Cell Cycle

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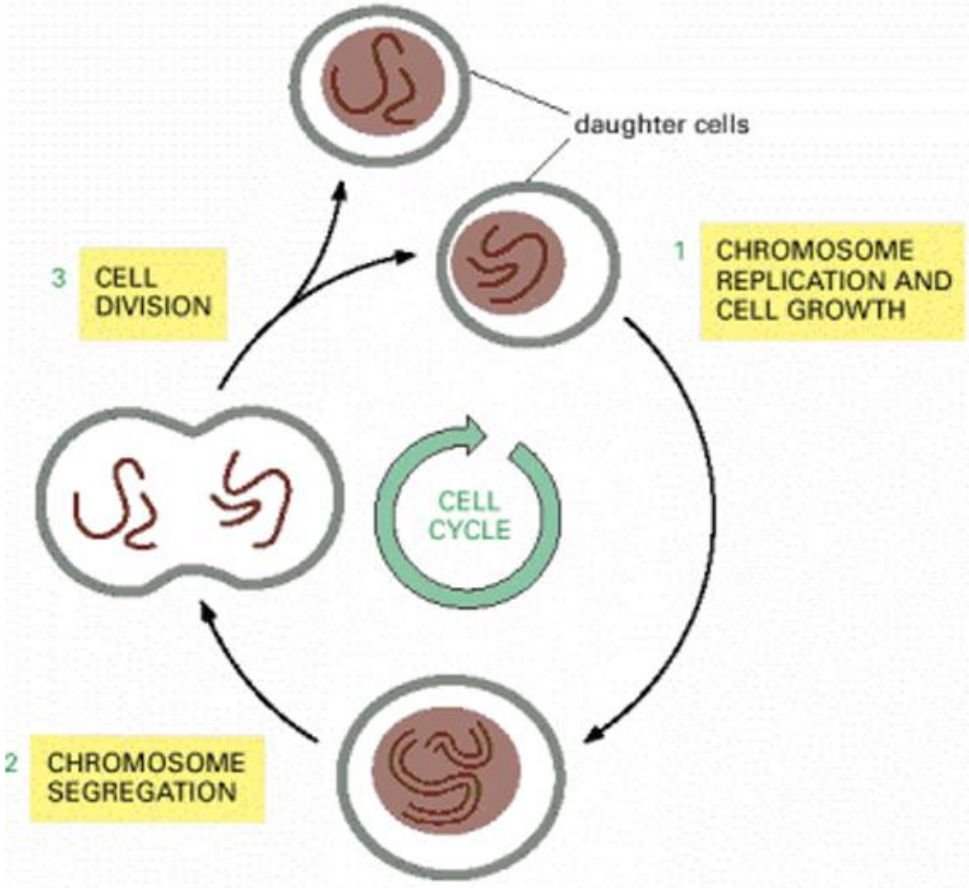
Cell Division & Cell Cycle

- Cells are generated from cells, and the only way to make more cells is by division of those that already exist.
- All living organisms, from the unicellular bacterium to the multicellular mammal, are products of repeated rounds of cell growth and division extending back in time to the beginnings of life on Earth over three billion years ago.
- The cell cycle is defined as the period between successive divisions of a cell. During this period, the contents of the cell must be accurately replicated.
- Howard and Pelc's work in the broad bean, *Vicia faba*, revealed that the cell goes through many discrete phases before and after cell division.
- Actively dividing eukaryote cells pass through a series of stages known collectively as the cell cycle: two gap phases (G1 and G2); an S (for synthesis) phase, in which the genetic material is duplicated; and an M phase, in which mitosis partitions the genetic material and the cell divides.
- This cycle of duplication and division, known as the cell cycle, is the essential mechanism by which all living things reproduce.
- Eucaryotic cells have evolved a complex network of regulatory proteins, known as the cell-cycle control system, that governs progression through the cell cycle.

Cell Cycle

- Inside the cell, the control system monitors progression through the cell cycle and delays later events until earlier events have been completed.
- In a multicellular animal, the system is highly responsive to signals from other cells, stimulating cell division when more cells are needed and blocking it when they are not.
- The cell-cycle control system therefore has a central role in regulating cell numbers in the tissues of the body.
- When the system malfunctions, excessive cell divisions can result in cancer.
- In addition to duplicating their genome, most cells also duplicate their other organelles and macromolecules; otherwise, they would get smaller with each division.
- To maintain their size, dividing cells must coordinate their growth (i. e., their increase in cell mass) with their division.



