

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ
(وَفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٌ)



Cytology & Molecular Biology | Lecture 14

The Cell-Cycle



Written by : NST

Quiz for the previous lecture



Lecture 9:

The cell cycle

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School of Medicine

Second year, First semester, 2025-2026

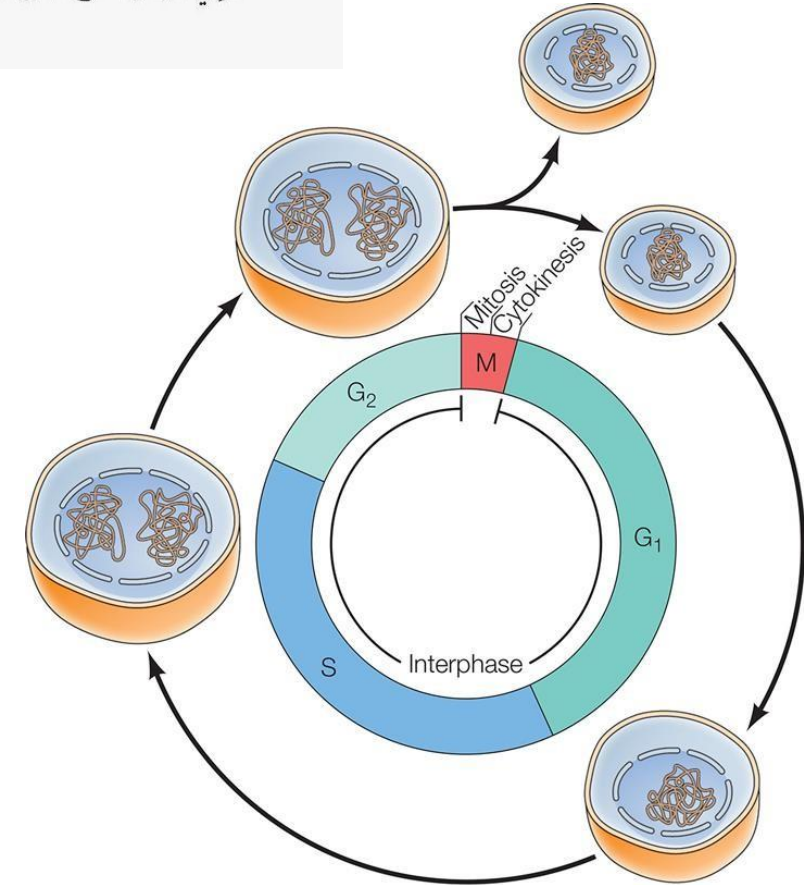
Some introduction

- Cells must divide for life to continue. **It undergoes mitosis then cytokinesis. During mitosis, chromosomes line up at the metaphase, then sister chromatids separate to opposite poles of the cell, then cytokinesis takes place and finally, the cell divides**
- **Before mitosis, the cell goes through a preparation phase (interphase) to get ready for division**
- Cell division involves: DNA replication and separation of chromosomes (mitosis).
- A typical eukaryotic cell cycle divides ~every 24 hours.
 - Mitosis (nuclear division) and cytokinesis (cell division) = ~1 hour
- Yeast cells **(a simple eukaryotic) : 90 minutes to divide**
- **Bacterial cells need 20-40 minutes to divide**

The cell cycle

أَسْتَغْفِرُ اللَّهَ الْعَظِيمَ
الَّذِي لَا إِلَهَ إِلَّا هُوَ، الْحَيُّ الْقَيُّومُ، وَأَتُوبُ إِلَيْهِ

- The cell cycle is divided into two basic stages:
 1. M phase: **includes** chromosomal segregation, nuclear and cell division (mitosis & meiosis). **Takes one hour.**
 2. Interphase: Interphase: cell growth and DNA replication occur in an orderly manner in preparation for cell division. **Takes 23 hours.**
- G₁: increased metabolism and cell growth; cells are diploid (2n). **The cell appears inactive, but in reality, it is metabolically active and preparing for DNA replication.**
- S: DNA replication; cells are 2-4n. **the cell makes an exact copy of its DNA so that each daughter cell will receive a complete set of chromosomes.**
- G₂: metabolism and cell growth; cells are 4n **there is another “gap,” which also appears inactive, but during this time, the cell continues to grow and prepare for mitosis. After G₂, mitosis begins, this completes the cell cycle.**



G = Gap

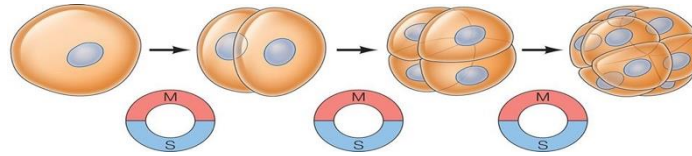
Special cases

لَا إِلَهَ إِلَّا اللَّهُ،
وَحْدَهُ لَا شَرِيكَ لَهُ، لَهُ الْمُلْكُ وَلَهُ الْحَمْدُ،
وَهُوَ عَلَى كُلِّ شَيْءٍ قَدِيرٌ.

Not all cells go through the full cell cycle. There are exceptions:

1. Zygote: no G1 or G2, but rapid S and M phases

The cells go directly from DNA synthesis (S phase) to mitosis, without G1 or G2 phases ;meaning there is no growth period. You can think of the “G” phases as growth phases where the cell grows and performs metabolism.



2. Some cells (nerve cells) enter a quiescent stage (G_0 phase).

The cell is metabolically active but does not divide or replicate DNA , it just performs its normal functions and sends signals.

3. Skin fibroblasts and cells of some internal organs divide as needed.

- These cells are arrested in G_0 and can re-enter the cell cycle in the presence of appropriate extracellular signals that stimulate cell division.

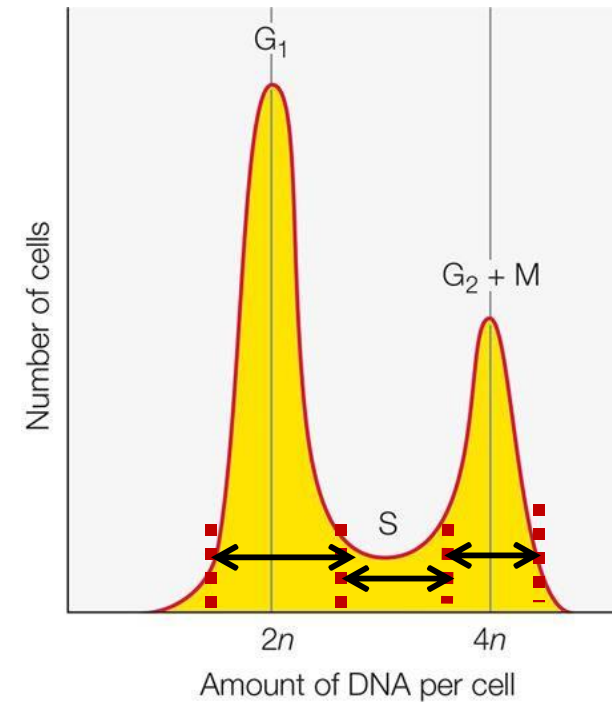
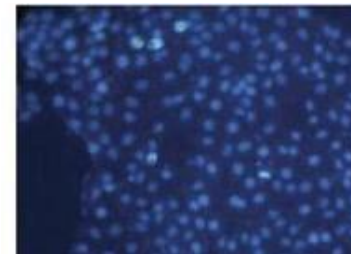
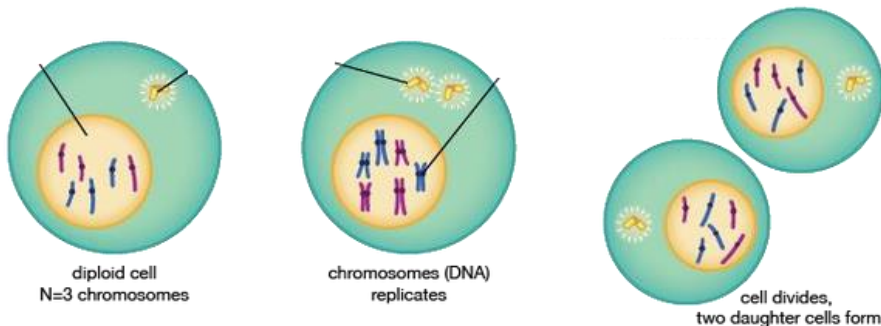
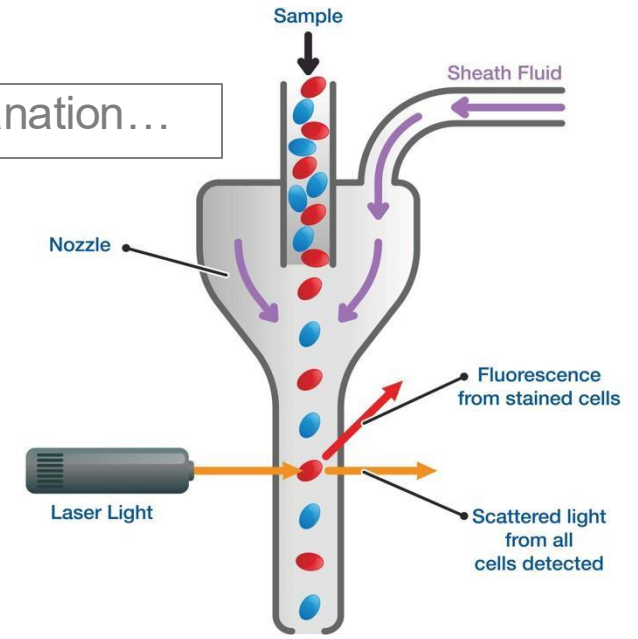
When the body sends signals for growth or tissue repair, these cells move back into G1 and continue through the rest of the cycle to divide.

