Nucleic Aid Structure

DNA (deoxyribonucleic acid) and RNA (ribonucleic acid) are polymers of nucleotides linked in a chain through phosphodiester bonds.
In biological systems, they serve as information-carrying molecules or, in the case of some RNA molecules, catalysts.
Nucleotides (building blocks of nucleic acids) have a distinctive structure composed of three components covalently bound together:

a **5-carbon sugar** - ribose or deoxyribose
a **nitrogen base** : pyrimidine (one ring) or purine (two rings)
a **phosphate group**

The combination of a base and sugar is called **a nucleoside**.
Nucleotides also exist in activated forms containing two or three phosphates, called **nucleotide diphosphates** or **triphosphates**. If the sugar in a nucleotide is deoxyribose, the nucleotide is called a **deoxynucleotide**; if the sugar is ribose, the term ribonucleotide is used.

There are five common bases, and four are generally represented in either DNA or RNA

Adenine, Guanine, Cytosine, Thymine and Uracil

DNA and RNA are synthesized in cells by **DNA polymerases** and **RNA polymerases** by forming **phosphodiester bonds** between the 3' carbon of one nucleotide and the 5' carbon of another nucleotide.

Most DNA exists in the famous form of a **double helix**, in which two linear strands of DNA are wound around one another. The major force promoting formation of this helix is complementary base pairing: Adenine form hydrogen bonds with Thymine (or Uracil in RNA), and Guanine form hydrogen bonds with Cytosine.

G-C base pairs have Three hydrogen bonds, whereas A-T base pairs haveTwo hydrogen bonds.

RNAs are usually single stranded and the base pairs that form are A-U and G-C.

Bacterial Genome

- The **genome** of an organism can be defined as the total DNA content of the cell , and as such it contains all the genetic information required to direct the growth and development of the organism.
- In prokaryotes, most of the genome (85-90%) is non-repetitive DNA, which means coding DNA mainly forms it, while non-coding regions only take a small part. In bacteria there is little repetitive DNA as seen in higher eukaryotes.

- *The relatively small genome size of bacterial genomes*, *together with the fact that they contain very little non-coding or repeat DNA* and *that they do not contain introns,* has made bacterial genomes ideal candidates for whole genome sequencing projects.
- Identification of potential genes within bacterial genomes is also much more reliable, because firstly bacterial genes do not contain introns and secondly the genes are much more closely packed.

Bacterial Chromosome in the Nucleoid

The Nucleoid

The **nucleoid** (meaning nucleus-like) is an irregularly-shaped region within the cell of a prokaryote that contains all or most of the genetic material. In contrast to the nucleus of a eukaryotic cell, it is not surrounded by a nuclear membrane.

The genome of prokaryotic organisms generally is a circular, double-stranded piece of DNA. The nucleoid is largely composed of about 60% DNA, plus a small amount of RNA and protein.

The Genophore

A **genophore** is the DNA of a prokaryote. It is commonly referred to as a prokaryotic chromosome. The term "chromosome" is misleading, because the genophore lacks chromatin. The genophore is compacted through a mechanism known as supercoiling, but a chromosome is additionally compacted through the use of chromatin.

