University of Jordan
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بسم الله الرحمن الرحيم

GENETICS & Molecular Biology

Number: 27

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Subject: The Cell Cycle (I)

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Price:

Date:

The Cell Cycle

- They are series of events that happen in different type of cells to regulate their division & constant tissue regeneration
- Cells that need to regenerate all the time include RBCs **&** Intestinal epithelium.
- Cell cycle consist of 2 main phases:

  -) Interphase (G1, S, G2)

  -) Mitotic phase (M)

➔ **G1 phase**: Before the cells divide, cells synthesize proteins and factors that mediate cell division.

- 2n chromosomes (46) **(In each cell)**

➔ **S phase**: Replicating DNA to 2 copies, ensuring that the two daughter cells will receive the same amount of DNA.

- 4n chromosomes (92)

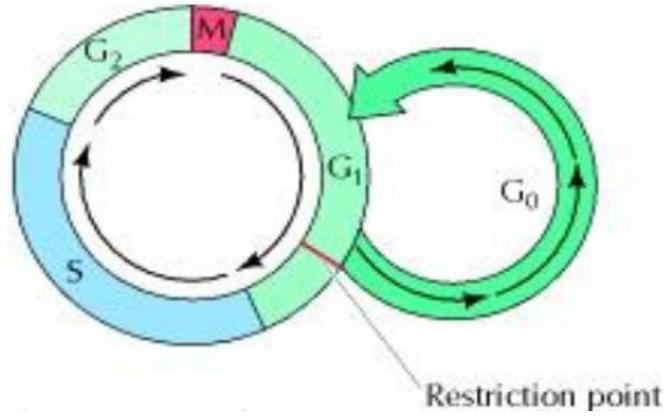
➔ **G2 phase**: Metabolism & production of proteins and factors needed for mitosis.

- 4n chromosomes (92)

➔ **M phase**: Mitosis, resulting in 2 copies of the cell.

- Each with 2n chromosomes (2 copies, each with 46)

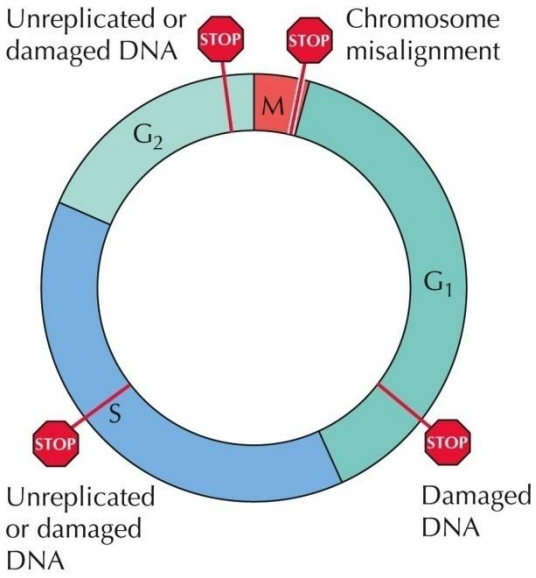
Extracellular growth factors are needed for cell cycle to proceed. (e.g. EGF, PDGF, Insulin-like GF, Growth Hormone, etc..).



In the absence of these growth factors, Some cell types will not divide, e.g. Nerve cells , They will proceed into the G1 phase but when they reach the restriction point in that phase, they will enter the "Quiescence state" (G0) and get stuck in it, thus, it will not replicate. (Proceeding into S phase will be inhibited).

Checkpoints

- There are several checkpoints in the cell cycle that verify & ensure there is no mistakes/mutations that would be transmitted to the next generations:-
  - 1st checkpoint → In **G1** (Before replication) → DNA damage
  - 2nd checkpoint → In **S** (During replication) → DNA damage
  - 3rd checkpoint → In **G2** (After replication) → DNA damage
  - 4th checkpoint → In **M** (Mitosis) → Spindle assembly



(P.S.) The 2nd checkpoint ensures that DNA is replicated only once and from one origin of replication, by masking all other origins of replication by the action of mainly Helicases & other proteins).

