

# BME 1532-CELL BIOLOGY

## Cell Cycle

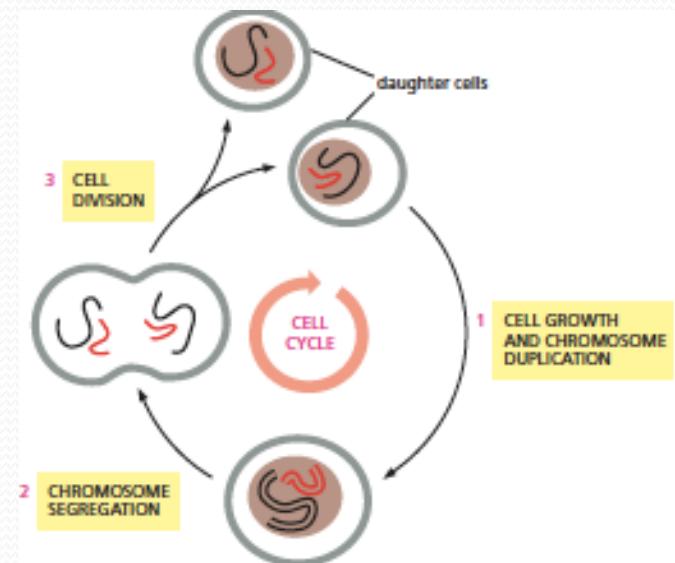
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Spring 2020

# Last week on BME 1532

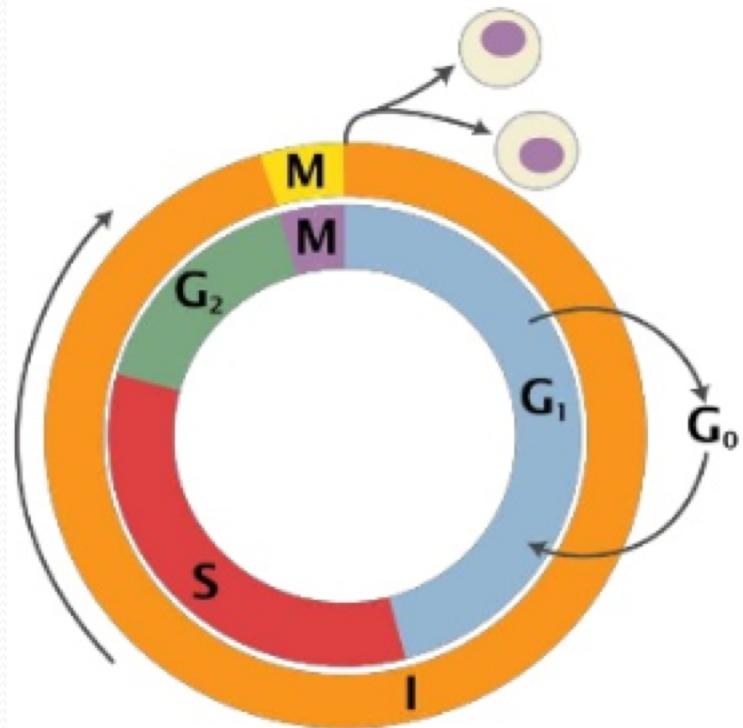
- Cell Signaling
  - Endocrine
  - Paracrine
  - Neuronal signaling through synapses via neurotransmitters
  - Contact dependent
- Sensing of the signals by the cells through receptors
  - Cell surface receptors
  - Intracellular receptors: Steroid hormones or gases
- Intracellular signal transduction via signaling molecules
- Activation of signaling molecules via phosphorylation activity of kinase enzymes
- Molecular cross-talk between different pathways and common molecules

- A cell reproduces by carrying out an orderly sequence of events in which it duplicates its contents and then divides in two.
- This cycle of duplication and division, known as the ***cell cycle***, is the essential mechanism by which all living things reproduce.
- The most basic function of the cell cycle is to duplicate accurately the vast amount of DNA in the chromosomes and then to segregate the DNA into genetically identical daughter cells such that each cell receives a complete copy of the entire genome.
- In most cases, a cell also duplicates its other macromolecules and organelles and doubles in size before it divides; otherwise, each time a cell split it would get smaller and smaller. Thus, to maintain their size, dividing cells coordinate their growth with their division.

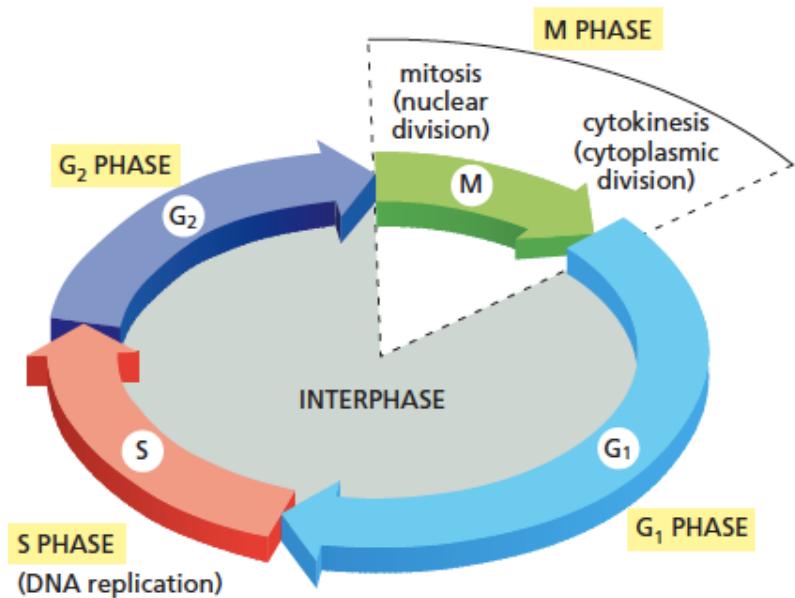


# Cell Cycle Phases

- The eukaryotic cell cycle includes 4 phases: G<sub>1</sub>, S, G<sub>2</sub>, M.
- The nucleus divides in a process called **mitosis**, and the cell later splits in two in a process called **cytokinesis**.
- These two processes together constitute the M phase of the cycle.
- In a typical mammalian cell, the whole of M phase takes about an hour, which is only a small fraction of the total cell-cycle time.
- The period between one M phase and the next is called **interphase** which consists of G<sub>1</sub>, S and G<sub>2</sub> phases.



- Interphase, is a very busy time for a proliferating cell, and it encompasses the remaining three phases of the cell cycle.
- During S phase (S = synthesis), the cell replicates its DNA.
- S phase is flanked by two “gap” phases—called G<sub>1</sub> and G<sub>2</sub>—during which the cell continues to grow.
- During these gap phases, the cell monitors both its internal state and external environment.
- This monitoring ensures that conditions are suitable for reproduction and that preparations are complete before the cell commits to the major upheavals of S phase (which follows G<sub>1</sub>) and mitosis (following G<sub>2</sub>).
- At particular points in G<sub>1</sub> and G<sub>2</sub>, the cell decides whether to proceed to the next phase or pause to allow more time to prepare.



- During all of interphase, a cell generally continues to transcribe genes, synthesize proteins, and grow in mass.
- Together with S phase, G<sub>1</sub> and G<sub>2</sub> provide the time needed for the cell to grow and duplicate its cytoplasmic organelles.

