

How Do We Understand Life?

The ultimate goal of molecular biology and biochemistry is to understand in molecular terms the processes that make life possible. In his “Lectures on Physics,” Richard Feynman famously remarked that in order to understand life “the most powerful assumption of all … (is) that everything that living things do can be understood in terms of the jigglings and wiggles of atoms.”¹ Feynman made this statement about 50 years ago, shortly after James Watson and Francis Crick had discovered the double-helical structure of DNA, and Max Perutz and John Kendrew were working out the first structures of proteins.

How do we even begin to make good on Feynman’s assertion that life can be understood in terms of the “jigglings and wiggles” of atoms? Our purpose in this book is to connect fundamental principles concerning the structure and energetics of biologically important molecules to their function. The concepts that we shall introduce provide a start towards establishing a complete understanding of the physical basis for life. As we move through these concepts, we shall assume that you are familiar with the essential principles of chemical structure, reactivity, and bonding, as covered in a typical introductory chemistry course. We shall also assume familiarity with basic concepts in molecular biology, again at the level encountered in introductory courses. If you find some of the material presented in the earlier chapters of this book difficult to follow, you may wish to consult elementary textbooks in chemistry and biology, such as those listed at the end of Chapter 1.

Any living cell is, ultimately, a collection of different kinds of molecules. The molecular structure of a particularly well-studied bacterium, *Escherichia coli*, is shown in Figure 1. This rendering of the cell, by the scientist and artist David Goodsell, is based on three-dimensional molecular structures that have been determined by many scientists, piece by piece, over the 50 years since Feynman’s assertion about life. The particular cell shown in Figure 1 is encapsulated by two lipid membranes that are coated by a layer of glycans (carbohydrates). The interior of the cell is densely packed with many different kinds of macromolecules, which are very large molecules consisting of thousands of atoms each. Prominent among these is DNA, which is depicted as long yellow strands. The macromolecules with irregular shapes are various kinds of proteins and RNAs, as well as glycans.

Like all living cells, *E. coli* takes in nutrients and catalyzes chemical reactions that release energy from the nutrients. The cell is able to harness this energy to grow and to reproduce. By dividing into two cells, the mother cell passes on the blueprint for life, encoded in the DNA, to its two daughter cells, along with all of the other kinds of molecules that are necessary for these cells to live. It is apparent, even from looking at this relatively simple bacterial cell, that it is a formidable challenge to work out how such a molecular system can grow and reproduce, using only information contained within itself. We shall start by understanding the properties and interactions of the biological macromolecules that are the nanoscale machines of the cell.

The four types of macromolecules in the cell (DNA, RNA, proteins, and glycans) are polymers—that is, they are constructed by forming covalent linkages between

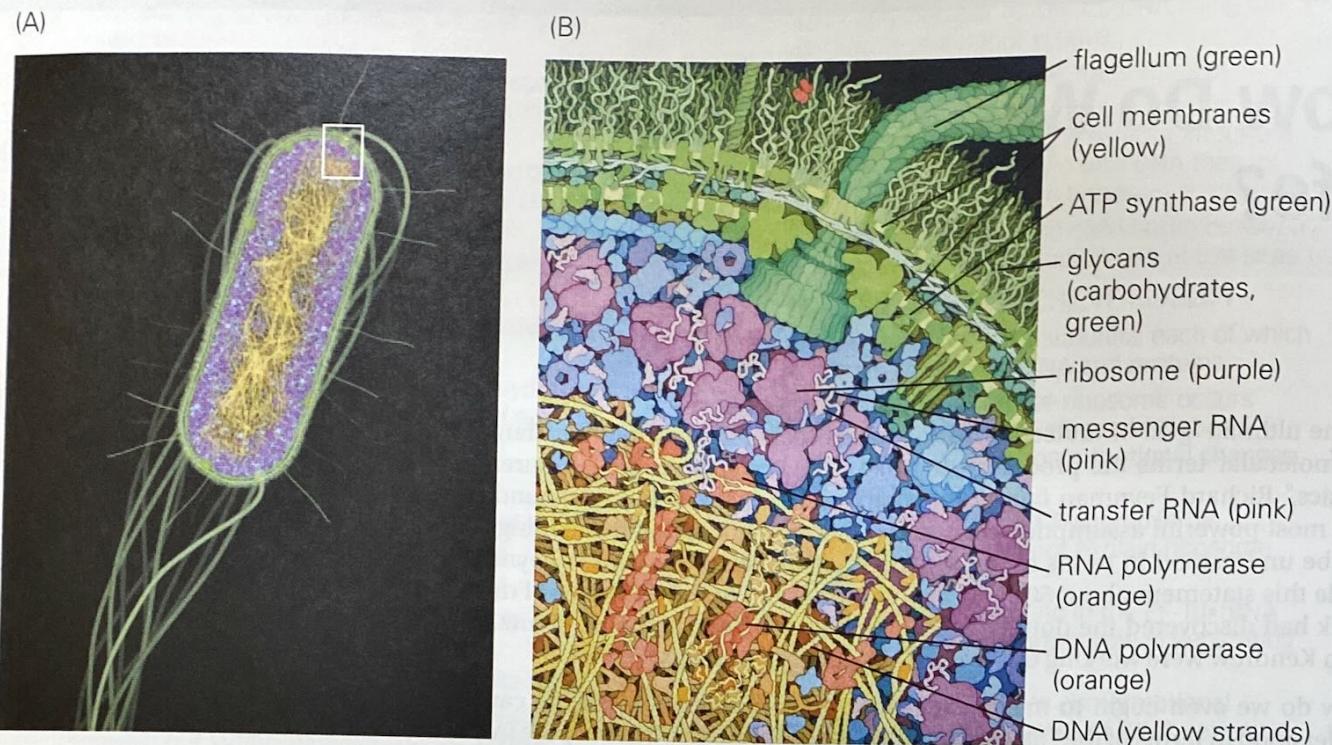


Figure 1 Molecular structure of a bacterial cell. Shown here is an *Escherichia coli* cell, illustrated by David Goodsell of The Scripps Research Institute. (A) Cross section of an *E. coli* cell. The main body of the cell is approximately 1 μm wide and has long whip-like flagella, which power the movement of the cell. (B) Expanded view of the region outlined in white in (A). Many of the macromolecules in the cell are shown here, drawn to scale. Some of the many protein machines in the cell are identified: DNA polymerase makes copies of DNA strands, RNA polymerase generates messenger RNA (mRNA) from DNA, and ATP synthase stores energy in the form of adenosine triphosphate (ATP). Transfer RNA (tRNA) is involved in the translation of the sequence of a messenger RNA to the sequence of a protein, by a particularly large machine called the ribosome. You can appreciate the scale of this drawing by considering that each ribosome is $\sim 300 \text{ \AA}$ in diameter. (From D.S. Goodsell, *Biochem. Mol. Biol. Educ.* 37: 325–332, 2009. With permission from John Wiley & Sons, Inc.)

smaller molecules. RNA and DNA are formed by linking nucleotides together, proteins are formed from amino acids, and glycans are polymers of sugars. Of these, DNA, RNA, and proteins are special because they are the three components of the process by which genetic information is translated into the machinery of the cell.

DNA, RNA, and proteins are linear polymers in which the linkage between the component units extends in only one direction without branching. The order of specific kinds of nucleotides in DNA or RNA, or of specific amino acids in proteins, is called the sequence of the polymer. All living cells store heritable information in the form of DNA sequences, which are copied through the process of DNA replication and transmitted to progeny cells. The sequences of particular segments of DNA are also copied during the process of transcription to make RNAs with different kinds of functions. Messenger RNAs (mRNAs) are used to synthesize proteins. Other kinds of RNAs carry out diverse functions in the cell.

Most of the molecular machines that carry out the various processes essential for life are proteins. These include enzymes that catalyze chemical reactions, motor proteins that move things inside the cell, architectural proteins that give the cell its dynamic shape, and regulatory proteins that switch cellular processes on and off. Two particularly important kinds of protein enzymes are DNA polymerases, which replicate DNA, and RNA polymerases, which make RNAs based on the sequence of DNA. Another important protein enzyme is ATP synthase, which stores energy by synthesizing adenosine triphosphate (ATP). Some enzymes are made of RNA. The ribosome, which synthesizes proteins based on the sequences of messenger RNAs, is made of both proteins and RNA, with RNA being the functionally more important part. These molecular machines are identified in Figure 1, and we shall study some of them in this book.

There are four kinds of nucleotides in DNA and also in RNA, and 20 kinds of amino acids in proteins. Although this basic set of molecular building blocks is limited, they can generate a vast number of possible sequences. The *E. coli* bacterium has ~ 4.5 million (4.5×10^6) nucleotides in its DNA. A DNA molecule of this length corresponds to $\sim 10^{2,700,000}$ possible sequences ($4 \times 4 \times 4 \times 4 \times \dots \times 4.5 \times 10^6$ times),

which is an unimaginably large number. A typical protein molecule is made from ~300 amino acids. The total number of different sequences possible for proteins of this length is $20^{300} \approx 10^{390}$, also an enormously large number. It is from this vast diversity of possible sequences that evolution is able to select the much smaller number of sequences of DNAs, RNAs, and proteins that are used in life.

There are two central themes underlying the concepts in this book. The first is that the function of a molecule depends on its structure and that biological macromolecules can assemble spontaneously into functional structures. The second theme is that any biological macromolecule must work together with other molecules to carry out its particular functions in the cell, and this depends on the ability of molecules to recognize each other specifically. Clearly, to understand the molecular mechanism of any biological process, we must understand the energy of the physical and chemical interactions that drive the formation of specific structures and promote molecular recognition.

You may be familiar with the concept of entropy, which is a measure of the likelihood of a particular arrangement of molecules. The flow of energy is governed by a very general principle, which is that the entropy of the universe always increases in any process. This statement is known as the second law of thermodynamics, and you have encountered it in some form in introductory chemistry. Another way of stating the second law is that a system always tends towards increased disorder, unless there is an input of energy. The relevance of the second law to living systems should become apparent if you study Figure 1 again. A living cell is a highly organized entity, with the cell membrane surrounding a specific collection of macromolecules that are where they need to be in order to function efficiently. Cells require a constant supply of energy to carry out the processes associated with living. Without energy, they would quickly go into a quiescent state and eventually disintegrate. The increase in entropy (disorder) upon disintegration overcomes the energetically favorable interactions that enable the cell to function.

In the first part of this book (Part I, Biological Molecules), we introduce the important classes of biological macromolecules and discuss the details of their structures. With the architectural principles of macromolecular structures in hand, we turn our attention to the physical principles that govern the interactions between molecules. As we explain in Part II of this book (Energy and Entropy), considerations of the energetics of interactions must always go hand in hand with consideration of the entropy (taken together, energy and entropy control the “jigglings and wigglings” of the atoms). By combining energy and entropy we arrive at a parameter known as the free energy, which allows us to predict whether a molecular process will occur spontaneously. This concept is developed in Part III of the book (Free Energy), and applied to processes such as the spontaneous adoption of specific structures by proteins and the transmission of electrical signals in nerve cells. In Part IV (Molecular Interactions), we focus on the idea that molecular interactions in living systems have to be highly specific. By drawing on the descriptions of protein and nucleic acid structure that we developed in Part I and the idea of free energy developed in Part III, we explain how molecules that need to interact find each other in the crowded environment of the cell.

Living systems change with time. Another way of saying this is that living systems are never at equilibrium: they would be dead if they were. In Part V (Kinetics and Catalysis), we turn to a study of kinetics, which describes the time dependence of molecular processes such as chemical reactions and diffusion. This part of the book provides us with several essential ideas about how enzymes work. Finally, in Part VI (Assembly and Activity), we focus on two particularly fascinating aspects of cellular processes: how proteins and RNA fold into specific three-dimensional structures, and how the processes of replication and translation achieve very high fidelity.

¹ Feynman RP, Leighton RB, and Sands ML (1963) The Feynman Lectures on Physics. Reading, MA: Addison-Wesley Publishing Co.

Miniseries: Illustrating the Machinery of Life

Escherichia coli*

Received for publication, August 21, 2009, and in revised form, September 15, 2009

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Diverse biological data may be used to create illustrations of molecules in their cellular context. I describe the scientific results that support a recent textbook illustration of an *Escherichia coli* cell. The image magnifies a portion of the bacterium at one million times, showing the location and form of individual macromolecules. Results from biochemistry, electron microscopy, and X-ray crystallography were used to create the image.

Keywords: Cellular biology, molecular biology, molecular visualization, textbook, diagrams.

