

LAB 09 – Cell Division

Introduction:

One of the characteristics of living things is the ability to replicate and pass on genetic information to the next generation. Cell division in individual bacteria and archaea usually occurs by binary fission. Mitochondria and chloroplasts also replicate by binary fission, which is evidence of the evolutionary relationship between these organelles and prokaryotes.

Cell division in eukaryotes is more complex. It requires the cell to manage a complicated process of duplicating the nucleus, other organelles, and multiple chromosomes. This process, called the cell cycle, is divided into three parts: interphase, mitosis, and cytokinesis (**Figure 1**). In the first growth phase (G₁), the cell grows and prepares to duplicate its DNA. In the synthesis phase (S), the chromosomes are replicated. In the second growth phase (G₂), the cell prepares to divide. In mitosis, the duplicated chromosomes are separated into two nuclei. In most cases, mitosis is followed by cytokinesis, when the cytoplasm divides and organelles separate into daughter cells. This type of cell division is asexual and is important for growth, renewal, and repair of multicellular organisms.

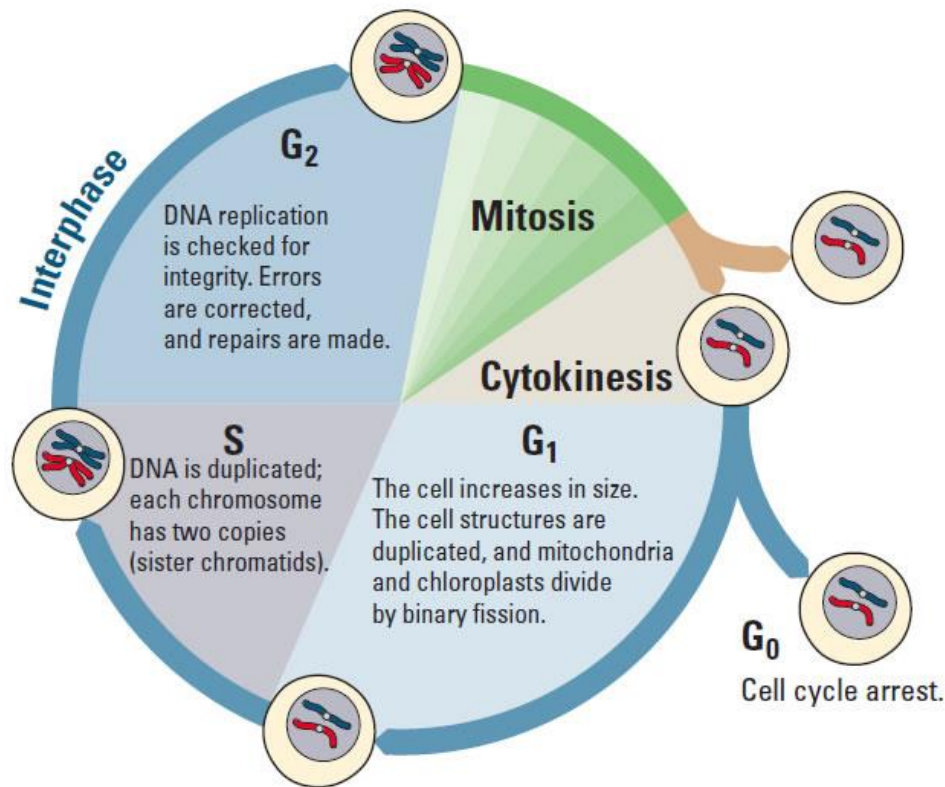


Figure 1: The Major Stages of the Cell Cycle

Cell division is tightly controlled by complexes made of several specific proteins. These complexes contain enzymes called cyclin-dependent kinases (CDKs), which turn on or off the various processes that take place in cell division. CDK partners with a family of proteins called cyclins. One such complex is mitosis-promoting factor (MPF), sometimes called maturation-promoting factor, which contains cyclin A or B and cyclin-dependent kinase (CDK) (**Figure 2a**). CDK is activated when it is bound to cyclin, interacting with various other proteins that, in this

case, allow the cell to proceed from G₂ into mitosis. The levels of cyclin change during the cell cycle (**Figure 2b**). In most cases, cytokinesis follows mitosis.