Cell Cycle Phases

- T he most basic function of the cell cycle is to duplicate accurately the vast amount of DNA in the chromosomes and then segregate the copies precisely into two genetically identical daughter cells.
- These processes define the two major phases of the cell cycle.

S- Phase: DNA duplication occurs during S phase (S for synthesis), which requires 10-12 hours and occupies about half of the cell cycle time in a typical mammalian cell.

M-Phase: After S phase, chromosome segregation and cell division occur in M phase (M for mitosis), which requires much less time (less than an hour in a mammalian cell).

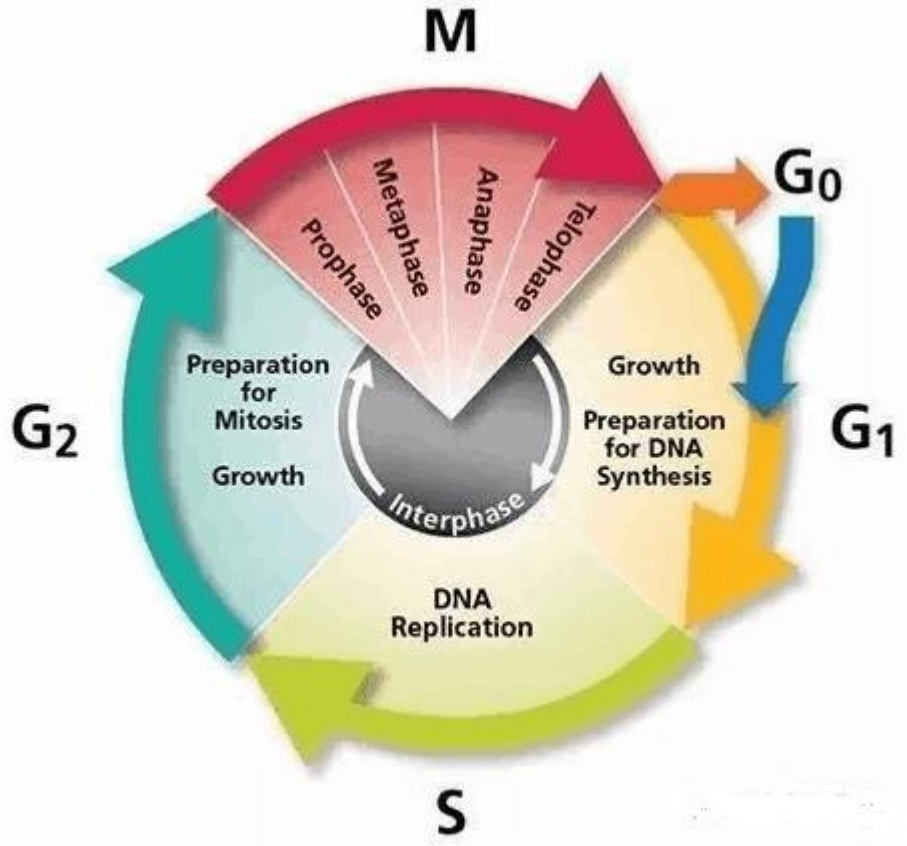
- M phase involves a series of dramatic events that begin with nuclear division, or mitosis.
- Mitosis begins with chromosome condensation: the duplicated DNA strands, packaged into elongated chromosomes, condense into the much more compact chromosomes required for their segregation.
- The nuclear envelope then breaks down, and the replicated chromosomes, each consisting of a pair of sister chromatids, become attached to the microtubules of the mitotic spindle.
- As mitosis proceeds, the cell pauses briefly in a state called metaphase, when the chromosomes are aligned at the equator of the mitotic spindle, poised for segregation.
- The sudden separation of sister chromatids marks the beginning of anaphase, during which the chromosomes move to opposite poles of the spindle, where they decondense and reform intact nuclei.
- The cell is then pinched in two by cytoplasmic division, or cytokinesis, and cell division is complete.

