- Topoisomerases are enzymes that can change the amount of supercoiling in DNA molecules. They make transient breaks in DNA strands by alternately breaking and resealing the sugar-phosphate backbone. For example, in *Escherichia coli*, DNA gyrase (DNA topoisomerase II) can introduce negative supercoiling into DNA.

Nucleosomes and Chromatin

High-Yield

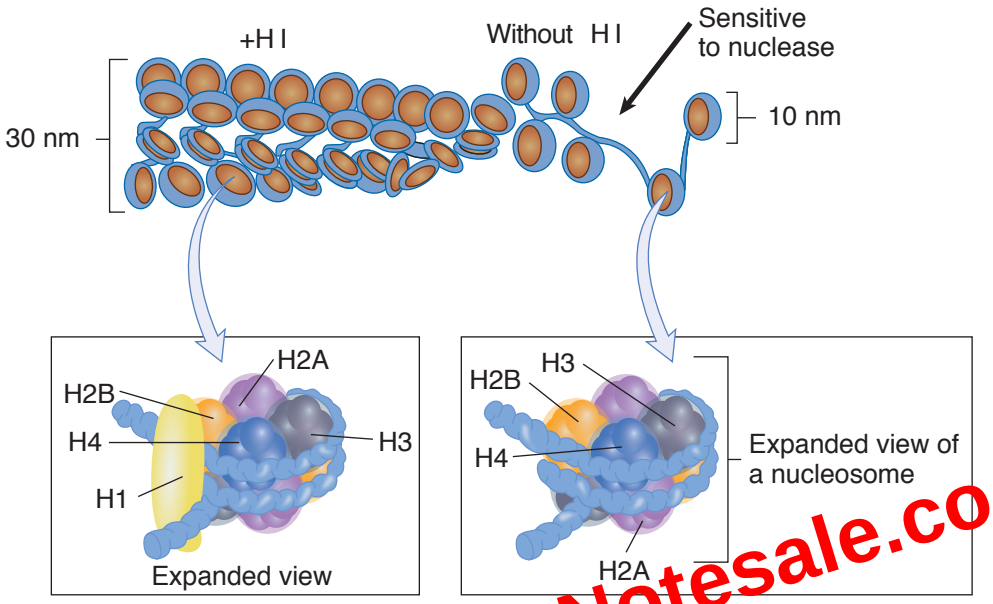
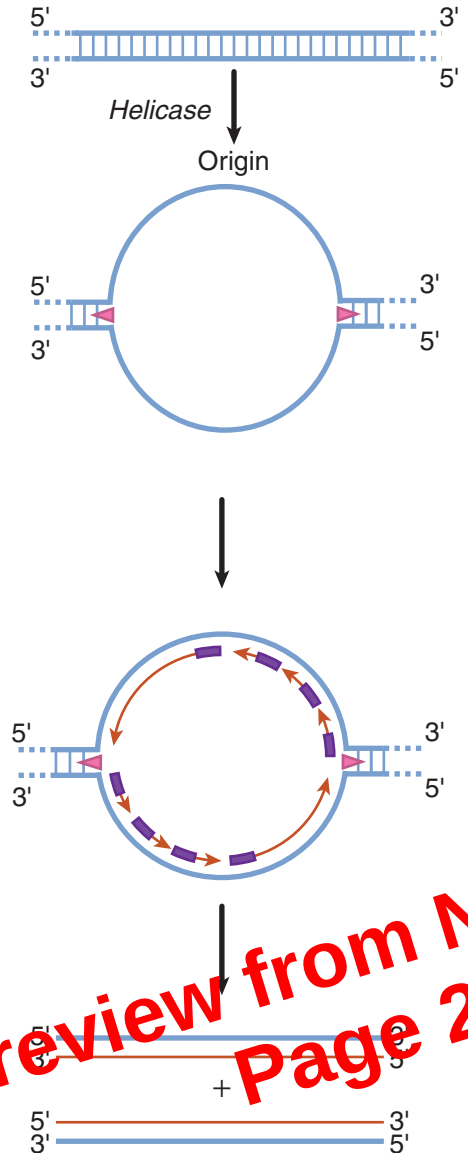


Figure I-1-10. Nucleosome and Nucleofilament Structure in Eukaryotic DNA

Nuclear DNA in eukaryotes is found in chromatin associated with histones and nonhistone proteins. The basic packaging unit of chromatin is the nucleosome.

