Chapter 13: The Cell Cycle and Its Regulation

# Introduction

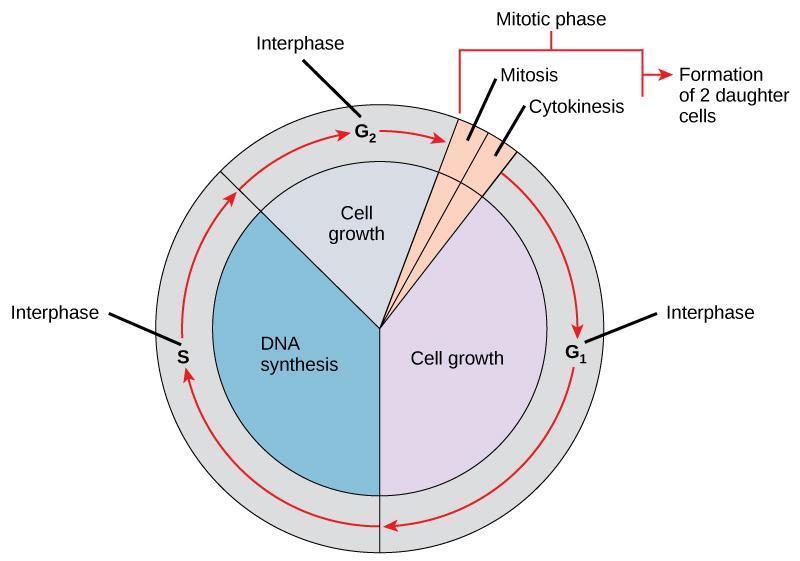
The cell cycle is an ordered series of events involving cell growth and cell division that produces two new daughter cells from one parent cell. Cells on the path to cell division proceed through a series of precisely timed and carefully regulated stages of growth, DNA replication, and nuclear and cytoplasmic division that ultimately produces two identical (clone) cells.

The frequency of cell division varies depending on cell type and developmental stage. For example, during embryogenesis, cells divide rapidly as the embryo grows in size. Conversely, in an adult human body, almost all cells are terminally differentiated and are not dividing (example: neurons) or divide very infrequently (example: liver cells that divide about once per year). These non-dividing cells are termed quiescent cells (They are in the Go stage, which is discussed later in the chapter). There are some populations of adult human cells that are actively dividing. Examples of these include intestinal epithelial cells and adult-derived stem cells, such as bone marrow stem cells (See Chapter 16 for more on stem cells).

Overall, the process of cell division is complex and highly regulated (regulation is discussed in the latter half of this chapter). To begin, we will explore the stages of the cell cycle:

## Interphase

The cell cycle has two major phases: interphase and the mitotic phase. During **interphase**, the cell grows, and DNA is replicated. During the mitotic phase, the replicated DNA and cytoplasmic contents are separated, and the cell cytoplasm is typically partitioned by a third process of the cell cycle called cytokinesis (Figure 13-1). During **interphase**, the cell undergoes normal growth processes while also preparing for cell division. For a cell to move from interphase into the mitotic phase, many internal and external conditions must be met. The three stages of interphase are called G1, S, and G2.



**Figure 13-1: The eukaryotic cell cycle.** The cell cycle in multicellular organisms consists of interphase and the mitotic phase. During interphase, the cell grows and the nuclear DNA is duplicated. Interphase is followed by the mitotic phase. During the mitotic phase, the duplicated chromosomes are segregated and distributed into daughter nuclei. Following mitosis, the cytoplasm is usually divided as well by cytokinesis, resulting in two genetically identical daughter cells.

**G1 Phase (First Gap)**

The first stage of interphase is called the G1 phase (first gap) because, from a microscopic point of view, little change is visible. However, during the G1 stage, the cell is quite active at the biochemical level. The cell is accumulating the building blocks of chromosomal DNA and the associated proteins as well as accumulating sufficient energy reserves to complete the task of replicating each chromosome in the nucleus. Furthermore, during the G1 stage, DNA is assessed for damage and repaired, if needed, (See Chapter 14 for DNA repair) and the cell also grows in size.

