CHAPTER 2

Biochemical Evolution

The authors have used evolution as the unifying theme of this book. The present chapter is an ambitious attempt to illustrate how all aspects of cellular functioning can be better understood from an evolutionary perspective. Truly mastering the breadth of information touched on here may be difficult but the reward will be a more basic, thorough, and intuitive grasp of the whole remainder of the book.

The origin of life is considered in four stages—generation of biomolecules, transition to replicating systems, interconversion of light and chemical energy, and adaptability to change. This discussion is theoretical, since the origins are obscure and hard data is lacking about actual mechanisms.

Evolution requires three properties: a system must reproduce, there must be variation, and there must be competition in a selective environment. Any system that satisfies these requirements will evolve, whether pure RNA in solution with a replicating enzyme, or a population of cells or higher plants and animals.

After touching on ribozymes as evidence that life passed through an "RNA World" stage, the authors illustrate how duplication and variation led to the many features of modern cells including DNA genes, ATP, lipid membranes, ion pumps, energy transducers, receptors with second messengers, etc. Cells have to move, either with flagella (procaryotes) or by changing shape using microfilaments, microtubules, and molecular motors (eucaryotes). Multicellular organisms require cells to differentiate according to developmental programming and signals from neighboring cells. All life on Earth came from a single progenitor, so we can learn about human biochemistry by studying any species, even simple single-celled organisms.

LEARNING OBJECTIVES

When you have mastered this chapter, you should be able to complete the following objectives.

Key Organic Molecules Are Used by Living Systems (Text Section 2.1)

1. List the four stages leading from inert chemicals to modern living cells.
2. Explain the Urey-Miller experiment, and diagram the apparatus. Describe the major products produced by this experiment.

Evolution Requires Reproduction, Variation, and Selective Pressure (Text Section 2.2)

3. Identify the three principles necessary for evolution to occur.
4. Describe Spiegelman's experiment with Qβ RNA. Understand how the three principles of evolution are included in this experiment.
5. Most enzymes are composed of protein. Explain how ribozymes differ from more normal enzymes.
6. Describe what is meant by a "hammerhead ribozyme."
7. Explain how RNA bases are derived from amino acids.
8. Explicate the advantages that polymers of amino acids have over nucleic acid polymers in providing catalysis for the cell.
9. Describe the roles of mRNA, tRNA, and rRNA in protein synthesis. Know that three mRNA bases are required to code for a single amino acid.
10. Ribosomal catalysis of peptide bond synthesis is mediated by regions of rRNA, and not by protein. Understand the implications of this catalysis for the concept of an RNA World.
11. Recall the three principles necessary for evolution as defined in Section 2.2. With these in mind, explain how the genetic code is ideally suited as a medium for evolutionary change.
12. Transfer RNAs all have very similar structures with minor variations that lead to significant differences in function. This is a common phenomenon in biochemistry. Describe how this situation would arise.
13. Explain the advantages of DNA compared to RNA for long-term storage of information.
14. The building blocks of DNA are made directly from the building blocks of RNA. Understand that this leads to the deduction that RNA must be older than DNA.
15. Define *transcription* and *translation.*

Energy Transformations Are Necessary to Sustain Living Systems (Text Section 2.3)

16. Describe the similarities between ATP production and use, and the function of money in society. You should appreciate the fact that this leads to the description of ATP as "energy currency" in the cell.
17. Describe the properties of a cell membrane that are responsible for keeping important cellular constituents (enzymes, nucleic acids, ATP, etc.) inside.
18. Define *osmosis, ion pump,* and *ion gradient.*

19 Describe the process of photosynthesis in general terms. Understand why photosynthesis must be membrane-associated.

20. Write the equation for the oxidation of water to oxygen.

21. Understand why oxygen is described as "toxic."

22. Know how many ATPs are produced per glucose consumed when using oxygen in glucose metabolism.

Cells Can Respond to Changes in Their Environments (Text Section 2.4)

23. Describe how E. coli responds when arabinose is the only source of carbon.

24. Define *second messenger* and *signal transduction.* Name two second messengers.

25. Distinguish between flagella, microfilaments, and microtubules.

26. Identify what happens on a molecular level when cells change shape.

27. Define *cell differentiation.*

28. Describe how the slime mold Dictyostelium uses signaling and changes in cell differentiation to respond to varying conditions. Understand that cAMP acts as a messenger (not a second messenger) for Dictyostelium.

29. Give a general description of how development is controlled in C. elegans. Notice the total number of cells in an adult human, and contrast that with the number of cells in C. elegans.

