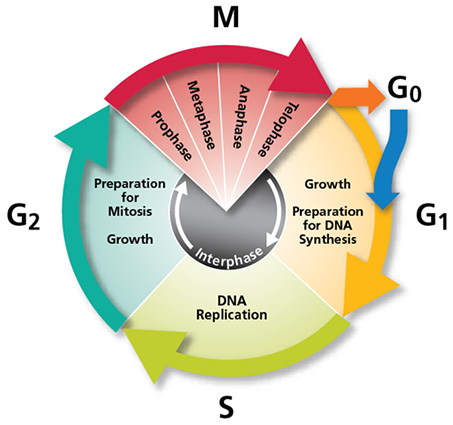
**PROLIFERATION AND DIFFERENTIATION**

The way in which the number of cells increases is similar for all somatic cells and involves the growth of all cellular components. Cell proliferation is an increase in the number of cells through cell reproduction- cell division. It is an essential process for all living organisms. During reproduction, one cell (mother) divides to form two cells (daughters).

In order for cell division to be complete, it is necessary to transfer intact genetic information to the next generation- the production of two genetically identical daughter cells. The DNA in each chromosome must be copied correctly and the newly formed chromosomes must be precisely separated into two daughter cells so that each daughter cell receives a copy of the same genome. Eukaryotic cells have evolved a complex network of regulatory proteins, known as the cell cycle control system, that controls the progression of the cell cycle. At the core of this system is a series of biochemical switches that trigger major cycle events including DNA replication and segregation. In most cells, additional levels of cell cycle control enable more precise cell division and enable the cell to respond adequately to both intracellular and extracellular signals. Inside the cell, control mechanisms monitor the progression of the cell cycle and delay later stages until the initial events are complete (for example, preventing preparation for segregation until DNA replication is complete). The cell "monitors" events in the external environment and responds to signals originating from surrounding cells. Thus, the cell responds to signals from the outside by dividing or stopping the cell cycle. The cell cycle control system plays a key role in regulating the number of cells in a tissue.

In 1951, Howard and Peltz, studying plant cell division, divided the process into four phases: GAP1, synthetic phase, GAP2 and mitosis (G1, S, G2 and M). This process is now called the cell cycle (Figure 1).

The cell cycle is a tightly coordinated program for cell growth and division. During one cell cycle, DNA replication takes place in the S-phase (phase of DNA synthesis) so that at the end of this phase the cell has two complete sets of DNA (one for each daughter cell). Upon entering the M-phase (phase of mitosis), the cell divides and two genetically identical daughter cells are formed, which completes the cell cycle. Two key events in the cell cycle are DNA synthesis, which mostly takes place in the S phase, and cell division, in the M phase. The phase of DNA synthesis and the phase of cell division are separated by the growth and reorganization phases, that is, by the G1 and G2 phases (from the English Gap 1 and Gap 2). The G1 phase is a preparatory phase characterized by gene expression and protein synthesis important for cell growth and DNA synthesis and it precedes the S-phase. It is the only phase of the cycle that is primarily controlled by extracellular stimuli such as various growth factors, but also oncogenes and tumor suppressor genes (anti-oncogenes) that mainly act in this phase of the cycle as endogenous regulators. Tumor cells are less dependent on extracellular signals than normal cells. The G2 phase is the second preparatory phase during which the cell continues to grow and prepares enzymes and other active molecules necessary for cell division. After division, the cell cycle can be stopped by entering the resting phase (G0 phase) when the cell does not divide but performs all the functions for which it is destined. Some cells in this phase, if adequately activated, can start the division cycle again. Cells in the G0 phase are smaller than those in the G1 phase and have less RNA and protein.

Figure 1: phases of the cell cycle

Entering the G1 phase, the cell divides again. Such a cell is in the proliferative phase and is part of the proliferative fraction in the tissue. On average, about 20% of the cells in typical cancers are in the proliferative phase at any given time. Some normal tissues, such as bone marrow and mucosa of the digestive tract, have a higher proliferative fraction than many tumors, even tumors of the same tissues. A cell that enters the prolonged G0 phase is in the resting phase (non-proliferative fraction). Some differentiated cells, such as neurons, are permanently non-proliferative. Most terminally differentiated cells, such as polymorphonuclear leukocytes, have a defined lifespan. Many non-proliferative cells are in an unstable G0 phase and can enter the G1 phase under appropriate extracellular signals. Stem cells, like neurons, have a long lifespan. However, stem cells, as well as cells in the unstable G0 phase, can proliferate periodically. If chemotherapy primarily kills cells in the proliferative phase, the ability of tumor cells to persist in the G0 phase for a longer period of time may be one of the reasons for the lack of response to therapy.

