Johns Hopkins Engineering

Molecular Biology 585.407

DNA, Chromosomes, and the Nucleus," Part 1

JOHNS HOPKINS
WHITING SCHOOL
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Information: DNA, Chromosomes, and the Nucleus
Part 1

Gregor Mendel

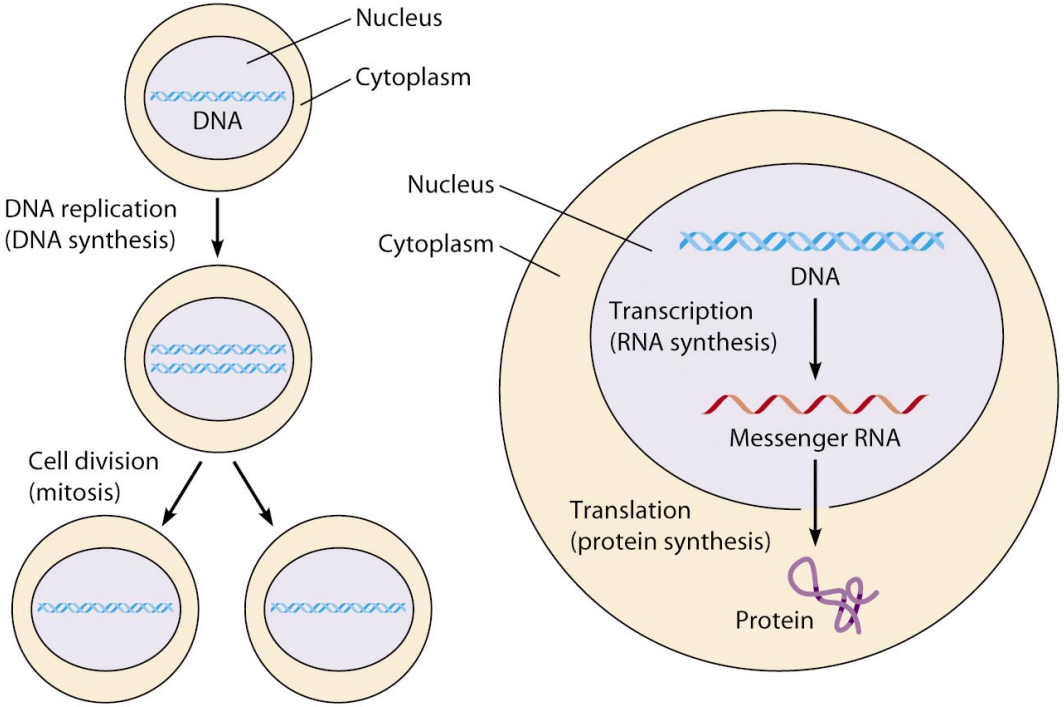
- More than a hundred years ago, the Augustinian monk **Gregor Mendel** worked out rules accounting for the inheritance patterns he observed in pea plants. He had little inkling of the cellular or molecular basis for these rules.
- These studies led Mendel to conclude that hereditary information is transmitted in the form of distinct units that we now call ***genes***.
- We now know that genes are made of DNA - we can tell a coherent genetic story starting with this important molecule.

Information and DNA

- Information carried by DNA flows both ***between*** generations of cells and ***within*** each individual cell.

- During the first of these two processes, information stored in a cell's DNA molecules undergoes ***replication***, generating two DNA copies that are distributed to the daughter cells when the cell divides.

Information and DNA



(a) The flow of genetic information between generations of cells

(b) The flow of genetic information within a cell: the expression of genetic information

Figure 18-1

The flow of information in cells

From Figure 18-1:

- The diagrams here feature eukaryotic cells (eukaryotic cells possess a clearly **defined** nucleus), but DNA replication, called division, transcription, and translation are processes that occur in prokaryotic cells as well.

- Genetic information encoded in DNA molecules is passed on to successive generations of cells by DNA **replication** and **cell division** (in eukaryotic cells, by **mitosis**)

- DNA is first duplicated and then divided equally between the two daughter cells. In this way, each daughter cell is assured of having the same genetic information as the parent.

The flow of information in cells

Figure 18-1

- Within each cell, genetic information encoded in the DNA is expressed through the processes of **transcription** (DNA synthesis) and **translation** (protein synthesis).
- **Transcription** involves the use of selected segments of DNA as templates for the synthesis of Messenger RNA and other RNA molecules.
- **Translation** is the process whereby **amino acids** are joined in a sequence dictated by the sequence of nucleotides in messenger RNA.

The flow of information within cells

Figure 18-1b:

- During **transcription**, RNA is synthesized in an enzymatic reaction that copies information from DNA.
- During **translation**, the base sequences of the resulting messenger RNA molecules are used to determine the amino acid sequences of proteins.
- This figure shows these events in eukaryotic cells, which carry out **transcription** within a membrane-bounded nucleus and transport messenger RNA to the cytoplasm for **translation**.
- It is the proteins synthesized by a cell that ultimately determine most of a cell's structural features as well as the functions it performs.
- Transcription and translation, which together constitute the **expression of genetic information**.

Johann Friedrich Miescher

- DNA was unwittingly discovered by **Johann Friedrich Miescher**, a Swiss physician.
- Miescher reported the discovery of the substance now known as DNA in 1869, just a few years before the cell biologist **Walther Flemming** first observed chromosomes as he studied dividing cells under the microscope.
- Miescher's Discovery of DNA led to conflicting proposals concerning the **Chemical Nature of Genes**.

Miescher flawed experiments

- Miescher isolated nuclei from white blood cells obtained from pus found on surgical bandages.
- Upon extracting these nuclei with base, he discovered an unusual substance, which he called "**nuclein**" and which we now know to have been largely DNA.
- Miescher then went on to study DNA from a more pleasant source, salmon sperm. Fish sperm may seem a somewhat unusual source material, until we realize that the nucleus accounts for more than 90% of the mass of a typical sperm cell and therefore DNA accounts for most of the mass of sperm cells.
- Miescher initially believed that DNA was involved in the transmission of hereditary information. He soon rejected this idea because his crude measuring techniques incorrectly suggested that egg cells contain much more DNA than sperm.
- Reasoning that sperm and egg must contribute roughly equal amounts of hereditary information to the offspring, it seemed to him that DNA could not be carrying hereditary information.

Botanist Eduard Zacharias

- In the early 1880s botanist **Eduard Zacharias** reported that extracting DNA from cells caused the staining of the chromosomes to disappear.

- Since evidence was already beginning to suggest a role for chromosomes in transmitting hereditary information, **Zacharias and others inferred that DNA is the genetic material**.

- This view prevailed until the early 1900's, when incorrectly interpreted staining experiments led to the false conclusion that the amount of DNA changes dramatically within cells.

- Because cells would be expected to maintain a constant amount of the substance that stores their hereditary instructions, these mistaken observations led to a repudiation of the idea that DNA carries genetic information.

DNA, a simple polymer

From around 1910 to the1940s, most scientists believed that **genes were made of protein** rather than DNA.

The building blocks of both proteins and nucleic acids had been identified by the early 1900s, and proteins were perceived to be more complex and hence more likely to store genetic information.

It was argued that proteins are constructed from 20 different amino acids that can be assembled in a vast number of combinations, thereby generating the sequence diversity and complexity expected of a molecule that stores and transmits genetic information.

DNA was widely perceived to be a **simple polymer** consisting of the same sequence of four bases (the tetra-nucleotide -ATCG-) repeated over and over, thereby **lacking the variability expected of a genetic molecule**.

Such a simple polymer was thought to serve merely as a structural support for the genes, which were in turn made of protein. This view prevailed until two lines of evidence resolved the matter in favor of DNA as the genetic material…..

Avery showed DNA is genetic material of bacteria

- 1928 British physician **Frederick Griffith**, who was studying a pathogenic strain of a bacterium, then called "pneumococcus" causes a fatal pneumonia in animals.