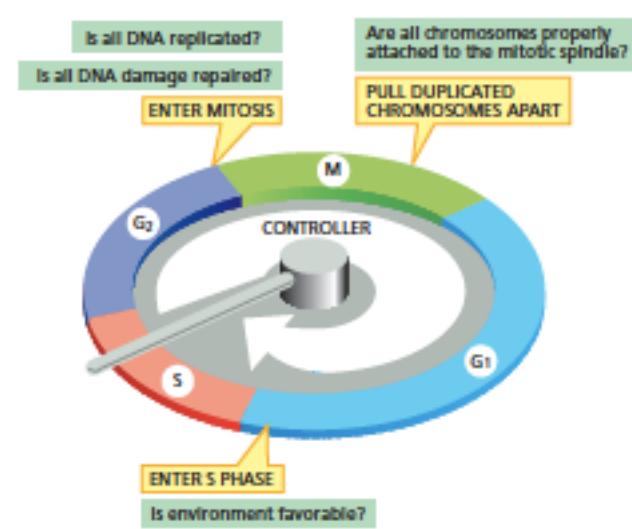
# Cell Cycle Control

- To ensure that they replicate all their DNA and organelles, and divide in an orderly manner, eukaryotic cells possess a complex network of regulatory proteins known as the cell-cycle control system.
- This system guarantees that the events of the cell cycle—DNA replication, mitosis, and so on—occur in a set sequence and that each process has been completed before the next one begins.
- To accomplish this, the control system is itself regulated at certain critical points of the cycle by feedback from the process currently being performed.

- All of the nuclear DNA, for example, must be replicated before the nucleus begins to divide, which means that a complete S phase must precede M phase.
- If DNA synthesis is slowed down or stalled, mitosis and cell division must also be delayed. Similarly, if DNA is damaged, the cycle must arrest in G<sub>1</sub>, S, or G<sub>2</sub> so that the cell can repair the damage, either before DNA replication is started or completed or before the cell enters M phase.
- The cell-cycle control system achieves all of this by employing molecular brakes, sometimes called checkpoints, to pause the cycle at certain transition points.
- In this way, the control system does not trigger the next step in the cycle unless the cell is properly prepared.

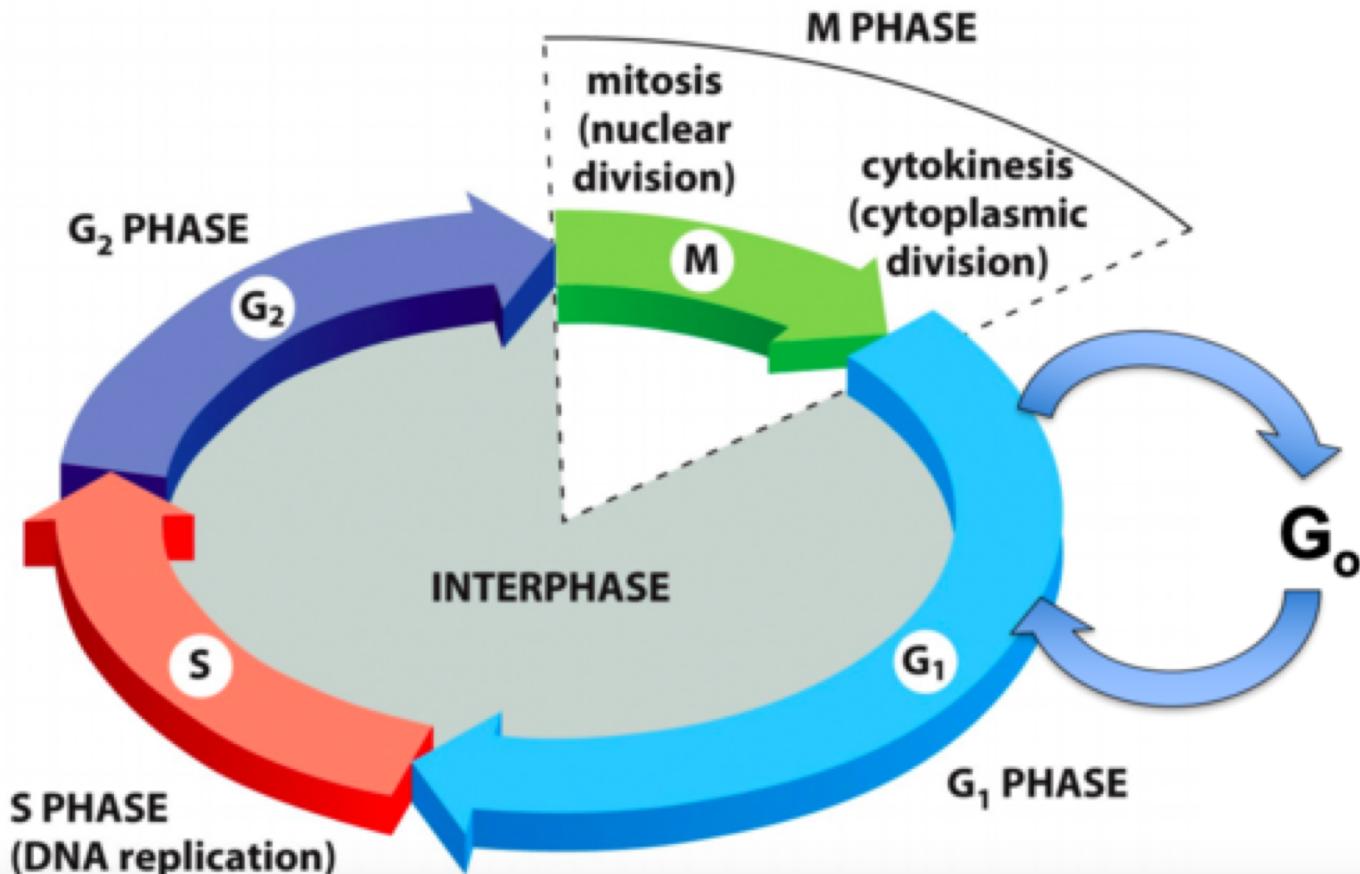
# Cell Cycle Check Points

- The cell-cycle control system regulates progression through the cell cycle at three main transition points.
- 1. G<sub>1</sub>-S checkpoint: At the transition from G<sub>1</sub> to S phase, the control system confirms that the environment is favorable for proliferation before committing to DNA replication.
- 2. G<sub>2</sub>-M check point: At the transition from G<sub>2</sub> to M phase, the control system confirms that the DNA is undamaged and fully replicated, ensuring that the cell does not enter mitosis unless its DNA is intact.
- 3. M check point: Finally, during mitosis, the cell-cycle control machinery ensures that the duplicated chromosomes are properly attached to a cytoskeletal machine, called the mitotic spindle, before the spindle pulls the chromosomes apart and segregates them into the two daughter cells.



- In animals, the transition from G<sub>1</sub> to S phase is especially important as a point in the cell cycle where the control system is regulated.
- Signals from other cells stimulate cell proliferation when more cells are needed—and block it when they are not.
- Cell proliferation in animals requires both sufficient nutrients and specific signal molecules in the extracellular environment; if these extracellular conditions are unfavorable, cells can delay progress through G<sub>1</sub> and may even enter a specialized resting state known as G<sub>0</sub> (G zero).
- The cell-cycle control system therefore plays a central part in the regulation of cell numbers in the tissues of the body; if the control system malfunctions such that cell division is excessive, cancer can result.

# Events of the cell cycle

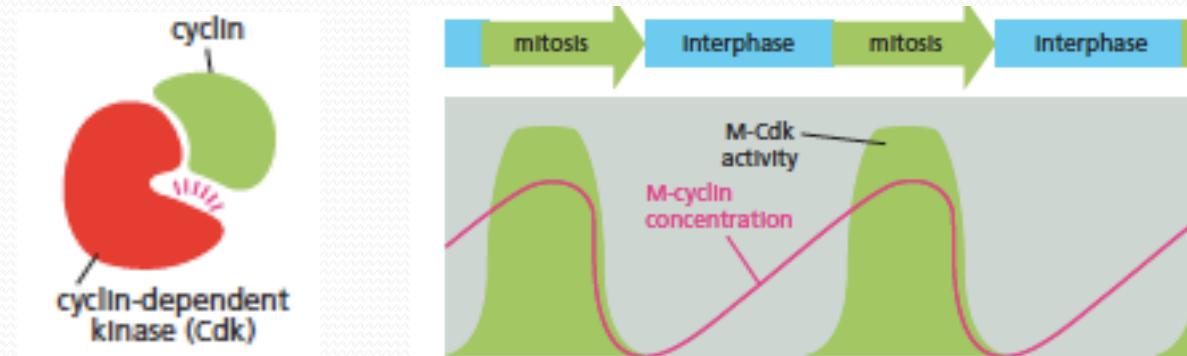


- Two types of machinery are involved in cell division: one manufactures the new components of the growing cell, and another hauls the components into their correct places and partitions them appropriately when the cell divides in two.
- The cell-cycle control system switches all this machinery on and off at the correct times, thereby coordinating the various steps of the cycle.
- The core of the cell-cycle control system is a series of molecular switches that operate in a defined sequence and orchestrate the main events of the cycle, including DNA replication and the segregation of duplicated chromosomes.