“A clear picture of the interior of a living cell that shows the average distribution of molecules at the proper scale, the proper concentration and with no missing parts, seems to me to be central to the understanding of the workings of life.” This is how I began my 1991 article that presented several illustrations of *Escherichia coli* [1]. At the time, there was just enough information to create a convincing picture of the environment inside living cells. Today, most of the important features of this cell have been revealed, although many intriguing mysteries remain. For the new edition of “The Machinery of Life” [2], I updated these illustrations. I found that I was able to create an illustration where every molecule is specified, unlike the 1991 images where many generic proteins were included as placeholders with the expected size and oligomeric state.

Escherichia coli is arguably the most comprehensively studied organism known to science. A truly daunting amount of information is available for constructing an illustration of this cell. This includes information that spans the entire range from atoms to cells, including a full description of the genome, many studies of the proteome, ultrastructural studies of entire cells, and atomic structures of most of the major macromolecules [3]. This has prompted a number of efforts to create models of the entire cell and its processes [4–6].

When I started gathering materials for this illustration, I had hoped that I could use proteomic studies to provide an exact recipe for the cell. Unfortunately, this did not prove to be possible. Many proteomic studies are available, but the protein composition that they reveal is

highly dependent on the environmental conditions of the cell. I settled on a hybrid approach. I took the concentrations of macromolecules from the same sources that I used in the 1991 article. This includes the overall value of 70% water for the cell, as well as the number of proteins, RNA, lipids, and other molecules. I also used the same values for the concentrations for the major players in protein synthesis, transport, and energy production. I then added information from two large-scale proteomic studies—the Gene-Protein Database [7] and a GeneChip approach that measures levels of mRNA [8]—to identify other proteins that are commonly present.

I created two images of *Escherichia coli* cells for the book. The first illustration, shown in Fig. 1, is an image of the entire cell. The magnification is such that molecules can barely be seen, but the illustration gives a feeling for the overall ultrastructure of the cell. The second illustration, shown in Fig. 2, enlarges a portion of the cell so that the shape and size of individual macromolecules may be more easily seen. A detailed key to Fig. 2 is included in Fig. 3.

Of course, there are many assumptions to be made when creating illustrations like this. For molecules that are present in high copy numbers, such as tRNA or ribosomes, I tried to give a representative distribution based on the concentration. However, many other molecules, such as DNA polymerase or flagellar motors, are found in much lower concentrations and would only be seen by chance in a randomly chosen view. I have chosen to include many of these in the illustration, however, to give a feeling for the diversity of function that is present in the cell.

Whenever possible, I based the shape and size of individual molecules on atomic structures of the protein from *Escherichia coli* or related bacteria. These structures were taken from the RCSB Protein Data Bank [9] and are referenced here using the four-character accession

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*This work is supported by the RCSB PDB (NSF DBI 03-12718), grant DUE 06-18688 from the National Science Foundation, and the Fondation Scientifique Fournementin-Guilbert.

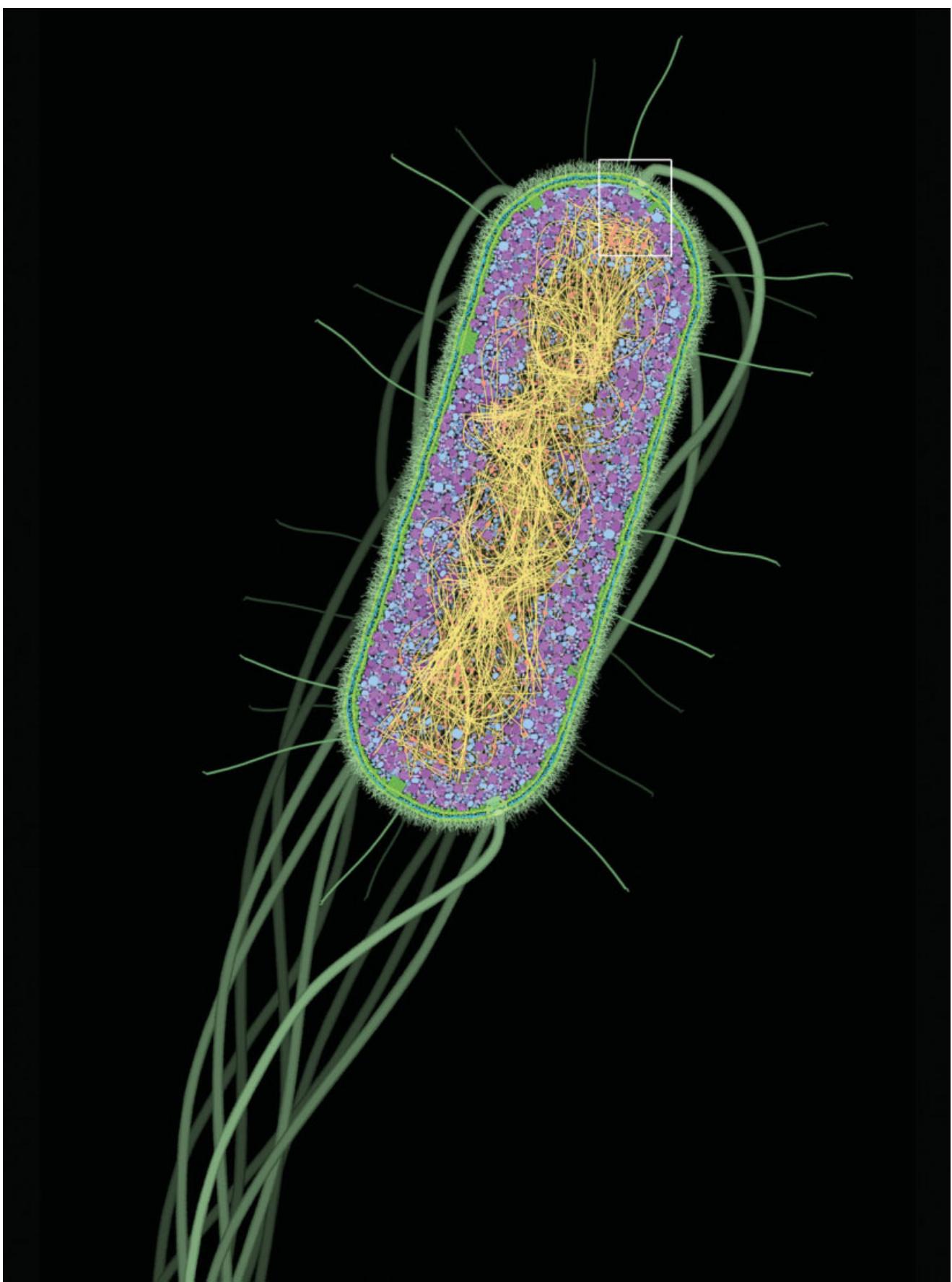


FIG. 1. Cross section through an entire *Escherichia coli* cell. The cell wall is in green, the cytoplasm is in magenta and blue, and the nucleoid is in yellow and orange. The boxed region is enlarged in Fig. 2. (magnification, $\times 70,000$).

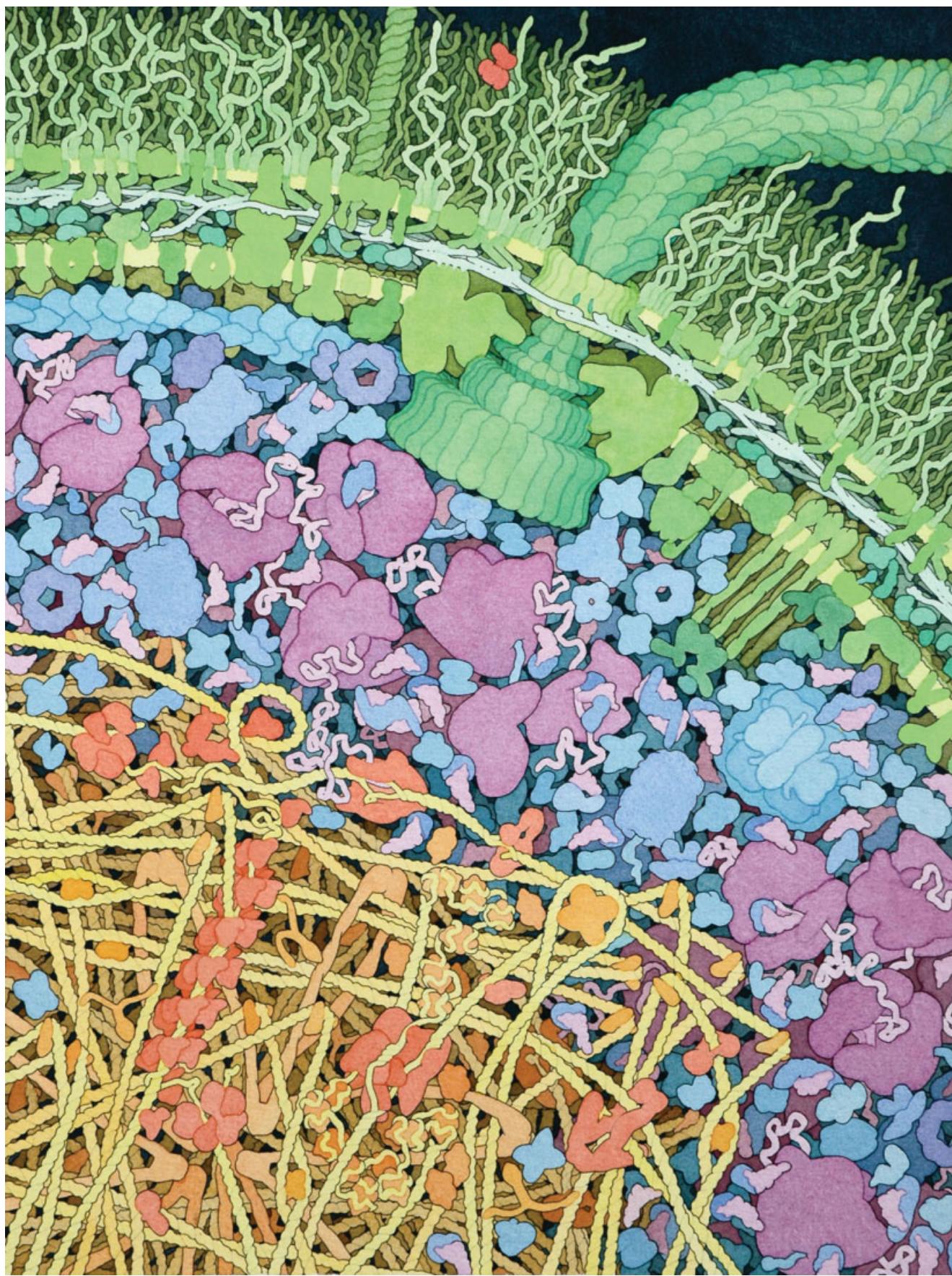


FIG. 2. Cross section through an *Escherichia coli* cell, showing all macromolecules at a magnification of $\times 1,000,000$. At this magnification, individual atoms are too small to resolve (about the size of a grain of salt). The cell wall is at the top, the cytoplasm runs through the middle, and the nucleoid is at the bottom.



codes. I have not included the primary references to these structures, as they are easily accessible at the RCSB PDB WWW site (<http://www.pdb.org>).

CELL WALL

Escherichia coli cells are surrounded by a complex cell wall composed of two concentric lipid bilayers, the outer membrane and the cytoplasmic membrane with a periplasmic space in between. This cell wall plays many functional roles in protection, transport, locomotion, sensing, detoxification, and energy production.

Many molecules contribute to the structural role played by the cell wall [10, 11]. The outer membrane is filled with lipopolysaccharides—molecules that include a long polysaccharide chain connected to a complex lipid with several fatty acid tails. I based the lipid core on the structure of lipopolysaccharide bound to the outer membrane protein FhuA (1fcp). The polysaccharide chains are heterogeneous, composed of 1–40 copies of a pentasaccharide repeat, each about 15 Å long. There are about 3.5 million per cell [10].

Several proteins dominate the outer membrane. These include porin OmpF (2omf), a trimeric protein that forms a pore through the membrane. Two other plentiful proteins, OmpA and lipoprotein, interact with the peptidoglycan layer. OmpA has a large membrane spanning region (1g90) and a periplasmic domain (1r1m). Lipoprotein (1eq7), on the other hand, is tethered on the inner face of the membrane by covalently attached lipids. Estimates place 200,000 Omp proteins and 720,000 lipoproteins per cell [10]. Assuming a surface area of about 6×10^6 nm², this places the lipopolysaccharide about 1.3 nm apart, Omp proteins about 9.4 nm apart, and lipoprotein (trimers) about 5 nm apart. Also included is an iron transporter FhuA (1fcp) connected to the inner membrane through TonB and a fimbrial usher, described later.

