

Cell cycle

Ahmed Salem, MBBCH, PhD, FRCR



The molecular basis
for cell cycle
regulation &
Cell cycle check
points

•**Describe the components and regulation of the cell cycle, understanding how the process of DNA replication is regulated throughout the cell cycle and what happens when DNA replication goes awry.**

- Point out DNA damage checkpoints.
- Describe The events that can trigger cell apoptosis & the main constituents of the apoptotic machinery



Cell cycle

- **Definition:** self-regulated sequence of events that lead to completion of cell division and formation of two daughter cells as a result of mitosis
- **Purpose:** to produce 2 identical daughter cells (each containing chromosomes identical to parent cell)
- Progression through cell cycle is regulated at points called checkpoints
- 2 principle phases: interphase (growth of cell) & mitosis (cell division)



Somatic cell classification

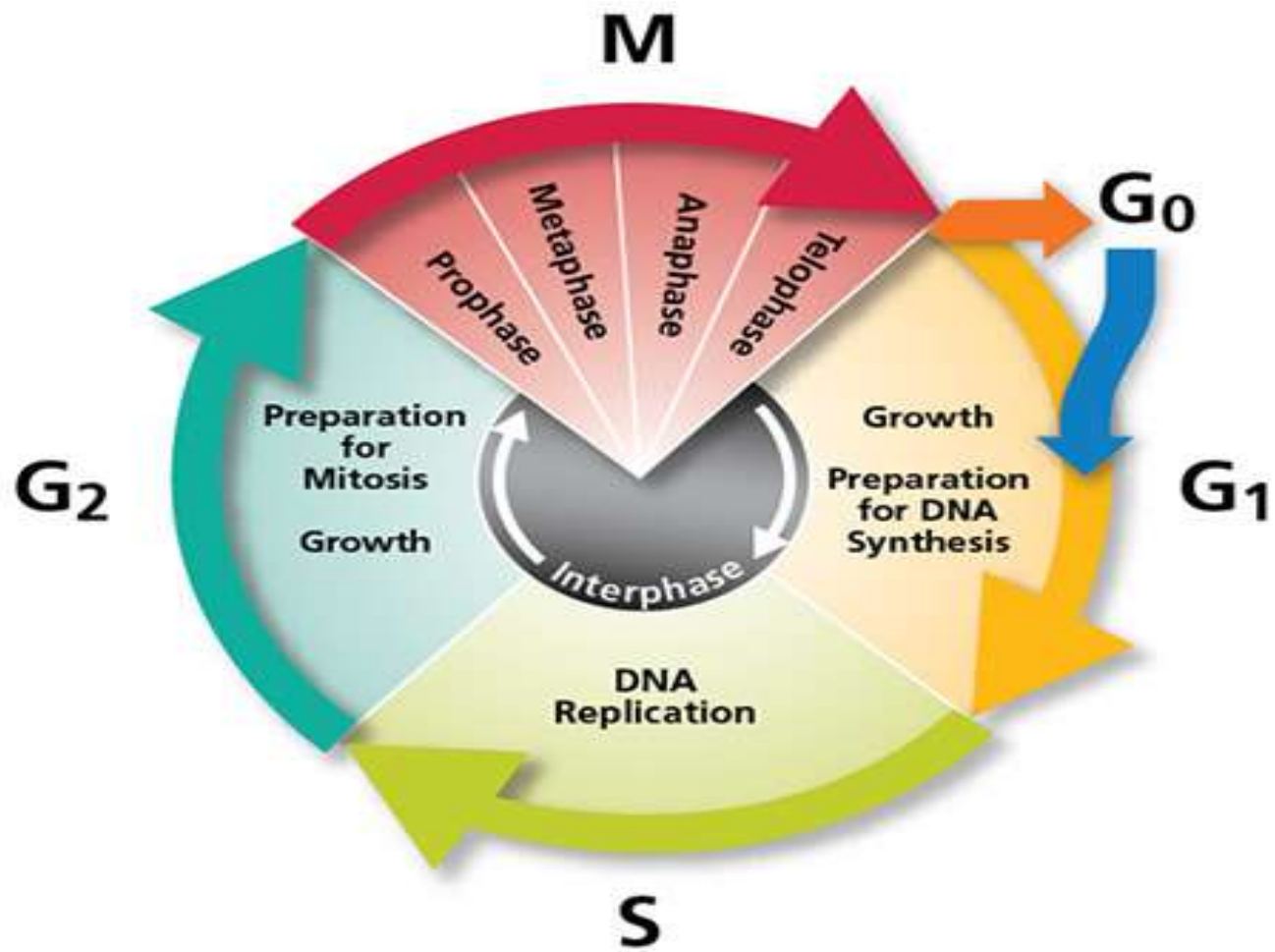
- **Stem cells** → self-perpetuate & produce cells for a differentiated cell population
- **Transit cells**, which are cells in movement to another population
- **Mature cells**, which are fully differentiated and do not exhibit mitotic activity e.g. neurones



