(A+G) and **pyrimidines** (T + C), but different ratios of A+T to G+C. These observations became known as **Chargaff's rules**. Chargaff's findings proved immensely useful when Watson and Crick were getting ready to propose their DNA double helix model! You can see after reading the past few pages how science builds upon previous discoveries, sometimes in a slow and laborious process.

# 14.2 DNA Structure and Sequencing

By the end of this section, you will be able to do the following:

- Describe the structure of DNA
- Explain the Sanger method of DNA sequencing
- Discuss the similarities and differences between eukaryotic and prokaryotic DNA

The building blocks of DNA are nucleotides. The important components of the nucleotide are a nitrogenous (nitrogen-bearing) base, a 5-carbon sugar (pentose), and a phosphate group (Figure 14.5). The nucleotide is named depending on the nitrogenous base. The nitrogenous base can be a purine such as adenine (A) and guanine (G), or a pyrimidine such as cytosine (C) and thymine (T).

👁 VISUAL CONNECTION
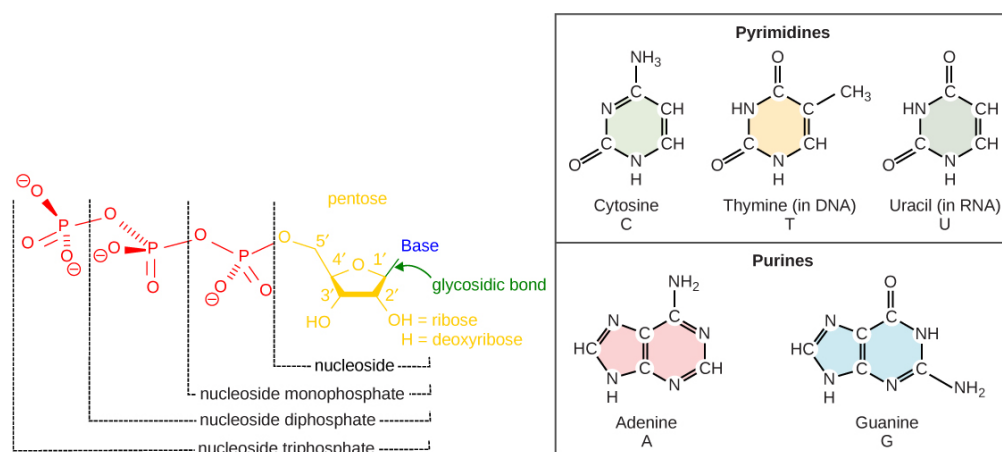
Figure 14.5 The purines have a double ring structure with a six-membered ring fused to a five-membered ring. Pyrimidines are smaller in size; they have a single six-membered ring structure.

The images above illustrate the five bases of DNA and RNA. Examine the images and explain why these are called "nitrogenous bases." How are the purines different from the pyrimidines? How is one purine or pyrimidine different from another, e.g., adenine from guanine? How is a nucleoside different from a nucleotide?

The purines have a double ring structure with a six-membered ring fused to a five-membered ring. Pyrimidines are smaller in size; they have a single six-membered ring structure.

The sugar is deoxyribose in DNA and ribose in RNA. The carbon atoms of the five-carbon sugar are numbered 1', 2', 3', 4', and 5' (1' is read as "one prime"). The phosphate, which makes DNA and RNA acidic, is connected to the 5' carbon of the sugar by the formation of an ester linkage between phosphoric acid and the 5'-OH group (an ester is an acid + an alcohol). In DNA nucleotides, the 3' carbon of the sugar deoxyribose is attached to a hydroxyl (OH) group. In RNA nucleotides, the 2' carbon of the sugar ribose also contains a hydroxyl group. The base is attached to the 1'carbon of the sugar.

