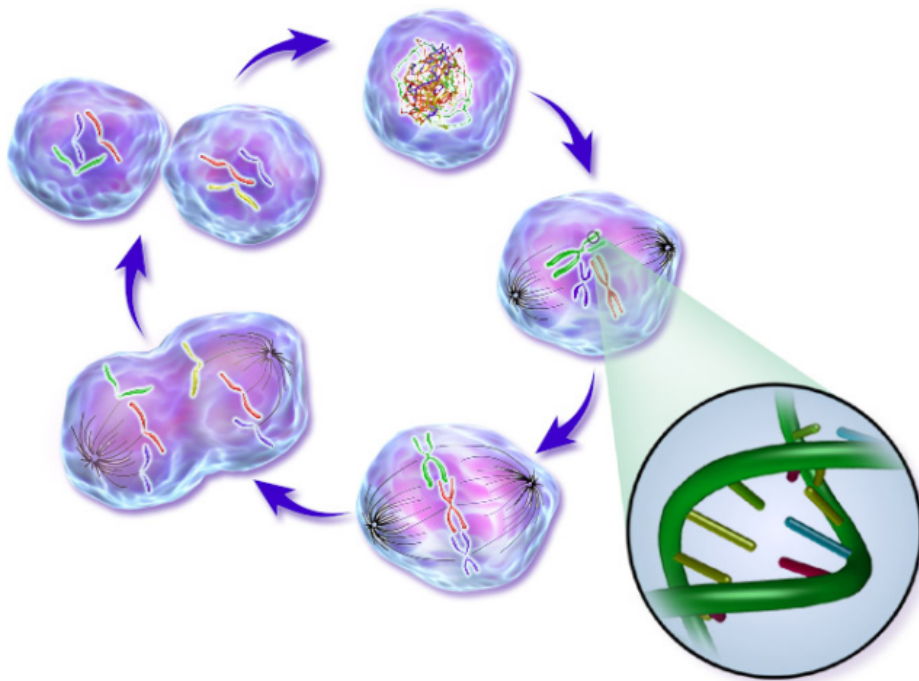


The Cell Cycle: Regulation, Apoptosis, Mitosis and Replication of DNA

[See online here](#)

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.

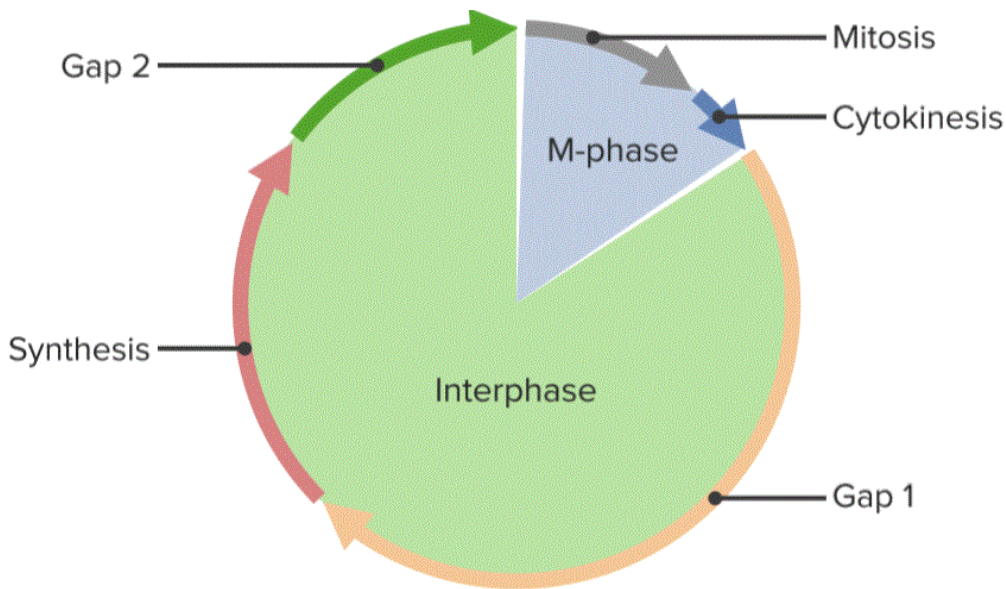


The Cell Cycle

The cell cycle describes the **cyclic sequence of events in eukaryotic, proliferating cells**. Its purpose is to enable the duplication of a cell into 2 identical daughter cells. The cell cycle consists of 4 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 the 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 2 daughter cells (0.5-1 hour)



"Cell Cycle. Divided into 5 Phases" Image created by Lecturio

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 are created.