Cells propagate through division

- **Mitosis:** Division of somatic cells → 2 genetically identical daughter cell
 - Chromosomes of daughter identical to original cell
- **Meiosis:** Division of germ cells → 4 sex cells each with half n of chromosomes





G1 phase

- Time gap btwn mitosis and DNA replication (S phase)
- Usually is longest and most variable phase of the cycle
- The following occurs in G1:
 - Active RNA & protein synthesis
 - Cell grows to parent size
 - Somatic cell contains 46 single threaded chromosomes
- G1/S checkpoint: present at end of G1
 - Some cells exit cycle → can enter G0 & become differentiated
 - Some can re-enter cycle (e.g. liver cells)
 - Some are terminally differentiated (muscle and nerve cells)
 - Some cells complete the cycle



S phase (DNA synthesis)

- The duration of this phase last about 6 to 8 hours
- DNA is replicated (chromosomes are duplicated to become tetraploid; 46 double threaded chromosomes)
- These events result in the formation of two sister chromatids attached at the centromere



G2 phase

- Gap btwn DNA replication and mitosis
- The duration of this phase last about 4 to 5 hours
- It is preparatory phase for M phase
 - RNA & protein synthesis are taking place as well as cytoplasmic enlargement
 - Most of the proteins synthesized are cytosolic proteins (needs to synthesize enough proteins for 2 cells)
- DNA repair

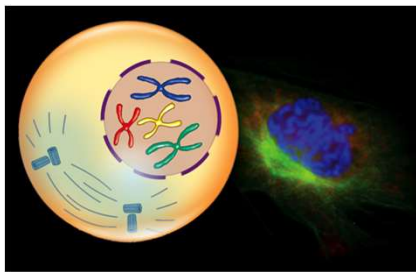


Explanation of each phase under slide in notes

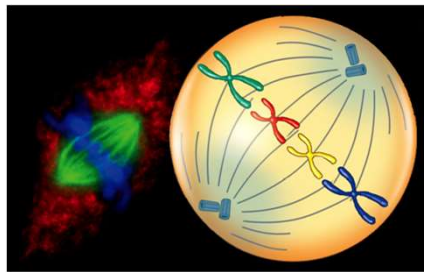
M phase (mitosis)



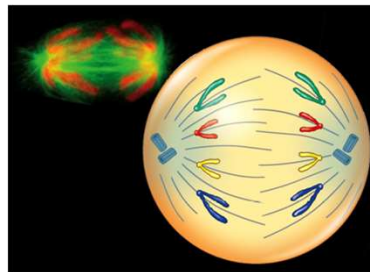
- The purpose of mitosis is cell division: making two cells out of one
- It is the shortest phase taking about 1-1.5 h
- Includes division of the cell nucleus (mitosis) and division of the cell cytoplasm (cytokinesis)