The nucleotides combine with each other to produce phosphodiester bonds. The phosphate residue attached to the 5' carbon of the sugar of one nucleotide forms a second ester linkage with the hydroxyl group of the 3' carbon of the sugar of the next nucleotide, thereby forming a 5'-3' phosphodiester bond. In a polynucleotide, one end of the chain has a free 5' phosphate, and the other end has a free 3'-OH. These are called the 5' and 3' ends of the chain.

In the 1950s, Francis Crick and James Watson worked together to determine the structure of DNA at the University of Cambridge, England. Other scientists like Linus Pauling and Maurice Wilkins were also actively exploring this field. Pauling

previously had discovered the secondary structure of proteins using X-ray crystallography. In Wilkins' lab, researcher Rosalind Franklin was using X-ray diffraction methods to understand the structure of DNA. Watson and Crick were able to piece together the puzzle of the DNA molecule on the basis of Franklin's data because Crick had also studied X-ray diffraction (Figure 14.6). In 1962, James Watson, Francis Crick, and Maurice Wilkins were awarded the Nobel Prize in Medicine. Unfortunately, by then Franklin had died, and Nobel prizes are not awarded posthumously.

(a)   (b)
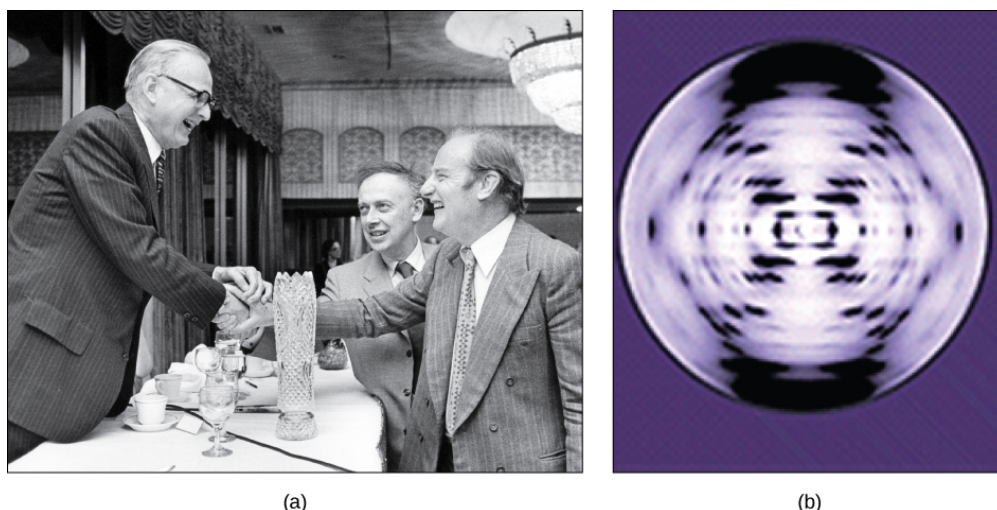
Figure 14.6 The work of pioneering scientists (a) James Watson, Francis Crick, and Maclyn McCarty led to our present day understanding of DNA. Scientist Rosalind Franklin discovered (b) the X-ray diffraction pattern of DNA, which helped to elucidate its double-helix structure. (credit a: modification of work by Marjorie McCarty, Public Library of Science)