30 Know why understanding enzymes and processes in single-celled organisms like yeast or E. coli help us understand how human cells work.

31. Examine the time line in Figure 2.27, and explain during what time frame single-celled anaerobes would have dominated life on Earth.

SELF-TEST

Key Organic Molecules Are Used by Living Systems

1. A reducing atmosphere as described in this chapter would not contain significant amounts of

   a. CH_4
   b. CO_2
   c. NH_3
   d. H_2O
   e. H_2

Evolution Requires Reproduction, Variation, and Selective Pressure

2. What would happen in Spiegelman's experiment with Qβ RNA if no selective conditions were imposed (inhibitors, limited time, etc.)? Would a variety of different RNAs still arise?

3. Does RNA self-replicate?

4. Which amino acid is not mentioned in textbook Figure 2.6 as a source for synthesis of RNA bases?

   a. glutamine
   b. glycine
   c. aspartic acid
   d. serine
   e. none of the above

5. Are ribozymes (RNA enzymes) theoretical or laboratory constructs, or are they present in cells today?
6. Which building block helps maintain the informational integrity of DNA?
   a. uracil
   b. adenine
   c. thymine
   d. cytosine
   e. guanine

Energy Transformations Are Necessary to Sustain Living Systems

7. Osmosis tends to equalize concentrations on both sides of a membrane. Any living cell will have protein and nucleic acid inside, which "draws" water inward. To prevent bursting, concentration of something inside the cell has to be made lower than the concentration outside. Concentration of what? How is this adjustment made?
8. Would the structure of an ion-driven ATP synthase have to be different from that of an ATP-driven ion pump?
9. What is the advantage to the use of oxygen in metabolism?

Cells Can Respond to Changes in Their Environments

10. What signal causes aggregation of Dictyostelium slime mold amoebae into mobile slugs?
11. Actin is an important part of human muscle. It is equally important in other species including amoebas and slime molds. Is it surprising to find the same protein in such diverse species?

ANSWERS TO SELF-TEST

1. b. CO_2. Interestingly, modern theories based on observations of atmospheres of other planets, and observations of the geochemistry of early minerals, hold that there was much more carbon dioxide (CO_2) than hydrogen (H_2) in the Earth's early atmosphere.
2. Yes. Variability should remain constant, but the variant RNAs would presumably remain in low concentrations or disappear, and the original RNA would probably remain dominant.
3. No. Despite much work to find a self-replicating RNA, the replication always requires the presence of protein. Recent work by David P. Bartel at MIT is showing some promise toward finding an RNA replicase ribozyme (*Science* **292**[5520]:1319). The fact that an RNA replicase may be produced in the laboratory does not, of course, prove that the ribozyme existed in nature.
4. d. Serine. In modern cells, the glycine plus two of the other carbons of the purine ring can originate as parts of the amino acid serine.
5. Ribozymes are easy to find in modern cells, and probably the most abundant one is the ribosome where peptide bonds are formed. Several others exist including certain ribonuclease enzymes.
6. c. Thymine. All of the other building blocks are found in RNA. Uracil is only found in RNA. Thymine replaces it in DNA.

7. Small ions including sodium and protons (H^+) are routinely pumped out of the cell. This allows the outward osmotic pressure generated by the ions to match the inward pressure generated by cellular macromolecules.
8. No. In fact, textbook Figures 2-16 and 2-17 depict the same system functioning inward or outward. And in living cells the structures are the same or very similar.
9. While aerobic cells have to have protection against oxygen damage, the rewards for dealing with oxygen are great. As stated in the text, glucose metabolism using oxygen affords 15 times as much ATP as anaerobic glucose metabolism. Thus anaerobes have to ingest 15 times as much sugar to do the same work as aerobes. Use of other fuels also produces much more ATP in aerobic cells. It is also true that using oxygen as an electron acceptor can aid in maintenance of a proton gradient.
10. Cyclic AMP causes the cells to aggregate into a multicellular organism. cAMP is found as a kind of "hunger signal" in many different organisms, from procaryotes to man.
11. Considering the "Unity of Biochemistry" perhaps it is more surprising that actin does not appear to play the same role in procaryotic cells. But actin is found in essentially all eucaryotes in a similar role, often paired with myosin as the contractile apparatus.