**S Phase (Synthesis of DNA)**

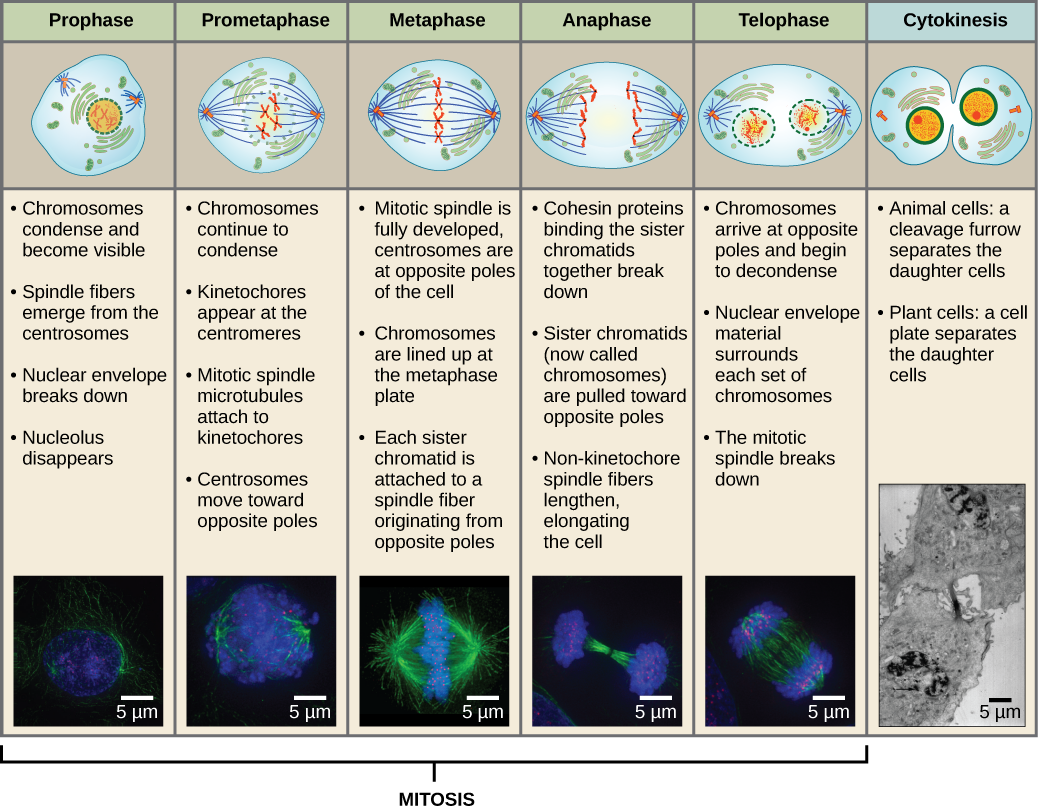
Throughout interphase, nuclear DNA remains in a semi-condensed chromatin configuration. In the S phase, DNA replication can proceed through the mechanisms that result in the formation of identical pairs of DNA molecules—sister chromatids—that are firmly attached to the centromeric region. Proteins called **cohesins** loop around sister chromatids to keep them connected. The centrosome is also duplicated during the S phase. The two centrosomes of homologous chromosomes will give rise to the mitotic spindle, the apparatus that orchestrates the movement of chromosomes during mitosis. For example, the centrosomes are associated with a pair of rod-like objects, the centrioles, which are positioned at right angles to each other. Centrioles help organize cell division. We should note, however, that centrioles are not present in the centrosomes of other eukaryotic organisms, such as plants and most fungi. DNA replication and S phase are discussed in more depth in Chapter 14.

**G2 Phase (Second Gap)**

In the G2 phase, the cell replenishes its energy stores and synthesizes proteins necessary for chromosome manipulation and movement. Some cell organelles are duplicated, and the cytoskeleton is dismantled to provide resources for the mitotic phase. As in the G1 stage, during G2, the size of the cell increases and DNA damaged is repaired, if needed. The final preparations for the mitotic phase must be completed before the cell can enter the first stage of mitosis.

## M-Phase

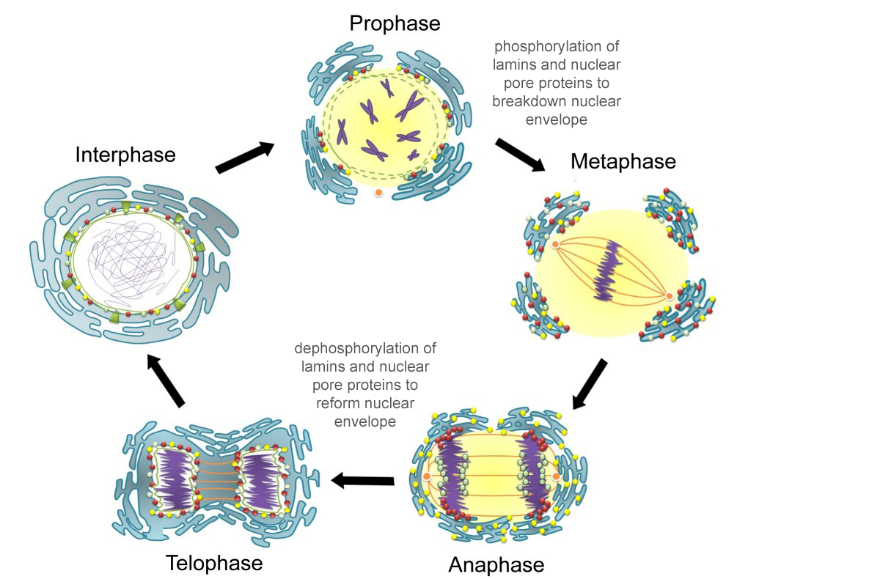
The **mitotic phase** (M-phase) is a multistep process during which the duplicated chromosomes are aligned, separated, and move into two new, identical daughter cells. M-phase has several sub-phases: prophase, prometaphase, metaphase, anaphase, and cytokinesis (Figure 13-2).