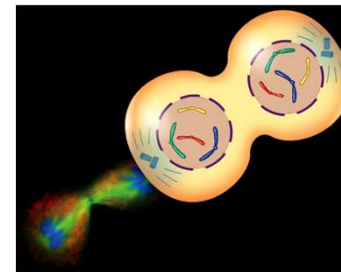
Prophase



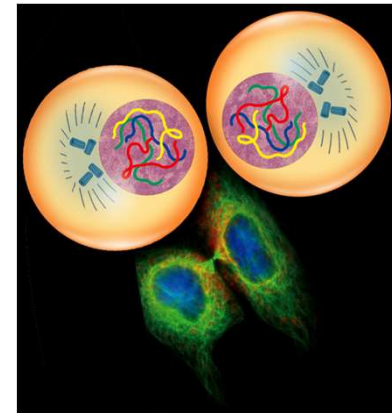
Metaphase



Anaphase



Telophase



Cytokinesis

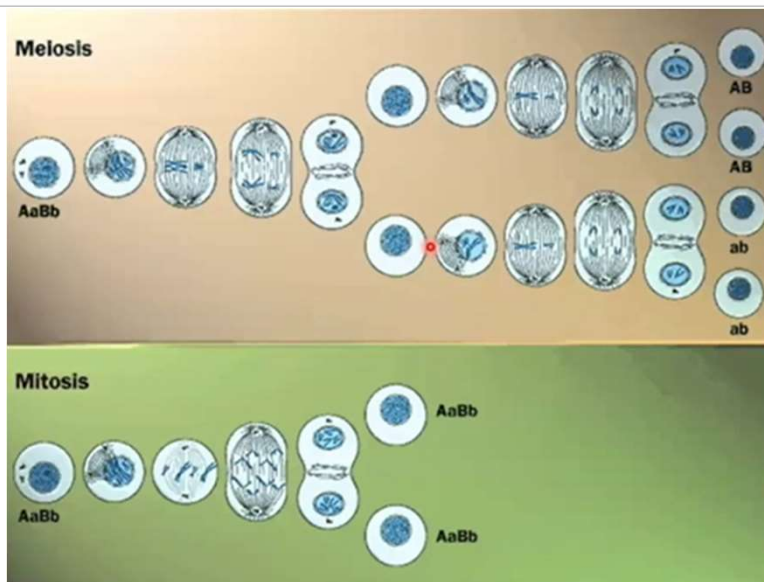
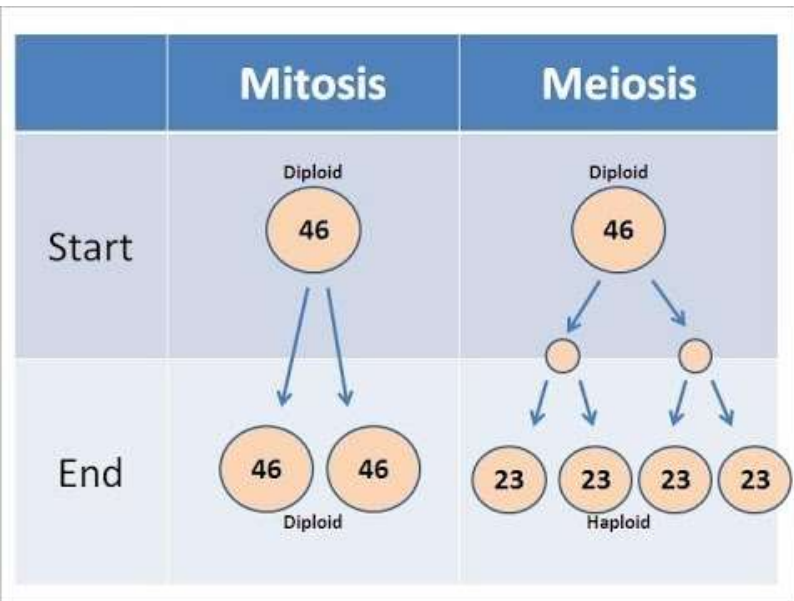