The use of flow cytometry

See the next slide for explanation...

To distinguish dividing cells by the amount of their DNA content

- DNA is stained and cells are analyzed by instruments known as flow cytometry or fluorescence-activated cell sorter (FACS).
- Cells in G₁ are **diploid** (containing two copies of each chromosome) and referred to as $2n$ (n = **haploid** DNA content).
- At the end of the S phase and the beginning of the M phase, the DNA content is $4n$.
- Cells in the S phase have DNA contents ranging from $2n$ to $4n$.
- At the end of the M phase, DNA decreases $2n$.



How do we study whether the cells are in the G₁ phase, S phase, G₂, or M phase?

We use an instrument called flow cytometry.

We call it **flow** because the cells are actually flowing continuously through the instrument in a fluid stream.

And we call it **cytometry** because the instrument is used to measure cellular properties, in this case, the DNA content of each cell.

Principle and Mechanism

Flow cytometry can distinguish dividing cells based on the amount of DNA they contain, which reflects the phase of the cell cycle.

Here's how it works:

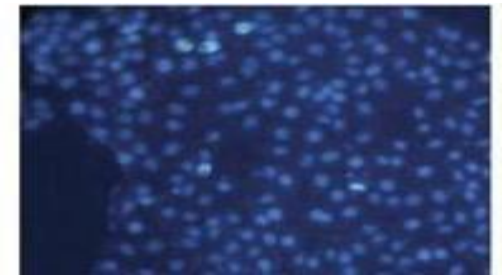
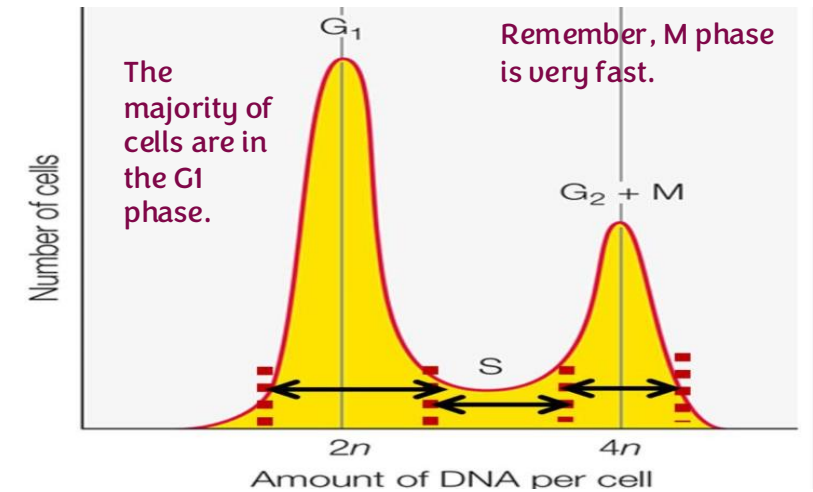
We stain the DNA with a special fluorescent dye that binds directly to DNA. When the stained cells pass through the instrument, they move one by one through a narrow tube

As each cell passes, it is hit by a laser beam.

The DNA-bound dye absorbs the laser light and emits fluorescence.

A detector on the other end measures how much fluorescence each cell gives; this tells us how much DNA that cell contains.

The data we generate is shown by chromatogram :



- NOTE: The intensity of the stain correlates with DNA content: cells in the G₁ phase have a specific, lower intensity because their DNA content is $2n$. In contrast, cells in the G₂ phase, which contain double the DNA, emit twice the signal intensity. Cells in the S phase show an intermediate intensity as their DNA content ranges between $2n$ and $4n$ during replication.

Let's say we are analyzing million cells .

As the cells flow one by one and the laser hits each of them, the detector measures the fluorescence intensity, which corresponds to DNA quantity.

- Cells with $2n$ DNA (normal diploid content) are in the G_1 phase.
- Cells with DNA between $2n$ and $4n$ are in the S phase, where DNA replication is actively occurring.
- Cells with $4n$ DNA are in the G_2 and M phases, where DNA replication has finished, and the cell is preparing to divide.

If the instrument detects cells with less than $2n$ DNA, this region is called sub- G_1 – it represents cells that have undergone DNA fragmentation, typically due to apoptosis (programmed cell death).

>>>From this, we can quantify how many cells are in G_1 , S, and G_2/M phases.

There's another related instrument called the Fluorescence-Activated Cell Sorter (FACS).

It works on the same principle as flow cytometry, but with an extra function which is sorting.

For example, imagine we have two cell populations:

- One is stained red
- The other blue

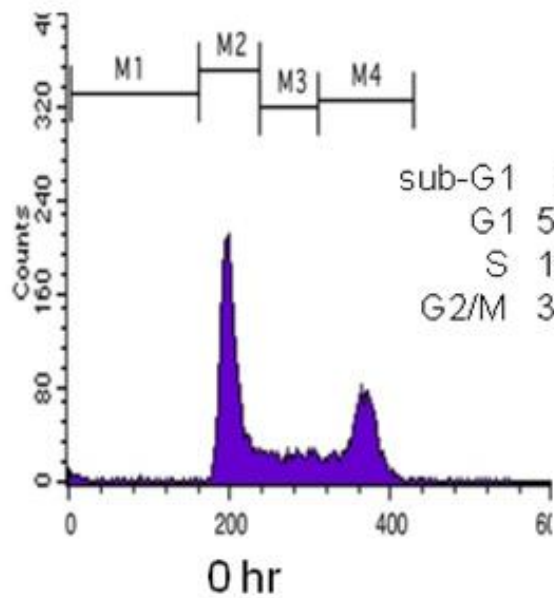
As they flow through the instrument, the laser detects their color (fluorescence signal).

Unlike the regular flow cytometer, the FACS machine can physically separate the two populations:

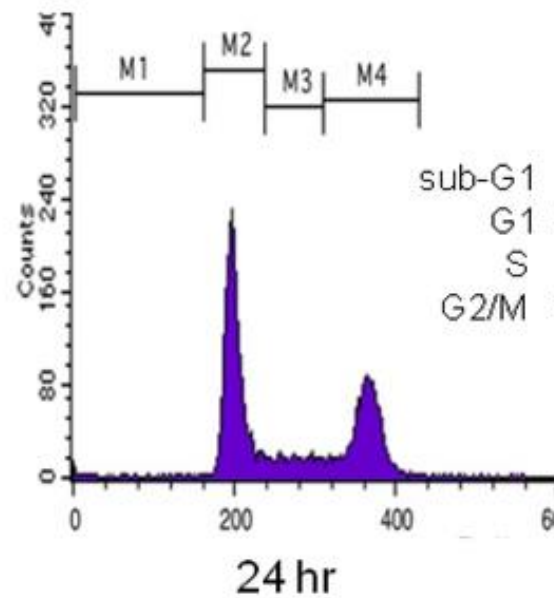
- It directs red cells into one collection tube
- And blue cells into another

>>>So FACS not only measures but also sorts, isolates, and collects specific cells .

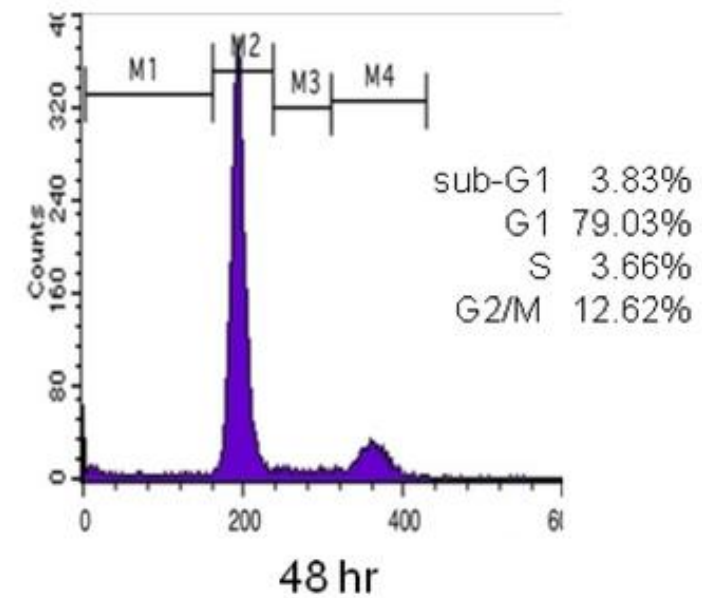
Imagine we are testing a drug to see how it affects cell division during time.



