The Cell Cycle: Regulation, Apoptosis, Mitosis and Replication of DNA

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This article explains the semiconservative replication of DNA and the role it plays in the cell cycle. The phases of the cell cycle and mitosis (prophase, metaphase, anaphase, and telophase), as well as the regulatory mechanisms associated with cyclins and their antagonists, are all relevant topics in medical exams.

The Cell Cycle

The cell cycle describes the cyclic sequence of events in eukaryotic, proliferating cells. Its purpose is to enable the identical duplication of a cell into two daughter cells. The cell cycle consists of four stages:

- **G1 phase (gap 1):** Cell growth, RNA and protein synthesis (3-12 hours)
- **S phase:** DNA replication, centriole separation, synthesis of histones; chromosomes double from a diploid set to a tetraploid set of chromosomes (8-12 hours)
- **G2 phase:** Quality assurance (ensuring accuracy of replication, DNA mismatch repair), preparation for mitosis (1.5-3 hours)
- **M phase:** Alongside prophase, metaphase, anaphase, and telophase, the creation of the spindle apparatus, chromatid separation and cell division
(cytokinesis also karyokinesis) also occurs; division of the tetraploid set of chromosomes into two daughter cells (0.5–1 hour)

The G1, S, and G2 phases are also grouped together as **interphase**.

In the reversible **G0 phase**, in which the cell can enter after an M phase, cells are said to be in a **quiescent state**. Triggers for this stage are a certain degree of differentiation, a low concentration of growth factors, or a high population density. In this state, which can be permanent, normal metabolism occurs but there is no cell division. With particular triggers, such as growth factors, some cells are able to return to the G1 phase.

The **G1 phase** is a growth phase during which proteins and cellular constituents required for mitosis, such as microtubules, for example, are created.

In the **S phase**, the synthesis phase, DNA is replicated (see DNA replication) to create a tetraploid set of chromosomes from a diploid set. In the tetraploid set, every chromosome is created from two sister chromatids that are attached to the kinetochore.

In the G2 phase that follows, specific proteins that are required for cell division, such as RNA molecules, are synthesized in preparation for mitosis. After replication checks and controls, and if necessary, the use of repair mechanisms, the cell completes the interphase stage and is ready for mitosis.

**Cell Division: Mitosis**
Mitosis is subdivided into prophase, metaphase, anaphase, and telophase.

In **prophase**, the chromosomes condense and become visible as a result of this condensation. Microtubules form the spindle structure along the centrioles, which are located on opposite poles of the cell. The nucleolus is no longer visible and the dissolution of the nuclear envelope begins.

During **metaphase**, the microtubules of the spindle apparatus connect to the kinetochores of the chromosomes, which are aligned along the equatorial region of the cell. The nuclear envelope completely dissolves.

**Anaphase** (from Greek *aná* = of each, in equal quantities) can be imagined as a cut through the cell along the kinetochores of the equatorially aligned chromosomes; double-chromatid chromosomes become single chromatid chromosomes which then migrate to the opposite poles of the cell along the spindle structures, thus dividing themselves into the two resultant daughter cells. Each daughter cell has a complete genome.

During **telophase**, both of the daughter cells grow new nuclear envelopes around the decondensing chromosomes. The spindle apparatus dissolves and each of the daughter cells develops its own complete cell membrane after which both daughter cells are completely separated from each other (**cytokinesis**).

**Regulation of the Cell Cycle**

A control system ensures that the cell cycle proceeds in a regulated and not uncontrolled manner: the cell cycle can only proceed to the next phase when specific signaling substances are present at each of the so-called **restriction points**. Regulatory errors can lead to unregulated cell growth and can result in serious illnesses such as cancer.

Below are the elements of the **cell cycle control system**:

- **Cyclins** are proteins released at specific stages that arrest the cell cycle if
they are not present in the right concentration and at the right time. The Nuclear Localization Sequence (NLS) allows cyclins to enter the cell nucleus as a heterodimer with CDKs.

- **Cyclin-dependent kinases CDKs**, (also known as cyclin activating kinases, CAKs) are protein kinases which are activated by cyclins and **protein kinases** (phosphorylation) and are inhibited by **protein phosphatases** (dephosphorylation) and **cyclin-dependent kinase inhibitors (CKIs)**.

At the **restriction points**

- in the later G1 phase,
- at the end of the G2 phase,
- during metaphase,

the cell cycle either makes its progress irreversibly in the presence of the correct endogenous and exogenous factors or is arrested in their absence.