- Griffith discovered that this bacterium (now called **Streptococcus pneumoniae***)* exists in two forms called the *S strain* and the *R strain.*

- When grown on a solid agar medium
  - S strain produces colonies that are smooth and shiny because of the mucous, polysaccharide coat each cell secretes
  - R strain lacks the ability to manufacture a mucous coat and therefore produces colonies exhibiting a rough boundary

(a) Living S (smooth) bacteria **(b)** Living R (rough) bacteria **(c)** Heat-killed S bacteria **(d)** Heat-killed S bacteria mixed with living R bacteria **(e)** Living S bacteria in blood from dead mice

Figure 18-2

Avery showed DNA is genetic material of bacteria

- When injected into mice, S-strain, but not R-strain bacteria trigger a fatal pneumonia. The ability to cause disease is directly related to the presence of the S strain's polysaccharide coat, which protects the bacterial cell from attack by the mouse's immune system.

- One of the most intriguing discoveries made by Griffith, however, was that pneumonia can also he induced by injecting animals with a mixture of live R-strain bacteria and dead S-strain bacteria (Figure 18-2).

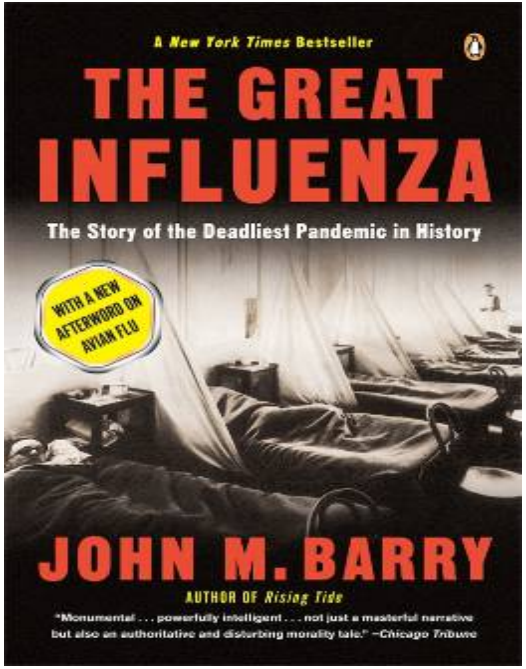
- This finding was surprising because neither live R-strain nor dead S-strain organisms cause pneumonia if injected alone. When Griffith autopsied the animals that had been injected with the mixture of live R-strain and dead S-strain bacteria, he found them teeming with live S-strain bacteria.

- Since the animals had not been injected with any live S-strain cells, he concluded that the nonpathogenic R bacteria were somehow converted into pathogenic S bacteria by a substance present in the heat-killed S bacteria that had been co-injected.

- He called this phenomenon genetic transformation and referred to the active (though still unknown) substance in the S cells as the "transforming principle."

Griffith’s discoveries

- Griffith's discoveries set the stage for 14 years of work by Oswald Avery and his colleagues at the Rockefeller Institute in New York.
- These researchers pursued the investigation of bacterial transformation to its logical conclusion by asking which component of the heat-killed S bacteria was responsible for the transforming activity.
- They fractionated cell-free extracts of S-strain bacteria and found that only the nucleic acid fraction caused transformation.
- Moreover, the activity was specifically eliminated by treatment with deoxyribonuclease, an enzyme that degrades DNA.
- This and other evidence convinced them the transforming substance of pneumoeoecus was DNA, a conclusion published by Avery, Colin MacLeod, and Maclyn McCarty in 1944.
- This was the first rigorously assertion that DNA can carry genetic information.



Skepticism in the science community

- Though the experiments of Avery and his colleagues were rigorous, the assignment of a genetic role to DNA did not meet with immediate acceptance.
- Skepticism was due in part to the persistent, widespread conviction that DNA lacked the necessary complexity for such a role.
- In addition, many scientists questioned whether genetic information in bacteria had anything to do with heredity in other organisms.
- Most remaining doubts were alleviated eight years later when DNA was shown to be the genetic material of a virus, the bacteriophage T2.