the cells are normal, healthy, and dividing naturally. This sample serves as our control



we start to see a slight increase in the number of cells in G₁ phase

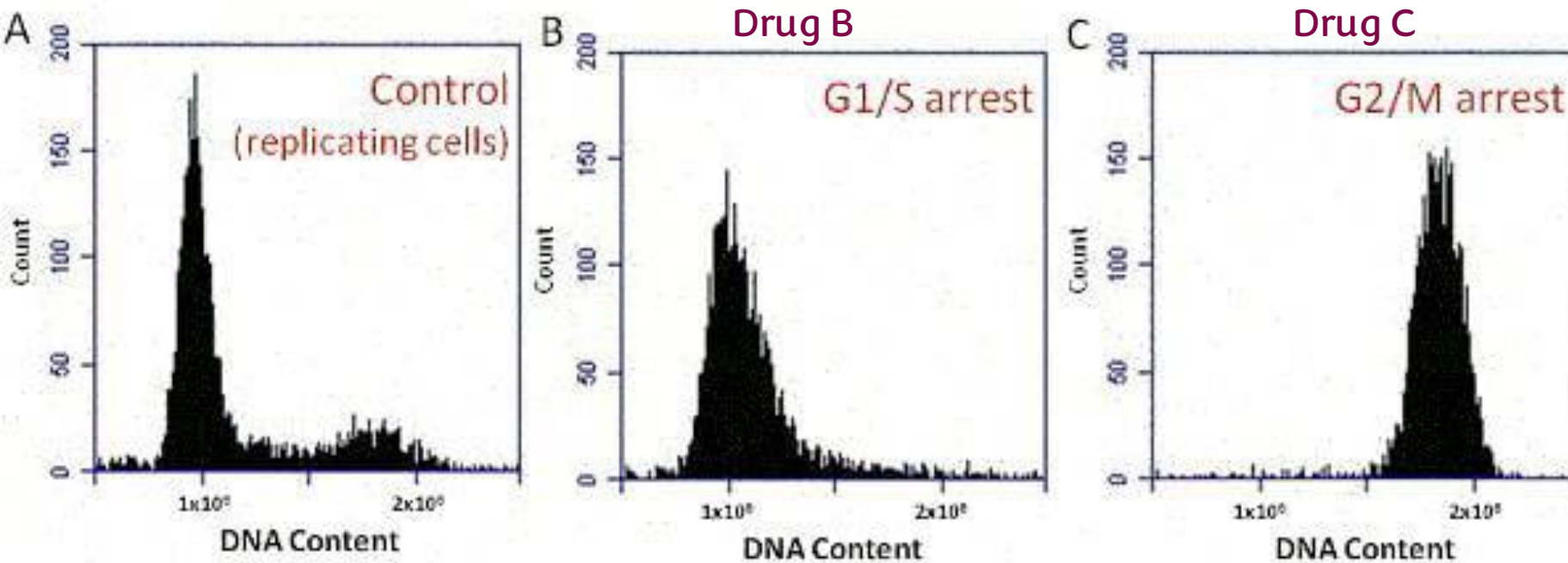


The G₁ peak increases dramatically while the S phase and G₂/M peaks decrease. This tells us that most of the cells have become “stuck” or arrested in the G₁ phase they are unable to proceed to the S phase

What Does the Drug Do?

From this data, we conclude that the drug inhibits DNA synthesis, preventing cells from entering the S phase.

As a result, the cells accumulate in G₁ phase and fail to reach the G₂/M phase.



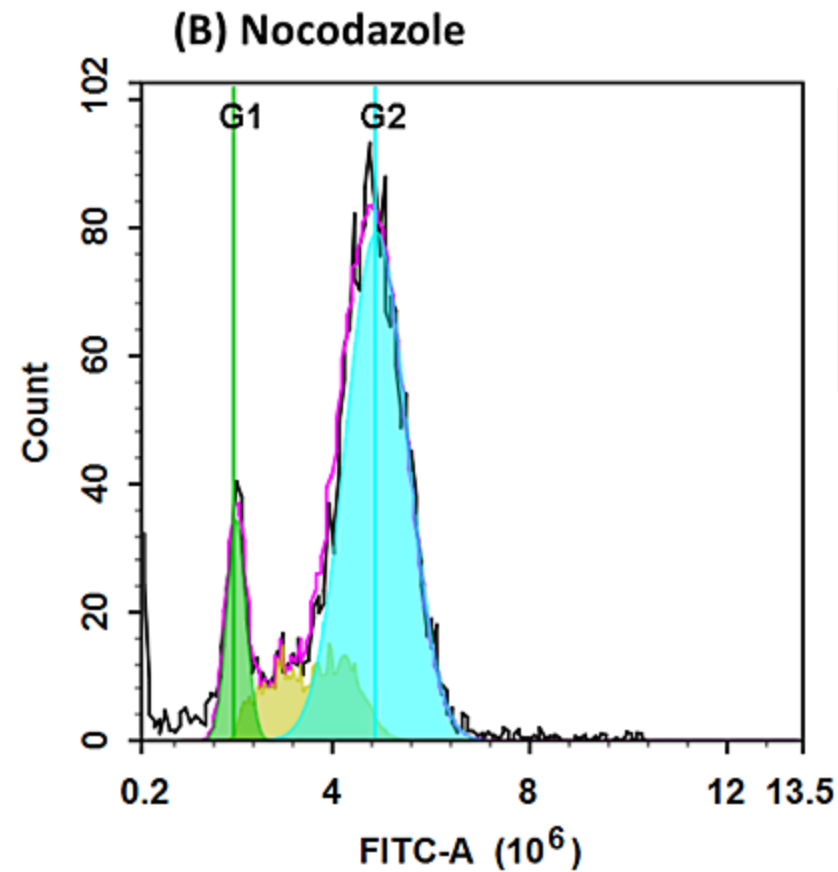
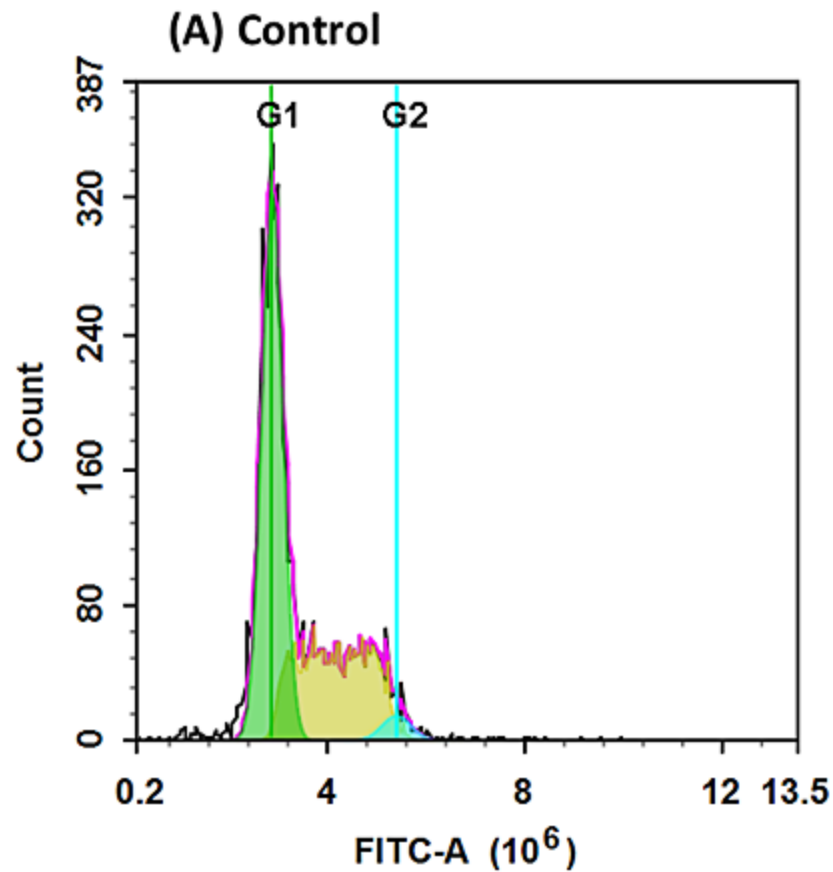
Drug B blocks cells at the G₁/S .

- This means cells cannot enter the S phase (where DNA replication occurs).
- As a result:
- Cells accumulate in G₁
- The S phase population decreases
- Consequently, the G₂/M population also decreases because cells cannot progress further.

Cells treated with Drug C can either:

1. Stop before entering mitosis (G₂ arrest), or
2. Enter mitosis but cannot complete cell division (M arrest).

When we look at the cells under the microscope, we can see that the chromosomes are aligned but the cells are unable to divide. This is because the drug interferes with the microtubules, which are essential for chromosome segregation during mitosis.



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There is a drug called Nocodazole. What it does is it inhibits microtubules

- Because microtubules are essential for mitosis, all the cells stop in G2 phase.
- They cannot enter M phase, so mitosis is blocked.
- Since the cells cannot proceed to divide, some of them start dying.
- This shows up as an increase in the sub-G₁ population because cells die by apoptosis

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Cell cycle checkpoints

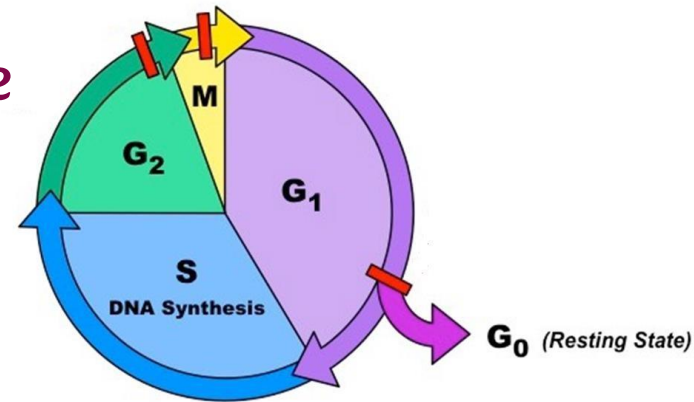
- Cells have several checkpoints to ensure that there are no mistakes before dividing.

checkpoints are stages in the cell cycle where the cell pauses to check its status.