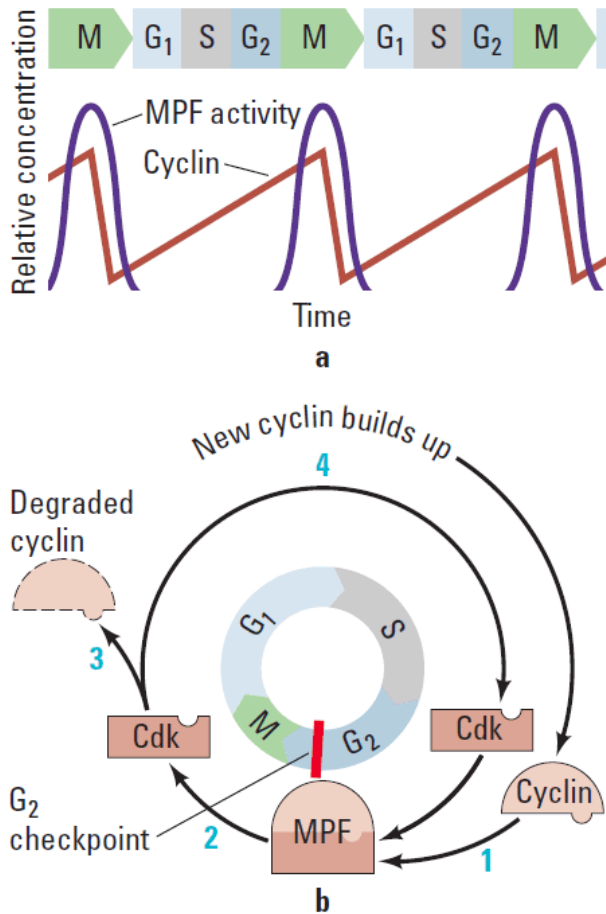


Figure 2: MPF Production During the Cell Cycle

As shown in **Figure 3**, different CDKs are produced during the phases. The cyclins determine which processes in cell division are turned on or off and in what order by CDK. As each cyclin is turned on or off, CDK causes the cell to progress through the stages in the cell cycle.

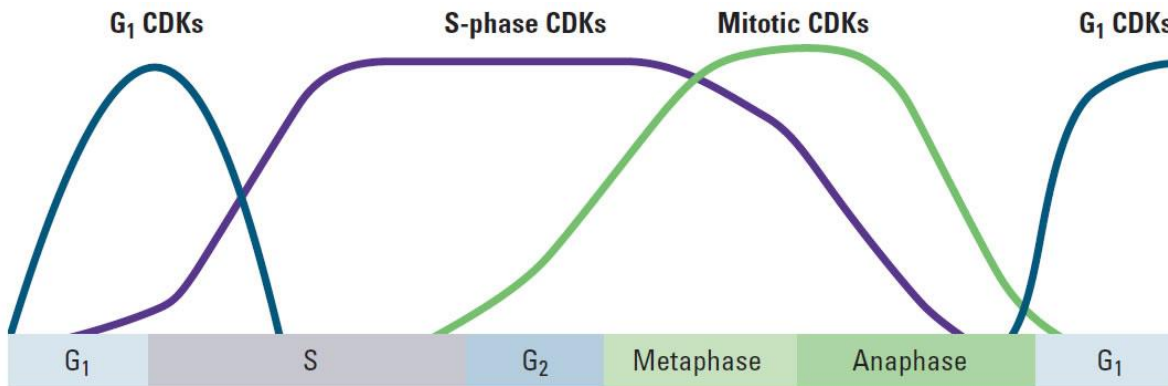


Figure 3: Levels of CDKs During the Cell Cycle

Cyclins and CDKs do not allow the cell to progress through its cycle automatically. There are three checkpoints a cell must pass through: the G₁ checkpoint, G₂ checkpoint, and the M-spindle checkpoint (**Figure 4**). At each of the checkpoints, the cell checks that it has completed all of the tasks needed and is ready to proceed to the next step in its cycle. Cells pass the G₁ checkpoint when they are stimulated by appropriate external growth factors; for example, platelet-derived growth factor (PDGF) stimulates cells near a wound to divide so that they can repair the injury. The G₂ checkpoint checks for damage after DNA is replicated, and if there is damage, it prevents the cell from going into mitosis. The M-spindle (metaphase) checkpoint assures that the mitotic spindles or microtubules are properly attached to the kinetochores (anchor sites on the chromosomes). If the spindles are not anchored properly, the cell does not continue on through mitosis. The cell cycle is regulated very precisely. Mutations in cell cycle genes that interfere with proper cell cycle control are found very often in cancer cells.