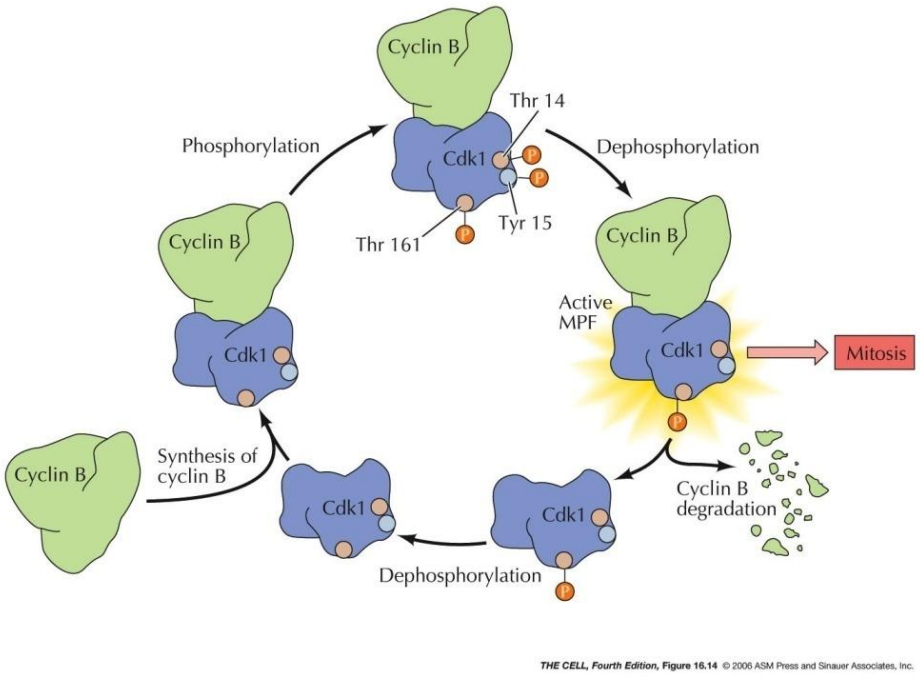
(P.S.) Spindle assembly checkpoint (4th) assures that both daughter cells receive the same amount of DNA).

At the molecular level, what regulate the work of these checkpoints are the changes and expressions of different proteins that work on (activate/inhibit) genes that code for other proteins that run the cell cycle. These other proteins that run the cycle are mainly "Cyclins" & "Cyclin-Dependent Kinases" (CDKs).

- Different types of cyclins will be activated in different stages of the cell cycle and each one will associate with a specific CDK forming Cyclin-CDK complexes (For example, "Cyclin **B**-CDK**1** complex").
- During interphase (G1, S, G2), the amount of these cyclins increase to the max then will drop during mitosis (M phase).

How does Cyclin-CDK complex work?

- For example, CyclinB-CDK1 complex in **G2 phase**: (CDK1 = CDC2 in yeast)



THE CELL, Fourth Edition, Figure 16.14 © 2006 ASM Press and Sinauer Associates, Inc.

Mechanism:-

1) CDK1 forms a complex with Cyclin B.
2) After the complex formation, CDK1 has 3 residues that get phosphorylated. (Threonine 14 / Tyrosine 15 / Threonine 161)

   Thr 14 –When phosphorylated → **Deactivates** kinase (Inhibitory)
   Tyr 15 – When phosphorylated → **Deactivates** kinase (Inhibitory)
   Thr 161 – When phosphorylated → **Activates** kinase ~~> (Activate cell cycle)

3) The complex is activated by dephosphorylating *Thr 14* & *Tyr 15.*
4) The active complex activates mitosis (proceeding to M phase) then Cyclin B dissociates from the complex and gets degraded.
5) The remaining *Thr 161* is dephosphorylated so that CDK1 can now reform a new complex with another Cyclin B. (Then all 3 residues in the complex get phosphorylated again and the cycle is repeated…)

- Cyclin-CDK complex has a binding site for the binding of the inhibitory molecules "Cyclin-Dependent Kinase Inhibitors" (CKIs).

Cell Signalling & Cell cycle

The signalling pathway of RAS/RAF/ERK:

- Once growth factors bind to receptors on the cell, they will activate RAS → Now, RAS phosphorylates RAF → RAF phosphorylates ERK → ERK goes into the nucleus & activate target genes (for Cyclin D).
- Once Cyclin D is expressed, it will bind to CDKs 4,6 forming complexes.
- Cyclin D-CDK4,6 complexes phosphorylate Retinoblastoma (RB) on its binding sites, allowing it to dissociate from E2F → Activating transcription of genes that regulate S phase.
  (RB protein is normally bound to E2F forming an "RB-E2F complex" that inhibits transcription of genes involved in preparation for S phase).

