Chapter 1

Introduction to the cell

Living cells are formed from a small number of the different types of molecules that make up the earth. Most biomolecules contain carbon and many contain nitrogen. Both carbon and nitrogen are very scarce in non-living entities. While cells may not always utilize the most abundant molecules, they do use molecules whose unique chemistry is capable of carrying out the reactions necessary for life.

There are three levels of organization to describe the molecules that make up living organisms (Fig. 1.1).

1 The simplest level is the individual elements such as carbon, nitrogen, or oxygen.

2 The basic elements can be arranged into a series of small molecules known as **building blocks**. Building blocks include compounds such as amino acids and nucleic acids.

3 The building blocks are organized into larger compounds, known as **macromolecules**. Macromolecules comprise the different structures that are found in cells.

Four different types of macromolecules are used to construct a cell:

- nucleic acids;
- proteins;
- lipids;
- carbohydrates.

Each type of macromolecule is used for a specific purpose in the cell. There are many examples of macromolecules being combined in different configurations to form larger cell structures.

The **nucleic acids** can be subdivided into **DNA** and **RNA**. DNA is composed of two kinds of building blocks, the bases (adenine, guanine, cytosine, and thymine) and a sugar–phosphate backbone. DNA is used by the cell as a repository for all of the information necessary to direct synthesis of the

FYI 1.1

Elements, ions, and trace minerals that make up living systems

Elements	Ions	Trace minerals
Oxygen	Sodium	Manganese
Carbon	Potassium	Iron
Nitrogen	Magnesium	Cobalt
Hydrogen	Calcium	Copper
Phosphorus	Chloride	Zinc
Sulfur		Aluminum
		Iodine
		Nickel
		Chromium
		Selenium
		Boron
		Vanadium
		Molybdenum
		Silicon
		Tin
		Fluorine

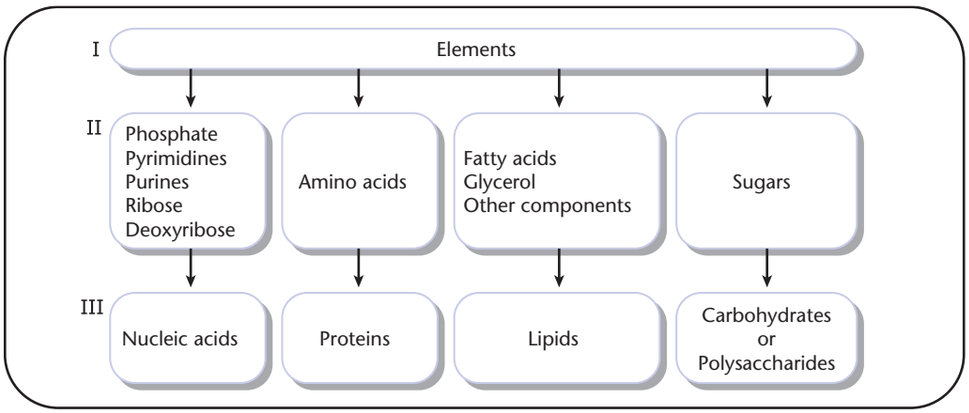

Fig. 1.1 Three levels of organization describe the compounds that make up living organisms.

macromolecules and to produce energy for this synthesis. DNA is also used to transmit information from one generation to the next. RNA has a very similar composition to DNA. The two major differences between DNA and RNA are in the sugar used in the sugar–phosphate backbone (ribose for RNA and deoxyribose for DNA) and in one of the bases (uracil for RNA and thymine for DNA). In *Escherichia coli*, DNA exists as a double-stranded molecule. Chapter 2 describes in detail both the structure and replication of DNA.

The RNA in the cell has at least four different functions.

1 Messenger RNA (mRNA) is used to direct the synthesis of specific proteins.

2 Transfer RNA (tRNA) is used as an adapter molecule between the mRNA and the amino acids in the process of making the proteins.

3 Ribosomal RNA (rRNA) is a structural component of a large complex of proteins and RNA known as the ribosome. The ribosome is responsible for binding to the mRNA and directing the synthesis of proteins.

4 The fourth class of RNA is a catch-all class. There are small, stable RNAs whose functions remain a mystery. Some small, stable RNAs have been shown to be involved in regulating expression of specific regions of the DNA. Other small, stable RNAs have been shown to be part of large complexes that play a specific role in the cell. In general, RNA is used to convey information from the DNA into proteins.

Proteins are composed of amino acids. Most proteins are made from a unique combination of 20 different amino acids (Fig. 1.2). The order in which amino acids appear in a protein are specified by the mRNA used to direct synthesis of the protein. All amino acids have a common core of repeating amino–carbon–carboxyl groups, with varying side chains on the central carbon. Proteins, therefore, have a repeating backbone with an amino terminus and carboxyl terminus. The amino acids can be grouped together and described by physical properties such as charge (acid or basic), size, interactions with water (hydrophobic—water "hating" or hydrophilic—water "loving"), a specific element (sulfur containing) or structure they contain (aromatic rings). The types of amino acids used to make up a protein specify what the protein is capable of. Proteins perform many duties in the cell, including functioning as structural and motor components, enzymes, signaling molecules, and regulatory molecules. Some proteins perform only one function while others are multifunctional.

Lipids are an unusual group of molecules that, in bacteria, are used to make the membranes that surround a cell. One type of lipid, known as a fatty acid, is composed

