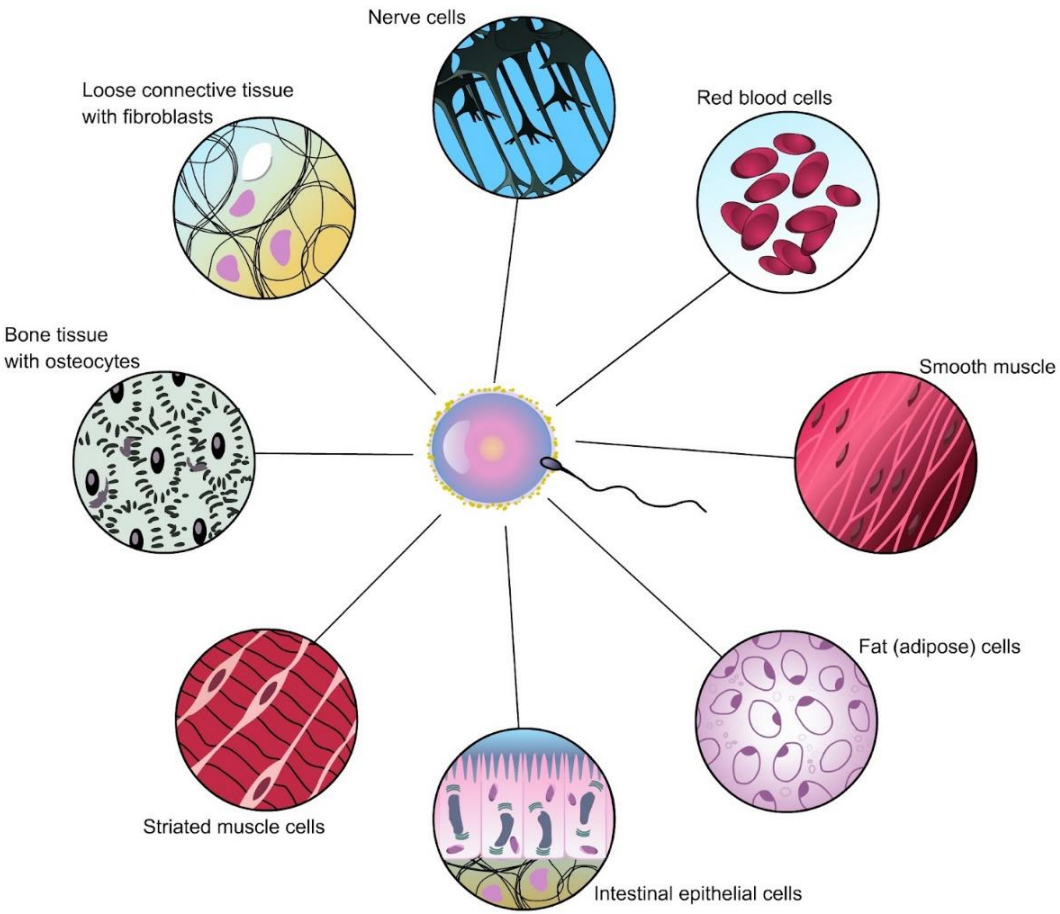
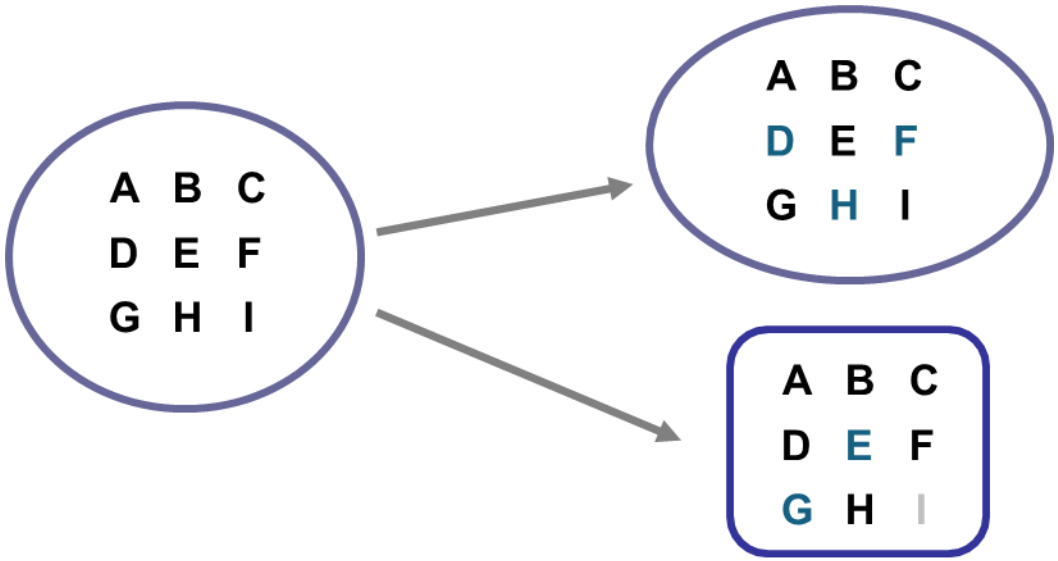
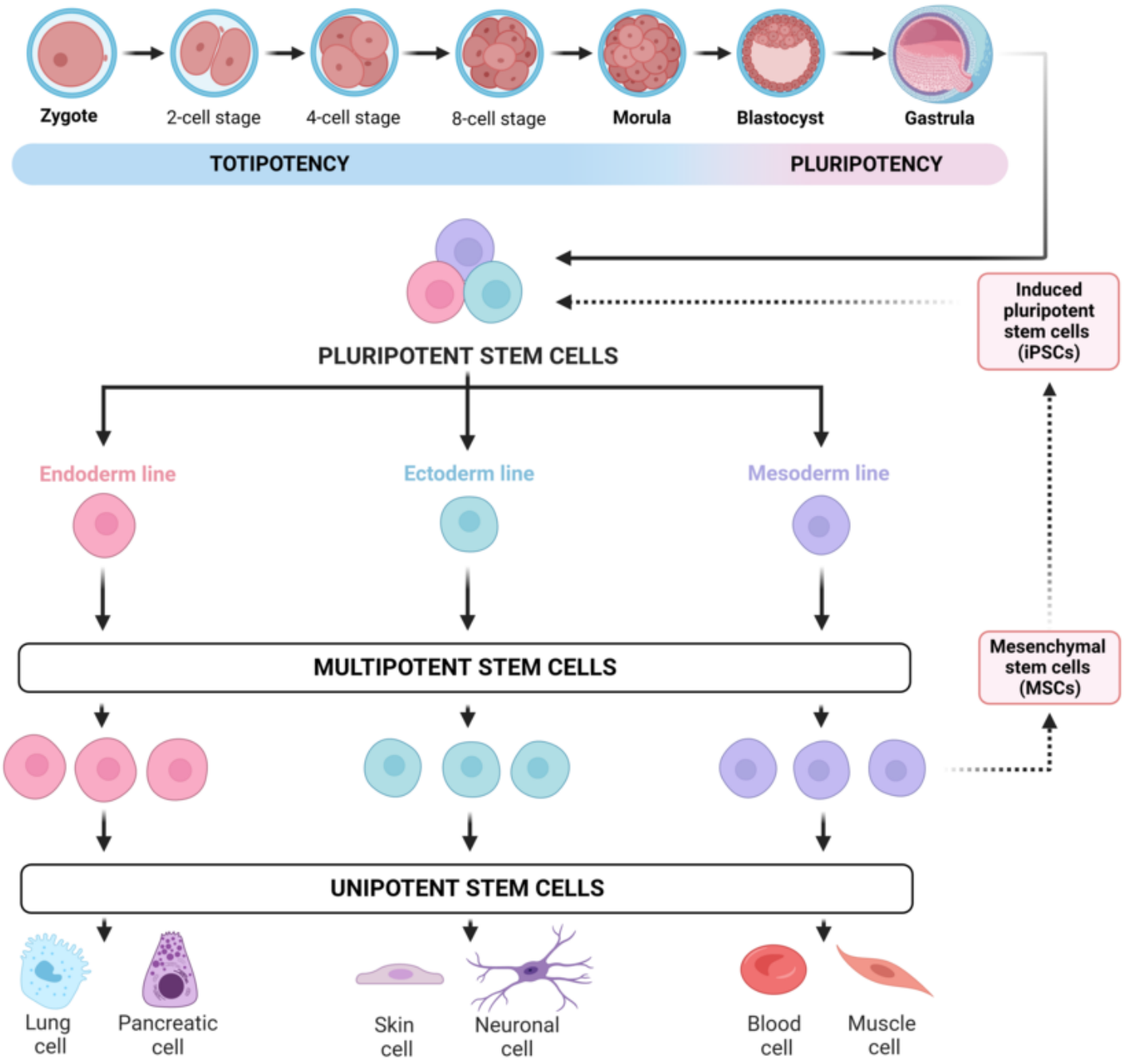
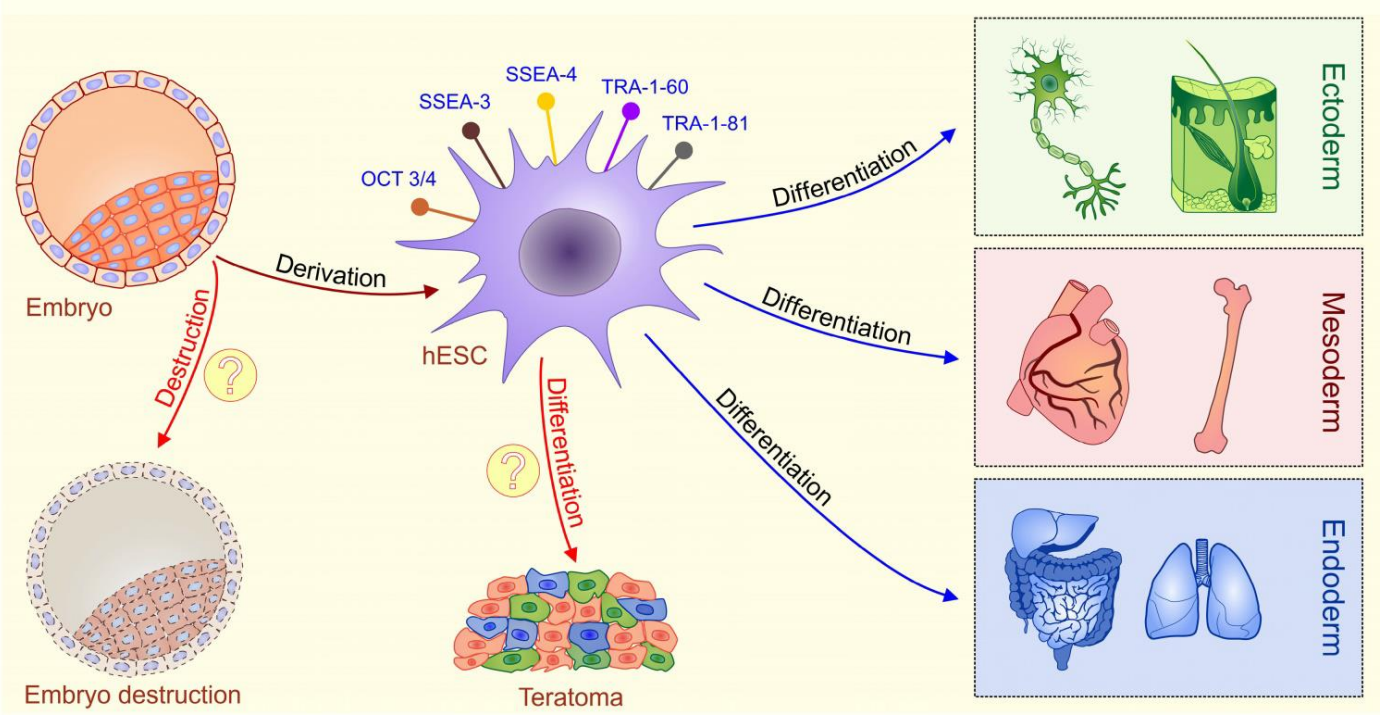
Cell differentiation



Cell differentiation is a phenomenon of gradual, structural, and functional cell specialization resulting from a change in the gene expression pattern of daughter cells.




Zygote
2-cell stage
4-cell stage
8-cell stage
Morula
Blastocyst
Gastrula
TOTIPOTENCY
PLURIPOTENCY
Induced pluripotent stem cells (iPSCs)
PLURIPOTENT STEM CELLS
Endoderm line
Ectoderm line
Mesoderm line
MULTIPOTENT STEM CELLS
Mesenchymal stem cells (MSCs)
UNIPOTENT STEM CELLS
Lung cell
Pancreatic cell
Skin cell
Neuronal cell
Blood cell
Muscle cell

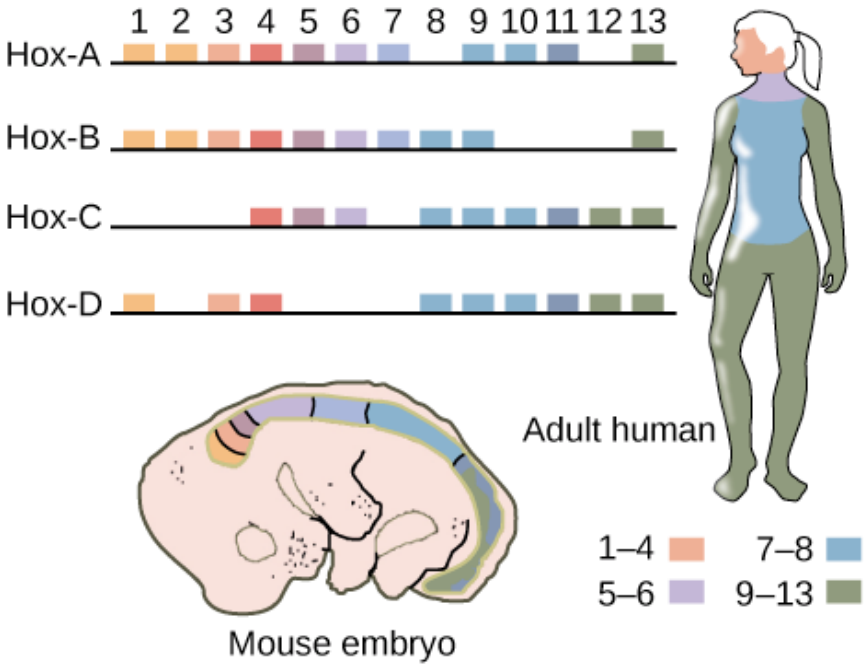


- **Oct-3/4** – (octamer- binding transcriptional factor) is active as a transcription factor with homeodomain in the oocyte and in the preimplantation period
- **NANOG** - transcription factor with a homeodomain that helps the ESC maintain pluripotency by suppressing cell-determining factors.

Transcription factors

Homeotic genes- regulatory genes that are responsible for the development of particular segments or structures of the body.

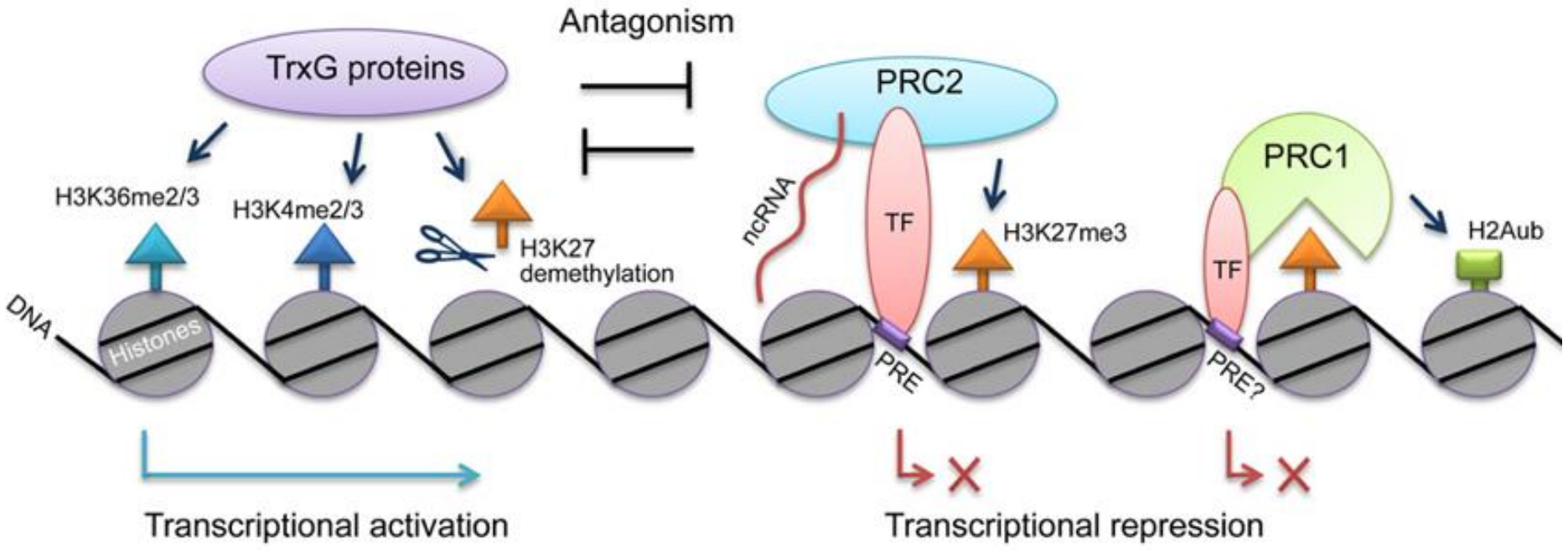
Homeotic genes (Hox genes) code for **transcription factors**, which contain a fragment called **homedomain** encoded by a specific sequence of nucleotides - the Homeobox



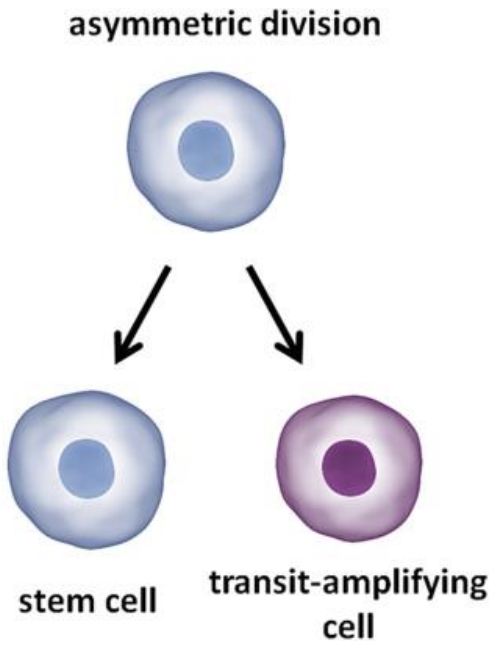
Epigenetic modifications

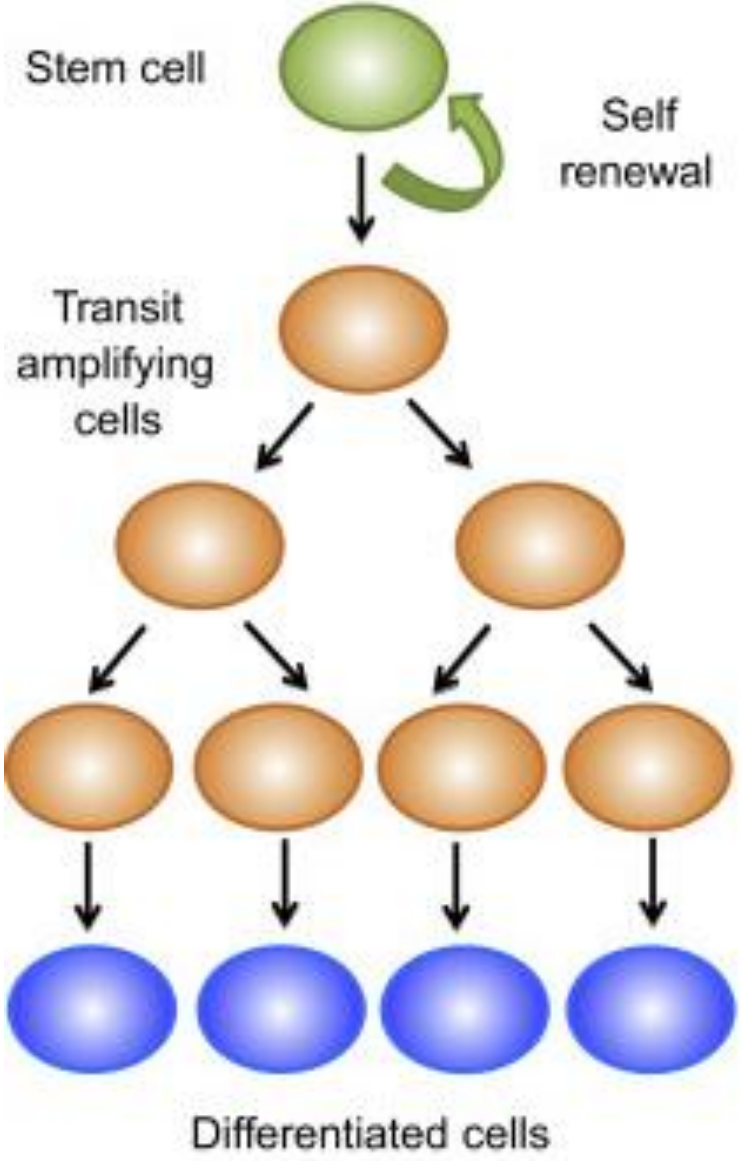
Complex of proteins **Trithorax** – Gene activation.