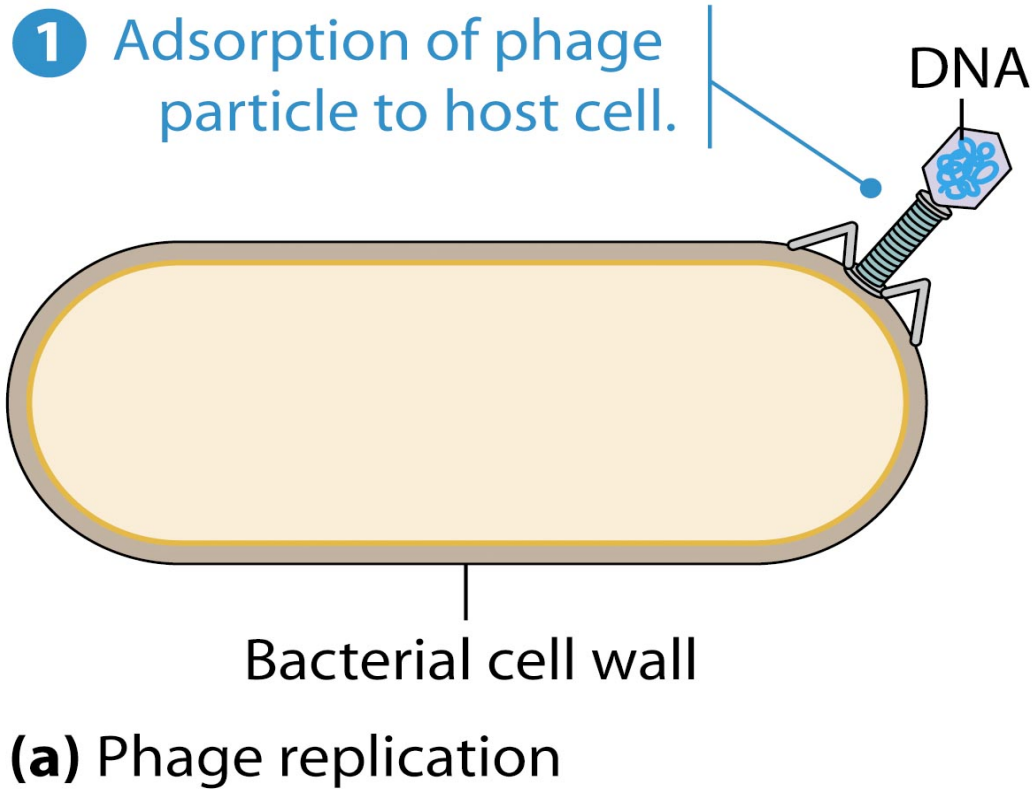


Figure 18A-2a

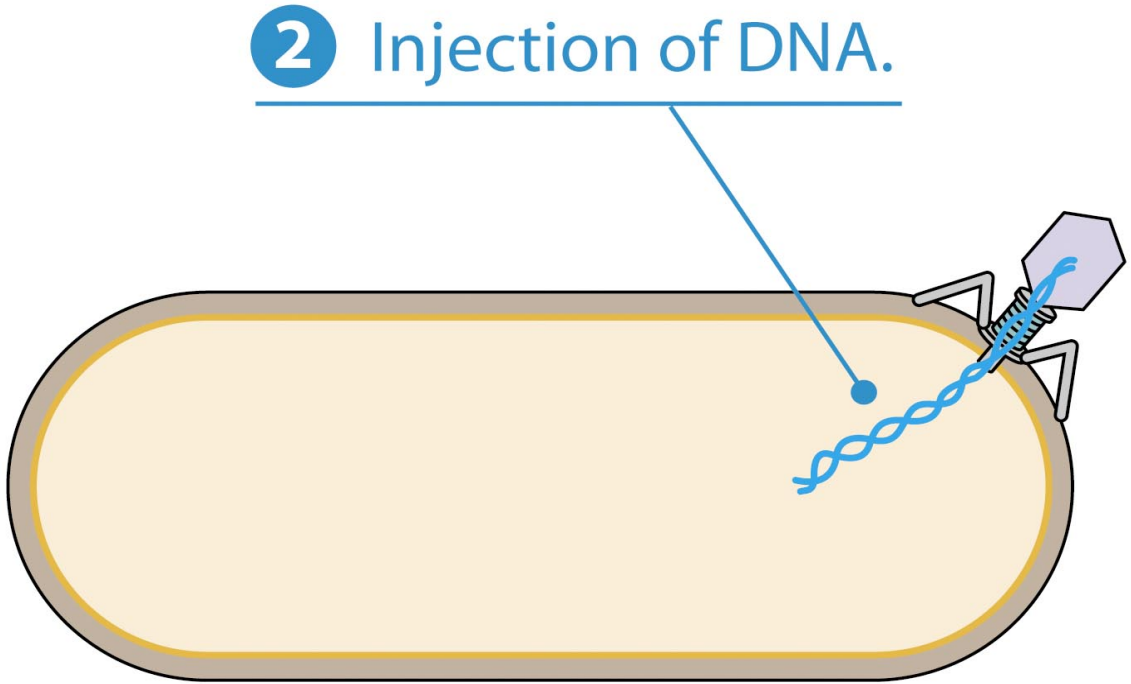


Figure 18A-2b

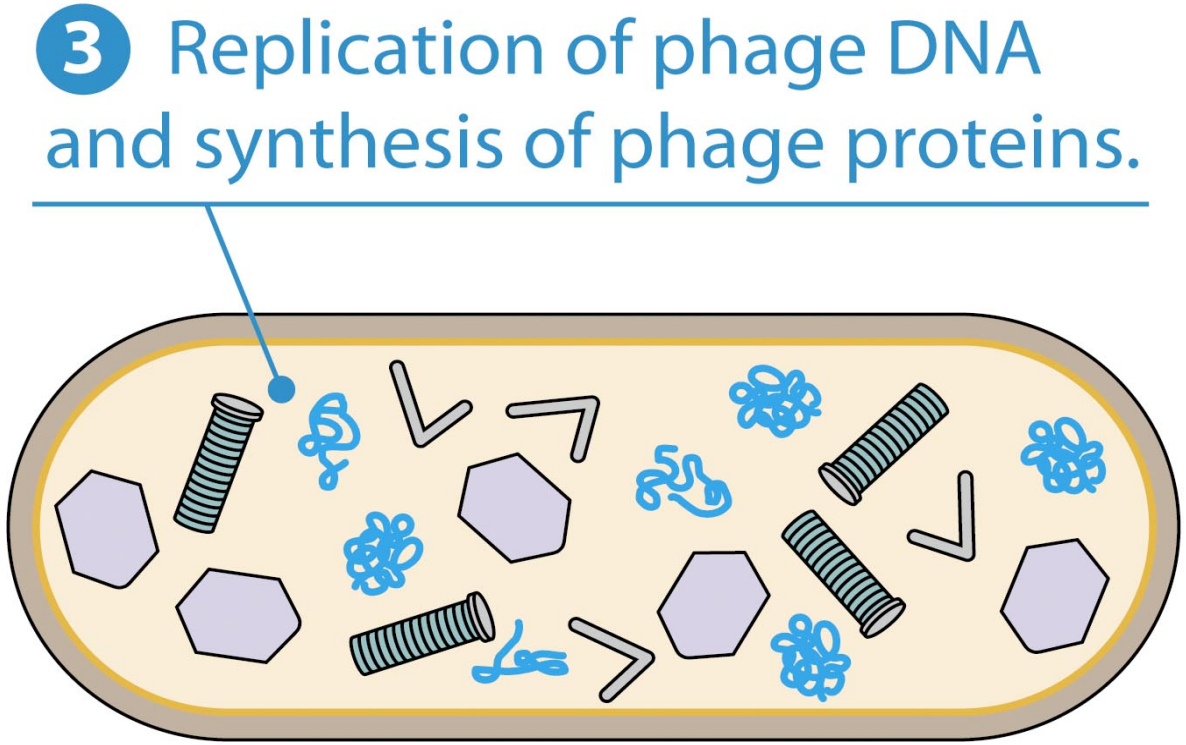


Figure 18A-2c

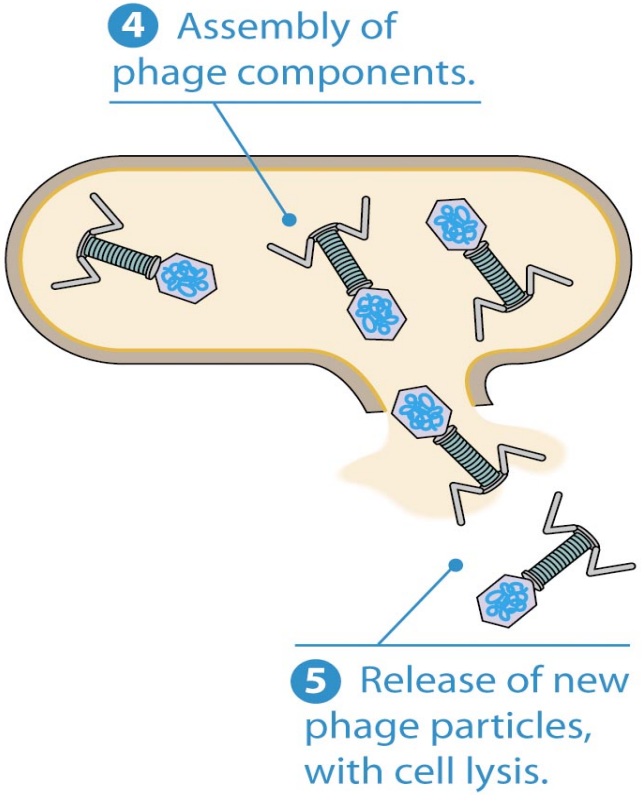


Figure 18A-2d

Hershey and Chase showed that DNA is the genetic material of viruses

- **Bacteriophages or phages** are viruses that infect bacteria. They have been objects of scientific study since the 1930s, and much of our early understanding of molecular genetics came from experiments involving these viruses. Box 18A describes the anatomy and replication cycle of some phages and highlights their advantages for genetic studies.

- One of the most thoroughly studied of the phages that infect bacterium *Estherichia coli is* bacteriophage T2. During infection, this virus attaches to the bacterial cell surface and injects material into the cell. Shortly thereafter, the bacterial cell begins to produce thousands of new copies of the virus.

- This scenario suggests that material injected into the bacterial cell carries the genetic information that guides the production of the virus. What is the chemical nature of the injected material?

- In 1952 Alfred Hershey and Martha Chase designed an experiment to address this question. Only two possibilities exist because the T2 virus is constructed from only two kinds of molecules: DNA and protein.

- To distinguish between these two alternatives, Hershey and Chase took advantage of the fact that the proteins of the T2 virus, like most proteins, contain the element sulfur (in the amino acids methionine and cysteine) but not phosphorus, while the viral DNA contains phosphorus (in its sugarphosphate backbone) but not sulfur.

- Hershey and Chase prepared two batches of T2 phage particles (as intact phages are called) with different kinds of radioactive labeling. In one batch, the phage proteins were labeled with the radioactive isotope ^{35}S; in the other batch, the phage DNA was labeled with the isotope ^{32}P.