Mammalian somatic cells contain a diploid number of chromosomes. Cells that do not enter the cell cycle and remain in the G0 phase have diploid DNA content.

**Cell cycle control**

The cell cycle control system functions as a trigger that allows the events in the cell cycle to occur in the correct order. It is independent of the events it controls and thus continues to control even if some of the cell cycle events fail to complete. However, in most cells the control system reacts to the information it receives about the process it controls. Sensors detect the final phase of DNA synthesis and if some malfunction prevents the successful completion of this process, signals are sent to the control system which then prevents the transition to the M phase. In this way, time is provided for the correction of the error and prevents a possible premature entry into the next phase of the cell cycle with an incomplete copy of the chromosome.

The cell cycle control system consists of a series of interconnected biochemical switches, each of which initiates a specific event in the cell cycle. Switches trigger the event completely, which is very important because it excludes the possibility of a partial start or a started but not completed process. The control system is highly adaptive and can be modified depending on the cell type and to respond to different intracellular and extracellular signals.

The cell cycle is regulated at several levels. The mechanisms underlying cell cycle control and regulation are remarkably conserved. The control of this process is achieved at the level of three large groups of genes such as proto-oncogenes, anti-oncogenes and genes of the DNA repair system. There are at least two types of cell cycle control mechanisms: a cascade of proteins whose phosphorylation allows the cell to move to the next phase of the cycle and a set of control points that serve to check possible errors during division, and in the event of an error delay the entry of the cell into the next phase of the cell cycle and enable their correction .

Three groups of proteins are most important for the continuation of the cell cycle:

* Cyclin dependent kinases (Cdk), enzymes that phosphorylate intracellular proteins and thereby activate them
* Cyclins, proteins that activate cyclin-dependent kinases
* Inhibitors of cyclin-dependent kinases, when present in sufficient quantity, prevent the activity of cyclin-dependent kinases

The main components of the cell cycle control system are members of a family of protein kinases known as cyclin-dependent kinases. There are four types of cyclin-dependent kinases: G1-CDKs, G1/S-CDKs, S-CDKs and M-CDKs. The activity of these kinases increases and decreases as the cell passes through different phases of the cell cycle, allowing cyclic changes in the phosphorylation of intracellular proteins that initiate or regulate major events in the cell cycle.

Cyclic changes in the activity of cyclin-dependent kinases are controlled by a special group of proteins called cyclins. For cyclin-dependent kinase to be activated, it is necessary to be tightly bound to cyclin. Cyclins are proteins divided into several classes: D (1, 2, 3), E, ​​A and B (Figure 2). They form complexes with cyclin-dependent kinases - CDKs, their catalytic partner (Figure 3).

Cyclin-dependent kinases are enzymes and their activation requires cyclins, whose expression is transient so that their concentration changes depending on the phase of the cell cycle (cyclin = cycle = circulate). Coordinated phosphorylation and dephosphorylation of the cyclin-CDK complex determines the clear transition of the cell to the appropriate phase of the cycle. The cyclin-CDK complex, i.e. its activity can be blocked by CDK inhibitors. Some oncogenes (eg Myc) block the action of CDK inhibitors and allow uncontrolled cell proliferation.

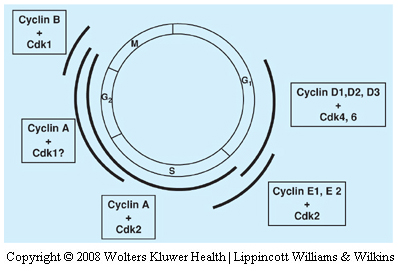


Figure 2. Distribution of cyclins by phases of the cell cycle

We distinguish four classes of cyclins, defined according to the stage of the cell cycle during which they bind to CDK. The three classes of cyclins essential to every eukaryotic cell are:

* G1/S cyclins, which bind to CDK in late G1 phase, allowing the cell to progress through the cell cycle restriction point. Their values ​​decrease in the S phase.
* S cyclins, binding CDK stimulate chromosome duplication. They are active until mitosis and contribute to the regulation of early mitotic events.
* M cyclins activate CDK at the level of the G2/M checkpoint, and stimulate cell entry into mitosis.

