CELL CYCLE, APOPTOSIS & NECROSIS

R. BEŇAČKA

Department of Pathophysiology

P.J. Šafarik University, KOŠICE

CELL CYCLE

A eukaryotic cell cannot divide into two, the two into four, etc. unless two processes alternate:

- **doubling** of its (**DNA**) in **S phase** (synthesis phase) of the cell cycle
- **halving** of that genome during **M phase** (mitosis)

The period between M and S is called $\mathbf{G_1}$; that between S and M is $\mathbf{G_2}$

CELL CYCLE

Cell cycle consist of:

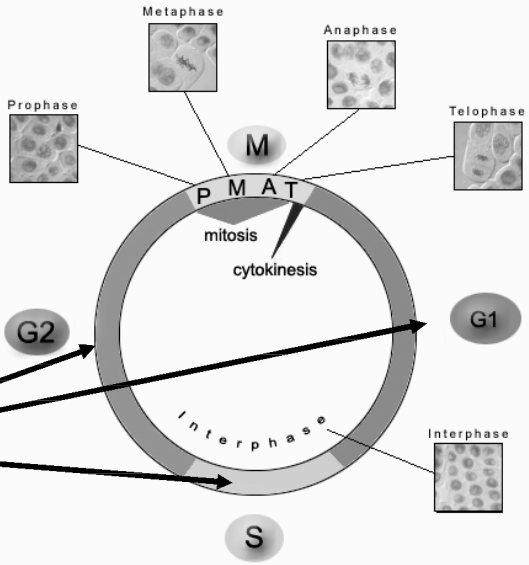
G_1 growth and preparation of the chromosomes for replication

S synthesis of DNA (and centrioles)

G_2 preparation for mitosis

M mitosis

Interphase - G1 + S+ G2



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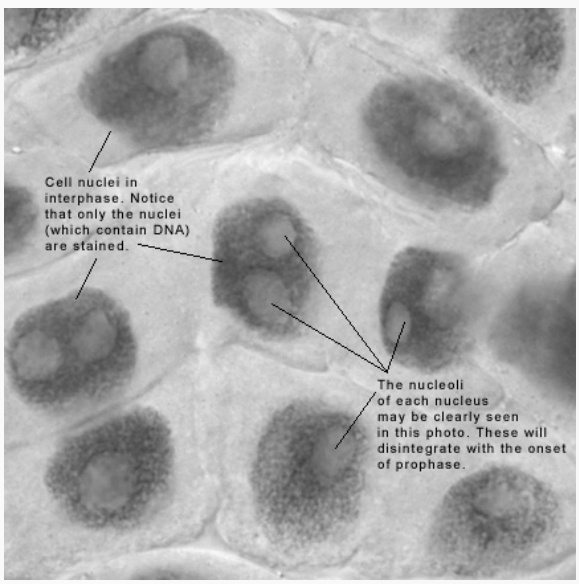
Some cells have shortened cell cycle allowing repeated S phases without completing mitosis and/or cytokinesis. This is called **endoreplication**

Interphase - G1, S and G2

- **most cells spent in interphase the majority of their life is and normal functions, such as growth and protein synthesis**
- **in tissues, such as in the brain, most mature cells will remain in interphase throughout their lives**

G1 stage "first Gap"

- long period of time following last mitosis, cell grows and carries out protein synthesis (cellular functions except DNA replication)
- chromosomes are all unreplicated, that is, each contains only one molecule of DNA **(monochromatide chromosome)**
- chromatin is very diffuse within the nucleus and so the individual chromosomes are not visible



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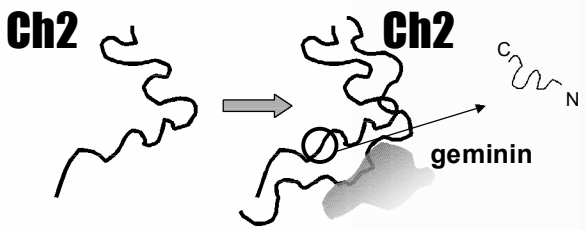
INTERPHASE - 2