The space between the membranes, the periplasmic space, is filled with peptidoglycan and a collection of small soluble proteins. The peptidoglycan appears as a dense layer in electron microscopy, 2–3 nm thick, and closer to the outer membrane than to the inner membrane. It is composed of polysaccharide chains composed of about nine disaccharide repeats (each repeat approximately 1 nm long), which are then extensively crosslinked by short peptides. The resultant network

interacts with lipoproteins and other proteins in the outer membrane, forming the primary structural support for the cell wall.

The periplasm also includes many small soluble proteins. These include periplasmic binding proteins that gather small molecules and deliver them to transporters in the cytoplasmic membrane. I have included two examples with their associated transporters: the molybdenum transporter ModA and ModBC (2onk) and the vitamin B₁₂ transporter BtuF and BtuCD (2qj9). Also included are several protective enzymes, including beta-lactamase (2bls), lysozyme inhibitor (1gpq), and a monomeric copper-zinc superoxide dismutase (1eso). Two chaperone proteins are also included: heat shock protease/chaperone DegP (1ky9) and proline isomerase FkpA (1q6u).

The inner membrane is filled with proteins of many different functions. I tried to include a variety of examples, including proteins from energy production, transport, peptidoglycan synthesis, sensing, and defense, to give a cross section of the processes that are occurring. These include several transporters: magnesium transporters CorA (2bbj) and MgtE (2yyv), sodium-proton antiporter NhaA (1zcd), zinc transporter YiiP (2qfi), a calcium pump (1su4), and the two small molecule transporters mentioned earlier. A secretory channel is shown on the right side of the image, including the SecY complex in the membrane and SecA and SecB inside the cell (1rhz, 2fsg, 1ozb). The large complex that spans both membranes at the left side is a drug efflux pump AcrAB (1iwg) and TolC (1ek9).

Several enzymes of energy production are also included, such as ubiquinol oxidase (1fft), nitrate reductase NarGHI (1y4z), tricarboxylic acid cycle enzyme succinate dehydrogenase (1nek), NADH dehydrogenase (based on results from electron microscopy [12]), and ATP synthase (1c17, 1e79, 1l2p, 2a7u). A synthetic enzyme is also shown: PBP2 (penicillin binding protein 2, 2olu) is involved in the synthesis of the peptidoglycan sheath. It is associated with the structural protein MreC, discussed later.

Several of the cytoplasmic membrane proteins are involved in sensing. MscL (2oar) is one of several mechanosensitive channels that monitor the internal pressure of the cell. On the right side of the illustration, a large assembly of chemotaxis receptors are modeled after the aspartate receptor. These include array of receptors,

Fig. 3. Key to Figure 2. Extracellular: 1, Enterotoxin. **Outer membrane:** 2, lipopolysaccharide; 3, lipoprotein; 4, porin; 5, OmpA; 6, fimbrial usher; 7, pilus; 8, iron transport protein FhuA. **Periplasm:** 9, peptidoglycan; 10, periplasmic binding proteins; 11, beta-lactamase; 12, superoxide dismutase; 13, heat shock protein/chaperone DegP; 14, proline isomerase FkpA. **Inner membrane:** 15, magnesium transporter MgtE; 16, vitamin B12 transporter BtuCD-F; 17, shape-determining proteins MreCD and penicillin-binding protein PBP2; 18, mechanosensory channel MscL; 19, molybdenum transporter ModBC-A; 20, drug efflux pump AcrAB and TolC; 21, magnesium transporter CorA; 22, sodium/proton antiporter NhaA; 23, nitrate reductase NarGHI; 24, succinate dehydrogenase; 25, ATP synthase; 26, ubiquinol oxidase; 27, aspartate receptor; 28, signaling proteins CheAY; 29, secretory channel SecAB; 30, NADH dehydrogenase; 31, zinc transporter YiiP; 32, calcium pump. **Flagellar motor:** 33, flagellum; 34, flagellar hook; 35, rotor; 36, motor. **Cytoplasm:** 37, cytoskeletal protein MreB; 38, ribosome; 39, transfer RNA; 40, elongation factor Tu; 41, elongation factor Ts; 42, elongation factor G; 43, initiation factors; 44, aminoacyl-tRNA synthetase; 45, chaperone GroEL; 46, proteasome HslVU; 47, glycolytic enzymes; 48, tricarboxylic acid cycle enzymes; 49, catalase; 50, Iron superoxide dismutase; 51, alkyl hydroperoxide reductase; 52, phosphoenolpyruvate: sugar phosphotransferase system; 53, nucleoside diphosphate kinase; 54, glycerol kinase; 55, acyl carrier protein system; 56, aspartate carbamoyltransferase; 57, aspartate aminotransferase; 58, glutamine synthetase. **Nucleoid:** 59, DNA; 60, RNA polymerase; 61, messenger RNA; 62, catabolite activator protein; 63, lac repressor; 64, topoisomerase; 65, HU; 66, H-NS; 67, IHF; 68, Fis; 69, Lrp; 70, condensin MukBEF; 71, RecA; 72, RecBCD; 73, DNA methyltransferase Hha1; 74, DNA glycosylase MutM; 75, DNA polymerase; 76, single strand binding protein.

modeled after two structures (1vlt and 2ch7), and the components of the Che system (1b3q), which deliver the messages inside the cell [13, 14].

FLAGELLAR MOTOR AND FIMBRIA

The flagellar motor is arguably the most impressive structure of the *Escherichia coli* cell. Typical cells have 5–10 flagella scattered at random points around the cell. The flagellum is composed of roughly 20,000 subunits, extending 5–10 μm from the cell surface [15]. It is connected to the motor with a tightly curved hook, which has roughly 130 subunits with a similar fibril structure as flagellin [16]. The motor is composed of a ring of Mota and MotB proteins surrounding a rotor composed of many different proteins. I based the structure on work from electron microscopy [17].

Fimbria is important for the attachment of cells to hosts. *Escherichia coli* can express several different types with different properties. The one shown here is a type I pilus, with a diameter of roughly 2 nm and length of 1–2 μm , composed of roughly 1,200–2,400 subunits [18]. The tip, not seen in this picture, has specialized subunits involved in adhesion. The fimbriae are extruded from the cell through an usher, shown here in the outer cell membrane [19]. The usher assembles new subunits to the end of the growing fiber, with the help of a FimC chaperone protein (1qun).

CYTOPLASMIC PROTEINS

Much of the cytoplasmic region of the bacterial cell is filled with molecules of protein synthesis. The remaining molecules are soluble enzymes performing diverse functions. As with my previous TIBS work, I included 130 glycolytic enzymes and 100 enzymes from the tricarboxylic acid cycle in each 100 nm^3 portion, which leaves about 100 enzymes of other types in this same volume. I included several of the most plentiful enzymes from proteomics studies in this collection, including aspartate carbamoyltransferase (2atc), aspartate aminotransferase (1ase), glycerol kinase (1bot), nucleoside diphosphate kinase (2hur), glutamine synthetase (2gls), alkyl hydroperoxide reductase (1n8j), catalase (1cf9), iron superoxide dismutase (1isa), and several enzymes of the phosphoenolpyruvate: sugar phosphotransferase system (2hwg, 1cm3, 1wcr) and the acyl carrier protein system (1dd8, 1i01).

There is also increasing evidence that bacterial cells have a significant cytoskeleton [20, 21]. I included the actin-like MreB protein (1jce), which is thought to form a large helical assembly inside the cytoplasmic membrane. MreC (2qf4) links this filament to the membrane and may be important for control of cell shape.

PROTEIN SYNTHESIS

Protein synthesis is one of the major tasks of the *Escherichia coli* cell, which is reflected in the composition of the cell: over a third of the molecules are involved in one way or another with production of proteins. Remarkably, atomic structures are available for most of the major molecules involved in protein synthesis. Unlike in eukaryotic

cells, transcription and translation are performed in the same cellular compartment in prokaryotes, and often occur simultaneously. I included one complex at the center of the image where ribosomes have started translating an mRNA that is in the process of being transcribed. RNA polymerase was modeled after the enzyme from *Thermus thermophilus* (1iw7). Based on information in my 1991 article, there would be approximately 6 RNA polymerase molecules per 100 nm^3 —I included one in foreground and one in the background of this view.

Amazingly, atomic structures are now available for ribosomes in many different states. I used two PDB files as the model for this illustration: 1yl3 and 1yl4. I used several structures for the elongation factors, including the tRNA/EF-Tu complex in 1ttt, the EF-Tu/EF-Ts complex in 1efu, and EF-G in 1dar. Structures are available for all classes of aminoacyl-tRNA synthetases—I used PDB entries 1asz, 1set, 1ffy, 1gax, 1euq, 1eiy, and 1qf6. As in my original TIBS report, I used a concentration of 30 ribosomes, 30 aminoacyl-tRNA synthetases, 340 tRNA per 100 nm^3 . EF-Tu is found to be one of the most plentiful proteins in most proteomics studies [7, 8]—I included a number roughly equal to the number of tRNA molecules. I also included several chaperonin proteins. GroEL and GroES show up as being particularly plentiful in proteomics studies. I included them based on PDB entry 1aon. A HslVU proteasome is also included from PDB entry 1e94.

DNA AND DNA PACKAGING

Escherichia coli cells do not have a discrete nucleus, but they do have a loosely defined area at the center termed the nucleoid that contains most of the DNA. It is generally seen to be less densely packed with proteins than the surrounding cytoplasm, presumably due to the sieve-like exclusion of proteins by the DNA strands. A simple calculation was used to estimate the packing of DNA in the nucleoid. The *Escherichia coli* K-12 genome has 4,639,221 base pairs [22], which is about 1.5 mm in length, and a typical growing cell has 2.3 genomes per cell. The volume of the nucleoid is roughly 0.14 μm^3 [1]. To get an estimate for the packing of the DNA, we may think of the nucleoid volume as a cube with sides of about 0.52 μm , then cut the DNA into 0.52 μm lengths and stack them uniformly in the cube. From this approximation, we obtain a spacing of about 6.4 nm between DNA strands.

To calculate the number of repressors that we might expect to see in this slice, I assumed that most operons were about the size of the lac operon. It is about 5,000 base pairs long or 1,700 nm. So, if our window is a 100 nm^2 , we might expect to see one repressor every 17 strands that pass through the view. I included one complex of lac repressor and catabolite activator protein in the foreground of this illustration, and we might expect to see one or two more in the background. The looped structure of the DNA is taken from the crystal structure analysis [23].

The bacterial genome is packaged by a variety of proteins that bend DNA and bridge neighboring portions of

the strand. Abundances for the DNA packaging proteins were taken from a review of the subject [24]: 50,000 histone-like protein HU, 20,000 H-NS (histone-like nucleoid structuring protein), 15,000 IHF (integration host factor), and 100,000 Fis (factor for inversion stimulation protein)—all values are the number of molecules per cell. Assuming that there are 2.3 genomes per cell, a protein with 10,000 copies per cell would be spaced about 1,000 base pairs, or 340 nm, apart. The structures of these proteins were taken from a recent review [25] and several PDB entries (3fis, 1p71, 1lr1). I also include one Lrp (leucine-responsive regulatory protein) in the view (2gqq), as well as a large star-shaped complex of condensin MukBEF [26].

I included two types of topoisomerases: a type I topoisomerase next to a transcribing RNA polymerase (1i7d) and a DNA gyrase caught in the middle of passing one DNA strand through another (1bgw, 1ei1). I also included several molecules involved in DNA repair. These include a long helical complex of RecA (2reb) and two RecBCD complexes acting on a DNA break (1w36). Also included are enzymes that correct damaged bases, such as the DNA glycosylase MutM (1kfv).

At the center of the nucleoid region, I included a replication fork. The DNA polymerase complex is based on several structures of the isolated components [27]—but the overall association of the components is still a matter of debate. The transient single-stranded regions are bound to SSB (single stranded DNA binding protein), modeled after the structure of the DNA-binding domain of the protein (1eyg).

