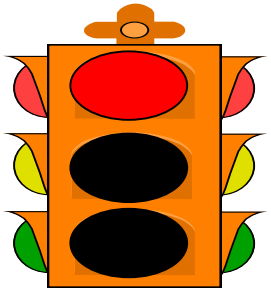
Acetylation

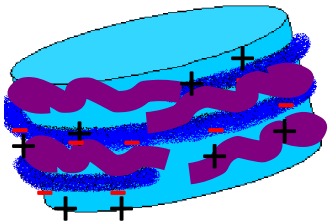
- Reversible phenomenon, consisting of the connection of the acetyl residue with the amino - terminal lysine (basic lysine residues - the positive charge)



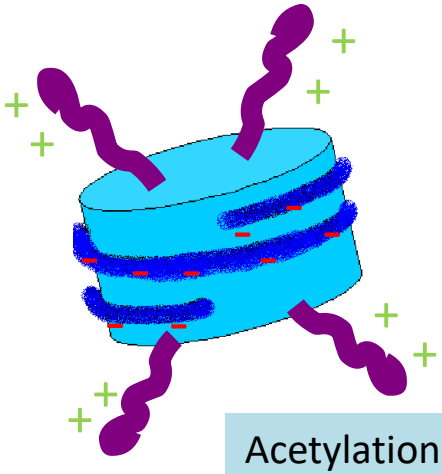
- Histone acetyltransferases (HATs) are enzymes that acetylate conserved lysine amino acids on histone proteins

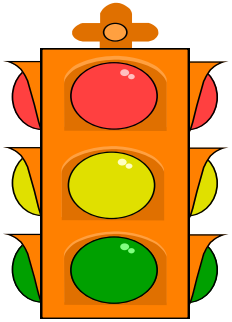
  enzymes turn-around this phenomenon are histone deacetylase (HDAC)





Lysine residues exhibit ion affinity an acidic (negatively charged) phosphate backbone of nucleosome - condensation of chromatin



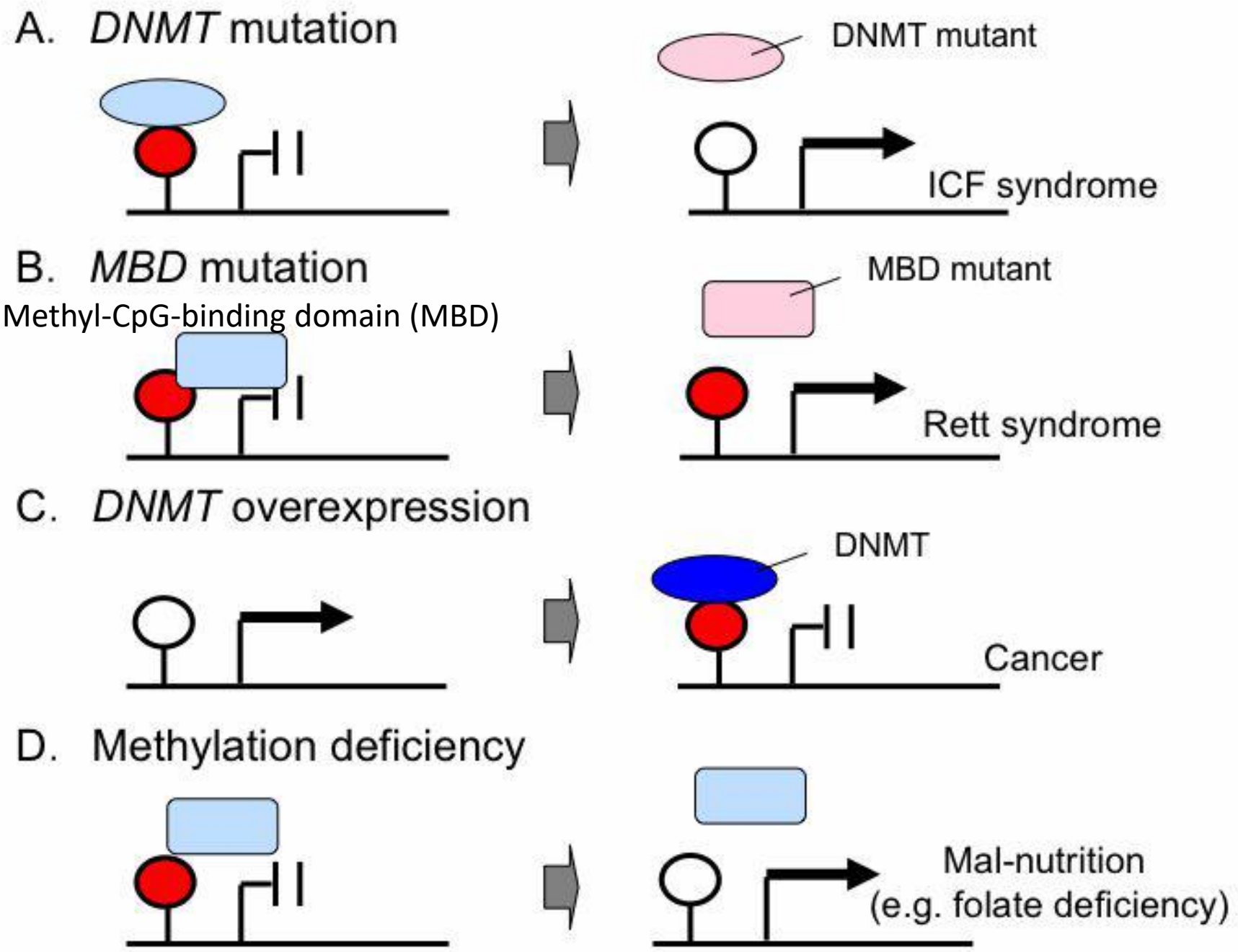


Acetylation neutralizes the positive charge of lysine residues

CONSEQUENCES of epigenetic ERRORS

RERSOR

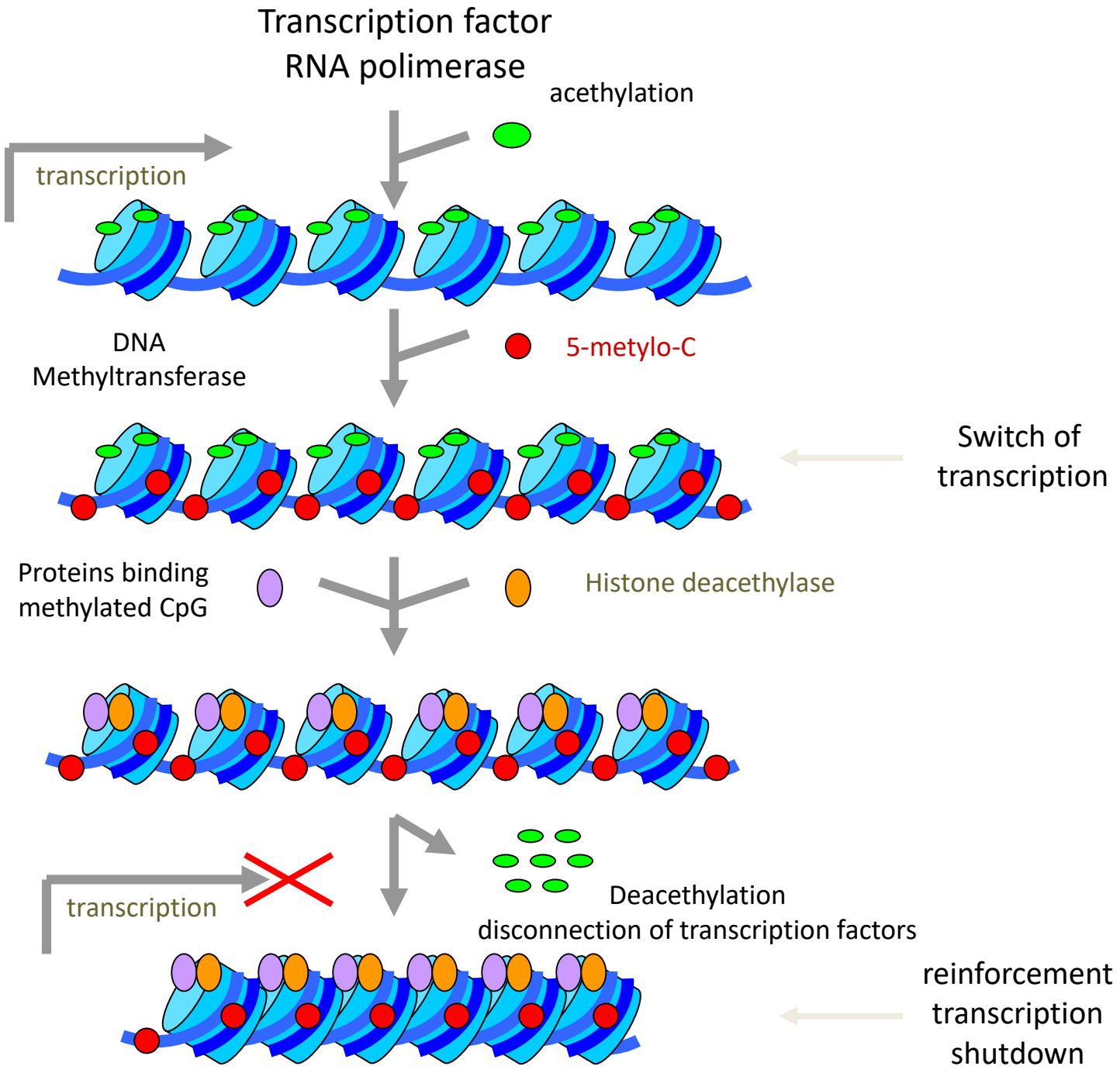
ERRORS

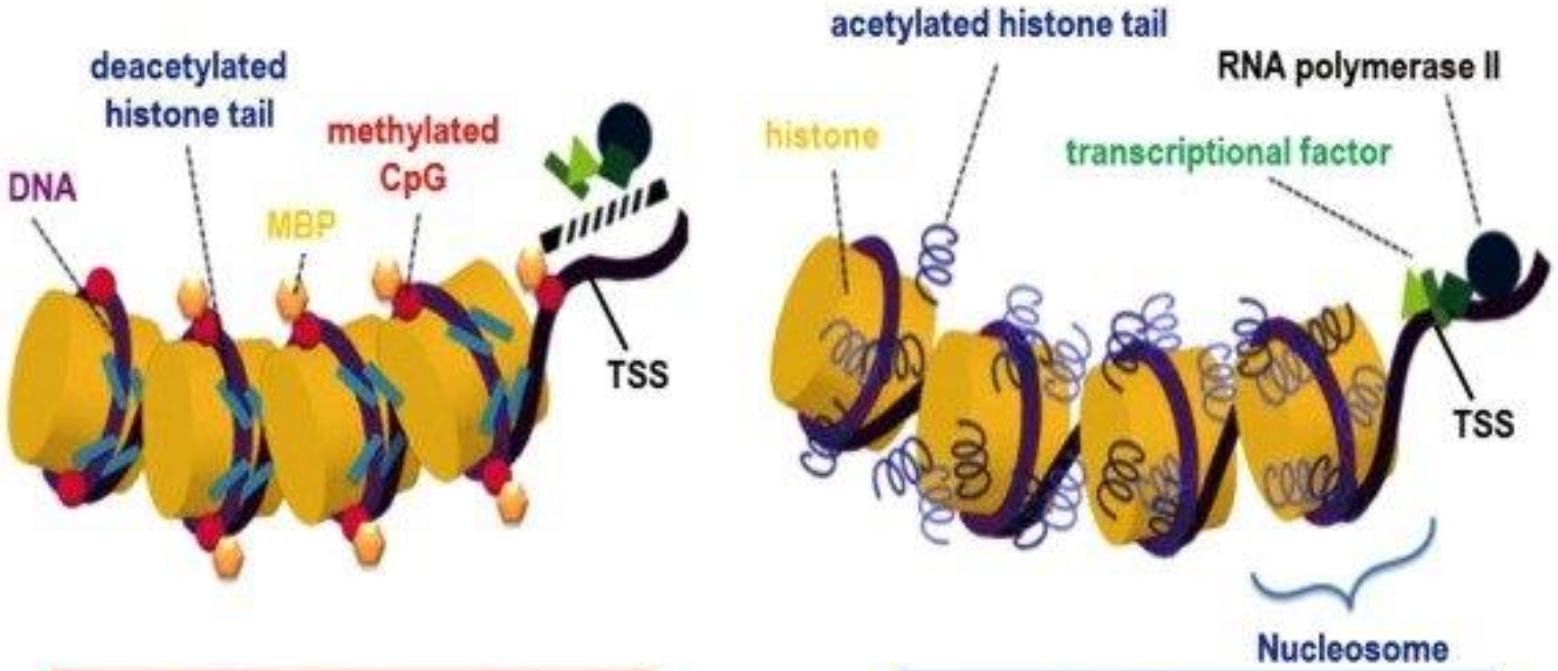

A. DNMT mutation
DNMT mutant
ICF syndrome
B. MBD mutation
Methyl-CpG-binding domain (MBD)
MBD mutant
Rett syndrome
C. DNMT overexpression
DNMT
Cancer
D. Methylation deficiency
Mal-nutrition
(e.g. folate deficiency)

Rett Syndrome

mutations in the gene MECP2 (methyl CpG binding protein 2) located on the X chromosome

a rare genetic postnatal neurological disorder of the grey matter of the brain that affects girls almost exclusively. It is characterized by normal early growth and development followed by a slowing of development, loss of purposeful use of the hands, distinctive hand movements, slowed brain and head growth, problems with walking, seizures, and intellectual disability.

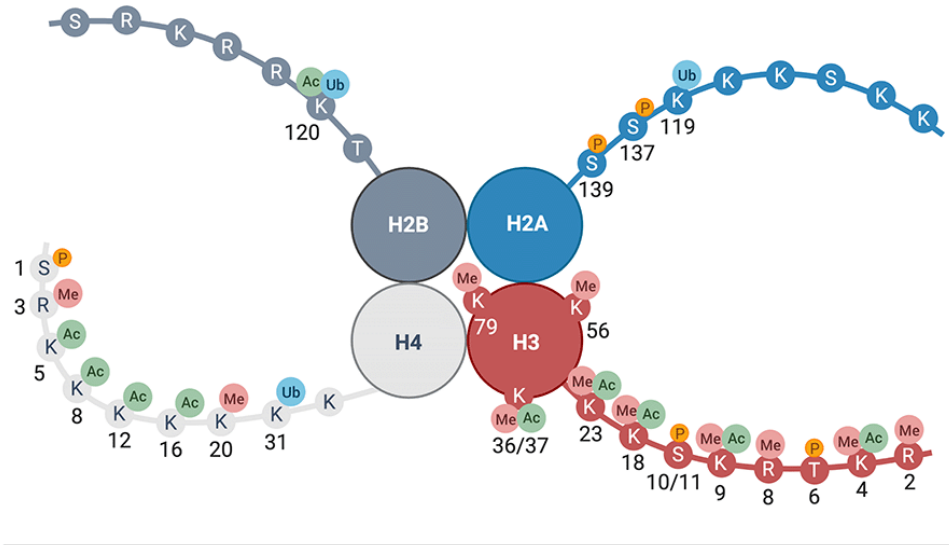

Transcription factor
RNA polimerase
acethylation
transcription
DNA
Methyltransferase
5-metylo-C
Switch of
transcription
Proteins binding
methylated CpG
Histone deacethylase
transcription
Deacethylation
disconnection of transcription factors
reinforcement
transcription
shutdown



Heterochromatin
closed chromatin
conformation
: repression

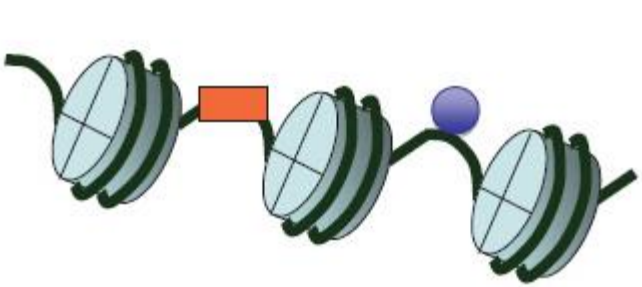
Euchromatin
open chromatin
conformation
: activation

TRANSCRIPTION



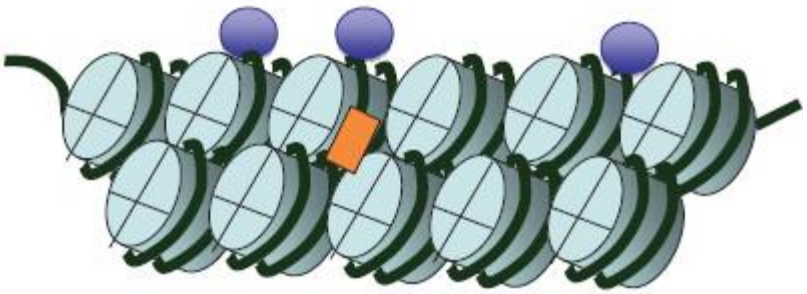
Epigenetic modification alters which genes are on or off

ON

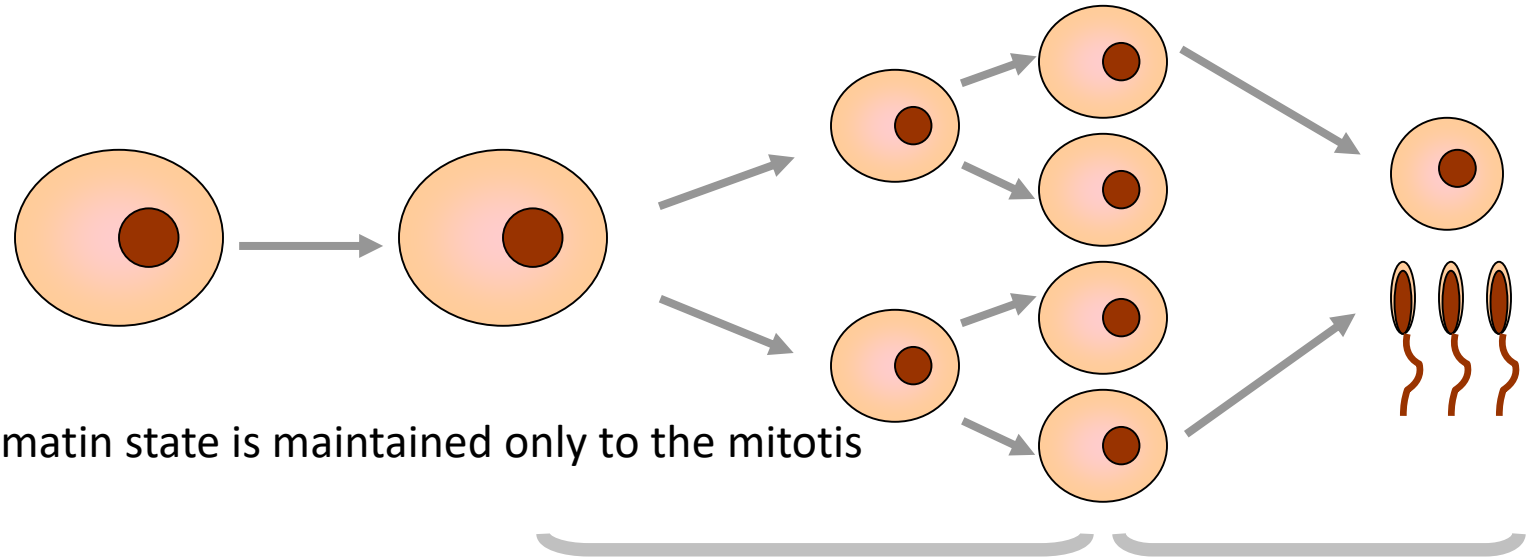


vs.