A fourth class of cyclins is the G1 cyclins that govern the activity of G1/S cyclins. In addition to activation, cyclins direct cyclin-dependent kinases to target proteins so that the cyclin-cyclin-dependent kinase complex phosphorylates the corresponding proteins. The same complex achieves different effects during different phases of the cell cycle, due to the change of the substrate of the complex during the cell cycle.

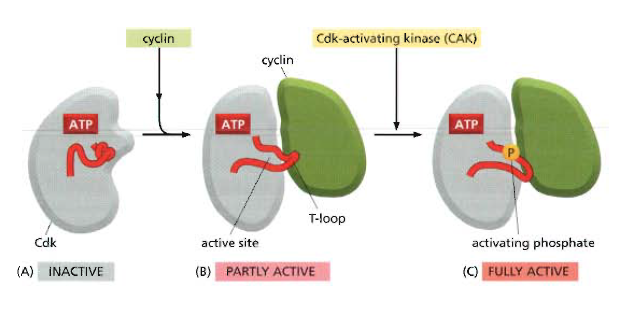


Figure 3: the active site of a cyclin-dependent kinase in the absence of cyclin is covered by a special protein called the T-loop. In the presence of kinase, the T-loop protein moves and partially activates CDK. Phosphorylation of CDK changes the shape of the T-loop, which enables better binding to cyclin and thus complete activation of CDK.

**Cell cycle inhibition**

The icrement and decrement of cyclin levels is a key factor that determines the activity of cyclin-dependent kinases during the cell cycle. Binding of a cyclin-dependent kinase inhibitor (CKI) regulates the activity of the cyclin-CDK complex. Binding of the inhibitor causes a rearrangement within the active site itself rendering it inactive. Cells use cyclin-dependent kinase inhibitors primarily to regulate G1/S-CDK and S-CDK activity early in the cell cycle.

**Extracellular control of the cell cycle**

Extracellular signaling molecules that regulate cell size and number are soluble, secretory proteins that bind to the cell surface or are components of the extracellular matrix. They are divided into three groups:

* Mitogens stimulate cell division by activating G1/S Cdk
* Growth factors stimulate cell growth (increase cell mass) by activating the synthesis of proteins and other macromolecules and inhibiting their degradation
* Survival factors enable cell survival by suppressing apoptosis.

Unlike unicellular organisms that grow and divide as fast as they can, multicellular organisms divide only when there is a need for it. For this to happen, it is necessary to have a stimulatory extracellular signal in the form of a mitogen from another cell, usually from the environment. Mitogens override intracellular inhibitory mechanisms that block the progression of the cell cycle.

Platelet-derived growth factor (PDGF) is one of over 50 proteins known to act as a mitogen. PDGF can stimulate various types of cells to divide, including fibroblasts, smooth muscle cells, and neuroglia. Epidermal growth factor (EGF) acts not only on epidermal cells but also on numerous other cells including epithelial and non-epithelial cells. However, there are also mitogens that exclusively act on one type of cell. Erythropoietin, for example, induces proliferation exclusively of red blood cell precursors. Many mitogens like PDGF have roles other than stimulating cell division- they can stimulate cell growth, survival, differentiation or migration depending on the circumstances or cell type.

Like mitogens, extracellular growth factors bind to receptors on the cell surface and activate intracellular signaling pathways, thereby enabling cell growth. These signaling pathways stimulate the accumulation of proteins and other macromolecules, which increases their synthesis and decreases their degradation. At the same time, they promote greater entry of nutrients into the cell and increased production of ATP, which is necessary for the synthesis of molecules.

In some tissues, inhibitory extracellular signaling proteins override positive regulators and thereby inhibit cell growth. One such is TGF-β, which acts to inhibit the proliferation of several types of cells by blocking the progression of the cell cycle in the G1 phase or by stimulating cell death.

In the absence of mitogens, various mechanisms inhibit CDK in the G1 phase and thereby block further cell cycle progression. In some situations, cells can enter a special G0 phase. The largest number of cells in our body is in the G0 phase. For example, neurons and skeletal muscle cells are in the resting G0 phase where the cell cycle is stopped. Expression of genes encoding CDK and cyclin synthesis is reduced and cell division occurs very rarely. The largest number of liver cells is also in the G0 phase, but in situations where the liver is damaged, the cells start dividing thanks to the stimuli that activate them. However, there are also cells such as fibroblasts and some lymphocytes that exit and immediately enter a new cell cycle throughout life. Therefore, the length of the cell cycle mostly depends on the period the cell spends in G0 and G1 phase.