- There are several key checkpoints points:

1. In G₁ phase
2. End of G₂ phase
3. Middle of mitosis

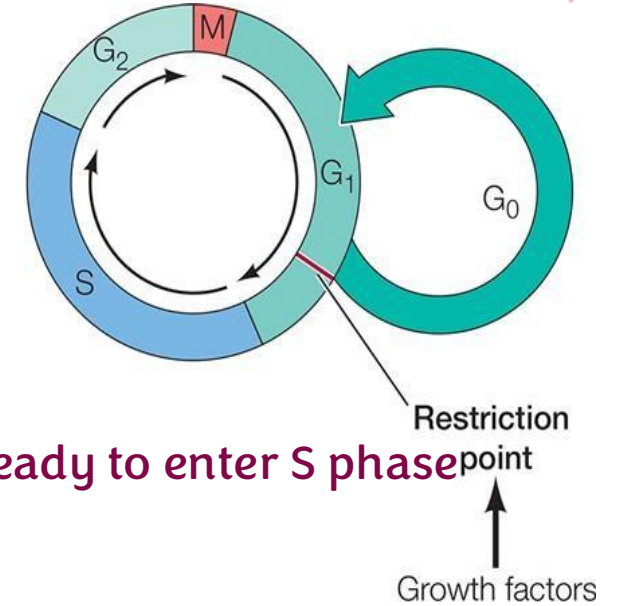
At each checkpoint, the cell assesses whether conditions are favorable to continue dividing.



Restriction point



1. Restriction point: a decision point in late G₁ is regulated by the extracellular growth factors.
- If not there, cells enter G₀ phase where they are metabolically active without growth.
 - Skin fibroblasts are arrested in G₀ **until they are stimulated to divide** by the platelets' platelet- derived growth factor, which is released during clotting to repair damaged tissues.



The restriction point is a critical checkpoint that determines whether the cell is ready to enter S phase and replicate its DNA.

- The cell evaluates the environment:
- Nutrients ,is there enough energy and building blocks to grow?
- Signals ,are there growth factors telling the cell to divide?
- If all conditions are favorable, the cell continues the cycle .

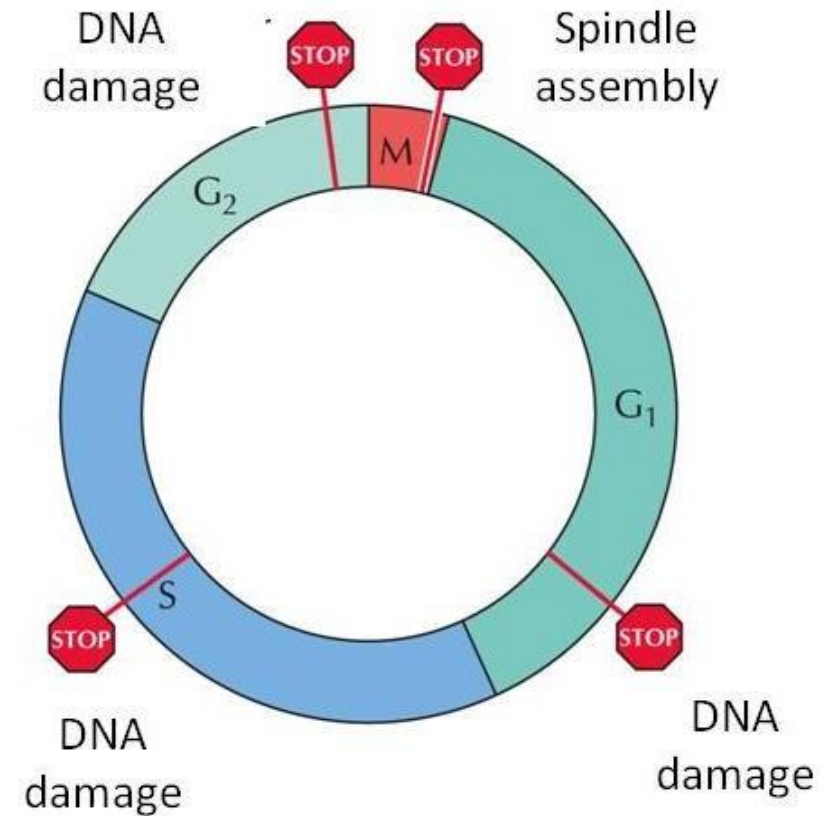
Growth factors are external signals that tell the cell: “It’s safe to grow and divide.”

- For example:
- A cell in G₀ (resting state) can re-enter G₁ if a growth factor signal is present.
- Once re-entering G₁, the cell prepares for DNA replication and division, provided there are sufficient nutrients and resources.

Checkpoints

DNA damage checkpoints:

- Cells are **arrested** at G₁, S, and G₂ and do not proceed to the next phase unless DNA is error-free.
- Spindle assembly checkpoints monitor the alignment of chromosomes on the mitotic spindle.



Checkpoints

G₁ Phase Checkpoint

- Before DNA synthesis, the cell checks the DNA.
- The DNA must be mutation-free, no breaks, no mistakes.
- If there is any problem, the cell pauses and repairs the DNA before continuing to S phase.

S Phase Checkpoint

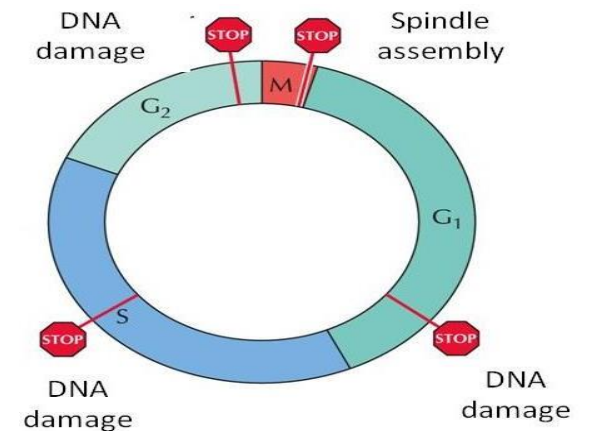
- During DNA replication, the cell keeps scanning the DNA.
- This ensures that the replication is going correctly and that no new mistakes are made.

G₂ Phase Checkpoint

- After replication, the cell checks everything before chromosome separation.
- It ensures that all DNA is correctly copied and ready for mitosis.
- If something is wrong, the cell stops before entering M phase to prevent errors.

M Phase Checkpoint (Spindle Checkpoint)

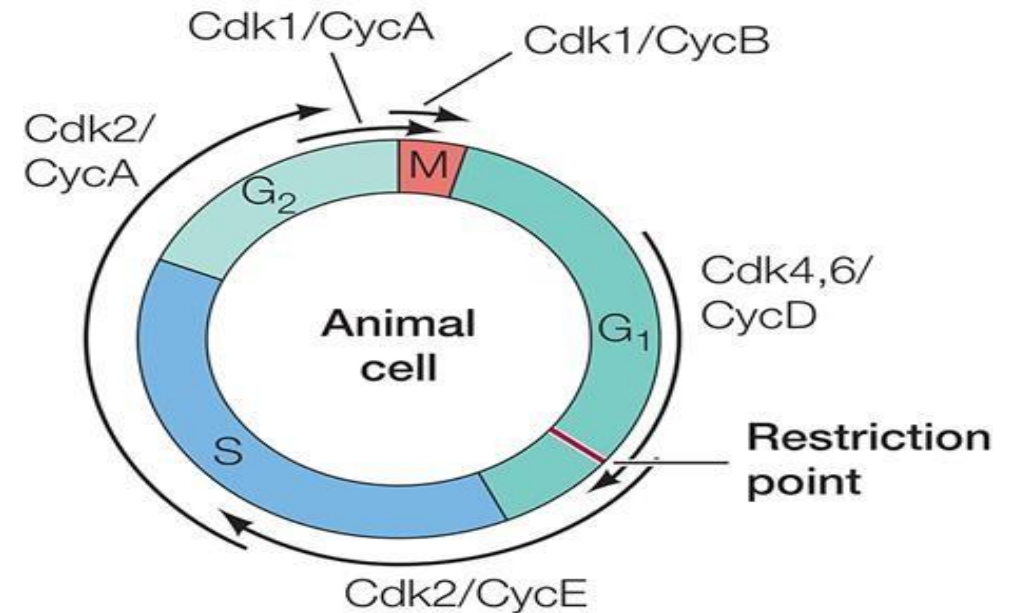
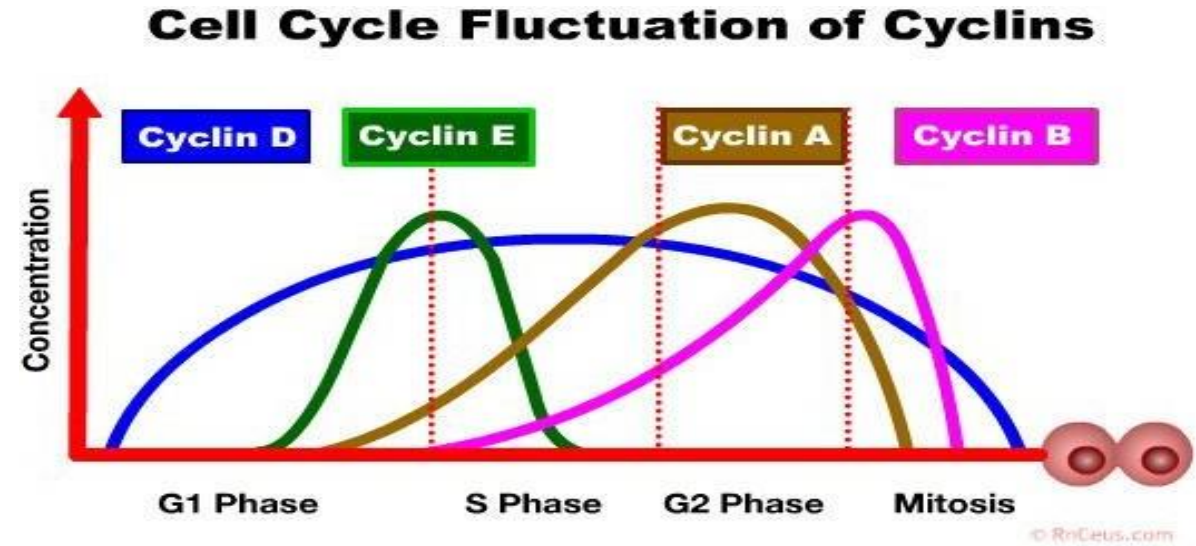
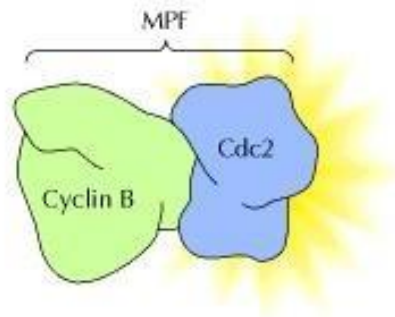
- During mitosis, the cell checks the microtubules and chromosome attachment.
- Why? Because if something goes wrong:
- Instead of each arm of a chromosome going to opposite sides, both arms might go to the same cell.
- Result: one daughter cell gets extra chromosomes, and the other loses chromosomes