- The cell-cycle control system governs the cell-cycle machinery by cyclically activating and then inactivating the key proteins and protein complexes that initiate or regulate DNA replication, mitosis, and cytokinesis.
- This regulation is carried out largely through the phosphorylation and dephosphorylation of proteins involved in these essential processes.
- The phosphorylation reactions that control the cell cycle are carried out by a specific set of protein kinases, while dephosphorylation is performed by a set of protein phosphatases.

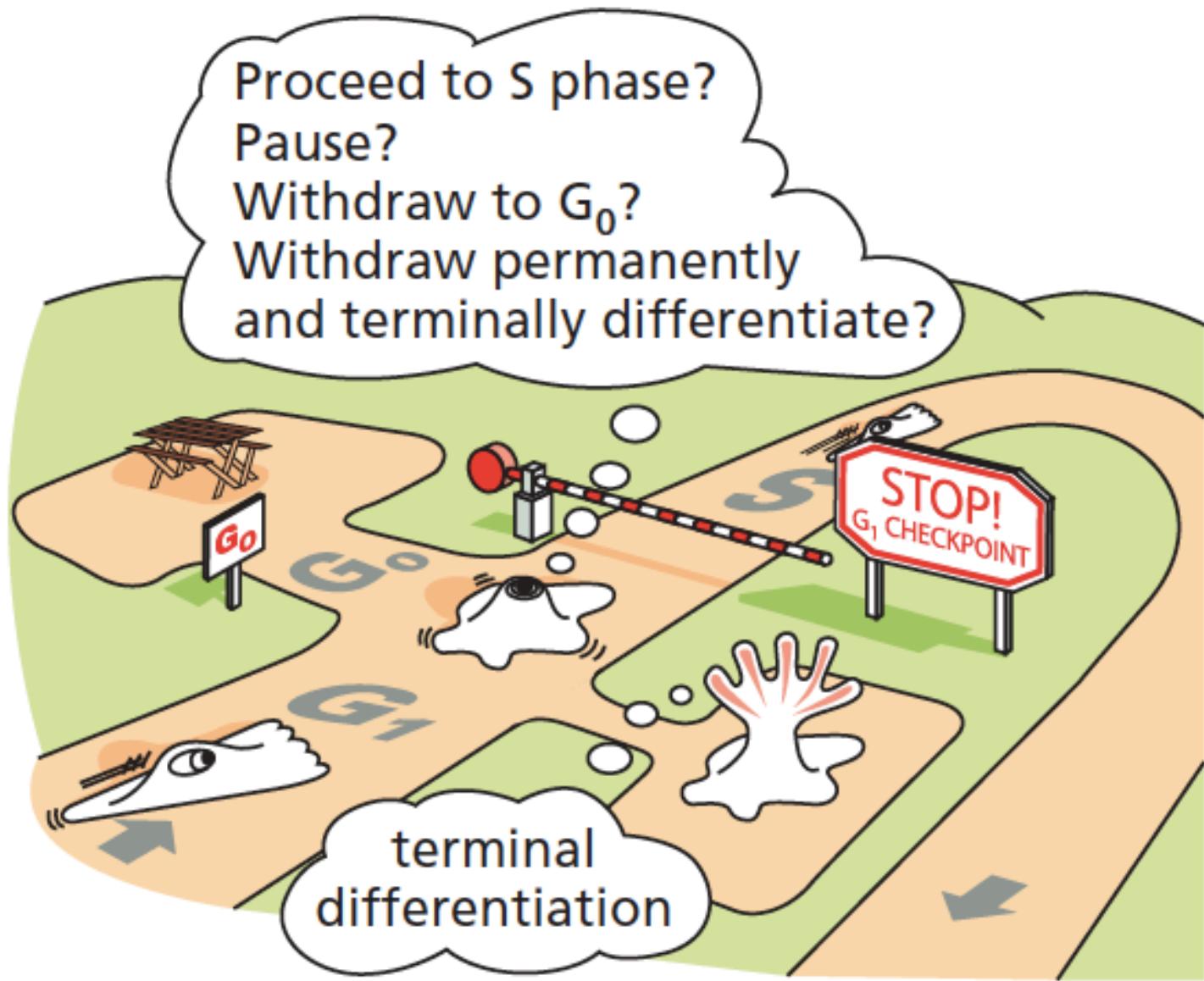
- The protein kinases at the core of the cell-cycle control system are present in proliferating cells throughout the cell cycle. They are activated, however, only at appropriate times in the cycle, after which they are quickly inactivated.
- Thus, the activity of each of these kinases rises and falls in a cyclical fashion. Some of these protein kinases, for example, become active toward the end of G<sub>1</sub> phase and are responsible for driving the cell into S phase; another kinase becomes active just before M phase and drives the cell into mitosis.
- Switching these kinases on and off at the appropriate times is partly the responsibility of another set of proteins in the control system—*the cyclins*.

- Cyclins have no enzymatic activity themselves, but they need to bind to the cell-cycle kinases before the kinases can become enzymatically active. The kinases of the cell-cycle control system are therefore known as cyclin-dependent protein kinases, or Cdks.
- The cyclical changes in cyclin concentrations help drive the cyclic assembly and activation of the cyclin-Cdk complexes. Once activated, cyclin-Cdk complexes help trigger various cell-cycle events, such as entry into S phase or M phase.



# G<sub>1</sub> Phase

- In addition to being a bustling period of metabolic activity, cell growth, and repair, G<sub>1</sub> is an important point of decision-making for the cell.
- Based on intracellular signals that provide information about the size of the cell and extracellular signals reflecting the environment, the cell-cycle control machinery can either hold the cell transiently in G<sub>1</sub> (or in a more prolonged nonproliferative state, G<sub>0</sub>), or allow it to prepare for entry into the S phase of another cell cycle.
- Once past this critical G<sub>1</sub>-to-S transition, a cell usually continues all the way through the rest of the cell cycle quickly.



# GO Phase

- As a general rule, mammalian cells will multiply only if they are stimulated to do so by extracellular signals, called *mitogens*, produced by other cells.
- If deprived of such signals, the cell cycle arrests in G<sub>1</sub>; if the cell is deprived of mitogens for long enough, it will withdraw from the cell cycle and enter a nonproliferating state (Go), in which the cell can remain for days or weeks, months, or even for the lifetime of the organism.
- Escape from cell-cycle arrest—or from certain nonproliferating states— requires the accumulation of cyclins. Mitogens act by switching on cell signaling pathways that stimulate the synthesis of cyclins, proteins involved in DNA synthesis and chromosome duplication.

- Cells can delay progress through the cell cycle at specific transition points, to wait for suitable conditions or to repair damaged DNA.
- They can also withdraw from the cell cycle for prolonged periods—either temporarily or permanently by going into G<sub>0</sub> phase.
- Many cells in the human body permanently stop dividing when they differentiate. In such *terminally differentiated cells*, such as nerve or muscle cells, the cell-cycle control system is dismantled completely and genes encoding the relevant cyclins and Cdks are irreversibly shut down.