Cell Cycle: Gap Phase

- Most cells require much more time to grow and double their mass of proteins and organelles than they require to replicate their DNA and divide.
- Partly to allow more time for growth, extra gap phases are inserted in most cell cycles a **G1phase** between M phase and S phase and a **G2phase** between S phase and mitosis.
- Thus, the eucaryotic cell cycle is traditionally divided into four sequential phases: G1, S, G2, and M.
- G1, S, and G2 together are called **interphase**.
- The two gap phases serve as more than simple time delays to allow cell growth.
- They also provide time for the cell to monitor the internal and external environment to ensure that conditions are suitable and preparations are complete before the cell commits itself to the major upheavals of S phase and mitosis.
- The G1 phase is especially important in this respect. Its length can vary greatly depending on external conditions and extracellular signals from other cells.
- If extracellular conditions are unfavorable, for example, cells delay progress through G1 and may even enter a specialized resting state known as **G 0 (G zero),** in which they can remain for days, weeks, or even years before resuming proliferation.

Cell cycle

- If extracellular conditions are favorable and signals to grow and divide are present, cells in early G1 or G0 progress through a commitment point near the end of G1 known as **Start** (in yeasts) or the **restriction point** (in mammalian cells).
- After passing this point, cells are committed to DNA replication, even if the extracellular signals that stimulate cell growth and division are removed.



Cell Cycle Control

- Each phase of the cell cycle is tightly regulated, with checkpoints in place near the end of G1, at the G2/M transition, and near the end of the metaphase stage of mitosis (spindle (M) checkpoint).
- During the G1 checkpoint, cellular conditions necessary for progression through the cell cycle are evaluated.
- A cell generally passes the G1 checkpoint if it is an appropriate size, possesses adequate energy, and does not have damaged DNA.
- The main function of the G2 checkpoint is to ensure that replication of all chromosomes is complete and without introductions of mutations or unrepaired DNA damage.
- In addition, appropriate cell size and protein reserves are also assessed during this checkpoint.
- The spindle/M checkpoint ensures that all sister chromatids are correctly attached to the spindle microtubules and that each cell has the correct number of chromosomes.
- These checkpoints halt cell cycle progression if the cell has not met each of the requirements being evaluated.
- Checkpoint mechanisms tend to act through negative intracellular signals that arrest the cell cycle