Hershey and Chase Showed that DNA is the genetic material of viruses

- By using radioactive isotopes, **Hershey and Chase** were able to trace the fates of both protein and DNA during the infection process (Figure 18-3a).
- They began the experiment by mixing radioactive phage with intact bacterial cells and allowing the phage particles to attach to the bacterial cell surface and inject their genetic material into the cells.
- Hershey and Chase found that the empty protein coats (or phage "ghosts") could be effectively removed from the surface of the bacterial cells by agitating the suspension in an ordinary kitchen blender and recovering the bacterial cells by centrifugation.
- They then measured the radioactivity in the supernatant liquid and in the pellet of bacteria at the bottom of the tube.
- The data revealed that most (65%) of the ^{32}P remained with the bacterial cells, while the hulk (80%) of the ^{35}S was released into the surrounding medium (Figure 18-3b). Since the ^{32}P labeled the viral DNA and the ^{35}S labeled the viral protein, **Hershey and Chase concluded that DNA, not protein**, had been injected into the bacterial cells and must function as the genetic material of phage T2.
- This conclusion received further support from the following observation: when the infected, radioactive bacteria were re-suspended in fresh liquid and incubated longer, the ^{32}P was transferred to some of the offspring phage particles, but the ^{35}S was not.

The Hershey-Chase Experiment: DNA as the Genetic Material of Phage T2

- **Figure 18A-3 (Right)**
- (a) T2 is radioactively labeled with *either* ***Sulfur 35*** (to label protein) or ***phosphorous 32*** (to label DNA) is used to infect duplicate batches of bacteria.
- The phages adsorb to the cell surface and inject their DNA.
- Agitation of the infected cells in a blender dislodges most of the ^{35}**S** from the cells in phage ghosts, whereas most of the ^{32}**P** remains with the cells.
- Immediate centrifugation causes the cells to form a pellet in the tube; any free phage particles, including ghosts, remain in the supernatant liquid.
- The radioactivity of the pellet and supernatant are measured separately.

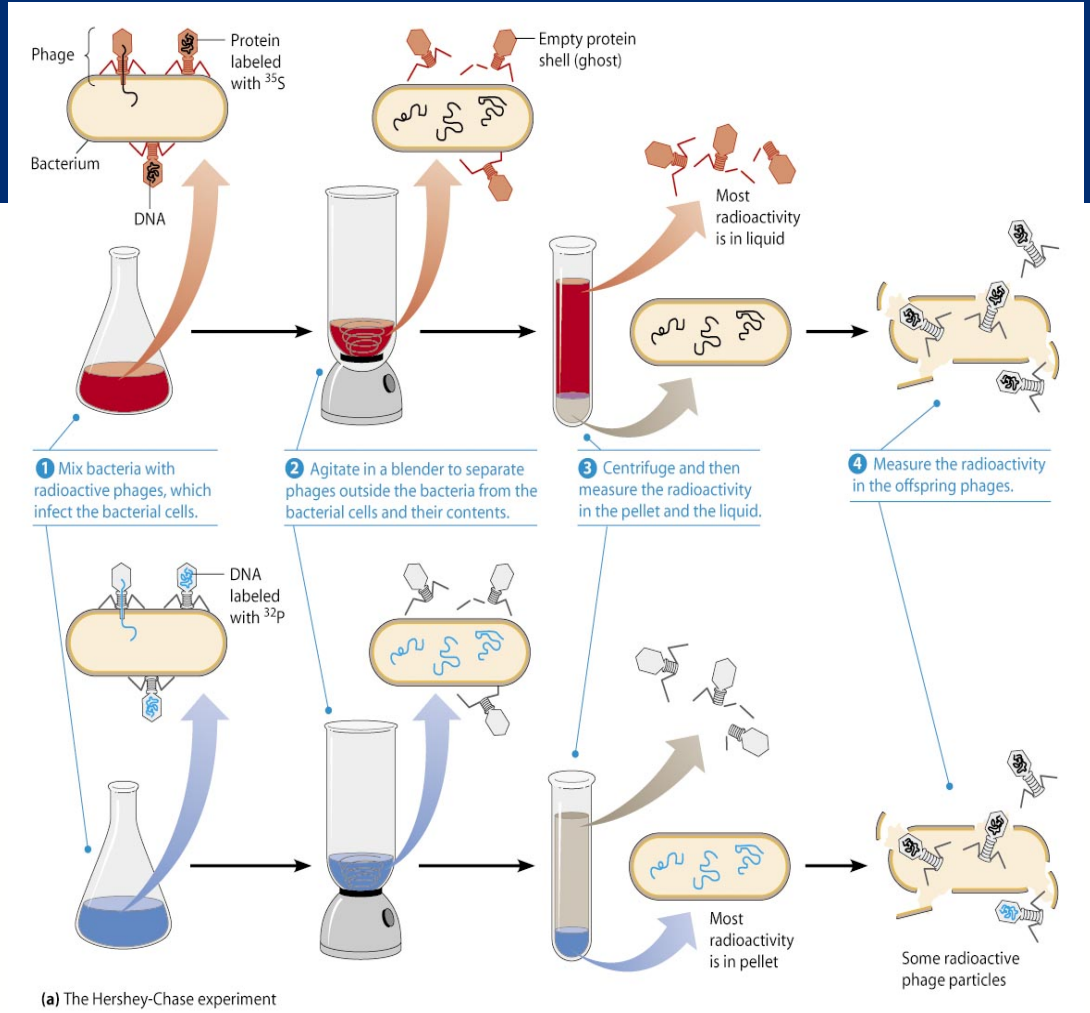


Figure 18-3a

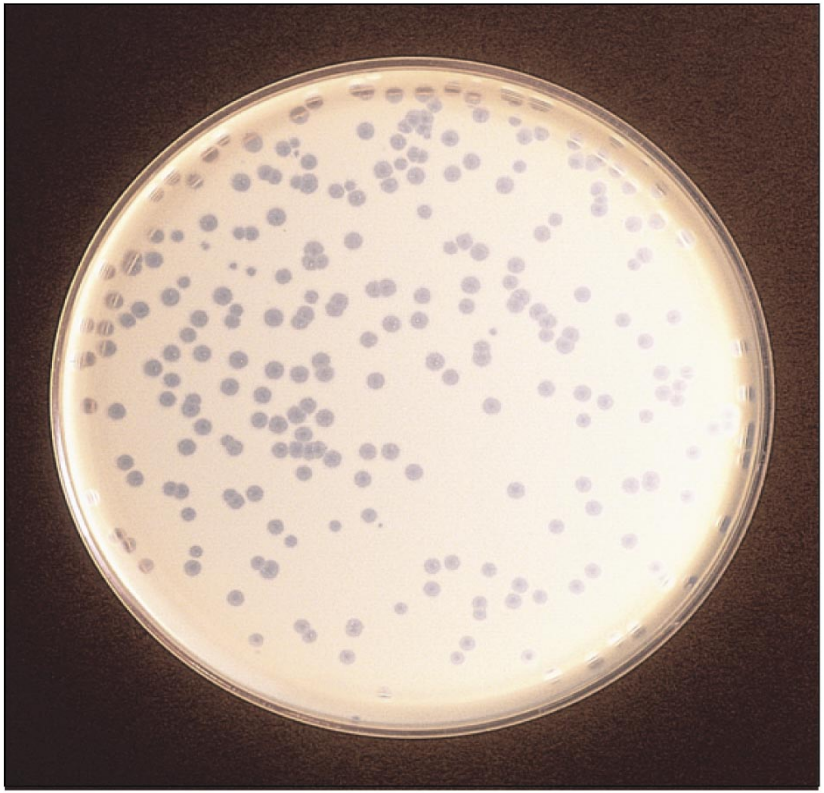
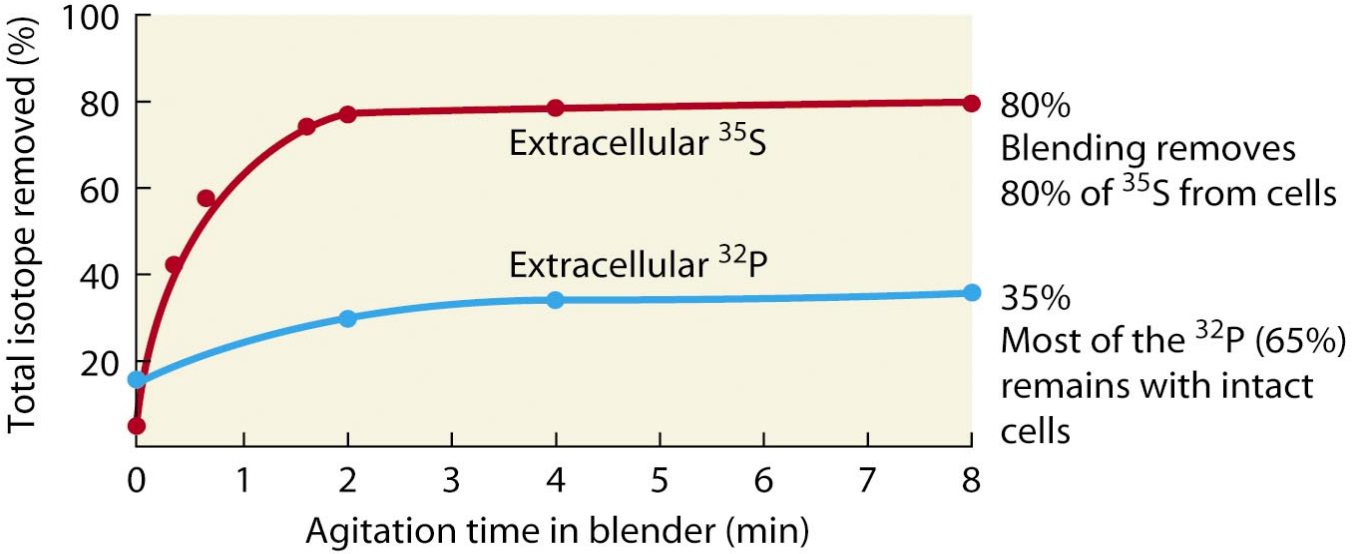