- Histones are rich in lysine and arginine, which confer a positive charge on the proteins.
- Two copies each of histones H2A, H2B, H3, and H4 aggregate to form the histone octamer.
- DNA is wound around the outside of this octamer to form a nucleosome (a series of nucleosomes is sometimes called "beads on a string" but is more properly referred to as a 10nm chromatin fiber).
- Histone H1 is associated with the linker DNA found between nucleosomes to help package them into a solenoid-like structure, which is a thick 30-nm fiber.
- Further condensation occurs to eventually form the chromosome. Each eukaryotic chromosome in G0 or G1 contains one linear molecule of double-stranded DNA.

Cells in interphase contain 2 types of chromatin: euchromatin (more opened and available for gene expression) and heterochromatin (much more highly condensed and associated with areas of the chromosomes that are not expressed).



Leading Strand Synthesis (Continuous)

1. *Primase* synthesizes the primer (▬) 5' to 3'.
2. *DNA polymerases* α and δ extend the primer, moving **into** the replication fork (Leading strand synthesis).
3. *Helicase* (◄) continues to unwind the DNA.

Lagging Strand Synthesis (Discontinuous)

1. *Primase* synthesizes the primer (▬) 5' to 3'.
2. *DNA polymerases* α and δ extend the primer, moving **away from** the replication fork (Lagging strand synthesis).
3. Synthesis stops when *DNA polymerase* encounters the primer of the leading strand on the other side of the diagram (not shown), or the primer of the previous (Okazaki) fragment.
4. As *helicase* opens more of the replication fork, a third Okazaki fragment will be added.

RNase H (5' exoribonuclease activity) digests the RNA primer from fragment 1. In the eukaryotic cell, *DNA polymerase* extends the next fragment (2), to fill in the gap.

In prokaryotic cells *DNA polymerase 1* has both the 5' exonuclease activity to remove primers, and the *DNA polymerase* activity to extend the next fragment (2) to fill in the gap.

In both types of cells *DNA ligase* connects fragments 1 and 2 by making a phosphodiester bond.

This whole process repeats to remove all RNA primers from both the leading and lagging strands.

Figure I-2-4. DNA Replication

Recall Question

Which of the following enzymes is the target of fluoroquinolones in replication of DNA strands?

A. DNA gyrase

B. DNA helicase

C. DNA ligase

D. DNA polymerase

Answer: A

Base excision repair: cytosine deamination

Cytosine deamination (loss of an amino group from cytosine) converts cytosine to uracil. The uracil is recognized and removed (base excision) by a uracil glycosylase enzyme.

- Subsequently this area is recognized by an AP endonuclease that removes the damaged sequence from the DNA
- DNA polymerase fills in the gap
- DNA ligase seals the nick in the repaired strand

A summary of important genes involved in maintaining DNA fidelity and where they function in the cell cycle is shown below.