**Figure 13-2: Sub-phases of Mitosis.** Mitosis is divided into five stages—prophase, prometaphase, metaphase, anaphase, and telophase. The pictures at the bottom were taken by fluorescence microscopy (hence, the black background) of cells artificially stained by fluorescent dyes: blue fluorescence indicates DNA (chromosomes) and green fluorescence indicates microtubules (spindle apparatus).

**Prophase**

During **prophase**, the “first phase,” several events must occur to provide access to the chromosomes in the nucleus. The nuclear envelope starts to break down. This is caused by phosphorylation of nuclear pore proteins and **lamins**, the intermediate filament cytoskeletal protein that provides structure to the nuclear envelope, by the cell cycle regulatory protein **M-cyclin/Cdk** (cyclins and Cdks are discussed in more detail later in the chapter). Later, in telophase, these proteins will be dephosphorylated to reform the nuclear envelope (Figure 13-3).



**Figure 13-3: The breakdown and reformation of the nuclear envelope.** During late prophase (prometaphase), lamins and nuclear pore proteins are phosphorylated. This causes the nuclear envelope to break down. In telophase, the nuclear envelope is reformed. This occurs when lamins and nuclear pore proteins are dephosphorylated, causing them to reassemble.

Additionally, the Golgi apparatus and endoplasmic reticulum fragment and disperse to the periphery of the cell. The nucleolus disappears, and centrosomes begin to move to opposite poles of the cell. The microtubules that form the mitotic spindle extend between the centrosomes, pushing them farther apart as the microtubule fibers lengthen, due to dynamic instability. The sister chromatids begin to coil more tightly and become visible under a light microscope. This process is facilitated by proteins called **condensins,** ring-shaped proteins that further condense chromatin. Condensins are phosphorylated by M-cyclin/Cdk to further condense chromatin.

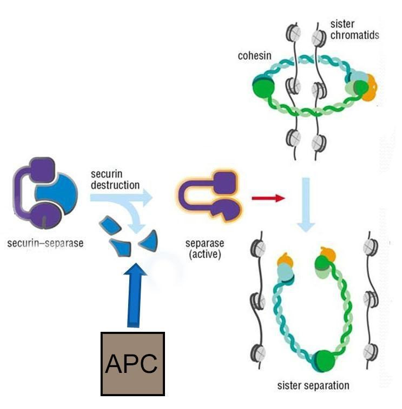
During the latter part of prophase (sometimes termed **prometaphase**), many processes that were begun in prophase continue to advance and culminate in the formation of a connection between the chromosomes and cytoskeleton. The remnants of the nuclear envelope disappear. The mitotic spindle continues to develop as more microtubules assemble and stretch across the length of the former nuclear area. Chromosomes become more condensed and visually discrete. Each sister chromatid attaches to spindle microtubules at the centromere via a protein complex called the **kinetochore**.

**Metaphase**

During **metaphase**, all of the chromosomes are aligned on the metaphase plate, or the equatorial plane, midway between the two poles of the cell. The sister chromatids are still tightly attached to each other, and the chromosomes are maximally condensed.

**Anaphase**

During **anaphase**, the sister chromatids at the equatorial plane are held together by cohesins. Before the chromatids can be separated, the cohesins must be removed. This is regulated by a protein called **Anaphase Promoting Complex (APC).** Prior to anaphase, APC is in its inactive (dephosphorylated) state. At the beginning of anaphase, APC is phosphorylated by M-cyclin/Cdk and becomes active. APC is a ubiquitin ligase, meaning it can add a small peptide called ubiquitin to proteins. When ubiquitin ligases add certain polymers of ubiquitin to proteins (called polyubiquitination), it serves to “tag” the proteins for degradation by the proteasome. At the beginning of anaphase, APC polyubiquitinates the protein **securin**, causing secruin to be degraded by the proteasome. The degradation of securing, releases the protein **separase**. Separase is the enzyme that physically breaks down the cohesin proteins that held the sister chromatids together (Figure 13-4).