In the **S phase** (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 2 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 after repairs, if necessary, the cell completes the interphase stage and is ready for mitosis.

Cell division: mitosis

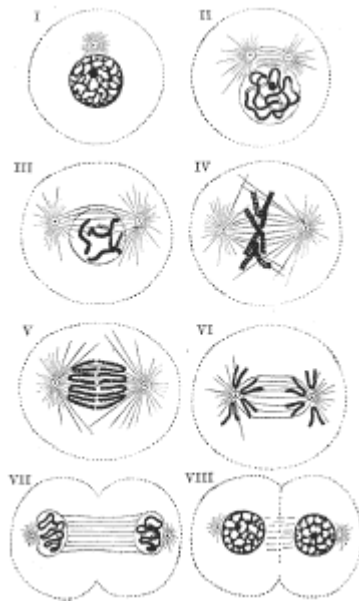


Image: "Diagram showing the changes which occur in the centrosomes and nucleus of a cell in the process of mitotic division. I to III, prophase; IV, metaphase; V and VI, anaphase; VII and VIII, telophase." by Henry Gray. License: Public Domain

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 2 daughter cells. Each daughter cell has a complete genome.

During **telophase**, both 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 cyclin-dependent kinases (CDKs).

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

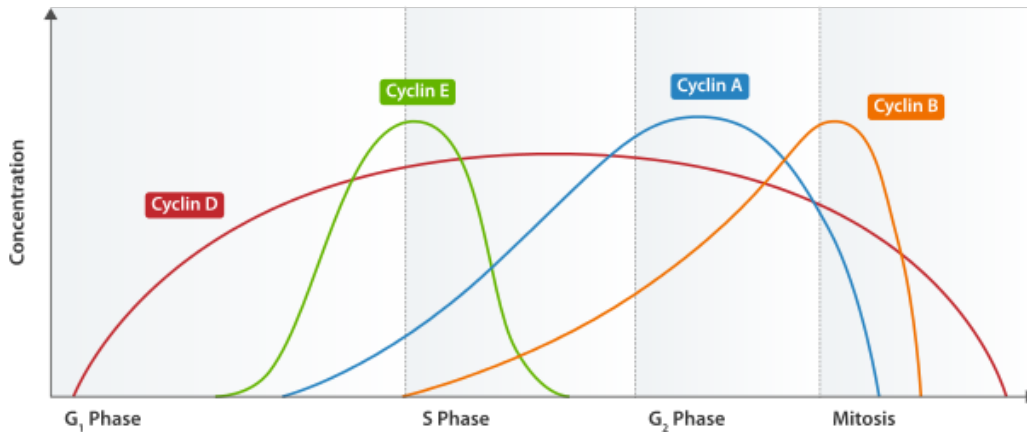


Image: "Cyclin expression cycle" by WikiMiMa. License: Public Domain

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.

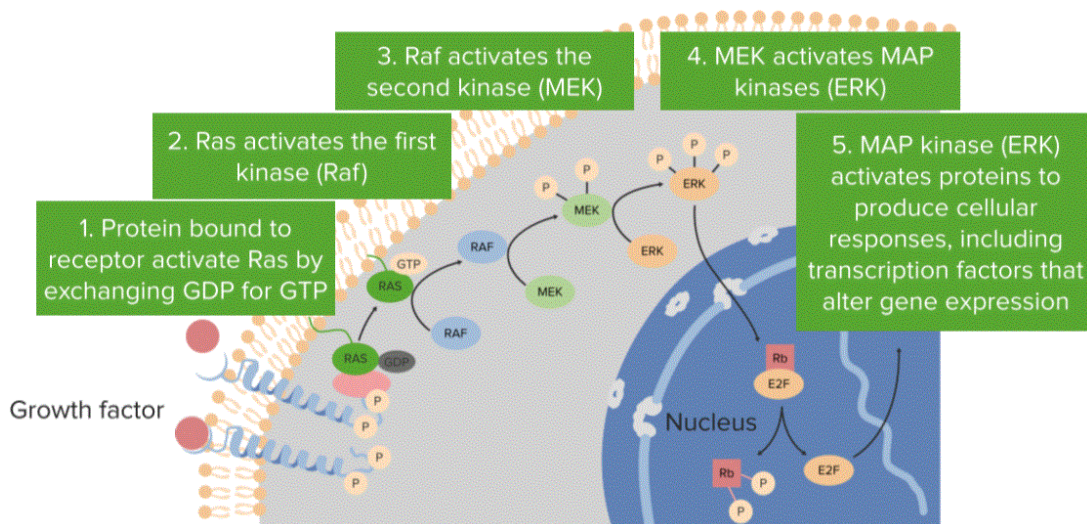
The **transition between the G₁ and S phases** is decisive in terms of whether the cell progresses to cell division or remains dormant in the G₀ phase. The cell cycle starts when the cell has a sufficient minimum size, appropriate nutritional conditions, and stimulation by growth factors in addition 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 play 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 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 have adhered to the microtubules of the spindle apparatus prior to their being separated in 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.