# G1-S checkpoint

- DNA damage can signal the cell-cycle control system to delay progress through the G1-to-S transition, preventing the cell from replicating damaged DNA.
- The mechanism that operates at the G1-to-S transition, which prevents the cell from replicating damaged DNA is called G1-S checkpoint.
- The arrest of the cell cycle in G1 gives the cell time to repair the damaged DNA before replicating it.
- If the DNA damage is too severe to be repaired, cells can be induced to kill itself by undergoing a form of programmed cell death called apoptosis.

- In the absence of appropriate signals, other cell types withdraw from the cell cycle only temporarily, entering an arrested state called Go.
- They retain the ability to reassemble the cell-cycle control system quickly and to divide again. Most liver cells, for example are in Go, but they can be stimulated to proliferate if the liver is damaged.
- Liver cells, normally divide only once every year or two, whereas certain epithelial cells in the gut divide more than twice a day to renew the lining of the gut continually.
- Many of our cells fall somewhere in between: they can divide if the need arises but normally do so infrequently.

# S Phase

- Before a cell divides, it must replicate its DNA.
- This replication must occur with extreme accuracy to minimize the risk of mutations in the next cell generation.
- Of equal importance, every nucleotide in the genome must be copied once—and only once—to prevent the damaging effects of gene amplification.

During G<sub>1</sub> DNA is first made replication-ready by the recruitment of proteins to origins of replication.

- Then S-Cdks promote the assembly of DNA replication machinery and pull the trigger for initiating DNA replication.
- Since G<sub>1</sub>-Cdk levels fall when cell cycle proceeds to S phase DNA synthesis can not be re-initiated so that DNA is replicated only once.

# G2 Phase

- If the DNA is not replicated correctly cell can delay entry into M phase.
- For the cell to progress into M phase, phosphorylation of M-Cdks should be removed by a phosphatase.
- When DNA is incompletely replicated that phosphatase is inhibited. Thus M-Cdk remains inactive and M phase is delayed until DNA damage is repaired or DNA replication is complete.
- Once a cell has successfully replicated its DNA in S phase, and progressed through G<sub>2</sub>, it is ready to enter M phase.

# M Phase

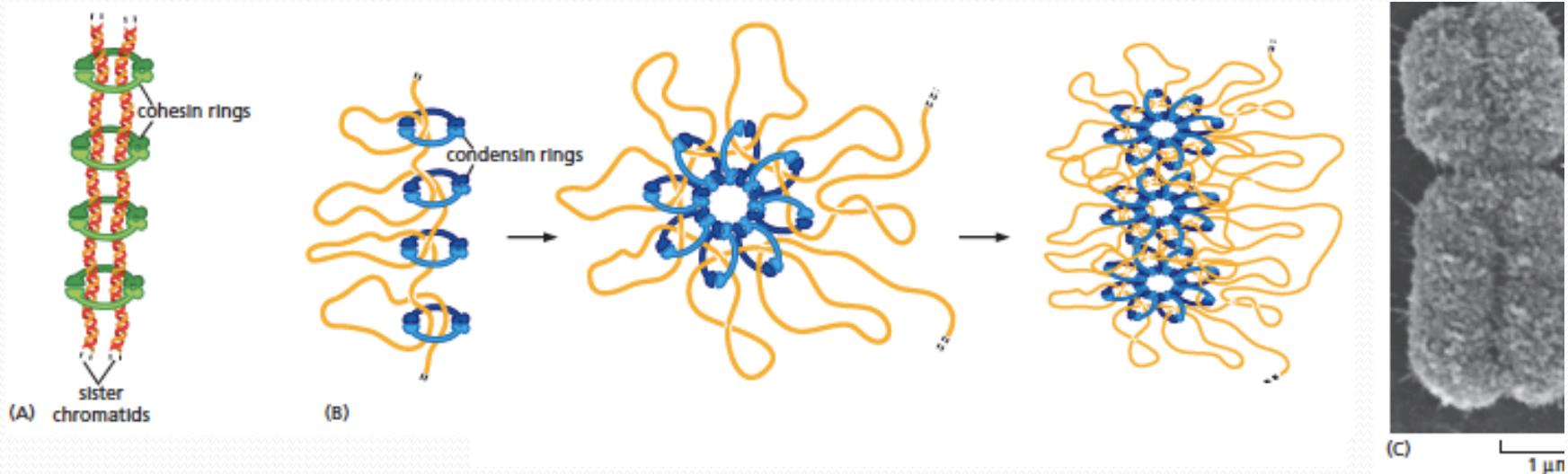
- During this brief period, the cell reorganizes virtually all of its components and distributes them equally into the two daughter cells.
- The earlier phases of the cell cycle, in effect, serve to set the stage for the drama of M phase.
- The central problem for a cell in M phase is to accurately segregate the chromosomes that were duplicated in the preceding S phase, so that each new daughter cell receives an identical copy of the genome.
- Eukaryotes solve this problem in a similar way: they assemble two specialized cytoskeletal machines, one that pulls the duplicated chromosomes apart (during mitosis) and another that divides the cytoplasm into two halves (cytokinesis).

- M-Cdk helps prepare the duplicated chromosomes for segregation and induces the assembly of the mitotic spindle—the machinery that will pull the duplicated chromosomes apart.
- Immediately after a chromosome is duplicated during S phase, the two copies remain tightly bound together. These identical copies—called sister chromatids—each contain a single, double-stranded molecule of DNA, along with its associated proteins.
- The sisters are held together by protein complexes called cohesins, which assemble along the length of each chromatid as the DNA is replicated. This cohesion between sister chromatids is crucial for proper chromosome segregation, and it is broken completely only in late mitosis to allow the sisters to be pulled apart by the mitotic spindle.
- Defects in sister-chromatid cohesion lead to major errors in chromosome segregation. In humans, such mis-segregation can lead to abnormal numbers of chromosomes, as in individuals with Down Syndrome, who have three copies of chromosome 21.

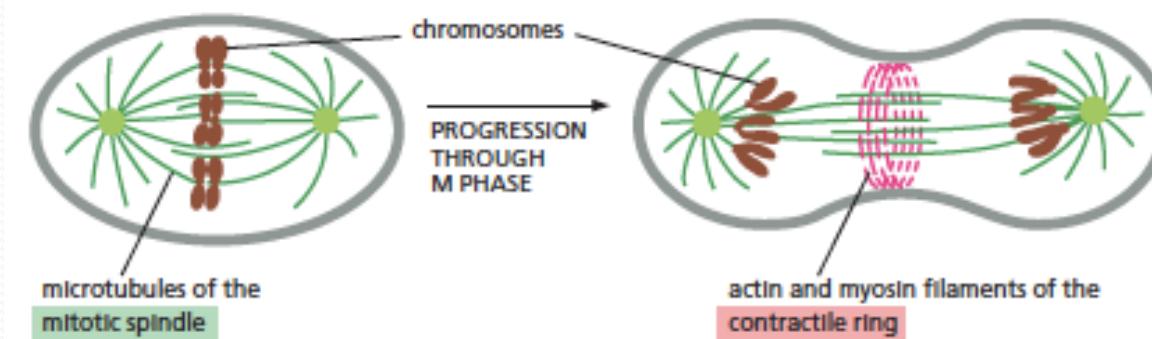
# Chromosome Condensation

- When the cell enters M phase, the duplicated chromosomes condense, becoming visible under the microscope as threadlike structures.
- Protein complexes, called *condensins*, help carry out this chromosome condensation, which reduces mitotic chromosomes to compact bodies that can be more easily segregated within the crowded confines of the dividing cell.
- The assembly of condensin complexes onto the DNA is triggered by the phosphorylation of condensins by M-Cdk.

- **Cohesins** tie the two sister chromatids together.
- Condensins assemble on each individual sister chromatid at the start of M phase and help each of these double helices to coil up into a more compact form.