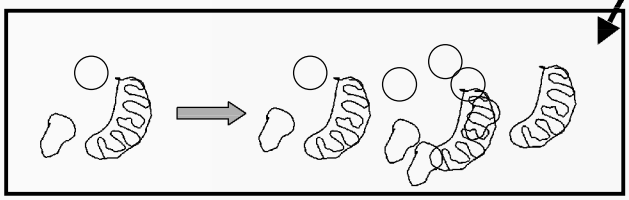
S stage

- cell replicates its DNA, every portion of genome is copied once - and only once - binding to geminin
- in the end of S all of chromosomes are composed of two identical molecules of DNA
- each chromosome thus has two so called chromatids **(dichromatide chromosome)**
- chromosomes are still decondensed and not distinctly visible



G2 stage "second Gap"

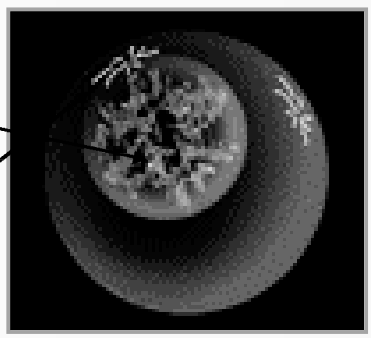
- cell continues in all normal functions, and continue growth
- genes are transcribed from one from two molecule of DNA in each chromosome
- near the end of G2, the cytoplasmic organelles replicate in preparation for the cell to divide during mitosis
- the nucleus is complete, bound by a membrane; nucleoli are clearly visible



MITOSIS - 1

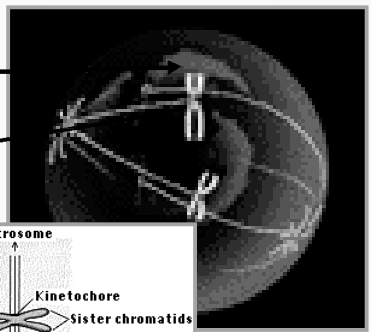
1. Prophase

- chromatin begins to condense and becomes visible in light microscope as chromosomes
- the nucleolus disappears
- centrioles move to opposite ends of the cell and fibers extend from the centromeres
- some fibers cross the cell to form the mitotic spindle



2. Prometaphase

- the nuclear membrane dissolves
- protein structure - kinetochore, appears at the centromere of each chromatid
- microtubules from centrosome attach at the kinetochores and the chromosomes begin moving

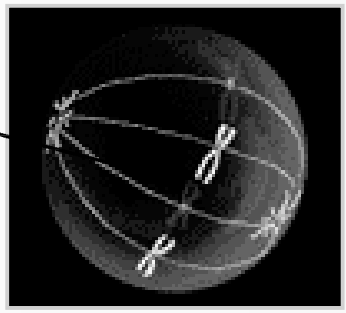


Failure of a kinetochore to become attached to a spindle fibers interrupts the process - **spindle checkpoint**

MITOSIS - 2

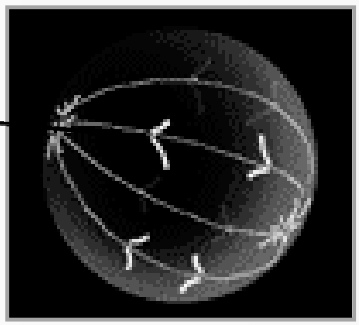
3. Metaphase

- spindle fibers align the chromosomes along the middle of the cell nucleus equatorial plate (metaphase plate)
- this organization helps to ensure that in next phase, when the chromosomes are separated, each new nucleus will receive one copy of each chromosome



4. Anaphase

- sister kinetochores suddenly separate and each moves to its respective pole dragging its attached chromatid (chromosome) behind it
- chromosome motion is a combination of kinetochore movement along the spindle microtubules and physical interaction of polar microtubules

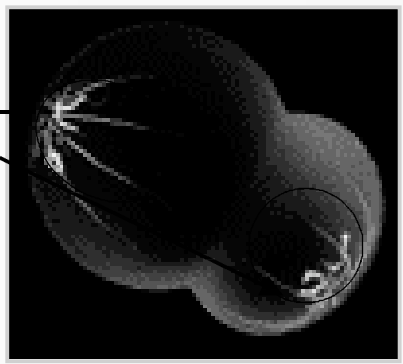


Separation of the sister chromatids depends on the breakdown of the cohesins. **Cohesin** breakdown is caused by separin (also known as separase). **Separin** is kept inactive until late metaphase by another protein called **securin**. Anaphase begins when **APT** destroys securin