THE CELL, Fourth Edition, Figure 16.8 © 2006 ASM Press and

Regulating dimers of the cell cycle

- Cyclins: proteins that go through cycles of synthesis (starting from the beginning of the interphase peaking at the beginning of mitosis) and degradation (by the end of mitosis).
- Cyclin-dependent kinases (Cdk's): get activated when dimerized with cyclins.



Regulating dimers of the cell cycle

Cyclins are named like this because their levels rise and fall in a cyclical pattern during the cell cycle.

Each cyclin appears and disappears at specific phases:

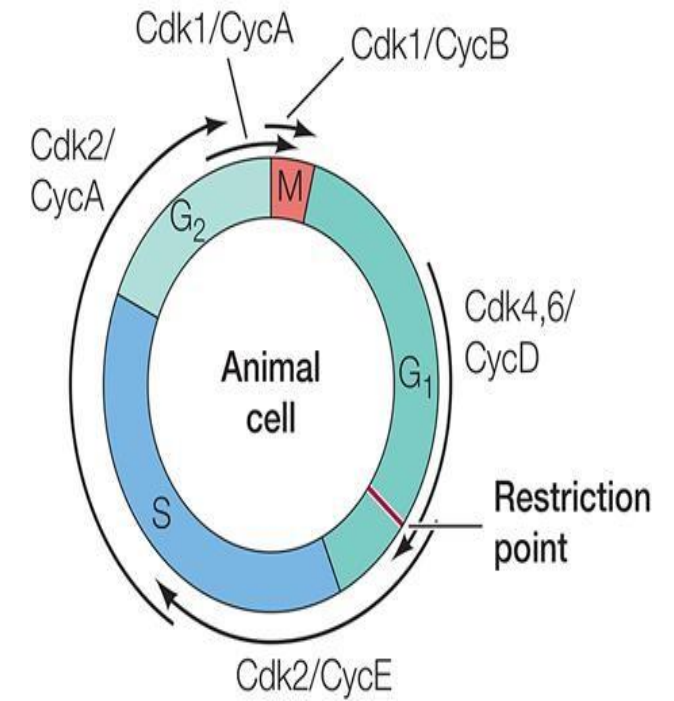
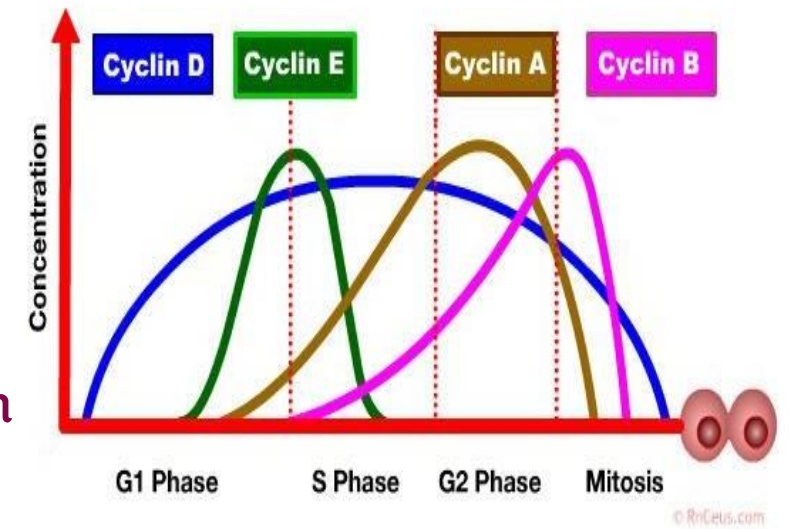
- Cyclin D appears in the G1 phase.
- Cyclin E appears at the end of the G1 phase.
- Cyclin A functions during the S phase and into the G2 phase.
- Cyclin B acts during the M phase

Cyclins form complexes with cyclin-dependent kinases (Cdks), which activate these kinases and drive the cell cycle forward.

- The **Cyclin D-Cdk4/6 complex** helps the cell pass the restriction point in G1.
- The **Cyclin E-Cdk2 complex** allows the cell to go through the S phase.
- The **Cyclin A-Cdk2 complex** helps the cell progress through the S phase into the G2.
- The **Cyclin B-Cdk1 complex** allows the cell to go through the M phase (mitosis).

At the end of the cycle, cyclins are degraded, and their absence ensures that the cycle does not proceed uncontrollably, this rise and fall in cyclin levels is what makes them “cyclic.”

Cell Cycle Fluctuation of Cyclins



Example of regulation of cell cycle progression

This shows how the concentration of cyclins increases, peaks, and then drops.

2 Cdk1 is phosphorylated

and dephosphorylated by CDC25 to become active

When Cdk1 becomes dephosphorylated, it is activated and can then phosphorylate proteins.

4 Cdk1 can phosphorylate a variety of target proteins that initiate entry into the M phase

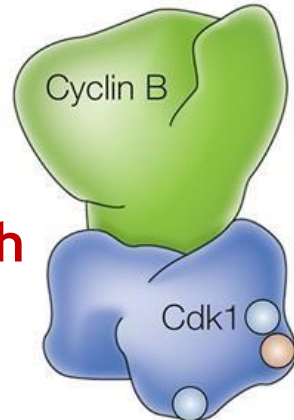
5 Proteolytic degradation of cyclin B terminates complex activity
So the CDK1 is inactive and waits the increasing of cyclin B concentration

1 Cdk1 complexes with cyclin B during G2.

Going into the M phase

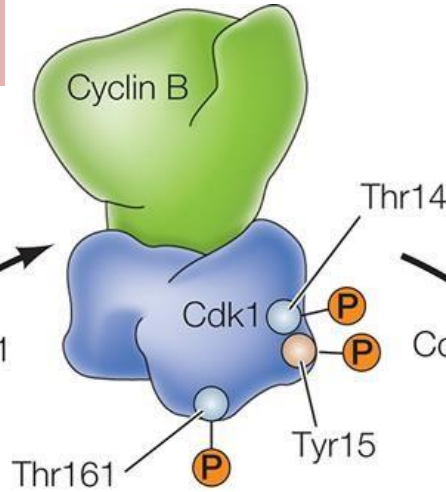


Synthesis of cyclin B



Phosphorylation

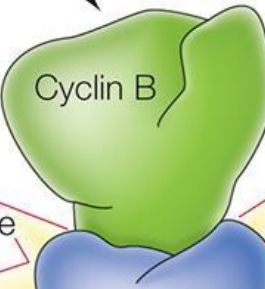
Wee1
CAK



Dephosphorylation

Cdc25

Active
MPF



Mitosis

APC/C

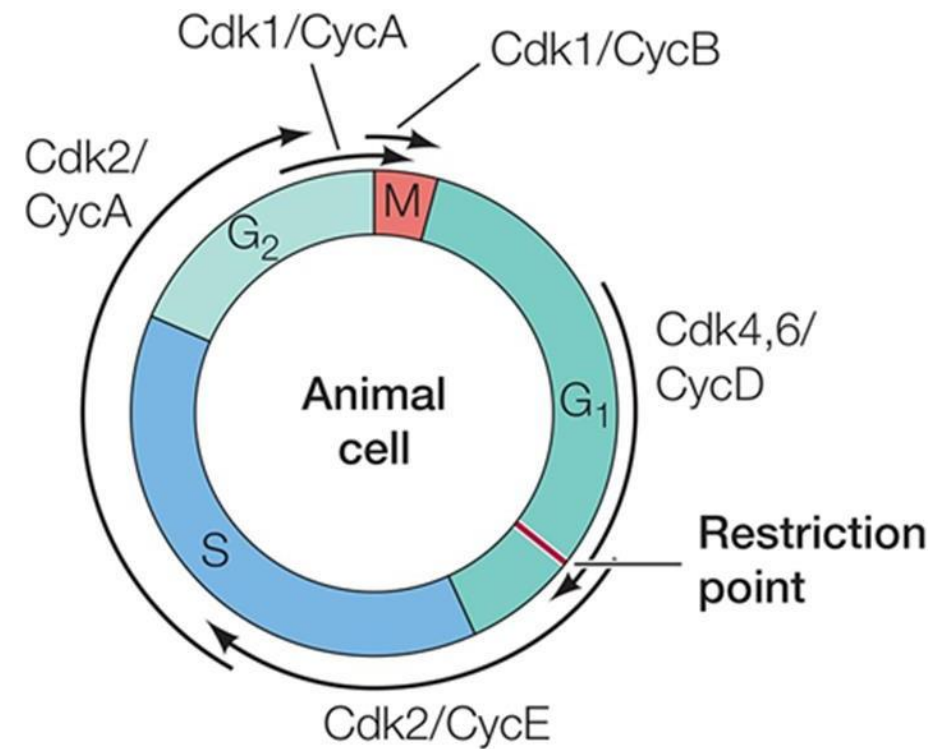
Cyclin B
degradation

Dephosphorylation

6 Cdk1 is dephosphorylated

The different regulatory complexes

- Complexes of Cdk4 and Cdk6 with cyclin D control progression through the G1 restriction point.
- Cdk2/cyclin E complexes are required for the G1 to S transition.
- Cdk2/cyclin A complexes are required for progression through S phase and G2.
- Cdk1/cyclin A and Cdk1/cyclin B complexes drive the G2 to M transition and progression through mitosis.