**Figure 13-4: Regulation of sister chromatid separation during anaphase**. APC (anaphase promoting complex) polyubiquitinates secruin, leading to the proteasomal degradation of secruin. This allows separase to become active. Separase cleaves cohesins, allowing sister chromatids to be pulled apart by microtubules.

Once the cohesins are removed, each sister chromatid, now called a chromosome, is pulled rapidly toward the centrosome to which its microtubule was attached. The cell becomes visibly elongated as the non-kinetochore microtubules slide against each other at the metaphase plate where they overlap.

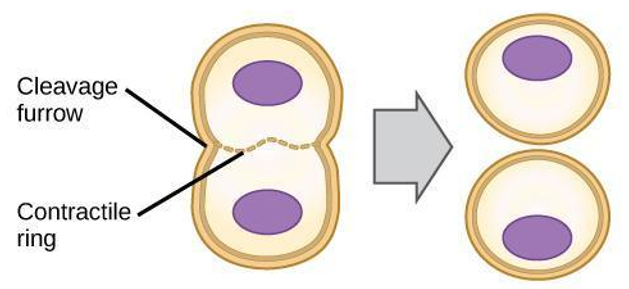
**Telophase**

During **telophase**, all of the events that set up the duplicated chromosomes for mitosis during the first three phases are reversed. The chromosomes reach the opposite poles and begin to decondense (unravel). The mitotic spindles are broken down into monomers that will be used to assemble cytoskeleton components for each daughter cell. As previously mentioned, lamins and nuclear pore proteins are dephosphorylated, leading to the reformation of the nuclear envelope chromosomes.

**Cytokinesis**

Cytokinesis is the second part of the mitotic phase during which cell division is completed by the physical separation of the cytoplasmic components into two daughter cells. Although the stages of mitosis are similar for most eukaryotes, the process of cytokinesis is quite different for eukaryotes that have cell walls, such as plant cells.

In animal cells, cytokinesis begins following the onset of anaphase. A **contractile ring** composed of actin filaments forms just inside the plasma membrane at the former metaphase plate. The actin filaments pull the equator of the cell inward, forming a fissure. This fissure, or “crack,” is called the **cleavage furrow.** The furrow deepens as the actin ring contracts, and eventually, the membrane and cell are cleaved in two (Figure 13-5).

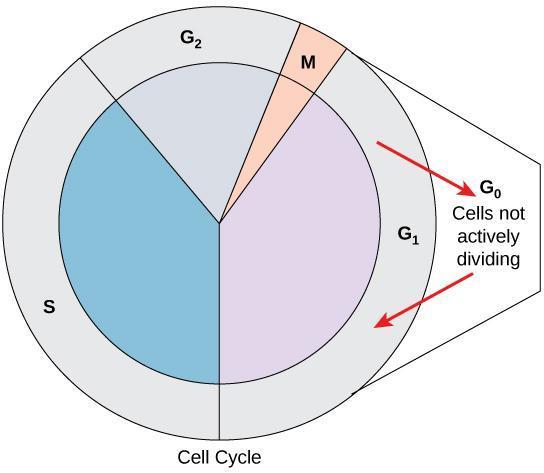


**Figure 13-5: Cytokinesis.** In the upper panel, a cleavage furrow forms at the former metaphase plate in the animal cell. The plasma membrane is drawn in by a ring of actin fibers contracting just inside the membrane. The cleavage furrow deepens until the cells are pinched in two.

## G0 Phase

As mentioned in the introduction, not all cells adhere to the classic cell-cycle pattern in which a newly formed daughter cell immediately enters interphase, closely followed by the mitotic phase. Cells in the G0 phase are not actively preparing to divide. The cell is in a quiescent (inactive) stage, having exited the cell cycle. Some cells enter G0 temporarily until an external signal triggers the onset of G1. Other cells that never or rarely divide, such as mature cardiac muscle and nerve cells, remain in G0 permanently.

The cell cycle is shown in a circular graphic, with four stages. Interphase accounts for the most time spent in the cell cycle with the G1 stage accounting for approximately 39% of the cycle, the S stage for about 40%, and the G2 phase for about 19%. Mitosis only accounts for approximately 2% of the cell cycle, thus demonstrating the importance of the preparation steps in interphase over the division in mitosis. An arrow is shown exiting the G1 stage that points to the G0 stage outside the circle, in which cells are not actively dividing. Another arrow points from the G0 stage back into the G1 stage, where cells may re-enter the cycle (Figure 13-6).