"Cell Cycle Controls. Platelet-Derived Growth Factors" Image created by Lecturio

Apoptosis

Apoptosis is 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, 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 with 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. Then the cell itself is digested within the membrane vesicle in **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), **development of immunotolerance** (e.g., elimination of hyperreactive T lymphocytes), **homeostasis of the cell count** (e.g., regular replacement of a mucous membrane), and **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.

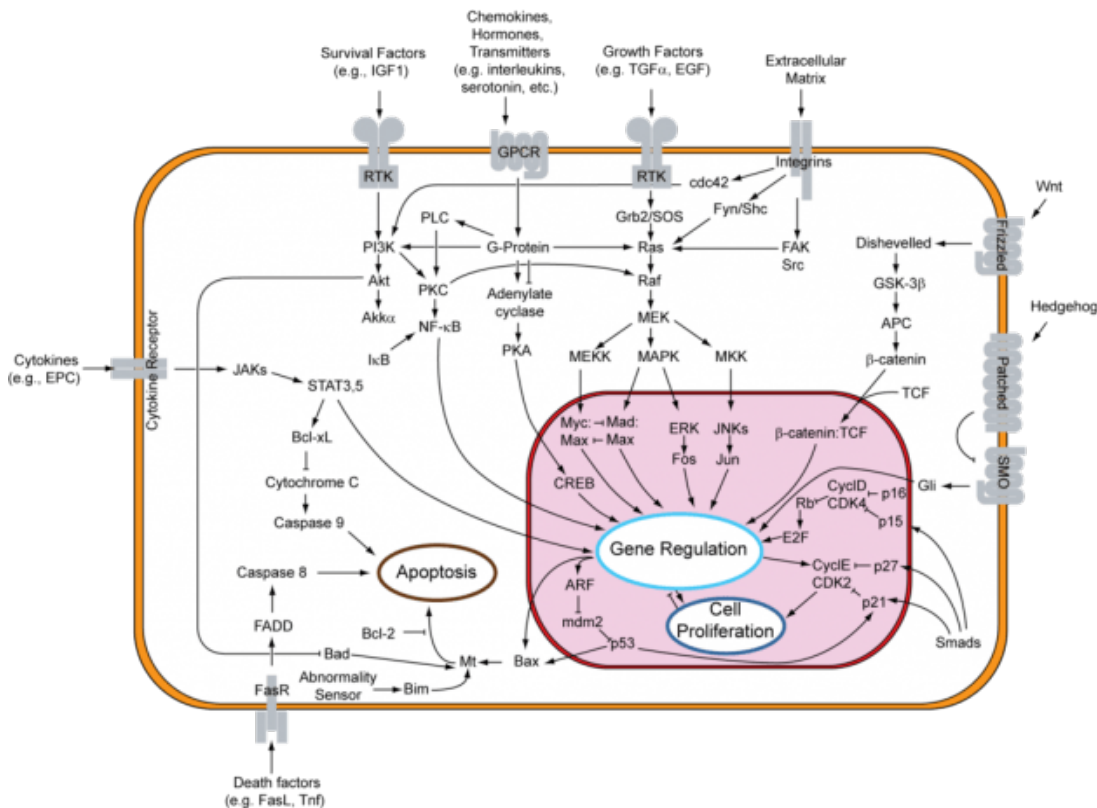


Image: "Signal transduction pathways" by Roadnottaken. License: [CC BY-SA 3.0](https://creativecommons.org/licenses/by-sa/3.0/)

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 **mediator proteins of mitochondrial apoptosis** (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

An additional 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 binds 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. 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 2 sets of chromosomes to be present in the subsequent metaphase, after which they are equally divided into 2 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.