Complx of proteins **Polycomb** remodel chromatin - epigenetic gene silencing.



Asymmetric division of stem cells

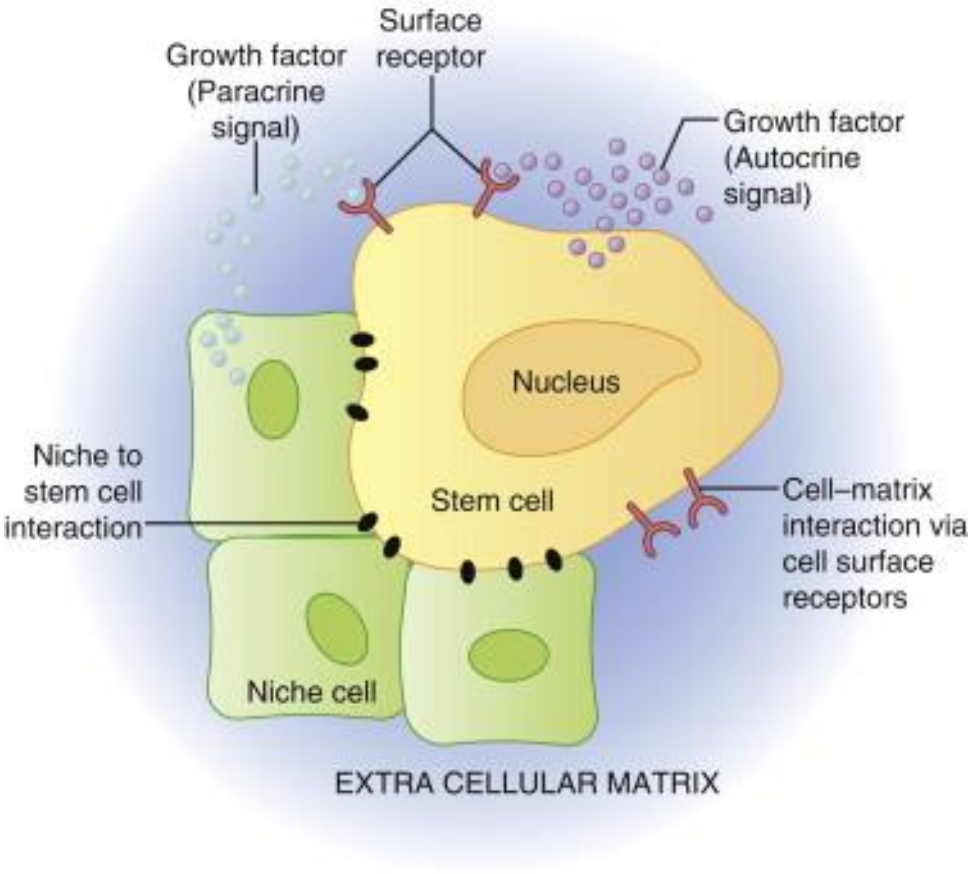




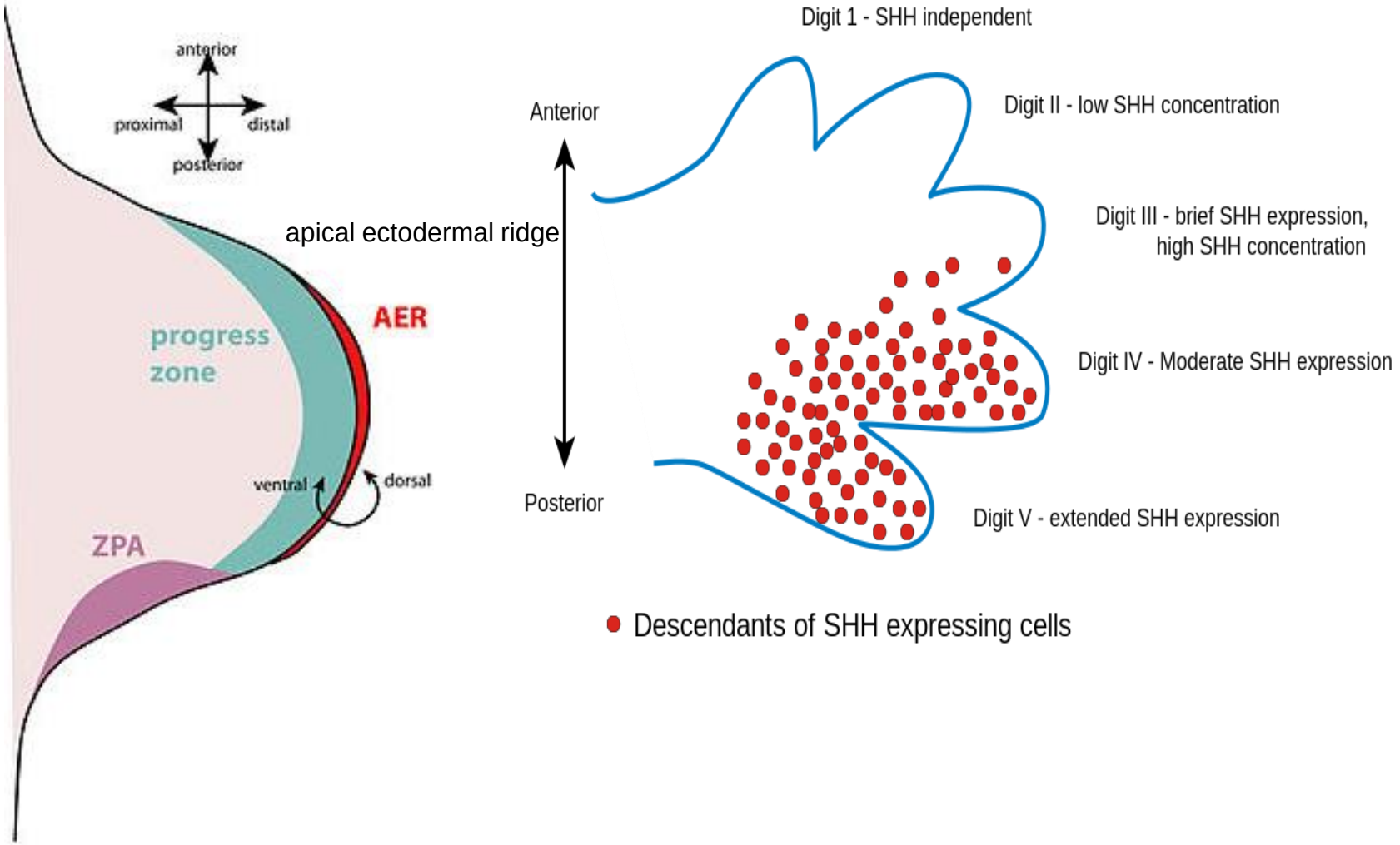
Stem cell niche

Extrinsic signaling:
- ECM – E-cadherin, β-catenin
- Growth factors
- Integrins
- Other soluble factors

Intrinsic mechanisms:
- Axis of polarity
- Asymmetric protein segregation



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



Adult stem cells

- Multipotent
- Can develop into cells that are closely related.
- Limited number of several cell types.
- Make all cell types from the tissue they come from.
- Found in many parts of the body.
- Can self-renew over a lifetime.

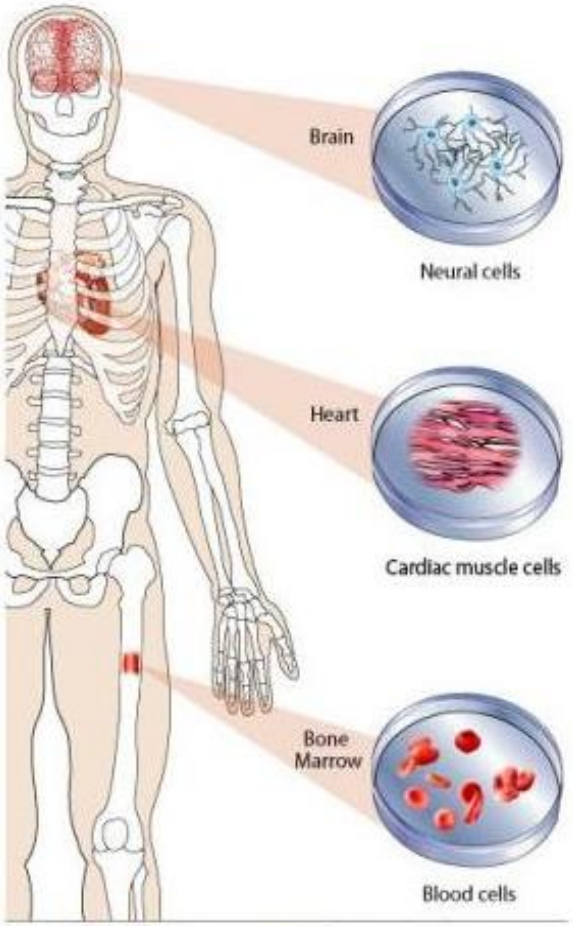
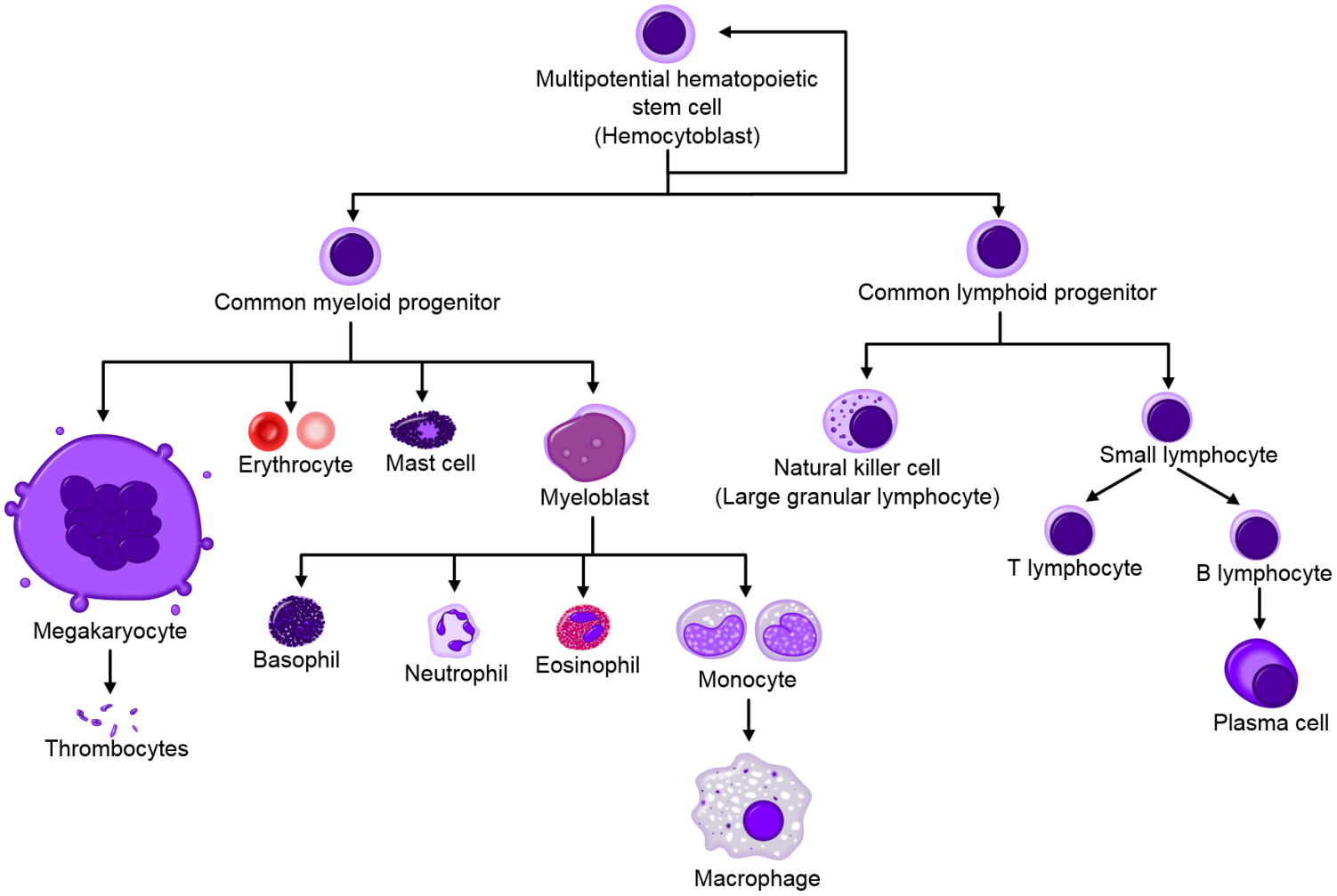
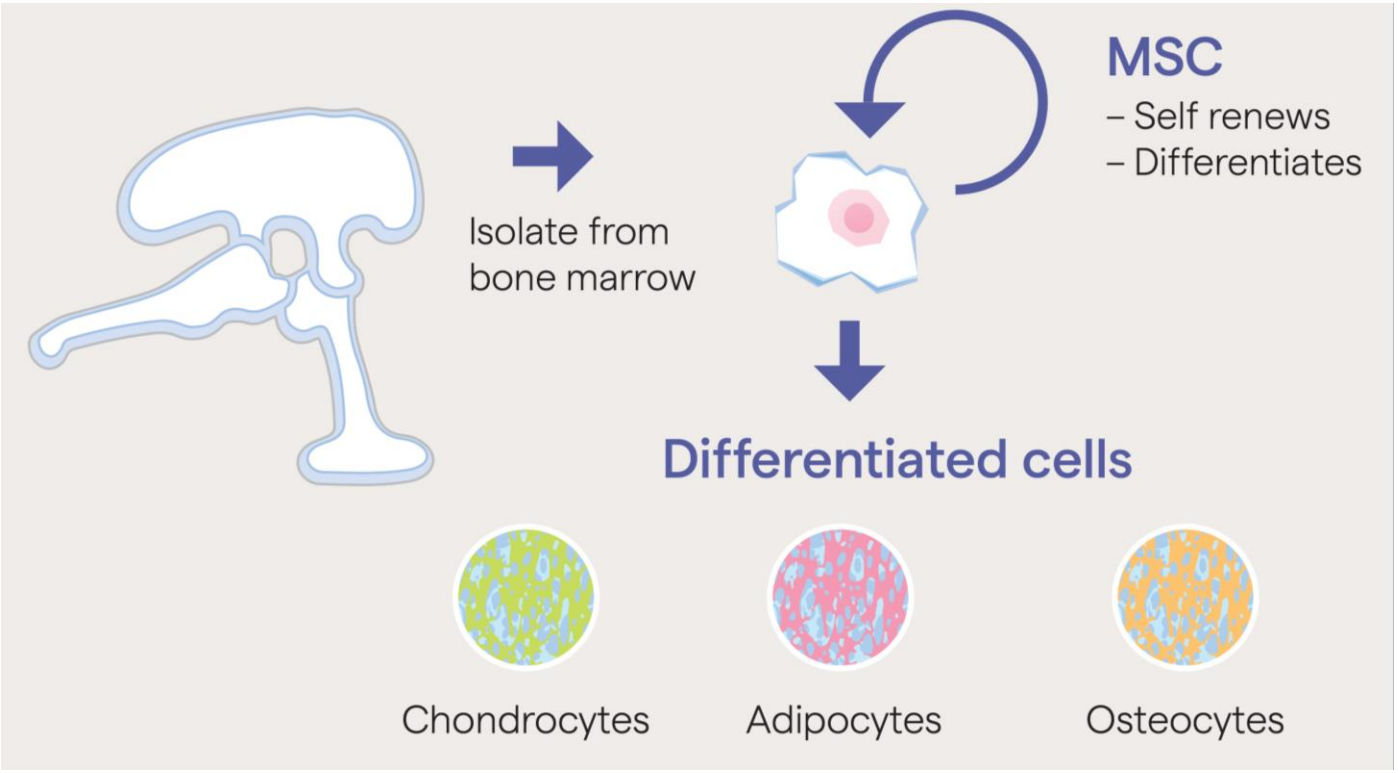


Illustration by Cell Imaging Core of the Center for Reproductive Sciences.

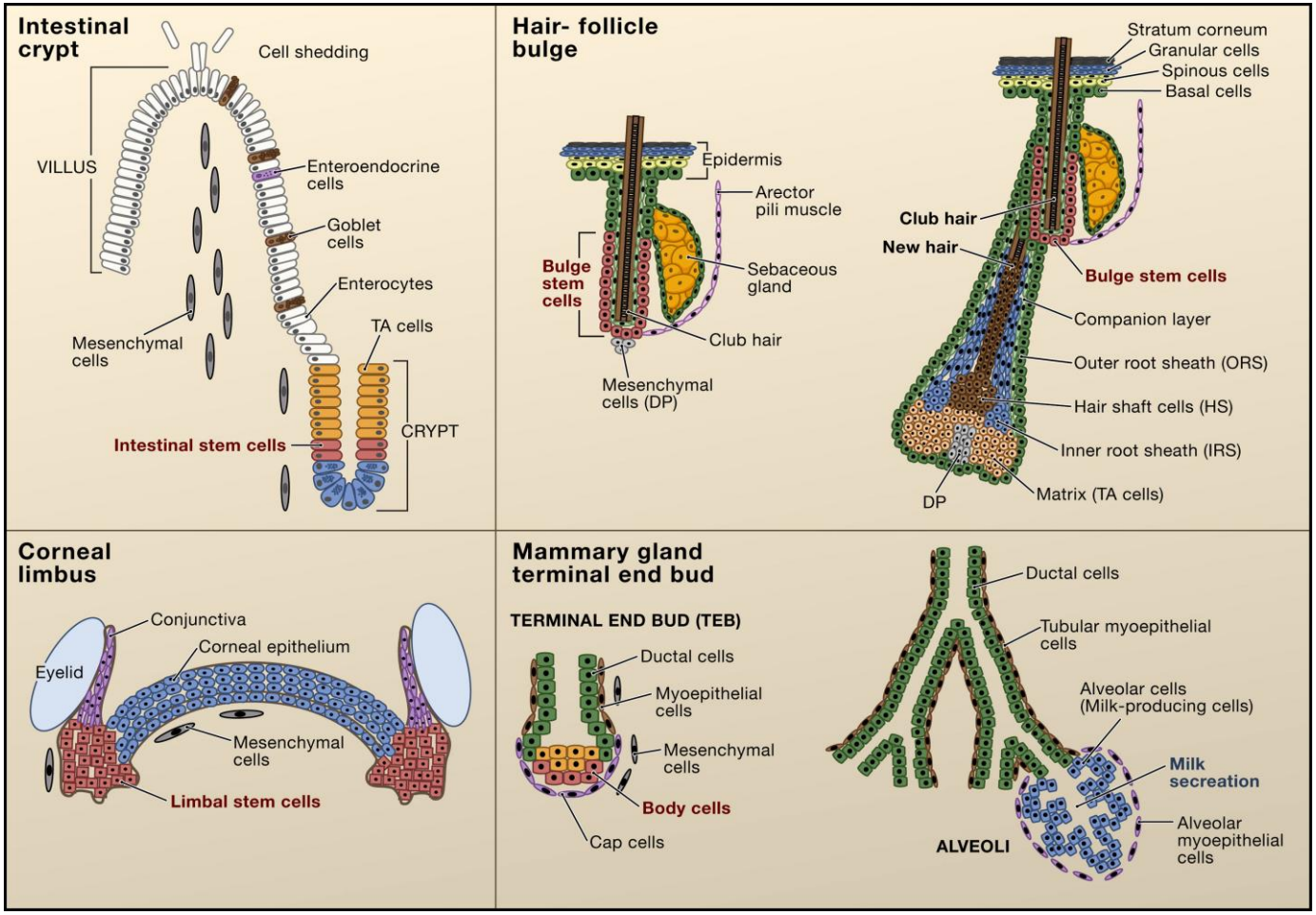
Hematopoiesis stem cells (HSC) - multipotent cells, differentiate into blood cells, megakaryocytes.