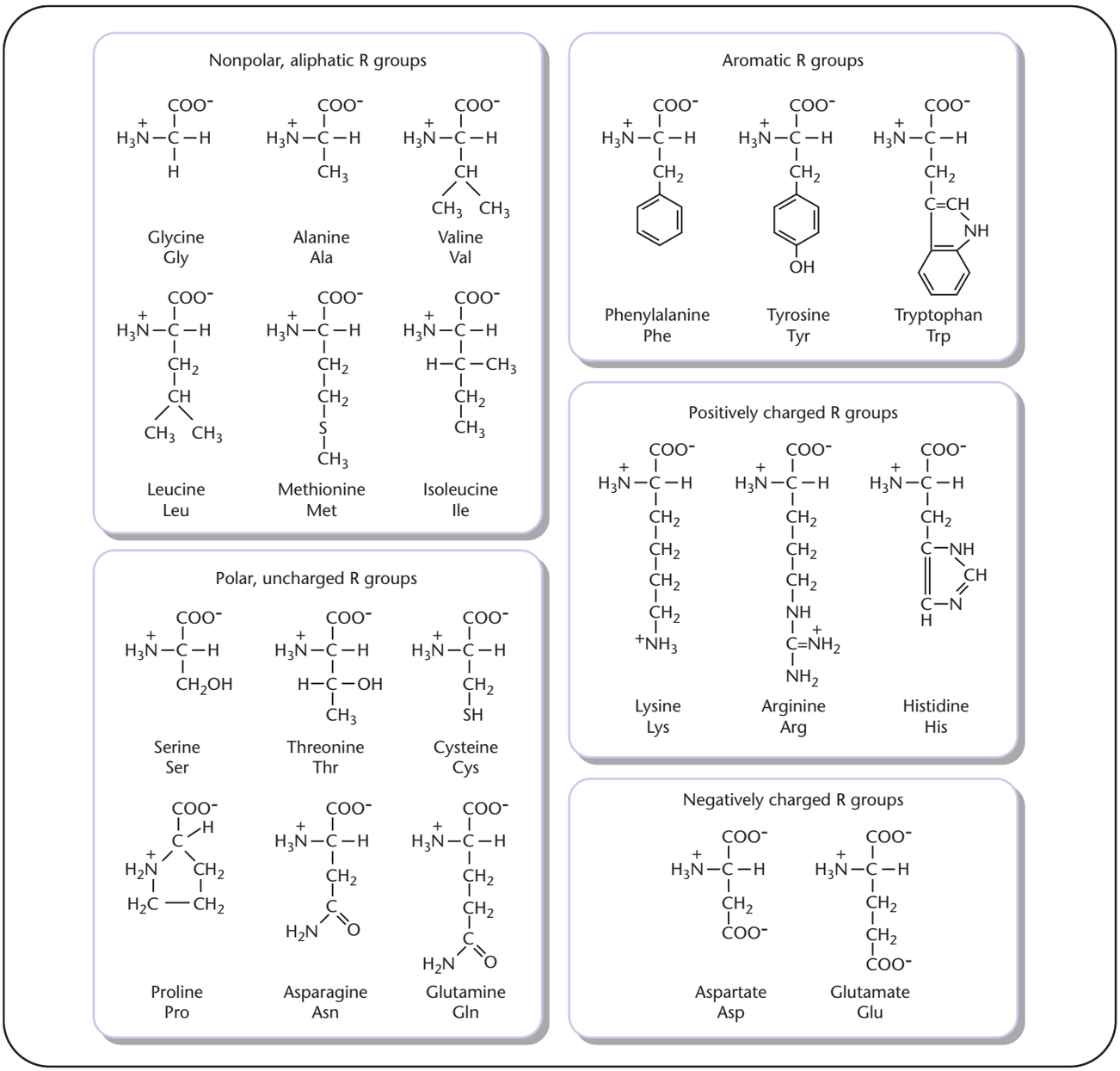

Fig. 1.2 The 20 common amino acids used to make proteins.

of long chains of carbon molecules attached to a smaller head group (Fig. 1.3a). The small head group is known as the polar head group. The fatty acid chains are extremely hydrophobic and line up with the long chains of carbons near each other. Because of the properties of the fatty acid chains, membranes are double-sided (Fig. 1.3b). One side of a membrane is known as a leaflet. The polar head groups face the outside surfaces of the membranes because the polar head groups are water soluble. Other chemical groups can be added to the head groups of the lipids but, in general,

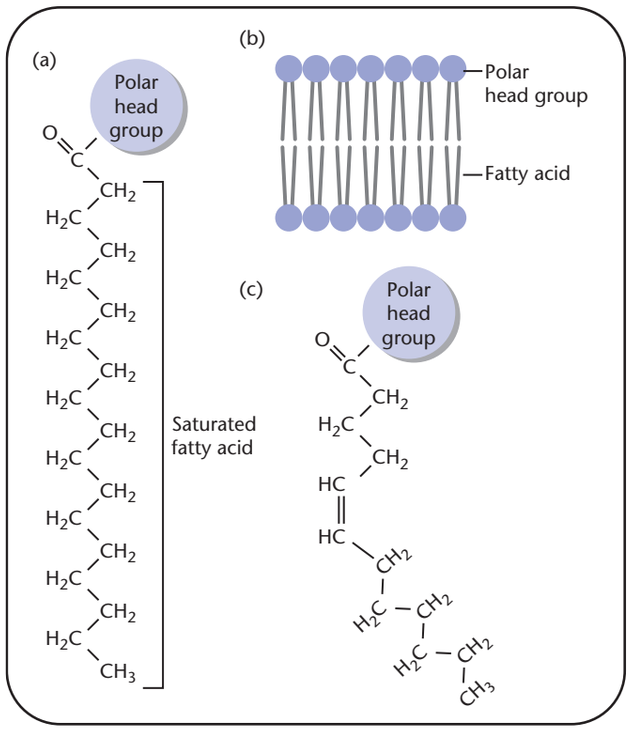

Fig. 1.3 Lipid molecules. (a) The general structure of a saturated fatty acid. (b) The structure of membranes with two leaflets. (c) The general structure of an unsaturated fatty acid.

other chemical groups cannot be added to the fatty acids. If the fatty acids contain only single bonds, they are known as saturated fatty acids. Saturated fatty acids are flexible and can be tightly packed. If the fatty acids contain any double bonds they are known as unsaturated fatty acids (Fig. 1.3c). Unsaturated fatty acids have a kink in them and cannot be packed as closely. Membranes usually contain a mixture of fatty acids to maintain the right packing density and fluidity.

Carbohydrates are composed of simple sugars (Fig. 1.4). They can be used as:

1 an immediate source of energy;
2 a stored source of energy;
3 structural components of the cell.

In bacteria, carbohydrates that are used as immediate sources of energy are the simple carbohydrates such as glucose, lactose, and galactose. Frequently, the carbohydrates that a bacterium utilizes as energy sources can be used to distinguish one species of bacteria from another. Energy is usually stored in a carbohydrate that is a long polymer of glucose known as glycogen.

Structurally, carbohydrates are used to make a protective covering for the outside of the

FYI 1.2

The composition of an average *E. coli* cell

Component	No. of different species	% of cell
Water	1	70
Proteins	3000 or more	16.5
Nucleic acids		
DNA	1	0.9
RNA	1000 or more	6.2
Lipids	40	2.7
Lipopolysaccharide	1	1.0
Peptidoglycan	1	0.8
Glycogen	1	0.8
Polyamines	2	0.1
Metabolites, cofactors, and ions	800 or more	1.0

Note: Although a nearly infinite number of different species of proteins, nucleic acids, polysaccharides, etc. are possible, only a tiny fraction of them are found in a given cell type.

cell called the **capsule** or capsular polysaccharide. Carbohydrates are also added to the polar head groups of the fatty acids on the face of the membrane that is exposed to the outside of the cell. The fatty acid attached carbohydrates help protect the cell from detergents and antibiotics. A complex mixture of carbohydrates is used to make the cell wall. Cell walls maintain the shape of the cell. In some species of bacteria, the cell wall is located outside the membrane. In other species, the cell wall is located underneath the outer membrane.

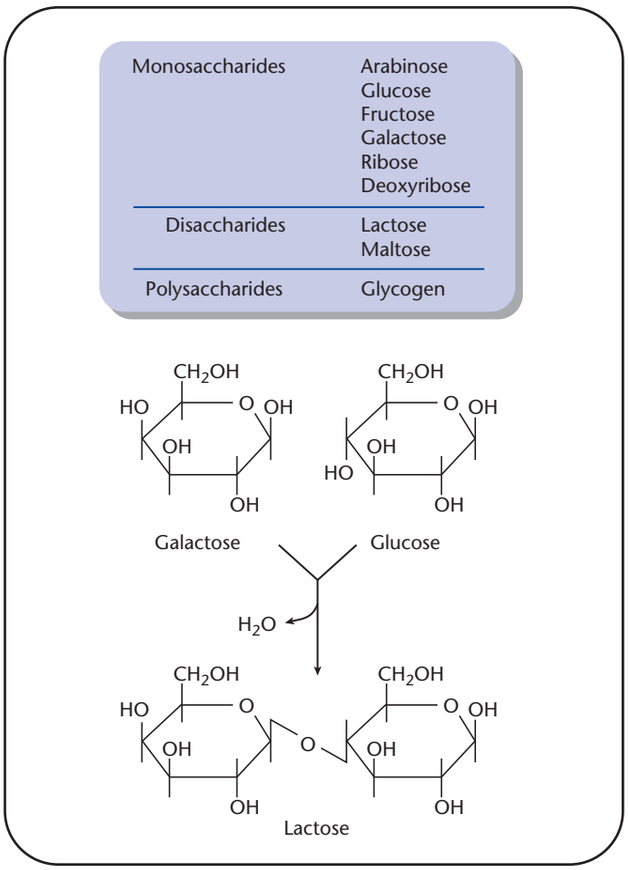

Fig. 1.4 Types and general structures of carbohydrates. The polymerizing of galactose with glucose results in the formation of lactose upon liberation of one H_2O molecule.