(Remember that):-

- *RAS – Oncogene. (Indirectly phosphorylates/inactivates RB)*
- *p53 – Tumor suppressor. (Inhibit over-proliferation)*
- *RB – Tumor suppressor. (Inhibit E2F)*
- *E2F – Activate transcription.*

At the molecular level, in DNA damage checkpoints (G1, S, G2):

- In a **single-strand** damage – ATR (protein kinase) identifies the site → Activates checkpoint protein 1 (CHK1)
- In a **double-strand** damage – ATM (protein kinase) identifies the site → Activates checkpoint protein 2 (CHK2)

Each of these checkpoint proteins (CHK) can inhibit CDC25 (CDK) phosphatase so CDC25 will remain phosphorylated, inactive, resulting in cell cycle inhibition.

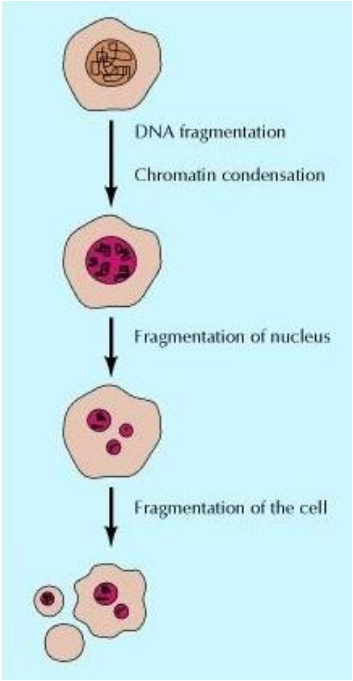
The signalling pathway of p53: (tumor suppressor/controls normal cell over-proliferation)

- ATM detects double-strand breaks.
- ATM stabilizes p53 protein (keep it working/activate it) by checkpoint proteins.
- p53 activates p21 (Cyclin-Dependent Kinase Inhibitor/"CKI").
- p21 inhibits CDKs, resulting in inhibition of cell division.

✓ p53 can also activate Apoptosis through the BAX pathway.

Apoptosis

A physiologically programmed cell death, happens when the cell is not functioning well so it decides to suicide. It needs to happen all the time in the body in order to regulate number of cells and keep them within the normal range & to maintain space for healthy cells.



- Stimulated by either intrinsic (DNA damage) **or** extrinsic signals (signals from other cells).
- Features of apoptosis:
  1) Fragmentation of DNA & Condensation of chromatin (darker nucleus).
  2) Fragmentation of the nucleus.
  3) Fragmentation of the whole cell, forming apoptotic bodies.
  4) Macrophages will recognize apoptotic bodies for phagocytosis.
     (Remember: Macrophages are phagocytic cells)

- How can macrophages distinguish apoptotic bodies that need to be phagocytosed?

- By targeting them with membrane lipids, specifically, Phosphatidylserine.

- Phosphatidylserine is normally found on the inner leaflet of cell membrane.
- During apoptosis, Phosphatidylserine in apoptotic bodies is going to be flipped to become on the outer leaflet so it could now be bonded by a receptor on the cell membrane of the macrophage, stimulating it for doing the engulfment.
- A very high marker for apoptosis is **Caspase** proteins. So inside the apoptotic bodies, caspases are highly present and active, digesting the components of the cell.
- Caspases are activated by Adapter protein (Apaf-1). Although, adapter proteins themselves need to get activated by conformational change due to binding with Cytochrome C.

The mechanism:-

- ❖ Multiple BAX protein domains are inserted into the membrane of the mitochondria, forming an oligomeric channel "Proapoptotic BAX pore channel".
- ❖ Cytochrome C particles leave the mitochondria toward the cytoplasm, where it binds to the adaptor (Apaf-1) and activate it.
- ❖ When the adaptor is activated, it will activate caspase 9.
- ❖ The complex of Apaf-1 + Caspases 9 + Cytochrome C molecules is called an **"Apoptosome"**.
- ❖ Caspase 9 subunits in the apoptosome will activate the zymogen pro-caspase 3, converting it into active caspase 3, thus, inducing apoptosis.