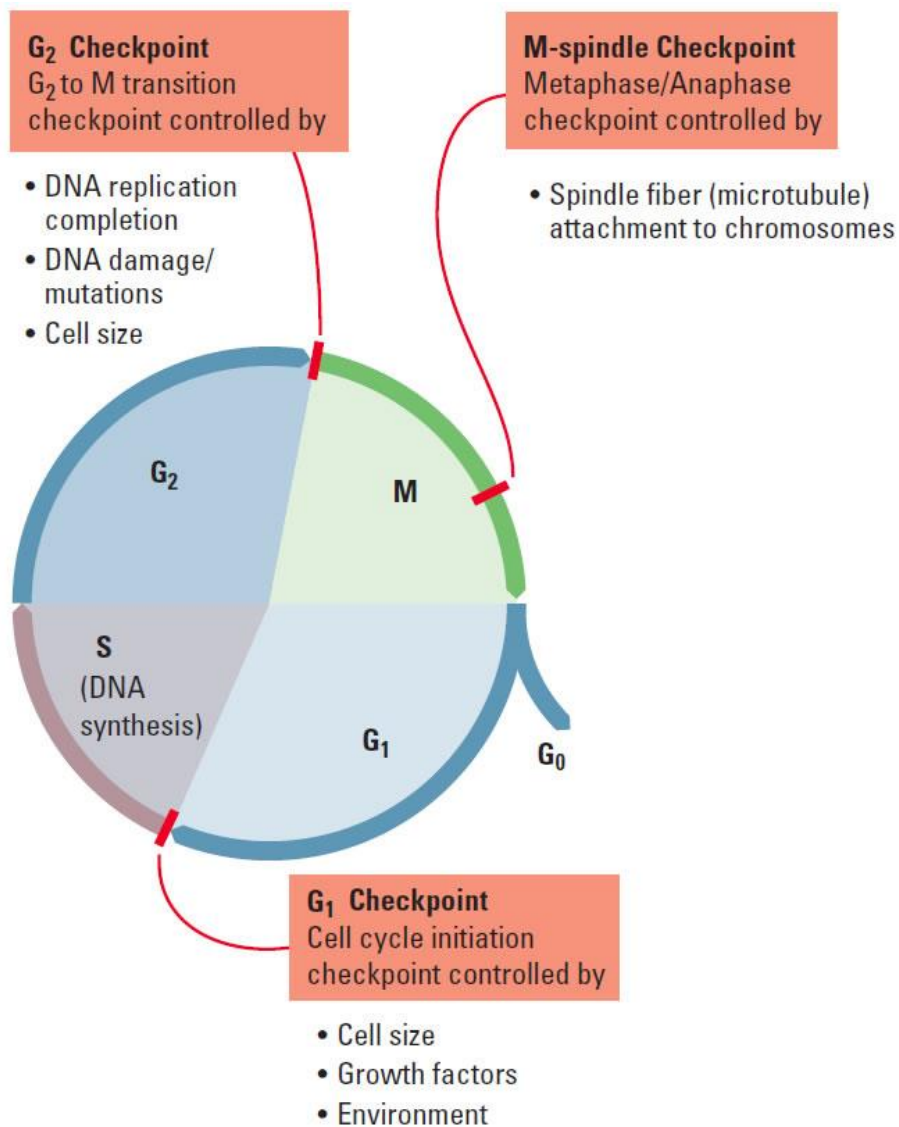


Figure 4: Diagram of the Cell Cycle Indicating Key Checkpoints

Figure 5 illustrates how the chromosomes move during mitosis. It is important to see how the duplicated chromosomes align, separate, and move into new cells. **Figure 6** is an onion root root tip showing various stages of the cell cycle.