Watson and Crick proposed that DNA is made up of two strands that are twisted around each other to form a right-handed helix. Base pairing takes place between a purine and pyrimidine on opposite strands, so that A pairs with T, and G pairs with C (suggested by Chargaff's Rules). Thus, adenine and thymine are complementary base pairs, and cytosine and guanine are also complementary base pairs. The base pairs are stabilized by hydrogen bonds: *adenine and thymine form two hydrogen bonds and cytosine and guanine form three hydrogen bonds.* The two strands are anti-parallel in nature; that is, the 3' end of one strand faces the 5' end of the other strand. The sugar and phosphate of the nucleotides form the backbone of the structure, whereas the nitrogenous bases are stacked inside, like the rungs of a ladder. Each base pair is separated from the next base pair by a distance of 0.34 nm, and each turn of the helix measures 3.4 nm. Therefore, 10 base pairs are present per turn of the helix. The diameter of the DNA double-helix is 2 nm, and it is uniform throughout. Only the pairing between a purine and pyrimidine and the antiparallel orientation of the two DNA strands can explain the uniform diameter. The twisting of the two strands around each other results in the formation of uniformly spaced major and minor grooves (Figure 14.7).
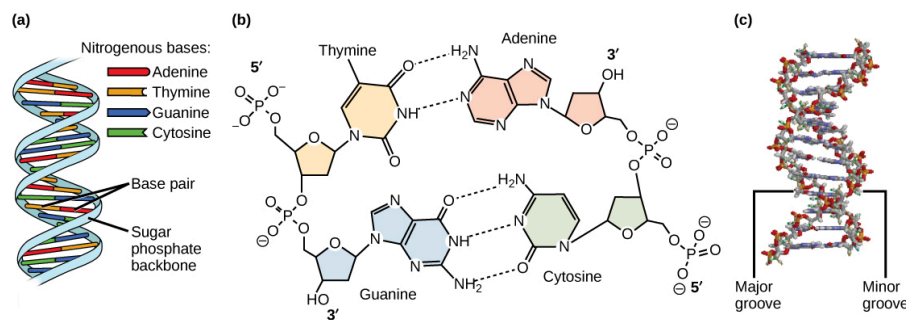
Figure 14.7 DNA has (a) a double helix structure and (b) phosphodiester bonds; the dotted lines between Thymine and Adenine and Guanine and Cytosine represent hydrogen bonds. The (c) major and minor grooves are binding sites for DNA binding proteins during processes such as transcription (the copying of RNA from DNA) and replication.

DNA Sequencing Techniques

Until the 1990s, the sequencing of DNA (reading the sequence of DNA) was a relatively expensive and long process. Using radiolabeled nucleotides also compounded the problem through safety concerns. With currently available technology and

automated machines, the process is cheaper, safer, and can be completed in a matter of hours. Fred Sanger developed the sequencing method used for the human genome sequencing project, which is widely used today (Figure 14.8).

🔗 LINK TO LEARNING

Visit this site (http://openstax.org/l/DNA_sequencing) to watch a video explaining the DNA sequence-reading technique that resulted from Sanger's work.

The sequencing method is known as the dideoxy chain termination method. The method is based on the use of chain terminators, the dideoxynucleotides (ddNTPs). The ddNTPSs differ from the deoxynucleotides by the lack of a free 3' OH group on the five-carbon sugar. If a ddNTP is added to a growing DNA strand, the chain cannot be extended any further because the free 3' OH group needed to add another nucleotide is not available. By using a predetermined ratio of deoxynucleotides to dideoxynucleotides, it is possible to generate DNA fragments of different sizes.

Dye–labeled dideoxynucleotides are used to generate DNA fragments of different lengths
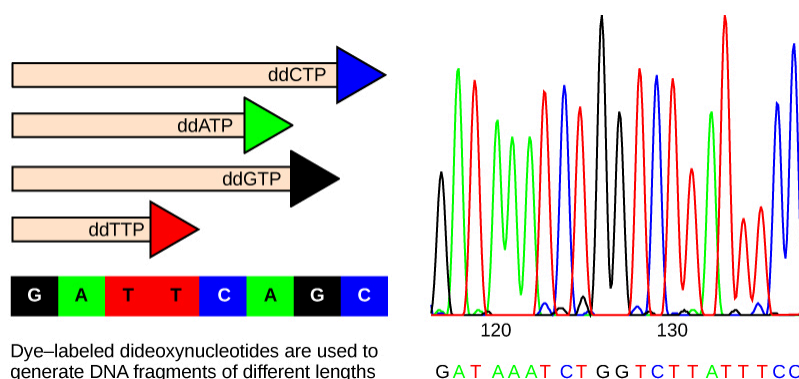
Figure 14.8 In Frederick Sanger's dideoxy chain termination method, dye-labeled dideoxynucleotides are used to generate DNA fragments that terminate at different points. The DNA is separated by capillary electrophoresis (not defined) on the basis of size, and from the order of fragments formed, the DNA sequence can be read. The DNA sequence readout is shown on an electropherogram (not defined) that is generated by a laser scanner.