The **transition between the G1 and S phase** is decisive in terms of whether the cell progresses to cell division or remains dormant in the G0 phase. The cell cycle starts when the cell has a sufficient minimum size, appropriate nutritional conditions, and stimulation by growth factors in addition to the absence of anti-mitogenic signals.

**Rb protein** plays an important role: if hyperphosphorylation occurs due to cyclin-D/CDK 4/6 dimers, the transcription of cyclin E and other proteins necessary for progression to the S phase begins. CKIs can have an inhibitory role in the transition between the G1 and S phases.

In the latter stages of the **G2 phase** of mitosis, the environmental conditions are checked once again along with the correct and complete replication of DNA. An incomplete genome at this stage will result in cell death.

During **metaphase**, checks are done to ensure all chromosomes are adhered to the microtubules of the spindle apparatus prior to them being separated in the anaphase.

During the G1 and G2 phases, there are further **DNA damage checkpoints**. This way, if the DNA has become damaged due to, e.g., ionizing radiation, the cell cycle can be arrested until the damage has been repaired or, if the DNA is irreparable, apoptosis is triggered.
Apoptosis

Apoptosis is the physiological, genetically programmed cell death, which allows for the targeted elimination of dysfunctional endogenous cells—a very different process from pathological necrosis, which is initiated by, e.g., certain pollutants or drugs or other external damaging mechanisms. Necrosis leads to osmotic swelling of the cells, the membrane bursting, and leakage of the cell’s contents into the intercellular space, causing an inflammatory reaction.

Typically, the initiation of apoptosis leads to cellular shrinkage, deformation, and a loss of contact to neighboring cells, but not to inflammation. The chromatin condenses in the nucleus of the cell, and endonucleases break the DNA into fragments of around 200 base pairs (bp). Then the cell itself is digested within the membrane vesicle in the so-called apoptotic bodies, which then undergo phagocytosis by macrophages.

Apoptosis is an essential part of many physiological processes. These include the differentiation of an organism (e.g., dissolution of the tissue between the fingers during the embryonic stages of development), the development of immunotolerance (e.g., elimination of hyperreactive T lymphocytes), the homeostasis of the cell count (e.g., regular replacement of a mucous membrane), and the removal of defective or infected cells (e.g., elimination of erythrocytes that get stuck in the spleen.)

Processes of Apoptosis

The following substances are part of apoptosis:

- **Caspases** (cysteine-dependent aspartate-directed proteases—they cut off aspartate behind the amino acid): They exist as inactive precursors (procaspases), which contribute as either initiator caspases, that help to initiate apoptosis, or as effector caspases, inducing the cell’s death.
- **Mitochondrial proteins of the Bcl-2 family**, which have either a pro- or anti-apoptotic effect on the state of the mitochondria, which in turn have a decisive influence on apoptosis.
- **Inhibitor of Apoptosis Proteins (IAPs)**, which modify certain caspases through ubiquitination and therefore lead to their restriction.
Distinctions can also be made between extrinsically initiated apoptosis, intrinsically initiated apoptosis and the perforin/granzyme pathway. All of these have a common final pathway via effector caspases.

Extrinsic Signaling Pathway of Apoptosis

The extrinsic signaling pathway is also called receptor-mediated apoptosis as ligands bind to death receptors in this process. These death receptors belong to the tumor necrosis factor receptor (TNFR) superfamily.

In order to activate the signaling pathway, cells like cytotoxic T cells, for example, can express matching ligands, such as the Fas Ligand. If the Fas molecules bind to the surface of the target cell, they become activated trimers and bind the intracellular adaptor molecules with their cytosolic section.

The complex of the receptor trimer and the adapter molecule is also known as DISC (death-inducing signaling complex), and it binds to the procaspase 8 molecule in such high concentrations that they activate themselves by autocatalytic cleavage. Caspase 8 then activates the proteolytic cleavage of procaspases 3, 6 and 7, i.e., the effector caspases of the common final pathway.

There are many substrates of effector caspases which are proteins that are vital for cell survival: proteins of the cytoskeleton and the nuclear membrane, endonuclease inhibitors, protein kinases, transcription factors, and complexes created from hnRNA and proteins (hnRNPs).
Intrinsic Signaling Pathway of Apoptosis

If the cell experiences intracellular stress, such as oxidative stress, then no receptors are necessary to initiate apoptosis as the mitochondria fulfill this role. When there is no stress on the cell, the mitochondria experience equilibrium of the pro-apoptotic proteins Bax, Bak or Bid, and the anti-apoptotic Bcl-2 protein.