OFF



Stability of epigenetic memory



level 1

chromatin state is maintained only to the mitotic division

level 2

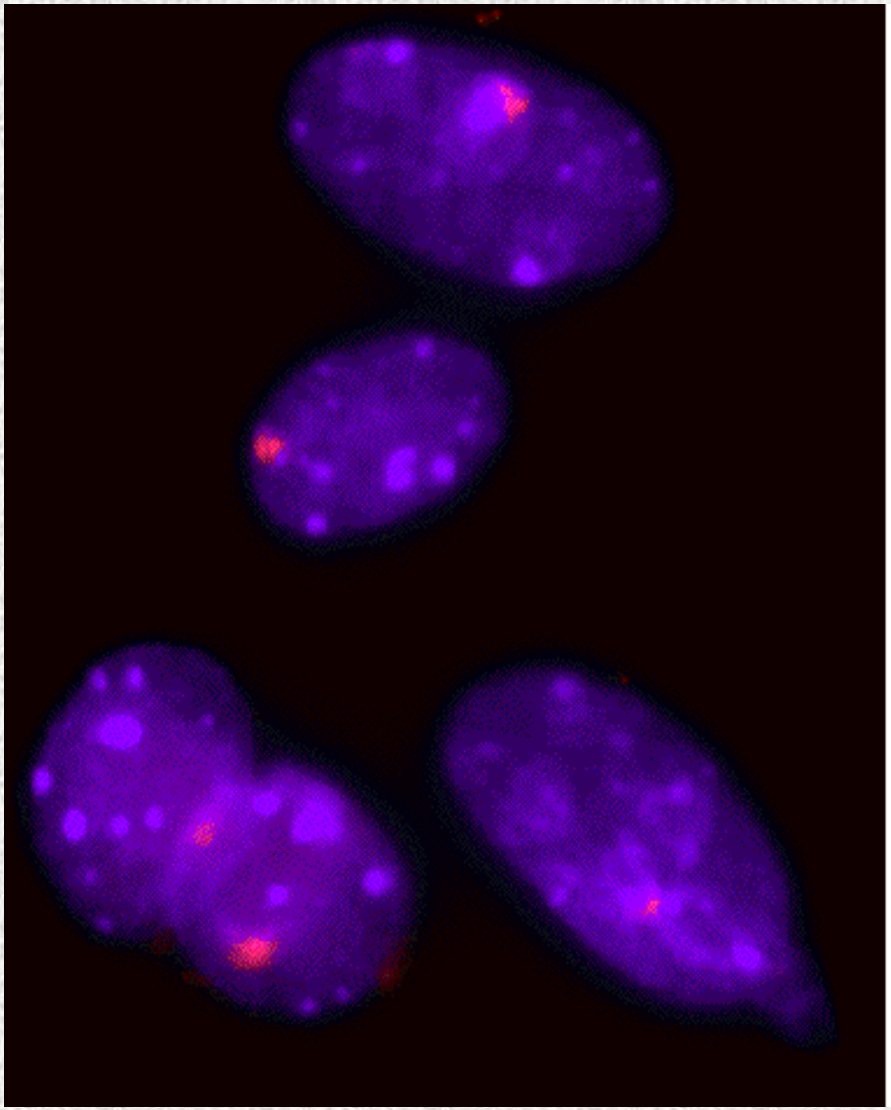
chromatin state is maintained by mitoses

Epigenetic inheritance is important in the development and differentiation

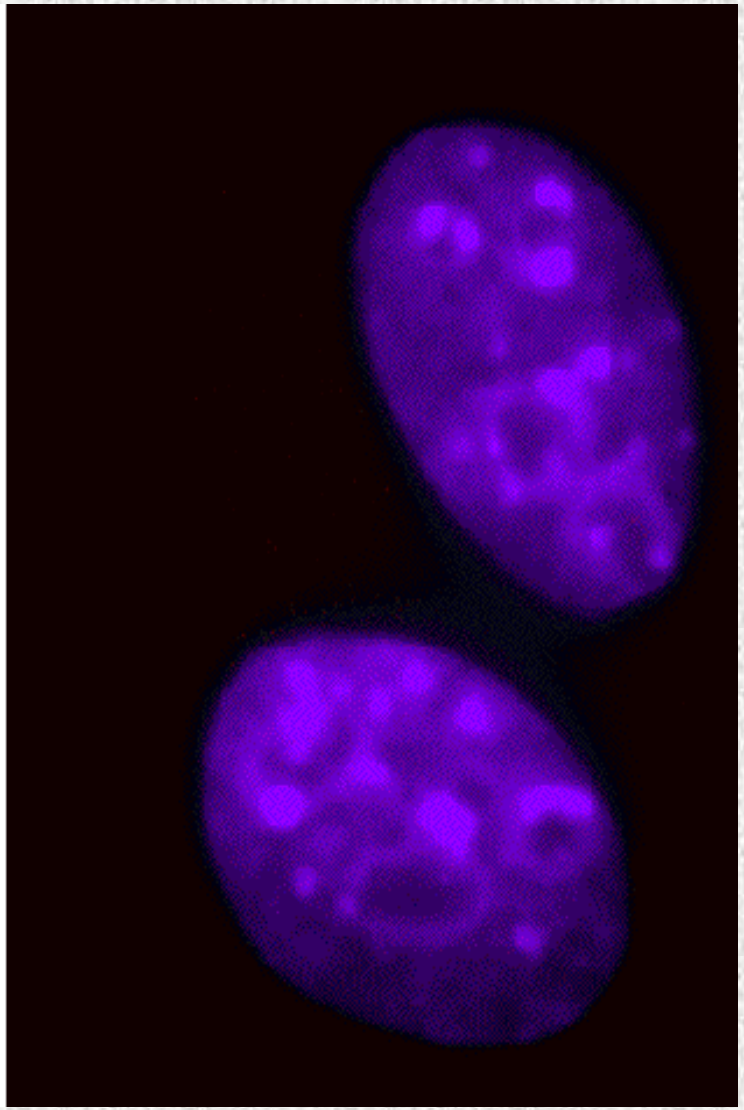
level 3

chromatin state is maintained from generation to generation

X chromosome - inactivation

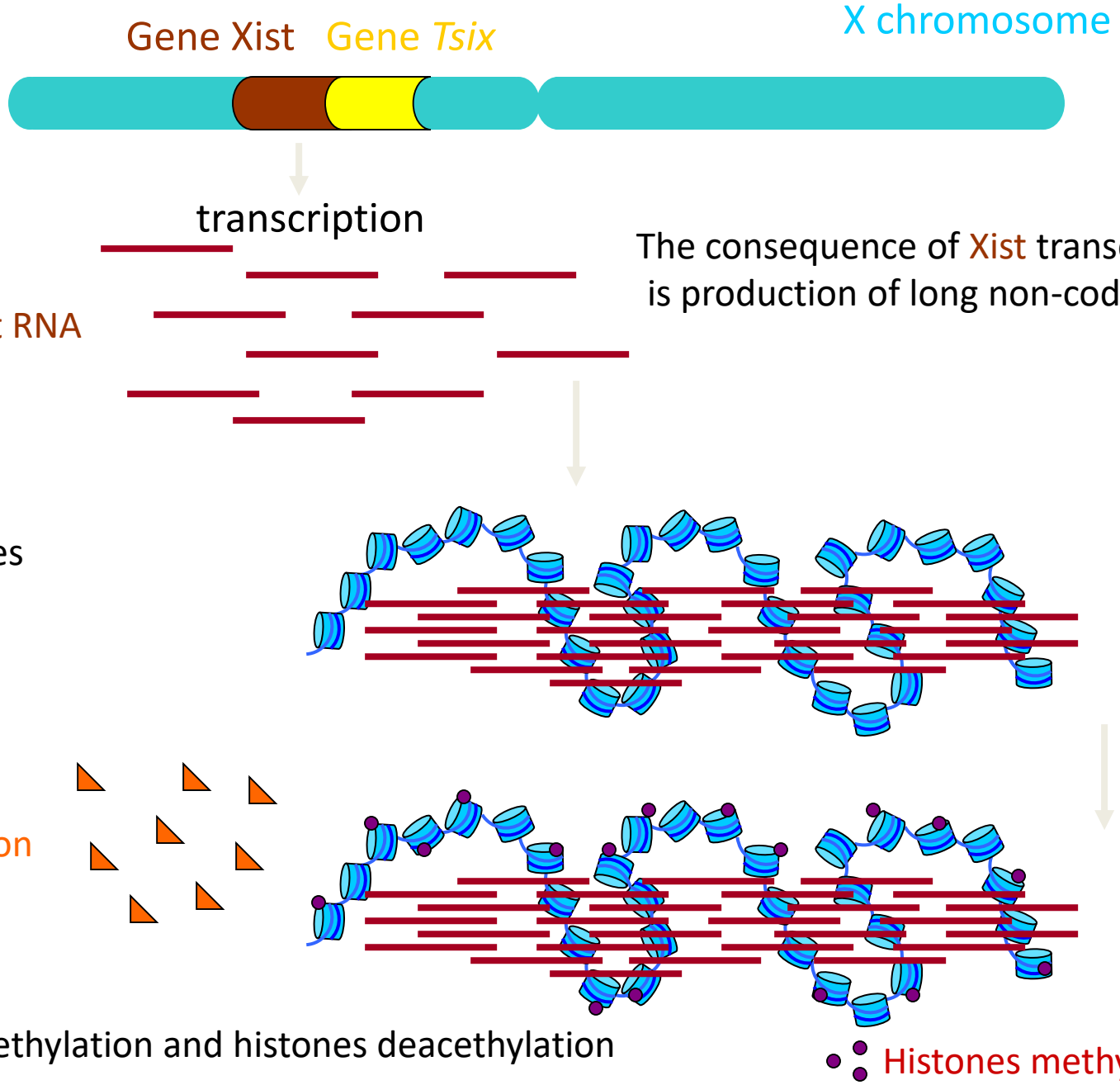


female



male

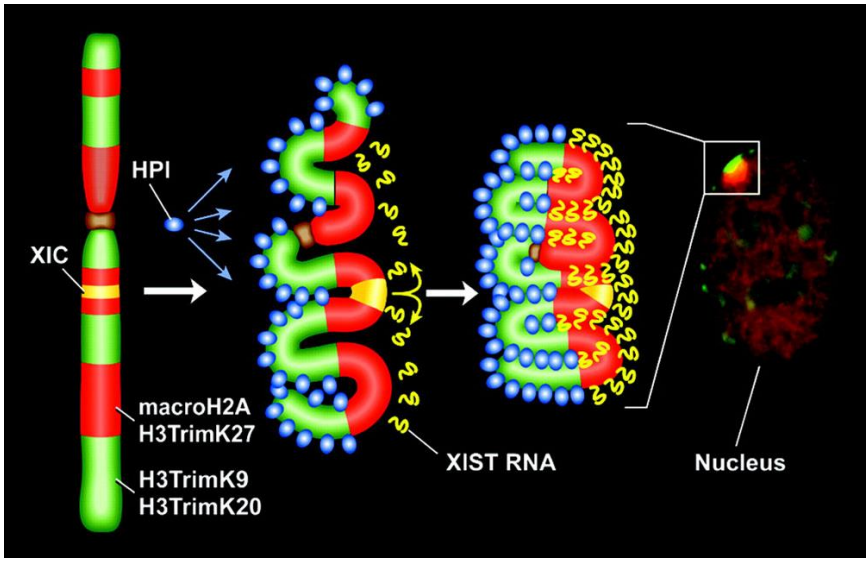
X CHROMOSOME INACTIVATION

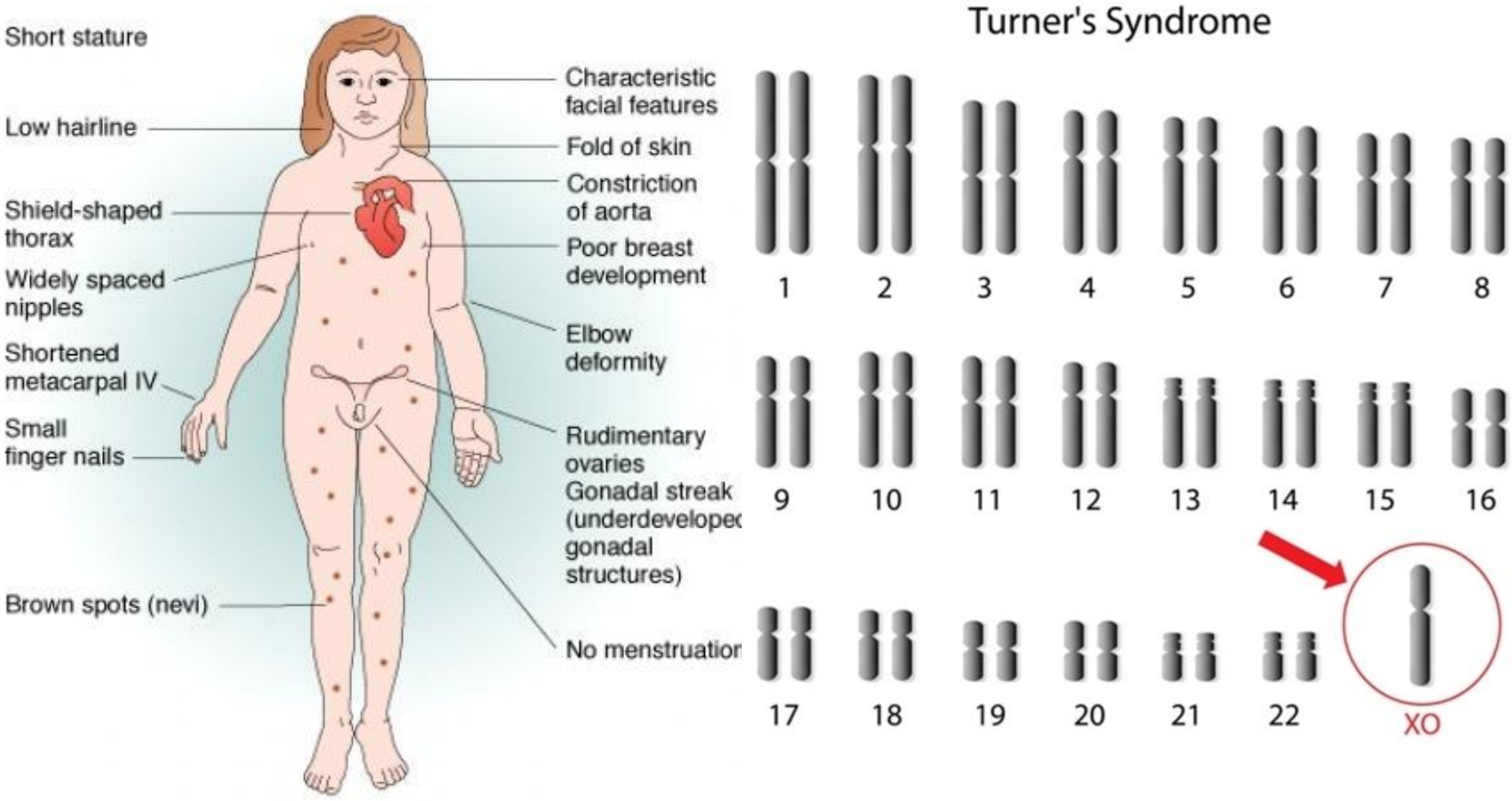


X CHROMOSOME INACTIVATION

Changes accompanying Xi heterochromatinisation:

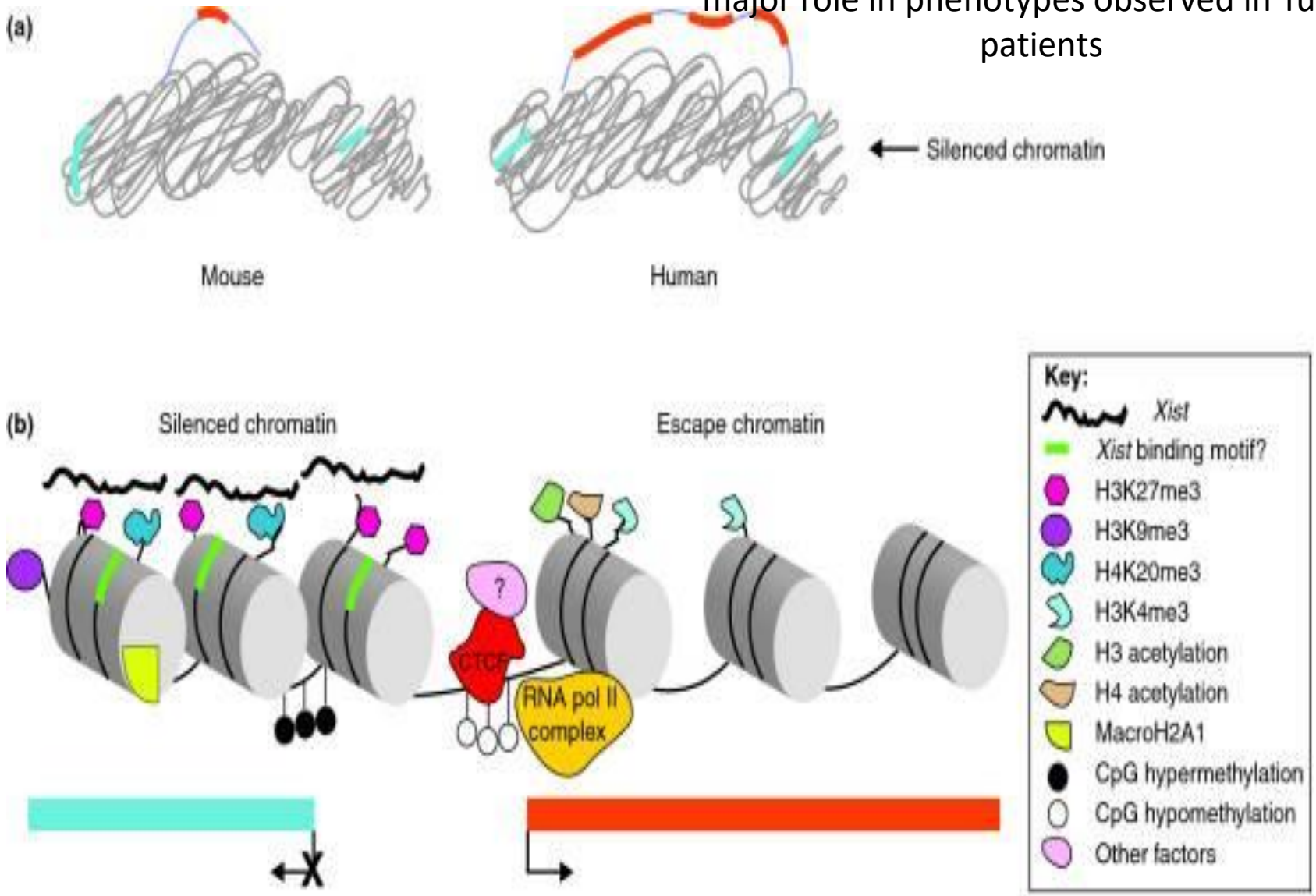
- Coating of X chromosome by Xist RNA
- H3K4 hypomethylation
- H3-K9 (H3-K27; H4-K20) methylation
- DNA hypermethylation
- Recruiting Polycomb proteins
- Late replication in S phase
- Hypoacetylation of core histone H4
- H2A-K119 mono-ubiquitinisation
- Exchange H2A histone by macroH2A

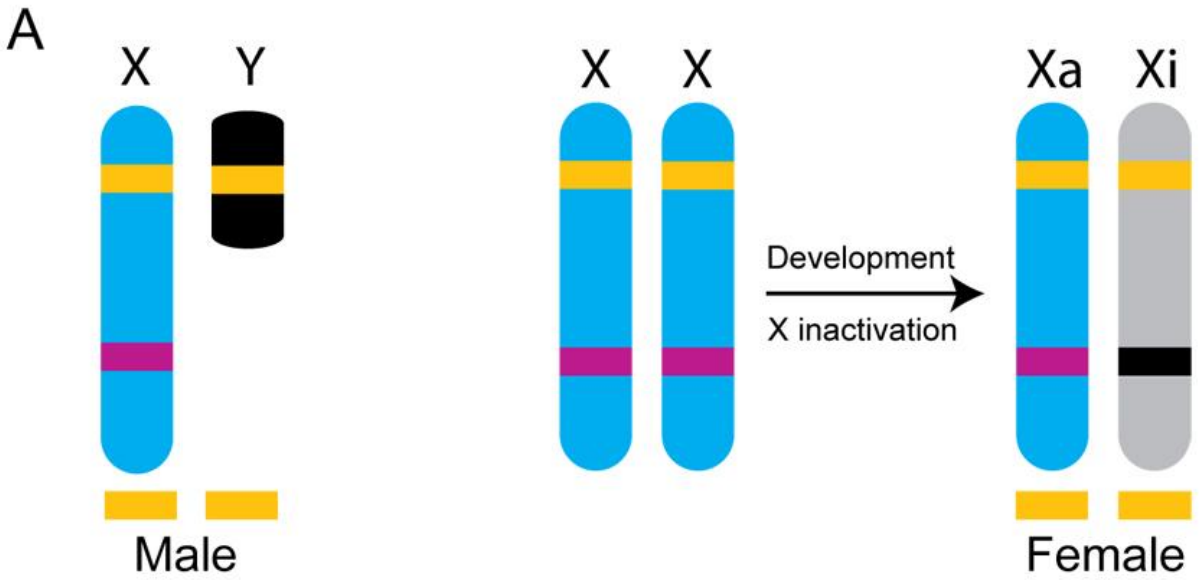



Short stature
Low hairline
Shield-shaped thorax
Widely spaced nipples
Shortened metacarpal IV
Small finger nails
Brown spots (nevi)
Characteristic facial features
Fold of skin
Constriction of aorta
Poor breast development
Elbow deformity
Rudimentary ovaries
Gonadal streak
(underdevelopec
gonadal structures)
No menstruation
Turner's Syndrome
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
XO