Figure 18A-3



(b) Experimental data from part a, step 3

Figure 18-3b

From figure 18-3b

- When the cells in each pellet are incubated further, the phage DNA within them dictates the synthesis and eventual release of new phage particles.
- Some of these phages contain ^{32}P in their DNA (because the old, labeled phage DNA is packaged into some of the new particles), but none contain in their coat proteins.
- The graph shows the extent to which ^{35}P and ^{32}S are removed from the intact cells at step 3, as a function of time in the blender.
- More than 90% of the cells remain intact, even after 8 minutes of blending, and even a few minutes of blending is enough to remove most (80%) of the ^{35}S, while leaving most (65%) of the ^{32}P with the cells.

Chargaff's Rules reveal that A = T and C = C

- Despite the lukewarm reaction initially received by Avery's work, it was an important influence on several other scientists.

- Among them was **Erwin Chargaff**, who was interested in the base composition of DNA. Between 1944 and 1952, Chargaff used chromatographic methods to separate and quantify the amounts of the four bases-adenine (A), guanine (G), cytosine (C), and thymine (T) found in DNA.

- From his analyses came several important discoveries. First, **he showed that DNA isolated from different cells of a given species have the same percentage of each of the four bases** (Table 18-1, lines 1-4), and that **this percentage does not vary with individual, tissue, age, nutritional state, or environment.**

- This is exactly what would be expected of the chemical substance that stores genetic information, because the cells of a given species would be expected to have similar genetic information.

- However, Chargaff did find that DNA base composition varies from species to species. This can be seen by examining the last column of Table 18-1, which shows the relative amounts of the bases A and T versus C and C in the DNAs of various organisms.

Chargaff's rules

- Comparison of such data revealed to Chargaff that DNA preparations from closely related species have similar base compositions, whereas those from very different species tend to exhibit quite different base compositions. Again, this is what would be expected of a molecule that stores genetic information.

- But Chargaff's most striking observation was his discovery that for all DNA samples examined, the number of adenines is equal to the number of thymines (A = T), and the number of guanines is equal to the number of cytosines (G = C).

- This meant that the number of purines is equal to the number *of* pyrimidines (A + T = C + G).

- The significance of these equivalencies, known as **Chargaff's rules,** was an enigma and remained so until the double-helical model of DNA was established by Watson and Crick in 1953.

Table 18-1 DNA Base Composition Data That Led to Chargaff's Rules

Source of DNA	Number of Each Type of Nucleotide*				Nucleotide Ratios**		
	A	T	G	C	A/T	G/C	(A + T)/(G + C)
Bovine thymus	28.4	28.4	21.1	22.1	1.00	0.95	1.31
Bovine liver	28.1	28.4	22.5	21.0	0.99	1.07	1.30
Bovine kidney	28.3	28.2	22.6	20.9	1.00	1.08	1.30
Bovine brain	28.0	28.1	22.3	21.6	1.00	1.03	1.28
Human liver	30.3	30.3	19.5	19.9	1.00	0.98	1.53
Locust	29.3	29.3	20.5	20.7	1.00	1.00	1.41
Sea urchin	32.8	32.1	17.7	17.3	1.02	1.02	1.85
Wheat germ	27.3	27.1	22.7	22.8	1.01	1.00	1.19
Marine crab	47.3	47.3	2.7	2.7	1.00	1.00	17.50
Aspergillus (mold)	25.0	24.9	25.1	25.0	1.00	1.00	1.00
Saccharomyces cerevisiae (yeast)	31.3	32.9	18.7	17.1	0.95	1.09	1.79
Clostridium (bacterium)	36.9	36.3	14.0	12.8	1.02	1.09	2.73

*The values in these four columns are the average number of each type of nucleotide found per 100 nucleotides in DNA.
**The A/T and G/C ratios are not all exactly 1.00 because of experimental error.

Chargaff's rules

First rule

- The first rule is that a double-stranded DNA molecule globally has percentage base pair equality: %A = %T and %G = %C. The rigorous validation of the rule constitutes the basis of Watson-Crick pairs in the DNA double helix model.

Second rule

- The second rule is that both %A = %T and %G = %C are valid for each of the two DNA strands. This describes only a global feature of the base composition in a single DNA strand..

DNA structure

As the scientific community gradually came to accept the conclusion that DNA stores genetic information, a new set of questions began to emerge concerning the way in which DNA performs its genetic function.

One of the first issues to arise was the question of how DNA is accurately replicated so that duplicate copies of the genetic information can be passed on from cell to cell during cell division, and from parent to offspring.

Answering this question required an understanding of the three-dimensional structure of DNA, which was provided in 1953 when Watson and Crick formulated their double-helical model of DNA.

Watson and Crick discovered that DNA is a double helix

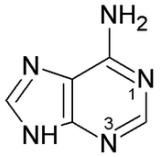
- In 1952, **James Watson and Francis Crick** were among a small number of scientists who were convinced that DNA was the genetic material and that knowledge of its three-dimensional structure would provide valuable clues as to how it functioned. Working at Cambridge University in England, they approached the puzzle by building wire models of possible structures. DNA had been known for years to be a long polymer with a backbone of repeating deoxyribose and phosphate units, and a nitrogenous base attached to each sugar.

- Watson and Crick were aided in their model building by knowing that the forms in which the bases A, G, C, and T exist at physiological pH permit specific hydrogen bonds to form between pairs of them. The crucial experimental evidence, however, came from an X-ray diffraction pattern of DNA determined by Rosalind Franklin, working in the laboratory of Maurice Wilkins at King's College in London. Franklin's picture told Watson and Crick that DNA was a helical structure composed of two strands-a double helix.