The DNA sample to be sequenced is denatured (separated into two strands by heating it to high temperatures). The DNA is divided into four tubes in which a primer, DNA polymerase, and all four nucleoside triphosphates (A, T, G, and C) are added. In addition, limited quantities of one of the four dideoxynucleoside triphosphates (ddCTP, ddATP, ddGTP, and ddTTP) are added to each tube respectively. The tubes are labeled as A, T, G, and C according to the ddNTP added. For detection purposes, each of the four dideoxynucleotides carries a different fluorescent label. Chain elongation continues until a fluorescent dideoxy nucleotide is incorporated, after which no further elongation takes place. After the reaction is over, electrophoresis is performed. Even a difference in length of a single base can be detected. The sequence is read from a laser scanner that detects the fluorescent marker of each fragment. For his work on DNA sequencing, Sanger received a Nobel Prize in Chemistry in 1980.

🔗 LINK TO LEARNING

Sanger's genome sequencing has led to a race to sequence human genomes at rapid speed and low cost. Learn more by viewing the animation here (https://openstax.org/l/DNA_and_genomes) .

Gel electrophoresis is a technique used to separate DNA fragments of different sizes. Usually the gel is made of a chemical called *agarose* (a polysaccharide polymer extracted from seaweed that is high in galactose residues). Agarose powder is added to a buffer and heated. After cooling, the gel solution is poured into a casting tray. Once the gel has solidified, the DNA is loaded on the gel and electric current is applied. The DNA has a net negative charge and moves from the negative electrode toward the positive electrode. The electric current is applied for sufficient time to let the DNA separate according to size; the smallest fragments will be farthest from the well (where the DNA was loaded), and the heavier molecular weight fragments will be closest to the well. Once the DNA is separated, the gel is stained with a DNA-specific dye for viewing it (Figure 14.9).
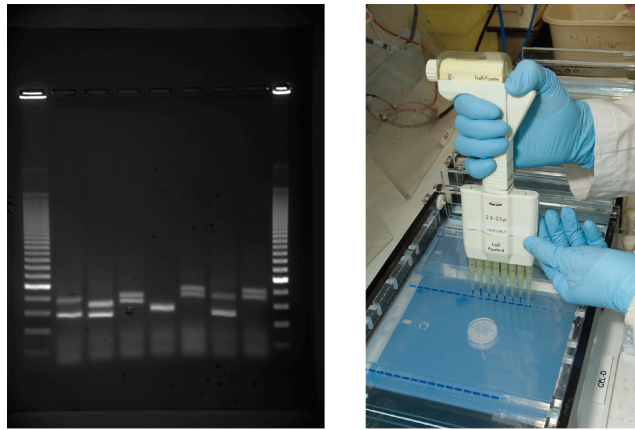
Figure 14.9 DNA can be separated on the basis of size using gel electrophoresis. (credit: James Jacob, Tompkins Cortland Community College)

EVOLUTION CONNECTION

Neanderthal Genome: How Are We Related?

The first draft sequence of the Neanderthal genome was recently published by Richard E. Green et al. in 2010.[1] Neanderthals are the closest ancestors of present-day humans. They were known to have lived in Europe and Western Asia (and now, perhaps, in Northern Africa) before they disappeared from fossil records approximately 30,000 years ago. Green's team studied almost 40,000-year-old fossil remains that were selected from sites across the world. Extremely sophisticated means of sample preparation and DNA sequencing were employed because of the fragile nature of the bones and heavy microbial contamination. In their study, the scientists were able to sequence some four billion base pairs. The Neanderthal sequence was compared with that of present-day humans from across the world. After comparing the sequences, the researchers found that the Neanderthal genome had 2 to 3 percent greater similarity to people living outside Africa than to people in Africa. While current theories have suggested that all present-day humans can be traced to a small ancestral population in Africa, the data from the Neanderthal genome suggest some interbreeding between Neanderthals and early modern humans.