Cells divide at different rates

- The rate of cell division varies with the need for those types of cells

FIGURE 5.2 CELL DIVISION	
CELL TYPE	APPROXIMATE LIFE SPAN
Skin cell	2 weeks
Red blood cell	4 months
Liver cell	300–500 days
Intestine—internal lining	4–5 days
Intestine—muscle and other tissues	16 years

- Some cells are unlikely to divide (G_0)



Mitosis

- 4 stages in total (plus interphase)
- Happens in somatic cells
- Purpose is cellular proliferation
- Produces 2 diploid daughter cells
- Chromosome number remains the same
- Genetic variation doesn't change

Same

- Produce new cells
- Similar basic steps
- Start with a single parent cell

Meiosis

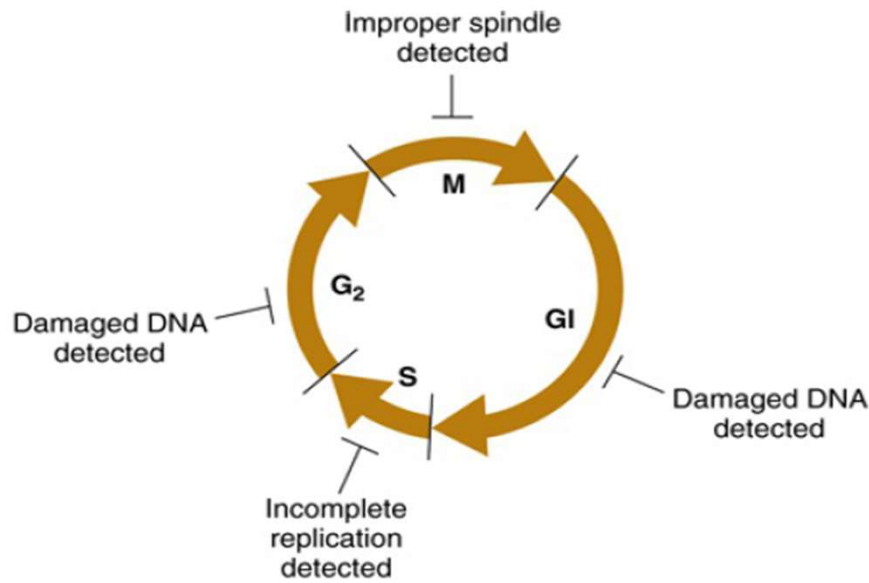
- 8 stages in total (plus interphase)
- Happens in germ cells
- Purpose is sexual reproduction
- Produces 4 haploid daughter cells
- Chromosome number is halved in each daughter cell
- Genetic variation increased



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Normal cellular behaviour



Source: Murray RK, Bender DA, Botham KM, Kennelly PJ, Rodwell VW, Weil PA: *Harper's Illustrated Biochemistry*, 29th Edition: www.accessmedicine.com



Possible outcomes at G₁ checkpoint

Delay progression

Go to G₀

Proceed to S 1

- Cells with proliferative capacity normally stop at certain checkpoints
- At these checkpoints, there are cellular mechanisms for detecting and repairing genetic defects