PEDAGOGIC AND ESTHETIC CONSIDERATIONS

I wanted to capture several concepts in this illustration: that the cell is a crowded environment, that many different processes are intermingled and occur simultaneously, and that there is compartmentation even in bacterial cells. To highlight compartmentation, I chose a coloring scheme that separated each compartment. The two membranes are colored greens, with the periplasmic space in turquoise. The cytoplasm is in blues and purples, and the nucleoid is in yellows and oranges. This coloring scheme highlights the physical compartmentation—the separation of spaces by membranes and the sieve-like separation provided by the DNA—but it hides the functional localization, for instance, the fact that the cytoplasmic membrane plays multiple roles in energy production, nutrient transport, and signaling. To show this intermingled function, I created a series of “keys” for the book that isolated just the molecules involved in a particular function. These keys are similar to Fig. 3, but show the molecules of a particular function in color and the rest in gray tones. As the reader progresses through the chapter, they can compare several of these keys and explore the mixture of different functions occurring in each space.

There is also a healthy dose of artistic license applied to this type of illustration. In particular, it is always a challenge to represent fibrous and planar components in a

way that is true to the actual three-dimensional geometry of the object. In this illustration, I purposefully aligned all of the DNA strands and the peptidoglycan strands approximately in the plane of the page, so that they would not be clipped. In reality, of course, we would expect a more tangled structure, with chains at all orientations. The section was chosen perpendicular to the membrane, so show the cross section through the entire cell wall. This unfortunately hides the continuous planar character of the membrane, but is necessary to reveal the different compartments.

I chose the overall view at the rounded end of the cell to give a feeling for the finite size of the cell. If I had chosen a view along one of the straight sides, there would not be any hints about the diameter of the cell. Of course, the relative size of molecules and cells is apparent in Fig. 1, which shows the whole cell, but I also wanted to capture this relationship in the enlarged cross-section. Figure 2 is a water color painting, created at twice the printed size. Figure 1 was created digitally entirely in Photoshop.

Acknowledgment—This is manuscript 20312 from the Scripps Research Institute.

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those points is given by

$$d = c^{-1/3}, \quad (2.12)$$

where c is the concentration of interest (measured in units of number of molecules per unit volume). Larger concentrations imply smaller intermolecular spacings. This idea is formalized in Figure 2.12(B), which shows the relation between the mean spacing measured in nanometers and the concentration in nanomolar units.

2.1.3 Looking Inside Cells

With our reference bacterium in mind, the remainder of the chapter focuses on the various structures that make up cells and organisms. To talk about these structures, it is helpful to have a sense of how we know what we know about them. Further, model building requires facts. To that end, we periodically take stock of the experimental basis for our models. For this chapter, the “Experiments Behind the Facts” focuses on how biological structures are explored and measured.

Experiments Behind the Facts: Probing Biological Structure

To size up cells and their organelles, we need to extract “typical” structural parameters from a variety of experimental studies. Though we leave a description of the design and setup of such experiments to more specialized texts, the goal is to provide at least enough detail so that the reader can see where some of the key structural facts that we will use throughout the book originated. We emphasize two broad categories of experiments: (i) those in which some form of radiation interacts with the structure of interest and (ii) those in which forces are applied to the structure of interest.

Figure 2.13 shows three distinct experimental strategies that feed into our estimates, all of which reveal different facets of biological structure. One of the mainstays of structural analysis is light microscopy. Figure 2.13(A) shows a schematic of the way in which light can excite fluorescence in samples that have some distribution of fluorescent molecules within them. In particular, this example shows a schematic of a microtubule that has some distribution of fluorophores along its length. Incident photons of one wavelength are absorbed by the fluorophores and this excitation leads them to emit light of a different wavelength, which is then detected. As a result of selective labeling of only the microtubules with fluorophores, it is only these structures that are observed when the cell is examined in the microscope. These experiments permit a determination of the size of various structures of interest, how many of them there are, and where they are localized. By calibrating the intensity from single fluorophores, it has become possible to take a single-molecule census for many of the important proteins in cells. For an example of this strategy, see Wu and Pollard (2005). A totally different window on the structure of the cell and its components is provided by tools such as the atomic-force microscope (AFM). As will be explained in Chapter 10, the AFM is a cantilever beam with a sharp tip on its end. The tip is brought very close to the surface where the structure of interest is present and is then scanned in the



EXPERIMENTS

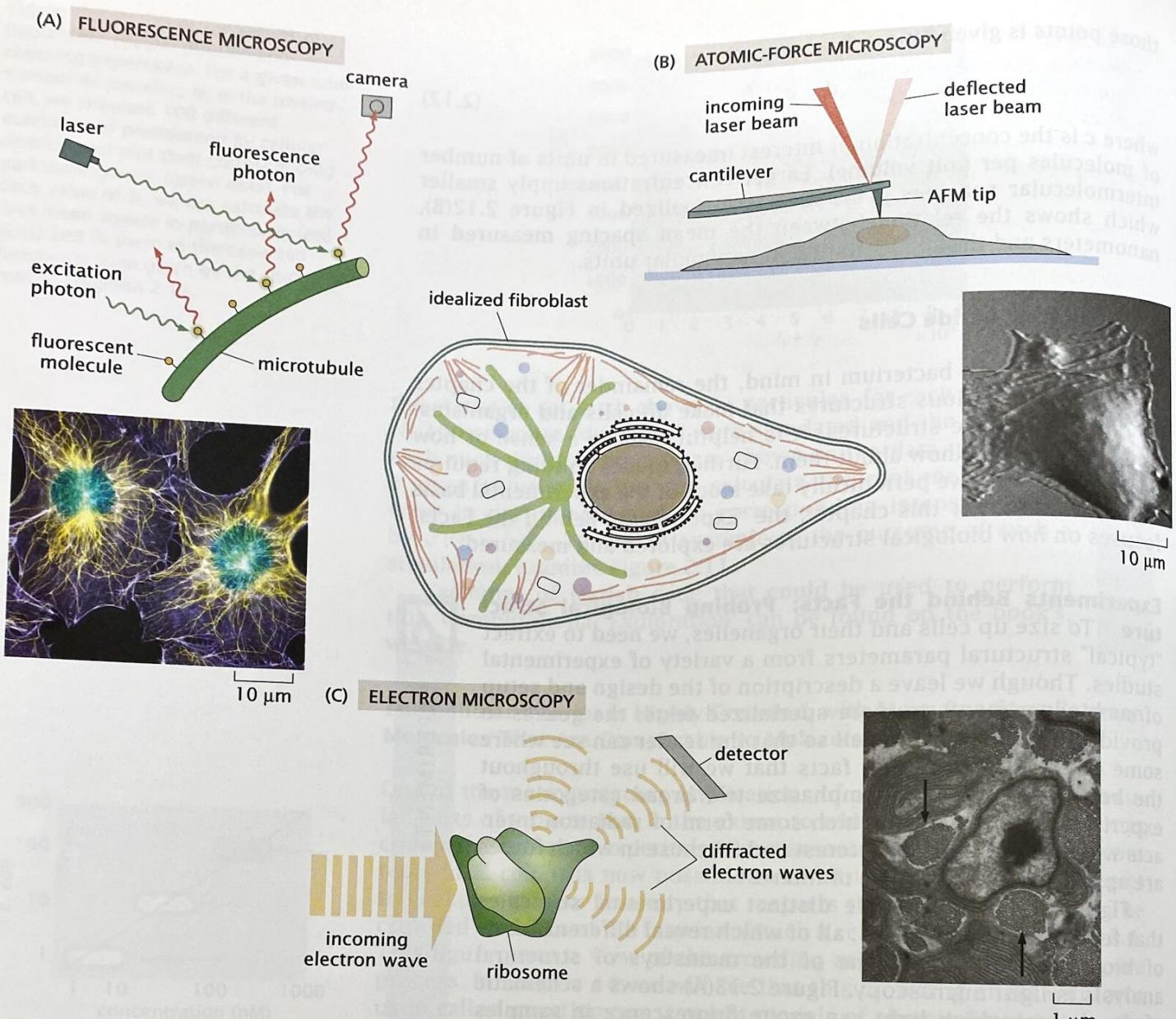


Figure 2.13: Experimental techniques that have revealed the structure of both cells and their organelles. (A) Fluorescence microscopy schematic and the associated image of a fibroblast with labeled microtubules (yellow) and DNA (green). (B) Atomic-force microscopy schematic and image of cross-section through a fibroblast in an animal tissue. Arrows indicate bundles of collagen fibers. (A, courtesy of Torsten Wittman; B, adapted from M. Radmacher, *Meth. Cell Biol.* 83:347, 2007; C, adapted from D. E. Birk and R. L. Trelstad, *J. Cell Biol.* 103:231, 1986.)

plane of the sample. One way to operate the instrument is to move the cantilever up and down so that the force applied on the tip remains constant. Effectively, this demands a continual adjustment of the height as a function of the x - y position of the tip. The nonuniform pattern of cantilever displacements can be used to map out the topography of the structure of interest. Figure 2.13(B) shows a schematic of an AFM scanning a typical fibroblast cell as well as a corresponding image of the cell.

Figure 2.13(C) gives a schematic of the way in which X-rays or electrons are scattered off a biological sample. The schematic shows an incident plane wave of radiation that interacts with the biological specimen and results in the emergence

Figure 2.12: Physical interpretation of concentration. (A) Concentration in *E. coli* cells: number of copies of a given molecule in a volume the size of an *E. coli* cell as a function of the concentration. (B) Concentration expressed in units of typical distance d between neighboring molecules measured in nanometers.

of radiation with the same wavelength but a new propagation direction. Each point within the sample can be thought of as a source of radiation, and the observed intensity at the detector reflects the interference from all of these different sources. By observing the pattern of intensity, it is possible to deduce something about the structure that did the scattering. This same basic idea is applicable to a wide variety of radiation sources, including X-rays, neutrons, and electrons.

An important variation on the theme of measuring the scattered intensity from irradiated samples is cryo-electron tomography. This technique is one of the centerpieces of structural biology and is built around uniting electron microscopy with sample preparation techniques that rapidly freeze the sample. The use of tomographic methods has made it possible to go beyond the planar sections seen in conventional electron microscopy images. The basis of the technique is indicated schematically in Figure 2.14, and relies on rotating the sample over a wide range of orientations and then assembling a corresponding three-dimensional reconstruction on the basis of the entirety of these images. This technique has already revolutionized our understanding of particular organelles and is now being used to image entire cells.

2.1.4 Where Does *E. coli* Fit?

Biological Structures Exist Over a Huge Range of Scales

The spatial scales associated with biological structures run from the nanometer scale of individual molecules all the way to the scale of the Earth itself. Where does *E. coli* fit into this hierarchy of structures? Figure 2.15 shows the different structures that can be seen as we scale in and out from an *E. coli* cell. A roughly 10-fold increase in magnification relative to an individual bacterium reveals the viruses that attack bacteria. These viruses, known as bacteriophages, have a characteristic scale of roughly 100 nm. They are made up of a protein shell (the capsid) that is filled with the viral genome. Continuing our downward descent using yet higher magnification, we see the ordered packing of the viral genome within its capsid. These structures are intriguing because they involve the ordered arrangement of more than 10 μm of DNA in a capsid that is less than 100 nm across. Another rough factor-of-10 increase in resolution reveals the structure of the DNA molecule itself, with a characteristic cross-sectional radius of roughly 1 nm and a length of 3.4 nm per helical repeat.

A similar scaling out strategy reveals new classes of structures. As shown in Figure 2.15, a 10-fold increase in spatial scale brings us to the realm of eukaryotic cells in general, and specifically, to the scale of the epithelial cells that line the human intestine. We use this example because bacteria such as *E. coli* are central players as part of our intestinal ecosystem. Another 10-fold increase in spatial scale reveals one of the most important inventions of evolution, namely, multicellularity. In this case, the cartoon depicts the formation of planar sheets of epithelial cells. These planar sheets are themselves the building blocks of yet higher-order structures such as tissues and organs. Scaling out to larger scales would bring us to multicellular organisms and the structures they build.