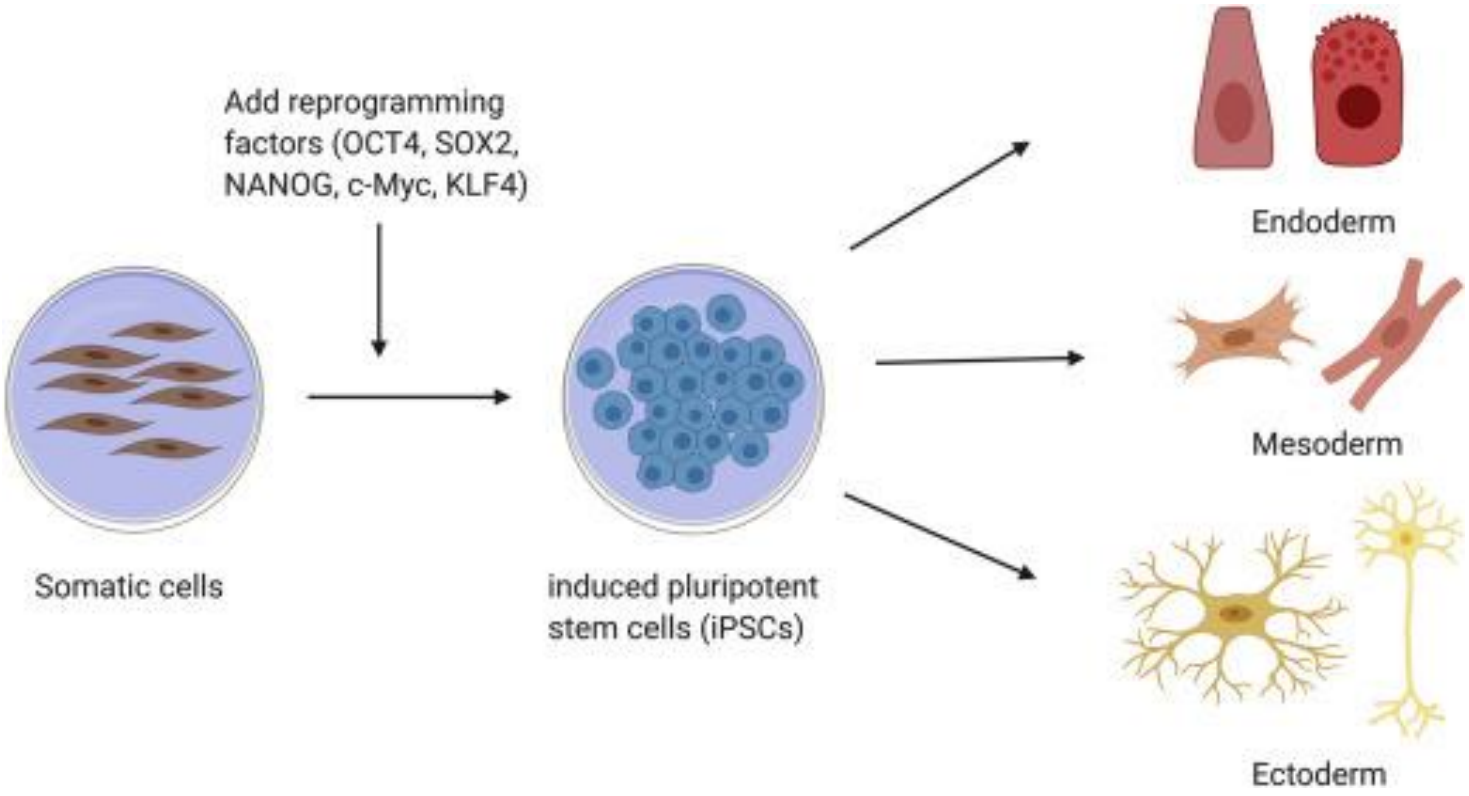
Mesenchymal stem cells (MSC) - multipotent cells, differentiate into osteoblast, adipocytes, chondroblasts



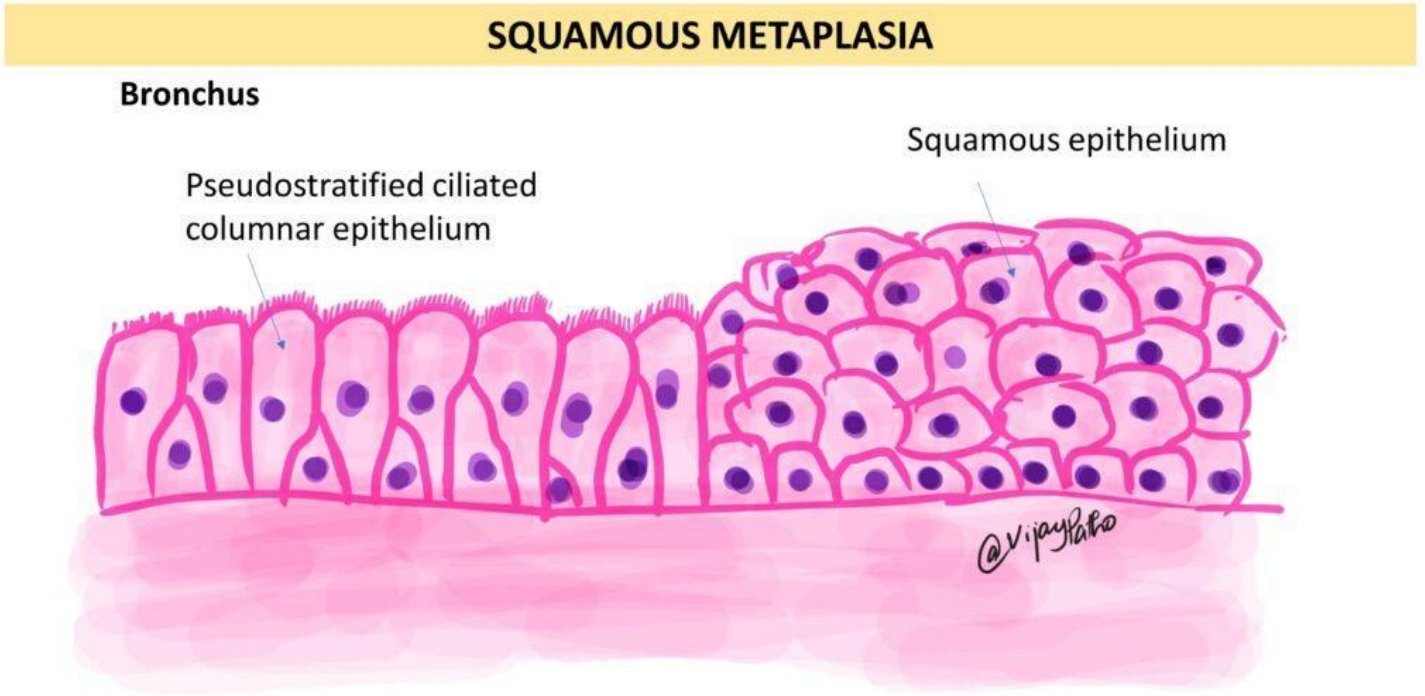
Epithelial stem cells (MSC) - multipotent cells, differentiate into keratinocytes of epidermis and hair follicle, enterocytes, pneumocytes



Induced pluripotent stem cells (iPSc)



Metaplasia is the transformation of a cell type to another cell type

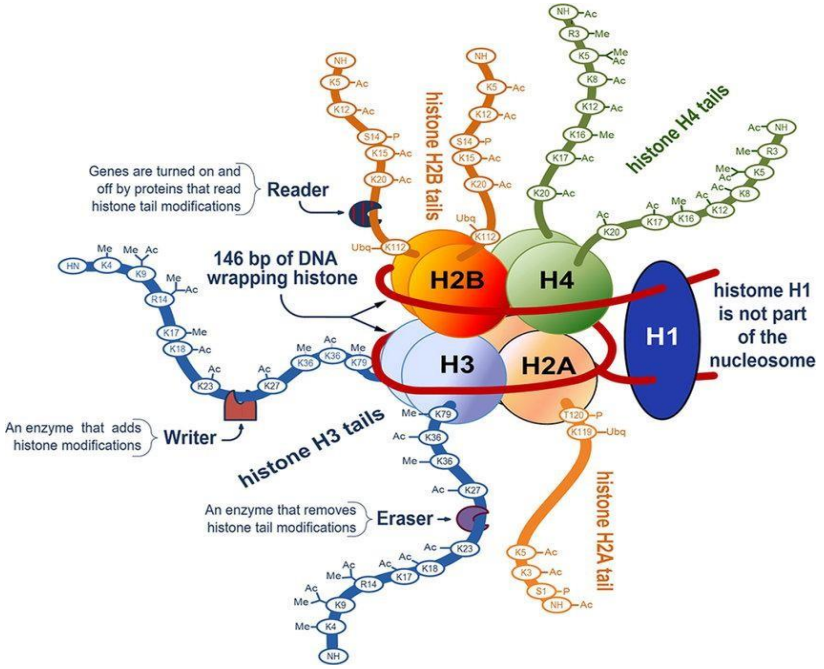


Epigenetics

- Changes in the genome that are not associated with changes in the sequence of nucleotides
- Essential for the development and maintenance of tissue-specific gene expression patterns in mammals
- Hereditary nature of modification – are restored after DNA replication– **cellular/epigenetic memory bookmarking**
- Mechanisms: **DNA methylation and histone modifications**, that alter the pattern of gene expression

BIOCHEMICAL MECHANISM OF EPIGENETIC MEMORY

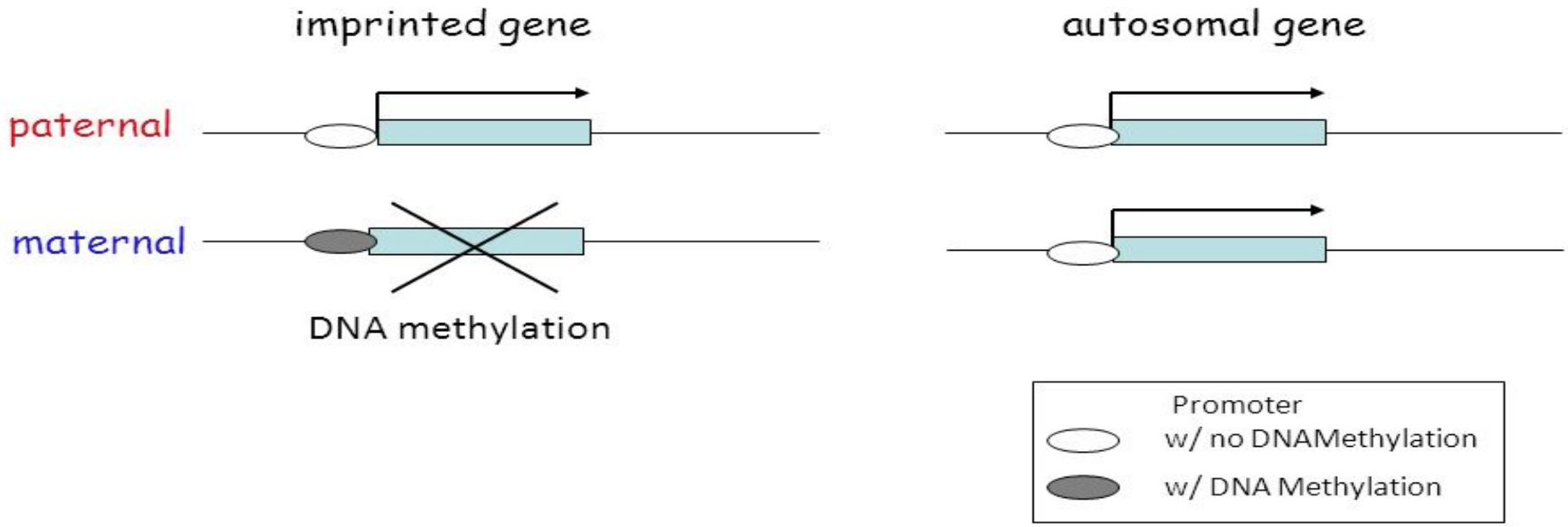
- DNA methylation
- Modifications of histone proteins: methylation acetylation phosphorylation
- lncRNA (long non-coding RNA)



Imprinting

Somatic (diploid) cells have two copies (alleles) of the gene (from the father and from the mother).

- Expression occurs from both alleles at the same time, a small part (<1%) of genes are imprinted genes. Their expression occurs from only one allele (the gene encoding IGF2 is expressed only from the paternal allele).
- Mechanism of epigenetic modification (DNA methylation)



Prader-Willi syndrome (PWS)

- Deletion of part of paternal chromosome 15 – maternal imprinting.

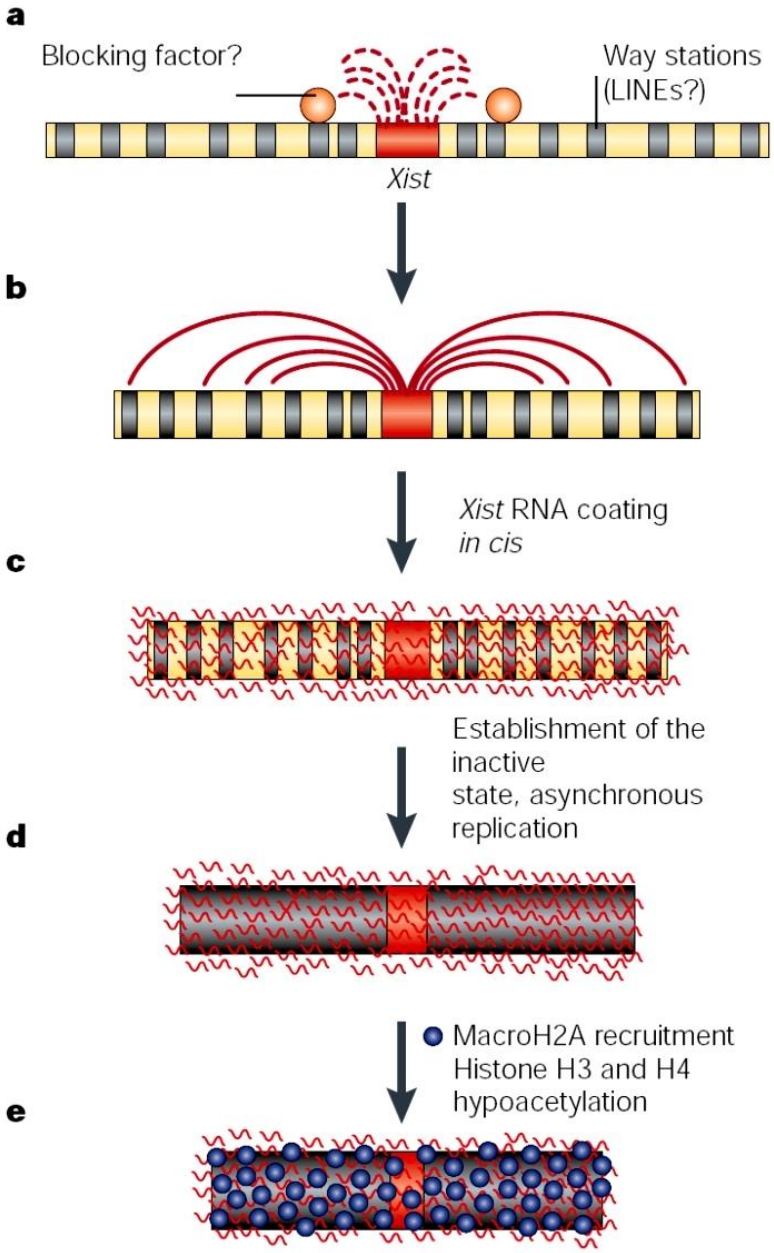
- There is a deletion of paternal copies of SNRPN and necdin genes together with snoRNA clusters - they play a role in pre-mRNA processing, possibly in alternative tissue-specific splicing.
- In childhood, constant hunger, which often leads to obesity and type 2 diabetes. Moderate mental disability.

Diego Velázquez
(1599–1660)
The Ladies-in-waiting.

Giovanni Francesco Caroto (1480 – 1555) Portrait of a Child with a Drawing

Angelman syndrome(AS)

- It consists of a dysfunction of a part of the maternal chromosome 15.
- This is due to a deletion or mutation of the UBE3A gene
- It mainly affects the nervous system. In most neurons, only the maternal copy is normally active - **paternal imprinting.**
- Symptoms: small head and specific facial appearance, severe intellectual disability, developmental disability, speech problems, balance and movement problems, and sleep problems. Children usually have a cheerful personality and are particularly interested in water



Xist – gene encoding (lncRNA)

- It acts as the main effector of the X chromosome inactivation process.

.

- The inactive X chromosome is covered with Xist RNA, X chromosomes devoid of Xist will not be inactivated.

- . X chromosome inactivation equalizes the number of genes in both sexes

- The choice of which X chromosome will be inactivated is random, but once inactivated, the X chromosome will remain inactive.