DNA Supercoiling

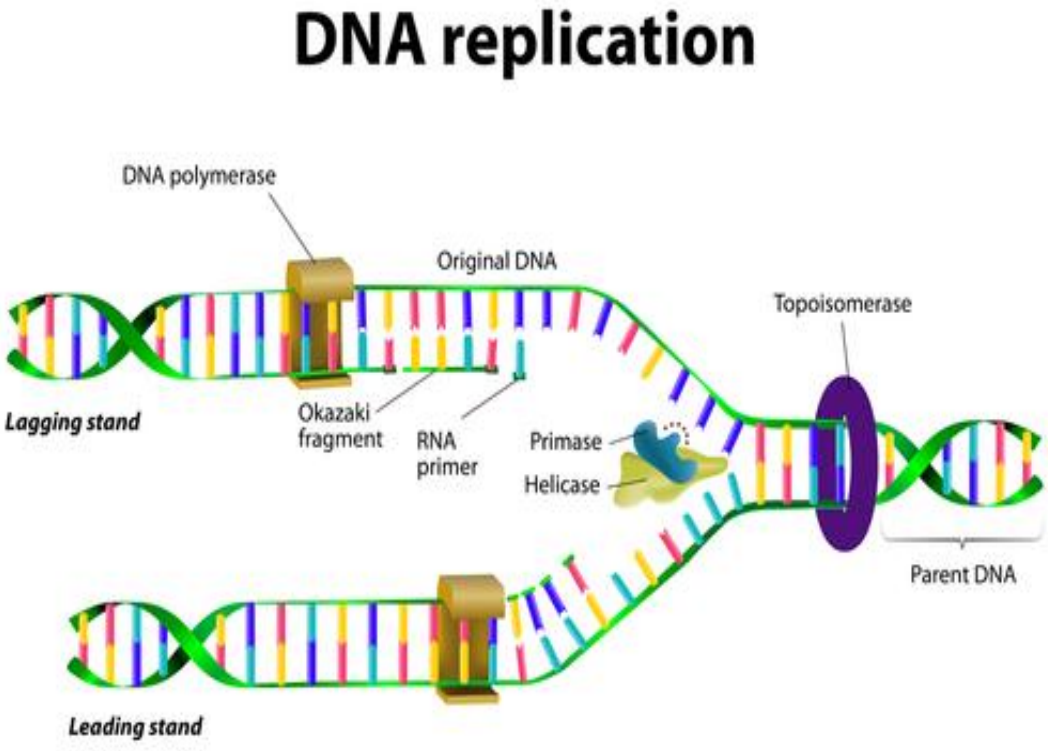
- DNA supercoiling refers to the over- or under-winding of a DNA strand, and is an expression of the strain on that strand. Supercoiling is important in a number of biological processes, such as compacting DNA.
- DNA supercoiling is important for DNA packaging within all cells. Because the length of DNA can be thousands of times that of a cell, packaging this genetic material into the cell or nucleus (in eukaryotes) is a difficult feat. Supercoiling of DNA reduces the space and allows for much more DNA to be packaged.

DNA Replication

- DNA replication employs a large number of proteins and enzymes, each of which plays a critical role during the process. One of the key players is the enzyme DNA polymerase, which adds nucleotides one by one to the growing DNA chain that are complementary to the template strand. In prokaryotes, three main types of polymerases are known: ***DNA pol I***, ***DNA pol II***, and ***DNA pol III.***

- DNA pol III is the enzyme required for DNA synthesis; DNA pol I and DNA pol II are primarily required for repair.
- There are specific nucleotide sequences called origins of replication where replication begins. The origin of replication is recognized by certain proteins that bind to this site.
- An enzyme called **helicase** unwinds the DNA by breaking the hydrogen bonds between the nitrogenous base pairs.

  ATP hydrolysis is required for this process. As the DNA opens up, Y-shaped structures called replication forks are formed. Two replication forks at the origin of replication are extended bi-directionally as replication proceeds. Single-strand binding proteins coat the strands of DNA near the replication fork to prevent the single-stranded DNA from winding back into a double helix. DNA polymerase is able to add nucleotides only in the 5' to 3' direction (a new DNA strand can be extended only in this direction). It also requires a free 3'-OH group to which it can add nucleotides by forming a phosphodiester bond between the 3'-OH end and the 5' phosphate of the next nucleotide. This means that it cannot add nucleotides if a free 3'-OH group is not available.



Another enzyme, **RNA primase**, synthesizes an RNA primer that is about five to ten nucleotides long and complementary to the DNA, priming DNA synthesis.

A primer provides the free 3'-OH end to start replication. DNA polymerase then extends this RNA primer, adding nucleotides one by one that are complementary to the template strand.