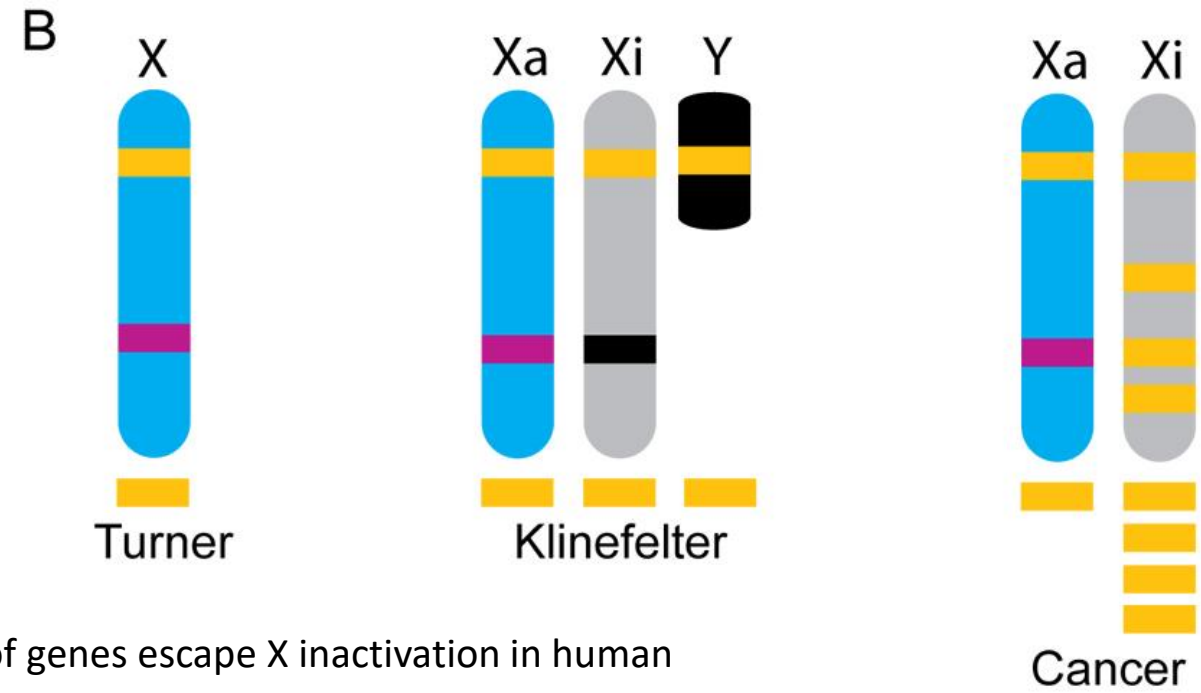
In both human and mouse, many of the genes that escape X inactivation are expressed more strongly in females

Deficiency in escape genes is thought to play a major role in phenotypes observed in Turner patients





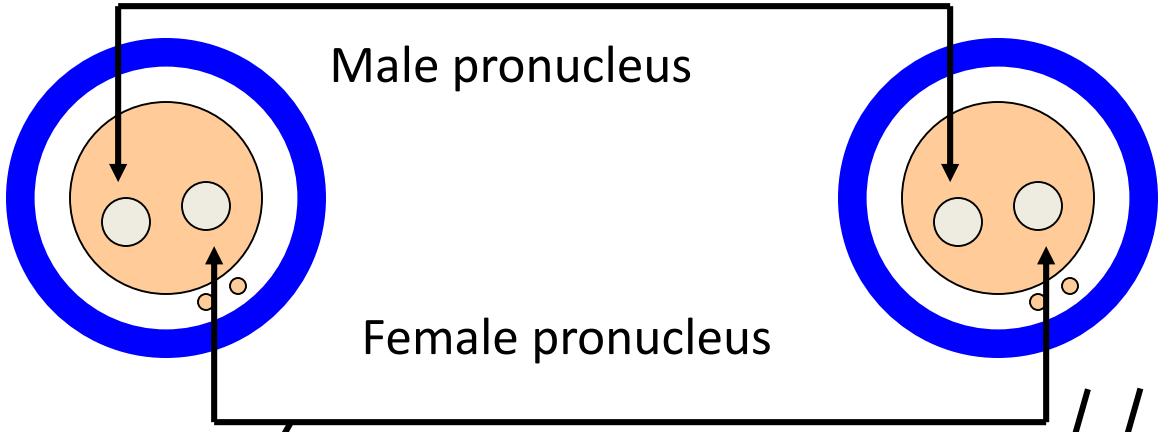
One specific gene implicated is SHOX (short stature homeobox), whose loss or gain explains the short stature of Turner patients or the tall stature of Klinefelter patients, respectively

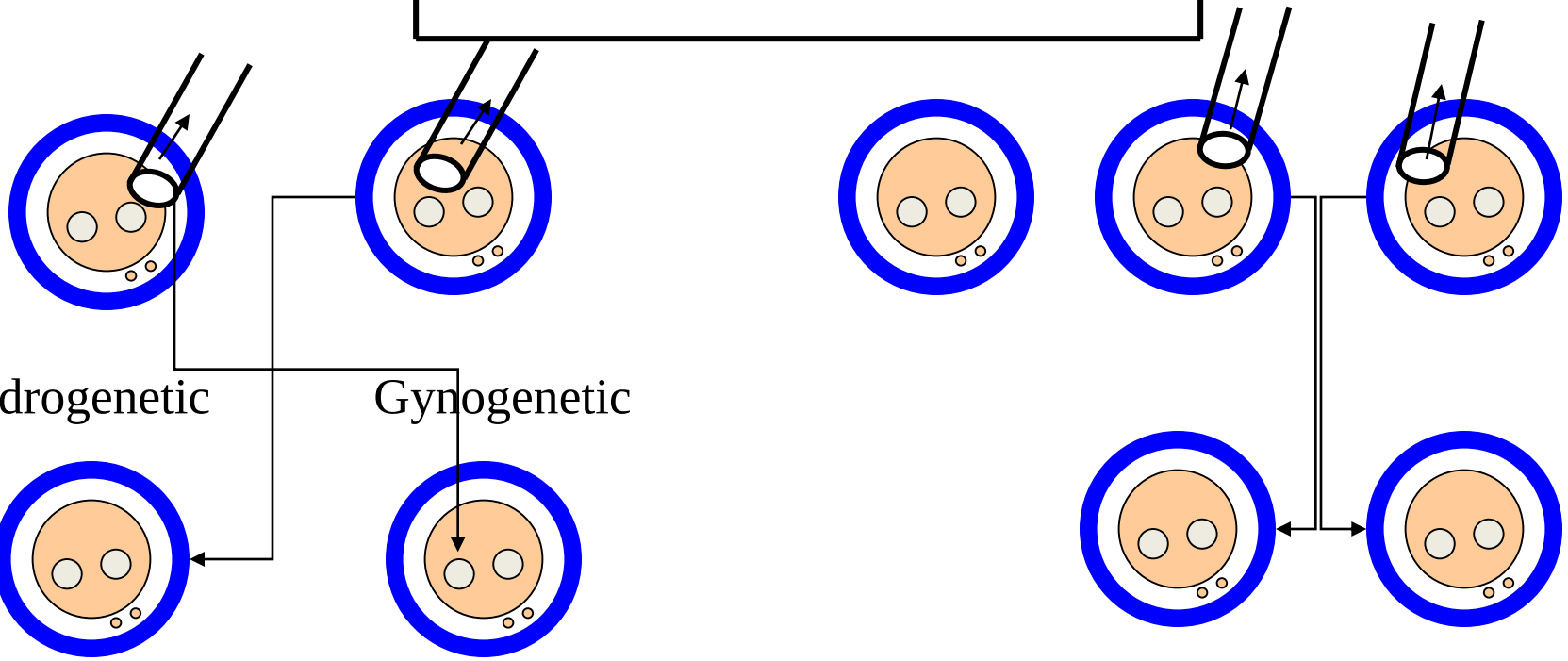


about 15% of genes escape X inactivation in human

J Genet. 2015 Dec

GENOMIC IMPRINTING
epigenetic phenomenon



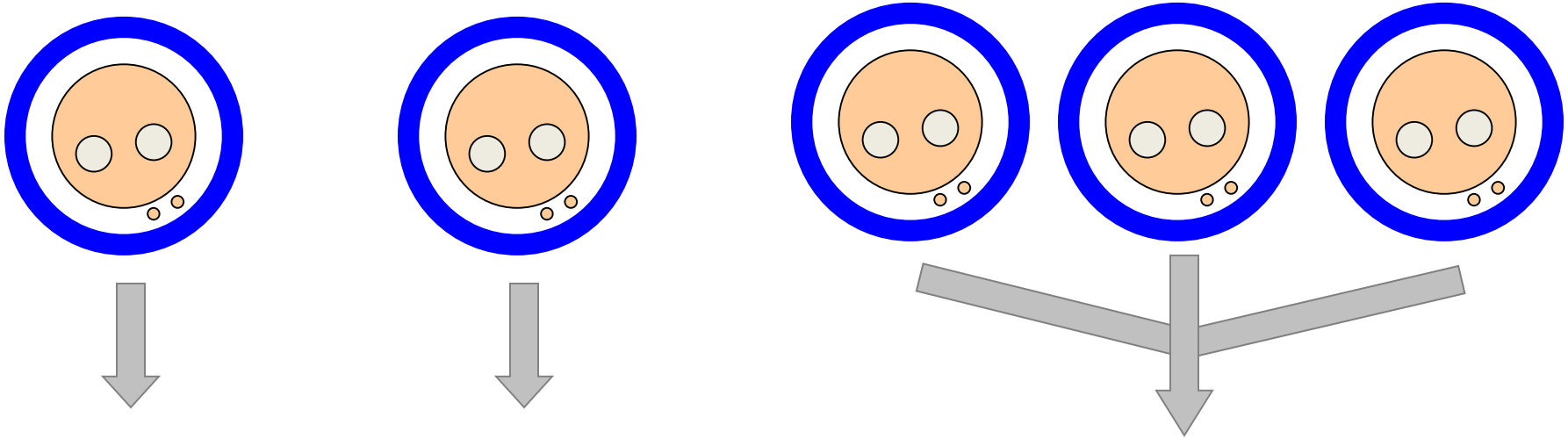


GENOMIC IMPRINTING
epigenetic phenomenon

control

Gynogenetic embryo

Androgenetic embryo

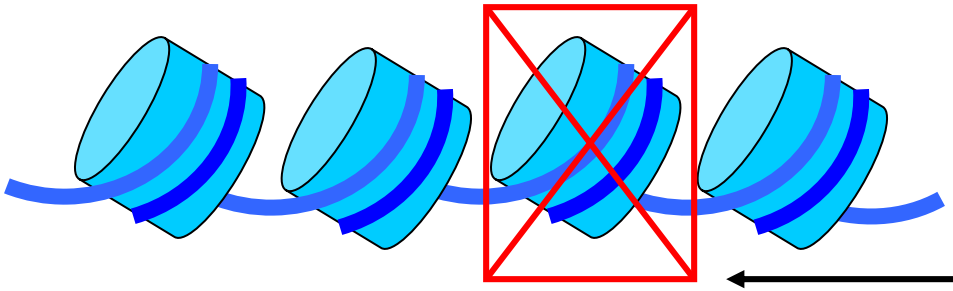


Normal fetus +
small placenta
decay
to mid-gestation

Embryoblast - lack
or underdevelopment
Normal placenta +
decay
in the early stages of pregnancy

Normal fetus+
Normal placenta+
Normal pregnancy

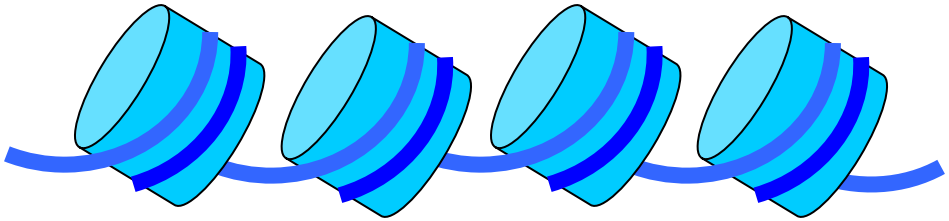
GENOMIC IMPRINTING



15 Chromosome maternal

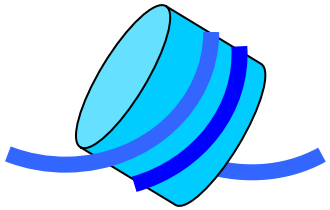
No expression
- Inactive gene

Imprinted genes are inactive

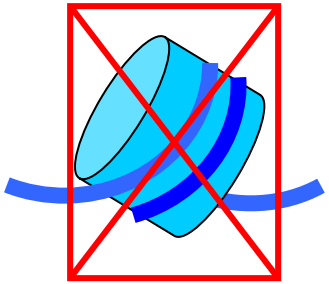


15 Chromosome paternal

Gene expression
- Active gene



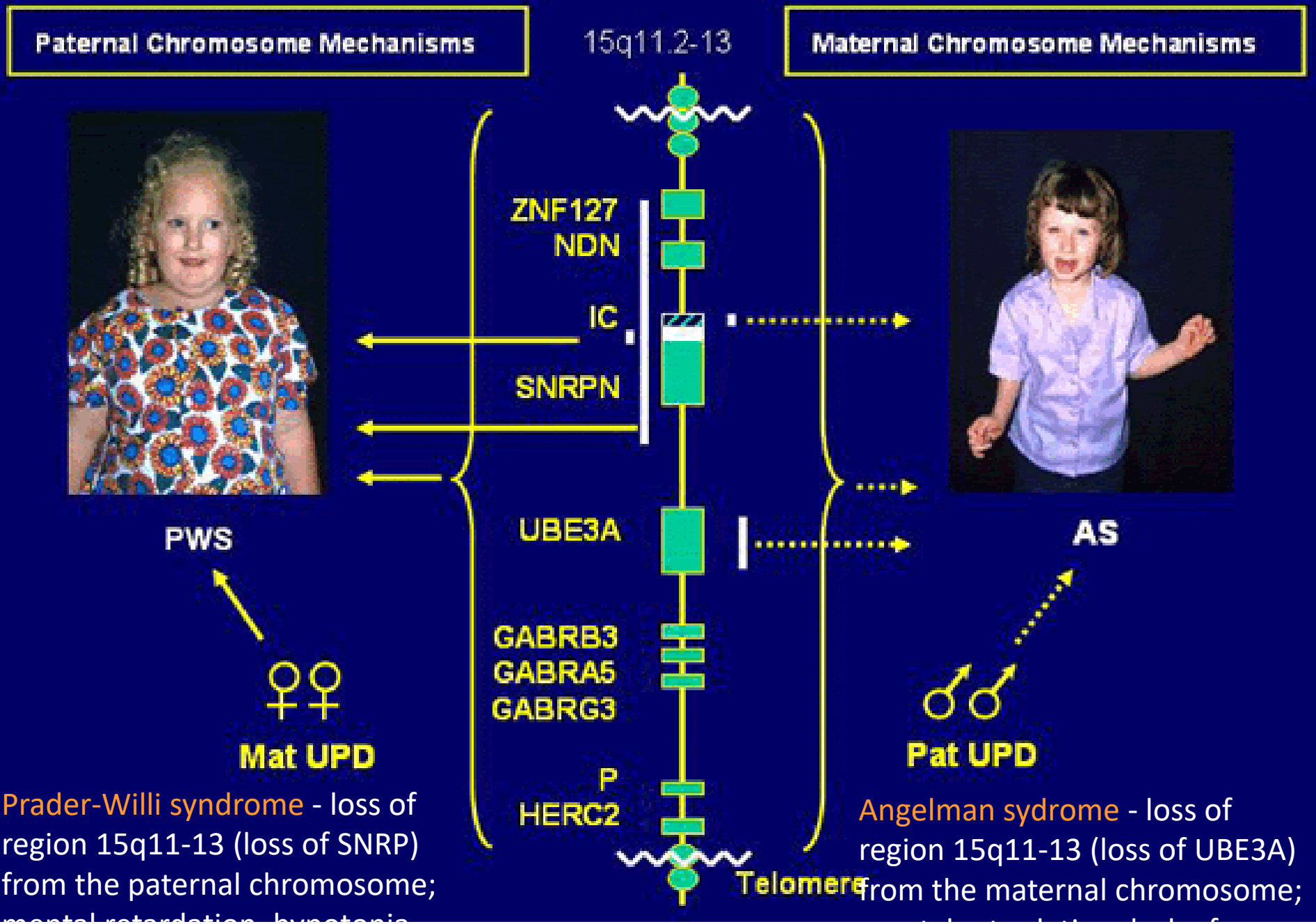
Active gene copy



Imprinted gene copy

For most genes, we inherit two working copies -- one from mom and one from dad. But with imprinted genes, we inherit only one working copy. Depending on the gene, either the copy from mom or the copy from dad is epigenetically silenced.

Uniparental disomy (UPD)



Prader-Willi syndrome - loss of region 15q11-13 (loss of SNRP) from the paternal chromosome;

Angelman sydrome - loss of region 15q11-13 (loss of UBE3A) from the maternal chromosome;

The red chromosomes represent the chromosome inherited from the mother while blue represents the father.