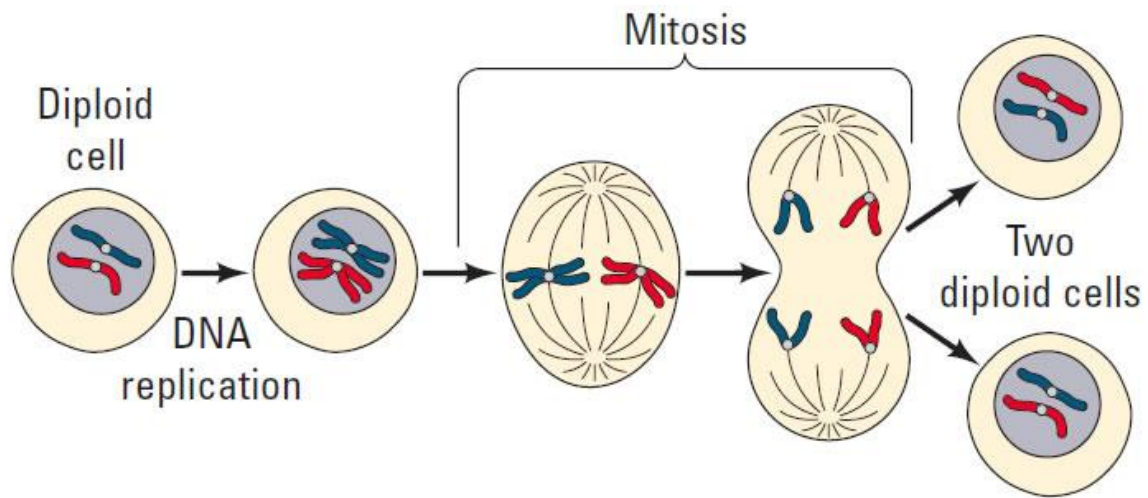


Figure 5: Mitotic Cell Division Emphasizing Chromosome Movement

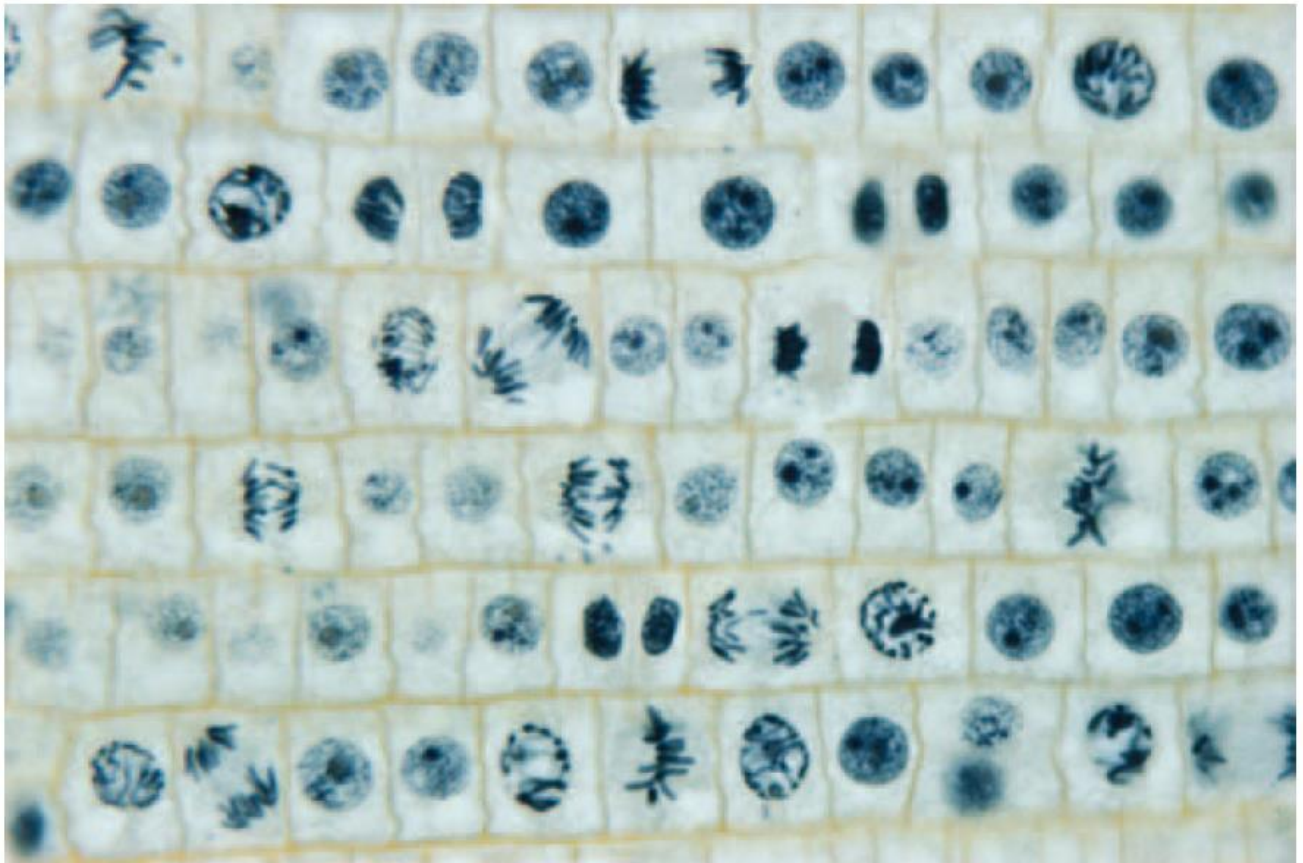


Figure 6: Onion Root Tip

Scientists reported that a fungal pathogen may affect the growth of soybeans (*Glycine max*). The soybean growth was decreased during three years of high rainfall. The soybean roots were poorly developed. *R. anaerobis* is another fungus that also is a plant pathogen that grows in the soil. *R. anaerobis* releases the protein lectin. A lectin-like protein was found in soil surrounding the affected soybean roots. Lectins accelerate mitosis in some root apical meristems (growth regions of plants – similar to stem cells in animals); however, in many instances, rapid cell division weakens plant tissues. We are using onions instead of soybeans since onion root tips are more easily grown and studied.