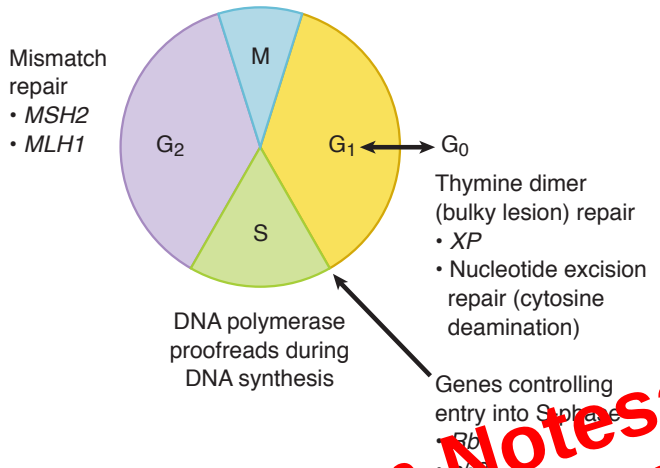


Figure I-2-6. Important Genes Associated with Maintaining Fidelity of Replicating DNA

Diseases Associated with DNA Repair

High-Yield

Inherited mutations that result in defective DNA repair mechanisms are associated with a predisposition to the development of cancer.

Xeroderma pigmentosum is an autosomal recessive disorder, characterized by extreme sensitivity to sunlight, skin freckling and ulcerations, and skin cancer. The most common deficiency occurs in the excinuclease enzyme.

Hereditary nonpolyposis colorectal cancer results from a deficiency in the ability to repair mismatched base pairs in DNA that are accidentally introduced during replication.

Transcription and RNA Processing 3

Learning Objectives

- Use knowledge of types of RNA
- Understand concepts of prokaryotic messenger RNA
- Understand concepts of eukaryotic messenger RNA
- Demonstrate understanding of alternative splicing of eukaryotic primary pre-mRNA transcripts
- Know key features of ribosomal RNA (rRNA)
- Know key features of transfer RNA (tRNA)

TRANSCRIPTION

The first stage in the expression of genetic information is transcription of the information in the base sequence of a double-stranded DNA molecule to form the base sequence of a single-stranded molecule of RNA. For any particular gene, only one strand of the DNA molecule (the template strand) is copied by RNA polymerase as it synthesizes RNA in the 5′ to 3′ direction. Because RNA polymerase moves in the 3′ to 5′ direction along the template strand of DNA, the RNA product is antiparallel and complementary to the template. RNA polymerase recognizes start signals (promoters) and stop signals (terminators) for each of the thousands of transcription units in the genome of an organism.

The figure below illustrates the arrangement and direction of transcription for several genes on a DNA molecule.

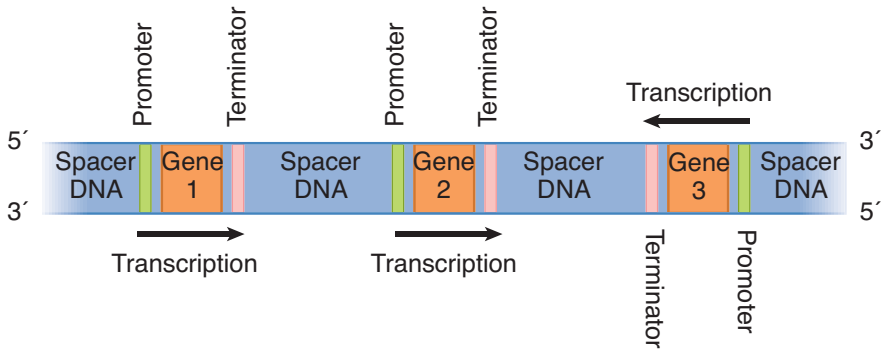


Figure I-3-1. Transcription of Several Genes on a Chromosome

TYPES OF RNA

RNA molecules play a variety of roles in the cell. The major types of RNA are:

- Ribosomal RNA (rRNA), which is the most abundant type of RNA in the cell. It is used as a structural component of the ribosome. Ribosomal RNA associates with ribosomal proteins to form the complete, functional ribosome.
- Transfer RNA (tRNA), which is the second most abundant type of RNA. Its function is to carry amino acids to the ribosome, where they will be linked together during protein synthesis.
- Messenger RNA (mRNA), which carries the information specifying the amino acid sequence of a protein to the ribosome. Messenger RNA is the only type of RNA that is translated. The mRNA population in a cell is very heterogeneous in size and base sequence, as the cell has essentially a different mRNA molecule for each of the thousands of different proteins made by that cell.
- Heterogeneous nuclear RNA (hnRNA or pre-mRNA), which is found only in the nucleus of eukaryotic cells. It represents precursors of mRNA, formed during its posttranscriptional processing.
- Small nuclear RNA (snRNA), which is also only found in the nucleus of eukaryotes. One of its major functions is to participate in splicing (removal of introns) mRNA.
- Ribozymes, which are RNA molecules with enzymatic activity. They are found in both prokaryotes and eukaryotes.

RNA POLYMERASES

There is a single prokaryotic RNA polymerase that synthesizes all types of RNA in the cell. The core polymerase responsible for making the RNA molecule has the subunit structure $\alpha_2\beta\beta'$. A protein factor called sigma (σ) is required for the initiation of transcription at a promoter. Sigma factor is released immediately after initiation of transcription. Termination of transcription sometimes requires a protein called rho (ρ) factor. The prokaryotic RNA polymerase is inhibited by rifampin. Actinomycin D binds to the DNA, preventing transcription.

There are 3 eukaryotic RNA polymerases, distinguished by the particular types of RNA they produce.

- RNA polymerase I is located in the nucleolus and synthesizes 28S, 18S, and 5.8S rRNAs.
- RNA polymerase II is located in the nucleoplasm and synthesizes hnRNA/mRNA and some snRNA.
- RNA polymerase III is located in the nucleoplasm and synthesizes tRNA, some snRNA, and 5S rRNA.

Transcription factors (such as TFIID for RNA polymerase II) help to initiate transcription. The requirements for termination of transcription in eukaryotes are not well understood. All transcription can be inhibited by actinomycin D. In addition, RNA polymerase II is inhibited by α-amanitin (a toxin from certain mushrooms).

Table I-3-1. Comparison of RNA Polymerases

Prokaryotic	Eukaryotic
Single RNA polymerase (α_2 ββ')	RNAP 1: rRNA (nucleolus) Except 5S rRNA RNAP 2: hnRNA/mRNA and some snRNA RNAP 3: tRNA, 5S rRNA
Requires sigma (σ) to initiate at a promoter	No sigma, but transcription factors (TFIID) bind before RNA polymerase
Sometimes requires rho (ρ) to terminate	No rho required
Inhibited by rifampin Actinomycin D	RNAP 2 inhibited by α-amanitin (mushrooms) Actinomycin D

TRANSCRIPTION: IMPORTANT CONCEPTS AND TERMINOLOGY

RNA is synthesized by a DNA-dependent RNA polymerase (uses DNA as a template for the synthesis of RNA). Important terminology used when discussing transcription is illustrated below.

- RNA polymerase locates genes in DNA by searching for promoter regions. The promoter is the binding site for RNA polymerase. Binding establishes where transcription begins, which strand of DNA is used as the template, and in which direction transcription proceeds. No primer is required.
- RNA polymerase moves along the template strand in the 3′ to 5′ direction as it synthesizes the RNA product in the 5′ to 3′ direction using NTPs (ATP, GTP, CTP, UTP) as substrates. RNA polymerase does not proofread its work. The RNA product is complementary and antiparallel to the template strand.
- The coding (antitemplate) strand is not used during transcription. It is identical in sequence to the RNA molecule, except that RNA contains uracil instead of the thymine found in DNA.
- By convention, the base sequence of a gene is given from the coding strand (5′→3′).
- In the vicinity of a gene, a numbering system is used to identify the location of important bases. The first base transcribed as RNA is defined as the +1 base of that gene region.
  - To the left (5′, or upstream) of this starting point for transcription, bases are –1, –2, –3, etc.
  - To the right (3′, or downstream) of this point, bases are +2, +3, etc.
- Transcription ends when RNA polymerase reaches a termination signal.