Healthy person

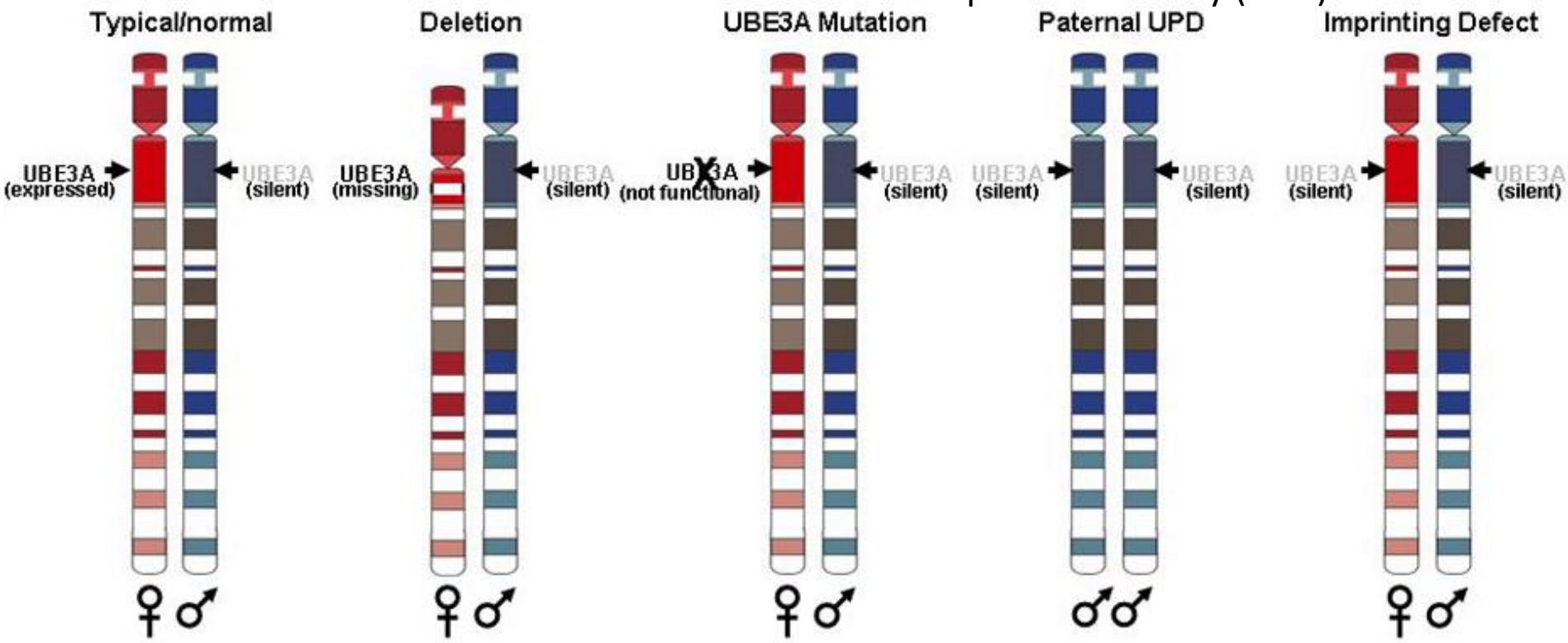
maternal CH15 paternal CH15

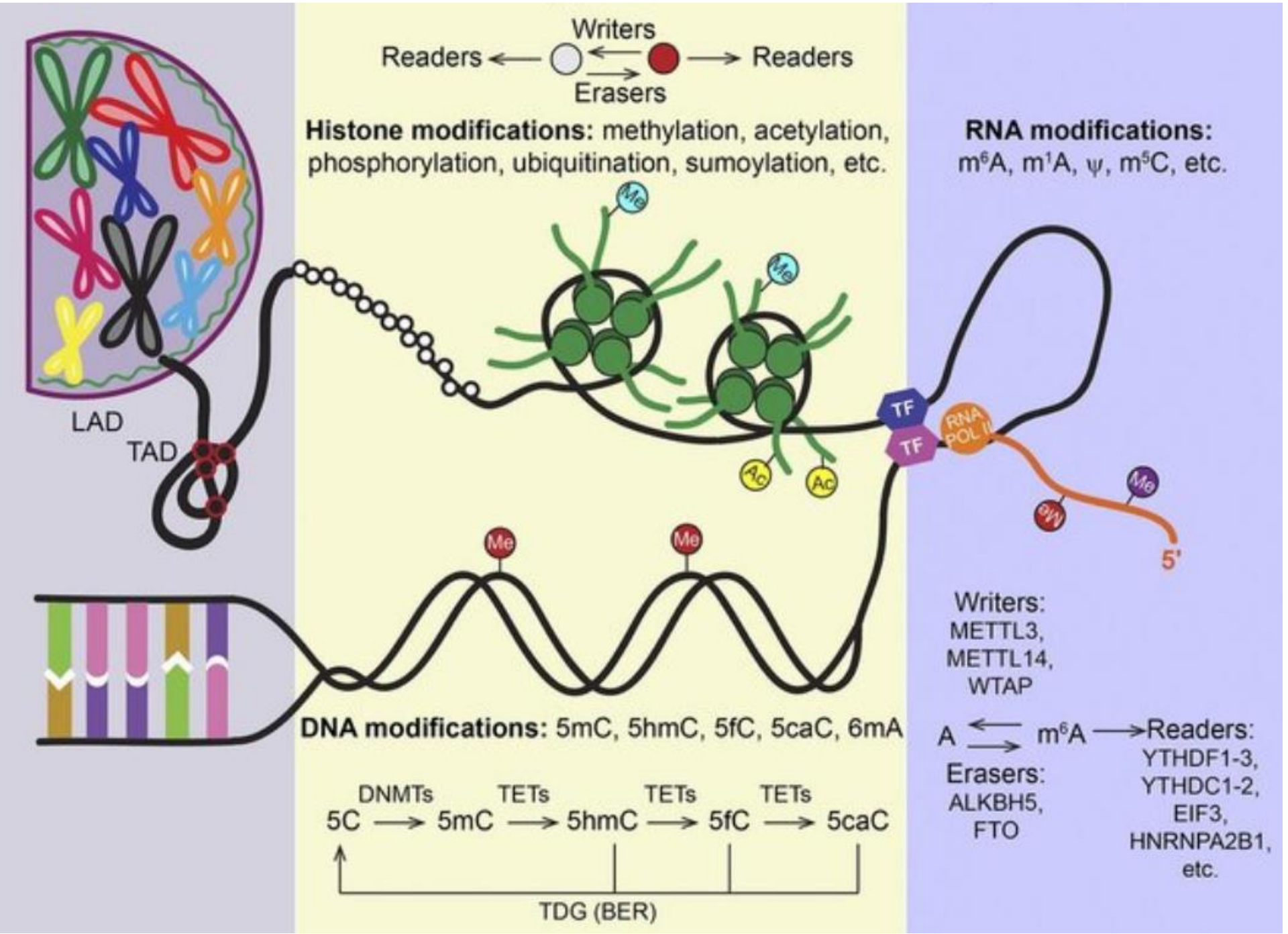
metSNRP SNRP

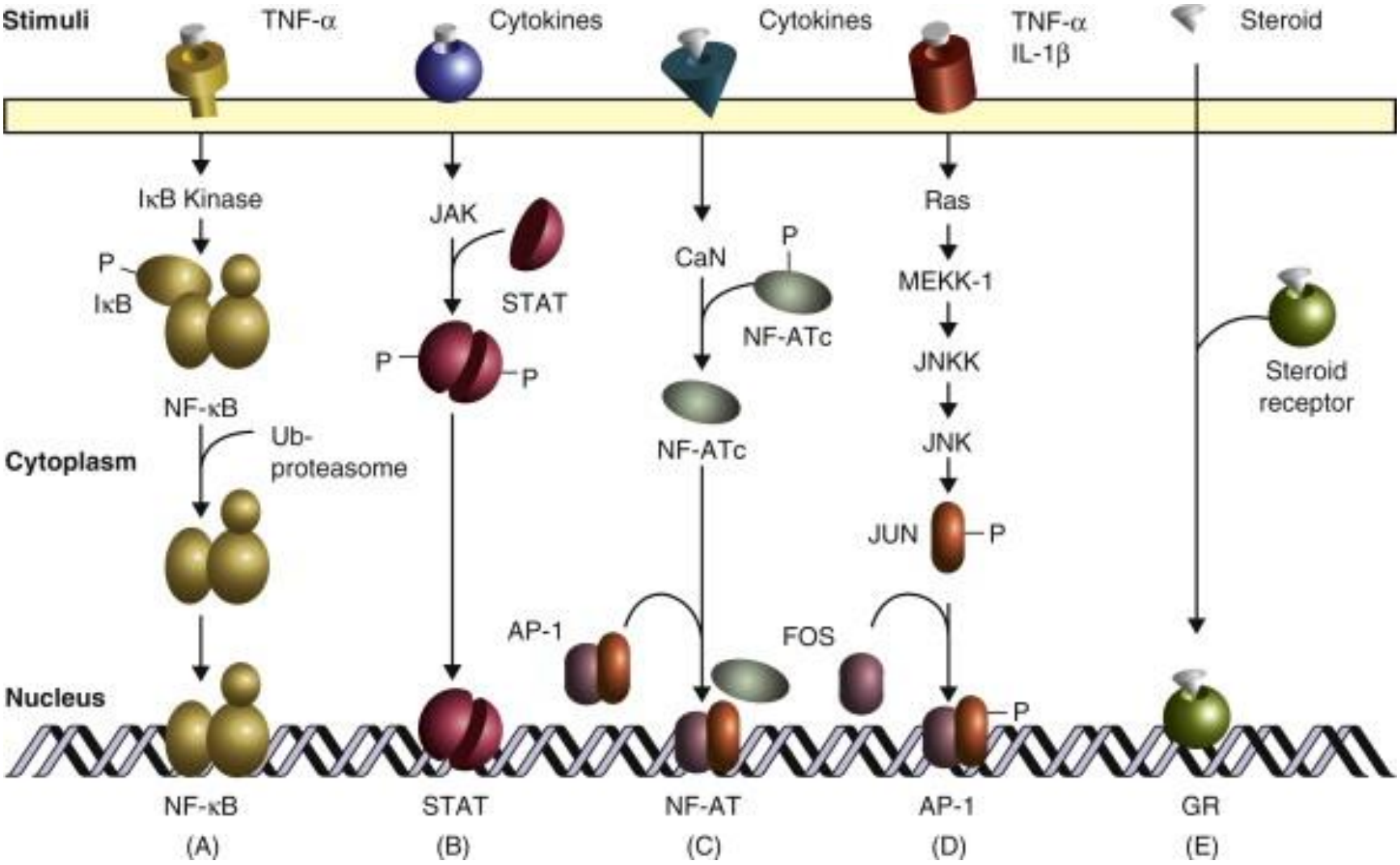
UBE3A metUBE3A

Angelman sydrome

Uniparental disomy (UPD)

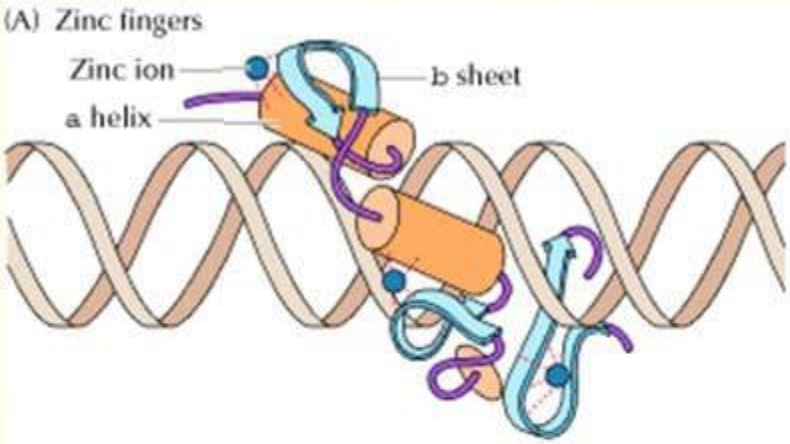


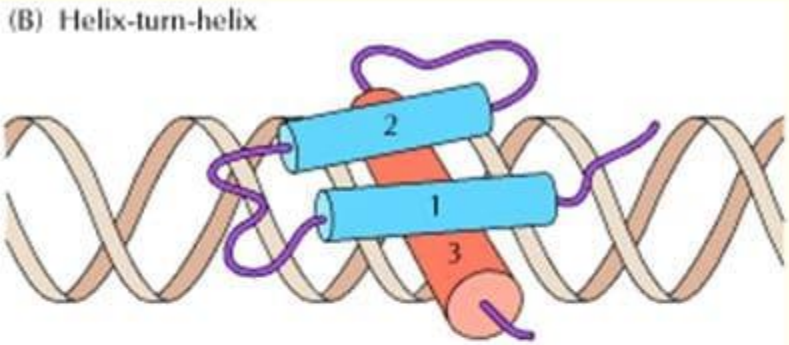

Writers
Readers
Readers
Erasers
Histone modifications: methylation, acetylation, phosphorylation, ubiquitination, sumoylation, etc.
RNA modifications:
m6A, m1A, ψ, m5C, etc.
LAD
TAD
Me
Ac
TF
RNA POL II
5'
DNA modifications: 5mC, 5hmC, 5fC, 5caC, 6mA
DNMTs
TETs
TETs
TETs
5C
5mC
5hmC
5fC
5caC
TDG (BER)
Writers:
METTL3,
METTL14,
WTAP
A
m6A
Readers:
YTHDF1-3,
YTHDC1-2,
EIF3,
HNRNPA2B1,
etc.
Erasers:
ALKBH5,
FTO

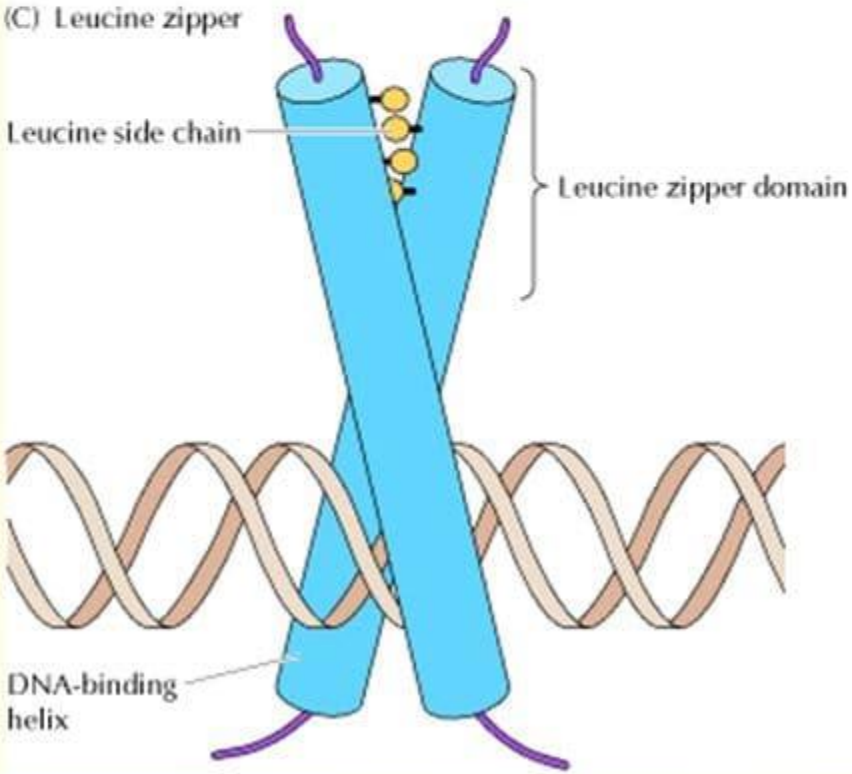


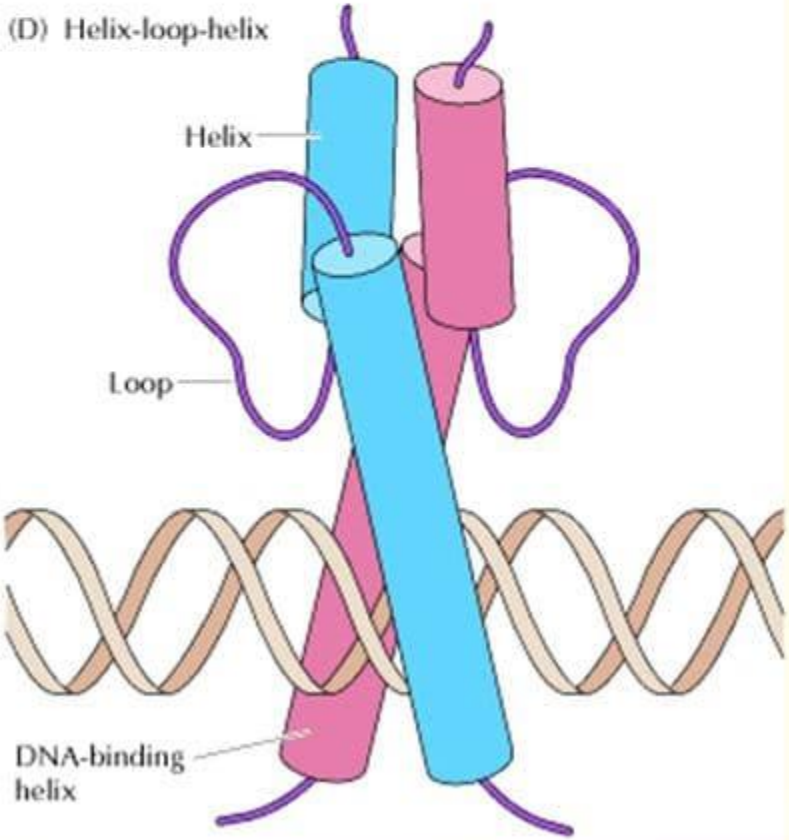
specyficzne czynniki transkrypcyjne

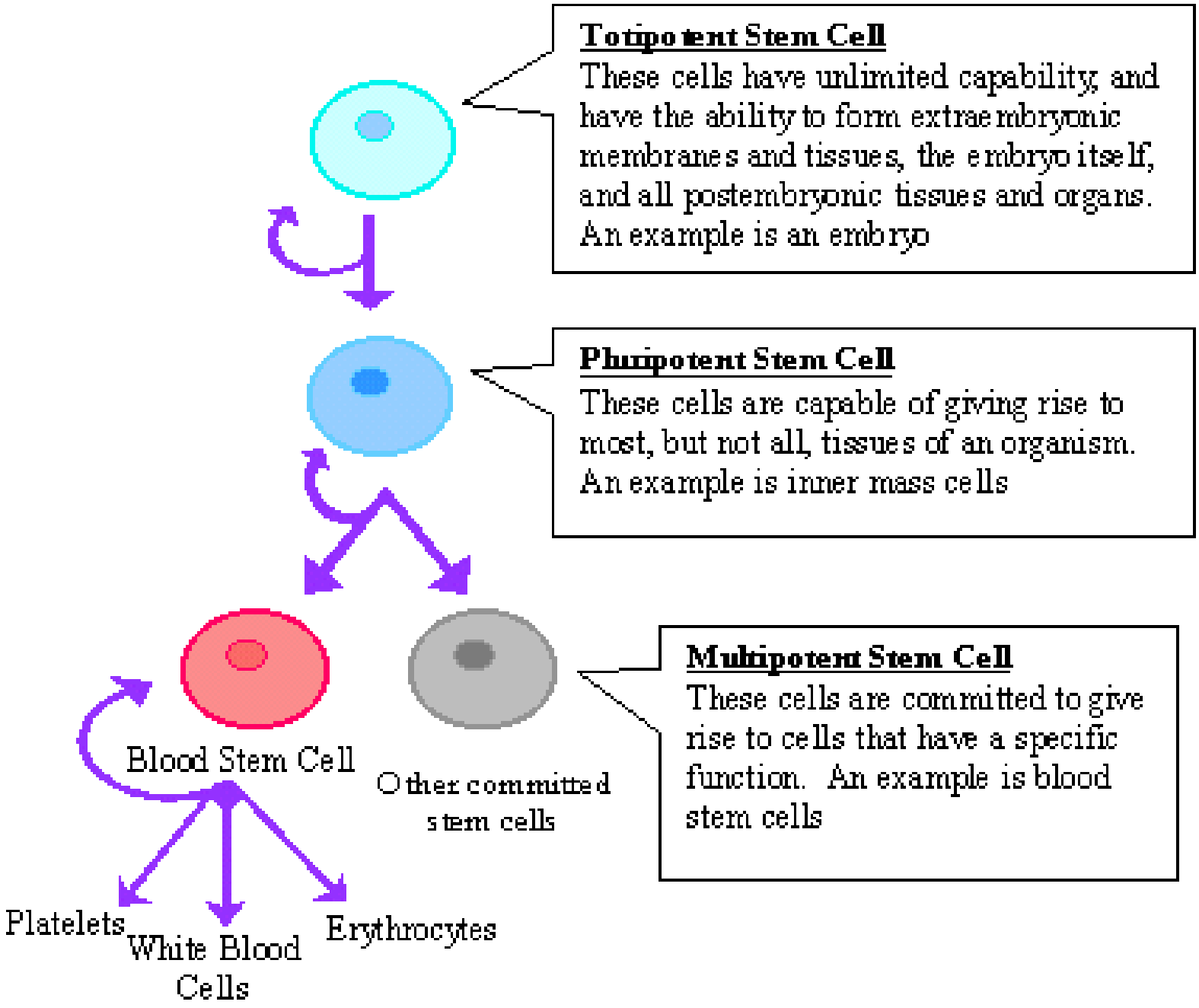
Families of DNA-binding domains










Totipotent Stem Cell
These cells have unlimited capability, and have the ability to form extraembryonic membranes and tissues, the embryo itself, and all postembryonic tissues and organs. An example is an embryo
Pluripotent Stem Cell
These cells are capable of giving rise to most, but not all, tissues of an organism. An example is inner mass cells
Multipotent Stem Cell
These cells are committed to give rise to cells that have a specific function. An example is blood stem cells
Blood Stem Cell
Other committed stem cells
Platelets
White Blood Cells
Erythrocytes