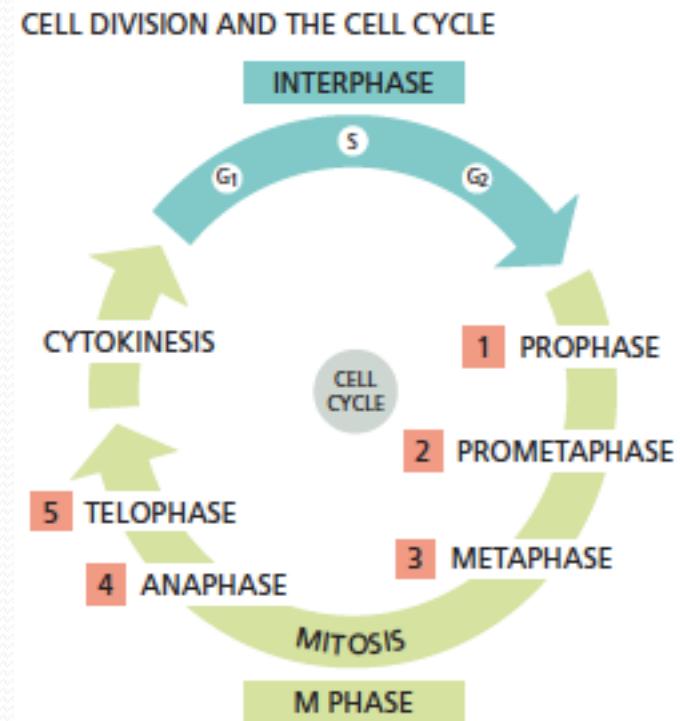


- After the duplicated chromosomes have condensed, two complex cytoskeletal machines assemble in sequence to carry out the two mechanical processes that occur in M phase.
- The ***mitotic spindle*** carries out nuclear division (mitosis), and, in animal cells and many unicellular eukaryotes, the ***contractile ring*** carries out cytoplasmic division (cytokinesis).
- The mitotic spindle is composed of microtubules and the various proteins that interact with them, including microtubule-associated motor proteins.
- In all eukaryotic cells, the mitotic spindle is responsible for separating the duplicated chromosomes and allocating one copy of each chromosome to each daughter cell.
- The contractile ring consists mainly of actin filaments and myosin filaments arranged in a ring around the equator of the cell .
- It starts to assemble just beneath the plasma membrane toward the end of mitosis. As the ring contracts, it pulls the membrane inward, thereby dividing the cell in two.



# Stages of Cell Cycle

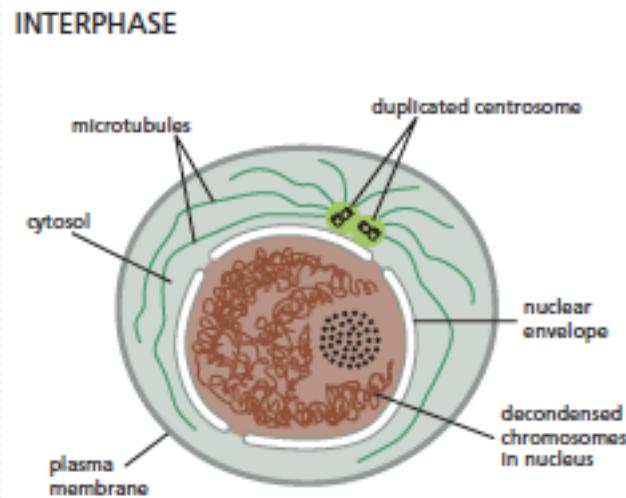
- Cell cycle includes 4 stages: G<sub>1</sub>, S, G<sub>2</sub> and M.
- G<sub>1</sub>, S and G<sub>2</sub> together form the interphase.
- Mitosis and cytokinesis together form the M phase.



The division of a cell into two daughters occurs in the M phase of the cell cycle. M phase consists of nuclear division, or mitosis, and cytoplasmic division, or cytokinesis. In this figure, M phase has been greatly expanded for clarity. Mitosis is itself divided into five stages, and these, together with cytokinesis, are described in this panel.

# Interphase

- It includes G<sub>1</sub>, S and G<sub>2</sub> stages of cell cycle.
- During interphase cell increases in size, DNA and the centrosomes are replicated.

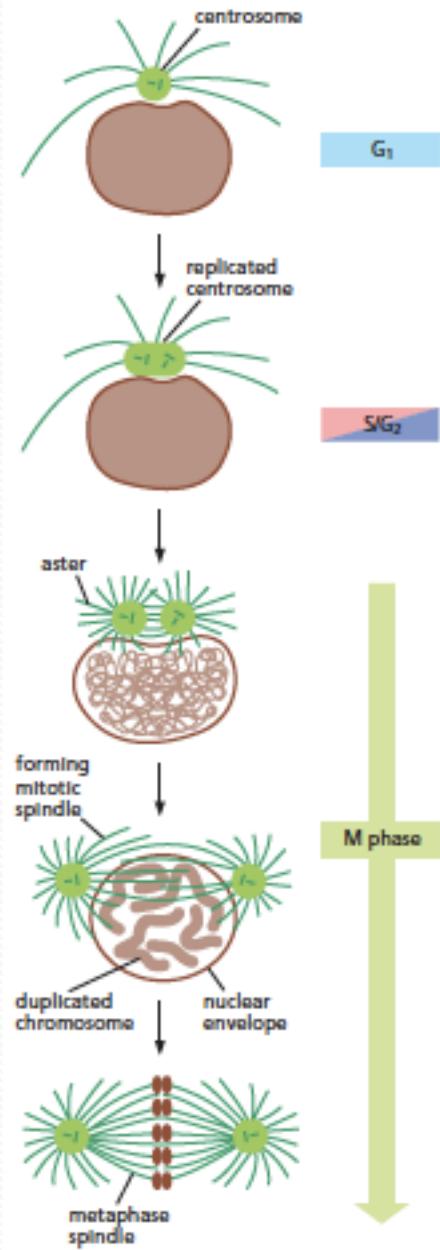


During Interphase, the cell increases in size. The DNA of the chromosomes is replicated, and the centrosome is duplicated.

# Stages of M phase

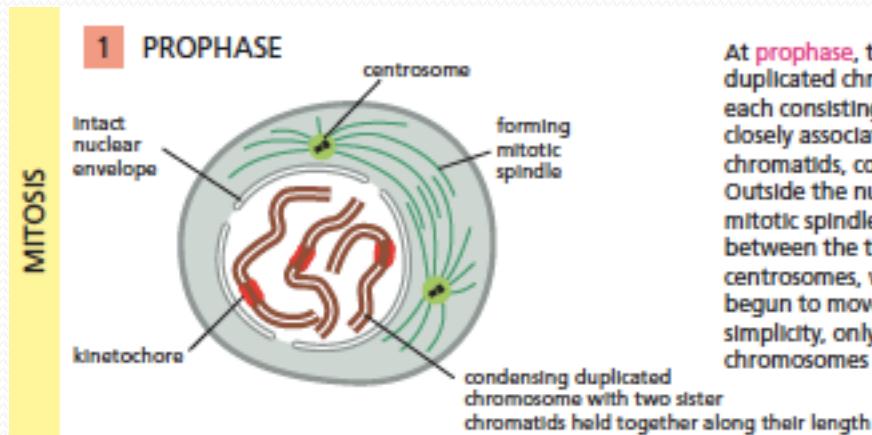
- Although M phase proceeds as a continuous sequence of events, it is traditionally divided into a series of stages.
- The first five stages of M phase—prophase, prometaphase, metaphase, anaphase, and telophase—constitute mitosis. Cytokinesis, which constitutes the final stage of M phase, begins before mitosis ends.
- Together, they form a dynamic sequence in which many independent cycles—involving the chromosomes, cytoskeleton, and centrosomes—are coordinated to produce two genetically identical daughter cells.

- Before M phase begins, two critical events must be completed: DNA must be fully replicated, and, in animal cells, the centrosome must be duplicated.
- The centrosome is the principal microtubule-organizing center in animal cells. It duplicates so that it can help form the two poles of the mitotic spindle and so that each daughter cell can receive its own centrosome.
- Centrosome duplication begins at the same time as DNA replication.
- As mitosis begins, however, the two centrosomes separate, and each nucleates a radial array of microtubules called an aster. The two asters move to opposite sides of the nucleus to form the two poles of the mitotic spindle.