PROBLEMS

1. Stanley Miller's experiments are called the "Primordial Soup Theory." There are other schools of thought not mentioned in the chapter, notably Günter Wächtershäuser's Pyrite World. He suggests that early life might have lived in the hot sulfur-rich environment near deep volcanic vents, and that precellular reactions could have taken place on the surface of pyrite crystals. One disadvantage is the extreme heat and pressure—over 110°C—but that environment is rich in life today. Can you think of other advantages or disadvantages of the Pyrite Theory versus the Soup Theory?
2. The RNA from Phage Qβ was shown to evolve in an artificial system with no membranes or cells. Why is it so important that organisms should have had membranes for them to evolve efficiently? What is the difference?
3. The antibiotic peptide, gramicidin, is assembled (in modern cells) without the use of RNA. Peptide bonds are formed after the amino acids are activated by attachment to sulfur on the enzyme surface. Does this suggest an alternative, or a precursor, to the RNA world described in the chapter?
4. RNA bases are built from amino acids. Thus amino acids (which are produced in the Urey Miller experiment) are older than RNA building blocks (which are not produced in this experiment). Is it reasonable that the only use to which amino acids were put was synthesis of RNA building blocks?
5. DNA has a remarkable ability to preserve complex information perfectly intact for millennia. Would it be a favorable situation if DNA could always be reproduced with absolutely no errors, and never had any mutations?
6. Theorists of the RNA World have debated whether the constituents of the cell arose in the sequence RNA-DNA-Protein or RNA-Protein-DNA. The universal use of ribonucleotide reductase enzymes provided an answer to this question. Can you see why?

7. If arabinose is the only source of carbon, E. coli cells utilize it for metabolism. The system described in this chapter apparently is driven only by the presence of arabinose. What if glucose and arabinose are present in equal concentrations? The arabinose would not be the "sole source." Is there an implication that the cell also checks for the absence of glucose?
8. Scientists know that the Earth's early oceans around three billion years ago were very rich in dissolved iron salts, including ferrous chloride ($FeCl_2$). Many ferric compounds, including ferric oxide (Fe_2O_3), are rather insoluble in water. Given these facts, what kind of hard evidence would you look for to prove that oxygen entered the atmosphere about two billion years ago, as shown in textbook Figure 2.27?

ANSWERS TO PROBLEMS

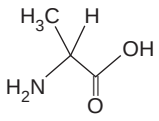
1. One major advantage of the "hot deep" origin of life is the fact that at volcanic vents, one finds metal sulfides that would be insoluble at cooler temperatures, and hydrogen sul fide gas (H_2S). These can combine to form pyrite or "Fool's Gold" ($H_2S + FeS \longrightarrow FeS_2 + H_2$). Thus a deep-sea volcanic vent is a reducing environment (with electrons from hydrogen [H_2]), and spontaneous synthesis of both amino acids and peptides has been observed in laboratory simulations of this environment. Much of the work and theory has come from the collaboration of Claudia Huber and Günter Wächtershäuser (recent papers published in *Science*). It is especially important to identify a terrestrial system with reducing properties now that the Earth's early atmosphere is thought to have been a CO_2 greenhouse and not reducing at all. The obvious disadvantage is that organic compounds can be destroyed by the extremely hot environment. But the fact that there are abundant living organisms at the vents illustrates that this is a problem that life has solved. (Huber & Wächtershäuser. *Science* **281**[5377]: 670.)
2. Spiegelman's RNA system with a replicase enzyme is very artificial; there is only one molecule being reproduced. A living cell has many constituents, and part of the competition in evolution involves which cell has the best mixture of constituents. The whole organism must evolve, with all its parts. This cannot happen in a "soup"; it requires individuals surrounded by a barrier, hence a membrane.
3. Yes, it does. While the thioester method of peptide synthesis used in making gramicidin is cumbersome compared to RNA-directed peptide synthesis, it does suggest that proteins might be able to self-replicate. Several prominent theoreticians including Graham Cairns-Smith, Freeman Dyson, Robert Shapiro, and the Nobel laureate Christian de Duve see a period before the "RNA World" in which proteins are the dominant cellular macromolecule, and many aspects of metabolism would resemble what is seen in modern cells. The use of ATP and other nucleotides as energy currency in a very primitive system would lead naturally to an environment where RNA synthesis could occur spontaneously. This is in contrast to the "Primordial Soup" where nucleotides would be unstable and probably quite rare.
4. Not really. A system rich in amino acids would have at least some peptides. And there are many processes that are easily catalyzed by simple proteins but have never been demonstrated using RNA ribozymes. An example would be the sort of electron transfer mediated by iron sulfur clusters. Cellular synthesis of purines and pyrimidines must be very ancient, but it would seem likely that these are merely representatives of many other processes involving amino acids and peptides.