Each of the four types of macromolecules provides unique functions to the cell. For some of the cell's requirements, a single type of macromolecule suffices. In other situations, a mixture of macromolecules is required. It is interesting that beginning with a limited number of elements and ending with only four major classes of large molecules, the 5000–6000 different compounds needed to make a cell can be constructed.

The bacterial cell: a quick overview

Escherichia coli is a simple, single-celled organism. It is most commonly found in one of two places, either as a normal, though minor, component of the human intestinal tract or outside the body in areas contaminated by feces. *E. coli* can grow in either the absence or presence of oxygen. It is easy to grow in the laboratory and grows in the absence of other organisms. It can be grown on chemically defined media and divides as quickly as every 20 to 30 minutes. Many of its genetic variants are non-disease causing. These attributes are the main reasons that *E. coli* has been so extensively and intensively studied. The last 50 years of research have made it one of the best-understood life forms.

E. coli is a Gram-negative, rod-shaped bacteria. It is 1 to 2 microns in length and 0.5 to 1 micron in diameter. The cell is surrounded by two membranes (the inner and outer membranes) and as such has four cellular locations, the **outer membrane**, the **inner membrane**, the space between the two membranes or the **periplasm**, and the space surrounded by the inner membrane called the **cytoplasm** (Fig. 1.5). Each

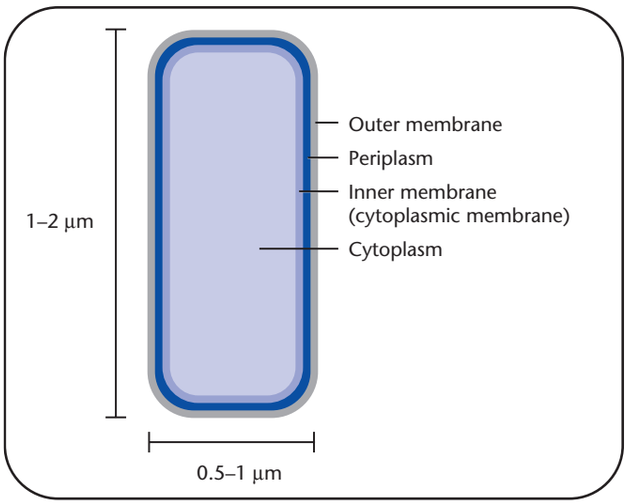

Fig. 1.5 The four cellular locations of the Gram-negative *E. coli* cell. The outer membrane contains lipids, lipopolysaccharides, and protein molecules. The periplasm or periplasmic space is an aqueous environment containing many kinds of proteins. The inner membrane contains lipids and proteins. The proteins used to generate energy are located here. The cytoplasm contains the DNA and is the site of synthesis for most of the macromolecules in the cell.

of these locations has unique contents and properties that provide specific functions to the cell.

The **outer membrane** is the main barrier of the cell to certain kinds of toxic substances. The outer membrane is the cellular surface that interacts with the outside environment. Detergents, dyes, hydrophobic antibiotics, and bile salts from the intestines that could be toxic to the cell cannot cross this membrane. It is composed of the very common double layer of lipids with the polar head groups of the fatty acids facing outward (Fig. 1.6a). One unusual aspect of the outer membrane is that the lipids on the outer leaflet of the membrane are different from the lipids on the inner leaflet. This means that the cell can distinguish the inside of the cell from the outside of the cell by the composition of the membrane leaflets.

Attached to the outer membrane and facing away from the cell are specialized lipids, known as **lipopolysaccharides** or LPS, that contain large carbohydrate side chains (Fig. 1.6b). LPS is essential for the cell to prevent toxic compounds from passing through the outer membrane. Because the LPS is very dense, with six to seven fatty acid chains per molecule, it is very hard for any compound to get through. LPS contains predominantly saturated fatty acids, which favor tight packing of the chains and a greater impermeability of the membrane. In mutants that are partially defective in LPS, the outer membrane is much more permeable and the cells become very fragile. The carbohydrate side chains of LPS are usually negatively charged. The side chains allow the cells growing in the intestinal tract to avoid being engulfed by intestinal cells, attacked by the immune system, or degraded by digestive enzymes. The side chains may also allow the bacterial cells to colonize the surface of human cells in the intestinal tract.

Anchored in the outer membrane and facing the outside of the cell are a number of specialized structures. Many different types of hair-like projections, known as **fimbriae** (singular = fimbria), can cover the cell surface (Fig. 1.7). A single cell can express several types of fimbriae at the same time and there can be between 100 and 1000 fimbriae per cell. Fimbriae are made of protein and are not generally associated with helping the cell move. Rather, fimbriae usually bind to a specific component of eukaryotic cells and allow the bacteria to adhere to the eukaryotic cell. Some of the more

FYI 1.3

The Gram stain

The Gram stain was discovered in 1884 by Christian Gram. It distinguishes two major groups of bacteria, Gram-positive and Gram-negative. The major difference in these groups of bacteria is the nature of their cell wall. In a Gram stain, actively growing cells are heat-fixed, stained with the basic dye, crystal violet, and then with iodine. They are briefly decolorized by treatment with a solvent such as acetone or alcohol. Gram-positive cells are not decolorized and remain stained a deep blue-black. Gram-negative cells are completely decolorized and appear red. The Gram stain remains one of the fixtures for classifying bacteria.