DNA polymerase can only extend in the 5' to 3' direction, which poses a slight problem at the replication fork. As we know, the DNA double helix is anti-parallel; that is, one strand is in the 5' to 3' direction and the other is oriented in the 3' to 5' direction.

One strand (**the leading strand**), complementary to the 3' to 5' parental DNA strand, is synthesized continuously towards the replication fork because the polymerase can add nucleotides in this direction.

The other strand (**the lagging strand**), complementary to the 5' to 3' parental DNA, is extended away from the replication fork in small fragments known as ***Okazaki fragments***, each requiring a primer to start the synthesis. Okazaki fragments are named after the Japanese scientist who first discovered them.

- The leading strand can be extended by one primer alone, whereas the lagging strand needs a new primer for each of the short Okazaki fragments. The overall direction of the lagging strand will be 3' to 5', while that of the leading strand will be 5' to 3'.
- **Topoisomerase** prevents the over-winding of the DNA double helix ahead of the replication fork as the DNA is opening up; it does so by causing temporary nicks in the DNA helix and then resealing it.
- The primers are removed by the ***exonuclease*** activity of DNA pol I, while the gaps are filled in by deoxyribonucleotides.

Gene Expression

Gene expression is the process by which information from a gene is used in the synthesis of a functional gene product. These products are often proteins, via Two main steps Transcription and translation, but in non-protein coding genes such as transfer RNA (tRNA) or small nuclear RNA (snRNA) genes, the product is a functional RNA.
In genetics, gene expression is the most fundamental level at which the genotype gives rise to the phenotype, i.e. observable trait.

RNA Transcription

- Prokaryotic transcription is the process in which messenger RNA transcripts of genetic material in prokaryotes are produced, to be translated for the production of proteins.
- Transcription like replication need free 3' end to add the complementary nucleotide, also the direction of movement and polymerization of RNA polymerase from 5' →3'-.

- The transcript sequence (complete gene) start with promoter region where the DNA will opened and the RNA polymerase bind to start transcription.
- **Promoter**: a regulatory nucleotide sequences of DNA (40-60 nts) at the beginning of every gene located upstream (towards the 5' region) of a gene. In prokaryotes, the promoter is recognized by RNA polymerase (δ sigma sub unite). The promoter consists of two short sequences know as -10 box and -35 box positions upstream from the transcription start site.
- **Coding region**: Nucleotide sequences which will detriment the genetic code then will translated to Amino acid. It start with ATG triplet initiation codon (AUG in m RNA). The length of coding region depend on type of produced protein.
- **Terminator**: Nucleotide sequences exist after the coding region rich with poly G followed by poly C then poly A .