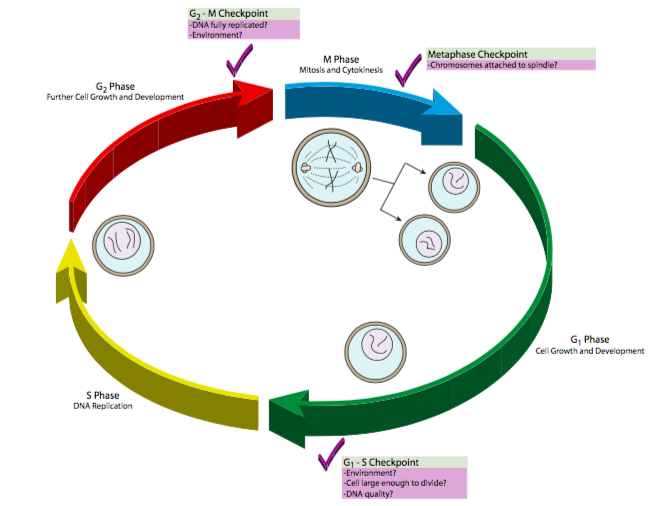
**Figure 13-6: G0 Phase.** Cells that are not actively preparing to divide enter an alternate phase called G0. In some cases, this is a temporary condition until triggered to enter G1. In other cases, the cell will remain in G0 permanently.

# Control of the Cell Cycle

The length of the cell cycle is highly variable even within the cells of an individual organism. In humans, the frequency of cell turnover ranges from a few hours in early embryonic development to an average of two to five days for epithelial cells or an entire human lifetime spent in G0 by specialized cells such as cortical neurons or cardiac muscle cells. There is also variation in the time that a cell spends in each phase of the cell cycle. When fast-dividing mammalian cells are grown in culture (outside the body under optimal growing conditions), the length of the cycle is approximately 24 hours. The timing of events in the cell cycle is controlled by mechanisms that are both internal and external to the cell.

## Regulation at Internal Checkpoints

Daughter cells must be exact duplicates of the parent cell. Mistakes in the duplication or distribution of the chromosomes can lead to mutations that may be passed forward to every new cell produced from the abnormal cell. To prevent a compromised cell from continuing to divide, there are internal control mechanisms that operate at three main cell cycle checkpoints (Figure. 13-7). The cell cycle can be stopped until conditions are favorable or errors have been corrected. These checkpoints occur at the end of G1 (G1-S checkpoint), at the end of G2 (G2–M checkpoint), and during metaphase (M checkpoint).



**Figure 13-7: Cell Cycle Checkpoints**. The cell cycle is controlled at three checkpoints. The integrity of the DNA is assessed at the G1-S checkpoint. Proper chromosome duplication is assessed at the G2-M checkpoint. Attachment of each kinetochore to a spindle fiber is assessed at the M checkpoint.

**The G1-S Checkpoint**

The G1-S checkpoint determines whether all conditions are favorable for cell division to proceed and for DNA replication to occur during S phase. The G1-S checkpoint, also called the restriction point, is the point at which the cell irreversibly commits to the cell division process. In addition to adequate reserves and cell size, there is a check for damage to the genomic DNA and ensure there is space for a near cell at the G1-S checkpoint. A cell that does not meet all the requirements will “arrest” (pause or become quiescent) in G1 and will attempt to correct these deficiencies (e.g., repair DNA damage). If, during cell cycle arrest, the cell can meet the G1-S checkpoint criteria, the cell cycle will proceed to the S phase. If not, the cell will undergo apoptosis (programmed cell death) (See Chapter 15).

**The G2-M Checkpoint**

The G2-M checkpoint bars the entry to the mitotic phase if certain conditions are not met. As in the G1-S checkpoint, cell size and protein reserves are assessed. However, the most important role of the G2 checkpoint is to ensure that all of the chromosomes have been replicated and that the replicated DNA is not damaged. Also similar to the G1-S checkpoint, the cell cycle will arrest at the G2-M transition if checkpoint criteria are not met and will attempt to repair the damage or undergo apoptosis if damaged severely.

**The M Checkpoint**

The M checkpoint occurs near the end of the metaphase stage of mitosis. The M checkpoint is also known as the spindle checkpoint because it determines if all the sister chromatids are correctly attached to the spindle microtubules. Because the separation of the sister chromatids during anaphase is an irreversible step, the cycle will not proceed until the kinetochores of each pair of sister chromatids are firmly anchored to spindle fibers arising from opposite poles of the cell. As with the previous checkpoints, cells that cannot proceed past this checkpoint will be eliminated by apoptosis.

## Regulator Molecules of the Cell Cycle

In addition to the internally controlled checkpoints, two groups of intracellular molecules regulate the cell cycle. These regulatory molecules either promote the progress of the cell to the next phase (positive regulation) or halt the cycle (negative regulation). Regulator molecules may act individually, or they can influence the activity or production of other regulatory proteins. Therefore, the failure of a single regulator may have almost no effect on the cell cycle, especially if more than one mechanism controls the same event. However, the impact of a deficient or non-functioning regulator can be wide-ranging and possibly fatal to the cell if multiple processes are affected.