Locus B – black, chocolate and cinnamon - eumelanin

B – the dominant gene in relation to the others, black in colour

b – gene recessive in relation to B, but dominant in relation to B1, chocolate colour

b1 – gene recessive to both B and b1, cinnamon colour

BB
Bb
Bb1

b1b1

bb
bb1

Locus O – red colors – X chromosome – pheomelanin **– EPISTATIC GENE**

O - is an allele that blocks the production of eumelanin, i.e. only pheomelanin is produced in a given cell

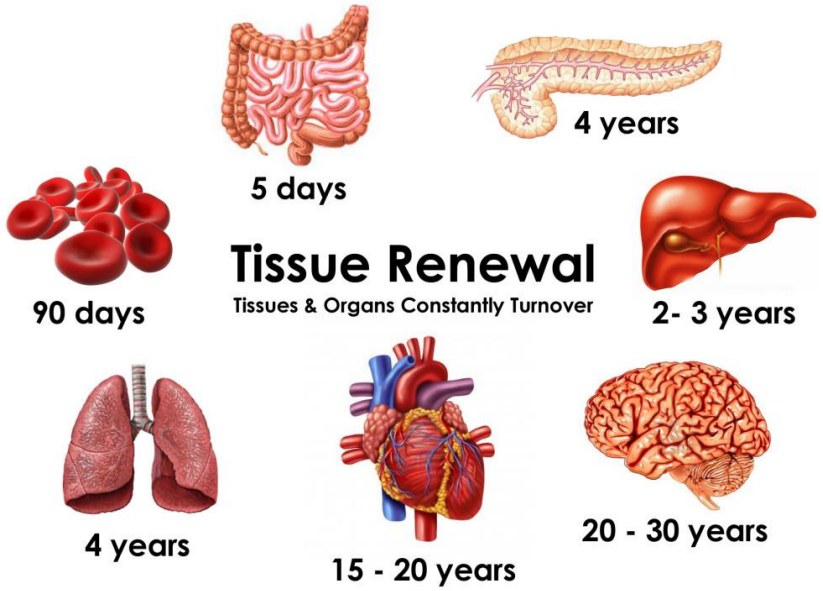
o - does not block eumelanin production

OO female

O- male

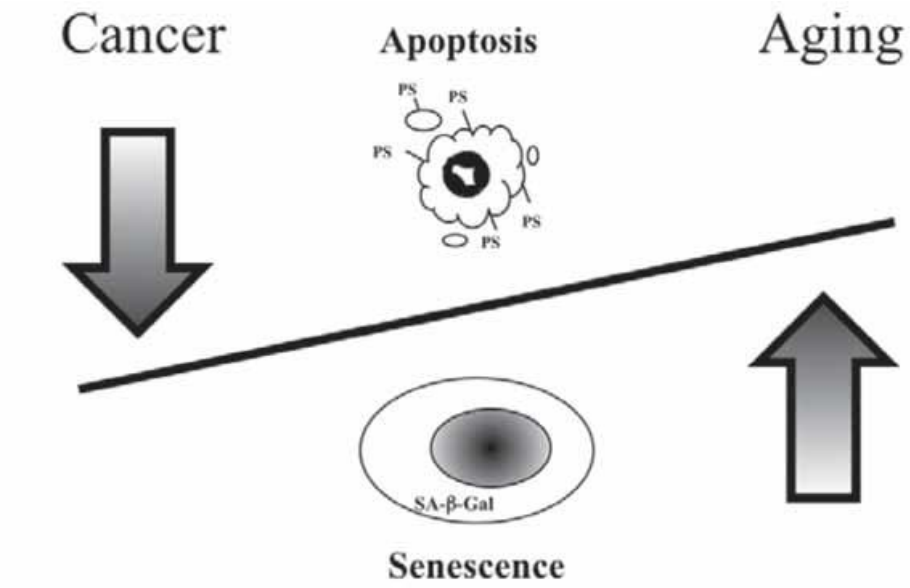
Oo
female

SENESCENCE AND APOPTOSIS



Tissue renewal is essential for the viability of complex multicellular organisms such as mammals, but cell proliferation is essential for tumorigenesis, and renewable tissues are at risk of developing cancer.

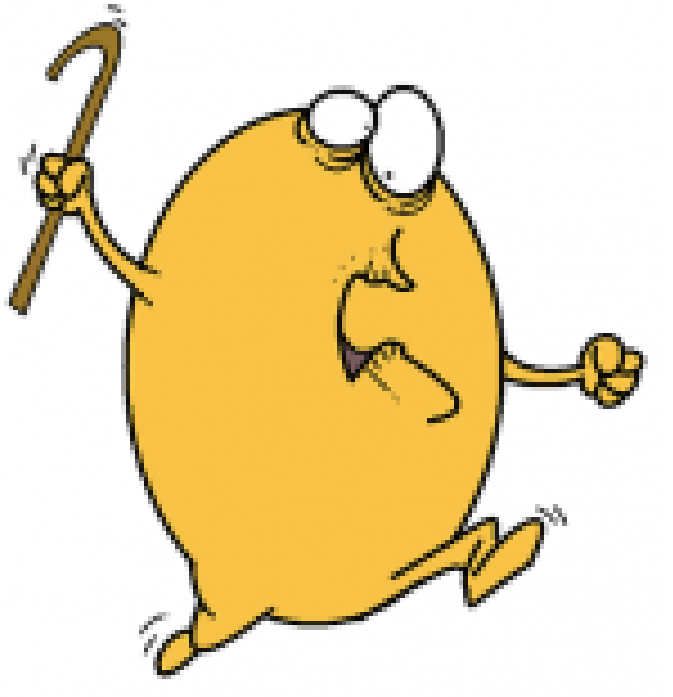
SENESCENCE AND APOPTOSIS



The danger that cancer posed to longevity was mitigated by the evolution of tumor-suppressor mechanisms. One of them is **cellular senescence**, which stops incipient cancer cells from proliferating, second is programmed cell death – **apoptosis**, which eliminates incipient cancer cells from organism

Senescent cell

mitotic cell no longer capable of dividing but still alive and metabolically active.



Senescent cell phenotype

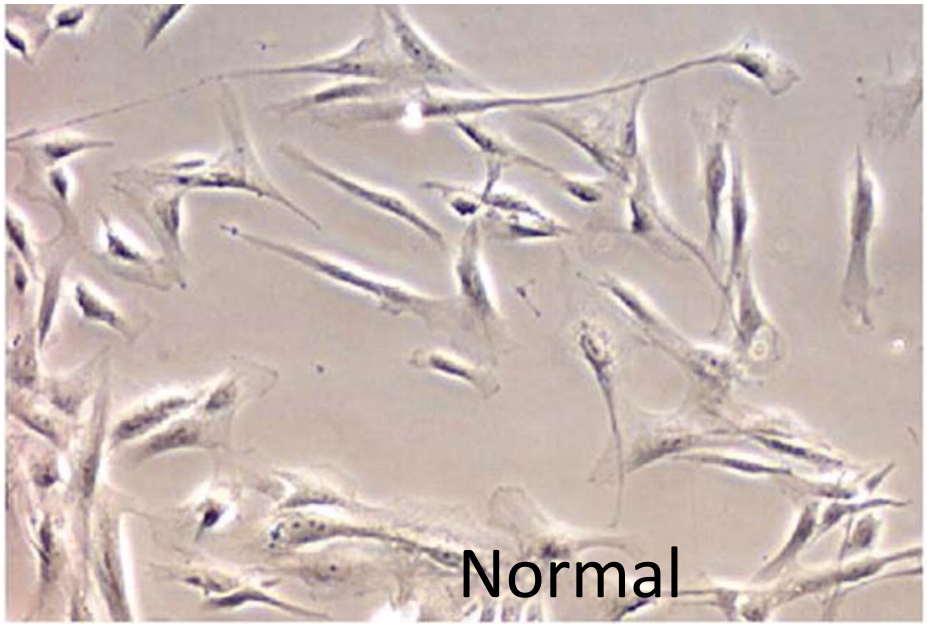
1. Express **SA- β-galactosidase** - senescence-associated β-galactosidase

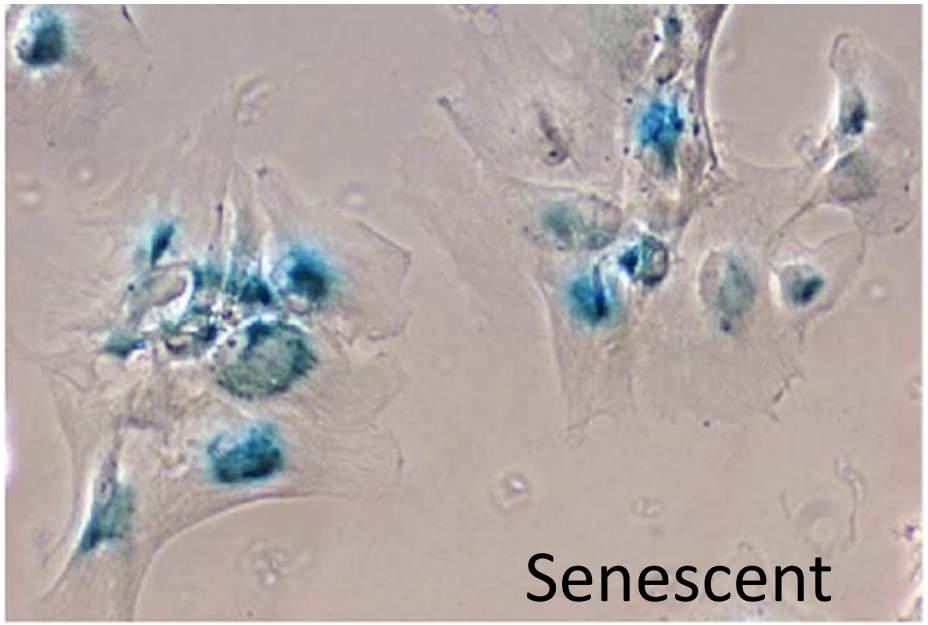
2. **Growth arrest** in G1 – p16 and p21

3. Chromatin modifications - Senescence Associated Heterochromatic Foci (**SAHF**)

4. Apoptotic resistance

Smooth muscle cells in culture labeled for senescence-associated β-galactosidase





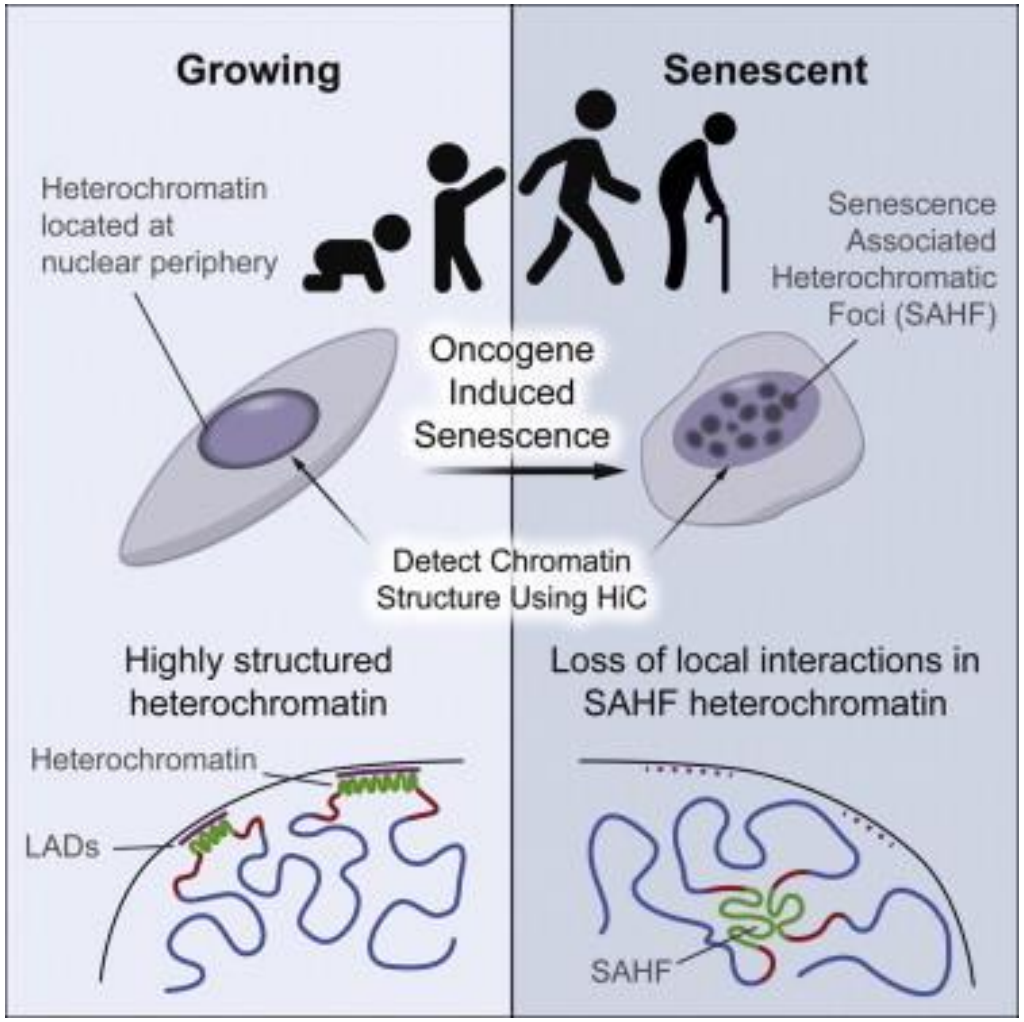
Senescence-associated secretory profile

SASP factors can be divided into the following categories: interleukins, chemokines, growth factors, proteases and ECM components.

These factors can affect surrounding cells by activating various cell-surface receptors and corresponding signal transduction pathways that may lead to multiple pathologies, including cancer, and can participate in degradation of ECM.

Senescent cells can modify the tissue microenvironment.

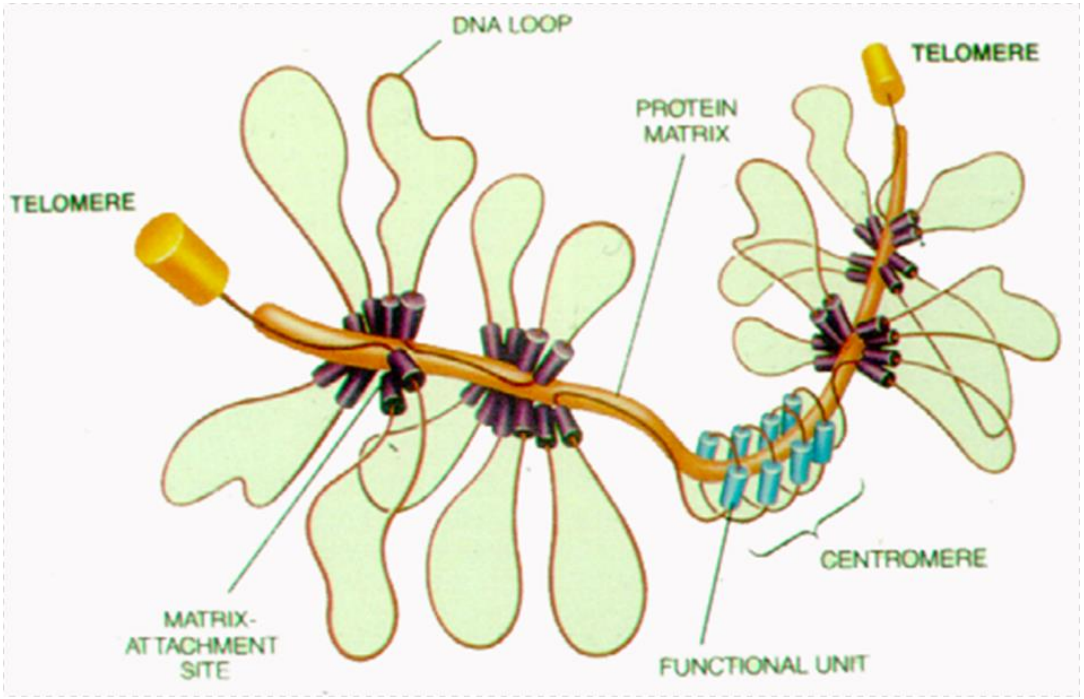
Senescence Associated Heterochromatic Foci (SAHF)

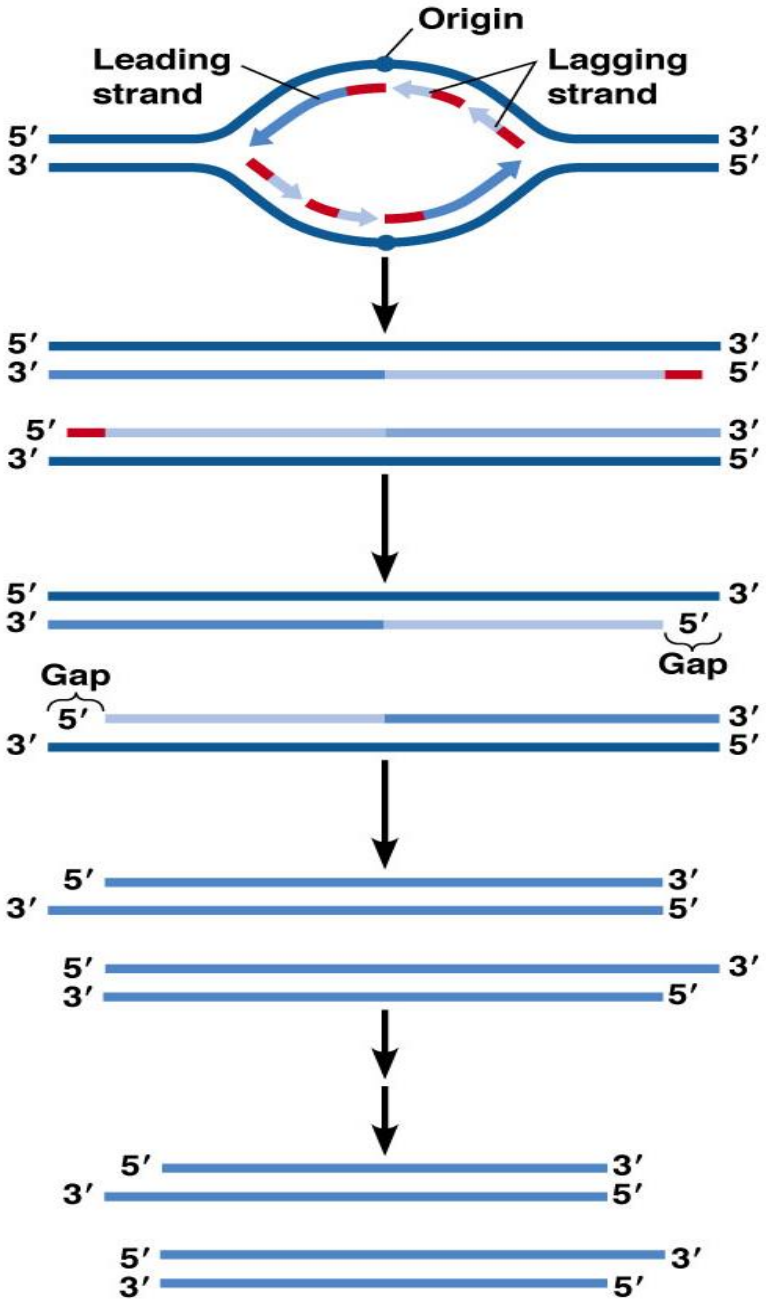


These foci are present in senescence but not in quiescent cells. SAHF embed genomic loci encoding pro-proliferative proteins into heterochromatin structures, thus preventing their transcription.

REPLICATIVE SENESCENCE

Dysfunctional, short telomeres trigger senescence or apoptosis through the p53 – p21 pathway.



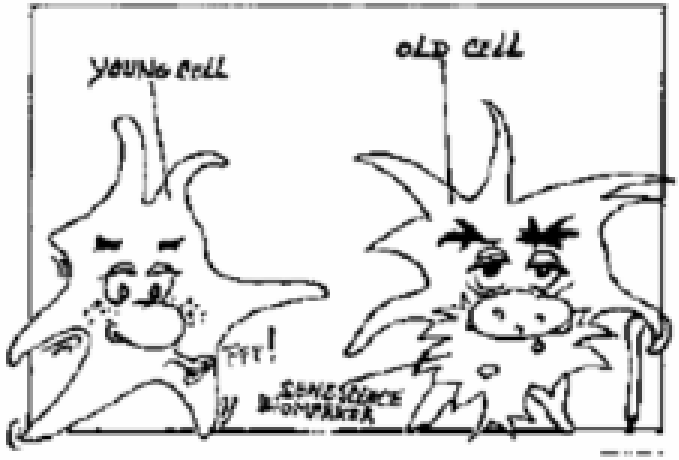


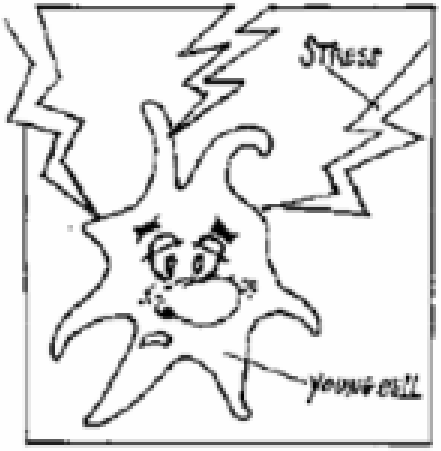
End replication problem - the shortening of telomeres during replication

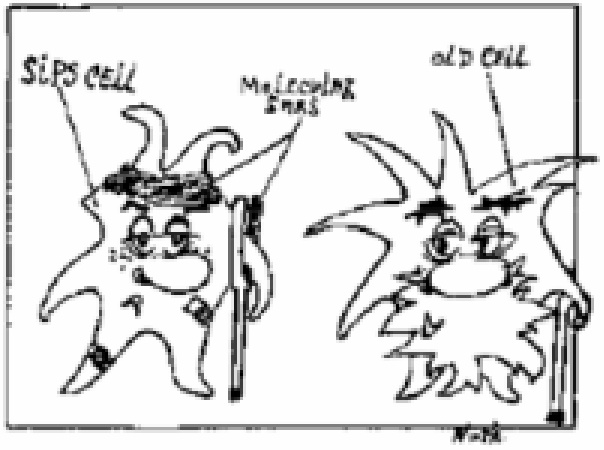
The DNA polymerase can read and synthesize the new strand of DNA in one direction, starting with the primer. Because the synthesis of DNA requires primers attaching ahead on the DNA strand, human chromosomes lose some telomere DNA in each cell division.