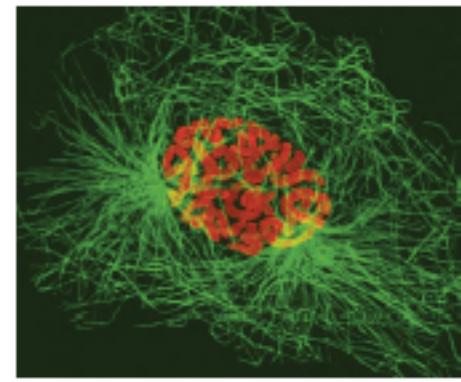


# Prophase

- Duplicated chromosomes begin to condense.
- The mitotic spindle begins to form.
- Centrosomes move apart towards the opposite poles.



At prophase, the duplicated chromosomes, each consisting of two closely associated sister chromatids, condense. Outside the nucleus, the mitotic spindle assembles between the two centrosomes, which have begun to move apart. For simplicity, only three chromosomes are drawn.



# Promethaphase

- Prometaphase starts abruptly with the disassembly of the nuclear envelope, which breaks up into small membrane vesicles.
- The spindle microtubules, which have been lying in wait outside the nucleus, now gain access to the duplicated chromosomes and capture them.

MITOSIS

2 PROMETAPHASE

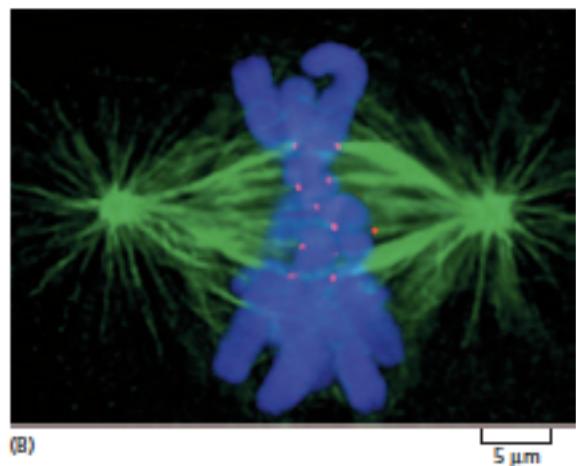
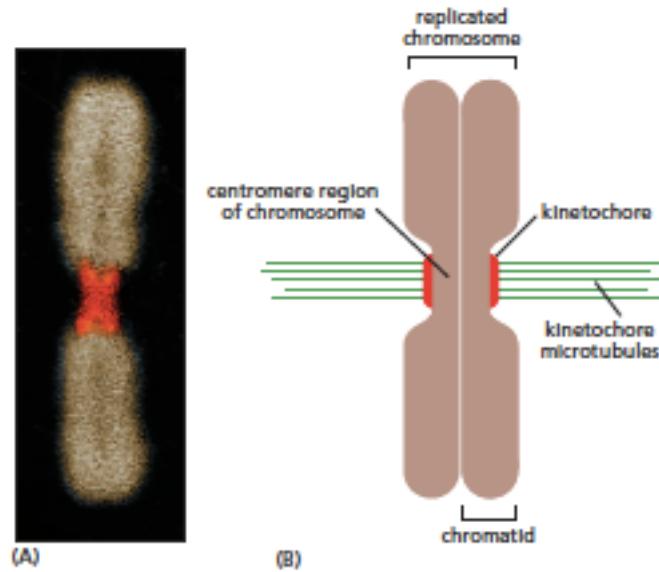
The diagram illustrates the transition from Prophase to Prometaphase. A grey circle represents the nucleus containing two red, V-shaped chromosomes. Green lines radiating from opposite sides represent the spindle poles. As the nuclear envelope breaks down, fragments of the envelope are shown as small green and grey shapes. One chromosome is labeled 'chromosome in motion' and is shown being pulled towards a spindle pole by a green line labeled 'kinetochore microtubule'. Another chromosome is labeled 'fragments of nuclear envelope'.

Prometaphase starts abruptly with the breakdown of the nuclear envelope. Chromosomes can now attach to spindle microtubules via their kinetochores and undergo active movement.

A fluorescence microscopy image showing a cell undergoing mitosis. The chromosomes are stained red and appear as several distinct, elongated structures. The spindle fibers are stained green and form a network around the chromosomes. The text 'time = 79 min' is visible at the bottom right.

time = 79 min

- The spindle microtubules grab hold of the chromosomes at ***kinetochores***, protein complexes that assemble on the centromere of each condensed chromosome during late prophase. Each duplicated chromosome has two kinetochores—one on each sister chromatid—which face in opposite directions.
- Kinetochores recognize the special DNA sequence present at the centromere.

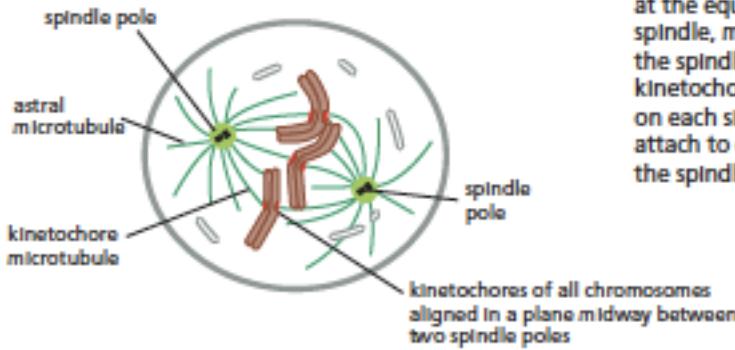


# Metaphase

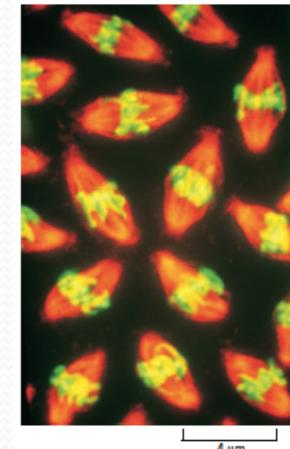
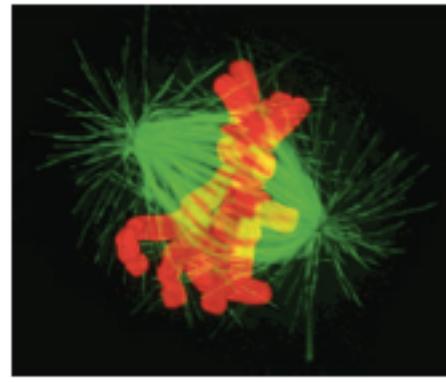
- During prometaphase, the duplicated chromosomes, now attached to the mitotic spindle, begin to move about.
- Eventually, they align at the equator of the spindle, halfway between the two spindle poles, thereby forming the metaphase plate. This event defines the beginning of metaphase.

## MITOSIS

### 3 METAPHASE

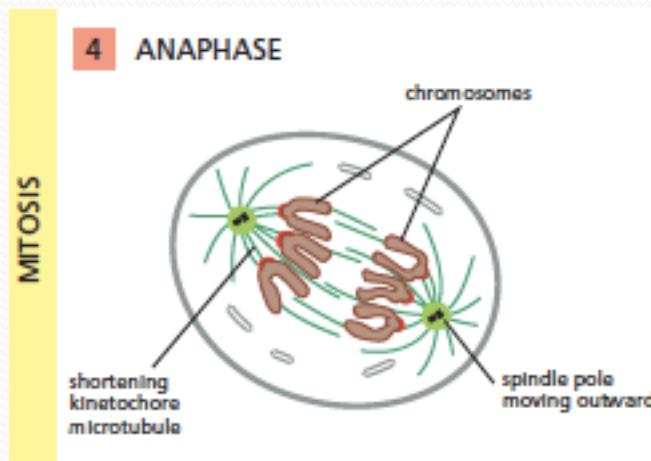


At metaphase, the chromosomes are aligned at the equator of the spindle, midway between the spindle poles. The kinetochore microtubules on each sister chromatid attach to opposite poles of the spindle.