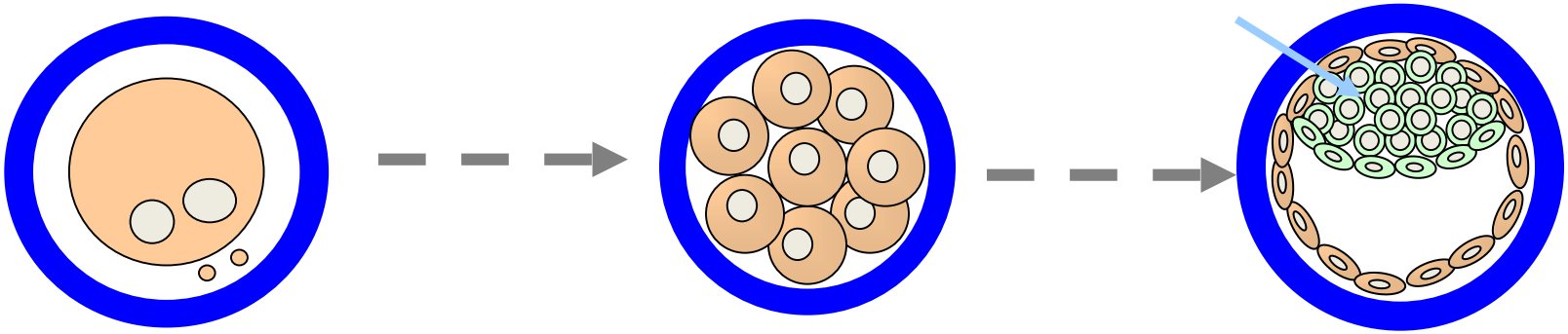
CELL POTENCY IS A CELL'S ABILITY TO DIFFERENTIATE INTO OTHER CELL TYPES

ZYGOTE

8-blastomers embryo

Inner mass cells

Blastocyst



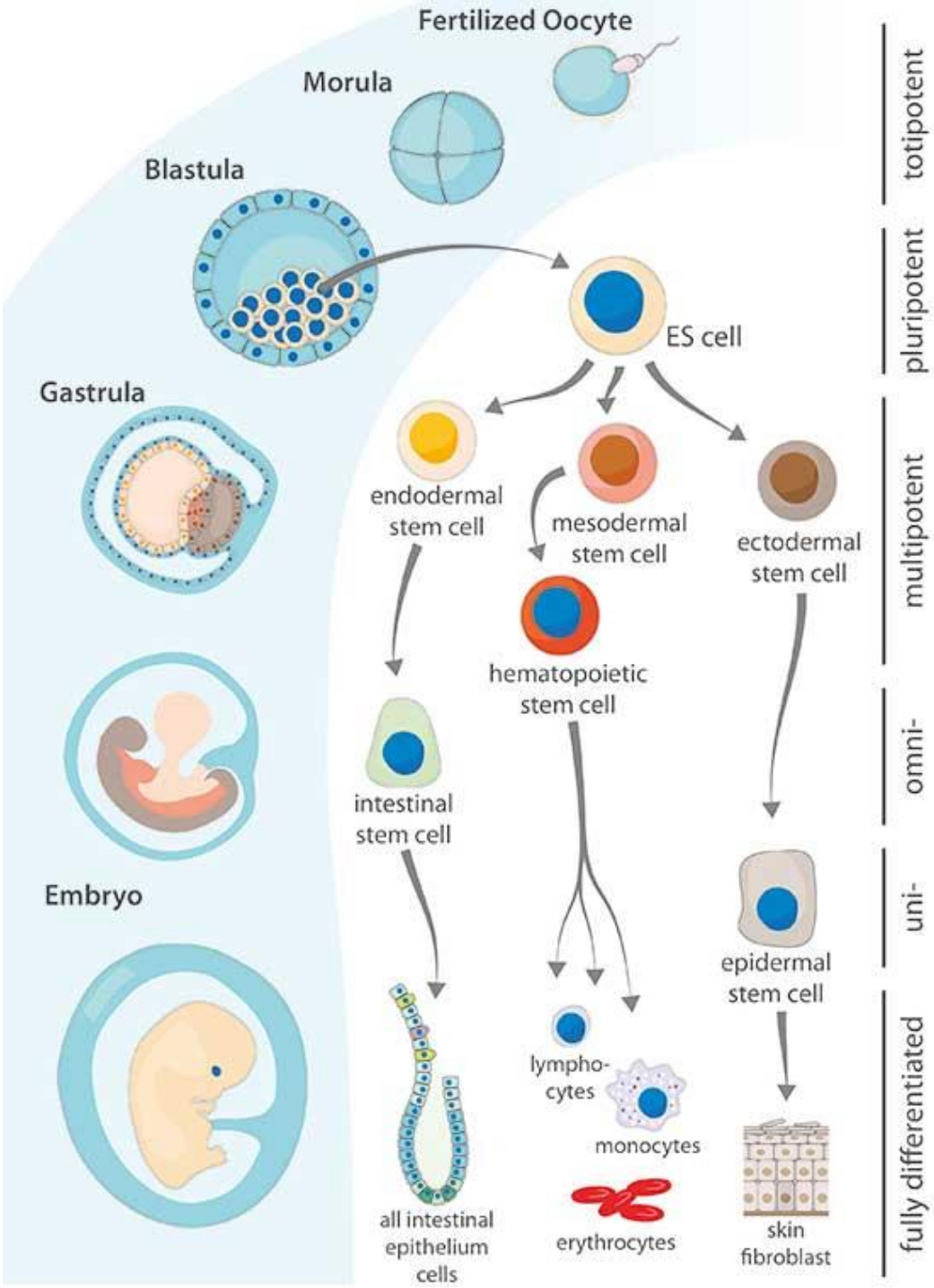
TOTIPOTENT

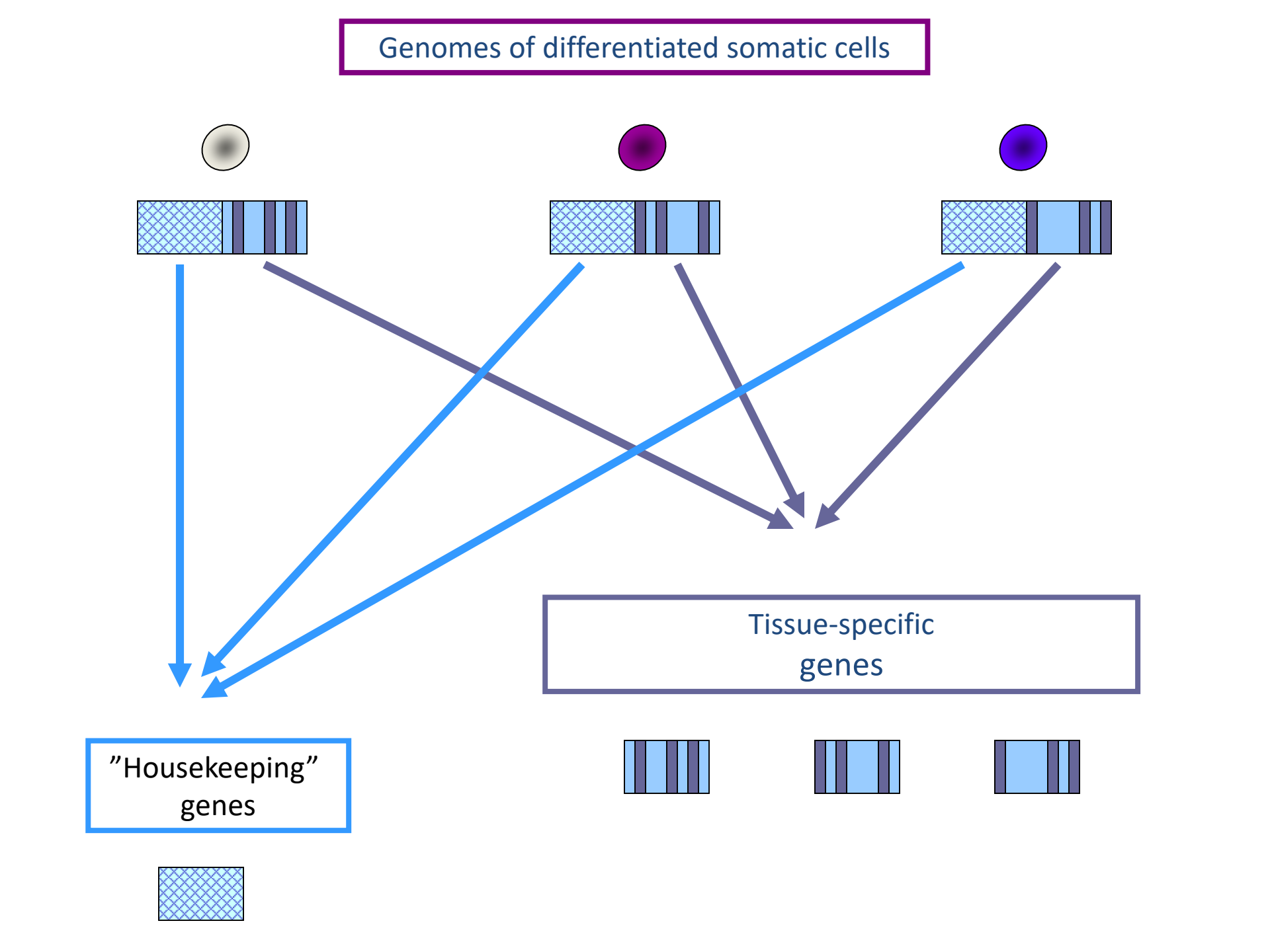
TOTIPOTENT
BLASTOMERS

PLURIPOTENT

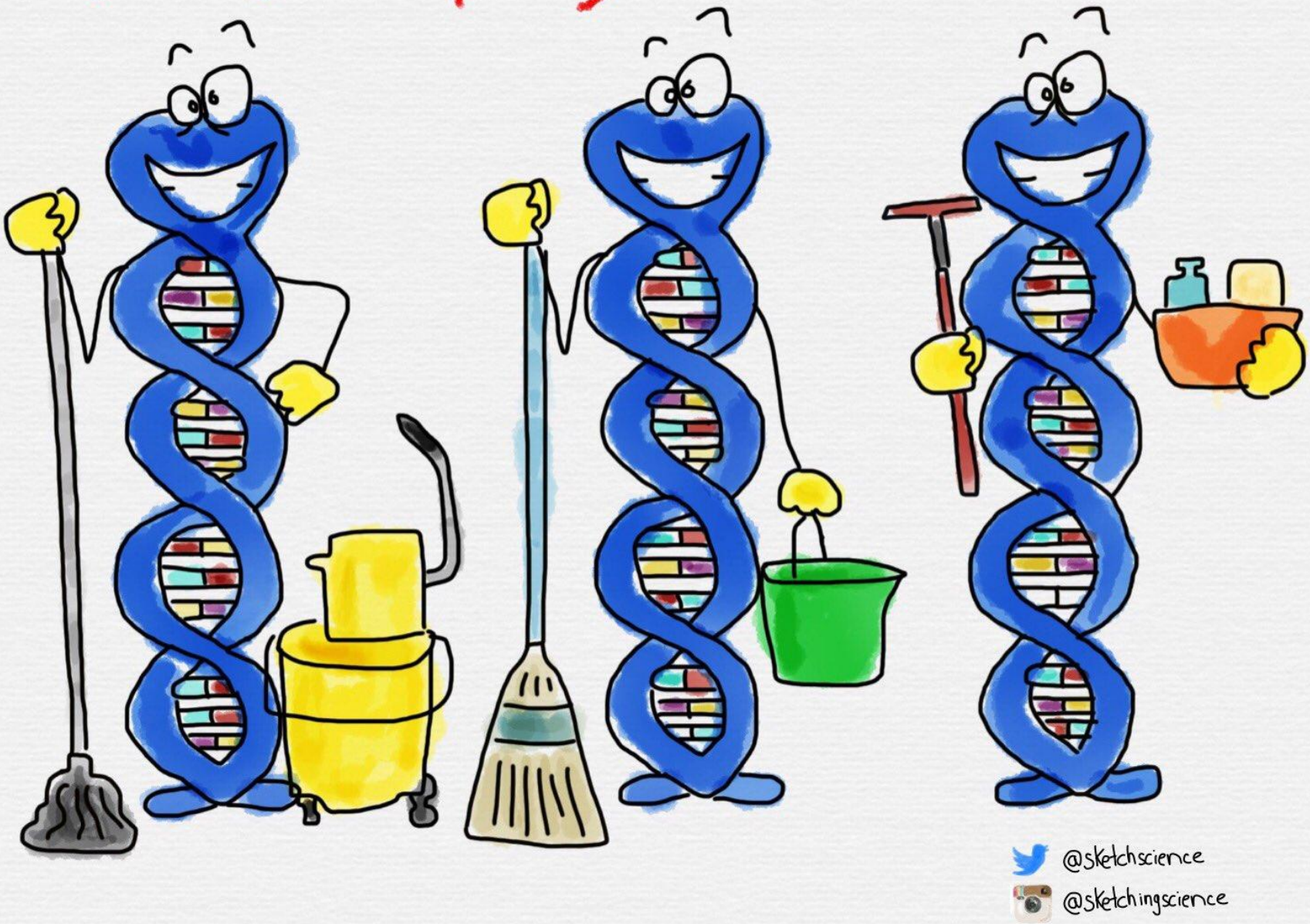
MULTIPOTENT

TERMINAL DIFFERENTIATED CELL


Fertilized Oocyte
Morula
Blastula
Gastrula
Embryo
ES cell
endodermal stem cell
mesodermal stem cell
ectodermal stem cell
hematopoietic stem cell
intestinal stem cell
epidermal stem cell
lympho-cytes
monocytes
erythrocytes
all intestinal epithelium cells
skin fibroblast
totipotent
pluripotent
multipotent
omni-
uni-
fully differentiated


Genomes of differentiated somatic cells
Tissue-specific genes
”Housekeeping” genes

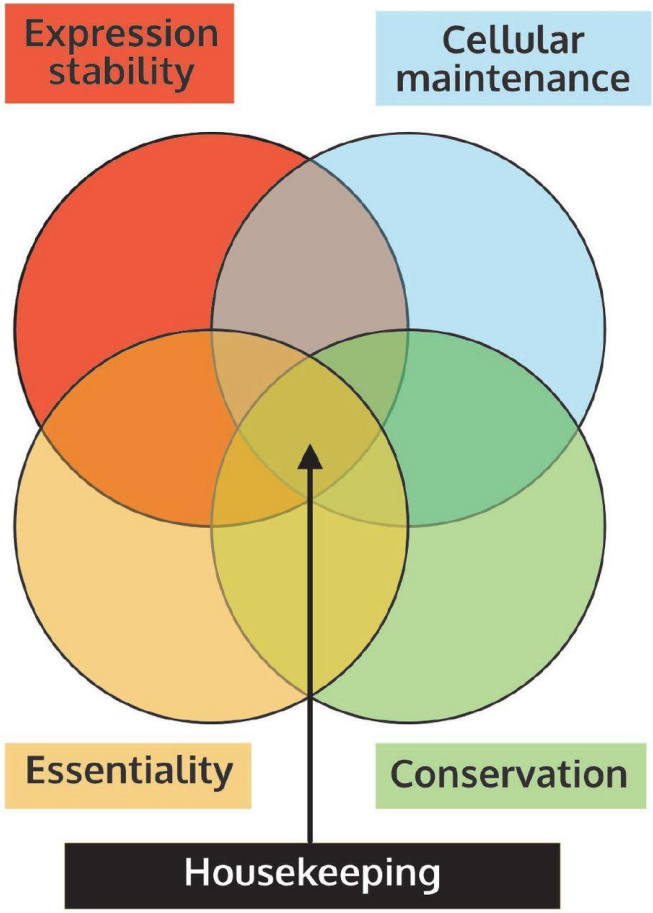
HouseKeeping Genes



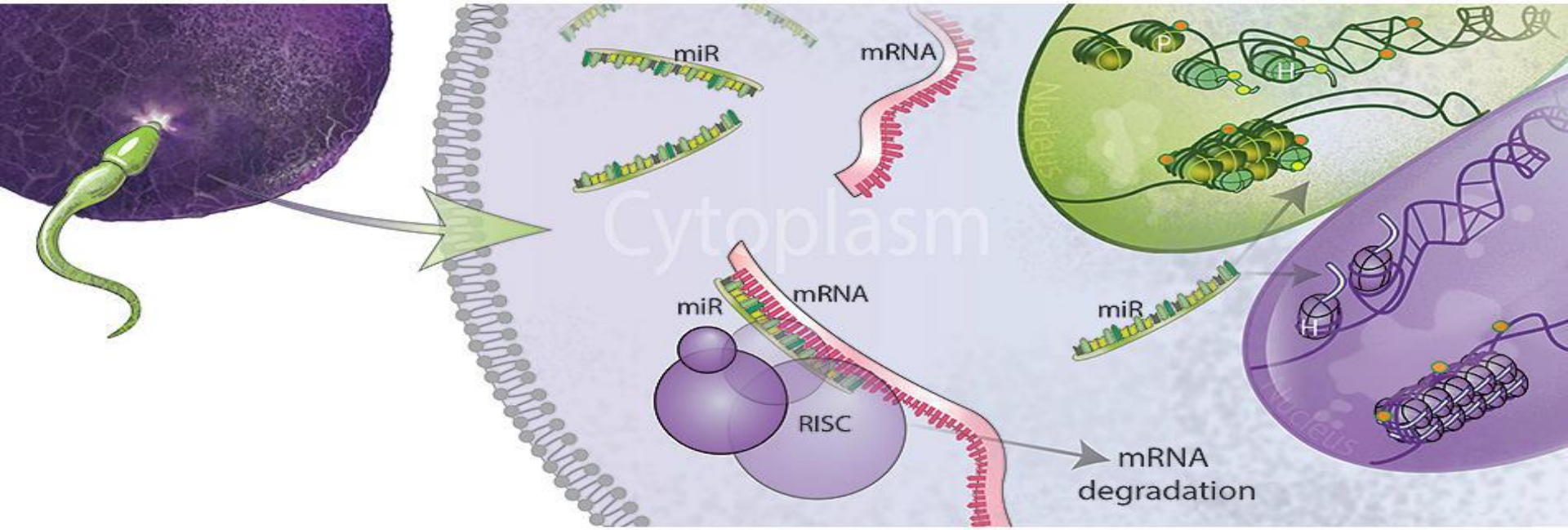
Housekeeping Genes vs Luxury Genes

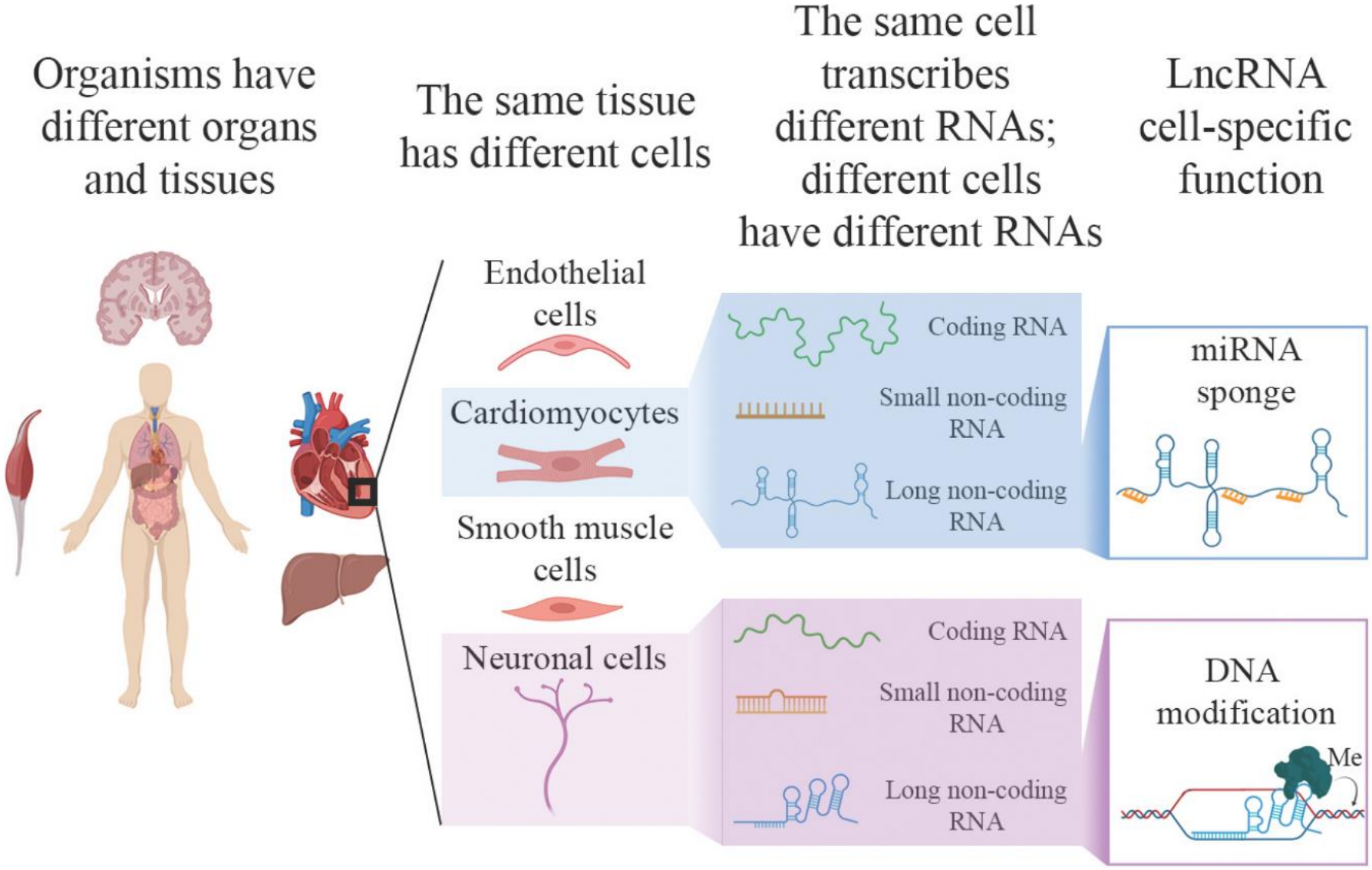
More Information Online WWW.DIFFERENCEBETWEEN.COM

	Housekeeping Genes	Luxury Genes
DEFINITION	Housekeeping genes are the genes constantly expressed in all cells	Luxury genes are the genes expressed only when their products are needed
SYNONYM	Constitutive genes	Non-constitutive genes or specialists genes
EXPRESSION OF GENES	Maintain a constant expression rate	These genes are not always expressed
EXPRESSION IN	All cells	Only in certain types of cells
PROTEIN PRODUCTS	Proteins that are constantly required by the cell	Specialized protein products
STATE	Remain 'on' all the time	Remain inactive most of the time
EXAMPLES	Genes for enzymes of glycolysis, ATAase	Gene for nitrate reductase in plants, lactose system in *E. coli*
EXPRESSION	Expression is not regulated	Expression is regulated

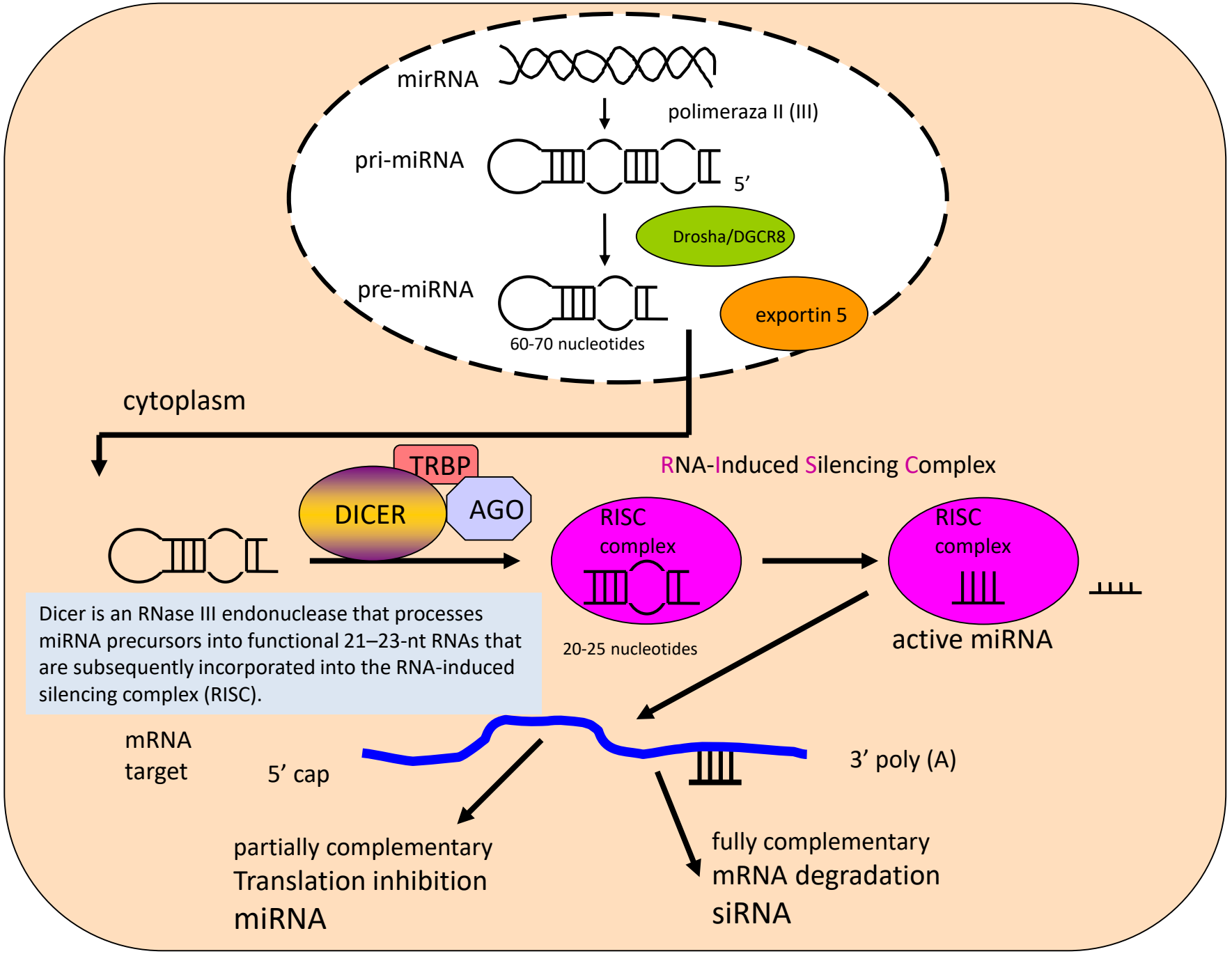


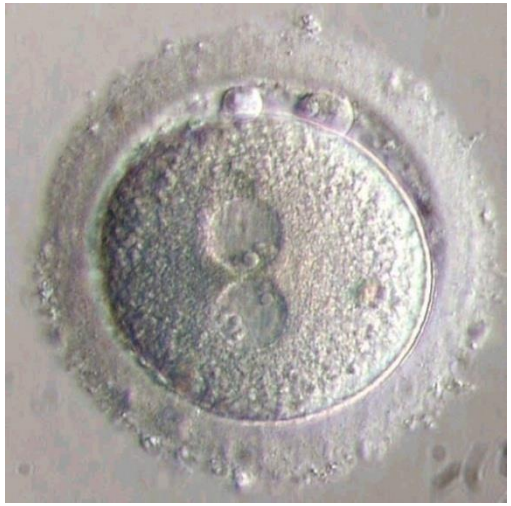
MicroRNAs in sperm target maternal mRNA for destruction to influence offspring development.