…Cell Cycle Control

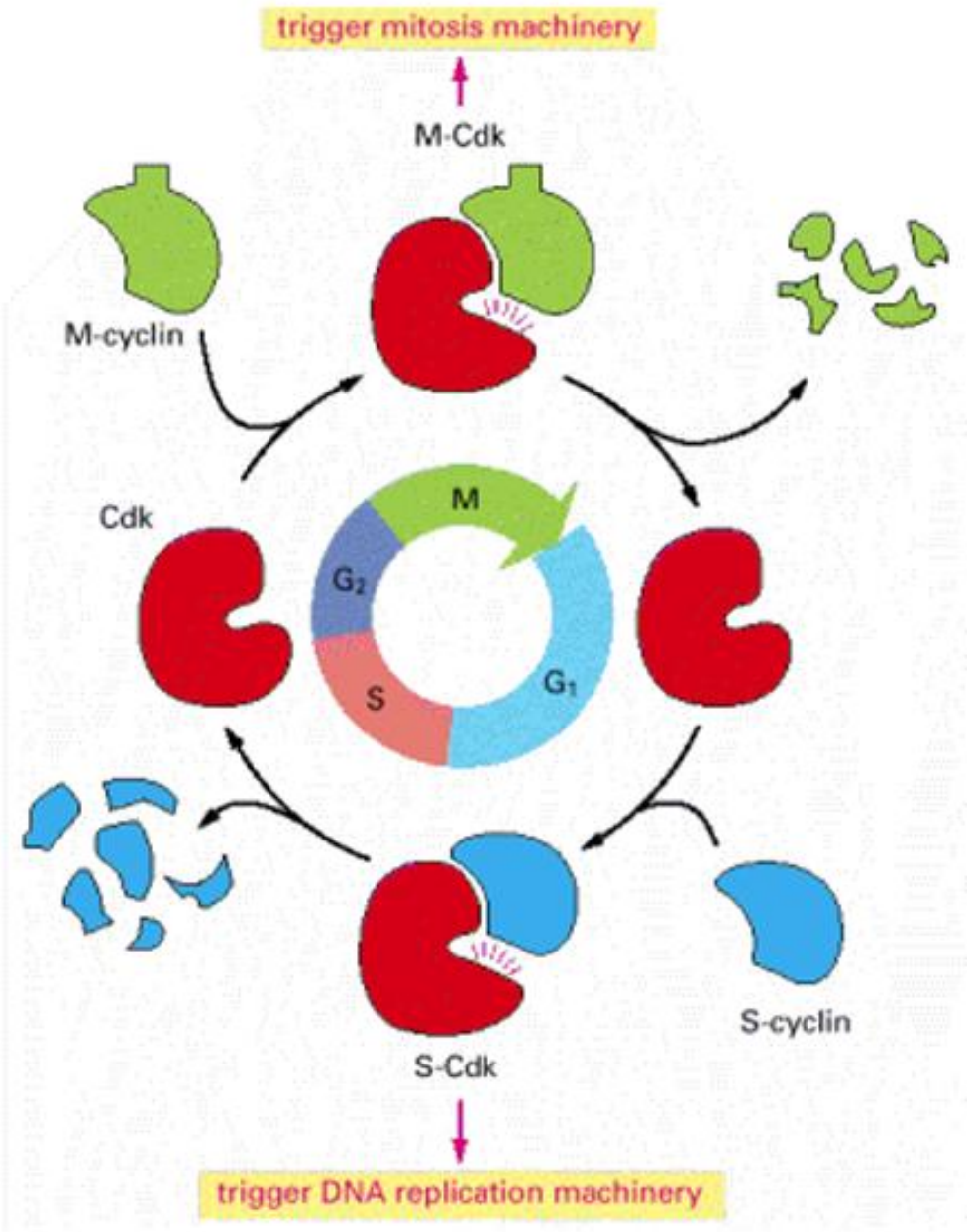
- At the heart of the cell-cycle control system is a family of protein kinases known as **cyclin-dependent kinases (Cdks)**.
- The activity of these kinases rises and falls as the cell progresses through the cycle.
- The oscillations lead directly to cyclical changes in the phosphorylation of intracellular proteins that initiate or regulate the major events of the cell cycle DNA replication, mitosis, and cytokinesis.
- An increase in Cdk activity at the beginning of mitosis, for example, leads to increased phosphorylation of proteins that control chromosome condensation, nuclear envelope breakdown, and spindle assembly.
- Cyclical changes in Cdk activity are controlled by a complex array of enzymes and other proteins.
- The most important of these **Cdk regulators** are proteins known as **cyclins**.
- Cyclical changes in cyclin levels result in the cyclic assembly and activation of the cyclin-Cdk complexes; this activation in turn triggers cell-cycle events.

Cyclins

- There are four classes of cyclins:
  - G1 /S-cyclins bind Cdks at the end of G1 and commit the cell to DNA replication.
  - S-cyclins bind Cdks during S phase and are required for the initiation of DNA replication.
  - M-cyclins promote the events of mitosis.
  - G1-cyclins, helps promote passage through Start or the restriction point in late G1.
- In yeast cells, a single Cdk protein binds all classes of cyclins and drives all cell-cycle events by changing cyclin partners at different stages of the cycle.
- In vertebrate cells, by contrast, there are four Cdks.
- Two interact with G1-cyclins, one with G1/S- and S-cyclins, and one with M-cyclins.
- Each cyclin-Cdk complex phosphorylates a different set of substrate proteins.
- Studies of the three-dimensional structures of Cdk and cyclin proteins have revealed that, in the absence of cyclin, the active site in the Cdk protein is partly obscured by a slab of protein.
- Full activation of the cyclin-Cdk complex then occurs when a separate kinase, the Cdk-activating kinase (CAK), phosphorylates an amino acid near the entrance of the Cdk active site.
- This causes a small conformational change that further increases the activity of the Cdk, allowing the kinase to phosphorylate its target proteins effectively and thereby induce specific cell-cycle events.