The goal is to prevent mutant cells from replicating

- DNA repair
- Apoptosis

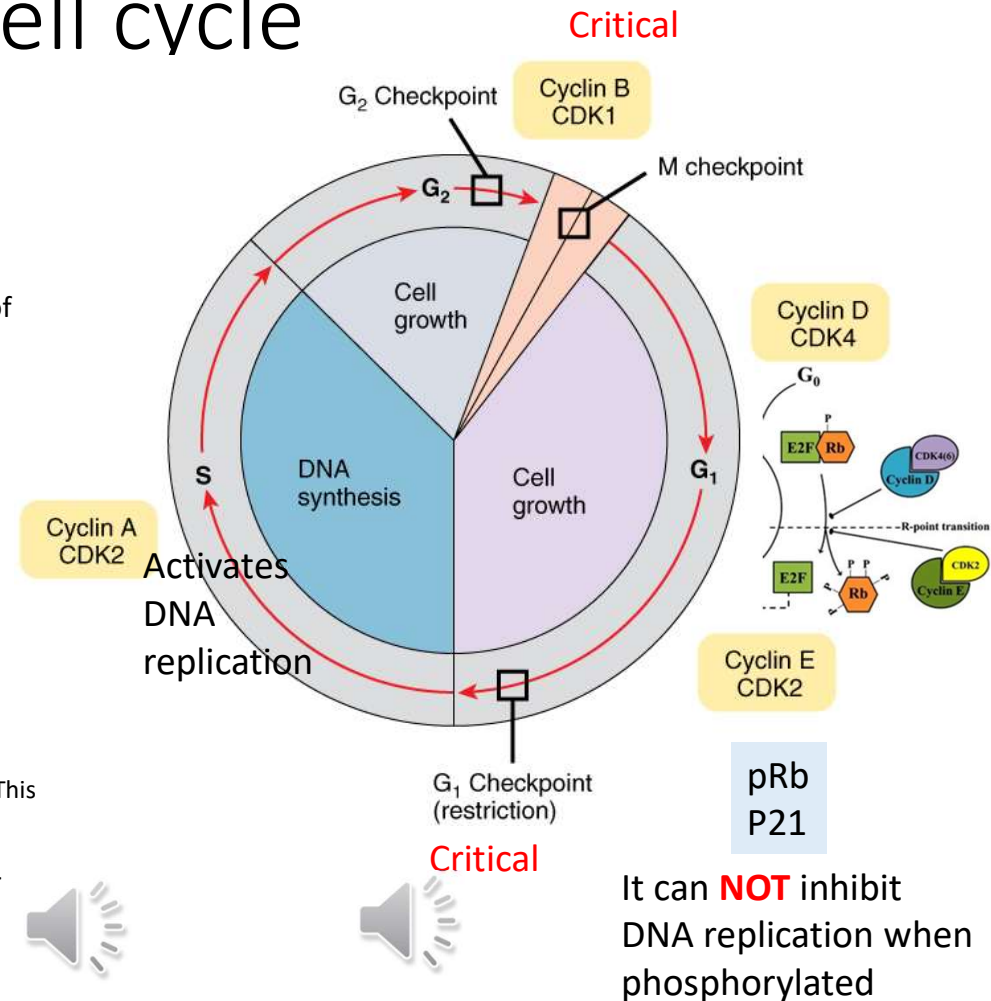
Cell cycle regulation: role of CDKs, cyclins

- A family of proteins called cyclins governs the transition of a cell from one phase to another
 - The concentration of different cyclins increases & decreases during different phases of the cell cycle, and hence their name
- Cyclin dependent kinases (CDK)
 - Adds a phosphate group on other proteins essential for the passage of the cell from one phase to another. →
 - In default status are inactive
- Cyclins activate CDKs
 - CDKs are only active if bound to cyclins



Transition through the cell cycle

- **Retinoblastoma (pRb)** is tumour suppressor protein that restricts $G_1 \rightarrow S$
 - pRb exists in 2 forms: active (dephosphorylated) and inactive (phosphorylated)
 - Active pRb $\rightarrow$ stop cell progress at G1/S by binding to & inactivation of E2F (transcription factor needed G1 to S progression)
 - CDK 4 and CDK 6 phosphorylate pRb
- p21 is a CDK inhibitor that regulates $G_1 \rightarrow S$
- P16^{Ink4a}(CDKN2A) is tumour suppressor protein that inhibits S phase
- Cyclin E & CDK2 form a complex in late G1
 - Cyclin E rapidly degraded, and the released CDK2 then forms a complex with cyclin A $\rightarrow$ This sequence is necessary for the initiation of DNA synthesis in S phase.
- The B cyclins are produced late in the G2 phase. A complex between cyclin B & CDK1 is rate- limiting for the G2/M transition in eukaryotic cells



Cell cycle check points:



- DNA, chromosome, chromosome segregation integrity is continuously monitored throughout the cell cycle in **4 check points**.
- DNA damage is detected in G1 & G2 phases
- Completeness of replication is detected late in the S phase
- Proper chromosomal segregation machinery is detected in the M phase.
- Cells will not progress through the phase of the cycle in which defects are detected. In some cases if the damage cannot be repaired, such cells undergo programmed cell death (apoptosis)



p53

- Is a tumour suppressor protein/ transcription factor
- Imp functions in cell regulation, DNA repair and apoptosis
- Activates several genes as p21, GADD45 and apoptotic genes
- In unstressed cells, p53 is –vly regulated by MDM2 which targets p53 for proteasomal degradation
- DNA DSBs activate ATM (or ATR) resulting in phosphorylation of p53, thereby preventing it's degradation by MDM2

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Apoptosis

- *Apo = off, ptosis = falling*
- The cells carry in their nucleus **genetic program for suicide** that can be turned on to get rid of a specific cell so it is called **cell suicide** or **programmed cell death**

Why do cells commit suicide?

- Kill unwanted cells for:
 - Precise regulation of cell number in embryo morphogenesis → e.g. digits are separate as cells btwn fingers apoptose
 - As a defence mechanism to removes unwanted cells which are potentially dangerous (e.g. virus infected cells or tumour cells)
 - In aging and tissue degeneration
 - Hormone dependent involution in adult (e.g. in endometrial cell breakdown during menstruation)



Mechanism of apoptosis

- Energy dependent cascade of events:
 1. **Signalling pathways** that initiate apoptosis
 2. **Control and integration phase [extrinsic or intrinsic pathway]**
 3. **Common execution phase** (actual death program): accomplished largely by caspase family of proteases
 4. **Phagocytosis** to remove apoptotic bodies



Extrinsic pathway

Receptor mediated apoptosis results from the interaction of a ligand with a specific transmembrane receptor, the most important are the Fas receptor and tumor necrosis factor receptor 1 (TNFR)

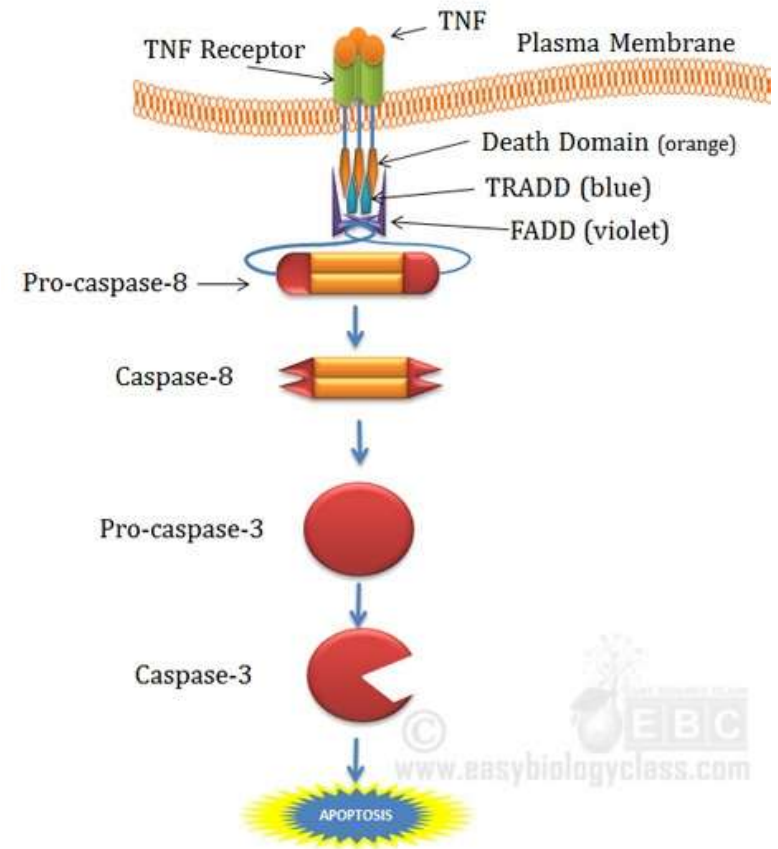
These (death) receptors are coupled with intermediate membrane proteins (adaptor proteins)