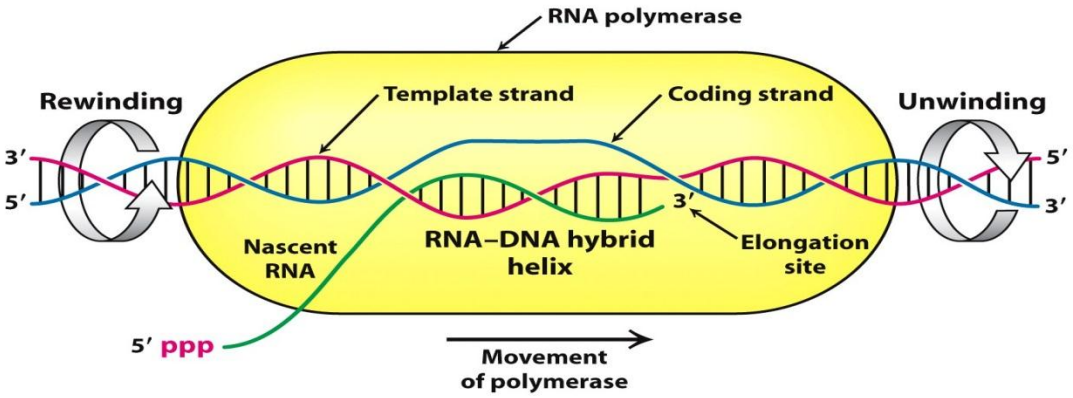


Figure 29.13
Biochemistry, Seventh Edition

- The RNA polymerase enzyme consist from core (5 units) and another 6^{th} unit called sigma δ, after core binding with sigma it will convert to **holoenzyme**.
- **sigma subunit** play significant role in recognition promoter region then it will released leaving core continue transcription RNA from template.
- The Promoter and Terminator are directions for RNA polymerase to indicate the location of the gene to be transcribed.
- The start and stop codons are directions for the ribosome to indicate where the amino acid information for translation begins and ends.

Stages of Transcription

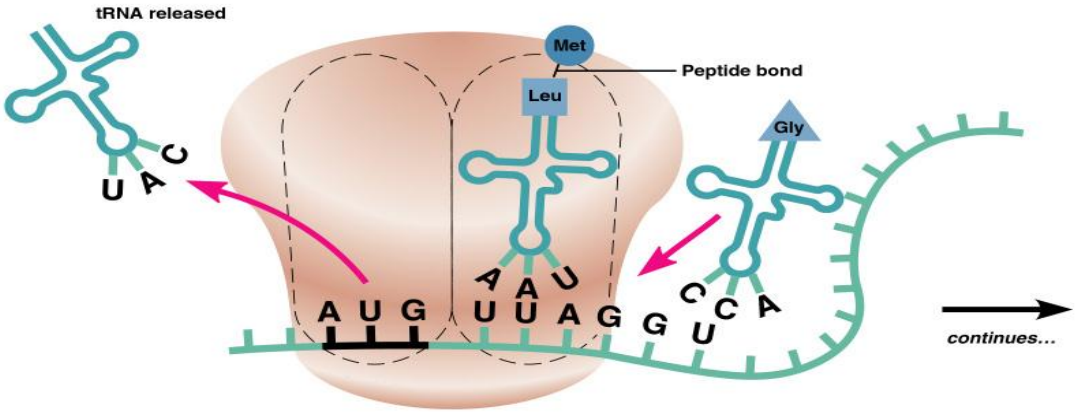
- **Initiation stage** involves recognizing core promoter region by sigma factor of RNA polymerase .
- **Elongation stage** involves the further addition of ribonucleotides

- **Termination stage**: When Transcription process reached a region called TERMINTATOR, termination stage start by: a mechanism called **Rho-independent termination**: is a mechanism in prokaryotes that causes RNA transcription to be stopped without the aid of rho protein.

  Rho-dependent termination: Rho factor is a prokaryotic ATP-dependent unwinding enzyme involved in termination transcription.

Translation

- The translation of genetic information from the 4-letter (A ,C ,G and T) of polynucleotides into the 20-amino acid of proteins is a complex process. The information in the sequence of a messenger RNA molecule is read out in groups of three nucleotides at a time: each triplet of nucleotides, or codon, specifies (codes for) a single amino acid in a corresponding protein.



4 The first amino acid joins to the second by a peptide bond, and the first tRNA is released.

- Initiation codon (AUG): signals the start of translation. Lies just downstream of the Shine Dalgarno sequence .
- Termination codon (UAG,UGA,UAA): signals the end of translation.
- mRNA stands for messenger RNA. It's the product of transcription and the template for translation.
- Prokaryotic mRNAs don't have the 5' cap or polyA tail.
- Both tRNA (transfer RNA) and rRNA (ribosomal RNA) are products of transcription. However, they do not serve as the template of translation.
- tRNA is responsible for bringing in the correct amino acid during translation. rRNA makes up the ribosome, which is the enzyme responsible for translation.
- Ribosomes are the sites of protein synthesis. Bacterial ribosomes differ from those of eukaryotic cells in both size and chemical composition. They are organized in units of 70S.