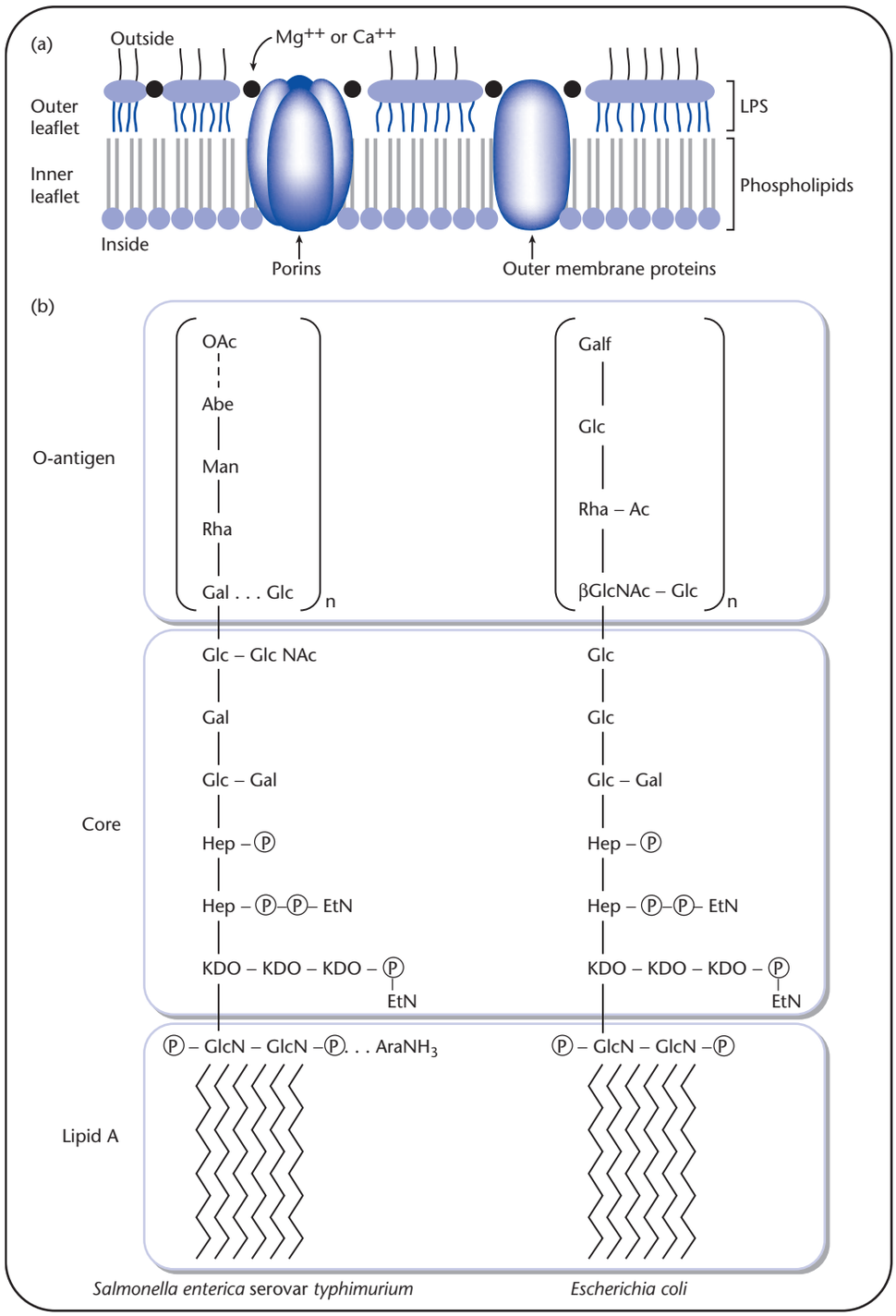

Fig. 1.6 (a) Structure of *E. coli*'s outer membrane. (b) The lipopolysaccharide of *E. coli* and *Salmonella*. Mutants defective in the O antigen lose their virulence. Mutants defective in the parts of the core closest to the lipid A are hypersensitive to dyes, bile salts, antibiotics, detergents, and mutagens. Mutants defective in lipid A are dead. OAc, O-acetyl; Abe, abequose; Man, D-mannose; Rha, L-rhamnose; Gal, D-galactose; GlcNAc, N-acetyl-D-glucosamine; Hep, L-glycero-D-manno-heptose; KDO, 2-keto-3-deoxy-octonic acid; EtN, ethanolamine; P, phosphate; GlcN, D-glucosamine; $AraNH_3$, 4-aminoarabinose.

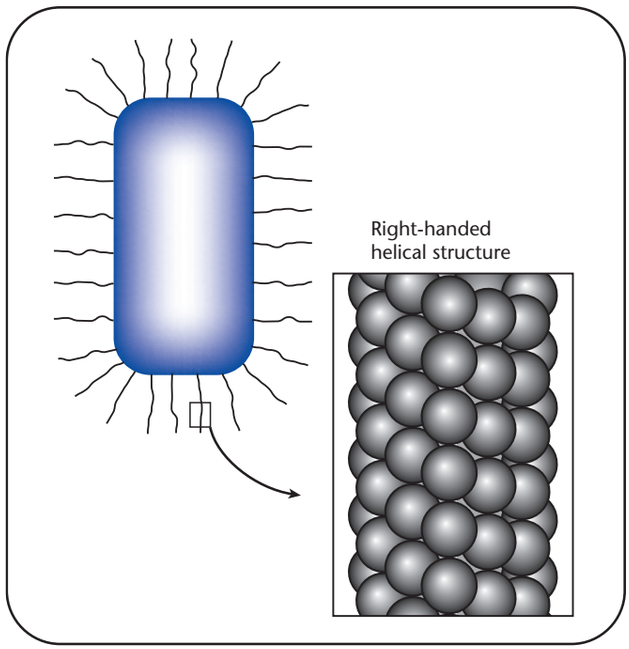

Fig. 1.7 Structure of *E. coli* fimbriae. Note the right-handed helical structure. Each subunit of the fimbriae is a single identical protein.

FYI 1.4

The colonization of human cell surfaces by bacteria

Virtually every exposed surface of the body is inhabited by some type of bacteria. Usually each exposed surface has a mixed population of many different species of bacteria. Different bacteria live on different cell surfaces: for example, the skin is widely inhabited by *Staphylococcus epidermis* while *E. coli* is a natural but minor resident of the colon. Problems for humans arise when either the number of a certain type of bacteria increases dramatically or the bacteria end up in a part of the body where bacteria do not normally reside. Some parts of the body contain extremely large numbers of bacteria. The colon contains ~10^9 bacteria per gram. A few simple calculations lead to the conclusion that there are about 10 times more bacterial cells than there are human cells in the human body!

common receptors for fimbriae are blood group antigens, collagens, and eukaryotic cell surface sugars.

The **flagella** (singular = flagellum) are anchored in the inner membrane and face the outside of the cell. They differ from the fimbriae in that they are corkscrew-like and 5 to 10 microns in length. Flagella are present in fewer numbers (1 to 10 per cell) and they have a motor at their base that is imbedded in the membrane (Fig. 1.8). The flagella rotate in both the clockwise and counter-clockwise directions. The result of flagella rotation is to move the cell. Flagella movement is coupled to a sensing system in the cytoplasm that results in the net movement of the cell towards a favorable environment and away from a harmful one. This directed movement of a bacterial cell is known as **chemotaxis**.

Some *E. coli* cells have an additional surface structure that has a unique function. It is also a hair-like structure 2 to 3 microns in length and is called the **F pilus** (plural = pili) (Fig. 1.9). It is used to build a bridge between two cells, the male that contains the F pilus and the female that does not. A single strand of DNA from the male chromosome is transferred to the female cell. The transferred DNA is replicated and the double-stranded DNA can be incorporated into the chromosome of the female cell. F pilus mediated transfer of genetic information between cells is called **conjugation** (see Chapter 10). Conjugation has been instrumental in our understanding of *E. coli* and continues to provide insights into exchange of DNA between cells. It has been shown that conjugation can be used to transfer DNA from *E. coli* to other bacterial species and even into eukaryotic cells.

A second surface structure that is loosely attached to the outer membrane and completely surrounds the cell is the **capsule** or **capsular polysaccharide**. The capsule is a thick slime layer that is sometimes present on the cell. Cells use the capsule to protect themselves from dehydration or osmotic shock when they are outside the intestines and to avoid being eaten by macrophages when they are inside the body.