The remainder of the chapter takes stock of the structures at each of these scales and provides a feeling for the molecular building blocks

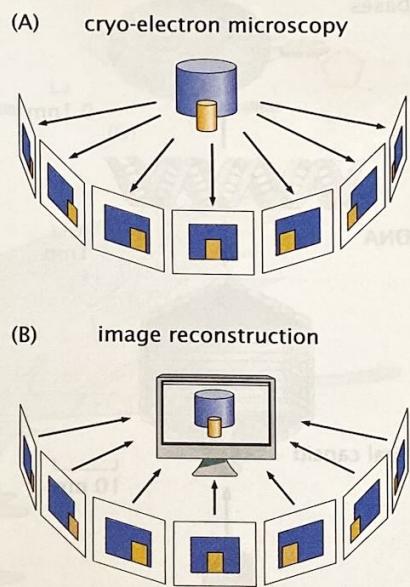
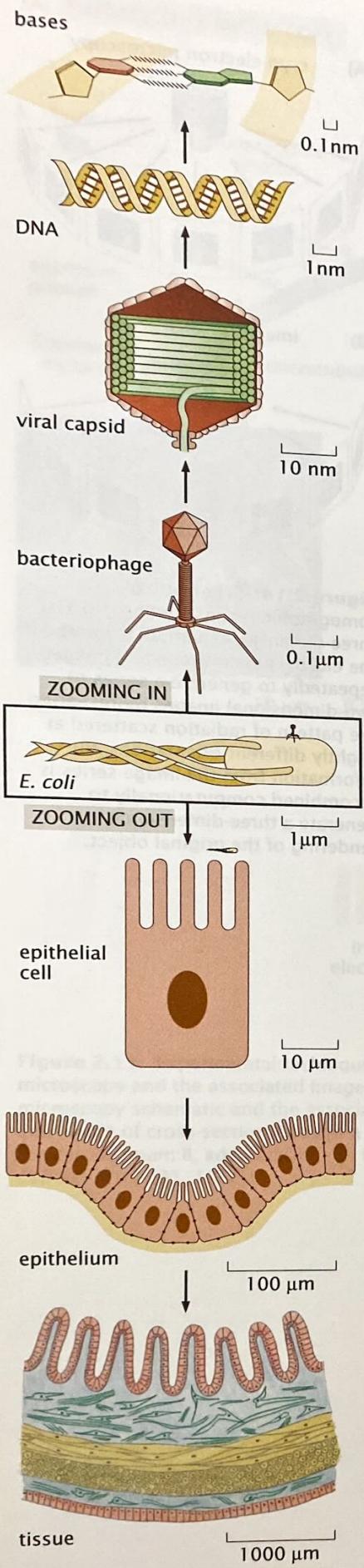


Figure 2.14: Schematic of tomographic reconstruction. (A) The three-dimensional sample is rotated in the electron microscope and imaged repeatedly to generate a series of two-dimensional images representing the pattern of radiation scattered at slightly different orientations. (B) Information from the image series is recombined computationally to generate a three-dimensional rendering of the original object.



that make up these different structures. Our strategy will be to build upon our cell-centered view and to first descend in length scale from that of cells to the molecules of which they are made. Once this structural descent is complete, we will embark on an analysis of biological structure in which we zoom out from the scale of individual cells to collections of cells.

2.2 Cells and Structures within Them

2.2.1 Cells: A Rogue's Gallery

All living organisms are based on cells as the indivisible unit of biological organization. However, within this general rule there is tremendous diversity among living cells. Several billion years ago, our last common ancestor gave rise to three different lineages of cells now commonly called Bacteria, Archaea, and Eukarya, a classification suggested by similarities and differences in ribosomal RNA sequences. Every living organism on Earth is a member of one of these groups. Most bacteria and archaea are small (3 μm or less) and extremely diverse in their preferred habitats and associated lifestyles ranging from geothermal vents at the bottom of the ocean to permafrost in Antarctica. Bacteria and Archaea look similar to one another and it has only been within the last few decades that molecular analysis has revealed that they are completely distinct lineages that are no more closely related to each other than the two are to Eukarya.

The organisms that we most often encounter in our everyday life and can see with the naked eye are members of Eukarya (individuals are called eukaryotes). These include all animals, all plants ranging from trees to moss, and also all fungi, such as mushrooms and mold. Thus far, we have focused on *E. coli* as a representative cell, although we must acknowledge that *E. coli*, as a member of the bacterial group, is in some ways very different from a eukaryotic or archaeal cell. The traditional definition of a eukaryotic cell is one that contains its DNA genome within a membrane-bound nucleus. Most bacteria and archaea lack this feature and also lack other elaborate intracellular membrane-bound structures such as the endoplasmic reticulum and the Golgi apparatus that are characteristic of the larger and more complex eukaryotic cells.

Cells Come in a Wide Variety of Shapes and Sizes and with a Huge Range of Functions

Cells come in such a wide variety of shapes, sizes, and lifestyles that choosing one representative cell type to tell their structural story is misleading. In Figure 2.16, we show a rogue's gallery illustrating a small segment of the variety of cell sizes and shapes found in the eukaryotic group, all referenced to the *E. coli* standard ruler. This gallery is by no means complete. There is much more variety than we can illustrate, but this covers a reasonable range of eukaryotic cell

Figure 2.15: Powers-of-10 representation of biological length scales. The hierarchy considers a succession of 10-fold increases in resolution as are shown in the figure.

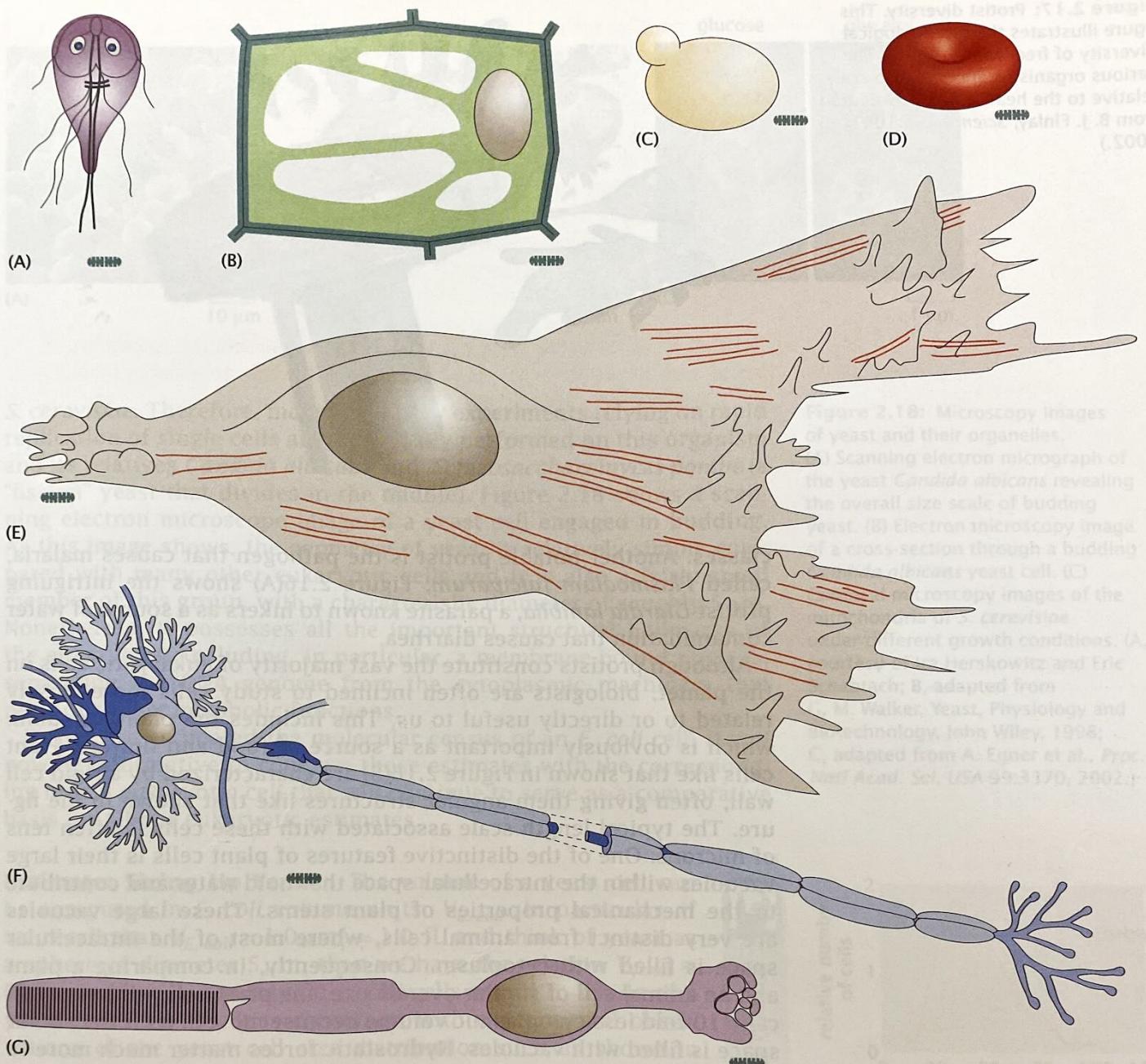


Figure 2.16: Cartoons of several different types of cells all referenced to the standard *E. coli* ruler. (A) The protist *Giardia lamblia*, (B) a plant cell, (C) a budding yeast cell, (D) a red blood cell, (E) a fibroblast cell, (F) a eukaryotic nerve cell, and (G) a retinal rod cell.

types that have been well studied by biologists. In this figure, we have chosen a variety of examples that represent experimental bias among biologists where more than half of the examples are human cells and the others represent the rest of the eukaryotic group.

The vast majority of eukaryotes are members of a group called protists, as shown in Figure 2.17. This poorly defined group encompasses all eukaryotes that are neither plants nor animals nor fungi. Protists are extremely diverse in their appearance and lifestyles, but they are all small (ranging from 0.002 mm to 2 mm). Some examples of protists are marine plankton such as *Emiliania huxleyi*, soil amoebae such as *Dictyostelium discoideum*, and the lovely creature *Paramecium* seen in any sample of pond water and familiar from many high-school biology

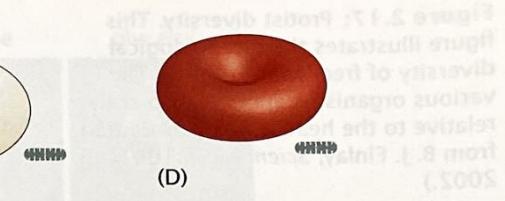


Figure 2.18: Microscopy images of yeast and their organelles. Scanning electron micrograph of the yeast *Candida albicans* revealing the overall size scale of budding yeast. (B) Electron microscopy image of a cross-section through a budding yeast cell. (C) Microscopy images of the yeast *Saccharomyces cerevisiae* under different growth conditions. (A) adapted from J. R. Sierskowicz and Eric B., adapted from C. Walker, Yeast: Physiology and Genetics, John Wiley, 1998; (B) adapted from A. Eigner et al., Proc. Natl. Acad. Sci. USA 99:3170, 2002; (C)

cell size distribution. Distribution of cell volumes measured for wild-type yeast cells. (Adapted from P. Jørgensen et al., Science 297:395, 2002.)

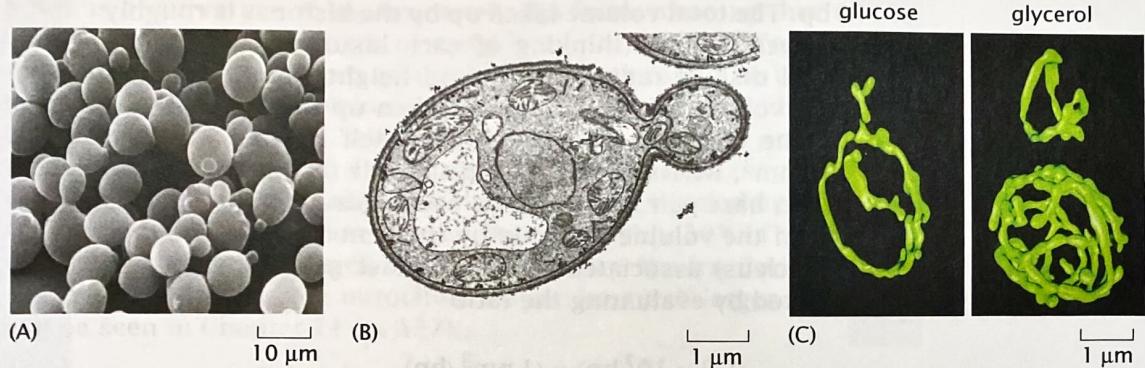
Figure 2.17: Protist diversity. This figure illustrates the morphological diversity of free-living protists. The various organisms are drawn to scale relative to the head of a pin. (Adapted from B. J. Finlay, *Science* 296:1061, 2002.)



classes. Another notable protist is the pathogen that causes malaria, called *Plasmodium falciparum*. Figure 2.16(A) shows the intriguing protist *Giardia lamblia*, a parasite known to hikers as a source of water contamination that causes diarrhea.