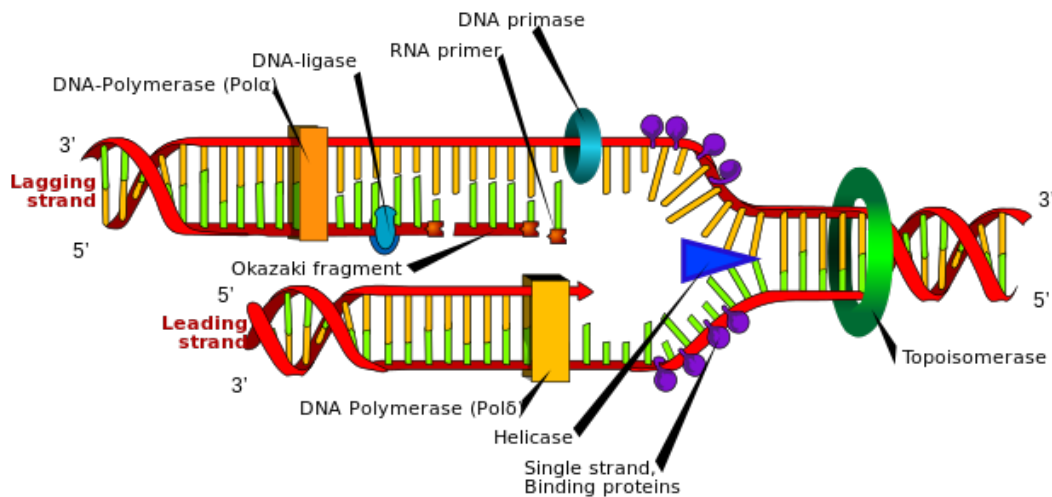


Image: "DNA replication" by LadyofHats. License: Public Domain

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 2 strands to expose 2 single strands. Helicase moves in the 5'-3' direction along the DNA molecule and forms the **replication fork** by forcing the complementary strand apart.

Single-strand binding proteins (SSB) bind to the unraveled single strands and prevent them from immediately re-attaching to 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, protecting 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). 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 in the same direction as you read a book—from page 3 to page 5.

The reaction is a **nucleophilic attack** by the 3'-OH end of the growing nucleic acid strand on 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 polymerases 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 an **exonuclease**. It 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.

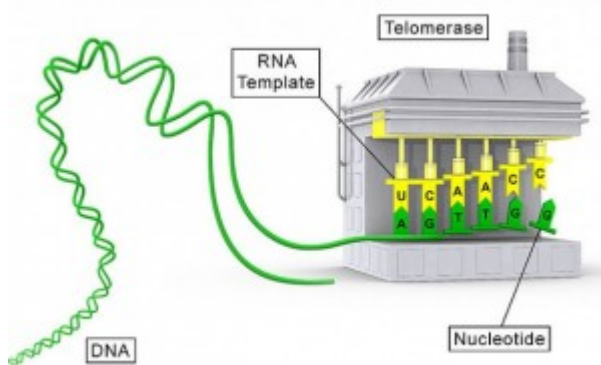
After the duplication of the DNA, there are 46 double-chromatid chromosomes (4n); following anaphase and cytokinesis, this is reduced to 46 single chromatid chromosomes (2n).

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](#)

References

Fitzgerald-Hayes, M., &Reichsman, F. (2009). *DNA and Biotechnology*. Burlington: Elsevier Science.

Goodman, S. R. (2007). *Medical Cell Biology*. San Diego: Elsevier Science.

Grafi, G. (1998). Cell Cycle Regulation of DNA Replication: The Endoreduplication Perspective. *Experimental Cell Research*, 244(2), 372-378. doi:10.1006/excr.1998.4213

Kurtz. (2007). *Medical cell biology*. Elsevier Health Sciences.

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Notes