A simplified view of the core of the cell-cycle control system.

- Cdk associates successively with different cyclins to trigger the different events of the cycle.
- Cdk activity is usually terminated by cyclin degradation.
- For simplicity, only the cyclins that act in S phase (S-cyclin) and M phase (M cyclin) are shown, and they interact with a single Cdk; as indicated, the resulting cyclin-Cdk complexes are referred to as S-Cdk and M-Cdk, respectively.



Cdk Activity Can Be Suppressed Both by Inhibitory Phosphorylation and by Inhibitory Proteins

- The activity of a cyclin-Cdk complex can be inhibited by phosphorylation at a pair of amino acids in the roof of the active site.
- Phosphorylation of these sites by a protein kinase known as **Wee1** inhibits Cdk activity, while dephosphorylation of these sites by a phosphatase known as **Cdc25** increases Cdk activity.
- Cyclin-Cdk complexes can also be regulated by the binding of **Cdk inhibitor proteins** (CKIs eg.p27).
- Most notably, cyclin-Cdk complexes are inactivated by regulated proteolysis of cyclins at certain cell-cycle stages.
- Cell-cycle control depends exclusively on post-transcriptional mechanisms that involve the regulation of Cdk activity by phosphorylation and the binding of regulatory proteins such as cyclins, which are themselves regulated by proteolysis.
- Two enzyme complexes, SCF and APC, induce the proteolysis of specific cell-cycle regulators by ubiquitylating them and thereby trigger several critical events in the cycle.
- In the more complex cell cycles of most cell types (eg. Budding yeast), however, transcriptional control provides an added level of regulation.
- Cyclin levels in most cells, for example, are controlled not only by changes in cyclin degradation but also by changes in cyclin gene transcription and cyclin synthesis.

Cell-Cycle Progression is Blocked by DNA Damage and p53: DNA Damage Checkpoints

- The cell-cycle control system can readily detect DNA damage and arrest the cycle at DNA damage checkpoints.
- Most cells have at least two such checkpoints one in late G1, which prevents entry into S phase, and one in late G2, which prevents entry into mitosis.
- When cells in G2 are exposed to damaging radiation, for example, the damaged DNA sends a signal that blocks the dephosphorylation and activation of M-Cdk, thereby blocking entry into mitosis.
- When the DNA damage is repaired, the inhibitory signal is turned off, and cell-cycle progression resumes.
- The G1 checkpoint blocks progression into S phase by inhibiting the activation of G1/S-Cdk and S-Cdk complexes.
- In mammalian cells, for example, DNA damage leads to the activation of the gene regulatory protein p53, which stimulates the transcription of several genes.
- One of these genes encodes a CKI protein called p21, which binds to G1/S-Cdk and S-Cdk and inhibits their activities, thereby helping to block entry into S phase.
- If DNA damage is so severe that repair is not possible, the decision to die in this way also depends on the activation of p53, and it is this function of p53 that is apparently most important in protecting us against cancer.

Cell Cycle Control (Summary)

- An ordered sequence of cyclin-Cdk activities triggers most of the events of the cell cycle.
- During G1 phase, Cdk activity is reduced to a minimum by Cdk inhibitors (CKIs), cyclin proteolysis, and decreased cyclin gene transcription.
- When environmental conditions are favorable, G1- and G1/S-Cdks increase in concentration, overcoming these inhibitory barriers in late G1 and triggering the activation of S-Cdk.
- The S-Cdk phosphorylates proteins at DNA replication origins, initiating DNA synthesis through a mechanism that ensures that the DNA is duplicated only once per cell cycle.
- Once S phase is completed, the activation of M-Cdk leads to the events of early mitosis, whereby the cell assembles a mitotic spindle and prepares for segregation of the duplicated chromosomes which consist of sister chromatids glued together.
- Anaphase is triggered by the destruction of the proteins that hold the sisters together.
- The M-Cdk is then inactivated by cyclin proteolysis, which leads to cytokinesis and the end of M phase.
- Progression through the cell cycle is regulated precisely by various inhibitory mechanisms that arrest the cell cycle at specific checkpoints when events are not completed successfully, when DNA damage occurs, or when extracellular conditions are unfavorable.