→ Activation of initiator caspase 8 → activates other effector caspases → apoptosis



- The tumor necrosis factor receptor superfamily (TNFRSF) is a protein superfamily of cytokine receptors characterized by the ability to bind tumor necrosis factors (TNFs) via an extracellular cysteine-rich domain

- The term death receptor refers to those members of the TNF receptor superfamily that contain a death domain, such as TNFR1, Fas receptor



Extrinsic Pathway of Apoptosis

Intrinsic pathway

Initiated from within the cell due to severe stress (e.g. radiation, hypoxia, free radicals and chemical agents as chemotherapy produce DNA damage)

Involves activation of p53 →

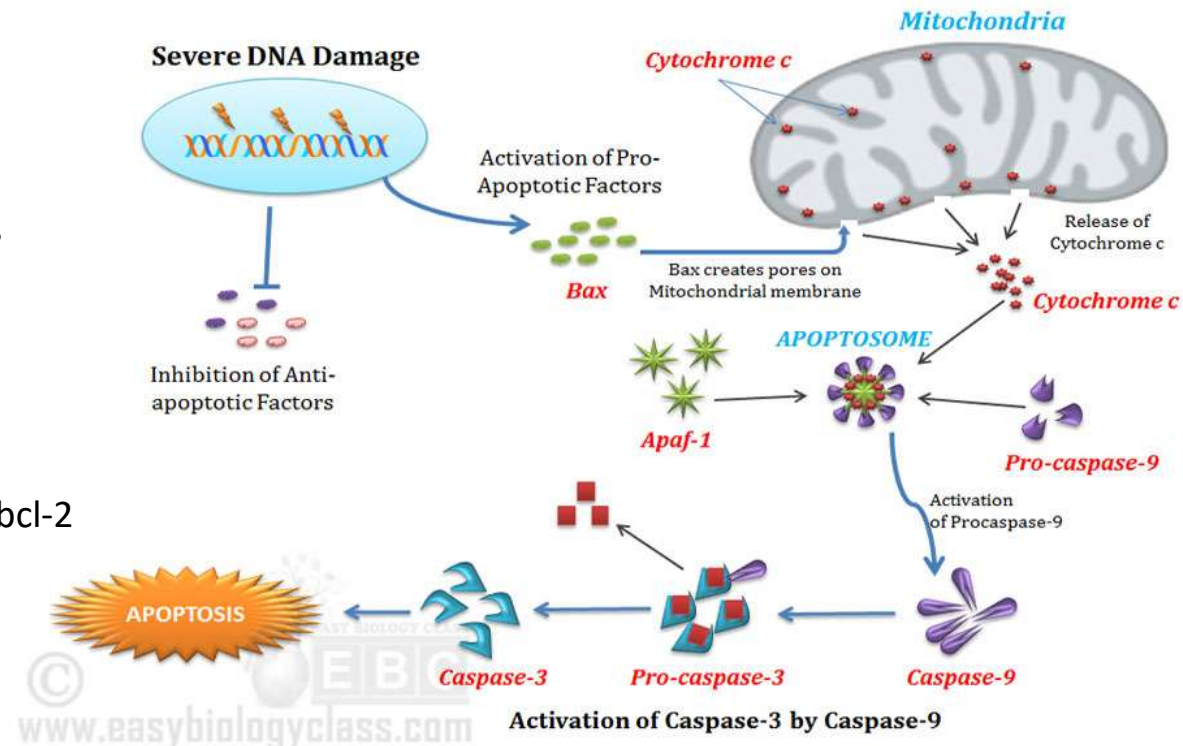
transcription of bcl-2 pro-apoptotic proteins (e.g. Bax (bcl-2 antagonist x) →

mitochondrial release of cytochrome C →

activate caspase enzymes

INTRINSIC PATHWAY OF APOPTOSIS

(Mitochondria Mediated Programmed Cell Death Pathway)