Roles of Caspases (They act on):

- ✓ **Nuclear Lamins** → No more support on the inner nuclear membrane, leading to fragmentation of nucleus.
- ✓ **Inhibitors of DNase (ICAD)** → No more inhibition of DNA fragmentation.
- ✓ **Cytoskeletal proteins** (either cytosekeletal proteins themselves or the binding protein which cross-links these structures together).
- ✓ **Golgi proteins**

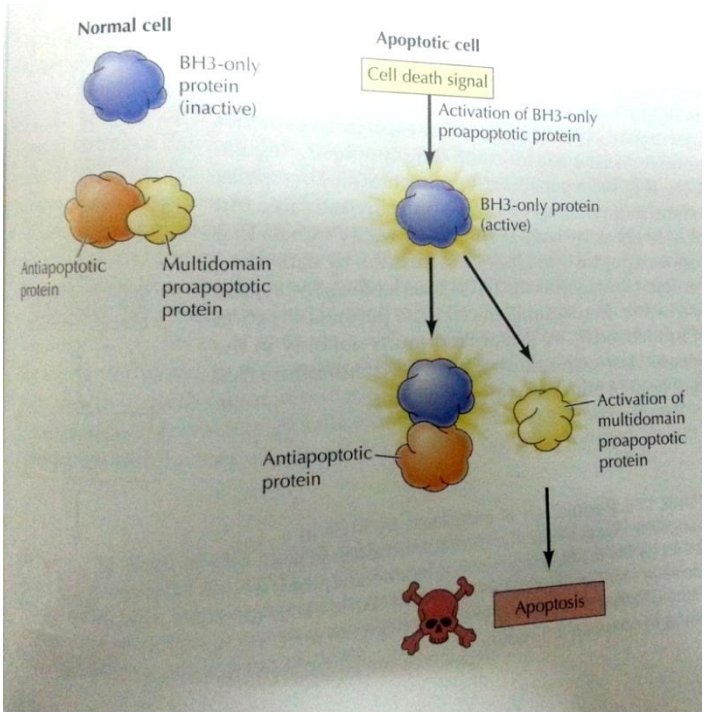
Bcl-2 Family: (3 proteins)

- **BH3** (proapoptotic when active) / (anti-apoptotic when inactive)
- **Multidomain proapoptotic protein**
- **Anti-apoptotic protein**

➢ **In a normal cell:**

- BH3 is in the inactive form. (In normal cell we don't need apoptosis to be activated)
- The "Multidomain proapoptotic protein" bound to the "Anti-apoptotic protein", forming an **anti-apoptotic complex**.

- **In an apoptotic cell (due to apoptotic signal):**
  - When BH3 is activated, the Anti-apoptotic protein dissociates from the complex & binds to the BH3, forming a **proapoptotic complex**.
  - The Multidomain proapoptotic protein is separated from the complex & becomes activated as well.



(Note: Apoptotic signals include: Lack of growth factors, lack of nutrients, detection of misfolded proteins, signals for renewing/regenerating the cell)

Intrinsic pathway (Internal):

*** Example) In double-stranded DNA damage:-**

-) The damage is detected by ATM.

-) ATM activates CHK2, which activates p53 by phosphorylating it.

-) Phosphorylated p53 will induce the expression of Bcl-2 gene specifically for BH3-only protein.

-) BH3 will be activated, resulting in stimulation of apoptosis.

P.S.) When DNA damage occurs, the cell will first try to fix the damage but if the damage was severe, **apoptosis will occur**.

Extrinsic pathway (External):

*** Example 1) '<u>Pro-survival</u>' by PI-3 Kinase/AKT signalling pathway:-**

-) The receptor "Tyrosine Kinase" is embedded on the cell membrane.

-) A ligand (growth factor) binds at the extracellular part of the receptor causing auto-phosphorylation of the tyrosine residues on the receptor itself.

-) The phosphorylated sites attract other proteins, like "PI 3-Kinase".

-) The activated PI 3-Kinase converts the PIP2 (Phosphatidylinositol diphosphate) into PIP3 (Phosphatidylinositol triphosphate).

-) PIP3 is a binding site for "Akt".

-) Once Akt is bound to the membrane by binding to PIP3, it will get phosphorylated by "PDK1".

-) The phosphorylated Akt now dissociates from the membrane and activate so many signalling pathways that will lead to cell proliferation.

➔ **Inhibition of this pathway will lead to apoptosis.**

*** Example 2) '<u>Pro-death</u>' by TNF pathway (Tumor Necrosis Factor):-**

-) When TNF binds to its receptor at the outer side, it will attract the adapter protein

-) Once the adapter is bound to the TNF receptor at the inner (cytosolic) side, it will now attract caspase 8 & activate it.

-) Once caspase 8 is activated, it will activate "BAD" which is a member of the proapoptotic BH3-only proteins, stimulating **apoptosis**.

➔ BAD can also stimulate BAX/BAK, stimulating the release of cytochrome C, activation of caspase 9 and thus, the intrinsic pathway as whole.

Sheet is over, sorry for any mistakes. (msh kol mara tafa2ol bs tfa2alu :p)
Good luck!