Under stress, caspase 8 enables the activation of Bid or strengthens the expression of Bax. The concentration of Bcl 2 is no longer high enough to protect the mitochondria, so Bax and Bid form permeability transition pores with other proteins. The membrane potential of the mitochondrial membrane is destroyed and mitochondrial apoptosis mediator proteins (i.e., cytochrome c, Smac/DIABLO) appear in the cytosol. The cascade of effector caspases 9, 3, 6, and 7 is also activated by the intermediates (e.g., Apaf-1 and the apoptosome formed from it).

Perforin/Granzyme Pathway of Apoptosis

A further method to initialize the extrinsic signaling pathway is performed by cytotoxic T cells and natural killer cells (NK) via the excretion of granzymes and perforins. Perforins form a pore in the membrane of the target cell through which proteolytic granzymes enter the intracellular space. Here, they can cleave to procaspase 3 or activate Bid.

Semiconservative Replication of DNA

During the S phase of the cell cycle, an identical copy of the whole DNA is made during replication. When Watson and Crick presented their model of DNA in 1953, showing complementary base pairs and the deriving base pairing rules (adenine joins to thymine/uracil, guanine binds to cytosine), the principle of replication could be extrapolated as well:

As both polynucleotide strands of DNA are complementary to each other, each strand contains the same information, independent of the other. A copy of one strand is also a copy of the other and each strand can serve as a template for re-synthesis.

Replication occurs prior to cell division in the S phase (synthesis phase) of mitosis in order to allow two sets of chromosomes to be present in the subsequent metaphase, after which they are equally divided into two new cells during separation (anaphase). Many interventions, point modification, and repair mechanisms are responsible for the continuity of the genetic material. The following section explains the replication step by step. Pay particular attention to the enzymes involved at each stage.
Step 0: Preparation and Prerequisites for Replication

The cell is in the **S phase** of mitosis. In humans, this phase takes about 8-12 hours. The DNA exists as 46 single chromatid chromosomes (2n).

Step 1: Recognition of the Ori and Initiation

Certain proteins recognize sections of DNA from which replication can begin, the so-called **origins of replication (ori)**. In prokaryotes, there is only a single ori; eukaryotes have multiple oris. Human DNA contains over 30,000 oris without which the S phase would last about 40 times longer.

Step 2: Unraveling of DNA and Separation of the DNA Strands

In prokaryotes, the protein DnaA binds to an ori (in eukaryotes, this is done by the origin recognition complex, ORC), and **ATP-dependent helicase** DnaB unravels the DNA double helix, then separates the two strands to expose two single strands. Helicase moves in the 5'→3' direction along the DNA molecule and forms the **replication fork** by forcing the complementary strand apart.

The so-called **single-strand binding proteins (SSB)** bind to the unraveled single strands and prevent them from immediately re-attaching onto each other behind the helicase molecule.

The section before the replication fork that is unwound during replication rotates during the process. **Topoisomerases** prevent torsional stresses and as a result, protect the DNA from unwanted breaks by unraveling supercoiled DNA and then separating one of the single strands of DNA (topoisomerase class I) or by changing the spatial structure of the double helix and utilizing ATP to separate the DNA double helix (topoisomerase class II).

**Clinical relevance:** In prokaryotes such as bacteria, topoisomerase II is also called gyrase and is the target of many **antibiotic drugs** (e.g., fluoroquinolones).
Step 3: Synthesis of the Primer – Beginning of Elongation

In order to synthesize a DNA daughter strand, a **primer** is necessary, which is a short piece of RNA (8-10 base pairs, bp). This primer is synthesized by **primase**, a DNA-dependent RNA polymerase that is a subunit of **DNA polymerase alpha** in eukaryotic cells. For this, DNA polymerase needs a free 3' OH end on the (deoxy-) ribose of a nucleotide to which it can attach further nucleotides.

Step 4: Synthesis of the Daughter DNA Strand

**DNA polymerase III** begins to synthesize a complementary daughter strand along the parent strand at the free 3'-OH end of the primer. Polymerases, including DNA polymerases, always read in the **3'-5' reading the direction** and synthesize the complementary daughter strand correspondingly in the **5'-3' synthesis direction**.

**Mnemonic aid:** Polymerases read the same direction as you read a book—from page three to page five.

The reaction is a **nucleophilic attack** by the 3'-OH end of the growing nucleic acid strand upon the phosphate residue of the attached NTPs (in RNA: ATP, UTP, GTP, CTP; see **nucleotide metabolism**) or dNTPs (in DNA: dATP, dTTP, dGTP, dCTP), and this process forms a **phosphoric acid-ester bond** due to the liberation of the attacked pyrophosphate.