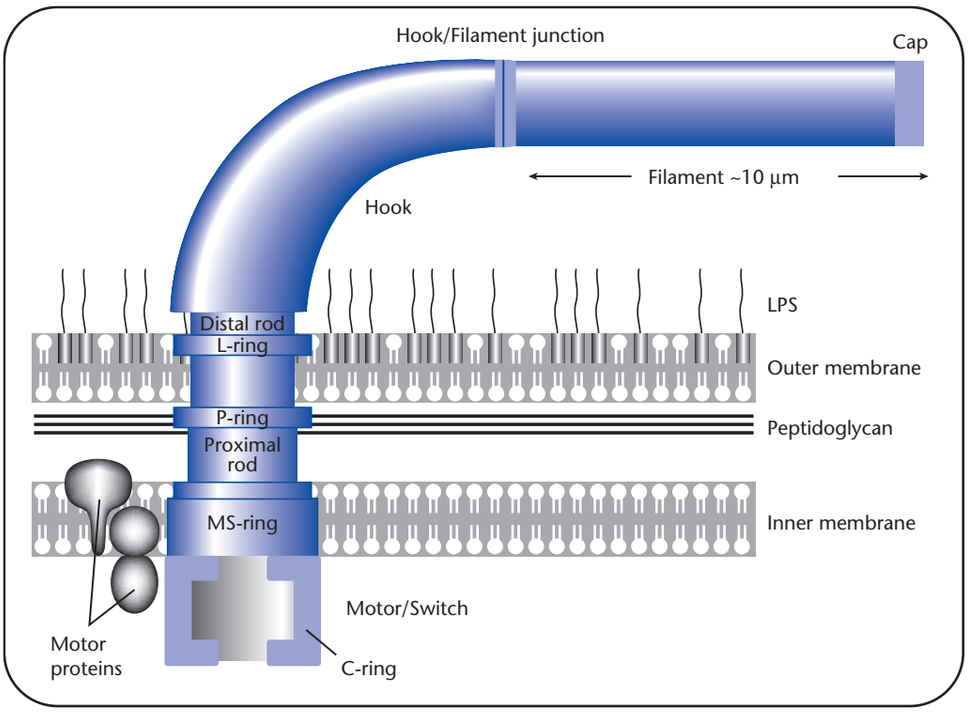

Fig. 1.8 The base of the *E. coli* flagella. Note the motor structure anchored in the membrane.

Imbedded in the outer membrane are a number of different types of proteins (Fig. 1.10). Some act as receptors for specific substances and are used to transport these substances through the membrane. Some of the proteins form gated holes or pores through the membrane. The pores allow passive diffusion across the outer membrane of chemicals required by the cell for growth. Pores that go all the way through the membrane must be gated or closed off when not in use. The pores must be gated so that small molecules do not leak out of the cell or toxic compounds do not leak into the cell. Other proteins have no transport function and appear to play a structural role. It is generally thought that the outer membrane must contain a certain amount of protein. If one of the protein species is missing from the membrane, the cell responds by increasing the amounts of the other proteins.

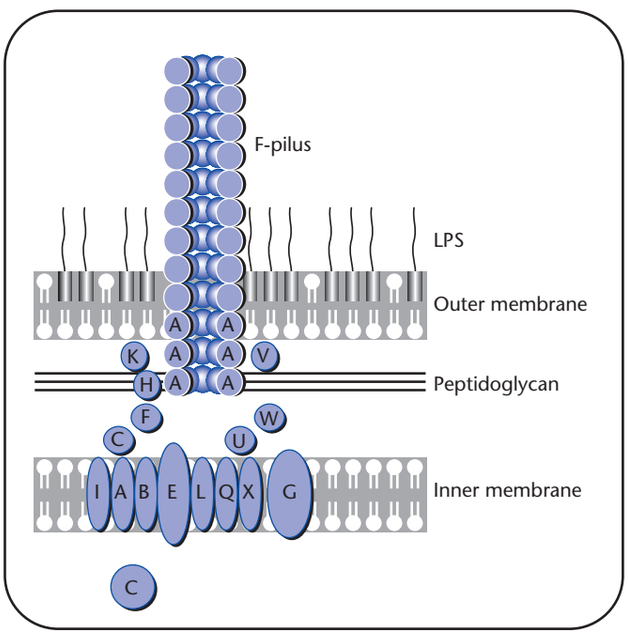

Fig. 1.9 Assembly of the F pilus. The proteins A, B, C, E, F, G, H, I, L, Q, U, V, and W are needed for F pilus biogenesis. Additional F encoded proteins are needed for mating pair stabilization (two proteins), surface exclusion (two proteins), and DNA synthesis (four proteins). There are 13 F encoded proteins with no known function.

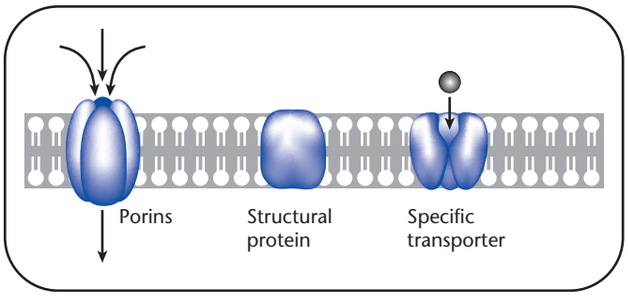

Fig. 1.10 The porins, structural proteins, and specific transport receptors spanning *E. coli*'s outer membrane. Porins are composed of three identical subunits with three channels, one through each subunit. They are responsible for passive diffusion of small molecules. Examples of porins are OmpF and OmpC. Structural proteins are required in the outer membrane. When they are missing, the outer membrane is destabilized. An example of a structural protein is OmpA. Specific transporters only allow specific compounds to pass through them. Many have structural similarities to porins. Examples include LamB that transports maltodextrin sugars, BtuB that transports vitamin B, and FadL that transports fatty acids.

FYI 1.5

KHO antigens

Many different subspecies of *E. coli* can be isolated. More than 50 years ago, Kauffman and coworkers described a system to classify *E. coli* based on their outer membrane surface structures. The system uses three major surface structures that are capable of eliciting an immune response in mammals: the lipopolysaccharide or O antigen, the flagella or H antigen, and the capsule or K antigen. To date, 173 different O groups, 56 H groups, and 80 K groups have been found. The most widely used laboratory strain is *E. coli* K12. In contrast to the innocuous, non-pathogenic *E. coli* K12 strain, outbreaks of *E. coli* O157:H7, a strain with very distinct O and H antigens, continue to arise in the United States each year, usually from contaminated food. Infection by *E. coli* O157:H7 can be lethal in very young, very old, or immunocompromised individuals.

Anchored to the inner face of the outer membrane and jutting out into the periplasm is a rigid structure called the **peptidoglycan** layer or cell wall (Fig. 1.11a,b,c). The peptidoglycan surrounds the cell and is required to maintain the rod shape of the cell. The peptidoglycan is arranged in rings that go around the short axis of the cell. The penicillin antibiotics interfere with the assembly of the peptidoglycan rings into the completed structure. When a cell is growing in the presence of penicillin, the rings are not connected to one another so that when the cell grows, the rings come apart and the cell explodes. Thus, the peptidoglycan maintains the cell shape and protects the cell from the pressure differences between the inside and outside of the cell.

The **periplasmic space** or periplasm is an aqueous compartment that resembles the aqueous environment outside the cell. It contains proteins to help concentrate nutrients from outside the cell to inside the cell. Other periplasmic proteins sense the external environment and transduce this information across the inner membrane to the cytoplasm. These sensing systems allow the cell to respond to the environment, either protecting itself from harmful conditions or taking advantage of beneficial conditions. Additional components of the periplasm include proteases to degrade abnormal proteins and a system to provide for the formation of disulfide bonds in specific proteins. Stable disulfide bonds are only found in the periplasm; they cannot be found in the cytoplasm. The periplasm monitors the outside of the cell and concentrates solutes to the inside of the cell.