Questions:

1. State your null and experimental hypotheses for this investigation. (Remember why it is called a *NULL* hypothesis...)

H₀: _____

H_a: _____

2. Complete the **Table 1** below. Practice identifying cells in various stages of the cell cycle by counting the cells in **Figure 6**.

Table 1: Count of cells in the various stages of the cell cycle.

	Interphase	Prophase	Metaphase	Anaphase	Telophase	Total
Number of Cells						
Percent of Cells in Phase						

Procedure:**Preparing Chromosome Squashes**

NOTE: Onion root tips have been grown over the past few weeks with and without lectin added to the tissues. After a 3 day growing period, they were harvested and preserved in 70% ethanol and then stored at 4°C. You will need to do the following for BOTH treatments.

1. Place the onion root tip in a few drops of 12 M HCl for 4 minutes.
2. Transfer the tip to Carnoy's fixative for 4 minutes.
3. Remove the slide from the Coplin jar containing 70% ethanol, dry with a scientific cleaning wipe, and label it.
4. Place the onion tip on the slide, and cut off the distal 2 mm portion of the tip.
5. Cover the root tip piece with carbol-fuchsin stain for 2 minutes.
6. Blot off excess stain and cover tip with 1–2 drops of H₂O.
7. Place the cover slip over tip and cover the cover slip with a scientific cleaning wipe. Firmly press down on the cover slip with your thumb or with the eraser end of a pencil. Do not twist the slide.

Counting Cells and Analyzing Data

8. Observe the cells under low power (100X).
9. Focus and center a good patch of cells.
10. Observe the cells at high magnification (400X).
11. Look for well-stained, distinct cells.
12. Within the field of view, count the cells in interphase and those in one of the mitotic stages. Enter these data in **Tables 2 and 3**. It will be important to have the count of cells in interphase and total of cells in any phase of mitosis. Repeat the counts in **at least two other root tip preparations**.
13. Find out from your teacher which group was the control and which was the lectin-treated group. Compare the number of cells from each group in interphase and in mitosis.
14. Use a chi-square distribution test to statistically analyze the data. (Use **Table 4**)
 - a. Enter the number of lectin-treated cells in interphase and mitosis as observed (o).
 - b. Calculate the percentage of cells in interphase and mitosis in the control group from **Table 2 or 3**.
 - c. Multiply the percentages by the total number of cells in the respective lectin-treated stage; this will give you the expected numbers (e) in the lectin-treated group IF THERE IS NO EFFECT ON THE CELLS FROM THE LECTIN! (Remember – we are testing our **null hypothesis**)
 - d. Finish up the chi-square analysis using your calculations and **Table 5** and then answer the question on page 9.

Name: _____

Table 2: Onion Root Tip Cell Phase Data; Treatment Group _____

Slide Preparation	Number of Cells		
	Interphase	Mitotic	Total
1			
2			
3			
4*			
5*			
Total			

Table 3: Onion Root Tip Cell Phase Data; Treatment Group _____

Slide Preparation	Number of Cells		
	Interphase	Mitotic	Total
1			
2			
3			
4*			
5*			
Total			

* minimum of 3 slide preparations

Table 4: Calculation of the Chi-Square Value

	o	e	(o - e)	(o - e)²	$\frac{(o - e)^2}{e}$
Interphase Cells					
Mitotic Cells					
X² total =					

Table 5: The Chi-square Distribution Table.

degrees of freedom	probability value (p-value)									
	ACCEPT NULL HYPOTHESIS								REJECT	
	0.99	0.95	0.80	0.70	0.50	0.30	0.20	0.10	0.05	0.01
1	0.001	0.004	0.06	0.15	0.46	1.07	1.64	2.71	3.84	6.64
2	0.02	0.10	0.45	0.71	1.30	2.41	3.22	4.60	5.99	9.21
3	0.12	0.35	1.00	1.42	2.37	3.67	4.64	6.25	7.82	11.34
4	0.30	0.71	1.65	2.20	3.36	4.88	5.99	7.78	9.49	13.28

Name: _____

Did the fungal pathogen lectin increase the number of cells in mitosis? Use your statistical analysis to completely and correctly answer this question.