>There are clinical trials targeting Cyclin D activity. They use inhibitors of Cyclin D to prevent cancer cells from proliferating and progressing through the cell cycle and these treatments have shown promising results in breast cancer.

Cdk's are selectively inhibited

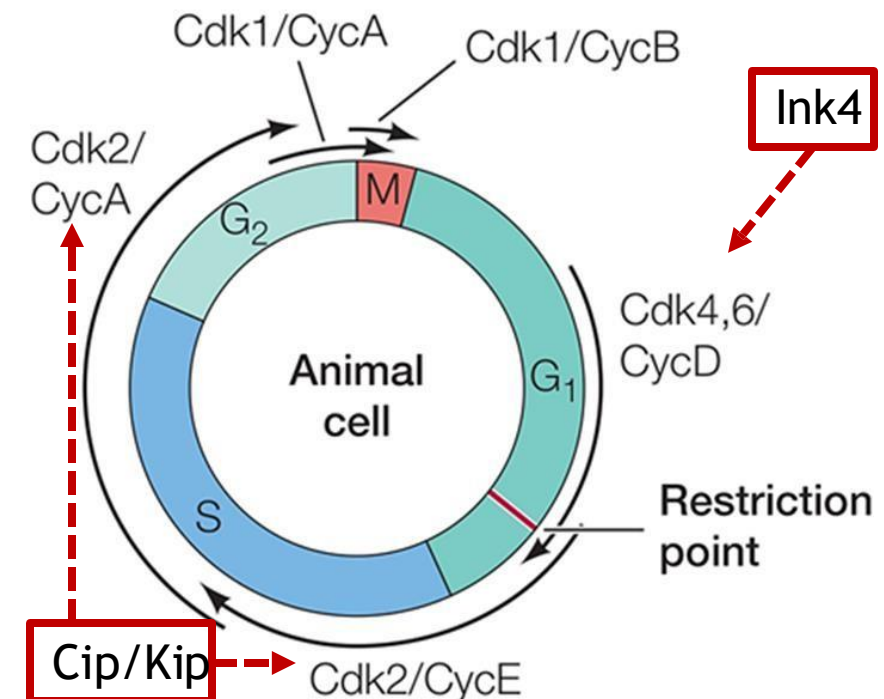
- The activities of Cdks are selectively regulated by Cdk inhibitors (CKIs). Cdk inhibitors (CKIs) also undergo activation and deactivation, meaning they are tightly regulated within the cell cycle.

Inhibitor	Cdk or Cdk/cyclin complex	Cell cycle phase inhibited
Ink4 family (p15, p16, p18, p19)	Cdk4 and Cdk6	G1
Cip/Kip family (<u>p21</u> , <u>p27</u> , p57)	Cdk2/cyclin E	G1
	Cdk2/cyclin A	S, G2

P27 and P21 : proteins are inhibitors of cyclin-dependent kinases, particularly Cdk2, thereby preventing the cell from going through the S phase and the G2 phase.

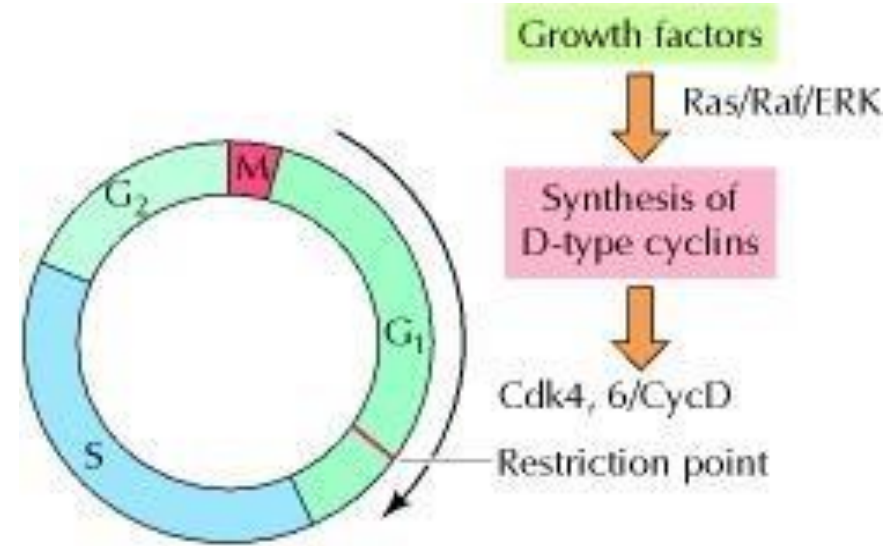
P: protein

Number: size of the protein=MW kDa



Cells signaling and cell cycle

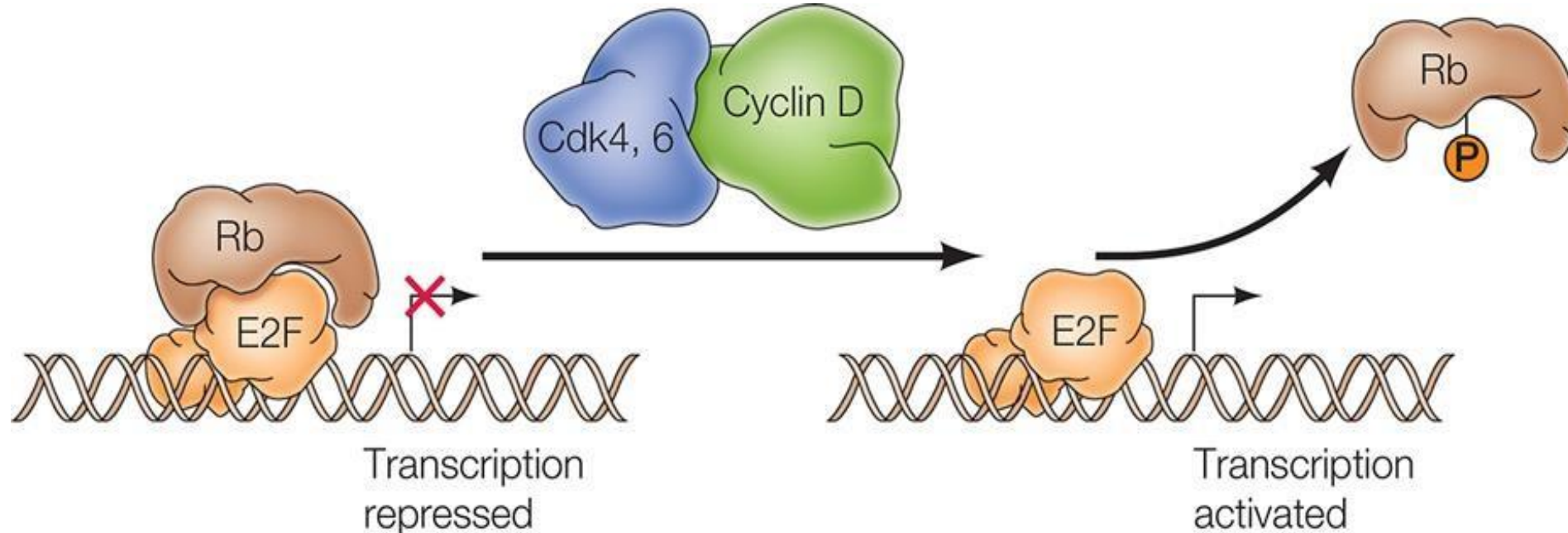
- Growth factors regulate cell cycle progression through the G₁ restriction point by inducing the synthesis of cyclin D via the Ras/Raf/ERK signaling pathway.
- Defects in cyclin D regulation lead to the loss of growth regulation characteristic of cancer cells.
- Also, mutations that inactivate the Ink4 Cdk inhibitors are commonly found in human cancer cells.
 - An example is Rb protein (a tumor suppressor gene).



Retinoblastoma



- When unphosphorylated, Rb protein binds to members of the E2F family of transcription factors repressing the transcription of many genes involved in cell cycle progression such as cyclin E.
- E2F is freed when Rb is phosphorylated by Cdk4,6/cyclin D stimulating cell cycle progression through restriction point.



لَا حَوْلَ وَلَا قُوَّةَ
إِلَّا بِاللَّهِ

Retinoblastoma

«اللَّهُمَّ صَلِّ وَسَلِّمْ عَلَى نَبِيِّنَا مُحَمَّدٍ»

Retinoblastoma (Rb) protein is a tumor suppressor gene, meaning it helps prevent uncontrolled cell growth and tumor formation.

A mutation in the Rb gene is one of the most important causes of cancer development.

When this gene is mutated, it can lead to retinoblastoma, a cancer of the eye that occurs mainly in children.