STRESS SENESCENCE

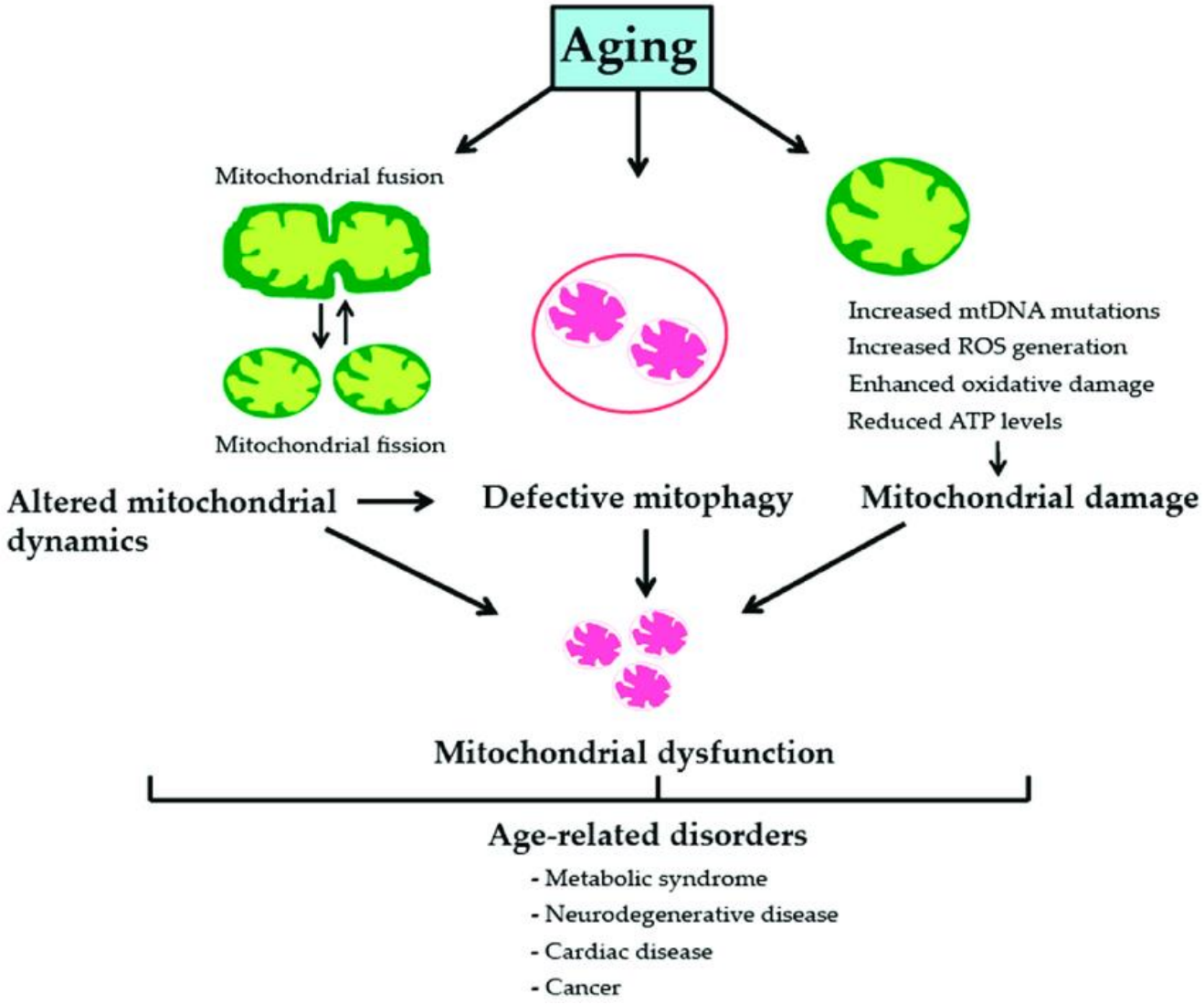
Some cells undergo replicative senescence independently of telomere shortening. This senescence is due to stress (oxidative stress). It increases **p16** (an inhibitor CDK4 and 6) expression and engages the p16–retinoblastoma protein (pRB) pathway. This response is termed stress-induced premature senescence (SIPS).







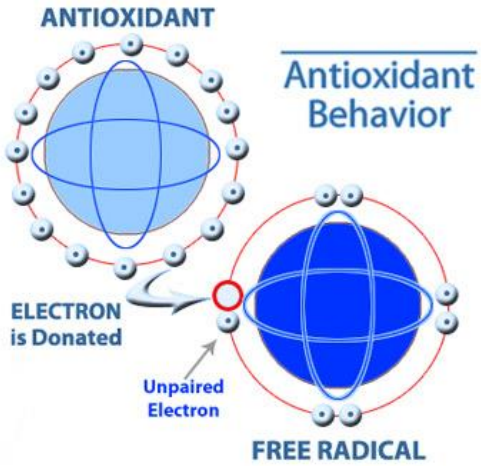
Mitochondria and reactive oxygen species

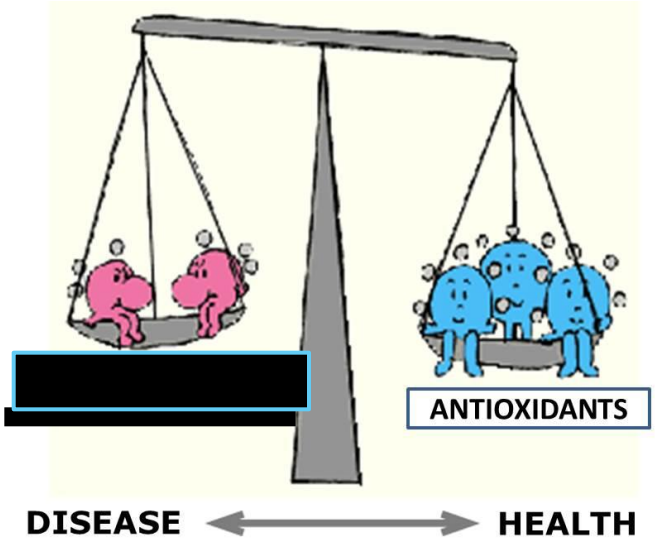


Antioxidants

In young organisms free radicals are efficiently neutralized by antioxidants (superoxide dismutase (SOD), catalase, glutathione peroxidase, peroxiredoxin1, β-carotene, folate, uric acid, vitamin A, C, E).

The activity and amount of antioxidants decrease with age. This situation leads to an excess of free radicals in tissues and oxidative stress. It causes progressive aging and diseases associated with this process.





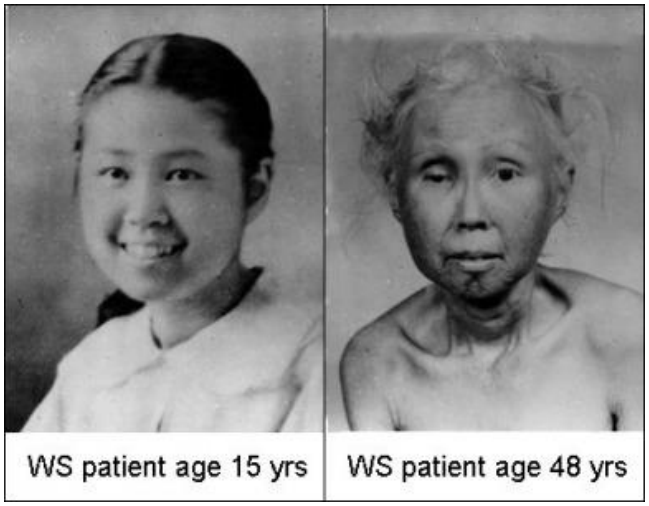
DNA mutations and senescence – replication stress

Unrepaired mutations, often in genes encoding DNA processing proteins, accumulate in cells and are responsible for process called **entropic aging**.

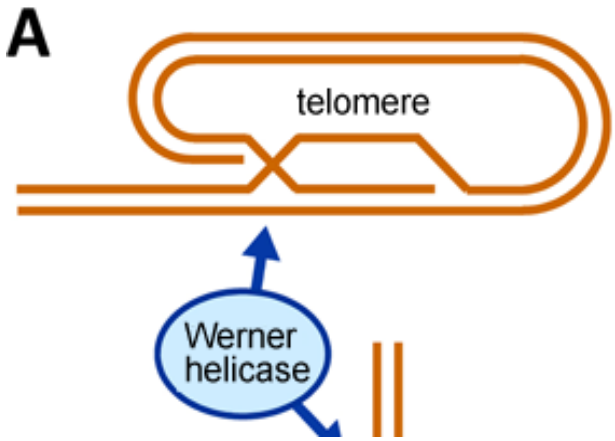
Embarrassing moments at gene parties

Werner Syndrome

Werner syndrome (WS), - adult progeria, autosomal recessive progeroid syndrome, characterized by the appearance of premature aging.

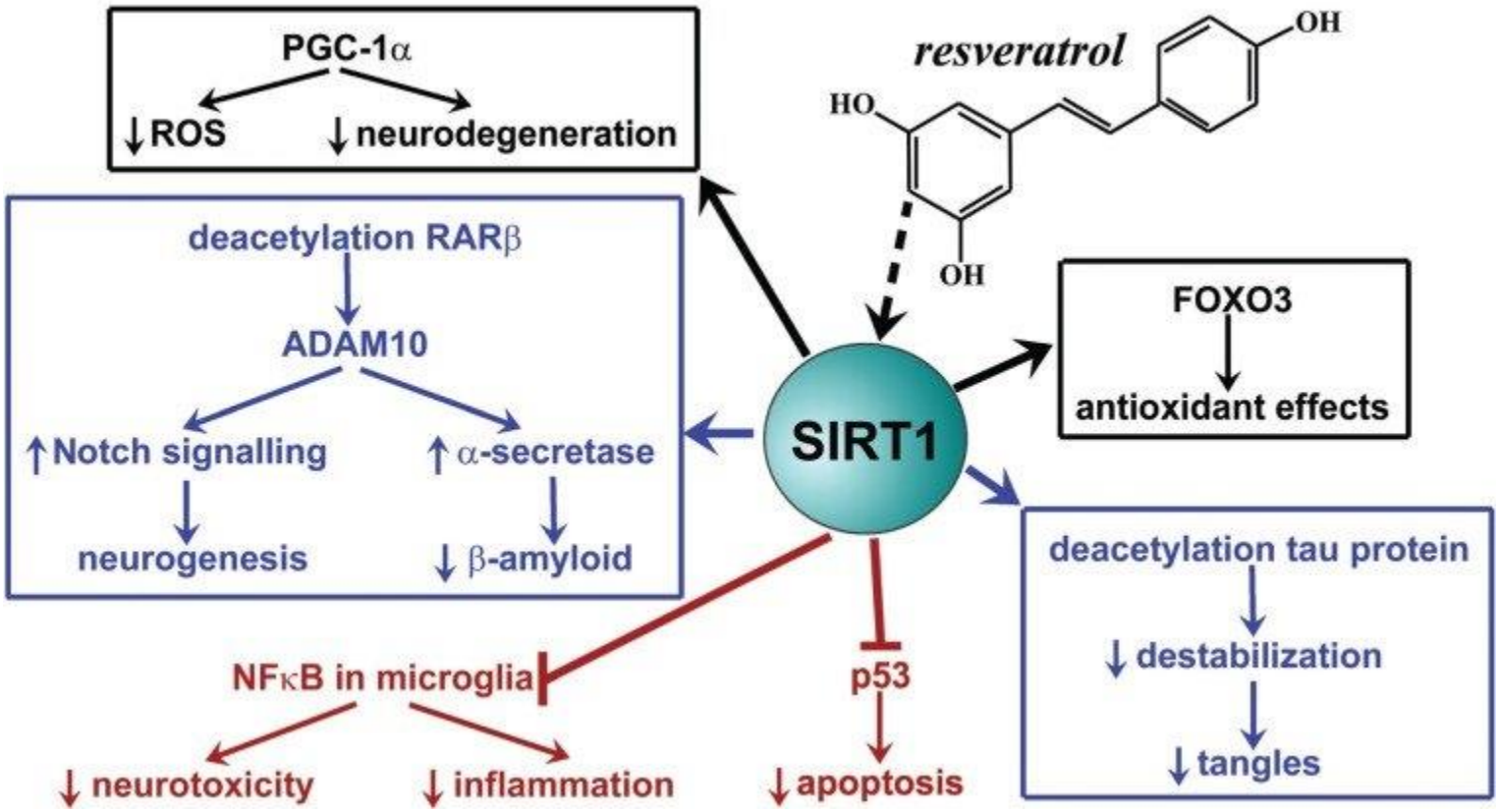


Different mutations in gene WRN, encoding, helicase, that functions in DNA repair of doubled stranded breaks.



SIRTUINS AND CELLULAR SENESCENCE

SIRT1 deacetylates p53 gene. The deacetylated form of p53 has decreased transcriptional activity. SIRT1 promotes the survival of cells. It also deacetylates NF-κB and suppresses immune response and inflammaging.

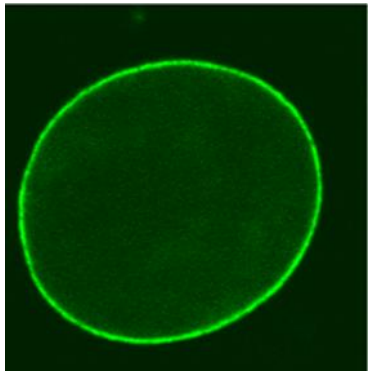


Activators of Sirtuin 1

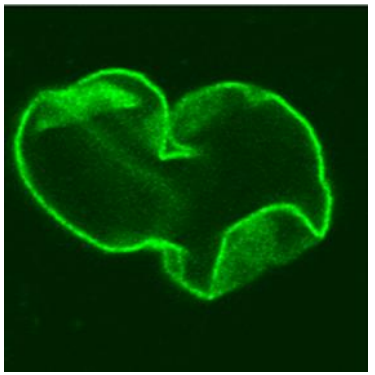
Resveratrol - natural phenol, produced by plants in response to injury or to pathogens, present in the skin of grapes, blueberries. Resveratrol increases the expression of SIRT1 and enhances the binding between Sirtuin 1 and Lamin A.

Hutchinson Gilford Progeria Syndrome

Genetic disease, which symptoms resemble aging. People born with progeria typically live to their mid teens and early twenties. Children with progeria usually develop the first symptoms during their first few months.



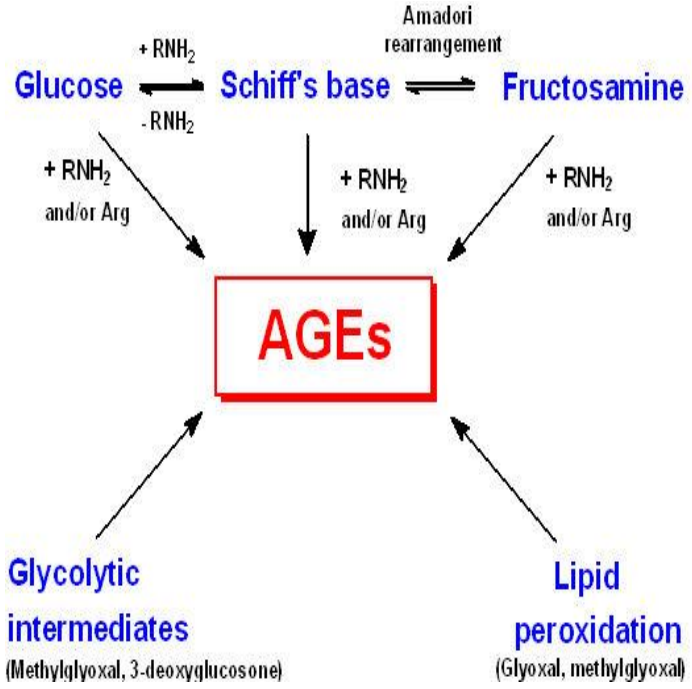
The cause of progeria - a point mutation in the **lamin** LMNA gene



Glycation and senescence

Glycation is the result of covalent binding of a protein, lipid or DNA molecule with a sugar molecule without the controlling action of an enzyme - a haphazard process that impairs the functioning of biomolecules.

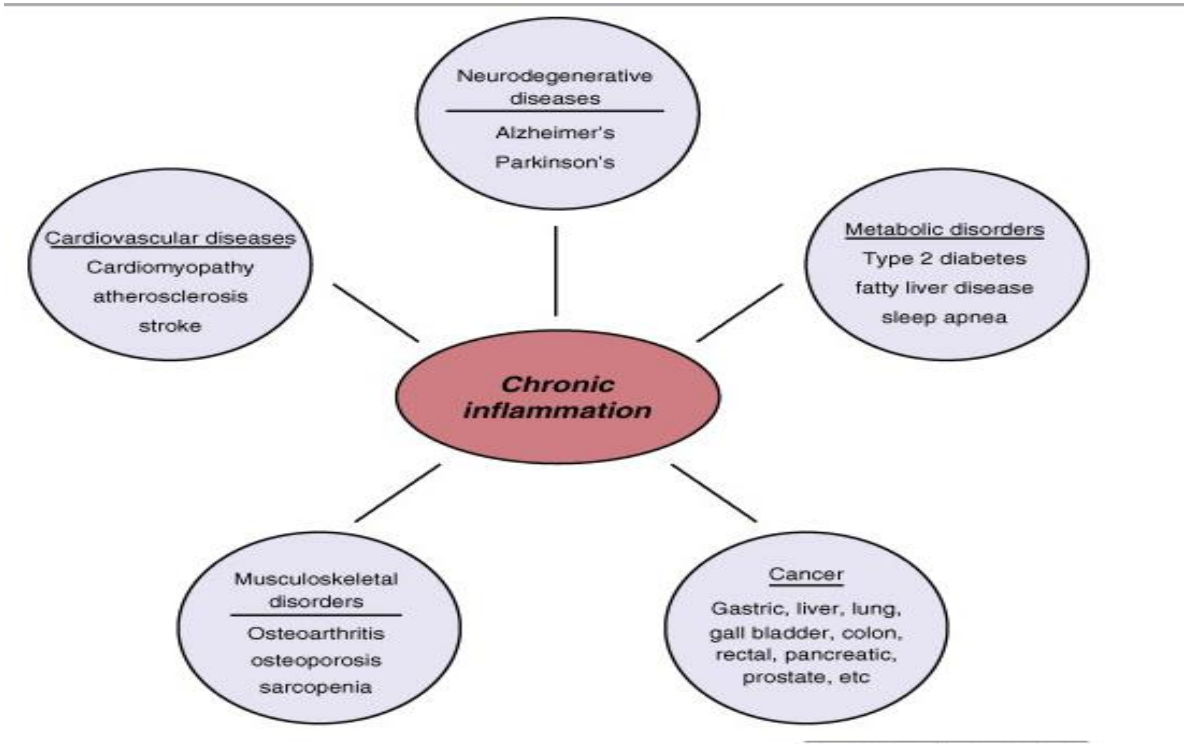
Glycation leads to formation of advanced glycation endproducts (AGEs).