5. No, it would not be favorable. While some critical genes, such as those for the histone proteins found in the nuclei of eucaryotes, appear to remain pristine and never change, in fact there must be variation that is ruthlessly trimmed by selection. A lack of variation, of mutation in the DNA, would lead to an end to evolution. We would be "stuck" with the species that lived millions of years ago, or more accurately, "we" would never have come into existence. Considering the fact that DNA must vary, it is quite interesting that some of the earliest microfossils found by J. William Schopf and others appear to be cyanobacteria, or blue-green algae, which are morphologically almost identical to pond-scum living today. This is despite the fact that one or two billion years separate the fossils from the living examples (e.g., Entophysalis, living today, and Eoentophysalis, 2.1 billion years old [see *Cradle of Life,* Schopf, Princeton 1999, p. 229]).
6. The mere fact that DNA building blocks are made from RNA building blocks shows that DNA is newer than RNA. But the fact that, universally, DNA building blocks are produced by a protein enzyme proves that protein also came before DNA. Note that it does not resolve the question of whether the correct sequence is RNA-Protein-DNA or Protein-RNA-DNA. There are several other indications that DNA is a "recent" development, including RNA genomes in some viruses, and inconsistencies in DNA structure and usage. There are some eucaryotic species (dinoflagellates) which use 5-Hydroxymethyluracil instead of Thymine, for example. The fact that DNA is bound to histones as chromatin in most eucaryotes is very different from the way DNA is handled in procaryotes.

   Freeland, Knight, & Landweber. *Science* **286**(5440): 690.
7. Yes, there is. If there were no mechanism to check for the presence of glucose, then arabinose or other sugars would be utilized whenever they were present, and not only when they were the "sole source." In fact a second messenger mentioned in this chapter, cyclic AMP, is generated when glucose is absent. This "hunger signal" then allows the use of other sugars.
8. The evidence is as hard as iron! Geologists know that the best iron ore is found in "banded iron formations," which are usually in layers that were part of the ocean floor around two billion years ago. These attractive red-layered formations represent the precipitation of most of the dissolved iron in the world's oceans as hematite, magnetite, and other insoluble ferric salts. The age of these layers can be clearly established by isotopic dating. The emergence of more abundant oxygen is the only possible explanation for this worldwide chemical reaction.

EXPANDED SOLUTIONS TO TEXT PROBLEMS

1. For alanine, the NH_2 would come from NH_3; CH_3, CH, and the other carbon from CH_4; OH and the other oxygen from H_2O. (Some hydrogens could also be replaced from H_2 if lost in earlier oxidation reactions.)



2. The lone fast-replicating molecule will complete three "generations" for every replication of the 99 other molecules. After n "generations," each of 15 minutes duration, therefore,

one population will be $(99)(2^n)$, while the other population will be $(1)(2^{3n})$. The results will be:

Generation	# Slow	# Fast	% Slow	% Fast
n	$(99)(2^n)$	$(1)(2^{3n})$		
0	99	1	99.00%	1.00%
1	198	8	96.12%	3.88%
10	1.0×10^5	1.1×10^9	0.01%	99.99%
25	3.3×10^9	3.8×10^{22}	0.00%	100.00%

3. The more tightly bound nucleotide monomers would be more available for RNA replication and could therefore cause a faster rate of replication. This advantage would be most important if the monomers were in short supply, that is, present only in low concentrations in the solution (environment).
4. Chemical or physical equilibrium between two compartments would require the same ion concentrations in both compartments (a state of high entropy). To establish a gradient with unequal ion concentrations in the two compartments would require work to impose more order on the system (and move the system to a state of higher energy and lower entropy). (Consider also a bag of 100 red marbles and another equivalent bag that has 100 green marbles. It requires less effort [energy] to allow the marbles to mix together in a single bag than it does to separate the mixture back into the original all-red and all-green compartments.)
5. If a "gate" is opened to allow protons to flow out of the cell, then energy will be released. If some of this energy could be captured for useful work, then the energy could be used for pumping a second type of ion out of the cell? (E.g., a proton ATPase would couple the synthesis of a high-energy bond in adenosine triphosphate (ATP) to the release of a proton gradient; the chemical energy stored in the ATP could then be used for another purpose, such as pumping the second ion.)
6. Eight protons, because the generation of hydroxide ion on one side is equivalent to the generation of a proton on the other side.
7. Very hydrophobic molecules could cross the cell membrane without the assistance of a transport protein. For these molecules, therefore, only a gene-control protein would be needed.
8. From the early part of the time scale in Figure 2.26, it appears that there are between five and seven cycles of approximately synchronous division before the respective cell division rates diverge.