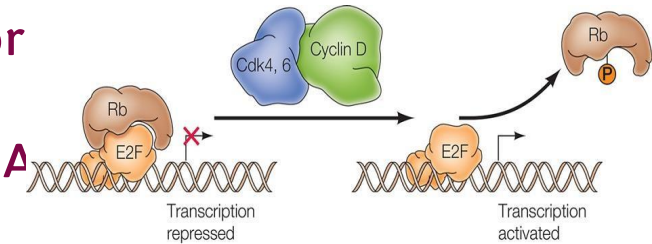


Here's how it normally works:

When a growth factor binds to its receptor, it triggers the production of Cyclin D. Cyclin D binds with Cdk4 and Cdk6, forming an active Cyclin D-Cdk4/6 complex. This complex phosphorylates the Rb protein, causing it to release E2F, a transcription factor.

Once E2F is free, it activates the expression of Cyclin E and other genes needed for DNA synthesis (S phase).

As a result, the cell moves from the G1 phase into the S phase and prepares for DNA replication.



However, if the Rb gene is mutated, the Rb protein can no longer bind to E2F, even without phosphorylation.

This means E2F remains constantly active, Cyclin E keeps being expressed, and the cell continues to divide uncontrollably, leading to cell proliferation and tumor formation.

Activation of Cdk2/cyclin E

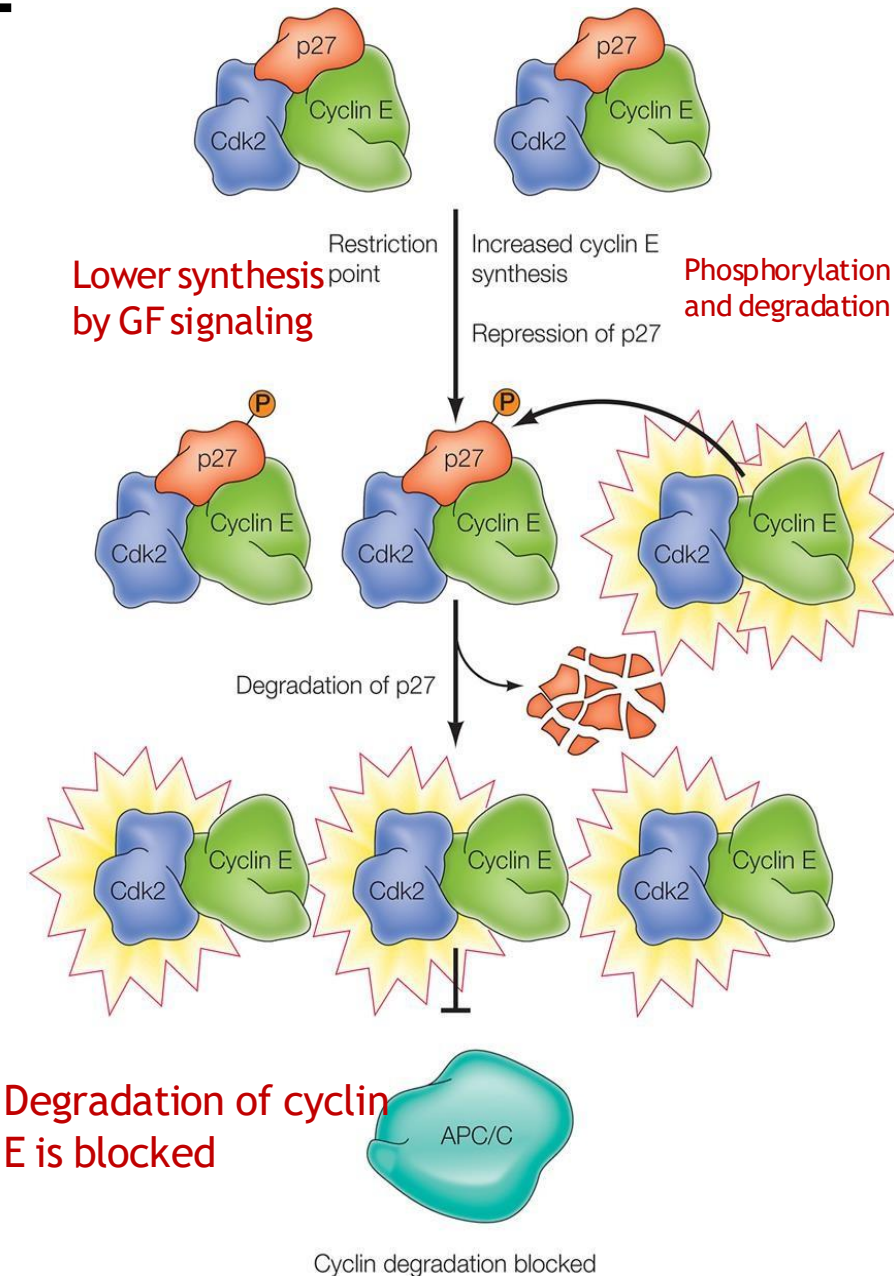
Even when Cyclin E is expressed, its activity is still regulated by an inhibitor, called p27 . The p27 protein can bind to the Cyclin E-Cdk2 complex and inhibit its activity, preventing the cell from progressing into the S phase.

When p27 is phosphorylated, it undergoes ubiquitination and degradation by the proteasome.

As a result, the inhibition on Cyclin E-Cdk2 is removed, allowing the complex to become active.

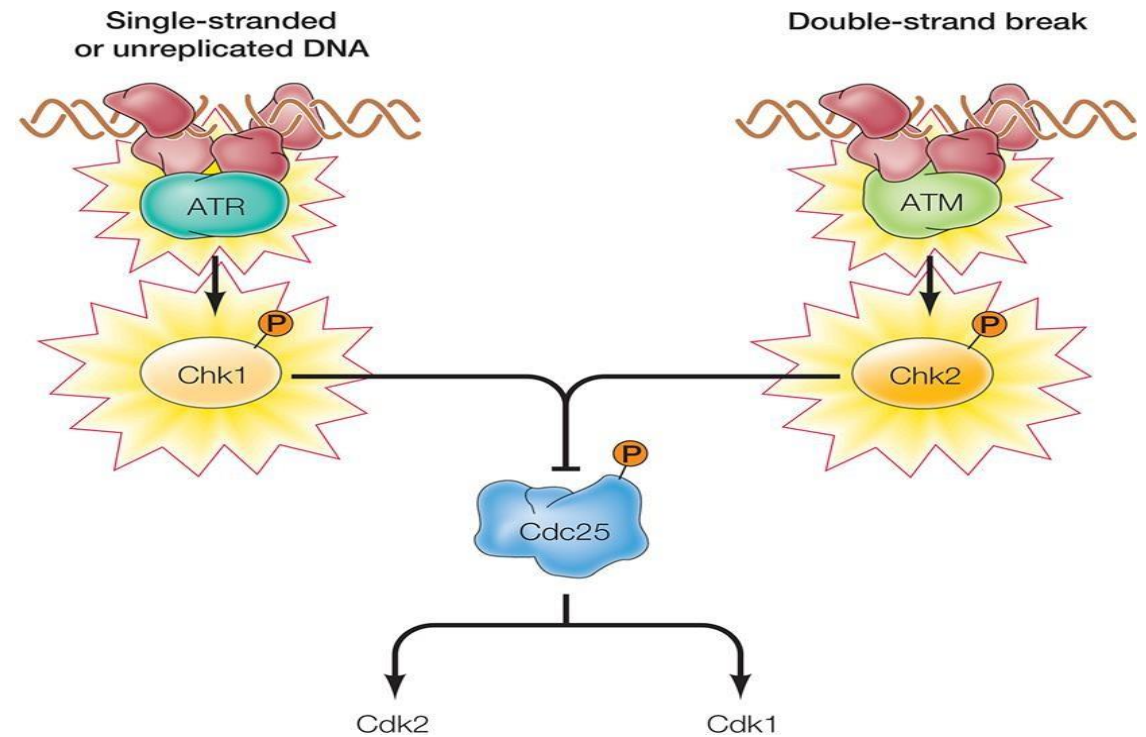
Once active, the Cyclin E-Cdk2 complex phosphorylates target proteins, enabling the cell to enter the S phase and begin DNA replication.

- The Cdk inhibitor p27 inhibits cdk2/cyclin E complexes.
- When cells pass through the restriction point, (1) the synthesis of cyclin E is induced via activation of E2F.
- Also, (2) growth factor signaling inhibits the synthesis of p27.
- (3) Activated Cdk2 phosphorylates and targets p27 for degradation, resulting in further activation of Cdk2/cyclin E complexes.
- (4) Cdk2/cyclin E also inhibits ubiquitination, and degradation of cyclin E.



ATR and ATM

- If DNA is damaged, the cells undergo cycle arrest mediated by two protein kinases, ATR and ATM.
- Both are activated in response to single- and double-stranded DNA damage and activate:
 - cell cycle arrest
 - DNA repair and,
 - in some cases, programmed cell death
- Their activation leads to inhibition of Cdk1 and 2.



ATR and ATM

اللَّهُ صَلِّ وَسَلِّمْ عَلَى نَبِيِّنَا مُحَمَّدٍ ﷺ

If DNA is damaged, the cell must not enter the S phase or M phase until the DNA is properly repaired.

When DNA damage occurs, two key proteins ATM and ATR become activated. These proteins act as sensors that detect DNA damage.

Once activated, they in turn activate the checkpoint kinases CHK1 and CHK2.