Green and his colleagues also discovered DNA segments among people in Europe and Asia that are more similar to Neanderthal sequences than to other contemporary human sequences. Another interesting observation was that Neanderthals are as closely related to people from Papua New Guinea as to those from China or France. This is surprising because Neanderthal fossil remains have been located only in Europe and West Asia. Most likely, genetic exchange took place between Neanderthals and modern humans as modern humans emerged out of Africa, before the divergence of Europeans, East Asians, and Papua New Guineans.

Several genes seem to have undergone changes from Neanderthals during the evolution of present-day humans. These genes are involved in cranial structure, metabolism, skin morphology, and cognitive development. One of the genes that is of particular interest is *RUNX2*, which is different in modern day humans and Neanderthals. This gene is responsible for the prominent frontal bone, bell-shaped rib cage, and dental differences seen in Neanderthals. It is speculated that an evolutionary change in *RUNX2* was important in the origin of modern-day humans, and this affected the cranium and the upper body.

LINK TO LEARNING

Watch Svante Pääbo's talk (http://openstax.org/l/neanderthal) explaining the Neanderthal genome research at the 2011 annual TED (Technology, Entertainment, Design) conference.
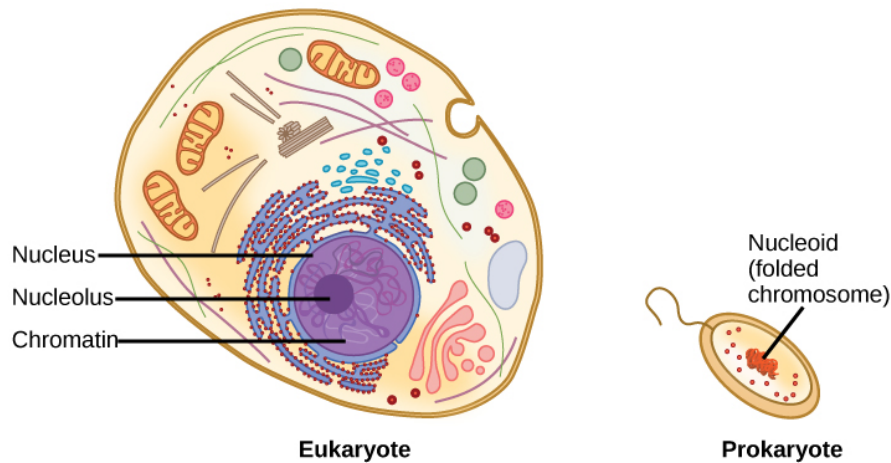
DNA Packaging in Cells

Prokaryotes are much simpler than eukaryotes in many of their features ([Figure 14.10](#)). Most prokaryotes contain a single, circular chromosome that is found in an area of the cytoplasm called the *nucleoid region*.

1Richard E. Green et al., "A Draft Sequence of the Neandertal Genome," *Science* 328 (2010): 710-22.