MITOSIS - 3

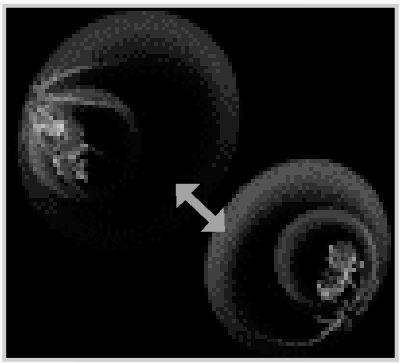
5. Telophase

- chromatids arrive at opposite poles of cell
- new membranes form around the daughter nuclei
- the chromosomes disperse and are no longer visible under the light microscope
- the spindle fibers disperse



6. Cytokinesis (animal cells)

- fiber ring composed of actin around the center of the cell contracts pinching the cell into two daughter cells



Controllers of the Cell Cycle

The passage of a cell through the cell cycle is controlled by cytoplasm proteins:

1. Cyclins

- G1 cyclins
- S-phase cyclins
- M-phase cyclins

← their levels in the cell fluctuate rise and fall with the stages of the cell cycle

2. Cyclin-dependent kinases (CDKs)

· G1 CDKs
· S-phase CDKs
· M-phase CDKs

← their levels in the cell remain stable
bind and activate the appropriate cyclin
fosforylate variety of proteins that control cell cycle

3. The anaphase-promoting complex (APC) & other proteolytic enzymes

· events leading to destruction of cohesins, thus allowing the sister chromatids to separate
· degrades the mitotic (M-phase) cyclins

Control of cell cycle - Course

G1 phase events

- **G_1 cyclins** signals the cell to prepare the chromosomes (DNA) for replication (occurs in S-phase)

S phase events

- **S-phase promoting factor** (**SPF**) (the complex of S-cyclins + S-phase CDK) prepares the cell to enter S phase and promote duplication of DNA (and its centrioles)
- as DNA replication continues, one of the cyclins shared by G_1 and S-phase CDKs (cyclin E) is destroyed

G2 phase events

- **M- cyclins** begin to rise in the end of G2 phase to prepare the cell into mitosis

Control of cell cycle - Course

M phase events - early stage

- **M-phase promoting factor (MPF)** (the complex of M-cyclins + M-phase CDK) initiates events leading the cell from **prophase to the metaphase** of mitosis:
  - –assembly of the mitotic spindle
  - –breakdown of the nuclear envelope
  - –condensation of the chromosomes

Meiosis (particularly meiosis I, meiosis II - is essentially a mitosis) requires special controls; some of them are similar to those in mitosis, e.g. **APC** and **MPF** (**m**aturation-**p**romoting **f**actor for its role in meiosis I and II of developing oocyte

Control of cell cycle - Course

M phase events - late stage

- **A-phase promoting complex (APC)** is activated by M-phase promoting factor and :
  - destroys the M-phase cyclins by ubikvitination (conjugating them with the protein **ubiquitin** which targets them for destruction by **proteasomes)**
  - allows the sister chromatids at the metaphase plate to separate and move to the poles during anaphase
  - turns on synthesis of G_1 cyclins for the next turn of the cycle
  - degrades **geminin**, a protein that has kept the freshly-synthesized DNA in S phase from being re-replicated before mitosis

Checkpoints of the Cell Cycle

The cell has several systems for interrupting the cell cycle:

(1) S-phase completion check - the cell monitor the presence of the Okazaki fragments on the lagging strand during DNA replication. The cell is not permitted to proceed in the cell cycle until these have disappeared

(2) DNA damage checkpoints

- before the cell enters S phase (G_1 checkpoint)
- during S phase (S checkpoint)
- after DNA replication, when cell leaves S phase (G_2 checkpoint)

(3) Spindle checkpoints

- detect any failure of spindle fibers to attach to **kinetochores** and arrest the cell in **metaphase** (M checkpoint)
- detect wrong alignment of the spindle itself and block cytokinesis
- trigger apoptosis if the damage in any event is irreparable

Checkpoints of the Cell Cycle

- complex of cytososlic proteins
- checkpoint failures allow the cell to continue dividing despite damage to its integrity
  - $\Rightarrow$ oncogenes - gain mutations in normal genes (proto-oncogenes)
  - $\Rightarrow$ tumor supressor genes - loss mutation of normal genes

Checkpoints - Protein p53

- senses DNA damage and can halt progression of the cell cycle in both G_1 and G_2
- **product of tumor suppressor** gene - both copies of the p53 gene must be mutated for this to fail (mutations *p53* are recessive)
- key element in forcing “unwanted" cells to commit suicide apoptosis (if the cell has only mutant versions of p53 protein, it can live on developing into a cancer)
- more than half of all human cancers do, in fact, harbor *p53* mutations and have no functioning p53 protein.