**Positive Regulation of the Cell Cycle**

Two groups of proteins, called **cyclins** and **cyclin-dependent kinases (Cdks),** are termed, positive regulators. They are responsible for the progress of the cell through the various checkpoints. The levels of **cyclin** proteins fluctuate throughout the cell cycle in a predictable pattern (This is the origin of their name. Cyclin levels “cycle” up and down throughout the cell cycle.). Both external and internal signals trigger increases in the trigger the expression of cyclins that are specific to each stage of the cell cycle. After the cell moves to the next stage of the cell cycle, the cyclins that were active in the previous stage are polyubiquitinated and degraded by the proteasome. One such cyclin, M-cyclin, which is associated with the M-phase of the cell cycle, is described in Figure 13-8.

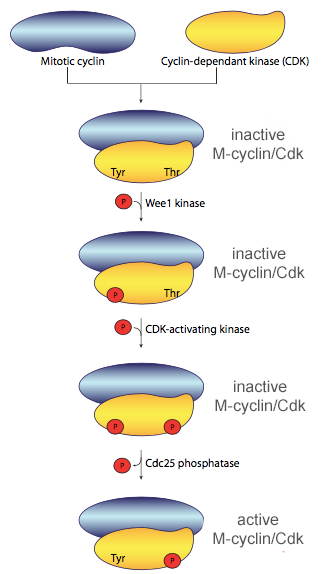

M-cyclin is the cyclin associated with the M-phase of the cell cycle. Cellular levels of M-cyclin steadily grow throughout the G1, S, and G2 phases. M-cyclin levels are at their highest during M-phase. After M-phase, there is a sharp decline of M-cyclin levels, as the cyclin is degraded by the proteasome


**Figure 13-8: M Cyclin**. M-cyclin is the cyclin associated with the M-phase of the cell cycle. Cellular levels of M-cyclin steadily grow throughout the G1, S, and G2 phases. M-cyclin levels are at their highest during M-phase. After M-phase, there is a sharp decline of M-cyclin levels, as the cyclin is degraded by the proteasome.

Cyclins regulate the cell cycle only when they are tightly bound to **Cdks**. To be fully active, the Cdk/cyclin complex must also be phosphorylated in specific locations. Like all kinases, Cdks are kinase enzymes that phosphorylate other proteins. Phosphorylation activates the protein by changing its shape. The proteins phosphorylated by Cdks are involved in advancing the cell to the next phase. The levels of Cdk proteins are relatively stable throughout the cell cycle; however, the concentrations of cyclin fluctuate and determine when active Cdk/cyclin complexes form. The different cyclins and Cdks bind at specific points in the cell cycle and thus regulate different checkpoints.

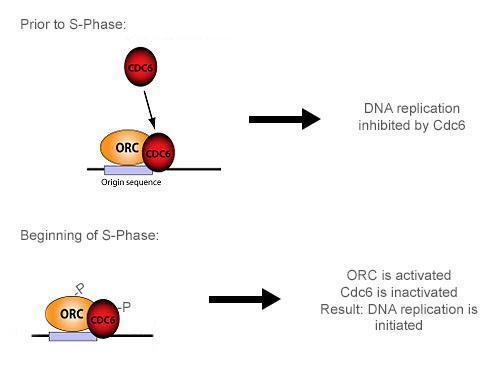
In eukaryotes, cyclins and Cdks are designated with letters and numbers, respectively (examples: Cyclin B and Cdk2). For the purposes of this text, cyclin and Cdks will be named after the stage of the cell cycle where they are active. For example, “M cyclin” refers to the cyclin that is at its highest level during M phase, and “G1-S cyclin/Cdk” refers to the cyclin/Cdk complex active at the transition between the G1 and S phases.

In some cases, cyclin/Cdk complexes must be further modified before Cdks have full kinase activity. Often Cdks are phosphorylated at “activating” sites on the protein and dephosphorylated at “inhibitory” sites. These sites are sidechains of specific serine, tyrosine, or threonine residues within the protein. One example is the activation of the **M-cyclin/Cdk** complex (Figure 13-9)**.** Before M-phase, the M-cyclin/Cdk complex is phosphorylated at an inhibitory site by the kinase **Wee1**, inactivating M-cyclin/Cdk activity. Next, M-cyclin/Cdk is phosphorylated at an activating site by **CDK-activating kinases (Cak)**. Lastly, at the beginning of M-phase, the phosphatase **Cdc25** removes the inhibitory phosphate, which leads to the activation of M-cyclin/Cdk. Active M-cyclin/Cdk can then phosphorylates target proteins needed in the progression of M-phase, such as APC and lamins.