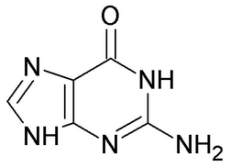
- Rosalind Elsie Franklin (25 July 1920 – 16 April 1958) was an English chemist and X-ray crystallographer who made contributions to the understanding of the molecular structures of DNA (deoxyribonucleic acid), RNA (ribonucleic acid), viruses, coal, and graphite. Although her works on coal and viruses were appreciated in her lifetime, her contributions to the discovery of the structure of DNA were largely recognized posthumously. https://en.wikipedia.org/wiki/Rosalind_Franklin

- Watson and Crick then put this information together with what they already knew to arrive at a model In their model, the sugar-phosphate backbones are on the outside of the helix, and the bases face inward toward the center of the helix, forming the "steps" of the "circular staircase" that the structure resembles.

Purines

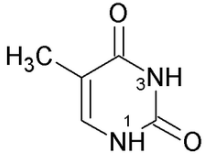


Adenine

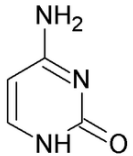


Guanine

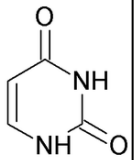
Pyrimidines



Thymine



Cytosine



Uracil

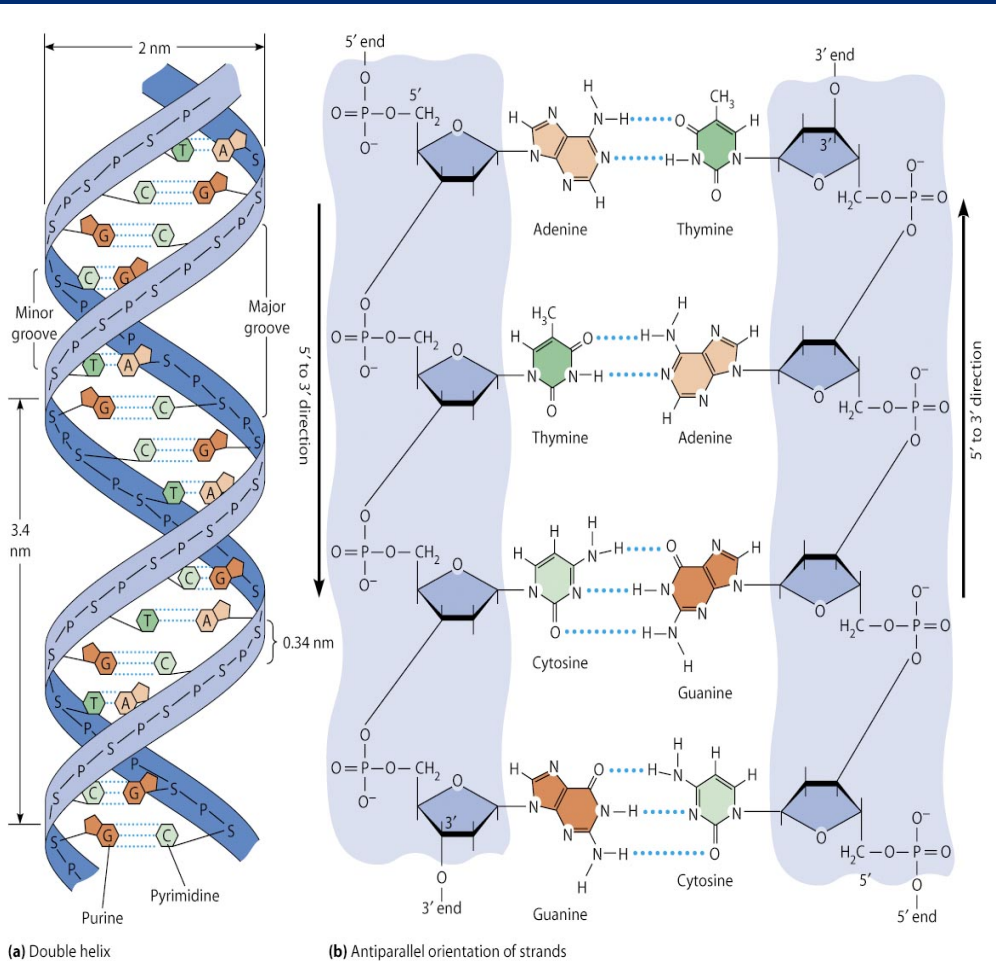


Figure 18-4

Structural features of the Watson-Crick double helix

- Figure 18-4a illustrates several structural features of the Watson-Crick double helix.

- The helix is right-handed, meaning that it curves "upward" to the right (notice that this is true even if you turn the diagram upside down). It contains ten nucleotide pairs per turn and advances 0.34 nm per nucleotide pair. Consequently, each complete turn of the helix adds 3.4 nm to the length of the molecule. The diameter of the helix is 2 nm. This distance turns out to be too small for two purines and too great for two pyrimidines, but it accommodates a purine and a pyrimidine well, consistent with Chargaff's rules.

- Pyrimidine-purine pairing, in other words, was necessitated by steric considerations. The two strands are held together by hydrogen bonding between the bases in opposite strands. Moreover, the hydrogen bonds holding together the two strands of the double helix *only fit when they form between the base adenine (A) in. one chain and thymine (T) in the other, or between the base guanine (G) in one chain and cytosine (C) in the other.* This means that the base sequence of one chain determines the base sequence of the opposing chain; the two chains of the DNA double helix are therefore said to be **complementary** to each other.

- Such a model explains why Chargaff had observed that DNA molecules contain equal amounts of the bases A and T and equal amounts of the bases G and C.

Watson-Crick model

- The most profound implication of the Watson-Crick model was that it suggested a mechanism by which cells can replicate their genetic information: The two strands of the DNA double helix could simply separate from each other prior to cell division so that each strand could function as a template dictating the synthesis of a new complementary DNA strand using the base-pairing rules.

- In other words, the base A in the template strand would specify insertion of the base T in the newly forming strand, the base C would specify insertion of the base C, the base T would specify insertion of the base A, and the base C would specify insertion of the base C. In the next chapter, we will discuss the experimental evidence for this proposed mechanism and describe the molecular basis of DNA replication in detail.

- Several other important features of the DNA double helix are illustrated in Figure 18-4. For example, the way in which the two strands are twisted around each other creates a *major groove* and a *minor groove.* These grooves play significant roles in the interactions of a variety of molecules with DNA. Another important feature is the *antiparallel* orientation of the two DNA strands, which is illustrated in Figure l8-4b.

- This diagram shows that as you move along one of the strands in a given direction, successive nucleotides are linked together by phosphodiester bonds that join the 5' carbon of one nucleotide to the 3' carbon of the next nucleotide; such a chain is said to have a *5' - 3' orientation.* But if you move along the opposing strand in the same direction, the order of the bonds exhibits a 3' - 5' orientation.

- In other words, the phosphodiester bonds of the two strands are oriented in opposite directions. The differing orientations of the two strands is a feature that has important implications for both DNA replication and DNA transcription.