Genetically-engineered mice that express extrahigh levels of p53 activity gain additional protection from cancer but show many signs of premature aging (and die sooner)

Checkpoints -other

- ***ATM*** (="**a**taxia **t**elangiectasia **m**utated") - detects DNA damage and interrupts the cell cycle (mutation in ATM leads at increased risk of cancer.
- ***MAD*** (="**m**itotic **a**rrest **d**eficient") - binds to each kinetochore **until** a spindle fiber (one microtubule will do) attaches to it. If there is any failure to attach, MAD remains and blocks entry into anaphase (mutations in *MAD* lead to falilure of mitosis - daughter cells with too many (polyploidy) or too few chromosomes (aneuploidy).
- **Human T cell leukemia virus-1** (**HTLV-1**) leads to a cancer (**ATL** = "adult T cell leukemia").HTLV-1 encodes a protein, called **Tax**, that binds to the MAD protein causing failure of the spindle checkpoint.
- K**inesin** - moves the kinetochore to the end of the spindle fiber is involved in the spindle checkpoint

CELL DEATH

(1) Cell death by chance - injury (necrosis)
(2) Cell death by suicide (apoptosis)

(1) DEATH BY INJURY (NECROSIS)

- **Reasons:**
  - mechanical damage, hypoxia, ischaemia, ROS
  - exposure to chemicals, toxins (in most cases)
  - large biological damage (bacteria)
  - loss of surface antigens, transplants (in many)

- **Manifestations:**
  - cells and their organelles swell
  - disordered disruption of organelles, break of membranes
  - the cell contents leak out (potassium, enzymes)
  - inflammation of surrounding tissues, phagocytosis

(2) Death by suicide (apoptosis)

- **Reasons:**

1. Physiological development & functioning

  - The formation of the fingers and toes of the fetus requires the removal of the tissue between them
  - The sloughing off of the endometrium of the uterus at the start of menstruation
  - The formation of the proper connections (synapses) between neurons in the brain requires that surplus cells be eliminated
  - Regress of hyperplastic responses and atrophy - striated muscles when untrainted, uterus after pregnancy, etc.
  - Reneval of polulations of cells (endocytes of intestine)

(2) Death by suicide (apoptosis)

2. Damaged cells

- **Cells infected with viruses**
  - **cytotoxic T lymphocytes** (CTLs) kill virus-infected cells

- **Cells of the immune system** (cell mediated - immune responses)
  - CTLs induce apoptosis in each other (defects are associated with autoimmune responses (lupus erythematodes, reumatoid arthritis)

- **Cells with genome damage which may**
  - disrupt embryonic development leading to congenital defects
  - disrupt somatic development leading to cancers
  - increasing their production of potent **inducer** of apoptosis p53 (mutations in *p53* gene are often found in cancer cells

- **Cancer cells**
  - radiation and chemicals used in therapy in some types of cancer cells

(2) Death by suicide (apoptosis)

- **Manifestation**
  - bubble-like blebbing of plasma membrane, phosphatidylserine, normally hidden, is exposed on the surface
  - shrinking of cell
  - break down of organelles (mitochondria)
  - membrane-wrapped fragmentation of chromatin (DNA and protein) in nucleus
  - This is bound by receptors on cells like and which then engulf the cell fragments.
  - This is bound by receptors on phagocytic cells like macrophages and dendritic cells which then engulf the cell fragments
  - The phagocytic cells secrete cytokines that **inhibit inflammation**
- The pattern of events in death by suicide is very orderly - **programmed cell death (PCD)** The cellular machinery of programmed cell death turns out to be as intrinsic to the cell PCD = **apoptosis.**

STIMULI FOR APOPTOSIS

- **Withdrawal of positive signals needed for continued survival**
  - continuous humoral stimulation from other cells (growth factors, cytokines, lymphokines, differentiation factors)
  - continued adhesion to the surface on which they are growing (basal membranes)
  - continuous communications to neighbour cells (gap junctions)

- **Receipt of negative signals triggering apoptotic programme**
  - oxidative damage of the cell (e.g. oxygen radicals)
  - damage to DNA by oxidants or some agents: UV, x-rays, drugs
  - binding of pro-apoptotic humoral or adhesive molecules that bind to specific receptors and signal the cell to begin the apoptosis program (FasL, Fas ligand, TNF-α, tumor necrosis factor - alpha; TNF-β, lymphotoxin)