Although protists constitute the vast majority of eukaryotic cells on the planet, biologists are often inclined to study cells more closely related to or directly useful to us. This includes the plant kingdom, which is obviously important as a source of food and flowers. Plant cells like that shown in Figure 2.16(B) are characterized by a rigid cell wall, often giving them angular structures like that shown in the figure. The typical length scale associated with these cells is often tens of microns. One of the distinctive features of plant cells is their large vacuoles within the intracellular space that hold water and contribute to the mechanical properties of plant stems. These large vacuoles are very distinct from animal cells, where most of the intracellular space is filled with cytoplasm. Consequently, in comparing a plant and an animal cell of similar overall size, the plant cell will have typically 10-fold less cytoplasmic volume because most of its intracellular space is filled with vacuoles. Hydrostatic forces matter much more to plants than animals. For example, a wilting flower can be revived simply by application of water since this allows the vacuoles to fill and stiffen the plant stem.

Among the eukaryotes, the group most closely related to the animals (as proved by ribosomal RNA similarity and many other lines of evidence) is, surprisingly, the fungi. The representative fungus shown in Figure 2.16 is the budding yeast *Saccharomyces cerevisiae* (which we will refer to as *S. cerevisiae*). *S. cerevisiae* was domesticated by humans several thousand years ago and continues to serve as a treasured microbial friend that makes our bread rise and provides alcohol in our fermented beverages such as wine. Just as *E. coli* often serves as a key model prokaryotic system, the yeast cell often serves as the model single-celled eukaryotic organism. Besides the fact that humans are fond of *S. cerevisiae* for its own intrinsic properties, it is also useful to biologists as a representative fungus. Of all the other organisms on Earth, fungi are closest to animals in terms of evolutionary descent and similarity of protein functions. Although there are no single-celled animals, there are some single-celled fungi, including



S. cerevisiae. Therefore, many laboratory experiments relying on rapid replication of single cells are most easily performed on this organism and its relatives *Candida albicans* and *Schizosaccharomyces pombe* (a “fission” yeast that divides in the middle). Figure 2.18 shows a scanning electron microscope image of a yeast cell engaged in budding. As this image shows, the geometry of yeast is relatively simple compared with many other eukaryotic cells and it is also a fairly small member of this group, with a characteristic diameter of roughly 5 μm. Nonetheless, it possesses all the important structural hallmarks of the eukaryotes, including, in particular, a membrane-bound nucleus, separating the DNA genome from the cytoplasmic machinery that performs most metabolic functions.

Earlier, we estimated the molecular census of an *E. coli* cell. It will now be informative to compare those estimates with the corresponding model eukaryotic cell that will continue to serve as a comparative basis for all our eukaryotic estimates.

Figure 2.18: Microscopy images of yeast and their organelles. (A) Scanning electron micrograph of the yeast *Candida albicans* revealing the overall size scale of budding yeast. (B) Electron microscopy image of a cross-section through a budding *Candida albicans* yeast cell. (C) Confocal microscopy images of the mitochondria of *S. cerevisiae* under different growth conditions. (A, courtesy of Ira Herskowitz and Eric Schabtach; B, adapted from G. M. Walker, Yeast, Physiology and Biotechnology, John Wiley, 1998; C, adapted from A. Egner et al., Proc. Natl Acad. Sci. USA 99:3370, 2002.)

CURRENT PROBLEMS IN RESEARCH

Organic Compound Synthesis on the Primitive Earth

Several questions about the origin of life have been answered, but much remains to be studied.

Stanley L. Miller and Harold C. Urey

Since the demonstration by Pasteur that life does not arise spontaneously at the present time, the problem of the origin of life has been one of determining how the first forms of life arose, from which all the present species have evolved. This problem has received considerable attention in recent years, but there is disagreement on many points. We shall discuss the present status of the problem, mainly with respect to the early chemical history of, and the synthesis of organic compounds on, the primitive earth.

Many of our modern ideas on the origin of life stem from Oparin (1), who argued that the spontaneous generation of the first living organism might reasonably have taken place if large quantities of organic compounds had been present in the oceans of the primitive earth. Oparin further proposed that the atmosphere was reducing in character and that organic compounds might be synthesized under these conditions. This hypothesis implied that the first organisms were heterotrophic—that is, that they obtained their basic constituents from the environment instead of synthesizing them from carbon dioxide and water. Horowitz (2) discussed this point further and outlined how a simple heterotrophic organism could develop the ability to synthesize various cell constituents and thereby evolve into autotrophic organisms.

In spite of the argument by Oparin,

numerous attempts were made to synthesize organic compounds under the oxidizing conditions now present on the earth (3). Various sources of energy acting on carbon dioxide and water failed to give reduced carbon compounds except when contaminating reducing agents were present. The one exception to this was the synthesis of formic acid and formaldehyde in very small yield (10^{-7} H₂CO molecules per ion pair) by the use of 40-million-electron-volt helium ions from a 60-inch cyclotron (4). While the simplest organic compounds were indeed synthesized, the yields were so small that this experiment can best be interpreted to mean that it would not have been possible to synthesize organic compounds nonbiologically as long as oxidizing conditions were present on the earth. This experiment is important in that it induced a reexamination of Oparin's hypothesis of the reducing atmosphere (5).

The Primitive Atmosphere

Our discussion is based on the assumption that conditions on the primitive earth were favorable for the production of the organic compounds which make up life as we know it. There are many sets of conditions under which organic compounds could have been produced. All these conditions are more or less reducing. However, before accepting a set

of conditions for the primitive earth, one must show that reactions known to take place will not rapidly change the atmosphere to another type. The proposed set of conditions must also be consistent with the known laws for the escape of hydrogen.

Cosmic dust clouds, from which the earth is believed to have been formed, contain a great excess of hydrogen. The planets Jupiter, Saturn, Uranus, and Neptune are known to have atmospheres of methane and ammonia. There has not been sufficient time for hydrogen to escape from these planets, because of their lower temperatures and higher gravitational fields. It is reasonable to expect that the earth and the other minor planets also started out with reducing atmospheres and that these atmospheres became oxidizing, due to the escape of hydrogen.

The meteorites are the closest approximation we have to the solid material from which the earth was formed. They are observed to be highly reduced—the iron mostly as metallic iron with some ferrous sulfide, the carbon as elemental carbon or iron carbide, and the phosphorus as phosphides.

The atmosphere under these reducing conditions would contain some hydrogen, methane, nitrogen, and ammonia; smaller amounts of carbon dioxide and carbon monoxide; and possibly small amounts of other substances such as higher hydrocarbons, hydrogen sulfide, and phosphine. These substances were probably not present in equilibrium concentrations, but compounds which are thermodynamically very unstable in this highly reducing atmosphere—such as oxygen, oxides of nitrogen, and oxides of sulfur—could not have been present in more than a few parts per million. This is true of compounds which are unstable in the present oxidizing atmosphere of the earth, such as hydrogen, ozone, methane, and nitrous oxide.

The over-all chemical change has been the oxidation of the reducing atmosphere to the present oxidizing atmosphere. This

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is caused by the loss of hydrogen, which results in the production of nitrogen, nitrate, sulfate, free oxygen, and ferric iron. As is discussed below, many complex organic compounds would have been formed during the course of this over-all change, thereby presenting a favorable environment for the formation of life. Whether the surface carbon of the present earth was all part of the initial atmosphere or whether it has been escaping from the earth's interior in a somewhat reduced condition is not important to the over-all picture.

Escape of Hydrogen

We have learned in recent years that the temperature of the high atmosphere is 2000°K or more, and there is no reason to suppose that the same temperature was not present in the past. One might expect that a reducing atmosphere would be cooler than an oxidizing atmosphere because methane and ammonia can emit infrared radiation while the diatomic molecules, nitrogen and oxygen, cannot. Curtis and Goody (6) have shown that carbon dioxide is ineffective in emitting infrared radiation in the high atmosphere. This is due to the low efficiency of energy transfer from the translational and rotational to the vibrational degrees of freedom, and it seems likely that this would apply to methane as well.

The loss of hydrogen from the earth is now believed to be limited by the diffusion of H₂ to the high atmosphere, since almost all the water is frozen out before it reaches the high atmosphere. Urey (7) has discussed this problem and finds that the loss is entirely due to these effects and not to the Jeans escape formula.

The present rate of escape is 10⁷ atoms of hydrogen per square centimeter per second, and it is proportional to the concentration of molecular hydrogen in the atmosphere, which is now 10⁻⁶ atm at the earth's surface. This rate would result in escape of hydrogen equivalent to 20 g of water per square centimeter in the last 4.5 × 10⁹ years. This rate is not sufficient to account for the oxygen in the atmosphere (230 g/cm²).

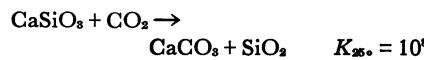
In addition, we must account for the oxidation of the carbon, ammonia, and ferrous iron to their present states of oxidation. The oxidation of the 3000 g of surface carbon per square centimeter present on the earth from the 0 to the +4 valence state (that is, from C or

H₂CO to CO₂) would require the loss of 1000 g of hydrogen per square centimeter. At the present rate of escape this would require 2.5 × 10¹² years. In order for this escape to be accomplished in 2.5 × 10⁹ years (that is, between 4.5 × 10⁹ and 2.0 × 10⁹ years ago), a pressure of hydrogen at the surface of the earth of 0.7 × 10⁻³ atm would have been required. In order for the nitrogen, sulfur, and iron also to be oxidized, even larger losses and a higher pressure of hydrogen would have been needed. We use a figure of 1.5 × 10⁻³ atm for the hydrogen pressure in the primitive atmosphere.

These calculations are greatly oversimplified, since methane and other volatile hydrogen compounds would be decomposed in the high atmosphere and therefore a higher concentration of hydrogen might exist in the high atmosphere than is indicated by surface partial pressures. However, the results of the calculation would be qualitatively the same for hydrogen pressures different from the chosen value by an order of magnitude.

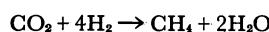
Equilibria of Carbon Compounds

The partial pressure of CO₂ in the atmosphere is kept low by two buffer systems. The first system, which is rapid, is the absorption in the sea to form HCO₃⁻ and H₂CO₃. The second, which is slow, is the reaction of carbon dioxide with silicates; for example



The partial pressure of CO₂ at sea level (3.3 × 10⁻⁴ atm) is somewhat higher than the equilibrium pressure (10⁻⁸ atm), but very much lower than would be the case without the formation of limestones (CaCO₃).

The equilibrium constant at 25°C in the presence of liquid water for the reaction



is 8 × 10²². Assuming that equilibrium was attained, and using partial pressures P_{CO₂} = 10⁻⁸ atm and P_{H₂} = 1.5 × 10⁻³ atm, we find that the pressure of CH₄ would be 4 × 10³ atm. In order to have a reasonable pressure of CH₄, the partial pressure of CO₂ would have to be less than 10⁻⁸ atm, and limestones would not form.

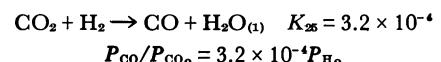
Complete thermodynamic equilibrium could not exist in a reducing atmosphere

because of the dependence of the equilibrium proportions of compounds on pressure and hence on altitude. It is more likely that the steady-state concentrations of CO₂ and CH₄ would be determined not by the equilibrium at sea level but rather by the equilibrium at higher altitude, where the ultraviolet light would provide the activation energy to bring about rapid equilibrium. Under these conditions water would be a gas, and the equilibrium constant would be 10²⁰, so

$$K_{25^\circ} = 10^{20} = P_{\text{CH}_4} P_{\text{H}_2\text{O}}^2 / P_{\text{CO}_2} P_{\text{H}_2}^4 \\ = X_{\text{CH}_4} X_{\text{H}_2\text{O}}^2 / X_{\text{CO}_2} X_{\text{H}_2}^4 \quad P^{-8}$$

where the X's are the mole fractions and P is the total pressure. If the surface partial pressures were P_{CH₄} = 1, P_{CO₂} = 3.3 × 10⁻⁴ (the present value), and P_{H₂} = 1.5 × 10⁻³, the X's would be equal to these partial pressures. We use X_{H₂O} = 10⁻⁶, which is the present value for H₂O above the tropopause. Equilibrium will be established under these conditions where P = 2.5 × 10⁻⁹ atm—the present atmospheric pressure at about 180 km. It is reasonable to assume that equilibrium was established at some high altitude; therefore, carbon dioxide and hydrogen could both have been present at small partial pressures and methane could have been present at a moderate partial pressure in a reducing atmosphere where the pressure of hydrogen was 1.5 × 10⁻³ atm.

Carbon monoxide should not have been an important constituent of the atmosphere, as can be seen from the following reaction



Using P_{H₂} = 1.5 × 10⁻³, we have the ratio P_{CO}/P_{CO₂} = 5 × 10⁻⁷, which is independent of pressure. Furthermore, carbon monoxide is a relatively reactive compound, and should any significant quantities appear in the atmosphere, it would react rather rapidly to give organic compounds, carbon dioxide and hydrogen, and formate.