**Figure 13-9: Activation of mitotic cyclin/Cdk complex.** The M-Phase cyclin/Cdk complex is inactive until inhibitory phosphates have been removed and activating phosphates have been added to the protein. This ensures the specific timing of M-Phase cyclin/Cdk kinase activity in the cell cycle.

Another example of cyclin/Cdk activity occurs at the transition between the G1 and S phases. In G1, proteins needed to initiate DNA replication and an inhibitory protein called **Cdc6** assemble at origins of replication (Figure 13-10) (refer to Chapter 13 for more on Cdc6 and its role in DNA replication). This is called the pre-replication complex and forms to prevent DNA replication until the appropriate time in the cell cycle. At the beginning of S-phase, the S-cyclin/Cdk complex phosphorylates proteins in the pre-replication complex to recruit helicases and DNA polymerases to the origin of replication. The S-cyclin/Cdk complex phosphorylates Cdc6, which inactivates the inhibitory protein. Collectively, this ensures that DNA replication occurs, and only once, during the cell cycle.



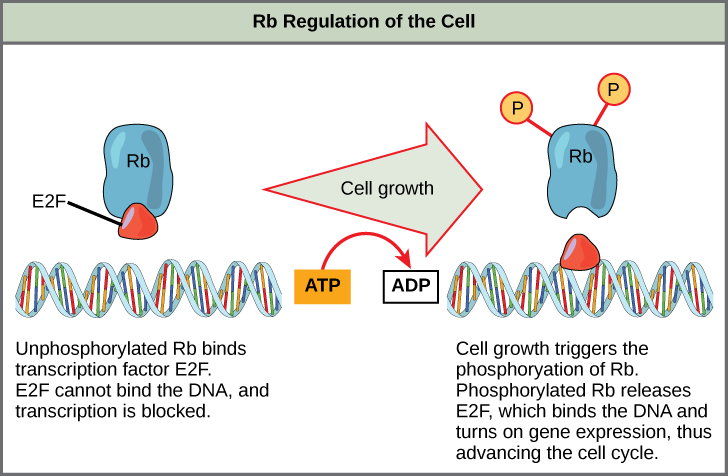
**Figure 13-10: Regulation of the initiation of DNA Replication by the S-Cdk/cyclin complex.** Cdc6 is a protein that binds to the pre-replication complex to prevent the start of DNA replication. At the beginning of S-phase, the S-cyclin/Cdk complex phosphorylates Cdc6 to inactive it. The S-cyclin/Cdk complex also phosphorylates proteins in the pre-replication complex to recruit enzymes needed for DNA replication.

**Negative Regulation of the Cell Cycle**

The second group of cell-cycle regulatory molecules is negative regulators, which stop the cell cycle in comparison to what we just discussed where positive regulators cause the cell cycle to progress. The best understood negative regulatory molecules are retinoblastoma protein and p53.

**Retinoblastoma (Rb)** proteins are a group of tumor-suppressor proteins, which function to prevent tumor growth by inhibiting cell division (refer to Chapter 15 for more on tumor suppressors). Much of what is known about cell-cycle regulation comes from research conducted with cells that have lost regulatory control. All three of these regulatory proteins were discovered to be damaged or non-functional in cells that had begun to replicate uncontrollably (i.e., became cancerous). In each case, the main cause of the unchecked progress through the cell cycle was a faulty copy of the regulatory protein.