Inflammaging

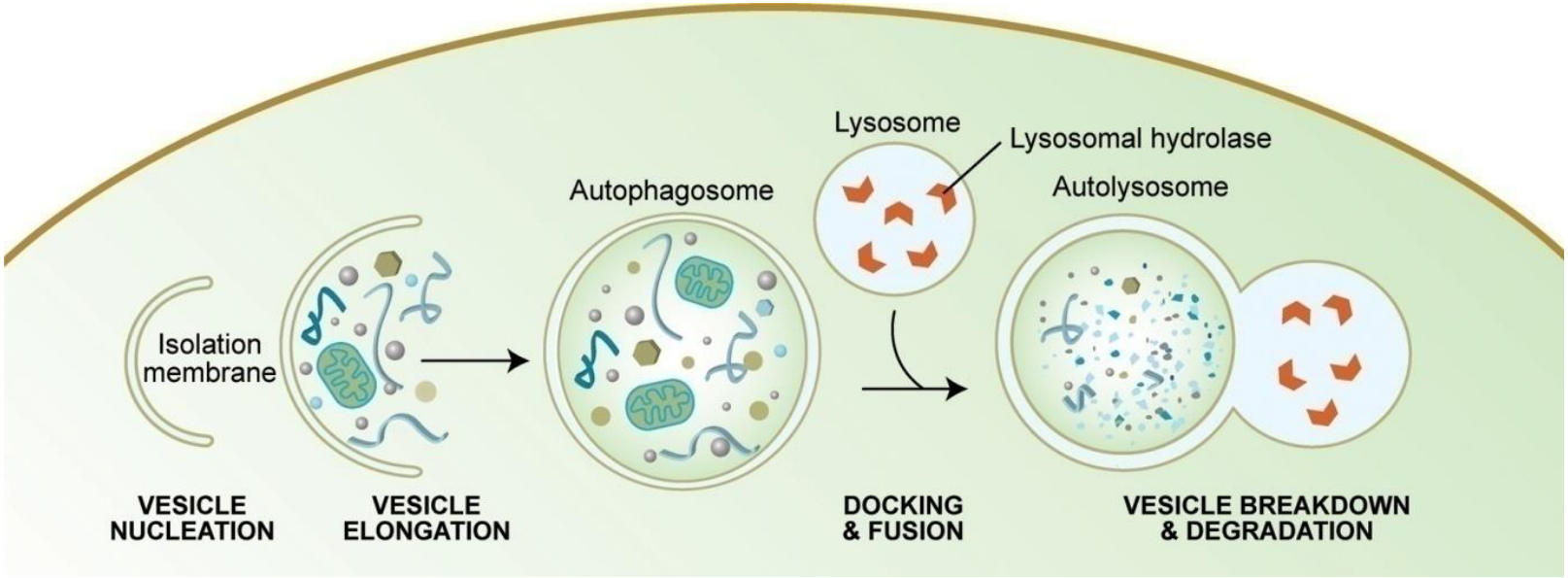
defined as low-grade **chronic systemic inflammation** established during physiological aging

Increased level of pro-inflammatory cytokines (e.g., IL-6 and TNF), acute-phase reactants (C-reactive protein), and decreased level of anti-inflammatory cytokines (IL-10) impair the maintenance of immunological homeostasis.



Autophagy

mechanism of degradation of cellular components through the actions of lysosomes

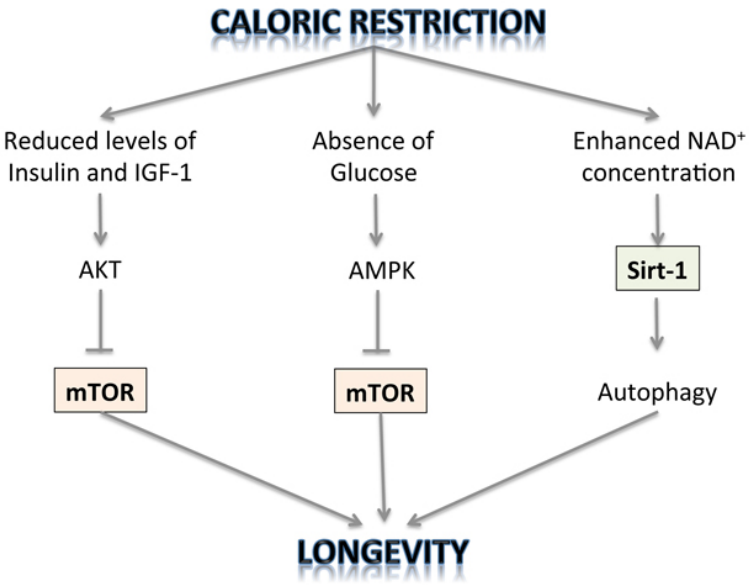


Autophagy promotes cellular survival during starvation and allows the degradation and recycling of cellular components.

Probably autophagy is required for the lifespan-prolonging effects of caloric restriction

Caloric restriction

dietary regimen that is based on low calorie intake.

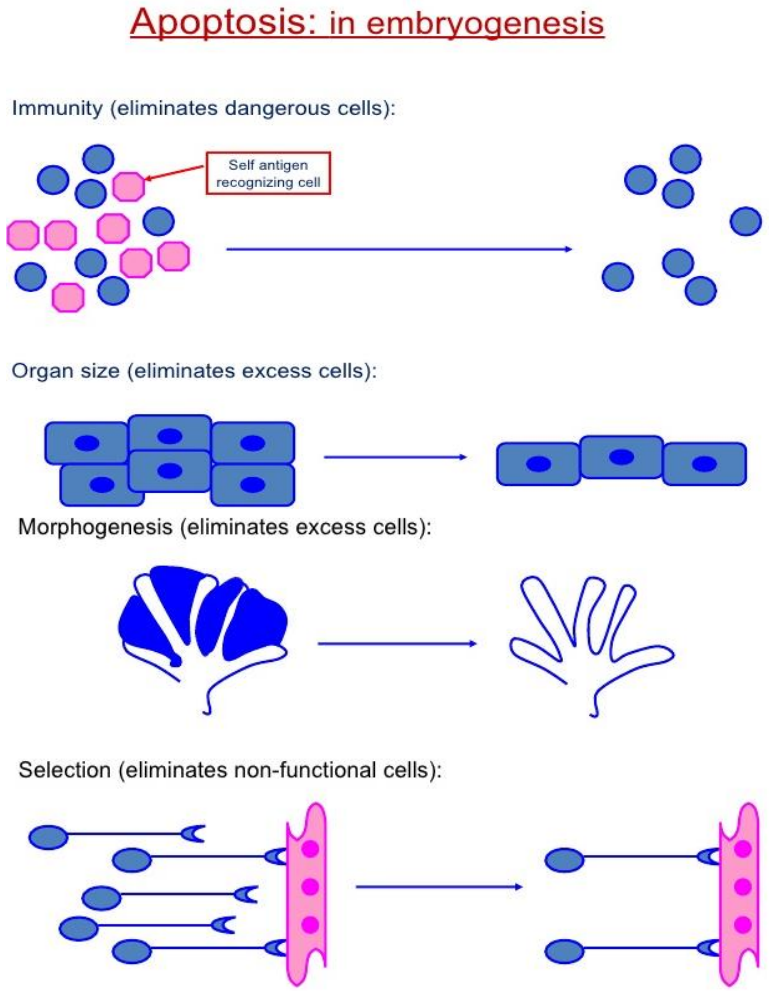


Caloric restriction (CR) without malnutrition has been shown to decelerate the aging process, resulting in longer maintenance of youthful health and an increase in maximum lifespan of yeast, fish, rodents and dogs.

Apoptosis

The differentiation of fingers and toes occurs because cells between the fingers undergo **apoptosis.**

Throughout the rest of life is necessary to maintain the balance between cell proliferation and cell death.

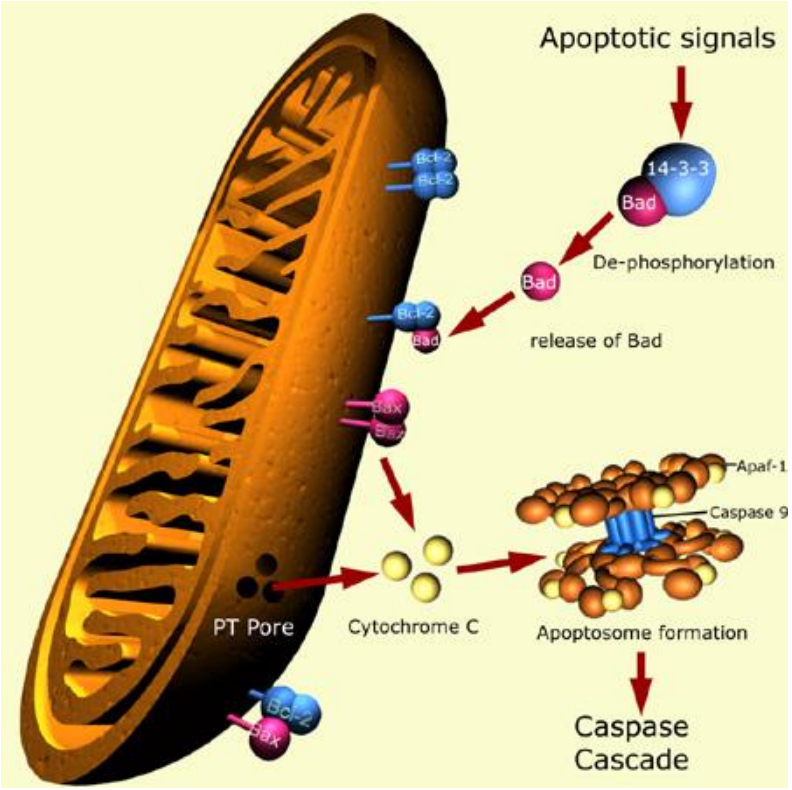


Apoptosis

is triggered by multi-signal pathways, regulated by extrinsic and intrinsic ligands

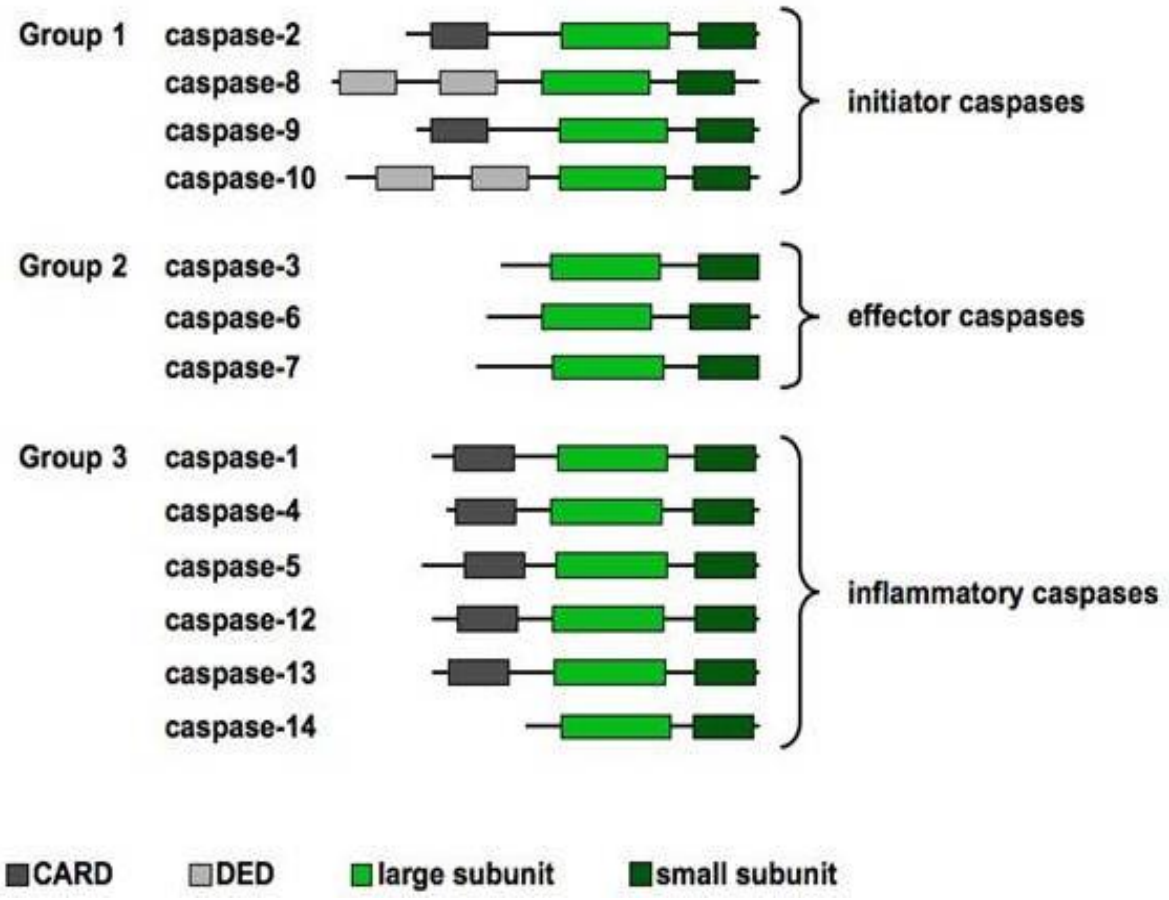
There are two major apoptosis pathways distinguished according to whether caspases are involved or not.

The mitochondria, as the cross-talk organelles, connect the different apoptosis pathways.



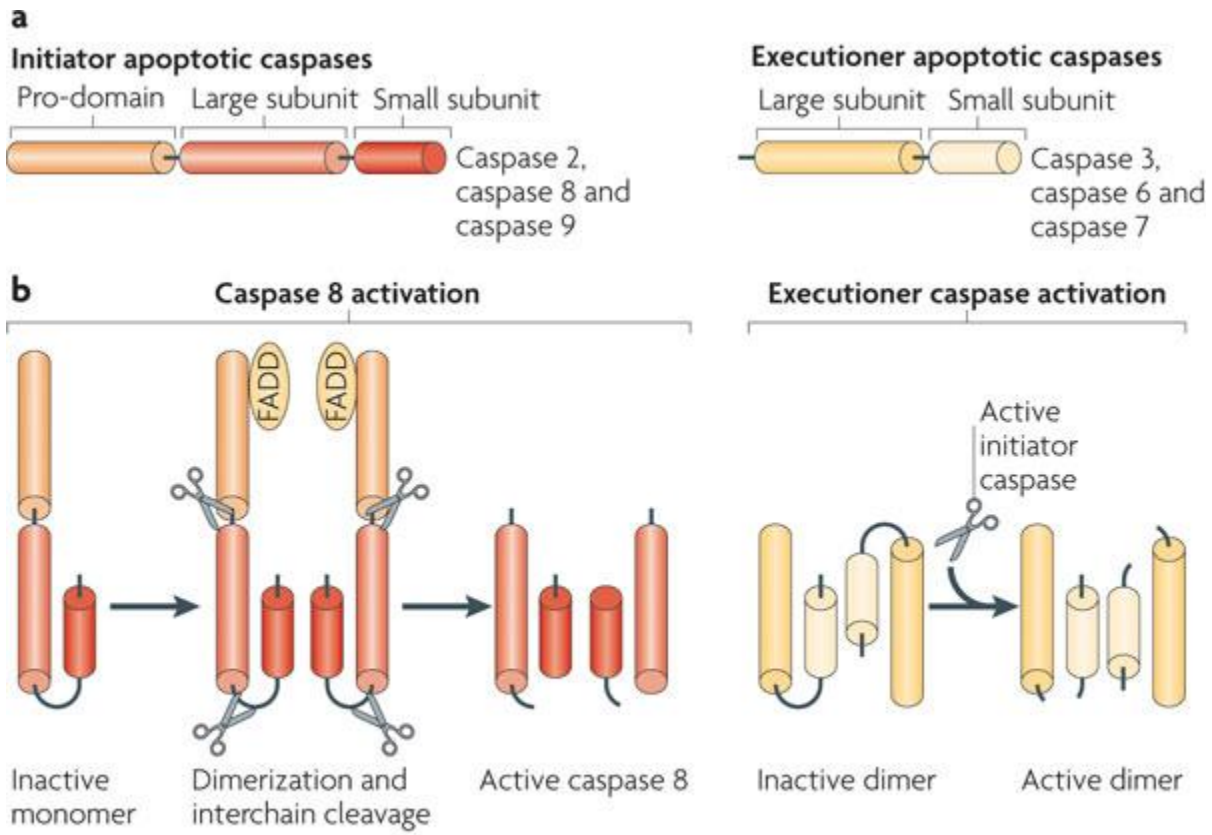
Caspases

cysteine-aspartic proteases or cysteine-dependent aspartate-directed proteases



Caspases

Apoptotic caspases are divided into two classes: initiator and executioner caspases. Initiator caspases (caspase 2, 8, 9 and 10) and executioner caspase (caspase 3, 6 and 7)



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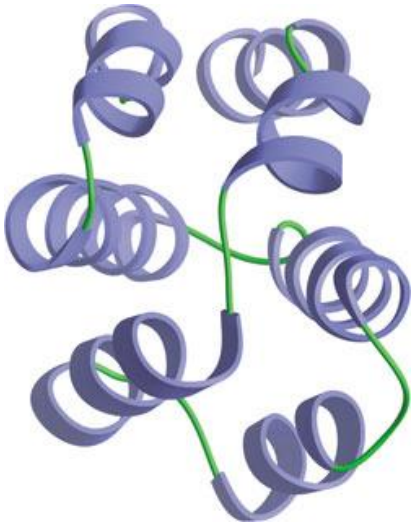
These enzymes are present in cytoplasm as inactive **proenzymes - proteolysis**

Initiator caspases

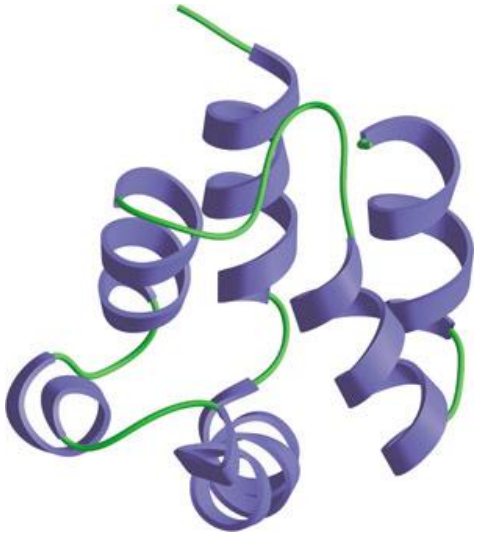
recruit executioner caspases

Initiator caspases 2 and 9 possess caspase activation and recruitment domains (CARDs). The CARD domain typically associates with other CARD-containing proteins, forming either dimers or trimers.

Initiator caspases 8 and 10 possess death-effector domains (DEDs). DED associates with other DED –containing proteins.