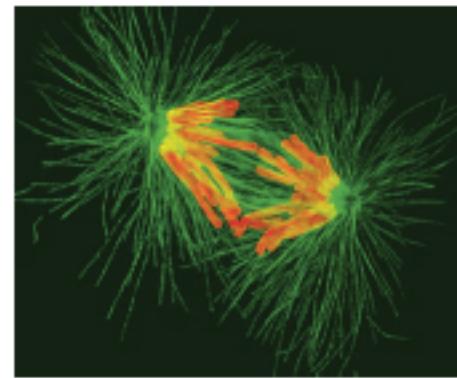


# Anaphase

- Anaphase begins abruptly with the breakage of the cohesin linkages that hold sister chromatids together .
- This release allows each chromatid—now considered a chromosome—to be pulled to the spindle pole to which it is attached.
- This movement segregates the two identical sets of chromosomes to opposite ends of the spindle.



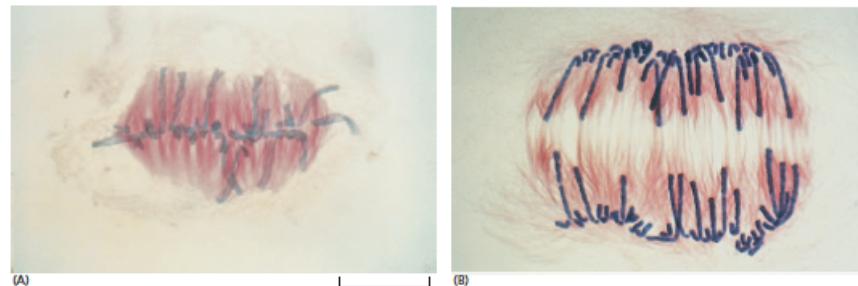
At **anaphase**, the sister chromatids synchronously separate and are pulled slowly toward the spindle pole to which they are attached. The kinetochore microtubules get shorter, and the spindle poles also move apart, both contributing to chromosome segregation.



time = 279 min

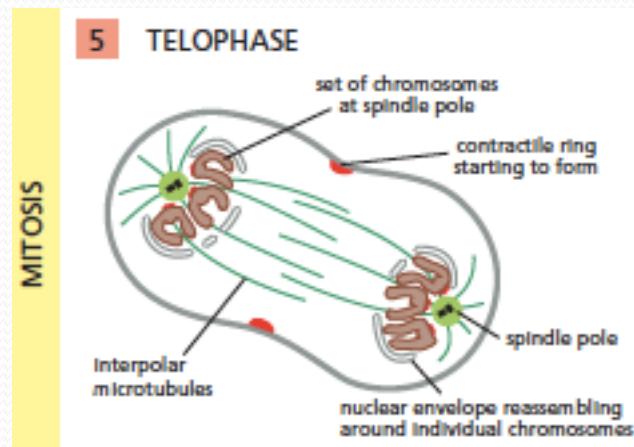
# Spindle Assembly Checkpoint

- If a dividing cell were to begin to segregate its chromosomes before all the chromosomes were properly attached to the spindle, one daughter cell would receive an incomplete set of chromosomes, while the other would receive a surplus. Both situations could be lethal.
- Thus, a dividing cell must ensure that every last chromosome is attached properly to the spindle before it completes mitosis.
- To monitor chromosome attachment, the cell makes use of a negative signal: unattached chromosomes send a “stop” signal to the cell-cycle control system.
- This so-called *spindle assembly checkpoint* thereby controls the onset of anaphase, as well as the exit from mitosis.

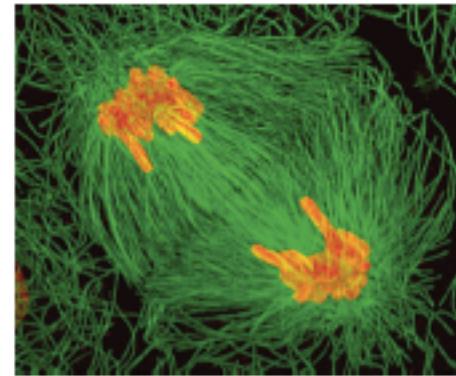


# Telophase

- During telophase, the final stage of mitosis, the mitotic spindle disassembles, and a nuclear envelope reassembles around each group of chromosomes to form the two daughter nuclei.
- The condensed chromosomes decondense into their interphase state. As a consequence of this decondensation, gene transcription is able to resume.
- A new nucleus has been created, and mitosis is complete.



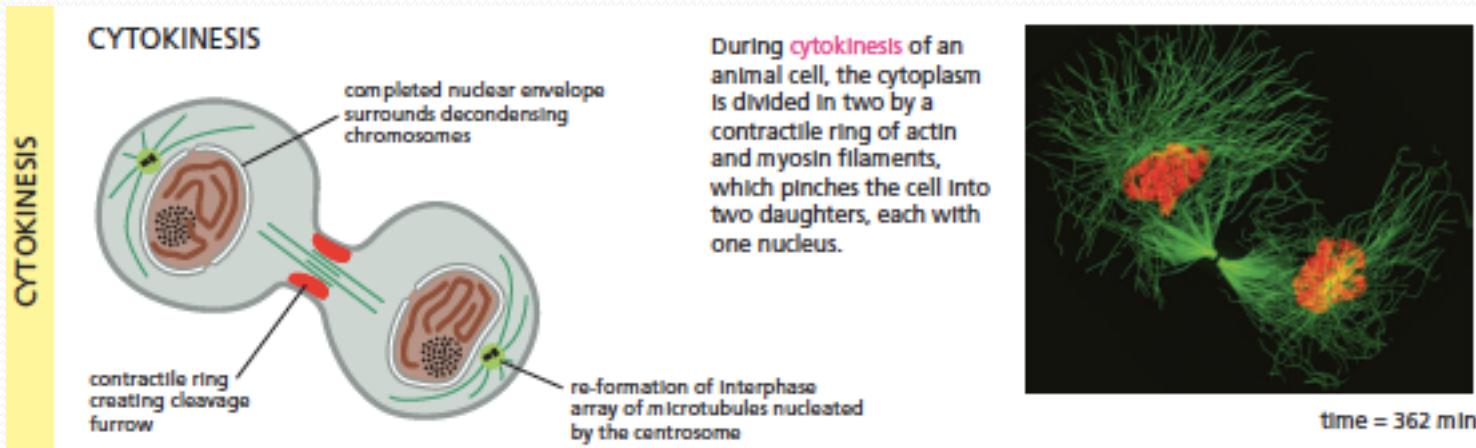
During **telophase**, the two sets of chromosomes arrive at the poles of the spindle. A new nuclear envelope reassembles around each set, completing the formation of two nuclei and marking the end of mitosis. The division of the cytoplasm begins with the assembly of the contractile ring.