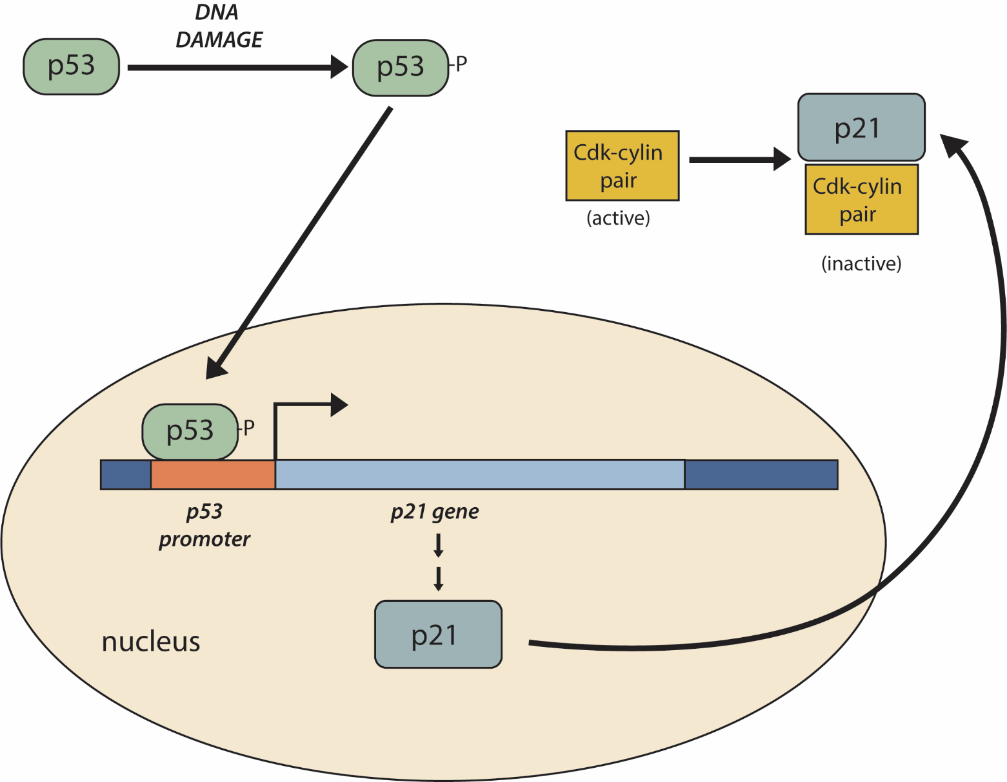
Rb primarily acts primarily the G1-S checkpoint. Rb, which largely monitors cell size, exerts its regulatory influence on other positive regulator proteins. In the active, dephosphorylated state, Rb binds to proteins called transcription factors, most commonly, E2F. Transcription factors “turn on” specific genes, allowing the production of proteins encoded by that gene. When Rb is bound to E2F, the production of proteins necessary for the G1/S transition is blocked. In response to growth factor signaling molecules (refer to Chapter 12 for more on growth factor signaling) the cell will increase in size. This triggers the phosphorylation of Rb by the G1-S cyclin/Cdk complex. Phosphorylation of Rb causes its inactivation, resulting in the release E2F and the expression of proteins needed to progress to S phase (Figure 13-11).



**Figure 13-11: Retinoblastoma.** Retinoblastoma (Rb) binds to and inhibits the transcription factor E2F. At the G1-S checkpoint, the G1-S cyclin/Cdk complex phosphorylates Rb in response to growth factor signaling. When phosphorylated Rb, “releases” E2F. E2F can now bind to its promoter region to stimulate the expression of genes needed to advance in the cell cycle, such as cyclin genes.

Another important negative regulator of the cell cycle is the **tumor suppressor p53**. p53 is a multi-functional protein that has a major impact on the commitment of a cell to division because it acts when there is damaged DNA in cells that are undergoing preparatory processes during G1 (and G2, to a lesser extent). p53 is a transcription factor that regulates the expression of over 50 genes that control cell cycle arrest, DNA damage repair, and cell death. Additionally, p53 has transcription factor-independent functions in cells. p53 is often referred to as the “guardian of the genome” because of the significant role it plays in regulating the cell cycle.

One example of the actions of p53 occurs at the G1-S checkpoint (Figure 13-12). In a resting cell, p53 is produced in the cytoplasm and rapidly degraded by the cell. If damaged DNA is detected, p53 is phosphorylated, which stabilizes p53 and prevents its degradation. Phosphorylated p53 translocates to the nucleus where it binds to its promoter to induce the expression of the gene **p21**. p21 is a cyclin inhibitor protein, meaning it can bind to and inhibit the activity of cyclin/Cdk complexes. Inhibition of cyclin/Cdks by p21 causes the cell cycle to arrest. This allows time for repair of DNA damage through the actions of other p53-controlled genes. If the DNA cannot be repaired, p53 can trigger apoptosis to prevent the duplication of damaged chromosomes.



**Figure 13-12: p53 and p21.** The tumor suppressor p53 is produced and then rapidly degraded by the cell. In response to DNA damage, p53 is phosphorylated. This stabilizes p53 and prevents its degradation. p53 is a transcription factor with over 50 target genes that collectively control cell cycle arrest, DNA damage repair, and apoptosis. One of these targets is the p21 which binds to and inhibits the kinase activity of Cdk/cyclin complexes. When DNA is damaged, the stabilized p53 increases the expression of p21. p21 then binds to Cdk/cyclin complexes, causing a “pause” in the cell cycle. This allows time for DNA damage repair to occur. If the damage cannot be repaired, apoptosis will be initiated by other p53-target genes.

Associated Videos:

* [Mitosis: The Amazing Cell Process that Uses Division to Multiply! Amoeba Sisters](https://www.youtube.com/watch?v=f-ldPgEfAHI)
* [Mitosis: Splitting Up is Complicated. Crash Course Biology](https://www.youtube.com/watch?v=L0k-enzoeOM)