MECHANISMS OF APOPTOSIS - 1

(1) Apoptosis triggered by internal signals: the intrinsic or mitochondrial pathway

- In a healthy cell, the outer membranes of its mitochondria express the protein **Bcl-2** bound to a molecule of the protein **Apaf-1**
- Bcl-2 releases Apaf-1 upon internal **damage** to the cell (e.g., from ROS)
- The released cytochrome c and Apaf-1 bind to molecules of **caspase 9**.
- Formation of **apoptosome (**complex **cytochrome c, Apaf-1, caspase 9, ATP)** and aggregation in the cytosol
- Caspase 9 cleaves and activates other caspases (proteases) - **caspase cascade**
  - $\Rightarrow$ digestion of structural proteins in the cytoplasm
  - $\Rightarrow$ degradation of chromosomal DNA and
  - $\Rightarrow$ phagocytosis of the cell

MECHANISMS OF APOPTOSIS-2

(2) Apoptosis triggered by external signals: the extrinsic or death receptor pathway

- **Fas** and the **TNF receptor** - integral membrane proteins bind **death activator** (**FasL** and **TNF** respectively)
- transmits a signal to the cytoplasm that leads to activation of **caspase 8**
- caspase 8 (like caspase 9) initiates a cascade of caspase cascade
- phagocytosis of the cell - the early steps are reversible
- final destruction of the cell is guaranteed only with its engulfment by a phagocyte.

When TC recognize (bind to) their target:

- they produce more **FasL** at their surface
- This binds with the **Fas** on the surface of the target cell leading to its death by apoptosis

Apoptosis and Cancer

- **Some cancer-causing viruses use tricks to prevent apoptosis of the cells they have infected** .
  - **Human papilloma viruses** (**HPV**) - cause of cervical cancer
    - **protein E6** binds and inactivates the apoptosis promoter p53
  - **Epstein-Barr Virus** (**EBV**) - cause of mononucleosis and a cause of
    - produces a protein similar to Bcl-2 and protein that causes the cell to increase its own production of Bcl-2 make the cell more resistant to apoptosis (thus enabling the cancer cell to continue to proliferate)
- **Cancer cells themselves may have mechanims to avoid apoptosis**
  - **B-cell leukemias and lymphomas** hyperexpress **Bcl-2** (translocation of the *BCL-2* gene into an **enhancer** region for antibody production)
  - **Melanoma** inhibite expression of the gene encoding **Apaf-1**
  - **Lung and colon cancer cells**, secrete elevated levels of a soluble **"decoy" molecule** that binds to FasL (it cannot bind Fas and cytotoxic T cells cannot kill the cancer cells)
  - **Other cancer cells** express high levels of **FasL**, and can kill any cytotoxic T cells (CTL) that try to kill them because CTL also express Fas

APOPTOSIS AND AIDS

- AIDS (**acquired immunodeficiency syndrome**) - decline in the number of the $CD4^+$ T cells (helper cells) due to invasion of **HIV** (**human immunodeficiency virus**) ?
- **Infection by HIV** does not case causes the main dying-off of $CD4^+$ T
- less than 1 in 100,000 $CD4^+$ T cells in the blood of AIDS patients are actually infected with the virus
- Infected T-cells kill **uninfected** $CD4^+$ cells by apoptosis
  - All T cells, both infected and **uninfected**, express **Fas**.
  - Expression of a HIV gene ***Nef*** in infected cell hyperexpress **FasL** in infected cells
  - infected T cell encounters an uninfected one (e.g. in a lymph node), the interaction of **FasL** with **Fas** on the uninfected cell kills it by apoptosis

Apoptosis and Organ Transplants

- certain parts of the body (the anterior chamber of the eye, the testes are "immunologically privileged sites“ (antigens within these sites fail to elicit an immune response)
  ⇒ cells in these sites differ from the other cells of the body - express high levels of **FasL** (antigen-reactive T cells, which express **Fas**, would be killed when they enter these sites)

- Perspectives:
  ⇒ high levels of FasL in cells on a transplanted kidney, liver, heart, etc. To protect the graft from attack by the T cells of the host's . treatment with immunosuppressive drugs for the rest of the transplant recipient's life might be reduced or eliminated