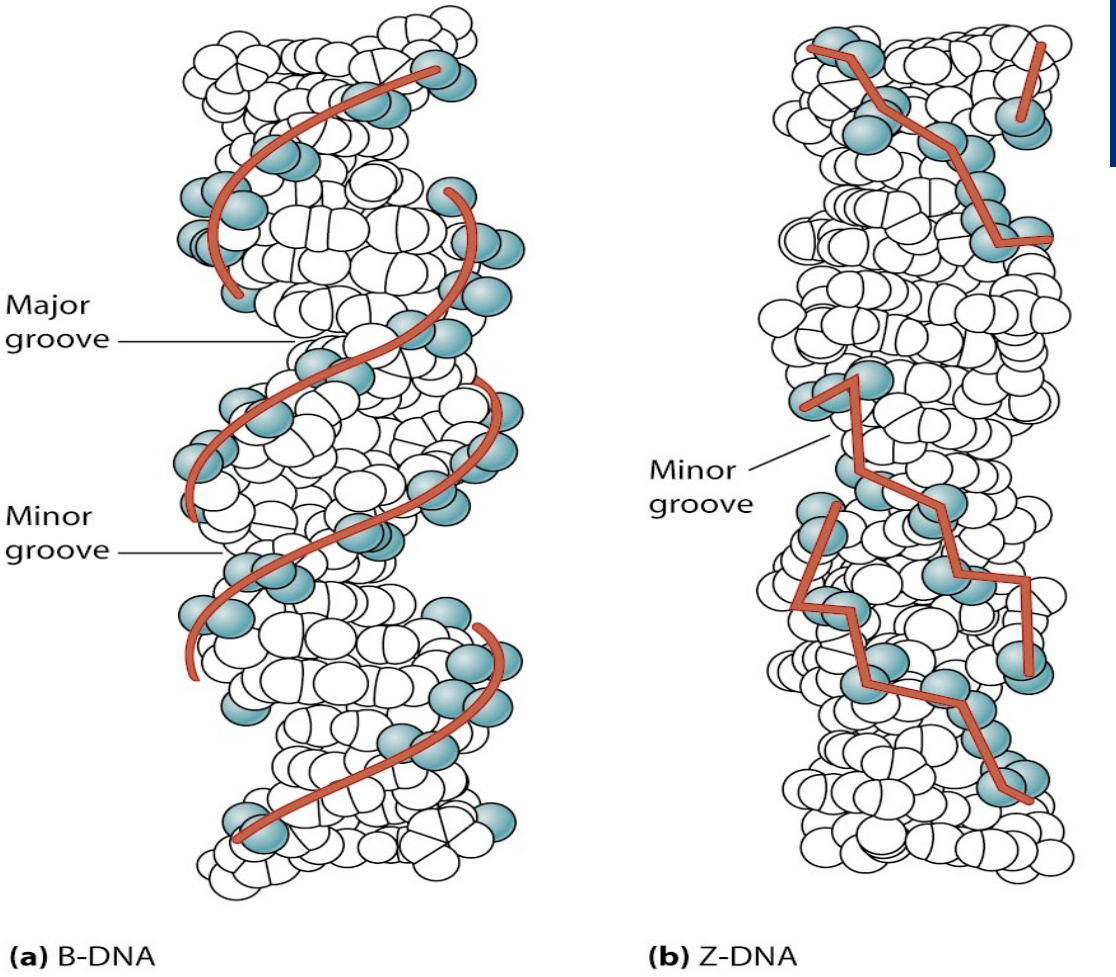


Figure 18-5

DNA can be interconverted between relaxed and supercoiled forms

- In many situations, the DNA double helix can be twisted upon itself to form **supercoiled DNA**.
- Although now known to be a widespread property of DNA, supercoiling was first identified in the DNA of certain small viruses containing circular DNA molecules that exist as closed loops.
- Circular DNA molecules are also found in bacteria, mitochondria, and chloroplasts.
- Although supercoiling is not restricted to circular DNA, it is easiest to study in such molecules.

DNA can be interconverted between relaxed and supercoiled forms

- A DNA molecule can go back and forth between the supercoiled state and the nonsupercoiled, or *relaxed,* state. To understand the basic idea, perform the following exercise.

- Start with a length of rope consisting of two strands twisted together into a right-handed coil; this is the equivalent of a relaxed, linear DNA molecule. Just joining the ends of the rope together changes nothing; the rope is now circular but still in a relaxed state. But if before sealing the ends you first give the rope an extra right-handed twist (i.e., another twist in the direction in which the strands are already entwined around each other), the rope becomes overwound and is thrown into what is called a *positive supercoil.*

- *Conversely, if the rope is given a left-handed twist before sealing (i.e., twisted in the direction opposite to that in which it is wound), it becomes underwound and is* thrown into a *negative supercoil.* Circular DNA molecules found in nature, including those of bacteria, viruses, and eukaryotic organelles, are invariably negatively supercoiled.

Supercoiling helps to make the DNA more compact

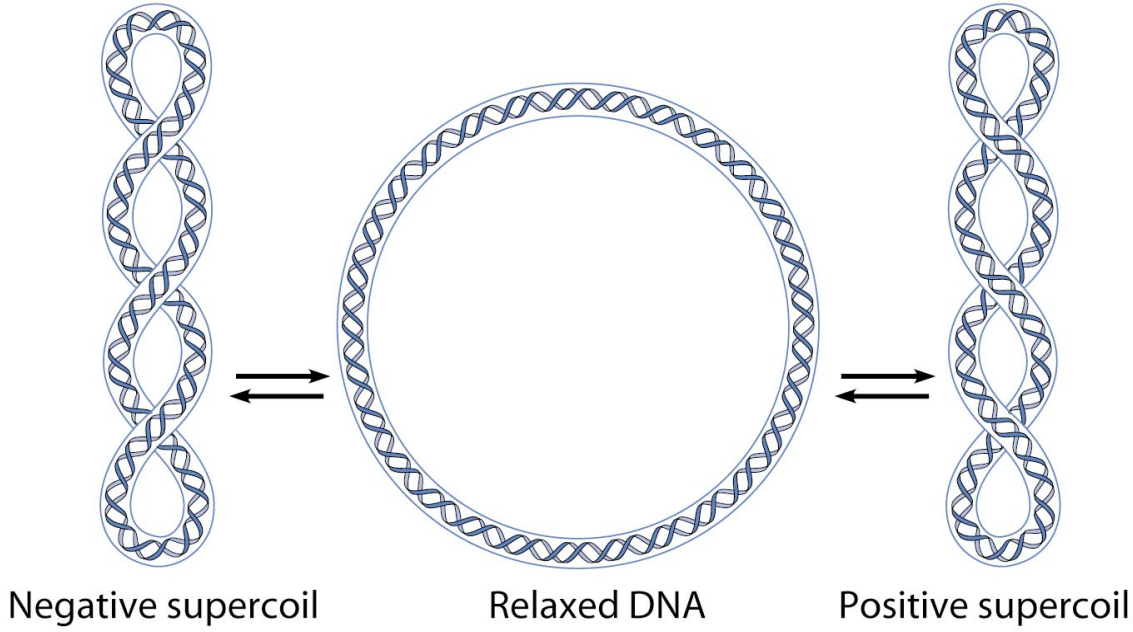


Figure 18-6a

Supercoiling helps to make the DNA more compact

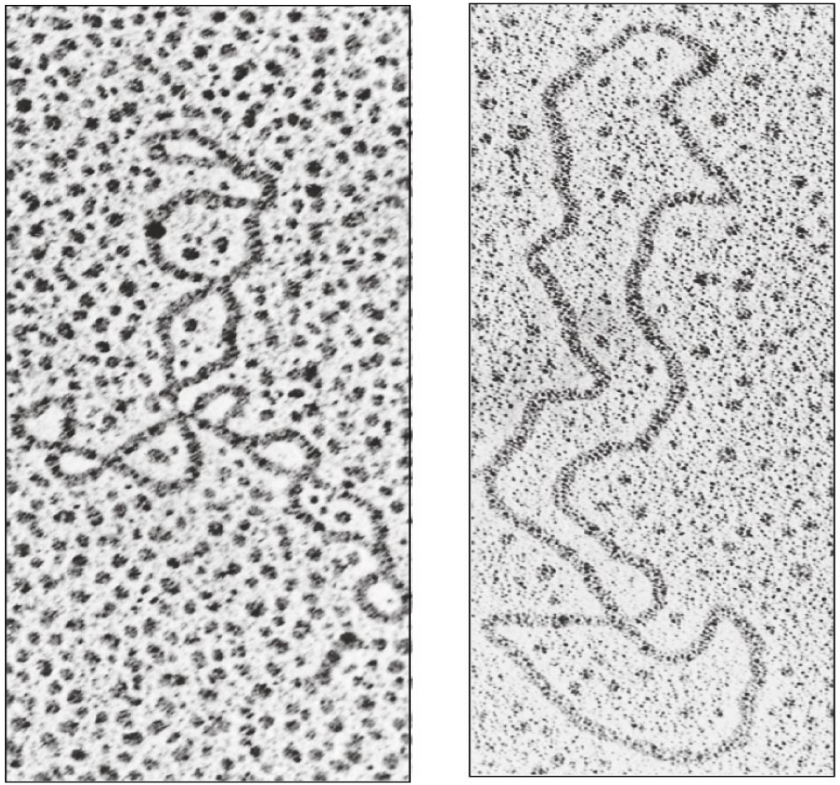


Figure 18-6b

One way to denature DNA in the laboratory is to raise its temperature

- The temperature at which one-half of the absorbance change has been achieved is called the DNA melting temperature (Tm). The value of the melting temperature reflects how tightly the DNA double helix is held together.