Int. J. Mol. Sci. 2020, 21(1), 302; https://doi.org/10.3390/ijms21010302


mirRNA
polimeraza II (III)
pri-miRNA
5’
Drosha/DGCR8
pre-miRNA
exportin 5
60-70 nucleotides
cytoplasm
TRBP
DICER
AGO
RNA-Induced Silencing Complex
RISC complex
RISC complex
active miRNA
20-25 nucleotides
Dicer is an RNase III endonuclease that processes miRNA precursors into functional 21–23-nt RNAs that are subsequently incorporated into the RNA-induced silencing complex (RISC).
mRNA target
5’ cap
3’ poly (A)
partially complementary
Translation inhibition
miRNA
fully complementary
mRNA degradation
siRNA



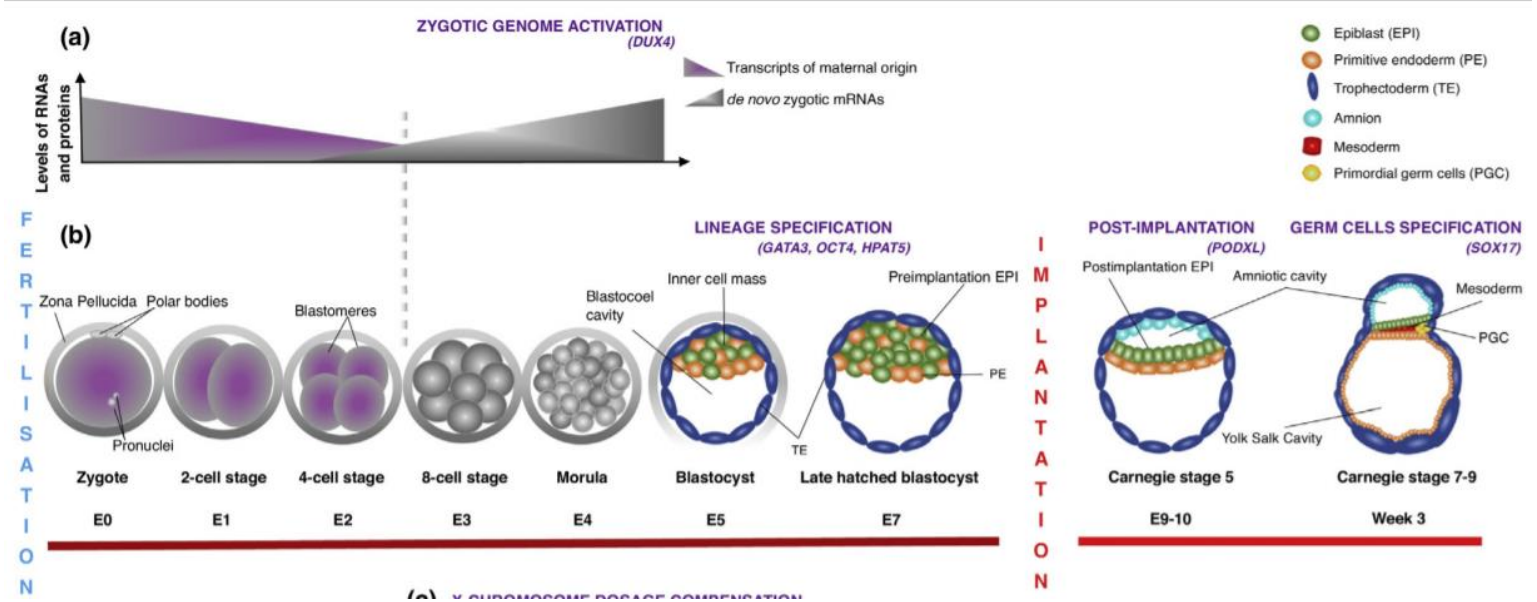
Maternal-Effect Genes

Most mRNAs of unfertilized oocyte remain unread

sperm does not provide

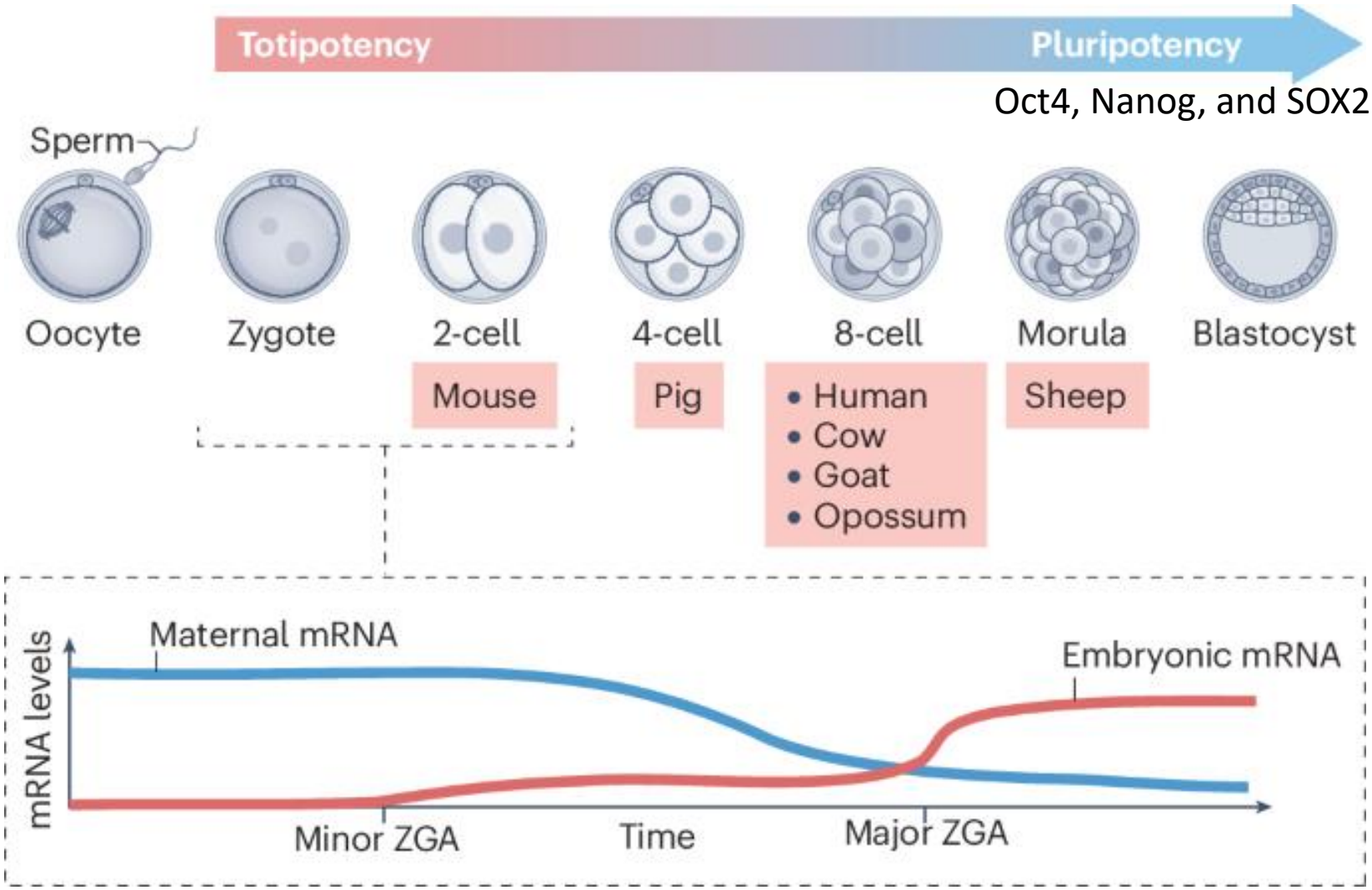
mRNA, or mitochondrial DNA to zygote

Sperm-borne miRNAs might regulate early embryonic development

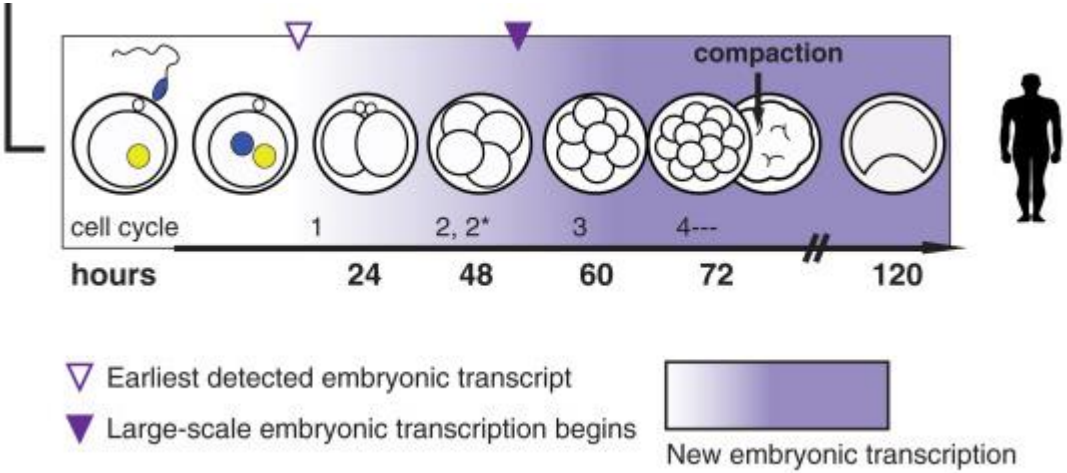


after fertilization mRNAs, miRNA of the oocyte are responsible for basic functions of the cell (e.g., control of the cell cycle)

maternal effect genes



Zygote genome activation



A significant shift in gene expression was observed during the transition from four- to eight-cell stages

transcription factors (TFs)

recognize specific DNA sequences to control chromatin and transcription, forming a complex system that guides expression of the genome

e.g.
ATF3, EN1, IFI16, IKZF3, KLF3, NPAS3, NR2F2, RUNX1, SOX2, ZBTB20, and ZSCAN4.

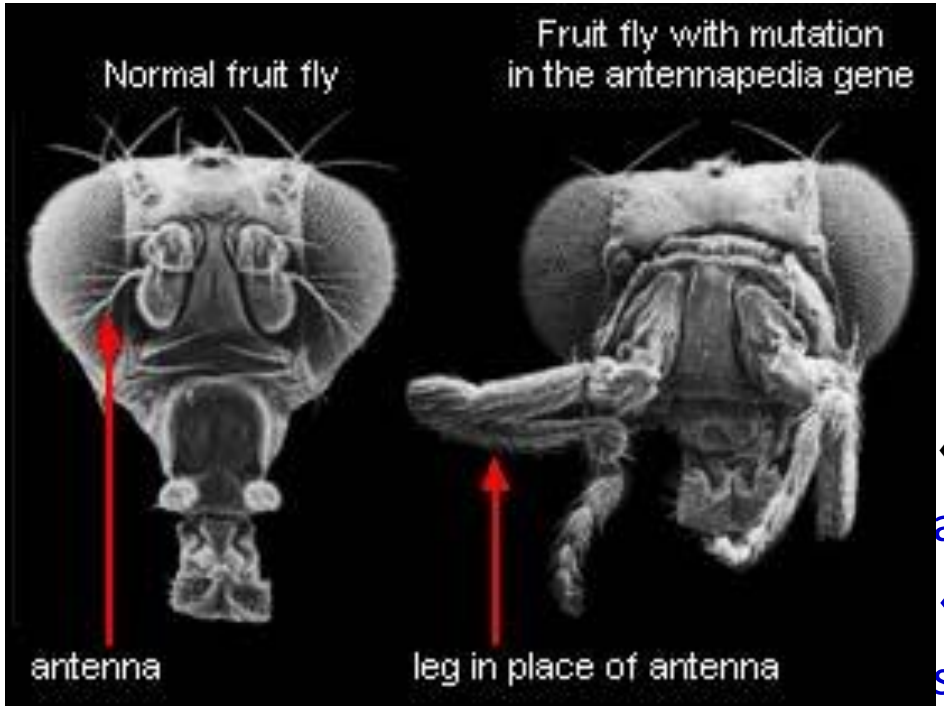
Homeotic genes (HOX)

morphogens

secreted signalling molecules that direct cell fate decisions during embryonic development
acts on cells directly to induce distinct cellular responses in a concentration-dependent manner

e.g.
BMP, FGF, WNT

Sonic hedgehog



Hox proteins
are transcription factors

- control of the identity

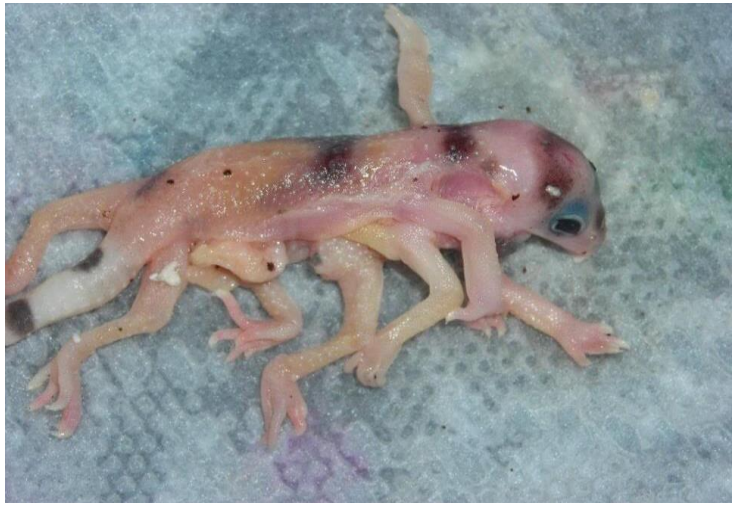
and the position of the segments,

- information about the specialization of segments

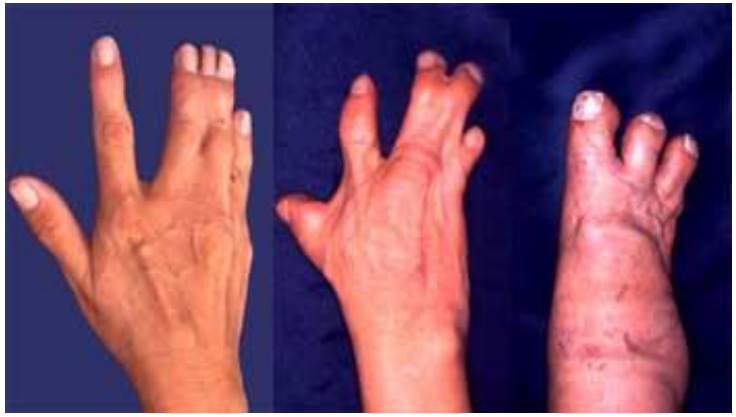
and creation of organs characteristic for these segments

- expression necessary

for the cells transition in the differentiation state and to maintain differentiation



lizard



human

Hox genes

Possess specific nucleotide sequence,

called cassette homeobox

portion of the protein encoded by homeobox called the homeodomain

Hox proteins are transcription factors

maintaining the state of differentiation of cells

determining the position of differentiated cells along the axis anterior – posterior

Homeotic genes

Over 170 genes with homeodomain

HOX clustered homeotic genes

Non-clustered homeotic genes

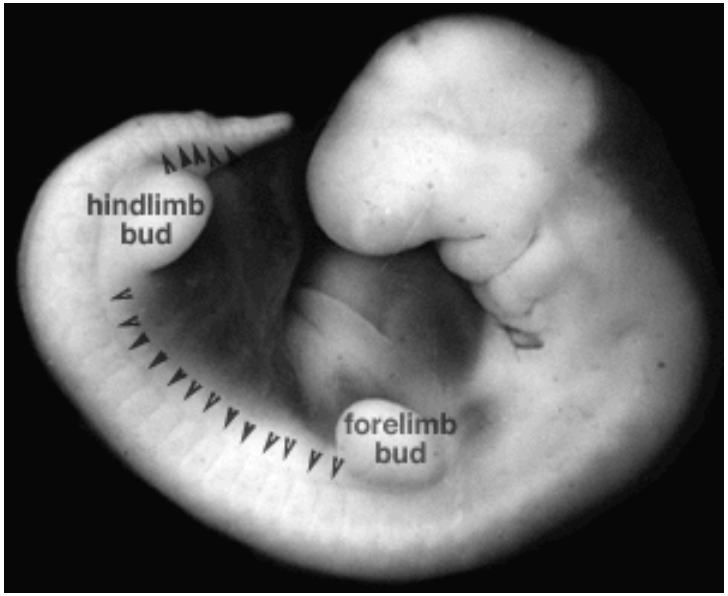
4 complexes with 9 -11 genes

HOX1 - short arm of chromosome 7

HOX2 - long arm of chromosome 17

HOX3 - long arm of chromosome 12

HOX4 - long arm of chromosome 2



- Mutations in homeotic genes lead to abnormalities in organogenesis (changes in the structure and location of systems or organs)