CARD



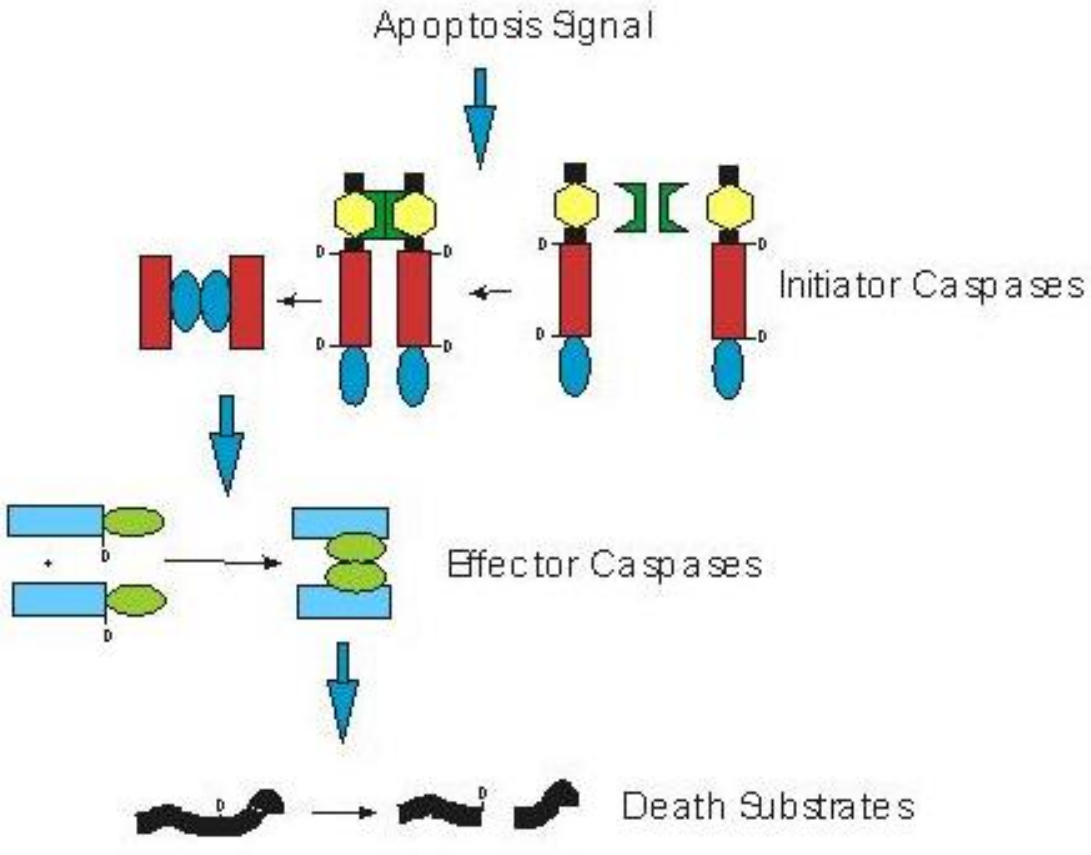
DED

Executioner caspases 3, 6, 7

coordinate the execution phase of apoptosis by cleaving multiple structural and repair proteins

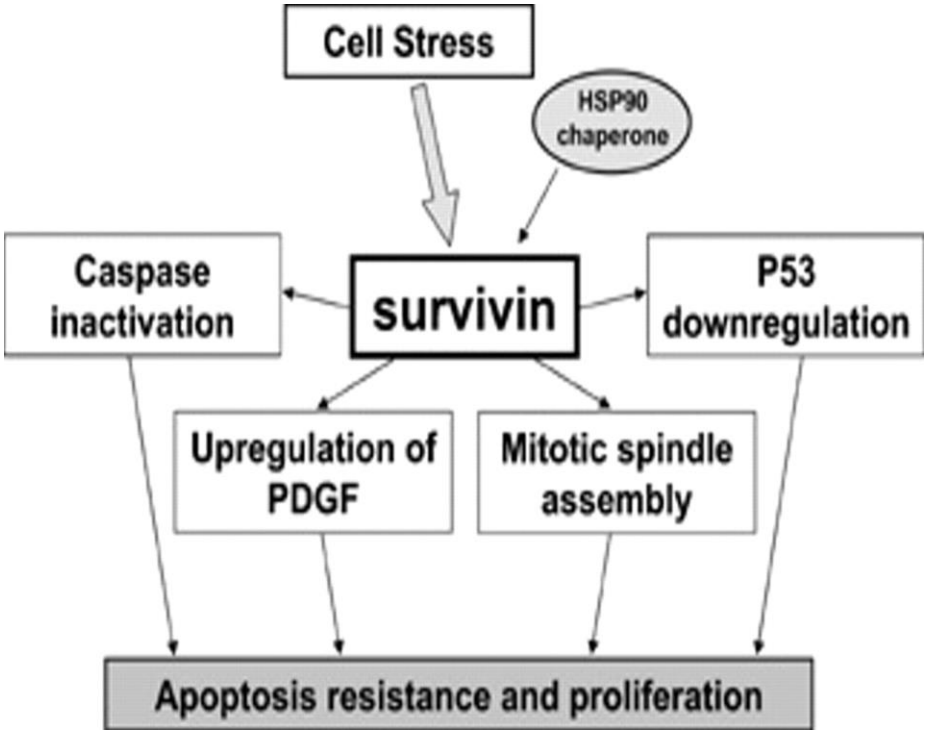
Main executioner of apoptosis is caspase 3

Substrates :
Numerous structural and regulatory



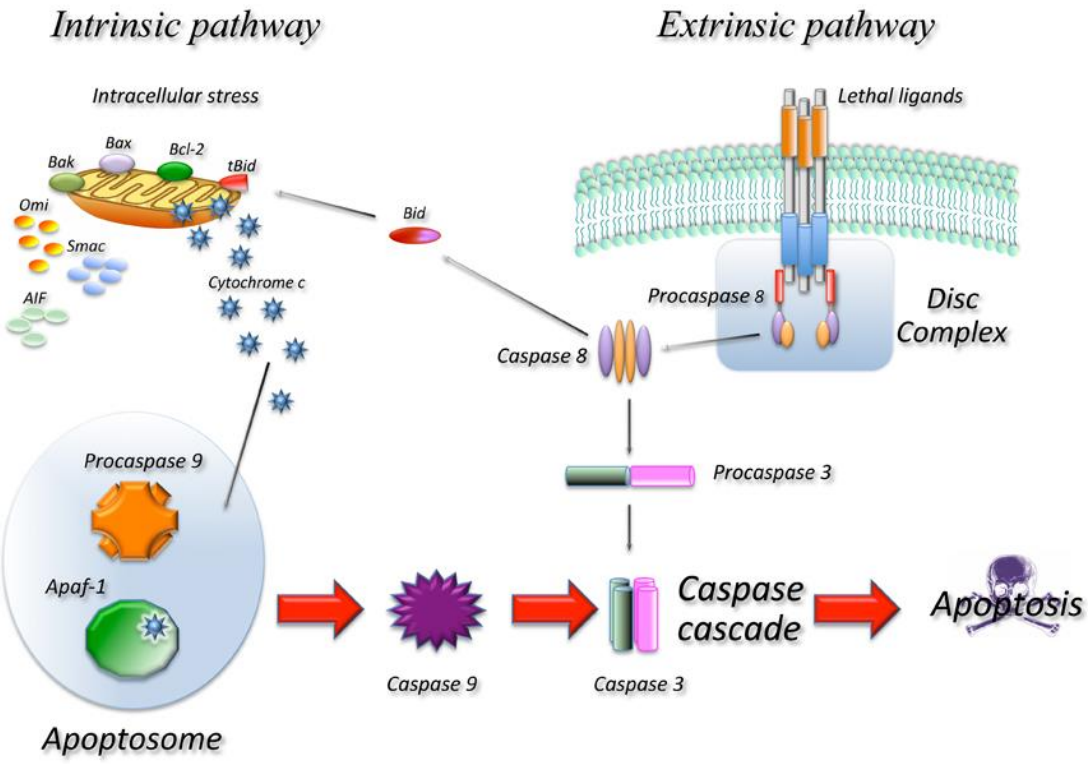
Inhibitors of apoptosis proteins (IAP)
Survivin

expressed in most human tumors and fetal tissue, but absent in terminally differentiated cells



Caspase dependent pathways of apoptosis

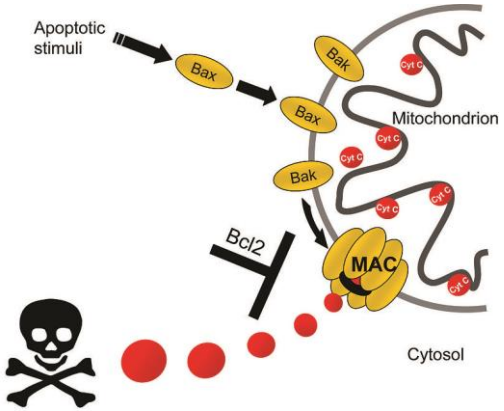
Intrinsic pathway
triggered by DNA damage, lack of trophic factors, lack of normal interactions with ECM.



Extrinsic pathway
triggered by action of other cells or their mediators – TNF, FAS-ligand (CD95L) and TRAIL (TNF-related apoptosis-inducing ligand)

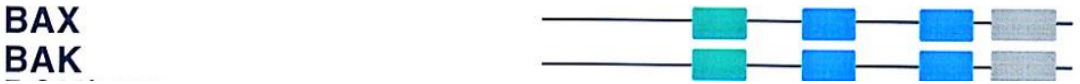
Intrinsic pathway of apoptosis

Apoptosis regulator **Bcl-2** (B-cell lymphoma) protein family.These family comprises anti- and proapoptotic proteins

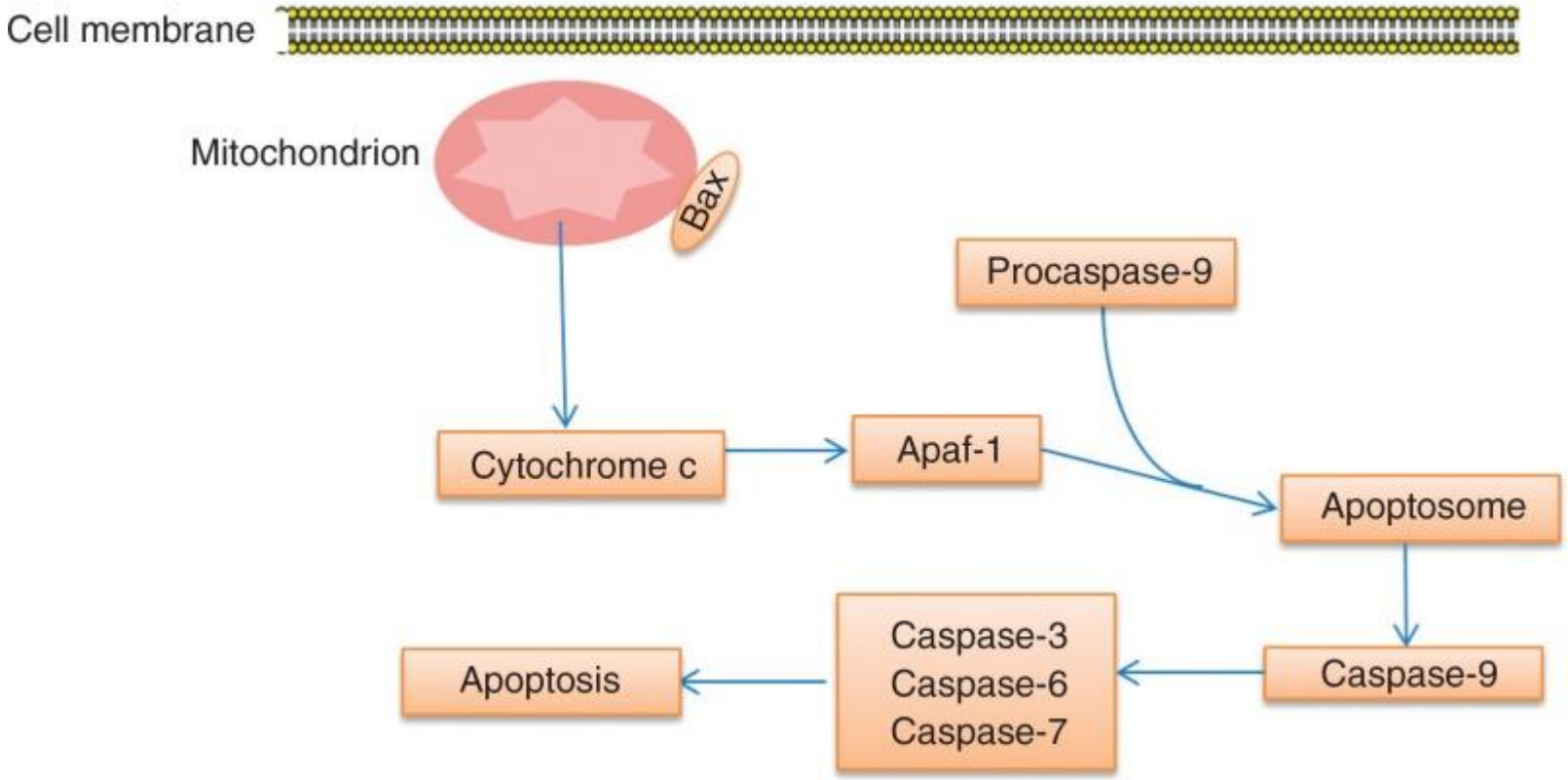


Antiapoptotic proteins: Bcl-2 and Bcl-XL

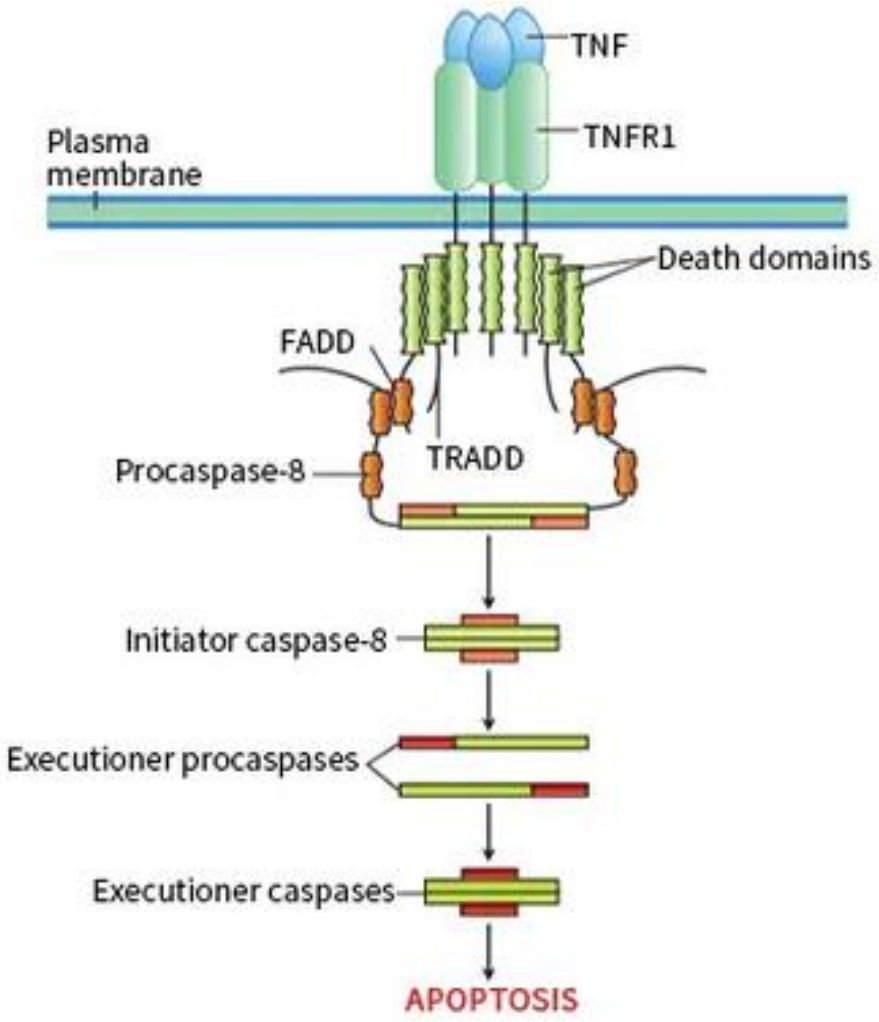
Proapoptotic proteins BAX and open mitochondrial channels



Intrinsic pathway of apoptosis

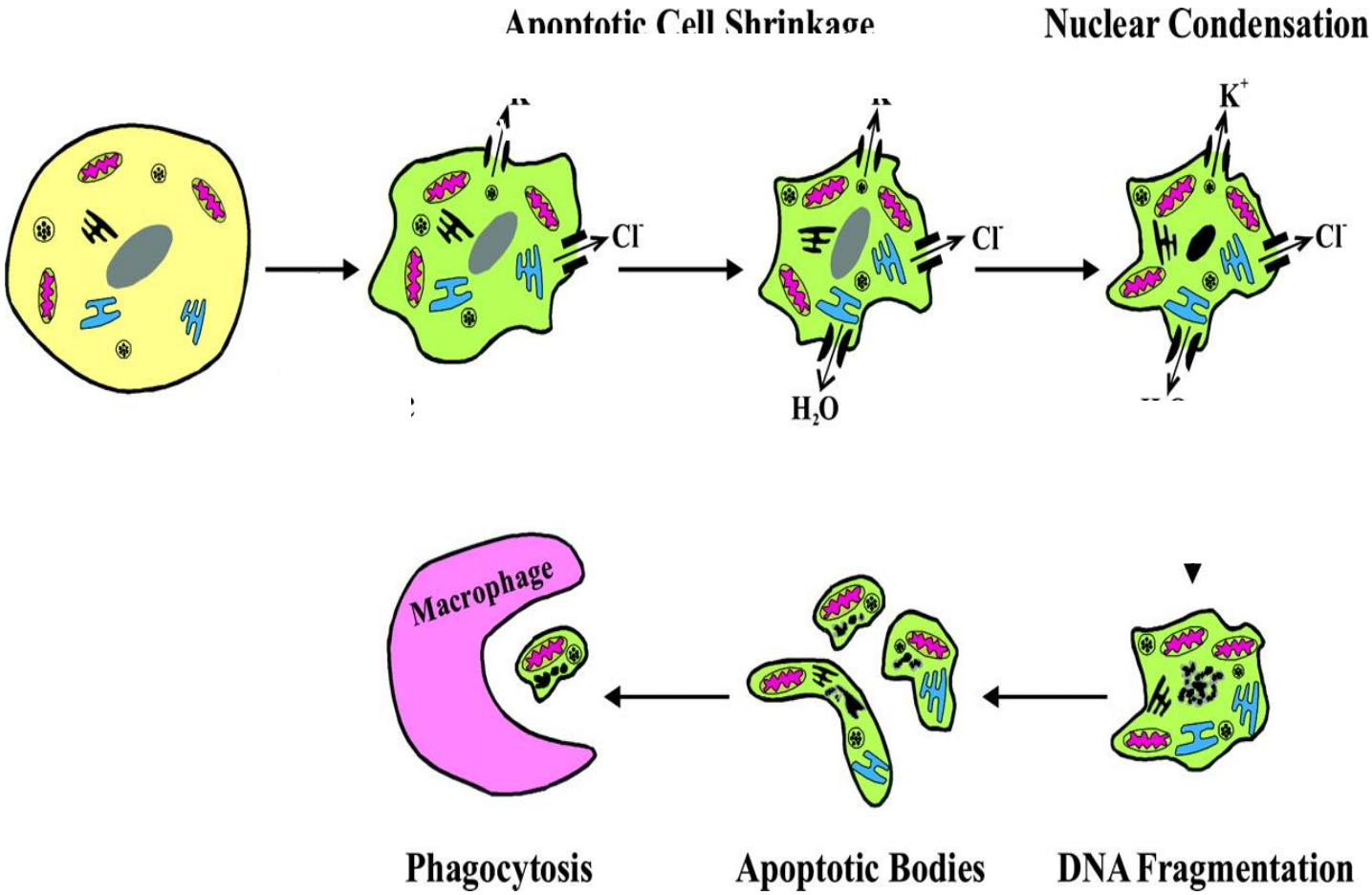


Extrinsic pathway of apoptosis

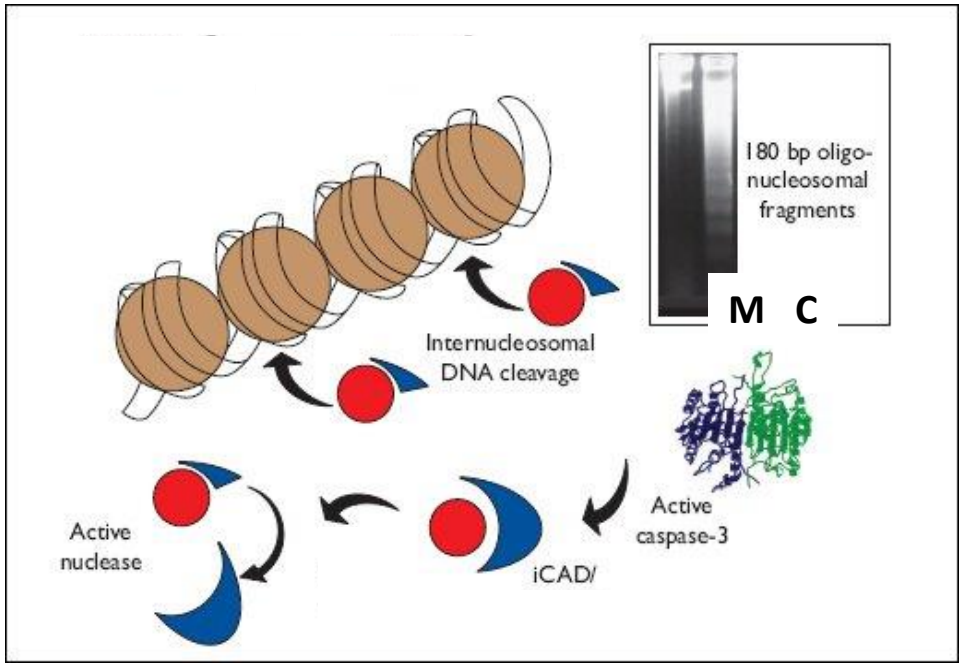


Apoptosis

Executioner caspases 3, 6, 7 coordinate the execution phase of apoptosis by cleaving multiple structural and rgulatory proteins (cascade of caspases).