- Loss of protein Bax function as a result of gene mutation is associated with cancer development **e.g.** gastric adenocarcinoma
- **Bcl-2** is an antiapoptotic protein that acts through binding with protein Bax and produces its inactivation
- Increased level of Bcl-2 is associated with cancer development
- In normal cells, both Bax and Bcl-2 are present in balance **(the ratio of apoptosis inhibitors to apoptosis stimulators makes death decision).**





Execution phase of apoptosis

- Mediated by family of proteolytic enzymes called **caspases**
- **Caspases:** name comes from “c” cysteine amino acid in catalytic site of enzyme, “aspase” ability to cleave substrate after aspartic acid AA
- Types:
 - Initiator caspases (8, 9)
 - Effector caspases (3)
- Caspases also produce activation of endonucleases that split DNA, termed caspase activated Dnase (CAD)
- CAD produces a series of DNA fragments varying in length by approximately 200 bp (nucleosomal fragments and their multiplies), these fragments have characteristic ladder appearance on gel electrophoresis

Phagocytosis to remove apoptotic bodies

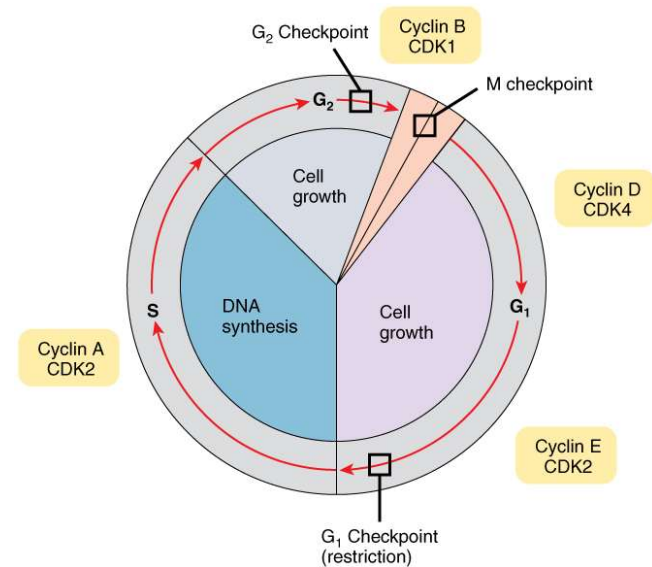
- Dead cells are removed by neighbouring phagocytes which recognises re-distributed phosphatidylserine in EC matrix (normally is a phospholipid in plasma membrane)
- Removal of dying cells occurs in orderly manner without eliciting an inflammatory response



Questions

CDK1/cyclin B plays an important role in the transition of cells from:

- A. G_0 into G_1
- B. G_1 into S
- C. S into G_2
- D. G_2 into M
- E. M into G_1



Answer: D The CDK1/cyclin B complex plays an important role in the transition of cells from G_2 phase into mitosis.

Which phase of the cell cycle has the most variable length of time?

- A. M
- B. S
- C. G1
- D. G2

Answer: G1

The typical cell cycle time (TC) for proliferating cells in human tumours is in the range of?

- A. <1 day
- B. 1-5 days
- C. 6-25 days
- D. 26-100 days
- E. >100 days

B The typical T_c for tumor cells *in vivo* is generally in the range of 1-5 days.

Terz et al. Analysis of the cell kinetics of human solid tumors. *Cancer*. 28(5):1100-10, 1971. [Pubmed](#)

Which cell in the human body has the shortest lifespan?

- A. Skin cells
- B. Neutrophils
- C. Skeletal muscle cells
- D. Pancreas cells
- E. Heart muscle cells

Answer: Neutrophils have a lifespan of 4 h or less

Cardiac: 40 years

Muscle: 15 years

Pancreas 1 year