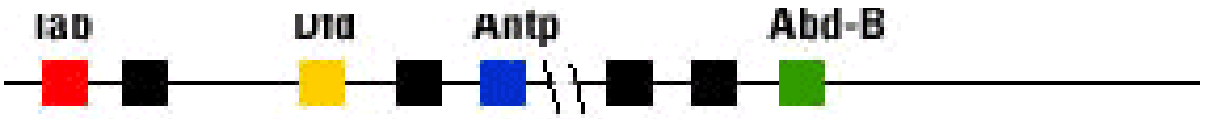
zarodek
Drosophila melanogaster

postać dorosła

Kompleks Antennapedia

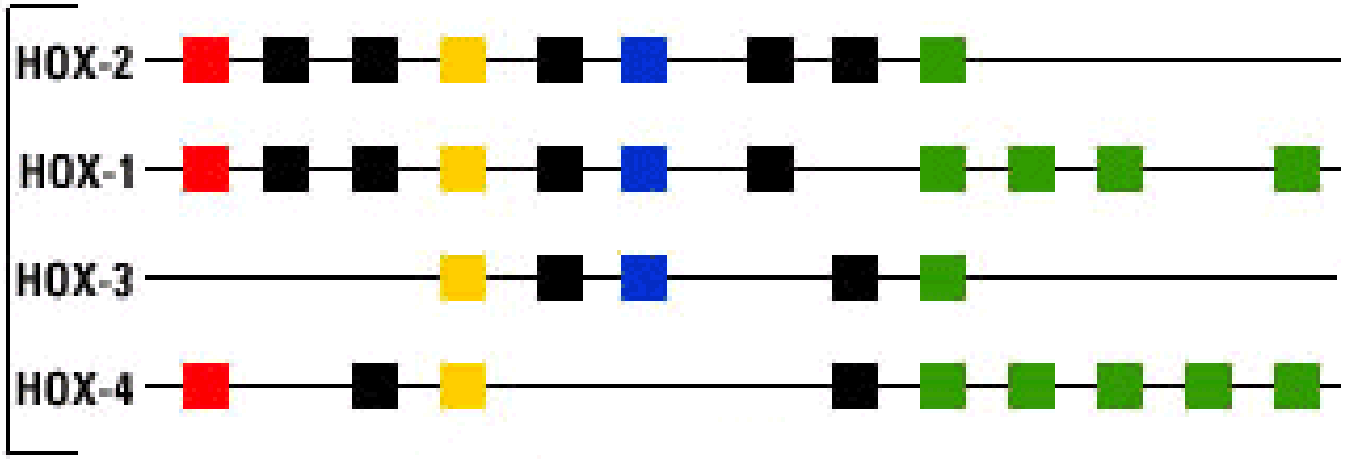
Kompleks Bithorax

chromosom owada

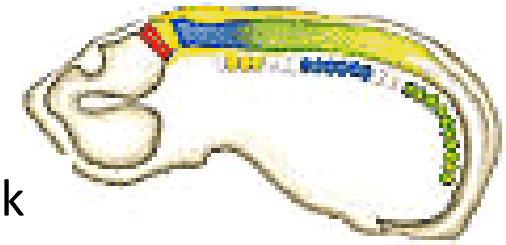


chromosom myszy

duplikacja genów bezkręgowców



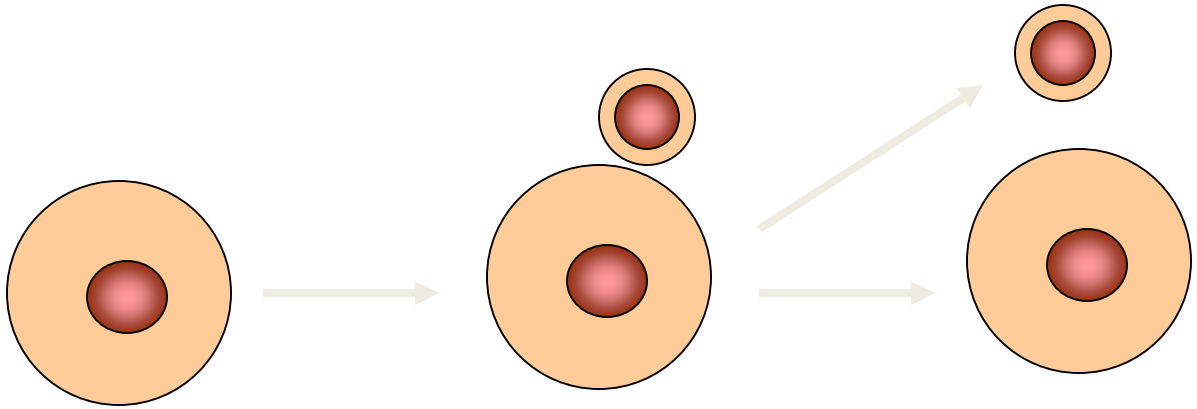
zarodek mysz



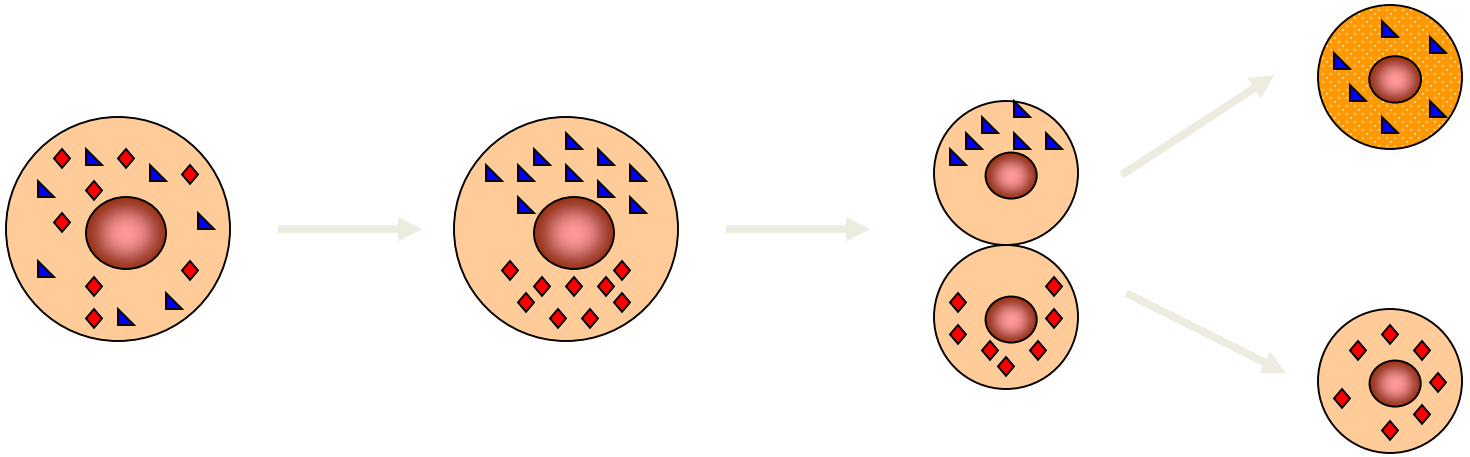
postać dorosła

Determination of cell fate

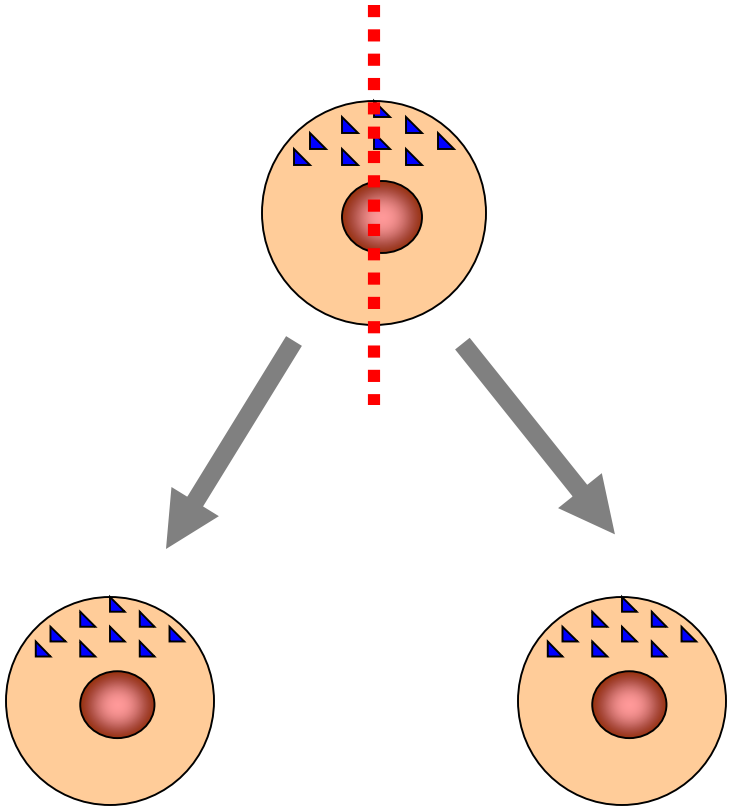
- nonequivalent division of the fertilized oocyte



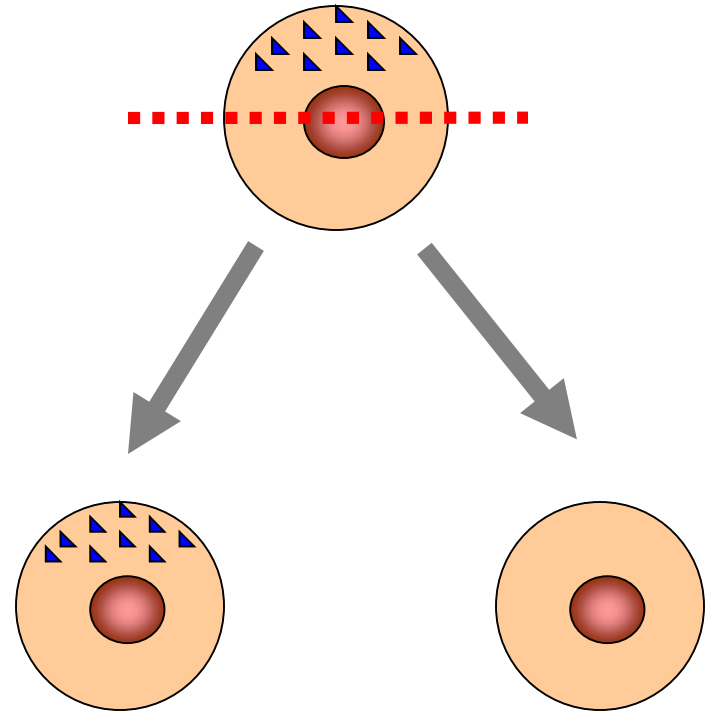
- uneven segregation of cytoplasmic determinants



Symmetric division

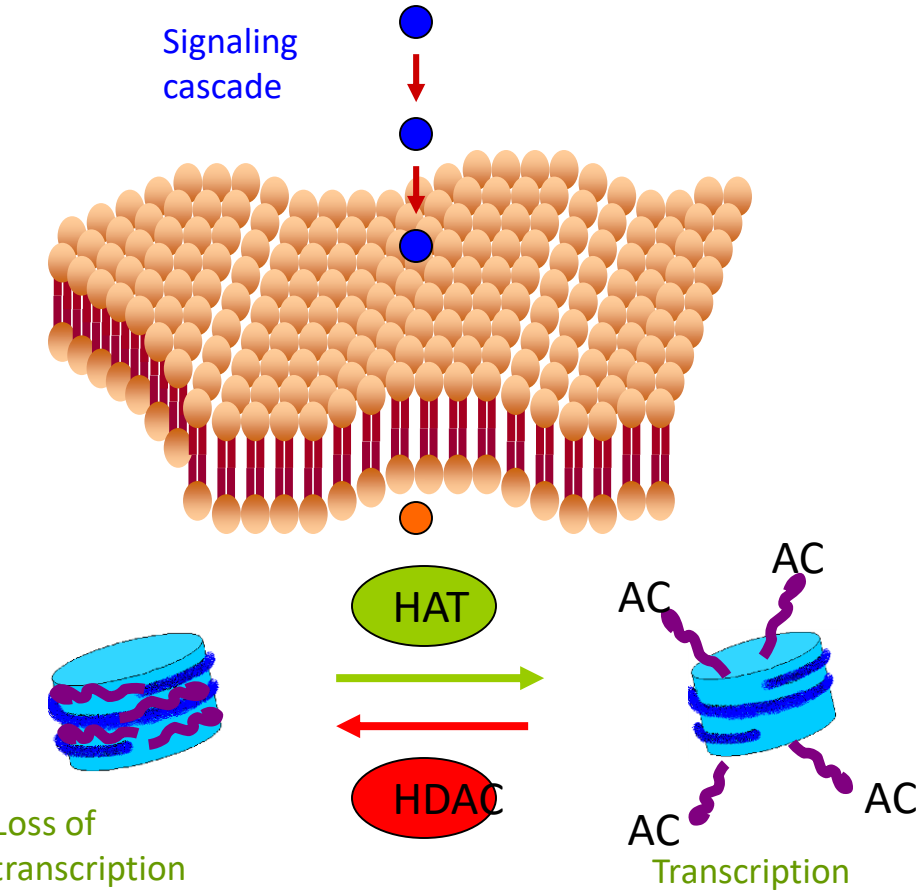


asymmetric division



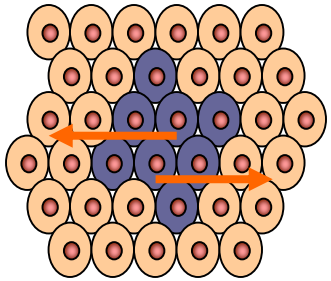
Inducing substances

- Proteins or peptides operating in low concentration
- The same inductor may be a participant of the various cell interactions at various stages of development
- Examples of such substances are: growth factors (FGF, TGFb; BMP)
  peptides of the hedgehog family
  Wnt glycoproteins
- Inducers whose effect in the process of differentiation depends on their concentration gradient is called morphogens



- Intracellular signaling
- Intercellular signaling
- Time dependencies
- Positional dependiences

inductive signaling between cells



Signalling center

morphogens

Cell secreting positional and informative signal

Signal transduction

Receptor for signal

Low activation level

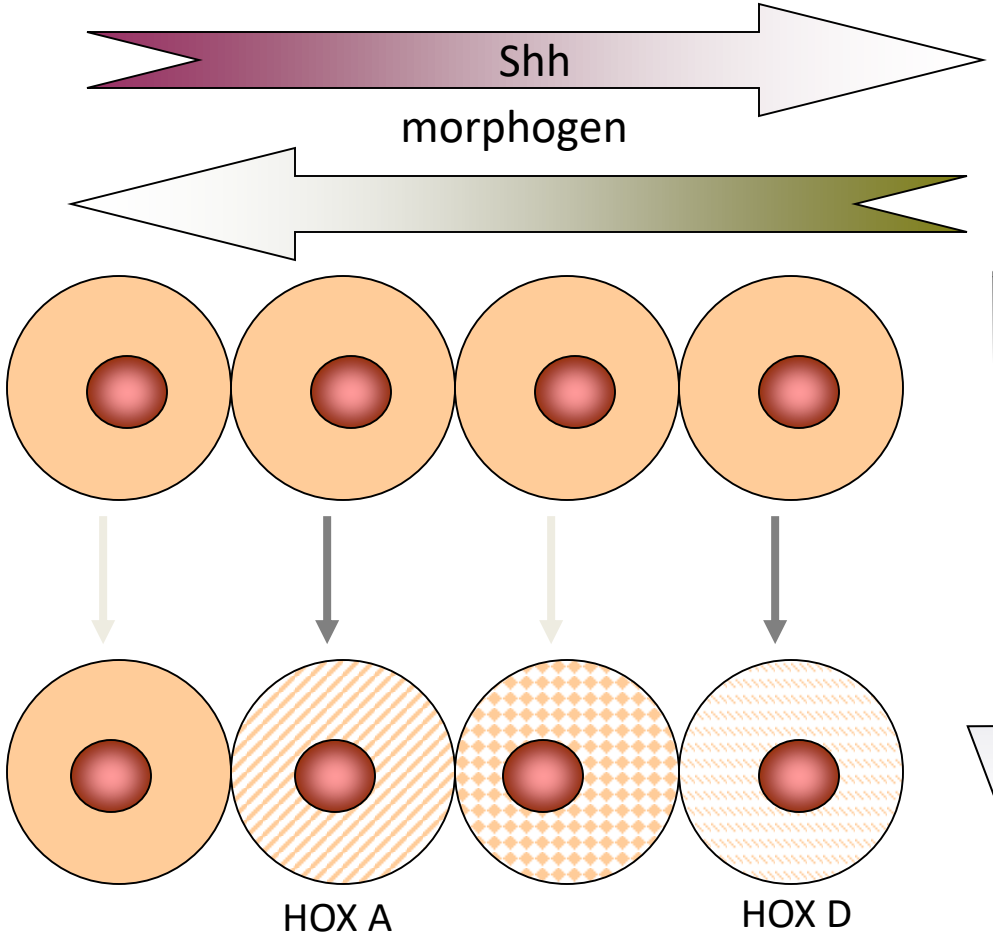
High activation level of transcription factor

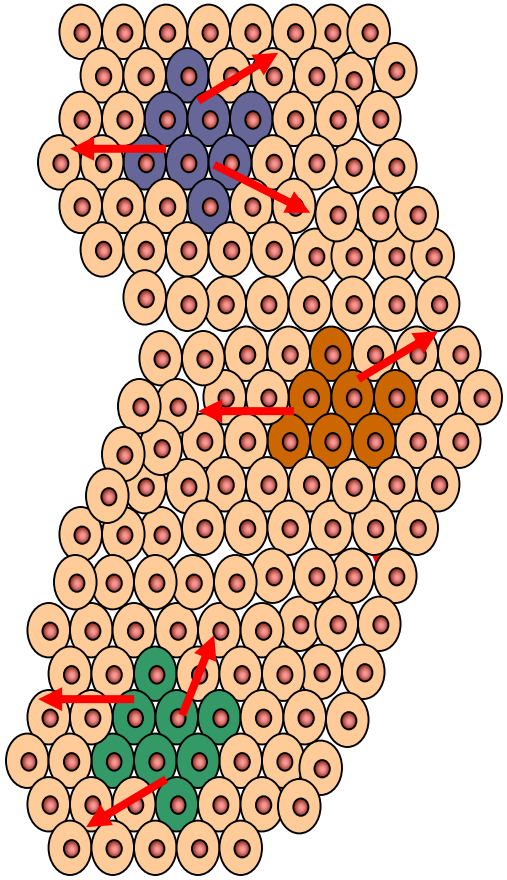
Low activation level

Inductive interactions are cascade, and cells can be under influence of many morphogens of different gradients, and mechanisms of action

positional signaling

cell differentiation depends on the location





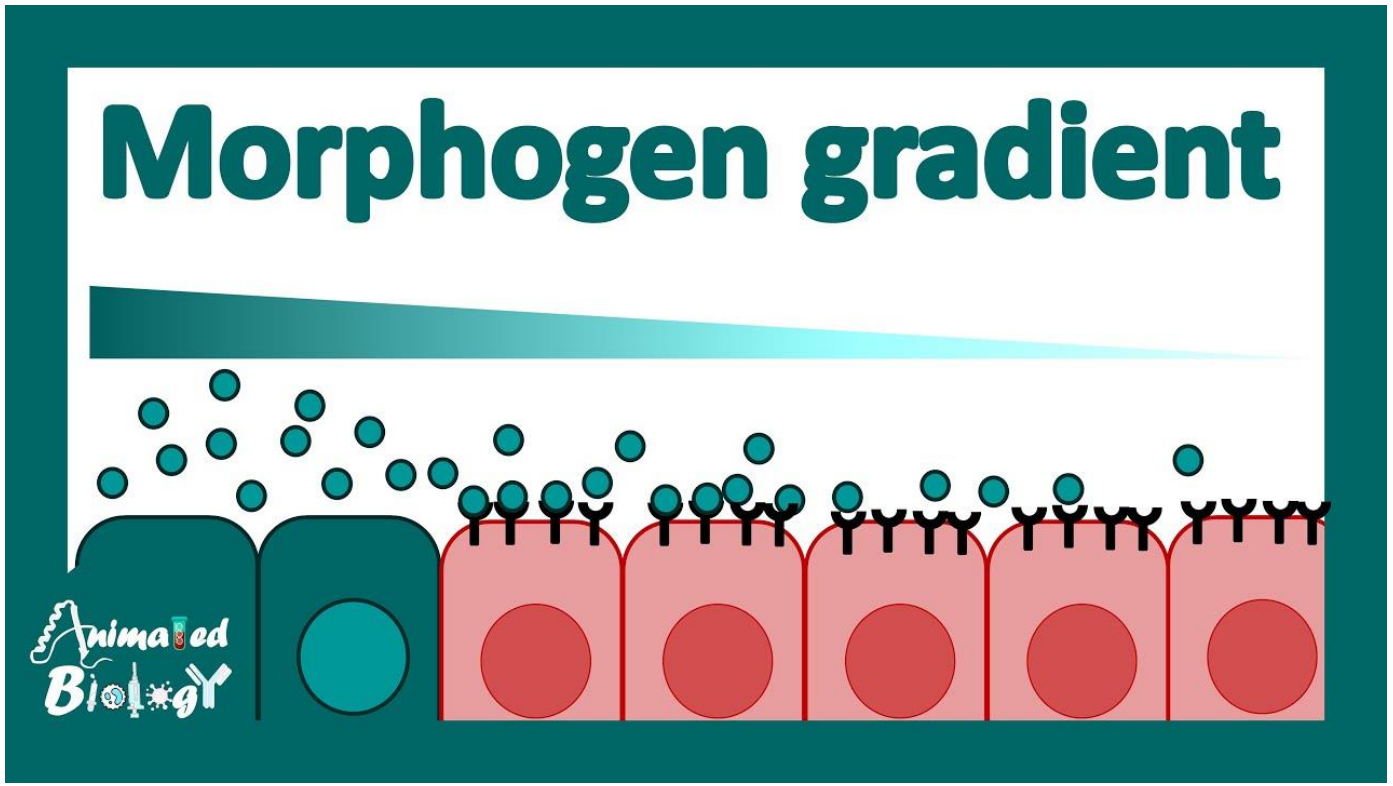
Spatial "models"
Distribution of
structurally and functionally specialized cells

cell fate map

Morphogenic areas

complex system of intercellular interactions:

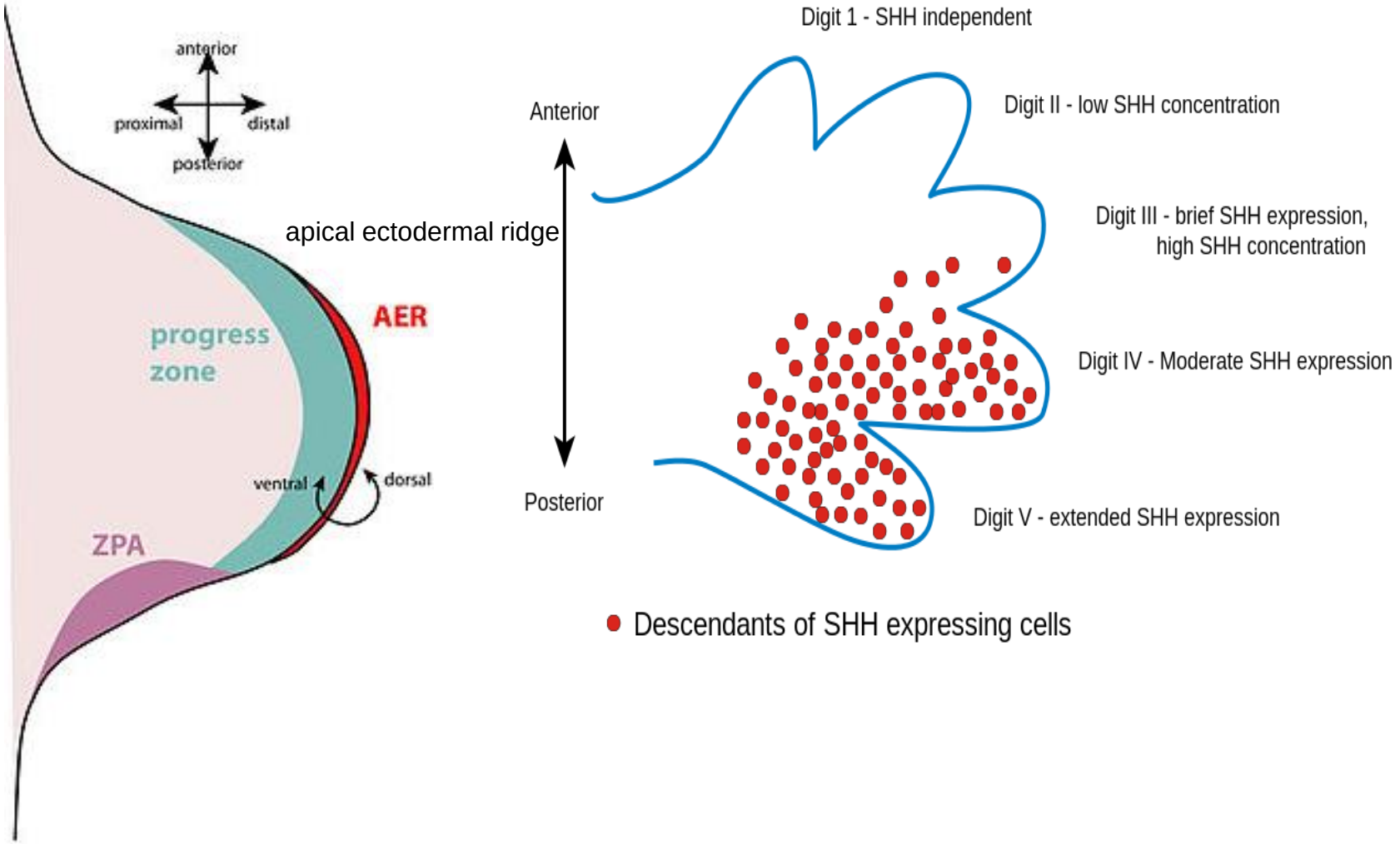
- overlapping gradients of morphogens,
- varied responses to the inductors,
- creation of new signaling centers



- Soluble
- Paracrine action
- Action along a concentration gradient

The anterior/posterior polarity of the vertebrate limb is regulated by a signaling center called the polarization activity zone (ZPA). Apical ectodermal ridge (AER).