Rubey (8) and Abelson (9) have argued that the surface carbon and nitrogen have come from the outgassing of the interior of the earth instead of from the remaining gases of the cosmic dust cloud from which the earth was formed. The carbon from the outgassing of the earth is a mixture of CO₂, CO, and CH₄, and hydrogen may be present. While outgassing may have been a significant process on the primitive earth, this does not

mean that the atmosphere was necessarily composed of CO_2 and CO. The thermodynamic considerations discussed above would still apply. The carbon dioxide would dissolve in the ocean to form bicarbonate, and CaCO_3 would be deposited, and the CO would be unstable, as is demonstrated above.

Many writers quote "authorities" in regard to these questions without understanding what is fact and what is opinion. The thermodynamic properties of C, CO, CO_2 , CH_4 , N₂, NH₃, O₂, H₂O, and other similar substances are all well known, and the equilibrium mixtures can be calculated for any given composition without question. The only point open to argument is whether equilibrium was approximated or whether a nonequilibrium mixture was present. A mixture of hydrogen and carbon monoxide or hydrogen and carbon dioxide is very unstable at 25°C, but does not explode or react detectably in years. But would such mixtures remain in an atmosphere for millions of years subject to energetic radiation in the high atmosphere? We believe the answer is "No." These mixtures would react even without such radiation in geologic times. Hydrogen and oxygen will remain together at low temperatures for long times without detectable reaction by ordinary methods. The use of radioactive tracers shows that a reaction is proceeding at ordinary temperatures nonetheless.

The buffer systems of the ocean and the calcium silicate-calcium carbonate equilibrium were of sufficient capacity to keep the partial pressure of the carbon dioxide in the atmosphere at a low value; hence, the principal species of carbon in the atmosphere would have been methane, even though the fraction of surface carbon in the oxidation state of carbon dioxide was continuously increasing. This would have been true until the pressure of H₂ fell below about 10^{-6} atm. It is likely that shortly after this, significant quantities of molecular oxygen would have appeared in the atmosphere.

Equilibria of Nitrogen Compounds

The equilibrium concentrations of ammonia can be discussed by considering the reaction



Using $P_{\text{H}_2} = 1.5 \times 10^{-3}$, we have $P_{\text{NH}_3}/P_{\text{N}_2}^{3/2} = 0.04$.

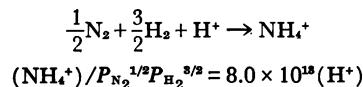
Ammonia is very soluble in water and

Table 1. Present sources of energy averaged over the earth.

Source	Energy (cal $\text{cm}^{-2} \text{yr}^{-1}$)
Total radiation from sun	260,000
Ultraviolet light	
λ < 2500 Å	570
λ < 2000 Å	85
λ < 1500 Å	3.5*
Electric discharges	4 †
Cosmic rays	0.0015
Radioactivity (to 1.0 km depth)	0.8‡
Volcanoes	0.13§

* Includes the 1.9 cal $\text{cm}^{-2} \text{yr}^{-1}$ from the Lyman α at 1216 Å (39). † Includes 0.9 cal $\text{cm}^{-2} \text{yr}^{-1}$ from lightning and about 3 cal $\text{cm}^{-2} \text{yr}^{-1}$ due to corona discharges from pointed objects (40). ‡ The value, 4×10^6 years ago, was 2.8 cal $\text{cm}^{-2} \text{yr}^{-1}$ (41). § Calculated on the assumption of an emission of lava of 1 km³ ($C_D = 0.25$ cal/g, $P = 3.0$ g/cm³) per year at 1000°C.

therefore would displace the above reaction toward the right, giving



which is valid for pH's less than 9. At pH = 8 and $P_{\text{H}_2} = 1.5 \times 10^{-3}$, we have



which shows that most of the ammonia would have been in the ocean instead of in the atmosphere. The ammonia in the ocean would have been largely decomposed when the pressure of hydrogen fell below 10^{-5} atm, assuming that the pH of the ocean was 8, its present value. A higher pH would have made the ammonia less stable; the converse is true for a lower pH.

All the oxides of nitrogen would have been unstable and therefore rare. Hydrogen sulfide would have been present in the atmosphere only as a trace constituent because it would have precipitated as ferrous and other sulfides. Sulfur would have been reduced to hydrogen sulfide by the reaction



It is evident that the calculations do not have a quantitative validity because of many uncertainties with respect to temperature, the processes by which equilibrium could be approached, the atmospheric level at which such processes would be effective, and the partial pressure of hydrogen required to provide the necessary rate of escape. In view of these uncertainties, further calculations are unprofitable at the present time. However, we can conclude from this dis-

cussion that a reducing atmosphere containing low partial pressures of hydrogen and ammonia and a moderate pressure of methane and nitrogen constitutes a reasonable atmosphere for the primitive earth. That this was the case is not proved by our arguments, but we maintain that atmospheres containing large quantities of carbon monoxide and carbon dioxide are not stable and cannot account for the loss of hydrogen from the earth.

Synthesis of Organic Compounds

At the present time the direct or indirect source of free energy for all living organisms is the sunlight utilized by photosynthetic organisms. But before the evolution of photosynthesis other sources of free energy must have been used. It is of interest to consider the sources of such free energy as well as the origin of the appropriate chemical compounds containing excess free energy which supplied the energy for chemical evolution prior to the existence of what should be called living organisms, and before the evolution of photosynthesis.

Table 1 gives a summary of the sources of energy in the terrestrial surface regions. It is evident that sunlight is the principal source of energy, but only a small fraction of this is in the wavelengths below 2000 Å which can be absorbed by CH_4 , H_2O , NH₃, CO_2 , and so on. If more complex molecules are formed, the absorption can move to the 2500-Å region or to longer wavelengths where a substantial amount of energy is available. With the appearance of porphyrins and other pigments, absorption in the visible spectrum becomes possible.

Although it is probable, it is not certain that the large amount of energy from ultraviolet light would have made the principal contribution to the synthesis of organic compounds. Most of the photochemical reactions at these low wavelengths would have taken place in the upper atmosphere. The compounds so formed would have absorbed at longer wavelengths and therefore might have been decomposed by this ultraviolet light before reaching the oceans. The question is whether the rate of decomposition in the atmosphere was greater or less than the rate of transport to the oceans.

Next in importance as a source of energy are electric discharges, such as lightning and corona discharges from pointed objects, which occur closer to

the earth's surface and hence would have effected more efficient transfer to the oceans.

Cosmic-ray energy is negligible at present, and there is no reason to assume it was greater in the past. The radioactive disintegration of uranium, thorium, and potassium was more important 4.5×10^9 years ago than it is now, but still the energy was largely expended on the interior of solid materials such as the rocks, and only a very small fraction of the total energy was available in the oceans and atmosphere. Volcanic energy is not only small but its availability is very limited. A continuous source of energy is needed. It contributes little to the evolutionary process to have a lava flow in one part of the earth at one time and to have another flow on the opposite side of the earth years later. For a brief time heat is available at the surface of the lava, but the surface cools and heat flows slowly from the interior for years, making the surface slightly warm. Only a very small contribution to the evolutionary process could be contributed by these energy sources.

Electric Discharges

While ultraviolet light is a greater source of energy than electric discharges, the greatest progress in the synthesis of organic compounds under primitive conditions has been made with electric discharges. The apparatus used by Miller in these experiments was a closed system of glass, except for tungsten electrodes. The water is boiled in a 500-ml flask which mixes the water vapor and gases in a 5-lit. flask where the spark is located. The products of the discharge are condensed and flow through a U-tube back into the 500-ml flask. The first report (10) showed that when methane, ammonia, water, and hydrogen were subjected to a high-frequency spark for a week, milligram quantities of glycine, alanine, and α -amino-n-butyric acid were produced.

A more complete analysis (11, 12) of the products gave the results shown in Table 2. The compounds in the table account for 15 percent of the carbon added as methane, with the yield of glycine alone being 2.1 percent. Indirect evidence indicated that polyhydroxyl compounds (possibly sugars) were synthesized. These compounds were probably formed from condensations of the formaldehyde that was produced by the

electric discharge. The alanine was demonstrated to be racemic, as would be expected in a system which contained no asymmetric reagents. It was shown that the syntheses were not due to bacterial contamination. The addition of ferrous ammonium sulfate did not change the results, and the substitution of N_2 for the NH_3 changed only the relative yields of the compounds produced.

This experiment has been repeated and confirmed by Abelson (13), by Pavlovskaya and Passynsky (14), and by Heyns, Walter, and Meyer (15). Abelson worked with various mixtures of H_2 , CH_4 , CO, CO_2 , NH_3 , N_2 , H_2O , and O_2 . As long as the conditions were reducing conditions—that is, as long as either H_2 , CH_4 , CO, or NH_3 was present in excess—amino acids were synthesized. The products were the same and the yields as large in many of these mixtures as they were with methane, ammonia, and water. If the conditions were oxidizing, no amino acids were synthesized. These experiments have confirmed the hypothesis that reducing atmospheres are required for the formation of organic compounds in appreciable quantities. However, several of these mixtures of gases are highly unstable. Hence the synthesis of amino acids in these mixtures does not imply that such atmospheres were present on the primitive earth.

Heyns, Walter, and Meyer also performed experiments with different mixtures of gases, with results similar to Abelson's. These workers also used CH_4 , NH_3 , H_2O , and H_2S . They obtained ammonium thiocyanate, thiourea, and thioacetamide as well as compounds formed when H_2S was absent.

The mechanism of synthesis of the amino acids is of interest if we are to extrapolate the results in these simple systems to the primitive earth. Two alternative proposals were made for the synthesis of the amino and hydroxy acids in the spark discharge system. (i) Aldehydes and hydrogen cyanide are synthesized in the gas phase by the spark. These aldehydes and the hydrogen cyanide react in the aqueous phase of the system to give amino and hydroxy nitriles, which are hydrolyzed to amino and hydroxy acids. This mechanism is essentially a Strecker synthesis. (ii) The amino and hydroxy acids are synthesized in the gas phase from the ions and radicals that are produced in the electric discharge.

It was shown that most, if not all, of the amino acids were synthesized accord-

ing to the first hypothesis, since the rate of production of aldehydes and hydrogen cyanide by the spark and the rate of hydrolysis of the amino nitriles were sufficient to account for the total yield of amino acids (12).

This mechanism accounts for the fact that most of the amino acids were α -amino acids, the ones which occur in proteins. The β -alanine was formed not by this mechanism but probably by the addition of ammonia to acrylonitrile (or acrylamide or acrylic acid), followed by hydrolysis to β -alanine.

The experiments on the mechanism of the electric discharge synthesis of amino acids indicate that a special set of conditions or type of electric discharge is not required to obtain amino acids. Any process or combination of processes that yielded both aldehydes and hydrogen cyanide would have contributed to the amount of α -amino acids in the oceans of the primitive earth. Therefore, whether the aldehydes and hydrogen cyanide came from ultraviolet light or from electric discharges is not a fundamental question, since both processes would have contributed to the α -amino acid content. It may be that electric discharges were the principal source of hydrogen cyanide and that ultraviolet light was the principal source of aldehydes, and that the two processes complemented each other.

Ultraviolet Light

It is clear from Table 1 that the greatest source of energy would be ultraviolet light. The effective wavelengths would be $CH_4 < 1450 \text{ Å}$, $H_2O < 1850 \text{ Å}$, $NH_3 < 2250 \text{ Å}$, CO $< 1545 \text{ Å}$, $CO_2 < 1690 \text{ Å}$, $N_2 < 1100 \text{ Å}$, and $H_2 < 900 \text{ Å}$. It is more difficult to work with ultraviolet light than with electric discharges because of the small wavelengths involved.

The action of the 1849-A Hg line on a mixture of methane, ammonia, water, and hydrogen produced only a very small yield of amino acids (16). Only NH_3 and H_2O absorb at this wavelength, but apparently the radical reactions formed active carbon intermediates. The limiting factor seemed to be the synthesis of hydrogen cyanide. Groth (17) found that no amino acids were produced by the 1849-A line of mercury with a mixture of methane, ammonia, and water, but that amines and amino acids were formed when the 1470-A and

1295-A lines of xenon were used. The 1849-A line produced amines and amino acids with a mixture of ethane, ammonia, and water. The mechanism of this synthesis was not determined. Terenin (18) has also obtained amino acids by the action of the xenon lines on methane, ammonia, and water.