While the outer membrane is the barrier for many toxic compounds, the **inner membrane** is the major physiological barrier between the outside of the cell and the cytoplasm (Fig. 1.12). It contains the proteins needed to generate energy and has an electrical gradient, called the proton motive force, across it. The proton motive force is used to generate ATP. In addition, the inner membrane contains proteins that transport substances into or out of the cytoplasm, proteins that transmit signals into the cytoplasm, and systems to transport proteins out of the cytoplasm. The inner

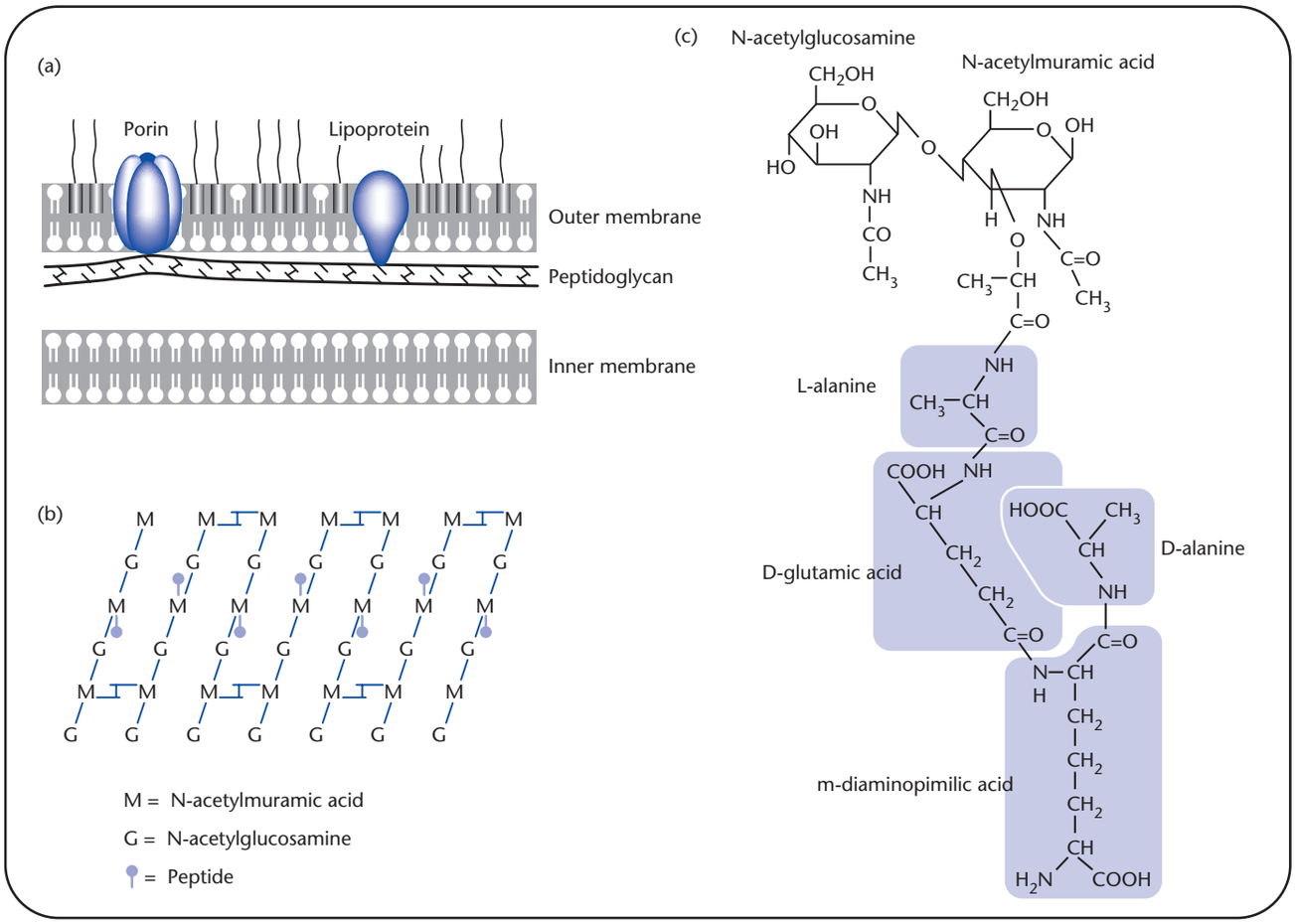

Fig. 1.11 (a) The location of peptidoglycan in *E. coli*. (b) The structure of peptidoglycan strands. (c) The chemical structure of peptidoglycan.

membrane is the location of many proteins involved in cell division. Some of these division proteins are only found in limited places in the inner membrane, such as in a ring around the center of the short axis of the cell. The inner membrane is the site of many and varied biochemical reactions, signaling reactions, and transport activities.

The **cytoplasm** is the hub of cellular activities. It is the location of the single *E. coli* chromosome, a circular double-stranded molecule of 4,639,221 base pairs. All DNA replication and repair of damaged DNA takes place here. **Transcription** of the DNA into messenger RNA (mRNA) and **translation** of the mRNA into protein occur in the cytoplasm. Decisions about which gene products to make are made in the cytoplasm. Signals from the environment are received and acted upon in the cytoplasm. In the cytoplasmic compartment, food sources are broken down or stored and energy is generated. Because the cytoplasm contains the DNA, it is the site of synthesis of the majority of the molecules that the cell makes.

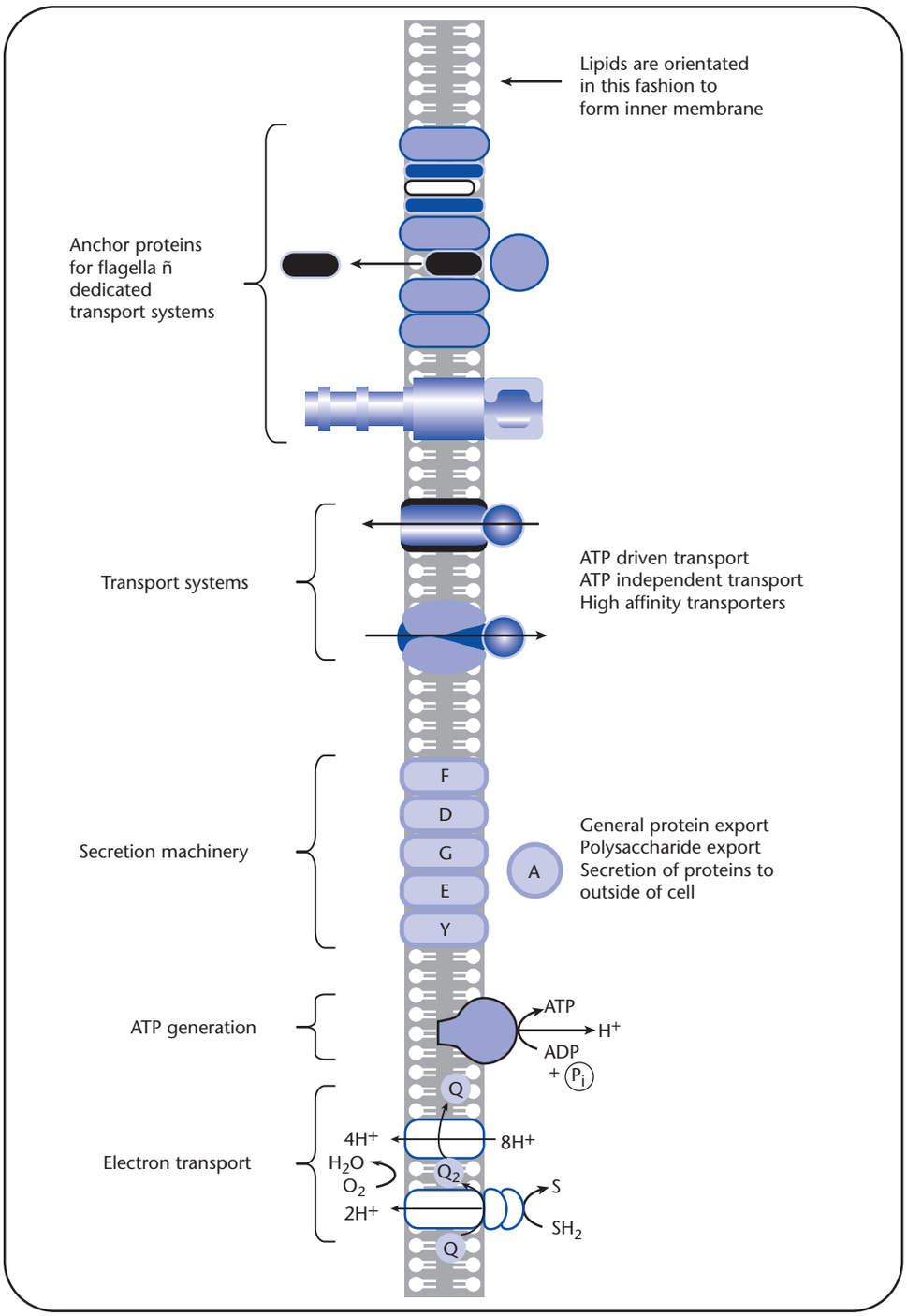

Fig. 1.12 The inner membrane of *E. coli* is composed of approximately 50% protein and 50% phospholipid. The major types of phospholipid are phosphotidylethanolamine, phosphotidylglycerol, and cardiolipin. The inner membrane contains the proteins for transport systems, the protein export machinery, ATP generation machinery, and electron transport, to name a few.