**DNA damage blocks the cell cycle. Regulation of G2/M transition.**

The second type of regulation implies the presence of control (surveillance) points in the cell cycle. In addition to mitogens and other extracellular and intracellular mechanisms, an extremely important mechanism for cell cycle control is DNA damage. Cells constantly suffer damage to the genetic material, which can originate from the cell itself as by-products of metabolism or from the environment such as chemical agents or radiation. Most often, damage to the genetic material occurs during the S phase as a result of an error in the DNA synthesis process. The cell cycle control system detects DNA damage and blocks the cycle at both checkpoints- at the Start point in the late G1 phase, which prevents entry into the S phase, and at the G2/M point, which prevents entry into mitosis. At these points it is possible to check for errors in the genetic material.

Although the responses to different forms of DNA damage are not identical, they are similar enough to generalize the principle of action. DNA damage of various forms is initially detected by a protein complex bound to DNA. In mammalian cells, two proteins (protein kinases) ATM and ATR are sensors that are activated by DNA damage in all stages of the cell cycle (Figure 4). ATM and ATR that bind to the damaged site and phosphorylate various target proteins including two protein kinases Chk1 and Chk2. Together, these different kinases phosphorylate other target proteins that arrest the cell cycle. The main target is the regulatory protein p53 which stimulates the transcription of genes encoding a CKI protein called p21. A high level of this inhibitor blocks the activity of CDK2, and most likely CDK4 and CDK6, which arrests the cell cycle in the G1 phase. The basic mechanism of action on damage registered in the G2 phase is independent of p53. It involves two effector proteins, protein kinases known as chk1 and chk2 (inhibitors of cyclin-dependent kinases). The mentioned proteins dephosphorylate cyclin-dependent kinase1 (CDK1). Thus, in response to DNA damage, inhibited cyclin B-CDK complexes accumulate in cells in the G2 phase of the cell cycle and prevent the continuation of the cell cycle. DNA damage registered during the S phase of cells is responded to by chk1 and chk2 kinases that dephosphorylate CDK2.

The cell cycle is stopped until the error is corrected, and in the case that it cannot correct it, other signals are activated that introduce such a genetically modified cell into cell death (apoptosis). Also, the cell will not enter the M phase if the S phase is not completed.

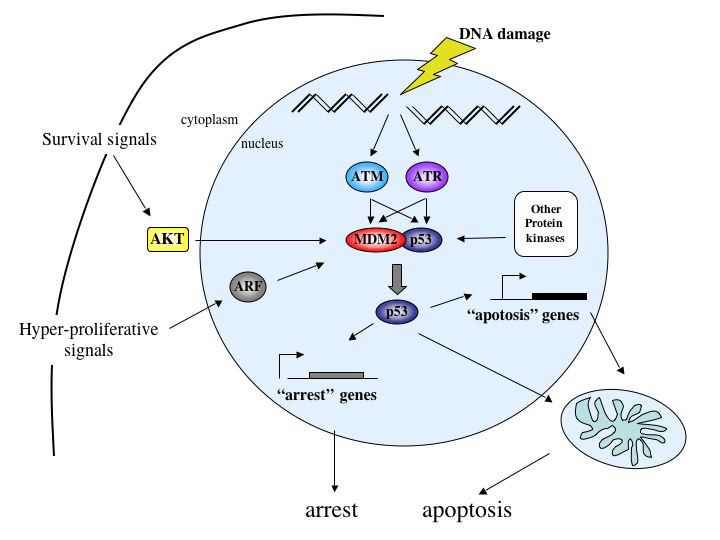


Figure 4. Cell response to DNA damage

**Cell differentiation**

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| Cell differentiation is an adaptive process characterized by the expression of certain genes that dictate the synthesis of a series of proteins forming a specific cellular phenotype. |

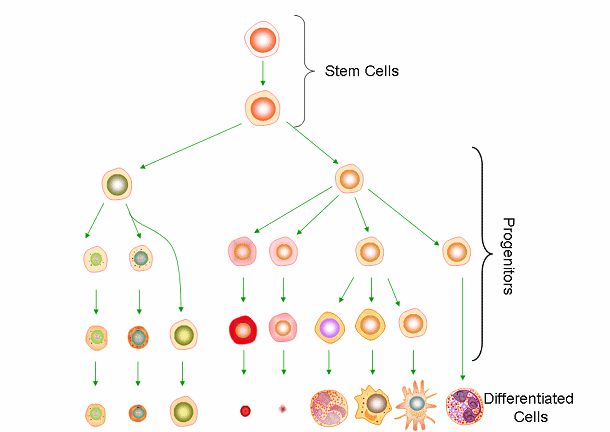
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Figure 5. Schematic representation of hematopoietic stem cell differentiation

Cell differentiation represents the maturation of cells and their progeny during several consecutive cell cycles. This biological process is accompanied by the expression of a part of the genome, that is, specific sets of genes that control protein synthesis, thus determining the structure and function of the cell. In the process of differentiation from non-specialized cells, specialized cells are created to perform one, and sometimes more, specific functions. Such cells are called differentiated cells. Between stem and differentiated cells there are several generations of increasingly specialized cells called transitional cells.