- For example, G-C base pairs, held together by three hydrogen bonds, are more resistant to separation than A-T base pairs, which have only two (see Figure 18-4b).

- Likewise, DNA molecules in which the two strands of the double helix are properly base-paired at each position will melt at higher temperatures than DNA in which the two strands are not perfectly complementary.

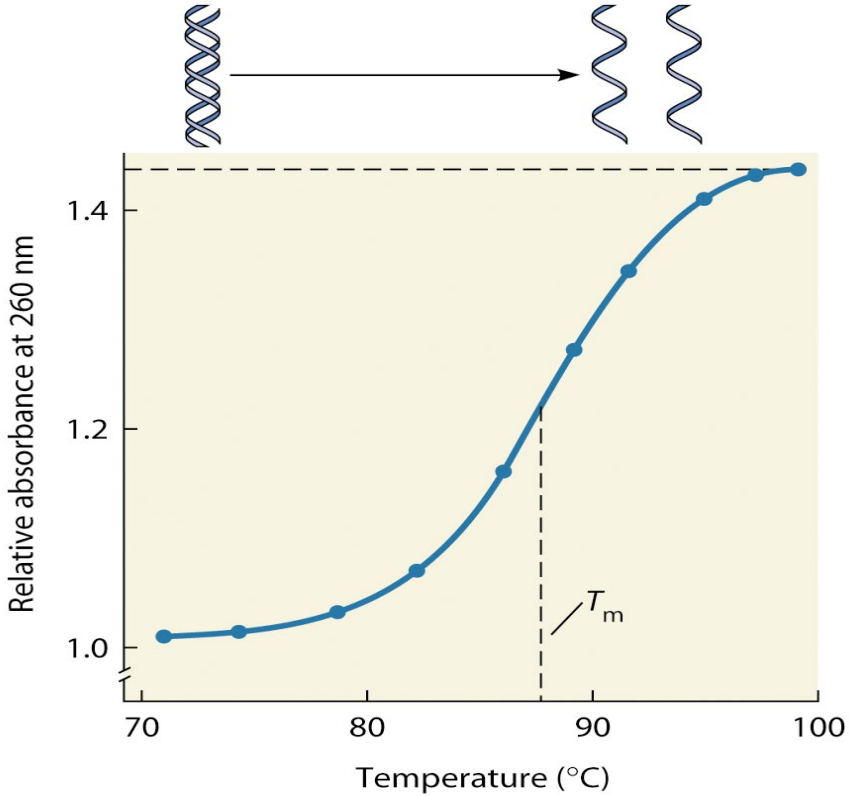


Figure 18-8

The DNA Double Helix can be separated by Denaturation and rejoined by Renaturation

- Because the two strands of the DNA double helix are **bound together by relatively weak, non-covalent bonds**, the two strands can he readily separated from each other under appropriate conditions.

- Strand separation is an integral part of both DNA replication and RNA synthesis.

- Strand separation can also be induced experimentally, resulting in DNA denaturation; the reverse process, which reestablishes a double helix from separated DNA strands, is called DNA renaturation.

- One way to denature DNA in the laboratory is to raise its temperature. If this is done slowly, the DNA retains its double-stranded, or native, state until a critical temperature is reached, at which point the duplex rapidly denatures, or "melts' into its component strands. The melting process is easy to monitor because double-stranded and single-stranded DNA differ in their **light-absorbing properties**.

- All DNA absorbs ultraviolet light, with an absorption maximum around 260 nm. When the temperature of a DNA solution is slowly raised, the absorbance at 260 nm remains constant until the double helix begins to melt into its component strands. As the strands separate, the absorbance of the solution increases rapidly because of the higher intrinsic absorption of single-stranded DNA (Figure 18-8).

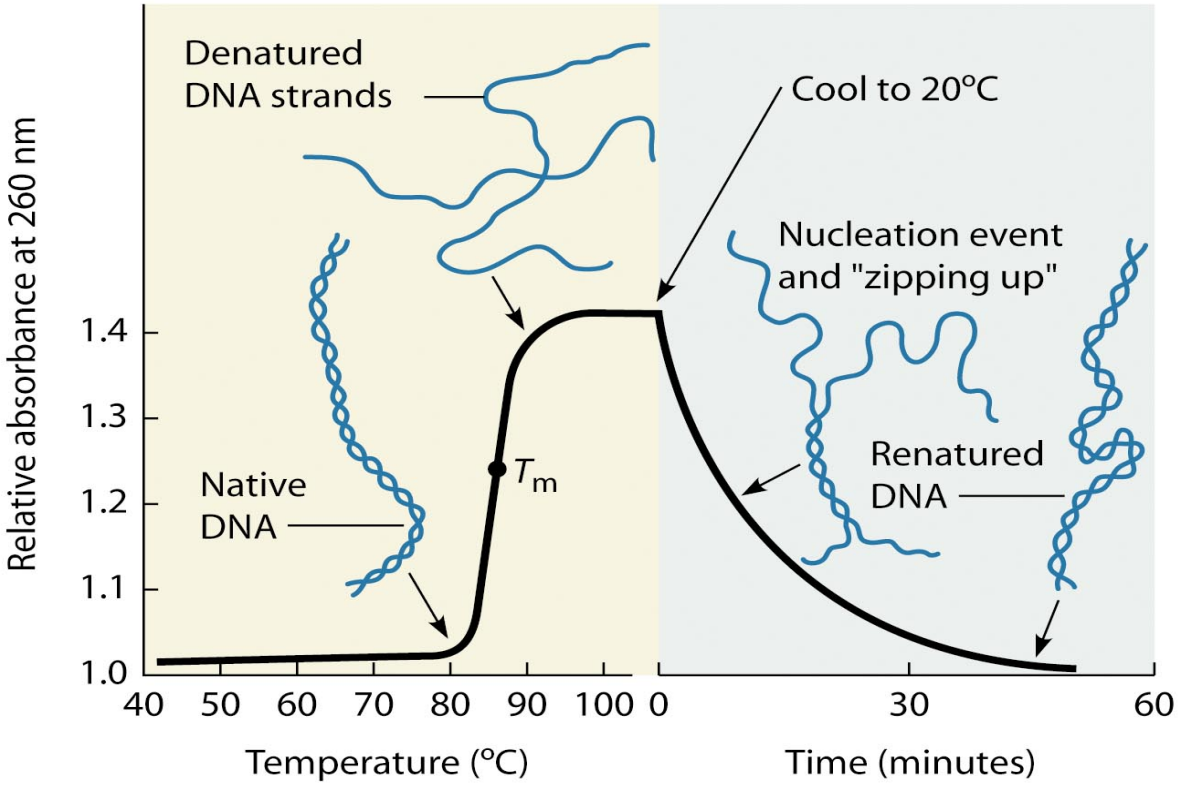


Figure 18-10

Denatured DNA can be renatured

- Denatured DNA can be renatured by lowering the temperature to permit hydrogen bonds between the two strands to reestablish (Figure 18-10).
- The ability to renature nucleic acids has a variety of important scientific applications.
- Most importantly, it forms the basis for **nucleic acid hybridization**, a family of procedures for identifying nucleic acids based on the ability of single-stranded chains with complementary base sequences to hind, or *hybridize, to each other.*