How do cells grow?

In order to reproduce, cells must grow and divide. Growth is not random, but rather follows a very regular pattern (Fig. 1.13). The diameter of most rod-shaped bacteria does not change during growth. It is the length of the cylinder that increases until the cell is approximately double in size. At this point, *E. coli* builds a physical barrier, called the **septum**, between the two daughter cells. Subsequently, the daughter cells are physically separated from one another.

During growth, all components of the cell must be

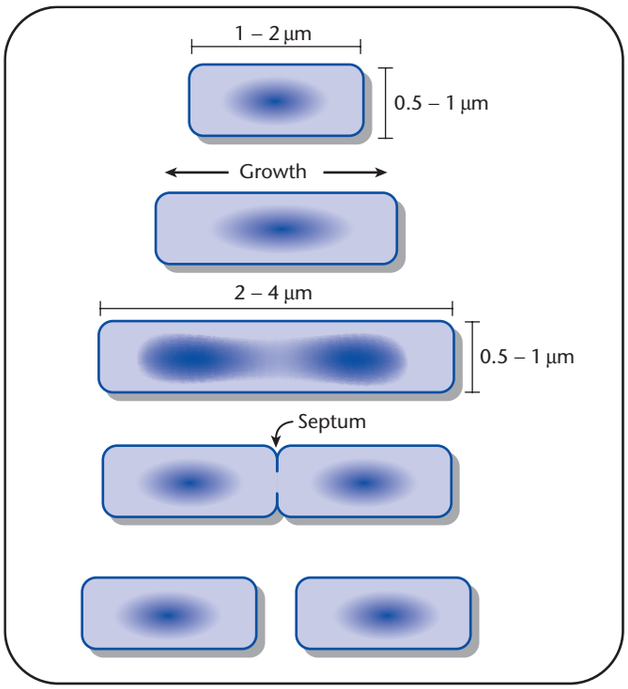

Fig. 1.13 Cell growth. During growth, all components of the cell must be doubled. The resulting daughter cells are physically separated from one another by a septum.

FYI 1.6

Measuring cell growth

Measuring cells as they grow and divide provides a reliable and quantitative indication of the health of the population. Cell growth is directly related to the nutrients the cells are consuming. Measurements of cell growth on different energy sources or in mutant cells can be directly compared. For energy sources, this has allowed the determination of which combinations of nutrients are most or least beneficial to the cells. For different mutant cells, it indicates how important the gene is to the cell and if there are conditions where it is more or less important.

Cell growth can be measured in three different ways. First, the **optical density (OD)** of cells growing in liquid media can be measured in a spectrophotometer. This technique is based on the fact that small particles (cells, in this case) scatter light approximately proportionally to their number. A suspension of cells is put into a holder or cuvette in the spectrophotometer and a beam of 600 nm wavelength light is passed through the suspension. The amount of light that comes out of the suspension is measured. The amount of light coming out is either the same as the amount that went in or it is less. If it is the same, there are not enough cells in the suspension to measure. If it is less it is because the cells have scattered the light. How much less is proportional to the number of cells. For example, if the OD600 is 0.25 in one culture and 0.5 in a second culture, the second culture has twice as many cells. OD600 is used to make measurements on populations of cells and to compare the relative numbers of cells in these populations.

The main drawback of this method is that it does not distinguish between live and dead cells or between cells and large particles of debris. It also relies on the cells being approximately the same size. If a mutant cell forms long filaments or other odd-shaped cells, the OD will not give a reading that is proportional to the number of cells. The strengths of this method are that it is rapid and easy so that many samples can be measured in a short amount of time. OD measurements do not kill cells so after an OD measurement is made, the cells can be used for further experimentation.

The second way to measure bacterial growth is by a **viable cell count (VCC)**. A sample is taken, diluted and spread on solid media, and incubated overnight. The next day, each colony, or group of cells, that form on the agar represents the growth from a single cell. The colonies are counted and the number of bacteria in each sample is determined. Frequently, the OD600 measurements and the VCC are both carried out. This allows determination of the correlation of an OD value with an exact count of the number of viable cells. The combination of OD and VCC measurements eliminates the drawbacks of only taking OD readings.

Both the OD600 and the VCC take measurements on populations of cells. If a change occurred at the level of a single cell, these measurements would miss it. A third method, called **flow cytometry**, was devised to measure the light scatter of single cells. A stream of media with bacteria in it is passed across a microscope slide field. Aimed at the field is a beam of visible light and opposite the beam is a detection system that is attached to a computer. As individual cells pass through the beam, the amount of light they scatter is recorded. Several thousand cells can be measured per second. This is a very powerful technique but the main drawback is the cost of the flow cytometer. For certain types of experiments where measurements on individual cells are required, it is the only way the data can be obtained.

FYI 1.7

The growth curve

When *E. coli*, or any bacteria, is placed in a nutrient-rich broth, they begin to grow. Invariably, they follow a simple pattern of growth. First, the cells must prepare for growth and make sure they have all the necessary proteins required for growth, this is called the lag phase. Next, cells begin growing logarithmically and double at a constant rate, this is log or exponential growth. Once cells have used all the available nutrients, they begin to slow down and prepare for a period of inactivity known as the stationary phase. If the nutrient deprivation lasts long enough, the cells will begin to die. Historically, most experiments have been carried out on exponentially growing cells at 37°C. In the past few years, the genes that respond to different growth conditions have been investigated. New families of genes have been discovered that are required for stationary phase, cold shock, and heat shock, to name a few.

doubled. For components that are present in very large numbers such as ribosomes (2×10^4 molecules/cell), membrane lipids (2×10^7 molecules/cell), or outer membrane pore proteins (2×10^5 molecules/cell), doubling is approximate. Because of the molecules' abundance, the numbers do not have to be exact. For cell components that are present in small numbers, doubling must be exact. In an *E. coli* cell that is dividing every 30 minutes, there is a single circular chromosome. The chromosome must be replicated only once so that each daughter cell can inherit one molecule. The duplication of the chromosome is a carefully controlled and regulated event. Different cell components are present in specific numbers with some being rare, some at intermediate levels, and some in very high numbers. Many different mechanisms are employed to make sure the cell has the correct number of any given component and that the daughter cells inherit the right amount. The rarer a component is, the more effort the cell invests to ensure that it is divided evenly.