All cells of an organism come from a fertilized egg cell. Almost all cells of an organism "carry" the same genetic material, originating from the mentioned fertilized egg cell. Cells are phenotypically very different from each other even though they have the same genetic material. During the embryonic development of the organism, cells localized in different parts of the embryo begin to adapt structurally, acquire different phenotypic characteristics and enter the process of differentiation. Differentiated cells form different tissues and contain the same set of genes.

Exit from the cell cycle is a component and the first step of cell differentiation. Although cell cycle exit and postmitotic differentiation differ from cell to cell in many aspects, from the perspective of cell cycle control they have much in common.

First, cell cycle exit is usually associated, at least initially, with the accumulation of the G1/S inhibitor cdk. The concentration of members of the INK4 family of proteins that target CDK4 and CDK6 and members of the Cip/Kip family as well as Rb protein- p130 that inhibits CDK2 increases significantly. This causes the cells to "lag" in the G1 phase from which the cells can exit the cell cycle.

The entry and exit of the cell from the cell cycle is mediated by growth factors and mitogens that interact with membrane receptors, which leads to the emergence of a cascade of intracellular signaling pathways that regulate the dynamics of protein synthesis, as well as the transcription of genes that promote proliferation (CDKs and cyclins).

Differentiation is characterized by the expression of certain genes characteristic of that type of cells. These genes dictate the synthesis of a series of proteins that form a specific cellular phenotype.

It can be concluded that the phenotype of each differentiated cell of the organism is basically determined by the specific set of genes that are expressed in that type of cell. All genes in mammalian cells can be classified into two large groups: housekeeping and tissue-specific genes. Most genes encode proteins that are necessary for the life of all types of cells or for the performance of certain biological functions common to all cells. These genes, expressed in all cells, are housekeeping genes. In each differentiated cell, housekeeping genes make up the vast majority of expressed genes. The minority of expressed genes in each differentiated cell are represented by tissue-specific genes. They are "responsible" for the protein synthesis and phenotype that are characteristic of cells of a certain differentiation. In one differentiated cell, 10,000 to 15,000 housekeeping genes are expressed, while some 1000 expressed tissue-specific genes are responsible for the specific characteristics of the differentiated cell. Simple math leads to the number of about 15,000 unexpressed genes of the human genome in one differentiated cell. These genes are not necessary for specific cell differentiation or for housekeeping needs.

In the process of differentiation, a large number of genes are expressed temporally and spatially synchronized, while others remain inactive, with the aim of the cell developing a specific phenotype. Transcription factors are responsible for such coordinated expression. These proteins bind to specific DNA sequences present in the control regions of each gene and determine whether the gene will be transcribed or not. In a still incompletely explained way, transcription factors allow the enzyme RNA polymerase to access genes. Other transcription factors, on the other hand, block access to genes and thus ensure the inactivity of those genes. Gene control regions contain series of short nucleotide sequences that are recognized by specific transcription factors, bind to them and "take control" of the transcription of a given gene. The presence or absence of these short DNA sequences (called enhancers) determines whether the transcription factor can bind to the control region of the gene. The control sequence is generally localized near the transcription start site. A gene can be divided into two functionally significant regions: a non-transcriptional control sequence and a transcriptional sequence. In many genes, control sequences are inserted into the transcriptional region of the gene, mostly in introns.

The cell controls gene expression at multiple levels from DNA to protein. When and how often a particular gene is expressed represents transcriptional control. The cell then controls the modifications of information RNA after transcription, its transport and localization in the cytosol, control of translation and degradation of RNA molecules, as well as protein activity.

Gene expression in a specialized cell can be modified by extracellular factors. In addition, after the termination of the action of the extracellular factor that induced changes in the gene expression of the cell of a multicellular organism, the cell continues the normal path of differentiation into a specific cell type. This phenomenon is called cellular memory and is the basis for the formation of stable differentiated cell lines and organized tissue