We can expect that a considerable amount of ultraviolet light of wavelengths greater than 2000 Å would be absorbed in the oceans, even though there would be considerable absorption of this radiation by the small quantities of organic compounds in the atmosphere. Only a few experiments have been performed which simulate these conditions.

In a most promising experiment, Ellerbogen (19) used a suspension of ferrous sulfide in aqueous ammonium chloride through which methane was bubbled. The action of ultraviolet light from a mercury lamp gave small quantities of a substance with peptide frequencies in the infrared. Paper chromatography of a hydrolyzate of this substance gave a number of spots with Ninhydrin, of which phenylalanine, methionine, and valine were tentatively identified.

Bahadur (20) has reported the synthesis of serine, aspartic acid, asparagine, and several other amino acids by the action of sunlight on paraformaldehyde solutions containing ferric chloride and nitrate or ammonia. Pavlovskaya and Passynsky (21) have also synthesized a number of amino acids by the action of ultraviolet light on a 2.5-percent solution of formaldehyde containing ammonium chloride or nitrate. These high concentrations of formaldehyde would not have occurred on the primitive earth. It would be interesting to see if similar results could be obtained with $10^{-4} M$ or $10^{-5} M$ formaldehyde. This type of experiment deserves further investigation.

Radioactivity and Cosmic Rays

Because of the small amount of energy available, it is highly unlikely that high-energy radiation could have been very important in the synthesis of organic compounds on the primitive earth. However, a good deal of work has been done in which this type of energy has been used, and some of it has been interpreted as bearing on the problem of the origin of life.

Dose and Rajewsky (22) produced

Table 2. Yields from sparking a mixture of CH_4 , NH_3 , H_2O , and H_2 ; 710 mg of carbon was added as CH_4 .

Compound	Yield [moles ($\times 10^5$)]
Glycine	63.
Glycolic acid	56.
Sarcosine	5.
Alanine	34.
Lactic acid	31.
N-Methylalanine	1.
α -Amino-n-butyric acid	5.
α -Aminoisobutyric acid	0.1
α -Hydroxybutyric acid	5.
β -Alanine	15.
Succinic acid	4.
Aspartic acid	0.4
Glutamic acid	0.6
Iminodiacetic acid	5.5
Iminoacetic-propionic acid	1.5
Formic acid	233.
Acetic acid	15.
Propionic acid	13.
Urea	2.0
N-Methyl urea	1.5

amines and amino acids through the action of x-rays on various mixtures of CH_4 , CO_2 , NH_3 , N_2 , H_2O , and H_2 . A small yield of amino acids was obtained through the action of 2 Mev electrons on a mixture of CH_4 , NH_3 , and H_2O (23).

The formation of formic acid and formaldehyde from carbon dioxide and water by 40 Mev helium ions was mentioned previously. These experiments were extended by using aqueous formic acid (24). The yield per ion pair was only 6×10^{-4} for formaldehyde and 0.03 for oxalic acid. Higher yields of oxalic acid were obtained from $\text{Ca}(\text{HCO}_3)_2$ and NH_4HCO_3 by Hasselstrom and Henry (25). The helium ion irradiation of aqueous acetic acid solutions gave succinic and tricarboxlic acid along with some malonic, malic, and citric acids (26).

The irradiation of 0.1- and 0.25-percent aqueous ammonium acetate by 2 Mev electrons gave glycine and aspartic acid (27). The yields were very small. Massive doses of gamma rays on solid ammonium carbonate yielded formic acid and very small quantities of glycine and possibly some alanine (28).

The concentrations of carbon compounds and the dose rates used in these experiments are, in all probability, very much larger than could be expected on the primitive earth, and the products and yields may depend markedly on

these factors, as well as on the effect of radical scavengers such as HS^- and Fe^{2+} . It is difficult to exclude high-energy radiations entirely, but if one is to make any interpretations from laboratory work, the experiments should be performed with much lower dose rates and concentrations of carbon sources.

Thermal Energy

The older theories of the formation of the earth involved a molten earth during its formation and early stages. These theories have been largely abandoned, since the available evidence indicates that the solar system was formed from a cold cloud of cosmic dust. The mechanisms for heating the earth are the gravitational energy released during the condensation of the dust to form the earth and the energy released from the decay of the radioactive elements. It is not known whether the earth was molten at any period during its formation, but it is clear that the crust of the earth would not have remained molten for any length of time.

Studies on the concentration of some elements in the crust of the earth indicate that the temperature was less than 150°C during this lengthy fractionation, and that it was probably close to present terrestrial temperatures (29).

Fox (30) has maintained that organic compounds were synthesized on the earth by heat. When heated to 150°C , malic acid and urea were converted to aspartic acid and ureidosuccinic acid, and some of the aspartic acid was decarboxylated to α - and β -alanine. The difficulty with these experiments is the source of the malic acid and urea on the primitive earth—a question not discussed by Fox. Fox has also synthesized peptides by the well-known reaction (31) of heating amino acids at 150° to 180°C , and the yield of peptides has been increased by using an excess of aspartic or glutamic acid (32). There is a difficulty connected with heating amino acids and other organic compounds to high temperatures. Geological conditions can heat amino acids to temperatures above 100°C over long periods of time, but it is not likely that this could occur over short periods. Abelson (33) has shown that alanine, one of the more stable amino acids, decarboxylates to methylamine and carbon dioxide. The mean life of alanine is 10^{11} years at 25°C but only 30 years at 150°C . Therefore, any extensive heating

of amino acids will result in their destruction, and the same is true for most organic compounds. In the light of this, and since the surface of the primitive earth was probably cool, it is difficult to see how the processes advocated by Fox could have been important in the synthesis of organic compounds.

Surface Reactions, Organic Phosphates, and Porphyrins

It is likely that many reactions were catalyzed by adsorption on clay and mineral surfaces. An example is the polymerization of aminoacetonitrile to glycine peptides in the presence of acid clays, by Akabori and his co-workers (34). Formaldehyde and acetaldehyde were shown to react with polyglycine adsorbed on kaolinite to give serine and threonine peptides. This field offers many possibilities for research.

Gulick (35) has pointed out that the synthesis of organic phosphates presents a difficult problem because phosphate precipitates as calcium and other phosphates under present earth conditions, and that the scarcity of phosphate often limits the growth of plants, especially in the oceans. He proposes that the presence of hypophosphites, which are more soluble, would account for higher concentrations of phosphorus compounds when the atmosphere was reducing. Thermodynamic calculations show that all lower oxidation states of phosphorus are unstable under the pressures of hydrogen assumed in this article. It is possible that stronger reducing agents than hydrogen reduced the phosphate or that some process other than reduction solubilized the calcium phosphate. This problem deserves careful attention.

The synthesis of porphyrins is considered by many authors to be a necessary step for the origin of life. Porphyrins are not necessary for living processes if the organism obtains its energy requirements from fermentation of sugars or other energy-yielding organic reactions. According to the heterotrophic theory of the origin of life, the first organisms would derive their energy requirements from fermentations. The metabolism of sulfate, iron, N₂, hydrogen, and oxygen appears to require porphyrins as well as photosynthesis. Therefore, porphyrins probably would have to be synthesized before free energy could be derived from these compounds. While porphyrins may have been present in the environment

before life arose, this is apparently not a necessity, and porphyrins may have arisen during the evolution of primitive organisms.

Intermediate Stages in Chemical Evolution

The major problems remaining for an understanding of the origin of life are (i) the synthesis of peptides, (ii) the synthesis of purines and pyrimidines, (iii) a mechanism by which "high-energy" phosphate or other types of bonds could be synthesized continuously, (iv) the synthesis of nucleotides and polynucleotides, (v) the synthesis of polypeptides with catalytic activity (enzymes), and (vi) the development of polynucleotides and the associated enzymes which are capable of self-duplication.

This list of problems is based on the assumption that the first living organisms were similar in chemical composition and metabolism to the simplest living organisms still on the earth. That this may not be so is obvious, but the hypothesis of similarity allows us to perform experiments to test it. The surprisingly large yields of aliphatic, hydroxy, and amino acids— α -amino acids rather than the other isomers—in the electric-discharge experiments, plus the arguments that such syntheses would have been effective on the primitive earth, offer support for this hypothesis. Further support can be obtained by demonstrating mechanisms by which other types of biologically important compounds could be synthesized.

Oparin (1) does not view the first organism as a polynucleotide capable of self-duplication but, rather, as a coacervate colloid which accumulates proteins and other compounds from the environment, grows in size, and then splits into two or more fragments, which repeat the process. The coacervate would presumably develop the ability to split into fragments which are very similar in composition and structure, and eventually a genetic apparatus would be incorporated which would make very accurate duplicates.

These two hypotheses for the steps in the formation of the first living organism differ mainly in whether the duplication first involved the relatively accurate duplication of nucleic acids, followed by the development of cytoplasm duplication, or whether the steps occurred in

the reverse order. Other sequences could be enumerated, but it is far too early to discuss profitably the exact nature of the first living organism.

It was probably necessary for the primitive organisms to concentrate organic and inorganic nutrients from their environment. This could be accomplished by means of a membrane or by absorption on rocks or clays (36). The development of optical activity in living organisms is another important problem. This has been discussed by many authors and is not taken up here.

Life on Other Planets

Life as we know it—and we know of no other variety of life than that existing on the earth—requires the presence of water for its chemical processes. We know enough about the chemistry of other systems, such as those of silicon, ammonia, and hydrogen fluoride, to realize that no highly complex system of chemical reactions similar to that which we call "living" would be possible in such media. Also, much living matter exists and grows actively on the earth in the absence of oxygen, so oxygen is not necessary for life, although the contrary is often stated. Moreover, the protecting layer of ozone in the earth's atmosphere is not necessary for life, since ultraviolet light does not penetrate deeply into natural waters and also because many carbon compounds capable of absorbing the ultraviolet light would be present in a reducing atmosphere.

It is possible for life to exist on the earth and to grow actively at temperatures ranging from 0°C, or perhaps little lower, to about 70°C. It seems likely that if hot springs were not so temporary, many plants and possibly animals would evolve which could live in such temperatures. Plants are able to produce and accumulate substances which lower the freezing point of water and hence they can live at temperatures below 0°C. At much lower temperatures the reactions would probably be too slow to proceed in reasonable periods of time. At temperatures much above 120°C, reaction velocities would probably be so great that the nicely balanced reactions characteristic of living things would be impossible. In addition, it is doubtful whether the organic polymers necessary for living organisms would be stable much above 120°C; this is prob-

ably true even when allowance is made for the amazing stability of the enzymes of thermophilic bacteria and algae.

Only Mars, Earth, and Venus conform to the general requirements so far as temperatures are concerned. Mars is known to be very cold and Venus may be too hot. Observations of the black-body emission of radio waves from Venus indicate surface temperatures of 290° to 350°C (37). The clouds of Venus have the polarization of water droplets. Clearing of the clouds occurs, and this indicates that the clouds are composed of some volatile substance, for nonvolatile dust could hardly settle out locally. However, no infrared bands of water have been observed. It is possible that this is due to a very dry, high atmosphere, such as is characteristic of the earth, and to a cloud level that rises to very near the tropopause, so that there is little water vapor above the reflecting layer.

Mars is known to be very cold, with surface temperatures of $+30^{\circ}\text{C}$ to -60°C during the day. The colors of Mars have been observed for many years by many people. The planet exhibits seasonal changes in color—green or bluish in the spring and brown and reddish in the autumn. Sinton (38) has observed an absorption at $3.5\ \mu$ in the reflected light of Mars. This corresponds to the C-H stretching frequency of most organic compounds, but many inorganic compounds have absorptions at this wavelength. The changing colors of Mars and the $3.5\ \mu$ absorption are the best evidence, however poor it may be, for the existence of life on the planet. One thing that can be stated with confidence is that if life exists there, then liquid water must have been present on the planet in the past, since it is difficult to

believe that life could have evolved in its absence. If this was so, water must have escaped from the planet, as very little water remains there now and no liquid water has been observed. Hence, oxygen atoms must escape from the planet. This is possible if the high atmosphere has a temperature of 2000°K , and this may well be the case in view of the high temperatures in the high atmosphere of the earth.

Surely one of the most marvelous feats of 20th-century science would be the firm proof that life exists on another planet. All the projected space flights and the high costs of such developments would be fully justified if they were able to establish the existence of life on either Mars or Venus. In that case, the thesis that life develops spontaneously when the conditions are favorable would be far more firmly established, and our whole view of the problem of the origin of life would be confirmed (42).

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42. One of the authors (S. M.) is supported by a grant from the National Science Foundation.