The growth of a cell is ultimately dictated by the growth of the membranes and the peptidoglycan layer that make up the cell's boundaries. For both the inner and the outer membrane, new lipids are added randomly into the existing membranes, there is no one specific zone of growth (Fig. 1.14). Practically, this means that the cell cannot use the growth of the membrane to mark specific places along the membrane or to move molecules along it. How the cell localizes specific structures along the membrane and how it measures its length are not known. The fact that daughter cells are born at approximately the same size and very rarely are mistakes made indicates that the measuring system is accurate.

Growth of the peptidoglycan cell wall occurs randomly around the peptidoglycan along the long axis of the cell. The peptidoglycan that forms the caps of the cells and the septum is not exactly the same as that on the long axis of the cell and is synthesized by different enzymes. For cells dividing every 48 minutes, 50,000 **muropeptides** or strands of peptidoglycan per minute are added to the growing cell wall. The new strands are inserted next to preexisting muropeptides at ~200 different sites in the cell wall (Fig. 1.15). The enzymes that synthesize the peptidoglycan reside in the inner membrane and move in a unidirectional fashion around the cell. They can circumnavigate the cell in about 8 minutes. Excluding the poles, a cell has ~1100 rings of muropeptides that maintain its shape.

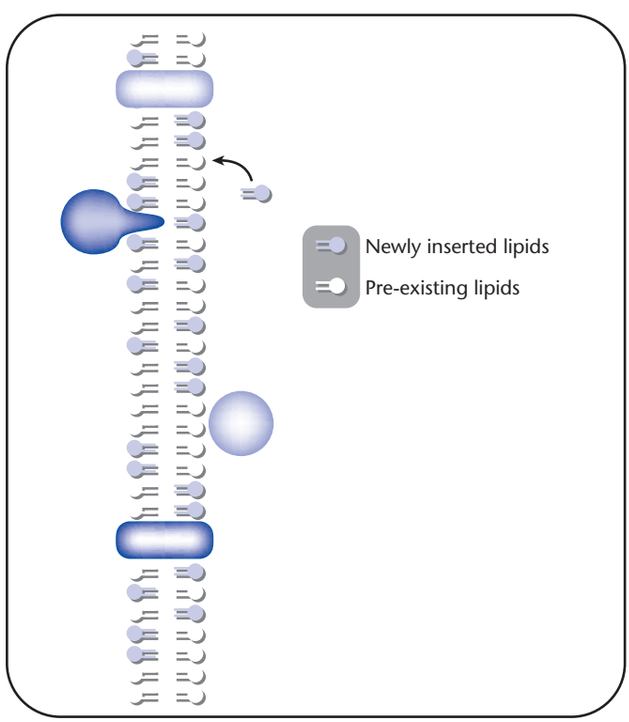

Fig. 1.14 Growth of membranes. Lipid insertion into growing membranes does not occur at a specific place on the membrane, rather it is random.

As the membranes grow, the volume of the cytoplasm also increases. The cytoplasmic contents including the DNA and the

several thousand different protein species must double in number. Because proteins are regulated independently of each other, there is no singular mechanism for their doubling. The vast majority of the proteins in the cytoplasm freely diffuse throughout the boundaries of the cytoplasm. When the division septum is laid down, approximately half of the contents of the cytoplasm are trapped on one side of the septum and half on the other side. There are a few known cytoplasmic proteins that are not freely diffusable but they are the exception rather than the rule. Aside from the DNA, the components of the cytoplasm are numerous enough to be divided approximately in half.

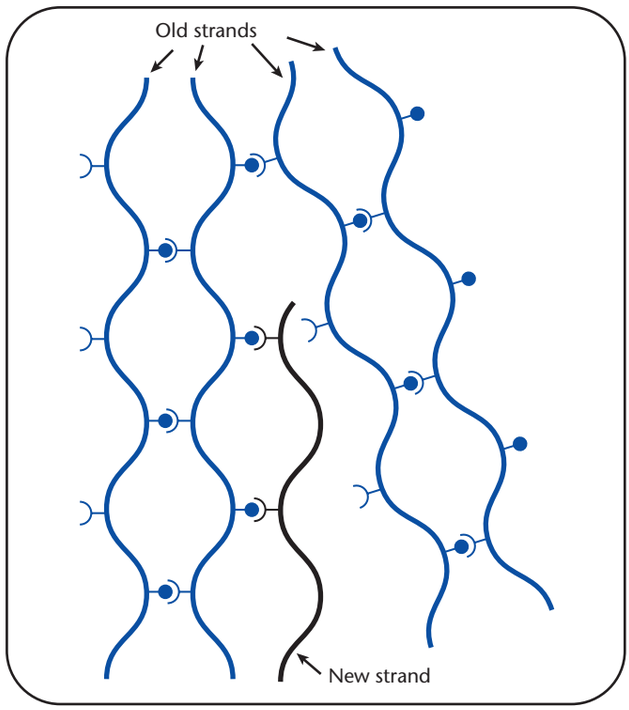

Fig. 1.15 Growth of peptidoglycan. Each strand is actually a ribbon that is in a helical conformation.

What is genetics?

The main goal of biology is to understand how a living cell works. Genetics is specifically concerned with the characteristics of the cell (size, shape, ability to grow on a certain sugar, specific internal structures, etc.) and how they are passed from one generation to the next. Early geneticists described physical characteristics or **phenotypes** of the cell and followed their inheritance without knowing that they were encoded by the DNA. After the discovery of DNA and the proof that it is the molecule used to transmit information from generation to generation, geneticists began to correlate the phenotype with the site in the DNA (or gene) that is responsible for it. The information in the DNA is referred to as the **genotype** and the characteristic it specifies is described as the phenotype.

One of the main characteristics defining genetics is that genetics studies the cell when the cell is intact and growing. Genetics does not attempt to separate each molecule and study the behavior of an individual molecule in a test tube. Rather, genetics focuses on a specific molecule and isolates that molecule in the cell by making a mutation in the DNA or gene that specifies the molecule. The behavior of the mutant cell is then compared to a **wild-type**, or normal cell. From the behavior patterns of the two cells, the function of the molecule is inferred. The greater the number of specific tests that can be devised for the wild-type and mutant cells, the more reliable is the assignment of the function for the gene being studied. Genetics always tries to find the simplest, logical argument to describe what has been observed.

Summary

E. coli is one of the best-understood life forms. As a simple, single-celled organism with a completely sequenced genome, much is known about *E. coli*. Our understanding of *E. coli*'s lifestyle and the molecular structures needed to support that lifestyle has contributed to *E. coli*'s widespread use as a model organism for techniques involving molecular genetic analysis. When undertaking a genetic analysis, the first goal is to make mutants that affect the specific pathway or molecule of interest. To make mutants, it is necessary to understand DNA metabolism, how mutants arise or can be induced, and what kinds of alterations are possible. Each of these topics can dramatically influence how to go about isolating mutants and what interpretations can be drawn from the mutants. Next, it is important to know what tools are available to study the mutants. How can they be moved from cell to cell? How can the mutants be cloned and subjected to the tools of molecular biology? In the chapters that follow, these subjects will be examined in more detail. Each of the chapters contributes one more piece to the puzzle of how we study biological systems using a genetic point of view.

Study questions

1 What are the building blocks for each of the four macromolecules?
2 What are differences between DNA and RNA? Similarities?
3 What are the functions of messenger RNA, transfer RNA, and ribosomal RNA?
4 What two features of a fatty acid molecule dictate how it is oriented in a membrane?
5 What are the differences between carbohydrates used as energy sources and carbohydrates used as structural components of the cell?
6 What is the function of the outer membrane? The inner membrane?
7 How many different cell surface structures can be present on the outer surface of a bacterial cell? What is the function of each?
8 In what cellular compartment are disulfide bonds formed?
9 What is the function of the septum and where is the septum located?

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