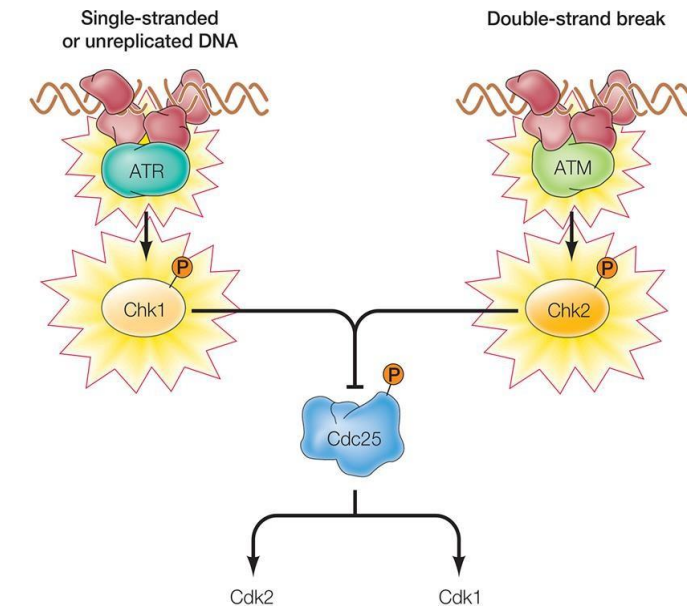
Normally, Cdc25 is a phosphatase that activates Cdk1 and Cdk2, allowing the cell cycle to proceed.

However, when CHK1 and CHK2 are activated by ATM and ATR, they phosphorylate and inhibit Cdc25, preventing it from activating Cdk1 and Cdk2. As a result, the cell cycle is paused giving the cell time to repair its DNA before continuing.

If ATM, ATR, CHK1, or CHK2 are mutated, this checkpoint control fails.

The cell no longer senses DNA damage properly, and it continues to divide even with genetic errors.

This unchecked division leads to the accumulation of mutations over time, which can ultimately cause cancer, for example, mutations in these checkpoint genes are seen in certain breast cancers.



NOTE: Cancer develops not from one mutation, but from the accumulation of many mutations over time

Ataxia-telangiectasia

Loss of muscle control-small ,dilated blood vessels

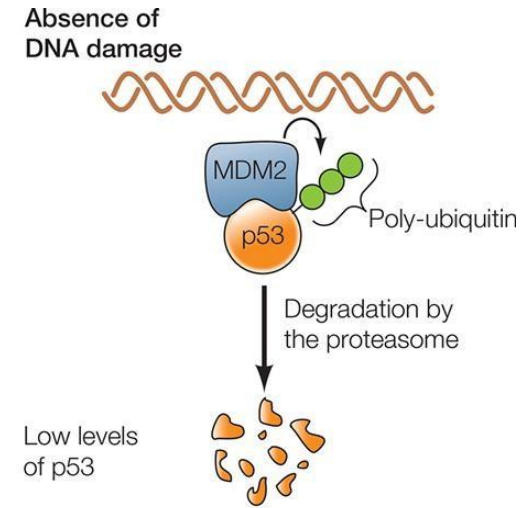
- Defective ATM is responsible for the ataxia-telangiectasia, (defective nervous and immune systems and a high frequency of cancer.
 - Ataxia: uncoordinated movements, such as walking.
 - Telangiectasias: enlarged blood vessels (capillaries) below the surface of the skin.

34) Ataxia Telangiectasia			
	❖ Clinical features ✓ Cerebellar ataxia ✓ Oculocutaneous telangiectasia ▪ Bulbar conjunctivae. ▪ Ears ▪ Neck ▪ Cubital fossae ✓ Recurrent infection ✓ Increase risk of malignancy	❖ Laboratory finding ? ✓ High serum alpha-fetoprotein (AFP) ✓ High carcinoembryonic antigen (CEA) ✓ Low IgA, IgG & IgE	
	❖ Mode of inheritance? ✓ Autosomal recessive ✓ ATM gene ✓ Due to chromosome instability	❖ What is the most consistent laboratory abnormality? ✓ High AFP	
		❖ Which part of immune system is impaired? ✓ Both cellular and humoral immunity	
▪ Associated with increase sensitivity to ionizing radiation			

Role of p53 in cell cycle arrest

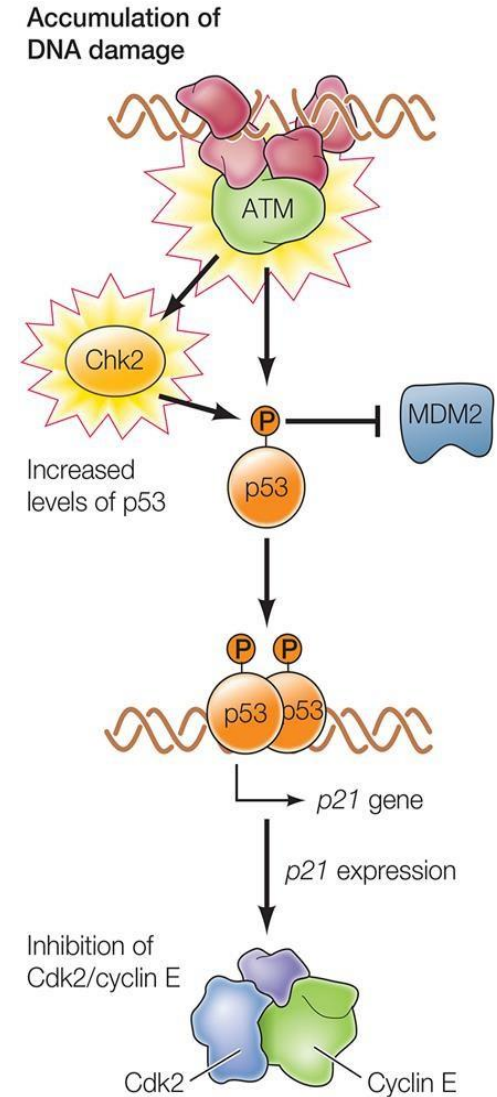
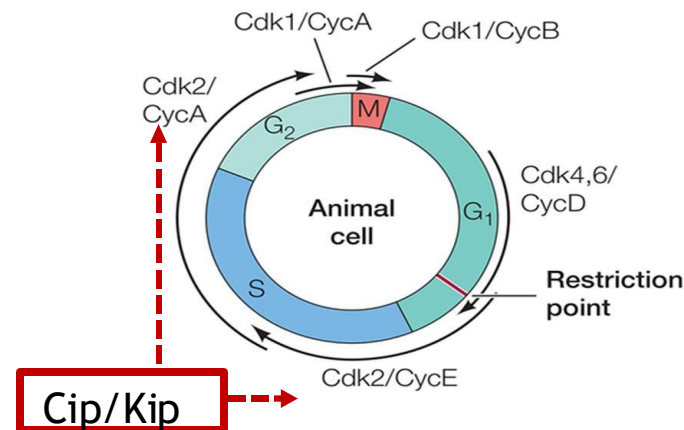
p53 was first discovered as a mutated gene in cancer cells. At first, scientists thought it was an oncogene (a gene that causes cancer). But after further research, they realized it is actually a tumor suppressor gene, which means it prevents cancer by controlling cell division and stopping the cell cycle when the cell is damaged or stressed. Mutations in p53 are found in many types of cancers, including breast, pancreatic, and colon cancers.

When DNA is damaged, ATM results in p53 phosphorylation blocking ubiquitination. In turn, p53 levels increase and p53 activates the transcription of the Cdk inhibitor p21, leading to inhibition of Cdk2/cyclin E complexes and cell cycle arrest.



In the absence of DNA damage, p53 is ubiquitinated proteasomal degradation, keeping p53 levels low.

Ubiquitination means labeling a protein for degradation by the proteasome.



Quick quiz

Q1:What is the primary role of p53 in the cell?

- A) Promote DNA replication**
- B) Suppress tumor formation**
- C) Degrade cyclins**
- D) Activate mitosis**

Answer: B) Suppress tumor formation

Q2:Which protein senses DNA damage and phosphorylates p53?

- A) Cdk2**
- B) Cyclin E**
- C) ATM**
- D) Ras**

Answer: C) ATM

Q3: What is the function of p21 in response to DNA damage?

- A) Activate Cyclin E**
- B) Inhibit Cdk2/Cyclin E complex**
- C) Stimulate S phase entry**
- D) Degrade p53**

Answer: B) Inhibit Cdk2/Cyclin E complex

Q4:Ubiquitination of p53 normally leads to:

- A) Activation of DNA repair**
- B) Transcription of p21**
- C) Proteasomal degradation**
- D) Cell cycle arrest**

Answer: C) Proteasomal degradation

Q5:If p53 is mutated and non-functional, what is likely to happen?

- A) DNA damage is repaired faster**
- B) The cell cycle proceeds unchecked**
- C) Cdk2/Cyclin E is inhibited**
- D) ATM is degraded**

Answer: B) The cell cycle proceeds unchecked

Q6:What phase does the cell typically arrest in when p21 inhibits Cdk2/Cyclin E?

- A) M phase**
- B) G2 phase**
- C) S phase**
- D) G1 phase**

Answer: D) G1 phase

رسالة من الفريق العلمي:

يا فتى ..

« تعاهد نقاء قلبك..وصفاء
سريرتك..وإخلاص نيّتك .. وصواب عملك..

وذكر نفسك !

« أنّ السّعي لله .. ولدين الله.. لا لشهرة ولا شهوة ولا منصب !
وأنّ السّعي بالخفاء ميدان الصالحين !

وأعلمها !

" أنّ لإبليسَ تلبيسًا.. يُطفئ نورَ البصيرة.. ويُبطئ النّفسَ عن إكمال المسيرة ..فيردي بها نحو
المهالك..ويُبعدُها عن أصوب المسالك ..

فإياها إياها أن تحيدَ بعد الثّبات!

وأن تستسلم قبل الممات !

• وأنتَ يا من حملتها بينَ جنبيك..

ستسأل عنها .. فكنَ خيرَ حاملٍ لها!

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Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1			
V1 → V2			