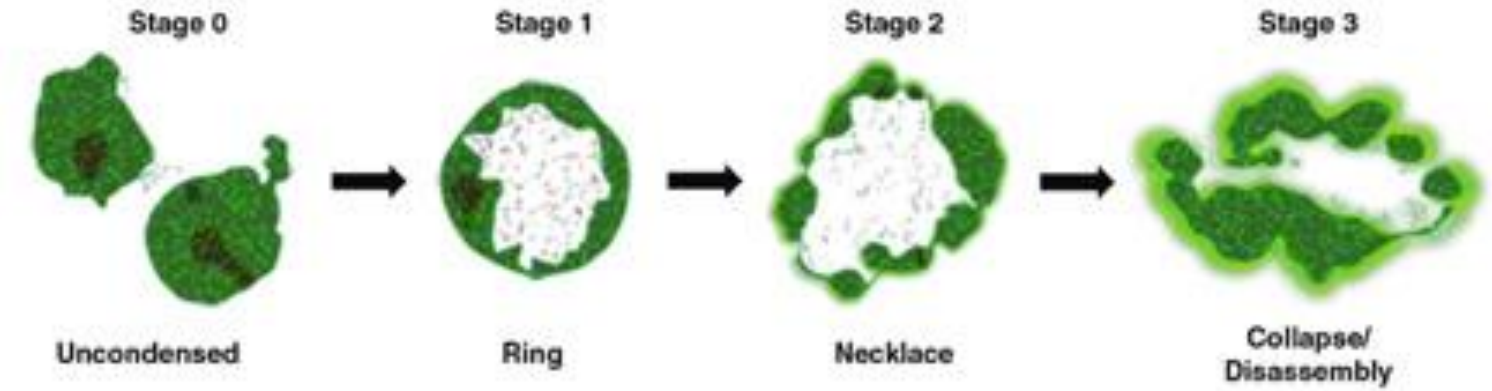


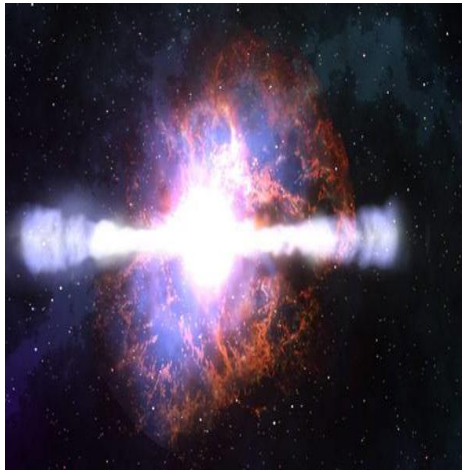
DNA fragmentation during apoptosis



CAD cleaves DNA at internucleosomal linker sites between nucleosomes, that occur at ~180-bp intervals. This is because the DNA is normally tightly wrapped around histones. The linker sites are the only parts that are exposed and accessible to CAD. Therefore the DNA fragments can comprise 180 or multiples of 180 bp (360, 540, 720 etc).

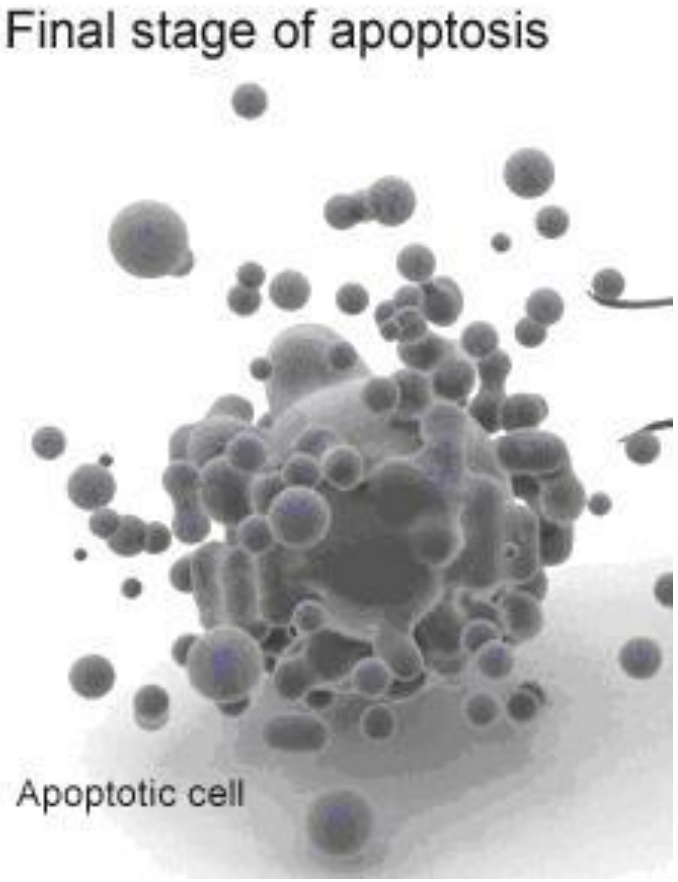
Changes in cell nucleus during apoptosis





Apoptotic bodies formation

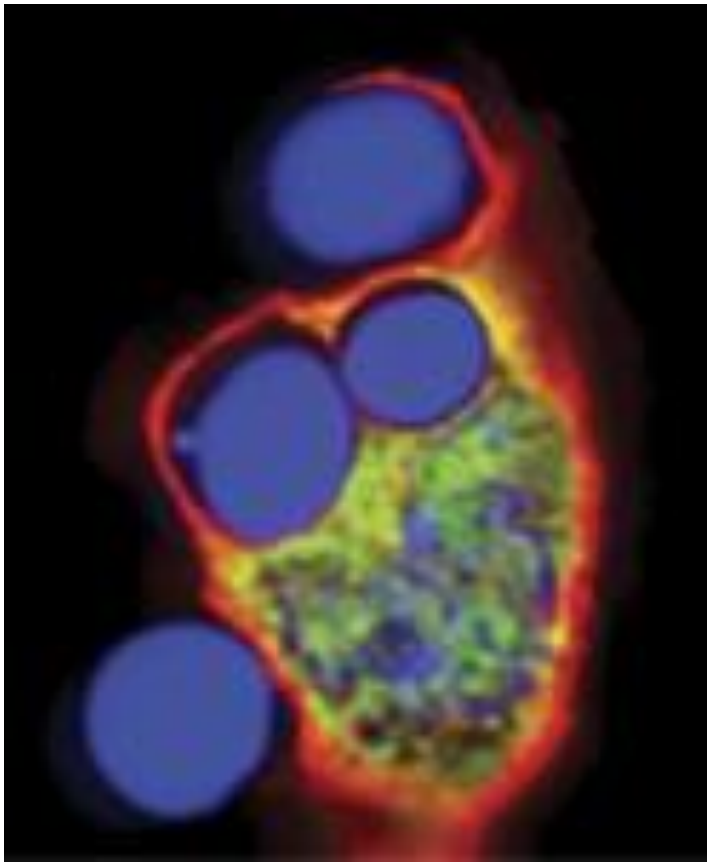
Apoptotic bodies - apobodies, are small membrane sealed vesicles. Because of the formation of apoptotic bodies the content of dead cells does not leak into the surrounding tissue.



Phagocytosis - efferocytosis

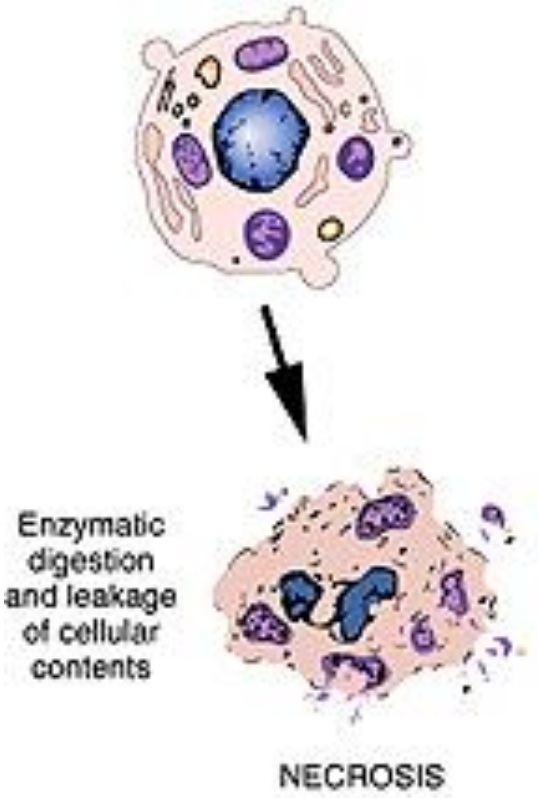
Efferocytosis is performed by macrophages, dendritic cells, epithelial cells and fibroblasts.

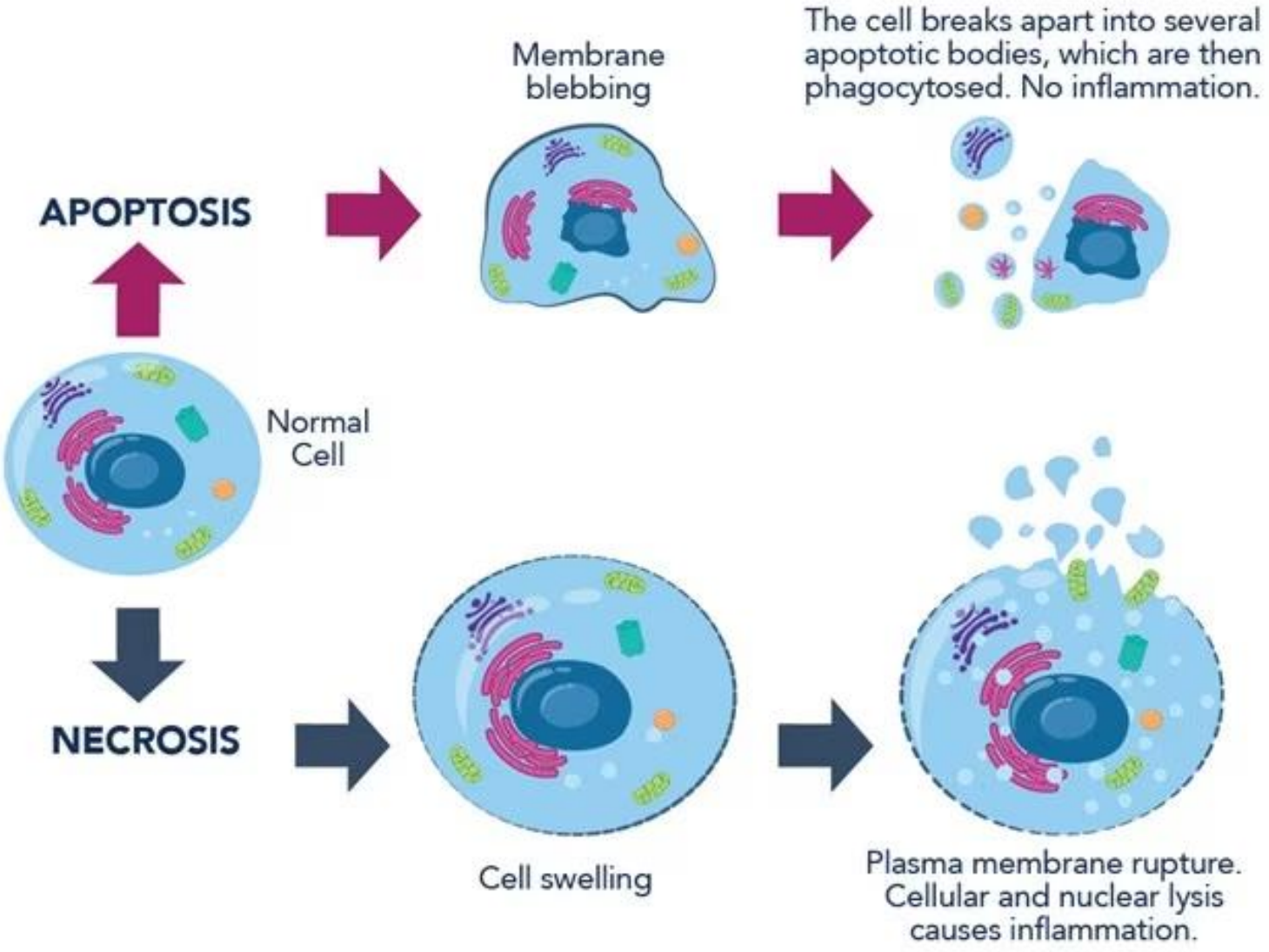
Phosphatidylserine



Necrosis

In necrosis cell or tissue destruction is due to **autolysis.** The dead cells are not actively digesting themselves.



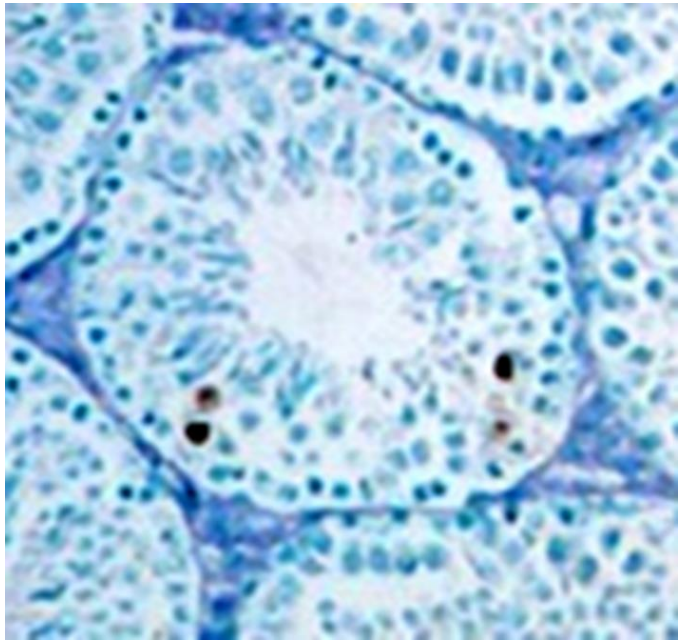

APOPTOSIS
Membrane blebbing
The cell breaks apart into several apoptotic bodies, which are then phagocytosed. No inflammation.
Normal Cell
NECROSIS
Cell swelling
Plasma membrane rupture. Cellular and nuclear lysis causes inflammation.

TUNEL ASSAY

(terminal deoxynucleotidyl transferase dependent nick end labeling)

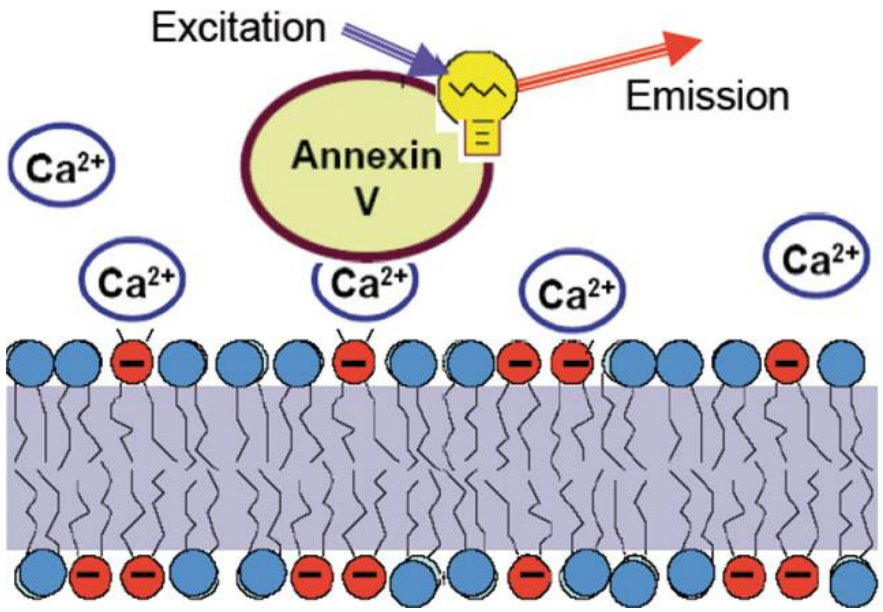
Common method for detecting DNA fragmentation in apoptosis.

The assay relies on the presence of nicks in the DNA which can be identified by terminal deoxynucleotidyl transferase - TdT, an enzyme that catalyzes the addition of labeled dUTPs (deoxyuridine 3-phosphate) or synthetic nucleoside BrdU, - bromodeoxyuridine (5-bromo-2'-deoxyuridine) to 3’-OH end of DNA strand breaks

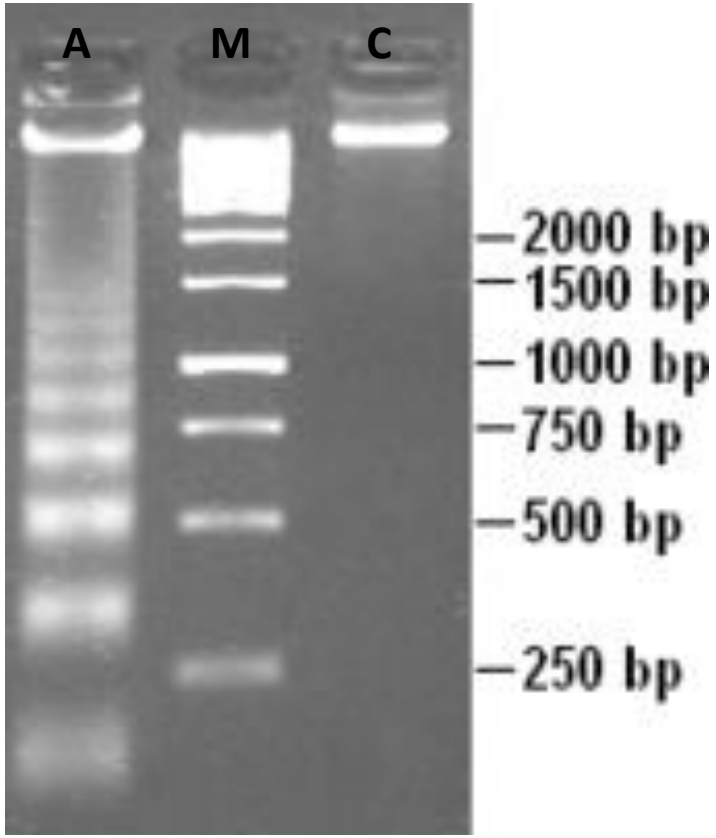


Annexin V - binding assay

The annexin V binding assay is used for the detection of apoptotic cells *via* annexin V binding to **phosphatidylserine** at cell surface of apoptotic cells.



Apoptotic DNA ladder assay



DNA ladder is observed when DNA fragments, resulting from apoptotic DNA fragmentation, are visualized after separation by gel electrophoresis.