Sonic hedgehog



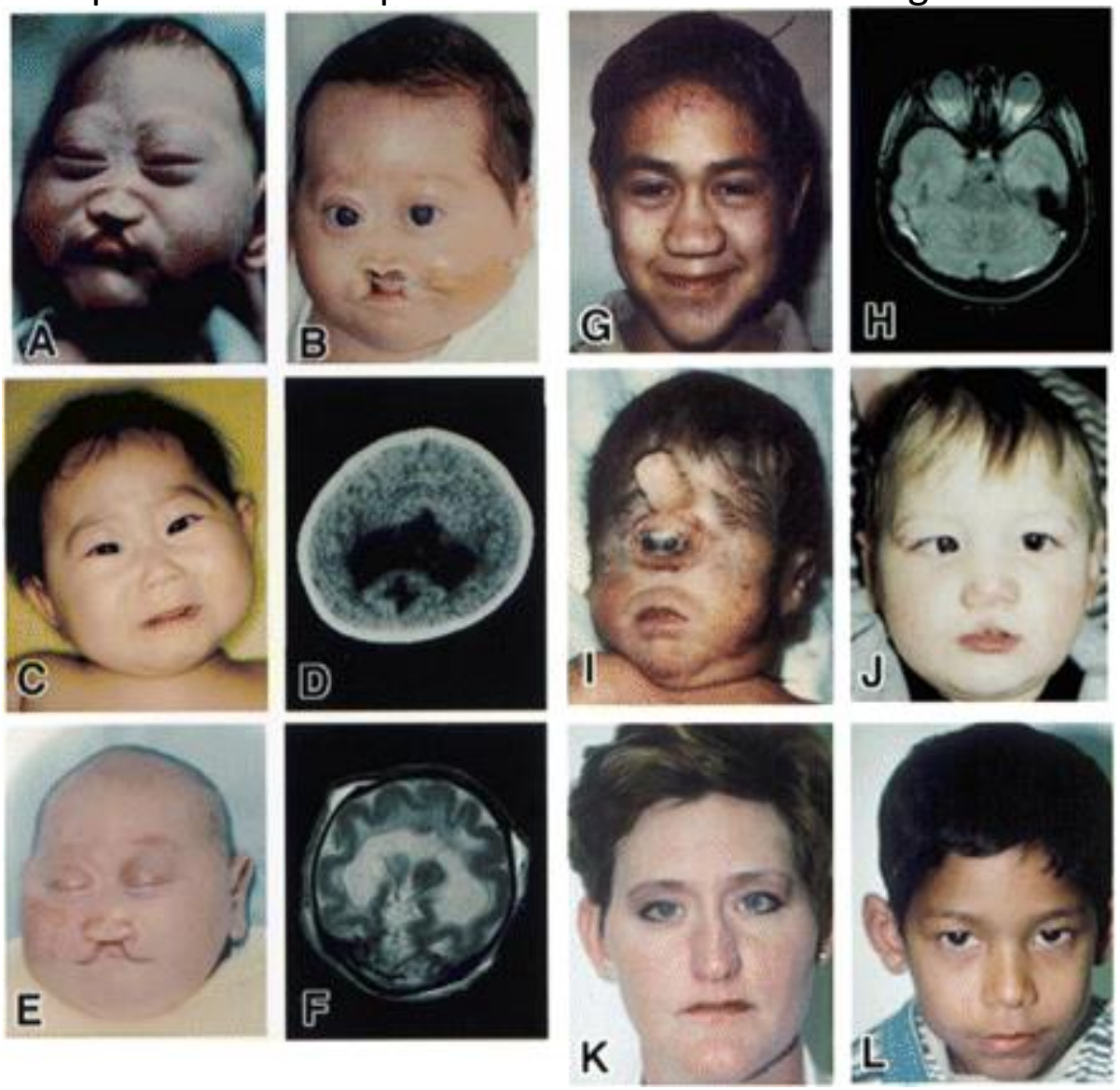
Holoprosencephaly (HPE)

Mutations - *Sonic Hedgehog* (*SHH*)

a common developmental anomaly of the human forebrain and midface where the cerebral hemispheres fail to separate into distinct left and right halves.

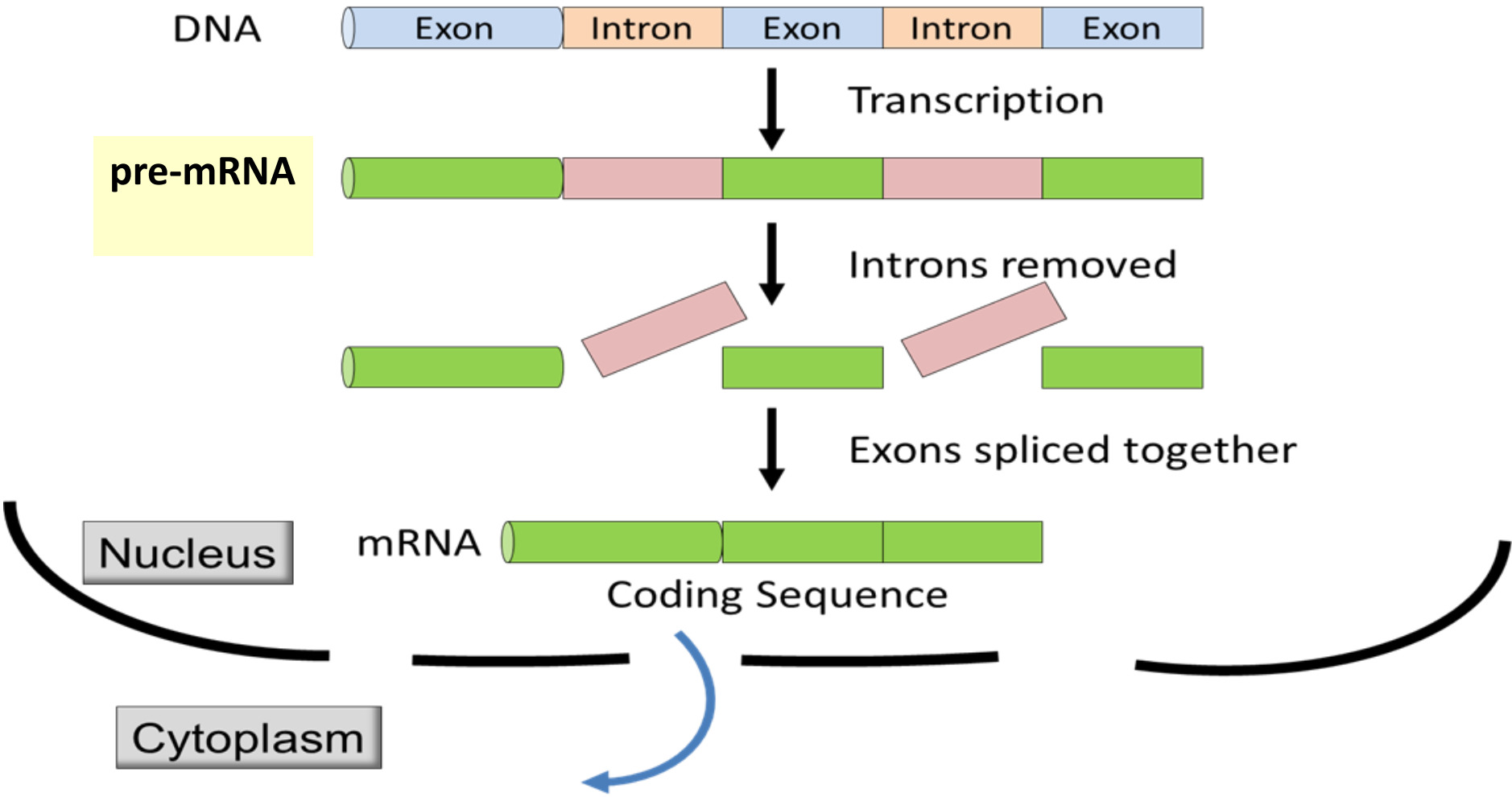
cyclopia

proboscis

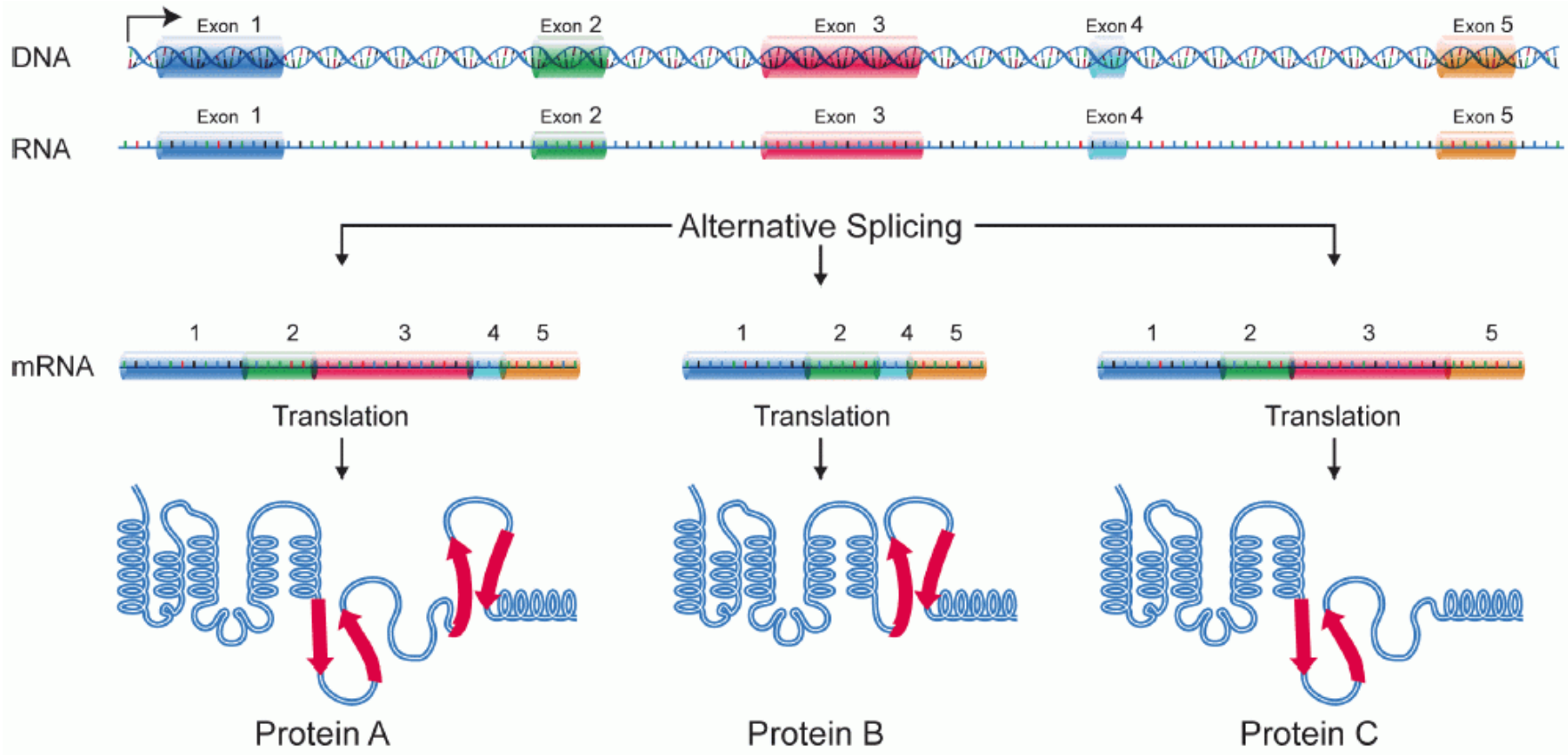


corpus callosum

Primary transcript (precursor messenger RNA (pre-mRNA) - coding segments - **exons** and noncoding segments – **introns**. Introns - removed, exons - spliced together - **splicing**



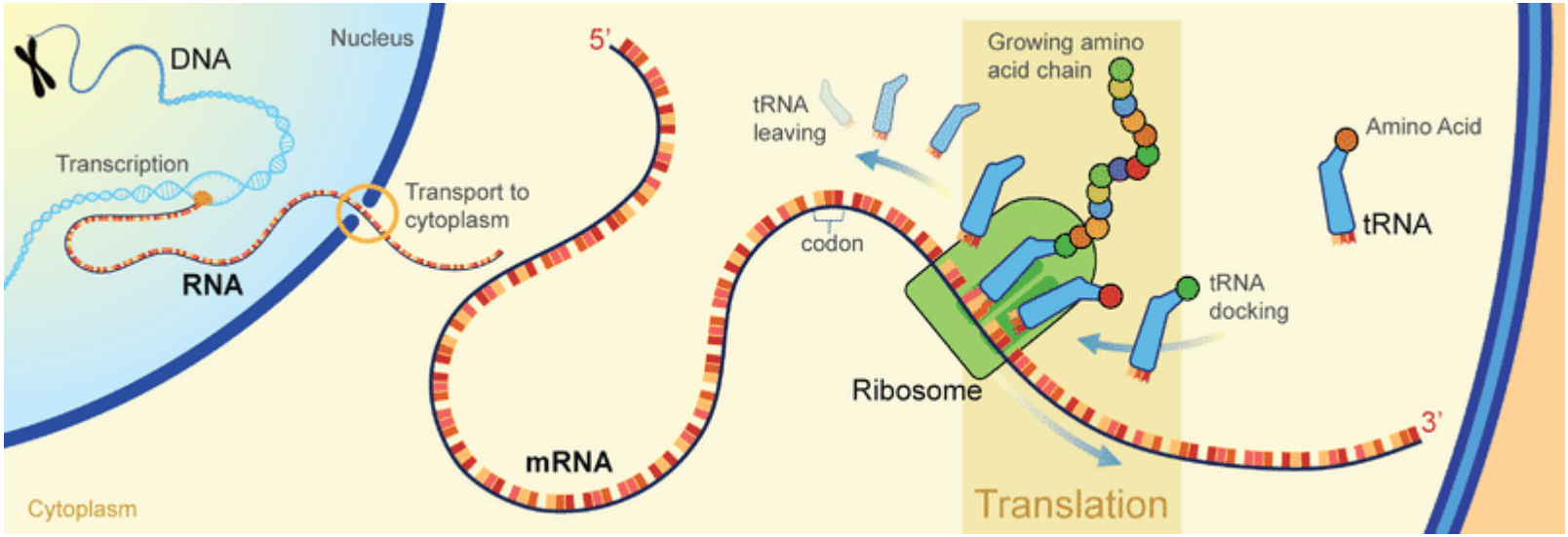
Alternative splicing - alternative forms of mRNA



Alternative splicing allows the synthesis of many more proteins than would be expected from the 20,000 protein-coding genes.

Translation

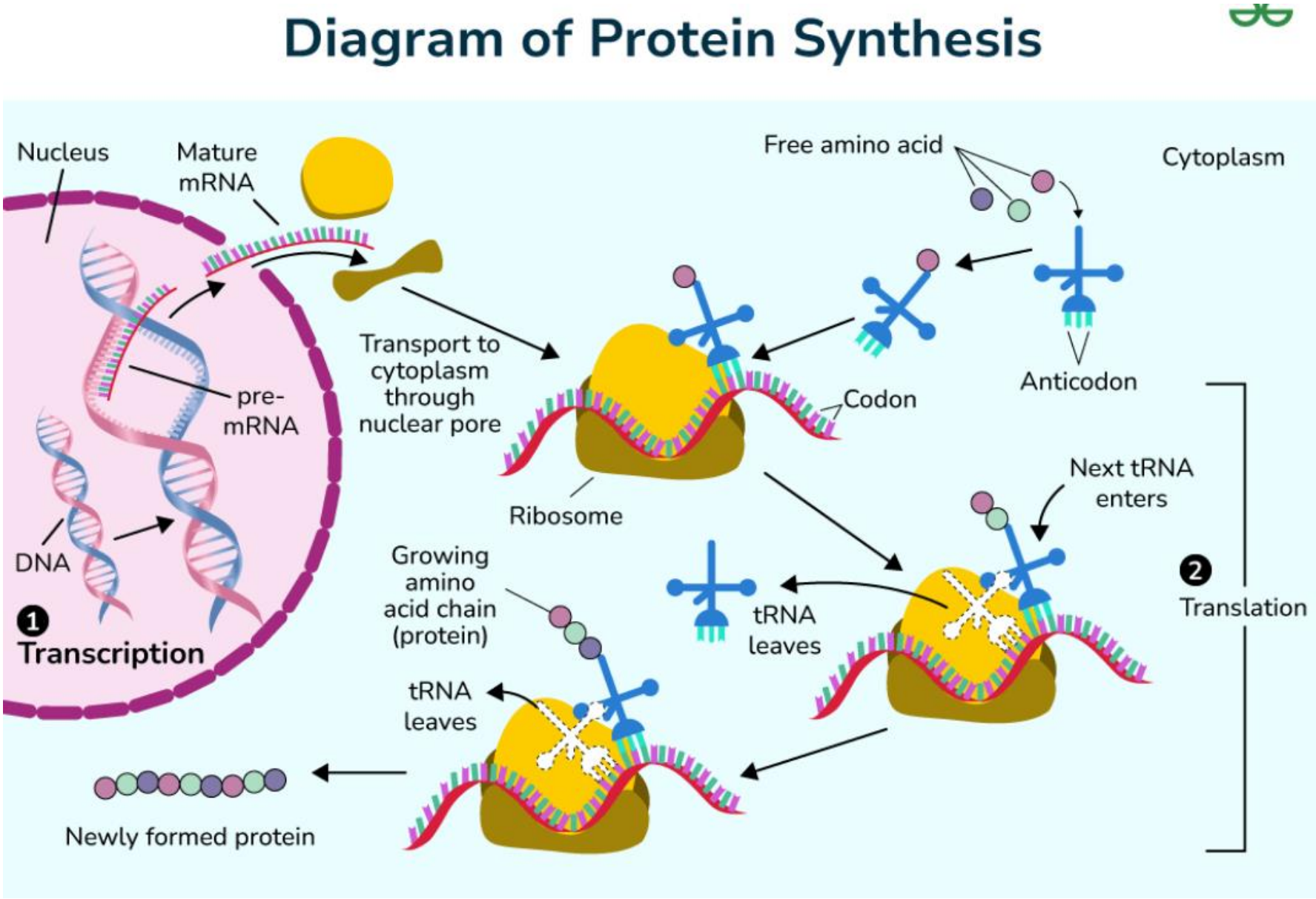
Synthesis of an amino acid chain from an mRNA.



• **Outside the nucleus** (nuclear processing of the pre-mRNA have been completed and the mRNA molecules have been transported to the cytoplasm via nuclear pores).

• **Facilitated by ribosomes** located on the rough endoplasmic reticulum (on the outer surface of the nuclear envelope, or in the cytoplasm).

Translation




THANK
YOU!