**Step 4a: Continuous Synthesis Along the Leading Strand of DNA in a 5'-3' Direction**

**DNA polymerase III** (or in eukaryotes, DNA polymerase α, and δ) **continuously** synthesizes in its reading and movement direction along with the 3'-5' parent strand of the new daughter strand. The daughter strand then becomes the **leading strand** and its parent strand is now referred to as the **template strand**.

**Step 4b: Discontinuous Synthesis Along the Lagging Strand in a 3'-5' Direction in Okazaki Fragments**

On the 5'-3' parent strand, the direction of DNA synthesis of **DNA polymerase III** (in eukaryotes, DNA polymerases α and ε) is opposite to the movement of the replication fork. This daughter strand, called the **lagging strand**, is therefore synthesized in a discontinuous manner into small segments called **Okazaki fragments**. In this case, unlike on the leading strand, numerous primers are necessary. The attached parent DNA strand is called the **coding strand**.

Both of these steps can occur simultaneously.

Step 5: Completion of the Daughter Strands

In order to ensure that the daughter strands consist solely of DNA, the RNA primers in the leading strand and the one between the Okazaki fragments of the lagging strand, have to be removed and the gaps filled with dNTPs. This and other tasks are completed by the important enzyme, **DNA polymerase I**.

DNA polymerase I removes the RNA primers as **ribonuclease**, recognizes defective base pairings and removes the erroneous nucleotides as **exonuclease**, then fills the resultant gaps with dNTPs as **DNA polymerase**. At this point in time, the Okazaki fragments are still not covalently bonded to each other.
Step 6: Bonding of the Okazaki Fragments – Ligation

The final step is the covalent bonding of the Okazaki fragments with each other by DNA ligase. This process uses ATP to esterify the 3’-OH end of one nucleotide with the 5’-phosphate end of the other.

After the duplication of the DNA, there are 46 double-chromatid chromosomes (4n); following anaphase and cytokinesis, this reduces to 46 single chromatid chromosomes (2n).

Synthesis at the End of Chromatin: Telomeres and Aging

The chromosomes of eukaryotes are linear, meaning they have a 3’-OH group at one end. If during replication the complementary primer is at the 5’-OH end of the daughter strand, there is no free 3’-OH end for ligation. The gap which is left by the primer cannot be filled—this means that after each replication, a small piece at the end of the DNA is missing.

This is the reason for a non-coding repetitive sequence (GGGTTA) with over 10,000 base pairs at the end of the eukaryotic chromosome (telomere). Coding sequences, genes, only stop being completely replicated after 30–50 cell cycles; this limits the life expectancy of most somatic cells.

In pathologically proliferating tissues and tumor cells, an enzyme called telomerase, can extend the life expectancy of cells: The RNA-dependent DNA polymerase (reverse transcriptase) contains an RNA matrix, with which it can attach to a complimentary 3’-OH end. The other part of the matrix is a repetitive sequence based on which DNA polymerase synthesizes a complementary strand of DNA, thus elongating the telomere.

Image: “An illustration of a telomerase molecule” by Sierra Sciences, LLC. License: CC BY 3.0

Review Questions

Solutions can be found below the references.

1. Plasmids...
A. ...require an origin of replication for duplication.
B. ...is the term used to refer to antibiotic-resistant proteins in prokaryotes.
C. ...are double-stranded RNA rings outside of the nucleus of the cell.
D. ...are membrane enveloped vectors for the transfer of proteins in cells.
E. ...form ring-shaped intermediates during splicing.

2. If methylated cytosine follows guanine at position 5 on a cytosine ring in a DNA sequence, this structure is also known as 5-methyl-CpG. 5-Methyl-CpG structures in chromosomal DNA...

A. ...restrict/prevent the transcription of a gene, if it occurs in the associated promoter segment of that gene.
B. ...define the starting point for transcription (initiator element, Inr).
C. ...usually, initiate the acetylation of histones.
D. ...mark the genes of RNA polymerase I in the nucleolus.
E. ...are normally only found in the oris (origins of replication).

3. Which of these statements relating to telomerase is correct?

A. The expression of telomerase is inhibited by a transcription factor c-Myc.
B. The catalytic TERT subunit of telomerase is an RNA molecule (a ribozyme).
C. Healthy cells normally have higher telomerase activity compared with the cells that are derived from them.
D. Telomerase completes overhanging single strand DNA ends and makes them double-stranded by synthesis from 3'→ 5' end.
E. Telomerase has RNA-dependent DNA polymerase activity.

References


Correct answers: 1A, 2A, 3E

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