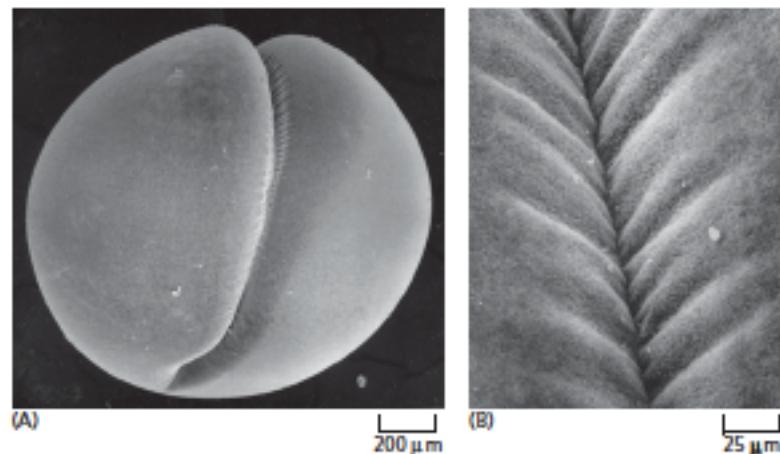
time = 315 min

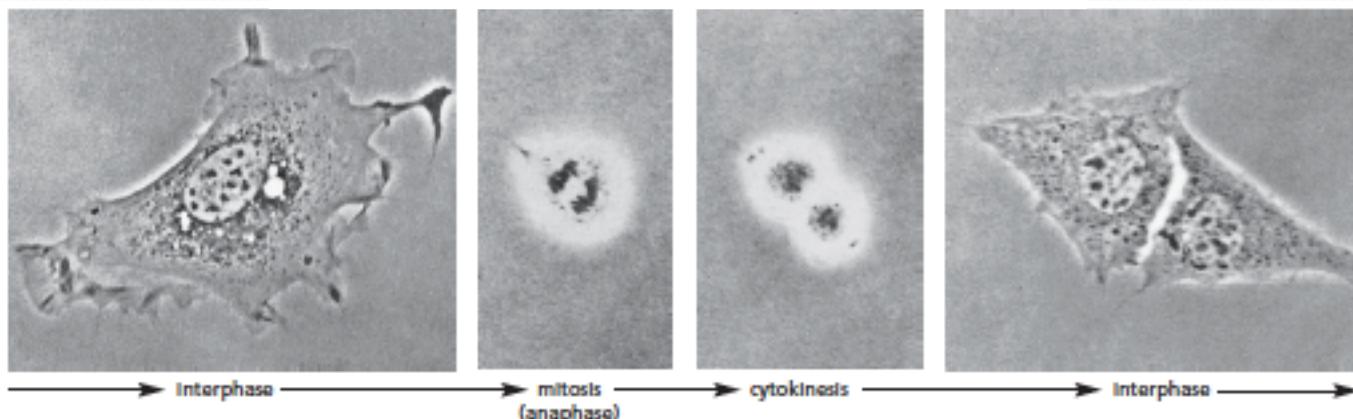
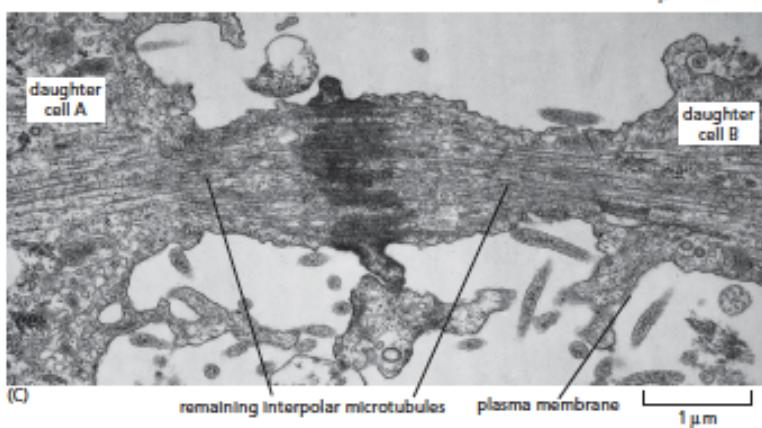
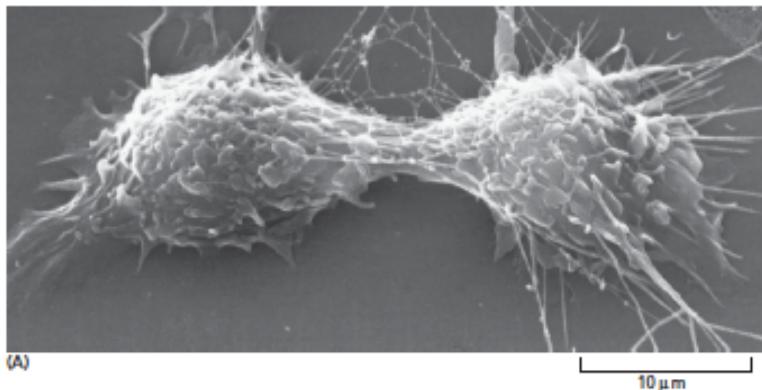
# Cytokinesis

- Cytokinesis, the process by which the cytoplasm is cleaved in two, completes M phase.
- It usually begins in anaphase by furrowing the plasma membrane but is not completed until the two daughter nuclei have formed in telophase.
- Whereas mitosis depends on a transient microtubule-based structure, the mitotic spindle, cytokinesis in animal cells depends on a transient structure based on actin and myosin filaments, the contractile ring.



- The furrowing of plasma membrane invariably occurs in a plane that runs perpendicular to the long axis of the mitotic spindle.
- This positioning ensures that the cleavage furrow cuts between the two groups of segregated chromosomes, so that each daughter cell receives an identical and complete set of chromosomes.
- When the mitotic spindle is located centrally in the cell—the usual situation in most dividing cells—the two daughter cells produced will be of equal size.





# Control of Cell Survival, Size and Number

- In a multicellular organism, the fate of individual cells is controlled by signals from other cells. For either tissue growth or cell replacement, cells must grow before they divide.
- Nutrients are not enough for an animal cell to survive, grow, or divide. It must also receive chemical signals from other cells, usually its neighbors. Such controls ensure that a cell survives only when it is needed and divides only when another cell is required, either to allow tissue growth or to replace cell loss.
- Most of the extracellular signal molecules that influence cell survival, cell growth, and cell division are either soluble proteins secreted by other cells or proteins that are bound to the surface of other cells or to the extracellular matrix.
- Although most act positively to stimulate one or more of these cell processes, some act negatively to inhibit a particular process.

# **Positive Regulators of Cell Growth and Division**

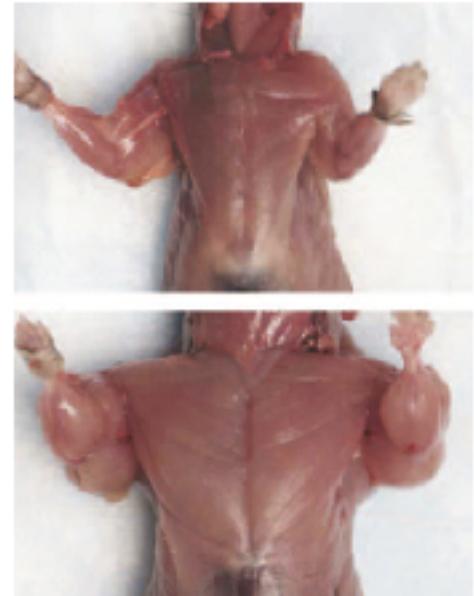
- The positively acting signal proteins can be classified, on the basis of their function, into three major categories:
  1. Survival factors promote cell survival, largely by suppressing apoptosis.
  2. Mitogens stimulate cell division, primarily by overcoming the intracellular braking mechanisms that tend to block progression through the cell cycle and by stimulating the progression through S phase.
  3. Growth factors stimulate cell growth (an increase in cell size and mass) by promoting the synthesis and inhibiting the degradation of proteins and other macromolecules.

# Negative Regulators of Cell Growth and Division

- The extracellular signal proteins that we have discussed so far—survival factors, mitogens, and growth factors—act positively to increase the size of organs and organisms. Some extracellular signal proteins, however, act to oppose these positive regulators and thereby inhibit cell growth and division.
- Positive and negative signals act in balance to control cell size and cell number in organism.
- Cancers are similarly the products of mutations that set cells free from the normal “social” controls operating on cell survival, growth, and proliferation.
- Because cancer cells are generally less dependent than normal cells on signals from other cells, they can out-survive, outgrow, and outdivide their normal neighbors, producing tumors that can kill their host.



(A)



(B)

Mutations in Myostatin gene which normally inhibits the cell growth and division in myoblasts (muscle cells) result in big muscles in cattle (A) and in mouse (B).