VISUAL CONNECTION

Figure 14.10 A eukaryote contains a well-defined nucleus, whereas in prokaryotes, the chromosome lies in the cytoplasm in an area called the nucleoid.
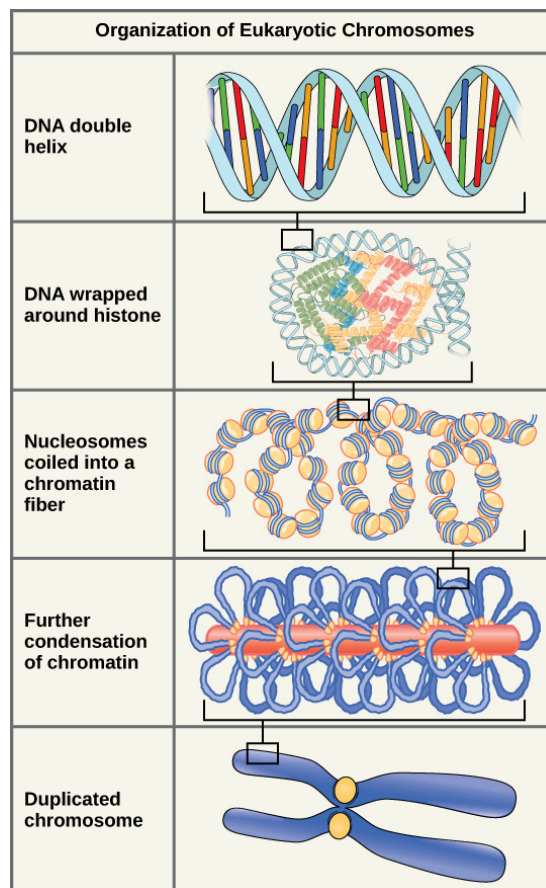
In eukaryotic cells, DNA and RNA synthesis occur in a separate compartment from protein synthesis. In prokaryotic cells, both processes occur together. What advantages might there be to separating the processes? What advantages might there be to having them occur together?

The size of the genome in one of the most well-studied prokaryotes, *E.coli,* is 4.6 million base pairs (approximately 1.1 mm, if cut and stretched out). So how does this fit inside a small bacterial cell? The DNA is twisted by what is known as supercoiling. Supercoiling suggests that DNA is either "under-wound" (less than one turn of the helix per 10 base pairs) or "over-wound" (more than 1 turn per 10 base pairs) from its normal relaxed state. Some proteins are known to be involved in the supercoiling; other proteins and enzymes such as DNA gyrase help in maintaining the supercoiled structure.

Eukaryotes, whose chromosomes each consist of a linear DNA molecule, employ a different type of packing strategy to fit their DNA inside the nucleus (Figure 14.11). At the most basic level, DNA is wrapped around proteins known as **histones** to form structures called nucleosomes. The histones are evolutionarily conserved proteins that are rich in basic amino acids and form an octamer composed of two molecules of each of four different histones. The DNA (remember, it is negatively charged because of the phosphate groups) is wrapped tightly around the histone core. This nucleosome is linked to the next one with the help of a *linker DNA.* This is also known as the "beads on a string" structure. With the help of a fifth histone, a string of nucleosomes is further compacted into a 30-nm fiber, which is the diameter of the structure. Metaphase chromosomes are even further condensed by association with scaffolding proteins. At the metaphase stage, the chromosomes are at their most compact, approximately 700 nm in width.

In interphase, eukaryotic chromosomes have two distinct regions that can be distinguished by staining. The tightly packaged region is known as heterochromatin, and the less dense region is known as euchromatin. Heterochromatin usually contains genes that are not expressed, and is found in the regions of the centromere and telomeres. The euchromatin usually contains genes that are transcribed, with DNA packaged around nucleosomes but not further compacted.

Organization of Eukaryotic Chromosomes

DNA double helix	
DNA wrapped around histone	
Nucleosomes coiled into a chromatin fiber	
Further condensation of chromatin	
Duplicated chromosome	

Figure 14.11 These figures illustrate the compaction of the eukaryotic chromosome.

14.3 Basics of DNA Replication

By the end of this section, you will be able to do the following:

- Explain how the structure of DNA reveals the replication process
- Describe the Meselson and Stahl experiments

The elucidation of the structure of the double helix provided a hint as to how DNA divides and makes copies of itself. In their 1953 paper, Watson and Crick penned an incredible understatement: "It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material." With specific base pairs, the sequence of one DNA strand can be predicted from its complement. The double-helix model suggests that the two strands of the double helix separate during replication, and each strand serves as a template from which the new complementary strand is copied. What was not clear was how the replication took place. There were three models suggested (Figure 14.12): *conservative, semi-conservative, and dispersive.*